Insect Resistant Maize

Recent Advances and Utilization

Proceedings of an International Symposium held at CIMMYT

John A. Mihm

CIMMYT

World Development

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John A. Mihm

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World Development

CIMMYT is an internationally funded, nonprofit scientific research and training organization. Headquartered in Mexico, the Center works with agricultural research institutions worldwide to improve the productivity and sustainability of maize and wheat systems for poor farmers in developing countries. It is one of 16 similar centers supported by the Consultative Group on International Agricultural Research (CGIAR). The CGIAR comprises over 50 partner countries, international and regional organizations, and private foundations. It is co-sponsored by the Food and Agriculture Organization (FAO) of the United Nations, the International Bank for Reconstruction and Development (World Bank), the United Nations Development Programme (UNDP), and the United Nations Environment Programme (UNEP).

Financial support for CIMMYT's research agenda currently comes from many sources, including the governments of Australia, Austria, Belgium, Canada, China, Denmark, the European Union, the Ford Foundation, France, Germany, India, the Inter-American Development Bank, Iran, Italy, Japan, the Kellogg Foundation, the Republic of Korea, Mexico, the Netherlands, Norway, the OPEC Fund for International Development, the Philippines, the Rockefeller Foundation, the Sasakawa Africa Association, Spain, Switzerland, the United Kingdom, UNDP, the USA, and the World Bank

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Abstract: This publication reports advances in worldwide research on the mechanisms and bases of insect resistance in maize; the genetics of resistance; on the biotechnological manipulation of resistance; on techniques for the mass rearing of pests, for scoring damage, for conducting bioassays, and for detecting resistance mechanisms; and on the verification and use of resistance. It also describes maize insect pests and related research in specific countries and regions.

AGROVOC Descriptors: Maize; Zea mays; Hybrids; Plant breeding; Pest resistance; Chilo; Diatraea; Sitophilus; Lepidoptera; Root eating insects; Stem eating insects; Leaf eating insects; Pest control; Biological control organisms; Research projects.

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Contents

The genetics of resistance

Biotechnological manipulation of resistance

Advances in techniques (rearing, rating bioassays, mechanism detection)

Resistance verification and utilization

Acknowledgments

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John A. Mihm

Foreword

D.C. Hess, Director, CIMMYT Maize Program

Future Opportunities for Host Plant Resistance Research in the CIMMYT Maize Program

First, let me say that I have personally enjoyed the past four days listening to the some 65 presentations concerning the various aspects of host plant resistance. There is no question but that there has been more experts and expertise on maize host plant resistance here at this conference than ever before in a similar gathering. It is obvious that since the last similar conference held here in 1987, many scientific disciplines have become involved in team efforts to understand the mechanisms and intensify the efforts in increasing the effectiveness of host plant resistance.

The Importance of Host Plant Resistance

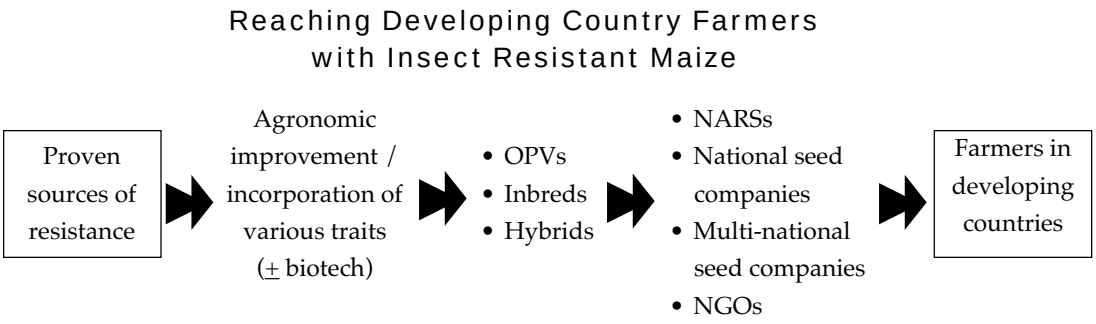
I would like to address the question of why insect host plant resistance is important to the CIMMYT Maize Program. Let me remind you that the mission of the Maize Program is "to help the poor of developing countries by increasing the productivity of resources committed to maize while protecting natural resources." Maize that can be grown by resource poor farmers without being vulnerable to attacks by insects and without needing the application of usually scarce, expensive and often dangerous insecticides help these farmers increase their production of an essential food product, while protecting the environment.

Another reason host plant resistance is important to the CIMMYT Maize Program is because it is a complex trait. National programs of the developing countries often find it beyond their capability to effectively manage this trait, although I hasten to say that there are some programs that have quite successful HPR programs. The trait is also not one that fits private seed companies very well, as they are often required to apply their resources on more short-term research projects. Smaller local seed companies usually find such complicated traits well beyond their very limited resources. It is also interesting to note that the areas of the developing world that need maize resistant to tropical insects are often the areas the multinational seed companies find less attractive markets. Since the trait is complex, it lends itself to the application of more advanced scientific techniques such as marker assisted selection.

As we have heard several times during the week, host plant resistance is an important component of integrated pest management (IPM). In fact some would contend that it is by far the most important component of integrated pest management programs. Some of you are aware that there is an effort to establish an IPM facility which will be a collaborative effort by important funders to insure that IPM activities are emphasized and well supported throughout the world. The organizations behind this movement are the World Bank, UNDP, UNCED and USAID. This initiative should certainly boost the IPM efforts and along with them the strengthening of host plant resistance work. I hope that you all agree with me that HPR is an important component of IPM and will be influential whenever possible in assuring that HPR is included in IPM projects.

Getting HPR Maize to Farmers

I know you have seen about as many slides and overheads this week as you can stand; however, I would like to show just one more:



The diagram represents a series of steps that must occur in order for us to fulfill the objective of making insect resistant maize available to farmers; in CIMMYT's case, to farmers of the developing world. Beginning at the left side of the diagram, I believe significant progress has been made in this area, largely due to the efforts of individuals in this room, especially those of John Mihm, Frank Davis, and Bill Wiseman. Through what we would now call conventional methods, these and other scientists have proven beyond a doubt that effective insect resistance maize is available and with enough effort the trait can be transferred to all types or genotypes of maize. This is not to say we are done with this part of the equation; there is certainly more work to do in this area and we do not yet know the limit that can be reached with host plant resistance.

As we move to the next step of improving the insect resistant lines or varieties for agronomic and other traits, we are not so far along, at least at CIMMYT. A tremendous amount of work will be required to accomplish this task and, even after resistance is available in more productive and acceptable genotypes, they will have to be tested in new open pollinated varieties, inbreds and hybrids. We at CIMMYT will be making a concentrated effort to move the present level of resistance into the mainline breeding programs to help with this step.

The next step is to deliver the products to those that can be effective in further research and evaluations, developing and recommending specific products for specific ecologies. These include national agricultural research programs, national and local seed companies as well as multinational seed companies, and non governmental organizations. And of course the final test is that of the farmers themselves. Unfortunately, at this time satisfactory insect resistant products have not been made available to farmers in any significant manner, especially in CIMMYT's target areas in developing countries.

The above challenges are far too large to be accomplished by a single organization but will require the efforts and linkages of all of our organizations. We think it is so important that we are contemplating developing a global special project that would enable us, working with others, to enhance the possibilities of success in accomplishing these goals. Certainly we at CIMMYT consider host plant resistance work one of our primary objectives. We believe that the time is right for host plant resistance to make significant impacts on the developing world , since the needs are so clear and the benefits of insect resistant maize are so great, for both productivity and the environment. We look forward to joining all of you in working on these very important tasks.

An Overview of the Mechanisms and Bases of Insect Resistance in Maize

C. M. Smith, Department of Entomology, Kansas State University

Abstract

Many insect resistant maize varieties have been developed during the past 50 years, due to the development of highly efficient techniques for maize insect pest rearing, artificial infestation and damage evaluation. Through the efforts of an international working group of scientists, maize genotypes developed primarily from the Antigua Group 2 gene bank and selected from it at CIMMYT have been shown to be resistant to many of the major lepidopterous pests of maize in the world. In several resistant varieties, resistance is controlled by different allelochemicals. The cyclic hydroxamic acid DIMBOA, and its decomposition product, 6-MBOA, occur in the foliage of some resistance sources. The flavone glycoside maysin and its related luteolin c-glucosides occur in the silks of other resistant varieties. These allelochemicals kill or impair the growth of many of the major insect pests of maize. Several morphological factors, including increased leaf fiber content, increased silica content, increased vascular bundle density, increased husk tightness and decreased leaf trichome density also contribute to some sources of resistance that do not have high levels of DIMBOA or maysin. Insect resistant maize greatly increases farming efficiency since insect control is available for the cost of only the seed. In addition, research on developing resistant varieties provides 100- to 300-fold greater returns on investment than research to develop insecticides. During the past 20 years, insect resistant maize in the United States has helped prevent the application of several million tons of insecticides onto croplands, reduced insecticide rates and applications, and encouraged the use of biological and cultural insect control practices in integrated maize insect pest management programs. Several examples demonstrate how insect resistant maize varieties act synergistically with both biological and chemical insect control tactics. National agricultural program staffs in many countries should work jointly with scientists located at centers that are members of the Consultative Group for International Agricultural Research to train farmers about the benefits of insect resistant maize varieties in insect pest management and incorporate insect resistance genes into locally adapted varieties which possess grain quality and yield desirable to specific localized conditions.

Introduction

Though there are few written accounts, early farmers in Africa, the Americas and Asia probably selected edible plants resistant to insect pests and saved seed of these plants to continue growing them in successive years. Crops with insect resistant properties have helped United States agriculture for over 200 years. Wheat varieties with resistance to the Hessian fly (HF), *Mayetiola destructor* (Say), were grown in New York around the beginning of the 1800's.

Studies of insect resistant maize began in the early 1900's, when Hinds (1914) demonstrated the value of maize husk tightness and thickness for corn earworm (CEW), *Helicoperva zea* (Boddie), resistance and Gernert (1917) demonstrated that corn leaf aphid (CLA), *Rhopalosiphum maidis* (Fitch), resistance existed in teosinte x yellow dent corn hybrids. The first maize varieties with resistance to the European corn borer(ECB), *Ostrinia nubilalis* (Hubner) were studied by Huber et al. (1928). In research at CIMMYT, Elias (1970) and Peairs (1977) identified resistance to the sugarcane borer(SCB) *Diatraea saccharalis* (F.) in Caribbean and Mexican maize populations, respectively. Peairs (1977) also identified resistance to the fall armyworm(FAW), *Spodoptera frugiperda* (J.E. Smith) in tropical Mexican maize populations.

Over 300 varieties of insect resistant alfalfa, corn, sorghum, and wheat are grown presently in Africa, Asia, Europe and the United States. Of these, over one-half are cereal grains and many were developed by scientists at the International Maize and Wheat Improvement Center (CIMMYT) or

scientists around the world cooperating with CIMMYT researchers. In Missouri, a major U. S. maize producing state, over 75% of all varieties grown possess some resistance to whorl, leaf and sheath collar feeding of the ECB (Barry and Darrah 1991). Today, entomologists and maize breeders continue to make global progress toward the release and production of multiple insect resistant maize varieties. Through the efforts of an international working group of scientists, maize genotypes derived primarily from the CIMMYT Antigua Group 2 germplasm have been shown to be resistant to several major lepidopterous pests of maize in Africa, Asia, Latin America and North America (Ampofo et al. 1986; Dabrowski 1990; Dabrowski and Nyrangiri 1983; Davis and Williams 1986; Davis et al. 1988; Mihm 1985; Smith et al. 1989).

In the first textbook on insect resistance in crop plants, Painter (1951) described methods to measure plant resistance to insects. Since then, gas and high pressure liquid chromatography, x-ray crystallography and mass spectral analysis have become routinely used to quantify allelochemicals involved in maize resistance to insects.

Transmission and scanning electron microscopy also permit the study of the cellular as well as the whole structure morphological bases of insect resistant maize.

Artificial diets and rearing methods have been developed for many of the major maize pest insects of the world (Mihm 1983a,b,c; Ortega et al. 1980). These accomplishments have greatly increased the rate at which new sources of insect resistance have been identified. The invention and widely accepted use of a very simple plastic device, the bazooka or plant inoculator, that is used to infest plants with neonate lepidoptera larvae mixed in cob grits (Davis and Williams 1980; Mihm et al. 1978; Wiseman and Widstrom 1980; Wiseman et al. 1980) has greatly improved the efficiency and accuracy of many insect resistance plant breeding programs and tremendously accelerated the rate of progress of identifying sources of resistance in maize to many foliage feeding Lepidoptera.

In this paper, I will provide some working definitions on plant resistance to insects, discuss the advantages to the use of insect resistant maize, and review the allelochemical and morphological mechanisms of insect resistance in maize.

Economic Advantages

There is a major economic advantage to the use of insect resistant varieties by farmers. Insect resistant crops greatly increase farming efficiency by reducing or eliminating the costs of insecticides and reduce or eliminate the risk of yield losses from insect damage. When insect resistant varieties are planted, insect control is available for little more than the cost of the crop seed, and there is often no need or in many cases, a greatly reduced need to purchase insecticides or the equipment to apply them for pest control. The advantages to the use of insect resistant varieties are especially important in developing countries, where farmers can rarely afford to purchase insecticides for crop protection. In this setting, they provide practical and economical ways to minimize losses to insect pests (Mihm 1989). Many of these farmers have a limited access to insecticides, because they lack the income to purchase them or because there are no organized systems of pesticide distribution. Potential human health hazards are high with insecticide use, due to limited farmer training about insecticide application methods and often inadequate water supplies. The need for insect resistant maize varieties is also high in the tropics, since pest incidence is greater than in temperate regions, due to rapid pest population increases, which lead to several continuous pest generations each year. (Smith et al. 1989).

The effects of plant resistance to insects are cumulative over time, and the longer resistance is employed and effective, the greater the benefits of its use. Panda (1979) demonstrated an average 12-fold population reduction among 25 different insect pests damaging 10 food and fiber crops. In a 10 year study of rice insect pest related-crop losses in the Philippines, Waibel (1987) determined that the 10-year average yield losses of insect resistant rice varieties were approximately one-half (14%) of the losses in susceptible rice varieties (26%).

Plant resistance research provides a substantially greater return (as much as 120-fold greater) on each research dollar invested, compared to research on the development of insecticides. Since the late 1960s, wheat varieties with HF resistance have been proven to return approximately $600 per research dollar invested, compared to a $5 return per dollar spent on insecticide development (Painter 1968).

Insect resistant cultivars of alfalfa, corn, barley and wheat have been proven to have marked economic advantages in United States agriculture (Luginbill 1969; Maxwell et al. 1972; Painter 1968).

Based on reductions in the costs of insecticide applications and reduced insect damage, the value of insect resistant cultivars of these crops during the 1970's was nearly $500 million each year (Schalk and Ratcliffe 1976). Though insect resistant crops are sound economic investments for the agricultural economy of any country, United States crop production using insect and mite resistant alfalfa, barley, corn, sorghum, and wheat cultivars currently returns an economic benefit of over $1.4 billion each year.

Compatibility with Integrated Pest Management

Insect resistant maize varieties generally compliment integrated pest management (IPM) tactics such as chemical and biological insect control (Table 1.). Improved maize varieties resistant to the CEW require much less insecticide (in some cases, as much as 28-fold less) than susceptible varieties to achieve equivalent control (Wiseman et al. 1975). Insecticides applied to maize varieties with intermediate and high levels of resistance to the ECB are of little benefit in reducing borer damage in the field (Robinson et al. 1978).

Isenhour and Wiseman (1987) found a synergistic interaction between genotypes of maize resistant to FAW and its parasite, *Campoletis sonorensis* (Cameron). Parasitism results in further reductions in FAW larval weights over those caused by FAW consumption of resistant foliage alone and has no adverse effects on parasite development.

In research with *Cotesia marginiventris* (Cresson), a naturally occurring parasite of FAW, Riggin et al. (1992, 1994) demonstrated in laboratory and field studies that FAW-resistant maize varieties have no negative effect on the rate of FAW parasitism and that FAW larvae feeding on resistant plants are more heavily parasitized than those feeding on susceptible plants.

Wiseman et al. (1976) demonstrated that higher levels of the predator *Orious insidiosus* Say, are found on maize hybrids tolerant to CEW during and after silking. This interaction contributes to a greater suppression of CEW larval populations on the resistant hybrid than on susceptible hybrids.

The interactions of viruses and fungi with insect resistant maize varieties are not well known. However, Hamm and Wiseman (1986) confirmed the existence of a synergistic interaction between maize varieties resistant to leaf feeding by the FAW and the nuclear polyhedrosis virus (NPV). The protozoan parasite, *Nosema pyrausta* and maize varieties resistant to leaf and sheath-collar feeding by the ECB, interact to significantly reduce ECB populations (Lynch and Lewis 1976; Lewis and Lynch 1976).

Moderately insect-resistant crop varieties are normally compatible with different types of biological control. However, some resistant varieties that possess high levels of toxic plant allelohemicals or dense levels of leaf or stem trichomes have been shown to have negative effects on beneficial insects (Campbell and Duffey 1979; Obrycki et al. 1983). Similarly, allelochemicals mediating insect resistance in plants may adversely affect the synergism of resistance with NPV (Felton et al. 1987).

Plant breeding goals, however, normally strive to incorporate moderate levels of insect resistance in varieties with yield, processing and cooking qualities acceptable to farmers and consumers. Such varieties also guard against the development of resistance-breaking insect biotypes and insure a longer useful life of resistant varieties that work synergistically with natural enemies. The numerous advantages of the compatibility of maize resistance to pests with other IPM tactics are sufficient to indicate that varieties produced by all maize improvement programs should possess some level of insect resistance. Unfortunately, many current maize varieties have limited, if any, insect resistance.

Table 1. Examples of synergistic interaction of insect resistant maize with various integrated pest management tactics.

IPM Tactic	Insect affected	Reference(s)
Insecticidal	Corn earworm	Wiseman et al. 1975
	European corn borer	Robinson et al. 1978
Biological		
Archytus marmoratus and *Ichneumon promissorius*	Corn earworm	Mannion et al. 1994
Campoletis sonorensis	Fall armyworm	Isenhour and Wiseman 1987
Cotesia marginiventralis	Fall armyworm	Riggin et al. 1994
Nosema pyrausta	European corn borer	Lewis and Lynch 1976
Nuclear polyhedrosis virus	Fall armyworm	Hamm and Wiseman 1986; Wiseman and Hamm 1993
Orious insidiosus	Corn earworm	Wiseman et al. 1976

Environmental and Social Advantages

In addition to being compatible with IPM tactics and economically advantageous to farmers, insect resistant crop varieties, including some maize varieties, improve the quality of the environment and the general health of agricultural producers and consumers. By reducing the amount of insecticides applied in maize production, as shown above, insect resistant maize varieties increase the safety of food produced for animal and human consumption, protect water supplies from insecticide contamination and help improve the general quality of water resources.

Definitions

"Plant resistance to insects" is the genetically inherited qualities that result in a plant of one variety or species being less damaged than a susceptible plant lacking these qualities. Resistance is a relative property, based on the comparative reaction of resistant and susceptible plants, grown under similar conditions, to the pest insect. "Pseudo"**-** or "false resistance" may occur in susceptible plants due to earlier than normal planting, low levels of insect infestation, or variations in temperature, day length, soil chemistry and plant or soil water content. "Associational resistance" refers to a normally susceptible plant growing in association with a resistant plant, and deriving protection from insect predation. "Induced resistance", the enhancement of a plant's pest defense system in response to external physical or chemical stimuli, (Kogan and Paxton 1983) occurs in many crops due to the elicitation of endogenous plant metabolites (Pearce et al. 1991). Induced resistance may last from a few to several days.

Categories of Resistance

In addition to the types of resistance described above, three categories have been referred to since their description by Painter (1951). Antibiosis and nonpreference resistance describe the reaction of an insect to a plant, while tolerance resistance describes the reaction of a plant to insect infestation and damage. In antibiosis resistance, the biology of the pest insect is adversely affected after feeding on the plant. With nonpreference resistance (now referred to by many researchers as antixenosis (Kogan and Ortman 1978)), the plant is as a poor host and the pest insect then selects an alternate host. Plant tolerance describes the inherent genetic vigor or growth capacity of a resistant plant that gives it the ability to withstand or recover from insect damage that a susceptible plant cannot survive.

In describing his attempts to classify causes of plant resistance to insects, Painter (1951) stated "I have attempted to work out a classification of those items suggested as 'cause(s)' of resistance so as to emphasize the insect-plant interrelations that are a feature of insect resistance." Painter then presented the now classic diagram of the three-fold basis of field plant resistance to insects, consisting of what he termed the three bases or mechanisms of resistance. However, in the legend explaining the diagram, he referred to these as mechanisms of resistance classifications.

Horber (1980) referred to Painter's triad of resistance as a ... "workable compromise between mere categorization of phenomena and the basic study of the causative factors or processes." In his discussion of the different types of plant resistance to insects, Horber (1980) chose to describe the three elements of the resistance triad as functional categories of resistance. Smith (1989) termed these categories functional modalities of resistance.

According to *Webster's 7th New Collegiate Dictionary*, a "category" is a general class or group, and a "modality" is a classification or form. Conversely, a "mechanism" is a fundamental physical or chemical process involved in or responsible for an action, reaction or other natural phenomenon. The term "basis" refers to the foundation or principal component of anything. Thus, the terms category and modality refer to the way a group of items are classified, while the terms basis and mechanism denote the principal process governing a natural phenomenon.

In applying these terms to the study of plant resistance to insects, many examples exist to show that insects are affected by resistant plants in ways we categorize or classify as antibiosis or antixenosis, while plants themselves demonstrate tolerance as a third type of resistance. In contrast to Painter's use of the term, I propose that the term "mechanisms" be used to describe the underlying chemical or morphological plant processes that, where known, are responsible for the (negative) reaction of insects to resistant plants. To describe the outcome of insect-plant interactions, I propose the use of the term "categories" to refer to antibiosis, antixenosis and other as of yet undefined types of plant-insect interactions, observed as responses of

insects to plant resistance mechanisms. I will use these definitions throughout the remainder of this manuscript.

Often, antibiosis and antixenosis resistance overlap because of the difficulty involved in designing experiments to delineate between the two. Horber (1980) stated that "all three categories, while workable, are arbitrary and vaguely delineated," since not all resistance can be assigned into one of these categories. An insect confined to a resistant plant may fail to gain weight at the rate it normally does on a susceptible plant, due presumably to the presence of antibiotic properties in the plant. However, reduced weight gain may also be due to the presence of an antixenotic physical or chemical feeding deterrent that causes aberrant behavior in the test insect, resulting in a weakened physiological condition.

Antibiosis exists in maize to the aphid *Metopolophium dirhodum* (Walker) (Argandona et al. 1980); the CLA (Long et al. 1977); the CEW (Waiss et al. 1979; Wiseman et al. 1992a,b) the ECB (Klun et al. 1970; Robinson et al. 1982b); the FAW (Hershey 1978; Wiseman et al. 1981) and the southwestern corn borer(SWCB), *Diatraea grandiosella* Dyar (Davis et al. 1989). Antixenosis exists in maize to the CEW (Wiseman et al. 1977), the ECB (Robinson et al. 1978), the FAW (Wiseman et al. 1981), the maize weevil (MW), *Sitotroga zeamais* Motchulsky, the rice weevil (RW), *Sitophilus oryzae* (L.) (Singh et al. 1972; Wiseman et al 1974) and the SWCB (Davis et al. 1989). Smith (1982) identified both antibiosis and antixenosis resistance to FAW in certain Caribbean maize germplasm.

Very detailed sets of experiments are normally required to delineate the actual contributions of plant factors to each category of resistance. From a practical standpoint, the absolute contribution of a given category may never need to be fully understood before a resistant variety is released.

From an ecological and environmental standpoint, tolerance has many advantages, since it does not adversely affect beneficial insects or exert sufficient selection pressure on pest insect populations to develop biotypes as does antibiosis alone. Often however, agricultural producers tend to prefer varieties with antibiosis and antixenosis resistance that reduce pest abundance. We, as conscientious agricultural researchers also often screen for antibiosis and antixenosis in developing maize varieties. However, tolerance in maize to the northern corn rootworm (NCRW), *Diabrotica barberi* Smith and Lawrence, the western corn rootworm (WCRW), *Diabrotica virgifera virgifera* LeConte, the CEW, the maize borer, *Chilo partellus* (Swinhoe) and ECB, are well documented (Dabrowski and Nyangiri 1983; Mollenbeck et al. 1994; Ortman et al. 1968; Wiseman and Widstrom 1992; Wiseman et al. 1972; Zuber et al. 1971). At CIMMYT, Hershey (1978) identified several progeny from three tropical maize populations with tolerance to the FAW and Smith (1982) developed moderate levels of FAW tolerance in selected lines of Tuxpeno germplasm.

Allelochemical and Morphological Mechanisms of Resistance

Both chemical and morphological maize defenses mediate resistance to insect pests. Resistance may be due to the presence of olfactory repellents, feeding or oviposition deterrents, and toxins, or, the absence of feeding or oviposition stimulants. In one instance, the lack of nutrients has been shown to affect insect resistance in maize. Penny et al. (1967) determined that maize resistant to ECB larvae had an ascorbic acid content that was inadequate to support normal ECB larval growth. Resistance may also be a result of the density of external or internal plant structural features that either alter insect behavior or reduce insect digestion. In some maize varieties, the content of silica containing cells is high enough to adversely affect ECB larval feeding and impart some resistance to ECB (Rojanaridpiched et al. 1984).

The lethal effects from both allelochemical and morphological factors may be acute, often affecting young larvae, or chronic, and lead to mortality in older larvae, prepupae, pupae, and adults, where larvae and pupae fail to pupate and eclose, respectively. Individuals surviving the direct effects of these plant defenses may exhibit the debilitating effects of reduced body size and weight, prolonged periods of development in the immature stages, and reduced fecundity as surviving adults.

Plant Allelochemicals

Organic acids were some of the first allelochemicals found to mediate antibiosis to insects in several maize varieties. An aglucone in maize foliage, 2,4-dihydroxy-7-methoxy-2H-1, 4-benzoxazin-3(4H)-one, (DIMBOA) is one of the more widely studied plant allelochemicals affecting crop resistance to arthropods. When normal, healthy maize foliage is mechanically damaged, the glucoside, 2-0-glucosyl-4-hydroxy-1, 4-benzoxazin-3-one, is enzymatically converted to DIMBOA

(Fig. 1) (Loomis et al. 1957; Smissman et al. 1957; Wahlroos and Virtanen 1959). DIMBOA and its decomposition product, MBOA have antibiotic effects on the ECB (Barry et al. 1994; Campos et al. 1988; Klun and Brindley 1966; Klun et al. 1967; 1970, Robinson et al. 1982b), and limited antibiotic effects on the SWCB and the FAW (Nicollier et al. 1982). Robinson et al. (1982a) developed an accurate, efficient thin layer chromatography (TLC) technique to identify maize lines with high concentrations of MBOA for ECB resistance. Barry et al. (1994) surveyed ECB leaf feeding resistance and DIMBOA content in progeny of crosses of resistant and susceptible maize varieties and found the two traits to be positively correlated. Their results and those of Sullivan et al. (1974) however, indicate that some maize germplasm that resists ECB leaf feeding does so without a high DIMBOA content.

The CLA and the aphid *Metopolophium dirhodum* (Walker) are also adversely affected by DIMBOA (Argandona et al. 1980; Long et al. 1977). CLA population levels sustained on various maize varieties are strongly correlated to the DIMBOA concentration of each variety (Beck et al. 1983). HMBOA (Fig. 1), another intermediate degradation product of DIMBOA (Feng et al. 1992; Kumar et al. 1994) may also have toxic effects on ECB. *N-O*-ME DIMBOA (2-hydroxy-4, 7-dimethoxy-1,4-benzoxazin-3-one), yet another related compound, exists in higher concentrations than DIMBOA or MBOA in the surface waxes of some SWCB-resistant maize varieties derived from CIMMYT germplasm (Hedin et al. 1993). Total surface wax content of these varieties is higher than in susceptible varieties.

Feng et al. (1990, 1992) demonstrated that ingestion of DIMBOA and MBOA by ECB greatly increases the levels of activity of several detoxification enzymes, including cytochrome b_5, NADH oxidase, NADH cytochrome c reductase and *o*-demethylase.

Xie et al. (1990, 1992) demonstrated that CIMMYT maize lines developed by Agriculture Canada with high DIMBOA root content negatively affect the emergence of WCRW adults and that one high DIMBOA line is significantly less damaged by CRW larvae than a low DIMBOA line. Although MBOA has been shown to be toxic or deterrent to several insects, Bjostad and Hibbard (1992) found that MBOA functions as a volatile attractant to WCRW in combination with carbon dioxide. Related research (Aboufakhr et al. 1994) has demonstrated that MBOA is non-toxic to WCRW larvae. Other major foliage or stem feeding lepidopterous pests of maize do not suffer significant adverse effects from DIMBOA or MBOA.

The flavone glycoside maysin (Fig. 2), is an allelochemical contained in the silks of maize varieties resistant to CEW and FAW (Waiss et al. 1979; Ellinger et al. 1980; Wiseman et al. 1992a). Increasing

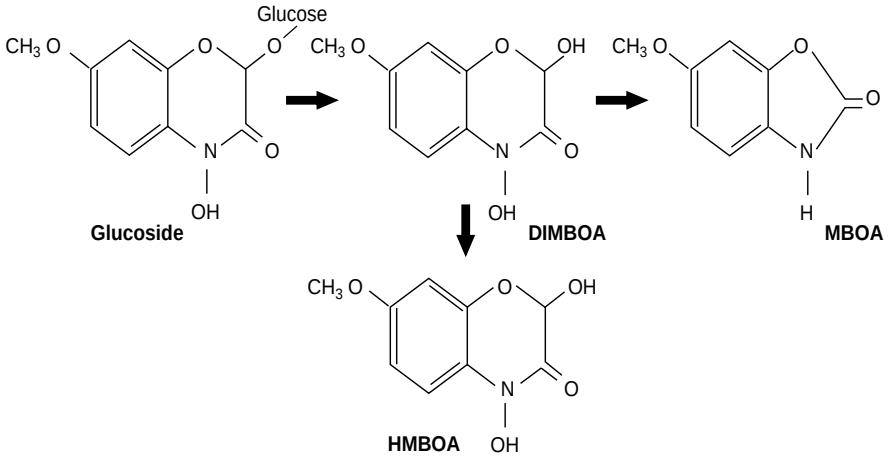


Figure 1. Production of DIMBOA (2,4-dihydroxy-8-methoxy-2H-1, 4-benzoxazin-3(4H)-one), MBOA (6-methoxyben-zoxazolinone) and HMBOA (2-hydroxy-7-methoxy-1,4-benzoxazin-3-one) by enzymatic hydrolysis of a glucoside of mechanically damaged maize foliage (from Campos et al. 1988; Feng et al. 1992; and Klun et al. 1967).

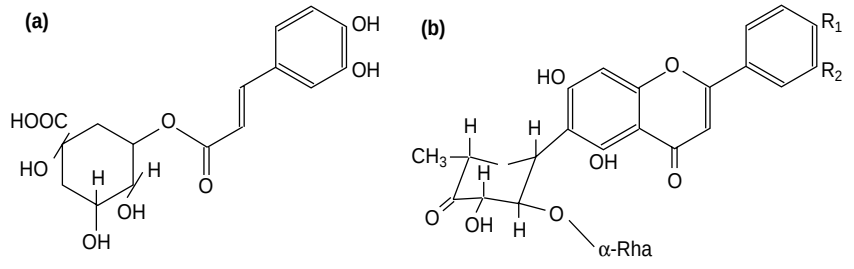


Figure 2. Chlorogenic acid (a) and the related flavonoid glycosides (b) maysin (R_1 = OH, R_2 = OH), apimaysin (R_1 = CH_3, R_2 = CH_3) and 3'-methoxymaysin (R_1 = CH_3, R_2 = OH) from foliage of insect resistant maize cultivars which inhibit growth of the corn earworm, *Helicoverpa zea* Boddie and fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Gueldner et al. 1991; Wiseman et al. 1992a).

the concentration of maysin in artificial diets inhibits the growth of these insects proportionally (Wiseman et al. 1992a). The related luteolin c-glycosides chlorogenic acid, apimaysin (the apigenin analogue of maysin) and 3' -methoxymaysin (Fig. 2) may also contribute to the resistance of maize to the CEW and the FAW (Gueldner et al. 1991,1992; Wiseman et al. 1992b).

Growth inhibition in insects feeding on resistant maize may also be related to altered nutrient levels. Early research conducted by Penny et al. (1967), determined that maize resistant to ECB larvae had an ascorbic acid content inadequate for larval growth.

Plant Morphology

Several types of morphological defenses in maize varieties deter insect feeding and oviposition (Table 2). As previously mentioned, increased leaf and stem silica content contribute to ECB resistance in some maize varieties (Rojanaridpiched et al. 1984). Tight-husked maize ears, a character also mentioned previously, continue to contribute the resistance of current varieties to the CEW (Wiseman and Widstrom 1992; Wiseman et al. 1977), the MW, (Wiseman et al. 1974) and the RW (Singh et al. 1972). Maize varieties with reduced trichome density and delayed development of pubescence have been shown to be less preferred for oviposition by CEW and are resistant to larval feeding (Wiseman et al. 1976; Widstrom et al. 1979). At CIMMYT, screening and breeding maize for oviposition nonpreference is avoided, since moth oviposition behavior can evolve to overcome the oviposition resistance of germplasm and because soil and environmental factors interact to make adult oviposition behavior measurements difficult to reliably predict (Mihm 1989).

Several maize inbred (Mp) lines developed jointly by scientists at the USDA Crop Science Research Laboratory at Mississippi State, Mississippi (including Mp496, 701, 704, 706, and 708) have morphological defenses related to their resistance to the CEW, the ECB, the FAW, the SWCB and the SCB (Davis et al. 1988). Hedin et al. (1984) demonstrated that Mp701 and Mp496 have higher hemicellulose and crude fiber content than susceptible inbred lines, and that crude fiber is negatively correlated with SWCB larval feeding damage. Ng (1988) found that Mp701 has more vascular bundles, thicker cuticle and a thicker outer epidermal cell wall than susceptible inbred lines. Recent results by Davis et al. (1995) with Mp496, 704, 706 and 708 confirm these findings and also demonstrate that inner whorl leaves of these Mp inbred lines have thicker leaves and thicker upper and lower leaf epidermal cell walls than susceptible inbred lines. Leaf feeding damage by SWCB and FAW larvae is highly correlated with epidermal cell wall thickness. In research with another Mp resistance source, MpSWCB-4, Yang et al. (1991, 1993) determined that removal of leaf cuticular lipids from whorl leaves removes resistance to FAW larval feeding. Gel electrophoresis of the total leaf protein extracts from field grown tissues of Mp496, 701, 707, and 708 has identified polypeptides which predict SWCB and FAW resistance (Callahan et al. 1992).

Many of these Mp lines have been used as resistance components to develop the CIMMYT multiple borer resistant (MBR) maize population 590 (Benson 1986). Bergvinson (1993) found significant correlations between the leaf fiber content and cell wall dehydrodiferulic acid content of MBR lines with ECB leaf feeding damage, and that leaf toughness was inversely related to leaf feeding damage.

Table 2. Morphological defenses of insect resistant maize.

Defense	Insect(s) affected	Reference(s)
Dense surface waxes	Southwestern corn borer Fall armyworm	Hedin et al. 1993 Yang et al. 1991,1993
High fiber, dense vascular bundles, high hemicellulose, thick cuticle	European corn borer Fall armyworm Southwestern corn borer Sugarcane borer	Bergvinson 1993, Davis et al. 1995, Hedin et al. 1984, Ng 1988
Low trichome density	Corn earworm	Widstrom et al. 1979, Wiseman et al. 1976
Silica	European corn borer	Rojanaridpiched et al. 1984
Tight husks	Corn earworm	Wiseman et al. 1977, Wiseman and Widstrom 1992
	Maize weevil Rice weevil	Wiseman et al. 1974 Singh et al. 1972

Genetically Transformed Maize

New discoveries in crop plant molecular genetics are occurring rapidly, and maize insect resistance research is currently moving molecular biology into maize production and protection (Koziel et al. 1993). Within

the next five years, hybrid maize containing transgenic insect resistance will be sold commercially in the United States. The resistance factor(s) in these hybrids is derived from the HD-1-delta-endotoxin gene that encodes plant DNA to produce a crystal protein from the bacteria *Bacillus thuringiensis* (*B.t.*). The protein is toxic to insects but not to mammals. Research during the next decade will attempt to develop gene release strategies that maximize the life span of different *B.t.* genes for insect resistance in maize and other crops. CIMMYT's varietal release strategy is to pyramid *B.t.* genes into maize populations with existing multigenic pest resistance, in order to enhance both the levels and durability of plant resistance to maize pests.

There is a real need for varietal release strategies that avoid promoting the development of resistance-breaking insect biotypes similar to those that have developed resistance to insecticides. Such strategies are necessary because of the high potential that exists for the selection of *B.t.*-resistant pest populations when seed of transgenic crops are marketed for production. Gene release strategies are especially necessary for highly polyphagous pest insects, such as migratory Lepidoptera that will be exposed to the *B.t.* toxin in maize and other crops in the same agroecosystem.

The development of successful gene release strategies will depend on the ability of researchers in government, industry and universities to cooperatively conduct field experiments that test several different types of gene release techniques. An additional factor that will directly affect the success of the development of transgenic plant release strategies will be the selection of well-defined, functional IPM systems in which to test different release strategies.

Induced Resistance

New discoveries in the area of induced plant resistance to arthropods indicate that this physiological process is likely a part of a general maize plant protection mechanism against insect damage. Guiterrez et al. (1988) demonstrated that in a maize variety with high DIMBOA content and resistance to the maize borer (MB), *Sesamia nonagrioides*, and in a variety with low DIMBOA content and susceptibility to MB, both varieties contained significantly increased leaf DIMBOA content within 3 days of MB infestation. Thus, the existence of the same physiological phenomenon in both insect-resistant and susceptible maize varieties indicates the possibility of using the inherent induced response of all maize genotypes to develop types of insect resistance to complement previously identified allelochemical and morphological based sources of maize resistance to insects.

Callus Tissue Culture

The callus tissues of some maize varieties exhibit resistance to the FAW, SWCB and CEW that closely resembles damage to whole plant foliage (Williams and Davis 1985; Williams et al. 1985, 1987a, 1987b; Isenhour and Wiseman 1988). Isenhour and Wiseman (1991) isolated somaclonal variant plants regenerated from callus tissues of maize genotypes resistant to FAW that have greater levels of FAW resistance than non-regenerated lines. The use of regenerated lines in a breeding program for enhanced insect resistance should proceed with caution, however, as field screening of the somaclonal variants indicated above did not prove to be highly resistant to FAW in field trials at CIMMYT (Mihm et al. unpublished manuscript).

Summary and Conclusions

During the past thirty years, numerous sources of multiple insect resistant maize germplasm have been developed, and a detailed understanding of the allelochemical and morphological mechanisms of some of this germplasm has begun to be understood. The production of maize varieties with genetically-expressed pest resistance has improved farming profitability and environmental safety in many developed countries. Techniques invented by maize researchers in developing these varieties have also provided many benefits to global agricultural research and production. These have all been truly remarkable developments.

However, these accomplishments are yet to result in a corresponding increase in the use of insect resistant maize by farmers in many developing countries of the world. In the next five years, Africa's population will grow at a rate of 3% annually, but food production will increase by only 2% each year, computing to an annual African food production shortage of about 250 million tons by the end of the century (Anonymous 1992). African food production capabilities have steadily eroded over the past 20 years, but there is limited use of IPM or insect resistant crop varieties in most of African agriculture.

With increasing demands for an abundant and safe world food supply, there are many countries where insect resistant maize can make an important

difference. What will the strategies be for the 21st century to ensure deployment of insect resistant maize varieties? I believe a real challenge now exists for International Agricultural Research Centers to work with National Agricultural Research Staffs (NARS) to deploy insect resistant maize varieties into the field in the same way that genes have been deployed around the world to be screened for resistance. In order for this cooperative effort to work, NARS will need to actively provide funding and personnel in this process. NARS, agricultural economists, rural sociologists and pest management workers must help farmers realize the benefits and limitations of insect resistant maize varieties in their fields. Farmers must be assisted to understand that insect resistant maize can lower yield losses from insect damage and increase their harvests and market profits.

What will the research agenda for maize insect resistance be in the next century? International research teams such as those mentioned in this paper must continue to develop and refine accurate and efficient maize insect pest bioassay techniques, continue to discover the functional categories and underlying mechanisms mediating resistance, and continue to develop and refine microanalytical techniques to determine resistance mechanisms. Although knowledge continues to accumulate at a rapid rate concerning the allelochemical and morphological bases of insect resistance in maize plants, in only a few cases such as DIMBOA, is the specific site of activity of a plant allelochemical on insect metabolism actually known.

The science of identifying, quantifying and developing insect resistant maize varieties is recognized as one of the most highly productive areas of modern agricultural research. Genes for resistance to most of the major maize insect pests have been identified and incorporated into maize breeding programs in many countries, and the future is bright for continuing success in many other parts of the world. New and emerging genetic technologies also promise to enhance the types and numbers of insect resistance genes available for placement into maize varieties. There is also a solid understanding or the major plant chemical and physical factors mediating maize resistance to certain major insect pests. With all of these factors in place, there are really no major reasons why varieties with resistance to all major insect pests of maize cannot be developed and cultivated. A key to this accomplishment will be to mesh the IPM needs of maize farmers at the local level with the sociological needs of farmers in each maize growing location (Peairs 1989). When this is accomplished, varieties with the necessary combinations of insect resistance, high yield and good grain quality can be "tailored" to fit the needs of farmers in specific geographic conditions.

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The Effect of DIMBOA Concentration in Leaf Tissue at Various Plant Growth Stages on Resistance to Asian Corn Borer in Maize

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Abstract

The chemical analytical values obtained for MBOA (6-methoxy-2-benzoxazolinone) were related to the labile cyclic hydroxamic acid precursors DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one) which is formed enzymatically from its glucosides when the leaf tissues are crushed or placed at high temperature. The results of chemical analysis revealed that the MBOA concentrations in leaf tissue decreased as the plants grew towards maturity, inversely the TLC plate ratings increased as the plants grew older. This showed that there were higher MBOA concentrations in the leaf tissue of earlier stage plants than in those of later ones. Leaf-feeding damage ratings caused by artificial infestation with Asian corn borer (ACB), Ostrinia furnacalis *(Guenée) egg masses and number of surviving borer larvae per plant increased as the plants grew older, indicating that younger plants were more resistant than older ones to cornborer feeding. Of 11 inbred lines tested JT 30-1-1-1-15-3 and CI31A had lower leaf-feeding ratings, lower number of surviving larvae per plant and higher MBOA concentrations than any other lines at various stages of plant development. This implies that these 2 lines possess a remarkable degree of resistance to leaf-feeding by corn borer. The correlation coefficients of MBOA concentrations with leaf-feeding ratings at the 4th, 6th, 8th, 10th and 12th leaf stages were as follows: -0.85, -0.84, -0.86, -0.82, and -0.84 respectively, while the correlation coefficients of MBOA concentrations with number of surviving larvae at the same leaf stages in order were as follows: -0.88, -0.83, -0.82, -0.78 and -0.80 respectively. The negative correlations of MBOA concentrations with leaf-feeding ratings and number of surviving borer larvae per plant were highly significant. This means that the higher the MBOA concentrations in leaf tissue, the lower the leaf-feeding ratings and number of surviving borer larvae per plant. The results prove that DIMBOA is an important chemical factor responsible for resistance in maize to the Asian corn borer.*

Introduction

DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)- one) was first associated with insect resistance in crop plants when Klun et al (1967) isolated it from corn seedlings and bioassayed it in artificial diets for the European corn borer (ECB), *Ostrinia nubilalis* (Hubner). They found that this compound inhibited larval development and caused 25% mortality. These results, and associated experimental evidence, revealed that the compound is a chemical factor in the resistance of maize to first brood ECB. As a result, DIMBOA concentration in leaf tissue was used as one of the indicators for selecting maize inbreds resistant to leaf-feeding by the ECB (Klun and Robinson 1969; Sullivan et al. 1974; Russell et al. 1975).

The concentration of DIMBOA in maize was found to vary between different plant tissues. Concentrations were generally highest in the root and then in decreasing order of concentration; the stalk, whole plant and leaf (Klun and Robinson 1969). Moreover, the concentrations in the various tissues were different for each inbred. Biosynthesis of the benzoxazinone took place throughout the development of the plant, but the overall concentration in the whole plant decreased as the plant matured (Klun and Robinson. 1969). The high concentration of DIMBOA in seedling corn may explain the apparent resistance of young corn to the ECB (Klun and Robinson 1969; Guthrie 1974).

The precursor to DIMBOA occurs as a glucoside in intact maize tissue. When plant tissues are crushed, the glucoside is hydrolyzed by a plant enzyme to the aglucone, 2,4-dihydroxy-7-methoxy-1, 4- benzoxazin-3(4H)-one (DIMBOA) (Klun and Robinson. 1969). DIMBOA is chemically labile and slowly

decomposes to 6-methoxy-2-benzoxazolinone (MBOA), which is chemically stable (Fig. 1). Thus, DIMBOA concentration in plant tissue could be estimated by analyzing for MBOA. The MBOA analytical value is interpreted as a stoichiometric measure of DIMBOA formed as the result of enzymatic cleavage of its glucoside precursor (Klun et al. 1967).

Klun et al. (1970) used a diallel set of 11 maize inbreds (55 single cross hybrids) to study the concentration of DIMBOA in whorl leaf tissue and the resistance to leaf-feeding by first-generation ECB. The correlation between concentration of DIMBOA in plant tissue and level of resistance was highly significant for the inbreds (r=-0.89) and the single crosses (r=-0.74). Genetic effects due to general and specific combining ability were highly significant for both traits, but general combining ability accounted for 84% of the variation in the resistance ratings and for 91% of the variation in the concentration of DIMBOA. These results provided further evidence that DIMBOA is a chemical factor in the resistance of maize to the ECB. However, most chemicals exhibit their specific properties only in host plant resistance (HPR) to insects (Beck 1965; Guthrie 1974). Hence, further studies were needed to determine whether the maize inbreds with high DIMBOA concentration would exhibit similar levels of resistance to the Asian corn borer (ACB) *Ostrinia furnacalis* (Guenée). This study was carried out to determine the changes of DIMBOA concentration in all stages of maize development and to evaluate any relationship between DIMBOA and resistance to leaf-feeding by ACB.

Materials and Methods

The 11 dent corn inbreds chosen in this study and their origins are listed in Table 1. The experiments were conducted at the Corn Research Center, Tainan DAIS, Potzu, Chiayi, using four replicates with a split-plot design — main plots : inbreds; subplots : plant growth stages; sub-subplots : infesting artificial ACB egg masses inside the whorl leaves (Tseng and Twu 1974) (Fig. 2) and cutting the whorl leaf for chemical analysis of DIMBOA

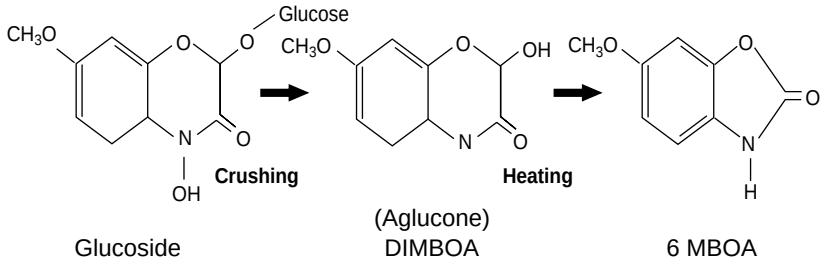

Figure 1. Formation of DIMBOA (Aglucone) and MBOA from a glucoside occurring in maize tissue.

Table 1. Eleven dent corn inbreds used in the study and their origins.

Inbred	Derivation	Origin
JT 30-1-1-1-15-3	JWL. 305 x Tainan DMR #2	CIMMYT
YT 148-2-1-1-2-1	Yellow hard endosperm x Tainan DMR #2	CIMMYT
ST 153-1-3-2-2-1	South African Yellow x Tainan DMR #2	CIMMYT
CT 139-5-1-1-1-1	Cogollero x Tainan DMR #2	CIMMYT
ANMT 55-1-3-2-2-1	(Amber x (B 57 x B 37) x Akbar) x Tainan DMR #2	CIMMYT
PT 169-1-1-4-1-1	Pendu x Tainan DMR #2	CIMMYT
ANT 176-1-3-5-13-3	Antigua Gr. x Tainan DMR #2	CIMMYT
B 49	Iowa 2 ear syn.	Iowa State
CI31A	Midland "A" O. P.	USDA
B 52	Midland	Iowa State
WF 9	Wilson Farm Reid	Indiana State

Figure 2. Infesting artificial ACB egg masses inside the whorl leaves.

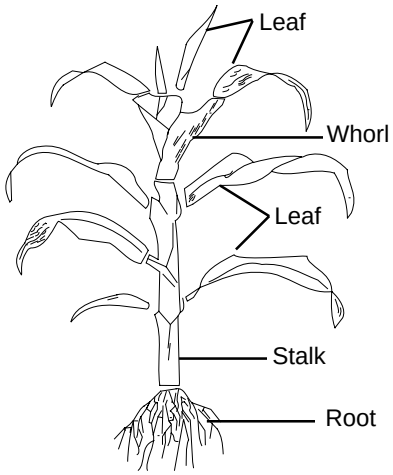

Figure 3. Samples of plant tissues taken for chemical analysis of DIMBOA concentration (after Klun and Robinson 1969).

concentration (Fig. 3). Samples analyzed were taken at the 4th, 6th, 8th, 10th, and 12th leaf stages, defined according to the uppermost leaf whose collar was visible (Ritchie and Hanway 1982).

The 11 inbreds were planted in 10-row plots (24 hills of two seeds/hill and thinned to one plant/hill) in 1985 and 1986. The distance between rows was 75 cm and between hills within row distance was 25 cm.

Five rows in each plot were infested with ACB egg masses at the 4th, 6th, 8th, 10th and 12th leaf stages of plant development, respectively. Infestations were made in 3 applications of 3 egg masses (Ca. 450 eggs/plant), each spaced 1 day apart. Leaf-feeding damage was rated on a plot basis, 21 days after egg hatching, using a scale of 1 to 9 (1 = no damage, 9 = extremely damaged) (Guthrie et al. 1960) (Fig. 4 and Fig. 5). After rating, 10 plants from each row were dissected to count the number of surviving borer larvae per plant (Fig. 6).

The other 5 rows in each plot were used for DIMBOA analysis. Whorl leaves from 10 plants in each row were collected at the 4th, 6th, 8th, 10th and 12th leaf stages of plant development, respectively. The whorl leaves collected were placed in plastic bags and stored

Figure 4. Resistant inbred line of dent maize rated 1 according to the visual rating system (Guthrie et al. 1960).

Figure 6. Dissecting the infested stalks to count the surviving Asian corn borer larvae

Figure 5. Susceptible inbred line of dent maize rated 9 according to the visual rating system (Guthrie et al. 1960).

at -23ºC prior to analysis. The frozen leaf tissue was then thawed, dried in an oven at 45ºC, and ground into a fine powder. The chemical determinations carried out on the ground tissue were actually for MBOA, expressed as mg MBOA/g of plant tissue (Brendenberg et al. 1962; Klun and Robinson; 1969; Klun et al. 1970; Klun, 1970; Tseng et al. 1984).

The procedures used to obtain quantitative measurement of MBOA in leaf tissue were modified from those used by Klun and Robinson (1969). For each sample, 20 ml of boiling water were added to a 70 ml jar containing 0.5g of dried ground leaf tissue; after shaking vigorously for 1 min, this solution was poured into a Buchner funnel (lined with filter paper), and an aspirator vacuum (Fig. 7) filtered the filtrate into a 500 ml flask. The filtrate was then poured into a 100 ml beaker and allowed to cool (the leaf residue was discarded). Four drops of concentrated hydrochloric acid were added to acidify the filtrate (pH 1.0). The acidified filtrate was poured into a separating funnel (Fig. 8) and 40 ml of diethyl ether were added. After vigorously shaking the funnel, the water and ether were allowed to separate, then each layer was drained into 100 ml beakers; the aqueous phase was then poured back into the separating funnel. To wash MBOA from the aqueous phase as completely as possible, the procedure involving the separating funnel was repeated twice, then the aqueous phase was discarded. Anhydrous calcium chloride was added to the ether layer to remove any water left in the ether. The ether was allowed to evaporate under a fume hood, and the ether soluble residue was dissolved in 1 ml ethyl acetate: benzene solution (1:1 vol./vol.).

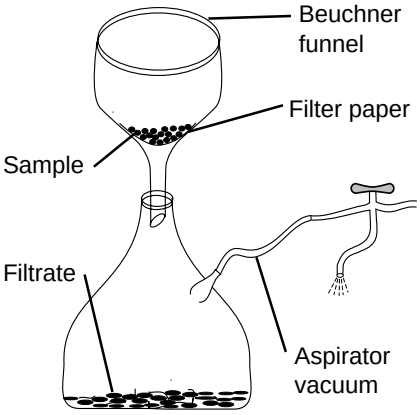

Figure 7. Buchner funnel and aspirator vacuum.

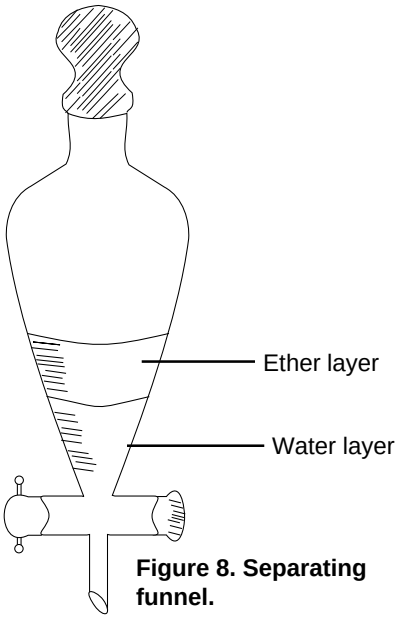

Figure 8. Separating funnel.

A 100 µl aliquot of this solution was then spotted on a 20 x 20 cm glass plate covered with a thin layer of silica gel (GF 254 Brinkmann Instruments , Westbury, NY). Six samples plus commercial MBOA (Calbiochem-Behring Corp., P. O. Box 12087, San Diego, California), as a control, were spotted on an individual plate. Each sample of the 4 replications was repeated twice. The 7 spots were placed along one edge of the plate (Fig. 9).

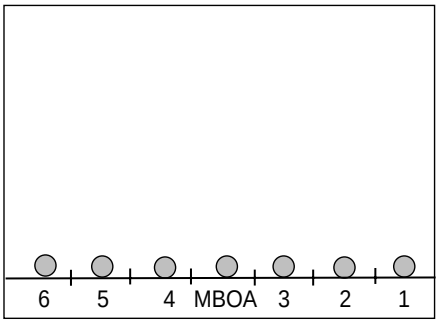

Figure 9. Thin layer chromatography (TLC) plate.

After spotting, the chromatogram was developed with chloroform: ethyl-acetate: cyclohexane (4:4:2 vol./vol.). After development, the plates were removed from the solution, dried, and then redeveloped in the same direction with cyclohexane : isobutanol (85 : 15 vol./vol.). The chromatogram was then air-dried and two observers visually rated, under short wave uv light (254 nm), the intensity of each MBAO spot from the extracts in classes of 1 to 5 (1=highest intensity, 5=lowest intensity) as described by Robinson et al. (1982).

When the intensity ratings were completed, the area of the silica gel corresponding to the reference MBOA spot was scraped from the chromatogram and transferred to a disposable pasteur pipette plugged with glass wool (Fig. 10). MBOA was then eluted from the silica gel with 6 ml of 95% ethanol and the uv absorbance of this solution was measured at 231 nm with a Beckman Model DB Spectrophotometer. The uv spectrophoto-metric percent transmission (T %) was read twice for

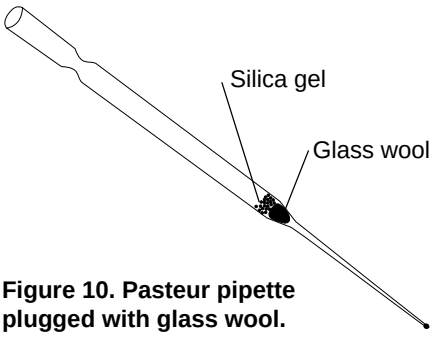

Figure 10. Pasteur pipette plugged with glass wool.

each sample. The MBOA concentration (mg MBOA/g dried leaf tissue) was then calculated from a MBOA standard curve.

Thus, we used two methods for measuring DIMBOA concentrations in maize leaf tissue: 1) Chemical analysis for mg MBOA/g of maize leaf tissue and 2) thin layer chromatography (TLC) to rate differences visually in the concentration of MBOA (TLC plate rating).

Data on leaf-feeding ratings, number of surviving larvae per plant, mg MBOA/g dried leaf tissue and TLC plate ratings collected from above experiments were statistically analyzed to elucidate significant differences between experimental results (Steel and Torrie 1960).

Results

MBOA leaf tissue content and TLC readings

The results of chemical analysis for MBOA concentration and TLC plate rating for MBOA spot intensity revealed that the highest MBOA concentrations were at the 4th leaf stage and the lowest were at the 12th leaf stage for all inbreds (Table 2). Of all inbreds tested JT 30-1-1-1-15-3 and CI31A had the highest and WF 9 had the lowest MBOA concentration in all plant growth stages. The former possessed about three times more MBOA than the latter. The TLC plate ratings showed just the inverse, as the highest ratings were at the 12th leaf stage and the lowest were at the 4th leaf stage. JT 30-1-1-1-15-3 and CI31A had the lowest rating throughout all growth stages and amongst all inbreds.

Leaf-feeding ratings and larval survival

Leaf-feeding rating, after artificial infestation with ACB egg masses, and the number of surviving larvae per plant both increased as the plants matured (Table 3), indicating that young plants were more resistant to leaf-feeding by ACB than older ones.

Among all the inbreds tested JT 30-1-1-1-15-3 and CI31A had both the lowest leaf-feeding rating and number of surviving borer larvae per plant in all leaf stages. CT139-5-1-1-1-1 and WF9 had the highest leaf-feeding ratings and numbers of surviving borer larvae per plant in all growth stages of plant development.

Table 2. Mean concentrations of MBOA in leaf tissue and TLC plate ratings at various leaf stages.

Inbred	Leaf stage									
	4	6	8	10	12	4	6	8	10	12
	mg MBOA/g dry weight					TLC plate ratings				
JT 30-1-1-1-15-3	3.60	3.25	2.85	2.50	2.15	1.7	1.8	2.2	3.0	3.5
YT 148-2-1-1-2-1	2.70	2.15	1.80	1.40	1.20	2.5	2.8	3.0	3.5	4.0
ST 153-1-3-2-2-1	2.51	1.81	1.65	1.10	0.85	3.5	4.0	4.5	4.5	4.5
CT 139-5-1-1-1-1	1.52	1.21	1.10	0.95	0.66	4.5	4.5	4.5	4.7	5.0
ANMT 55-1-3-2-2-1	2.01	1.80	1.41	1.20	0.95	4.0	4.5	4.5	4.8	5.0
PT 169-1-1-4-1-1	1.81	1.23	0.90	0.80	0.70	4.5	4.5	4.5	4.7	5.0
ANT 176-1-3-5-13-3	1.68	1.70	1.15	0.96	0.80	4.5	4.5	4.5	4.6	5.0
B 49	2.90	2.20	1.75	1.40	1.40	2.0	2.5	3.0	3.5	4.0
CI31A	3.56	2.90	2.60	2.45	2.10	1.7	2.0	2.5	3.0	3.5
B 52	2.31	1.80	1.45	1.30	1.25	3.5	3.5	4.0	4.5	4.5
WF 9	1.28	0.92	0.75	0.66	0.48	4.5	4.6	5.0	5.0	5.0

LSD (0.05)
Any two means of MBOA concentrations between leaf stages for the same inbred is 0.64
Any two means of MBOA concentrations between inbreds for the same leaf stage is 0.85
Any two means of TLC plate ratings between leaf stages for the same inbred is 0.45
Any two means of TLC plate ratings between inbreds for the same leaf stage is 0.55

Table 3. Mean leaf-feeding ratings after artificial infestation with ACB egg masses and number of surviving larvae per plant at various leaf stages.

Inbred	Leaf stages									
	4	6	8	10	12	4	6	8	10	12
	Leaf-feeding ratings					No. of surviving larvae				
JT 30-1-1-1-15-3	1.5	1.5	2.0	2.5	3.0	1.2	1.6	2.4	2.8	2.5
YT 148-2-1-1-2-1	2.5	3.0	3.6	4.5	5.5	2.2	3.0	5.0	7.5	6.5
ST 153-1-3-2-2-1	3.0	3.5	5.0	6.0	6.5	2.8	3.5	6.5	7.5	8.0
CT 139-5-1-1-1-1	5.0	4.5	6.0	6.5	7.5	5.6	6.5	7.0	8.5	8.8
ANMT 55-1-3-2-2-1	3.5	4.0	5.0	6.0	6.5	4.0	4.3	5.5	6.5	7.0
PT 169-1-1-4-1-1	4.0	4.5	6.0	6.0	7.5	4.4	6.2	7.6	8.4	8.6
ANT 176-1-3-5-13-3	4.0	4.5	6.0	6.0	6.5	4.8	5.0	6.5	7.3	6.8
B 49	2.5	3.0	4.0	5.0	5.5	2.8	3.5	5.0	5.5	4.5
CI31A	1.5	2.0	2.5	2.5	3.0	1.5	1.3	2.6	3.0	2.4
B 52	3.0	3.5	4.5	5.0	5.5	3.1	3.5	5.5	4.5	5.4
WF 9	5.0	5.5	6.5	7.5	8.5	6.5	8.0	8.5	9.8	10.5

LSD (0.05)
Any two means of leaf-feeding ratings between leaf stages for the same inbred is 1.0
Any two means of leaf-feeding ratings between inbreds for the same leaf stage is 1.5
Any two means of numbers of surviving larvae per plant between leaf stages for the same inbred is 1.1
Any two means of numvers of surviving larvae per plant between inbreds for the same leaf stage is 1.9.

Correlation of MBOA leaf tissue content with leaf-feeding ratings at various leaf stages

The correlation coefficients of MBOA concentrations in leaf tissue with leaf-feeding ratings of 11 inbreds at the 4th, 6th, 8th, 10th and 12th leaf stages, were -0.85, -0.84, -0.86, -0.82 and -0.84, respectively (Fig. 11). The correlation of MBOA concentration with leaf-feeding rating was highly significant throughout all growth stages of plant development.

Correlation of MBOA leaf tissue content with larval survival at various leaf stages

The correlation coefficients of MBOA concentrations in leaf tissue with the number of surviving larvae per plant, of 11 inbreds at the 4th, 6th, 8th, 10th and 12th leaf stages, were -0.88, -0.83, -0.82, -0.78 and -0.80 respectively (Fig. 12). The correlation of MBOA concentration with number of surviving larvae per plant was also highly significant in all growth stages of plant development.

Discussion

It is imperative to have resistant germplasm available for breeding insect resistant crop varieties; this holds true in developing maize that possesses resistance to ACB. Resistant germplasm can be obtained through introductions or exchanges with foreign or domestic research institutes and through identifying resistance sources in materials in stock or locally available. However, since ACB resistance mechanisms in maize are unclear, it is difficult to know where to collect or how to identify the resistant germplasm (Beck 1965; Dahms 1972). The breeding of maize varieties resistant to ACB would be more efficient, if we knew more about the resistance mechanisms. In this study we used 11 inbreds to determine the relationship of MBOA concentration in leaf tissue with resistance to ACB at various growth stages of plant development, and also to provide more information to identify resistant inbreds. The results from leaf-feeding ratings after artificial infestation with ACB egg masses at the 4th, 6th, 8th, 10th and 12th leaf stages (Table 3) indicated that the lowest leaf-feeding ratings and number of surviving borer larvae per plant were at the 4th leaf stage for all inbreds tested. However, leaf-feeding ratings and number of surviving larvae per plant increased as plants matured. This indicated that the young plants were more resistant to ACB than the older ones. Therefore, if the inbreds could maintain the leaf-feeding rating and number of surviving larvae per plant at a low level throughout all growth stages of plant development, they would possess the high resistance level to ACB. Among all inbreds tested, JT30-1-1-1-15-2 and CI31A had the lowest leaf- feeding ratings and numbers of surviving larvae per plant at all leaf stages. This showed that JT30-1-1-1-15-3 and CI31A were more resistant to ACB than other inbreds.

DIMBOA (2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3(4H)-one) is chemically labile and slowly decomposes to 6-methoxy-2- benzoxazolinone (MBOA), which is chemically stable (Brendenberg et al. 1970; Klun, 1970).

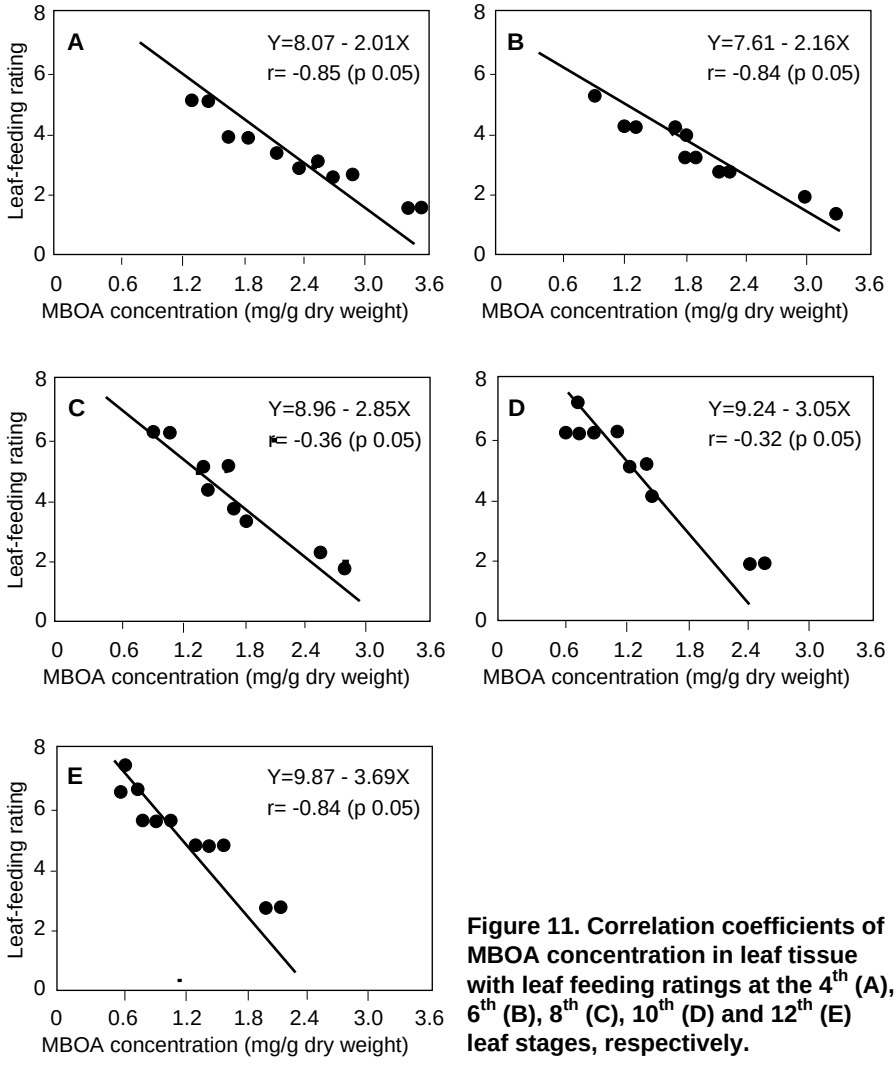

Figure 11. Correlation coefficients of MBOA concentration in leaf tissue with leaf feeding ratings at the 4th (A), 6th (B), 8th (C), 10th (D) and 12th (E) leaf stages, respectively.

Thus, DIMBOA concentration can be determined by chemical analysis of dried plant tissue for MBOA (Klun and Robinson, 1969; Klun et al. 1970; Klun, 1970). The results of chemical analysis of MBOA concentrations and TLC plate ratings (Table 2) revealed that MBOA concentration decreased as plants grew toward maturity. Inversely, TLC plate rating increased as plants grew older. This showed that young plants contained a higher MBOA concentration than older ones. Among 11 inbreds tested, JT 30-1-1-1-15 -3 and CI31A had the highest MBOA concentrations and the lowest TLC plant ratings. The correlation coefficients of MBOA concentrations with leaf-feeding ratings at the 4th, 6th, 8th, 10th and 12th leaf stages were -0.85, -0.84, -0.86, -0.82 and -0.84 respectively (Fig. 11), whereas the correlation coefficients of MBOA concentrations with numbers of surviving borer larvae per plant at the same leaf stages were -0.88, -0.83, -0.82, -0.78 and -0.80 respectively (Fig. 12). These results clearly indicate that the relationship between MBOA concentration and both leaf-feeding rating and number of surviving borer larvae per plant was highly significant. In other words, the higher the MBOA concentration in leaf tissue, the lower the leaf-feeding rating and the number of surviving larvae per plant. This means that the inbreds with greater MBOA concentrations will possess greater resistance to ACB. The experimental data proved that DIMBOA was a significant biochemical factor in maize responsible for ACB resistance.

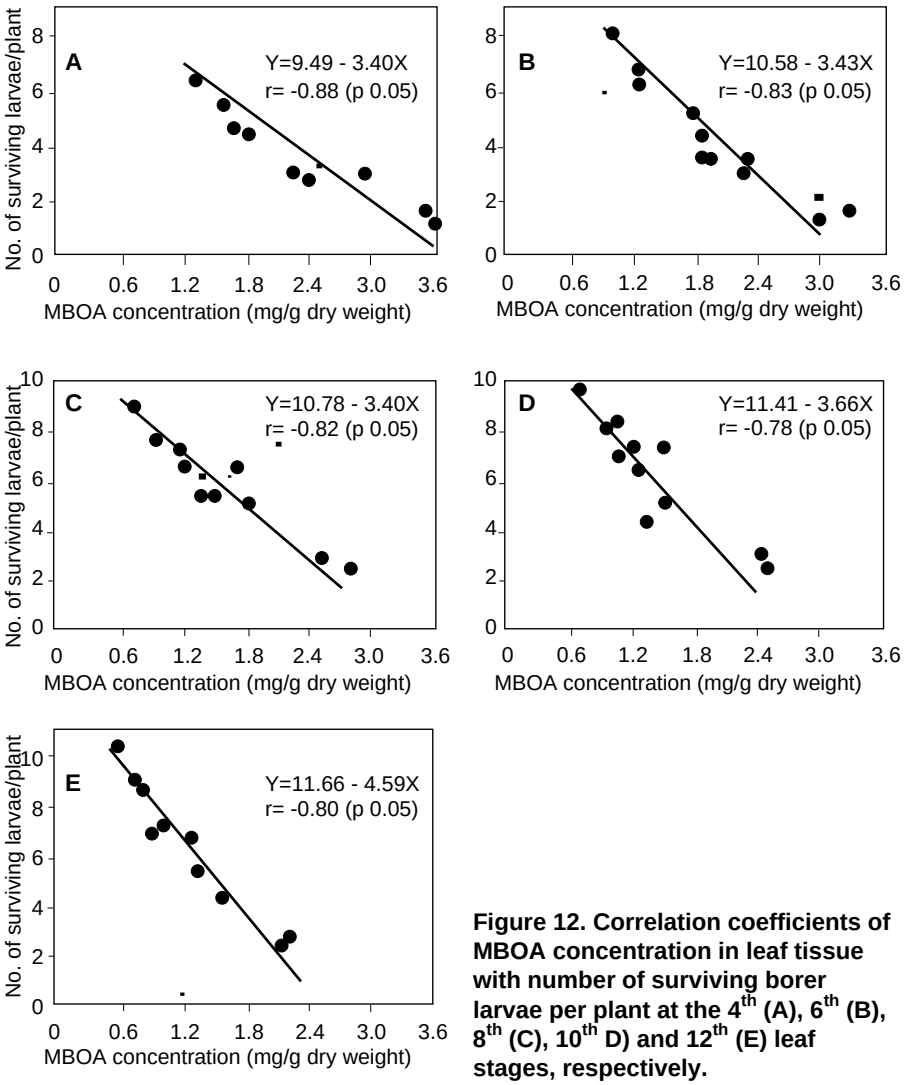

Figure 12. Correlation coefficients of MBOA concentration in leaf tissue with number of surviving borer larvae per plant at the 4th (A), 6th (B), 8th (C), 10th D) and 12th (E) leaf stages, respectively.

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Impact of Mechanisms of Resistance on European Corn Borer Resistance in Selected Maize Hybrids

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Abstract

Four commercial maize hybrids plus a susceptible and a resistant check were compared in experiments to determine which mechanism(s) of resistance — i.e., 1) preference, 2) antibiosis and/or 3) tolerance to first and second generation European corn borer (ECB) — contributed to overall resistance. Preference evaluations were made under natural ECB infestations in an area where ECB is endemic using six replications and counting shot holes for the first generation and number of egg masses and tunnel length (stalk splitting) for the second generation. Antibiosis was determined by manual infestations and no infestation and using Guthrie's (1960) scale for first generation ECB. second generation ECB antibiosis was determined by splitting stalks of manually infested and non-infested hybrids and estimating the amount of tunneling. Tolerance was measured with leaf-feeding ratings and the amount of tunneling and all hybrids were infested with 0, 30, 120, and 240 larvae per plant. In all six experiments, yields were measured to relate the effects of resistance mechanisms and infestation levels.

Introduction

Our report concerns the effects of mechanisms of resistance on European corn borer (ECB), *Ostrinia nubilalis* (Hübner), resistance in selected maize, *Zea mays* (L.), hybrids. The ECB is an Old World insect and before maize was introduced into Europe, the ECB was of limited economic importance except in hemp, *Cannabis sativa* (L.), and hops, *Humulus lupulus* (L.). This borer probably arrived in the United States about 1914 in a shipment of hemp and was first described as a pest of maize (Fig. 1, photograph taken in 1918 by B. E. Hodgson, Medford, MA) in 1917 (Vinal 1917). Estimated losses in the Corn Belt due to this insect during 1981 were 190 million dollars (Table 1), but the most frequently quoted figures for average annual losses are between 200 and 500 million dollars. The Illinois Entomology Extension Service continues to monitor ECB populations and their data suggest losses in Illinois

Figure 1. European corn borer damage to maize in 1918, four years after ECB was introduced into the United States (photograph by B. E. Hodgson, Medford, MA).

Table 1. Estimated economic losses due to second generation ECB damage in field corn harvested for grain in 1982.

Location	Area (000 ha)	Yield (t/ha)	ECB per 100 plants	Losses[†] (US$000'000)
Missouri	797	6.53	141	21.23
Iowa	5,322	7.60	51	59.64
Illinois	4,605	8.42	26	29.14
United States	29,604	7.21	31[‡]	191.35

[†] 1982 price of corn averaged $2.45.
[‡] Calculated from average statistics for the above three states.

for 1991, 1992, and 1993 of 324, 33, and 101 million dollars, respectively. The ECB population in Illinois in 1991 was the highest since 1949 (personal communication, Mike Gray, Illinois Extension Service, 1994). If we extrapolate from the Illinois data for the acreage of maize for the U.S. Corn Belt, losses would have been 2,197; 236; and 706 million dollars for 1991, 1992, and 1993, respectively (Table 2).

The ECB has been the most studied economic pest of maize in the United States and the majority of studies have dealt with control. As early as the 1920s, Huber et al. (1928) proposed host-plant resistance (HPR) as a means of control. L.H. Patch worked with field maize; and his colleague, M. Schlosberg, another entomologist, worked with sweet maize (personal communication, Orlo Vance, retired, 1994). These scientists made many contributions toward our current HPR programs for ECB resistance. Ideas and techniques for manually infesting plants for screening, laboratory rearing, and a damage rating scale began with these scientists. Guthrie et al. (1960) developed a rating scale for ECB (Figs. 2 and 3) that is currently used for several lepidopteran pests of maize. Of course, the father of HPR was R.H. Painter from Kansas State University (Fig. 4). Painter began promoting HPR as early as 1923 and published his first book on the subject in 1951 (Painter 1951). He defined resistance and the mechanisms of resistance: antibiosis, non-preference, and tolerance:

- "Resistance" refers to the heritable qualities of the host which allow infested (with insects) cultivars to produce more than similar cultivars without these qualities.
- "Antibiosis" refers to adverse biological consequences to the life history of an insect due to the feeding on a resistant host. The effects may be death, small size, low weight, reduced fecundity, extended life cycle, and/or abnormal behavior.
- "Non-preference" refers to the lack of attractiveness of a host for food and shelter for an insect. The non-preference attribute of a host could

Table 2. Statistics for maize and estimated ECB damage in Illinois from 1991-1993 and extrapolations for the United States.

	Year		
	1991	1992	1993
Illinois			
Hectares planted (millions)	4.5	4.5	4.2
Yield (t/ha)	8.16	9.36	6.72
Price ($/t in Iowa)	96.85	87.40	107.48
Plants infested with ECB (%)	91.4[†]	30.9	50.3
Average number of borers/plant	3.3	0.3	1.1
Total tons produced (millions)	36.98	42.39	28.54
Loss due to ECB (millions of tons)	3.35	0.38	0.94
Loss in dollars (millions)	324.0	33.3	101.2
United States			
Hectares planted (millions)	30.6	32.1	29.7
Loss in dollars (millions, extrapolated)	2,197.1	235.9	706.5

[†] Second highest recorded infestation (highest was during the 1940s). Entomologists in Illinois surveyed 10 fields in 35 counties by examining samples of 25 plants in each field.

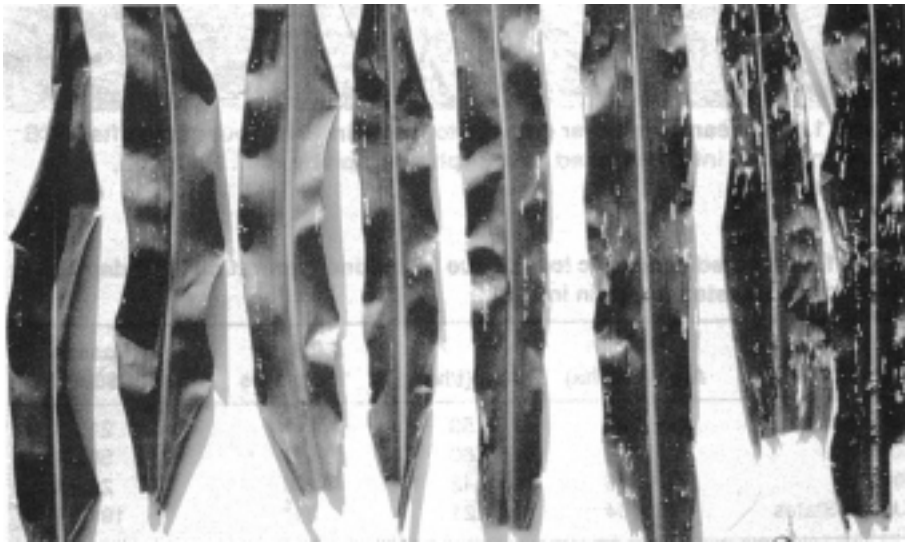

Figure 2. Leaf damage corresponding to Guthrie et al.'s 1-9 rating scale for first generation ECB damage.

Class 1. No visible leaf injury or a small amount of pin or fine shot-hole type of injury on a few leaves.

Class 2. Small amount of shot-hole type lesions on a few leaves.

Class 3. Shot-hole injury common on several leaves.

Class 4. Several leaves with shot-hole and elongated lesions.

Class 5. Several leaves with elongated lesions.

Class 6. Several leaves with elongated lesions (about 2.5 cm).

Class 7. Long lesions common on about one-half of the leaves.

Class 8. Long lesions common on about two-thirds of the leaves.

Class 9. Most leaves with long lesions.

Figure 3. Description for leaf damage corresponding to Guthrie et al.'s 1-9 rating scale for first generation ECB damage.

also discourage continued habitation even though it had served as shelter and/or as an oviposition site.

- "Tolerance" is the ability of the host plant to support a certain population level of insects due to plant vigor, or the ability to repair the damaged tissue without loss of quality or yield. This mechanism of resistance may be rendered ineffective, however, if the pest population is too large.

Research focused towards insect resistance has primarily been concerned with antibiosis, although some scientists have studied adult non-preference and tolerance for a number of insects. Wiseman and his colleagues at the U.S. Department of Agriculture, Agricultural Research Service, Insect Biology and Population Management Research Laboratory, Tifton, GA, have been the most prolific researchers in this work (Chang et al. 1985; Waiss et al. 1979; Widstrom et al. 1979; Wilson et al. 1984; Wiseman 1985; Wiseman and Bondari 1992; Wiseman and McMillian 1980; Wiseman and Widstrom 1986; Wiseman et al. 1967, 1972, 1977, 1981, and 1983) and they have demonstrated both non-preference and tolerance in maize for corn earworm, *Helicoverpa zea* (Boddie), and fall armyworm *Spodoptera frugiperda* (J.E. Smith). Barry and Darrah (1988) have shown that adult non-preference and antibiosis resistance for ECB can exist within a single cultivar (Table 3).

We designed six experiments to study the three mechanisms of resistance for both generations of ECB by using manual and natural infestations. Our objective was to evaluate the mechanisms of resistance for their contribution to overall ECB resistance in selected maize hybrids.

Materials and Methods

Four widely-grown commercial maize hybrids (one from each of four companies) plus two hybrid checks were selected primarily for tolerance and/or antibiosis for ECB. Each hybrid was entered twice, once for manual or natural infestation and once as a control protected from insects. A research site, approximately 1 km north east of Grand Pass, Missouri, was chosen because of having an endogenous population of ECB. Planting, fertilization, herbicide treatment, and other cultural practices for the 1994 evaluation were those used by farmers in the area. Experiments were planted on 18 May 1994 in a field fertilized with 168 kg/ha N, 67 kg/ha P_2O_5, and 78 kg /ha K_2O. Atrazine and metolachlor were applied at rates of 1.8 and 2.2 kg a.i./ha, respectively, for weed control. No insecticides were applied at planting. All plots were four rows wide (0.91 m [36"] between rows) and 4.9 m (16' long). Antibiosis and tolerance experiments had four replications and

Figure 4. R.H. Painter and colleagues: R.H. Painter, E.C. Ortman, and E.L. Sorenson (left to right).

Table 3. Comparisons of means of first and second generation ECB activities on three maize cultivars as they relate to host plant resistance in Missouri (Barry and Darrah 1988).

	1984-1985†		1986†	
Insect activity	**Mo-2 ECB**	**MFA 5802**	**Mo-2 ECB**	**Wf9 x W182E**
First generation				
Egg masses/plant‡	0.7a	0.9b	8.3a	11.5b
Larvae/plant	0.3a	0.6b	3.2a	12.8b
Larvae/eggmass	0.6a	1.1b	0.4a	1.2b
Second generation				
Egg masses/plant‡	0.4a	1.5b	7.9a	4.3b
Tunnel (cm)/plant	0.9a	2.7b	12.0a	29.0b
Tunnel (cm)/egg mass	6.0a	7.0a	1.6a	9.6b

† Means for insect activity (horizontally between two cultivars) followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 0.05 probability level. Data for the 1984-1985 study were derived from observations of 150 plants and from 50 plants for the 1986 study.

‡ Each egg mass contained 20-25 eggs.

the non-preference experiments had six replications because we were depending upon natural infestation in the latter.

Data were collected from the center two rows of each plot. In each experiment, control plots were treated with *Bacillus thuringiensis (Bt)* (Dipel, Abbott Lab., North Chicago, IL., or Bio-bit, E.I. Dupont, Wilmington, DE[1]) on a 7-10 day schedule beginning at about the eight-leaf stage and continuing to one interval beyond anthesis. Plots infested with ECB for first generation studies received *Bt* treatments beginning about 21 days after manual infestations were made, and the second generation plots were treated from about the eight-leaf until mid-whorl stage of plant development. The experiments were:

- **Experiment #1:**
  First generation ECB antibiosis
  All plants in the center two rows of the four-row plots for the infested treatment were manually infested by using a bazooka (Mihm, 1983a, 1983b) during the whorl stage of plant development with approximately 100 live, neonate ECB larvae. Twenty-one days after infestation the plots were rated for ECB leaf damage using Guthrie et al.'s (1960) scale of 1 to 9 (1 = no damage and 9 = severe damage; Figs. 2 and 3).
- **Experiment #2:**
  Second generation ECB antibiosis
  All plants of the center two rows of the four-row plots for the infested treatment were manually infested during anthesis in the leaf axils, within one leaf above or below the top ear zone, with approximately 100 live, neonate ECB larvae. Before harvest, but at least 60 days after infestation, five stalks from each plot were randomly selected and split from the node above the ear to ground level, and centimeters of tunneling were estimated.
- **Experiment #3:**
  First generation ECB nonpreference
  This experiment was dependent on natural ECB populations to infest plants. Six replications were used. About 10 days after the first egg masses from overwintering ECB were observed on plants in ECB-treatment plots , one center row of each plot was checked for the number of plants exhibiting shot holes (Fig. 5).
- **Experiment #4: Second generation ECB non-preference**
  Natural populations of ECB were depended upon for infestation. Four days after moths were seen and the first egg masses were found in the plots during anthesis, egg masses were counted by examining 10 plants selected at random from the two center rows of each plot. Measurement of the amount of stalk tunneling was done as in Experiment #2.
- **Experiment #5: First generation ECB tolerance**
  The center two rows of each plot for the infested treatment were all manually infested during the whorl stage of plant development with 0, 30, 100, and 240 neonate ECB larva and leaf damage was rated as in Experiment #1.
- **Experiment #6:**
  Second generation ECB tolerance
  During anthesis, hybrids were manually infested with 0, 30, 100, and 240 neonate ECB larvae for the infested treatment. Larvae were applied in the leaf axils near the ear zone. As in Experiment #2, five stalks were randomly selected from the center two rows of each plot, then split to measure ECB tunneling.

Results and Discussion

The results of our field evaluations of four commercial maize hybrids and two hybrid checks to determine which mechanisms of resistance are responsible for first and second generation ECB resistance are presented in Tables 4 to 6. In these tables, letter subscripts are used to indicate significant differences in the vertical direction, and superscripts are used to indicate significant differences in the horizontal direction. The yields

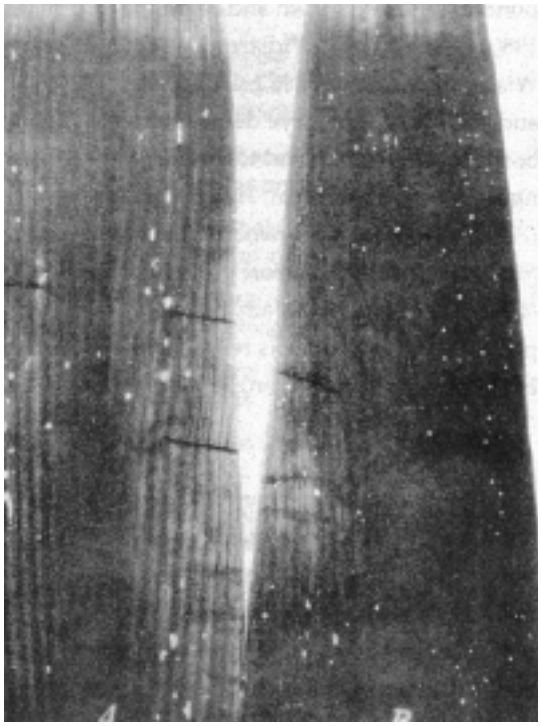

Figure 5. Shot holes; taken from Patch (1943).

[1] Mention of a trademark or proprietary product does not constitute a guarantee, warranty, or recommendation of the product by the U.S. Department of Agriculture or the University of Missouri and does not imply its approval to the exclusion of other products that may also be suitable.

among hybrids and within treatments for all experiments were highest for Pioneer Brand 3184 (resistant check) and ICI Seeds 8326, with more than 12.1 t/ha. DeKalb Genetics 623 and Ciba 4666 yields were 10.9 to 12.1 t/ha. Pioneer Brand 3471 (unadapted in Missouri) yielded in the range of 9.1 to 10.6 t/ha, where as the susceptible check hybrid yielded slightly over 6.0 t/ha. Although manual insect infestations were made to enhance insect damage, they were not as successful as we had anticipated (this was not unusual in ECB research plots in the Midwest in 1994).

Experiment #1

This experiment was done to evaluate antibiosis to first generation ECB (Table 4). Leaf-feeding ratings among hybrids within the infested plots were the same except for Pioneer Brand 3471, which was significantly better (lower rating). Several significant differences in leaf-feeding damage for the non-infested hybrids were found, although the ratings were too low to have biological meaning. DeKalb Genetics 623 and Pioneer Brand 3184 had the lowest ratings, where as Pioneer Brand 3471 and Ciba 4666 had significantly higher ratings. However, when comparing leaf-feeding damage ratings between infested and non-infested treatments within hybrids, all were significantly different except for Pioneer Brand 3471, which indicated antibiosis resistance for first generation ECB. There were no significant differences in yield between infestation treatments within hybrids. Even though this experiment was not designed to look at tolerance, all hybrids appeared to have some tolerance, particularly since survival and damage were low this year. There was also a trend noted for lower yields in the infested plots.

Experiment #2

This experiment was done to evaluate antibiosis to the second generation ECB (Table 4). The amount of tunneling was very low, which indicated a poor survival following infestation, particularly because the susceptible check hybrid did not have much tunneling. There were some significant differences among hybrids within the non-infested treatment, but the amounts of tunneling were small. Stalk tunneling differences observed in this experiment had no biological meaning. There were no significant differences between infested and non-infested treatments within any hybrid. No conclusions were made about antibiosis for second generation ECB in these hybrids.

Experiment #3

This experiment was done to determine if non-preference was a mechanism for first generation ECB resistance (Table 5). For comparison, naturally infested vs. protected treatments were used and number of shot holes were counted as

Table 4. Evaluation of four commercial hybrids plus two check hybrids for antibiosis by the first and second generation of ECB in Missouri in 1994.

	First generation			
	Infested		Non-infested	
Hybrid	Rating† (1-9)	Yield (t/ha)	Rating† (1-9)	Yield (t/ha)
ICI Seeds 8326	$3.5^{a}_{a‡}$	12.41^{a}_{b}	1.2^{b}_{bc}	12.36^{a}_{b}
DeKalb Genetics 623	2.8^{a}_{a}	11.21^{a}_{c}	1.0^{b}_{c}	11.61^{a}_{b}
Ciba 4666	3.0^{a}_{a}	11.11^{a}_{c}	1.8^{b}_{ab}	11.79^{a}_{b}
Pioneer Brand 3471	1.8^{a}_{b}	8.73^{a}_{d}	2.0^{a}_{a}	8.82^{a}_{c}
Pioneer Brand 3184 (Check)	2.8^{a}_{a}	13.61^{a}_{a}	1.0^{b}_{c}	14.05^{a}_{d}
Wf9 x W182E (Check)	3.0^{a}_{a}	5.81^{a}_{e}	1.2^{b}_{bc}	6.34^{a}_{d}
Average rating	2.8^{a}		1.4^{b}	
LSD 0.05 = 0.5				
Average yield		10.46^{a}		10.83^{a}
LSD 0.05 = 0.47				

	Second generation			
	Infested		Non-infested	
Hybrid	Tunnel§ (cm)	Yield (t/ha)	Tunnel§ (cm)	Yield (t/ha)
ICI Seeds 8326	$0.25^{a}_{a¶}$	12.99^{a}_{a}	0.51^{a}_{b}	12.59^{a}_{a}
DeKalb Genetics 623	0.51^{a}_{a}	10.73^{a}_{c}	1.40^{a}_{a}	11.14^{a}_{b}
Ciba 4666	0.64^{a}_{a}	12.00^{a}_{b}	0.38^{a}_{a}	12.47^{a}_{a}
Pioneer Brand 3471	0.38^{a}_{a}	10.25^{a}_{c}	0.76^{a}_{b}	9.84^{a}_{c}
Pioneer Brand 3184 (Check)	0.25^{a}_{a}	12.76^{a}_{ab}	0.13^{a}_{b}	12.52^{a}_{a}
Wf9 x W182E (Check)	0.25^{a}_{a}	8.73^{a}_{d}	0.25^{a}_{b}	9.85^{a}_{c}
Average tunnel length/plant	0.38^{a}		0.57^{a}	
LSD 0.05 = 0.38				
Average yield		11.25^{a}		11.40^{a}
LSD 0.05 = 0.49				

† Rating is according to Guthrie's (1960) scale of 1-9 (1 = no damage, 9 = severe damage).

‡ For first generation ECB data, superscript letters indicate significance horizontally (LSDs 0.05, rating = 1.1; yield = 1.16) and subscript letters indicate significance vertically (LSDs 0.05, rating = 0.8; yield = 0.82) in the table. If letters are different, the numerical values are significantly different.

§ Average tunnel length/plant.

¶ For second generation ECB data, superscript letters indicate significance horizontally (LSDs 0.05, tunnel = 0.91; yield = 1.20) and subscript letters indicate significance vertically (LSDs 0.05, tunnel = 0.64; yield = 0.85) in the table. If letters are different, the numerical values are significantly different.

an indicator of attractiveness. There were no significant differences between treatments for either yield or number of plants having shot holes. The natural populations of ECB did not develop in synchrony with the maize hybrids. However, there was a trend for increased yield for hybrids with protected treatment, except for Pioneer Brand 3184.

Experiment #4

This experiment determined whether non-preference was a mechanism for second generation ECB resistance (Table 5). Naturally infested vs. protected plots were used as the treatments; and egg mass counts, tunneling, and yield were observed. There was no significant difference between the treatments for any of the traits observed. A few significant differences were noted among hybrids within treatments, but they were not consistent. Again, natural ECB populations did not develop in good synchrony with the crop.

Experiment #5

This experiment determined whether tolerance was important for first generation ECB resistance by observing ECB leaf-feeding damage and yield (Table 6). A significant difference in ECB leaf-feeding damage was observed for every hybrid when no infestation was compared with the infestation rate of 240 larvae per plant. There were generally no significant differences in yields across treatments within hybrids, indicating good tolerance. Additionally, these results suggested that we should have used 240 larvae per plant for our first generation ECB infestations in Missouri in 1994.

Experiment #6

This experiment determined the importance of tolerance to second generation ECB resistance by observing tunneling and yield (Table 9). However, the manual infestations were not effective and no conclusions about tolerance for second generation ECB could be made.

Our experiments indicated that Pioneer Brand 3471 has antibiosis as a resistance mechanism for first generation ECB when manually infested with 120 larvae per plant. Because of very low second generation ratings (including our susceptible check), no significant antibiosis was determined. The preference studies were dependent upon natural infestations of ECB and since these populations were very low, no preference or non-preference was found for either generation. In tests for

Table 5. Evaluation of four commercial hybrids plus two check hybrids for preference by the first and second generation of ECB in Missouri in 1994.

	First generation			
	Natural		Protected	
Hybrid	Shot holes† (no. plants)	Yield (t/ha)	Shot holes† (no. plants)	Yield (t/ha)
ICI Seeds 8326	$1.6^{a}_{b‡}$	12.61^{a}_{a}	2.0^{a}_{b}	12.90^{a}_{a}
DeKalb Genetics 623	6.5^{a}_{a}	11.15^{a}_{b}	3.2^{a}_{b}	11.41^{a}_{b}
Ciba 4666	1.8^{a}_{b}	12.14^{a}_{a}	6.5^{a}_{a}	12.34^{a}_{a}
Pioneer Brand 3471	3.0^{a}_{b}	9.47^{a}_{c}	2.7^{a}_{b}	10.19^{a}_{c}
Pioneer Brand 3184 (Check)	1.8^{a}_{b}	12.73^{a}_{a}	2.3^{a}_{b}	12.57^{a}_{a}
Wf9 x W182E (Check)	1.2^{a}_{b}	6.85^{a}_{d}	1.8^{a}_{b}	7.46^{a}_{d}
Average number of plants/plot with shot holes LSD 0.05 = 1.7	2.6^{a}		3.1^{a}	
Average yield LSD 0.05 = 0.39		10.83^{a}		11.14^{a}

	Second generation					
	Natural			Protected		
Hybrid	Egg masses§ (no.)	Tunnel¶ (cm)	Yield (t/ha)	Egg masses§ (no.)	Tunnel¶ (cm)	Yield (t/ha)
ICI Seeds 8326	$3.2^{a\#}_{ab}$	0.51^{a}_{ab}	12.10^{a}_{a}	2.8^{a}_{a}	0.69^{a}_{ab}	12.53^{a}_{a}
DeKalb Genetics 623	3.3^{a}_{ab}	0.76^{a}_{ab}	11.16^{a}_{b}	1.8^{a}_{a}	0.91^{a}_{a}	11.18^{a}_{c}
Ciba 4666	5.0^{a}_{a}	0.51^{a}_{ab}	11.18^{a}_{ab}	3.7^{a}_{a}	0.33^{a}_{b}	11.45^{a}_{bc}
Pioneer Brand 3471	5.2^{a}_{a}	0.25^{a}_{b}	9.99^{a}_{cd}	2.8^{a}_{a}	0.33^{a}_{b}	9.71^{a}_{d}
Pioneer Brand 3184 (Check)	1.6^{a}_{b}	0.84^{a}_{a}	10.46^{b}_{bc}	3.7^{a}_{a}	0.94^{a}_{a}	12.17^{a}_{ab}
Wf9 x W182E (Check)	4.0^{a}_{a}	0.69^{a}_{ab}	9.07^{a}_{d}	3.7^{a}_{a}	0.76^{a}_{ab}	9.45^{a}_{d}
Average no. of egg masses/ plant LSD 0.05 = 1.4	3.7^{a}			3.1^{a}		
Average tunnel length/plant LSD 0.05 = 0.33		0.59^{a}			0.66^{a}	
Average yield LSD 0.05 = 0.54			10.66^{a}			11.08^{a}

† Average number of plants/plot with shot holes.

‡ For first generation ECB data, superscript letters indicate significance horizontally (LSDs 0.05, shot holes = 4.03; yield = 0.97) and subscript letters indicate significance vertically (LSDs 0.05, shot holes = 2.85; yield = 0.68) in the table. If letters are different, the numerical values are significantly different.

§ Average number of egg masses/plant.

¶ Average tunnel length/plant.

For second generation ECB data, superscript letters indicate significance horizontally (LSDs 0.05, egg masses = 3.5, tunnel = 0.81; yield = 1.34) and subscript letters indicate significance vertically (LSDs 0.05, egg masses = 2.5, tunnel = 0.56; yield = 0.94) in the table. If letters are different, the numerical values are significantly different.

tolerance, no significant differences were found for yield. The infestation rate of 240 larvae per plant, however, showed significant differences in leaf feeding (antibiosis) for all hybrids. Those hybrids with higher leaf-feeding ratings [ICI Seeds 8326 (4.0 rating), DeKalb Genetics 623 (3.8 rating), and Ciba 4666 (5.0 rating)] would be considered intermediate in resistance, and the check Wf9xW182E (6.0 rating) was susceptible. Pioneer Brand 3471 and Pioneer Brand 3184 (resistant check) were resistant when using Guthrie et al.'s 1 to 9 scale (Guthrie et al., 1960). During the 1994 growing season, in our plots, 100 or fewer larvae per plant were inadequate for making good evaluations.

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Table 6. Evaluation of four commercial hybrids plus two check hybrids for tolerance by the first and second generation of ECB in Missouri in 1994.

	First generation							
	Noninfested		30 larvae per plant		100 larvae per plant		240 larvae per plant	
Hybrid	Rating† (1-9)	Yield (t/ha)	Rating† (1-9)	Yield (t/ha)	Rating† (1-9)	Yield (t/ha)	Rating† (1-9)	Yield (t/ha)
ICI Seeds 8326	$1.0^{c‡}_{b}$	12.96^{a}_{a}	1.8^{bc}_{b}	12.79^{a}_{a}	2.2^{b}_{bc}	13.05^{a}_{a}	4.0^{a}_{c}	13.15^{a}_{a}
DeKalb Genetics 623	1.0^{c}_{b}	11.16^{a}_{b}	1.0^{c}_{c}	11.83^{a}_{b}	2.8^{b}_{b}	12.18^{a}_{b}	3.8^{a}_{c}	11.51^{a}_{b}
Ciba 4666	2.2^{b}_{a}	12.60^{a}_{a}	2.8^{b}_{a}	12.43^{ab}_{ab}	2.5^{b}_{bc}	11.20^{b}_{c}	5.0^{a}_{b}	12.89_{a}
Pioneer Brand 3471	1.2^{b}_{b}	9.39^{a}_{c}	1.5^{ab}_{bc}	9.27^{a}_{c}	2.0^{ab}_{c}	9.34^{a}_{d}	2.8^{a}_{d}	9.54^{a}_{c}
Pioneer Brand 3184 (Check)	1.2^{b}_{b}	13.04^{a}_{a}	1.5^{ab}_{bc}	12.26^{a}_{ab}	2.0^{ab}_{c}	13.03^{a}_{a}	2.2^{a}_{e}	11.98^{a}_{b}
Wf9 x W182E (Check)	2.2^{c}_{a}	6.79^{ab}_{d}	3.0^{c}_{a}	6.16^{b}_{d}	4.5^{b}_{a}	7.43^{a}_{e}	6.0^{a}_{a}	7.35^{ab}_{d}
Average rating		1.9^{c}		1.9^{c}		2.7^{b}		4.0^{a}
LSD 0.05 = 0.4								
Average yield	10.99^{a}		10.79^{a}		11.04^{a}		11.07^{a}	
LSD 0.05 = 0.51								

	Second generation							
	Noninfested		30 larvae per plant		100 larvae per plant		240 larvae per plant	
Hybrid	Tunnel§ (cm)	Yield (t/ha)	Tunnel§ (cm)	Yield (t/ha)	Tunnel§ (cm)	Yield (t/ha)	Tunnel§ (cm)	Yield (t/ha)
ICI Seeds 8326	$0.38^{a¶}_{cd}$	12.38^{a}_{ab}	0.51^{a}_{ab}	12.90^{a}_{a}	0.13^{a}_{b}	12.41^{a}_{ab}	0.51^{a}_{a}	12.72^{a}_{ab}
DeKalb Genetics 623	1.27^{a}_{a}	11.76^{a}_{b}	0.13^{b}_{c}	10.81^{a}_{c}	0.51^{b}_{a}	11.53^{a}_{cd}	0.76^{ab}_{a}	11.51^{a}_{c}
Ciba 4666	0.76^{a}_{b}	13.09^{a}_{a}	0.25^{a}_{bc}	11.91^{a}_{b}	0.64^{a}_{a}	13.13^{a}_{a}	0.13^{a}_{b}	13.07^{a}_{a}
Pioneer Brand 3471	0.13^{a}_{d}	9.59^{a}_{c}	0.25^{a}_{bc}	10.49^{a}_{c}	0.13^{a}_{b}	10.70^{a}_{d}	0.00^{a}_{b}	10.26^{a}_{d}
Pioneer Brand 3184 (Check)	0.64^{a}_{bc}	12.06^{a}_{b}	0.38^{a}_{bc}	12.18^{a}_{ab}	0.00^{b}_{b}	12.06^{a}_{bc}	0.51^{a}_{a}	11.75^{a}_{bc}
Wf9 x W182E (Check)	0.64^{a}_{bc}	9.41^{a}_{c}	0.76^{a}_{a}	8.33^{a}_{d}	0.64^{a}_{a}	9.16^{a}_{e}	0.76^{a}_{a}	9.36^{a}_{e}
Average tunnel length/plant		0.64^{a}		0.38^{a}		0.34^{b}		0.44^{a}
LSD 0.05 = 0.30								
Average yield	11.38^{a}		11.10^{a}		11.50^{a}		11.38^{a}	
LSD 0.05 = 0.70								

† Rating is according to Guthrie's (1960) scale of 1-9 (1 = no damage, 9 = severe damage).

‡ For first generation ECB data, superscript letters indicate significance horizontally (LSDs 0.05, rating = 1.0; yield = 1.25) and subscript letters indicate significance vertically (LSDs 0.05, rating = 0.5; yield = 0.62) in the table. If letters are different, the numerical values are significantly different.

§ Average tunnel length/plant.

¶ For second generation ECB data, superscript letters indicate significance horizontally (LSDs 0.05, tunnel = 0.76; yield = 1.70) and subscript letters indicate significance vertically (LSDs 0.05, tunnel = 0.56; yield = 1.20) in the table. If letters are different, the numerical values are significantly different.

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Mechanisms and Bases of Resistance in Maize to Southwestern Corn Borer and Fall Armyworm

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Abstract

Maize, Zea mays *L., germplasm lines with resistance to leaf feeding by the southwestern corn borer (SWCB),* Diatraea grandiosella *Dyar, and fall armyworm (FAW),* Spodoptera frugiperda *(J.E. Smith), have been developed and released. A series of experiments were conducted to determine the mechanisms and bases of this resistance. Field experiments have shown that antibiosis is a mechanism of resistance to both insects. When resistant and susceptible maize genotypes were infested with either SWCB or FAW neonates, the larvae that were recovered 10 to 14 days later from susceptible genotypes weighed twice as much as those recovered from resistant genotypes. Laboratory experiments using excised leaf tissue and liquid pressed from leaves demonstrated that larval non-preference is also a mechanism of resistance of these germplasm lines. When experiments were conducted using callus tissue of susceptible and resistant maize genotypes, both SWCB and FAW larvae preferred to feed on callus of susceptible genotypes. Larvae that fed on the susceptible calli weighed twice as much as those that fed on resistant calli. Similar differences in size were observed when larvae were fed on lyophilized leaf tissue of susceptible and resistant genotypes. Factors responsible for these differences in growth are not fully known; however, leaf tissue from the inner whorls of resistant genotypes tends to be tougher than that from susceptible genotypes. The cuticle and epidermal cell wall of resistant genotypes is generally thicker. Leaves of resistant plants have a higher fiber content and lower total protein content. A 33kD polypeptide found in callus tissue appears to be associated with resistant genotypes. Electrophoretic analysis of whorl leaf tissue also indicated a possible association of resistance to SWCB and FAW with 21kD and 36kD polypeptides.*

Introduction

Identifying germplasm with resistance to a pest is critical to the success of any plant resistance research program. Therefore, developing methods of evaluating damage to the pest and locating suitable germplasm to evaluate receive a high priority at the inception of a new program. However, once germplasm with resistance has been identified, other questions quickly arise. Why is the germplasm resistant? What mechanisms of resistance are operating? How is the resistance inherited? How effective is the resistance in reducing yield losses?

In our work with SWCB and FAW resistance in maize, we have not only posed these questions, but have undertaken research to try to answer them. Scott and Davis (1981) released the first germplasm with resistance to SWCB and FAW in 1974. Soon thereafter, attempts to determine how the resistant plants differed from susceptible plants and to compare the responses of larvae to resistant and susceptible plants began. As we developed and released additional germplasm lines, we have continued our investigations in these areas using field and greenhouse experiments, chemical analyses, microscopy, and laboratory bioassays.

Field and Greenhouse Experiments

One of the first experiments conducted to determine mechanisms of resistance operating in germplasm released from our program compared MpSWCB-4 and Antigua 2D-118 (FAW resistant germplasm identified at Tifton, GA) with susceptible genotypes (Wiseman et al. 1981). Choice tests to determine larval preference were conducted by randomly placing leaf sections of 5 genotypes along the outer edge of 25.4-cm-diam. dishes, then 200 first instar larvae were placed in the center. The dishes were maintained in darkness for 18 hours; larvae on or

under each leaf section were then counted. Fewer larvae were found on the resistant genotypes than the susceptible genotypes (Table 1). Tests were also conducted to compare growth of FAW larvae on leaf sections of the same genotypes (Table 1). After 8 days, larvae fed on leaf tissue of MpSWCB-4 were significantly smaller than those fed on any other genotype. Larvae fed on Cacahuacintle X tissue consumed 72.4 cm^2 leaf tissue while those fed on MpSWCB-4 consumed only 21.5 cm^2 tissue. MpSWCB-4 showed the highest level of resistance with both antibiosis and non-preference expressed. The resistance of Antigua 2D-118 appeared to be primarily non-preference.

Further evidence of the high degree of non-preference of Antigua 2D-118 was provided by a field cage test designed to determine if FAW larvae were crawling off resistant plants (Wiseman et al. 1983). Test plants of 3 genotypes were planted approximately 120 cm apart and each was surrounded by plants of a susceptible hybrid. The test plants were infested with 10, 20, or 40 newly hatched larvae per plant. At 3, 5, 7, and 11 days after infestation, the larvae that had moved from test plants were counted (Table 2). Significantly more larvae crawled from Antigua 2D-118 than from MpSWCB-4 or Cacahuacintle Xs, which did not differ. An additional investigation was conducted to determine survival and growth of FAW larvae under field conditions using some of the same genotypes (Williams et al. 1983b). Again, larval weights and survival were lower on Antigua 2D-118 and MpSWCB-4 than on Ab24E x Mp305 and Cacahuacintle Xs, indicating antibiosis and possibly non-preference as resistance mechanisms in the 2 resistant types of germplasm.

Several field experiments have also indicated that SWCB survival and growth are reduced on plants of leaf-feeding resistant genotypes (Davis and Williams 1986; Davis et al. 1991; Williams et al. 1989). Data from one of these (Davis et al. 1991) are given in Table 3. In this experiment, plants were infested in the mid-whorl stage of growth with 30 neonates per plant. Larvae were counted and weighed 14 days later. The significantly lower weights of larvae recovered from the resistant hybrids provide evidence for antibiosis as a mechanism of resistance in these hybrids. Differences in larval survival between resistant and susceptible hybrids were less distinct,

Table 1. Number of FAW larvae present on leaf sections of different maize genotypes after 18 hours in a choice test, and mean weights of larvae fed for 8 days on the same genotypes in a no-choice test (Wiseman et al. 1981).

Genotype	Field rating[a]	No. of larvae[b] (18 hr) choice	Wt. of larvae (mg)[c] (8 days) no-choice
Cacahuacintle X s	S	17.7 a	333.5 a
Ab24E x Mp305	S	13.0 b	263.3 b
Mp4008	R	8.5 c	193.3 c
Antigua 2D-118	R	5.8 cd	229.6 bc
MpSWCB-4	R	2.1 d	151.8 d

[a] S, susceptible; R, resistant.
[b] Means (based on 30 replicates) followed by the same letter do not differ significantly (P = 0.05, Duncan's Multiple Range Test).
[c] Means (based on 50 replicates) followed by the same letter do not differ significantly (P - 0.05, Duncan's Multiple Range Test).

Table 2. Mean number of FAW larvae moving from test maize genotypes surrounded by susceptible trap plants at various time intervals after infestation (Wiseman et al. 1983).

Genotype	Field classification[a]	Days after infestation[b] 3	5	7	11
Antigua 2D-118	R	0.6 a	5.6 a	5.9 a	8.0 a
MpSWCB-4	R	0.1 b	2.1 b	3.7 b	5.0 b
Cacahuacintle X s	S	0.2 b	2.1 b	3.3 b	4.5 b

[a] R, resistant; S, susceptible.
[b] Means within a column followed by the same letter do not differ (P = 0.05, Duncan's Multiple Range Test.

Table 3. Number and weights of SWCB larvae 14 days after infestation of hybrids with 30 larvae/plant at Mississippi State, MS (Davis et al. 1991).

Hybrid	Classification[a]	No. larvae 1988	1989	Larval wt (mg) 1988	1989
Ab24E x Va35	S x S	4.1	4.9	60.1	55.5
T202 x Va35	S x S	2.7	4.7	62.1	57.1
Ab24E x Tx601	S x S	3.4	5.6	53.3	47.7
Mp496 x Mp701	R x R	2.1	2.2	15.6	7.0
Mp701 x Mp705	R x R	2.8	1.6	13.2	8.4
Mp703 x Mp704	R x R	1.8	1.3	11.3	10.2
Mp704 x Mp707	R x R	2.5	0.6	10.0	7.6
LSD (0.05)		1.7	1.6	7.8	10.5

[a] S, susceptible; and R, resistant, to SWCB leaf feeding.

especially in 1988. The differences in number of larvae recovered from resistant and susceptible hybrids could be attributed to either non-preference or antibiosis.

Chemical Analyses, Bioassays, and Anatomical Observations

While field and greenhouse experiments yielded information on the mechanisms of resistance operating in this germplasm, it became obvious to us that other types of experiments would be necessary for a more thorough understanding of what factors are responsible for the resistance. This led us into the area of chemical analyses and laboratory bioassays. Whorl tissue from resistant and susceptible genotypes was analyzed. The tissue from the resistant genotype was at least 25% higher for crude fiber, acid detergent fiber, lactic acid, calcium, and glutamate-oxalacetate transaminase. The susceptible genotype was at least 25% higher for crude protein, crude lipid, ash, stearic and oleic acids, and silica (Hedin et al. 1984). It appeared that components associated with nutrition, such as protein, minerals, and lipids, were higher in the susceptible genotype, whereas fiber was higher in the resistant genotype. Subsequent analyses of tissue from additional genotypes generally supported this conclusion.

In another facet of this investigation, whorl tissue was freeze-dried, ground, and extracted by Soxhlet with cyclohexane/ethyl acetate/acetic acid, 500/500/1 (CHEA). The tissue was subsequently extracted at boiling reflux with methanol/water, 7/3 (mw). The extracts were incorporated into the artificial diet used in our rearing program (Davis 1989). The diet is based on wheatgerm, casein, sucrose, vitamins, salts, agar, and antimicrobial agents. Newly hatched SWCB larvae were fed on the test diets for 5 days and then weighed. The extracts from both the susceptible and resistant tissue caused only limited inhibition of larval growth that did not appear to be biologically significant (Hedin et al. 1984). Analyses to determine the composition of the residue did not yield any information that suggested a basis of resistance. We concluded that the celluloses and hemicelluloses making up the higher fiber content of the resistant genotypes could contribute to leaf toughness, indigestibility, and intractability to metabolism by the insect and this might be at least a part of the basis of resistance.

Because of the ambiguity of our results at this point, we were somewhat discouraged and were unsure as to whether we should look for an anatomical or biochemical basis of resistance. Investigations were begun to determine whether anatomical differences exist between a leaf-feeding resistant line, Mp704, and a susceptible line, Ab24E. Ng (1988) found that the number of vascular bundles per unit area was greater in Mp704 whorl leaf tissue than in Ab24E whorl leaf tissue. The cuticle and outer cell wall of the epidermis on both the upper and lower leaf surfaces of Mp704 leaves were found to be thicker.

In a follow-up study, 4 resistant and 6 susceptible lines were included (Davis et al. 1994). Again, both the upper and lower cell wall complexes of the resistant lines were thicker. The thickness of the upper and lower cell wall complexes were also highly correlated with SWCB and FAW leaf feeding damage ratings (Table 4). Larval weights were negatively correlated with thickness. In a second part of this investigation, the pressure required to split whorl tissue of the same lines was determined. Although the required pressure differed between years and there was a significant interaction with years, greater pressure was generally required to split the whorl tissue of resistant genotypes.

Tissue Culture

At about the same time the anatomical studies commenced, we undertook investigations to determine whether the reductions in larval weight expressed on plants of resistant genotypes in the field would also be expressed if larvae were fed undifferentiated maize callus tissue. Initially, we conducted experiments to determine whether SWCB larvae would feed and develop on callus tissue and whether larval growth was affected by callus genotype (Williams et al. 1983a). We found that the larvae did indeed feed and develop on callus tissue and those fed on callus of resistant maize genotypes were generally smaller.

Table 4. Correlation coefficients (r) between anatomical characteristics and SWCB and FAW damage ratings and larval weights for susceptible and resistant maize lines (Davis et al. 1994).

	Cell wall complex thickness	
Insect	**Upper**	**Lower**
Southwestern corn borer		
Damage score	0.92**	0.92**
Larval weight	- 0.85**	- 0.85**
Fall armyworm		
Damage score	0.91**	0.91**
Larval weight	- 0.71*	- 0.71*

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.

Encouraged by the results of our initial tests, we designed experiments to measure both SWCB and FAW growth on resistant and susceptible genotypes. We evaluated a diallel cross for leaf feeding by SWCB in the field and for larval growth on callus in the laboratory (Williams and Davis 1985). Both the leaf feeding ratings and the larval weights clearly delineated resistant and susceptible hybrids (Table 5). The differences in larval growth indicated that antibiosis was acting as a mechanism of resistance. Significant differences in larval weight were also expressed when FAW larvae were fed for 7 days on callus of resistant and susceptible hybrids (Table 6) (Williams et al. 1985). As with SWCB, antibiosis was apparently operating as a mechanism of resistance to FAW.

Additional investigations were conducted to determine whether non-preference might also be operating as a mechanism of resistance. To determine whether FAW larvae, upon hatching, fed preferentially on callus of different maize hybrids, approximately 500 mg of callus of 4 hybrids was placed in the corners of six plastic containers (130 x 130 x 55 mm). Approximately 50 blackhead-stage eggs were placed in the center of each container. Containers were placed in complete darkness for 24 hours; larvae present on each portion of callus were then counted (Williams et al. 1985). Twice as many larvae were attracted to the callus of susceptible hybrids (Table 7), indicating non-preference for the callus of resistant hybrids.

A similar experiment was conducted to determine whether SWCB larvae exhibited a preference for callus of some genotypes (Williams et al. 1987a). In this experiment, callus of 4 hybrids was equally spaced around the perimeter of Petri plates (150 mm diameter) and 50 eggs, just prior to hatch, were placed in the center of the plate. Larvae were counted after 24 hours. As with FAW, the SWCB larvae strongly preferred callus of the leaf-feeding susceptible hybrids (Table 8).

We have also conducted similar experiments with corn earworm (CEW), *Helicoverpa zea* (Boddie), using our leaf feeding resistant and susceptible lines (Williams et al. 1987b). The results were similar to those obtained with SWCB and FAW.

The differences we observed in larval growth and preference associated with maize exhibiting different levels of

Table 5. Mean ratings of SWCB leaf-feeding damage in the field and weights of larvae grown on callus initiated from resistant and susceptible maize hybrids (Williams and Davis 1985).

Hybrid	Classification[a]	Leaf feeding damage[b]	7-Day larval wt (mg)[c]
Ab24E x Tx601	S x S	7.1	17.8
Ab24E x GT112	S x S	6.9	19.2
Tx601 x GT112	S x S	6.7	16.5
Mp496 x Mp704	R x R	5.5	11.1
Mp496 x Mp78:518	R x R	4.9	10.3
Mp704 x Mp78:518	R x R	4.8	11.0
LSD (0.05)		0.5	2.3

[a] S indicates susceptibility and R, resistance.
[b] Damage was visually rated 14 days after infestation with 30 larvae per plant on a scale of 0 (no damage) to 9 (heavy damage) in 1982 and 1983.
[c] Larvae were weighed after feeding on callus for 7 days.

Table 6. Weights of FAW larvae fed on callus of resistant and susceptible maize hybrids for 7 days (Williams et al. 1985).

Hybrid	Classification[a]	No. of larvae[b]
Pioneer Brand 3369A	S	52 a
Ab24E x Va35	S	48 a
Mp496 x Mp704	R	34 b
Mp703 x Mp704	R	25 b

[a] S indicates susceptible; R, resistant to leaf feeding in field tests.
[b] Means not followed by the same letter differ at the $P = 0.05$ level of significance (Student-Newman-Keuls test).

Table 7. Mean number of FAW larvae present on different maize hybrids 24 hours after infestation with 50 black-head-stage eggs (Williams et al. 1985).

Hybrid	Classification[a]	No. of larvae[b]
Pioneer Brand 3369A	S	13 a
Ab24E x Va35	S	15 a
Mp496 x Mp704	R	6 b
Mp703 x Mp704	R	7 b

[a] S indicates susceptibility to leaf feeding; R indicates resistance.
[b] Means not followed by the same letter differ at the $P = 0.05$ level of probability (Student-Newman-Keuls test).

Table 8. Number of SWCB larvae feeding on callus 24 hours after infestation with 50 blackhead-stage eggs (Williams et al. 1987a).

Hybrid	Classification[a]	No. of larvae[b]
Ab24E x Va35	S	6.4 a
SC229 x Tx601	S	7.6 a
Mp496 x Mp701	R	2.7 b
Mp704 x Mp706	R	1.3 b

[a] S indicates susceptibility to leaf feeding; R indicates resistance.
[b] Means (10 replications) followed by the same letter differ at the $P = 0.05$ level of significance (Student-Newman-Keuls test).

resistance to FAW and SWCB leaf-feeding in the field renewed our interest in trying to find a chemical basis for this resistance. Chemical analyses of callus of susceptible and resistant genotypes indicated that the most obvious difference between the two was a higher amount of aspartic acid in the resistant callus (Hedin et al. 1990). We also found that when choice tests were conducted in which a series of amino acids were compared in attractiveness with water, only aspartic acid elicited less response than water.

Laboratory Bioassays and Chemical Analyses

Because of the difficulty of producing callus in the quantities needed by the chemist with whom we were working, we sought other approaches for investigating the chemical basis of resistance. To determine whether larvae discriminated among extracts from leaves of different genotypes, plant whorls were pressed in a hydraulic press. Filter paper disks were saturated with extracts from different genotypes and randomly placed around the perimeter of 150-mm diameter Petri plates. Forty neonate larvae were placed in the center of the dish, and the dishes were placed in darkness for 4 hours. The number of larvae on each filter paper were then counted. We found that larvae were attracted to susceptible genotypes twice as often as to resistant genotypes (Williams et al. 1987b). Choice tests conducted following fractionation of leaf extracts indicated that FAW were more strongly attracted to the amino acids of extracts from susceptible genotypes than to those from resistant ones (Hedin et al. 1990). Further experimentation also indicated that SWCB larvae exhibited a preference for the amino acids alanine and valine, but aspartic acid was non-preferred (Hedin et al. 1993).

In other investigations, lyophilized leaf tissue of corn genotypes with varying levels of resistance to FAW and SWCB was added to an artificial diet. FAW larvae grew well on all diets into which lyophilized tissue had been incorporated, and weights of larvae fed on resistant and susceptible genotypes did not differ (F. Davis, unpublished). This indicated to us that the diet was either masking differences that would have been expressed in growth of larvae fed on susceptible or resistant genotypes in the field or lyophilization destroyed genotypic differences among tissue samples.

We, therefore, designed a bioassay based primarily on lyophilized leaf tissue (Buckley et al. 1991; Williams and Buckley 1992; Williams et al. 1990a). For these bioassays, whorl leaves were harvested when plants reached the mid-whorl stage of growth. They were trimmed to approximately 15 cm in length, placed in plastic freezer bags, and frozen at -18°C. The whorl tissue was later lyophilized and ground to a fine powder. Diets were prepared by combining 250 ml distilled water, 2400 mg agar, 12.5 mg gentamicin sulfate, 132 mg sorbic acid, and 528 mg ascorbic acid. The mixture was heated to 82°C while stirring, and 11 g lyophilized tissue was then added. The mixture was then dispensed in 10-ml aliquots into 30-ml cups.

Experiments were carried out by infesting cups with neonates, covering them with insert paperboard caps, and placing them in an environmental chamber maintained at 28°C with a photoperiod of 12:12 (L:D). FAW larvae were weighed after 10 days and SWCB larvae after 14 days. For both insects, the larvae weighed significantly less when fed on diets containing tissue of resistant genotypes (Table 9). The bioassay was also used successfully with CEW (Table 10) (Buckley et al. 1991).

This was the first bioassay that we had used which appeared to have promise as a way of comparing various fractions of susceptible and resistant maize genotypes. In one investigation, we evaluated FAW larval growth, not only on susceptible and resistant inbred

Table 9. Weights of FAW and SWCB larvae reared for 10 and 14 days, respectively, on diets containing lyophilized whorl tissue of various inbred lines (Williams et al. 1990a).

	Larval weight (mg)[a]	
Inbred line	**FAW**	**SWCB**
Susceptible		
Ab24E	172 b	116 b
SC229	185 b	161 a
Tx601	239 a	112 b
Va35	150 b	126 b
Resistant		
Mp701	62 c	65 c
Mp705	86 c	71 c
Mp704	97 c	69 c
Mp707	53 c	46 d

[a] Means in a column followed by the same letter do not differ (P <0.05) (Student-Newman-Keuls test).

Table 10. Weights of CEW larvae reared for 11 days on diets containing only lyophilized whorl tissue of corn inbreds (Buckley et al. 1991).

Inbred	Classification[a]	Larval wt (mg)
Ab24E	S	223
Tx601	S	139
Va35	S	59
Mp704	R	11
Mp707	R	26
Mp708	R	12
LSD (0.05)		28

[a] S indicates susceptibility to leaf feeding; R indicates resistance.

lines, but also on mixtures of tissue from resistant and susceptible lines (Williams and Buckley 1992). We found generally that larvae fed on mixtures of tissue of susceptible and resistant genotypes exhibited weight gains less than those of larvae fed on susceptible tissue alone, but greater than those fed only resistant tissue. The mean weight of those fed on susceptible tissue alone was 238 mg, while those fed on only resistant tissue weighed 114 mg, and those fed on a mixture of the two weighed 185 mg. This would be consistent with the presence of reduced amounts in resistant genotypes of substances essential for larval growth.

We have also carried out experiments using methods similar to those described in the previous experiment (Williams and Buckley 1992) except various combinations of water extracts and residues of lyophilized tissue of resistant, Mp708, and susceptible, Ab24E, genotypes replaced the 10 g lyophilized tissue in our usual bioassay diet. This was done to help us determine whether the extraction process itself affected larval growth and to determine whether the factors causing differences in larval growth on resistant and susceptible genotypes occur in the extracts or residues. The results (Table 11) indicated that the water extracts provided substances essential to growth, but the resistant and susceptible genotypes provided these equally well. Larval growth indicated that genotypic differences between residues are responsible for differences in growth.

We have not yet been able to capitalize on this. In further fractionation of the residue, we apparently have either lost or changed substances essential for growth. We are, however, still working on this problem. We do make these conclusions about the basis of resistance from our research:

- Resistant genotypes probably do not contain a highly toxic substance.
- Such characteristics as leaf toughness may be a part, but not the complete, basis of resistance.
- Nutritional differences between tissue of resistant and susceptible genotypes may be associated with resistance.
- There are likely several factors responsible for resistance in the lines we have released.

Table 11. Weights of FAW larvae fed on test diets composed of various combinations of residues and extracts of lyophilized leaf tissue from resistant and susceptible maize genotypes (Williams and Buckley 1992).

Diet composition	Larval wt. (mg)
Lyophilized tissue (S)[a]	165
Lyophilized tissue (R)	20
Water extract (S) + residue (S)	106
Water extract (R) + residue (S)	106
Water extract (S) + residue (R)	13
Water extract (R) + residue (R)	10
Residue (S)	11
Residue (R)	1
LSD (0.05)	22

[a] S indicates leaf feeding susceptible Ab24E; R indicates leaf feeding resistant Mp708.

Identification of Proteins Associated with Resistance

Because we have been unable to definitely identify specific substances responsible for resistance in the lines we have released, we have attempted to identify proteins associated with resistance (Paiva 1988; Callahan et al. 1992; Jiang 1994). We assumed that although proteins *per se* might not affect larval growth, it should be possible to find differences in proteins that were involved in some way in the synthesis of those substances that affect larval growth. This work has involved the electrophoretic analyses of proteins extracted from whorl leaf tissue (Callahan et al. 1992) and callus tissue (Jiang 1994) of resistant and susceptible maize genotypes.

In a comparison of polypeptides present in the leaf-feeding resistant line Mp708 with the lines from which it was developed, Mp704 and Tx601 (Williams et al. 1990b), Callahan et al. (1992) found 8 polypeptides present in both Mp708 and its resistant parent, Mp704, which were absent in the susceptible Tx601. The full complement of polypeptides was not present in 1 other resistant line nor completely absent from 3 other susceptible lines (Table 12). The combined presence of polypeptides 5(36kD) and 7(21kD) was, however,

Table 12. Summary of two-dimensional gel data of 8 maize lines with regard to presence (+) or absence (-) of 8 polypeptides (Callahan et al. 1992).

	Polypeptide number							
Line[a]	1	2	3	4	5	6	7	8
Mp708 (R)	+	+	+	+	+	+	+	+
Mp704 (R)	+	+	+	+	+	+	+	+
Mp707 (R)	-	-	-	-	+	-	+	-
Mp496 (R)	-	-	+	+	+	-	+	-
Tx601 (S)	-	-	-	-	-	-	-	-
Ab24E (S)	-	+	+	+	+	+	-	-
GT106 (S)	-	-	-	+	-	-	+	-
SC229 (S)	-	-	-	+	+	-	-	-

[a] R indicates resistance to leaf feeding; S indicates susceptibility.

specific to the resistant lines. Further research will be needed to reveal the significance of these findings.

Extensive analyses by Jiang (1994) of the proteins of callus of resistant and susceptible maize lines revealed one protein (33kD) that was consistently present in callus of resistant, but not susceptible, genotypes. The n-terminal amino acid sequence of the 33kD protein suggests that it may be a cysteine proteinase. In F_2 progeny of the resistant by susceptible cross, Mp704 x Tx601, concentration of the 33kD protein and weight of larvae feeding on those callus lines were negatively correlated.

One interesting fact was observed during this investigation. Callus of Mp704 is normally not friable, but after culturing for extended periods of time, it sometimes becomes friable or easily crumbled. In one insect feeding trial, both friable and non-friable Mp704 callus was included. The FAW larvae that fed on friable callus were much heavier than those fed on non-friable callus. After this was observed, an experiment was designed to compare growth on callus with friable and non-friable morphology. The results indicated that FAW larvae fed on friable Mp704 callus and friable callus of the hybrid, Mp704 x Tx601, were not only heavier than those fed on non-friable callus of the same genotypes, but also heavier than those fed on callus of the susceptible line, Tx601 (Table 13). Analysis of the proteins of the friable and non-friable callus revealed that loss of the 33kD protein accompanied the change in morphology. This provides additional evidence that this protein may play a role in resistance. The indication that the 33kD protein may be associated with resistance represents one of our more definitive findings. We are now attempting to identify the protein and determine its function in the plant.

From the many experiments we have conducted, we conclude that both antibiosis and non-preference are operating as mechanisms of resistance. We have not found a single factor, such as a strong toxin, to which resistance can be attributed. It may well be that the resistance we have found is conditioned by several factors, such as leaf toughness, increased fiber, and reduced nutritional quality of the resistant plants. If this is true, it would explain our difficulties in identifying those factors.

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Table 13. Mean weights of FAW larvae fed nonfriable and friable calli of resistant and susceptible maize lines for 7 days (Jiang 1994).

Genotype	Callus morphology	Larval wt. (mg)
Mp704 (R)[a]	Nonfriable	59
	Friable	130
Mp704 x Tx601 (RxS)	Nonfriable	93
	Friable	131
Tx601 (S)[a]	Nonfriable	100
LSD	(0.05)	16

[a] S indicates susceptibility to leaf feeding; R indicates resistance.

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Chemicals Associated with Maize Resistance to Corn Earworm and Fall Armyworm

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Abstract

The resistance of certain corn silks to the corn earworm, Helicoverpa zea *(Boddie) and fall armyworm,* Spodoptera frugiperda*, is due to the presence in the silks of one major luteolin-C-glycoside called maysin. A recent HPLC screening of over 1,100 corn inbreds, populations, Plant Introductions, and various unassigned lines for maysin content has resulted in the discovery of a number of lines with high levels of maysin. This screening also led to the discovery of several lines with relatively high levels of flavone-C-glycosides, other than maysin. Laboratory bioassays showed a high correlation between antibiosis activity and flavone content and type. Compounds identified include 3'-methoxymaysin, the apigenin-analogue of maysin (apimaysin), and 4"-hydroxy-maysin. Several lines were found to contain large levels of isoorientin, 6-C-glucosylluteolin. Bioassays determined that it was almost as active as maysin, while apimaysin and 3'-methoxymaysin were about half as active in reducing corn earworm growth. Chlorogenic acid was also found in silks and was shown to be almost as active as maysin in the bioassay. Incorporation of these new compounds into corn silks of new germplasm should greatly increase corn earworm and fall armyworm resistance.*

Introduction

The control of the corn earworm, *Helicoverpa zea*, (Boddie) (CEW) and fall armyworm, *Spodoptera frugiperda* (FAW) (J.E. Smith), in corn by increased natural resistance would result in higher yields and decreased agrochemical expenses. Zapalote Chico silks were first reported to possess an antibiosis factor to the CEW by Straub and Fairchild (1970). After several attempts were made to isolate the factor (Starks et al. 1965; McMillian et al. 1970), Waiss et al. (1979) and Elliger et al. (1980a) successfully characterized a C-flavone glycoside called maysin [2"-*O*-L-rhamnosyl-6-C-(6-deoxy-xylo-hexos-4-ulosyl)-luteolin] (Fig. 1) that was shown to possess high antibiosis activity. Wiseman et al. (1992) showed

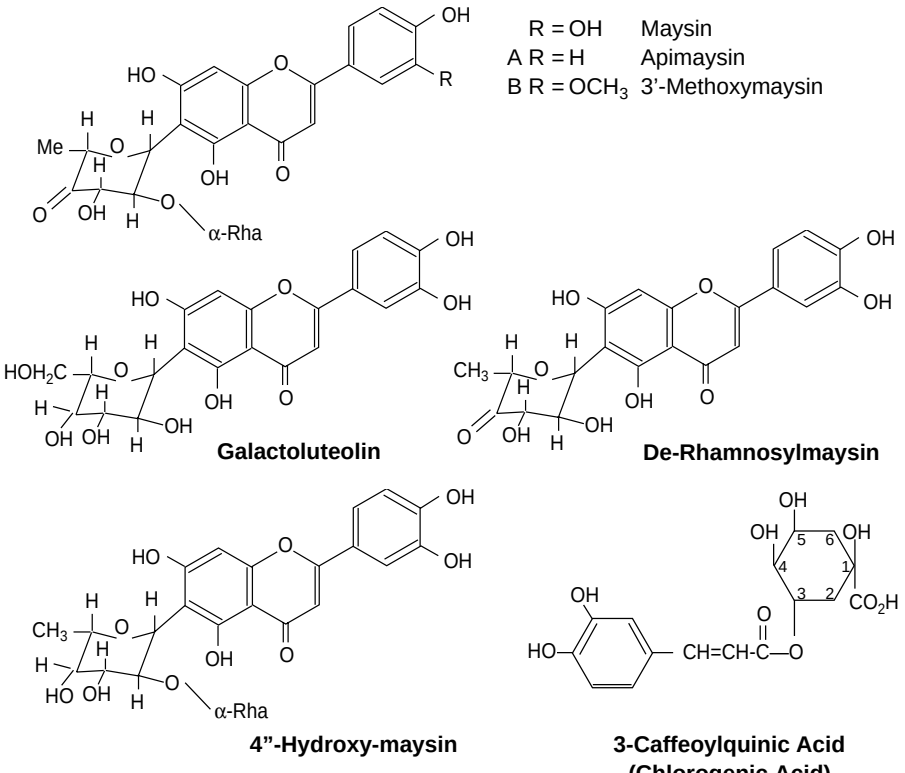

Figure 1. Structures of corn silk polyphenols.

that maysin was also active against FAW. Elliger et al.(1980b) tested a number of flavonoids for growth inhibition against CEW and demonstrated that the presence of adjacent hydroxyl groups on the B ring of the flavone was essential for activity, a structural feature exhibited by maysin (Fig. 1). Maysin can thus be considered a natural insecticide that is target specific (CEW) and is present at the right place (silk) and right time (first instar) to stop insect infestation.

We have recently developed a high performance liquid chromatographic (HPLC) method for the determination of maysin in corn silks (Snook et al. 1989). Besides allowing us to monitor maysin levels accurately, the HPLC has provided a more complete profile of the flavonoid contents of the silks than was previously possible. To date, we have surveyed the maysin content of silks from 1,129 corn inbreds, populations, plant introductions (PI), and various unassigned collections. In addition to discovering many new sources of corn with high silk maysin levels, several lines were identified that contained high levels of related flavonoids. We report here the identification of these new flavonoids and their biological activity towards CEW in a laboratory bioassay.

Materials and Methods

Plants

Plants were grown between 1989 and 1994 at the Coastal Plain Experiment Station, Tifton, GA, under standard cultural practices of fertilizer and weed control. Silks were covered to prevent pollination and were sampled when 3-5 days old.

HPLC analysis

Sufficient numbers of plants were sampled to give approximately 30 g of silk/sample. The silks were weighed, placed immediately in 8 oz jars (Teflon-lined cap) and the jars were filled with 100% MeOH (approx. 180 mL). Samples were stored at 0ºC until analysis. Chrysin was added as internal standard. After ultrasonication for 20 min, aliquots of the solution were analyzed by reversed-phase HPLC, as previously described (Snook et al. 1989), using an H_2O/MeOH linear gradient from 10% to 90% MeOH in 35 min, a flow rate of 1 mL/min, and detection at 340 nm. Each solvent contained 0.1% H_3PO_4. Most analyses were performed with an Altex Ultrasphere C18, 5 micron (4.6 X 250 mm, Beckman Instruments, Norcross, GA) column. Additional analyses for apimaysin and 3'-methoxymaysin were made with a Hypersil Phenyl, 5 micron (4.6 x 250 mm, Alltech Associates, Deerfield, IL) column.

Isolation of flavone glycosides

Typical isolation procedures, following the methodology of Snook et al. (1993, 1994) for silk flavone glycosides, were as follows:

Extraction. Silk/methanol extracts were filtered, concentrated, extracted with CH_2Cl_2, followed by extraction with n-butanol. The n-butanol was evaporated to dryness (a small amount of water, added at the end of the evaporation, facilitated the removal of the last traces of n-butanol). The residue was dissolved in 40% MeOH/H_2O and submitted to preparative reversed-phase column chromatography.

Isolation. Isolation was mainly by preparative reversed-phase, silicic acid column chromatography followed by a second preparative reversed-phase separation. The n-butanol residue (dissolved in water) was chromatographed on a column packed with Waters PrepPAK 500 C18 cartridge material (Millipore Corp., Milford, MA) and eluted with water and 50% methanol/water. The latter fraction was evaporated to dryness and submitted to silicic acid (SA) (Mallinckrodt, 100 mesh, washed with methanol and activated at 155°C for 1 hr) column chromatography. The column was packed in CH_2Cl_2 and after applying the sample to the top of the column (as a SA/sample deposited mixture), eluted with CH_2Cl_2 followed by ethyl acetate or acetone/ethyl acetate mixtures. Most of the flavonoids of interest were found in the ethyl acetate eluant. After evaporation to dryness, the SA separated flavonoids were dissolved in 40% MeOH/H_2O and submitted again to reversed-phase chromatography using the following linear solvent program: 40-60% MeOH/H_2O in 400 min. 8 mL fractions were collected and column effluents were monitored at 340 nm.

Identification. Isolated flavonoids were identified by UV, NMR (1H and 13C), and FAB/MS spectrometric methods.

Bioassay procedures

Silk extract bioassay. Silk/methanol extracts were bioassayed by the method of Wiseman et al. (1992) and Snook et al. (1994). A small aliquot of the extracts was analyzed for maysin content and the remaining solution filtered into a 1 L roundbottom flask, 5 g of celufil (US Biochemical, Cleveland, OH) was added and the solvent was evaporated to deposit the extract onto the celufil. The dried celufil/extract mixture was then added to 100 g of diluted pinto

bean diet (3 mL diet:2 mL water), 10 mL were dispensed into plastic diet cups and one neonate CEW added. After 8 days the weights of the worms were recorded. Appropriate MeOH/celufil blanks were used. The experiment was arranged in a randomized complete block design with 15 replications.

Model compound bioassay (Microbioassay method). Isolated flavonoids or commercially available compounds (chlorogenic acid) were deposited onto celufil as above. Concentrations for each compound were 240, 120, 60, and 30 mg/2 g celufil. Each compound/celufil mixture was added to 25 g of diluted pinto bean diet. Detached, disposable plastic pipette bulbs were filled with 2 g of the diet/celufil mixture, allowed to solidify, and one neonate CEW was placed on the diet. The bulbs were placed in diet cups and larval weights measured after 8 days. There were ten replications for each compound concentration.

Results and Discussion

New sources of high maysin germplasm

Waiss et al. (1979) determined that a level of maysin of 0.15% (wt/wt of diet) in laboratory bioassays reduced CEW larval weights by 50%. We have bioassayed the silk methanol extracts of 50 different corn lines and crosses and compared the growth of CEW to the maysin level (Fig. 2). A highly significant negative relationship (r =-0.81) was found between maysin concentration in fresh silks and 8-day larval weights of CEW. This study showed that silk maysin concentration of 0.2% (fresh wt.) reduced larval weights to about 50% and that higher maysin levels (>0.3%) inhibited larval growth by about 80%. Larval growth reduction reached a plateau at maysin levels >0.4%. From these data, 0.2% has been deduced as the minimum level needed for resistance, with levels >0.3% most desirable, because of possible yearly variation.

These results have prompted us to survey other corn inbreds, populations and plant introductions (PIs) for maysin content. Other high maysin lines may have more desirable agronomic characteristics than Zapalote Chico for development of new, stable corn inbreds with a sufficient level of maysin for resistance. Flavonoid analyses were performed on the methanol extracts of the silks of 497 inbreds and 295 populations of corn, selected as representing a broad genetic base. In addition, 337 PIs and unassigned germplasm sources of corn from the North Central Regional PI Station, Ames, Iowa, were also analyzed. The results of these analyses are given in Tables 1-4 and showed that there is a wide range in silk maysin levels, from <0.01% to >0.5% fresh weight.

As expected, the majority of lines tested (82.6%) contained levels of maysin below that considered necessary for activity (<0.2% fresh wt.). Fully 50% of the inbreds were completely devoid of maysin or only possessed trace amounts of maysin (Table 4). In this study, we found a number of corn inbreds and populations with high silk maysin levels above the 0.2% fresh weight threshold, considered significant for CEW antibiosis. Approximately 1/5 of both the inbreds and populations were found to have maysin levels >0.2%. Most of these fall in the 0.2-0.5% range. Fully 1/3 of the high inbred maysin lines contained silk-maysin, at a greater concentration than Z. Chico, based on the amount of maysin per quantity of silk. Only 5% of the populations had maysin levels >0.2%. Relatively few PIs (12%) had high maysin levels in their silks. However, the silks of PI340856 averaged 0.743% maysin, over 3 years. This PI is a popcorn from the Eldredge collection. Crosses of PI340856 with a number of inbreds produced high levels of maysin in the progeny (Wiseman et al. 1992). Prior to our analyses, only Z. Chico was known to contain maysin although several other lines possessed maysin type UV absorptions in their methanol silk extracts (Waiss et al. 1979). We have now identified almost 200 inbreds,

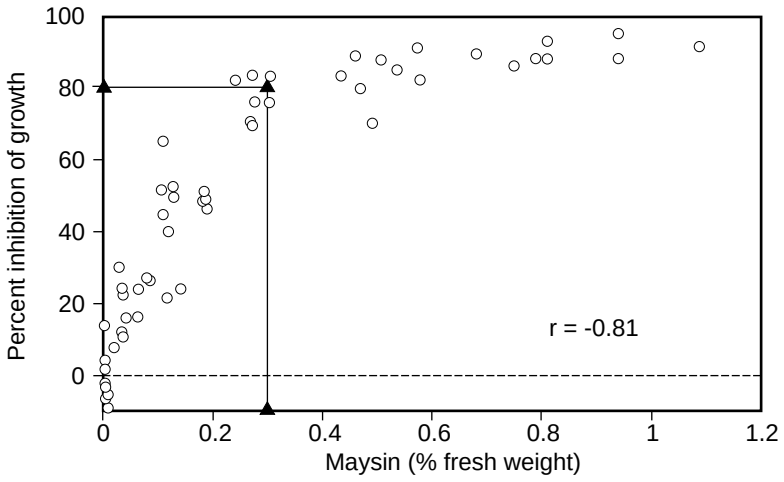

Figure 2. Percent growth inhibition of CEW versus silk maysin levels.

Table 1. Silk maysin levels in inbreds (percent fresh weight).

(Silk Maysin Levels > 0.2%)											
A102	0.307	C1-5	0.271	GE84	0.299	Mp704	0.392	SC250A	0.473	0-1836	0.325
Ab16	0.443	C.I.37B	0.368	GE295	0.245	Mp707	0.274	SC265R	0.336	1-1566	0.377
Ab416	0.244	C.I.64	0.356	GEC100	0.240	NC24	0.278	T226	0.523	2-07A	0.214
Ab602	0.463	C.I.83A	0.307	GT106	0.200	NC45	0.362	T238	0.358	2-043	0.278
Ab604B	0.229	C.I.317B	0.314	GT114	0.259	NC64	0.310	T315	0.296	2-635	0.360
Ab608A	0.464	E239S	0.204	GT154	0.246	NC264	0.274	Tx501	0.215	9-96A	0.533
Ab612A	0.778	E2629P	0.321	GT169a	0.510	R4	0.223	Tzi30	0.226	9-502B	0.290
Ab616	0.233	F45	0.270	Gu54-5	0.493	SC90	0.233	W22	1.125	9-676A	0.228
AB618	0.204	F54	0.257	H31	0.566	SC102	0.301	W23	0.260	9-928A	0.266
Akd24	0.305	F98	0.296	H45	0.246	SC114	0.380	WF-038B	0.521	49-1201B	0.284
Akd26	0.230	GE37	0.897	L90	0.293	SC229	0.205	WT23	0.514	57-163	0.300
Akd34	0.206	GE58	0.523	L329	0.459	SC243	0.263	0-835	0.328	79:295-2	0.216
Akd52	0.239	GE70	0.279	Mo10	0.890	SC245A	0.313	0-909	0.390	79:301-2	0.267
B1138T	0.220	GE74	0.302	Mo14W	0.200	SC249	0.298	0-1480A	0.238	8940C	0.420
B14(T)	0.322	GE80	0.847	Mp311	0.249	SC249A	0.219	0-1566	0.206	91201Y	0.521
(Silk Maysin Levels 0.1-<0.2%)											
A103	0.185	E263S	0.196	L601	0.192	SC54	0.138	T115	0.116	WT31	0.129
Ab20	0.194	F2L	0.161	Lahue0-514W	0.112	SC60	0.169	T139	0.130	WVLFPC1x7	0.181
Ab28E	0.131	F44	0.140	Lahue9-032A	0.107	SC84E	0.168	T204D	0.138	YT14	0.174
Ab30	0.192	F47	0.181	M68	0.111	SC144	0.179	T208	0.127	0-102	0.129
Ab44B	0.173	F101	0.122	May79	0.110	SC229	0.162	T212	0.185	0-641	0.151
Ab418	0.108	GE62	0.130	Mo15W	0.124	SC229MH	0.186	T236	0.118	1-1072	0.119
Akd36	0.181	GE82	0.138	Mo47	0.112	SC235R3	0.124	T244	0.166	2-717	0.195
Akd38	0.181	GE86	0.164	Mo102	0.135	SC254	0.138	TGY2	0.168	49-1684	0.198
B64	0.170	GE109	0.158	Mp426	0.192	SC277	0.186	Tx501	0.169	9-032A	0.105
C103	0.169	GEC8	0.131	Mp464	0.118	SC343	0.115	Tzi1	0.108	9-110C	0.102
CK3W	0.155	GEC116B	0.181	NC254	0.177	SC357	0.106	Tzi8	0.114	9-201	0.197
C.I.85B	0.167	GT107	0.154	Oh26F	0.154	SC401	0.117	Tzi15	0.115	9-213F	0.112
C.I.287	0.166	GT114	0.259	R101	0.179	SC413	0.202	Tzi24	0.199	9-238	0.107
C.I.38B	0.172	GT166	0.107	Rec.38-11	0.109	Syn36	0.129	WF9	0.174	9-880	0.125
ESDJ1	0.111	Hy2 (normal)	0.166	SC46	0.170	T105	0.140	WH	0.194		
A286Y	0.051	E226S	0.068	GT3	0.099	Mp337	0.098	SC256	0.058	T246	0.093
Ab59	0.050	E241S	0.070	H21	0.095	N6	0.093	SC311A	0.050	Tx601	0.099
Ab412A	0.077	FF8	0.097	H30	0.059	NC7	0.058	SC346	0.070	Tx46139	0.065
Ab424	0.093	GA221	0.093	L708	0.055	NC232	0.063	SC353	0.099	0-514A	0.085
Akd32	0.085	GA222	0.099	L765	0.076	Oh7B	0.066	SEG	0.091	0-1325A	0.091
B41	0.081	GE76	0.060	Lahue9-213D	0.069	Oh422	0.059	SH258	0.070	0-1432	0.099
B504	0.077	GE90	0.077	Lahue9-996	0.089	Pa36	0.085	Syn15	0.064	0-11830	0.081
C1-11	0.095	GE92	0.075	M14(Mo)	0.068	Pa884P	0.054	T8	0.057	1-40A	0.061
C.I.21	0.079	GE291	0.076	Mo12	0.80	R227	0.50	T220	0.099	2-673	0.071
C.I.84B	0.097	GE331	0.079	Mo13	0.060	SC16	0.078	T240	0.077	7-104	0.050
D160	0.099	GT112 (old)	0.097	Mo426	0.095	SC228A	0.053	T242	0.050	9-886	0.053
(Silk Maysin Levels < 0.05%)											
A239		ES1W		GE311		L503		Mp113		Oh45B	
Ab12A		ES2W		GE317		L578dd CEW		MP303		Oh56	
Ab18		ESN		GE321		L578		Mp307		SC253R3	
Ab26		F1D		GE325		L605		Mp309		SC2-3	
Ab408		F6		GE333		L609		Mp313E		Sa4(W)	
Ab454		FF3		GE335		L615		Mp317		SC15	
Ab610		GA152		GE337		L617		Mp335		SC44	
AC454		GA209		GE339		L621		MP339		SC73	
AC455		GA212		GE341		L668		MP410		SC91	
AC456		GA215		GE440		L678		Mp412		SC152	
AC543		GA219		GE247-205B		L690		MP420		SC212	
Akd40		GCP9A		GEC40		L699		MP446		SC213R	
B2		GE10		GEC119A		L709		Mp448		SC214	
B37		GE19		GT9		L764		Mp460 (Miss.66)		SC225	
B539		GE25		GT11		L814		Mp462		SC233	
BJ28		GE38		GT102		M6		Mp466		SC235	
BJ30		GE54		GT119		M102		MP496		SC246C	
C.I.82B		GE68		GT150		Miss.Ace996.3		MP708		SC257	
C.I.88A		GE72		H84		Mo1W		N20		SC260R	
C.I.90A		GE78		JLM1		Mo5		N101		SC270RS	
C.I.91C		GE88		K44		Mo6		N104		SC273	
C.I.121		GE129		K5Y2-3		Mo16W		N106		SC276R	
D113		GE205		KY21		Mo17NSyn		N132		SC278DY	
D187		GE275		KyWS1		Mo20W		NC220		SC279-4	
D287		GE281		L317		Mo45		NC222		SC285	
E199S		GE293		L317(la)		Mo46		NC224		SC301	
E2667P		GE297		L501		Mp1D		NC605		SC310	
SC324		T11		T222		0-115		0-1290		8-12A	
SC333		T101		T224		0-145		1-34B		9-54C	
SC335		T111		T234		0-159A		1-222A		9-218	
SC344		T125		T331		0-177		1-278		9-220	
SC359		T127		Tx44-91		0-190		1-759		9-230B	
SC375		T129		Va35		0-509		1-837		9-245	
SC402		T133		W48FSK		0-530A		1-919		9-908A	
SC403		T135		WPT4		0-538A		1-977A		9-971	
SC441		T137		WT12		0-572A		2A12		9-1028	
SC444		T141W		WT34		0-677B		2A44		33-16	
Su4(Red)		T143W		WT46		0-708A		2-12B		48-1166	
Syn3B		T202		YT14		0-956A		2-535A		49-1166	
Syn23		T206		YT23W		0-1032		3L2		49-1170	
Syn49		T210		YT27W		0-1130		5-666A		49-1550	
Syn52		T216		YT37		0-1243		8HL6		936-2179	

Table 2. Silk maysin levels in populations (percent fresh weight).

(Silk Maysin Levels > 0.2%)							
AERD (C1)	0.297	CB65	0.252	Kyle Late Syn	0.299	133#	0.224
Amar. Salv. X's	0.243	CC-MIO	0.203	Kyle Long Ear Syn	0.221	142#	0.342
Ant.2D-118	0.211	Chis Group X's	0.365	MWSA	0.235	1439x 4T	0.243
ANTB-EP	0.457	Coah Group	0.200	Oax. Comp Group	0.565	1487#	0.210
ANTB-EPDS	0.564	Colorado Manfredi GP	0.295	Panama Gpo 84A 1	0.256	14x 3T#	0.306
ANTB-EPM	0.934	Cow Corn	0.278	PR 70B 602-604	0.359	1520	0.235
ANTB-SIDS	0.532	Cuba III	0.200	RFC-FI(C9)	0.564	1889x 2PR	0.392
ANTB-SIM	1.031	Dial-4 P28	0.263	Salvadoreno	0.255	1973x 2T	0.215
Azteca X's	0.263	Dial-5 P43	0.262	Strawberry Dent (Tex)	0.203	2280x 1T	0.327
Azul	0.238	Ducle Ja 1	0.255	Tabloncillo X's	0.343	37x 7PR	0.242
B-20#	0.241	ETO X's	0.233	Tbly Syn	0.321	524x 2T	0.240
B-70#	0.303	Florident White	0.217	Trinidad X's	0.271	762#	0.275
B-81#	0.229	Gourdseed Dent	0.297	Z. Chico (2451)#(P)C3	0.350	78x 1T	0.808
B-219#	0.200	GT CEW-RSB	0.220	123#	0.419	891x 3T#	0.243
BlueK.M.	0.200	Guat. Gp030-1A	0.295	1243x 2PR#	0.289	984x 1PR	0.213
Catito Limon	0.392	Hond. Group	0.344	1299x 1T	0.337	998x 1T	0.452
(Silk Maysin Levels 0.1-<0.2%)							
Ant 20xTxp	0.115	Caribbean #	0.183	Granada X's	0.131	Shumway's Goli	0.149
ANTB-SI	0.186	Chiapas 138 Ear 1...	0.111	Guad X's	0.115	Snow's St. Croix	0.118
Arroc. Amao X's	0.170	Chiapas Gp0 41 Ear...	0.111	Harinoso Sudan	0.145	St. Crush Syn. A#	0.121
Azul	0.121	Coe G12#in	0.171	Indian Chief	0.101	SWCB Syn X	0.152
Blandito sonora	0.176	Comiteco X's	0.168	Jamaica X's	0.105	Syn F	0.124
Bofo	0.161	Comp (Va)	0.144	MAS (pwnf)	0.106	Syn Kby	0.140
Bolita	0.123	Compuesto Am. Caribe	0.102	Mexican ? #1	0.200	S. African Syn #1	0.120
B-116#	0.102	Costa Rica X's	0.179	MOM Syn #3	0.164	S.A. Yellow Syn	0.135
B-12#	0.122	Crillo de Cat. X's	0.120	Mosby's Prol	0.110	Tamps Gpo #1	0.145
B127#	0.145	Cuba X's Low Ear Syn	0.125	MWSB	0.137	Tepecintle X's	0.106
B-137#	0.157	Dial 4 Suwan 1	0.133	M-A[MoSQA(S7-H)C12]	0.140	Tuxpeno No. 3	0.105
B-18#	0.190	Dial 5 P22	0.156	Neal's Pay	0.191	Vandeno X's	0.105
B-200#	0.104	Dial 8 P63QPM	0.150	N.L. Group	0.100	Ver X's	0.197
B-208#	0.127	Dial 8 P64QPM	0.115	Oloton No. 1	0.173	Z. Chico X's	0.137
B-220#	0.105	Diallel (Late)	0.165	Pencil Cob (Tex)	0.107	10LDD Sel. Rec.	0.126
B-260#	0.127	Dial-4 P24	0.176	Peru X's	0.118	44x 4PR	0.154
B-40#	0.127	Diente de Caballo	0.143	PR69A 42	0.135	117#	0.161
B-46#	0.105	Duloillo Noroeste	0.108	PR70A 475	0.180	121#	0.102
B-50#	0.103	FAW-CC [C5]	0.133	Puerto Rico #	0.114	203#	0.148
B-80#	0.118	FLA 767 Syn	0.126	RS 10(C3)	0.135	210#	0.103
B-94#	0.120	Fla Comp	0.155	San Croix X's	0.194	238#	0.126
Camp. Group	0.156	FSC 662-25	0.139	SC Syn.	0.120	362#	0.100
365#	0.180	706x 2T	0.113	960x 2PR	0.102	2302x 5PR	0.106
408X 2PR	0.161	713x 2T	0.147	1603#	0.102	2375x 3PR	0.159
415x 3PR	0.128	721x 3PR	0.121	1762x 1T	0.194	2377x 5PR	0.192
538x 1T	0.106	824x 1T	0.170	1858x 1T	0.162	3146x 1T	0.125
581x 1T	0.174	917#	0.179	2110x 1T	0.112	3296x 1T	0.145
690x 1T	0.164	933x 4PR	0.157	2300x 1PR	0.118	3371x 4PR	0.137
(Silk Maysin Levels 0.05-<0.1%)							
Amar. Wh. Flint	0.054	B-178#	0.065	MEX. (VA.)	0.063	697x 1T#	0.073
Antigua 2D-109	0.054	B-181#	0.084	Pepitilla No. 1	0.053	958#	0.093
Antigua X's	0.052	B-193#	0.071	PEX (VA)	0.075	1007x 1T	0.076
AntiguaGp2(blanco)	0.052	Barbadox X's	0.077	Robyn	0.051	1218x 3PR	0.077
Argentina	0.074	Cacahuacintle X's	0.053	San Vic x's	0.056	1455#	0.089
Ark CB	0.054	Canilla	0.074	Seneca Ind. Mix	0.064	1508#	0.082
B-8#	0.065	Chiapas Gp0 41...	0.067	Spykepit X's	0.053	1515#	0.054
B-10#	0.056	Chih Group X's	0.084	Syn TW	0.067	1548x 2T	0.075
B-16#	0.080	Clavilla #	0.056	Tuxpan	0.071	1953 1PRA#	0.081
B-23#	0.097	Dom. Rep.	0.050	VMX	0.054	2019x 2PR	0.086
B-31#	0.088	FLA C62 Syn	0.080	Yellow Hickory King	0.052	2041x 4PR	0.050
B-60#	0.071	Gobi Yell	0.059	Yellow Jellicorse	0.053	2745x 2T	0.096
B-101#	0.059	Golden Beauty	0.098	Yuc. Group	0.065	3316x 3PR	0.064
B-133#	0.072	GT-MAS: gk	0.087	38x 5PR	0.056	3457x 3PR	0.053
B-160#	0.074	Homedale	0.052	111A Comp	0.086	8056#	0.097
B166#	0.080	Legg Prol	0.060	500x 1T	0.054		
(Silk Maysin Levels < 0.05%)							
Alapaha		CB So		Long Ear Syn		Y&W-16 Lines Syn	
Altiplano		Celaya		Mayorbella		Z. Grande X's	
B-1#		Chantelpa Chaparro...		Mic's Success		3x 1T#	
B-15#		Chapalate X's		MPCS-1A		42x 5PR	
B-25#		Coroico		Natal Wh. Horsetooth		126#	
B-63#		Douthit Prol		OP24#		135#	
B-109#		Farmer's Comp		OP60-9#		234x 1PR	
B-120#		FSH MR		San Pedro 1		997X 1T	
B-140#		Gaspe Flint		SGP-MIO		1113x 2PR	
B-144#		Guat. Gp013-5		SI1285 Syn A High		1208x 1T	
B-155#		Guat. Gp021-11		Syn A. High 3rd CYC		1968x 2PR	
B-240#		IK		Syn L		2116x 3T	
B-252#		Jarvis		Syn Mdy		2206x 4T	
BSP2CI		Jellicorse x South		Teko Yellow		2370X 1T	
Caribe Salvadoreno OP		Knightin 8-Row		Yellow Neals Pay			

Table 3. Silk maysin levels in plant introductions (PI) (percent fresh weight).

(Silk Maysin Levels > 0.2%)											
PI 172328	0.211	PI 219889	0.520	PI 340840	0.336	PI 340870	0.356	PI 445630	0.258	PI 571793	0.611
PI 194791	0.972	PI 221839	0.242	PI 340844	0.276	PI 340872	0.609	PI 474214	0.284	AMES 10585	0.421
PI 208473	0.295	PI 222319	0.394	PI 340856	0.823	PI 340873	0.531	PI 515375	0.261	AMES 10587	0.914
PI 213742	0.972	PI 222497	0.251	PI 340859	0.207	PI 438942	0.239	PI 515551	0.320	AMES 10589	0.914
PI 217404	0.374	PI 278722	0.228	PI 340865	0.581	PI 444142	0.370	PI 516037	0.313	AMES 10590	0.975
PI 217460	0.411	PI 340837	0.225	PI 340867	0.201	PI 444443	0.324	PI 516120	0.233	AMES 14099	0.238
PI 219874	0.544	PI 340838	0.297	PI 340869	0.753	PI 445235	0.222	PI 540777	0.229	AMES 8177	0.292
(Silk Maysin Levels 0.1-<0.2%)											
PI 165457	0.150	PI 340863	0.124	PI 444364	0.177	PI 503727	0.163	PI 515408	0.138	AMES 8462	0.152
PI 180359	0.105	PI 340866	0.175	PI 444562	0.100	PI 503728	0.192	PI 515425	0.132	AMES 8473	0.119
PI 184282	0.182	PI 340871	0.132	PI 444686	0.156	PI 503794	0.180	PI 515428	0.135	AMES 8482	0.117
PI 193655	0.131	PI 347252	0.102	PI 444785	0.198	PI 503806	0.141	PI 515461	0.157	AMES 10358	0.117
PI 194386	0.109	PI 414182	0.183	PI 444868	0.118	PI 503832	0.187	PI 515558	0.156	AMES 10501	0.141
PI 197094	0.116	PI 414184	0.198	PI 444872	0.135	PI 514923	0.103	PI 516061	0.137	AMES 10538	0.130
PI 219885	0.185	PI 430456	0.135	PI 445002	0.153	PI 515065	0.133	PI 516155	0.197	AMES 10551	0.139
PI 220065	0.142	PI 443442	0.131	PI 445056	0.118	PI 515076	0.175	PI 532310	0.107	AMES 10579	0.191
PI 224083	0.108	PI 443762A	0.110	PI 445248	0.135	PI 515078	0.101	PI 532319	0.112	AMES 10623	0.146
PI 227937	0.176	PI 443859	0.192	PI 445377	0.104	PI 515126	0.152	PI 532324	0.126	AMES 10665	0.182
PI 245138	0.155	PI 444010	0.177	PI 445422	0.152	PI 515213	0.162	PI 540779	0.186	AMES 10672	0.106
PI 257626	0.112	PI 444042	0.126	PI 445504	0.102	PI 515219	0.185	PI 571795	0.102	AMES 15695	0.145
PI 257629	0.194	PI 444217	0.102	PI 445514	0.121	PI 515302	0.149	PI 571899	0.104		
PI 331441	0.118	PI 444331	0.128	PI 474215	0.102	PI 515326	0.116	AMES 8426	0.167		
(Silk Maysin Levels 0.05-<0.1%)											
PI 162927	0.088	PI 357120	0.050	PI 444320	0.065	PI 503725	0.064	PI 515106	0.067	PI 532315	0.097
PI 181988	0.080	PI 357125	0.084	PI 444607	0.060	PI 503731	0.081	PI 515107	0.050	PI 571801	0.059
PI 183753	0.080	PI 367115	0.075	PI 444859	0.082	PI 503764	0.093	PI 515112	0.063	PI 572066	0.095
PI 218174	0.077	PI 430455	0.071	PI 444923	0.073	PI 503793	0.073	PI 515114	0.056	AMES 8428	0.061
PI 221826	0.071	PI 443779	0.065	PI 445299	0.084	PI 503849	0.080	PI 515115	0.067	AMES 8477	0.088
PI 257619	0.062	PI 443794	0.063	PI 445307	0.064	PI 503863	0.084	PI 515134	0.067	AMES 8491	0.058
PI 331455	0.089	PI 443805	0.058	PI 445432	0.092	PI 514735	0.073	PI 515205	0.094	AMES 8493	0.098
PI 331456	0.078	PI 443827	0.077	PI 445585	0.076	PI 514768	0.053	PI 515355	0.051	AMES 8497	0.065
PI 331708	0.067	PI 443849	0.072	PI 445641	0.075	PI 514848	0.058	PI 515464	0.065	AMES 8503	0.059
PI 340836	0.084	PI 443992	0.074	PI 474213	0.066	PI 514947	0.096	PI 515997	0.066	AMES 8515	0.089
PI 340843	0.073	PI 444029A	0.069	PI 483495	0.061	PI 514987	0.095	PI 516039	0.070	AMES 8521	0.081
PI 347251	0.080	PI 444029B	0.065	PI 484435	0.063	PI 514995	0.066	PI 520631	0.060	AMES 10363	0.087
PI 357097	0.067	PI 444174	0.098	PI 484535	0.051	PI 515003	0.064	PI 520691	0.061	AMES 10446	0.091
PI 357098	0.055	PI 444239	0.067	PI 503667	0.084	PI 515009	0.051	PI 520693	0.088	AMES 10465	0.061
PI 357112	0.097	PI 444282	0.065	PI 503678	0.086	PI 515064	0.085	PI 522309	0.076	AMES 10635	0.050
PI 357115	0.069	PI 444292	0.075	PI 503722	0.052	PI 515097	0.077	PI 532312	0.068	AMES 10638	0.066
(Silk Maysin Levels <0.05%)											
PI 174416		PI 331440		PI 444731		PI 514843		PI 515528		AMES 8248	
PI 186221		PI 331442		PI 444991		PI 514858		PI 515529		AMES 8429	
PI 193653		PI 331443		PI 445401		PI 514896		PI 515529		AMES 8488	
PI 193658		PI 331452		PI 484506		PI 514921		PI 515531		AMES 8498	
PI 194390		PI 331709		PI 485139		PI 514932		PI 520626		AMES 8501	
PI 194741		PI 340853		PI 485256		PI 514994		PI 520702		AMES 8573	
PI 213796		PI 347253		PI 485257		PI 515008		PI 521313		AMES 8577	
PI 213807		PI 347254		PI 485316		PI 515108		PI 532321		AMES 10024	
PI 219871		PI 357094		PI 490973		PI 515111		PI 532327		AMES 10042	
PI 219886		PI 357101		PI 501124		PI 515113		PI 540767		AMES 10074	
PI 219888		PI 357121		PI 501126		PI 515116		PI 571493		AMES 10075	
PI 221825		PI 357122		PI 503660		PI 515117		PI 571506		AMES 10076	
PI 221831		PI 357129		PI 503669		PI 515122		PI 571511		AMES 10362	
PI 221844		PI 390837		PI 503688		PI 515411		PI 571582		AMES 10382	
PI 222307		PI 443931		PI 503697		PI 515436		PI 571754		AMES 10436	
PI 222309		PI 443997		PI 503720		PI 515457		PI 571767		AMES 10560	
PI 233007		PI 444000		PI 503723		PI 515462		PI 571803		AMES 13932	
PI 303850		PI 444125		PI 503736		PI 515467		PI 571897			
PI 303851		PI 444139		PI 503861		PI 515467		PI 572049			
PI 317330		PI 444223		PI 504301		PI 515490		AMES 8225			

Table 4. Distribution of maysin in corn germplasm.

Maysin levels % fresh weight	Inbreds	Populations	Plant introductions	Total	% of total
> 0.2	90	64	42	196	17.4
> 0.05-<0.2	155	172	178	505	44.7
< 0.05	252	59	117	428	37.9
Total # of lines	497	295	337	1,129	100.0

populations, and PIs with high maysin silk levels. These lines form an important, new genetic base for breeding studies to produce agronomically acceptable CEW resistant germplasm.

Isolation and identification of new corn silk flavones

In addition to identifying corn germplasm with high maysin contents, the survey resulted in the discovery of several inbreds, populations and PIs with very high levels of flavone glycosides related to maysin. Some of these lines showed high activity towards CEW and therefore, it was of interest to identify the compounds responsible. The compounds were isolated by a combination of solvent partitioning and column chromatography (silicic acid followed by preparative reversed-phase). Elliger and co-workers (Elliger et al. 1980a) previously identified an apigenin-analog of maysin (called apimaysin) and 3'-methoxymaysin (Fig. 1) from Zapalote Chico, in which they occur in minor amounts. Our analysis of Zapalote Chico showed apimaysin and 3'-methoxymaysin to be present in only 0.019% and 0.045% fresh weight, while maysin was at the 0.35% level (averaged over 4 years). We have determined that most corn lines with high maysin levels have minor levels of apimaysin and 3'-methoxymaysin. However, our survey identified several lines that had very high levels of apimaysin (Fig. 3) and 3'-methoxymaysin (Fig. 4). One line, the inbred NC7, was unique in that it produces 0.614% fresh weight apimaysin along with only a trace amount of maysin. Recently, inbred Mp416 was found to produce 0.72% apimaysin and only 0.088% maysin. SC353 is another good source of maysin and apimaysin (0.40% and 0.22% respectively). Only one population line was found to contain high apimaysin (3146x 1T#). An unassigned line, Ames 1903, contained maysin, apimaysin and 3'-methoxymaysin (Fig. 3).

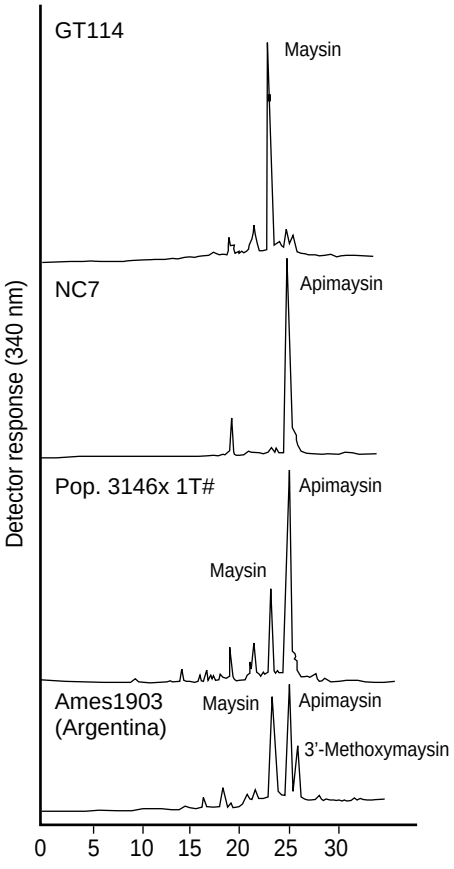

Figure 3. Corn lines with high levels of apimaysin.

Very few lines contained high levels of 3'-methoxymaysin. Inbred Tx501 was the best source of this compound, containing 0.19% (Fig. 4). Other good sources are lines 9-201 (0.297%), and SC144 (0.293%). Populations with high 3'-methoxymaysin are 891x 3T# (0.243%), Kyle Late Syn (0.155%), 998x 1T# (0.132), and Oloton No.1# (0.109%).

Of the other maysin analogues identified in corn silks, only two occur in amounts to be significant for CEW resistance. One of these compounds is isoorientin (6-C-glucosylpyranosyl-luteolin) (Fig. 1), first found in inbred T218 (Snook et al. 1994) (Fig. 5). Our previous report (Snook et al. 1993) on the identification of this compound as galactoluteolin was based on preliminary NMR data. Further studies have shown that the compound is isoorientin (glucosylluteolin). Other lines where isoorientin occurs are T315 and Mo6. T218 also contained de-rhamnosylmaysin (Fig. 1), which has lost the ether-bonded rhamnose.

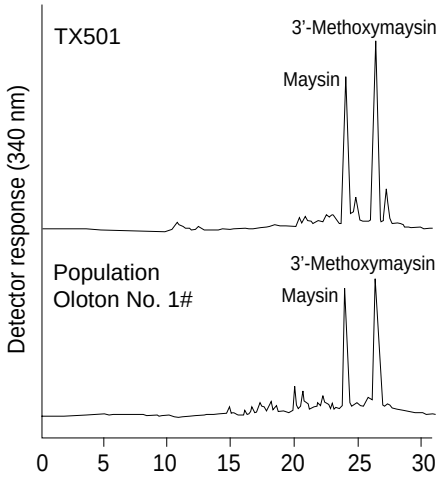

Figure 4. Corn lines with high levels of 3'-methoxymaysin.

However, the level of this compound was rather variable from year to year. The other maysin analogue, which was found in appreciable quantities in only 3 lines, is 4"-hydroxymaysin (4"-OH-maysin). Lines containing 4"-OH-maysin in levels sufficient to be considered resistant are A103, ESDJ1 and CML131.

HPLC characterization of corn silk flavones.

The HPLC analyses of such a large number of inbreds, populations and PIs revealed that practically all silks could be classified into seven major groups based on the presence of specific flavonoids. The first group is characterized by lines low levels (>0.05-0.1%), medium levels (>0.1-<0.2%) or high levels (>0.2%) of maysin. Examples of these lines are given in Figure 6. They comprise fully 62.1% of all lines tested (Table 4). It thus appears that maysin is widespread in corn germplasm, but, as mentioned before, only 17% of corn lines have maysin levels high enough to be considered resistant. However, many of the lines between 0.05 and 0.2 have the potential for maysin to be increased to >0.2% with a minimum of effort.

The second group of silks is characterized by low flavonoid containing lines (<0.05%, which is equivalent to trace levels) and represent almost 38% of lines (Fig. 6). The third and fourth groups of corn lines are those that contain apimaysin and 3'-methoxymaysin respectively. Although only 1% of lines contained these compounds in high levels, they were found in measurable quantities in 12% of the lines. The fifth and sixth types of corn flavone profiles are those containing isoorientin flavones (Fig. 6). One line, T218, has high levels of isoorientin while others, such as the Eldridge Popcorn Collection PI340853, contain rhammosylisoorientin. The corn line Azul was also found to contain this compound, along with maysin. The seventh type of corn flavone profile is typified by ESDJ1 (Fig. 6), where relatively large amounts of 4 -hydroxymaysin are found.

Biological activity of maysin and maysin-analogues

Isolated flavonoids were submitted to laboratory bioassays against CEW and FAW. As shown in Figure 7, maysin produced larval weights that were only 16% of controls (at 12.6 mM conc.). FAW was more sensitive to the effects of maysin, producing larvae weighing only 6% of controls at only 11.5 mM concentration of maysin.

The isolated corn flavones- maysin, apimaysin, 3'-methoxymaysin, isoorientin and 4"-hydroxymaysin, were tested in the microbioassay method. In this test, maysin reduced the weights of CEW by 92% (15.4 mM) while isoorientin gave worm weights about 76% of controls at the highest level tested (19.85 mM) (Fig. 8). 4"-

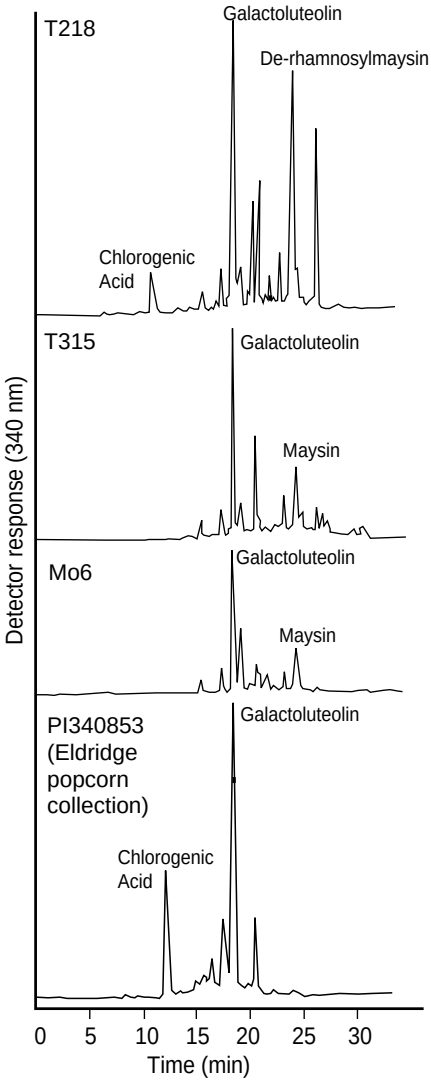

Figure 5. Corn lines with high levels of isoorientin.

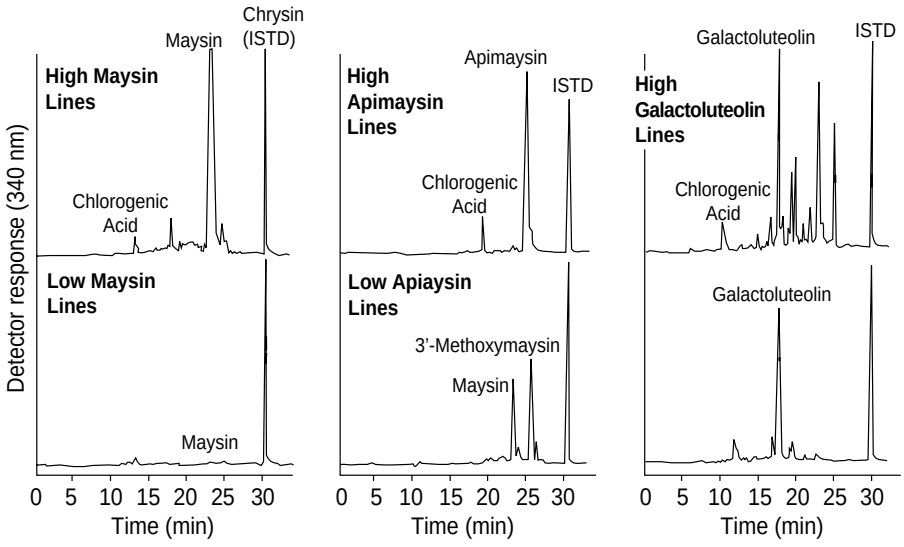

Figure 6. Characteristic HPLC polyphenolic profiles of major corn silk types.

Hydroxymaysin was found to be just as active as maysin in the test. Apimaysin and 3'-methoxymaysin both gave about 50% inhibition of growth at the maximum concentrations tested (15.9 and 15.1 mM respectively). Elliger et al. (1980b) reported 3'-methoxymaysin as about half as active as maysin based on ED50 concentrations (mM/kg to retard growth to 50% of control). Chlorogenic acid also has an ortho-dihydroxybenzene structure and is found in small amounts in corn silk. It was found to be active against CEW, resulting in an 80% reduction of growth at 20.5 mM concentration (Fig. 8).

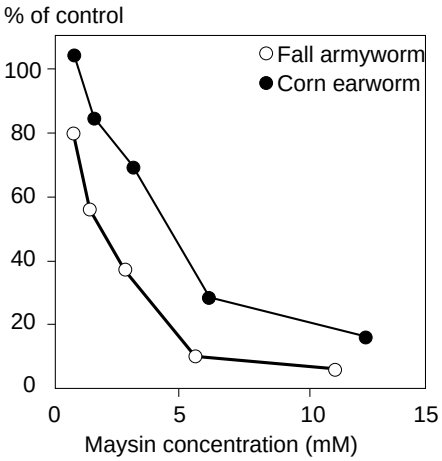

Figure 7. Growth of fall armyworm (FAW) and corn earworm (CEW) versus concentration of maysin.

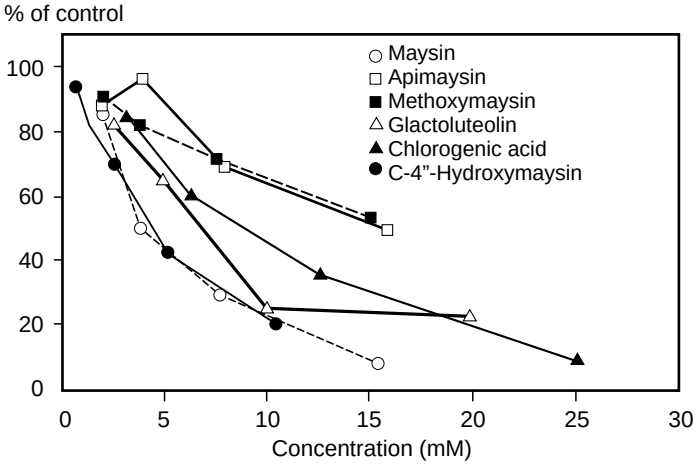

Figure 8. Growth of corn earworm (CEW) versus concentration of corn flavonoids and chlorogenic acid.

The bioassay data show that maysin, isoorientin, and chlorogenic acid are comparable in activity against CEW. Breeding experiments are currently underway to incorporate all three active compounds into one line that, hopefully, will possess high antibiosis activity against CEW and FAW and be useful for production of naturally resistant hybrids.

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Mechanisms of Maize Resistance to Corn Earworm and Fall Armyworm

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Abstract

Tolerance, non-preference, and antibiosis, the mechanisms of resistance in maize, Zea mays *L., to* Helicoverpa zea *(CEW) (Boddie) and* Spodoptera frugiperda *(FAW) (J. E. Smith) have been described for some maize cultivars. The behavior of larvae and, to a lesser extent, of adults of these pest insects as it relates to non-preference has been delineated for a few cultivars. CEW moths preferred to oviposit on the adaxial over abaxial surface of young maize leaves of both resistant and susceptible genotypes. Foliage of Antigua 2D-118 is less pubescent and less preferred than Cacahuacintle X's. FAW larval behavior on both leaf surfaces with and without cuticular lipids was monitored by video camera. Larvae showed more non-acceptance behavior on the untreated foliage than that with cuticular lipids removed. The effects on the insect's life history of maize cultivars with antibiotic resistance have been shown and include reduced size of larvae, prolonged length of both the larval and pupal cycle, reduced pupal weights, reduced fecundity, increased number of instars, and decreased head capsule size. Tolerance to FAW was shown as a resistance mechanism in some commercial hybrids. The 12-leaf stage tolerated damage by the FAW larvae better than the 8-leaf stage. Yield reduction was 32.4% at the 8-leaf stage compared to 15.4% at the 12-leaf stage. Two predictive models of maize resistant to CEW and FAW illustrate the value and impact of resistance on developing populations of pest insects.*

Introduction

Maize, *Zea mays* L., resistance to corn earworm (CEW), *Helicoverpa* (=*Heliothis*) *zea* (Boddie) and fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) may be defined as "the relative amount of heritable qualities possessed by the plant which influence the ultimate degree of damage done by the insect" (Painter 1951). Painter further classified resistance into three mechanisms: non-preference, antibiosis, and tolerance (Painter 1951, 1968). Non-preference results when a plant does not possess the normal attractive substances or qualities for oviposition, establishment and/or feeding, or possesses repellent or deterring substances. There are two types of non-preference: relative (non-preferred plants or cultivars in the presence of susceptible plants or cultivars) and absolute (plants or cultivars not preferred even when plants or cultivars are grown or tested alone) (Owens 1975). Antibiosis denotes adverse biological effects (e.g., larval mortality, extended development time, etc.) on the insect pest as it uses the resistant plant for food. On the other hand, tolerance describes a plant or cultivar that is able to yield well despite infestations that seriously damage and reduce yield of susceptible plants. Generally one or more of these three mechanisms may occur in the same resistant cultivar. Researchers often fail to recognize this possibility because of a lack of ingenuity in designing experiments to separate the mechanisms of resistance or to understand the importance of the biological phenomena involved with each resistance mechanism.

A case in point is the resistance of certain maize genotypes to the CEW. Painter (1951) described an "unclassified resistance mechanism" in which the importance of long husks of maize was discussed in relation to its resistance to CEW. This concept of the "unclassified resistance mechanism" lingered for several years. In fact, most of the early works on maize resistance to CEW involved mechanical factors: long, tight husks, and such factors as silk-balling or husk protection (Luckman, et al. 1964; Wiseman, et al. 1970; McMillian and Wiseman 1972). Most workers omitted studies on the mechanisms of resistance, instead researching the broad-based chemical factors (Walter 1957; Knapp et al. 1965, 1967; McMillian and Wiseman 1972) or correlating CEW resistance in maize with plant physical factors (Widstrom and McMillian 1967; Widstrom et al. 1970).

Anderson (1944) stated "it is a fundamental principle, too often ignored, that before a biological phenomenon is to be investigated on the mathematical level it must be thoroughly analyzed on the biological level." This principle may be applied to premature studies on the biochemical basis of resistance factors. Knapp et al. (1967) and Straub and Fairchild (1970) were among the first to study the mechanisms of resistance in maize to CEW, but their studies delineated only antibiosis. The early progress in identifying FAW resistant maize was much slower because of inadequate rearing and/or infestation procedures. However, Wiseman et al. (1966, 1967) found resistance in an Antigua race of maize. With the advent of artificial rearing of FAW (Burton 1967; Burton and Perkins 1989) and infestation procedures (Mihm 1983; Wiseman et al. 1980), sources of FAW resistance in maize have been found, developed and released (Wiseman and Davis 1990).

The basic triad of the resistance mechanisms proposed by Painter (1951) is usually elucidated by specifically designed experiments to demonstrate the independence of the three components; however, resistant cultivars often possess combinations of these mechanisms, especially non-preference and antibiosis (Wiseman 1990). With this combination of mechanisms, a cultivar that is non-preferred does not require the same level of antibiotic resistance. Thus, different cultivars may possess the same levels of resistance with different mechanisms of resistance and/or levels of the resistance components. The remainder of this paper will be devoted to recent elucidations of the mechanisms of resistance of maize to FAW and CEW.

Tolerance

Tolerance resistance is associated with the plant's ability to recover and yield satisfactorily, despite insect damage. Tolerance also can mean that the resistant plant simply tolerates the pest insect in the presence of a population of insects equal to that which damages a susceptible plant or cultivar. In 1972, Wiseman et al. reported that when plants were planted early in the growing season, two resistant maize hybrids, Dixie 18 and 471-U6 X 81-1, supported numbers of CEW larvae on the ear that were similar to those on ears of susceptible hybrids but suffered much less damage (Table 1). At a later planting date the number of CEW larvae in the ears of the resistant hybrids was greater, yet the damage to the ears was significantly less than that on the susceptible hybrids. Thus, the resistance of Dixie 18 and 471-U6 X 81-1 was identified as tolerance. Later studies, Wiseman et al. (1976, 1981a), where CEW larvae were fed fresh silks of Dixie 18 and 471-U6 X 81-1, supported these findings. Larvae and percent mortality of larvae that were fed fresh silks of Dixie 18, 471-U6 X 81-1, or silks of susceptible cultivars did not differ for 6- and 10-day weights or % mortality. Ears of tolerant maize hybrids were described by Wiseman et al. (1977) as having tight husks, long silk channels, and large amounts of silk that maintained a high moisture content over the period of development of CEW larvae. In addition, these tolerant hybrids or cultivars were found to have little or no maysin content (Waiss et al. 1979), later found to be a major factor for the basis of antibiosis resistance (Wiseman et al. 1992a,b).

The establishment of FAW tolerance in maize had not been achieved until the last 20 years, though many observers have suggested that maize cultivars do tolerate large numbers of larvae and damage (Brett and Bastida 1963; Wiseman and Davis 1979; Ortega et al. 1980; Mihm 1989). However, Wiseman and Isenhour (1993) did show that tolerance existed in some commercial hybrids. They showed that the 12-leaf stage tolerated damage by the FAW larvae better than the 8-leaf stage. Yield reduction was 32.4% at the 8-leaf stage compared to 15.4% at the 12-leaf stage.

Non-preference

Few studies have been conducted to determine the mechanism of non-preference in maize to either CEW or FAW. Ovipositional non-preference against Antigua 2D-118 by CEW was reported by Widstrom et al. (1979). CEW moths preferred to oviposit on the adaxial as compared to abaxial surface of young maize leaves of both

Table 1. Tolerance as a mechanism of resistance in maize to the corn earworm (CEW).

	CEW injury in indicated plantings[a]		Larvae per ear in indicated planting[a]	
Hybrid	Early	Late	Early	Late
Dixie 18 (R)	3.6b	2.5a	0.8b	1.7c
Asgrow 200 B (S)	6.1d	4.6c	0.8b	1.3b
Ioana (S)	5.7c	7.3d	0.7a	1.0a
471-U6 X 81-1 (R)	2.9a	3.6b	0.7a	1.4b

[a] Means in columns followed by the same letter are not significantly different ($P < 0.01$). CEW injury means are the depth of penetration into the ear in cm (Wiseman et al. 1972).

resistant and susceptible genotypes. Antigua 2D-118, which is less pubescent than Cacahuacintle X's, was less preferred than Cacahuacintle X's. Subsequent studies have shown progress in selecting within Antigua 2D-118 for a more pubescent type and within Cacahuacintle X's for a less pubescent type to demonstrate the ovipositional behavioral preferences of the female CEW (Wiseman et al. 1988).

Non-preference by CEW larvae for silks of resistant maize was reported by Wiseman et al. (1983a). They found in laboratory choice tests that significantly more larvae had fed on silks of 'Stowell's Evergreen' sweet maize after 4 days than on silks of 'Zapalote Chico'. But, when larvae were placed on silks of these two maize cultivars in both choice and no-choice situations in the laboratory, many larvae had crawled off the Zapalote Chico silks after 4 days, and significantly more larvae were found on Stowell's Evergreen silks (Table 2). Thus, it was concluded that the resistant feeding responses of CEW larvae observed in the field (Wiseman et al. 1978) could be due in part to non-preference. Larvae which fed in the silk channel of Zapalote Chico for 3 to 6 days girdled the silks to the point where the exposed silk mass was detached from the silk channel. Exposed larvae then faced the behavioral decision of leaving the silk channel (non-preference) or attempting to penetrate deeper into the silks, which would retard their development (antibiosis).

Non-preference by larvae of FAW has been studied using both leaves and silks of the maize plant. Wiseman et al. (1983b) found that significantly more FAW larvae crawled off resistant plants than off susceptible plants in the whorl-stage. The data from this test confirmed that Antigua 2D-118 had a higher level of non-preference than MpSWCB-4 (Table 3). Yang et al. (1993a) reported similar results, as there were fewer larvae on resistant genotypes than on susceptible ones, indicating the cuticular lipids are involved in resistance.

Wiseman and Isenhour (1988) speculated from studies where they fed green or yellow whorl tissue to FAW larvae that the presumed antibiotic resistance of 'Antigua 2D-118' and 'MpSWCB-4' could actually be behavioral resistance (i.e., non-preference), due to the fact that larvae fed yellow whorl tissue were smaller than those fed green whorl tissue, regardless of whether plants were resistant or susceptible. Yang et al. (1991, 1993b,c) performed a chemical and ultrastructural analysis of maize leaves and studied the effect of cuticular lipids on feeding by FAW larvae. Larval behavior on the adaxial and abaxial leaf surfaces with and without cuticular lipids was monitored by video camera. Larvae showed more non-acceptance behavior on the untreated foliage containing cuticular lipids than on foliage from which the cuticular lipids were removed. Larvae traveled greater distances and crawled faster when they were on upper leaves rather than lower leaves and when they were on the abaxial leaf surface than on the adaxial surface. Yang et al. (1993c) found that larvae weighed more and developed faster when they were reared on diet containing maize foliage from which the cuticular lipids had been removed than when they were fed untreated foliage.

Resistance of maize silks to FAW larvae was first reported by Wiseman and Widstrom (1986). This resistance manifested itself as non-preference and

Table 2. Mean percent corn earworm (CEW) larvae on silks of Zapalote Chico (ZC) and Stowell's Evergreen (SEG) after 4 days of laboratory infestation.

	% larvae					
	Large dish			Small dish		
Initial larval placement	ZC		SEG	ZC		SEG
Zapalote Chico	19.4	*	80.6	7.5	*	92.5
Stowell's Evergreen	11.1	*	88.9	7.5	*	92.5
Center				5.6	*	94.4

Mean percent comparing ZC vs SEG with an asterisk between are significantly different ($P < 0.01$). Large dish = 25.5 cm dia. and small dish = 8 cm dia. (Wiseman et al. 1983a).

Table 3. Mean number of fall armyworm (FAW) larvae moving from resistant or susceptible corn genotypes to surrounding trap plants (common hybrid) at varying intervals after infestation, 1981.

	Larval numbers on surrounding trap plants at days after infestation[b]			
Genotype[a]	3	5	7	11
Antigua 2D-118	0.6a	3.6a	5.9a	8.0a
MpSWCB-4	0.1b	2.1b	3.7b	5.0b
Cacahuacintle X's	0.2b	2.1b	3.3b	4.5b

[a] Antigua 2D-118 and MpSWCB-4 = resistant and Cacahuacintle X's = susceptible.
[b] Means within a column followed by the same letter are not significantly different ($P < 0.05$) (Wiseman et al. 1983b).

antibiosis. Larvae moved off or away from Zapalote Chico silks regardless of whether larvae had a choice or not. Overall, a 6 to 1 ratio of larvae preferred silks of the susceptible entry (83%) to silks of the resistant entry (15%). All of the silks of the susceptible cultivar were consumed, while only about 10% of the silks of the resistant cultivar were consumed when larvae were presented with a choice (Table 4). Similar differences were found when the larvae were placed initially on the resistant or susceptible silks (Table 5). However, more silks of the resistant cultivar (20%) were fed upon when the larvae were initially placed on the susceptible silks compared with those initially placed on the resistant silks (10%). Yet, when the silks of the two cultivars were mixed, about 90% of the silks of the susceptible cultivar were fed upon as compared with no feeding on the resistant silks.

The non-preference mechanism of resistance against both CEW and FAW associated with maize silks or whorl-stage plants prompts the larvae to move about on the resistant plant in search of a more appropriate feeding site. Non-preference such as reported here could be a valuable tool by itself or when used with certain other components of pest management that could take advantage of these characteristics of larval behavior.

Antibiosis

Walter (1957) was one of the first to demonstrate that the resistance in silks of some maize lines was due to antibiosis. Straub and Fairchild (1970) and Wiseman et al. (1976 and 1981a) showed that silks of Zapalote Chico possessed a CEW larval growth inhibitor. Wiseman and Isenhour (1990) found additional adverse biological characteristics associated with the antibiotic responses when CEW were fed on resistant silk-diets (such as prolonged developmental time, reduced weight of pupae, and reduced fecundity reduced by as much as 65% over 4 generations) (Table 6). Wiseman et al. (1991) also associated the production of additional instars and reduced head capsules with antibiotic factors in the silks. Waiss et al. (1979) suggested that a portion of the antibiotic factor in Zapalote Chico was "maysin", a luteolin-C-glycoside. Henson et al. (1984) found no relationship between maysin concentration in maize silks and ear penetration by CEW larvae. Also, Wiseman et al. (1985) found no significant relationship between growth of CEW larvae that were fed on silks and/or silk diets and maysin concentration of silks. However, Wiseman et al. (1992a) later found a significant ($P < 0.01$) relationship in four separate tests between reduced growth of CEW and increased maysin concentration, whether maysin was fed directly in meridic diets or fed as silk-diets. A biological relationship must be established between the suspected chemical basis of resistance in the silks and the insect.

Recently two additional cultivars (GT114 and PI340856; Wilson et al. 1991) have been identified with high levels of antibiosis as well as high levels of maysin (Wiseman and Widstrom 1992; Wilson and Wiseman 1988; Wiseman et al. 1992a,b). PI340856 has some of the highest levels of maysin found to date, and is highly resistant, while the resistance of PI340853 is high, but the silks do not contain maysin (Wiseman et al. 1992b). The resistance of PI340856 is governed by a single dominant gene (Wiseman and Bondari 1995), whereas the inheritance of PI340853 silk resistance is not known to date.

Antibiosis to FAW was discovered in whorl-stage maize by Wiseman et al. (1981b). They found that FAW larvae

Table 4. Preference of neonate fall armyworm (FAW) larvae for either silks of Stowell's Evergreen or Zapalote Chico.

Silks	Mean % larvae on	% silks consumed	% feeding on mixed silks
Stowell's Evergreen	80a	100a	90a
Zapalote Chico	20b	10b	0b

Means within a column followed by the same letter are not significantly different ($P < 0.05$; least significant difference test) (SAS Institute 1982). Mixed silks are a mixture of Stowell's Evergreen and Z. Chico silks. (Wiseman and Widstrom 1986)

Table 5. Mean percent of fall armyworm (FAW) found on silks of Zapalote Chico (ZC) or Stowell's Evergreen (SEG) four days after infestation.

Initial placement of larvae	Mean % of larvae on			% silks consumed		
	ZC		SEG	ZC		SEG
Zapalote Chico	17.5	*	80	10	*	70
Stowells' Evergreen	10	*	90	20	*	90

Percents separated by * are significantly different ($P < 0.05$; least significant difference test (SAS Institute 1982). About 2.5% of the larvae placed initially on ZC were not accounted for after 4 days. (Wiseman and Widstrom 1986).

that fed on resistant genotypes were significantly smaller than those fed on susceptible maize genotypes (Table 7), and that consumption of leaves of resistant plants was also significantly less than consumption on more susceptible plants (Table 8). Resistance in maize silks has been demonstrated at a much higher level (Wiseman and Widstrom 1986). When FAW larvae were fed for 10 days on a complete meridic diet containing fresh silks of Zapalote Chico (200 mg/ml diet), their final weight averaged 4 mg compared to 361 mg for larvae fed on the control meridic diet without corn silks (Table 9).

Table 6. Mean growth, development time, and egg production of corn earworm (CEW) after having fed on susceptible, low resistance, intermediate-resistance, and high resistance diets over four generations.

Treatment[a]	9-day larval weight (mg)	Pupation (d)	Weight of pupae (mg)	Adult eclosion (d)	Relative egg production
Generation 1					
Lab C	—	—	—	—	—
Susceptible	399b	14.3a	542a	24.8a	21a
Bean diet	494a	14.3a	562a	24.9a	21a
Low-resistant	148c	16.9b	475b	27.6b	20a
Intermediate-resistant	26d	22.5c	471b	32.5c	22a
High-resistant	6d	30.0d	302c	39.3d	10b
MSDc	30	0.63	32	0.49	3
Generation 2					
Lab C	821a	12.2a	530ab	22.4a	27a
Susceptible	691b	13.0b	554a	23.2a	24b
Bean diet	692b	12.8b	537ab	23.3a	27a
Low-resistant	310c	14.7c	503b	25.5b	25ab
Intermediate-resistant	20d	25.2d	431c	35.9c	18c
High-resistant	11d	25.6d	420c	38.3d	14d
MSDc	42	0.53	39	0.96	3
Generation 3					
Lab C	840a	11.3a	565a	21.8a	26a
Susceptible	715b	12.3b	526bc	22.9a	23a
Bean diet	708b	11.4a	537b	22.2a	29a
Low-resistant	325c	16.3c	512c	27.6b	23a
Intermediate-resistant	74d	21.2d	376d	31.0c	13b
High-resistant	7e	35.1e	263e	46.5	2c
MSDc	39	0.64	20	0.64	5
Generation 4					
Lab C	781a	12.8a	546a	23.4a	25a
Susceptible	673b	13.5ab	556a	24.1b	24a
Bean diet	609c	13.8bc	555a	24.3b	19bc
Low-resistant	400d	14.6c	517b	25.9c	23ab
Intermediate-resistant	165e	17.3d	502b	28.5d	16c
High-resistant	11f	37.8e	249c	49.1e	6d
MSDc	49	0.77	21	0.68	4

[a] Lab C, laboratory control larvae from the laboratory culture on bean diet; susceptible, diet of Stowell's Evergreen sweet corn, 25 mg dry silk/ml of dilute bean diet; bean diet, larvae on pinto bean diet; low resistance, 25 mg dry Zapalote Chico silk/ml of dilute pinto bean diet; intermediate-resistance, 50 mg dry Zapalote Chico silk/ml of dilute pinto bean diet; high resistance, 75 mg dry Zapalote Chico silk/ml of dilute pinto bean diet. Relative egg production was based on the system used by Perkins et al. 1973. (Wiseman and Isenhour 1990).

Means within a column for each generation not followed by the same letter are significantly different (P < 0.05, k-ratio = 100; Waller and Duncan 1969).

c MSD = Minimum significant difference.

Table 7. Mean weight of fall armyworm (FAW) larvae after 8 days of a no-choice test involving leaf sections of resistant and susceptible maize entries, 1980.

Genotype	Field rating[a]	Mean larval wt. (mg)[b]
Cacahuacintle X's	S	333.5a
Ab24E X Mp305	S	263.3b
Antigua 2D-118	R	229.6bc
Mp4008	R	193.3c
MpSWCB-4	R	151.8d

[a] S, Susceptible; R, resistant.

[b] Means followed by the same letter are not significantly different at P < 0.05. Means of 50 replications. (Wiseman et al. 1981b).

Table 8. Total leaf consumption, percentage consumption, and mean weight of fall armyworm (FAW) larvae after 8 days of a no-choice feeding test involving leaves of a resistant and a susceptible maize, 1980.

Genotype	Field rating	Total consumption (cm^2)	% consumption	Mean larval wt. (mg)
Cacahuacintle X's	S*	72.4a	37.1a	294.2a
MpSWCB-4	R	21.5b	10.9b	77.5b

Total consumption, percent consumption, and mean larval weight followed by the same letter are not significantly different at P < 0.05. Means of 12 replications.

* S, susceptible; R, resistant. (Wiseman et al. 1981b).

Table 9. Weight of fall armyworm (FAW) larvae after feeding 10 days on silks of maize mixed in meridic diets, 1984.

Amount of silks (mg) per ml diet	Mean wt. of larvae (mg)[a]		
	Stowell's Evergreen		Zapalote Chico
0	357		361
25	394	*	271
50	337	*	150
100	23	*	41
200	246	*	4
Slope [b]	-16	*	-43

[a] Cultivar means separated by * are significantly different (P < 0.05; least significant differences) (SAS Institute 1982).

[b] Expressed per 25 mg of silk per ml diet. (Wiseman and Widstrom 1986).

Plant Resistance and Integrated Pest Management (IPM)

Plant resistance to insects in each crop-insect relationship should be the hub of integrated approaches to pest management (Fig. 1). Though the effects of the resistant cultivar are specific, cumulative and persistent, it can be used safely and compatibly in combination with any one or more of the conventional integrated components that radiate outward from the central hub of insect pest management. The effects of the resistant cultivar have been demonstrated over and over again in crops such wheat, alfalfa, grapes, sorghum, maize and grasses. Thus, it is our responsibility to keep on promoting and demonstrating the benefits of plant resistance to insects so that the next generation of scientists can appreciate its true value and also have materials and ideas to build on.

Losses by CEW larvae in field maize have been estimated at 2.5% annually for the USA. Losses in recent years in Georgia have ranged from 1.5 to 16.7%. Losses for popcorn and sweet maize for human consumption can be as high as 50%, but these high losses rarely occur because the crops are protected by as many as 29 applications of insecticides for the control of insects (Personal Communication, J. Coppedge). Large CEW populations develop on susceptible maize hybrids from May through mid-September in the southern and southeastern United States, and through the northern areas of the USA. Populations of female moths emerging from 200,000 ha of maize in the southern USA have been estimated at 148 to 716 million (74 to 358 million females/200,000 ha, assuming a 50:50 sex ratio) which could produce economic infestations on 3.0 to 14.3 million ha of other crops (Raulston et al. 1992).

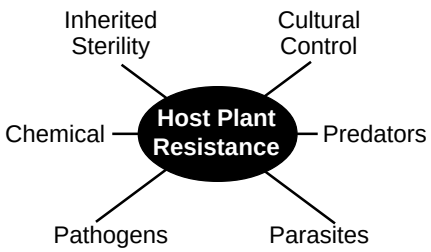

Figure 1. Integrated components of pest management that could be used in a sustainable system for agricultural production.

For many years field maize was protected from damage by CEW larvae by growing tolerant hybrids (Wiseman et al. 1984). However, the commercial maize industry and growers changed from full season hybrids, which gave the husk protection (mechanical resistance) or tolerance to CEW larvae, to the open, short husk hybrids. This change by growers, industry and users is probably the main reason we have seen increased losses in field maize in recent years.

High levels of antibiosis in the silks of some maize cultivars have been identified in recent years (Wiseman and Davis 1990; Wiseman and Isenhour 1990; Wilson et al. 1991; and Wiseman and Widstrom 1992). Wiseman and Isenhour (1990) demonstrated the effects of antibiosis on several biological parameters of larvae of CEW. They showed that a low level of resistance reduced CEW larval growth and extended its life cycle by ca. 3 days. An intermediate level of antibiosis reduced the larval growth, extended its life cycle by ca. 8 days and reduced the fecundity of females by ca. 30 percent. A high level of antibiosis in the silks caused a drastic reduction in larval growth, extended the life cycle by ca. 20 days and reduced fecundity by ca. 65 percent. The very high level of resistance found in the silks of the popcorn, PI340856, resulted in total larval mortality (Wilson et al. 1991 and Wiseman et al. 1992b).

Resistance to CEW larvae in silks of commercial maize hybrids could reduce CEW populations, keeping them from developing into huge populations which cause tremendous economic losses in not only maize but in cotton, soybeans, peanut, sorghum, and vegetables (Table 10).

Table 10. Cumulative effects of various levels of resistance in maize silks on numbers of corn earwom (CEW) larvae and generations per year.

	Number of larvae	Number of generations
Susceptible	1.6 x 106	6
Low resistance	3.1 x 105	5
Intermediate resistance	1.8 x 104	5
High resistance	1.7 x 102	4
Very high resistance	0	0

Assuming an initial corn earworm population of 100 moths, a 50:50 sex ratio, beginning May 1 with an egg production of 1000 eggs per female moth and each generation egg to adult mortality of 99 percent due to natural causes, 27 days/generation on a susceptible host 30, 35, and 47 days on a host with a low, intermediate, and high level of resistance, and no development on the low, intermediate, high and very high silk resistant maize hybrids. Also based on the findings of Wiseman and Isenhour (1990) of no additional mortality of larvae on the low resistant hybrid, 25 percent additional mortality of larvae on the intermediate, 50 percent additional mortality of larvae on the high resistant silks (Wiseman et al. 1978) and total larval mortality on the silks of the very high resistant hybrid silks (Wiseman et al. 1992b). Wiseman and Isenhour (1990) also showed that the intermediate and high resistant silks could cause a reduction in female fecundity of 30 and 65 percent, respectively. An assumption is made that silking maize was available from May 1 in the south to September 20 in the more northern areas of the U.S.

Over 1.6 million CEW larvae would survive after 6 generations, as a result of the constant build-up on a susceptible maize hybrid. With a low level of resistance (i.e., one that extends the life cycle by 3 days per generation), 312 thousand larvae would survive after 5 generations — 5 times less than the number produced on the susceptible hybrid. An intermediate level of silk resistance that extends each generation by 8 days, increases mortality by 25 percent, and reduces fecundity 30 percent could reduce surviving to 17,800 the number of CEW larvae after 5 generations, 17.6 times fewer than those surviving on the low-resistance hybrid and 87.8 times fewer than the larvae that result from feeding on the susceptible hybrid. A high level of resistance in the silks of maize hybrids could reduce the number of generations per season to 4 and the number of surviving larvae to 168 — 106, 1,860, and 9,301 times fewer larvae than those produced on the intermediate, low-resistance or susceptible hybrids, respectively. The very high silk resistant hybrid would not permit any increase in CEW populations. By integrating high levels of silk resistance with other forms of pest management, populations of surviving CEW larvae could be reduced to negligible levels. Thus, this safe, nonpolluting, persistent, specific, and cumulative control method is a feasible alternative to chemical pesticides and can be implemented by farmers.

Plant resistance to FAW may be viewed as another model system where the resistant cultivar is the hub (Fig. 1) for an integrated approach to pest management (Wiseman 1996). Plant resistance alone would have a tremendous impact on FAW populations (Table 11). There would be 196.8 thousand times more larvae produced in the 6 generations on the susceptible maize than by the 4 generations completing their life cycle on the resistant maize. On susceptible sorghum, there would be 13.3 million times more larvae produced by the end of the 6 generations compared to the 3 generations completing their life cycle on resistant sorghum. However, on susceptible millet there would only be 544 times more larvae produced by the end of the 4 generations as compared to the number produced at the end of the 3 generations on resistant millet. But there would be 24 million times more larvae produced by the end of the third generation on susceptible grass compared to none produced on the highly resistant grasses. Multiple resistance in cultivars of maize, sorghum, and millet attacked in sequence would result in 6.9 times fewer larvae, 3.4 times fewer moths or 6.7 times fewer eggs on resistant cultivars than on susceptible cultivars by the end of the third generation. Integration of plant resistance with other control tactics would produce an even greater impact on FAW populations.

Scientists and the general public are becoming increasingly aware of the need to reduce our reliance on fossil fuels and lessen the contamination of air, rivers, and lakes associated with applying more and more pesticides to produce crops. It is now clear that society's needs can be met using techniques based on ecological principles, techniques that lie within our grasp and which minimize detrimental effects on the environment. Likewise, current trends in entomology, both at the state and federal level, are emphasizing area-wide management of pests, reduced use of pesticides, improved food safety, and more sustainable systems of agriculture. The safety and compatibility of resistant cultivars helps reduce pesticide use, poses no hazard to production workers, increases food safety, and boosts profits by reducing production costs. Resistant cultivars should be the focal point for the area-wide management of insect pests.

Table 11. Cumulative effects of resistance in maize, sorghum, millet, and grass on number of fall armyworm (FAW) and generations per year on each host.

	Number of FAW larvae and (generations)[a]			
Crop	**Susceptible cultivar**		**Resistant cultivar**	
Maize	6.1 x 1013	(6)	3.1 x 108	(4)
Sorghum	6.1 x 1012	(6)	4.6 x 105	(3)
Millet	9.8 x 108	(4)	1.8 x 106	(3)
Grass	2.4 x 107	(5)	0	(1)
Sequence[b]	2.0 x 107	(3)	2.9 x 106	(3)

[a] Assumptions: 100 moths in the initial infestation; 50:50 sex ratio; 1000 eggs/female (Lynch et al. 1989); 95% natural mortality; additional mortality on resistant maize (50%; Wiseman et al. 1981c), sorghum (66%; Diawara et al. 1991), millet (50% reduction in oviposition, 10% larval mortality; Leuck 1970), and grass (100%; Wiseman et al. 1982; Lynch et al. 1983; Chang et al. 1985); 27 days/generation on a susceptible host and 35 days on a resistant host; and an unlimited food supply.

[b] Depicts population increases on a sequence of hosts, i.e., maize, sorghum, and millet. Grass is not included because of the present confusion on host strains. (from Wiseman 1996).

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Mechanisms of Resistance in Maize to Southwestern Corn Borer and Sugarcane borer

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Introduction

Maize is an important cereal crop used for food and fodder. CIMMYT has an active program that has developed maize germplasm with a desirable level of resistance to insect pests. The objective of this study was to generate quantitative information on the components of resistance in the single cross hybrids and their parents to the stem borers, *Diatraea grandiosella* (Dyar) and *Diatraea saccharalis* Fabricius. The mechanisms of resistance, non-preference, antibiosis and tolerance are studied in two ways:

- Responses of insects to plants (orientation, feeding, metabolism of the ingested food, larval growth and development, fecundity and oviposition).
- Plant characters determining responses of the insects (biochemical and physical).

Material and Methods

The following three single cross hybrids and the five inbreds developed at CIMMYT were used for the present study:

- Hybrids - Ki3 x CML131 (susceptible), CML67 x CML135 (resistant) and CML135 x CML139 (resistant).
- Inbreds - CML131 (S), CML135 (I), CML139 (R), CML67 (R), and Ki3 (S).

For studying non-preference and antibiosis in maize to insects, the plants were grown in the fields at Tlatizapan and Poza Rica and infested with *D. grandiosella* and *D. saccharalis.* At varying intervals, the plants were examined for leaf damage and larval survival and growth. In some experiments, the excised parts of the plants were also infested with *D. grandiosella* in the laboratory and lesions on leaves due to larval feeding were measured using graph paper. For determining tolerance in hybrids, the plants of each of the three hybrids were grown in Tlaltizapan in four row plots, replicated three times. Two rows of each plot were infested with 60 larvae of *D. grandiosella* while the other two rows were protected with insecticides. Data on foliar damage ratings and grain yield loss suffered by each hybrid were used to separate the components of resistance into tolerance and antibiosis.

The quadrants created by the intersection of the line for mean foliar damage and the regression line of foliar damage vs. yield reduction were used to separate the components of resistance into tolerance and antibiosis.

Results and Discussion

When the single cross hybrids Ki3 x CML131, CML67 x CML135 and CML135 x CML139 were infested with *D. grandiosella* larvae for 21 days, leaf feeding ratings on the resistant hybrids (67 x135 & 135 x139) were significantly lower than the susceptible hybrid (Fig. 1). Larval survival and weight gained by the surviving larvae on the resistant hybrids were also low (Fig. 1). Similar results were obtained when the hybrids and inbreds were infested with *D. saccharalis* (Figs. 2 and 3.). In a separate experiment, when the three hybrids and their inbreds were infested with

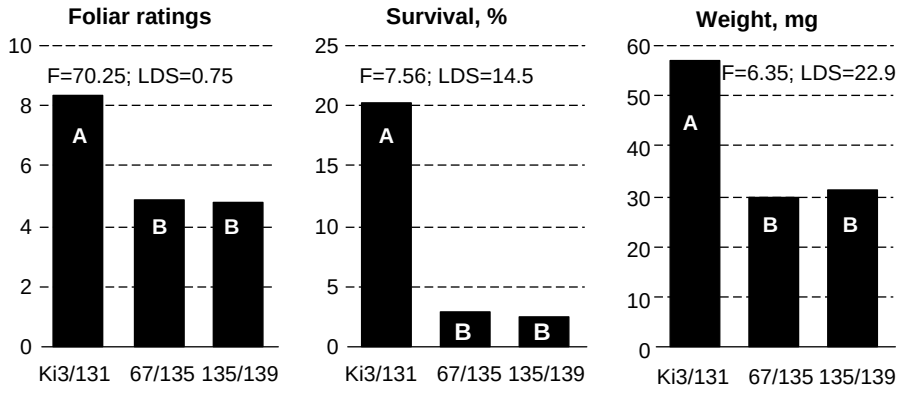

Figure 1. *Diatraea grandiosella* larval damage, survival and weight gained on 3 hybrids in 21 days.

D. grandiosella for 96 hours, the larval survival on all the hybrids and inbreds was the same (Fig. 4). However, 21 days after infestation, larval survival on the resistant hybrids and inbreds was significantly lower than the susceptible checks (Fig. 4). In the laboratory bioassays, when the excised leaf whorls of the resistant and susceptible inbreds (CML67 and CML131) and hybrids (67 x 135 & 135 & 139) were offered to *D. grandiosella*, larval survival was 100% on all genotypes (Fig.5). However, larval feeding as indicated by the area of the leaves eaten was significantly lower on the resistant hybrids and inbreds (Fig. 5). This showed that feeding non-preference and antibiosis were the mechanisms of resistance in hybrids developed at CIMMYT.

A significant correlation between the foliar damage caused by *D. grandiosella* and the yield reduction indicated a possible partitioning of the resistance components into antibiosis and tolerance (Fig.6). The points for the susceptible hybrid Ki3 x CML131 fell distinctly into "antibiosis absent/tolerance" absent quadrant (Fig.6). For the hybrid CML67 x CML135 and CML135 x CML139, the tolerance and antibiosis types of resistance mechanisms were operating against *D. grandiosella* because all the points were scattered in "antibiosis absent/tolerance present" and "antibiosis present/tolerance present" quadrants.

Our data show that CIMMYT has developed potentially useful single-cross hybrids which have adverse effects on the larval feeding and growth/development of stem borers. In addition to resistance, the hybrids possess tolerance to stem borers; i.e., surviving larvae would not cause any yield reduction in the resistant hybrids.

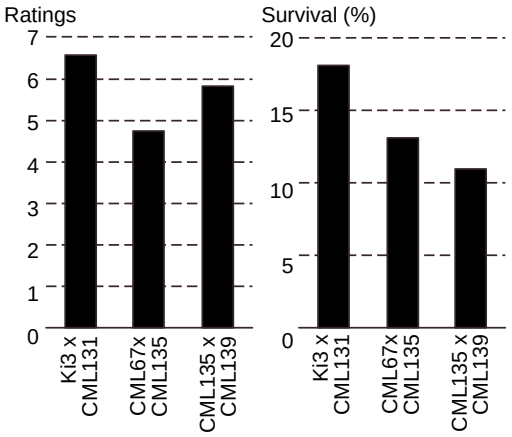

Figure 2. *Diatraea saccharalis* damage and survival on three maize hybrids.

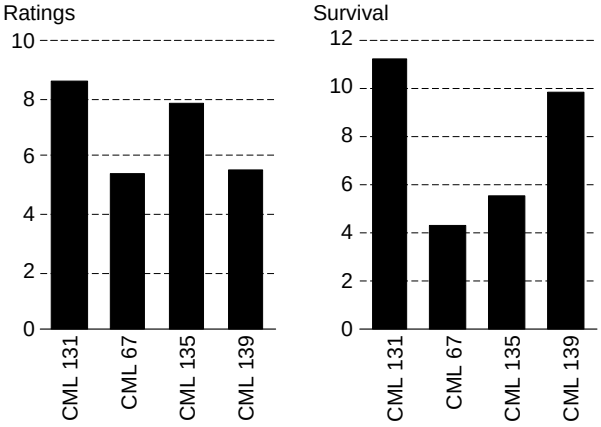

Figure 3. *Diatraea saccharalis* damage and survival on five maize inbred lines.

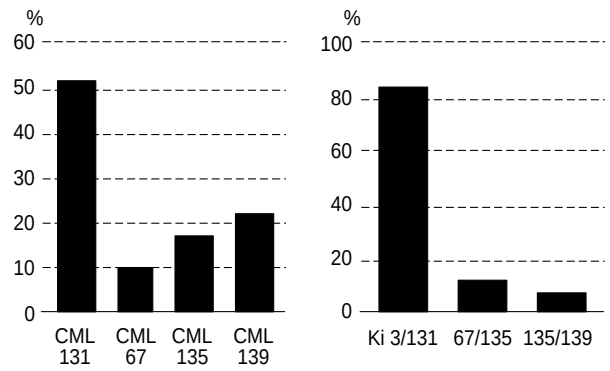

Figure 4. *Diatraea grandiosella* larval survival on 4 inbreds and 3 hybrids at 21 days after infestation.

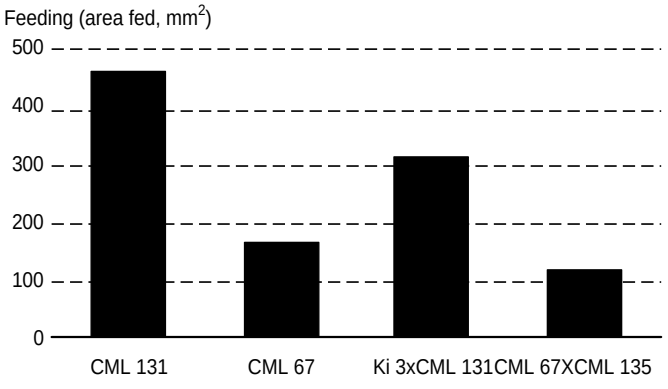

Figure 5. Feeding response of *Diatraea grandiosella* on 2 inbreds and 2 hybrids in the laboratory for 48 hours.

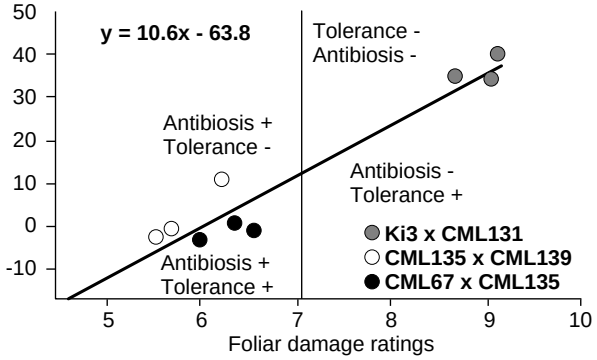

Figure 6. Correlation between foliar damage and yield reduction on maize hybrids and inbred lines by *Diatraea grandiosella*.

Variability for Maysin content in Maize Germplasm Developed for Insect Resistance

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Abstract

First described in the Mexican maize landrace Zapalote chico, the flavone maysin has been identified as a potent factor in antibiosis to corn earworm Helicoverpa zea *(Boddie). This study was conducted to determine maysin content in 20 inbreds and populations of maize which were being developed for resistance to insects. This genetic material and checks were evaluated in the field for corn earworm injury and leaf feeding damage by larvae of fall armyworm,* Spodoptera frugiperda *(J.E. Smith). Maysin levels in silks and young leaves were measured using HPLC. Maysin levels in silks ranged from 0 to 4 mg/g of fresh weight. The main part of the studied material contained maysin below 1.5 mg/g, the concentration considered to be necessary for resistance based on larval toxicity. Several populations reach the resistance level of Zapalote chico, but a few other populations also possess minor levels of the substance. Among other things, maysin level can be used as a selection criterion to increase the diversity of resistance mechanisms in source germplasm.*

Introduction

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), and corn earworm (CEW), *Helicoverpa zea* (Boddie) (Lepidoptera, Noctuidae), are the major pests of maize, *Zea mays* L., in the southeastern United States, Central America and the Caribbean. For FAW, damage to maize is caused by leaf feeding during the whorl stage (Bunting 1986). The CEW larvae usually feed on the whorl tissue leaves, emerging tassel and silks in the tips of the ears. While feeding on silks, larvae often mine into the silk channels and damage the accompanying kernels (Wiseman and Isenhour 1992). Development of plant resistance to insects is one of the most promising methods for controlling insect pests in maize (Wiseman and Davis 1990).

Several maize populations have been identified, including Zapalote chico, with high antibiosis against CEW (Straub and Fairchild 1970; Wiseman *et al*. 1976; Wiseman *et al*. 1977). Waiss *et al.* (1979) suggested that a part of the resistance in silks is due to maysin, a flavonol-C-glycoside compound. The biological relationship between maysin concentration in the silks and its effect on the growth of CEW and FAW is now clearly demonstrated (Wiseman *et al*. 1992, Wiseman *et al*. 1993).

The French National Institute for Agronomical Research (INRA) and the Centre for International Cooperation in Agricultural Research for Developement (CIRAD) carry out in Guadeloupe a maize breeding program focused on insect resistance and adaptation to Caribbean farming conditions. Base populations constituted from local maize landraces and introduced, resistance source germplasm are being improved through recurrent selection (Welcker, 1993).

The three major objectives of the present study were to determine 1) the maysin content in maize populations used and developed in Guadeloupe for resistance to fall armyworm and corn earworm; 2) the potential interest of some populations; 3) the potential usefulness of maysin content as a selection criterion.

Materials and Methods

Materials

The study included maize lines and populations with agronomic characteristics related to host plant resistance and with potential interest

for our breeding program (Table 1.) At first, we used Zapalote chico, a CEW resistant population with high maysin levels, as a positive check for maysin concentration in silks. Zapalote grande, two populations from the USDA-Georgia (GTDDSA and GTRI4), and at least one population from Central America (Maïa XXIX) were chosen for their resistance to CEW. The cultivar Stowell Evergreen was used as a negative check for maysin concentration. The susceptible local population Fond'or and a very susceptible line (Mo17) were also described.

Material resistant to FAW was also included in this study because, on the one hand, as described by Wiseman *et al.* (1992), FAW larvae development could be affected by maysin and, on the other hand, we wanted to detect maysin in organs fed on by FAW. Therefore, we studied the two main sources of resistance to FAW: MpSWCB4 and Antigua and the lines Mp705 and CML67, derived from these sources (USDA Mp, CIMMYT). We chose also the well adapted, intermediate resistance cultivar, 'Spectral', and PopG-C1a, issued from the first cycle of recurrent selection for FAW resistance in a composite of Guadeloupean maize ecotypes showing good performance under FAW and CEW (Welcker *et al.*, these proceedings).

Additional sources included TZBR-E3, a population with resistance to African sugarcane stem borer (SSB), *Eldana saccharina* Walker, introduced from IITA (Kling and Bosque-Perez, 1995); Desirade, a local, early maturing population; KI3, a susceptible check line for borers; B86, a line resistant to European corn borer, *Ostrinia nubilalis* (Hübner); CI66; and Sure Sweet, a sweet corn selected for resistance to insects at the Tropical Agricultural Research Station, Mayaguez, Puerto-Rico.

Table 1. Maize germplasm studied.

Genotype	#	Origin	FAW	CEW
Zapalote chico	1	CIMMYT	-	R
Zapalote Grande	2	CIMMYT	-	r
GTDDSA	3	USDA	-	r
GTRI4	4	USDA	-	r
Sure Sweet	5	USDA	-	r
Maïa XXIX	6	EMBRAPA	-	r
MpSWCB4	7	USDA	R	-
PopG-C1a	8	INRA	r	-
Desirade	9	INRA	-	-
Fond'or	10	INRA	-	s
Spectral	11	INRA	r	-
TZBR-E3	12	IITA	-	-
Antigua 2D-118	13	CIMMYT	R	-
Mo17	14	USDA	S	S
Mp705	15	USDA	R	-
Ki3	16	CIMMYT	S	-
CML67	17	CIMMYT	R	-
B86	18	USDA	-	-
Stowell Evergreen	19	USDA	-	S
Ci66 91-27-63	20	USDA	-	-

R: resistant material
S: susceptible material
r: population with intermediate level of resistance

Methods

Resistance to CEW was evaluated in a replicated trial in 1993 at INRA Domaine Duclos (Basse Terre, 110 masl, latitude 16°12"N, average annual rainfall 2,840 mm, average temperature 24.5°C), an area where frequent and heavy natural CEW infestations occur. CEW injury was rated for depth of kernal feeding on random sampled individual ears.

Resistance to FAW was visually rated plot by plot, 14 days after infestation with 25 larvae per plant (at the 5-leaf stage), on a scale of 0 (no damage) to 9 (heavy damage) (Davis *et al.*, 1992). The trial was carried out in 1993 at INRA Domaine Godet (Grande Terre, latitude 16°20"N, 35 masl, average annual rainfall 1,300 mm, average temperature 25.8°C).

Silk and leaf extracts were analysed for maysin following the procedure described by Snook *et al*. (1989) using reversed-phase HPLC. The silk analyses were conducted (5 to 25 replicates by genotype) on silk mass removed from the husk channel (3 to 7 g) of individual ears (1 day after silk emergence). For the leaf analyses (5 to 30 replicates by genotype), the plants were at mid-whorl stage and the tissue sampling was restricted to the furl leaves (4 to 9 g) of individual plants. Maysin was identified by its retention time, measured with a standard kindly supplied by Neil Widstrom (USDA GA). Maysin concentrations are expressed as mg/g of plant tissue, fresh weight.

Results

Corn earworm injury

Mean CEW injury ratings for the studied material are given in Table 2. These results highlight the potential of TZBR-E3 and POPG-C1a as new sources of resistance to CEW. These are improved populations developed for resistance to SSB and FAW, respectively. So the indications are that they exhibit multiple resistance. For TZBR-E3, this could be explained by the similarity in the feeding behaviour of both insects. Sure Sweet is a sweet corn which was improved for resistance to CEW at the Tropical Agricultural Research Station, Puerto-Rico. The results prove the effectiveness of this selection. On the other hand, no information can explain the level of resistance observed for KI3.

Response to leaf-feeding by fall armyworm

Mean FAW leaf-feeding ratings are given in Table 3. These data show the high level of resistance to FAW for MpSWCB4, Antigua, and derived lines. Of potential interest also are PopG-C1a and Spectral, which showed an intermediate level of resistance to FAW. Other local materials and germplasm improved for resistance to CEW appeared susceptible.

Table 2. Mean corn earworm injury rating on maize populations.

Genoytpe	Rating[a]	Replicates	Statistical grouping
Mo17	10.1	19	a
CML67	4.4	66	b
B86	3.8	19	b
Stowell evergreen	2.7	3	c
Fond'or	2.4	63	c
MpSWCB4	2.3	67	c d
GTDDSA	2.1	35	c d
CI66	2.1	8	c d e
GTRI4	1.9	20	c d e
Antigua 2D 118	1.3	41	d e
Mp705	1.2	35	d e
Spectral	1.1	51	e
Desirade	1.0	67	e
Z. Chico	1.0	45	e f
Z. Grande	1.0	13	e f
Ki3	0.6	24	e f
PopG-C1a	0.4	68	e f
Maia XXIX	0.3	54	f
Sure Sweet	0.2	56	f
TZBR.E3	0.1	61	f

[a] Mean of individual ratings of depth of kernal feeding (cm)

Table 3. FAW leaf-feeding rating in maize germplasm.

Genotype	Mean rating [a]
Mo17	8.5
Z. Grande	7.6
Ki3	7.3
GTRI4	7.1
Maia XXIX	7.1
TZBR.E3	7.0
GTDDSA	6.8
B86	6.7
Z. Chico	6.7
Desirade	6.6
Fond'or	6.5
PopG-C1a	6.5
Spectral	6.5
Mp705	4.4
Antigua 2D.118	4.2
MpSWCB4	3.8
CML67	2.6

[a] Visually rated on a 0-9 scale (0 = no damage; 9 =heavy damage).
SD Line = 0.33
SD Pop. = 0.55

Maysin content in silks

A wide range of responses was detected for maysin in silks, indicating the usefulness of maysin measurements. Figure 1 provides an example of contrasting (Zapalote chico and Stowell Evergreen) and intermediate (PopG-C1a) spectra. Maysin levels in silks ranged from 0 to nearly 4 mg/g of plant tissue, fresh weight (Table 4). As expected, maysin concentration in the silks of Zapalote chico were significantly higher than in the other genotypes.

Several laboratory bioassays have shown that a maysin concentrations of 1.5 mg/g of fresh weight reduce CEW larvae growth by 50% (Waiss *et al*. 1979; Wiseman *et al*. 1992). In this study, we found several maize populations and lines with silk maysin content below this threshold — considered to be significant for CEW antibiosis. Among these low maysin populations and lines were the negative control Stowell Evergreen and two populations

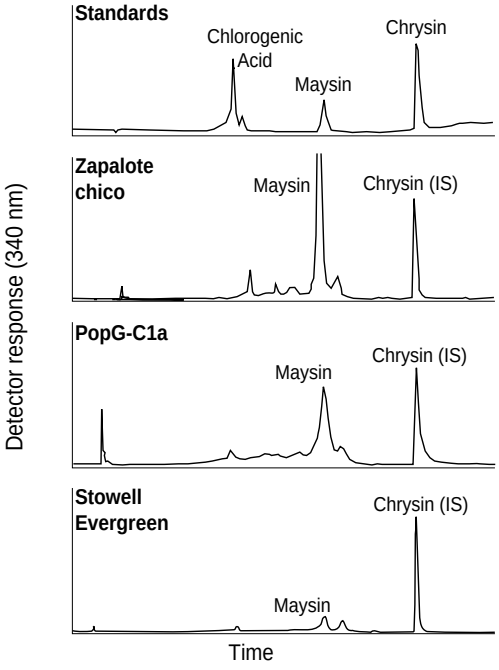

Figure 1. HPLC spectra of silks.

Table 4. Maysin concentrations in silks (mg/g of fresh weight).

Genotype	Maysin content		Replicates	Statistical grouping
	Mean	+/- SE		
CI66	0.00	+/- 0.00	5	a
B86	0.02	+/- 0.01	5	a
GT DDSA	0.08	+/- 0.03	7	a
Fond'or	0.11	+/- 0.02	7	a
Ki3	0.34	+/- 0.11	5	a b
Antigua 2D.118	0.36	+/- 0.06	24	a b
Stowell evergreen	0.44	+/- 0.38	5	a b c
CML67	0.87	+/- 0.21	4	a b c d
Maia XXIX	0.93	+/- 0.18	15	b c d
Desirade	0.99	+/- 0.44	10	b c d
Sure Sweet	1.00	+/- 0.26	4	b c d e
Mp705	1.04	+/- 0.13	15	b c d e
PopG-C1a	1.07	+/- 0.28	10	b c d e
Mo17	1.08	+/- 0.13	5	b c d e
Spectral	1.11	+/- 0.21	11	b c d e
MpSWCB4	1.20	+/- 0.23	11	c d e
Z. Grande	1.52	+/- 0.10	3	d e
TZBR E3	1.60	+/- 0.29	10	d e
GTRI4	2.08	+/- 1.11	3	e
Z. Chico	3.71	+/- 0.44	8	f

improved for resistance to insects, GTDDSA and Antigua. However, some populations showed a maysin concentration above this threshold: Zapalote grande, GTRI4 (known to have antibiosis) and TZBR-E3 apparently new germplasm with antibiosis. The maysin concentration of many entries (MpSWCB4 and Mp705, Spectral, PopG-C1a, or Maïa) was not significantly different from this arbitrary threshold. For such genotypes we could expect a 30% reduction in larval growth.

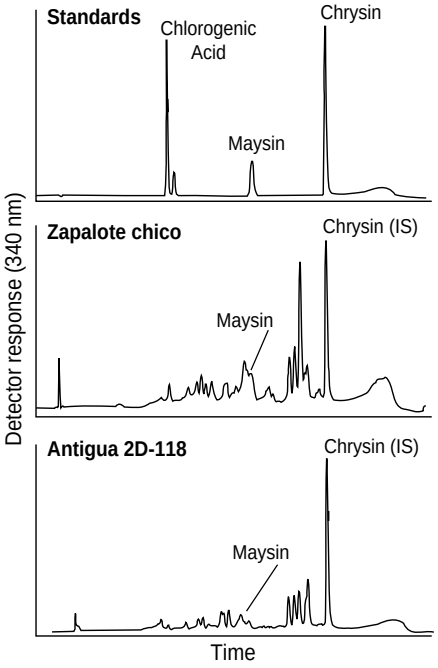

Figure 2. HPLC spectra of leaves.

HPLC spectra of leaves

In contrast to the clean HPLC traces obtained from silks, many unidentified peaks were observed in extracts from young leaves (Fig. 2). Hence, maysin measurements were more difficult. The maximum level of maysin (0.1mg/g plant tissue, fresh weight) was found in Zapalote Chico leaves.

Discussion

Correlation between maysin concentration and resistance

No significant correlation was found between maysin concentration and the level of resistance to CEW in the studied material (Fig. 3), suggesting the involvement of other resistance factors or mechanisms. For instance, Widstrom *et al*. (1992) showed that the resistance from GTDDSA is primarly tolerance. However, maysin appears to contribute to antibiosis resistance to CEW in Zapalote chico and probably in TZBR-E3, Zapalote grande and GTRI4 (provided that samples were representative, in the latter case). Field resistance to CEW is also present in other populations: PopG-C1a, Maïa XXIX and, at a lower level, Spectral. These populations possess a minor level of antibiosis which could be of interest in breeding programs. Maysin has been found at an intermediate level in silks of material improved for FAW resistance (MpSWCB4, PopG and Spectral), suggesting this as a possible selection criterion to maintain variability in resistance mechanisms in source germplasm.

Plant to plant variation for silk maysin concentration

We have reported in Figure 4 the inter-population variability of maysin concentration versus its intra-population variation. Some genotypes, in particular guadeloupean materials, present an intermediate level with interesting intra-population variation. This new variability would be worth studying, assuming it is not simply the expression of environmental effects.

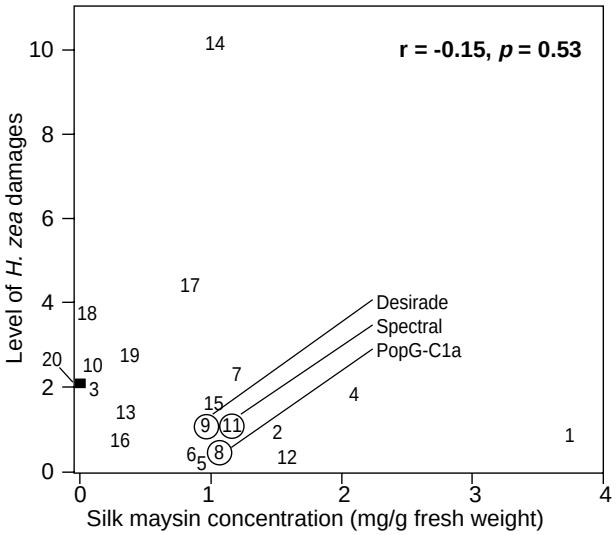

Figure 3. Correlation between silk maysin concentration and damages.

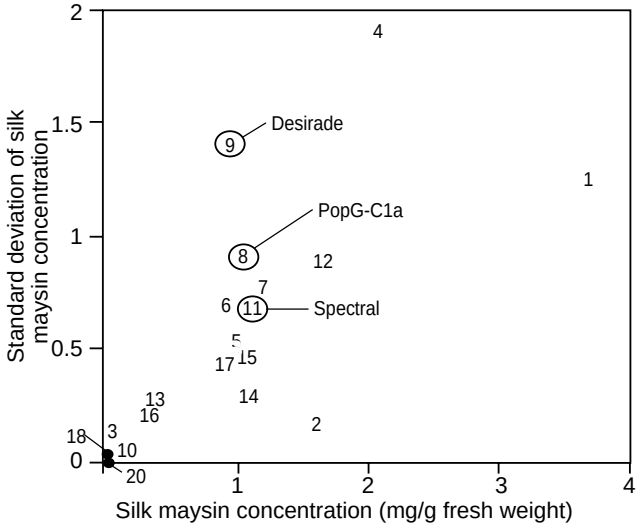

Figure 4. Variability for maysin concentration in silks within and between maize populations.

Maysin in leaves

Maysin concentrations in leaves were 30 times less than those in silks — below the concentration threshold required to significantly reduce FAW growth as reported in the literature (Wiseman *et al*. 1992). However, the characterization of material improved for resistance to FAW is still of interest, as it could reveal maysin in some populations with multiple insect resistance and could be a way to maintain genetic variability when improving populations.

This study reports new available variability for antibiosis resistance to CEW, partly resulting from maysin content. Use of maysin as a criterion could be of special interest for describing initial variability and for breeding genotypes which combine several resistance factors.

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A Review of Entomological Techniques and Methods Used to Determine Mechanisms and Bases of Stem Borer Resistance in Maize

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Abstract

Among numerous insects which attack maize, Zea mays *L., stem borers are ubiquitous and major pests. These lepidopterous insects infest the maize crop throughout its growth from seedling emergence to maturity. Maize in every country and type of crop cultivation is usually infested by one or more stem borer species. The use of borer-resistant maize varieties is an ideal method of managing these pests. Breeding for stem borer resistance has become a major research objective in most of the maize growing countries of Africa, Asia, and the Americas. Success in breeding for stem borer resistance depends upon the development of effective and efficient techniques for screening germplasm for sources of resistance. Screening techniques were presented at the first CIMMYT symposium on developing insect resistant maize in 1987. After sources of resistance have been identified and developed to some usable form (i.e., inbreds), the mechanism(s) and bases of resistance should be determined to fully understand the nature of the resistance and how to best use the resistance source in breeding programs and the resistant cultivars in integrated pest management programs. This paper serves as a review of some entomological techniques which have been used to determine mechanism(s) operating in resistant plants and to elucidate the chemical and/or physical factors (bases) responsible for resistance.*

Mechanisms of Resistance

The mechanisms of resistance in plants to insects have been divided by Painter (1951) into three categories: non-preference, antibiosis, and tolerance. Antixenosis has been suggested as a replacement for the term 'non-preference' (Kogan and Ortman 1978). Non-preference reduces the insects' three major behavioral responses, i.e., oviposition, orientation, and/or feeding (Saxena 1985). Antibiosis adversely affects the biology of insects (e.g., survival, developmental time, and fecundity). Tolerance is the ability of plants to compensate for insect damage without adversely affecting the insects' biology and/or behavior.

To determine which mechanism or combination of mechanisms are operating in a resistant plant, experiments must be carefully designed that prove or disprove the involvement of each of the three mechanisms (Davis 1985). Different experimental test procedures are necessary to differentiate among non-preference, antibiosis, and tolerance. Studying non-preference requires testing with insects under choice and no-choice conditions; for antibiosis, testing must take place under no-choice conditions and, for tolerance, pest infested and uninfested conditions (Davis 1985).

Non-preference

Non-preference denotes the presence of morphological and/or chemical plant factors that adversely affect insect behavior. To confirm non-preference, plants are planted together, infested with test insects, and left until the susceptible control has acquired a heavy population accumulation. Plants are then evaluated for insect settling response, feeding damage and/or oviposition. Techniques for measuring non-preference in resistant maize are as follows.

Larval orientation and settling - The female moths are usually responsible for selecting the plants for their larvae or progeny to feed upon. However, upon emergence the larvae must find a suitable site to initiate feeding. The larvae do have the option of accepting the plant as a host or not. Orientation and settling responses of an insect to a plant are generally measured in choice tests by observing the number of individuals which initially orient toward a plant (orientation), and then remain settled for some time for

feeding or oviposition. While the orientation response is measured within a few minutes to an hour of the release of the test insect, settling responses are generally measured at longer time intervals. The following methods have been used to measure orientation.

Attraction test - The method used by Saxena (1990) to determine the attraction of larvae of *Chilo partellus* (Swinhoe) to various susceptible and resistant sorghum cultivars can be used with maize also. Plants of each test cultivar were grown in 3.0 m x 2.5 m plots in five rows parallel to the wind direction. A rectangular tray (40 cm long x 25 cm wide), with two longer sides continuing upward as 10 cm high vertical walls, was lined with filter paper. The tray was placed 20 cm from the downwind end of each plot with its long axis parallel to and in line with the central row of plants so that distance-perceivable stimuli from the plants could reach the tray. 20 neonate *C. partellus* larvae were released across the middle of the tray and the number of larvae that moved to the two ends of the tray in 30 min were recorded. The percentage of larvae reaching the end nearest the plants reflected the level of larval attraction to the plants.

Olfactometer - The orientational responses of neonate larvae to the odor of plants can be studied using various kinds of olfactometers. The response can be measured in two-choice or multi-choice olfactometers depending upon the number of test samples. A simple Y-shaped olfactometer, used by Chang et al. (1985) for fall armyworm, *Spodoptera frugiperda* (J.E. Smith), can be used for studying orientational responses of maize stem borers. The olfactometer was constructed from two plastic rearing cups (4 cm diameter, 4.5 cm high) connected with a 10 cm, Y-shaped tube. Test materials were kept in one or both of the rearing cups and the cups were capped. Twenty neonate larvae were placed in the entrance of the Y-tube after which the tube is sealed with a cork and kept in darkness. The number of larvae reaching each rearing cup was recorded after 3 h.

To test orientation of lesser cornstalk borer, *Elasmophalus lignosellus* (Zeller) (Fig. 1) to multiple samples, an eight-sample olfactometer was constructed by Huang et al. (1990). Eight sample tubes were connected in a circle equidistantly to the bottom of a Petri dish (15 cm diameter). The Petri dish served as a bioassay arena. A small air-inlet pore was made on the outside of each tube to eliminate the differences in airflow rate. Small holes were drilled in clusters in the center of the dish to which an exhaust tube was attached and connected to a vacuum pump. All air-inlet pores were enclosed in an air-inlet chamber formed by gluing another Petri dish of the same size, with holes for the corresponding tubes and exit tube, to the bottom of the bioassay arena. Air (100 ml/min) passed into the inlet tubes was distributed in the air-inlet chamber, entered each tube, passed into the arena, and was exhausted at the base of the bioassay arena. A cotton ball was placed above each test sample. Test insects were placed in the bioassay arena and the number of *E. lignosellus* crawling into each tube was recorded after 30 min or at 5 min intervals for 30 min.

Choice test - Using a choice test, Davis et al. (1989) determined the presence of non-preference mechanism in selected maize hybrids to *Diatraea grandiosella* Dyar and *Ostrinia nubilalis* (Hübner). Inner whorl leaf tissue disks (2 cm diam.) from the test plants were randomly placed equidistant from each other on a piece of paper towel in a plastic dish containing 2% agar for providing moisture to prevent tissue drying. Each tissue disk was held flat to towel surface by inserting two small pins. The pins keep the disks from folding and allows a thigmotoxic condition that is favored by these stem borer larvae. 50 to 75 blackhead stage eggs were placed in the center of each dish. The dishes were then held in complete darkness to prevent effects of light on larval distribution within chambers. The larvae on each tissue disk were counted 24 h after egg hatch. Leaf tissue from resistant hybrids was significantly less preferred by both species of stem borers than leaf tissue from susceptible hybrids.

To determine whether neonate larvae of stem borers orient and settle preferentially on callus initiated from susceptible or resistant plants, larval orientation and settling responses were measured following the methodology of Williams et al. (1987). Five pieces of callus (0.5 g) of each test

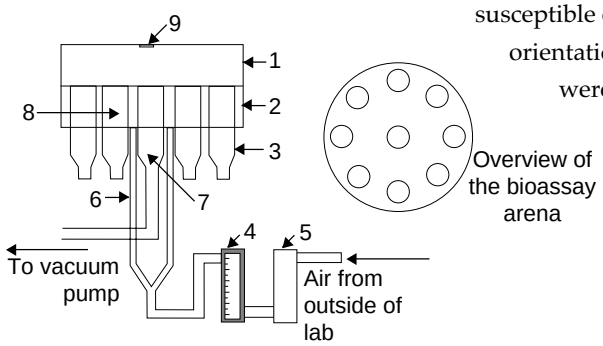

Figure 1. A multi-choice olfactometer. 1, bioassay arenas; 2, air-inlet chamber; 3, sample tube; 4, airflow meter; 5, filter fitted with activated carbon; 6, air-inlet tubes; 7, exit tube; 8, air-inlet pore; 9, insect releasing hole (Huang et al. 1990).

cultivar were placed in a circular manner equidistantly from the center of a Petri dish. 50 to 100 blackhead stage eggs or freshly hatched larvae were transferred carefully to the center of each Petri dish. The Petri dish was kept in complete darkness and number of larvae present on each callus was recorded at 1, 6, 12, 24, and 48 hours after infestation. Williams et al. (1987) reported significantly more *D. grandiosella* than *D. saccharalis*, and *O. nubilalis* larvae preferred the callus originating from maize hybrids which were susceptible to leaf feeding.

Arrest and dispersal - The settling response of lepidopterous larvae to different cultivars can be compared with respect to their arrest and dispersal on plants or plant parts. Robinson et al. (1978) placed a sticky trap around maize plants in the laboratory and field to measure arrestment or dispersal of *O. nubilalis*. Thirty first-instar larvae were placed in the whorl of each plant. The number of larvae that moved off the plant was recorded daily for 4 days, then each plant was dissected and the remaining larvae were counted. Robinson et al. (1978) reported that more larvae consistently settled on the susceptible, inbred WF9 than on the highly resistant inbred CI31A. Using similar methodology, Kumar et al. (1993) studied larval arrestment of *C. partellus* on 3-week-old plants of susceptible and resistant maize cultivars. The mean number of larvae recovered from resistant genotypes MP 704 and Poza Rica 7832 was significantly lower than the number recovered from the susceptible control.

Ampofo (1986) studied arrestment and dispersal of *C. partellus* larvae on susceptible and resistant maize plants in field plots. The experiment was planted in 3-row plots 5 m long with a spacing of 75 cm and 30 cm between and within rows, respectively. The central row was planted with a cultivar different from the two adjoining rows. Twenty days after germination, each plant in the middle row was infested with an egg mass. All plants were dissected 7 days after infestation and the number of larvae recovered from each plant was recorded and mapped. Dispersal of first-instar larvae increased twofold when infested resistant cultivar, IC22-CM, was surrounded by a susceptible cultivar, and decreased when an infested susceptible cultivar was surrounded by IC22-CM plants (Ampofo 1986).

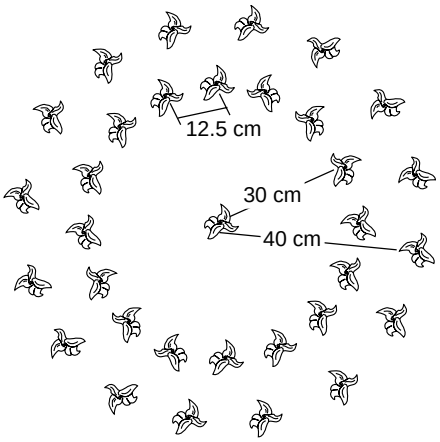

Figure 2. Arrangement of test plants to measure arrest and dispersal of lepidopterous larvae (Wiseman et al. 1983).

The field cage experiment designed by Wiseman et al. (1983) for fall armyworm can also be used to evaluate stem borers' movement from susceptible and resistant maize plants. A susceptible or resistant plant was surrounded by susceptible plants spaced alternately at 30 cm and 40 cm from the central test plant (Fig. 2). The surrounding plants were spaced about 12.5 cm apart. The test plant was infested with a known number of neonate larvae and the number of larvae present on the surrounding plants 4, 6, 8, and 10 days after infestation served as an indicator of larval movement.

Feeding - Techniques that record subtle changes in insect feeding behavior on susceptible and resistant plants can be useful in identification of resistant germplasm. Such changes in insect feeding behavior can be determined either through the measurement of damaged plant parts, or in terms of amount of food ingested. Insect feeding in a choice assay (Fig. 3) involves the determination of insect feeding preference among multiple plant genotypes. Choice experiments can be useful in the preliminary evaluation of plants. However, no-choice

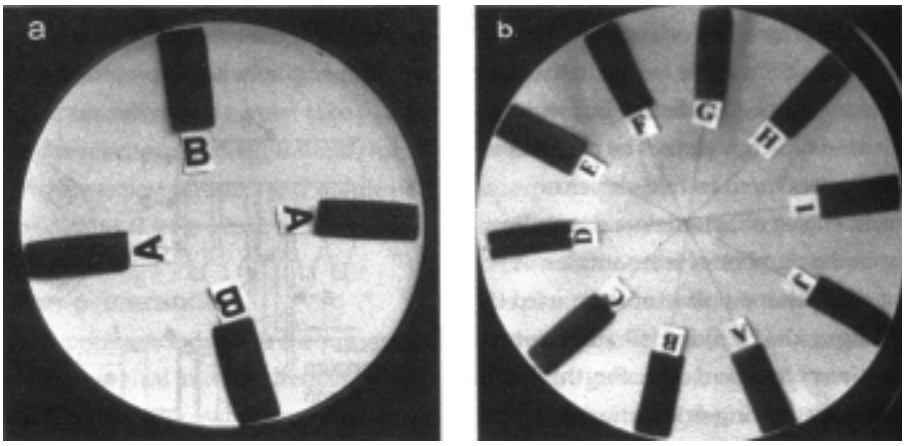

Figure 3. Experimental set-up to measure stem borer larval feeding on leaf cuts of susceptible and resistant plants in (a) two-choice and (b) multi-choice tests.

experiments are necessary to verify the degree of resistance. Insect feeding can be measured either on excised or on intact plants.

In a no-choice feeding bioassay, Saxena (1990) offered a 7 cm long basal segment of a leaf whorl to 20 neonate *C. partellus* larvae, or an internode segment of a stem to a single 4th instar *C. partellus* larva in a glass vial. After 72 h, the area of feeding lesions on the leaf was measured using a dotted paper sheet or graph paper. The stem segment was removed after 24 h, split open and the length and width of the cavities resulting from larval feeding were measured. In a similar no-choice feeding experiment with excised leaves Kumar et al. (1993) used a photometric device (leaf area meter) for measuring area of leaves before and after insect feeding. For the bioassay with stems, a pre-weighed, 6 cm long segment of a cultivar was offered to a 4th instar *C. partellus* larva in a glass vial. After 48 h, the uneaten stem was weighed again after the excreta was separated. Stems of each cultivar were also kept alongside the experiment to determine the weight loss from evaporation. The difference between the initial and final weights of stem after adjustment for weight loss from evaporation indicates stem feeding by the larvae.

Oviposition **-** For many phytophagous insects, the selection of an oviposition site is a critical stage in their choice of a host. For most stem borers and other lepidopterous pests, only the adult female has a large and direct influence on host preference/non-preference; therefore, understanding the details of the insect's oviposition preference is valuable when attempting to identify resistant germplasm in a plant breeding program. However, since an insect's ovipositional preference under a choice situation could be influenced by a more attractive plant, the relative non-preference of a host plant could often be misconstrued for true or genetic resistance. To ascertain the presence of true resistance in a cultivar, and not the relative preference existing only in a choice situation, insect ovipositional response in a no-choice situation must also be measured. Without it, oviposition preference studies are of very little utility in predicting pest response under field situations.

Saxena (1990) developed and used a three-compartment chamber (Fig. 4) to evaluate the ovipositional response of *C. partellus* under field conditions. Tests were conducted in a field with a constant number of females in the sector between two equal-sized end sectors on either side. The chamber's roof and two vertical end-walls were of glass but open below, the floor being formed by the test arena. The front and rear walls of the central sector were of glass and those of the two end sectors were of removable screen. In the field the chamber was aligned with its long axis at right angle to the wind direction. Test plants were arranged inside one end compartment in a row along the wall. The opposite end compartment had a similar row of plants of another cultivar in a two-choice test, or contained no plants, but had wax paper sheets. Gravid stem borer females were released in the central compartment and the eggs laid on the plants and on the wax paper sheets were counted.

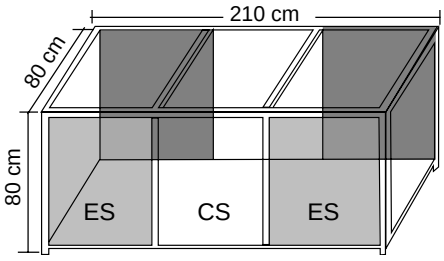

Figure 4. A three-compartment chamber for studying ovipositional response of *Chilo partellus* to sorghum plants in a field. ES, end compartment; CS, central compartment (Saxena 1990).

Ovipositional preference of stem borer adults to susceptible and resistant maize cultivars can be measured in two-choice tests following the method of Ng et al. (1990), Kumar (1993) and Kumar et al.(1993), or in a multiple choice bioassay as described by Ampofo et al. (1986). Ovipositional response in a no-choice bioassay can be tested following the methodology of Ampofo (1985). Khan (1994, unpublished) presented cut maize stems(20 cm long) in choice bioassays to ovipositing *B. fusca* females (Fig. 5).

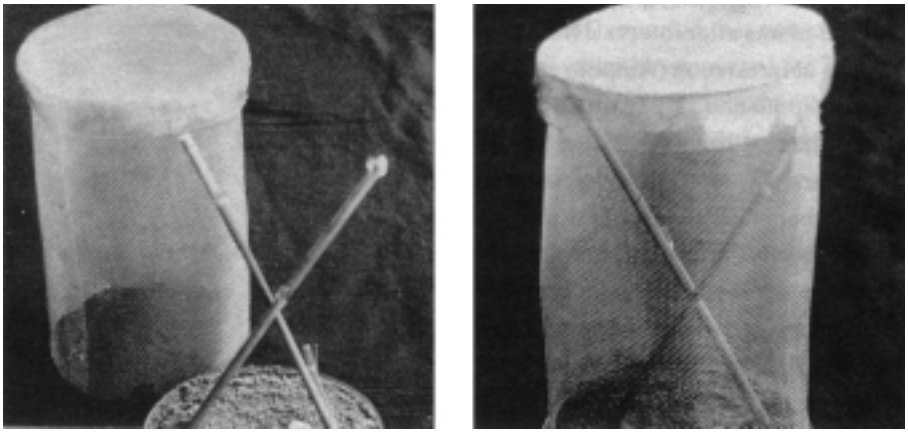

Figure 5. Experimental set-up to measure *B. fusca* oviposition on stem cuts from susceptible and resistant plants in two-choice test.

Antibiosis

Both chemical and morphological plant defenses mediate antibiosis, and antibiotic effects of these resistant plants on the insect pests can range from weak to strong. Field and/or laboratory experiments have been designed to determine if the mechanism antibiosis is operating within the resistant plant. The biological criteria used most commonly to determine if antibiosis is present or not is growth, which includes both weight gain and developmental time of the insect. Other criteria include survival of the various insect stages, morphological normality of growth stages, and fecundity. Techniques for evaluating for antibiosis as related to growth utilize intact plants, excised plant tissue, callus tissue, and artificial diets are discussed as follows.

Growth of an insect on susceptible or resistant plants is commonly determined by measuring the weight gain of the larvae, and the development of larvae into pupae. The latter is quantified as the percentage of larvae transforming into pupae, and the average time period required to do so by those that pupate. Growth rate of stem borers on resistant and susceptible varieties of maize has been frequently measured by infesting intact plants of 3 to 4 weeks of age and by removing infested plants after intervals from 7 to 42 days of infestation (Ampofo et al. 1986; Ampofo and Kidiavai 1987; Davis and Williams 1986; Kumar et al. 1993). Each plant was carefully dissected and the number of surviving larvae and their respective growth stages and weights were recorded. Insect growth was also measured on freshly excised leaves and/or stems in laboratory assays of susceptible and resistant plants (Davis et al. 1989; Saxena 1990).

Even callus tissue from insect-susceptible and resistant maize have been used to determine growth of stem borer larvae feeding on them. To determine the growth of larvae feeding on callus initiated from susceptible and resistant plants, Petri dishes containing approximately 500 mg to 1 g of callus were infested with 3 to 5 neonate larvae. The larvae were weighed after 7 to 15 days after infestation (Williams et al. 1983; Williams and Davis 1985; Williams et al. 1987). Williams et al. (1983) reported that *D. grandiosella* larvae reared for 7 days on calli of resistant maize genotypes were significantly smaller than larvae reared on calli from susceptible maize genotypes. Williams et al. (1987) also reported that *D. grandiosella* and *O. nubilalis* larvae reared for 7 days on callus initiated from resistant maize hybrids weighed significantly less than those reared on callus from susceptible hybrids. Figure 6 shows differences in growth of fall armyworm larvae after feeding for 7 days on callus of resistant and susceptible maize hybrids.

Artificial diets have been widely used to detect the presence of stem borer larval growth inhibitors in maize plants (Zhou et al. 1983; Wilson and Wissink 1986; Durbey and Sarup 1988; Williams et al. 1990; Saxena 1992; Kumar 1993). Fresh, oven-dried or lyophilized leaf powder, or plant extract is thoroughly blended with a known amount of artificial diet. First-instar larvae are fed the amended diets and comparisons of insect growth on diets incorporated with different susceptible and resistant plant materials can be used effectively to assay for antibiosis resistance. By placing eggs or newly-emerged larvae on control and treated diets, differences in feeding, weight gain, survival, and developmental rate can be detected.

Also, some researchers have studied the ingestion, digestion, and assimilation of plant tissue by the larvae to determine how the resistant plant affects its metabolism. Kumar (1993) and Ng et al. (1993) used the gravimetric method described by Weldbauer (1968) to calculate approximate digestibility (AD), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) of *D. grandiosella, C. partellus,* and *B. fusca.* The calculations are done as follows:

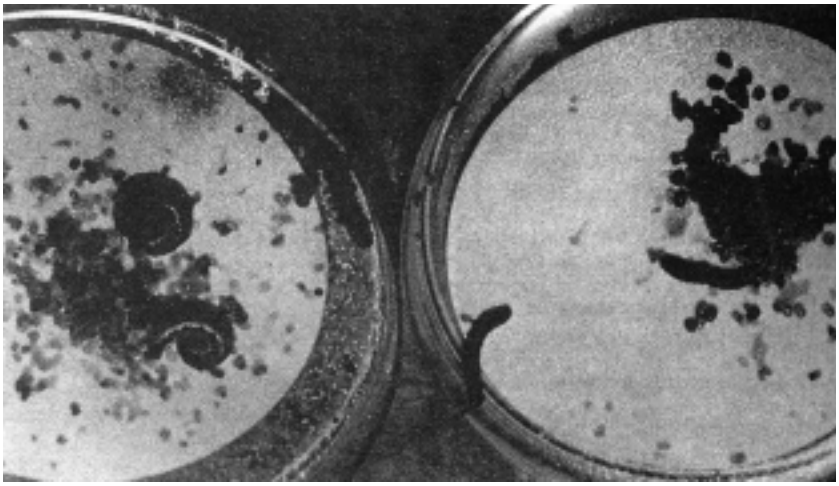

Figure 6. Fall armyworm larvae after feeding for 7 days on callus of resistant (left) and susceptible (right) maize hybrids (Williams et al. 1985).

AD = (DWF - DWE) / DWF
ECI = (DWG / DWF) x 100
ECD = [DWG / (DWF - DWE)] x 100

Where :

DWF = Dry weight of food ingested;
DWE = Dry weight of excreta; and
DWG = Dry weight gained by insect

Tolerance

Tolerance is unlike non-preference and antibiosis in that the plant does not adversely affect the behavior or biology of the insect pest. Tolerance is a response by the plant to compensate for damage inflicted by the herbivore. Tolerance can occur in combination with the other two mechanisms. Because of its unique nature in plant resistance to insects, the quantitative measurement of tolerance is accomplished by using entirely different experimental procedures from those used to study antibiosis or non-preference. The study of tolerance usually involves comparing yields or plant growth characters (e.g. height) among genotypes by using infested and uninfested plots.

Chiang and Holdway (1965) studied the relationship between plant height and yield of field maize as affected by *O. nubilalis.* The resistant cultivar Oh43 x Oh51A suffered less reduction in plant height and yield than the susceptible cultivar WF9 x M14 with the same degree of initial infestation suggesting that the resistant cultivar had tolerance to borer injury in addition to its well recognized antibiosis which reduces borer survival. Ajala (1992) estimated tolerance levels of seven maize cultivars against *C. partellus* using the following formula:

Tolerance = 100 x [(YC-YI) / YC] / ST

where, YC = Yield of control plants, YI = Yield of infested plants, and ST = stem tunneling.

Bases of Resistance

There is ample evidence to suggest that plant morphological and chemical characters affect normal feeding and establishment of stem borers on maize plants. It is therefore important to elucidate the causal factors and their role in insect resistance and susceptibility.

Morphological bases

Trichomes, also known as hairs or pubescence, are one of the more important morphological bases of plant resistance to insects. In numerous species, a negative correlation has been established between trichome density on the plant surface and insect feeding and oviposition. Long and dense trichomes hinder normal feeding and oviposition. However, the relative contribution of trichome and nontrichome based resistance to insects may not be well understood unless trichomes are removed to detect insect resistance. Without the removal of trichomes, the effects of plant allelochemicals can also be mistakenly ascribed to trichome based resistance. Ampofo (1985) studied the influence of trichomes of certain maize genotypes on *C. partellus* oviposition. Trichomes on the upper and lower surface of odd numbered leaves were counted and classified. Generally trichome density was highest on the resistant genotype ICZ2-CM and lowest on the susceptible Inbred A. Kumar (1992) reported that significantly more trichomes on the upper and lower surfaces of leaves of resistant maize cultivar ICZ-T were responsible for deterring oviposition by *C. partellus*. Using a thoroughly washed muslin cloth, Kumar (1992) removed the trichomes from one side of the central midrib of the lower surface of ICZ-T leaf. On the other side of the leaf, the trichomes were left intact. The leaf was then presented to ovipositing females, and the number of eggs laid by the females on the hairless side was compared with that on the intact side. The moths laid significantly more eggs on the hairless side than on the side with trichomes.

Chemical bases

Artificial diets have been widely used to bioassay the activity of allelochemicals against maize stem borers. Water extracts of host plants are generally added directly to the diet solution, whereas phytochemicals soluble in organic solvents are coated onto alphacel, the solvent is then removed from the material under vacuum, and the remaining material is added to the diet as a portion of the alphacel component.

Zhou et al. (1984) developed a technique for bioassaying water soluble maize extracts against *O. nubilalis*. Diet plugs weighing 3 g were cut, frozen at -10°C for 24 h and lyophilized. The shriveled, lyophilized plugs were dipped in plant extractables and were allowed to absorb the extracts for 12 h at 4°C. After each of the diet plugs had thoroughly absorbed the extract, the surplus extract on the outside of each plug was removed and they were infested with larvae of *O nubilalis*. Zhou et al. (1984) reported that neonate and second-instar larvae reared for 7 days on plugs of diets absorbed with extract of resistant maize cultivar weighed less than the larvae on susceptible plugs.

Durbey and Sarup (1988) assessed the antibiotic effects of resistant maize cultivars on *C. partellus* by incorporating their water, ethyl alcohol or acetone plant extracts into an artificial diets. The ethyl alcohol fraction

from a resistant Mex-17 cultivar was the most active in reducing larval and pupal survival, larval weight, and fecundity of females.

For *O. nubilalis*, Czapla and Lang (1990) used artificial diets to study the effects of plant lectins on larval development, and Houseman et al. (1992) studied the effects of DIMBOA and MBOA on their growth and digestive processes.

Torto et al. (1991) applied test samples in solvents to both sides of cellulose acetate disks to study feeding responses of *C. partellus*. Test disks were dried and then dampened with double distilled water and offered for feeding to third-instar larvae in a no-choice bioassay. Control disks were dipped into solvent only. Test and control disks were weighed before after larval feeding to calculate the amounts of feeding.

Summary

As a result of efficient entomological techniques and methods, progress in the development of insect resistant cultivars of maize has recently occurred. A good understanding of the mechanism(s) and bases of resistance is needed for establishing differences among resistant genotypes for these characters and for making intelligent decisions about using resistant germplasm in a breeding program and in integrated pest management. However, it is not unusual to find that combinations of each mechanism contribute to insect resistance, and the absolute contribution of a given resistance mechanism may never be fully elucidated. Similarly, in some cases, it is difficult to demonstrate if a certain morphological character contributes towards insect resistance. Simple bioassays, involving insect responses on plants with different morphological characters, may not provide sufficient evidence to prove the real role of plant morphology in insect resistance. Therefore, appropriate techniques are needed to prove that a true relationship exists between physical factor(s) and resistance rather than just a simple correlation.

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An Overview of Research on Mechanisms of Resistance in Maize to Spotted Stem Borer

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Abstract

The spotted stem borer Chilo partellus *(Swinhoe) (Lepidoptera:Pyralidae) is an important pest of maize in several countries of Asia and Africa. Serious crop losses have been reported, mostly in experiments conducted under artificial infestations at experimental stations. In order to develop economical and environmentally friendly methods of pest management, a large number of maize genotypes with varying level of resistance to* C. partellus *have been identified. In the identified resistant germplasm, the three components of resistance, namely, non-preference, antibiosis, and tolerance, have been identified. In Asia, various studies have been conducted to elucidate the mechanism of resistance/ susceptibility in the two maize genotypes, Antigua Group 1 (Resistant) and Basi Local (Susceptible), against* C. partellus*. Several biological parameters including* C. partellus *larval and pupal survival, larval and pupal weights, larval and pupal period and fecundity were adversely affected due to unknown factors in the resistant source, but not on the susceptible one. An ethanolic extract of Mex 17 has also been reported to inhibit growth and development of* C. partellus *in comparison to the susceptible genotypes. The studies conducted in Africa show that ovipositional non-preference by* C. partellus *on maize genotypes was due to trichomes and surface waxes. A genotype, ICZ-T, with trichomes on both the leaf surfaces was also developed. In some studies, using regression of grain yield reduction on foliar injury due to* C. partellus *attack on maize genotypes, namely, ICZ1-CM and ICZ2-CM, antibiosis and tolerance were reported to be the components of resistance. In more detailed studies in Africa, non-preference, antibiosis and tolerance types of resistance mechanisms have been reported to be operating within maize genotypes Mp704, Poza Rica 7832 and ER - 29SVR. The resistance mechanisms operating within these sources have also been reported to be expressed in the crosses with agronomically desirable sources.*

Introduction

Maize, *Zea mays* L., is an important staple food for millions of people in Africa, Asia and Latin America where it serves as a human subsistence crop. However, the grain yield per hectare is low (2.2 t/ha) in comparison to the developed countries (5-6 t/ha). Of the various major constraints responsible for the low maize production in the developing countries (Table 1), insect pests are the most destructive and unmanageable because the chemical control tactics are inaccessible to the farmers. Host plant resistance, which is the cheapest and biologically, ecologically, economically and socially feasible method of crop protection, is absent in the commercial maize varieties in the developing countries. There are several reasons for the unpopularity of host plant resistance in these countries:

- Intense competition between the multinational insecticide manufacturing companies and the resource poor national programs of the developing countries.
- Lack of active collaboration among different members of the multidisciplinary team needed for the development of resistant varieties with good agronomic background.
- Unawareness among the farmers regarding the existence of plant resistance to insects and the cost/ benefits ratios of using plant resistance as a control tactic.

Notwithstanding the above problems, exhaustive information has been generated on screening of maize

Table 1. Major constraints to maize production.

1.	Inaccessibility to expensive fertilizers
2.	Costs of certified seeds of the improved commercial varieties/hybrids
3.	Unreliable and erratic rainfall in the major maize growing areas (>80%)
4.	Pests (insects and non insects), diseases, weeds

genotypes for resistance to insects as well as on the mechanisms of resistance in selected maize genotypes to insects.

In Asia and Africa , three major species of *Chilo* infest maize (Table 2). Of these, the spotted stem borer *Chilo partellus* (Swinhoe) is the most important. In the literature, the common name of this stem borer has been too variable. The spotted stem borer should be used irrespective of the crop it infests. It is distributed widely in India, Pakistan, Indonesia, Sri Lanka, Thailand, Ethiopia, Kenya, Somalia, Tanzania and South Africa (Seshu Reddy 1983; Hamburg 1979) (Table 3). In India, the pest is active during July to September and remains dormant during November to April (Fletcher and Ghose 1920; Rahman 1944) (Table 4). In Africa, *C. partellus* remains active throughout the year (Ampofo 1985). Until harvest, the maize plant suffers damage by two generations of *C. partellus*. (Fig. 1). The first generation *C. partellus* attack on maize commences at the early whorl stage. Neonates hatching from the eggs laid by *C. partellus* on the basal leaves of the early whorl stage maize, disperse and enter the leaf whorl where they feed and cause damage to the leaves (Figs. 2 and 3). Because of the extensive feeding by the larvae in the leaf whorl, the central shoot dries up and the plant can not grow any more. This type of damage is termed as 'dead heart' (Kumar and Asino 1993) (Fig. 3). The older larvae leave the leaf whorl and bore into the stem to cause stem tunneling. The second generation *C. partellus* attack commences at anthesis. Neonates feed inside the leaf sheaths

Table 2. Major species of *Chilo* infesting maize.

Chilo partellus (Swinhoe)	Asia and Africa
Chilo agamemnon (Blezynski)	Egypt
Chilo orichalcociliellus Strand	Coastal Kenya and Madagascar

Table 3. Distribution of *C. partellus.*

1. India, Pakistan, Indonesia, Sri Lanka, and Thailand (CIA Map 184)
2. Ethiopia, Kenya, Somalia, Sudan, Tanzania and Uganda (Seshu Reddy 1983)
3. South Africa (Hamburg 1979)

Table 4. Seasonal occurrence of *C. partellus.*

	India (Fletcher and Ghose 1920)	**Kenya** (Ampofo 1985)
Peak activity	July-September	June, August, Dec., January
Dormant Period	Nov.-April	None

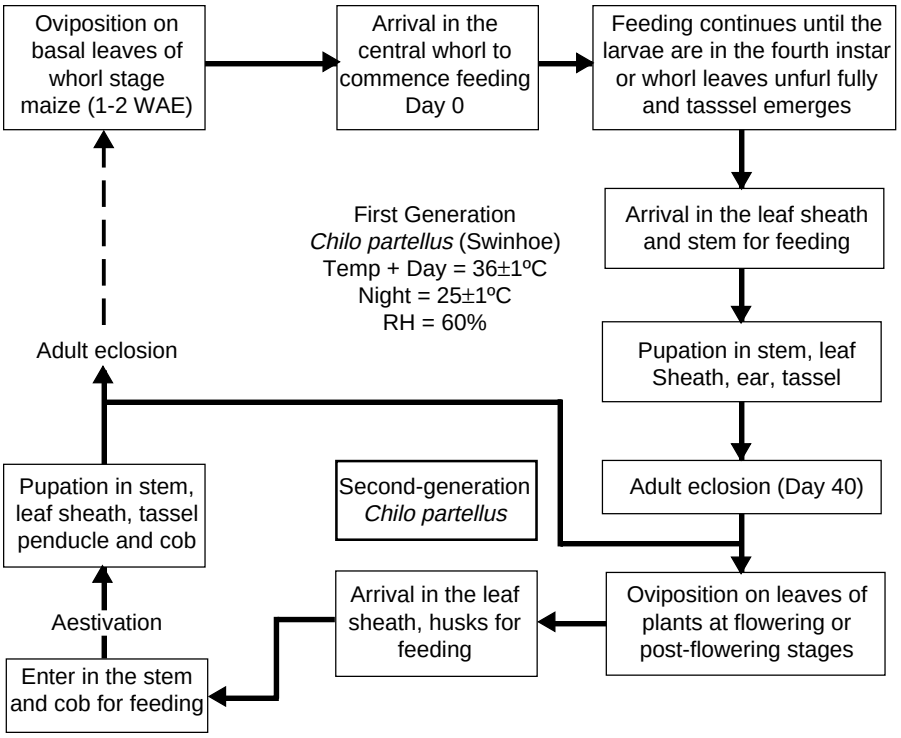

Figure 1. Biological relationships between *C. partellus* and the maize plant.

Figure 2. Foliar damage on maize by stem borers.

Figure 3. Dead heart caused by the stem borers to the maize plants.

and ear husks (Kumar 1992b). Older larvae bore into the stem and ear to cause stem tunneling and ear damage. Reference to the literature shows that maximum mating by *C. partellus* occurs the first night after the emergence and that maximum oviposition occurs the first night after mating (Kumar and Saxena 1985b). The mating is confined to the second half of the night (after midnight) while oviposition is restricted to the first half of the night (before midnight). Unnithan and Saxena (!990) monitored *C. partellus* populations by using live females in the traps. The complete chemical nature of *C. partellus* sex pheromone has not been elucidated yet, although some components have been reported to attract males over short distances (Lux et al. 1994).

Grain yield losses due to *C. partellus* attack on whorl stage maize have been reported to vary with the cultivars (Ampofo 1986; Kumar 1988a; Seshu Reddy and Sum 1992), infestation levels (Chatterji et al. 1969; Kumar 1988a; Sarup et al. 1977; Seshu Reddy and Sum 1992) and crop phenology at infestation (Sarup et al. 1977; Seshu Reddy and Sum 1992). Yield losses due to *C. partellus* attack at anthesis have also been reported to vary with cultivars and infestation levels (Kumar and Asino 1994).

A meridic diet for *C. partellus* has been developed and used successfully to rear this stem borer (Siddiqui et al. 1977; Seshu Reddy and Davies 1978; Ochieng et al. 1985). The egg masses at the black head stage or neonates have been used to infest maize genotypes to determine their resistance or susceptibility to *C. partellus.* (Sarup 1983; Kumar 1993a,b, 1994; Kumar et al. 1993). Some researchers have also used *C. partellus* adults to infest maize (Ampofo et al. 1986), because under natural conditions, oviposition by the adults on the plants is the first step to start the infestations. However, this method is less practical because large arenas are needed to confine the flying adults on the plants. All these methods are useful to elucidate the mechanisms of resistance in selected maize genotypes to *C. partellus*.

Kumar (unpublished data) also conducted trials to simulate the natural infestation by planting border rows of the susceptible genotype and infesting with *C. partellus*. The planting dates of the test genotypes were then adjusted in such a way that the plants were at 6-8 leaf stage when the adults emerged from the infested border rows and started to infest the test genotypes. However, this method was not very successful in the tropical environment, because the survival of the larvae on border rows was not sufficient to be transformed into adults to infest test genotypes adequately. However, this method would be suitable on a small scale in the screen house. Nevertheless, to distinguish resistant and susceptible maize genotypes, infestation with larvae has been reported as more effective than the egg masses (Kumar, in press). The hand operated device called "bazooka" can be adapted by the entomologist to screen maize germplasm for resistance to *C. partellus* (Fig. 4).

A number of parameters have been used by various workers to assess damage by *C. partellus.* Ampofo et al. (1986) used number of egg masses, foliar damage, percentage of stem length tunneled, number of entry and exit holes and stalk breakage by *C. partellus* to distinguish resistant and susceptible genotypes. The ratio of each parameter's value for a test cultivar to that for the susceptible check was computed. The relative ratios of all the parameters for each genotype were then averaged to give the overall resistance/susceptibility index (ORSI). The lower the ORSI value of a genotype, the greater would be the resistance to *C. partellus* and vice versa. However, such a method is not suitable for rapid screening of maize germplasm in a breeding program. Plus, the secondary damage parameters, like entry holes or stalk breakage, are considered on a par with the primary damage parameters. Kumar and Asino (1993) suggested foliar damage, dead heart and stalk damage on maize by *C. partellus* to clearly distinguish the resistant and susceptible genotypes. Various workers have used different plant growth stages to screen maize for resistance to *C partellus.* Ampofo et al. (1986) used 4 week old plants to infest and screen maize for resistance to *C. partellus*. Kumar and Asino (1993) demonstrated that resistant and

Figure 4. Bazooka for the artificial infestation of maize plants by stem borers

susceptible genotypes were clearly distinguished when infested at 2 weeks after the germination of the plants. Resistance to *C. partellus* at the early whorl stage is desirable because economic losses have been reported to decline with the advance in the age of the plant (Sarup et al. 1977; Seshu Reddy and Sum 1992).

Based on the information generated above, many maize genotypes with resistance to *C. partellus* have been identified (Sarup et al. 197; Ampofo et al; 1986; Kumar 1991; Kumar and Saxena 1992; Kumar 1994a,b,c). The most notable sources of resistance to *C partellus* are Antigua Group 1, Population 590 (Multiple Borer Resistant, MBR) of CIMMYT, Population 390 (Multiple Insect Resistant Tropical, MIRT) of CIMMYT (Table 5), and several inbred lines from Mississippi and CIMMYT. Several lines with a high level of resistance to European Corn Borer, *Ostrinia nubilalis*, have been found susceptible to *C partellus* (Ampofo et al. 1986). Several land races and commercial maize hybrids from Kenya have also been found susceptible to *C. partellus* (Kumar 1994a). Little information is available on sources of resistance to second-generation *C. partellus*. Kumar (1992b) studied the larval establishment and damage by *C. partellus* on plants at anthesis. Severe yield losses can occur at anthesis because *C. partellus* attacks maize directly in the growing ear. Kumar and Asino (1994) and Kumar (1994c) identified a few sources of resistance to second-generation *C. partellus.*

Components of Resistance in Maize to *C. partellus*

Painter (1951) proposed three main categories of resistance in plants to insects:

- Preference subsequently referred to as non-preference (Painter 1958) and antixenosis (Kogan and Ortman 1978).
- Antibiosis affecting insects survival, development and egg production on the plants.
- Tolerance in plants involving repair and regeneration of their damaged tissues.

To establish the above three components of resistance in plants to insects, certain responses of the insects to the plants can be studied as explained by Saxena (1969, 1985) and are summarized in Table 6. The responses are:

- Orientation.
- Feeding.
- Metabolism of the ingested food.
- Development of larva.
- Egg production in the adults.
- Oviposition.
- Hatching.

Orientation, feeding and oviposition responses by the insects are involved in the non-preference type of mechanisms of resistance in plants which possess characteristics to inhibit these responses. The metabolic responses of the insect would involve antibiosis type of mechanisms of resistance in plants which will provide inadequate nutrients or metabolic inhibitors to cause failure of larval development , survival, egg production and hatching of the eggs.

Table 5. A comparison of infestation and damage (mean ± se) caused by *C. partellus* among maize cultivars from Kenya and CIMMYT (Mexico).

Maize cultivar	Source	No. of larvae recovered	Foliar damage ratings	% plants showing dead hearts	% stem length tunneled
Inbred A	Kenya	—	9 ± 0[a]	74 ± 10	—
Mp 704	Mississippi	4 ± 0.6	4 ± 0.5	11 ± 11	12 ± 0.9
EV SR BC 4/8429	CIMMYT[a]	12 ± 1.5	8 ± 0.3	5 ± 25	47 ± 3.5
EV SR BC 6/8430	CIMMYT[a]	8 ± 2.3	6 ± 0.4	10 ± 10	39 ± 1.5
EV SR RSF /8343	CIMMYT[a]	7 ± 1.3	7 ± 0.4	13 ± 13	42 ± 4.5
EV SR BC 6/8744	CIMMYT[a]	6 ± 1.0	8 ± 0.6	6 ± 6	41 ± 2.1
EV SR BC 5/8749	CIMMYT[a]	4 ± 0.8	7 ± 0.1	0	39 ± 2.5
Tuxpeño Sequia	CIMMYT[a]	7 ± 0.4	8 ± 1.1	47 ± 9	39 ± 3.5
La Posta Sequia	CIMMYT[a]	9 ± 2.0	7 ± 1.3	0	56 ± 5.5
Pool 16 Sequia	CIMMYT[a]	5 ± 0.9	6 ± 1.0	0	29 ± 1.0
Hybrid 622	Kenya	11 ± 0.6	8 ± 0.8	29 ± 11	54 ± 5.5
Pwani hybrid	Kenya	5 ± 0.8	7 ± 1.2	30 ± 30	48 ± 8.5
Hybrid 511	Kenya	8 ± 1.3	8 ± 0.2	18 ± 18	37 ± 2.5
MIRT[b] FAM. 1	CIMMYT[a]	4 ± 1.2	4 ± 0.2	0	19 ± 1.0
MIRT[b] FAM. 2	CIMMYT[a]	4 ± 0.1	4 ± 0.2	0	29 ± 1.5
MIRT[b] FAM. 18	CIMMYT[a]	8 ± 2.5	4 ± 0.6	0	32 ± 7.0
MIRT[b] FAM. 99	CIMMYT[a]	6 ± 2.0	4 ± 1.0	0	15 ± 1.5
MIRT[b] FAM. 136	CIMMYT[a]	4 ± 0.7	4 ± 0.7	0	16 ± 4.5
MIRT[b] FAM. 170	CIMMYT[a]	7 ± 2.2	4 ± 0.9	0	27 ± 3.0
F (df = 17.17)		4.9	4.4	3.06	11.5
LSD (P = 0.05)		3.2	1.8	37.60	11.5

[a] International Maize and Wheat Improvement Center.
[b] Multiple Insect Resistant Tropical.

Responses of Insects to Plants

Orientation

This insect response determines the establishment of the insect on the plant in two ways. Firstly, an insect may be

attracted to a plant or repelled from it because of a certain attractant or repellent, respectively. If the insect is attracted to a plant, the chances of its establishment on the plant would be enhanced. On the contrary, if the insect is repelled from the plant, the chances of its establishment on the plant would be reduced. The attraction/repulsion could be for feeding in the case of larvae or oviposition in the case of adults. The role of larval orientation in determining resistance/susceptibility of maize genotypes has not been studied, but *C. partellus* adults have been reported to be attracted equally by the resistant and susceptible genotypes for oviposition (Kumar and Saxena 1985a; Kumar 1994b). Secondly, *C. partellus* larvae emerging from the eggs laid on the leaves may continue to stay on the plant and reach the feeding sites in the leaf whorls, or may depart from the plant during their movements from the oviposition site (basal leaves) to the feeding site (leaf whorl) due to various morphological and biochemical factors. Kumar et al. (1993) compared four maize genotypes for larval orientation from oviposition to feeding sites (Table 7). The maize genotypes Mp704 and Poza Rica 7832 seem to possess characteristics which suppressed the movements of larvae from oviposition to the feeding sites.

Feeding response

After the arrival of *C. partellus* larvae in the leaf whorls, the establishment of its population on the plants would depend on larval feeding in the leaf whorls. Feeding responses of *C. partellus* on plants can be studied in the laboratory, as well as in the field, as described by Kumar et al. (1993) and Kumar and Saxena (1992). In the laboratory, the yellow green portions of the unfurled whorl leaves of 3 week old plants can be offered to neonates of *C. partellus* in glass vials (7.5 cm x 2.5 cm) filled to a depth of 2 cm with 2% agar gel. After 24-48 hours, the area eaten by the larvae on resistant and susceptible maize genotypes can be measured with an area meter or by a dotted paper sheet (Letra set International Ltd., UK). Using this technique, Kumar et al. (1993) demonstrated that *C. partellus* larvae fed less on the resistant genotypes (Mp704, V-37 and Poza Rica 7832) in comparison to the susceptible genotype (Inbred A) (Table 8). In the field, the resistant and susceptible genotypes can be grown and 2-3 weeks after plant emergence, the plants are infested with 20 neonates in the leaf whorls. After 24-48 hours, the feeding lesions on the plants are measured as described above. Kumar and Saxena (1992) reported significantly more feeding by *C. partellus* on the susceptible than the resistant genotypes.

Table 6. Mechanisms of resistance as related to the responses of insects or plants.

Categories or mechanisms of resistance (Painter 1951, 1958)	Responses of insects or plants involved		Differences between resistant (R) and susceptible (S) plants
Non-preference	Orientation of insects		
[= antixenosis,	Repulsion:	avoidance/departure from plants	R > S
Kogan and	Attraction:	arrival and stay on plants	R < S
Ortman (1978)]	Feeding:	inhibition	R > S
		stimulation	R < S
	Oviposition:	inhibition	R > S
		stimulation	R < S
Antibiosis	Metabolism of food ingested by insects:		
		nutrition	R < S
		metabolic disturbance	R > S
	Development in the larval stage*		R < S
	Survival and egg-production in the adult stage*		R < S
Tolerance	Repair, regeneration of damaged tissues of plants		R > S

* All failures of insects, survival, development and egg-production (fecundity) need not represent antibiosis; such failures caused by inadequate food-intake would correctly belong to the category non-preference for feeding.

Table 7. *Chilo partellus* larval arrest on different maize cultivars.

Cultivar	% First instars arrested in 72 h
Inbred A	39.3 ± 9.7a
Mp 704	21.7 ± 3.8b
V-37	38.7 ± 2.1a
Poza Rica 7832	20.0 ± 4.0b

Means ± SE. Means followed by the same letters are not significantly different ($P > 0.05$; Duncan's multiple range test).
One-way ANOVA of arcsin-transformed data ($F = 10.88$; df = 3.6; $P < 0.01$).

Table 8. *Chilo partellus* larval feeding responses to different maize cultivars, each offered alone.

Cultivar	Fresh wt (mg) of Leaf area (mm^2) eaten by 10 first-instars/ 24 h [a, c]	food ingested by fourth instars/ 48h [b,d]
Inbred A	20.2 ± 4a	1,227 ± 254a
V-37	2.3 ± 1b	652 ± 107a
Poza Rica 7832	3.3 ± 2b	766 ± 86a
Mp 704	6.0 ± 3b	1,149 ± 158a

Means ± SE. Means within a column followed by different letters are significantly different ($P < 0.05$).
[a] Disk of unfurled whorl leaf of 3-wk-old plant offered to larvae.
[b] A 6-cm-long basal internode of stem of 5-6-wk-old plant offered to larvae for feeding.
[c] One-way ANOVA ($F = 31.7$; df = 3, 9; $P < 0.01$).
[d] One-way ANOVA (F = 2.60; df = 3, 27; P > 0.05; LSD = 514.3).

Metabolism of the ingested food

The next step which determines the establishment of *C. partellus* population on the plant and its successful colonization is the efficient metabolism of the food ingested by the larvae. The experiments are conducted in the laboratory with excised leaves of the resistant and susceptible genotypes. Only the yellow-green portions of the unfurled whorl leaves (minus midrib) of 3 week old plants are used. The experiments are conducted in plastic vials (3 cm high by 4 cm diameter) filled to a depth of about 1 cm with 2% agar gel. The gel keeps the paper toweling placed beneath the leaf tissue moist and prevents the tissue from wilting. The leaf tissue in each vial is presented to 10 neonates of *C. partellus* in the form of a disk (2 cm diameter). The initial fresh weights of the larvae and leaf disk are measured on a Sartorius (R200D) balance (Sartorius GMBH, Goltingen, Germany). After 60 h, the larvae, the uneaten part of the leaf disk, and larval frass are collected, dried separately, and re-weighed. These measurements are taken on five replicates of 10 larvae each.

To determine the initial dry weight of food offered to the larvae, the fresh weights of 10 leaf disks (2 cm diameter) are measured separately for each cultivar. They are then dried at 60ºC for 24 h, and the mean weight per unit fresh weight is calculated and is used to calculate the initial dry weight of each leaf disk offered to the larvae for feeding. The fresh weight of 200 neonates in four replicates of 50 each is measured and dried without feeding.

Using this information the initial dry weight of 10 experimental larvae offered leaf disks is estimated. The quantity of food ingested by 10 larvae in each replicate, I, is calculated on a dry weight basis as:

$$\mathbf{F_I\ (FC_2/FC_1) - F_2}$$

Where F_I = initial fresh weight of food, F_2 = dry weight of uneaten food, FC_1 = fresh weight of control food, and FC_2 = dry weight of control food.

The weight gain of the insect, G, is calculated on a dry weight basis as:

$$\mathbf{W_2 - W_1\ (WC_2/WC_1)}$$

Where W_1 = initial weight of the insect before feeding W_2 = dry weight after feeding, and WC_1 = fresh weight of control insects.

The relative consumption rate (RCR), the amount of feeding relative to time and to the mean weight of larvae during the feeding period, is calculated as:

$$\mathbf{RCR = I/(T * W)}$$

Where I = dry weight of food ingested, T = duration of feeding period in days, and W = mean weight of larva during feeding period.

The relative growth rate (RGR) is calculated as:

$$\mathbf{RGR = G/(T * W)}$$

Where G = dry weight gained by larva, T = duration of feeding period in days, W = mean weight of larva during feeding period.

Utilization of food consumed is calculated by the methods of Waldbauer (1964) and Okech and Saxena (1990) using the data obtained on food intake described above.

Approximate digestibility (AD) is calculated as:

$$\mathbf{(I - E/I) * 100}$$

Where E = dry weight of frass produced.

The efficiency with which digested food is converted to body matter (ECD) is calculated as:

$$\mathbf{ECD = (G/I\text{-}E) * 100}$$

$$\mathbf{ECI = AD * ECD}$$

The only notable report which describes the metabolism of food ingested by *C partellus* on the resistant and susceptible genotypes is that of Kumar (1993a). According to this report, the dry weight of the food ingested by *C partellus* larvae on the resistant inbred Mp704 and the single cross hybrid Mp704 x Inbred A was lower than the susceptible check Inbred A (Table 9). The ECI and ECD on the resistant cultivars were also lower than the susceptible check. Thus the resistant inbred and the cross involving a resistant parent had a deleterious effects on the ingestion of the food and its subsequent utilization by the larvae. The larvae gained less weight on the resistant cultivars in comparison to the susceptible ones.

Survival, growth and development

This aspect can be studied in the screen house or field. Under field conditions, it is difficult to avoid natural infestation of the borers and the data gets confounded. Hence, experiments in the screen house can help avoid natural infestation of the stem borers. The plants of the resistant and susceptible genotypes are grown in the screen house in a replicated trial. The plants are infested at the 6-8 leaf stage with 20 larvae per plant. At 15-20 days after infestation, the percentage of larvae recovered from each genotype is recorded. On the basis of head capsule widths, the recovered larvae are then classified in their respective instars. A greater percentage of larvae advancing to older instars on the susceptible,

compared to the resistant genotype, would reflect the suitability of former and unsuitability of the latter for the development of the larvae. Using this technique, Kumar et al. (1993) reported that the percentage of larvae recovered from the resistant cultivars Mp704, V-37 and Poza Rica 7832 at 15 days after infestation was significantly lower than the susceptible genotype (Fig. 5). Of the larvae recovered from the susceptible genotype, most were in the fourth instar and a few had advanced to the fifth instar. On the resistant cultivars, the percentage of the larvae in fourth instar was significantly lower than the susceptible genotype (Fig. 5).

Similarly, several workers in Asia studied the survival and development of *C. partellus* in the laboratory (Sharma and Chatterji 1971, 1972; Lal and Pant 1980; Durbey and Sarup 1984; Sekhon and Sajjan 1987). According to these workers, survival, growth and development of *C. partellus* on Antigua Group 1 was lower than the susceptible check.

Survival, growth and development of *C. partellus* can also be studied in the laboratory by incorporating dry leaf powders of resistant and susceptible maize genotypes into the artificial diet. The maize lines are grown in a screenhouse. When the plants are 3 weeks old, the leaf whorls are harvested. After discarding the outer leaf, the whorls are trimmed to 20 cm, dried in an oven at 60°C for 24 h, and ground in an electric blender.

The standard artificial diet of *C. partellus* (Ochieng et al. 1985) is modified to study the effects of the dry leaf powders on growth of *C. partellus*. After preliminary experiments, it was found that there was no larval survival if two ingredients of the standard diet, sorghum leaf powder and bean powder, were removed and compensated with an equivalent amount of cellulose powder (Avicel, E. Merck, Germany). Hence, to this sorghum leaf and bean powder deficient diet, dry maize leaf powders of the maize cultivars are incorporated. The following test diet has been devised: 130 ml distilled water, 1.7 g brewer's yeast, 200 mg sorbic acid, 500 mg ascorbic acid, 300 mg methyl-p-hydroxybenzoate, 200 mg vitamin E, 83 ml distilled water, 2.12 g agar, and 32 g dry maize leaf powder. The artificial diet is dispensed into glass vials, (7.5 by 2.5 cm diameter) fitted with plastic lids with 40 mesh screen.

For each cultivar, eight glass vials filled with diet to a depth of 6 cm are prepared. On the following morning, each of the eight glass vials is infested

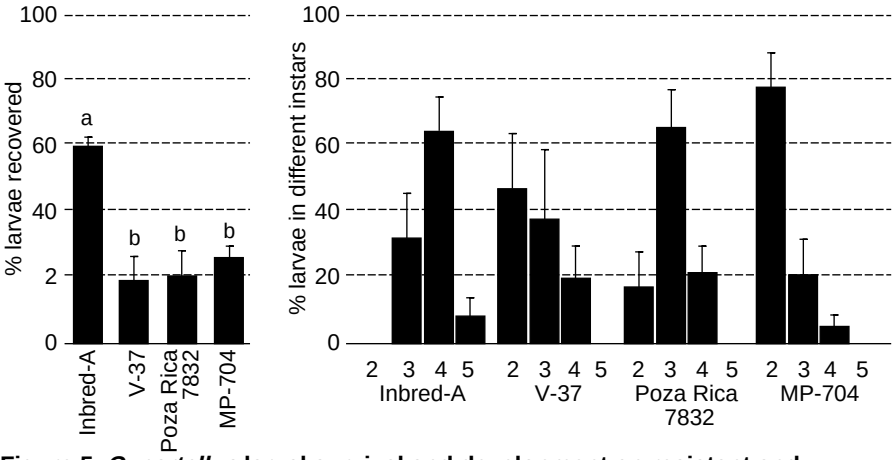

Figure 5. *C. partellus* larval survival and development on resistant and susceptible maize plants.

Table 9. The utilization of leaf tissue from two inbred maize cultivars and their reciprocal crosses by first-instar *C. partellus*.

Cultivar	I[a]	(RCR)[b]	AD[c]	ECD[d]	ECI[e]	G[f]	(RGR)[g]
'Inbred A'	5.2 ± 0.3a	(5.0[a])	78.2 ± 1.2a	9.2 ± 0.2b	7.1 ± 0.2b	0.40 ± 0.02a	(0.4a)
'Mp704'	2.5 ± 0.2b	(4.2[a])	89.4 ± 2.9a	1.5 ± 0.4c	1.4 ± 0.4c	0.03 ± 0.006c	(0.05c)
'Mp704' x 'Inbred A'	1.8 ± 0.2b	(2.5[b])	64.2 ± 6.0b	21.7 ± 6.1a	12.5 ± 2.4b	0.21 ± 0.02b	(0.27b)
'Inbred A' x 'Mp704'	0.6 ± 0.2c	(1.3[b])	53.2 ± 3.0b	5.7 ± 2.0c	3.1 ± 0.7c	0.02 ± 0.01c	(0.04c)

Mean ± SE (n = 4 replicates of 10 larvae). Means in a column followed by the same letter are not significantly different (P > 0.05) by LSD test.

[a] Dry weight of food ingested. (ANOVA test: F = 62.8; df = 3, 12; P <0.01) LSD = 0.75

[b] Relative consumption rate. (ANOVA test: F = 17.5; df = 3, 12; P <0.01) LSD = 1.18

[c] Approximate digestibility. (ANOVA test: F = 13.60; df = 3, 12; P <0.01) LSD = 13.24

[d] Efficiency of conversion of digested food. (ANOVA test: F = 3.68; df = 3, 12; P <0.05) LSD = 9.48

[e] Efficiency of conversion of ingested food. (ANOVA test: F = 3.60; df = 3, 12; P <0.05) LSD = 3.92

[f] Larval growth. (ANOVA test: F = 96.13; df = 3, 12; P <0.01) LSD = 0.043

[g] Larval growth rate. (ANOVA test: F = 97.92; df = 3, 12; P <0.001) LSD = 0.015

with 15 neonates of *C. partellus*. The glass vials are arranged in a completely randomized design in a room maintained at 27-29ºC, 40-50% RH and a photoperiod of 12:12(L:D)h. Each vial with 15 larvae is considered a replicate. After 20 days, the percentage of larvae recovered from each vial is recorded. The head capsules of the recovered larvae are measured at their greatest widths, with a stereomicroscope fitted with a calibrated eye piece micrometer. Larvae are measured at a magnification of 40X. The head capsule widths can be used to determine the instar of the larvae collected. Using this information, the average instar of *C. partellus* in each treatment is calculated. Weights of the surviving larvae are also measured. Using this technique (Kumar 1993a) demonstrated that when first instars of *C. partellus* were reared on the standard artificial diet, almost 95% of the larvae survived for 20 days after the infestation (Table 10). When dry maize leaf powders of different cultivars were incorporated into the sorghum leaf and bean powder-deficient diet, larval survival was equally high on the diets except for deficient diet + 'Inbred A' x 'Mp704' (Table 10). Of the surviving larvae on the diets containing leaf powders of the maize cultivars, the average instar was significantly lower than that on the standard diet (Table 10). Among the diets containing dry leaf powders of the maize cultivars, the average instar on the deficient diet containing 'Inbred-A' leaf powder was significantly higher than that on the diets containing leaf powders of 'Mp704'or the F_1 hybrids . The mean weight of the larvae reared on the diet containing the leaf powder of 'Inbred A' was significantly greater than that of those reared on the diet having 'Mp704' leaf powder . Larval weights on 'Mp704' x 'Inbred A' and 'Inbred A' x 'Mp704' were intermediate between those of the two parental lines. This technique can be used only to establish the mechanisms of resistance in maize to *C. partellus,* but can not replace the conventional screening techniques in the fields. The level of resistance in a genotype established with this technique may not conform with that in the field because of the absence of strong genotype x environment interactions.

Egg production in the adult and their viability

This aspect can be studied by rearing *C. partellus* neonates on the susceptible and resistant genotypes. Single pairs of adults emerging from the pupae reared on these genotypes are confined in the oviposition cages to determine the number of eggs laid by the female until it dies. Durbey and Sarup (1984) and Sharma and Chatterji (1971) reported that fewer eggs were laid by *C. partellus* females which were reared on the resistant Antigua Group 1 in comparison to the susceptible Basi Local. Sekhon and Sajjan (1987), on the other hand ,did not find any difference in the fecundity of *C. partellus* reared on these two genotypes.

Ovipositional responses

This aspect can be studied in the field by growing the resistant and susceptible genotypes in the field under natural infestation (Ampofo 1985; Kumar 1988b) or by growing and exposing the genotypes in the specially constructed cages to the ovipositing females (Kumar and Saxena 1985) Field tests by Ampofo (1985) revealed differences in *C. partellus* oviposition on the resistant and susceptible genotypes. Durbey and Sarup (1982) reported ovipositional non-preference for certain resistant genotypes. In field conditions, the differences observed between the resistant and susceptible genotypes may not necessarily be due to plant characteristics alone because certain non-plant characteristics have also been reported to influence *C. partellus* orientation and subsequent oviposition by the females (Kumar 1994b). In more detailed studies, Kumar and Saxena (1985a) compared ovipositional responses of *C. partellus* to different susceptible and resistant genotypes in the field or screen house in such controlled conditions that the differences in the responses were clearly shown to be caused by the plant characteristics and not influenced by the environment or other stimuli. These workers experimentally demonstrated that variation in the humidity stimuli in the vicinity of the plants was capable of influencing oviposition by *C. partellus*.

Table 10. Growth and development of *C. partellus* larvae on artificial diets containing dry maize leaf powders of two inbred cultivars and their reciprocal crosses.

Treatment	% survival	Instar	Wt (mg)
Base diet	95.1 ± 3.5a	4.9 ± 0.03a	59.0 ± 2.0a
SLBPDD[a] + 'Inbred A'	91.7 ± 2.7a	4.4 ± 0.08b	57.0 ± 1.0ab
SLBPDD + 'Mp 704'	86.6 ± 6.7ab	4.0 ± 0.06cd	34.0 ± 1.0d
SLBPDD + 'Mp 704' x 'Inbred A'	83.4 ± 5.2ab	4.1 ± 0.08c	54.0 ± 2.0b
SLBPDD + 'Inbred A' x 'Mp704'	79.3± 3.9b	3.8 ± 0.06d	49.0 ± 1.0c

Mean ± S.E. (n = 8 containers of 15 neonates) measured 20 d after inoculation.
Means in a column followed by the same letter are not significantly different. ($P > 0.05$).
ANOVA tests: % survival ($F = 5.4$; df = 4, 28; $P < 0.05$; LSD = 5.2), Instar ($F = 47.28$; df = 4, 28; $P < 0.01$; LSD = 0.19), Larval weight ($F = 50.49$; df = 4, 28; $P < 0.01$; LSD = 4.13).
[a] SLBPDD, sorghum-leaf and bean powder deficient diet.

The reduced number of eggs laid by the females on the resistant maize genotypes was due to contact-perceivable characters (surface waxes, trichomes, etc.) (Table 12) rather than due to distance-perceivable ones (hygro, visual and olfactory stimuli) (Table 11).

Tolerance in Maize to *C. partellus*

This aspect has not been studied adequately well in maize resistance to *C. partellus* although this is the most desirable type of resistance in plants. With tolerance as a mechanism of resistance to insects, the insects are relieved of the strong selection pressure evident in the case of strong antibiosis in plants to insects. The most notable reports are those of Ampofo (1986) and Kumar (1994c). Using regression of grain yield reduction on foliar damage ratings due to *C. partellus*, Ampofo (1986) demonstrated the presence of tolerance in resistant genotypes ICZ1-CM and ICZ2-CM (Fig. 6). Kumar (1994c) used regression of functional plant loss index (FPLI) on leaf feeding damage by *C. partellus* to elucidate the presence of tolerance of in maize genotypes, ER-29SVR, MBR8637 and Poza Rica 7832 (Fig. 7). There was a significant biomass loss by the plant with unit increase in the larval biomass on the susceptible Inbred A. Besides displaying a moderate degree of antibiosis against *C. partellus*, the plants of ER-29SVR, MBR8637 and Poza Rica 7832 lose very little plant biomass per unit larval weight gain in comparison to the susceptible Inbred A. The field tests revealed that *C. partellus* infestation caused a significant reduction in the grain yield of the susceptible cultivar, but not that of resistant cultivars (Kumar 1994c).

Role of Plant Characteristics in Determining Responses of Insects

After determining the components of resistance in plants to insects, the next step in understanding the mechanisms of resistance in plants to insects is to examine the role of plant characteristics in determining the responses of the

Table 11. Ovipositional responses of *C. partellus* to distance-perceivable characters of a susceptible and a resistant maize genotype.

Test material		Percentage of eggs laid (mean ± SE)*	
A	B	A	B
Inbred A	None	69 ± 4.5	31 ± 4.5
ICZI-CM	None	73 ± 8.0	27 ± 8.0
Inbred A	ICZI-CM	53 ± 4.0^{NS}	47 ± 4.0

* Data based on 40-50 females in 4-5 replicates of 10 each. Significantly different from 'B' at $P = 0.05$. NS = not significantly different from 'B' at $P = 0.05$.

Table 12. Oviposition responses of *C. partellus* to contact-perceivable characters of a susceptible and a resistant maize genotype.

Test material		Leaf portion*	Percentage of eggs laid (mean ± SE)	
A	B		A	B
Inbred A	Glass	TL	81 ± 7	19 ± 7
ICZI-CM	Glass	TL	73 ± 5	27 ± 5
Inbred A	ICZI-CM	BU	67 ± 8	33 ± 8
		TU	72 ± 9	28 ± 9
		BL	58 ± 11^{NS}	42 ± 11
		TL	74 ± 7	26 ± 7

NS = not significantly different from B.

* BU and TU = basal and terminal portions of the upper leaf surface respectively. BL and TL = basal and terminal portions of lower leaf surface respectively.

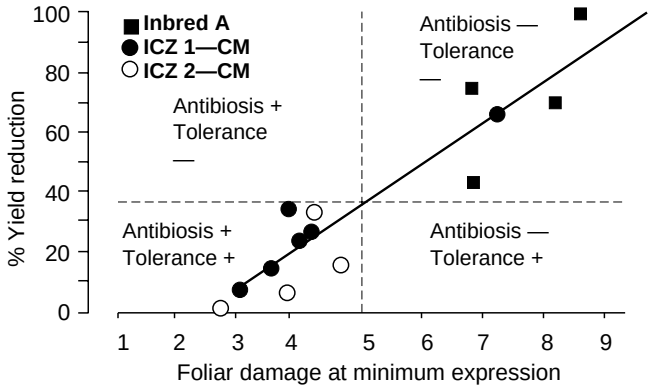

Figure 6. Components of resistance in maize to *Chilo partellus* taking % yield reduction and the foliar damage by the stem borers as the parameters.

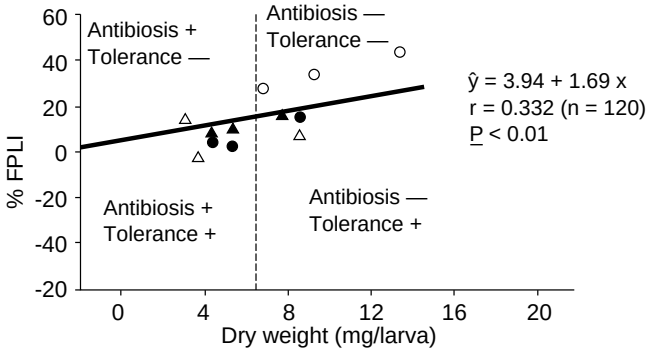

Figure 7. Components of resistance in maize to *Chilo partellus* taking larval weight as an indicator of antibiosis and FPLI as an indicator of tolerance. Empty circles represent Inbred A (susceptible); solid circles represent MBR 8637 (resistant); empty triangles represent ER-29SVR (resistant); and solid triangles represent Poza Rica 7832 (resistant).

insects. Excellent reviews are available on the role of plant characteristics in determining the resistance/susceptibility of the plants to insects (Beck 1965; Thorsteinson 1960; Norris and Kogan 1980; Pathak and Dale 1983). However, with reference to resistance in maize to *C. partellus,* the information is scattered.

Morphological characters

Trichomes on the upper leaf surfaces of the resistant genotypes have been reported to be related with low oviposition by *C. partellus* (Durbey and Sarup 1982; Ampofo 1985). The role of trichomes in inhibiting oviposition by *C. partellus* has been experimentally demonstrated by Kumar and Saxena (1985a). When the trichome studded leaf of the resistant genotype was compared with a wax paper, the females preferred to lay eggs on the wax papers. Even when the trichomes on one side of the midrib of a leaf were shaved off leaving the other side intact, oviposition by the moths on the hairless side was greater than the hairy side of the leaf.(Fig. 8). Kumar (1992a) developed an inbred line, ICZ-T which had trichomes on both the leaf surfaces and these trichomes were equally effective in inhibiting oviposition by the females.

Chemical characters

Plant chemicals influence the resistance/susceptibility of the plants in several ways: either by determining the orientation, feeding and oviposition behavior of the insects, or by determining the metabolism of insects serving as (a) toxins interfering with the metabolic processes of insects causing failure of the insect survival, development and egg production on the plant; or (b), nutrients promoting normal metabolic processes resulting in the insect's normal survival, development and egg production. Detailed studies conducted by Kumar and Saxena (1985a) and Kumar (1994b) showed that plant volatiles from the resistant and susceptible maize genotypes were equally effective in eliciting oviposition by *C. partellus*. However, distance-perceivable stimuli from the *C. partellus* infested plants were much more effective than those from the uninfested plants in eliciting oviposition by *C. partellus* (Kumar 1986; Kumar 1994b). After arrival on the plants, leaf surface waxes of the resistant genotype Mp704 were less effective than those of the susceptible genotype Inbred A (Fig.9) in eliciting oviposition by *C. partellus*.

To elucidate the basis of antibiosis in maize to *C. partellus*, Durbey and Sarup (1988) found that alcoholic extracts of the resistant genotype Mex. 17 adversely affected growth and development of *C. partellus*. Kumar (unpublished data) demonstrated that *C. partellus* larval development on the artificial diet containing hexane extracts of the resistant genotype Mp 704 was adversely affected (Table 13), but the diet containing methanolic extracts of the resistant genotype did not inhibit the growth of *C. partellus* (Table 14).

Inheritance of Resistance in Maize to *C. partellus*

Both additive and non-additive gene effects are important in the inheritance of resistance in maize to *C. partellus* (Pathak and Othieno 1990). Based on the studies of Kumar (1993), performance of F_1 hybrids between susceptible and resistant inbreds was satisfactory. The non-preference and antibiosis types of resistance operating

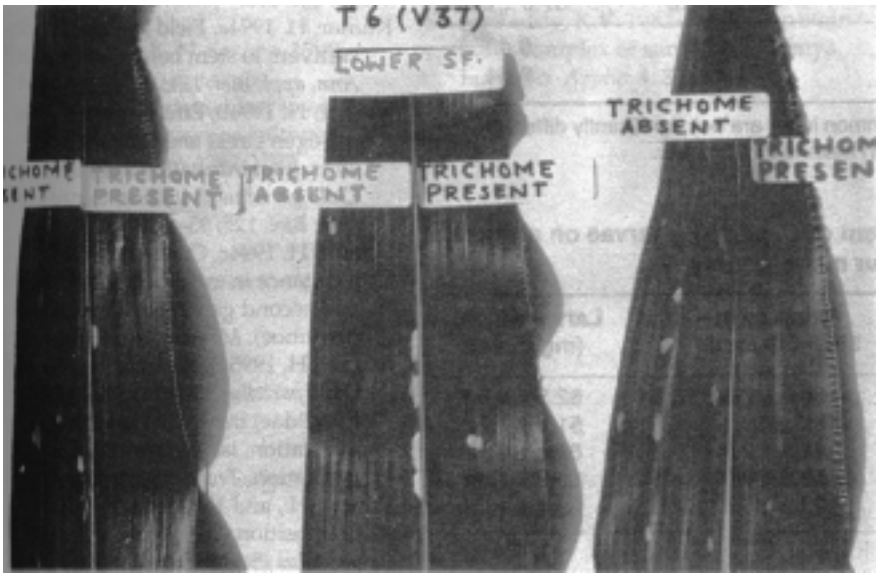

Figure 8. Evidence for the inhibition of *C. partellus* oviposition by trichomes on the maize leaves.

Figure 9. Role of chloroform extracts of a resistant (Mp704) and a susceptible (Inbred A) maize inbreds in determining oviposition by *C. partellus.*

within the resistant inbred Mp704 was clearly manifested in the single cross hybrids. The accumulation of the desirable additive alleles at loci in a breeding population through S_1 or S_2 recurrent selection is highly desirable (Pathak and Othieno 1990).

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Table 13. Survival, growth and development of *C. partellus* larvae on artificial diet containing hexane extracts of four maize genotypes.

	% larvae surviving	% larvae in 5th + 6th instar	Larval weight (mg/larva)
Diet + H	83.3 ± 6.0a	74.7 ± 4.6a	53.8 ± 3.9a
Diet + EA	64.2 ± 5.5b	47.8 ± 9.1b	58.9 ± 3.1a
Diet + EMp	92.5 ± 2.7a	24.7 ± 3.1c	53.8 ± 2.2a
Diet + EPR	64.2 ± 4.9b	27.0 ± 7.2bc	56.9 ± 3.5a
Diet ± EV37	5.9 ± 6.0b	46.5 ± 9.1b	57.6 ± 2.2a
F value (df = 4, 28)	8.48**	7.58**	0.49^{NS}
LSD at *P* = 0.05	14.66	21.21	9.54

Mean ± SE in a column, means followed by a common letter are not significantly different.
100 g of whorl leaves of 3 week old plant dipped in hexane for 72 hours.

Table 14. Survival, growth and development of *C. partellus* larvae on artificial diet containing methanolic extracts of four maize genotypes.

	% larvae surviving	% larvae in 5th + 6th instar	Larval weight (mg/larva)
Diet + M	74.2 ± 7.8	56.8 ± 7.5	62.5 ± 3.3b
Diet + EA	81.4 ± 4.8	45.6 ± 10.1	51.3 ± 1.2c
Diet + EMp	89.0 ± 4.9	41.4 ± 2.9	65.8 ± 1.7ab
Diet + EPR	80.8 ± 6.1	50.4 ± 6.0	69.7 ± 1.3a
Diet + EV37	82.5 ± 3.3	38.2 ± 5.5	63.4 ± 1.3b
F value (df = 4, 28)	0.92^{NS}	1.11^{NS}	13.04**
LSD at *P* = 0.05			5.50

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Phytochemical Basis for Multiple Borer Resistance in Maize

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Abstract

One of the major research emphasis's of the CIMMYT maize improvement program has been the development of germplasm with resistance to multiple generations and species of insects as well as resistance to disease pests. During the past decade, CIMMYT entomologists and breeders in collaboration with Cornell University have developed multiple borer resistant (MBR) populations for the major leaf feeding and stalk-boring pests of maize in temperate, subtropical and tropical areas. Identifying the phytochemical mechanisms of resistance employed by MBR genotypes would serve entomologists, breeders and biotechnologists in identifying new sources of resistance and locating major resistant genes within the genome. For MBR genotypes, the resistance mechanism appears to be nutritional in nature. Leaf tissue of MBR genotypes is tough, which may restrict feeding by early instar larvae. MBR genotypes also tend to have reduced nutritional value (lower nitrogen content), and elevated levels of fiber and cell wall phenolics which may account for the elevated leaf toughness. Cell wall phenolics can cross-link the hemicellulose of the cell wall by the action of peroxidase to produce diferulic acid. Approximately 80% of the variation in field leaf ratings for Ostrinia nubilalis *could be accounted for by protein, fiber and diferulic acid content in leaf tissue at the mid-whorl stage in plant development.*

Introduction

Lepidopteran stalk boring larvae cause economically significant losses to maize production throughout the world (Dicke and Guthrie 1988). Host plant resistance is an effective and environmentally safe means of control for these pests. A source population with multiple borer resistance (MBR) was developed by recombination and recurrent selection under infestation with southwestern corn borer (SWCB), *Diatraea grandiosella*, sugarcane borer (SCB), *D. saccharalis*, European corn borer (ECB), *Ostrinia nubilalis*, and fall armyworm (FAW), *Spodoptera frugiperda* (Mihm 1985; Benson 1986; Smith et al. 1989). Tropical maize resistance to lepidopteran pests appears to be polygenically controlled and involves primarily additive variation (Hinderliter 1983). Recent diallel experiments with MBR inbreds have determined that general combining ability is the most important source of variation among F_1s for leaf feeding resistance and yield (Thome et al. 1992, 1994).

Although the mechanism of MBR resistance has not been determined, other tropical maize resistant to both generations of ECB were found to have low levels of the conventional resistance factor 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA) (Sullivan et al. 1974). Silica and lignin content appear to be important in antibiosis-type resistance in tropical maize (Rojanaridpiched et al. 1984). Fiber and hemicellulose content of the whorl tissue was associated with SWCB resistance in Caribbean germplasm which again did not involve a DIMBOA based resistance (Hedin et al. 1984).

The other major group of secondary compounds in maize, the hydroxycinnamic acids, have received relatively little attention as plant defense chemicals. Biological activity of soluble hydroxycinnamic acids towards insects has been investigated (Dowd 1990); however, cell wall bound hydroxycinnamic acids have only been studied in relation to storage insect pests (Classen et al. 1990). Rumen digestion of grass leaf tissue has demonstrated reduced breakdown of cell wall materials which have elevated

levels of the hydroxycinnamic acids, p-coumaric and ferulic acid (Akin et al. 1990; Jung and Casler 1990). In addition, recent research has shown that cell wall bound phenolic acids can strengthen the cell wall through a peroxidase mediated dimerization that cross-links adjacent arabinoxylan molecules with diferulic acid (Fig. 1) (Bergvinson 1993). Another phenolic-based cross-linking mechanism involves UV-mediated dimerization of phenolic acids to produce compounds known as truxillic and truxinic acids which may also strengthen plant cell walls (Hartley et al. 1988; Hartley and Ford 1989). Fortification of structural components would render energy and nutrients less accessible and possibly less desirable to herbivorous insects (Scriber and Slansky 1981).

Some of the tools that are available for studying and identify host plant resistance mechanisms are depicted in Fig. 2. Having access to germplasm with a broad range in field resistance is essential for studying HPR. Having identified the tissues, timing and conditions when insect feeding is most severe, then plant sampling practices can then be established to obtain ecologically relevant data on HPR. The most prominent analytical tools of HPR work are gas chromatography-mass spectroscopy (GC-MS) and high performance liquid chromatography (HPLC). These tools enable the identification and quantification of a broad range of secondary metabolites in plants from which inferences can be made on the relative importance a particular secondary metabolite has on HPR. For soluble defense compounds like DIMBOA, synthetic standards can be made to test the antibiotic and antixenosis effects on the pest organism. For structural defense compounds, the only methods to substantiate their importance is through correlations using a broad range in germplasm or by recurrent selection for these structural components.

The primary objective of this study was to conduct a phytochemical screening of MBR varieties developed at CIMMYT and commercially available checks to elucidate the phytochemical components that best predict the observed field resistance, insect bioassay studies and leaf toughness of maize. Although not exhaustive, the list of parameters measured included resistance parameters studied to date such as DIMBOA, lignin, fiber, and protein. Bound phenolic acid-carbohydrate complexes and associated dimers received special attention. The

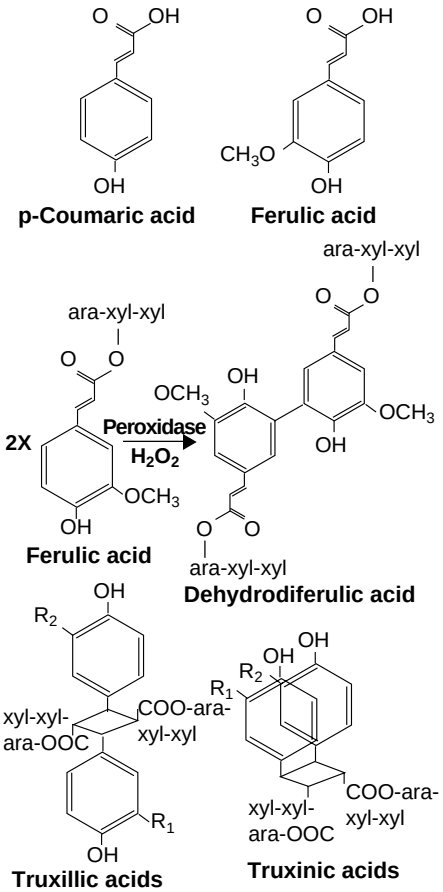

Figure 1. Structures of the major phenolic acids, ferulic and p-coumaric acids, and their associated dimers through the action of peroxidase (diferulic acid) or by the absorption of ultraviolet light (truxillic/truxinic acids).

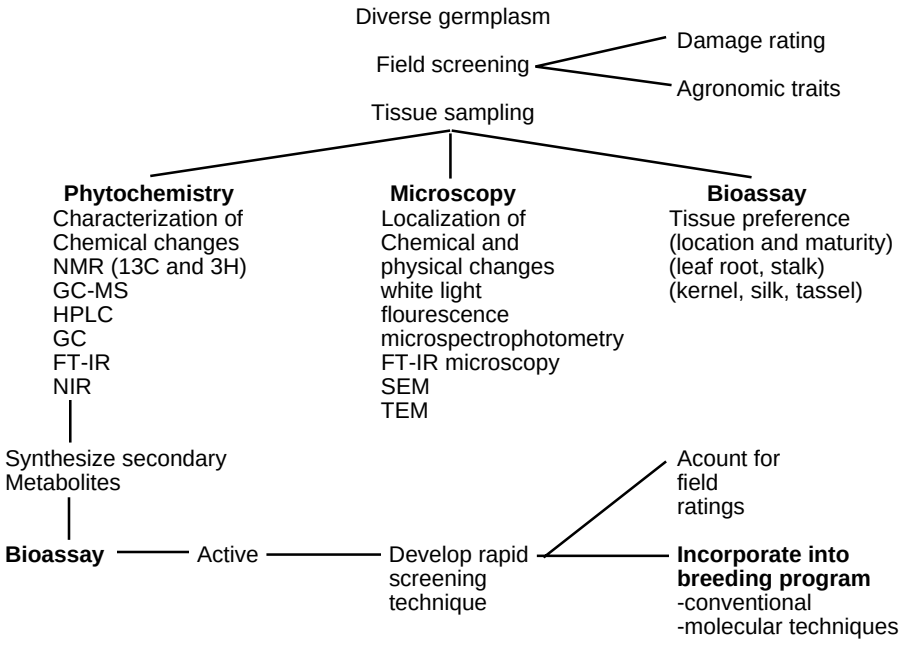

Figure 2. Schematic of the tools and protocol used for identifying host plant resistance mechanisms. Abbreviations: NMR, nuclear magnetic resonance spectroscopy; GC-MS, gas chromatography-mass spectroscopy; FT-IR, Fourier transform-infrared spectroscopy; NIR, near infrared spectroscopy; SEM, scanning electron microscopy; TEM, transmission electron microscopy.

second objective was to develop a simple phytochemical model that would account for the field resistance to the ECB.

Materials and Methods

Germplasm and screening

MBR varieties included Across 86590(IR), Mbita 86590 (Chilo), Poza Rica 86590 (SCB), Across 86590-2 (ECB), Tlaltizapán 85590 (SWCB), CML-135 x CML-139, and Ki-3 x Tx601. MBR adapted progeny included 6796-13, -49, and -48. Four commercial checks included Fontanelle 6230, Pioneer 3184, Dekalb 435 and Pickseed 4533. MBR varieties were developed at CIMMYT and provided by J.A. Mihm, northern adapted inbreds were developed at Agriculture Canada, Ottawa and provided by R.I. Hamilton. Planting occurred in mid-May of 1990 at the Plant Research Centre, Agriculture Canada, Ottawa, Ontario, Canada. The rows contained 30 plants spaced over 4.5 m with 0.9 m spacing between rows. The soil type was a sandy loam. Four replicates were planted in a complete randomized block design. Plants were infested using the larval infestation method developed by Mihm (1983) with ca. 80 larvae per plant. Three weeks after infestation plants were rated according to Guthrie's et al. (1960) 9 point scale (1=resistant, 9=very susceptible). Plants were dissected from late September through early October to count the number of larvae, number and length of tunnels, position of tunneling and estimated cross-section of pith excavated by larval feeding.

Sample collection

Since ECB females tend to oviposit on the undersurface of the upper whorl leaves, these tissues were collected for phytochemical analysis. At the mid-whorl stage the 13th leaf was harvested from uninfested plants by pulling the 10th leaf and above whorl out of the plant and unwrapping the leaves to expose the 13th leaf which was green along the exposed half and yellow along the basal half of the leaf length. The 13th leaf was used for insect bioassays in the laboratory. Leaf tissue for phytochemical analysis consisted of the green portion of leaves 10, 11, and 12. The midribs of these leaves were removed and the leaves were cut into paper bags. Immature tissue within the whorl was also cut, placed in paper bags, frozen on dry ice, and held at -20°C. Three plants per row were pooled as one phytochemical sample. Frozen tissue was thawed for 1 h to hydrolyze hydroxamic glucosides and then refrozen for lyophilization. Samples were milled on a UD cyclone mill (UD Corp., Bolder, CO) with a 1 mm screen. Milled samples were stored at -20° C until analyzed.

Bioassays

Two leaf sections (3x7 cm) were taken from the middle of the green and yellow portions of the 13th leaf. Tissue was stored in water to prevent desiccation and incorporated into insect bioassays within 6 h of harvest. A bioassay apparatus was used to measure the area consumed in mm^2 from a 1.2 cm diam. disk of the leaf tissue exposed to two third-instar larvae (for details see Bergvinson et al., these Proceedings). Mean area consumed was determined for 40 leaf disks for each genotype and tissue type.

Leaf toughness

Using the method reported in Bergvinson et al. (1994), force measurements were taken from the abaxial leaf surface between veins using a 1 mm diam., rounded probe. A standard Instron (model TM-M, Instron Corp., Canton, Mass) was equipped with a 2 Kg load cell (Lebow load cell, model 3108, Eaton Corp., Troy Mich.) and a 9 mm chuck to hold the probe. The probe was lowered at a rate of 1 cm/s until the probe had punctured the leaf (Fig. 3). The leaf was orientated with the undersurface facing up and held firmly in place using a stainless steel platform with threaded bolts to secure the leaf between the platform and a Plexiglas plate (Fig. 3). A typical force profile is shown in Figure 4, with the force required to puncture the lower epidermis being recorded. Leaf toughness is significantly correlated with field damage ratings for the MBR

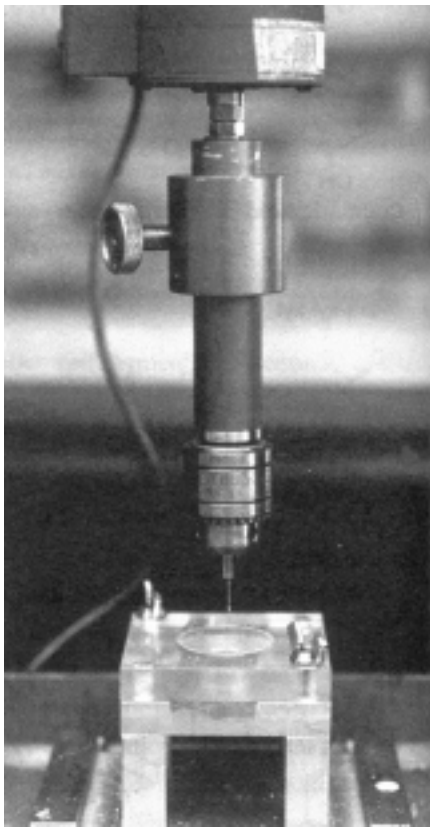

Figure 3. Instron apparatus for determining leaf toughness. Stainless steel stage has a 2 cm dia. hole through the plate's center. Leaf is placed on the stage and covered with a Plexiglas plate with a 2 cm dia. hole through its center. Leaf is pulled taut and Plexiglas plate is tighly secured against stage by wing-nuts. Drill chuck is attached to a 2 Kg load cell.

hybrids ($r = -0.82$, $P < 0.001$). For this study, 20 ear leaves from each genotype were harvested at flowering for toughness measurements.

Protein determinations

Protein content was estimated by an automatic micro-Kjeldahl nitrogen analyzer (Tecator model 1030, Höganäs, Sweden) on 0.3 g samples using the conversion factor 6.25 to estimate protein from nitrogen (McKenzie and Wallace, 1954). One measurement from each of 3 replicates were taken for both mature and immature tissue for each genotype.

Phytochemical analysis

Soluble phenolic conjugates and hydroxamic acids were extracted from a 0.5 g sample of dry leaf tissue. Samples were extracted for 20 s in 70% methanol (4 x 20 mL) and mixed with a polytron mixer (Brinkmann model TC-1200, Westbury, NY). After centrifugation at 500 g for 10 min. the supernatants were pooled, methanol was removed by rotary evaporator (35ºC), and the pH lowered to 2.0 using 1N HCl. The pH must be lowered to enable phenolic and hydroxamic acids to move from a water phase into ethyl acetate. The water fraction was extracted with ethyl acetate (4 x 50 mL) (BDH, Omni-Solv grade). Ethyl acetate fractions were pooled and dried by rotary evaporator and stored at -20ºC until HPLC analysis.

After extraction, the pellet that remained was washed in a Büchner funnel with 30 mL each of water, methanol and ethyl acetate to remove chlorophyll and provide a crude cell wall preparation. Cell wall samples were dried in a desiccator for four days. This preparation was weighed and the weight loss was used as the gravimetric measure of soluble metabolites. Cell wall preparations were shaken in 20 mL of 2N NaOH for 4 h under N_2 and wrapped in foil to hydrolyze phenolic ester linked to hemicellulose. Nitrogen was required to prevent oxidation of phenolics and a foil wrapping was required to minimize photoisomerization of phenolic acids. Samples were neutralized with 6N HCl and the pH lowered to 2.0. After centrifugation the supernatant was extracted with ethyl acetate (3 x 50 mL). The pellet was resuspended in water and centrifuged twice with both fractions pooled and extracted with ethyl acetate (3 x 50 mL). Ethyl acetate fractions were pooled and dried by rotary evaporator and stored at -20°C until HPLC analysis. The pellet that remained after extraction was dried and weighed to provide an estimate of fiber content.

HPLC analysis

All analyses were performed with a Perkin-Elmer system consisting of an LC 480 diode scan array detector and a Perkin-Elmer LC250 binary pump fitted with 10 µL injection loop. Separations were achieved using a C18 ODS reverse phase column (250 x 4.6 mm, 5 µm particle size, Beckman, Fullerton, CA).

Soluble extracts were suspended in 4 mL of 50% methanol and centrifuged at 500 g for 5 min. The supernatant was filtered and injected onto the column. The solvent system was comprised of methanol (A) and 10 mM H_2PO_4, pH 2.4 (B) at a flow rate of 1.5 mL/min and a gradient as follows: 25 to 55% A in 15 min, 55 to 80% A in 5 min, 80 to 100% A in 2 min, 100% A for 8 min, 100 to 25% A in 2 min and 25% A for 3 min. DIMBOA (Rt = 10.5 min) and 6-methoxybenzoxazolinone (MBOA) (Rt = 13.6 min) standards were synthetically prepared according to Atkinson et al. (1991). Peak identity was confirmed by on-line UV spectra and spiking of extracts with authentic standards.

Cell wall bound phenolic acids were suspended in 1 mL of methanol, diluted 10 fold in methanol, filtered and injected onto the column. The solvent system was the same as above except the starting mixture was 15% methanol. Standards of E-p-coumaric (Rt = 15.2 min) and E-ferulic acid (Rt = 15.6 min) were purchased from Sigma. A typical chromatogram from a cell wall extraction of maize leaf tissue is illustrated in Figure 5 as well as the

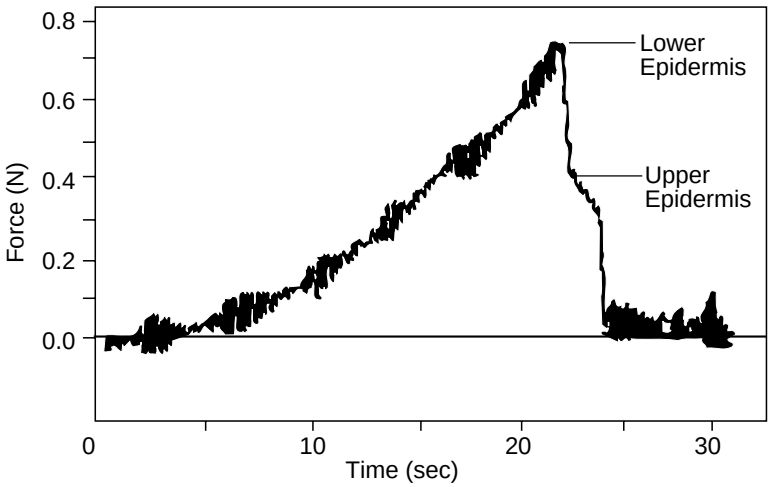

Figure 4. Computer plot of the force profile for a mature maize leaf. Force recorded in newtons (N).

characteristic absorption spectra for the phenolic dimers that cross-link the cell wall carbohydrates.

Lignin determinations

A modified acetyl bromide procedure outlined by Iiyama and Wallis (1990) was used. After base hydrolysis, the fiber pellet that remained was dried in a dessiccator and 50 mg was used for lignin analysis. Tissue was digested with 25% acetyl bromide and 4% perchloric acid in acetic acid at 70°C for 30 min. After digestion the samples were cooled on ice and transferred to a volumetric flask containing 10 mL of 2 M sodium hydroxide and 12 mL of acetic acid and the volume brought to 50 mL using distilled water. The absorption at 280 nm was taken and the value of 20.0 g/L/cm was used for lignin calculations.

Statistical analysis

All statistical analyses were performed on SAS V. 6.03 (SAS 1988). Leaf rating data, bioassay consumption, and leaf toughness were transformed by ln (x+1) to satisfy the assumptions of the general linear model. Forward regressions were done using the forward option in PROC REG.

Results and Discussion

The mean range of leaf parameters associated with resistance for the MBR genotypes tested are shown in Table 1. Leaf feeding damage after artificial infestation ranged from a low of 2 to a high of 6, with all plants showing signs of feeding. Leaf bioassay consumption ranged from 14-78 mm^2 but was too variable despite the large number of replicates. Attempts were made to use neonate larvae for the bioassay but because the ECB is not voracious, the variability for a given genotype was excessive. Immature leaf tissue was almost entirely consumed within the 48 h bioassay and was not used for further analysis. Although the spread in leaf toughness was not large (0.59 to 0.89 N), the standard error of the mean was low (LSD0.05= 0.083). Leaf feeding damage and bioassay consumption of green tissue by ECB larvae were both negatively correlated (r=-0.58* and -0.81**, respectively) with leaf toughness. Likewise, the number of larvae, number of stalk tunnels, length of tunneling and cross-section of pith excavated were negatively correlated with leaf toughness (data not shown). These correlations indicate a possible reduction in the capacity of larvae to establish on genotypes that have tougher leaf tissue. Neonate mortality often exceeds 80% for the first two days post-eclosion (Ross and Ostlie 1990). One mortality factor is desiccation (Lee 1988), whereby larvae that cannot penetrate leaf tissue shortly after eclosion may desiccate. Although later instars have little difficulty penetrating mature leaf tissue as observed in leaf bioassays, neonates may not have the mandibular strength to penetrate tougher leaves. This incapacity may account for their migration into the whorl of the plant. This reasoning has been used to explain the feeding behavior of the SWCB (Hedin et al. 1984).

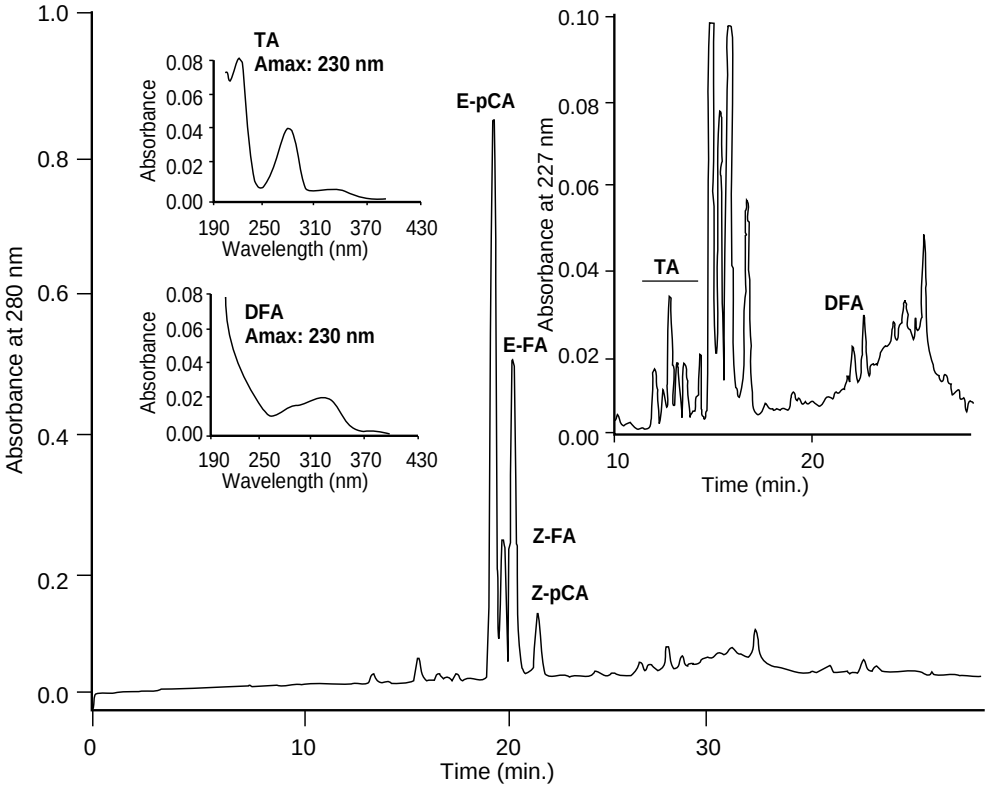

Figure 5. High performance liquid chromatography (HPLC) run of a cell wall extraction from mature maize leaf tissue. Abreviations: p-CA, p-coumaric acid; FA, ferulic acid; DFA, diferulic acid; TX, truxillic and truxinic acids.

Plant nitrogen is a major determinant of insect growth and development, with low nitrogen possibly serving as a plant resistance strategy (Scriber and Slansky 1981). Leaf protein content correlated positively with number of larvae (r=0.55*), length of

tunneling (r=0.56*) and with cross-sectional consumption of pith (r=0.53*) (data not shown). These observations suggest that more resistant genotypes with lower leaf-protein content may not provide sufficient accessible protein to facilitate larval development beyond early instars. This hypothesis is supported by field observations of differential size and biomass of SWCB larvae grown on resistant (MBR) and susceptible plants (Davis et al. 1988).

Mean concentration of major phytochemicals in maize leaf tissue is also reported in Table 1. Cell wall constituents included estimated fiber content and, hemicellulose bound p-coumaric, ferulic, diferulic and truxillic acids. With the exception of the light activated truxillic acids, cell wall phenolics occurred at higher concentrations in immature whorl tissue. Weight of soluble components was greatest for the immature leaf tissue, as were the levels of soluble secondary metabolites such as DIMBOA and the glycosides of phenolic acids (Table 1).

Table 1. Means for biochemical and physical resistance factors in mature and immature leaf tissue of maize harvested at the midwhorl stage, 1990.

	Leaf	Bio-	Leaf	Protein	mg/g dry wt.[†]							μg/g dry wt.	
Genotype	Rating	assay[†]	Force[†]	(%)	PCA	FA	Tx	DFA	Soluble	Fiber	Hx	sPCA	sFA
Mature Leaf													
Across													
86590(IR)	2.46	20	0.83	15.59	2.65	2.8	0.85	0.42	300	320	0.89	36	67
Mbita													
86590 (Chilo)	2.56	22	-	16.98	1.81	2.46	0.97	0.35	240	360	1.13	25	17
Poza Rica													
86590 (SCB)	2.14	29	0.82	15.89	2.40	2.52	0.73	0.58	340	300	1.45	36	47
Across													
86590-2 (ECB)	2.12	19	0.83	16.07	2.00	2.81	1.04	0.60	300	280	1.17	41	52
Tlaltizapán													
85590 (SWCB)	2.22	27	0.79	15.65	2.14	2.68	1.13	0.58	300	300	0.95	73	80
CML135x													
CML139	2.22	14	0.89	16.11	2.53	3.16	1.37	0.40	320	300	0.39	30	37
Ki3xTx601	3.61	20	0.68	16.47	2.07	2.14	0.73	0.41	340	260	1.2	40	15
Pioneer 3184	3.9	27	0.77	15.39	2.57	2.62	1.01	0.40	280	300	1.48	115	64
Fontanelle 6230	5.71	40	0.72	18.64	1.31	1.25	0.51	0.20	320	280	.23	136	100
6796-48	5.11	70	0.64	18.37	1.76	2.48	1.52	0.18	280	300	0.65	80	92
6796-49	5.36	78	0.65	19.17	1.73	2.41	0.89	0.23	300	300	1.06	31	122
6796-13	2.41	44	0.74	15.61	1.62	1.90	0.54	0.25	380	260	0.73	25	32
Dekalb 435	3.47	41	0.67	16.28	1.78	1.65	1.20	0.28	320	300	1.29	190	139
Pickseed 4533	4.11	62	0.59	17.60	1.63	2.45	1.26	0.30	300	300	2.12	140	173
Immature Leaf													
Across													
86590(IR)	2.46	20	18.54	0.83	4.07	4.44	0	0.49	440	200	1.06	53	61
Mbita													
86590 (Chilo)	2.56	21	18.79	-	3.37	4.17	0.01	0.64	500	160	1.82	70	57
Poza Rica													
86590 (SCB)	2.14	29	18.28	0.82	2.86	3.24	0.01	0.42	500	200	2.36	109	28
Across													
86590-2 (ECB)	2.12	19	12.38	0.83	4.73	6.93	0.05	0.49	340	240	1.54	35	68
Tlaltizapán													
85590 (SWCB)	2.22	27	12.84	0.79	5.76	6.97	0.08	0.57	340	200	1.66	60	35
CML135x													
CML139	2.22	13	12.68	0.89	5.59	6.45	0.01	.039	380	220	0.87	56	53
Ki3xTx601	3.61	20	14.01	0.68	3.43	4.27	0	0.28	560	160	0.88	53	20
Pioneer 3184	3.90	27	11.14	0.77	5.95	7.49	0.08	0.58	380	220	0.94	453	50
Fontanelle 6230	5.71	39	15.27	0.72	3.82	4.24	0	0.51	400	220	1.65	653	623
6796-48	5.11	70	19.42	0.64	2.48	3.7	0.02	0.08	500	160	1.62	30	40
6796-49	5.36	78	21.72	0.65	2.42	3.58	0	0.20	480	180	1.75	31	46
6796-13	2.41	44	16.27	0.74	2.49	2.98	0	0.21	500	180	1.83	32	41
Dekalb 435	3.47	41	15.46	0.67	3.10	3.76	0.13	0.35	460	180	4.90	79	117
Pickseed 4533	4.11	62	17.02	0.59	3.68	5.49	0.02	0.26	460	180	3.07	345	153

[†] Leaf rating is Guthrie's (1960) 1-9 scale, bioassay is mm2 tissue consumed, laf toughness of mature ear leaf at tasseling, PCA=p-coumaric acid, FA=ferulic acid, Tx=total cyclobutane dimers, DFA=dehydrodiferulic acid, soluble is gravimetric determination of soluble metabolites on a dry weight basis, fiber is estimated dtergent fiber, Hx=DIMBOA equivalents, sPCA and SFA are soluble conjugates of PCA and FA.

Soluble phytochemicals such as DIMBOA, ferulic and p-coumaric acid conjugates and flavonoids were positively correlated with leaf feeding and negatively correlated with leaf toughness, with the stronger correlations being observed for mature leaf tissue (Table 2). One possible explanation for this is the possibility that soluble secondary metabolites are acting as host recognition factors and as such are phagostimulants. Semipurified extracts of ferulic and p-coumaric acid glycosides from Mbita 86590 (Chilo) acted as phagostimulants at ecologically relevant dosages (10 µg/cm^2) and became phytotoxic and antifeedants at 100 µg/cm^2 (Bergvinson 1993). In addition to the phenolic glycosides, DIMBOA can increase consumption by inhibiting digestive proteases in the insect, thus requiring greater consumption of leaf tissue to assimilate sufficient nitrogen for larval development (Houseman et al. 1992). All these factors probably contribute to the higher consumption observed for genotypes with higher levels of soluble components.

Cell wall weight and cell-wall-bound phenolic acids in mature leaf tissue were negatively correlated with field leaf damage ratings and bioassay feeding and positively correlated with leaf toughness (Table 2). Maize-grain resistance to storage pests has been previously correlated with cell wall ferulic acid levels and kernel toughness (Classen et al. 1990). Cell-wall-bound p-coumaric acid (PCA) showed stronger correlations with the dependent variables than ferulic acid (FA). This may be attributed to PCA being more prevalent in secondary cell wall tissue and its prominent role in lignin linkage to polysaccharides (Goto et al. 1991; Lam et al. 1990). Such lignin linkages likely contribute to cell wall fortification and tissue toughness.

The most consistent relationship was observed between DFA and variables of insect resistance with $|r| > 0.66$ for mature tissue and $|r| > 0.42$ for immature tissue (Table 2). Diferulic acid, like the cyclobutane dimers, can cross-link cell wall carbohydrates and increase the mechanical strength of the cell wall (Ishii 1991; Fry 1986; Markwalder and Neukom 1976). This is evident in the positive correlation between DFA content and leaf toughness (r=0.68**, Table 2). Perhaps because cyclobutane dimer (Tx) production is largely under environmental control a poor correlation was observed for both leaf toughness and leaf damage rating for this photoactivated dimer (Table 2). On the other hand, diferulic acid is produced by a cell-wall-bound peroxidase which is under genetic control and could be manipulated in the future to increase the production of phenolic dimers and cell wall toughness.

Forward regressions between biochemical parameters as independent variables and plant resistance parameters as dependent variables are shown in Table 3. All regression models exceeded an R^2 value of 0.7 with only three independent variables in the models. The most common independent variables within these models include protein content (PRO), fiber content (CW) and DFA content. Incorporating protein, fiber and DFA content into a fixed regression model

Table 2. Correlations of biochemical parameters with plant damage parameters for 13 maize genotypes, 1990.

Tissue Type	Independent Variable	Field Leaf Ratings†	Bioassay Feeding†	Leaf Toughness†
Mature	Protein	0.82 ***	0.67 **	-0.65 **
	Wt. Solubles	-0.13	0.08	0.06
	DIMBOA	0.24	0.27	-0.45
	pCA (sol.)	0.39	0.37	-0.51
	FA (sol.)	0.63 *	0.69	-0.64 *
	Flavonoids	0.16	0.31	-0.45
	Fiber	-0.14	-0.13	0.12
	pCA (CW)	-0.55 *	-0.65	0.69 **
	FA (CW)	-0.52 *	-0.44	0.51
	Tx	0	0.08	-0.12
	DFA	-0.76 ***	-0.66	0.68 **
	Lignin	0.06	-0.07	0.16
Immature	Protein	0.32	-	-0.46
	Wt. Solubles	0.22	-	-0.54
	DIMBOA	0.11	-	-0.47
	pCA (sol.)	0.54 *	-	-0.19
	FA (sol.)	0.51	-	-0.14
	Flavonoids	0.31	-	-0.22
	Fiber	-0.36	-	0.83 ***
	pCA (CW)	-0.34	-	0.59 **
	FA (CW)	-0.25	-	0.42
	Tx	-0.14	-	0.05
	DFA	-0.42	-	0.66 **

*,**,*** P < 0.05, P < 0.01, P < 0.001.

† Field rating, bioassay feeding and leaf toughness were transformed by ln(x +1) prior to statistical analysis.

for each tissue type accounts for a reasonable amount of the variation, with the more consistent and more significant models being observed for mature leaf tissue (Table 4). Although not providing direct evidence for the mechanism of host plant resistance employed by MBR varieties, it is apparent that much of the variability of field leaf ratings and leaf toughness can be accounted for by these three parameters. These regressions support the hypothesis that MBR varieties employ a nutritional resistance mechanism whereby lower protein content acts in concert with increased cell wall mechanical strength, manifested through higher fiber content and higher levels of cell wall phenolics, to reduce nutrient availability to early instar larvae. This model is consistent with earlier reports of FAW showing reduced digestion of bermudagrass with high cell wall content (Quisenberry and Wilson 1985) and may explain the reduced growth rate of SWCB larvae feeding on MBR cultivars compared to those feeding on susceptible cultivars (Davis et al. 1988).

Inheritance of multiple borer resistance appears to be polygenically controlled, and involves primarily additive variation (Smith et al. 1989). This proposed inheritance is consistent with our proposed mechanism of resistance which involves three polygenic components, namely protein, fiber and cell wall phenolic acid content. A fourth component of this resistance model is the peroxidase-mediated production of DFA which likely involves only a single gene product. The main advantages of polygenic resistance are the reduced likelihood of resistant pest populations developing and an effective resistance over a broader spectrum of pest organisms. To date, MBR resistance has not broken down and some MBR germplasm is effective against several borers belonging to different genera.

Table 3. Forward multiple regressions of biochemical parameters with resistance parameters for resistance to European corn borer in 13 maize genotypes, 1990.

Tissue Type	Dependent Variable	Regression Equation†	r^2
Green	Leaf Rating	LLR = -0.621 + 0.129(PRO) - 0.541(DFA) + 0.0015(pCA sol.)	0.78**
	Bioassay	LBA = 0.605 + 0.223(PRO) - 0.458(FA) + 0.0043(FA sol.)	0.79**
	Leaf Toughness	LLT = 0.408 + 0.124(DFA) + 0.061(pCA) - 0.0004(FA sol.)	0.78**
Yellow	Leaf Rating	LLR = 2.236 - 0.0033(CW) - 0.850(DFA) + 0.0011(pCA sol.)	0.71**
	Leaf Toughness	LLT = -0.167 + 0.0029(CW) + 0.0419(SOL) - 0.0001(pCA sol.)	0.89**

*,**,*** P < 0.05, P < 0.01, P < 0.001.
† Field rating, bioassay feeding and leaf toughness were transformed by ln(x +1) prior to statistical analysis.

Table 4. Multiple regressions of protein, fiber and dehydroxydiferulic acid levels with plant resistance parameters for 13 maize genotypes, 1990.

Tissue Type	Dependent Variable	Regression Equation†	r^2
Green	Leaf Rating	LLR = 0.108 +0.136(PRO) - 0.0023(CW) - 0.683(DFA)	0.78**
	Bioassay	LBA = 1.794 + 0.188(PRO) - 0.0032(CW) - 1.30(DFA)	0.58*
	Leaf Toughness	LLT = 0.664 - 0.015 + 0.0003(CW) + 0.146(DFA)	0.54
Yellow	Leaf Rating	LLR = 1.869 + 0.0075(PRO) - 0.0018(CW) - 0.512(DFA)	0.22
	Leaf Toughness	LLT = 0.221 - 0.00038(PRO) + 0.00179(CW) - 0.016(DFA)	0.69**

*,**,*** P < 0.05, P < 0.01, P < 0.001.
† Field rating, bioassay feeding and leaf toughness were transformed by ln(x +1) prior to statistical analysis.

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Mechanisms of Resistance in Maize Grain to the Maize Weevil and the Larger Grain Borer

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Abstract

The mechanism of resistance in maize to the stored product insects such as the maize weevil (MW), Sitophilus zeamais *Motsch and the larger grain borer (LGB),* Prostephanus truncatus *Horn has been investigated in relation to secondary chemistry and other biochemical and physical characteristics of maize genotypes. Performance parameters of weevils (number of eggs laid, number of progeny, Dobie index, grain consumption) were negatively and significantly correlated (r = -0.8, P = 0.05) to the most abundant phenolic of grain, E-ferulic acid. With* P. truncatus*, the weight loss of grain also showed a negative correlation with E-ferulic acid while percent damage of kernels by insects was negatively correlated to p-coumaric acid. These phenolic acids were found in highest concentration in the pericarp and cell walls of the endosperm by fluorescence microscopy. Phenolic acid content was also found to correlate strongly with hardness of the grain, which may be related to the mechanical contributions of phenolic dimers to cereal cell wall strength. In the aleurone layer phenolic acid amines have been detected that have toxic effects on insects.*

Mechanisms of Resistance to *Sitophilus zeamais*

A decade ago, while working with Maya farmers of Belize, we noted the substantial resistance of traditionally used landraces of maize as well as some maize varieties released by the International Maize and Wheat Improvement Center (CIMMYT) to the maize weevil, *Sitophilus zeamais* as compared to introduced commercial hybrids (Fortier et al. 1982). A remarkable feature of the resistant maize was the intense fluorescence of the kernel pericarp as observed by fluorescence microscopy (Serratos et al. 1987). This suggested the role of hydroxycinnamic acids (phenolics) which are fluorescent and highly concentrated in the pericarp, as factors in resistance, in addition to previously described nutritional and mechanical factors (Fig. 1). Subsequently, a study of 15 CIMMYT pools showed that developmental parameters of weevils (number of eggs laid, number of progeny, Dobie index, grain consumption) in standardized tests were negatively and significantly correlated (r > -0.8, P = 0.05) to the E ferulic acid content of grain varieties (Classen et al. 1990).

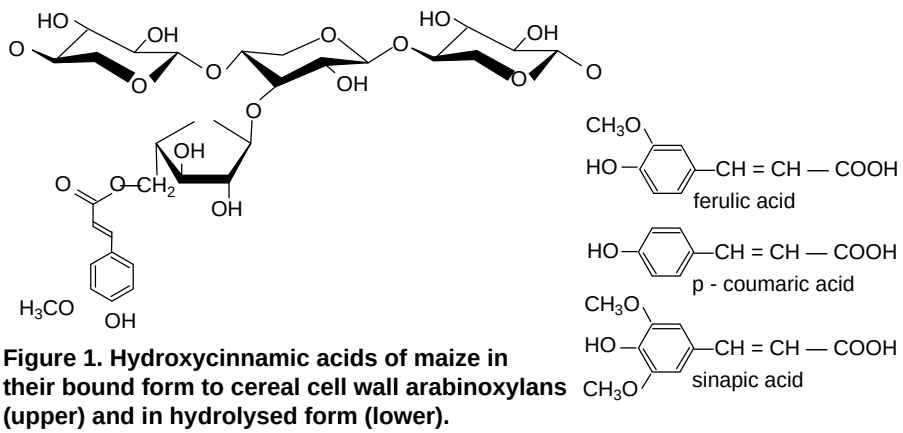

Figure 1. Hydroxycinnamic acids of maize in their bound form to cereal cell wall arabinoxylans (upper) and in hydrolysed form (lower).

Protein content and kernel hardness of varieties were also negatively correlated with susceptibility in this (Table 1) and other studies by our group. These have been reported as resistance factors by other authors. In a subsequent study (Arnason et al. 1993) of 31 quality protein maize genotypes from CIMMYT with approximately twice the lysine and tryptophan content of normal maize, we found no indication that these genotypes were any more susceptible on average than backcrossed material expressing normal protein in the endosperm (Table 2). We also examined a group of 30 Mexican landraces in an attempt to define sources of resistance for future studies. The ancient indigenous landraces were the group showing least susceptibility to weevils (Arnason et al. 1994).

Our work on the role of phenolics in resistance has recently been reviewed (Arnason et al. 1992). In particular it has been demonstrated that phenolic fluorescence (Sen et al. 1991) can be used as a rapid indicator for resistance and may be useful for breeders who wish to make a biochemical pre-screening of material for resistance. In addition, progress has been made on defining the inheritance of resistance parameters and phenolics in a generation means analysis (Serratos et al. 1993).

Recent work has shown that the phenolics are probably important in resistance in two ways: through mechanical resistance and antibiosis. The major hydroxycinnamic acids of maize kernels are ferulic and *p*-coumaric acid, which are not found as free acids but are esterified to the cereal cell wall hemicelluloses in compounds such as feruloyl and *p*-coumaroyl arabinoxylans (Fig. 2). These compounds can be cross-linked by extracellular peroxidases forming a mechanical cross-link in the cereal cell wall. Resistant genotypes have higher concentrations of these diferulic acids than susceptible materials (Arnason et al. 1994). A second bound form of phenolic acids has recently been localized in the aleurone layer of cell walls. Our preliminary results suggest that these phenolic amides, such as diferuloyl and dicoumaroyl putrescine may be antibiosis factors to *S. zeamais.* Together these phenolic acid conjugates can be detected by new fluorescence imaging techniques which clearly show the phenolic barrier to insects in the outer tissues (Fig.3)

Table 1. Pearson correlation coefficients (P=0.05) of grain parameters with maize weevil development parameters for 10 genotypes of maize.

Grain characteristic	Oviposition (egg plugs/ 100seeds)	Weight Loss of Grain (g/100g)
E-ferulic acid content	-0.71	-0.63
protein	-0.81	-0.75
instron hardness (N)	-0.62	-0.71
shape index	0.67	0.76

Note: lignin, sugar and lipid content were not significant.

Table 2. Susceptiblity of quality protein maize (QPM) and backcross to normal (QPM x NOR) genotypes to maize weevil.

Cultivar type	Dobie Index of Suceptibility (S.D)
QPM	8.03 (4.6)
QPM x NOR	9.13 (1.3)

Note: A Dobie Index of 14 indicates very susceptible grain and an Index of 0 is totally resistant.

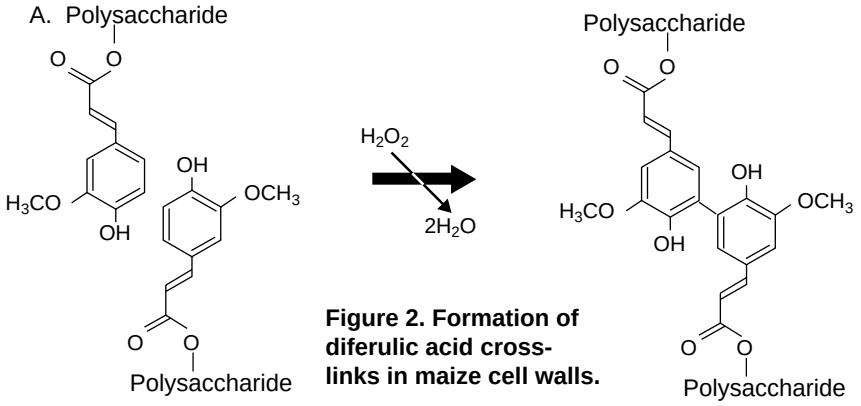

Figure 2. Formation of diferulic acid cross-links in maize cell walls.

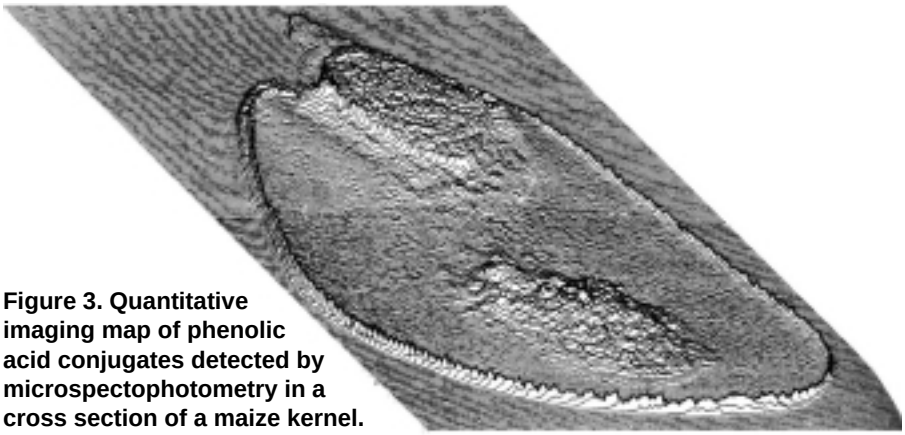

Figure 3. Quantitative imaging map of phenolic acid conjugates detected by microspectophotometry in a cross section of a maize kernel.

Resistance to the Larger Grain Borer (LGB), *Prostephanus truncatus* Horn

Despite the widespread destruction of grain in Africa by this pest, little was known about grain characteristics correlated to susceptibility of grain to the LGB. Seven cultivars from CIMMYT's program were assessed for the relative susceptibility of maize varieties to the LGB by studying grain damage parameters in standardized tests (% of grains attacked, loss of grain weight, powder produced), insect development parameters (mortality, weight of adults, consumption). Development was assessed on five replicate 100g samples of grain equilibrated at 70% relative humidity and 30°C which were infested with 100 unsexed adults for 2 weeks before assessment. Choice tests were performed by releasing 100 insects into an arena with 100g of grain samples of each variety (Table 3). The grain was analyzed for characteristics which may be correlated to resistance are such as hardness and deformation as measured with by instron, % vitreous endosperm (by quantitative imaging), total sugars and phenolics (by HPLC), total lipids (gravimetrically) protein (estimated by Kjeldahl) and water content (gravimetrically). Methods are described in detail elsewhere (Conilh de Beyssac 1991, 1992). The results were analyzed by ANOVA and Tukey's or the Kruskal-Wallace tests for the comparison of the means (Tables 3-5).

The Pearsons's correlation coefficients between grain characteristics and insect performance parameters are shown in Table 6. Some of the susceptibility parameters for LGB show the same pattern of correlation as MW. For example, humidity and partial water content are positively correlated to several indices of LGB damage as observed in the case of MW. Also several LGB development parameters were negatively correlated to hardness measurements as was found for MW. The amount of vitreous endosperm is negatively correlated to the amount of powder produced by adults. Powder produced by LGB adults is important for the development of early larval instars as well as in determining the total weight loss of the grain which is an important measure of economic damage.

The importance of hydroxycinnamic acids in resistance is also evident with this insect. Weight loss of grain is negatively correlated with ferulic acid content of grain, which may be associated with its importance in cross-linking and strengthening the

Table 3. *Prostephanus truncatus* development parameters on maize varieties.

Name of variety	Damaged kernals (%)	Weight loss (g/100g)	Powder produced (g/100g)	Choice test (%/variety)	Consumption (mg/insect/day)
Ilonga 8032	43.70b,c	5.02c	3.6b,c	8.76	2.50
Muneng 8128	46.16a,b	7.19a,b	3.74b,c	7.26	1.64
Cacahuacintle	54.48a	8.62a	8.38a	14.36	3.23
Poza Rica 8121	53.88a	5.62b,c	3.98b,c	10.56	2.61
Across 7740	42.42b,c	7.92a	4.78b	7.45	1.87
Across 8035	40.42b,c	6.99a,b,c	3.14c	5.25	1.67
Ratray-Arnold 8149	36.40c	5.47b,c	3.26c	10.08	2.41

Values followed by the same letter are not significantly different.

Table 4. Physical characteristics of maize varieties.

Variety	Hardness (peak force) (N)	Deformation before breakage (mm)	Kernel volume (cm^3)	Kernel weight (g)	Vitreous endosperm (%)
Ilonga 8032	430.4 (a)	0.433 a,b	0.405	0.317	44.5ab
Muneng 8128	418.5 a	0.476 a	0.560	0.304	47.4a
Poza Rica 8121	318.7 b	0.346 c,d	0.714	0.504	41.1bc
Ratray-Arnold 8149	289.0 b	0.313 d	0.376	0.307	33.9d
Across 8035	285.5 b,c	0.329 d	0.336	0.286	36.8cd
Across 7740	232.7 c,d	0.286 d	0.418	0.286	22.0e
Cacahuacintle	201.3 d	0.398 c	0.395	0.323	7.84f

Values followed by the same letter are not significantly different (P = 0.05).

Table 5: Biochemical characteristics of maize varieties.

Name of variety	Partial moisture (%)	Total moisture (%)	Estimated Protein content (%)	Lipid Content (%)	Total sugar (mg/g)	Total phenolics (mg/g)
Ilonga 8032	10.45	14.4b	11.14c	3.91ab	2.36g	2.10a
Muneng 8128	10.55	14.3c	11.78b	3.31ab	4.36f	1.64bcd
Cacahuacintle	10.84	14.8a	8.99g	4.98a	5.81d	1.53cd
Poza Rica 8121	10.42	14.3c	10.82d	3.82ab	5.05e	1.93ab
Across 7740	11.01	14.3c	12.14a	3.64ab	7.53c	1.35d
Across 8035	10.76	14.2d	9.85f	2.56b	8.24b	1.77a
Ratray-Arnold 8149	10.79	13.9e	10.43c	4.24ab	10.20a	2.03a

Values followed by the same letter are not significantly different (P = 0.05).

hemicelluloses of the outer pericarp of the kernel. *P*-coumaric acid content was correlated to % damage of kernels, as well as to the physical parameters of hardness and vitreous endosperm content in this data set. The importance of *p*-coumaric acid may be involved with its association with lignin in both pericarp and endosperm cell walls.

Clear differences with the MW situation also are evident. Protein content was never significant for resistance correlations for LGB, although it is negatively correlated with MW performance (Arnason et al. 1994). Sugar content was positively correlated to LGB mortality but was not significant for MW in our trials (Classen et al. 1990). Total lipids were positively correlated to insect choice and consumption parameters of LGB, suggesting they are attractants or phagostimulants which is the reverse of MW (Serratos et al. 1987). Some of the statistically significant relationships for LGB resistance correlation's are presented in Figures 4-7.

Table 6. Pearson correlation coefficients (and P values) of LGB susceptibility parameters to physical and biochemical characteristics of seven genotypes.

	Damaged kernals (%)	Powder produced (g/100g)	Weight loss (g/100g)	Choice test (#/variety)	Consumption (mg/day)	Weight adults (mg)	Mortality (%)
Kernels/100g		-0.88 (.009)		-0.90 (.006)	-0.84 (.02)		
Hardness-instron (J)							-0.79 (.03)
Plasticity of grain (mm)						-0.77 (.04)	-0.94 (.001)
Partial water content (%)	0.93 (.002)						
Humidity (%)	0.75 (.05)	0.87 (.01)					
Vitreous endosperm (%)		-0.87 (.01)	-0.72 (.066)				
E-ferulic acid (mg/g)			-0.88 (.01)				
p-coumaric acid (mg/g)	-0.70 (.006)						
Total phenolics			-.093 (.002)				
Total lipids (%)				0.94 (.001)	0.89 (.007)		
Total sugars (%)							0.87 (.01)
Dobie Index *Sitophilus zeamais*	0.90 (.006)						

The significance of these results is that they confirm the importance of the newly discovered phenolic factors in resistance to a second insect pest of grains as well as defining the importance of moisture, hardness,

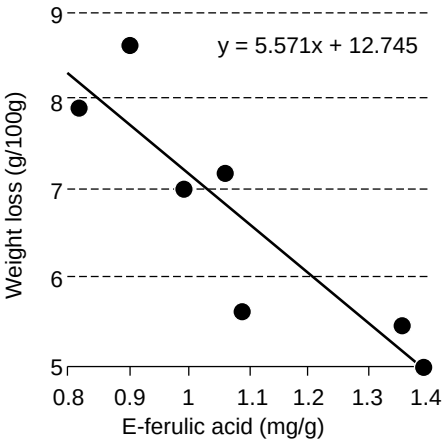

Figure 6. Relation of grain weight loss due to LGB and ferulic acid content of grain.

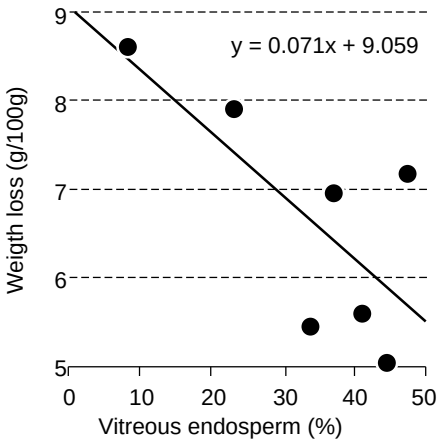

Figure 4. Relation of grain weight loss due to LGB and vitreous endosperm content of grain.

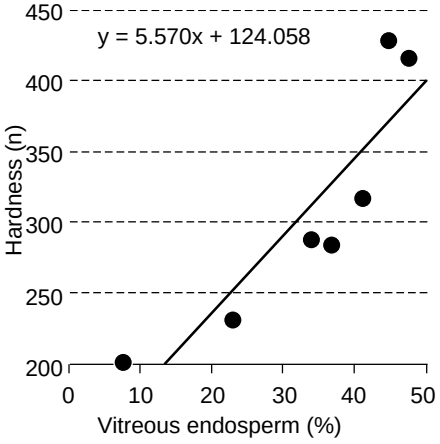

Figure 5. Relation of grain hardness and vitreous endosperm content of grain.

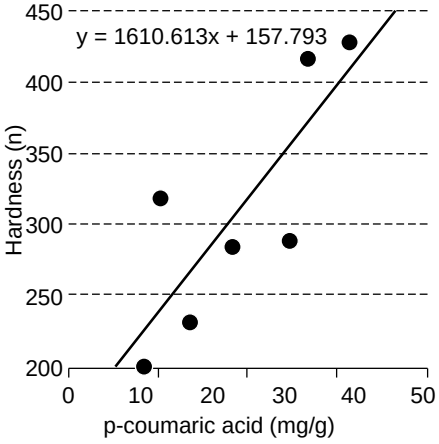

Figure 7. Relation of grain hardness and *p*-coumaric acid content of grain.

vitreous endosperm and nutritional factors such as lipids in LGB development or behavior.

Acknowledgment

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Mechanisms of Resistance in Maize to Western Corn Rootworm

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Abstract

The western corn rootworm (WCR), Diabrotica virgifera virgifera *LeConte, is considered a primary pest threatening maize cultivation in North America. Branson et al. (1983) indicated the existence of an unidentified antibiosis factor in resistant germplasm from South Dakota, in addition to the well known tolerance. Our laboratory has identified the hydroxamic acids (Hx): DIMBOA, DIM$_2$BOA, HMBOA and MBOA as antibiosis factors in maize roots. These substances induce larval mortality and delay development of the insect. Behavioral data suggest that Hx also reduce acceptability of maize roots as hosts. Using high pressure liquid chromatography (HPLC), these biochemicals have been located in maize tissue at the rootworm feeding sites. A greenhouse study demonstrated that maize varieties with high Hx content were less damaged than varieties with low Hx content when artificially infested with WCR larvae. This result has been confirmed in the field with 7 inbreds of varying Hx content which were artificially infested with WCR eggs. Pre-screening methods for selection of genotypes based on Hx content are currently being evaluated. Chromosome mapping of resistance and phytochemistry is also being undertaken.*

Introduction

In many areas of the US and Canada, the Western corn rootworm (WCR) *Diabrotica vigifera virgifera* LeConte has become the most important insect threat to maize cultivation. Chemical control is currently the major strategy to suppress the insect and the amount of insecticide used is greater than that for any other pest (Metcalf 1986). Government policy in both countries calls for the reduction of pesticide use. Alternative strategies of rootworm management including host plant resistance are required for widespread use. Although there has been interest in this area, most previous work has focused on field evaluation of tolerance, the ability of damaged roots to re-grow after pruning by rootworm larvae. The antibiosis (toxic) and antixenosis (behavior modifying) resistance components have received little attention. A study by Branson et al. (1983) reported antibiosis in several experimental maize hybrids. Our studies since 1988 have firmly established the role of maize secondary metabolites in antibiosis and antixenosis.

Phytochemistry of Maize Roots

We hypothesized that phytochemicals in maize roots may be contributing to the reported antibiosis in resistant varieties. The characteristic secondary chemicals of maize roots are hydroxamic acids. In preparation for these studies, J. Atkinson of our group developed a synthesis of the major hydroxamic acids of cereals (Atkinson et al. 1991) that allowed an evaluation of their role in maize roots. Using these synthetic materials, Y. Xie investigated the major hydroxamate compounds found in maize roots and their possible role in resistance. Although they are stored as glycosides *in vivo*, they are released as the free aglycones by b-glucosidases after damage of tissues, such as maceration or insect feeding. The protocol used for extraction and release of free hydroxamates is described in Figure 1. A gradient HPLC method was developed that conveniently resolved three hydroxamic acids from the root extracts (Fig. 2). The main compounds found are 2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one (DIMBOA), and its degradation product 6-methoxy-benzoxazoline (MBOA), while the lactam of DIMBOA, 2-hydroxy-7-

methoxy-1,4(2H)-benzoxazin-3-one (HMBOA) and 2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3(4H)-one (DIM_2BOA) are also present in significant quantities (Xie et al. 1991b).

A study (Xie et al. 1990) of the phenology of these hydroxamates indicated that HMBOA and DIM_2BOA were maximal at about 2 weeks after germination, while DIMBOA reached its peak at 4 weeks (Fig. 3). The concentration of these materials is then diluted by growth of the maize seedling. The time course of maximal production coincides with the early development of CRW larvae. Studies of the localization showed that they are generally found in higher concentrations in the cortex, which is the site of CRW feeding, than the steele (Xie et al. 1991a).

In Vitro Toxicity and Antixenosis of Hydroxamates to CRW Larvae

The major hydroxamic acid of maize roots, DIMBOA was found to be toxic to WCR larvae with an LC_{50} of 153 (108-209) mg/ml and LD_{50} of 917 (560-2297) mg/ml (n = 450). These concentrations are relevant to natural levels found in maize inbreds developed at Agriculture Canada from CIMMYT latitudinal pools (Table 1).

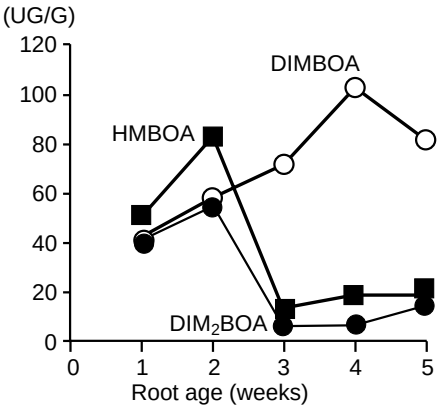

Figure 3. Phenology of hydroxamic acid concentration in young maize seedlings.

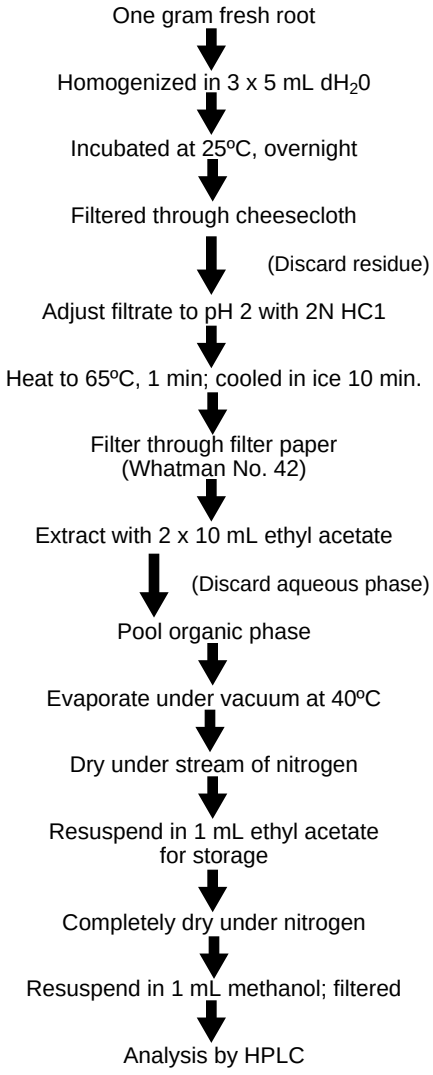

Figure 1. Extraction procedure for hydroxamic acids from maize roots.

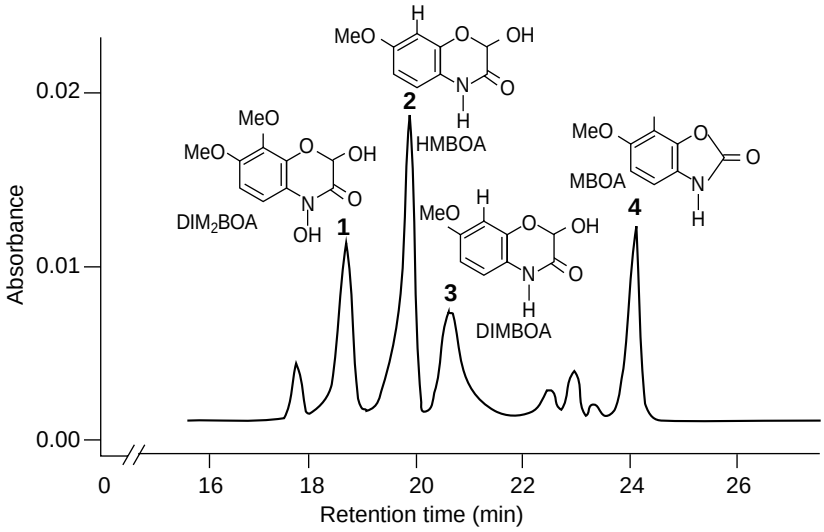

Figure 2. HPLC separation of hydroxamic acids from maize root extracts.

Table 1. Concentrations of hydroxamate compounds in roots of maize germplasm of various geographic origins (μg/g fresh wt.)

Maize line	Total	DIMBOA equiv.	HMBOA	DIM_2BOA
ITR 3872	1140.5 A	921.1 A	86.9 A	120.9 A
NTR-1 3983	444.3 B	327.1 B	68.5 B	35.1 CDE
ITR 3865	392.8 BC	296.4 BC	30.1 EF	61.7 B
NTR-1 3946	359.0 BCD	248.7 BCD	30.1 EF	70.1 B
NTR-1 3962	296.0 CDE	186.5 DEF	32.6 DE	69.5 B
NTR-2 4071	281.0 CDEF	215.0 CDE	22.3 EFGH	38.8 CD
ARGEN 2032	218.5 EFGH	177.9 DEFG	21.4 GH	11.7 FGHI
STR 3794	191.2 EFGHI	120.0 EFGH	39.7 CD	26.5 DEFG
STR 3815	184.3 EFGHI	115.2 EFGH	41.5 C	22.5 DEFGH
STR 3805	163.2 EFGHI	103.2 EFGH	31.4 E	22.9 DEFGH
ITR 3862	143.0 FGHI	99.8 EFGH	28.7 EFG	10.2 GHI
MEXICO 5	135.6 GHI	100.5 EFGH	19.1 HI	12.3 FGHI
NTR-2 4021	56.8 I	44.9 H	6.1 KL	4.0 I

Means followed by the same letter are not significantly different in Duncan's multiple range test (P= 0.05).

A behavioral study was also undertaken to determine the effect of naturally occurring and synthetic Hx on the characteristic search pattern of WCR larvae as they locate maize roots (Fig. 5). Strand and Dunn (1990) demonstrated that a decreased search area and locomotory rate and increased number of turns occurred after WCR larvae contacted host roots as compared with non hosts (Fig. 5). A comparison of behavior of larvae towards Hx treated roots and controls (Table 2) indicated that these compounds reduced the host suitability of the roots. In particular, locomotory rate and search area increased while number of turns decreased after treatments of the roots with HMBOA, DIMBOA, DIM_2OA or MBOA.

In Vivo Effects of Hydroxamates

A greenhouse study of the role of Hx in CRW resistance was undertaken with two elite maize lines with widely varying Hx content (Xie et al. 1990). Under artificial infestation with WCR eggs at four different levels, the high DIMBOA line ITR 3872 showed significantly less damage as indicated by plant growth parameters such as plant height, stem thickness, plant fresh weight, and root fresh weight, than the low DIMBOA line NTR-2 Germany 4042 (Fig. 6). Significantly fewer adult WCR emerged from the high DIMBOA line and they had lower mean weights and head capsule widths (Fig. 7).

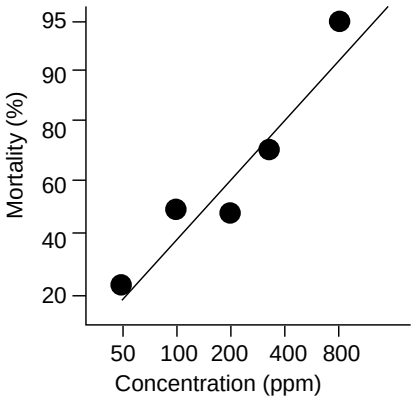

Figure 4. Probit plot of rootworm mortality as a function of DIMBOA concentration (from Xie et al. 1990).

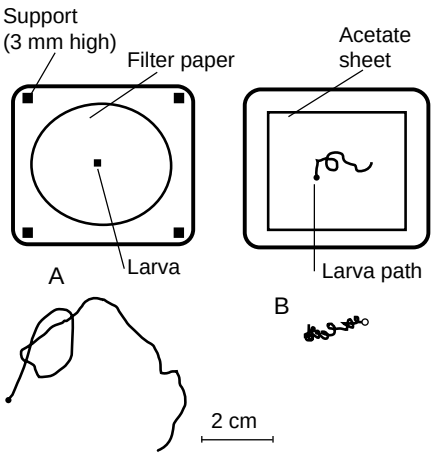

Figure 5. Apparatus for larval host seeking experiments (upper) and larval paths (lower) of insects near (A) unsuitable host or (B) suitable host.

Table 2. Behavioral parameters of rootworm larvae during a 5 min host searching period after removal from treated and control roots.

	Number of turns	Area searched (mm^2)	Locomotor rate (mm/min)
Control	58.5 a	117 ef	20.1 b
HMBOA	36.2 bc	158 cde	27.5 a
DIMBOA	30.4 bc	166 bcd	31.9 a
DIM_2BOA	26.9 cd	157 cd	28.1 a
MBOA	25.4 cd	204 ab	22.5 a

Means followed by the same letter are not significantly different in Duncan's multiple range test (P= 0.05)

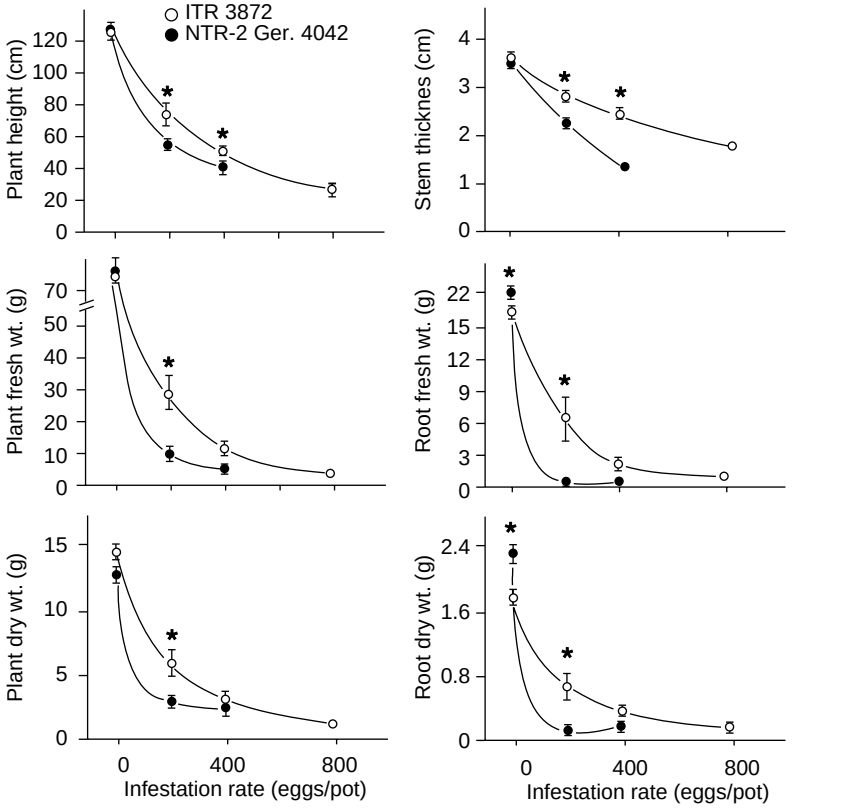

Figure 6. Mean plant 'performances' of maize lines with different DIMBOA contents infested at different rootworm egg concentrations. Significant difference (P = 0.05) between corn line performance is indicated by (*). (Data from Xie et al. 1990).

A subsequent greenhouse study of seven maize lines with varying DIMBOA content artificially infested with WCR larvae showed significant negative correlation's between larval 'performance' and root DIMBOA content (Table 3). Usually insect performance is a balance of nutritional factors such as protein or simple carbohydrates against anti-nutritional factors such as DIMBOA. We were surprised to find no positive correlation between nitrogen and insect performance, but the results are possibly confounded by the nitrogen content of DIMBOA. The negative correlation with sugar content suggests complex interactions with other factors.

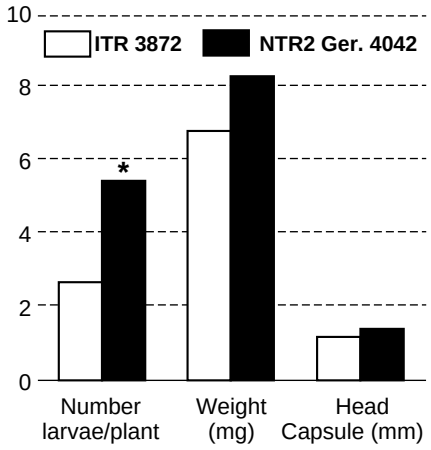

Figure 7. Mean 'performance' parameters for rootworms emerged from corn lines in Figure 6. Significant difference (P = 0.05) between insect 'performance' on corn lines is indicated by (*).

Field Verification of Antibiosis Results

While the laboratory and greenhouse trials had given us some confidence that hydroxamic acids of maize are an antibiosis and antixenosis factor to WCR, these results could not be considered useful in an agronomic context until verified in the field. Two years of field trials were conducted at the Central Experimental Farm in Ottawa in 1992-3 (Assabgui et al. 1994). Seven maize inbreds with varying root levels of DIMBOA were selected and grown in a randomized block design with 4 replicates. They were infested with 0, 500, 1000 or 1500 WCR eggs per 30.5 cm of row and damage was assessed at 8 and 16 weeks after infestation, by digging roots and assessing rootworm damage on the 9 class rating scale of Welch (1977). The relationship between root damage rating and the total Hx content of the roots was significant and negative (Fig. 8). The results demonstrate antibiosis due to Hx in a field context.

Screening for Antibiosis

The correlation results in greenhouse and field studies suggested an important application of biochemical pre-screening to WCR resistance assessment. Rootworm field trials are very labor intensive because of the effort of digging and washing the roots.

A prediction of resistance performance can be made on the basis of Hx content. Using biochemical screening, we assessed 18 Ontario check hybrids for the levels of Hx in roots (Assabgui et al. 1993). The results suggested that only two cultivars would be predicted to have significant antibiosis, nine to be moderately susceptible and seven susceptible. Two of the extremes were tested in field trials and performed as expected. While the method is promising, the results highlight the rootworm resistance problem that most germplasm is not resistant. This may be a result of limited selection for rootworm resistance in the past. However, we are now using biochemical pre-screening on a larger number of crosses of temperate inbreds to tropical and subtropical germplasm, in order to increase the probability of introducing and selecting phytochemically based resistance in elite cultivars.

Inheritance of Rootworm Resistance Factors

During the 1993 growing season at the Central Experimental Farm in Ottawa, a study was conducted by J. Larsen on the inheritance of WCR resistance and

Table 3. Correlation of larval rootworm performance parameters with nutritional and anti-nutritional factors in seven inbreds.

Rootworm performance	DIMBOA content of roots	Sugar content of roots	Nitrogen content of roots
Mean number of surviving larvae	r = -0.81 P = 0.02	n.s P > 0.05	n.s P > 0.05
Mean weight of larvae	r = -0.95 P = 0.0013	-0.895 P = 0.05	n.s P > 0.05
Mean head capsule width	r = -0.94 P = 0.0016	n.s. P > 0.05	n.s P > 0.05

Note: n = 7

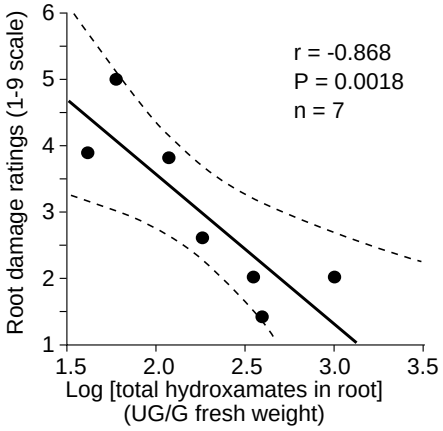

Figure 8. Relation between mean root damage rating and total root Hx content performed under field conditions for seven inbreds.

Hx. A diallel analysis was conducted involving seven inbred maize lines, varying in both Hx content and WCR resistance. The genotypes used were SD10, CM7, CO272, ITR3872, ITR3865, NTR3983 and NTR4034. Root damage was assessed according to the nine point rating scale of Welch (1977) and Hx levels were determined by HPLC according to Xie (1991b). The study found that for root resistance, the general combining ability (GCA) was highly significant and specific combining ability (SCA) was non-significant, and for root Hx content GCA and SCA were both significant. Plots of combining abilities against their respective traits showed that those varieties that combine well are also the varieties that perform well for the trait in question. The data for hydroxamates is shown (Fig. 9).

The diallel analysis was a preliminary study of the inheritance of WCR resistance and root Hx content and has led to an ongoing study intended to undertake the mapping of quantitative trait loci (QTLs) that significantly affect resistance to WCR.

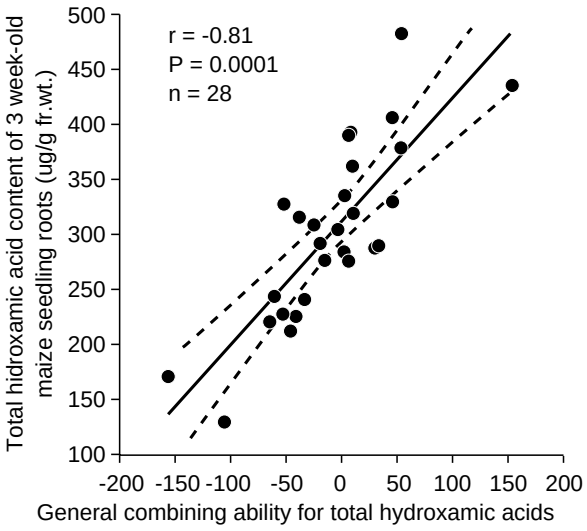

Figure 9. Correlation between total Hx of 7 inbreds and general combining ability for Hx content.

Acknowledgments

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Mechanisms and Bases of Resistance in Maize to Mites

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Abstract

Maize resistance to mites was isolated using recurrent selection, in a population of tropically adapted exotic germplasm accessions, that were crossed with temperately adapted NB 611. Since mite damage is greatest to maize during and following pollination, resistance research must be conducted in the field using plants in the reproductive growth stages. Methods for infesting maize with mites, rating damage, and making selections for resistance are discussed. Also, procedures for determining the mechanisms of resistance in maize to mites are described. Nine sources of resistance to mites have been identified. Preliminary research indicates that mite resistance in maize identified to date is primarily tolerance with some antibiosis involved.

Mite Biology

Mite pests on maize in the United States include the Banks grass mite, *Oligonychus pratensis* (Banks), two-spotted spider mite, *Tetranychus urticae* Koch, and carmine mite, *Tetranychus cinnabarinus* (Boisduval), (Ehler 1973). On maize, mite development from egg to adult is completed in <2 weeks when temperatures are 23 to 25ºC and <1 week when temperatures are over 30ºC (Perring et al. 1984). Mites usually are found on the underside of leaves and feed in the epidermal and sometimes outer mesophyll cells by sucking out dissolved nutrients (Jeppson et al. 1975). Mite feeding produces chlorotic spots on leaves, and may kill all or portions of leaves and reduce yield (Archer and Bynum 1990, 1993). Infestations begin on the lowest leaves of plants and spread upwards on the plant as mite abundance increases. Rate of increase and damage by mites are greatest when weather is hot and plants are water stressed (Perring et al. 1986). Mite densities are generally greatest from maize anthesis to maturity as mites exploit changes in plant physiology associated with seed production and leaf senescence (Perring et al. 1983; Archer et al. 1986, 1988).

Selection for Resistance

There are several biological considerations when designing maize resistance to mite research:

- Maize is most susceptible to mite damage and yield loss from pollination until dent.
- Mites have limited dispersal ability (walking or being blown by the wind), which results in uneven distribution in a field.
- Premature senescence of leaves from abiotic stress (e.g. low fertilizer or water stress) may mask mite damage.
- Very early or late maturing maize cultivars may escape mite damage without being resistant.

Mansour et al. (1993) reported that plants should be at least pollinating before resistance in maize can be determined for mites. It would be very difficult to grow and screen large numbers of plants in a greenhouse to grain filling stage. Therefore, screening for resistance should be done in the field. It is best to create uniform mite infestations in the field, because natural mite distribution is too clumped for reliable evaluation of plants for resistance. We have found that the best way to obtain large numbers of mites for infestation is to collect leaves from a commercial maize field that is heavily infested with mites. We obtain infested leaves as early in the season as possible to avoid collecting predators of mites and to get plants, in breeding blocks, infested by mid to late vegetative growth stages. Only heavily infested leaves are collected to assure rapid increase in mite densities on plants used for research. Infested leaves are placed in paper sacks and immediately transported to the research field. These leaves are laid across leaves in the lower third of plants to be infested. A single infested leaf will usually extend across two to four plants in a row. We

prefer to infest every row in the nursery, although one to two rows can be left between infested rows and mites will spread across rows after their densities become high on the originally infested plants. Usually 4 to 6 weeks are required to produce an infestation large enough to provide enough damage for resistance selection when infesting every row. If predators of mites are found on plants, plots are sprayed with chlorpyrifos at 0.28 kg ai/ha or permethrin at 0.22 kg ai/ha to kill the predators. Water and fertilizer stress should be avoided in mite resistance blocks because the symptoms of these stresses can confound accurate ratings and may affect plant resistance or rate of mite increase. Early maturing maize should be infested early in the season or with very heavy numbers of mites to allow time for mite increase and damage to develop before a significant amount of leaf senescence occurs. Late maturing maize should be planted early so that the susceptible growth stages occur while weather is best for mite increase.

When we were selecting lines for resistance, we used single row plots that were 5 m long and replicated three times. During breeding, we do not replicate plots, but select individual plants in a row. Because mite ratings cannot be made on plants prior to pollination, we self plants before we select for resistance. We infest every plant in a row and self 5 to 10 plants. During the dent growth stage, every selfed plant in a row is rated for mite damage using the 1-10 scale (Table 1). Chlorotic spots on leaves are symptomatic of mite damage. These small spots are caused by mite feeding which drains all nutrients and chlorophyll from individual epidermal cells. Mite infestation and damage will begin on lower leaves on the plant. Mites and damage spread up the plant over time. Under very heavy mite infestations, areas of a leaf or whole leaves may die from mite feeding. Death usually begins at leaf margins on the distal portions of leaves and spreads across and down the leaf. One must be careful not to rate leaves dead from senescence as killed by mites. Therefore, plants should be rated before a significant number of leaves senescence naturally as plants approach physiological maturity. Since mite feeding does not cause yield losses after maize kernels dent, we usually make ratings shortly after denting unless mite damage is slow in developing and ratings have to be delayed. The 10 to 20% of the plants receiving the lowest damage ratings are advanced to the next cycle.

Mite Resistant Maize

We have identified inbreds from nine sources of maize resistant to mites (Table 2). These inbreds have been advanced to the F_7 generation. Test

Table 1. Mite damage rating scale used to estimate leaf damage from mite feeding on maize.

Rating	% leaf area damaged/plant	Description of damage
1	1 - 10	A few small mite colonies and associated damage (chlorotic spots) along the midrib of the lowest leaves.
2	11 - 20	Mite colonies and damage spread along the midribs on the lowest leaves on a plant.
3	21 - 30	Mite colonies and damage spreading out from the midrib on the lowest leaves and small colonies may occur on leaves up to the ear.
4	31 - 40	Mites and damage cover most of the leaf area on the 1-2 lowest leaves and mite colonies and damage extend along the midrib to the ear leaf.
5	41 - 50	Mites have killed one leaf, bottom 2-3 green leaves heavily infested and damaged, and mite colonies on 1-2 leaves above the ear.
6	51 - 60	Mites have killed or nearly killed the bottom two leaves and colonies and damage extend beyond the midribs on two leaves above the ear.
7	61 - 70	Mites have killed or nearly killed the bottom three leaves, all leaves up to the ear significantly damaged, and mite colonies and damage found on most to all leaves on the plant.
8	71 - 80	Mites have killed or nearly killed all leaves up to the ear and mites and damage occur on most to all leaves on the plant.
9	81 - 90	Most leaves on the plant killed by mite feeding and only leaves in upper third of plant alive.
10	91 -100	Very little green area left on plant or plant dead.

Table 2. Pedigrees of maize resistant to mites.

Source	Pedigree	Races
1	(NB 611 X Valle 411) X (NB 611 X VEN 733)	(Comun.) (Guaribero)
2	(NB 611 X LOR 9) X (NB 611 X VEN 604)	(Piricinco) (Canilla)
3	(NB 611 X Arizona 8601) X (NB 611 X VEN 414)	(?) (Tuxpeno)
4	(NB 611 X VEN 426) X (NB 611 X Valle 411)	(Negrito) (Comun.)
5	(NB 611 X Sin 2) X (NB 611 X Valle 411)	(Chapalote) (?)
6	(NB 611 X KS 2301) X (NB 611 X Arizona 8601)	(?) (?)
7	Bahia Gpo 3	Tuson 9
8	Chiapas 26	Tepeci 19
9	Ecuador 569	Tusilla

Key: VEN = Venezuela; Sin = Sinaloa

crosses have been made between inbreds for most of the sources and MO17 and B73. These crosses were screened in experiments replicated three times. Mite damage ratings were about 10 to 20% higher in crosses than in the inbreds (Table 3). In most cases, yield and 100 seed weight were as good in crosses between mite resistant inbreds and B73 or MO17 and the susceptible checks, B73 X MO17 or Deltapine 4673B. The cross between source 6 and either MO17 or B73 did not yield well. The Deltapine 4673B was damaged by mites which may have reduced yield. B73 X MO17 was not infested by mites.

Mechanisms of Resistance

Research was begun recently to determine the mechanisms of resistance in maize to the Banks grass mite.

Mansour et al. (1993) indicate that this research has to be done on older maize plants beginning at pollination. This makes mechanism research difficult because plants have to be grown for over two months to reach pollination before research can be started. When we began this research, plants were grown in 4 liter pots until 4 to 6 leaves were free from the whorl and then transplanted into 19 liter pots. We found that plants grew faster and were more robust if grown from seed in 19 liter pots and not transplanted. Plants were grown in the greenhouse and research was conducted in the laboratory at 27ºC under florescent lights.

Antibiosis

Antibiosis is the measure of the effect of the plant on herbivore development, survival, and reproduction. Life tables are developed and compared for mites on resistant and susceptible lines to determine antibiosis.

Development - Young female mites are collected from the mite culture which is grown on a mite susceptible maize hybrid. Maize is used as the culture medium because if another plant species is used, the change of host could affect mite development in experiments on maize. Young females can be separated from the rest of the mites in a colony by placing uninfested maize plants among culture plants. In 24 hours, mostly young females and a few nymphs will migrate from heavily infested plants to the previously uninfested plants. These females can be transferred individually from the new plants to development cages on test plants with the aid of a small artist's brush. We used a cage similar to the one described by Perring (1983) to confine mites during development and survival research (Fig. 1). This cage consists of three pieces of 0.3 cm thick Plexiglas. One piece, 18 cm x 5 cm fits on the top of the leaf. Eight 1 cm diameter holes are drilled into the Plexiglas. A piece of photograph mounting tape with adhesive on both surfaces is attached to the bottom side of the Plexiglas and a 1 cm diameter cork borer is used to drill the eight holes through the tape. The cage is attached to the top surface of the leaf with the mounting tape adhesive. Two 18 cm x 2.5 cm Plexiglas strips are placed on the bottom side of the leaf on either side of the midrib. The sections of Plexiglas

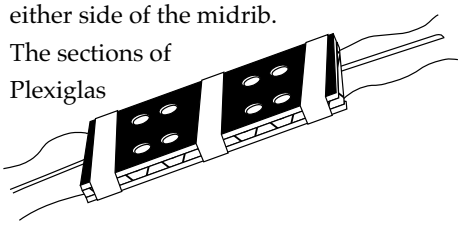

Figure 1. Cage used to contain mites to study development for determining antibiosis.

Table 3. Mite damage ratings for inbreds and crosses with MO17 and B73 and yields for crosses.

		Mite damage rating[1]			
Resistant (R) source	Susceptible (S) entry	R or S inbred	R X S cross	Yield (gm) per ear	100 seed weight (gm)
1	MO17	2.1	4.9±0.4 c-f[2]	144±14 ab[2]	24.9±1.3 abc[2]
	B73	—	4.4±0.3 c-g	137± 7 a-d	26.5±1.2 abc
2	MO17	1.5	3.7±0.3 e-h	110±12 a-d	21.4±1.8 c
	B73	—	3.7±0.8 e-h	123±16 a-d	24.3±2.1 bc
3	MO17	2.0	4.0±0.0 d-h	132±10 a-d	26.8±1.6 abc
	B73	—	3.0±0.6 gh	167± 3 a	26.7±1.5 abc
4	MO17	2.0	6.0±0.0 bc	135± 7 a-d	30.7±0.5 a
	B73	—	—	—	—-
5	MO17	3.0	5.8±0.4 bcd	131± 8 a-d	25.2±0.7 abc
	B73	—	5.5±0.2 b-c	133±16 a-d	26.2±0.5 abc
6	MO17	2.0	3.8±0.2 e-h	85±11 cd	15.8±0.9 d
	B73	—	2.5±0.2 h	79± 2 d	12.8±1.4 d
7	MO17	3.0	6.0±0.0 bc	146±11 ab	27.5±1.0 abc
	B73	—	3.6±0.8 fgh	143±18 ab	24.7±2.5 abc
8	MO17	3.0	—	122±37 a-d	27.7±0.7 abc
	B73	—	—	138± 4 abc	26.6±0.4 abc
9	MO17	3.0	5.6±0.5 b-e	117±10 a-d	25.5±1.1 abc
	B73	—	4.9±0.3 c-f	123± 8 a-d	24.8±1.3 abc
—	MO17	7.0	—	—	—-
	MO17 X B73[3]		7.0±0.0 ab	101± 3 bcd	29.9±0.8 ab
	Deltapine 4673B[3]		8.2±0.9 a	113± 3 a-d	25.5±0.8 abc

[1] Damage rated using the 1 - 10 scale listed in Table 1.
[2] Means in a column followed by the same letter are not significantly different according to Student-Newman-Kuels multiple range test (P=0.05, SAS PROC GLM).
[3] MO17 X B73 and Deltapine 4673B are a cross and a susceptible commercial hybrid, respectively.

sandwich the leaf and are held together with three strips of masking tape (one on each end of the cage and one in the middle).

Three to four adult female mites are placed into each cell and the cell opening is covered with a single layer of dialysis membrane held in place with contact cement. Dialysis membrane is used because air and moisture will move through it but mites cannot escape through it. We found that mites died in the dialysis membrane covered cells at high temperatures (over 32ºC). Therefore, fine mesh cloth covers are used when temperature is high, but mites can escape through cloth. After 24 hours, female mites are removed from each cell and the number of eggs are standardized to 15 per cell. Eggs are examined with the aid of a stereomicroscope. Sixteen cells are infested for each run of an experiment. We determined that the earliest hatch occurred 4 days after females were removed. Therefore, we begin daily observations for egg hatch 3 days after female removal. Eggs are observed for hatch or collapse. Eggs that do not hatch or have collapsed are considered dead. The number of larvae produced are counted and immature mites are observed every other day until the first male is detected. Males complete development about a day before females. Then mites are observed daily for the last nymphal molt. The number of immatures living to adult, number of days to reach adult, and sex of each adult are recorded. The number of dead or missing mites is recorded at each observation. Female mites produced in the development study are used for the oviposition study. If males are not seen in a cell as nymphs reach the last molt, some are added to mate with females.

Oviposition - Each freshly molted female (<24 hours old) is removed from the cells and placed in an oviposition arena consisting of a leaf section (3 x 3 cm) in a Petri dish. Females are transferred to leaf sections of the same inbred that they were reared on in the development experiment. Two females are placed on each section of leaf which rests on a cotton ball in a pool of water to keep the leaf fresh. The oviposition arena consisted of the bottom or lid of a 100 x 15 mm Petri dish. We attempt to set up at least 25 Petri dishes per run. Every 3 days until female death, females are transferred to a new leaf section. At transfer, the number of females on each leaf section is recorded as live, dead, or missing. Also, the number of eggs oviposited on a leaf section is recorded. The leaf section is held in the Petri dish with water until egg hatch. The number of larvae or nymphs (indication of hatch), or eggs that do not hatch are recorded. The experiment is terminated when all females die.

Tolerance

Tolerance is the most difficult mechanism to determine because it is a subjective measurement. For an accurate measure of tolerance, it is important that leaf damage percentages be related to pest density and leaf area available to mites. Five young female mites are placed in each of 20 clip-on leaf cages per inbred. Clip-on cages (clear plastic pill boxes) provide a means to restrict mite feeding to a limited area (2.5 x 2.5 cm) on each line (Fig. 2). Mites are collected from the culture as described in the antibiosis experiment, placed into each clip-on cage, and the cage is attached to a leaf so that mites have access to either the top or bottom leaf surface. A susceptible line has to be included in each experiment to compare mite densities and damage between susceptible and resistant lines. We use MO17 as our susceptible check. Weekly the leaf area in the susceptible check cages is observed for mite damage without removing the cages. When the average damage in susceptible check cages is > 80% of the leaf area, all caged leaf sections are removed. All leaf sections in cages are removed at the same time. The percentage of each leaf section damaged by mites is estimated and mite densities determined by stage. It is essential to relate the percentage of the leaf area damaged to the number of mites within the cage when determining tolerance. If a plant has some antibiosis, the number of mites present might be low and damage would then be correspondingly low. This would provide a false indication of tolerance.

Antixenosis

These experiments measure the comparative acceptability of resistant and susceptible leaf sections. One must be sure to include all resistant sources

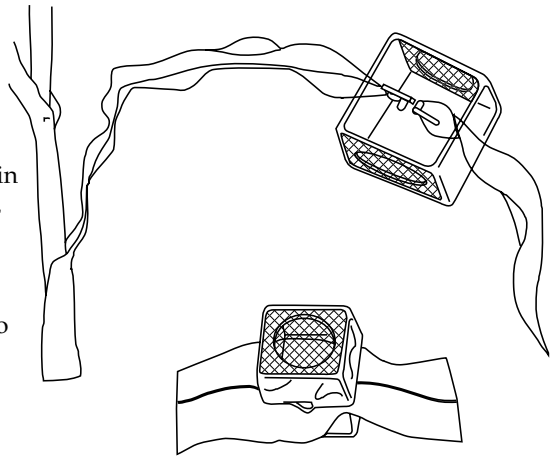

Figure 2. Clip-on leaf cage used for determining tolerance attached to a maize leaf (top and side views).

and a susceptible check in the choice test. The order of arrangement of leaf sections must be randomized each time an experiment is set up. We conduct this research in 100 x 15 cm Petri dishes with a layer of agar about 1 cm thick. Each leaf section used in our choice experiments is ca. 0.5 cm wide x 3 cm long. Both ends of the leaf section are inserted into the agar to keep the leaf viable for 48 hours. The leaf sections are bowed so that they do not touch the agar surface, because mites on the under side of leaves can get onto the wet agar and die. The agar and leaf sections remain viable longer when a thin layer of water is kept on the agar surface. A wax paper disc is placed in the center of the Petri dish so that its edge touches each leaf section. Ten young adult female mites from the culture are placed on the wax paper disc and allowed to disperse to leaf sections. Ten Petri dishes are used in each run of the antixenosis experiment. Three runs provide enough individuals to determine antixenosis. We allow mites 48 hours to choose a leaf based on data by Foster et al. (1977).

Future Directions

Inbreds of the nine maize sources resistant to mites have been selected. Test crosses of the inbreds and MO17 or B73 have been made. Yield equal to or better than the susceptible cross, B73 X MO17, was maintained by eight of the sources. There is little reduction in mite resistance in these crosses compared to the corresponding inbreds. We have begun random mating with resistant inbreds to combine genes for greater resistance. Random mating will be conducted for three generations and then we will begin selfing to extract further resistant inbreds. Since mite pest problems are usually most severe when maize is water stressed, research has been started to combine mite resistance and drought tolerance.

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Mechanisms and Bases of Resistance in Maize to Spotted Stem Borer

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Abstract

Spotted stem borer, Chilo partellus *(Swinhoe) is a serious pest of maize,* Zea mays *L. The mechanisms (antibiosis, antixenosis and tolerance) and bases of resistance to this pest have been investigated in India. Many materials were evaluated for antibiosis and about 20 were reported to manifest this mechanism of resistance. Among these, seven maize materials, namely, Antigua Gr. 1, A1 x Antigua Gr. 1, Antigua Compuesto, Ganga 5, J 22, J 605 and Mex. 17 manifested a higher level of antibiosis. The use of plant materials from this germplasm, as food for rearing* C. partellus, *adversely affected some vital parameters of the insect's biology. It reduced larval survival, larval and pupal weight, fecundity and egg viability, prolonged the larval and pupal period, and ultimately reduced the progeny of the pest. A cumulative effect of antibiosis was also observed. Among different plant parts, minimum antibiosis was recorded in ears and maximum in the tassel. Antibiosis was observed to develop and become operative when the plants were 10-15 days old and it increased with plant age. Antixenosis for oviposition occurred in Antigua Gr. 1, A1 x Antigua Gr. 1, Ageti 76, Caribbean Flint Composite and Cuba 11J. The 4-week old plants were less preferred than 2-week old plants. Antigua Gr. 1 and A1 x Antigua Gr. 1 exhibited both antibiosis and antixenosis. Among nine maize varieties tested for tolerance, Vijay ZFS3 appeared to possess this mechanism. Some chemical constituents of maize plants were evaluated in relation to the level of resistance. The germplasm having higher resistance, compared to those possessing lower resistance, had higher contents of silica and iron but lower nitrogen, phosphorous, potash and sugar. Furthermore, the studies showed that the resistance may be due to some toxins. The implications of the results obtained on mechanisms and bases of resistance are discussed.*

Introduction

Spotted stem borer, *Chilo partellus* (Swinhoe) is a serious pest of maize, *Zea mays* L., in India. It is one of the limiting factors in the successful cultivation of this crop. It is reported to cause 24 to 83% loss in maize yield (Chatterjee et al.; 1969; Sarup 1973; Mathur and Rawat 1981). The development and use of resistant varieties is the most useful approach to manage pests. Plant resistance could be explained through three fundamental mechanisms of resistance; antibiosis, antixenosis and tolerance in plants to insects (Painter 1951). The knowledge of the mechanisms and bases of resistance is useful in breeding cultivars having insect resistance. The research work carried out in India on the mechanisms and bases of resistance in maize to *C. partellus* is reviewed in this paper.

Mechanisms of Resistance

Antibiosis

This is the most evident, desirable and long lasting mechanism of resistance. It includes all the adverse effects of a temporary or permanent nature on the insect biology resulting from the ingestion of a plant by an insect. Studies have been conducted on antibiosis in different maize germplasm, its expression in relation to plant age and in different plant parts and its cumulative effects on *C. partellus*.

Antibiosis in maize germplasm - Antibiosis has been evaluated on the basis of larval survival by Pant et al. (1961), Kalode and Pant (1966), Mathur and Jain (1972) and Lal and Pant (1980), and development period by Panwar and Sarup (1980). The promising germplasm that exhibited antibiosis are presented in Table 1. These include indigenous collections, indigenously developed hybrids and composites and introductions from the Caribbean and the USA.

Sharma and Chatterji (1971b), Sekhon and Sajjan (1987) and Durbey and Sarup (1984) evaluated different populations and hybrids. In addition to larval survival they studied the

antibiotic effect of these germplasm on other biological parameters, namely larval and pupal weight, larval and pupal period, pupal survival fecundity, egg viability, sex ratio and multiplication rate. They reported that the resistant varieties, namely Antigua Gr. 1, A1 x Antigua Gr. 1, Antigua Compuesto, Ganga 5, J 22, J 605 and Mex. 17 reduced larval survival, larval weight and pupal weight, prolonged larval and pupal period as compared to the susceptible variety Basi Local. The pest multiplied at a slower rate when reared for one generation on resistant varieties than on Basi local. Further, the production of males out numbered the females on resistant varieties and the reverse was the case of Basi Local. The results obtained by Sekhon and Sajjan (1987) are presented in Table 2. Durbey and Sarup (1985, 1988) observed a similar antibiotic effect on *C. partellus* when the pest was reared on a diet that contained powdered dry material and the ether extract of resistant populations, Antigua Gr. 1 and Mex. 17. It may be added that Antigua Gr. 1, was used in all six studies on antibiosis.

Table 1. Maize germplasm showing antibiosis to *C. partellus.*

Germplasm	Reference
AES 805, Ill 1656, K41, nc 27, yellow no. 2	Pant et al. (1961)
Ganga 101, Arbhavi Local, Jalandhar Local, Rudrapur Local	Kalode and Pant (1966)
Antigua Gr. 1, A1 x Antigua Gr. 1	Sharma and Chatterji (1971)
Antigua Compuesto	
Vijay, J 12	Mathur and Jain (1972)
Antigua Gr. 1	Lal and Pant (1980)
Jawahar, Ganga 5	Panwar and Sarup (1980)
Antigua Gr. 1, Mex. 17	Durbey and Sarup (1984, 1985, 1988)
J. 605, J 22	Sekhon and Sajjan (1987)

Cumulative effect of antibiosis - Information on the cumulative or additive effect of antibiosis in maize germplasm on *C. partellus* reared continuously on a particular variety for more than one generation is of practical utility. This will help in identification of the germplasm which may suppress the population build-up of the pest in its active season. In the Punjab, *C. partellus* multiplies on the spring sown maize fodder crop for 2-3 generations before it shifts to the main rainy season crop.

Sajjan and Sekhon (1992a) studied the rate of multiplication of *C. partellus* on six maize materials over two generations. The results with respect to two resistant (Antigua Gr. 1 and Ganga 5) and one susceptible line (Basi Local) are presented in (Table 3).

The pest multiplied slowly on resistant materials in comparison to the susceptible one during the first generation. The cumulative effect of antibiosis for two generations caused a drastic reduction in the multiplication rate of the pest, which was only 0.4 times on Antigua Gr. 1, 1.8 times on Ganga 5 and about 10.0 times on Basi Local. Apparently, resistant or susceptible materials can markedly influence the build-up of the population in the field.

Antibiosis in different plant parts - The antibiotic effect of four plant parts; stem, whorl, ear and tassel on the biological parameters of *C. partellus* has been investigated by Sharma and Chatterji (1971b) (Table 4). The percentage survival of the larvae, larval weight, pupal weight, sex ratio (female/male), fecundity and egg viability were found to be relatively higher in the case of the larvae reared on ears than those on other parts of plant. Also, larval and pupal period and incubation period were relatively less when reared on ears. The results suggested that tassel and ear had the maximum and minimum antibiotic effect, respectively.

Table 2. Antibiotic effect of maize germplasm on different biological parameters of *C. partellus.*

Germplasm	Larval survival (%)	Larval weight (mg)	Larval period (d)
Antigua Gr. 1	19.5	43.6	29.8
Ganga 5	15.2	59.9	25.9
J 22	17.3	-	-
J 605	13.1	48.4	26.7
Basi Local	27.7	66.7	23.5

Germplasm	Pupal weight (mg)	Pupal period (d)	Multiplication ratio	Sex ratio Male	Sex ratio Female
Antigua Gr. 1	45.4	7.0	1:0.6	1	1.1
Ganga 5	53.3	7.5	1:1.3	1	1.0
J 22	44.3	6.8	-	1	0.7
J 605	42.6	7.3	1:0.6	1	0.8
Basi Local	52.6	5.9	1:2.7	1	1.8

Source: Sekhon and Sajjan (1987)

Table 3. Cumulative effect of antibiosis in maize germplasm on *C. partellus*.

Germplasm	Multiplication rate (times) One generation	Multiplication rate (times) Two generations
Antigua Gr. 1	1.19	0.37
Ganga 5	1.38	1.81
Basi Local	2.92	9.96

Source: Sekhon et al. (1992a)

Antibiosis in relation to plant age - Plant age has been reported to influence antibiosis (Kalode and Pant 1967). Sekhon and Sajjan (1990) evaluated larval survival of *C. partellus* on the plants of different ages (5, 10, 15, 20 and 25-days old) in Antigua Gr. 1, Ganga 5 and Basi Local (Table 5). There were very small differences among the lines for larval survival on 5 and 10-day old plants. The borer survival, however, sharply declined on 15-day old plants of resistant populations (Antigua Gr. 1 and Ganga 5) and the decline continued up to 25 days, but at a lower rate. Thus, the most critical time for the development of antibiosis may be when the plants are 10 to 15 days old. Sharma and Chatterjee (1971a) also reported more antibiosis in 27-day old plants than that in 15-day old ones. The lack of expression of antibiosis in resistant germplasm during their early growth period has also been observed by Mathur and Jain (1972), and Singh and Sandhu (1979).

Antixenosis

Antixenosis, or non-preference, denotes the plant characteristics and insect responses that lead to avoidance of a particular plant or variety, for oviposition, food or shelter or a combination of the three. Kogan and Ortman (1978) have proposed this new and appropriate term to replace Painter's term non-preference.

Antixenosis in maize for oviposition - Differential preference for oviposition by *C. partellus* in maize has been reported by Singh (1967), Sharma and Chatterji (1971a), Lal and Pant (1980) and Sekhon and Sajjan (1980) and Sekhon and Sajjan (1985). Sharma and Chatterjee (1971a) found Caribbean Flint Comp. and A1 x Antigua Gr. 1 to be relatively less preferred for oviposition than Basi Local and Antigua Gr. 1. However, Lal and Pant (1980a) and Sekhon and Sajjan (1985) reported Antigua Gr. 1 also to be less preferred than Basi Local. It may be added that Antigua Gr. 1 and A1 x Antigua Gr. 1 have been reported to exhibit antibiosis also. Thus, these may be usefully exploited in the breeding program. Antigua Gr. 1 is a parent of double top-cross hybrid cultivar, Ganga 5. In the study of Sekhon and Sajjan (1985), Ganga 5 manifested a fairly high amount of antibiosis but showed a little antixenosis for oviposition (Table 6), in contrast Ageti 76 expressed a little antibiosis but relatively more antixenosis.

Antixenosis in relation to plant age - According to Sekhon and Sajjan (1985) 5 day old plants were not preferred at all, but 15 day old plants were the most preferred for oviposition by *C. partellus* (Table 7). As the plant age increased from 15 days onward, the number of eggs laid by *C. partellus* went on decreasing so much that it was reduced to one-fourth.

Table 4. Antibiotic effect of different plant parts of maize germplasm on different biological parameters of *C. partellus*.

Plant part	Larval survival (%)	Larval weight (mg)	Larval period (d)	Pupation (%)	Pupal weight (mg)
Stem	40.9	49.6	18.7	34.5	54.3
Whorl	39.1	45.3	21.1	31.7	55.1
Ear	47.4	75.0	15.6	40.2	66.7
Tassel	34.7	31.7	22.0	28.6	44.4

Plant part	Pupal period (d)	Moth emergence (%)	Sex ratio (f/m)	Fecundity (no.)
Stem	6.0	76.0	1.13	200.6
Whorl	6.4	71.9	1.02	188.6
Ear	5.4	83.0	1.23	260.1
Tassel	7.9	80.6	0.88	163.0

Source: Sharma and Chatterji (1971b)

Table 5. Antibiotic effect of different maize germplasm in relation to plant age on *C. partellus.*

Plant age (d)	Larval survival (%)		
	Antigua Gr. 1	Ganga 5	Basi Local
5	7.3	77.5	78.5
10	65.0	76.3	80.0
15	40.0	52.5	75.6
20	31.3	41.3	76.3
25	28.8	38.8	74.4

Source: Sekhon and Sajjan (1990)

Table 6. Antixenosis in maize germplasm against oviposition by *C. partellus*.

Germplasm	Eggs/ plant (No.)	Egg masses (No.)
Antigua Gr. 1	11.9	0.9
Ganga 5	39.0	2.9
J 22	25.2	1.8
Vijay	25.2	1.6
Ageti 76	20.6	0.9
Basi Local	22.7	1.8

Source: Sekhon and Sajjan (1985)

Table 7. Antixenosis in maize in relation to plant age against oviposition by *C. partellus*.

Plant age (d)	Eggs/plant (No.)
5	0.0
10	34.8
15	82.8
20	36.4
25	21.6
30	18.4

Source: Sekhon and Sajjan (1985)

Sharma and Chatterjee (1971a) recorded relatively more oviposition on 15 day old plants than on 27 day old plants. Singh and Sandhu (1978) reported maximum oviposition on 16 to 18 day old plants and no oviposition on plants of age less than 10 days. According to Durbey and Sarup (1982) the maximum egg laying occurred on plants of 7 to 15 day old plants, with maximum egg laying on 7 day old plants. From these studies, it seemed that generally the most preferred plant age for oviposition was 7-15 days. Therefore, the application of insecticides for the control of this pest must be made on a 10 to 15-day old crop. Plants of about 15 days age should be used for studying antixenosis in maize germplasm for oviposition.

Tolerance

This is the ability of the plant to repair injury or grow to produce an adequate yield, despite supporting an insect population at a level capable of damaging a more susceptible crop. The cultivars exhibiting a moderate level of antibiosis and higher level of tolerance are considered ideal, as they allow the survival of an adequate pest population, large enough to maintain the parasites and predators, but prevent the build up of new biotypes (Horber, 1972). However, little work has been done on this mechanism of resistance. Sekhon and Sajjan (1992) evaluated genotypes having variable resistance to *C. partellus* (Table 8). The studies revealed that only Antigua Gr. 1 and Ganga 5 resisted the attack of the borer consistently. Both these materials showed low values for damage grade and the coefficient of harmfulness, but the resistance in these materials is more due to antibiosis as revealed by significantly less larval survival on these varieties than that of the others. Vijay ZFS3, however, appeared to show tolerance to this pest in one of the experiments, due to the high larval survival nearly equal to that on the susceptible Basi Local, and the relatively low coefficient of harmfulness. In the case of internal feeders like the spotted stem borer it may be difficult to maintain a relatively uniform population on the test varieties, whilst minimizing the antibiotic effects of the less susceptible varieties. Maxwell and Jennings (1980) also remarked that out of the three mechanisms of resistance, tolerance is perhaps the most difficult to quantify. But this mechanism of resistance needs further investigation.

Bases of resistance

Chemical constituents of maize

Chemical constituents of the plant affect the survival and developmental behavior of the insect in many ways. These may affect the normal feeding of an insect, its physiology or may act as an inhibitor or toxin. In the studies of Kalode and Pant (1967), Sharma and Chatterji (1971b) and Uma Kanta and Sajjan (1989), resistance was associated with lower nitrogen content. Furthermore, resistant germplasm, in comparison to the susceptible, had lower sugar (Kalode and Pant 1967a; Sharma and Chatterji 1971c), phosphorous and potash content, but higher silica and iron content (Sharma and Chatterji, 1971b). Uma Kanta and Sajjan (1989) reported that the nitrogen content in the plant decreased with plant age, even in the susceptible material (Table 9).

Nutritional deficiency in maize

Sharma and Chatterji (1972) carried out studies on the nutritional deficiencies in maize in relation to *C. partellus* resistance under both field and laboratory conditions. In field studies, they applied solutions of diet ingredients and diets lacking some

Table 8. Tolerance mechanism of resistance in maize germplasm to *C. partellus*.

	Experiment 1			Experiment 2		
Germplasm	Damage score*	Number of larvae	Loss coefficient (%)	Damage score	Number of larvae	Loss coefficient (%)
Antigua Gr. 1	4.7	2.6	26.2	4.7	3.3	-6.9
Ganga 5	5.2	3.3	19.0	4.5	2.9	14.8
Vijay ZFSC3	7.5	2.8	100.0	5.4	5.2	24.5
Basi Local	8.6	5.0	100.0	7.0	5.7	84.3
LSD (0.05)	0.6	1.2		1.2	0.9	

* 1-9 scale, where 1 = healthy, 9 = dead heart.

Table 9. Nitrogen concentration of plants of maize populations.

	Nitrogen (%)				
	Whole plant	Stem		Leaf	
Treatment	12 DAG	24 DAG	36 DAG	24 DAG	36 DAG
Antigua Gr. 1	2.27	1.96	1.56	2.13	1.55
Basi Local	3.18	2.31	0.79	2.45	0.73
LSD (0.05)	0.21	0.27	0.14	0.20	0.14

DAG = days after germination
Source: Uma Kanta and Sajjan (1989)

nutrients to the whorl of resistant plants. In the laboratory, they evaluated supplemented and deficient diets. Under field conditions, the addition of dextrose, ascorbic acid or salt mixture No.2 increased larval survival whereas the absence of these nutrients had an adverse effect. However, opposite effects were obtained in laboratory experiments.

Nature of antibiosis in maize

Sharma and Chatterji (1972a) reported that the addition of juice or an ether soluble extract from the susceptible plants to resistant plant whorls did not improve the survival of borer larvae in field studies. This indicated that the differential resistance is probably not due to the lack of feeding stimulants. On the other hand, addition of juice or ether soluble extracts to the whorl of susceptible material from the resistant one decreased larval survival (Table 10). This suggested that the resistance is probably due to the presence of some toxin in the resistant plants. Furthermore, laboratory studies revealed that larval establishment and survival were less in the diet with juice and ether soluble fraction from the resistant germplasm (Table 11 and 12). The ether soluble fraction was however more potent. From this it was inferred that the toxins probably have a feeding deterrent or even repellent action on the first instar larvae.

The data given in Table 13 show that the effect of the toxin was suppressed by some dietary components. Dextrose and ascorbic acid were the most potent suppressers of the toxin. These findings suggested that the resistance in maize to *C. partellus* is probably the result of the amount of toxin present and the suppression effect of the nutrients in a particular germplasm.

Relationship Between Plant Traits and Resistance

Sharma and Chatterji (1971, 1971a) evaluated the relationship of some plant characters with resistance (Table 14). The germplasms having vigorous plants, compact whorl, soft stem and long internode were more susceptible. Further, the *C. partellus* moths preferred to lay eggs either on leaves having a glabrous surface, trichome density of

Table 10. Effect of susceptible maize populations treated with juice and ether extract of Antigua Gr. 1 on *C. partellus*.

Susceptible population	Larvae (No.)		
	Antigua Gr. 1		Dist. Water
	Juice	Extract	
Basi Local	1.05	0.97	5.12
K T-4	1.02	0.87	3.52

Source : Sharma and Chatterji (1972)

Table 11. Effect of diets containing the juice and ether extracts of maize populations on the larval establishment of *C. partellus*.

Germplasm	Larval establishment (No.)	
	Juice	Extract
Antigua Gr. 1	0.5	0.0
A1 x Antigua Gr. 1	0.8	0.3
Antigua Comp.	4.4	3.5
Basi Local	35.3	19.4

Source: Sharma and Chatterji (1972)

Table 12. Effect of diets containing the juice and ether extracts of maize populations on the larval survival of *C. partellus*.

Germplasm	Larval survival (No.)	
	Juice	Extract
Antigua Gr. 1	4.0	0.1
A1 x Antigua Gr. 1	6.4	1.0
Antigua Comp.	4.3	5.3
Basi Local	38.4	29.6

Source: Sharma and Chatterji (1972a)

Table 13. Effect of diets containing ether extract of Antigua Gr. 1 and variable concentration of nutrient on the larvae number of *C. partellus*.

Nutrients	Larvae (No.)			
		Concentration		
	Absent	Normal	Triple	Diet only
Ascorbic acid	0.0	1.5	7.87	14.25
Dextrose	0.0	1.8	10.62	18.00
Casein	1.5	1.5	1.87	14.75
Salt mixt. # 2	2.0	1.6	2.75	15.75

Source: Sharma and Chatterji (1972)

Table 14. Plant traits in maize populations.

Population	Internode length (cm)	Stem hardness (kg/cubic cm)	
Antigua Gr. 1	11.5	2.6	
A1 x Antigua Gr. 1	11.1	2.3	
Basi Local	15.5	0.79	
Population	**Plant vigor**	**Whorl index**	**Leaf width**
Antigua Gr. 1	54.4	0.5	3.8
A1 x Ant. Gr. 1	60.3	0.7	3.6
Antigua Comp.	57.8	0.7	3.8
Basi Local	71.1	1.3	3.3

Source: Sharma and Chatterji (1971)

1.7 mm^2, or short, erect trichomes (Durbey and Sarup, 1982b). Durbey and Sarup (1982a) observed that the abaxial leaf surface of the tip portion was the most preferred ovipositional site by the moths.

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Maize Resistance to the Lesser Cornstalk Borer and Fall Armyworm In Brazil

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Abstract

Maize, Zea mays, *is an important cereal crop in Brazil. It is extensively grown throughout the country for food grain, feed, and fodder purposes. Among many factors, insects pests play a major role in limiting maize yields. The lesser cornstalk borer (LCB) and the fall armyworm (FAW) have been considered the most important field pests, being key pests in many of the areas where the crop is grown. The FAW and the LCB have been reared at EMBRAPA/CNPMS to undertake artificial infestation for large-scale studies, including screening for resistance. Several genetic materials were selected for resistance. Sources of resistance such as CMS 23 and CMS 24 to FAW, CMS 15 and CMS 454 to LCB are being used in breeding for resistance. The resistance mechanisms to FAW were studied on four selected maize genotypes. Larvae reared on CMS 14C required longer to develop to the pupal and adult stages and had reduced larval and pupal weights. The genotype Zapalote Chico had fewer larvae feeding on leaf sections than other genotypes tested. The analysis of a diallel cross indicated that gene action conditioning resistance to the FAW appears to be due to additive and non-additive effects.*

Introduction

Maize, *Zea mays,* is an important cereal crop in Brazil. It is extensively grown throughout the country for food grain, feed, and fodder purposes. The total area under cultivation in the country during 1992-93 was 11.2 million hectares, with a production of 26.8 million tons of grain, an average yield of 2.4 t/ha (Carrieri et al. 1993).

In Brazil, among many factors, insect pests play a major role in limiting maize yields. A list of insects attacking maize in Brazil is shown in Table 1. Among the insects attacking maize, the fall armyworm (FAW), *Spodoptera frugiperda* and the lesser cornstalk borer (LCB), *Elasmopalpus lignosellus* have been considered the most important field pests, being key pests in many of the areas where maize is grown.

Damage and Economic Importance

The FAW larvae attack maize at all stages, although the most serious damage occurs at the mid-whorl stage (Cruz 1980). According to Carvalho (1970), depending on the stage of the plant when the damage is done, the yield reduction ranges from 15 to 34%.

The LCB larva is a semi-subterranean feeder, usually attacking a seedling plant at or just below the soil surface. Larvae bore into the stem and during feeding, produce tunnels upward and downward from the entrance hole. Feeding usually kills the young plant. According to All et al. (1982), when plants are killed and desiccated, LCB larvae move to adjacent plants. Several

Table 1. Insects damaging maize in Brazil.

Scientific name	Common name	Pest status
Spodoptera frugiperda	Fall armyworm	***
Elasmopalpus lignosellus	Lesser cornstalk borer	***
Sitophilus sp	Weevils	***
Helicoverpa zea	Corn earworm	**
Diabrotica speciosa	Corn rootworm	**
Diatraea saccharalis	Sugarcane borer	**
Mocis latipes	-	*
Agrotis ipsilon	Black cutworm	*
Rhapalosiphum madis	Corn leaf aphid	*
Deois flavopicta	Leaf hoppers	*
Scaptocoris castanea	-	*
Sitotroga cerealella	Angoumois grain moth	*
Several species	Wireworms	*
Several species	White grubs	*

*** Key pest; ** occasional; * secondary

plants may be killed by one larva in this way. Damage caused by this insect is reported to be from 20 to 50% of the planted area (Sauer 1939; Viana 1991) or even the entire crop (Jacobsen 1928).

Techniques for Mass Rearing, Artificial Infestations and Evaluation Procedures

The Maize and Sorghum National Research Center/EMBRAPA at Sete Lagoas, MG, Brazil, has mass reared FAW and LCB since the early 1980s, enabling the Institute to undertake artificial infestation for large-scale studies — including screening for resistance and developing biological, cultural and chemical control tactics for pest management programs.

Fall armyworm

The FAW is reared at EMBRAPA/CNPMS on a modified black cutworm diet described by Reese et al. (1972) (Table 2). The moths lay eggs on paper napkins, placed into a oviposition cage (62 x 62 cm), which are cut into strips and placed in plastic jelly cups to be incubated at 28º C. After incubation, one small larva is transferred to an individual plastic jelly cup, containing the diet, and then sealed with flexiglas lids. The cups are placed into trays that hold 32 cups and are kept undisturbed until adult emergence. The adults are transferred daily from cups to oviposition cages and are fed with sugar solution through a cotton wick in a 50 ml plastic jelly cup. Recently, we are testing split cell modules placed into the boxes (29 x 29 x 4 cm), as used at CIMMYT and described by Mihm (1989a), to rear FAW larvae.

Table 2. Ingredients for the FAW diet used at EMBRAPA/CNPMS.

Ingredients	Amount
Pinto beans	333.0 g
Torula yeast	101.4 g
Wheat germ	158.4 g
Ascorbic acid	10.2 g
Methyl p-hidroxy benzoate	6.3 g
Sorbic acid	3.3 g
Agar	41.0 g
40% Formalin	8.3 ml
Water	2400.0 ml

Artificial infestation with FAW is done at EMBRAPA/CNPMS at the 4 to 5 fully expanded leaf stage. The technique used is similar to that described in detail by Mihm (1989b). The larval infestation of every plant to be screened is done with 30-40 hatched larvae mixed with maize cob grits, using a "bazooka" to deliver the neonate larvae into the plant whorl. Evaluation for resistance to leaf feeding is made 14 days after infestation using a visual leaf feeding damage scale varying from 0 to 9 as suggested by Davis and Williams (1989). For an initial screening of materials we usually plant one 10 m row where half of each row is protected with insecticide. Two replications are usually planted.

Table 3. Ingredients for the LCB diet used at EMBRAPA/CNPMS.

Ingredients	Amount
Agar	40 g
Water	1280 ml
Pinto bean	420 g
Water (hot)	1300 ml
Yeast	128 g
Wheat germ	200 g
Mold inhibitor	10 ml
Ascorbic acid	13 g
Methyl paraben	8 g
Sorbic acid	4 g
40% formalin	8 ml
55% linolenic acid	10 ml
Tetracycline	1 capsule (250 mg)
Vanderzaant's vitamin mixture	5 g
Mold inhibitor ingredients	
Propionic acid	418 ml
Phosphoric acid (conc.)	42 ml
Water (dist.)	540 ml

Lesser cornstalk borer

A modificiation of Burton's (1969) pinto bean diet cited by Chalfant (1975) (Table 3) is used to rear LCB larvae at EMBRAPA/CNPMS. The moths lay eggs singularly on napkins placed on the top and bottom of the oviposition cage (cylinder of 20 cm diam. x 20 cm). Napkins with eggs are placed inside a small plastic bag and kept at 28º C until hatch. Newly hatched larvae are mixed with fine (# 4) vermiculite and poured into plastic jelly cups containing diet. Larvae average 3 to 5 per cup using this method. Preformed trays holding 32 cups, are left undisturbed until adult emergence. The number of adults per oviposition cage is 30 pairs. The adult food (beer) is supplied through 4 medicine droppers inserted in the middle of the oviposition cage. The oviposition cage is maintained at 28º C with a 16 hour photoperiod.

Screening trials to evaluate maize germplasm for LCB resistance are conducted in the greenhouse. Ten maize seeds are planted in 5 L plastic pots. When the seedlings emerge, each pot is infested with 50 eggs. Plants attacked, number larvae alive and weight of larvae are recorded 15 days after infestation.

Genetic Sources of Resistance and Breeding Methodologies

In the mid-1980s research was intensified by EMBRAPA/CNPMS, with a large amount of indigenous and exotic germoplasm and elite lines being tested for resistance to FAW and LCB. The screening work identified several sources of resistance to these insect pests (Viana 1992a; 1992b). The materials selected are presented in

Tables 4 and 5. During the last 8 years, many maize genotypes were infested and the subsequent leaf damage and percentage of plants alive were evaluated for resistance to FAW and LCB, respectively. Some material that appeared to sustain less damage than others and showed good agronomic traits was selected for breeding for resistance. Sources of resistance such as CMS 23 and CMS 14C to FAW, CMS 15 and CMS 454 to LCB are being used in breeding for resistance.

A recurrent selection scheme and mass selection have been used to accumulate desirable genes for resistance to the FAW and LCB, respectively. A summary of the procedures of selection for resistance against these pests at EMBRAPA/CNPMS is presented in Table 6.

Mechanisms and Inheritance of Resistance

The resistance mechanisms to FAW have been studied in the laboratory, greenhouse and field at EMBRAPA/CNPMS. Four maize genotypes, CMS 23, CMS 14C, CMS 24 and Zapalote Chico were selected for study in the laboratory and greenhouse. Larvae reared on CMS 14C required longer to develop to the pupal and adult stages. Also, larvae reared on leaf tissue of CMS 14C presented reduced larval and pupal weights.

Both choice and non-choice tests were used to determine if resistant genotypes were less preferred by the larvae for

Table 4. Maize genotypes selected for resistance to FAW at EMBRAPA/CNPMS.

Year	Genotypes	Damage range	Mean ratings
1986/87	CMS 23		4.0
	CMS 14C		5.4
	CMS 24		5.5
	Zapalote Chico	4.0 to 7.5	5.5
1987/88	CMS 23		4.9
	CMS 24		4.9
	Zapalote Chico		4.1
	CMS 456		5.0
	BA 03		5.2
	SE 20		5.3
	CMS 451		5.4
	SE 14		5.5
	CMS 467	4.1 to 7.2	5.5
1988/89	Amarillo Cristalino		1.1
	WP 1		1.1
	RR 060		1.4
	MG 05	1.1 to 3.7	1.5
1989/90	BR 108 Tuxpeño		5.5
	Comp. Tuxpeño Veracruzano		5.4
	Mata Hambre X Guajira 314		5.5
	Nõdzob Torê		4.8
	Oaxaca 250		5.5
	Puerto Rico 5		5.0
	WP 33		5.5
	Cuba 45		5.5
	WP 18		5.4
	Zapalote Chico	4.8 to 7.0	5.3
1990/91	077 R2		2.2
	Guatemala 786		2.5
	Nõdzob Prê		2.5
	Puerto Rico 13		2.5
	Composto Arco Iris		2.5
	Guatemala 73		2.5
	139 R2	2.2 to 5.5	2.5
1991/92/93	PB 11		4.4
	WP 16		4.8
	Rep.Dominicana 248		5.2
	Zapalote Chico		5.3
	BA 22		5.5
	PA 008	4.4 to 7.0	5.5

Table 5. Maize genotypes selected for resistance to LCB at EMBRAPA/CNPMS.

Year	Genotypes	Damage range	Plants attacked (%)
1986/87	CMS 454		42
	CMS 15		42
	Baier		50
	Zapalote Chico	42 to 100	50
1987/88	RN 01		50
	BA III Tucson	50 to 100	50
1988/89	BA 60		50
	Guadeloupe 16		50
	SE 10	40 to 100	50
1989/90	CMS 472		30
	Jalisco 274	30 to 100	50
1990/91	Cateto Colômbia VII		40
	Cohauila 56		50
	CMS 15	40 to 100	50
1991/92	PB 13		40
	Zapalote Chico		42
	PAG VI - Moroti		45
	EW 3151 V.S.C.	40 to 100	54
1992/93	AC 84		45
	Centralmex J-VIII		45
	Composto Jaíba IV		45
	Cateto Prolífico IX		50
	Composto Cerrado I		50
	PB 11	45 to 100	50

Table 6. Schemes of selection for resistance used to FAW and LCB at EMBRAPA/CNPMS.

Population	Pest	Breeding methods	Year	Number of progenies screened	Number of progenies selected	Cycles of selection (1994)
CMS 14C	FAW	FS-S_1	87/88	200	20	4
CMS 23	FAW	Inbreeding Synthetics	88/89	200	20	1
MIRT	FAW	FS-S_1	91/92	180	35	2
CMS 15/ CMS 454	LCB	Mass Sel.	90/91	1000	128	3

feeding than susceptible genotypes. The results demonstrated that the genotype Zapalote Chico had fewer larvae preferring to feed on leaf sections than other genotypes tested. An additional test was conducted to determine adult oviposition preference using the same genotypes. The genotype CMS 14C was less preferred for oviposition compared with the remaining genotypes.

A tolerance study was conducted in yield trials where performance under both infested and protected split plots was evaluated. The results presented in Table 7 show a few materials indicating some tolerance to FAW leaf feeding damage.

We have conducted only limited investigations into the inheritance of leaf-feeding resistance to the FAW. The analysis of a diallel cross of 10 populations (Table 8) grown under artificial infestation indicated that both general and specific combining ability were significant sources of variation (Guimarães and Viana 1994). Gene action conditioning resistance to FAW appears to be due to additive and non-additive effects. The mean ratings of FAW damage on the 0 to 9 scale were 2.5 for crosses of resistant populations (Zapalote Chico x CMS 14C) and 4.35 for crosses between susceptible populations (CMS 01 x CMS 02).

Table 7. Maize genotypes showing tolerance to FAW at EMBRAPA/ CNPMS.

Genotypes	Mean rating	Grain weight (g)	
		Infested	Protected
Amarelo Sertão	6.9	2487	2125
CMS 21	6.6	2313	1962
Palha Roxa Mantena	6.2	2961	2534
CMS 04	6.1	3474	3174

Results obtained with 180 S_1 progenies of the MIRT population tested for resistance to the FAW showed a genetic heritability of 53% (superior limit) and 42% (low limit) (Viana and Guimaraes 1994), indicating a good range of genetic variability present in these materials which can be useful to a breeding program for resistance to this pest.

Conclusion

In summary, the plant resistance program to maize pests with emphasis on FAW and LCB at EMBRAPA/ CNPMS has been focussed on the following aspects:

- Locating new and better sources of resistance.
- Properly maintaining the resistant genotypes.
- Determining the mechanisms and inheritance of resistance.
- Developing suitable breeding methodologies for incorporating genetic resistance in agronomically suitable cultivars.

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Table 8. Diallel cross of 10 population tested for resistance to FAW at EMBRAPA/CNPMS. 1990/91/92.

Genetic Material	Mean rating	SCA [1]	GCA [2]
Zapalote Chico	3.2		-0.56
Z. Chico x CMS 01	3.1	-0.06	
Z. Chico x CMS 02	3.3	0.27	
Z. Chico x CMS 05	2.4	-0.53	
Z. Chico x CMS 06	3.2	0.19	
Z. Chico x CMS 11	2.7	-0.30	
Z. Chico x CMS 14C	2.1	-0.90	
Z. Chico x CMS 15	2.1	-0.82	
Z. Chico x CMS 23	3.2	0.37	
Z. Chico x CMS 28	3.4	0.22	
CMS 01	4.2		0.19
CMS 01 x CMS 02	4.3	0.57	
CMS 01 x CMS 05	3.4	-0.33	
CMS 01 x CMS 06	3.5	-0.21	
CMS 01 x CMS 11	3.9	0.15	
CMS 01 x CMS 14C	3.7	-0.10	
CMS 01 x CMS 15	3.6	-0.11	
CMS 01 x CMS 23	3.1	-0.52	
CMS 01 x CMS 28	3.9	0.02	
CMS 02	3.5		0.57
CMS 02 x CMS 05	3.7	0.16	
CMS 02 x CMS 06	3.4	-0.23	
CMS 02 x CMS 11	3.7	0.03	
CMS 02 x CMS 14C	3.4	-0.21	
CMS 02 x CMS 15	3.7	0.12	
CMS 02 x CMS 23	3.2	-0.24	
CMS 02 x CMS 28	3.6	-0.19	
CMS 05	3.4		0.01
CMS 05 x CMS 06	3.4	-0.13	
CMS 05 x CMS 11	4.0	0.38	
CMS 05 x CMS 14C	3.1	-0.46	
CMS 05 x CMS 15	3.9	0.42	
CMS 05 x CMS 23	3.7	0.26	
CMS 05 x CMS 28	4.1	0.41	
CMS 06	3.7		0.04
CMS 06 x CMS 11	3.1	-0.50	
CMS 06 x CMS 14C	3.5	-0.10	
CMS 06 x CMS 15	3.7	0.14	
CMS 06 x CMS 23	4.0	0.53	
CMS 06 x CMS 28	3.8	0.02	
CMS 11	3.9		0.08
CMS 11 x CMS 14C	3.6	-0.04	
CMS 11 x CMS 15	4.0	0.40	
CMS 11 x CMS 23	3.6	0.14	
CMS 11 x CMS 28	3.1	-0.67	
CMS 14C	4.0		0.08
CMS 14 x CMS 15	3.6	0.00	
CMS 14 x CMS 23	3.7	0.24	
CMS 14 x CMS 28	4.6	0.84	
CMS 15	3.6		-0.01
CMS 15 x CMS 23	3.3	0.13	
CMS 15 x CMS 28	3.4	-0.28	
CMS 23	3.0		-0.01
CMS 23 x CMS 28	3.5	-0.09	
CMS 28		3.8	0.21
Avg.	3.5		
LSD (0.050)	0.9		
Dp (G_i - G_i)			0.13
Dp (S_{ij} - Skl)		0.43	

[1] SCA Specific combining ability.
[2] GCA General combining ability.

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Windows of Maize Resistance

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Abstract

Breeding for maize resistance to insects and disease has been made possible by the broad genetic range in host plant resistance (HPR). However, within a given genotype, resistance can vary considerably over the course of plant development as well as between the different plant tissues for a given stage in development. These temporal and spatial changes in HPR are also reflected in the phytochemical composition of the plant. Using leaf bioassays, the feeding preferences of European corn borer larvae for certain portions of the leaf and stages of maturity were identified. These preferences were then related to phytochemcial composition which included nitrogen, fiber, phenolic acid, and DIMBOA content as well as leaf toughness and epidermal cell wall absorption of ultraviolet light. Disease pests of maize are also influenced by changes in host plant chemistry, in particular the silk and kernel chemistry as it relates to Fusarium sp. *Inferences on HPR strategies can be made from these types of studies which in turn can further our understanding of heritable resistance and how to screen germplasm in an efficient manner. This paper also serves to show the importance of sample position and timing when studying phytochemical mechanisms of HPR to insects and disease.*

Introduction

In the extensive cultivation of maize on large tracts of land, not all maize plants are of equal quality or suitable for insect and disease pests. Variation in host-plant quality can result from intrinsic factors, such as genetic or ontogeny, or from extrinsic factors such as soil conditions or environmental variation. Studies on maize resistance have documented extensive genetic variation in biochemical defenses against insect pests (Russell et al. 1975; Reid et al. 1991; Xie et al. 1992; Arnason et al. 1994), although the inheritance of specific resistance mechanisms is still not well defined. In addition to genetic variability, ontogeny generates both spatial and temporal heterogeneity in plant resistance (Kearsley and Whitham 1989). This is true for maize which exhibits biochemical changes between different tissues and different stages in development (Reid et al. 1992; Guthrie et al. 1986a; Argandona and Corcuera 1985). This temporal and spatial variation in biochemical resistance mechanisms may be heritable and subject to selection. Interdisciplinary teams consisting of breeders, entomologists, pathologists, physiologists and phytochemists can define the different biochemical strategies for HPR which are employed for different stages in plant development. With this understanding of HPR, screening and breeding for resistance can be accelerated.

Additional variation may be introduced by environmental factors — such as nutrients, light, water availability and temperature — which affect plant quality and suitability to insect pests (Mattson and Haack 1987). The proportion of variation in field resistance explained by environmental factors in contrast to genetic variation is unknown for most insect-plant systems but is beginning to be understood in maize.

This heterogeneity of host plant resistance in space and time effectively increases defense longevity by two important mechanisms:

- Feeding activity is concentrated on a restricted set of preferred tissues, thus lowering the contact rate with defensive compounds and reducing selection pressure for the evolution of detoxification mechanisms (Schultz 1983).
- The concentration of herbivores makes non-random searching by biological control agents much easier (Feeny 1976).

Upon assessing food plant quality through olfaction, gustatory or tactile responses, herbivorous insects decide if the plant possesses antixenosis or antibiosis characters, then, if undesirable or unpalatable, relocate and sample again (Renwick 1983). If the plant tissue is a poor food source, the insect will seek a more desirable food source in the case of motile herbivores,

or tolerate the reduced growth associated with poor sites as in the case of sessile herbivores (e.g. scales or aphids) (Schultz 1983). The out come is the same for both strategies: reduced insect growth.

Since its introduction to North America over 75 years ago, the European corn borer (ECB), *Ostrinia nubilalis* (Hübner) (Lepidoptera: Pyralidae), has been extensively researched and germplasm with resistance has been released, e.g. the synthetic BS9 (Russell and Guthrie 1982). Leaf resistance to first generation ECB has been attributed largely to 2,4-dihydroxy-7-methoxy-2H-1,4 benzoxazin-3 (4H)-one (DIMBOA) which affects ECB feeding (Robinson et al. 1978) as well as growth and development of larvae by noncompetitive inhibition of digestive proteases (Houseman et al. 1992; Campos et al. 1989). Other resistance factors that have been studied less extensively include silica, lignin and fiber content which may act by reducing the nutritional quality of the leaf or increase tissue toughness and thereby rendering nutrients less accessible (Buendgen et al. 1990; Rojanaridpiched et al. 1984).

The secondary compounds most studied in maize have been the hydroxamic acids, and a few studies did investigate their temporal and spatial distribution. The hydroxamic acids are known to decline with plant age (Morse et al. 1991; Gutierrez et al. 1988; Guthrie et al. 1986a), but these changes have not been related to insect performance on tissue of varying ages. Plant interaction studies using aphids and lepidopteran larvae have shown induction of hydroxamic acid production during insect feeding (Niemeyer et al. 1989; Gutiérrez et al. 1988). Hydroxamic acid levels are also influenced by the environment. Greenhouse grown plants have higher levels of DIMBOA than field grown plants but are more susceptible to leaf feeding damage by the ECB (Guthrie et al. 1986b). New mechanisms for maize resistance to ECB leaf feeding have recently been proposed that are based on phenolic acid - cell wall carbohydrate complexes that act by increasing the mechanical strength of the leaf (Bergvinson et al. 1994a). One mechanism that has been shown is ultraviolet (UV) light which influenced field resistance by facilitating the formation of cyclobutane dimers of phenolic acids esterified to cell wall hemicellulose (Bergvinson et al. 1994b). A second mechanism involved the action of peroxidase in the presence of peroxide to form dehydrodiferulic acid (Bergvinson et al., these Proceedings).

The objective of this study was to demonstrate the importance of spatial and temporal variation of biochemical resistance factors in maize leaf tissue, using the leaf feeding resistance to ECB as a model system. Resistance factors investigated included foliar nitrogen content, leaf toughness, fiber content, soluble and cell-wall-bound hydroxycinnamic acids, hydroxamic acids and cell wall absorbance using a microspectropho-tometer to localize cellular biochemical resistance factors. Biochemical changes over space and time also extend to many other tissues in maize. Results from ear rot studies for *Fusarium* sp. will be used to illustrate temporal changes in biochemical resistance factors of reproductive tissue (silk and kernel) which may find application to pests such as the corn ear worm (CEW), *Helicoverpa zea* (Boddie). Although the environment influences biochemical mechanisms in maize resistance (Bergvinson et al. 1994b) it will not be discussed in this paper. The main objective is to illustrate the dynamic changes in biochemical content that occur within the plant for a given tissue during the course of development (both vegetative and reproductive tissue) and the windows of resistance or susceptibility that result from these changes.

Materials and Methods

Germplasm

The maize synthetic BS9(CB)C4 was used which has the following inbreds in its genetic background: B49, B50, B52, B54, B55, B57, B68, CI31A, Mo17, and SD10 (Russell and Guthrie 1982).

Plant maturity and leaf profile study

BS9 (C4) was planted in mid-May of 1992 at the Plant Research Center, Agriculture Canada, Ottawa, Ontario, Canada. The rows were spaced 0.9 m and plants spaced 0.15 m apart with 30 plants per row. The soil was a sandy loam. Plants were harvested at full leaf extension for the 3, 5, 7, 9, and 10 leaf stages of development. Leaf tissue from several plants was bulked to obtain a minimum of 10 g fresh weight of tissue for each of three replicates. To study the variation in resistance along the length of the leaf, the 13th leaf from plants at the 14th leaf stage in development was harvested according to a previously reported method (Bergvinson et al., these Proceedings). For this study the leaf was aligned with other leaves and portioned into eight equal zones, with a minimum of 10 g wet weight per zone per replicate and three replicates per zone. The midribs were removed from all samples prior to processing, the tissue was placed in perforated paper bags, frozen at -20° C, and lyophilized. Samples were milled on a Wiley® mill

fitted with a 1 mm mesh screen. Milled samples were stored at -20ºC until analyzed.

Insect bioassay

Leaf tissues described above (time study and leaf profile) were incorporated into a bioassay apparatus illustrated in Figure 1. The percent consumption of a 1.2 cm^2 disk by two, third-instar ECB larvae in a 48 h period was determined. The bioassay apparatus consisted of three halves of plastic Petri plates (6 cm dia.) with the bottom plate having a 1.2 cm diam. hole. The top plate is inverted and wet cotton is placed inside and covered with Whatman #1 filter paper. Leaf tissue is placed with the top surface down onto the filter paper as the under-surface of the leaf is the preferred substrate in the field. The bottom plate with the hole is centered onto the leaf to expose the feeding surface. Plates were taped together and two early third-instar larvae were placed into the apparatus. Neonate ECB did not feed sufficiently to provide accurate consumption measurements. Third-instar larvae that molt during the bioassay are also not desirable due to purging of the gut prior to molting which interferes with the bioassay. A small plastic covering was placed inside to shade the larvae and the third plate was taped over the top to seal the larvae into the apparatus. Larvae were reared and tested under a 16:8 (L:D) photoperiod at 85% R.H. and a 25°C/19°C (L:D) temperature. Area consumed was measured using 1 mm2 graph paper. Forty bioassays were performed for each leaf stage and growth environment.

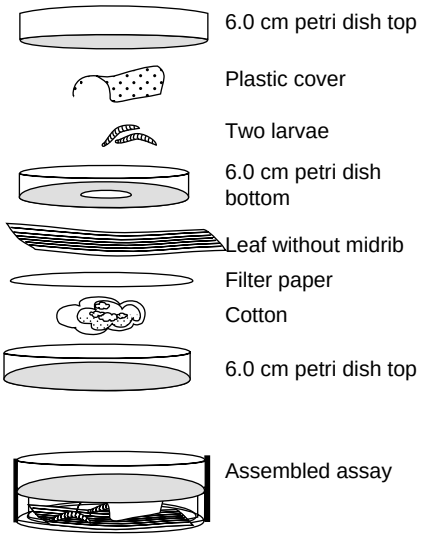

Figure 1. Bioassay apparatus used to study localized feeding on maize leaf tissue by the European corn borer. Plastic Petri dishes are modified by making a 1.2 cm diam. hole in the Petri dish bottom. Top of Petri dish is inverted and fitted with a wet ball of cotton and filter paper to keep the leaf moist. Leaf tissue is oriented with the top surface facing down. The bottom of the Petri dish is secured with tape to expose only a portion of the leaf. Two third-instar ECB larvae are added and covered by a plastic shelter to enhance feeding. Another top to a Petri dish is secured by tape to seal larvae into the apparatus.

Nitrogen determination

An automatic micro-Kjeldahl nitrogen analyzer (Tecator model 1030, Höganäs, Sweden) was used to determine percent nitrogen and estimate protein content of 0.3 g dry wt. leaf samples using the conversion factor 6.25 for estimating percent protein from nitrogen content.

Leaf toughness

Fifteen leaf samples were taken from each leaf stage of development and from each leaf section for the leaf profile study. The protocol for the Instron has been described (Bergvinson et al., these Proceedings).

Microspectrophotometer

A computer-controlled Zeiss UMSP-80 microspectrophotometer equipped with a high-pressure xenon lamp (XBO 75W) and a series connecting grating monochromator (bandwidth 10 nm) was used to measure transmittance spectra at wavelengths between 230 and 350 nm (5 nm steps). This microscope can emit a specific wavelength of light that is then absorbed by particular groups of phytochemicals based on differential absorbance spectra. Phenolics have a strong absorbance at 280 nm in addition to specific absorbances of 311 nm for p-coumaric acid and 326 for ferulic acid (see Bergvinson et al., these Proceedings for structures). Sections for microspectrophotometry (8 µm thick) were cut from leaf tissue, embedded in ice and mounted on quartz slides which do not absorb ultraviolet light. The microscope was fitted with a 100X ultrafluar Zeiss quartz objective and a 10X ultrafluar Zeiss quartz condenser lens. The measuring aperture placed over the middle of the cell wall was 0.32 mm, which provided a measuring field diameter of 2 µm. The microspectrophotometer was adjusted for 326 nm which gave a high signal to noise ratio for taking readings of epidermal cell walls. More details of the microspectrophotometer technique have been reported in Bergvinson et al. (1994c).

Phytochemical analysis

The phytochemical analyses has been previously described (Bergvinson et al., these Proceedings).

Extraction and quantification of silk waxes

Preliminary work using the scanning electron microscope identified silk waxes as a possible mechanism for resistance to ear rot, *Fusarium graminearum.* A time study was conducted on one resistant (CO272) and two susceptible (CO265, CO266)

inbred lines to relate changes in silk wax composition, from the time of first silk emergence till 12 days post emergence, to observed field resistance by artificial fungal inoculation (Hamilton et al., these Proceedings). The husk was peeled and the silk removed at 2, 4, 6, 8, 10, and 12 days post silk emergence. A second study was conducted to investigate changes in wax composition along the silk length of a resistant inbred (CO272) and two commercial hybrids (resistant Pride K127, susceptible Dekalb 435). Samples were stored at -20ºC until processed. Silk waxes were extracted from 1.5 g fresh weight samples of silk with 2 x 3 ml of chloroform. Each sample was mixed in a vortex mixer for 5 s and then decanted into a clean vial. Chloroform was purged with nitrogen and the dry sample was stored at -20ºC until analyzed by gas chromatography. Wax analysis was done on a Varian 3400 gas chromatograph with a flame ionization detector (FID) and a Varian model 8100 autosampler. A 15 m x 0.53 mm ID column was packed with 0.1 µm film of SPB-1 (Supelco, Bellefonte, PA. A 25 min. temperature gradient program starting at 120ºC and increasing at 5ºC/min. to 220ºC and holding at 220ºC for 5 min was used to separate wax components. The flow rate was 24 ml He/min. Eicosene (C20) was used as an internal standard. Routinely, 45 samples can be extracted and analyzed per day.

Statistical analysis

All statistical analyses were performed on SAS V. 6.03 (SAS, 1988). Data were transformed to satisfy the assumptions of the general linear model. Analysis of variance (ANOVA) was used to determine significant differences in biochemical factors for different stages in plant development ($P < 0.05$). The Student-Newman-Keuls (SNK) test was used to compare means between different plant development stages. Regression analyses between larvae consumption and phytochemical parameters were done using the mean values for each of the eight leaf sections for the profile study.

Results and Discussion

Recently eclosed ECB larvae generally move towards the center of the whorl during day light hours (unpublished data). Similar observations have been reported for other stem borers such as *Chilo partellus* (Swinhoe) (Lepidoptera: Pyralidae) in which 95 to 100% of live larvae are within the whorl (Ampofo and Kidiavai 1987). A possible explanation for this behavior is avoidance of the hot, dry micro-environment on the exposed whorl leaves, which can desiccate neonate larvae. This explanation is supported by the fact that egg mortality is higher at lower relative humidities (Lee 1988) and larval mortality often exceeds 80% during the first 48 h after eclosion (Ross and Ostlie 1990).

A profile of leaf consumption by ECB on BS9(C4) is depicted in Figure 2. ECB larvae show the highest consumption rate on immature leaf tissue within the whorl (sections 6 to 8). The leaf section with the lowest consumption was at the point where the leaf subtends from the whorl (section 4). By conducting leaf feeding bioassays in growth chambers the effect of relative humidity over the leaf length is controlled and the degree of feeding on mature tissue is obviously lower even when relative humidity is not a restricting factor. This suggests that factors other that micro-environment are influencing larval preference for feeding within the whorl of maize.

Other parameters thought to be associated with feeding behavior are shown in Figure 3. Protein was lower for the sections around the green-yellow interface of the leaf (section 4), reaching a level as low as 17%. Given the low protein levels at this location along the leaf length, one would expect consumption to be higher for this tissue so as to fulfill nutritional requirements for development (Scriber and Slansky 1981), but this was not observed during the 48 h bioassay (Fig. 2). Soluble metabolites washed from milled leaf tissue included sugars, soluble proteins, chlorophyll, phenolic conjugates such as flavonoids and hydroxycinnamic acids, and hydroxamics such as DIMBOA. The trend for soluble metabolites is similar to leaf consumption (Figs. 2 and 3). Phenolic conjugates of maize have been shown to be phagostimulants (Bergvinson 1993) and may account for higher consumption as the level of sugar conjugates of p-coumaric acid are higher for immature whorl tissue (Fig.

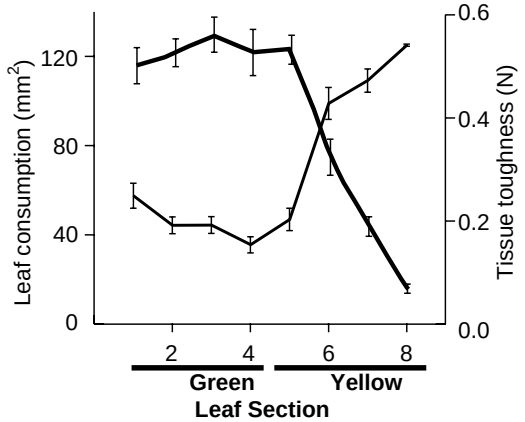

Figure 2. Line graphs of BS9 leaf toughness profile (dashed line) in relation to leaf consumption (solid line) using the bioassay illustrated in Figure 1. Force is measured in newtons (N). n=4 for each leaf section.

3). Other soluble secondary metabolites such as ferulic acid conjugates or HMBOA fluctuate and showed no consistent trend (data not shown).

DIMBOA was found to be at the highest levels within the yellow whorl tissue which was also the most preferred by ECB larvae (Fig. 3). Based on previous feeding preference studies, the converse would be expected (Robinson et al. 1978). Nutritional studies have shown that DIMBOA incorporated into meridic diet increased larval consumption while reducing the efficiency of nutrient assimilation and various fitness parameters (Houseman et al. 1992). This in part may explain the higher consumption rate of immature, yellow whorl tissue with elevated DIMBOA levels. Elevated levels of DIMBOA did not appear to be a significant deterrent to larval feeding during a 48 h bioassay, but may have affected insect performance through reduced fecundity and prolonged development if feeding was restricted to this tissue throughout larval development (Campos et al. 1989).

The high feeding preference for tissue with elevated levels of DIMBOA can be rationalized by observing the relative absence of physical defense mechanisms in immature whorl tissue. Fiber content in immature tissue is very low (Fig. 3) and the relative absence of phenolic fortification in epidermal cell walls, as demonstrated by staining and low microspectrophotometer readings (data not shown), render nutrients within the leaf more accessible and hence make the tissue more desirable (Scriber and Slansky 1981; Bernays and Barbehenn 1987). The tissue toughness profile found in Figure 1 best illustrates the absence of mechanical resistance factors *vis-à-vis* fiber and hydroxycinnamic acid fortification of cell walls (Fig. 4).

The toughness profile could account for field observations of neonate behavior. Immature whorl tissue would be easier to consume by neonates than tougher, mature leaf tissue. By migrating to the inner whorl, larvae would not only be in a higher humidity micro-environment, but would also be able to easily ingest water and nutrients to avoid desiccation and starvation during early stages of development.

The major cell wall bound phenolic acids are E-ferulic and E-p-coumaric acids which are attached to hemicellulose through pentose sugars (Kato and Nevins 1985). Both phenolic acids reach their highest levels in sections 5 and 6 (Fig. 4). Cell-wall-bound ferulic and p-coumaric acids can form dimers to cross-link cell wall carbohydrates either enzymatically through peroxidase to form 5, 5'-diferulic acid (Hartley and Jones 1976) or through photochemical reactions to form truxillic and truxinic acids (Hartley et al. 1988; Ford and Hartley 1989). From the profiles in Figure 4 no individual biochemical component provided a suitable explanation for leaf consumption or toughness. However, when taken together, hydroxycinnamic acids provide a biochemical explanation for feeding performance, with photodimers cross-linking the hemicellulose of mature tissue to provide structural resistance. For sections 4 through 6, elevated levels p-coumaric, ferulic and diphenolic acids

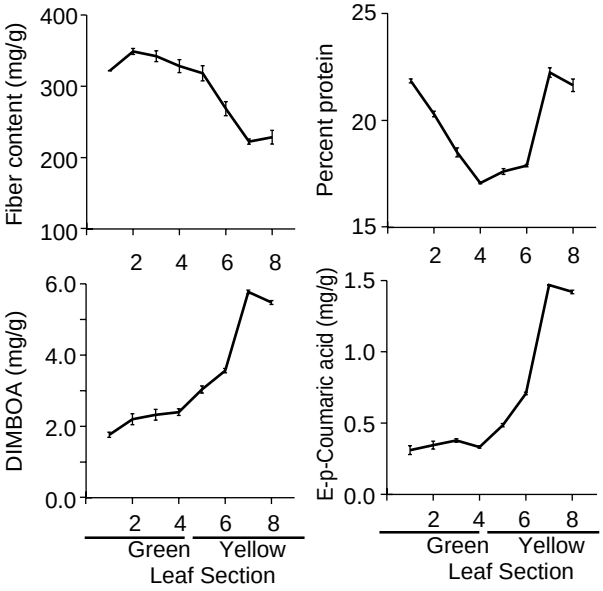

Figure 3. Line graphs of BS9 leaf profile for various biochemical factors that are considered important in host plant resistance. n=4 for each leaf section.

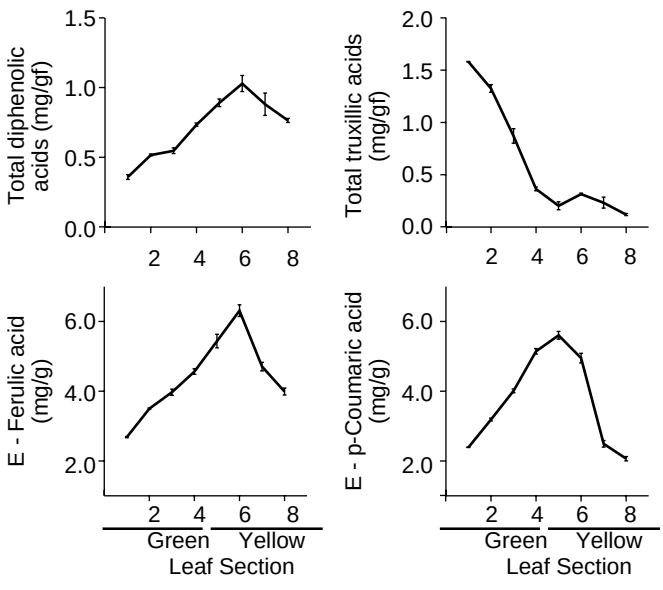

Figure 4. Line graphs of BS9 leaf profile for cell-wall-bound phenolics that are thought to be involved in host-plant resistance by fortifying the cell wall. n=4 for each leaf section.

sustain leaf toughness. For sections 7 and 8 all cell wall phenolics are at their lowest levels, corresponding to increases in leaf consumption (Fig. 1).

Regression analysis of leaf consumption against the biochemical parameters identified three parameters that could account for over 90% of the variation in leaf consumption. These included epidermal cell wall absorbance, toughness and fiber content which are all components or indicators of mechanical resistance. Fiber not only increases the bulk density of the insect's diet to make nutrient and water requirements less attainable (Bernays 1986), but would also increase the substrate for phenolic cross-linking. Acting in concert, fiber and hydroxycinnamic acid fortification in epidermal cell wall tissue could increase leaf toughness of mature leaf tissue. Neonate larvae would then be forced to feed on softer, immature whorl tissue which is defended by high levels of DIMBOA. As the insect matures, its mandibles may be better able to cope with tougher, mature tissue (Bernays, 1986) which has lower levels of DIMBOA. Based on within-leaf variation of feeding preference and biochemical factors, leaf toughness and the biochemical factors responsible for leaf toughness appear to be the predominant factors that influence ECB feeding behavior within maize during the mid-whorl stage of plant development.

When considering the biochemical changes over time and their relation to leaf resistance to herbivorous insects, the same trends that were observed in the profile study are evident. ECB larvae prefer plants younger than the 7th leaf stage (Fig. 5). This preference is demonstrated in artificially infested field plots with *Diatraea saccharalis* in which damage ratings are most severe when infestations are made early in plant development (Maredia and Mihm 1991).

The hydroxamic acid DIMBOA dropped to significantly lower levels after the three leaf stage (Table 1). Since the older leaf stages had lower levels of DIMBOA than the younger leaf stages, it is evident that reduced consumption of older leaves (Fig. 5) cannot be explained by non-preference for DIMBOA. Similar observations have been reported in other greenhouse studies which have made comparisons to field-grown maize (Guthrie et al. 1986b) or to plants grown under elevated artificial light conditions in the greenhouse (Manuwoto and Scriber 1985). From both studies it appears that in addition to DIMBOA, there are other biochemical resistance mechanisms.

Soluble phenolic acids conjugated to various sugars varied but generally showed a reduction with increasing leaf number (Table 1). This trend in soluble phenolic levels has been observed for *Sorghum bicolor* (Woodhead 1981). For some insects, phenolic acids can act as feeding deterrents (Dowd 1990), but based on ECB feeding bioassays it appears that soluble phenolic acids conjugated to sugars act as phagostimulants (Bergvinson 1993).

Protein content dropped significantly from the third to the fifth leaf and then gradually declined with subsequent leaves as the plant aged (Table 1). The highest protein content leaves were subjected to the most feeding, in contrast to low protein content leaves which would have been expected to have elevated feeding to sustain the insect's protein/growth requirements. Gravimetric determination of soluble metabolites provides a crude estimate of sugars, soluble secondary metabolites, proteins and chlorophyll (Table 1). The same trend as for protein

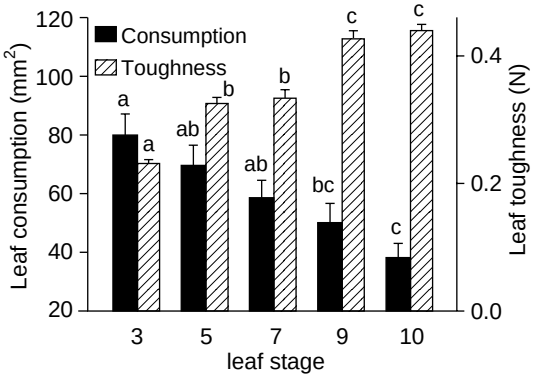

Figure 5. Bar graph of leaf consumption with the leaf bioassay and leaf toughness using the Instron for BS9 leaves at different stages in development. n=30 for the bioassays and n=15 for toughness measurements of each development stage. Bars topped with different letters within the same development stage are significantly different, SNK (P<0.05).

Table 1. Levels of soluble phenolic acid conjugates in BS9(C4) at different stages of development.

Leaf	DIMBOA mg/g dry wt.‡	p-Coumaric acid mg/g dry wt.‡	Ferulic acid mg/g dry wt.‡	Percent protein (dry wt.)	Soluble metabolites (g/g dry wt.)
3	2.96 ± 0.04 a	1.09 ± 0.21 a	0.67 ± 0.10 a	29.2 ± 1.2 a	0.356 ± 0.013 a
5	1.43 ± 0.07 d	0.53 ± 0.02 b	0.73 ± 0.03 a	24.5 ± 0.5 b	0.319 ± 0.009 a
7	2.32 ± 0.10 b	1.06 ± 0.12 a	1.48 ± 0.15 b	23.7 ± 0.1 bc	0.267 ± 0.036 a
9	1.91 ± 0.09 c	0.78 ± 0.03 b	1.55 ± 0.01 b	23.5 ± 0.1 bc	0.248 ± 0.043 a
10	2.03 ± 0.06 c	0.89 ± 0.02 ab	0.92 ± 0.04 a	21.5 ± 0.1 cd	0.283 ± 0.041 a

‡ Means within the same column and treatment followed by the same letter are not significantly different, SNK (P<0.05).

was observed, suggesting that younger leaves provide more accessible nutrients and water to larvae than do older leaves. Fiber content has been hypothesized to increase the bulk density of the insect's diet to the point that insects cannot ingest sufficient nutrients (Peterson et al. 1988). Fiber content did not change significantly within the leaf age range tested and would not account for the observed feeding preferences (Table 2).

The major cell-wall-bound phenolic acids, *E-ferulic and E-p-coumaric acids*, have been studied as digestibility-reducing factors in ruminants (Hartley and Ford 1989) and have been correlated with maize resistance to storage pests (Classen et al. 1990) and to leaf feeding by the ECB (Bergvinson 1993). Both phenolic acids reached their highest levels at the 9- and 10-leaf stages showing levels 2- to 3-fold higher than younger leaves (Table 2), which correlate with the observed reduction in ECB consumption on mature leaves.

Dimers formed by peroxidase (dehydrodiphenolic acids) or through UV absorption (truxillic and truxinic acids) may increase the mechanical strength of the cell wall by cross-linking hemicellulose (Hartley and Ford 1989). Here again a sharp increase in cyclobutane dimers and dehydrodiphenolic dimers occurs at the 9-leaf stage which coincided with reduced insect feeding (Table 2, Fig. 5). Similar results are reported for sorghum, with cyclobutane dimer levels being highest at later stages in plant development (Goto et al. 1991). Lignin content did not differ significantly (SNK, $P > 0.05$) between different development stages (Bergvinson 1993). Buendgen et al. (1990) showed that between different cycles of S1 selection for BS9, lignin content of whorl tissue did not increase as insect resistance increased which is consistent with these results.

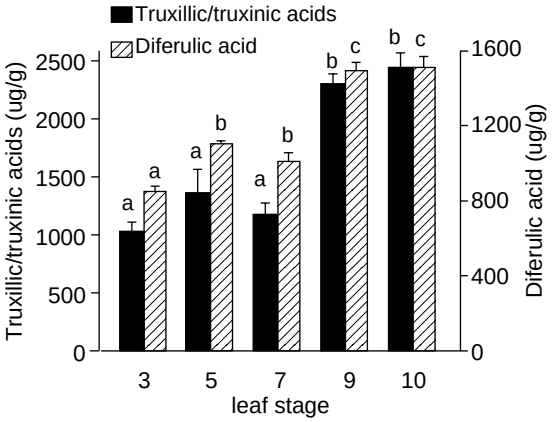

Figure 6. Bar graph of phenolic dimers in BS9 leaves at different stages in development. Truxillic and truxinic acids are dimers produced by ultraviolet light and diferulic acid is produced by peroxidase in the presence of peroxide. Both types of dimers are thought to be involved in cell wall fortification as a host plant resistance mechanism in mature leaf tissue.

The overall impact that cell wall phenolic acid - carbohydrate complexes have on leaf toughness is shown in Figure 5. Leaf stages with the lowest levels of cell wall phenolics have the lowest leaf toughness measurements, with the 3-leaf stage being the softest of all stages. It is hypothesized that leaf toughness *vis-à-vis* phenolic fortification of cell wall tissue is the primary defense for mature maize tissue against the ECB and is likely operating against other lepidopteran pests of maize given the structural nature of the proposed resistance mechanism.

Changes in phenolic acids within the epidermal cell wall can be estimated using the microspectrophotometer (Akin et al. 1990). Absorbance readings showed that the least fortified epidermal cell walls were found at the 3-leaf stage with a dramatic jump in absorbance occurring at the 9-leaf stage of development (Table 2). Based on absorbance changes in the epidermal cell wall this tissue appears to be the site of major phenolic changes. Leaf tissue which is most susceptible to ECB feeding tended to have the lowest epidermal cell wall absorbance and presumably lowest cell wall phenolic content.

From these spatial and temporal studies of resistance mechanisms in the leaf it appears that leaf toughness is of major importance to ECB larvae in controlled bioassays with environmental variability removed from the HPR equation. Larvae consume immature tissue (whether temporal or spatial) at a higher rate than mature tissue. The differences between different tissues of most significance were leaf toughness

Table 2. Levels of cell wall bound phenolic acids in BS9(C4) at different stages of development.

Leaf	µg / g dry weight‡		Epidermal cell wall absorbance at 326 nm
	p-Coumaric acid	Ferulic acid	
3	483 ± 14 a	981 ± 20 a	0.18 ± 0.04 a
5	590 ± 5 a	1229 ± 27 b	0.36 ± 0.08 a
7	615 ± 5 a	1490 ± 14 c	0.41 ± 0.08 b
9	1163 ± 47 b	2057 ± 82 d	0.83 ± 0.07 b
10	1557 ± 66 c	1986 ± 27 d	0.98 ± 0.11 b

‡ Means within the same column and treatment followed by the same letter are not significantly different, SNK ($P<0.05$).

and epidermal cell wall absorbance. An understanding of the variability associated with these biochemical resistance factors of the temporal and spatial changes in HPR to leaf feeding lepidopteran pests will assist in the development of resistant lines.

Temporal and spatial changes in biochemical resistance mechanisms are not exclusive to vegetative tissue. HPR to insect and disease pests of reproductive structures is also subject to changes over space and time. One case in point is silk resistance to the ear rot pathogen, *Fusarium graminearum.* By making morphological comparisons between the resistant inbred (CO272) and susceptible inbreds it was observed that the silk wax deposits on CO272 were much greater. Extracting the waxes in chloroform and analysis by gas chromatography revealed that the composition of silk waxes were less complicated than leaf waxes (Fig. 7) and that CO272 did have elevated levels of wax in comparison with susceptible inbreds (Fig. 8).

Temporal changes in silk wax load was of particular interest in that CO272 showed a peak load which corresponded to the time silks are most susceptible to infection by *F. graminearum* inoculation in the silk channel (Fig. 8). Susceptible inbreds showed an earlier and much reduced peak in wax load which dropped to basal levels at the time of artificial inoculation. This data illustrates that even structural components such as wax — which may be considered static — can change rapidly, and so timing of phytochemical sampling is as important as the time of artificial inoculation. Other biochemical constituents that change with silk maturity include soluble phenolics and flavonoids (Reid et al. 1992); these may also have an impact on the window of pest susceptibility. For example, the flavone glycoside maysin has been shown to inhibit CEW larval growth (Wiseman et al. 1985). By understanding the biochemical changes that are occuring over time, artificial screening techniques can be developed and used to identify a greater genetic variation in resistant and susceptible genotypes.

√	Pentacosane	C_{25}	
√	Heptacosane	C_{27}	
	Octacosane	C_{28}	Alkanes
√	Nonacosane	C_{29}	
√	Hentriacontane	C_{31}	
√	9-Nonacosanol	C_{29}	Alcohols
	11--Hentriacontanol	C_{31}	
	Eicosanal	C_{20}	
	Docosanal	C_{22}	Aldehydes
	Tetracosanal	C_{24}	
√	hexadecanoic acid	C_{16}	Acids
	9, 12 Octadecadienoic acid	C_{18}	

Figure 7. Phytochemical composition of silk wax in maize. Wax composition is largely simple alkanes. Ticks indicate major components in silk wax.

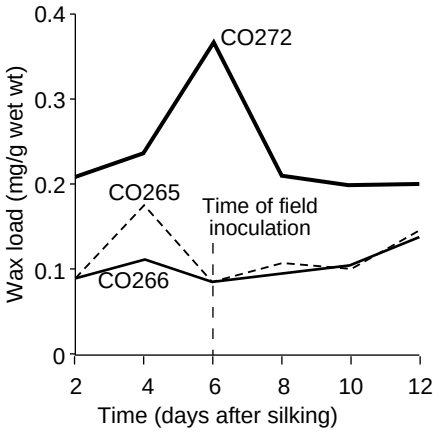

Figure 8. Line graph of the temporal changes in silk wax load for one resistant (CO272) and two susceptible (CO265, CO266) inbreds. The greatest differential between resistant and susceptible lines occurs at 6 days when the wax peaks for the resistant inbred line. n=3 for each sampling date.

As with vegetative tissue, the wax chemistry of the silk changes over its length (Fig. 9). Although no commercial hybrid has been found which matches the wax levels found in CO272, one commercial hybrid (Pride K127) does have moderately high levels, but only at the point where the silk extends outside the husk. If the silk sample were to be taken at the point of attachment to the kernel, the resistant hybrid would be the most susceptible based on wax content. It is essential for all studies on the biochemical mechanisms of HPR to consider the time and location of the sample and that it reflects the interaction which occurs in the field between the pest organisms and the host.

Although silk wax may not be a mechanism for CEW resistance, this study does show the rapid change that occurs in silk biochemistry and which should be considered with reproductive structures. Kernel chemistry has also been investigated in relation to disease resistance and we have found that major changes in soluble and cell wall chemistry occur approximately three weeks post-silking (unpublished data). These changes are

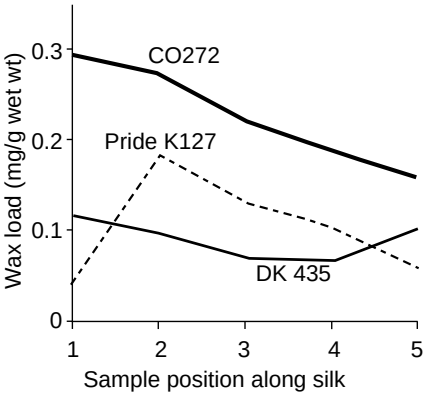

Figure 9. Silk wax profile along the length of the silk channel for one resistant inbred (CO272) and two commercial inbreds (Pride K127 resistant, DK435 susceptible).

most dramatic in the aleurone layer, a factor which may be important when studying HPR to lepidopteran larvae that feed on the ear. Artificial diet supplemented with sorghum panicles at different stages in development was shown to reduce FAW performance (time required to complete development, and larval and pupal weight) when more mature panicles were incorporated into the diet (Wiseman 1986). Similar studies in maize may indicate the time at which biochemical resistance mechanisms are being expressed in maize kernels against FAW and CEW.

From these studies of temporal and spatial changes in biochemical composition, it is evident that maize resistance is toxin-related during early stages of tissue development and structurally-related in mature tissue. The transition from one resistance strategy to another represents a continuum that is illustrated in Figure 10. The immature or young tissue is rather ephemeral and would likely employ qualitative defenses such as DIMBOA or toxic compounds localized in susceptible tissue. As the plant matures and the tissues remain exposed for prolonged periods to pests, more quantitative resistance mechanisms are employed such as the phenolic fortification of cell wall carbohydrates. Consideration of biochemical changes that are occurring within the plant over time and within a given tissue will assist in identifying HPR mechanisms for plant improvement programs in the future.

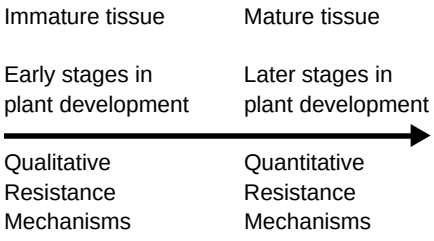

Figure 10. Proposed host plant resistance strategy used by maize during different stages of development. Based on Feeny's (1976) theory of quantitative and qualitative defense, maize tissues appear to employ different resistance strategies depending on tissue maturity and stage of plant development. Changes in defense strategy from qualitative to quantitative defenses represent a continuum with both types of defenses being present but varying with plant/tissue maturity.

Acknowledgments

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Genetic Basis of Silk Resistance (Antibiosis) to the Corn Earworm in Six Crosses of Maize Lines: Statistical Methodology

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Abstract

The genetic basis of resistance (antibiosis) in maize silks to larvae of corn earworm (CEW), Helicoverpa zea *(Boddie), was studied in six crosses of resistant and susceptible inbred lines of maize,* Zea mays *(L). For each breeding line, crosses were made between parental (P1 and P2) lines to produce F1 seed. The F1 plants were selfed to produce F2 seed and backcrossed to each parent to produce BC1 (F1 x P1) and BC2 (F1 x P2) seed. No attempt was made to produce reciprocal crosses since no evidence of significant maternal effects for these crosses existed. Silk from plants of all six generations (P1, P2, F1, F2, BC1, and BC2) was evaluated by recording 8-day weight of CEW larvae fed on a silk diet. A three-parameter additive-dominance model and a six-parameter digenic additive-dominance/ epistatic model were used to analyze generation means by the method of variance-weighted least squares. The genetic control of resistance to CEW larvae was determined in terms of additive-dominance gene action as well as contributions due to epistatic effects of genes at different loci. Results of the simple and joint scaling tests indicated genetic control for silk resistance to CEW, but the gene action differed from one type of cross to another. In the cross Zapalote chico x PI340856, the three-parameter additive-dominance model proved adequate and genes controlling resistance in PI340856 are dominant to those of Z. chico. However, in most crosses, non-allelic interactions were present, thus the fit of the additive-dominance model to the data was considered inadequate.*

Introduction

The biological effects (antibiosis) of resistant corn silks on CEW may be measured in several ways, including:

- Population reduction via mortality of eggs, larvae and pupae (Widstrom et al. 1977; Wiseman et al. 1978, 1983; Wilson et al. 1984) and decreased fecundity over several generations (Wiseman and Isenhour 1990).
- Reduced larval weight (8-10 days after hatching) and pupal weight (Wiseman and Isenhour 1990, 1991; Wiseman and Bondari 1992, 1995).
- Increased larval period (Wiseman and Isenhour 1990).

Although the biological effects on CEW of antibiosis in corn silks are well documented, the genetic basis of resistance remains elusive. According to Widstrom et al. (1992), maize breeders will seldom practice selective breeding in maize with the primary objective of enhancing its genetic resistance to insects, since any progress made will most likely be at the expense of other agronomic traits.

Wiseman and Bondari (1992, 1995) studied the genetic basis of antibiosis to CEW in maize silks from several crosses of resistant and susceptible inbred lines of maize and concluded that the additive-dominance three-parameter model may not accurately describe the underlying genetic control in most cases. The authors further concluded that in several crosses the genetic resistance was complex and perhaps controlled by several pairs of genes at different loci.

The primary objective of this study was to determine the genetic basis of antibiosis resistance via the 8-day weights of CEW larvae fed on silk diet (dry silk mixed in diluted pinto bean diet). The study also determined the relative importance of additive and non-additive genetic effects. The results should have important implications, contributing among other things to

reduced CEW damage and a reduced need for pesticides to control CEW.

Materials and Methods

The study involved six crosses among seven parent lines:

- Ab18 (P1, susceptible silk) x Zapalote chico (P2, resistant silk). Zapalote chico 2451# (PC3) originates from a collection of Z. chico from Mexico maintained in Tifton, Georgia, and Ab18 was used in a cross reported by Wiseman and Bondari (1992).
- GT114 (P1, resistant silk) x GT119 (P2, susceptible silk), both inbred lines recently released (Widstrom et al. 1988).
- Z. chico (P1, resistant silk) x PI340856 (P2, resistant silk). PI340856 (PI) is a popcorn plant introduction that has shown dominance in the F1 with over 20 dent maize inbreds for low larval weight (Wiseman et al. 1992; Wiseman and Bondari 1995).
- Z. chico (P1, resistant silk) x GT114 (P2, resistant silk).
- Z. chico (P1, resistant silk) x CI64 (P2, resistant silk). CI64 was developed from a cereal introduction (Wiseman et al. 1992; Wiseman and Bondari 1995).
- GT3 (P1, susceptible silk) x PI340856 (P2, resistant silk), GT3 was developed in Georgia (Wiseman et al. 1992; Wiseman and Bondari 1995).

In addition to P1 and P2 (parental generations), F1, F2, BC1 (backcross to P1 or F1 x P1), and BC2 (backcross to P2 or F1 x P2) generation seeds from each cross were produced in the maize breeding nursery. Bulk plantings of seed of each generation were made using a completely randomized design to provide sufficient silk for evaluating individual ear samples in a feeding trial using CEW larvae. Two-day-old unpollinated silks (ear covered with shoot bag) were excised from the ear tip of each experimental plant, oven dried at 41°C for about 10 days, ground (1 mm) by using a Cyclotec® 1093 sample mill, and stored at -10°C. Weights of individual larvae were determined after 8 days of feeding on silk diets (for further details see Wiseman and Bondari 1992; Wiseman and Widstrom 1992; Wiseman et al. 1993; Wiseman and Bondari 1995).

Statistical analysis

Generation means and standard errors (SE) of the means for each cross were computed from a one-way analysis of variance using PROC GLM of SAS (SAS Institute Inc. 1989). Within-cross comparisons of generation means were made using the PDIFF option of SAS. Generation means, SE of means, and number of observations associated with each mean were used in the variance-weighted least squares procedure to perform three-parameter and six-parameter scaling tests (Mather and Jinks 1982). Each generation mean was weighted by the reciprocal of the variance of the mean for that generation. Genetic parameters estimated from this procedure were used in genetic models to determine the adequacy of the additive-dominance model for resistance to CEW larvae.

Following the notation of Fisher et al., (1932) and Mather and Jinks (1982), genotypes of AA, Aa, and aa are assigned quantitative phenotypes +d, h, and -d, respectively and the origin of measurement is the mid-homozygote (m) which is the mid-point value from which measurements can be expressed as deviations. These genetic parameters are commonly designated as [d] = net additive deviation and [h] = net dominance deviation. The method of variance-weighted least squares, employing three- and six-parameter models, was used to estimate these parameters from the generation means. The goodness-of-fit of each genetic model was tested by a weighted chi-square (χ^2) comparing observed and expected generation means. The t-test was used to test the significance of each estimated genetic parameter and contrast (linear relationship among generation means). The three contrasts among generation means computed were A = 2BC1 - (P1 + F1) B = 2BC2 - (P2 + F1) C = 4F2 - (2F1 + P1 + P2). Standard errors of these contrasts were computed assuming independence of generation means included in each contrast.

We developed a computer program using several PROCs from SAS to perform generation means analyses, employing both three- and six-parameter models, based on the method described by Mather and Jinks (1982). Other computer programs (e.g., a BASIC program by Mosjidis et al. 1989) are also available to perform these analyses. The method of weighted least squares used in the analysis of generation means has been described by several authors, including Mather and Jinks (1982), Rowe and Alexander (1980), and Beaver and Mosjidis (1988). The procedure uses weights that are equal to the reciprocals of the standard errors of the generation means. The analysis method is based on Fisher (1941), with the average effect of a gene substitution using expected coefficients of the gene effects proposed by Hayman and Mather (1955) and Hayman (1958). For instance, the expected coefficients of gene effects for

the three-parameter model (m=mean, [d]=additive, and [h]=dominance) are presented below:

Gen	Cross	m	[d]	[h]
P1	P1 x P1	1	1.0	0
P2	P2 x P2	1	-1.0	0
F1	F1 x P2	1	0	1.0
F2	F1 x F1	1	0	0.5
BC1	F1 x P1	1	0.5	0.5
BC2	F1 x P2	1	-0.5	0.5

Generation means and appropriate weights (reciprocals of squared standard error of the means) are added to these coefficients to perform the weighted least-squares analysis. The generation means were used as the dependent variable and the coefficients of the genetic parameters as the independent variables. PROC MATRIX (SAS version 5) or PROC IML (SAS version 6) was used to obtain solutions for the unknown parameters. A test of goodness of fit described by Cavalli (1952) and Mather and Jinks (1982) was performed to verify the adequacy of the three-parameter additive-dominance model. For all six crosses, a three-parameter model was fitted first, but even when a good fit was observed, a six-parameter model was also fitted. In the presence of significant epistatic effects, fitting a six-parameter model alone would not provide meaningful estimates of the main effects.

Results and Discussion

The distribution of 8-day weights of CEW larvae over six generations (parents, P1 and P2; F1; F2; and backcrosses, BC1 and BC2) of each cross is presented in Figure 1. The 8-day weights of larvae fed the silk-diet are classified into three groups (<100, 100-200, and >200 mg). Distributions of these weight classes clearly indicate a genetic control of the 8-day larval weight by the host plant. It is evident that variation exists among generations within each cross and that variation exists among crosses possessing various degrees of CEW resistance.

Generation means and their standard errors and the results of the joint scaling test and estimates of gene effects based on a three-parameter additive-dominance genetic model for each cross assuming A,a alleles (m = mean, d = additive gene effect, and h = dominance gene effect) are presented in Table 1. The A, B, and C contrasts among generation means and χ^2 computed for the test of goodness-of-fit of the additive-dominance model are presented in Table 2. These results indicate that:

At least one of the three contrasts and the χ^2 statistic were significant for five of the six crosses (Table 2), indicating that the three-parameter additive-dominance model does not provide an adequate description of genetic control for the 8-day CEW larval weight.

None of the [h], [i], [j], or [l] parameters were significant for the two crosses involving PI as the parental line (Tables 1 and 3). PI340856 is a highly resistant maize line and when crossed with Z. chico, also possessing resistant silk, F1 and P2 (PI inbred line) means do not differ (Table 1). When F1 is backcrossed to PI340856, the BC2 mean does not differ from P2 or F1 (Table 1). Furthermore, [h] is negative and almost of equal magnitude to [d]. These findings indicate that the PI340856 genes controlling silk resistance are dominant to the Z. chico genes and that the three-parameter model seems adequate to predict generation means for this cross.

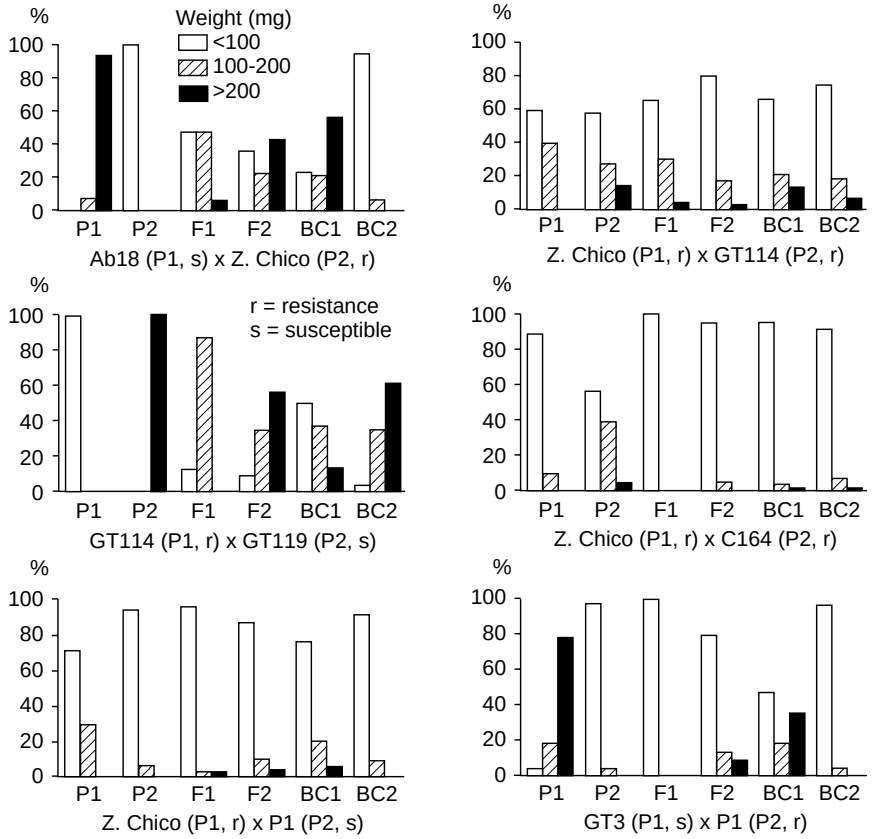

Figure 1. Distribution of eight-day weight of corn earworm larvae.

Results of the second cross involving PI340856 (GT3 x PI340856) differs from the first one. GT3 possesses susceptible silk and the significant B and C contrasts and χ^2 statistic (Table 2) indicate that the three-parameter model is inadequate for this cross. However, the six-parameter digenic model does not detect any significant non-allelic interaction effect (Table 3). Neither F_1 nor BC_2 means differ from the PI340856 mean (Table 1), [h] is negative and similar in magnitude to [d] and, thus, PI genes for this cross also act dominant to GT3 genes, as they did in PI340856 x Z. chico. However, the number of loci involved in the genetic resistance is not clear for this cross; thus the inheritance may involve a different genetic mechanism.

The remaining four crosses involve a significant contrast and a significant χ^2 (Table 2) and some types of non-allelic interactions (Table 3), indicating that genetic control for resistance in these crosses may involve two or more loci. The type of non-allelic interaction varied from one cross to another. For instance, GT114 x GT119 involved an additive x additive genetic effect; Z. chico x CI64 involved additive x dominance non-allelic interaction and more than one interaction effect was significant for Ab18 x Z. chico and Z. chico. x GT114. For these four crosses, both additive and non-additive genetic effects were found to play a significant role in the inheritance of antibiosis resistance in corn silks to CEW larvae.

Although resistance to CEW larvae varied among inbred lines used in these crosses, the biometric genetic procedures employed seemed best suited for crosses of parents quite diverse in degree of resistance. Another important consideration in this study was the ability to control environmental variations. All generations of each cross were grown under as similar environmental conditions as possible and the diet trial was conducted under controlled environmental conditions.

In conclusion, the generation means analysis indicates that resistance to silk-feeding by CEW larvae is under genetic control of the host plant, but gene

Table 1. Number of observations (n), mean corn earworm larval weight, and standard error of the mean (SE) for parental (P1 and P2), F1, F2, and backcross (BC1=F1 x P1 and BC2=F1 x P2) generations of six maize crosses.

	Ab18 x ZC[y]			GT114 x GT119			ZC x PI[y]			ZC x GT114[y]			ZC x CI64[y]			GT3 x PI[y]		
Gen[z]	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE	n	Mean	SE
P1	15	388.9[a]	22.1	15	38.7[d]	3.9	48	72.6[a]	5.9	30	82.2[b]	7.0	54	56.1[b]	5.5	49	358.0[a]	23.7
P2	15	22.5[d]	3.4	15	358.9[a]	12.8	36	38.6[c]	5.3	52	106.7[a]	10.3	45	100.7[a]	8.8	36	43.3[de]	4.6
F1	15	116.9[c]	10.1	15	143.4[c]	9.3	48	35.1[c]	6.5	49	82.2[b]	7.9	50	26.8[d]	2.1	51	60.9[cd]	17.0
F2	78	179.8[b]	14.4	80	244.7[b]	13.5	93	51.6[b]	5.4	97	64.2[b]	5.2	103	33.5[cd]	3.0	101	93.1[c]	12.8
BC1	48	225.2[b]	19.1	48	113.5[c]	9.7	100	65.9[a]	5.6	97	105.3[a]	9.2	87	38.3[cd]	3.8	101	176.8[b]	15.9
BC2	48	36.8[d]	4.9	48	271.2[b]	17.4	84	36.8[c]	7.1	95	75.9[b]	6.6	98	41.7[c]	5.1	94	23.2[e]	2.7

Genetic Effects from Joint Scaling Test (monogenic 3-parameter model)

P[z]	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
m	207.7**	9.4	207.2**	6.2	57.9**	3.6	85.4**	5.3	60.6**	3.5	194.0**	9.5
[d]	188.7**	9.3	-166.0**	6.2	19.4**	3.6	-2.0	5.3	-11.0*	3.9	154.0**	9.1
[h]	-120.2**	12.8	-44.2**	10.8	-17.5**	7.2	-13.0	9.8	-36.2**	4.5	-183.6**	13.0

[y] ZC=Zapalote chico 2451 # PC3, and PI=PI340856.
[a,b,c,d,e] Generation means, within a column, bearing different superscript letters differ (P<0.05).
*,** Estimated genetic parameter significant at the 0.05 (*) or 0.01 (**) probability level.
[z] Parameters are: m = mean, [d] = additive, and [h] = dominance effects.

Table 2. Generation means contrasts (C) from the joint scaling test using the three-parameter additive-dominance model and standard error of the contrasts (SE) for six maize crosses.

	Ab18 x ZCy		GT114 x GT119		ZC x Ply		ZC x GT114y		ZC x CI64y		GT3 x Ply	
C	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
A	-55.4	45.3	44.9*	21.9	24.1	14.2	46.1*	21.2	-6.3	9.7	-65.3	43.2
B	-65.8**	14.5	40.3	38.2	-0.1	16.4	-37.0*	18.5	-44.1**	13.6	-57.9**	18.4
C	74.0	65.0	294.4**	58.7	24.8	26.4	-96.3**	28.9	-76.6**	16.4	-150.6*	66.0
χ^2_3	26.9**		27.4**		3.2		22.3**		24.4**		10.7*	

[y] ZC=Zapalote chico 2451 # PC3, and PI=PI340856.
*,** Estimated contrast (A, B, or C) or chi-square (χ^2) with 3 df significant at the 0.05 (*) or 0.01 (**) probability level.

action controlling resistance may differ from one type of cross to another. Because of the dominance nature of the gene action, genetic resistance associated with the PI340856 inbred line may be easily transmitted to other commercial inbred lines. Using a breeding program that relies on pedigree and backcrossing should result in progress toward breeding maize resistant to CEW larvae. The end result would be reduced CEW damage, enhanced food safety, reduced pesticide use, and more environmentally sound agronomic practices for maize production. Industry has already made progress in transferring the resistant gene from this popcorn line to one of their "elite" dent inbred lines through 3-4 generations of backcrossing. The new inbred has been further crossed with several other inbred lines to produce hybrid combinations without ill effects from genes from the popcorn line. Furthermore, increasing silk resistance to CEW larvae may lead to the enhancement of resistance to some other maize pests or toxins as well.

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Table 3. Estimates of genetic parameters (P) and standard error of the estimates (SE) from a six-parameter digenic model for 8-day weights of corn earworm larvae fed silk diets from six maize crosses.

	Ab18 x ZCy		GT114 x GT119		ZC x Ply		ZC x GT114y		ZC x CI64y		GT3 x Ply	
Px	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE	Estimate	SE
m	401	70.7	409	67.4	57*	28.4	-11	31.4	52*	18.2	173**	61.7
[d]	183**	11.2	-160**	6.7	17**	4.0	-12*	6.2	-22**	5.2	157**	12.1
[h]	-600**	162.2	-390*	162.4	2	69.4	208**	80.8	-50	46.3	-208	137.7
[i]	-195**	69.8	-210**	67.1	-1	28.1	105**	30.7	26	17.5	27	60.6
[j]	10	45.3	6	42.0	24	19.7	83**	25.8	38*	16.5	-7	40.4
[l]	316**	102.2	124	98.9	-23	44.7	-115*	53.7	24	30.3	96	92.4

*,** Genetic parameter estimate differs from zero at the 0.05 (*) or 0.01(**) probability level (t-test).

[x] Parameters are: m = mean, [d] = additive (Add) and [h] = dominance (dom) effects and [i] = add x add, [j] = add x dom, and [l] = dom x dom epistatic effects.

[y] ZC = Zapalote chico 2451 # PC3, and PI = PI340856.

Genetics of Maize Grain Resistance to Maize Weevil

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Abstract

The genetics of maize grain resistance to the maize weevil, Sitophilus zeamais *Motsch., infestation was analyzed by means of additive linear models which considered genetic contributions of maize caryopsis through embryo, endosperm and pericarp. Specific traits associated with these grain tissues were: phenolic acids (pericarp, embryo), proteinase inhibitors (endosperm, embryo) and hardness of grain (pericarp, endosperm, embryo). The susceptibility of the grains to weevil infestation was measured by feeding, consumption and reproductive activities of insect populations. Inbred lines of quality protein maize (QPM), contrasting in resistance to maize weevil infestation, were used for the genetic analysis of resistance. Concentrations of phenolic acids in grain have a highly negative and significant correlation with indices of susceptibility of maize to the maize weevil. However, the correlation between susceptibility of grain and contents of proteinase inhibitors in the endosperm is low, although negative and significant. Resistance of pericarp-testa to compression forces was the only rheological trait of grain inversely correlated with susceptibility of maize to colonization by maize weevils, but neither the correlation coefficient nor the significance was high. The negative relationship of biochemical and biophysical traits of maize grain with feeding and reproductive activities of insects on the grain, suggests detrimental effects of these grain characteristics on the colonization success of insect populations. The estimated genetic parameters for additivity of endosperm and dominance of pericarp associated with the expression of phenolic acid concentration in the grain were highly significant and inversely correlated to estimated susceptibility parameters of genetic action. Estimated parameters of genetic action for proteinase inhibitor concentration in endosperm were non-significant, likewise estimated parameters for rheological traits of maize grains had very low significance.*

Introduction

Quantitative genetic analysis of any seed trait is a difficult task because of the complexity of the seed structure. In maize, as with all cereals, the caryopsis contains seed coat, endosperm-aleurone and embryo tissues which correspond to two different generations. Pericarp belongs to the *n* generation whereas endosperm and embryo represent the *n+1* generation within the grain. Also, there are two types of zygosity in grain: the caryopsis, embryo and seed coat are diploid while the endosperm is triploid. Several models have been proposed, but most of them have oversimplified or ignored the intricate genetic interaction among maternal, cytoplasmic, endosperm and embryo structures (Mather and Jinks 1982; Mosjidis et al. 1989). Although, recent studies (Huidong 1988; Foolad and Jones 1992) have paid more careful attention to the genetics of maternal, cytoplasmic and endosperm variation.

Resistance of maize grain to maize weevil (*S. zeamais* Motsch.) infestation is a trait connected to the whole caryopsis because the selection, colonization, feeding and reproductive activities of the insect take place entirely on and within the maize grains. Adult weevils feed, mate and oviposit on the maize grain, whereas the larvae feed, grow and develop inside the grains. Thus, the genetic analysis of resistance of maize grains to *S. zeamais* infestation, implies the analysis of all grain components.

The role of plant secondary products on plant-insect interactions is well documented (Fraenkel 1959; Dethier

1980; Guthrie and Russell 1989), and many instances of well studied cases of phytochemistry, ecology and biochemistry of plant secondary compounds and their significance to herbivorous insects exist (Berenbaum 1978, 1981; Waiss et al. 1979).

Molecular biology investigations into the mechanisms and modes of action of plant defenses, focusing on regulation of phenylalanine ammonia lyase, which is the key enzyme in the phenylpropanoid pathway, have been undertaken (Lamb et al. 1989; Xiaowu et al. 1989). However, these molecular genetic studies have concentrated on the plant-plant pathogen microorganism interaction.

In maize grain, phenolics are an indicator of resistance to maize weevil infestation (Serratos et al. 1987), and sources of resistance have been traced to "Ancient Indigenous" and "Prehistoric Mestizos" groups of maize landraces containing high concentrations of hydroxicinnamic acids (Arnason et al. 1994).In addition, phenolic acids seem to be related to the resistance of maize to other pests and pathogens (Reid et al. 1992; Xie et al. 1991).

Proteinase inhibitors have often been referred to as protective substances of plants to pathogens and insect pests (Ryan et al. 1986; Broadway et al. 1986; Ryan 1990). The theory of plant defense based upon induced synthesis of proteinase inhibitors, and their action against proteinases of insects, represents a dynamic plant-insect interaction (Ryan et al. 1986; Ryan 1992). The presence of constitutive proteinase inhibitors in dormant tissue (e.g. seed) represents an interesting recent discovery in plant-insect interactions.

Some types of proteinase inhibitors have been described and characterized in maize grain (Blanco-Labra and Iturbe-Chinas 1981; Baker 1982; Richardson et al. 1987), although their effect upon stored grain insect pests have not been well established.

The objective of this paper is to attempt the genetic analysis of maize grain resistance to maize weevil infestation by estimating genetic variation in biochemical and biophysical characters and susceptibility indices of selected genotypes of maize kernels, through three types of linear genetic models.

Materials and Methods

Maize material

Maize generations were derived from controlled crosses between quality protein inbred maize lines, resistant and susceptible to maize weevil infestation, as described in Serratos et al. (1993). These crosses yielded 14 generations as follows: P_1, P_2, F_{11}, F_{12}, BC_{11}, BC_{12}, BC_{21}, BC_{22}, RBC_{11}, RBC_{12}, RBC_{21}, RBC_{22}, F_{21} and F_{22} (Fig. 1). Pools of grains shelled from maize ears harvested at random in entries from 10-row plots represented the generations. Samples of 3 to 5 g from maize ears of

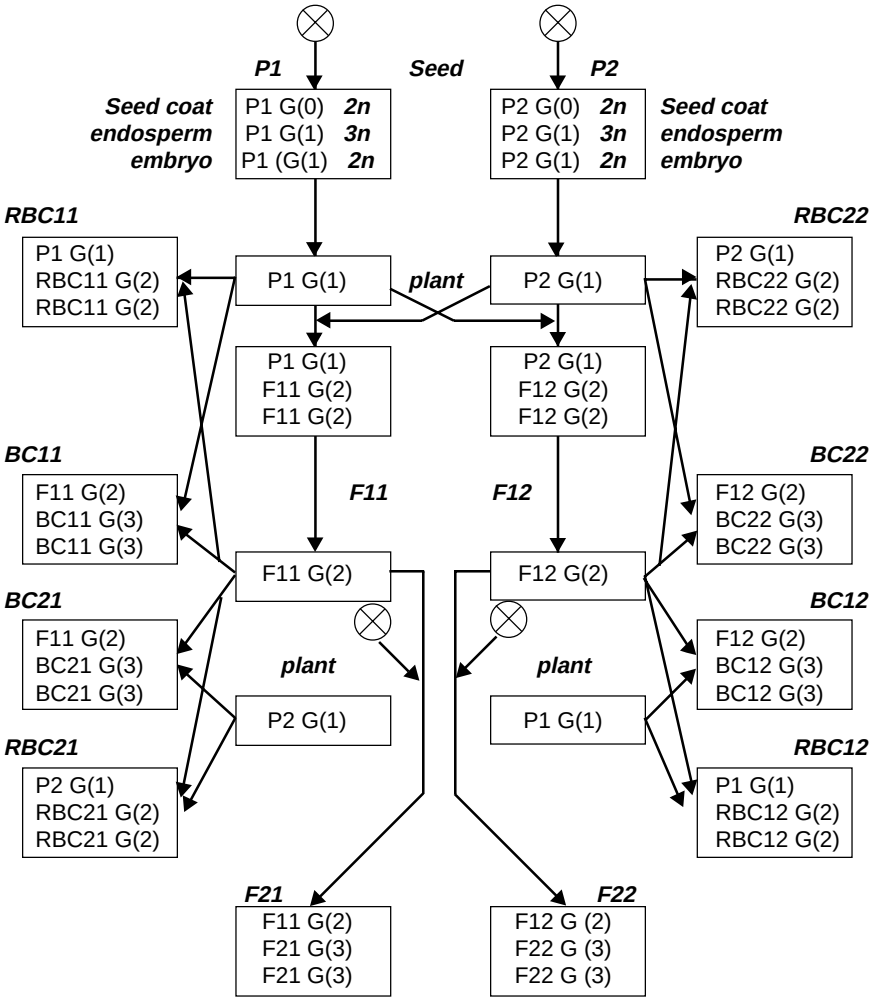

Figure 1. Generations derived from a cross between two inbreed lines of maize differing in resistance to infestation to maize weevil. Caryopsis is represented by the three compartment block. G(*n*) indicates the *n*th generation, while 2n and 3n stands for diploid and triploid zygosity.

each generation were used for biochemical determinations. Fifty grains from each of 5 to 10 ears harvested were used in the analysis of biophysical-hardness of grain.

Biochemical analysis of maize grain traits

Maize grain phenolics - Phenolics were determined gravimetrically by high performance liquid chromatography (HPLC) and quantitative imaging of phenolics was carried out by microspectrofluorimetric methods using a Carl Zeiss UMSP80 microspectrophotometer, as described in Sen et al. (1991), Serratos et al. (1993) and Arnason et al. (1994).

Assay of insect proteinases and maize proteinase inhibitor - Proteinase inhibitor was extracted from 1 g of ground defatted grains. The flour was sieved with a 1 mm mesh sieve and extracted with deionized water at 4°C for 12 hours. The crude extract was used to determine inhibitory activity of insect proteinase inhibitors from grain.

Weevil larvae were collected from infested grain under controlled infestation schedules to obtain 10 g of third-instar larvae. Whole larvae were homogenized in a 0.2 M Succinate buffer solution at pH 4.5 (1.5 p / v) using a Ultra-turrax homogenizer at maximum speed for 1 min at 4°C. Homogenates were clarified by centrifugation at 15,000 rpm for 25 min at 4°C.

Proteolytic and inhibitory activities of proteinases, that function in acid medium, were assayed as indicated in Sandoval (1991). This method requires hemoglobin as a substrate, with 0.2 M citrate buffer at pH 2.5. The extracts of enzymes from weevil larvae and the inhibitor from maize grain were pre-incubated for 10 min, the substrate was then added to this enzyme-inhibitor complex and the mixture incubated at 30 °C for 2 h. The reaction was stopped by adding an alkaline reagent (Sandoval, 1991).

Proteolytic and inhibitory activities were measured using routine spectrophotometric methods in a Beckman DU-50 spectrophotometer. Inhibition was directly correlated to inhibitor concentration for the aliquot tested. Amounts required for 50% inhibition were determined from the linear portion of percent inhibition plots. One unit of enzymatic activity was defined as the amount of enzyme that catalyzed an increase of 0.01 absorption units under the described assay conditions. One unit of inhibitory activity was defined as the amount of inhibitor that inhibited one unit of enzyme activity.

Rheological methods - Rheological characteristics of grain were determined using an universal texturometer, INSTRON (Instron Corp., Canton Massachussets, USA). A compression cell (strainsert 1000 lb) together with a force indicator (Daytronic-3278) integrated to a transducer of mechanical signal (Daytronic 9000) were used. The Texture Program Analysis Software package used to analyze the data was developed at the Institute of Engineering and Food Science, Ottawa Research Station, Central Experimental Farm, Agriculture Canada (Buckley et al. 1984).The 50 kernels from each maize ear were tested individually.

Indices of susceptibility of maize to weevils - Grain samples were prepared as described in Serratos et al. (1993). The index of susceptibility to weevil infestation ($I = 100 \times (\ln F)/D$) was determined as described previously (Dobie, 1974; Classen et al. 1990). Weight loss of grain was the difference in weight of grain samples before and after the infestation of weevils in no-choice trials (Serratos 1987). A parameter of resistance (b) was calculated as described in Serratos (1987). This parameter compares the rate of consumption of grain by insect populations in a confinement test.

Genetic analysis - The estimation of additive and dominance genetic parameters were carried out applying weighted multiple linear regression to three linear genetic models described in Mather and Jinks (1982), Huidong (1988), Foolad and Jones (1992), and modified by Serratos et al. (1993). The matrices of coefficients assigned to the generations for each one of the linear models are described in Table 1. In the present study, the 14 generations derived (Fig. 1) were pooled into 6 generations (P_1, P_2, F_1, BC_1, BC_2, and F_2) for the model of Mather and Jinks (1982). To accomodate the genetic model for expression of endosperm traits as descibed in Huidong (1988), the 14 generations were also pooled into 9 generations (P_1, P_2, F_1, F_{1R}, BC_1, BC_{1R}, BC_2, BC_{2R}, and F_2). Because of missing data for proteinase inhibitor and maximum force of compression of grain, the regression analysis was carried out directly on the generation means of Table 2, together with the coefficients of Table 1. The linear genetic models were as follows:

(1) $p_i = m + [a] + [d]$ (Mather and Jinks, 1982)

(2) $p_i = m + [a] + [d_{e1}] + [d_{e2}]$ (Huidong, 1988)

(3) $p_i = m + [a] + [a_e] + [a_{pc}] + [d_{ee}] + [d_p]$ (Serratos et al., 1993)

In these models p_i is the expected phenotypic value of a generation, m is

the midparent of two homozygous parents, *a* indicates the disomic additive effect, a_e and a_{pc} (equation 3) are additive parameters of the endosperm and pericarp-cytoplasm. The disomic dominance effect is represented by *d*, whereas d_{e1}, d_{e2} (equation 2), d_{ee} and d_p (equation 3) represent, the first and second dominance effect in the endosperm, the main effects of dominance attributed to embryo and endosperm and dominance effects of pericarp, respectively.

Results and Discussion

The generation means of seven traits analyzed for the maize generations specified in each model are summarized in Table 2. The matrix of correlations (Table 3) between grain traits and indices of susceptibility to the maize weevil show that there exists an excellent negative connection for phenolics and proteinase inhibitor concentration with susceptibility of maize to maize weevil. To explore further the relationship between phenolic acids, proteinase inhibitor of grain, and the index of susceptibility of grain to the maize weevil, the estimated values for these variables, as generated by each model, were plotted in three dimensional graphs as shown in Figure 3. The values in the graphs were smoothed by means of an inverse regression function to represent a response surface of maize generations with different levels of resistance, as related to different concentrations of phenolic acids and proteinase inhibitor.

Combining the values in Table 2 and the matrix of coefficients for each model, genetic parameters were estimated using weighted multiple linear regression. The estimated genetic parameters for each model are shown in Table 4. All three models adequately describe the observed results since more than 90% of variation in each model is explained by the regression and F ratios are significant. The estimated *m* values for all variables are highly significant for all models. With Mather and Jinks (1982) (MJ) and Huidong's (1988) (HU) models, estimated additive parameters are significant for phenolics, proteinase inhibitor, and the three indices of susceptibility to maize weevil infestation, whereas rheological traits of grain were non-significant. Estimated parameter of additivity of endosperm for phenolics in grain was the only significant additive parameter in the Serratos et al. (1993) (SE) model. On the contrary, none of the dominance parameters for all variables in either MJ or HU models were significant, whereas dominance of endosperm-pericarp for phenolics in grain, and dominance of pericarp for phenolics, maximum force of compression and index of susceptibility were highly significant in the SE model (Table 4).

The physiological and metabolic processes occurring during development of seeds have an enormous impact on the presence and accumulation of metabolites such as phenolics and proteinase inhibitors. The enzymes producing and accumulating these substances in the different tissues of the grain are coded by their specific

Table 1. Matrix of coefficients used with multiple linear regression to estimate parameters of genetic action for 3 linear genetic models. Parameters of genetic action are specified in the materials and methods section.

(Mather and Jinks, 1982)

Generation	Crosses	m	a	d
P_1	P_1 self	1	1	0
P_2	P_2 self	1	-1	0
F_1	$P_1 \times P_2$	1	0	1
BC_1	$F_1 \times P_1$	1	1/2	1/2
BC_2	$F_1 \times P_2$	1	-1/2	1/2
F_2	F_1 self	1	0	1/2

(Huidong, 1988)

Generation	Crosses	m	a	d_{e1}	d_{e2}
P_1	P_1 self	1	1 1/2	0	0
P_2	P_2 self	1	-1 1/2	0	0
F_1	$P_1 \times P_2$	1	1/2	1	0
F_{1R}	$P_2 \times P_1$	1	-1/2	0	1
BC_1	$F_1 \times P_1$	1	1/2	0	1/2
BC_{1R}	$P_1 \times F_1$	1	1	1/2	0
BC_2	$F_1 \times P_2$	1	-1/2	1/2	0
BC_{2R}	$P_2 \times F_1$	1	-1	0	1/2
F_2	F_1 self	1	0	1/4	1/4

(Serratos et al., 1993)

Generation	Crosses	m	a	a_e	a_{pc}	d_{ee}	d_p
P_1	P_1 self	1	1	1	2	0	0
P_2	P_2 self	1	-1	-1	-2	0	0
F_{11}	$P_1 \times P_2$	1	0	1/3	2	2	0
F_{12}	$P_2 \times P_1$	1	0	-1/3	-2	2	0
BC_{11}	$F_{11} \times P_1$	1	1/2	1/3	1	1	1
BC_{12}	$F_{12} \times P_1$	1	1/2	1/3	-1	1	1
BC_{21}	$F_{11} \times P_2$	1	-1/2	-1/3	1	1	1
BC_{22}	$F_{12} \times P_2$	1	-1/2	-1/3	-1	1	1
RBC_{11}	$P_1 \times F_{11}$	1	1/2	2/3	2	1	0
RBC_{12}	$P_1 \times F_{12}$	1	1/2	2/3	2	1	0
RBC_{21}	$P_2 \times F_{11}$	1	-1/2	-2/3	-2	1	0
RBC_{22}	$P_2 \times F_{12}$	1	-1/2	-2/3	-2	1	0
F_{21}	F_{11} self	1	0	0	1	1	1
F_{22}	F_{12} self	1	0	0	-1	1	1

genes. In this sense, enzymes catalyzing phenolics or proteinases are the same regardless of the grain tissue. However, endosperm, embryo or pericarp have different metabolic environments which imply different substrate concentrations and differences in activities and inductions for the catalytic activities of these enzymes — all of which necessarily affects the additive and dominance behavior of polygenes. In this context, the lack of significance for most of the dominance parameters of biochemical traits with all models should be considered with some caution.

In conclusion, although the information generated in this report contributes to a better design and efficiency of plant breeding strategies, due to the estimation of genetic parameters useful for plant breeders, it should be emphasized that more detailed molecular and biochemical knowledge of maize mechanisms of resistance is required.

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Table 2. Mean values of maize kernel traits grouped according to generations used for genetic analysis of three linear models. All the F ratios from the analysis of variance for all variables are significant at P < 0.001.

Generations	Phenolic acids [μg/g]	Proteinase inhibitor [PIU/g]	Maximum force of compression [Newtons]	Time of resistance to breakage of seed coat [seconds]	Index of susceptibility [I]	Weight loss of grain [grams]	Parameter of resistance [b]
(Mather and Jinks 1982)							
P_1	404.50	187.24	92.63	.958	9.51	1.78	0.56
P_2	54.39	76.67	126.08	.717	13.28	5.59	1.20
F_1	146.83	100.00	132.88	.772	11.59	4.32	0.94
BC_1	361.92	197.92	180.47	.607	11.11	4.54	0.99
BC_2	184.72	119.63	174.02	.562	11.94	4.93	1.08
F_2	292.85	164.06	158.55	.813	10.27	4.51	0.99
(Mo Huidong 1988)							
P_1	404.50	187.24	92.63	.958	9.51	1.78	0.56
P_2	54.39	76.67	126.08	.717	13.28	5.59	1.20
F_1	201.86	111.39	146.14	.828	10.40	4.58	1.00
F_{1R}	91.81	65.83	119.61	.717	12.78	4.06	0.89
BC_1	353.45	161.83	209.59	.481	11.01	4.46	0.97
BC_{1R}	369.97	258.06	125.95	.783	11.21	4.44	0.98
BC_2	243.05	123.50	185.97	.591	11.08	4.12	0.91
BC_{2R}	111.80	114.79	159.08	.524	13.02	5.94	1.29
F_2	292.85	164.06	158.55	.813	10.27	4.51	0.99
(Serratos et al. 1993)							
P_1	404.50	187.2	92.63	.958	9.51	1.78	0.56
P_2	54.39	76.7	126.08	.717	13.28	5.59	1.20
F_{11}	201.86	111.4	146.14.	.828	10.40	4.58	1.00
F_{12}	91.81	65.8	119.61	.717	12.78	4.06	0.89
BC_{11}	337.67	177.5	203.38	.478	11.14	5.03	1.11
BC_{12}	373.18	138.3	217.36	.484	10.86	3.73	0.81
BC_{21}	247.36	132.5	173.05	.695	10.36	4.00	0.88
BC_{22}	238.73	110.0	198.89	.488	11.80	4.23	0.93
RBC_{11}	369.97	258.1	125.95	.783	11.21	4.44	0.98
RBC_{21}	99.64	78.3	162.90	.454	13.07	6.18	1.34
RBC_{22}	132.08	151.3	152.73	.642	12.94	5.52	1.21
F_{21}	280.03	155.4	162.81	.953	10.32	4.61	1.02
F_{22}	305.68	172.7	154.3	.673	10.22	4.41	0.97

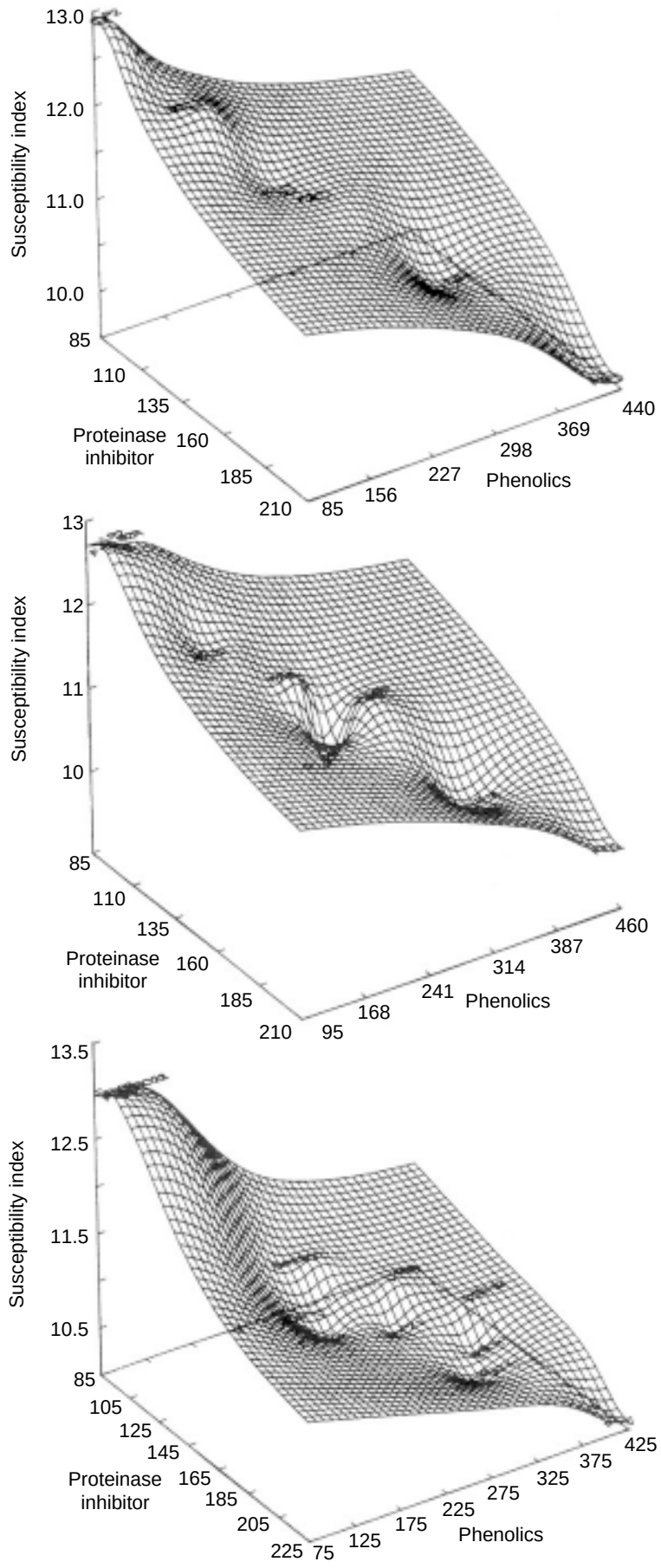

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Figure 2. Three dimensional graph of the estimated values of phenolic acids, proteinase inhibitor and index of susceptibility, obtained applying three linear genetic models. a) model of Mather and Jinks (1982); b) model of Huidong (1988); c) model of Serratos et al. (1993). Axis X represents phenolic acid concentration [μg/g], axis Y is the concentration of proteinase inhibitor [PIU/dg], and in axis Z the estimated values of susceptibility index have been plotted [I].

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Table 3. Matrix of correlation coefficients for biochemical and biophysical traits of the maize kernel against indices of susceptibility to maize weevils for three genetic models. Correlation coefficients were obtained using data from Table 2.

	Model[a]	Phenolic acids [μg/g]	Proteinase inhibitor [PIU/g]	Maximum Force of compression [Newtons]	Time of resistance to breakage of seed coat [seconds]
Index of susceptibility [I]	MJ	- 0.901**	- 0.830**	0.230	- 0.620*
	HU	- 0.844**	- 0.590*	0.001	- 0.524
	SE	- 0.823**	- 0.564*	- 0.030	- 0.467
Weight loss of grain [g]	MJ	- 0.738*	- 0.605*	0.632*	- 0.752*
	HU	- 0.653*	- 0.366	0.413	- 0.602*
	SE	- 0.655*	- 0.340	0.268	- 0.462
Parameter of resistance [b]	MJ	- 0.750*	- 0.619*	0.603*	- 0.747*
	HU	- 0.642*	- 0.340	0.363	- 0.577*
	SE	- 0.648*	- 0.306	0.194	- 0.421

[a] Abbreviations are: MJ (Mather and Jinks 1982); HU (Huidong 1988); SE (Serratos et al. 1993)

Table 4. Estimated genetic parameters using three linear genetic models. ** indicates significance at P < 0.001; * indicates significance at P < 0.05. The values without asterisk are non significant.

Estimated genetic parameters	Phenolic acids [μg/g]	Proteinase inhibitor [PIU/g]	Maximum force of compression [Newtons]	Time of resistance to breakage of seed coat [seconds]	Index of susceptibility [I]	Weight loss of grain [grams]	Parameter of resistance [b]
(Mather and Jinks 1982)							
m	261.80**	147.68**	126.97**	.787**	11.26**	3.92**	.92**
a	175.48**	59.88*	-12.09	.105	-1.68**	-1.60**	-.27**
d	-50.25	-16.23	41.13	-.116	.06	0.87	.10
(Huidong 1988)							
m	277.27**	162.05**	135.31**	.745**	11.30**	4.07**	.95**
a	118.75**	44.75*	-8.49	.070	-1.05**	-0.97**	-.17*
d_{e1}	-80.68	-33.04	27.15	-.002	-0.37	0.99	.12
d_{e2}	-84.54	-53.64	19.89	-.129	0.75	0.28	.0
(Serratos et al. 1993)							
m	251.26**	160.96**	119.19**	.777**	11.60**	4.15**	-.97**
a	-33.58	6.21	-7.71	.051	1.16	0.69	.18
a_e	224.67**	42.56	7.37	-.111	-2.33	-2.45	-.52
a_{pc}	-8.76	7.40	-5.22	.080	-0.18	0.21	.05
d_{ee}	-41.30**	-21.67	11.76	-.033	0.10	0.32	.03
d_p	87.15**	8.45	54.01**	-.116	-0.91**	-0.13	-.05

Improving Two Tropical Maize Populations for Resistance to Stunt Complex

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Abstract

Caused by mycoplasmas, spiroplasmas, and maize fine stripe virus, maize stunt complex is endemic throughout the tropical lowlands of Central America and poses a potential danger for maize production in the region. To counteract the damaging effects of the disease in commercial maize plots, the Regional Maize Program for Central America and the Caribbean (Programa Regional de Maíz, PRM/CAC) has undertaken a collaborative stunt resistance breeding project, with the principal objective of developing high yielding, disease resistant cultivars. A tropical late white dent population (Pop. 73) and a tropical intermediate white flint population (Pop. 76), in their fifth and third improvement cycle, respectively, are being improved using an S_1-S_2 recurrent selection scheme. Research conducted independently in El Salvador and Nicaragua is aimed at developing S_1 lines, advancing them to S_2, recombining the best segments of each population, and forming experimental synthetic varieties. Lines are evaluated in both countries during normal crop cycles under heavy disease pressure. Lines developed each cycle are tested in countries in the region facing stunt problems. Synthetics developed during the latest breeding cycles (SC3P73 N, SC2P76 N and SC3P73 R) out-yielded resistant cultivar NB-6 by 15.5%, 11.7%, and 17% respectively. A variable percentage (1-20.5%) had fewer stunted plants and ears. In disease free environments, performance of resistant cultivars was statistically similar to that of susceptible high yielding hybrids used as reference checks. Resistant cultivars show outstanding performance under disease pressure in less favored environments, without any loss in yield potential in favored ones.

Introduction

One of the most devastating maize diseases, stunt is a production constraint in tropical and subtropical environments of the American continent. It is found in areas situated from sea level to mid- and high altitudes, between 40º N to 30º S latitude (De León, 1981). In Central America and the Caribbean, the disease can reach critical levels of incidence, principally in regions where farmers sow local varieties with low input levels, and where climatological conditions such as low rainfall, high temperatures, and low relative humidity favor development of the disease vector.

The effects of stunt on commercial maize plots were quantified for the first time in Nicaragua in 1986. That year, area lost or partially affected totaled 27,682 ha; the foregone grain (not produced on this area) was 29,445 tons, equivalent in economic terms to a loss of US$5,005,700 (DGB-MIDINRA 1986).

In regions where stunt is endemic, the risk of loss increases when farmers delay planting due to a late-starting rainy season. Disease resistant cultivars must be planted to counteract the detrimental effect of the disease on commercial maize cropping and to ensure sustainable production.

Because of this problem, in 1975 the CIMMYT Maize Program and the national maize programs of Nicaragua and El Salvador initiated a collaborative breeding project aimed at developing stunt resistant cultivars. Comparing the stunt response of three selection cycles with cycle 0 at sites in Nicaragua and El Salvador, average reductions of 16, 28, and 19% were observed in the number of stunted plants in populations 73, 76, and 79, respectively (De León et al. 1984).

Collaborative efforts begun in 1975 led to the 1984 release in Nicaragua of variety NB-6 (Santa Rosa 8073), released subsequently under the name Lujosa B-101 in Honduras and Santa Rosa in Mexico and Venezuela (Córdova et al.1986). Reports from Nicaragua indicate that NB-6, planted

on 2,000 ha, yielded 3.5 t/ha, whereas stunt susceptible hybrids yielded only 1.5 t/ha (Urbina 1991).

Obvious progress has been achieved in breeding for stunt resistence using an S_1-S_2 recurrent selection scheme. Therefore in the second improvement phase, begun in 1985 by the PRM/CAC, the same methodology with certain variations is being used in the short term to:

- Eliminate or reduce the frequency of deleterious recessive genes in breeding populations;
- Increase the frequency of favorable alleles involved in stunt resistance.
- Develop high yielding stunt resistant cultivars.

Materials and Methods

The second phase of the collaborative stunt resistance breeding project was re-initiated by the PRM/CAC in 1985 in El Salvador and the Dominican Republic, with Nicaragua joining in 1986.

Germplasm

This phase began with two white and two yellow populations, but this paper refers only to white populations improved in El Salvador and Nicaragua. Both populations were formed based on S_1 lines derived from the following experimental varieties:

Pop. 22 (Bulk Tropical White)	Pop. 73 (Tropical Late White Dent)
Across 8222	Cuyuta 8073
Los Baños 8222	Porrillo 8073
Los Baños (1) 8222	Santa Rosa 8073
Gwibi(1) 8222	Tlatizapán 8073
Gwibi (2) 8222	Bulk of Pop. 73
Cycle IV (50%)	
Maracay 8222	
Suwan 8222	
Suwan (1) 8222	

Population 22 was eliminated from the project after two selection cycles when it failed to achieve any significant gains in resistance, likely due to a lack of a high frequency of resistance genes. A group of lines derived from Santa Rosa 8576 was substituted for Pop. 22; the genetic background of Santa Rosa 8576 included improved germplasm from the Taiwanese Technical Agricultural Mission and the Nicaraguan Maize Program. From the time it was incorporated into the project it was referred to as Population 76, because it contained a good percentage of the TIWF population.

Breeding methodology

Populations are being improved using an S_1-S_2 recurrent selection scheme. Since this is a collaborative project, breeding is carried out in the participating countries, but responsibility for managing each population resides with one country. Thus El Salvador is handling Population 73, and Nicaragua handles Population 76.

At the beginning of the first cycle, 400 S_1 lines of each population were generated. Four nurseries with lines from each population were formed for testing in Nicaragua and El Salvador using two sowing dates (one normal, and the other late with high disease incidence). A simple 20 x 20 lattice design with two replications was used. Plot size was a 5 m row. For each line, data were recorded on agronomic traits, stunt response (number of stunted plants and ears, and disease severity score), and grain yield.

Pooled data of all test variables were used to select the superior fraction of each population (40 lines), which was then planted the following cycle in each country to recombine S_1 lines through full-sibbing. Likewise, each cycle the 10 best lines were selected to form a synthetic experimental variety. Efforts are made to select unrelated lines to avoid narrowing the population's genetic base.

Full-sib families are planted during the period of high disease incidence, and each family is selfed. Fifteen days after flowering is completed, selfed plants showing stunt symptoms are eliminated. The remaining plants are harvested and used for further testing in the next breeding cycle.

Changes in the recurrent selection scheme

First change - In cycle 3 of Pop. 73 and cycle 2 of Pop. 76, S_1 lines were recombined through half-sibbing. This was done to break up undesirable linkage groups that in the future might obscure the selection of favorable traits and, at the same time, to reduce inbreeding in the population. Full-sibs of half-sib families were formed through direct and reciprocal crosses for testing in international trials in different countries in two seasons: one normal and the other under disease pressure. Once the international testing of full-sib families was completed, a normal cycle of recurrent selection was begun.

Second change - Starting with cycle 4 of Pop. 73 and cycle 3 of Pop. 76, S_1 lines were advanced to S_2. The S_1 lines were sown in 6 m rows during the period of stunt incidence. A high seeding rate (15 cm between plants) is used on half the row to evaluate the line, and a low seeding rate (30 cm between plants) is used on the other 3 m to allow selfing. Undesirable families are eliminated before and after flowering; at harvest, only healthy plants are selected for inclusion in the following cycle's yield and phytosanitary trials. As a result of this change, 225 S_2 lines are being evaluated in 2.5 m rows with two

replications in two sites and using two planting dates.

Breeding progress in both populations is measured indirectly by testing experimental synthetics developed in the latest selection cycles, along with composite varieties from each cycle and resistant varieties and hybrids developed in previous years, using susceptible high yielding commercial hybrids as reference checks.

Trials including these materials are evaluated in Guatemala, El Salvador, Nicaragua, Panama, and the Dominican Republic during normal sowing cycles and periods of high disease incidence. Complete randomized blocks with four replications are used; plot size is four 5 m rows. Data are recorded on agronomic traits, stunt response, and grain yield of each entry.

Statistical analyses

Analysis of variance (site specific and combined), stability analysis, mean comparison using the Tukey test, orthogonal contrasts, simple regression analysis and class frequencies, calculation of selection and stunt indices, were performed on the data.

Results and Discussion

Selection based on inbred progenies (S_1, S_2, etc.) is theoretically effective for bringing about changes in the frequency of additive genes (Hallauer and Miranda 1981). Recurrent selection of both populations was effective as evidenced by grain yield, and numbers of stunted plants and ears. Tables 1 and 2 show the selection differential for grain yield increasing over the test cycles for Pop.73 and Pop. 76, respectively, the differential for percent stunted plants was negative but variable due to erratic disease incidence. Negative values indicate that lines selected for recombination in the following cycle have higher levels of disease resistance than the population as a whole.

Over the cropping cycles, advances on economically important characteristics indicate that recurrent selection of S_1 lines is effective for eliminating deleterious recessive alleles that limit selection progress (Córdova et al. 1986). Considerable gains were observed in the selected fraction in terms of grain yield and reduced disease damage to plants and ears, as a result of capitalizing on favorable alleles conferring resistance (Tables 1 and 2).

Results from the previous stunt resistance breeding program (De León et al. 1984) confirmed that a scheme combining recurrent selection, evaluation, and recombination of S_1 lines is effective for accumulating stable resistance levels.

Selection improvement of Population 73, shown in Table 3, has increased grain yield in environments with high disease pressure by an average of 306 / kg / ha / cycle (10.4% per cycle). This increase is associated with a 10% reduction in the number of stunted plants each cycle. The regression between yield and number of stunted plants (Table 4.) indicated that for each diseased plant, yield is reduced by approximately 75 grams (Aguiluz and Urbina 1992).

After three selection cycles and under moderate stunt incidence, per cycle gains of 11% in disease resistance and 4.3% in yield were achieved. These results show that selection has been effective for improving varietal performance under disease pressure although at the expense of slightly lower yield potential in optimal environments.

Table 1. Mean yields and stunt response of a selected fraction of Population 73. Combined data analysis from El Salvador and Nicaragua, 1989

	Cycle 1		Cycle 2		Cycle 3	
	kg/ha	% st.[1]	kg/ha	% st.[1]	kg/ha	% st.[1]
Population mean	3670	27	3252	75	1924	23
Selected fraction mean	3697	18	3829	38	2717	12
Exp. variety mean	4257	12	3898	28	3027	10
Selection differential	27	-9	577	-37	793	-11

[1] Percent stunted plants.

Table 2. Statistical data for 225 full-sib families from Population 76, cycle 2, Nicaragua, 1991.

	1991-A			1991-B		
	kg/ha	%st. pts.[1]	% st. ears[1]	kg/ha	% st. pts.[1]	% st. ears[1]
Population mean	3827	48	22	3749	54	11
Selected fraction mean	4488	35	12	4035	41	5
Selection differential	662	-13	-10	556	-13	-6
Maximum	5825	93	65	5170	91	53
Minimum	2097	9	0	1177	1	4
Standard deviation	695	15	13	734	16	8
Checks						
NB-12	4226	55	26	3627	76	27
B-833	911	100	94	1389	97	92

[1] Percent stunted plants and ears (respectively).

Comparing the best synthetics from both populations developed during the last breeding cycle with stunt resistant commercial varieties and high yielding hybrids clearly shows that synthetics SC3P73 N, SC2P76 N, and SC3P73 R produced higher grain yields and had lower percentages of stunted plants and ears than check varieties NB-6 and H-53 (Table 4). These results objectively demonstrate the progress achieved in that test cultivars performed better than the check varieties, which are widely used by farmers.

It is important to note that the synthetics show improved performance under high disease incidence in unfavored environments, but do not lose their yield potential in favorable ones. Synthetics SC3P73 N, SC2P76 N, and SC3P73 R yielded the same as hybrids B-833 and HN-879 in disease-free environments, while significantly outperforming them under high disease conditions, sometimes by more than 2.0 t/ha (>100%) (Tables 4 and 5).

Cultivars improved for stunt resistance during the last breeding cycles showed marked performance differentials compared to hybrids and resistant varieties under severe disease conditions. Synthetics SC3P73 R and NB-12 had the lowest yield reductions when shifted from an environment with stunt incidence to another with high disease incidence.

Given this evidence, there is no doubt that cultivars now available for farmer use are superior to the ones currently being grown.

Table 3. Grain yield and stunt response of synthetic and composite lines derived from Population 73 evaluated in seven locations of Central America, 1991

Genotype	Yield (kg/ha)[1]	% over NB-6	% st. plants
Composite C_3	4247 a	23	37.6
Synthetic C_3	4175 a	21	33.2
Composite C_2	4093 a	18	39.6
Synthetic C_2	3175 a	7	42.4
Synthetic C_1	3634 b	5	38.2
Synthetic C_0	3559 b	3	47.7
NB-6	3459 b	0	35.7
B-833	2708 c	-22	58.8

[1] Yields with the same letter are statistically similar at 5% probability using the Tukey test.

Table 4. Mean yields and stunt response of maize cultivars evaluated in Nicaragua, Panama, and El Salvador, 1991.

Cultivar	Yield kg/ha[1]	% over NB-6	Regression dev S^2di	Regression coeff Bi	% st. pts.[2]	% st. ears
SC3P73 N	4221 a	15	0.24 **	0.66 ns	38.5	21.7
SC2P76 N	4219 a	15	0.05 ns	0.51 *	42.3	17.0
SC3P73 R	4055 ab	11	0.12 *	0.36 *	42.8	18.3
NB-12	4014 ab	9	0.04 ns	0.55 *	49.5	20.9
H-53	3730 ab	2	0.13 *	1.19 ns	56.8	50.4
NB-6	3666 b	0	0.15 *	1.20 ns	54.0	38.8
B-833	2990 c	-18	0.79 **	1.78 *	78.8	72.8
HN-879	2795 c	-24	0.81 *	1.75 **	82.6	76.2
Mean yield	3711		0.29	1.00	55.7	39.5

[1] Yields with the same letter are statistically similar at 5% probability using the Tukey test.
[2] Mean of four environments with stunt stress.

Table 5. Effect of stunt on grain yield of maize cultivars evaluated at the H. Tapia B. experiment station, Managua, Nicaragua, June and September, 1991.

Cultivar	Favorable environment (kg/ha)	Unfavorable environment (kg/ha)	% yield reduction	Stunt resistance index[1]
SC3P73 N	4896	2873	41.3	0.59
SC2P76 N	4593	3026	34.1	0.66
SC3P73 R	4326	3298	23.8	0.76
NB-12	4053	2902	28.4	0.72
H-53	4607	1379	70.3	0.30
NB-6	4831	1747	63.8	0.36
B-833	3524	1234	65.0	0.35
HN-879	2827	789	72.1	0.28

[1] Stunt resistance index = $1-(Y_1-Y_2)/(Y_1)$, where, Y_1 = Yield in favorable environments; and Y_2 = Yield in unfavorable environments.

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Response to Selection for Resistance to Leaf Feeding by Fall Armyworm in PopG, a Guadeloupe Maize Population

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Abstract

Fall armyworm, (FAW) Spodoptera frugiperda *(J.E. Smith) is a serious insect pest on maize,* Zea mays *L., in the Central American tropical lowlands and the Caribbean. Development of populations of maize with effective levels of resistance to damage by FAW larvae appears essential for sustainable maize farming. In the Guadeloupe Archipelago, recurrent S_1 selection for resistance to leaf feeding by FAW larvae was conducted with a local maize composite, PopG. Genetic variability, heritability and predicted genetic gain were estimated from S_1 progeny performance tests, and response to selection following three selection cycles was evaluated. Genetic progress was determined from a multilocal, replicated evaluation of populations per se, which were generated by recombinations from each selection cycle. Heritability estimates reached 0.22 for C1 and C2 cycles, whereas S_1s and predicted genetic gains were 0.10 and 0.35 respectively. The regression of leaf damage ratings on selection cycles gave a significant b value of -0.16 units per cycle of selection. Advanced cycle PopG should be a good source of resistance with intermediate level to leaf feeding by FAW larvae.*

Introduction

In the Guadeloupe archipelago, an effort to enhance maize germplasm and to develop adapted varieties to the Caribbean was initiated by the French National Institute of Agricultural Research (INRA) at the end of the 1970s. For the last five years, in collaboration with the Center for International Cooperation in Agricultural Research for Development (CIRAD), France, the research program has focused on resistance in maize to leaf feeding by fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith, one of the main pest contraints in the Caribbean.

Caribbean maize has long been recognized as an important breeding material for lowland tropics and as a source of resistance to insects. Several populations and inbreds, derived from Caribbean genetic germplasm with resistance to FAW have been identified (Widstrom et al. 1972; Wiseman et al. 1979; Scott et al. 1981). The development and control of artificial infestation has enabled the screening of a large number of original populations (Mihm 1983) Techniques such as: selfing within populations and crosses among populations; recurrent selection among S_1 and half-sib families within broad-based populations; have opened up selection possibilities (Mihm 1989; Smith et al. 1989; Williams and Davis 1989; Widstrom et al. 1992).

Native maize samples were collected in the Guadeloupe Archipelago in 1983 and several samples, showing relatively good performance under insect pressure, were bulked in an original population. This population (PopG), which was well adapted to Caribbean conditions, was then subjected to a recurrent breeding scheme for FAW resistance (Welcker 1993).

Our main objectives were to evaluate actual progress for resistance to FAW after 3 cycles of recurrent S_1 selection in this population PopG, and to estimate genetic variance, heritability and expected gain from S_1 progenies of PopG-C2.

This approach enabled us to estimate available genetic variation in PopG, to assess expected selection effectiveness, and eventually to redirect selection scheme parameters.

Material and Methods

The plant material chosen was a composite formed from local ecotypes identified as the most resistant samples to FAW and / or corn earworm, *Helicoverpa zea* Boddie (CEW), in 1993 and 1985 in the Guadeloupe Archipelago. After three generations of recombined mating, and one of random mating, the population was labeled PopG.

Three hundred self pollinations of PopG were made in 1989 (plant selection based on resistance and vigor) and evaluated as S_1 progenies in 1990 in a randomized experiment under natural infestation. Plants were rated 20, 30 and 40 days after sowing on a scale of 1 (no damage) to 5 (heavy damage). The 50 best performing progenies were recombined to form a C_1 population in 1991. Crosses were realized using a bulk of the different families of the population, as male plants. Then, 500 self-pollinations were made in 1992 and tested as S_1 progenies in 1993 using 10 lattice linked trials with 2 replications under heavy natural infestation. Forty-six progenies were selected and recombined to form a C_2 population as shown in Figure 1. This half-sib family structure allowed a maternal link to be maintained, so inbreeding development could be controlled.

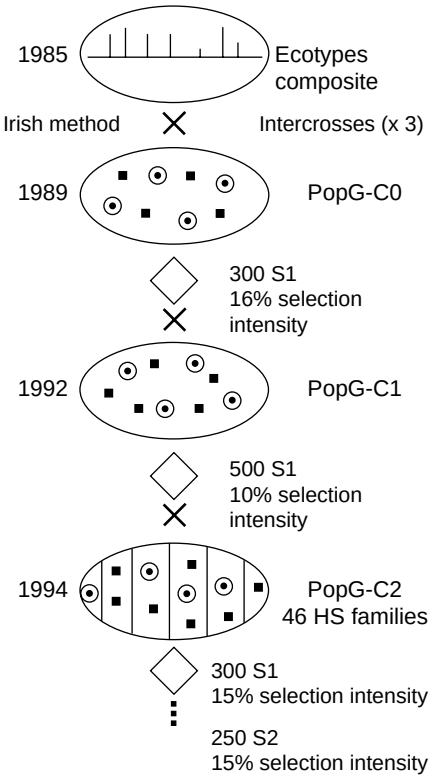

Figure 1. PopG recurrent selection scheme.

The third cycle was initiated in 1994. Plant and family selections were made in C_2 based on a performance rating scale of 0 to 9 (Williams and Davis 1989) of plants growing under artificial infestation. 300 S_1 progenies were sown in June 1994 and the best ones were selfed for evaluation at the S_2 level.

The three cycles of selection C_0, C_1 and C_2, are considered as varieties-populations formed by mass multiplication of a natural population and well adapted to their selection environment.

Plant selections were based not only on resistance parameters (under heavy natural infestation during the first cycles C_0 and C_1, and under artificial infestation for the cycle C_2), but also on agronomic characters such as vigor, plant height, ear productivity. A significant improvment was obtained for resistance evaluation in the last cycle with the development of artificial infestation and individual plant to plant observations.

The initial population C_0, and populations issued from the two cycles of selection, C_1 and C_2, were evaluated in a multilocal test which included three different environments in Guadeloupe: first, at Godet experimental station, on black cotton soil, during two different seasons (dry and warm season) and, second, at Duclos experimental station, on ferralitic soil in a wet area, during the warm season. This multilocation test was designed to obtain the optimal screening of the three cycles of selection and to characterize their behavioral variabilities in different locations.

The experimental design was a randomized complete block experiment with 10 replications at each location (2 rows of 5 m per plot). This design takes into account genotype x location interaction effect, commonly observed in host-plant resistance experiments (Mihm 1989; Widstrom et al. 1992).

The S_1 progenies of PopG-C2 were evaluated at Godet in the warm season, on six connected 7 x 8 lattices with two replications (10 plants per plot, resistant and susceptible checks randomly included) This structure was chosen to control potential location heterogeneity. Artificial infestations were applied to these trials (5 leaves, 25 larvae). The stage of 5-7 leaves appears to be the most susceptible one to FAW (Davis et al. 1989). Larval damage was rated for each plant on a scale of 0 (no damage) to 9 (heavy damage), as reported by Williams and Davis (1989). Plant and family selections were based on 7 and 14 Days After Infestation damage ratings (DAI).

Response to selection was evaluated from standard regression procedures of damage rating on selection cycles from the C_0 through to C_2 (Widstrom et al. 1992). This regression procedure permits estimation of the effective genetic gain obtained from the beginning of recurrent S_1 selection.

Standard analyses of variance, used to analyse leaf damage ratings at each location, were combined based on homogeneity of error variances. Both populations and locations were assumed to be random variables.

Components which estimated genetic variance and phenotypic variance were obtained from the software package SELECT (developed by INRA). We calculated genetic parameters from a statistical model of the genetic value based on maternal plant effects within PopG-C2 i.e. genetic variance-covariance components, heritabilities, and, Best Linear Unbiaised Predictor of genetic value (BLUP) of each of the 300 S_1 of popG-C2. Heritability was estimated according to the formula: $h^2 = s^2_G / s^2_P$. Additionally, genetic gains were estimated according to the formula $G_S = k\, s^2_P\, h^2$, in which k=1.76 for 10% selection intensity.

Results and Discussion

Genetic gain after three cycles of selection

Regression results, based on performances of the three cycles of selection *per se* and on the most discriminant environment, indicated significant progress for resistance to larvae feeding by FAW, at 7, 14 DAI, and with our selection index (mean of 7 and 14 DAI ratings) (Fig. 2). In these conditions, the response of 0.23 units reduction in damage per cycle attests effectiveness of the selection process.

After location adjustment, the multilocal regression b value, based on the three populations *per se* test environments, indicated a reduction of 0.16 units in damage per cycle (Fig. 3) This reduction indicates significant additive genetic variation and is comparable with FAWCC progress (0.18 units reduction on the two first selection cycles) obtained by Widstrom et al. (1992).

These results confirm that three cycles of S_1 recurrent selection seem sufficient to obtain a good level of resistance, as mentioned by Hallauer (1992).

Genetic parameters estimated from S_1 progenies test

Although the analysis of our data (based on individual observations and taking into account plant-to-plant variation) show significant genetic variation within the populations of PopG for resistance to larval feeding by FAW, it is possible that the high genotype by environment interaction effects, observed in multilocal analysis, might be contributing to the observed differences. For this reason, the S_1 progenies test could provide useful information. (Table 1).

Heritability, based on genetic estimates reached 0.22. This value seems to be similar to results obtained by Widstrom et al. (1992) on FAWCC. This result appears to be low, but it should be borne in mind that when environment interactions are considered, no appropriate experimental design could significantly reduce the estimate of h^2.

The low genetic variances of PopG-C2 were not encouraging, even though we later determined a larger mean

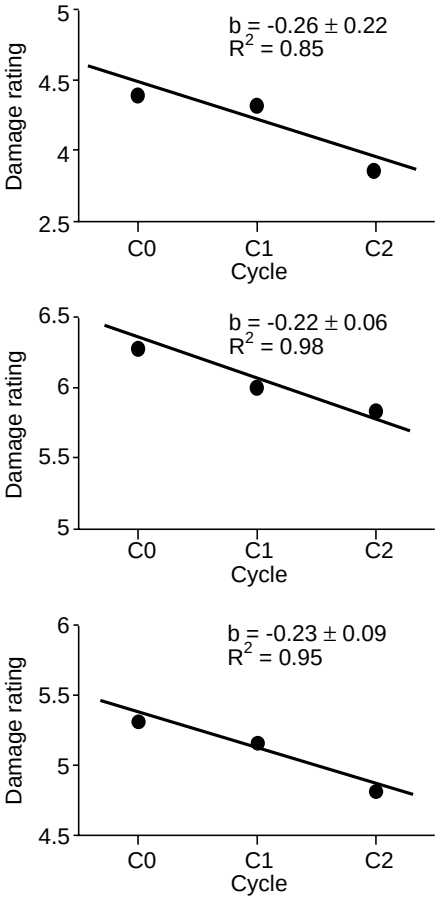

Figure 2. Selection response for reduced leaf-feeding damage by FAW to two cycles of recurrent selection in PopG within the most discriminant environment.

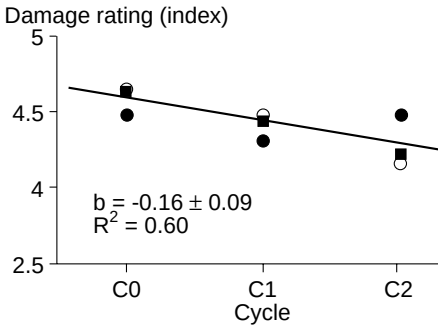

Figure 3. Selection response for reduced leaf-feeding damage by FAW to two cycles of recurrent selection in PopG at different locations.

Table 1. Heritability, genetic variance component estimates and predicted responses to S_1 selection for resistance to leaf feeding by FAW in PopG-C2.

	σ^2_G	$\bar{H}^2_G$	Predicted responses
7 DAI	0.15	0.24	0.33
14 DAI	0.18	0.22	0.35
Index 7 + 14 DAI			0.63

$r_G = 0.58$ **

** 7DAI rating - 14DAI rating genetic correlation estimated from the S_1 progenies of PopG-C2.

selection response by using a genetic gain test. However, the main fact was the increasing of genetic variance for PopG-C2 from the initial pool. This variance seems to be sufficient to suggest that recombination generated additional genetic variance.

Expected genetic gain, estimated from the genetic variance and heritability for resistance evaluated 7 DAI and 14 DAI appears to be promising. Its high level (0.35) and variability within PopG allow us to conclude that sufficient genetic variation remained in PopG to justify additional selection. This result affirms the benefits of artificial infestation and experimental design in cycle C_2, when used to aid the selection process in an S_1 testing procedure. This result confirms also the interest of individual-family combined selection and the maintaince of a maternal link. Results obtained using the SELECT software seem to confirm that great progress was obtained, from the initial pool and the first selection steps.

We selected the 10% best S_1 based on our selection index. From the estimations of their BLUP, the expected progress reaches 0.32 units, confirming the first SELECT evaluation based on the 300 S_1s. Hence, highlighting the value of BLUP estimations in the evaluation of the last S_1 selection, and the use of these estimates in the potential organization of further selection schemes (Fig. 4). These results demonstrate the effectiveness of the selection process for reduced leaf feeding, but also its slowness. This is probably a consequence of:
A compromise between variability preservation and selection intensity on the main character and,
Lower quality of the estimation of this character during the first steps of the selection scheme.

Analysis of variance of S_1 progenies indicated the presence of significant variation for resistance to larval feeding by FAW within PopG-C2. Widstrom et al. (1992) indicated similar values of genetic variance for FAWCC Cycle 3 and Cycle 4, which allowed significant progress in this population in the further cycles.

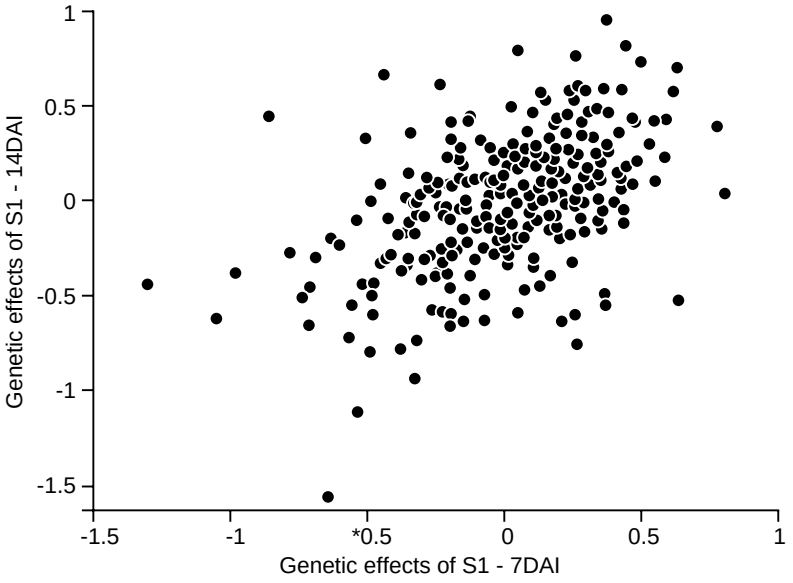

Figure 4. Genetic variation for leaf-feeding damage by FAW between S_1 of PopG-C2 - INRA - Godet 1994.

Variation within families appeared to be 10 times higher than the variation between families. High variation between S_1 plants was observed. Therefore, self-pollinations were made advancing the selected families to the S_2 level. The objective of this was also to get a more precise evaluation of their resistance levels, taking into account high environmental variance. This environmental variation, estimated from the residual value of inbred checks, underlines the importance of the check choice and the necessity to increase the number of test sites or replications, to improve the accuracy of genetic parameters.

Our results tend to show that faster progress could be obtained, if more importance is given to the 14 DAI rating in the index estimation. However, this could increase the risk of lost information on resistance mechanisms, potentially characterized by the 7 DAI rating. This was confirmed by a 7 DAI rating-14 DAI rating genetic correlation of 0.58 estimated from the S_1 progenies (Fig.4).

It does appear that continued progress should be possible in PopG. These results underline the interest of this original Caribbean population as a new source of resistance to insects, with high adaptability to the Caribbean. Therefore, PopG appears to be a promising source of inbreds with an intermediate to high level of resistance to FAW.

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Location and Effect of Quantitative Trait Loci for Southwestern Corn Borer and Sugarcane Borer Resistance In Tropical Maize

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Abstract

Development of multiple insect resistance in tropical and subtropical maize represents a major effort of the maize breeding program at CIMMYT. Resistance to the southwestern corn borer (SWCB), an aggressive feeder, appears to be polygenically controlled and has been widely considered to involve primarily additive gene action. Some of the components of resistance to SWCB seem to confer resistance to other important Lepidopteran maize pests, including the sugar cane borer (SCB). Our objective was to map, using restriction fragment length polymorphism (RFLP) markers, the quantitative trait loci (QTL) involved in the resistance to SWCB and SCB as a first step towards the use of marker-assisted selection in the breeding for such complex traits. Two distinct F_2 populations were developed, each from a cross between a susceptible (S) and a resistant (R) line: the population derived from the Ki3 (S) and CML139 (R) cross was comprised of 476 F_2 individuals and was evaluated for SWCB. The population derived from the CML131 (S) and CML67 (R) cross consisted of 215 individuals and was rated for SWCB and SCB. F_2 individuals were genotyped using close to 100 genomic and cDNA maize probes. F_3 families were rated for leaf-feeding damage (1-10 scale) after artificial infestation for two or three consecutive years at one or two locations. The QTL analyses were conducted using single-factor ANOVAs and a maximum likelihood approach (MAPMAKER/QTL). Several chromosomal regions were found to be involved in the resistance to SWCB and SCB. Not all regions were shared by the two populations for SWCB and some QTL were common in the resistance to both insects. Most of the QTL showed additive and dominance effects.

Introduction

About 30 out of 55 million hectares planted with maize in developing countries are seriously affected by insect problems. Lepidopteran insects are among the most important pests affecting this crop worldwide. For instance, typical annual losses estimated at over 4 million tons in Brazil and 1 million tons in Mexico result in an overall cash loss of more than US$ 600 million (CIMMYT, 1988). Improved germplasm resistant to some or all of these insects would provide an effective way of increasing maize production in affected areas, while keeping down the cost to the farmer and reducing the impact of chemicals on the environment.

Development of multiple insect resistance in tropical and subtropical maize represents a major effort of the maize breeding program at CIMMYT. Resistance to the southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar, one of the most aggressive feeders, appears to be polygenically controlled and is thought to involve primarily additive variation (Scott and Davis 1978; Williams et al. 1989; Thome et al. 1992). Moreover, some of the components of resistance to SWCB seem to confer resistance to other insect species, including the sugarcane borer (SCB), *Diatraea saccharalis* F., and to other Lepidopteran species against which Caribbean materials were tested (Smith et al. 1989). Breeding for resistance to SWCB and SCB has been laborious and time consuming because

it has required recurrent selection with at least four to five cycles of infestation in order to recover and verify a desirable level of resistance. This has also implied the need for insect mass-rearing facilities. In order to assist in the breeding efforts for borer resistance, our goal was to map, using restriction fragment length polymorphism (RFLP) markers, the quantitative trait loci (QTL) involved in the resistance to SWCB and SCB as a first step towards the use of marker-assisted selection (MAS) in the breeding for such complex traits.

Materials and Methods

Populations

Four maize lines, two susceptible to SWCB and SCB and two resistant ones, were used to form the two populations used in this study (Table 1). Crosses were made between the susceptible and the resistant lines: Ki3xCML139 (AxB), and CML131xCML67 (CxD) and two F_1 ears from each cross were selfed to produce the F_2 populations. For the RFLP analysis, leaf tissue was harvested from single F_2 plants which were then selfed to produce F_3 seeds. F_3 plants of each family were sib-mated and seeds pooled for planting in replicated trials for the evaluation of insect leaf feeding damage.

Genotyping the F_2 individuals

RFLP genotyping was done on 475 and 190 F_2 individuals for the cross AxB and CxD, respectively, using the protocols described in Hoisington et al. (1994). DNA was extracted from lyophilized ground leaf tissue then digested with one of two restriction endonucleases, *Eco*RI and *Hin*dIII. DNA fragments were separated by gel electrophoresis in 0.7% agarose gels then transferred onto non-charged nylon membranes by Southern blotting. Genomic and cDNA maize clones from the University of Missouri, Columbia (UMC), Brookhaven National Laboratory (BNL) and the Native Plants Inc. (NPI) collections were used as probes to detect RFLPs. These clones were amplified by PCR and labeled with 2.5% digoxigenin-dUTP. After overnight hybridizations, RFLPs were detected with the antidigoxigenin-alkaline phosphatase-AMPPD chemiluminescence system. The same blots were hybridized to several consecutive probes by first stripwashing the last probe off the blot. RFLP data were captured and verified using HyperMapdata, software developed at CIMMYT.

Insect damage rating of the F_3 families

SWCB and SCB infestation trials were conducted at CIMMYT's Tlaltizapán station in the State of Morelos, Mexico (18.41ºN, 940 masl, 830 mm average rainfall). In addition, one SCB trial was planted at the Poza Rica station in the tropical part of the State of Puebla, Mexico (20.34ºN, 60 masl, 1200 mm average rainfall) during the winter cycle of 1993 (PR93A). The AxB trials consisted of 619 entries: 476 F_3 families, 36 of parent A and 35 of parent B used as parental checks. In addition, 72 entries of an S_1 bulk of a white seeded hybrid (CML61xCML62) were used as a physical check to control planting errors in the field and / or loading and handling errors in the lab. The trials were grown in a RCBD with two replications in the summer of 1990 (Tl90B), and the winters of 1991 (Tl91A) and 1992 (Tl92A). The CxD trials consisted of 240 entries: 215 F_3 families, 12 of parent C and 13 of parent D, which were grown in a 24x10 a-lattice design with two replications during the winter seasons of 1992 (Tl92A) and 1993 (Tl93A).

Entries were grown in 2.5 or 5 m single-row plots, 0.75 m apart. Plants were thinned to a distance of 25 cm and were infested at the mid-whorl stage with 30-40 neonate SWCB or SCB larvae. These were applied as a larvae-grit mixture with a mechanical dispenser (Mihm, 1983). Leaf feeding damage by the insects was assessed 15-24 days after infestation using the 1 (no visible leaf damage) to 10 (dead plant) rating scale (1-9 as in Davis and Williams 1989).

Data analyses

Insect damage ratings from the individual plants were averaged to give a mean value per F_3 family. Lattice analyses of variance were performed for the CxD field trials on the data from each experiment. Adjusted entry mean squares and effective errors were then

Table 1. Some characteristics of the maize lines used to generate the populations for the mapping of SWCB and SCB resistance (DR=Dominican Republic)

Designation	Line	Reaction to SWCB, SCB	Origin	Adaptation	Maturity	Grain type
A	Ki3	Susceptible	Suwan1	Tropical	Late	Yellow, flint
B	CML139	Resistant	DR Grp. 1/ Antigua Grp.2	Subtropical	Intermediate	Yellow, semi-flint
C	CML131	Very susceptible	Pop. 42	Subtropical	Intermediate	White, dent
D	CML67	Very resistant	Antigua Grp.2	Tropical	Late	Red/yellow semi-dent

used to compute the combined analyses of variance and covariance across environments for SWCB and SCB experiments. For the AxB 1990 and 1991 trials, SWCB leaf feeding damage was evaluated in only one replication, therefore, only a combined analysis of variance was performed on the data from the three experiments. Heritabilities were computed according to Hallauer and Miranda (p. 90, 1981):

$$h^2 = \frac{\hat{\sigma}_g^2}{\hat{\sigma}_g^2 + \frac{\hat{\sigma}_{ge}^2}{e} + \frac{\hat{\sigma}^2}{re}},$$

where r = no. of reps, e = no. of environments, $\hat{\sigma}$ = error variance, $\hat{\sigma}_g^2$ = genotypic variance, and $\hat{\sigma}_{ge}^2$ = genotype x environment variance.

An RFLP linkage map was constructed for each population using the software package MAPMAKER (Lander et al. 1987). For declaration of linkage, a LOD ($\log_{10}$ of the likelihood ratio) threshold of 3.00 and a maximum recombination frequency of 0.40 were used. Genetic distances between markers were estimated with the Haldane mapping function. A combined map was also constructed by pooling the genotypic data from the two populations.

Mapping of QTL and estimation of their genetic effects were performed according to interval mapping using the package MAPMAKER/QTL (Lander and Botstein 1989). The presence of a putative QTL in a given genomic region was declared when the LOD score exceeded a threshold of 2.5. Gene action was determined based on the ratio of dominant to additive genetic effects and the criteria used by Stuber et al. (1987). The AxB data was also analyzed by one-way ANOVA using the SAS PROC GLM (SAS Institute, 1988).

Results and Discussion

Mean ratings of insect leaf feeding damage on the two F_3 populations exhibited near normal distributions with apparent transgressive segregation in the case of the population derived from AxB. The mean parental values and the range and mean for the F_3's in the separate trials are shown in Table 2 for SWCB and in Table 3 for SCB.

For the AxB population, unfortunately there is data from only one replication for the 1990 and 1991 trials. This was due to insufficient insects at the time of the artificial infestation and to poor growing conditions in the 1990B trial. Therefore, neither variance components nor heritabilitites were computed for these two trials (Table 2). Although the three trials were artificially infested, the damage was most severe in 1991, less severe in 1990 and a very light damage resulted in 1992. These differences are expressed by a significant GxE interaction and consequently a medium low heritability, h^2=0.39. It is important to note that the 1990 and 1991 AxB trials were sown in poor soil in the station, and plants were seen to be affected by iron deficiency particularly in the rainy season (Tl90B trial).

In contrast, the CxD SWCB trials were grown on better soils using a more efficient experimental design and therefore the results were more similar across seasons, although the 1993 trial showed slightly more severe damage. However, albeit the GxE interactions were significant, h^2 was moderately high at 0.64 (Table 2). The SCB trials were also similar in terms of distribution and resulted in a h^2 estimate of 0.64 across the three trials (Table 3).

Table 2. Means and standard errors for SWCB ratings of the four parents and the 476 F_3 families in the AxB population and the 215 F_3 families in the CxD population in the individual trials. Variance components and heritabilities were computed for the individual trials and across trials.

	AxB				CxD		
Parameter	**Tl90B[1]**	**Tl91A[1]**	**Tl92A**	**Combined**	**Tl92A**	**Tl93A**	**Combined**
Means ± SE							
P1	8.5 ± 0.13	8.9 ± 0.03	6.2 ± 0.06	—	9.1 ± 0.07	8.6 ± 0.14	8.9 ± 0.18
P2	6.1 ± 0.11	7.0 ± 0.10	4.2 ± 0.04	—	3.6 ± 0.06	5.5 ± 0.14	4.6 ± 0.16
F_3 lines	6.8 ± 0.06	8.0 ± 0.03	4.8 ± 0.02	—	6.2 ± 0.07	7.5 ± 0.05	6.9 ± 0.05
Range, F_3's	4.0 - 10.0	5.9 - 9.5	3.5 - 6.3	—	4.0 - 8.4	4.8 - 8.8	—
Variance components and heritabities (F_3 lines)							
s^2g	—	—	0.11**	0.12**	1.17**	0.54**	0.33**
s^2ge	—	—	—	0.42**	—	—	0.20**
s^2	—	—	0.19	0.19	0.50	0.20**	0.35
h^2	—	—	0.54	0.39	0.70	0.73	0.64

[1] Data from only one replication
** Significant at the 0.01 probability level.

Phenotypic correlations between SWCB and SCB mean leaf ratings on the F_3 families of the CxD cross was 0.5 (significant at the 0.01 probability level) for both the Tl92A and Tl93A trials. As shown by earlier work (Thome et al. 1992), this relatively high correlation between the damage caused by SWCB, a very aggressive feeder, and SCB may allow some progress to be made in breeding for multiple borer resistance by selecting only under infestation with SWCB. The selections could then be verified for multiple resistance by subsequent testing with other insects.

A total of 128 and 97 RFLP loci were placed on the AxB and CxD linkage maps respectively. The two maps were consistent in locus order with each other and also with other published maize maps (e.g., Maize Genetics Cooperation Newsletter no.68, 1994). The combined map included 166 loci (60 loci in common between both populations) and spanned a distance of 2041 cM resulting in an average marker distance of 12.4cM (Fig. 1). The individual maps provided a relatively dense framework for mapping QTL, as discussed below.

Results of the interval mapping analyses for QTL responsible for SWCB resistance are presented in Table 4. In the three AxB trials, several putative QTL were detected, most explaining a small portion of the total variance for SWCB leaf feeding damage. These were spread throughout the genome and only three regions on chromosomes 3 and 8 were common to two or three trials. The QTL exhibited both additive and dominance effects. With the exception of the QTL on chromosome 4 detected in the 1990 trial, all additive effects contributing to increased resistance came from the resistant parent. Most dominance effects contributed to increased resistance. A few of the effects were from Ki3, the susceptible parent in the AxB cross, and this was reflected in the transgressive segregation observed for leaf feeding ratings in this population. Results from the one-way ANOVA were very consistent with those from the interval mapping analysis in determining regions of the genome containing putative QTL with the exception of the QTL on chromosome 2 (Tl91A) and the one on chromosome 4 (Tl90B) where the F-test did not show any locus to be significantly correlated with the SWCB damage rating.

For the CxD cross, a smaller number of putative QTL, each explaining a larger portion of the genetic variance, were detected. These were located on chromosomes 1, 5, 7 and 9. One of the QTL on chromosome 1 was detected in both trials as well as in the AxB cross (Tl90B). Both additive and dominance effects were present and all additive effects contributing to the increased resistance came from the resistant parent. Surprisingly, dominance effects were almost as important as the additive ones and contributed to an increase in the rating scale or a decrease in resistance (Table 4).

The QTL for SCB resistance are summarized in Table 5. Putative QTL were located on chromosomes 1, 2, 5, 9, and 10. Again, the variance at each of these QTL included both additive and dominant effects and most alleles for increased resistance to SCB were contributed by CML67. In this case, dominance effects also were exhibited as an increased resistance.

Most of the gene action at the putative QTL detected in both populations for both insects ranged from partial to overdominance with the exception of the QTL on chromosome 3 in the AxB SWCB Tl92A trial and the QTL on chromosome 9 in the CxD SCB Tl92A trial. These results do not fully agree with results from the combining ability studies for SWCB and SCB resistance where additive gene action was found to be more important (Scott and Davis 1978; Williams et al. 1989; Thome et al. 1992). Up to 53% of the genetic variance of any one trait in any one trial could be explained in terms of the set of regions detected for resistance to SWCB or to SCB. The estimated heritabilities do not appear to provide a valid criterion to predict how many QTL will be detected in particular environments and which percentage of the phenotypic variance

Table 3. Means and standard errors for SCB ratings of the two parents and the 215 F_3 families in the CxD population in the individual trials. Variance components and heritabilities were computed for the individual trials and across trials.

Parameter	Tl92A	Tl93A	PR93A	combined
Means ± SE				
P1	8.3 ± 0.09	8.6 ± 0.19	8.1 ± 0.20	8.3 ± 0.20
P2	4.3 ± 0.17	4.3 ± 0.18	5.2 ± 0.25	4.6 ± 0.10
F_3 lines	6.2 ± 0.05	6.3 ± 0.06	6.6 ± 0.05	6.4 ± 0.00
Range, F_3's	4.2 - 8.1	4.0 - 8.5	4.4 - 9.3	—
Variance components and heritabilities (F_3 families)				
s^2g	0.59**	0.95**	0.47**	0.24**
s^2ge	—	—	—	0.22**
s^2	0.37	0.36	0.37	0.36
h^2	0.62	0.73	0.56	0.64

** Significant at the 0.01 probability level.

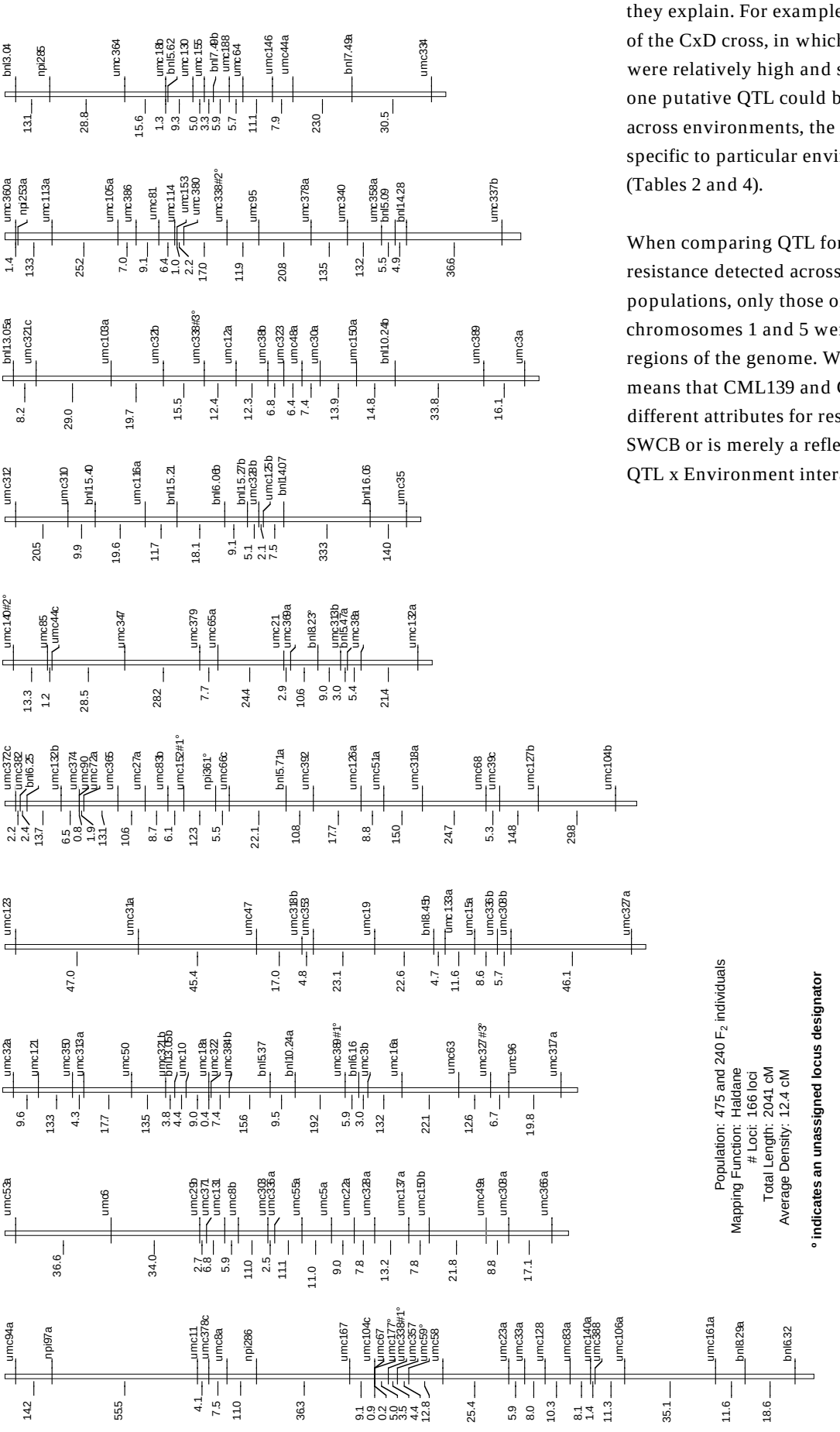

Figure 1. Combined RFLP linkage map of the genome of tropical maize Ki3 x CML139 and CML67 x CML131 (loci names on the right and distances in cM on the left of each linkage group).

they explain. For example, in the case of the CxD cross, in which heritabilities were relatively high and similar, only one putative QTL could be detected across environments, the rest being specific to particular environments (Tables 2 and 4).

When comparing QTL for SWCB resistance detected across the two populations, only those on chromosomes 1 and 5 were in the same regions of the genome. Whether this means that CML139 and CML67 have different attributes for resistance to SWCB or is merely a reflection of the QTL x Environment interactions is not

clear. When looking across insects, QTL on chromosomes 1, 5 and 9 were detected for both SWCB and SCB resistance in the CxD population and on chromosomes 1 and 5 with the AxB population. This indicates that at least some of the factors controlling resistance to one borer also control resistance to the other, and is in agreement with results reported by Thome et al. (1992).

We are now in the process of analyzing these data for QTL detection using alternative methods such as composite interval mapping where some markers are used as cofactors in order to reduce the noise produced and better define the location of the QTL.

Prospects for marker-assisted selection

These data, as many other in the literature (Schön et al. 1993), confirm the complexities of analyzing QTL inheritance and expression patterns, and raise many questions as to the practical approaches needed for the successful application of marker-assisted selection (MAS).

For a given population and trait, there was wide variation in the detection of some regions from one trial to another; this may indicate a highly plastic genotype-environment interaction with some regions only becoming "active" under certain conditions. In a MAS scheme, it will be critical to ascertain which are the most important regions enhancing the trait of interest under a given environment. These may be enough to provide an economically sufficient level of resistance, while other, minor regions, which may in some cases be false positives, may be ignored for practical purposes.

Table 4. Putative QTL for SWCB resistance and their genetic effects in the separate trials of the AxB (476 F_3 families) and the CxD (215 F_3 families) populations. Genetic effects are expressed as the change in the leaf feeding damage scoring due to the contribution of an allele from the resistant parent (a=additive, d=dominant, p=partial, od=overdominant).

Chromosome	Flanking markers	Position in interval (cM)	Max. LOD score	Phenotypic variance explained %	Genetic effects		
					Additive	Dominant	Gene action
AxB TI90B							
1	umc23a - umc83a	10	2.52	3.8	-0.33	-0.38	d
3	bnl10.24a - umc389	8	2.80	3.8	-0.37	0.08	pd
3	umc16a - umc63	16	2.80	4.0	-0.37	-0.18	pd
4	umc123 - umc31a	18	3.69	14.4	0.29	-1.64	od
5	umc382 - bnl6.25	0	2.54	2.7	-0.29	-0.28	d
5	umc318a - umc68	12	4.09	5.6	-0.44	-0.36	d
7	bnl6.06b - umc328b	8	2.57	3.1	-0.22	-0.66	od
8	umc103a - umc32b	12	4.24	5.8	-0.39	-0.58	od
			Total	**43.2**	**-2.12**	**-4.00**	
AxB TI91A							
1	umc388 - umc161a	18	3.20	5.7	-0.22	-0.14	pd
2	umc6 - umc371	0	2.93	3.0	-0.10	-0.36	od
3	bnl10.24a - umc389	6	4.32	5.9	-0.23	0.10	pd
3	umc16a - umc63	12	3.87	5.3	-0.21	-0.16	pd
5	bnl6.25 - umc90	0	2.84	2.8	-0.14	-0.20	od
8	bnl13.05a - umc321c	2	2.59	3.2	-0.11	0.36	od
9	bnl5.09 - umc337b	12	2.60	3.8	-0.17	-0.24	od
			Total	**29.7**	**-1.18**	**-0.64**	
AxB TI92A							
1	bnl8.29a - bnl6.32	12	2.53	4.1	-0.06	-0.34	od
3	umc16a - umc63	20	8.93	10.5	-0.21	-0.02	a
5	umc392 - umc126a	10	4.61	7.1	-0.12	-0.34	od
6	umc65a - umc21	12	5.02	6.5	-0.14	-0.20	od
8	umc103a - umc32b	10	3.96	5.7	-0.13	-0.24	od
9	umc95 - umc378a	8	11.40	18.4	-0.25	-0.32	od
			Total	**52.4**	**-0.91**	**-1.46**	
CxD TI92A							
1	umc67-umc357	0	5.01	13.9	-0.22	0.27	od
1	umc58-umc33a	18	5.86	19.3	-0.37	0.36	d
			Total	**33.2**	**-0.59**	**0.63**	
CxD TI93A							
1	umc33a-umc128	0	3.66	9.9	-0.21	-0.08	pd
5	umc318a-umc68	4	3.12	9.7	-0.24	0.30	od
7	bnl14.07-bnl16.06	6	2.94	10.2	-0.28	0.14	pd
9	umc380-umc340	0	2.53	6.7	-0.23	0.50	od
			Total	**36.5**	**-0.96**	**0.86**	

We have now embarked on a pilot experiment, reported elsewhere in these proceedings (Willcox et al.), in which we are examining the relative efficiency of MAS in transferring insect resistance from CML67 (parent D) into African elite germplasm. We believe that some of the intricacies of the expression of regions detected in the study reported here may well be clarified as we backcross them in specific combinations into susceptible backgrounds. Thus, in a very pragmatic fashion, we shall determine the feasibility and value of MAS for such complex traits as insect resistance. These traits have required many years of intensive, laborious and costly breeding to advance to the current levels of resistance and MAS may well prove to increase the speed and effectiveness of transfers to a wider germplasm pool.

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Table 5. Putative QTL for SCB resistance and their genetic effects in the separate trials of the CxD population (215 F_3 families). Genetic effects are expressed as the change in the leaf feeding damage scoring due to the contribution of an allele from the resistant parent (a=additive, d=dominant, p=partial, od=overdominant).

Chromosome	Flanking markers	Position in interval (cM)	Max. LOD score	Phenotypic variance explained %	Genetic effects		
					Additive	Dominant	Gene action
TI92A							
5	umc126a-umc318a	6	3.61	11.5	-0.30	-0.19	pd
9	umc105a-umc153	16	8.32	26.3	-0.51	-0.01	a
			Total	**37.8**	**-0.81**	**-0.20**	
TI93A							
2	umc53a-umc6	0	4.09	10.8	-0.18	-0.60	od
9	umc340-umc358a	6	7.66	21.7	-0.45	-0.69	od
10	umc44a-bnl7.49a	24	2.75	9.1	-0.30	-0.13	pd
			Total	**41.6**	**-0.93**	**-1.42**	
PR 93A							
1	umc167-umc67	2	2.63	7.4	-0.11	0.13	d
1	umc58-umc33a	12	3.61	13.2	-0.25	-0.33	od
2	umc131-umc22a	10	5.32	20.6	-0.31	-0.65	od
9	umc113a-umc105a	24	3.52	11.3	-0.28	-0.24	d
			Total	**52.5**	**-0.95**	**-1.09**	

Developing Insect Resistant Germplasm Using RFLP Aided Breeding Techniques

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Abstract

The molecular markers known as restriction fragment length polymorphisms (RFLPs) can be utilized to identify the chromosomal locations of genes controlling traits of agronomic importance. Among the traits that ICI Seeds has mapped are those responsible for resistance to European corn borer, Ostrinia nubilalis *Hübner, stalk tunneling, (ECB2). This information can be used to develop elite resistant germplasm. Families derived from a resistant x susceptible cross were utilized to map the chromosomal locations of the genes for resistance. The families of plants were screened against the specific insect damage, ECB2. Leaf tissue was taken from the families for DNA extraction and RFLP characterization. Insect damage ratings were regressed against RFLP data to map gene locations and identify gene action. Using this molecular marker data concurrently with insect screening, the technique was successfully used to introduce ECB2 resistance into elite ICI Seeds inbreds.*

The development of insect resistance in maize, *Zea mays L.*, for the US corn belt has been an ongoing process. Some of the first studies that tried to map the genes controlling resistance to European corn borer, *Ostrinia nubilalis*, (ECB) used reciprocal translocation studies to locate genes controlling resistance. The studies identified six chromosome arms associated with first generation resistance (ECB1) (Scott et al. 1966) and seven chromosomal arms associated with second generation resistance (ECB2), (Onukogu et al. 1978). This information confirmed the complexity of the trait but use of information, in particular reciprocal translocations, in commercial breeding programs to develop ECB2 resistance was limited. The objective of commercial hybrid development programs is to develop elite high yielding stable products. Rapid conversion of elite inbreds to either ECB1 or ECB2 resistance would be highly beneficial. Molecular markers, such as restriction fragment length polymorphisms (RFLPs), have given breeders the ability to fine map quantitative trait loci (QTLs), and use this information to integrate traits into elite germplasm with minimal linkage drag from donor sources. To have a viable marker assisted selection program for insects, the following steps should be taken for each pest for which resistance is to be developed. The basic components any breeding project needs for developing new insect resistant germplasm can be summarized as follows (Mihm 1983):

- Establish reliable production of mass reared insects for infestation that mimics the vigor and variability of the naturally occurring population.
- Develop screening techniques for large scale germplasm evaluation and become familiar with the rating scales used to rate resistance and classify germplasm.
- Screen germplasm: Identify resistant and susceptible germplasm. Determine whether resistance exists in adapted and / or exotic germplasm. Identify how the germplasm responds. Evaluate germplasm over time, define the rate of progress over time for each entry. Is the resistance tolerance, antibiosis or non preference?

When resistant germplasm is identified, there is additional information to obtain before efficient product development can take place:

- Identify the gene action of resistance. What is the dominance and / or additive nature of resistance?
- How does the resistance act in inbreds vs. hybrids?
- What is the inheritance of resistance?

Once these questions have been answered one can select appropriate breeding methodologies for developing the desired end product. At this point, breeding new resistant elite germplasm can commence. The development of new germplasm requires use of the

appropriate breeding techniques coupled with infestation, rating and selection. For hybrid development programs, infestation and rating of testcross hybrids is essential.

If new resistant germplasm cannot be developed, one must identify the reasons why and evaluate the feasibility and costs of alternative approaches. It is at this point that the use of molecular markers, RFLPs, should be considered. Remember that the use of markers is only possible when reliable infestation and rating of germplasm can be obtained.

Marker assisted selection using RFLPs requires mapping the chromosomal location of the gene(s) controlling the trait of interest (Greaves et. al. 1993). This requires:

- A segregating mapping population of plant families derived from a resistant x susceptible cross. The parents need to be fixed for the trait and have a maximum number of polymorphic RFLP loci.
- The evaluation requires approximately 200 or more segregating families. This number of families gives enough replication of the genetic classes for good data quality. Plant and collect leaf tissue for DNA extraction from each family. For insects, infest and rate each family.
- With insects it is wise to use families planted ear to row where the row is infested and the genetic structure of the family is evaluated against the mean rating of the family.
- Selection of RFLP probes to generally cover the genome; spacing of 20-30 centimorgans is sufficient.
- Identify the chromosomal regions responsible for resistance using the RFLP linkage analysis. Fine map those regions identified with resistance to precisely locate the genes controlling resistance.
- Identify gene number, gene action and the contribution of each loci to the trait.

Once the mapping and gene action studies have been completed, the appropriate breeding strategy can be selected to transfer the resistance genes into elite germplasm, although each trait introgression program carries its own specific challenges.

ICI Seeds has successfully used marker assisted selection (MAS) to develop lines and hybrids with resistance to insects, diseases and herbicides. Two cases will be discussed: First, for a single dominant gene, second, for a multigenic trait.

Backcrossing a desired trait into an elite line can be accomplished rapidly. This was the case with ICI Seeds introgression of the IT (ALS2) gene, a single dominant gene, into an elite inbred. This example of the impact of biotechnology on plant breeding used an interdisciplinary approach which involved molecular markers, combined with plant breeding and physiology. The project developed elite IT inbreds in as few as four generations, including the F_1. This was possible because of three factors. First the trait can be screened for in the seedling stage, greatly reducing the number of families that need to be mapped. Second, the molecular marker data can be obtained on individual plants pre-anthesis. This allows the selection of plants with opportunistic crossovers near the IT gene and the recipient parent background on all other chromosomes. The third is that pre-anthesis selection of plants allows continuous backcrossing. This minimizes the meiotic events and therefore reduces the chances of introgressing donor line DNA into the developing line. Once the desired genetic arrangement has been achieved, one generation of selfing is required to fix the trait in a homozygous state. (Greaves et. al. 1993)

The approach for introgressing multiple genes into an elite background is similar. However it requires more knowledge of gene action and the effect each gene has on the trait. Resistance to stalk boring by the ECB2 has been shown to be dominant or partially so (Guthrie et al. 1971). In other cases additive factors play a significant role and heterosis for resistance was also shown (Jennings et al. 1974). Seven chromosomal arms were shown to contain genes for resistance to ECB2 (Onukogu et al. 1978). Relying on this published data and internally generated information, ICI Seeds decided to initiate studies on using MAS for introgressing ECB2 resistance into elite germplasm. This program was initiated in 1987.

The initial F_1 cross of a resistant (R) source inbred to an elite susceptible (S) inbred was selfed to generate an F_2 population. Leaf tissue was taken from the F_2 plants for RFLP analysis. The F_3 families derived from the sampled F_2 plants were infested with ECB2. The linkage map was generated by regressing F_3 family data on the F_2 plant RFLP marker data. The linkage data indicated that there were more than five major and many minor loci associated with resistance. Additionally, some of the loci for resistance had close linkage with

unfavorable alleles from the resistant parent. Families were selected for advancement based on the presence of resistance loci, a favorable elite background from the RFLP data, the resistance data from field infestations and hybrid testcross data. Repeating the process of selection with RFLP markers and field infestations, an inbred with a favorable elite background and resistance to ECB2 was developed in four generations of selfing. The inbred contained some but by no means all of the mapped resistance loci.

In 1992, testing of the new inbred in hybrid combination and *per se* was initiated. The data are presented as follows: Table 1 and Table 2 present testcross data using different testers. Table 3 is the inbred data *per se*.

Table 1 indicates that the new inbred has the ECB2 resistance of the resistant source. Additionally, the yield of the new hybrid was equal to the susceptible hybrid. Table 2 indicates that for the new inbred with a different tester the ECB2 resistance was equal to the resistant source. Yield was intermediate between the resistant source and the susceptible inbred. The agronomics for the new line were improved over the susceptible line though not significantly so. Data for the inbreds *per se*, Table 3, shows that the new inbred has resistance to ECB2 that is equal to the resistant source and significantly different from the susceptible line selected for conversion.

In 1993, EXP 1 had higher stalk lodging and lower yield than comparable checks (data not shown). This was due to anthracnose stalk rot, *Colletotrichum graminicola,* introduced into the stalk at the point of initial ECB2 feeding. Other researchers have also reported this interaction between insects and disease as well (Keller et. al. 1986; Carruthers et. al. 1986).

When an initial linkage map is developed for any multigenic trait, there may be a desire to introduce the trait into other elite backgrounds. This could be accomplished by crossing plants, selected with RFLPs for resistance loci and a high level of favorable background from the mapping populations, to selected lines. The new F_1s can be backcrossed to the selected elite lines and / or selfed. The subsequent families could then be analyzed with RFLPs and screened for the trait. Selection with RFLPs should be used to retain favorable crossovers. Trait screening data can be used to pull through the alleles for the desired trait. Further development can be accomplished without using RFLPs by selecting with trait screening and yield trials.

From the development of the ECB2 resistant hybrids and the IT hybrids the following conclusions can be drawn:

- It is essential to screen for the trait in the field at every generation possible. This eliminates the possibility of selecting developing lines that have crossovers occurring between the selection markers and the gene controlling the trait.
- The interaction of the trait with other factors, such as yield or disease, can significantly limit the usefulness of the newly developed germplasm.

Table 1. 1992 yield trial data. EXP 1 is the newly developed resistant hybrid.

Entry	ECB2	Yield	% moisture	% SL	% RL	% DE
EXP 1	2.3	11.196	21.0	4.9	1.4	0.0
Check 1	4.0	11.762	20.4	3.5	1.7	0.0
Check 2	3.9	11.447	21.5	1.9	0.8	0.0
Susceptible testcross	4.4	11.133	20.9	4.2	1.2	0.0
Resistant testcross	2.4	11.447	23.3	3.4	1.1	0.0
LSD	1.3	0.881	1.1	3.0	2.4	0.1

ECB2 rating = cm of tunneling per internode for the four internodes above and four internodes below the ear.
Yield = t/ha.
Moisture, stalk lodging (SL), root lodging (RL), and dropped ears (DE) are in percent.
Check 1 and Check 2 are commercial hybrids and share a common tester with EXP 1.

Table 2. 1992 yield trial data. EXP 2 is the newly developed resistant hybrid.

Entry	ECB2	Yield	% moisture	% SL	% RL	% DE
EXP 2	2.3	11.951	22.9	5.5	1.2	0.0
Check 3	2.9	11.951	19.9	5.4	3.7	0.0
Check 4	3.8	12.328	20.5	1.9	0.7	0.0
Susceptible testcross	4.0	13.334	20.5	8.1	7.2	0.0
Resistant testcross	2.4	10.504	22.7	2.3	8.9	0.3
LSD	1.2	1.258	1.3	7.7	7.5	0.3

Check 3 and Check 4 are commercial hybrids and share a common tester with EXP 1.

Table 3 Resistance rating for ECB2 damage of the newly developed resistant inbred, the elite susceptible and the resistant source.

Inbreds	ECB2
NEW	2.7
Elite susceptible	5.8
Resistant source	2.5
LSD	1.3

- With a trait controlled by multiple alleles it may not be necessary to have all the alleles present in the finished line. An economically significant level of resistance can be achieved with only a portion of favorable alleles with a large effect present.
- Selection of plants with RFLPs for opportunistic crossovers and elite background early in the development and using field screens and testcrosses to fix the trait can greatly increase the probability of developing useful germplasm with a multigenic trait.

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Construction of a Bioinsecticidal Strain of *Pseudomonas fluorescens* Active Against Sugarcane Borer

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Abstract

A cryIA(c) gene was cloned from a native Bacillus thuringiensis *strain which showed activity against the sugarcane borer* Eldana saccharina. *The sequence of the cloned gene was very similar to that of the* B. thuringiensis *subsp.* kurstaki *HD-73 cryIA(c) gene. The gene was introduced into an isolate of* Pseudomonas fluorescens *capable of colonizing sugarcane, on two broad host range plasmids, pDER405 and pKT240, having copy numbers of 13 and 28 respectively. The cry gene was introduced into the chromosome of* P. fluorescens *isolate 14 using an artificial transposon-carrying vector, Omegon-Km. Bioassays on* Eldana *larvae showed that the strain carrying the gene integrated into the chromosome was as toxic as the one carrying it on pKT240. Glasshouse trials indicated that sugarcane treated with* P. fluorescens *14::Omegon-Km-cry were more resistant to* Eldana *damage than untreated sugarcane.*

Introduction

Many strains of *Bacillus thuringiensis* produce crystalline inclusions during sporulation which contain proteins exhibiting highly specific insecticidal activity (Höfte and Whiteley 1989). The inclusions dissolve in the larval midgut, releasing one or more insecticidal proteins called δ-endotoxins. Most are protoxins which are proteolytically converted into smaller toxic polypeptides. The activated toxins appear to generate pores in the midgut epithelium cells of susceptible insects, thus disturbing the osmotic balance. The cells swell and lyse, resulting in larval death. In some instances, specific high-affinity binding sites have been shown to exist in the midgut epithelial cells of susceptible insects which may explain the specificity of the toxins (Höfte and Whiteley 1989; Van Rie et al. 1990).

Eldana saccharina Walker (Lepidoptera:Pyralidae) is an endemic species in Africa, the larvae of which bore into the stalks of sugarcane and can cause considerable crop loss. It was decided to screen local isolates of *B. thuringiensis* for activity against *E. saccharina* larvae and develop a biological control agent.

Materials and Methods

Bacterial strains and growth conditions

Strains of *B. thuringiensis* were isolated from soil samples around insect-infested sugarcane and from dead *E. saccharina* larvae by growth on PEMBA medium (polymixin pyruvate egg yolk mannitol bromothymol blue agar [Holbrook and Anderson 1980]). *Pseudomonas* strains were isolated from sugarcane by growth on King's Medium B (King et al. 1954) and confirmed by API tests using the API 2ONE identification strips. Spontaneous nalidixic acid (Nal) and rifampicin (Rif) resistant mutants were isolated.

Laboratory toxicity bioassays

Two-week-old *E. saccharina* larvae were fed on an artificial insect diet in which different concentrations of freeze-dried bacteria were incorporated (Black and Snyman 1991). Larvae were incubated in plastic 32-cell trays for five days at 30°C after which mortality was recorded.

Purification of the δ-endotoxin

δ-Endotoxin crystals from *B. thuringiensis* isolate 234 were isolated from cultures grown on nutrient agar for 48 to 72 h at 30°C using gradient centrifugation through Urografin 60% (Schering) following the method of Gonzalez et al. (1982).

Isolation of DNA from *B. thuringiensis* isolate 234, construction and screening of a genomic library, immunological detection of δ-endotoxin production, and molecular techniques, These were as described by Herrera et al. (1994).

Colonization assays

Three-month old sugarcane plants were dipped in stationary phase cultures of *P. fluorescens* strains containing one drop of Tween 80 per 50 ml culture. Plants were harvested at various time intervals by cutting off at ground level, weighing, cutting into pieces and shaking vigorously on a wrist-action shaker in sterile flasks containing glassbeads and sterile water for 5 min. Bacteria were enumerated by plating on King's Medium B containing Nal (100 mg / ml) and Rif (50 mg / ml).

Effect on *E. saccharina* of sugarcane inoculated with *P. fluorescens* 14::Omegon-Km *cry*

Six-month-old sugarcane plants grown in pots in the glasshouse were sprayed with 100 ml of a suspension of either *P. fluorescens* 14 or *P. flourescens* 14::Omegon-Km-*cry* at 2 x 10^9 cfu / ml. After two weeks each plant was inoculated with 300 *E. saccharina* eggs placed by hand behind a leaf sheath at the base of the stalk. Stalks were sampled four weeks after egg placement, and larval numbers and the number of internodes that had been bored were recorded.

Results and Discussion

Cloning the δ-endotoxin gene of *B. thuringiensis* isolate 234

More than 50 local isolates of *B. thuringiensis* were subjected to screening assays on *E. saccharina* larvae and isolate 234 was identified as a potential candidate for the isolation of a *cry* gene. Crystals isolated from *B. thuringiensis* isolate 234 were bipyramidal and the d-endotoxin had an apparent M_r of 135 kDa (results not shown). A gene library was screened by colony hybridization using a ^{32}P-labelled 2.1-kb *Pvu*II fragment from pES1 as a probe, as *B. thuringiensis* subsp. *kurstaki* HD-1, from which pES1 was derived (Schnepf et al. 1987), also showed some toxicity towards *Eldana* larvae (results not shown). Plasmid pGH37 was chosen for further analysis. Comparisons between the DNA and deduced amino acid sequence of its *cry* gene and other d-endotoxin genes showed that the 234 *cry* was almost identical to that found in *B. thuringiensis* subsp. *kurstaki* HD-73, *cryIA*(*c*) (Adang et al. 1985). There were only 4 different nucleotides at positions 978 (A to C), 981 (G to T), 1102 (T to G) and 1020 (T to C), but these did not lead to any amino acid changes. The *cry* gene, an allele of *cryIA*(*c*), will shortly be given a number by the Cry Gene Nomenclature Committee.

Isolation of sugarcane-colonizing *Pseudomonas fluorescens* and construction of *P. fluorescens cry*$^+$ strains

Colonization studies showed that a number of isolates of *P. fluorescens* were able to survive on sugarcane. Isolate 14 was selected as one of the strains which, after 60 days, showed only a decrease in titer from 8 x 10^7 to 9 x 10^5 cfu per plant despite a 42% increase in plant mass. This corresponded to a decrease from 1 x 10^7 to 8 x 10^4 cfu / g fresh mass. None of the other isolates tested showed more efficient colonization. The *cry* gene from pGH37 was cloned into pKT240 (Rawlings et al. 1986) and introduced into isolate 14 by tri-parental conjugation. The resultant strain was found to express the *cry* gene (Herrera et al. 1994).

As horizontal spread of the *cry* gene could occur when it is carried on a mobilizable plasmid, we decided to integrate it into the chromosome of isolate 14 using the artificially generated interposon Omegon-Km (Fellay et al. 1989). The Omegon module consists of the W interposon, flanked with synthetic inverted 28-bp ends of IS*1*, which can transpose if IS*1* gene products are supplied. Omegon-Km is carried on the plasmid pJFF350 which has an origin of transfer allowing mobilization into Gram-negative bacteria. The 'disabled' IS*1* element on pJFF350 cannot itself transpose, but enables transposition of the Omegon-Km module. Thus *P. fluorescens* carrying the *cry* gene in the chromosome is stable *cry*$^+$. DNA sequence analysis of the *cry* gene showed that it was carried on a 3.7-kb *Nde*I fragment. This fragment was cloned into the *Nde*I site of the integration vector, pJFF350. pJFF350-*cry* was conjugally mobilized into isolate 14, selecting for Km^R exconjugants. As the plasmid cannot replicate in this host, Km selects for integration of the Omegon-Km-*cry* cassette into the chromosome.

Southern blot analysis of isolate 14 carrying the *cry* gene integrated into the chromosome showed that the gene could be integrated at single sites (Herrera et al. 1994). It was of interest

to note that a strain carrying the integrated gene was as toxic to *E. saccharina* as a strain carrying the gene on pKT240, despite the fact that the copy number of pKT240 in isolate 14 is 28. It is possible that the increased expression of the *cry* gene integrated into the chromosome was due to the deletion of 1.4 kb of DNA 5' to the gene which occurred during the cloning of the 3.7-kb *Nde*I *cry* fragment into pJFF350. Two AT-rich regions of dyad symmetry occur upstream of the *Nde*I site of the 234 *cry* gene and were removed during the subcloning into pJFF350. Support for our hypothesis comes from a previous experiment in which we cloned the entire 6.7 kb *Bam*HI fragment carrying the *cry* gene and the upstream region into pJFF350 and integrated it into the chromosome of isolate 14. No detectable toxin was found on Western blot analysis (data not shown).

Western blot (immunoblot) analysis confirmed the expression of the *cry* gene in the exconjugants (Herrera et al. 1994). *P. fluorescens* isolate 14 carrying pKT240-*cry* and Omegon-Km-*cry* were toxic to *E. saccharina* larvae (Fig. 1).

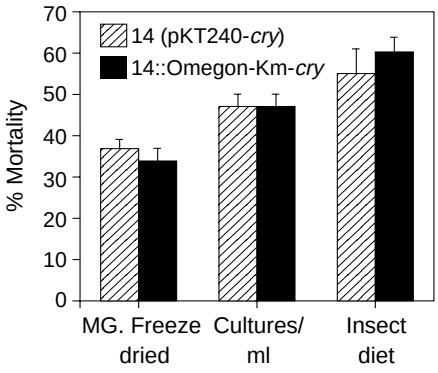

Figure 1. Toxicity of *P. fluorescens* 14 (pKT240-*cry*) and *P. fluorescens* 14::Omegon-Km-*cry* against *E. saccharina* larvae. Results are the means of three replicates. Bars above the histograms represent standard deviations.

Quantification of δ-endotoxin production in triplicate cultures using ELISA indicated that it represented 3.5% (SD 0.185%) and 3.7% (SD 0.153%) of the total dissolved protein in isolate 14 carrying pKT240-*cry*, and Omegon-Km-*cry* respectively.

The effect of *P. fluorescens* 14::Omegon-Km-*cry*-inoculated plants on *E. saccharina*

As the toxicity of isolate 14::Omegon-Km-*cry* was similar to that of the strain carrying pKT240-*cry*, it was used in glasshouse trials. Apart from the *cry* gene being a stable integration into the chromosome in this strain, it is more acceptable from a bio-safety consideration as the *cry* gene is not on a mobilizable plasmid. A comparison of the number of *Eldana* larvae recovered and the damage to stalks between plants sprayed with isolate 14 and 14::Omegon-Km *cry* is shown in Figure 2. These glasshouse trials showed that there was a decrease in the presence of larvae and consequent damage of approximately 60% after 4 weeks compared with the control strain. These results are promising. A further improvement to the biocontrol strain, in which the *cry* gene will be cloned downstream of the efficient *tac* promoter (Ge at al. 1990) and the construct introduced into the chromosome, is underway. In addition the potential of an obligate sugarcane endophyte, *Acetobacter diazotrophicus* (Cavalcante and Dobereiner 1988), as a recipient for the *cry* gene is being investigated.

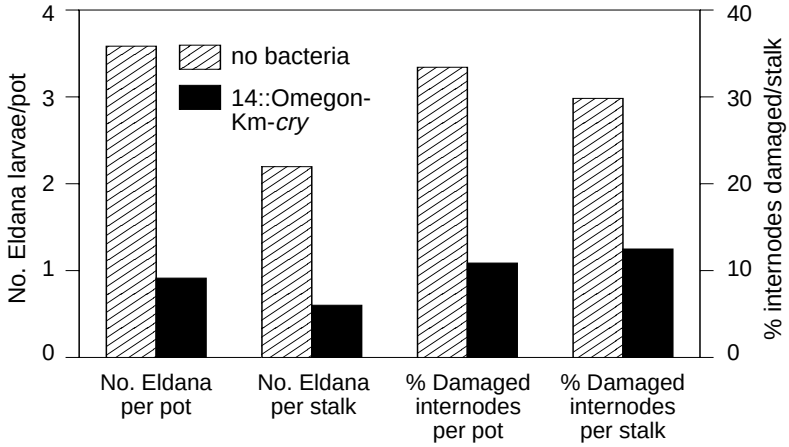

Figure 2. *Eldana* damage to sugarcane pretreated with no bacteria or with *P. fluorescens* 14::Omegon-Km-*cry*.

Acknowledgments

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Developing Maize with Resistance to European Corn Borer

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Abstract

The European corn borer (ECB), Ostrinia nubilalis *Hübner, causes hundreds of millions of US dollars in crop losses in the United States and Europe. With these large losses in mind, Northrup King began a multifaceted approach to develop commercial hybrids with resistance to ECB damage. A combination of conventional breeding tactics, molecular marker assisted breeding and transgenic technology have been employed to develop long lasting, effective resistance to this pest. Successes have been made using conventional pedigree breeding with an emphasis on ECB resistance, high yield, and good agronomic health. However, conventional breeding relies on artificial infesting with ECB, and it is resource intensive. Thus, we have actively pursued molecular-marker assisted breeding for stalk tunneling resistance to ECB. Molecular marker assisted selection allows 1) advances in selection in years with low ECB damage in the field; 2) more than one selection cycle in a year; 3) use of effective backcross breeding tactics for complexly inherited traits; and 4) reduced field evaluation. Transgenic technology has allowed the production of hybrid corn containing an insecticidal gene from* Bacillus thuringiensis Kurstaki. *During three years of field testing, corn plants containing this gene have provided excellent full-season control of ECB larvae. The combination of conventional breeding, molecular marker assisted breeding, and transgenic technology will result in stable, highly insect resistant hybrids. These should help us manage ECB and perhaps other lepidopteran pests into the future.*

Introduction

The European corn borer (ECB), *Ostrinia nubilalis* Hübner, reportedly causes hundreds of millions of US dollars loss in maize (corn), *Zea mays L.*, each year in Europe and the US. During the 1991 growing season, losses of $196 million were estimated in Minnesota (MN) alone (K. Ostlie, Personal Communication, 1992). MN growers planted slightly less than 10 percent of the total US corn acreage in 1991 (1991 USDA Annual Crop Summary, January 1992). Therefore, in years with high ECB populations such as 1991, loss due to ECB damage could surpass one billion US dollars throughout the world corn growing regions. Losses to ECB are extensive including:

- Physiological yield loss due to leaf, sheath, stalk, ear shank and kernel feeding damage.
- Harvest losses due to dropped ears or lodged plants.
- Costs associated with application of chemical insecticides to prevent damage.

In addition, stalk rot pathogens are often associated with damage by the corn borer. These pathogens further compromise the yield and standability of maize (Showers et al. 1989).

With this huge potential loss in mind, Northrup King Company has aggressively pursued the development of ECB resistant hybrid corn. We have used a multifaceted approach that includes conventional breeding, molecular marker-assisted selection, and transformation technology. Through combined research efforts, it is our primary goal to develop stable, high yielding, durable ECB resistant hybrids.

Conventional Breeding for Insect Resistant Maize

Various conventional breeding techniques have facilitated significant improvements in resistance to European corn borer. Often the breeding method of choice is a form of recurrent selection. Using recurrent selection, the selected resistant

progenies are intercrossed to increase the frequency of favorable resistance alleles. Barry et al. (1983, 1984, 1985) and Klenke et al. (1986a) reported successful use of recurrent selection to produce improved sources of resistance to ECB. Various modifications of pedigree breeding systems also have been used to develop ECB resistant lines and hybrids. Russell and Guthrie (1979) reported success using pedigree breeding to develop inbred line B86. Also, Hawk (1985) developed ECB resistance source DE811 using a pedigree breeding approach. There are many effective conventional breeding methods that may be used to improve resistance to insect pests. However, the source of resistance utilized and the exact goal of the breeding program must be considered.

Figure 1 shows a general pedigree breeding procedure for developing lines with improved levels of ECB resistance. We often use this or a similar method when crossing an elite (adapted) insect-resistant inbred line to an elite susceptible inbred line. The goal is to develop inbred lines with improved resistance levels relative to the susceptible elite line. Ultimately, through insect efficacy testing and yield testing procedures, a useful hybrid product may result. Figure 1 is self-explanatory for the most part, but some details that are not evident include:

- Artificial infesting with 300-600 ECB larvae begins at the F_3 (S_1) generation.
- Progeny may be screened against both leaf (first generation) and stalk (second generation) ECB damage.
- Winter nurseries may be used to produce testcross hybrid seed and sometimes to advance generations without ECB selection pressure.
- Testcross hybrids are evaluated for resistance under artificial ECB pressure.
- Testcross hybrids are evaluated for yield performance across multiple locations, throughout the testing procedure.

Principal selection criteria include:

- Improved insect resistance as a "line" *per se*.
- The ability to convey resistance to hybrid progeny produced using the "line".
- High general combining ability and ultimately specific combining ability with one or more other inbreds.
- Good agronomic appearance.
- Agronomic appearance includes features such as late season intactness, strong root systems, late season staygreen, disease resistance, and high grain quality. Numerous variations of this pedigree breeding protocol may be implemented according to personal preference and the goals of the breeding project.

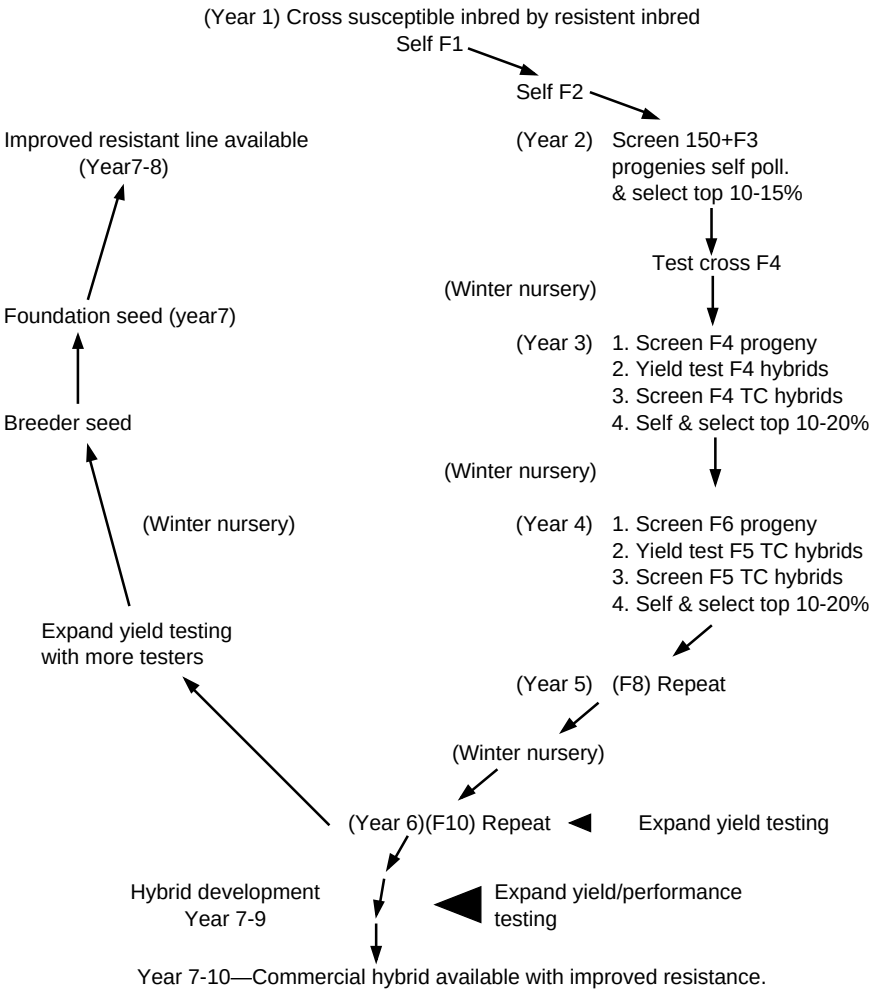

Figure 1. Conventional pedigree breeding for ECB resistant lines.

Results of Conventional Breeding Efforts

Conventional breeding technology has contributed greatly to reducing loss to the European corn borer. Barry et al. (1991) tested 400 commercial corn hybrids over a four year period. They found that 90% of the hybrids had intermediate or better resistance to leaf feeding damage by ECB. Of the tested hybrids, 75% had intermediate or better resistance to sheath and stalk tunneling damage.

Figure 2 displays an inbred line with resistance to ECB leaf feeding damage. This inbred displays a leaf feeding rating of 3, using Guthrie's 1-9 scale, where 1 represents no damage or a few pinholes and 9 represents severe leaf damage on several leaves (Guthrie et al. 1960). This line also has strong resistance to ECB stalk tunneling damage. Damage remains consistently below 5 centimeters per plant on average. Compare this to a susceptible inbred line with a leaf damage rating of 9 shown in Figure 3. The resistant inbred line was developed using a conventional pedigree breeding technique with selection under ECB feeding pressure. Both natural ECB pressure and artificial ECB pressure aided selection as this line was developed.

Figure 4 displays an example of ECB stalk tunneling resistance. This figure displays variation seen in segregating F_3 progeny rows. These F_3 progenies were the result of a cross between a susceptible inbred line and the resistant inbred line shown in Figure 2. Both of the displayed plants were infested with over 250 ECB larvae around anthesis. The resistant F_3 progeny row (top of photo) displayed an average of only 2.5 cm of tunneling damage per plant. In contrast, the susceptible F_3 progeny (bottom of photo) displayed an average of 36.6 cm of damage. The variability that exists in early generations of a cross between a resistant and susceptible parent allows useful selection for more resistant genotypes.

Maintaining stalk damage resistance in agronomically acceptable genotypes throughout the inbreeding process is labor

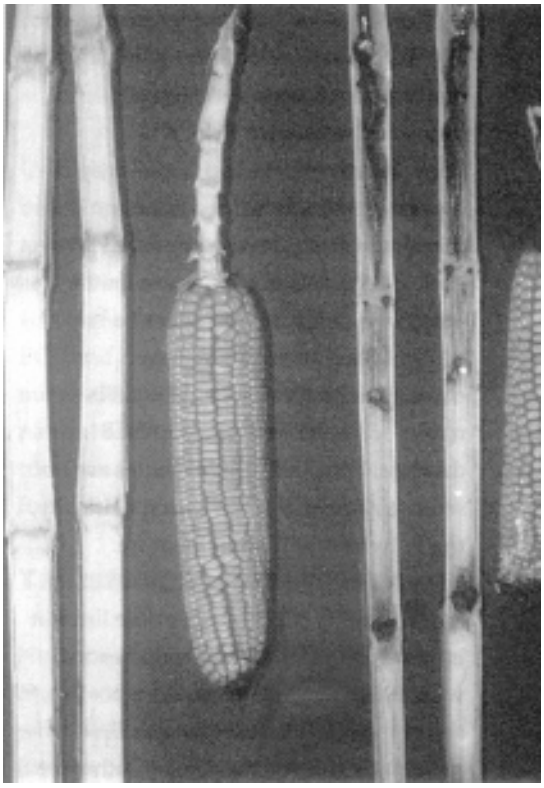

Figure 4. F_3 segregants produced by crossing susceptible inbred line by resistant line shown in Figure 2. Segregants show variation in levels of resistance to stalk and ear shank tunneling damage by European corn borer. Left plant is resistant to both types of damage. Right plant is susceptible to both types of damage. Each plant artificially infested with over 250 neonate ECB larvae at anthesis.

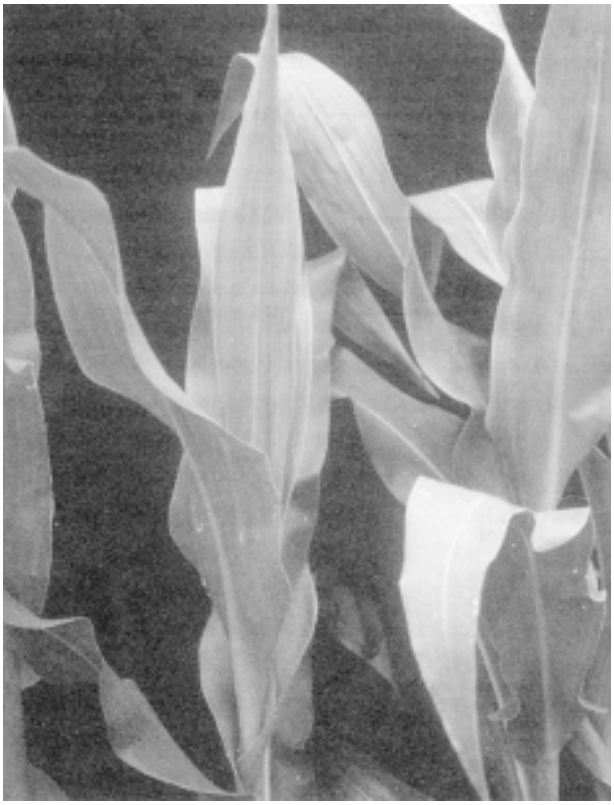

Figure 2. Inbred line with natural European corn borer leaf feeding resistance. Rates a "3" on 1-9 scale, where 1 = no damage or a few pinholes.

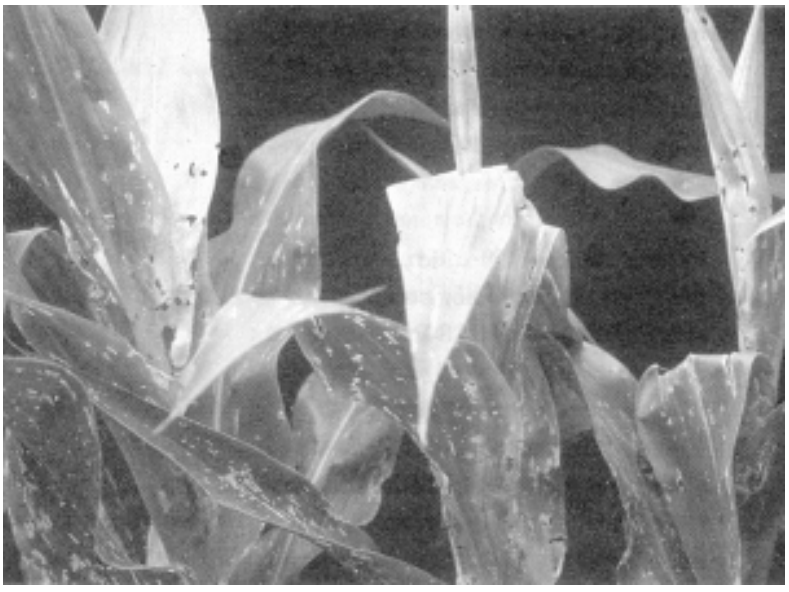

Figure 3. Inbred line showing high susceptibility to European corn borer leaf feeding damage. Rates a "9" on 1-9 scale, where 1 = no damage and 9 = several leaves shredded by ECB.

intensive and difficult. Often resistance alleles are lost during inbreeding and selection processes. Sometimes improved resistance to ECB is negatively correlated with grain yield, especially if yield is not a selection criterion during development (Klenke et al. 1986a). Often crosses are made between a resistant inbred and a susceptible inbred to produce F_1 commercial hybrid seed. Heterosis masks some susceptibility to ECB damage, but if hybrid progenies are not screened for ECB resistance specifically, the F_1 hybrid will often be more susceptible than desired. When crossing resistant by susceptible lines, it is preferable that resistance genes act with at least partial dominance to convey useful resistance to the F_1 hybrid progeny of the cross (Guthrie et al. 1985, 1989).

Finally, labor demands associated with developing ECB stalk damage resistance conflict with other essential operations in plant breeding programs. Artificial infesting for stalk tunneling damage evaluations occurs at anthesis, the same time hand pollinating activities typically occur in a breeding nursery. Damage evaluation (stalk splitting) also conflicts with hand harvest, and occurs after students (seasonal assistants) have returned to school.

In spite of these difficulties, plant breeders and entomologists can successfully reduce ECB stalk tunneling damage to approximately one-third that sustained by the original susceptible parent (D. Mies, Personal Communication, 1994). Guthrie et al. (1985, 1989) demonstrated that resistant inbred lines B86 and DE811 conveyed improved leaf feeding and stalk damage resistance to hybrids produced using them. Therefore, substantial gains have been made using conventional breeding methodology. The future holds additional improvements through conventional breeding to develop improved resistance sources. After repeated cycles of inbreeding, selection, yield testing, and advance, both inbred lines and commercial hybrids with improved resistance can be developed.

Molecular Marker-Assisted Breeding for ECB Resistance

Scientists have demonstrated that resistance to second generation ECB stalk and sheath damage is a quantitatively inherited trait which is conditioned by at least five alleles (Schön et al. 1993; Onukogu et al. 1978; Northrup King Company research, 1987-present). Therefore, backcross breeding would not normally be considered a practical approach for developing plants with improved resistance. However, with the assistance of molecular probes to track movement of both favorable resistance alleles and recurrent parent alleles, feasibility of backcross breeding for a complexly inherited trait improves. Figure 5 shows a typical backcross breeding procedure which may be used in conjunction with molecular marker-assisted selection.

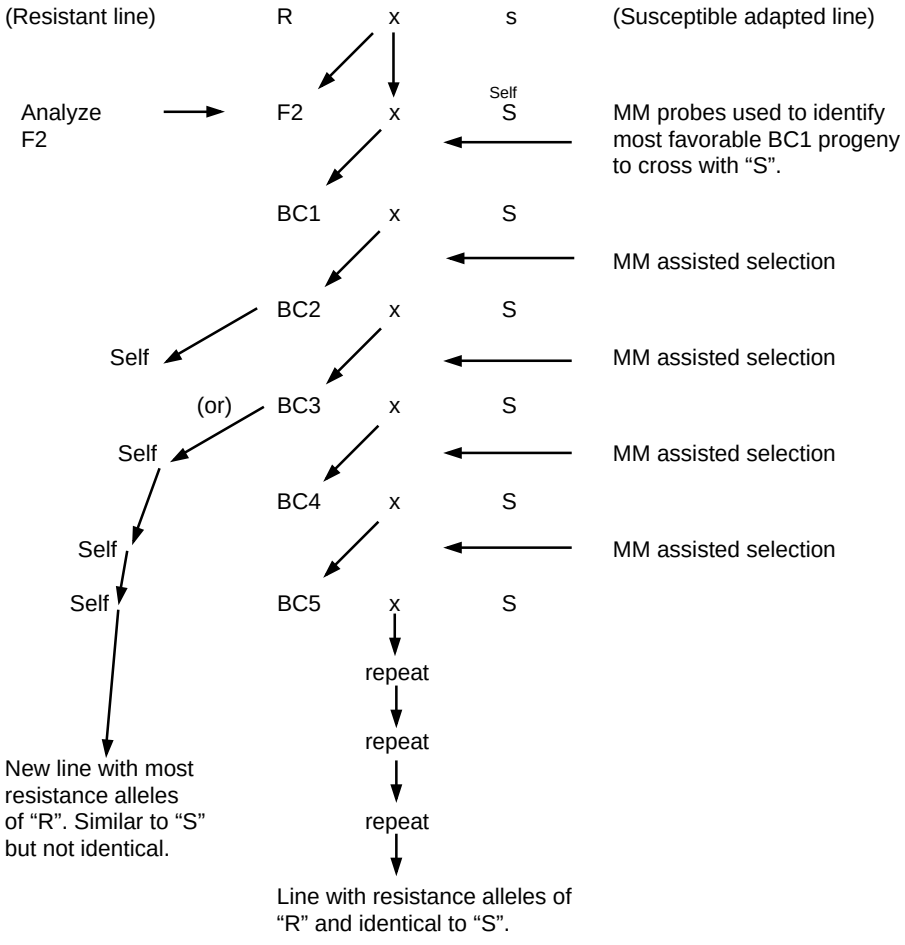

Figure 5. Molecular marker assisted backcross breeding procedure to select for ECB resistant lines.

Using artificially infested field trials, molecular markers (probes) are identified that are associated with resistance to stalk damage by ECB larvae. This is the process of developing a quantitative trait loci (QTL) model. To date, we have developed several QTL models for various sources of ECB resistance. These QTL models are currently being used to help develop lines and hybrids with improved natural resistance to ECB damage.

Identification of molecular probes is typically performed as follows:

- 200 (or more) F_2 (or later generation) progeny of a cross between a resistant parent and a susceptible parent are analyzed for ECB stalk tunneling resistance.
- DNA samples from the same progeny are cut into fragments using restriction enzymes.
- Fragments are analyzed using a broad set of molecular marker probes developed by Northrup King and assorted public and private institutions.
- Polymorphic probes that distinguish between the two parental genotypes are identified.
- Regions that are significantly associated with resistance to ECB feeding damage are identified using least squares analysis (e.g. regression analysis) and computer programs such as Mapmaker QTL (a software program designed to link molecular markers to phenotypic traits).
- Lander and Botstein (1989) describe details of Mapmaker QTL software use for these types of analyses. Lee et al. (1989) and Schön et al. (1993) describe specific details of methodology surrounding restriction fragment length polymorphism (RFLP) analysis.

Benefits of Molecular Marker-Assisted Selection for ECB Resistance

Although molecular marker technology is not likely to replace conventional techniques and field testing altogether, it may enhance these efforts significantly. Molecular marker assisted selection may:

- Allow advance in resistance development even in years with low natural ECB pressure (or low pressure from artificially infested ECB).
- Allow two or more selection cycles per year, since field evaluation is not essential each cycle.
- Allow the use of more efficient backcross breeding strategy for quantitatively inherited multigenic traits.
- Reduce workload associated with artificial infestation (which coincides with breeding nursery hand-pollinating) and fall damage evaluation (stalk splitting, which coincides with harvest).

Scott et al. (1967), Jennings et al. (1974), and Sadehdel-Moghaddam et al. (1983) demonstrated that resistance is conditioned predominantly by additive gene effects. However, the exact number and location of resistance factors (loci) vary according to the source of resistance utilized. Therefore, for each different resistance source utilized, molecular marker probes must be identified that are associated specifically with that source's ECB resistance alleles. These probes need to be polymorphic so they differentiate between the alleles of the resistant and susceptible genotypes in chromosome regions linked to resistance genes. Provided these conditions are met, molecular marker probes can be used to follow resistance alleles in progeny of a cross between a resistant parent and the susceptible parent you wish to improve.

Using a combination of conventional breeding tactics, artificial infestation, and molecular markers, plant breeders and entomologists have the tools to successfully reduce damage caused by ECB and other lepidopteran pests of maize. These improved sources of natural resistance combined with transgenic technology should provide a formidable source of ECB resistance.

Transformation Technology

Northrup King Company's corn plant transformation research began in 1987, when we obtained the first genes from *Bacillus thuringiensis Kurstaki*. Between 1987 and 1990, Northrup King and other private organizations invested significantly in the development of insect resistant transgenic plants. During that period several obstacles had to be overcome. They included:

- Cloning the *Bt* gene.
- Construction of functional expression vectors.
- Improving protein expression in transformed plant tissues.
- Modification of the gene itself (changing the nucleotide sequences to make them more plant like.
- Developing successful maize transformation techniques.

Between 1990 and 1992 ballistic and protoplast transformation methods became available which allowed successful recovery of fertile transformed maize plants. Figure 6 schematically displays two common methods of transformation. In the ballistic method, microscopic tungsten particles coated with foreign DNA are forcefully propelled through the cell

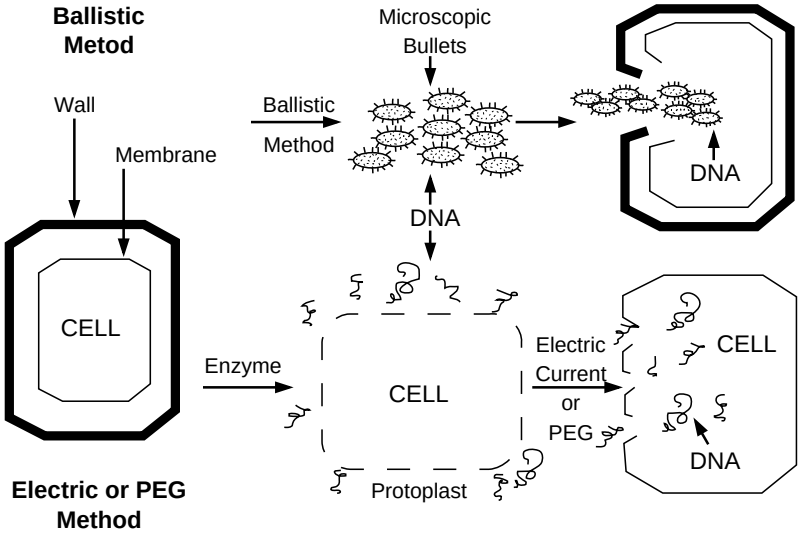

Figure 6. Two methods of plant transformation. Ballistic method shown on top. Protoplast method shown on bottom.

wall into the cytoplasm and nuclei of cells. In the protoplast method, the cell walls are first removed. Then the cell membrane becomes readily permeable to foreign DNA. Movement of foreign DNA through the cell membrane is facilitated either by applying an electrical current (electroporation) or adding Polyethylene Glycol (PEG). Foreign DNA in solution surrounding the cells passes through the cell membrane, with some of it being incorporated into the nuclei of cells.

Following either method of transformation, approximately 120-210 days pass prior to harvesting seed from the primary transformed plants. Photographs that demonstrate an approximate timeline of critical steps following protoplast transformation are shown in Figure 7. First, the transformed cells are placed on nurse cell cultures. These nurse cell cultures supply nutrition and provide a suitable osmotic environment for the fragile transformed cells. Often, a selective agent is included in the cell culture medium to kill non-transformed cells. After approximately 3 months growth on selective culture medium, calli are transferred to regeneration medium. Approximately 4 months following transformation, small seedlings are transferred to magenta boxes, which allow upright growth and normal development of roots, shoots, and leaves. During the last days in growth chambers, magenta boxes are opened so plantlets are exposed to air. This helps the leaves adapt to the less protected environment they will be exposed to in the greenhouse. Approximately 4.5 months post-transformation, seedlings are transplanted to soil and moved to greenhouses to grow to maturity. As soon as anthesis begins, plants are either self-pollinated or crossed to other elite non-transformed lines. Depending on which transformation technique is used this entire process, from transformation of cells to seed production, requires approximately 4-7 months.

Following initial transformation and production of fertile plants, *Bt* genes were backcrossed into elite parental lines to develop commercial hybrids expressing resistance from *Bt* genes. Throughout backcrossing and breeding procedures, selective herbicides acted as highly effective tools for selecting

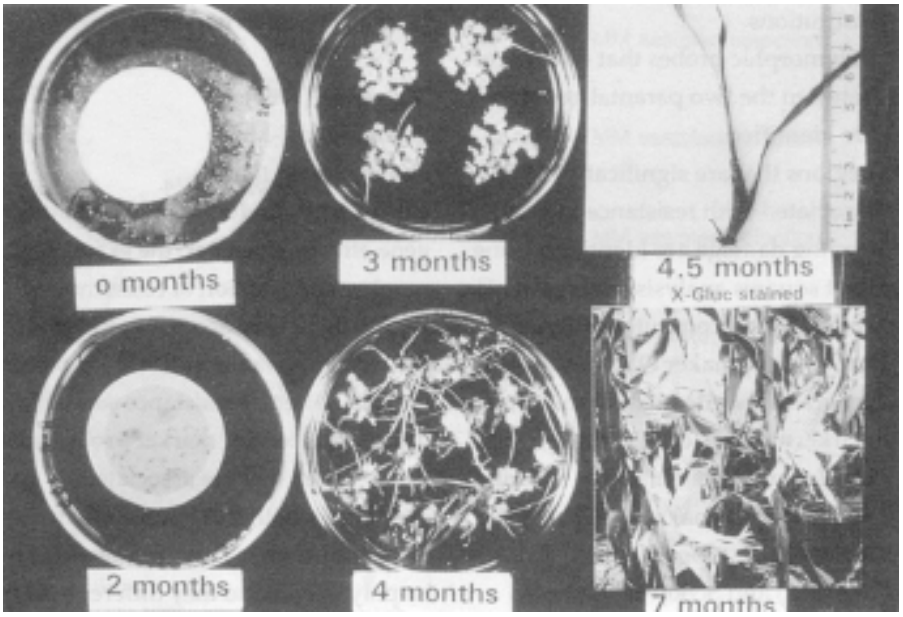

Figure 7. Approximate timeline for recovery of seed following polyethylene glycol mediated protoplast transformation. 0 months, transformed cells placed on nurse cell culture containing selectable agent; 2 months, transformed calli multiply; 3 months, healthy calli transferred to regeneration medium; 4 months, plantlets regenerate and are transferred to magenta boxes; 4.5 months, upright plantlets are transferred to soil in greenhouse; 7 months, transformed plants produce seed.

transformed plants. Plants grown in the greenhouse or field were sprayed with appropriate selective herbicides to eliminate those that were not transformed.

After several years of developmental research by Northrup King and contributions by several other private companies, we conducted our first field trials in 1992. Transgenic corn plants were field tested against ECB, the primary lepidopteran pest of U.S. maize.

Materials and Methods for Field Evaluation of Transformed Corn Plants

Artificially infested ECB efficacy evaluation

During the past three years, transformed maize has been screened against ECB using similar protocols each year. Seeds were planted to result in a final plant stand of 30 plants per 5.7 meter row (0.77 m row width). Two-row plots were planted to leave an uninfested buffer row between infested rows. Typically, 2 or 3 replicates of each entry were planted in randomized complete block design experiments. Replicate trials were planted at multiple locations. To evaluate leaf feeding damage, approximately 250 neonate larvae were applied to each plant in the first row of the 2-row plot. Infestation began as the plants reached the fifth leaf of development. A modified "bazooka" (Davis and Oswalt 1979; Mihm 1983) was used to infest approximately 50 larvae per plant per application. Larvae were applied every 3 days over a 2 week period. Plants were infested again at anthesis to simulate infestation for stalk damage. Approximately 250 larvae were applied directly to the leaf axils around the ear zone of plants. Multiple applications were spread over a 2 week period.

Leaf feeding damage was evaluated using a 1-9 whorl leaf damage rating, where 1 represents no damage and 9 represents several leaves with severe leaf shredding (Guthrie et al. 1960). Stalk tunneling damage was evaluated by dissecting stalks from approximately 4 nodes above the primary (top) ear down to the ground. Total ECB tunnel length was estimated in inches and converted to centimeters.

Natural pressure ECB efficacy trials

To gain information on the effects of *Bt*-maize on natural populations of ECB, an observation range was planted at all 1994 field test sites. At each site, we planted approximately eight *Bt* hybrids and eight representative non-*Bt* control hybrids. At each site, natural ECB feeding pressure was monitored by dissecting 10 plants each, of 2 different non-*Bt* hybrids (20 plants in total). If either control hybrid displayed an average tunneling score $\geq$ 5.1 cm, 10 plants were dissected from each plot at that site. Only six locations met this minimal damage threshold throughout the Midwest testing region. However, these trials provided useful stalk damage data that were analyzed across all trials of similar maturity. These trials were divided into two groups; northern US Corn Belt adapted hybrids or southern US Corn Belt adapted hybrids.

Bt-Transgenic Field Trial Results versus ECB

Field testing of transformed corn against ECB, the target pest, has been conducted during the past three years. Excellent full season ECB control has been the result each year. Leaf feeding damage has been limited to a few pinholes on one or two leaves. Figure 8 compares a non-transgenic plant (left) to a *Bt*-transgenic plant (right). Neonate ECB larvae only took a few bites of the transgenic tissue before they stopped feeding. Within 24 hours the neonate insects were dead. In artificially infested

Figure 8. *Bt* corn (right) shows no leaf feeding damage by ECB. Non-*Bt* control plant (left) shows first symptoms of severe leaf feeding damage. (Photo taken 10 days following infestation with approximately 200 neonate ECB larvae).

trials over the past two years, average leaf feeding ratings on transgenic hybrids have been 1.06. In contrast, non-transgenic control hybrids have displayed an average leaf damage rating of 3.71 (LSD = 0.58, a=0.05)(Fig. 9).

Larvae infested at anthesis to simulate ECB stalk tunneling damage also died quite rapidly. Very few live larvae were found in the stalks of thousands of *Bt* hybrids dissected over the past three years. Average tunneling damage was only 0.15 cm per *Bt*-hybrid versus 4.53 cm per non-transgenic hybrid control (LSD 2.16 cm, a=0.05) (Fig. 9).

In naturally infested ECB observation trials, results were equally dramatic (Fig. 10). *Bt* hybrids adapted to northern U.S. Corn Belt growing regions displayed only 0.10 cm of stalk tunneling damage on average. The non-*Bt* control hybrids displayed 5.20 cm of damage on average. Southern U.S. Corn Belt *Bt* hybrids displayed 0.3 cm of tunneling damage on average, compared to 9.7 cm of damage in non-*Bt* control hybrids. *Bt* hybrids have displayed tremendous reductions in stalk tunneling damage and improvements in late season plant intactness, relative to non-*Bt* hybrids. Figures 11 and 12 display these improvements, respectively.

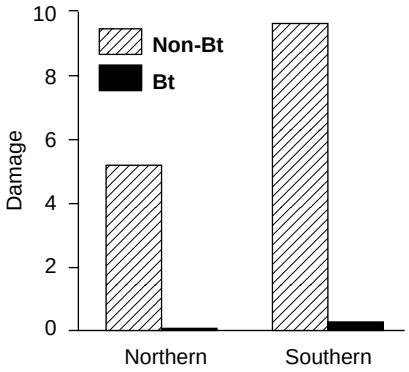

Figure 10. Stalk tunneling damage on plants under natural ECB pressure. Combined multi-location data. Hybrids were divided into two groups: those adapted to the northern U.S. corn belt and those adapted to the southern U.S. corn belt. Stalk damage expressed as average centimeters tunneled per plant. LSD (a=0.05): northern hybrids = 1.8 cm; southern hybrids = 5.6 cm.

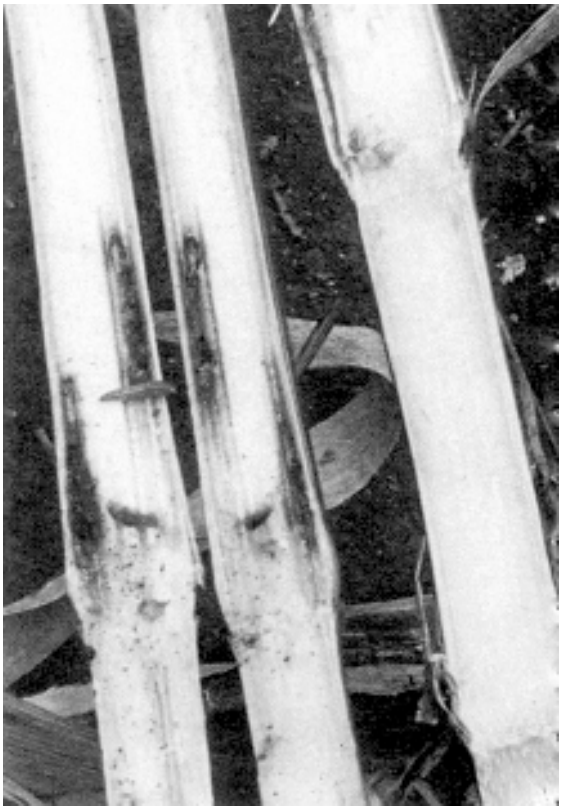

Figure 11. *Bt* hybrid (right) displays no stalk damage by ECB. Non-*Bt* hybrid (left) with live larva and associated stalk tunneling damage.

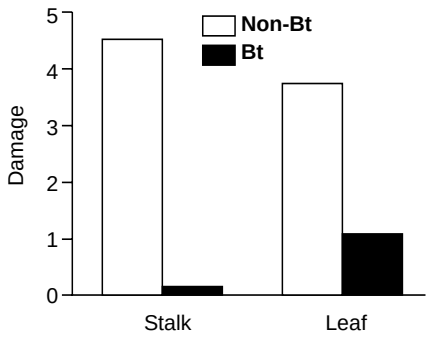

Figure 9. 1993 and 1994 combined ECB trial results across multiple locations and hybrids. Hybrid plants artificially infested during whorl stage of growth and at anthesis. Stalk damage expressed as average centimeters tunneled per plant. Leaf damage expressed on 1-9 scale where 1 = no damage or a few pinholes.

Figure 12. *Bt* hybrid (left) shows substantial improvement in late season plant intactness relative to non-*Bt* hybrid (right). Natural ECB feeding pressure at SW Iowa trial site, 1993.

Discussion

Tremendous gains have been made in developing natural sources of ECB resistance. Additional gains remain to be made using conventional breeding techniques. Also, the future holds a range of new tools to aid selection for resistance (e.g. molecular marker technology) and complementary, novel sources of resistance incorporated through transformation technology. Plant breeders and entomologists should be able to develop durable sources of plant resistance using a combination of:

- Conventional resistance sources and breeding procedures.
- New resistance sources from other species or novel proteins.
- Molecular marker technology to track resistance genes.

These new tools and sources of resistance will enhance the efficiency with which we can breed for resistance to insect pests. In turn this should help us manage insect pests of maize into the future.

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The Expression of a Synthetic *CryIA(b)* Gene in Transgenic Maize Confers Resistance to European Corn Borer

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Abstract

Pest control constitutes a major area of interest for the biotechnology industry. Genes encoding insecticidal proteins have been cloned and they are being introduced in crop plants. At CIBA Agricultural Biotechnology, we have introduced a truncated form of the cryIA(b) *gene obtained from* Bacillus thuringiensis *into an elite line of maize. A synthetic version of the gene was made to increase CryIA(b) protein levels in transgenic maize. The expression of the* cryIA(b) *gene was targeted to the pollen, pith, and green tissues by using appropriate tissue specific promoters. The resulting transgenic maize plants were evaluated for resistance to European corn borer (ECB),* Ostrinia nubilalis, *under field conditions. Plants with high levels of the CryIA(b) protein exhibited excellent resistance to repeated heavy infestations of the pest.*

Introduction

Propagation of plant varieties for the purpose of improving certain traits has been the main goal of plant breeding. Successful breeding programs consist of multi-step processes where plants are crossed and crossed again until the desired character(s) is obtained. Recently, genetic engineering has provided the means to obtain genetic information about those favorable traits. One of the most important applications of genetic engineering to crops has been the production of insect-resistant plants in one step.

Bacillus thuringiensis (*Bt*) is a Gram-positive, spore-forming bacterium, which produces parasporal crystals during sporulation. These crystals are formed by proteins (known as δ–endotoxins) which posses insecticidal activities when ingested by certain insects. Indeed, *Bt* strains have been used since 1938 as insecticidal sprays and, considering their record in efficacy and safety, they are now prime targets for plant biotechnology. δ–Endotoxins are the product of single genes and they constitute the seminal tool to engineer plants resistant to insects.

The first generation of transgenic *Bt* plants is represented by transgenic tobacco (Vaeck et al. 1987; Barton et al. 1987; Adang et al. 1987) or tomato (Fischhoff et al. 1987) where the expression of native *cryIA(b)* genes was driven by constitutive promoters. The resulting transgenic plants conferred good protection towards tobacco hornworm, *Manduca sexta*. However, it was clear that higher levels of *cryIA(b)* expression would be needed to achieve control of other agronomical important pest such as tomato fruitworm, *Heliocoverpa zea*, and tomato pinworm, *Keiferia lycopersicella*.

The second generation of transgenic *Bt* plants involves expressing δ-endotoxin genes whose sequences have been optimized for plants under the control of new promoters including tissue-specific promoters. Advances in transformation techniques has allowed to expand insect-control programs to monocots, in particular maize.

Material and Methods

Transformation vectors

Vectors used to transform maize are all derivatives of pUC18 or pUC19. They contain a truncated-synthetic version of the *cryIA(b)* gene from *Bacillus thuringiensis* var. *kurstaki* placed under the control of either the CaMV 35S promoter or tissue-specific promoters (Fig. 1).

Transformation and embryo rescue

Immature embryos (maize inbred CG00526) were excised 2 weeks after pollination and plated scutellum up on 2DG4 + 5 mg/l chloramben. Plasmid

DNA was deposited onto microprojectiles as described in the DuPont Biolistic manual. Generally, 6 mg of DNA are used per 50 ml of microcarrier. Delivery of the microprojectiles is performed using the PDS-1000He Biolistic Gun with rupture disks of 1550 psi. After bombardment, embryos are kept for one day in the dark at 25ºC, and then transferred to a callus initiation medium containing 3 mg/l of phosphinothricin (PPT). Resultant embryogenic callus was transferred to 2DG4 supplemented with 0.5 mg/l of 2,4-dichlorophenoxyacetic acid. About twelve weeks later, tissue was transferred to MS medium containing 3% sucrose and hormones (Koziel et al. 1993). Transformed plants were identified by PCR for sequences in the promoters and the synthetic *cryIA(b)* gene. Positive plants were moved to the greenhouse for additional tests and crosses with various inbreeds. Sixteen days after pollination, the ear tips were removed, the embryos excised and plated on B5 medium containing 2% sucrose.

Insect infestations

Lab-grown ECB larvae were used to infest plant material. Infestations started when maize plants were about 40 cm high. For four weeks, 300 neonates each week were mixed with corn cob grits and introduced into the whorl of each plant. When plants reached anthesis, a second round of infestations took place. 300 neonates per plant each week for four weeks were applied to emulate a second generation infestation. One hundred were deposited into the leaf axil of the primary ear, one node above and below the primary ear. The extent of the internal ECB tunneling damage was assessed in 90 cm sections of the stalk.

CryIA(b) protein determinations

Quantitative determinations of the levels of CryIA(b) protein were performed by ELISA (Clark et al. 1986). Immunoaffinity-purified polyclonal rabbit and goat antibodies specific for the CryIA(b) protein were used. The sensitivity of the double sandwich-ELISA is 1-5 ng CryIA(b) per mg of soluble protein from crude plant extracts. Extracts were prepared as described by Carozzi et al. (1992).

Results

Biology of the target

The principal target pest for CIBA has been European Corn Borer (ECB), *Ostrinia nubilalis*. ECB is a major pest in Europe and North America causing yield losses ranging from 3 to 20%. ECB has two generations annually, but three or even four generations can occur depending on the area of distribution. ECB larvae migrate into the whorl and feed on leaf material. First-instar larvae tunnel into the stalk where they will feed and pupate. Adult moths emerge over the summer period and deposit their egg masses on the abaxial side of the leaves close to the ear node. Neonates generally move to the leaf axils and feed on accumulated pollen, sheath and collar tissue. Larvae begin to tunnel into the stalk after three to six weeks, often in the ear region and this is where the feeding causes severe yield losses from stalk breakage and/or ear dropping.

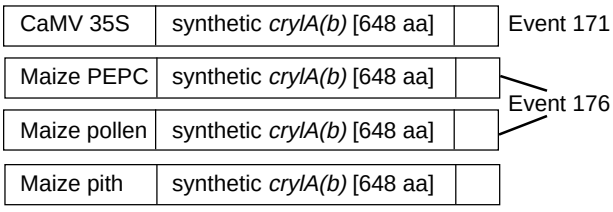

Figure 1. Versions of the maize optimized *cryIA(b)* gene under different promoters. The synthetic gene encodes the amino terminal 648 amino acids of CryIA(b) protein from *Bacillus thuringiensis* var. *kurstaki* HD-1. The promoters driving *cryIA(b)* gene expression are a green tissue-specific, a pollen-specific, and a pith-preferred promoter.

Optimizing δ–endotoxin expression in transgenic maize

Increasing the GC content of *B. thuringiensis* insecticidal protein genes leads to better expression in plants (Perlak et al. 1991). The Insect Control Group at CIBA decided to make a synthetic version of the *cryIA(b)* gene increasing the GC content from 38% in the native gene to 65% in the synthetic version. The gene encodes the first 648 amino acids (aa) of the 1155 aa protoxin and it produces the same active insecticidal toxin as the full-length protoxin, once it is processed in the insect gut. The expression of the synthetic *cryIA(b)* gene is driven either by a constitutive promoter (35S), or by tissue-specific promoters (see Fig. 1): a maize phosphoenolpyruvate carboxylase (PEPC) promoter, which expresses in green tissues, a maize pollen specific promoter, which expresses in pollen (Estruch et al. 1994), and/or a pith-preferred promoter.

Chimeric *cryIA(b)* genes were introduced into proprietary inbred lines by microprojectile bombardment of immature embryos (Koziel et al. 1993). The *bar* gene, used to confer resistance to PPT, was used as selectable marker. The material obtained was then analyzed thoroughly for PPT resistance, CryIA(b) levels, and ECB resistance.

Evaluation of transgenic maize plants in the field

Germination of immature embryos was used to produce the F_1 hybrid plantlets for planting in the field. When plants

reached about 40 cm in height, they were infested with neonate ECB. A total of 2,400 larvae per plant were applied during the eight week treatment (300 per week). This represents 10 to 100 fold the economic threshold of second generation ECB. As indicated by the severe foliar and stalk damage produced in the control plants, the ECB pressure employed was strong enough to evaluate the performance of the transgenic maize for *cryIA(b).* Of the different transgenic maize lines, the offspring coming from the cross CG00554 x 176 provided the best resistance, where no leaf damage could be observed (see also Koziel et al. 1993). Concerning the second generation of ECB, whose principal target is the stalk, transgenic maize and in particular CG00554 x 176, offered an excellent resistance against ECB. For example, while a control plant had 59 cm of tunneling damage on average, the transgenic line had less than 2 cm.

The best performers among the transgenic maize plants were thoroughly analyzed for *cryIA(b)* gene expression and CryIA(b) protein levels. Transgenic maize for the *cryIA(b)* gene under the PEPC and pollen specific promoter produced over 1000 ng CryIA(b) protein per mg of total protein (they could contain up to 4 times more) in leaves and up to 400 ng / mg in pollen. While the expression of the pith-preferred promoter led to lower levels of CryIA(b) protein (around 35 ng / mg in pith), it was sufficient to control ECB. In addition, the CryIA(b) protein could not be detected in kernels in these plants expressing the gene under tissue-specific promoters.

Discussion

Chimeric genes were introduced into elite inbreds of maize via microprojectile bombardment of immature embryos. The possibility of transforming inbred lines represents a significant advantage over the regular breeding programs.

The *Bt* gene encoding the δ–endotoxin CryIA(b) has been optimized for expression in maize plants. Maize plants transgenic for the *cryIA(b)* synthetic gene are protected from heavy infestations of European Corn Borer. This protection is observed in plants hemizygous as well as homozygous for the *cryIA(b)* gene, so hybrid maize obtained from a transgenic parent will inherit the protection trait.

Tissue-specific expression of the cryIA(b) gene is achieved by using green, pollen and pith-preferred tissue specific promoters. The use of these promoters allows expression of the insecticidal protein in parts of the plant where ECB feeds while minimizing expression of the insecticidal gene in seeds. The presence of the CryIA(b) protein in pollen is particularly important because it constitutes the main diet during the first and second instar of the ECB (Showers et al. 1989). The effectiveness of the transgenic maize plants against ECB infestation has also been tested under field conditions. Several transgenic maize lines have been produced, in particular line 176, that are very resistant to ECB even under infestation pressures several orders of magnitude higher than those occurring naturally.

Our group at CIBA Agricultural Biotechnology has created the framework to introduce traits into maize. Transgenic maize plants resistant to ECB are now a reality, and as improved insecticidal genes become available, they can be rapidly introduced into commercial maize lines. Transgenic plants will therefore represent an invaluable tool to use in integrated pest management strategies.

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Sustaining Host Plant Resistance Derived Through Conventional and Biotechnological Means

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Abstract

Globally, during the last four decades, large investments and long-term research efforts have been put into plant breeding to develop pest resistant varieties and hybrids of crop plants to substitute for the use of toxic chemical pesticides. More recently, new tools of biotechnology have been added to the plant breeding programs to speed up this process. Many pest resistant varieties and hybrids have been released, and in a few years, genetically engineered transgenic varieties and hybrids are expected to be commercialized and released worldwide. Pests can adapt to any management tactic depending on the selection pressure exerted on them, so deployment strategies must be designed and implemented to delay or prevent the breakdown of resistance. Some of these strategies may include use of multiple genes, combining the host plant resistance (HPR) derived through conventional and biotechnological means to pyramid or stack resistance genes, rotation or alteration of genes, use of different gene promoters, and manipulation in the levels of expression (spatial and temporal) of genes. In addition, these HPR deployment strategies must be integrated into an overall integrated pest management (IPM) program that incorporates multiple tactics (cultural, biological, mechanical, chemical, etc.) to diversify pest mortality sources and reduce subsequent selection pressure on the pests. Pest resistance management must be viewed within the context of IPM. If IPM is successfully adopted and implemented at a community or landscape level, the objective of resistance management will be automatically achieved. Hence, IPM should become a part of national agricultural policy.

Introduction

Food losses due to insect pests represent a major threat to global food security. Sustaining global food security will be of even greater concern in the future as the world's population continues to grow. With the advent of the insecticide, DDT, in the late forties, toxic chemical pesticides have been extensively used in agricultural landscapes to manage pests and help reduce food losses. However, due to a negative impact of pesticides on the environment and human health, the global community has been actively looking for alternatives to toxic chemical pesticides.

Several different approaches such as biological control, breeding for host plant resistance (HPR), cultural control and mechanical control have been investigated to substitute for chemical pesticides. Globally, during the last four decades, large investments and long-term research efforts have been put into plant breeding to develop pest resistant varieties and hybrids in both the public and private sector.

Many pest resistant varieties and hybrids developed through conventional plant breeding have been released; and undoubtedly this will continue in the future. More recently, the new tools of genetic engineering have been added to plant breeding programs to speed up this process. These new tools of biotechnology allow us to incorporate alien genes into crop plants to impart resistance to insect pests. The development of transgenic plants has given a new dimension to HPR.

In a few years, genetically engineered transgenic varieties and hybrids are expected to be commercialized and released worldwide. However, pests can adapt to a host plant resistance mechanism if sufficient selection pressure is exerted on them. In this paper we discuss different strategies for delaying or preventing the breakdown of resistance.

Host Plant Resistance (HPR) as a Tool of Pest Management

Plant resistant to insects is composed of genetically inherited qualities that result in a plant of one cultivar of a

species being less damaged than a susceptible plant, which lacks this quality (Smith 1989). Three types of resistance are recognized: non-preference (for shelter, food and oviposition), antibiosis (adverse effects of the plants on the biology of insects), and tolerance (ability of the plant to withstand damage or recover from damage caused by populations of insects that would decimate a susceptible plant). Non-preference prevents insects from occurring, antibiosis prevents them from establishing at high levels, and tolerance protects the host from large yield reductions.

The use of resistant varieties or hybrids offers an economic, stable and ecologically sound approach to minimizing losses from insect pests. This method is particularly appropriate for subsistence farmers in sub-tropical and tropical regions of developing countries who often have limited resources and inadequate knowledge of, or access to pesticides. In addition, HPR, unlike pesticides, is compatible with all other pest management tactics.

These arguments have justified large investments and long-term efforts by the global community in developing pest resistant varieties through conventional and biotechnological means. Through conventional breeding, resistance genes have been identified from plants within the same species and wild relatives. Some of these sources have been successfully incorporated into elite germplasm and varieties. In the case of maize in developing countries, excellent progress has been made in identifying sources of resistance to many important pests through conventional means.

More recently transgenic plants have been developed by incorporating alien genes such as *Bacillus thuringiensis* (*B.t.*) from bacteria, and trypsin inhibitor genes into crop plants. *B.t.* is an aerobic, gram positive, spore forming bacterium commonly found in the environment (McGaughey and Whalon 1992). The presence of a number of insect toxins in *B.t.* has been well documented. The most distinctive of these are protein crystals formed during sporulation (Feitelson et al. 1992). Gene transformation offers a potential method of delivery for the toxin. Using genetic engineering techniques, the *B.t.* genes have been inserted into many plant species including maize, tobacco, tomato, potato, and cotton.

Problem of Pest Resistance

Pest resistance is the adaptation of pests to management tactics. Pests can adapt to any management tactics depending on the selection pressure exerted on them. Although all living organisms have an ability to respond to their environment, arthropods are among the most successful. The ability of insects to utilize a variety of niches also allows them to compete with human beings for food and fiber. In response, humans have used a variety of tactics to reduce the impact of pest insects. However, as with any selection pressure placed on a population, the insect's response has been to adapt to an altered environment.

Pest resistance is a consequence of natural evolutionary processes and is not limited to a particular agricultural system. Thus, it has become a global phenomenon. Examples of pest resistance abound. Insects have developed behavioral, physiological, or metabolic mechanisms of resistance to HPR factors (Kogan 1976; Smith 1989), cultural control (Ostlie 1987), biological control agents (Maund & Hsiao 1991), and insect controlling pathogens (Dunn 1986).

In the case of HPR developed through conventional plant breeding, rice brown plant hopper, *Nilpervata lugens*, have been reported to have overcome the resistance in varieties developed by the International Rice Research Institute (IRRI) and many national agricultural research programs in Asia (Heinrichs 1986; Saxena 1987). In the case of maize, the only reported case of pest overcoming HPR is corn leaf aphids *Rhopalosiphum maidis* (Smith 1989). There is optimism for the future of agriculture due to developments in plant biotechnology (e.g. new crop varieties that use *B.t.* genes to impart plant defense mechanisms). But this new technology is already at risk since resistance to *B.t.* toxins has developed in the field (Tabashnik et al. 1991).

In the USA, the DIMBOA mechanism of HPR to European Corn Borer in maize has remained stable for the last three-four decades and has not broken down. This is mainly due to the fact that in the USA, not all varieties planted are resistant and maize is only grown once a year which limits the number of generations of corn borers to 2-3 per year. Hence, the selection pressure has been very low and slow. However, the warm and humid climates of tropics and subtropics (where most developing countries are located) are more conducive to pest development. Pests reproduce rapidly and produce multiple generations in a given season or a year, exerting higher selection pressure. At present, few pest resistant maize varieties have been released in developing countries.

However, when resistant varieties are more common, the chances of pests overcoming resistance will be higher than in temperate countries, although the effects are likely to be mediated if multiple genes are involved in conferring the resistance.

Management of the Pest Resistance Problem

Widespread development of pest resistance could seriously diminish the economic value of HPR and force continued reliance on chemical pesticides. This is particularly true for polyphagous insect pests, where breakdown of *B.t.* genes in one crop will diminish the value of the same genes in other crops. The deployment strategies must therefore be designed and implemented in HPR programs to delay or prevent the breakdown of resistance.

Resistance management strategies

Pest resistance management prevents or delays the adaptation of pest species to any defense mechanisms. Resistance management strategies must be based on the following five principles:

- Reduction of selection pressure from each mortality mechanism to the target pests.
- Diversification of mortality sources so that a selection pressure is divided between multiple mortality mechanisms; it is known that single gene traits are quickly overcome.
- Maintenance of susceptible pest individuals by providing refuges or promoting immigration of susceptibles.
- Development of resistance level estimation and / or prediction through the development of diagnostic tools and monitoring.
- Making pest resistance management a part of the national biosafety policy.

Strategies to Integrate HPR in Integrated Pest Management (IPM) Programs

Preventing pests from overcoming HPR will require reduction in selection pressure on pests. This can be accomplished by adopting an integrated system of pest management. IPM is a comprehensive approach to pest management that uses multiple tactics to avert or reduce the pest problems in agroecosystems. Conventional and biotechnological derived HPR must be used along with other means of pest management (cultural, biological, mechanical, chemical etc.). For example, in the case of maize stem borer, coupling HPR with biological, cultural and chemical controls can be accomplished to reduce the selection pressure due to the intensive use of any one tactic. Overall, HPR sources of mortality should be just one component of a stem borer management scheme (Table 1).

Deployment strategies must be designed from the onset of HPR programs to delay or prevent the problem of pest resistance. The following HPR strategies may be deployed:

- Use of multiple genes.
- Combining the HPR derived through conventional and biotechnological means to pyramid or stack resistance genes.
- Rotation or alteration of genes.
- Use of different gene promoters.
- Manipulation in the levels of expression (spatial and temporal) of genes.
- Preservation of susceptible pest genes through refuges.
- Integration of HPR deployment strategies into an overall IPM program

IPM: A National Policy

Pest resistance management must be viewed within the context of IPM. In order for both conventional and biotechnological means of pest management to last longer, they must be integrated and utilized within the context of IPM. This will reduce the

Table 1. Integrated management program for European Corn Borer in the Mid-Western United States.

Cultural Control	
	Adjustment of planting date
	Destruction of stubble's (use of animals)
	Design of landscape
Biological Control	
	Egg parasites (e.g. *Trichogramma*, Minute pirate bug)
	Egg predators (e.g. Spotted lady beetles)
	Larval parasites (e.g. *Eriborus terebrans*)
	Larval predators (e.g. Big eyed bug)
	Larval pathogens (e.g. *Nosema pyrausta*, *Beauvaria bassiana*)
Host Plant Resistance	
	DIMBOA mechanism
	Antigua sources
	B.t. genes (Transgenic hybrids)
Pesticides	
	Biopesticides
	Chemical pesticides (Reduced rate of less toxic chemicals based on monitoring and economic threshold levels)

selection pressure on the pest and hence help increase the life span of new innovations. This will not only help in the management of resistance to these strategies, but also to other IPM tactics by diversifying the pest mortality mechanisms. If IPM is successfully adapted and implemented at a community or landscape level, the objective of resistance management will be automatically achieved. Hence, IPM should become part of national agricultural policy. Also, many national programs are revising their national biosafety frameworks to incorporate biotechnology innovations. Pest resistance management must also become an integral component of any national biosafety framework.

International Initiatives in IPM

From the experience with DDT and synthetic pyrithroids, the global community needs to be made aware that no single management tactic can provide lasting solutions to the pest problem. A large investment has been made in HPR (derived through conventional and biotechnological means) and other ecologically sound pest management tools (biological control, biopesticides, etc.) to substitute for toxic chemical pesticides. It is in the interest of the global community that these tools of pest management endure. Otherwise, the world's farmers will be forced to continue to rely on toxic chemical pesticides. In this context, during the last few years, many international initiatives have been started to integrate these tools into an overall IPM program. These initiatives are designed to strengthen national program capabilities in IPM and influence policy-makers to integrate IPM in national agricultural policies:

- *USAID IPM CRSP*: The United States Agency for International Development (USAID) has established a collaborative research program in IPM. The program includes a consortium of several public and private institutions, NGOs and national programs of selected countries in Asia, Africa and Latin America. The goal of the program is to reduce use of chemical pesticides through non-chemical approaches based on ecological principles.
- *CGIAR IPM Task Force*: This task force consists of CGIAR centers and is coordinated from IITA's Biological Control Center in Benin. The goal is to design and implement IPM programs that will be based on farming systems rather than specific crops. This program will also foster interactions and information exchange across centers.
- *The International Organization of Pest Resistance Management (IOPRM)* is a Washington, D.C. based non-profit organization developed to assist the global community in pest resistance management. The IOPRM extends its membership to all institutions, including public and private sectors and international development agencies.
- *Global IPM Service*: The Consortium for International Crop Protection (CICP) and the USDA's National Biological Impact Assessment Program (NBIAP) has formed a strategic partnership to assemble and support global information and communication on IPM research, teaching, training, and implementation of technology and policy. This program has initiated an international IPM electronic data base and communication service which can accessed *via* the internet.
- *GPRM: The Global Pest Resistance Management (GPRM)* program has been developed at Michigan State University. Using the "train the trainers" approach, this program conducts an annual two-week summer institute in pest resistance management and provides training to scientists from around the world (Wierenga et. al. 1994).
- *USAID ABSP*: The USAID Agricultural Biotechnology for Sustainable Productivity (ABSP) project at Michigan State University is assisting developing countries in the use and management of agricultural biotechnology's with emphasis on insect and disease resistance. The ABSP project has incorporated resistance management strategies in its product oriented research programs in potatoes and maize.
- *B.t. working group*: This U.S. based group consists of members from industry with an advisory panel from academia. This group is developing deployment strategies for *B.t.* (used both conventionally and transgenically) to delay or prevent the development of resistance to these new and expensive technologies.
- *The World Bank/Rockefeller Foundation/UNDP Initiative*: In October 1993 the World Bank, Rockefeller foundation and United Nations Development Program (UNDP) sponsored an international workshop on biotechnology and IPM in Italy. The purpose was to assist the likelihood of new biotechnology's being usefully incorporated into pest management programs. The workshop also discussed the types of new biotechnology which would be most useful to facilitate the wider use of IPM strategies.

- *National IPM centers*: Several national programs have taken initiatives and formed national IPM centers to foster networking, provide training and facilitate information exchange related to IPM. As an example, India has formed a national center of Integrated Pest Management under the Indian Council of Agricultural Research, which plays an active role in promoting IPM at the national level and tries to influence policy makers in this area.
- *Regional IPM programs*: During the last few years, several regional programs in IPM have been initiated. For example, the Cooperative Program for the Development of Agricultural Technology in the Southern Cone (PROCISUR) region of Latin America has formed a regional collaborative program in IPM. FAO has also successfully implemented a regional IPM program in southeast Asia.

The Need for Regional and Global Cooperation

Since insects do not respect political boundaries, implementation of pest resistance management strategies will require both regional and global approaches and cooperation. In this context, the need for global networking to foster cooperation, and structural adjustments in institutions to encourage multi-disciplinary and systems approaches to pest management will become critical.

It is hoped that the initiatives at national and international levels will foster this philosophy and sustain HPR technologies. It is also hoped that HPR would play a key role in the pest management programs of the 21st century and contribute to the enhancement of global food security and long-term sustainability of agroecosystems.

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Insect Resistant Maize: A New Paradigm for Conducting Research

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Abstract

A paradigm is defined as a "model, pattern, or example." Our thesis is that the model for conducting maize research is changing. In this presentation we look at past models for conducting maize research, review some of the current models and introduce a suggestion for a future model. We are convinced that the team approach will be the hallmark of the twenty-first century, as it has been for the past several decades. Defining the team will be critical. Biotechnology offers a scientific paradigm shift that we in maize research and agriculture can use to our advantage.

Introduction

A paradigm has been defined as: "A set of rules and regulations that does two things. Firstly it establishes or defines boundaries; and secondly it tells you how to behave inside the boundaries so as to be successful" (Barker 1992). Barker describes three keys to the future of any organization. We believe these principles apply to researchers and research organizations, both formal and informal, including maize researchers. The keys to the future are: excellence, innovation and anticipation. All keys three are necessary, as shown below.

Excellence is the basis for research. It has been in the past and will be even more important in the future. Excellence provides the competitive edge for awhile and then it becomes the ticket price of entry into research. The basic components of excellence are continuous improvement, bench marking, the continuous pursuit of excellence, and the capability of knowing how to do the right thing the first time.

Innovation is the way teams gain a competitive edge. As researchers we have all taken pride in our innovative accomplishments. Innovation coupled with excellence is a powerful combination. Excellence and innovation, however, are not enough.

Anticipation provides teams with the information that allows them to be in the right place at the right time with an excellent innovative idea or service. Anticipation is the final key element of this triad. This triad allows us to predict our future needs, to provide innovative products or services, and to produce those products and services in an excellent manner. These three team attributes are necessary for us to survive in the twenty-first century.

Models

We believe there are minimally three models to conduct research: the past, the present and the future. The three models will be discussed briefly and then will be drawn on for comparative purposes to illustrate our point and support our arguments.

Past model

The past model was "one scientist - one project", and most work of this type involved one insect and one crop. Research under this model has provided excellent data and results, as evidenced by even a casual review of the literature. Compelling arguments can made against this model: for example, it lifted-up the excellence of an individual; obviously, though, one scientist cannot possess all the information, knowledge or skills for success. Furthermore, this model sometimes promoted the dominance or importance of one academic discipline over all others. Such a model was in our opinion doomed to fade into the past and it essentially has become part of our history.

Present model

The second model, the present, has been and continues to be very successful. The hallmark of this model promotes the "team approach". It is uncertain where or when this approach started with respect to insect resistance studies. Historically, teams of two or more people have probably existed

since the time of the first co-authored paper. Though Painter may have actually served as the catalyst for the promotion of this approach with the publishing of the first book on plant resistance to insects (Painter 1951). However, Painter was quick to give credit to earlier work, as he pointed to research on the woolly apple aphid, "A case for team research" (Hatton et al. 1937). This model, at least initially, promoted the basic research team of an agronomist or horticulturist, an entomologist and a plant breeder. The plant breeder had already successfully worked as a team member with plant pathologists. From this model a number of successes in several cereal crops, including maize, have resulted in the release of a large number of high yielding germplasms, varieties, and hybrids. It has continuously evolved and today many disciplines are involved. It is also responsible for the growth of the sub-discipline of entomology we call plant resistance to insects. The importance of cooperation between investigators working on the plant was central to the model, and it detailed the work to be handled by each investigator, outlined the facilities for the work and even suggested the division of labor assigned to each discipline. We believe it is unfortunate that some plant breeders interpreted their role to be more important because of being central.

Worthy of note in Painters' detailed effort to outline such a program for breeding for insect resistance was the relationship between breeders (being central) and entomologists, plant pathologists, and the United States Department of Agriculture-Agriculture Research Service. It is also interesting to note that in Painters' model the USDA was positioned to serve in a support and collaborative capacity. That role has certainly changed over time to one of full research partner; in many cases serving as the main thread to cooperative efforts between states. Some researchers have gone so far as to say that the USDA-ARS has been the mainstay, holding disparate state efforts together by providing collaborate leadership. Missing in Painters' depiction of a model team was a defined role for private and or corporate breeding programs. Correspondent to the efforts of state and federal research teams being formed, private or corporate breeding programs such as Pioneer Hi-bred Inc., Dekalb Inc. and others were established. Their efforts have grown with amazing rapidity, adding not only an ever increasing number of desirable traits to maize but always increase yield. The trait package of corporate breeding programs is a true success story in agricultural research. As a result of state, federal and corporate efforts, the collective grain yield increases for maize in the USA have averaged about 2-3 % per year over the last 50 years.

A parallel success story is the development of the international research centers such as CIMMYT. It is interesting to note that Painter pointed out that two crop teams had excelled using this team model, namely the corn insects and wheat insects research teams (Painter 1951). We are pleased to have served as a member of the wheat insects research efforts, in collaboration with CIMMYT, and to now be a part of the effort on maize.

The scenario we have just gone through is relevant in the following way. We offered up the definition of a paradigm as described by Barker(1992). We propose that most scientists conducting research on maize know the rules of team work and have shared in one or more aspects of its success. Further, we would venture that many have not contemplated the possibility of a shift in the way we conduct research. Kuhn (1962), stated, "Men whose research is based on shared paradigms are committed to the same rules and standards for scientific practice". Paradigms give us a set of expectations about what probably will occur based on our shared set of assumptions. Those committed to team work on maize know how we work and get things done. Smith (1975) says, " When we are in the middle of the paradigm, it is hard to imagine any other paradigm". We are in the middle of the present model; i.e., we are in the middle of a paradigm that we know and have become familiar with. The present model provides us with the, "basic way of perceiving, thinking, valuing and doing associated with a particular vision of reality" (Harmon 1970). The ability of the maize researchers to do team research is well documented. We know the boundaries and how to perform within those boundaries as defined by our paradigm; i.e., we know what we can expect from universities, the USDA-ARS, CIMMYT and other centers conducting corn research. Further, we each know and have developed linkages with corporate breeding and improvement entities. "The dominant paradigm is seldom if ever stated explicitly; it exists as an unquestioned, tacit understanding that is transmitted through culture and to succeeding generations through direct experience rather than being taught."(Harmon 1970). Our dominant paradigm or model is one of cooperative team research. We propose that maize researchers consider that we

are in a very changing research environment. The name of the game is changing . “A paradigm shift, then, is a change to a new game, a new set of rules”(Barker 1992).

Future model

The pertinent question is, what is the new game? Before that can be answered let us take a quick look at a few selected forces that will impact on us as maize researchers working in the future. These forces are not exclusive, but rather are a selected minimal number that will make our research lives more complex.

Firstly, there is a trend toward regionalization of world economics and reduced funding for research. No matter what state, country or region we are from, we will be affected in research by a relative decline in monetary resources. Research administrators express it in at least two ways. We will be doing more with less and we will be doing it with fewer people, i.e. “rightsizing”.

Secondly, biotechnology may be the most immediate and observable influence on maize researchers in the future. For example, take a quick look at the Proceedings of the International Symposium on Methodologies for Developing Host Plant Resistance to Maize Insects held here in Mexico in 1987. The term biotechnology did not appear in a single title listed in the table of contents! Today, it is highly probable that transgenic corn will be on the market in a couple of years. This is a powerful technological accomplishment in agriculture. Biotechnology offers us the genetic diversity that we as maize researchers have so long sought.

The third and obvious force that will affect the way we conduct future maize research concerns intellectual property rights. Obviously, the way we share and exchange both information and genetic resources will be affected by this issue.

The fourth force, and one of potentially enormous impact for maize research, is the use of fiber optics. Our ability to communicate, share information, the ease of moving data, our ability and ease to publish faster, and even distance learning and conferences will all be enhanced by this technology.

All of the above, when combined, will have hitherto unimagined effects on the way we conduct research programs.

We indicated that the keys to the future are excellence, innovation, and anticipation. The first two of these keys are evidenced by the accomplishment of many. The last key will be determined by how we answer the question of the new game. Convincing arguments can be made for one of many positions. The structure of new team efforts will be as varied as the number of teams and their objectives, however all will have to take into consideration the factors that affect the new model.

The future model will be built on the existing models, with change occurring in the third key, anticipation. Components of the new team(s) will comprise university researchers, the USDA-ARS, corporate breeding entities, international centers, and now a whole new set of players, such as biotechnology firms, lawyers and regulatory agencies. The linkages we make with all components of the new team or teams will determine our success.

A challenge for future teams will by necessity be budgetary constraints. How to finance research will be a serious consideration. The answer may be hidden in the key of excellence. In that key we noted that “bench marking” and “knowing how to do it right the first time” are basic components of that key. Bench marking is more than recording our successes and reporting them. Listing our accomplishments is not enough. Somehow we must do a better job of discussing impacts and the return on investment. The returns on team research investment are often substantial (Roberts et al. 1983,1988), yet there is a dearth of reports for most crops. Returns on investment are both direct and indirect. Direct measures are difficult, but not impossible to estimate. For example, Roberts et al. (1988) discussing wheat research, conservatively estimated the direct return on research dollars invested to be $4.6 million per person-year input. Indirect measures can be determined by number of publications such as refereed scientific journal articles, bulletins, published abstracts and graduate theses. Also, informal exchanges of information can be tallied by newsletters, conference records, invitational seminars, and numerous reports from regional efforts and working groups.

The second hidden message in the key of excellence may be in the phrase, “knowing how to do it right the first time”. Recently, Nelson (University of Nebraska Agricultural Research Division Newsletter) stated,” we are

answering questions that nobody is asking". He was referring to a luxury that we at universities can no longer afford. We must be accountable to our research financiers.

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Improved Technologies for Rearing Lepidopterous Pests for Plant Resistance Research

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Abstract

Two major advances in rearing lepidopterous insects have recently been made at the Crop Science Research Laboratory (USDA/ARS) located at Mississippi State, Mississippi. First, a multicellular tray made of 15 mil polyvinyl chloride plastic with a perforated polyester heat seal lid has replaced the 30 ml plastic cups with paperboard insert caps for rearing larvae to pupation. The new rearing container with 32 individual rearing cells is cheaper and saves time and space. Second, a solution to the human health hazard created by loose moth scales inherent in lepidopterous rearing programs has been obtained. This second technology involves a separate facility to house the moth colonies, large moth cages designed to allow free exit of scales, an improved air filtration system, and appropriate sanitation procedures to deal with trapped and residual scales.

Introduction

We have reared lepidopterous insects for plant resistance research for 25 years at the Crop Science Research Laboratory (USDA/ARS, Mississippi State, MS). Our goals have been to:

- Have the capability and reliability to produce the number of insects required.
- Rear an insect which is physiologically and behaviorally equivalent to its feral counterparts.
- Rear the insects in as efficient and cost effective manner as possible.

Our rearing program has evolved through three distinct eras. During the first (before 1976), our rearing system was simple and capable of producing only small numbers of southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar. The rearing container was a 30 ml clear plastic cup with a paperboard insert cap coated on one side to prevent moisture loss. It was chosen primarily because of its availability and the need to separate the SWCB larvae because of their strong cannibalistic nature. Procedures used during this era were described by Davis (1976).

In 1976, significant support was obtained for increasing research on plant resistance to SWCB. This required a dramatic increase in the number of SWCB for the program. In the early 1980's we also began artificial rearing of fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), for use in providing uniform infestations to screen maize for leaf feeding resistance. We continued using the cup and cap rearing containers, but developed semi-automatic equipment to increase rearing efficiency and allow for increased production. This rearing system, used during the 'second era' (1976 to 1987), was described in detail at the previous international symposium at CIMMYT on insect resistance in maize (Davis 1989).

In the early 1980's, we anticipated the need for less expensive, more efficient rearing containers. Large plastic containers, such as the dishes described by Guthrie et al. (1971) for rearing the European corn borer (ECB), *Ostrinia nubilalis* (Hübner), were tried. Our experience with production of SWCB and FAW in large, common containers was highly variable. Contamination of the diet by microbes and larval cannibalism were major problems.

The 'third era' (since 1988) features the development and use of a new rearing container and the completion of our system for managing loose moth scales and body fragments. I report herein on the origin of the new rearing container, its use and benefits, and the system that we now use to manage loose moth scales in the building where the adult colonies are housed.

Origin of New Rearing Container

In the fall of 1985, we were asked by personnel of the Gast Insect Rearing Facility (Southern Field Crops Insect Management Laboratory, USDA/ARS) at Mississippi State, MS to join them on a research project to improve an existing multicellular rearing container.

This container had been developed by Sparks and Harrell (1976) for use in their in-line form-fill-seal machine that was modified for mass rearing of lepidopterous insects. It consisted of a tray formed from 20 mil thick, high polystyrene. The tray contained 32 individual rearing cells. The top, or lid, for the tray was a commercially available product (Tyvek®), commonly used for many purposes (e.g., to control moisture in homes). Tyvek®, with an adhesive on one side, was sealed to the top of the plastic tray by applying heat and pressure. Because this rearing container consisted of separate rearing cells, we considered it potentially useful for rearing SWCB.

The problems with their multicellular rearing container were:

- The diet dried out too fast, resulting in poor larval development.
- Many of the larvae exited the rearing cells by chewing through both the lid and plastic tray under our rearing environment (27.6°C and 50-60% RH).
- The plastic used to form the tray was opaque, so one could not see clearly what was happening inside the rearing cells.

Technicians of the Gast facility made a new die for forming the plastic tray that was similar to the one used by Sparks and Harrell (1976). Their tray is 15.24 cm wide by 27.94 cm long. It consists of 32 individual cells that are 3.0 cm deep by 3.8 cm wide. A search was then made to find a suitable plastic to form the trays. I found a polyvinyl chloride (PVC), clear plastic (15 mil thick) that formed a tray strong enough to prevent larval escape. Developing a lid strong enough to prevent larval escape but porous enough to permit the diet to dry down slowly as the larvae developed was difficult. Oliver Products Company (445 Sixth St., N.W., Grand Rapids, MI 49504), a vendor that specialized in various types of lidding material, including Tyvek®, helped us develop a suitable lid.

Many types of lidding materials were tested including perforated and non-perforated papers, paper with tin foil backing that had been perforated, and polyesters of various thickness. Polyesters were emphasized because Ignoffo and Boening (1970) reported some success in rearing an array of insects, including lepidopterans, in compartmentalized disposable plastic trays (used in the food industry to provide individual servings of jelly and other foods) that had a lid made of the polyester, Mylar®. Their lidding material was a clear 0.5 mil Mylar® film with one side coated with a heat sensitive adhesive. The lid was sealed to the plastic tray using a Teflon®-coated tacking iron. Since Mylar® film is nonporous, they punctured the film, after sealing, with a specially constructed board containing a series of nails. Ignoffo and Boening (1970) encountered problems with lepidopterans that had a strong tendency to leave their compartments prior to pupation. They solved this problem by placing 0.16 cm mesh wire screening or 0.3 cm plywood covers between trays.

We tested Mylar® of 1, 2, 3, and 5 mil thickness as lidding material. The 1 mil Mylar® was not thick enough to prevent larval exit. Larvae of the FAW and two other test species, the tobacco budworm (TBW), *Heliothis virescens* (F), and the corn earworm (CEW), *Helicoverpa zea* (Boddie), were unable to exit their rearing cells when 2 mil Mylar® lidding was used, but SWCB larvae were able to exit. None of the above species could cut through the 3 mil Mylar®. We decided that the 2 mil Mylar® would be adequate for rearing the above species because we observed that most of the SWCB larvae exiting this thickness of Mylar® did so just before pupation and returned to their rearing cells to pupate.

Two additional steps were required after a suitable lidding material was selected. First, we needed to determine the spacing between pinholes which would provide sufficient air exchange so that the diet would dry slowly during larval development. Secondly, we worked with personnel of Oliver Products Company in testing for an adhesive that would hold its seal to the plastic tray for several weeks and peel back easily from the tray at pupal harvest. Different spacing of the pinholes was tested to determine the best for desired dry down of diet. Lidding with pinholes arranged 5 mm apart was selected based on developmental data for the four lepidopterans. Diet moisture requirements specific to a species were further adjusted by the time the diet was allowed to dry before infesting and sealing the lid. For example, FAW larvae do not develop satisfactorily if the diet is too moist during the latter instars. Extra drying time of diet in unlidded trays, under a clean air hood, solves this problem. By the end of 1987, an improved multicellular rearing container had been created (Fig. 1). Also, data to support its suitability as a rearing container for the SWCB, FAW, CEW, and TBW had been generated (Davis et al. 1990).

In 1987, Oliver Products Company made the polyester lidding (with perforations and adhesive coating) available to the public for use in insect rearing. In the same year, James White, an entomologist with CIBA-GEIGY Seed Division (Bloomington, IL)

assisted us in getting Dixon Paper Company (4402 Locust Avenue, Lubbock, TX 79408) to be a vendor for the 32-cell tray formed from 15 mil PVC plastic. Since then, Stephen Gould Corp. (91480 Deerecho Road, Lutherville, MD 21093) has also become a vendor for the 32-cell PVC plastic tray.

Use of New Rearing Container

The 32-cell tray with its polyester film lid replaced cups and caps as our standard rearing container in 1987. The only new equipment that we had to purchase was a sealer for securing the lid onto the plastic tray. Oliver Products Company fabricated a hand-operated sealer for us (Fig. 2). Over the years, we have made adjustments to the sealer to improve its seal. Oliver Products Company is now marketing a semi-automatic sealer with improved sealing capabilities.

The diet is prepared and dispensed into each rearing cell of the tray using the equipment described by Davis (1989). After dispensing, the diet-filled trays are placed under clean air hoods for cooling and drying of diet, after which the rearing cells are infested with neonate larvae mixed in autoclaved and medicated maize-cob-grits using a bazooka (Davis et al. 1990). The infested trays are then placed individually in the sealer's tray well (Fig. 2, see arrow). Just before initiating the sealing process, a moist sponge is lightly wiped over the top surface of the tray and the underside of the lidding film to eliminate static electricity which causes the maize-cob-grits to be strongly attracted to the lid surface. The lidding material is then placed over the tray and the top of the lidder containing the heating pad is brought down onto the lid and held for about 10 seconds. After sealing, the lidding material on the tray is cut from the unsealed film with a sharp knife.

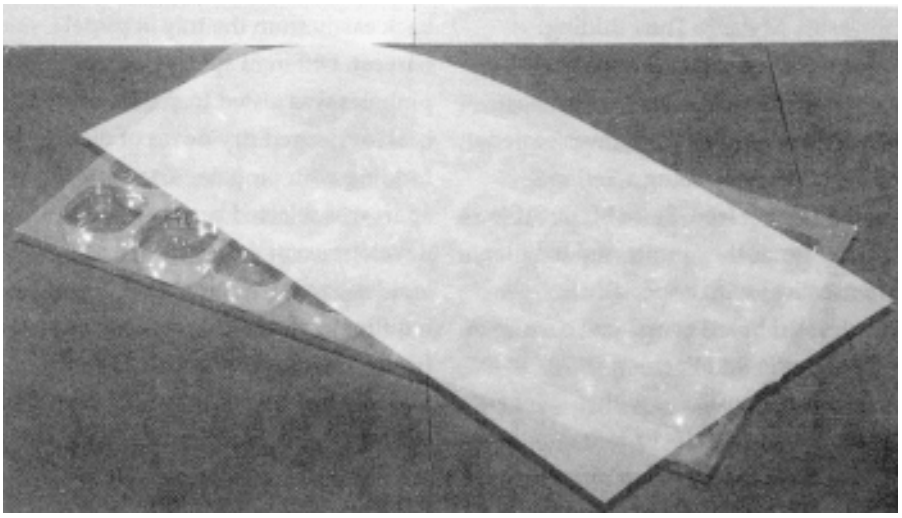

Figure 1. New rearing container consisting of a 32-cell clear plastic tray with a perforated Mylar® lid.

Figure 2. Semiautomatic lidder to seal Mylar® film to the top of the plastic tray.

After the lids have been sealed onto the trays, the containers are stacked in upright, portable racks (Fig. 3). Each rack holds 20 rearing containers. Fabrication of these racks was described by Davis et al. (1990). Pupae are removed from the containers by simply peeling back the lidding material (Fig. 4) and emptying the contents of the tray cells into a large plastic container. The pupae are then hand picked from the residual diet and frass.

Benefits of the New Rearing Container

Cost savings

Multicellular trays and Mylar® lidding material cost significantly less than an equivalent number of the previously used cups and caps. For example, we use approximately 10,000 multicellular containers at a cost of approximately $3,800 (including transportation) to rear FAW and SWCB. The cost of an equivalent number of rearing cells (320,000 30 ml cups and paperboard caps) is approximately $11,600, a saving of $7,800 that can now be used to offset other costs, such as diet and labor.

Time savings

The time required to process the new container with 32 rearing cells from dispensing the diet to harvesting the pupae is significantly less than that required for cups and caps. This is because it is much more efficient to handle a single container with 32 rearing cells than to handle 32 cups and caps, individually. This time saving has allowed us to significantly reduce our permanent rearing personnel and allowed us to increase our rearing to include some cooperative rearing for the Cotton Host Plant Resistance Research Unit, within our Crop Science Research Laboratory. Presently, we rear for them about the same number of TBW and CEW as our own lepidopterous species.

One rearing technician maintains the 4 colonies of insects primarily alone during the off-season. During the spring, when colony size must be increased to provide eggs for the peak rearing period, 1 to 2 additional part-time workers are needed. During peak production, the technician, plus three full-time, temporary employees, comprise the rearing work force. The bottom line is that it is more efficient to process the multicellular containers than cups and caps, and this results in savings in personnel requirements and, ultimately, in research dollars.

Space savings

Rearing containers must be stored prior to use. Storage can require substantial space, especially when purchasing large quantities to receive a volume discount. Multicellular trays and Mylar® lidding require significantly less space than storing cups and caps. For example, 320,000 cups and caps require 2.5 times more storage space than 10,000 multicellular rearing containers (trays and lids).

Space in environmentally controlled rooms to hold rearing containers during larval development is often a factor limiting increased production. Type of rearing container and type of structure to stack or hold containers are important considerations. Again, the multicellular rearing containers require significantly less space than a comparable number of 30 ml plastic cups. The multicellular rearing containers are held in racks that are 30.5 cm wide by 30.5 cm long by 30.5 cm high (Fig. 3). Each rack holds 20 containers or trays that contain a total of 640 individual rearing cells. In about the same space, only 210 cups can be stacked in Styrofoam cup holders (30 cups per holder). Savings on cost, labor, and space by using the multicellular rearing containers have made our rearing program more efficient and cost effective.

Moth Scale Collection System

Moth scales and other body fragments are well known allergens and pose a serious health hazard for sensitive workers in artificial rearing programs (Wirtz 1980, 1984; Bellas 1981; Lugo et al. 1994). For years we tried to develop a system to manage loose scales generated by the moths, but only recently has the system evolved into one that solves the problem.

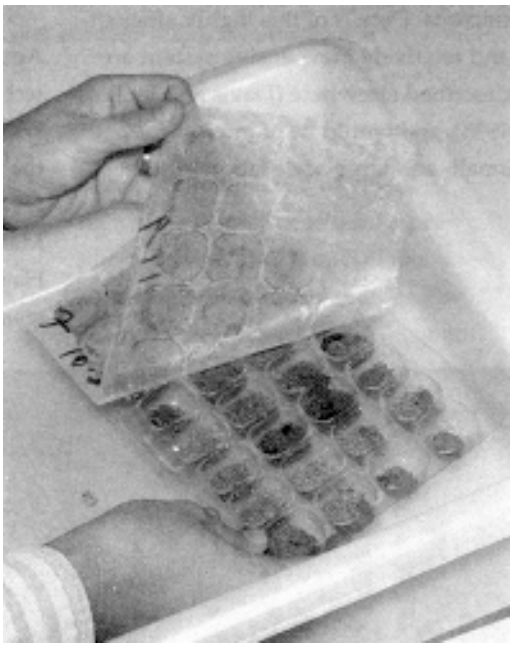

Figure 4. Removal of Mylar® lidding material from the plastic tray.

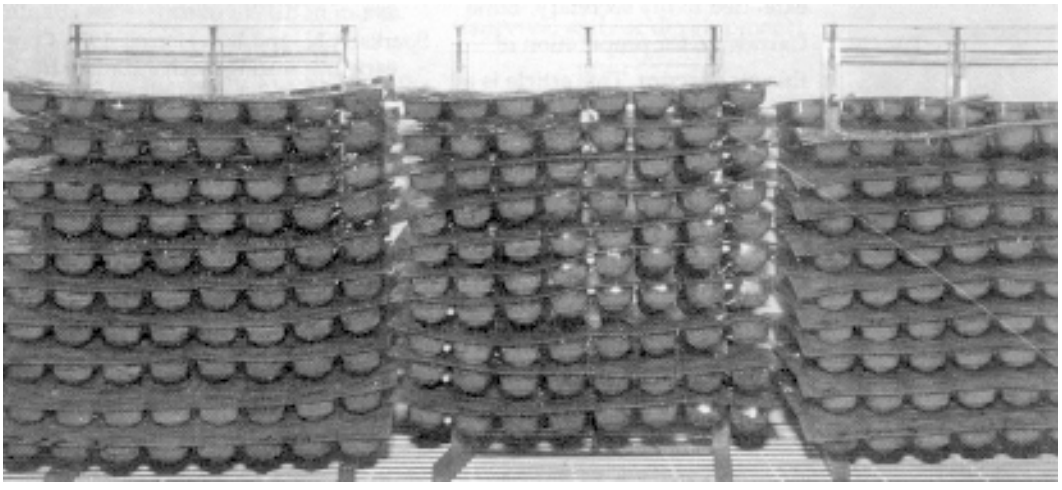

Figure 3. Portable rack for holding multicellular rearing containers.

Our present system involves a separate facility for housing the moth colonies, oviposition cages that facilitate the exit of scales and other body fragments, an improved air filtration system, and sanitation procedures to eliminate trapped and residual scales. Even during peak moth production (20,000 or more individuals), the air in our 'moth house' is lower in suspended particulate matter than the air just outside the building. The air filtration system (Fig. 5) takes in literally millions of scales and other debris particles created by the moths, especially during the scotophase cycle. Our tests show that the filtration system removes 95 to 100% of particles from 0.5 to 5.0 microns. Details of this highly efficient and relatively inexpensive system are described elsewhere (Davis and Jenkins 1995), and would be of interest for both small- and large-scale laboratories.

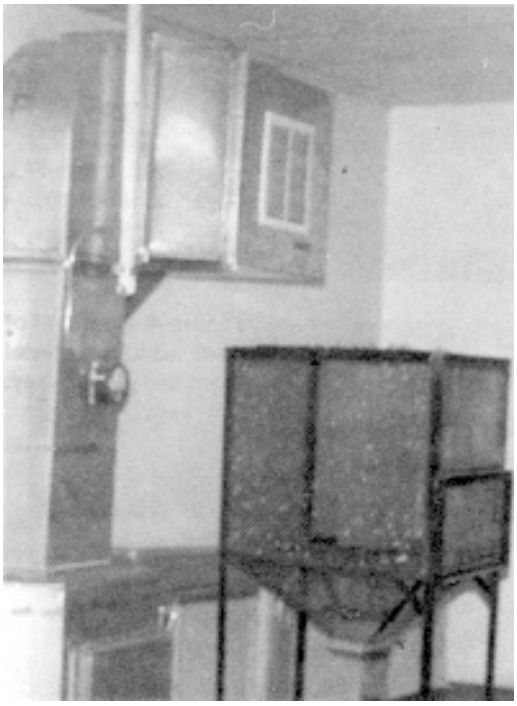

Figure 5. Air filtration unit used to remove moth scales and other debris from the air.

Status of Rearing Program

We have at last attained our goals of capability and reliability, efficiency and cost effectiveness, and the production of high quality insects. Given the excellence of the present rearing system, no further substantial research efforts are envisaged in this area. This does not mean, however, that we do not have to monitor carefully each rearing phase (i.e., production build-up plans, infusion of wild genes into the laboratory colony, diet contamination by microbes and diseases) to ensure standards are maintained.

Acknowledgments

Appreciation is expressed to my technicians, Thomas Oswalt and Susan Wolf, for their assistance in developing the rearing system. I am also thankful for the opportunity to work with Stan Malone, the late Bill Jordan, and Dan Harsh of the Gast Rearing Facility in creating an improved multicellular rearing container. Also, I appreciate the cooperation of the employees of Oliver Products Company (especially Eloy Cantu and David Haines) for helping us develop a suitable lid for the multicellular tray and making it available to the public. Also, appreciation is extended to my secretary, Edna Carraway, for preparation of this manuscript. This article is a contribution of the Crop Science Research Laboratory, Agricultural Research Service, U.S. Department of Agriculture in cooperation with the Mississippi Agricultural and Forestry Experiment Station. It is published with the approval of both agencies as Journal no. *PS-8637* of the Mississippi Agricultural and Forestry Experiment Station.

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A New Technique for Evaluating Southwestern Corn Borer Damage to Post-Anthesis Maize

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Abstract

An effective and efficient technique for evaluating plants' susceptibility to an insect pest is essential to screening for resistance. For many years, the accepted method of evaluating the resistance of maize, Zea mays (L.) at post-anthesis to southwestern corn borer (SWCB), Diatraea grandiosella Dyar, has been to measure the extent of stalk tunneling damage 35-to-40 days after infestation with eggs or neonate larvae. Given the failure thus far to identify and develop germplasm that possesses resistance after anthesis using this method, we developed a new technique. Studies have shown that SWCB larvae up to 14 days old feed primarily on leaf sheath and ear tissue (especially husk–leaves) on post-anthesis-stage plants. The larvae make feeding lesions on these tissues similar to those made on whorl leaves. A visual rating scale was developed utilizing type and number of feeding lesions on the outer three husk–leaves of the top ear and its associated leaf sheath. The leaf sheath and husk–leaf rating scales and their utility as an evaluation technique are discussed.

Introduction

At the previous international symposium on insect resistant maize at CIMMYT in 1987, we stated that we had made significant progress in identifying and developing maize with resistance to leaf feeding by SWCB. By 1990, we had released nine germplasm lines and one population with leaf–feeding resistance (Williams and Davis 1989; Williams et al. 1990). We also stated at the last symposium that we had not made progress in identifying sources of post-anthesis resistance to SWCB in maize. We felt that progress had been hampered by inadequate techniques for identifying resistance and a lack of significant resistance in the germplasm we have screened. In the last seven years, we have devoted much time and effort to improving our screening techniques. We report herein on:

- Problems in screening for resistance to SWCB in maize after anthesis.
- A new approach to evaluation using visual ratings of leaf sheath and husk–leaf damage.
- Progress in identifying reliable susceptible check genotypes and potentially resistant genotypes.

Screening Problems

Screening involves two components: 1) infesting plants with the test insect; and 2) evaluating the insect/plant interaction after a selected period of time. Evaluation can be done by determining either the effect of the insect on the plant (damage estimate) or the effect of the plant on the insect (survival and/or development).

A problem that occurs with infestation at post-anthesis (as opposed to the whorl stage) is that maturity differences result in plants of various genotypes not all being at approximately the same state of development. In dealing with this, the researcher has two options: to infest all plants in an experiment after all genotypes have reached a pre–selected growth stage, such as 7 days after 50% silking, or to infest genotypes separately as each reaches the pre–selected stage. Either option has advantages and disadvantages. For example, advantages of infesting all genotypes on the same day include the fact that all larvae come from the same group of eggs and survive and develop on the plants under the same environmental conditions. A disadvantage is that there may be a 2–to-3-week difference between when the first and last genotype reach the pre–selected growth stage. An advantage of infesting genotypes when each reaches the pre–selected growth stage is that the larvae have the opportunity to survive and grow on plants of the same physiological stage. Disadvantages are that the larvae used for infesting originate from different groups of eggs and the larvae must survive and grow on the plants under different environments. In this approach, staggered plantings of susceptible check genotypes could be used to provide a series of rows in different

physiological stages for comparison with test genotypes. Experiments should be conducted to compare damage and/or survival/growth of larvae on different physiological stages of the plant and thus indicate which approach is better. When known, genotypes with similar maturities should be screened together. Unfortunately, maturity (days to anthesis) is not known for many genotypes prior to planting in the screening nursery, and environment significantly influences maturity.

When analyzing our old technique of evaluating stalk damage (primarily by splitting stalks and measuring extent of tunneling 35-to-45 days after infestation), we realized that the behavior of non-diapausing and diapausing larvae influences the degree of stalk damage. SWCB larvae in the early instars feed primarily on leaf sheath and ear tissues, regardless of diapause status. The behavioral difference is that some non-diapausing larvae will continue feeding on ear tissue and pupate there without entering the stalk to tunnel, thus their damage is not reflected in stalk tunneling measurements. On the other hand, almost all later-stage diapausing larvae enter the stalk to tunnel and prepare an overwintering site at the base of the stem. Another potential problem with measuring stalk tunneling is the delay of 5-to-8 weeks between infestation and evaluation that allows other biotic (predators, intraspecific cannibalism) and abiotic factors to confound the insect/plant interaction. Finally, splitting stalks and measuring tunnels is slow, boring, and costly.

A problem which is not related to screening techniques, but can influence rate of progress in temperate zones is that germplasm can be screened only once each year. Even then, infestations can be made over a period of only a few weeks, thus limiting the number of genotypes that can be screened. If a growing season is missed because of inclement weather, then it is necessary to wait another year unless the researcher has access to a winter nursery or collaborates with someone outside of the temperate zone.

A New Evaluation Technique

Before describing the new evaluation technique involving leaf sheaths and husk–leaves, we want to discuss its origin briefly. For many years we had known that SWCB larvae feed on leaf sheath and ear (primarily husk–leaves) tissues prior to entering the stalks of maize plants at post-anthesis (Davis et al. 1972). During this study we failed, however, to describe the larval feeding lesions made on these tissues.

One day in the late 1980s, I (Davis) was walking through some maize plots in which plants had been infested for about two weeks with SWCB larvae released as neonates in the axil of the top ear leaf. Some interesting-looking, large, elongated lesions on the leaf sheath caught my attention (Fig. 1). Upon investigation, I found that these were caused by the SWCB larvae and that these feeding lesions were similar to those made by this insect on leaves of whorl-stage plants. Also, I observed different types of lesions on the husk–leaves. These observations stimulated us to begin evaluating post-anthesis maize by visually rating the extent of damage on the leaf sheaths and husk–leaves, similar to the technique described by Guthrie et al. (1978) for evaluating leaf sheath collar damage by European corn borer (ECB), *Ostrinia nubilalis*, Hübner.

The first step in developing this approach was to characterize the feeding lesions from different-aged SWCB larvae on leaf sheaths and husk–leaves. Also, information was needed on:

- Larval establishment sites on the plant after releasing neonates in the axil of the top ear leaf.
- Whether damage varied among the different husk–leaves of the top ear.
- The degree of damage from larvae of different ages, to determine how long the insect/plant interaction should last before evaluating larval feeding.

We observed that SWCB larvae make different feeding lesions depending on their age. Lesion types were the same on both husk–leaves and leaf sheaths.

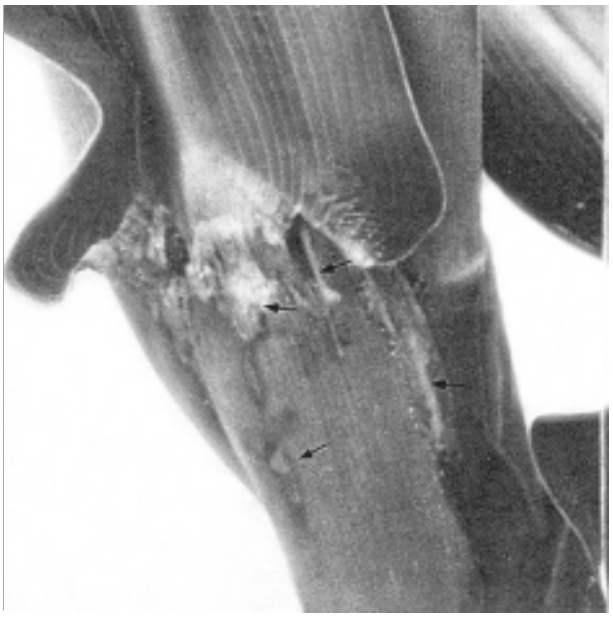

Figure 1. Lesions made by SWCB larvae feeding on the inner surface of the maize sheath.

Larvae up to 3 days old made only pinhole and small circular-to-elongated (rectangular) lesions. By the time the larvae were 7 days old, they made elongated lesions of from 1.3 to 2.5 cm. Larvae 10 days old made elongated lesions that exceeded 2.5 cm, plus some rather small lesions that were wider (>3mm) and which varied in shape from uniform (i.e., squares and rectangles) to irregular. By the time the larvae were 14 days old, they made significantly larger, uniform–to–irregular lesions. Additionally, the 10-to-14-day-old larvae often ate through the husk–leaves, leaving clean holes. Occasionally, larvae would eat a hole through the leaf sheath.

From these observations, we classified lesions into four types by shape (pinhole, circular, elongated, and uniform–to–irregular) and then by size (Fig. 2). Within the elongated and the uniform–to–irregular shaped lesions, we established three size groups (small, mid–sized, and large). For elongated lesions, size was based on length (small = <1.3 cm, mid–sized = 1.3–2.5 cm, and large = >2.5 cm), whereas for the uniform–to–irregular lesions, size was based on diameter. Lesions 17 mm in diameter or smaller (about the size of a US dime — the $0.10 coin) were considered "small;" lesions up to 22 mm in diameter, "mid–sized;" and those 23 mm (about the size of a U.S. quarter) in diameter or larger, "large". Lesions at the base of the husk–leaves were considered as belonging to the elongated lesion group.

Seven and 14 days after releasing neonates in the axil of the top ear leaf of anthesis stage plants, approximately 70% or more of the larvae recovered from these plants were feeding on tissues of the top ear and its associated leaf sheath. These findings indicate that neonates begin feeding very near this release site and continue feeding on these leaf sheath and ear tissues through at least the third-instar. Seven–day–old larvae were feeding primarily on husk–leaf tissue. The next preferred tissue was the leaf sheath. The larvae were found feeding on all ear tissues, including kernels, cob, and shank at 14 days after infestation. However, leaf sheaths and husk–leaves were still the preferred tissues.

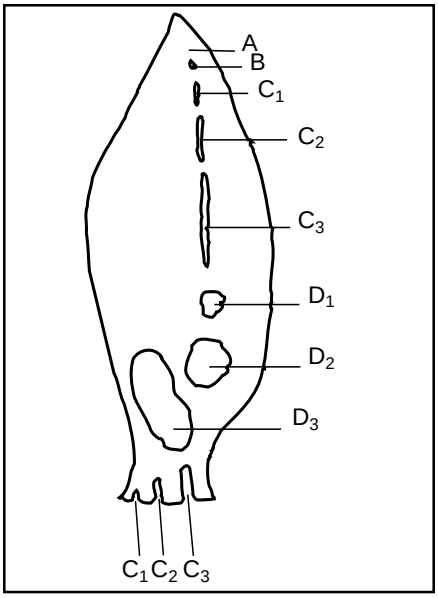

Figure 2. Types of lesions made by SWCB larvae feeding on ear husk–leaves and leaf sheaths: A, pinhole; B, small circular; C_1, small, elongated; C_2, mid–sized, elongated; C_3, large, elongated; D_1, small, uniform–to–irregular; D_2, mid–sized, uniform–to–irregular; and D_3, large, uniform–to–irregular.

The larvae feed on the inner surface of the leaf sheath below the collar. They feed on all areas of the husk–leaves, especially in the lower portion where the husk–leaf attaches to the ear shank. When the husk–leaves from top ears were compared for extent of damage, invariably the three outer husk–leaves suffered the most damage. The small outermost husk– leaf was a primary site for initiation of neonate feeding.

From this baseline information, we developed a new screening technique utilizing rating scales for visually scoring larval feeding damage on the leaf sheath and husks (Tables 1 and 2, respectively). Rating scores are based on the type and number of lesions observed 14 days after infestation, and separate degrees of damage but also

Table 1. Visual scale for rating the degree of damage caused by SWCB larvae to leaf sheaths of post-anthesis stage maize plants.

Score	Description
0	No visible damage.
1	Only pinhole lesions.
2	Pinholes plus a few small, circular lesions.
3	Pinholes and small, circular lesions or a few small, elongated lesions, or both.
4	Several to many small, elongated lesions or up to several mid-sized, elongated lesions, or both.
5	Mid-sized, elongated lesions plus a few large, elongated lesions or small uniform-to-irregular lesions or a combination.
6	Several large, elongated lesions or several small to a few mid-sized, uniform-to- irregular lesions, or both.
7	Many large, elongated lesions or small, uniform-to-irregular lesions, or several mid-sized to a few large, uniform-to-irregular lesions, or a combination.
8	Elongated lesions of all sizes or small to mid-sized, uniform-to-irregular lesions, or several large, uniform-to-irregular lesions, or a combination.
9	Many lesions of all types present.

Lesion numbers: Few = 1 to 3; several = 4 to 6; many = 7 or more.
Taken from Davis and Williams (1994).

reflect the plant's effects on insect survival and growth. The scales were modeled on the 1–9 scale developed by Guthrie et al. (1960) for evaluating maize for leaf feeding resistance to the ECB. The rationale for having separate rating scales for leaf sheaths and husk–leaves was that the tissues are different and that one tissue might possess resistant factors while the other might not. Also, the rating of husk–leaves involves multiple husk–leaves from an ear.

Because resistant genotypes were unknown, we had to test the utility of the new rating scales for separating resistant from susceptible plants by conducting experiments that simulated different rates of larval survival and growth. The methodology and results of these experiments have been published (Davis and Williams 1994). Here is a brief summary of the results and conclusions from these studies. Highly significant correlation's were found between larval survival and growth (weights) and both rating scales. The r^2 values were as follows: between survival and leaf sheath ratings, 0.63; survival and husk–leaf ratings, 0.72; growth and leaf sheath ratings, 0.40; and growth and husk–leaf ratings, 0.56. Also, significant differences in larval survival and growth were found among larvae reared on some test hybrids. When this occurred, differences in rating scores among hybrids also were found to be significant. Therefore, we concluded that the leaf sheath and husk–leaf ratings were successfully measuring differences in damage as reflected by rates of larval survival and growth and that this evaluation technique had potential. Rating leaf sheath and husk–leaf damage on plants 2 weeks after infestation solves two of the aforementioned problems. Evaluation occurs sooner after infestation, thus minimizing the confounding effects that abiotic and biotic factors may have on larval numbers, growth, and damage when evaluation occurs 5–7 weeks after infestation. Also, this evaluation technique measures feeding damage to sheath and husk–leaf tissue by larvae without the influence of their diapause state.

Table 2. Visual scale for rating the degree of damage caused by SWCB larvae to husk– leaves of the top ear.

Score	Description
0	No visible damage.
1	Only pinhole lesions.
2	Pinholes plus a few small, circular lesions.
3	Pinholes and small, circular lesions common on husk-leaves or a few small, elongated lesions, or both.
4	Several to many small, elongated lesions or up to several mid-sized, elongated lesions.
5	Many mid-sized, elongated lesions or a few large, elongated lesions, or a few small, uniform-to-irregular lesions, or a combination.
6	Several large, elongated lesions or a few small or mid-sized, uniform-to-irregular lesions, or both.
7	Many large, elongated lesions or small to mid-sized, uniform-to-irregular lesions, or a few large, uniform-to-irregular lesions, or a combination.
8	Many lesions of all types on two of the three husk-leaves.
9	Many lesions of all types on each of the husk-leaves.

Lesion numbers: Few = 1 to 3; several = 4 to 6; many = 7 or more.
Taken from Davis and Williams (1994).

We conducted another experiment to determine the effect of the physiological age of the reproduction-stage plant on larval survival and growth (Davis and Williams 1994). Significant differences were found in larval survival and growth when maize hybrids were infested on the same day, but at different physiological stages. We have decided to infest our test genotypes as each reaches a pre–selected physiological stage. This procedure of timing infestations is especially important when genotype maturities are unknown.

Based on our experience in Mississippi, our protocol for screening hybrid genotypes using the new technique is as follows:

- Genotypes are infested 7 days after 50% of the plants in a row reach anthesis.
- Each plant is infested with 60 neonates (preferably split applications of 30 neonates on consecutive days) released in the axil of the top ear leaf using the 'bazooka' method (Mihm 1983; Davis et al. 1989).

Larval damage on the three outermost husk–leaves of the top ear and its associated leaf sheath of each plant is evaluated 14 days after infestation by visual scoring using the rating scales presented in Tables 1 and 2. When the top ear is accompanied by small immature ears that also originate from the primary ear node, the rater must consider the extent of damage to them before arriving at a final score for the husk–leaves. Damage to these small ears is determined by counting entrance and exit holes and may or may not influence the final score. However, if the larvae preferred feeding within these ears instead of the

husk–leaves of the main ear, the score reflects the extent of damage to these ears.

Evaluation data can be taken directly in the field 14 DAI or delayed by collecting the leaf sheath, husk–leaves and small inner ear samples from the plants, and placing these tissues in pre–labled plastic bags and freezing them. The samples are thawed, when convenient, and rated visually for damage by placing them on a light table similar to those used to view photographic slides. The light table helps the rater see the larval feeding signs clearly. When rating leaf sheaths or husk–leaves, the rater should first identify the lesion types present and then consider lesion numbers. The most severe lesion type(s) immediately indicates to the rater the approximate score. A final score can be obtained quickly by estimating the numbers of each lesion type. The amount of time required for an experienced rater to score a plant in the field is ca. 30 seconds and, in the laboratory, approximately 1 minute (includes removing tissues from plastic bags and arranging them on the light table).

Our protocol for screening inbred genotypes differs slightly from that used for hybrids, primarily because the inbreds senesce rapidly after anthesis. Inbreds are infested with 45 instead of 60 neonates per plant when most of the plants in a row are in the anthesis stage. Normally, infestations are split over 2 consecutive days (30 larvae the first day and 15 larvae the next day). This mediates the effects of unusual environmental stresses or unfavorable events.

Our experimental design of choice for screening post-anthesis stage plants is a RCB with two or three replications. Each genotype is represented in each replication by a single row of 15 plants. Rows are 5.08 m long with 0.97 m between rows. Data on leaf sheath and husk–leaf ratings are taken from 10 plants per row. These data are analyzed using ANOVA and means are separated using the least significant difference test (P=0.05).

Progress

We are presently screening maize during post-anthesis using leaf sheath and husk–leaf ratings (Davis and Williams 1994). Additionally, we (primarily Williams) have developed a laboratory bioassay using lyophilized husk diets for screening. These two techniques should complement each other.

A few inbred lines have been identified as consistently susceptible (e.g. GE333). Also, a few candidates (e.g. Mp89:5459) have shown potential resistance. Ratings for test inbreds GE333 and Mp89:5459 are presented in Table 3. These data indicate the range of differences that we have observed among inbreds.

We feel we are making progress since susceptible checks and potential resistant genotypes have been identified. If the potentially resistant genotypes are confirmed as having resistance, the breeding process for developing germplasm for release will continue using techniques appropriate for that germplasm.

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Table 3. Evaluation of effects of feeding by 45 SWCB neonate larvae on 2 maize inbred lines at anthesis , 14 days after infestation.

	Means		Damage ratings (1-9)	
Inbred	**Survival/plant**	**Larval weight (mg)**	**Leaf sheath**	**Husk–leaves**
GE333	4.5	69.3	4.6	6.4
Mp89:5459	3.0	35.1	3.6	4.2
LSD (P=0.05)	1.3	13.7	0.8	0.9

Experimental design: RCB with 3 replications. Data were taken on 10 plants per genotype per replicate.
Plants of these inbreds were infested on the same day.

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Assessing Damage by Second-Generation Southwestern Corn Borer and Sugarcane Borer and Development of Sources of Resistance in Tropical and Subtropical Maize

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Abstract

In 1992, having achieved adequate levels of resistance to first-generation (whorl stage attack) Diatraea spp. borers, Population 391 was formed to attempt to identify sources of resistance to second-generation (post anthesis stage) attack. The ultimate objective is to develop complete cycle (planting to harvest) resistance to the two most important stem borer species that attack maize in the American subtropical and tropical growing areas. Plants were infested at anthesis +/- 1 week with Sugacane borer (SCB), Diatraea saccharalis *Fabricius, at Poza Rica (CIMMYT's tropical lowland station) or Southwestern Corn Borer (SWCB),* Diatraea grandiosella *Dyar, at Tlaltizapan (CIMMYT's subtropical station) in the ear zone (Ear leaf, one leaf above and below the ear) with 60-65 larvae larvae per plant. Selection was carried out over the next three cycles using one or several criteria (ear damage, sheath damage, stalk damage - indicated by the number of internodes tunneled) and compared and correlated with data from sub-samples rated for sheath and husk damage. For SCB in tropical environments, there was a marked and obvious preference for the larvae to attack the developing ears. The correlations between sheath damage and stalk damage were not significant, but those between ear damage and stalk damage were significant. However, the relationships were highly genotype specific. For SWCB, in subtropical environments, damage directly to the ears, sheath and husks was not so striking, so selection was based on stalk damage. The best lines were recombined at S_3 levels, and the second cycle of S_1 recurrent selection has begun, while the elite fraction is now available as S_4 lines for further testing. Our data show sufficient variability to forestall concluding that there is a single best method to select for multiple species, second-generation resistance in tropical maize.*

Introduction

Maize, *Zea mays* L., is an important food and fodder crop throughout the world. In several developing countries of Africa and Asia, maize is a major staple food of millions of people. Of the various insect pests attacking maize, stem borers are the most important, causing severe yield losses at the whorl (Sarup et al. 1977; Smith et al. 1989, Seshu Reddy and Sum 1991) and anthesis (Kumar and Asino 1994) stages of maize. Many maize genotypes resistant to first-generation stem borers have been developed through the joint efforts of breeders and entomologists (Williams and Davis 1989; Smith et al.1989). The International Maize and Wheat Improvement Center (CIMMYT) has an active program which has developed maize germplasm with a desirable level of resistance, in whorl stage maize, to first-generation stem borers. However, information on resistance in maize to second-generation stem borers is limited, and sources are few. Stem borer attack at anthesis is complicated, because damage is caused to several different parts of the plants (i.e., leaf sheath, stalk, husk, ear peduncle and ear). To screen maize for resistance to second-generation stem borers, we did not know whether damage to all parts attacked by stem borers should be assessed or whether the selections could be based solely on damage to the most important part of the plant. Davis and Williams (1994) developed a rating scale based on damage by stem borers to leaf sheath for selecting maize genotypes for resistance to second-generation stem borers. However, given the multi-faceted nature of stem

borer attack it seems prudent to determine, firstly, if there is any correlation among different parts of the plant damaged by the borers and secondly, to assess which tissue, if damaged, leads to maximum loss of grain yield. The first objective of this study was to examine the relationships between damage to different parts of the plants by Southwestern corn borer (SWCB), *Diatraea grandiosella* (Dyar) and sugarcane borer (SCB), *Diatraea saccharalis* Fabricius. Recently, work was also initiated to identify sources of resistance to second-generation stem borers and the second objective of this study was to provide information on the progress made in this area.

Materials and Methods

Experiments for this study were conducted at CIMMYT's research stations at Tlaltizapan (18º 41'N; 940 m elevation) and Poza Rica (20º 30' N, 50 m elevation) in the summer and winter cycles of 1993 and 1994. In order to examine relationships between different types of damage caused by stem borers, two single cross hybrids Ki3 x CML131 (susceptible) and CML67 x CML135 (resistant) with known level of resistance to first-generation stem borers were used. Two experiments were conducted at each location. For each experiment, the seeds of each hybrid were treated with the insecticides, Carbofuradan 27.5% (FMC Agroquimica de Mexico), Semevin (a.i. Thiodicarb 31.5%, Rhone- Poulenc Agro. Mexico) and Gaucho (a.i. Imidacloprid 70%, Bayer, Mexico). The seeds were treated at the rate of 350 g. a.i./ ha to protect seedlings from the attack of soil insects. In all the experiments, "zero tillage" was used and the trials were planted with a ALMACO planter (Model CTS, EODF, Nevada, U.S.A).

Experiment 1

For this experiment, the two hybrids were planted in a split plot design with variety as the main plot and the treatment as the subplot, with three replicates. The treatment involved infestations at three leaves, the ear leaf (EL), the leaf below the ear (-EL) and the leaf above the ear (+EL) (Fig. 1). Plots consisted of single row plots, 2.5 m long with 12 plants. Row-to-row and plant-to-plant spacing was 75 cm and 25 cm, respectively. Plots were fertilized with phosphorous at the rate of 50 kg/ha before planting and nitrogen at the rate of 150 kg/ha in split doses of half before planting and half 6 weeks later.

When 50% plants of each hybrid had reached anthesis, each plant was infested with 60-65 neonate larvae per plant. The plants of the two hybrids at Tlaltizapan were infested with SWCB and those at Poza Rica were infested with SCB. The insects used in this study were obtained from laboratory cultures of SWCB and SCB maintained on artificial diets as described by Mihm (1989). After every 10 generations, field collected adults were infused into the colony to maintain the vigor of the laboratory reared insects. The neonates were mixed with maize cob grits and placed in the axil of the EL, -EL and +EL of the plant with a mechanical dispenser called a 'bazooka' (Fig. 2). At the time of harvest, 10 plants from each plot were uprooted. The ear leaf, leaf below the ear and leaf above the ear were removed from each plant. The damage caused by the stem borers to

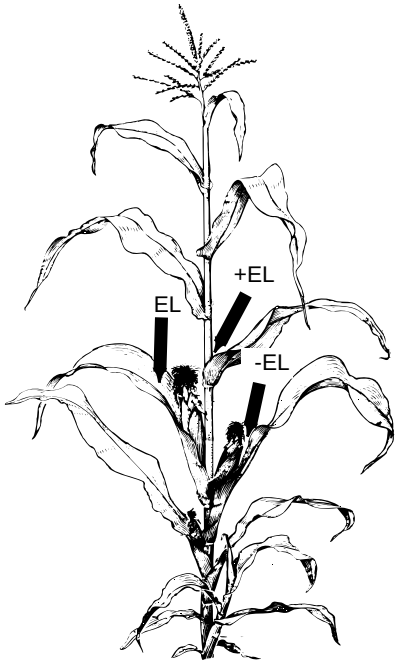

Figure 1. Maize plant showing three sites of infestation by the borers.

Figure 2. "Bazooka" used for infesting anthesis stage maize with stem borers.

the three leaf sheaths was assessed using sheath damage rating scale of 1-9 modified from that devised by Davis and Williams (1994) as follows: 1 = no visible damage; 2 = only pinhole lesions; 3 = pinholes plus a few small circular lesions; 4 = small circular lesions and a few elongated lesions (< 1 cm in size); 5 = mid-sized elongated lesions plus a small irregular shaped lesions; 6 = few elongated lesions (1 cm long) with a few mid-sized irregular shaped lesions; 7 = several elongated (1 cm long) and several mid-sized irregular shaped lesions; 8 = elongated lesions of all sizes and a few large irregular shaped lesions; 9 = elongated lesions of all sizes and large irregular shaped lesions spread on the whole leaf sheath.

The primary ear (counting from the top) of each plant was removed and the damage caused by the borers to the husks was assessed on the basis of a rating scale of 1-9 as described above, but the assessment of damage was based on feeding lesions of the borers on 2-3 husk leaves rather than only one (Davis and Williams 1994). The ear damage was evaluated on the basis of a rating scale 0-10, with 0 indicating no damage to the ear by the insects and 10 indicating 100% of the grains damaged by the borers. The stalk of each plant was split and the length of the tunnels made by the borers was measured. The sheath damage, husk damage, ear damage and the stalk damage of each plant were measured together, so keeping the data for each plant separate from the others. Two years of data were combined and subjected to factorial analysis (MSTAT-C, 1989).The correlation's were calculated between sheath damage and stalk damage, ear damage vs. stalk damage and husk damage vs. stalk damage.

Experiment 2

The objective of this experiment was to examine whether damage caused by the stem borers to two hybrids would vary under artificial infestation applied at different silking stage or time of day. The hybrids Ki3 x CML131 and CML67 x CML135 were planted in a split-split plot design in a randomized complete block design. The variety was the main plot, the silking stage was the sub plot and the time of day was the sub-sub plot. The three silking stages utilized for this experiment were pre-silk (emergence of ear shoots), green silk (a week after the silk emergence) and the brown silk (the drying of the silks). Each silking stage of a hybrid was infested at 8:00 a.m., 12:00 noon and 4:00 p.m. The larvae of SCB and SWCB were used at Poza Rica and Tlaltizapan, respectively. The plants were infested with 60-65 larvae per plant, as described above. At the time of harvest, the sheath damage, husk damage, ear damage and stalk damage was assessed for each plant separately, as described above. Data were subjected to factorial analysis and correlation's were calculated between sheath damage and stalk damage, husk damage and stalk damage, ear damage and stalk damage.

Breeding for resistance to second-generation stem borers

The source germplasm used for the development of resistance to second-generation stem borers was genetically diverse, with known level of resistance to first-generation stem borers and good agronomic traits. The notable sources used were the best lines from population 390 (MIRT), selections of the Antigua landrace from the germplasm bank, the variety Across 90390, Pop. 8523, Dekalb hybrids 810, 830,833, 840, 844, 555, SMC-305, Guatemalan hybrids, best hybrids from CIMMYT's lowland hybrid program, hybrids Ki3 x CML 131, CML67 x CML135, CML135 x CML139, CML61 x CML69, Pop. 590 (MBR) and Pop. 590B (MBR-MDR). These source materials were planted at Poza Rica station in the summer cycle of 1992 in two replications. Trials were planted in zero tillage plots, using a ALMACO planter (Model CTS, EODF, Nevada, USA.). Single row plots were 2.5 m long The plants were infested with 40-50 SCB larvae at the time when 50% plants had flowered. The larvae were placed in the leaf axil with a bazooka as described above. About 5-6 plants from each row were selfed to generate S_1 lines. At the time of harvest, the stems of selected plants were split along their length and the number of internodes tunneled in each plant was recorded. The plants with less damage (< 4 internodes tunneled) were selected and planted in the subsequent planting cycle . The S_2 lines selected under insect infestation were then planted both at Tlaltizapan and Poza Rica and infested with SWCB and SCB, respectively. The S_3 lines selected at the two locations were advanced to S_4 and recombinations were also made among the S_3 lines to start another cycle of selection. The S_3 lines were also evaluated for sheath damage, stalk damage, husk damage, and ear damage to examine the correlation's among the parameters.

Results and Discussion

Experiment 1

When the two hybrids were infested with SCB at Poza Rica with 60-65 larvae per plant at the ear leaf (EL), leaf below the ear (-EL) and leaf above the ear (+EL), the ANOVA showed that genotype x site of infestation interaction was not significant. When

infested at three leaves, damage by SCB to leaf sheath above the ear (+ EL) and the stalks of the two hybrids differed significantly (Table 1). When infested at the axil of the ear leaf, the differences in damage by SCB to the three leaf sheaths were significant. The damage by SCB to the leaf sheath of the infested leaf axil was always greater than that of the other two leaf sheaths. The husk and ear damage also differed significantly when infested at three leaves of the maize plant at anthesis. However, the stalk damage remained the same at each of the three infestations. These results indicate that the SCB larvae move to the leaf sheath of the leaf where they hatch from the eggs laid by the females and feed therein. When infested at the ear leaf, the correlation between ear damage and the stalk damage was highly significant for the hybrid Ki3 x CML131, but not for CML67 x CML135 (Table 2) indicating that ear damage can replace the tedious procedure of maize evaluation by assessing stalk damage. Also, infestations of the maize plants at the leaves below and above the primary ear did not give significant correlation's between the sheath damage and the stalk damage in any of the hybrids. There was a significant correlation between leaf sheath above the ear and the stalk, but this was also not consistent between the two hybrids (Table 2).

When the two hybrids were infested at EL, -EL and +EL with SWCB at Tlaltizapan, the factorial ANOVA did not show genotype x site of infestation interaction for any of the damage parameters (Table 3). Genotypes differed significantly in terms of ear sheath damage, ear damage and stalk damage. The sheath damage and the stalk damage also differed when the hybrids were infested at the three leaves. The correlation's between different parameters were again varied according to the parameters and the hybrid (Table 4). These observations indicate that assessment of damage on different parts of the plants at anthesis is quite independent of one another.

Experiment 2

When the two hybrids were infested with SCB at different silking stage and time of the day, genotype x silking stage x time of day interaction was not significant (Table 5). Genotypes differed significantly in terms of ear damage and stalk damage. Stalk damage also differed according to the silking stage at infestation and time of day. The correlations between the ear damage and the stalk damage were significant irrespective of the silking stage at infestation. (Table 6).

When the two hybrids were infested with SWCB at the three silking stages and time of day, genotype x silking stage x time of day interaction was not

Table 1. ANOVA for damage by *D. saccharalis* to maize hybrids, infestation on three leaves at anthesis.

Source	Mean squares for damage					
	Ear sheath	-Ear sheath	+Ear sheath	Husk	Ear	Stalk
Genotype (A)	0	0.04NS	4.00*	1.73*	1.14NS	568.03**
Site of infestation (B)	6.38*	7.93*	19.62**	1.82*	3.70*	11.13NS
AB	0.36	0.77NS	1.06NS	0.44	1.03NS	48.77NS
Error	0.55	0.66	1.12	0.40	0.87	29.78

Table 2. Correlation matrices of damage by *D. saccharalis* on two hybrids, infestation on three leaves at anthesis.

Genotype	Site of infestation	Correlation coefficients: Ear sheath vs stalk	-Ear sheath vs stalk	+ Ear sheath vs stalk	Husk vs stalk	Ear vs stalk
Ki3 x CML131	Ear leaf	0.016	-0.16	0.27*	-0.12	0.44**
	- Ear leaf	0.041	0.15	0.07	0.18	0.08
	+ Ear leaf	-0.12	0.08	0.27*	0.29*	0.21
CML67 x CML135	Ear leaf	-0.14	-0.19	0.04	0.15	0.08
	- Ear leaf	0.14	0.04	0.05	0.12	0.20
	+ Ear leaf	0.19	-0.14	0.09	-0.06	0.38**

[a] n = 60 plants.

Table 3. ANOVA for damage by *D. grandiosella* to maize hybrids, infestation on three leaves at anthesis.

Source	Mean squares for damage to different parts					
	Ear sheath	-Ear sheath	+Ear sheath	Husk	Ear	Stalk
Genotype (A)	0.04NS	0.28NS	1.69*	0.94NS	1.44*	146.8*
Site of infestation (B)	2.27**	7.57**	6.84**	0.24NS	0.30NS	111.7*
AB	0.39NS	0.28NS	0.29NS	0.33NS	0.17NS	37.9NS
Error	0.28	0.43	0.18NS	0.35NS	0.89NS	20.1

significant (Table 7). The two hybrids differed significantly in terms of husk, ear and stalk damage. The damage caused by SWCB to the hybrids also differed according to the silking stage at infestation. Infestations at different times of day did not affect damage by SWCB, except for sheath damage.

Thus, in the absence of clear-cut, consistent correlations between sheath damage and stalk damage or between ear damage and stalk damage, the selection of maize genotypes for resistance to second-generation stem borers should continue to be based on stalk damage, which has been demonstrated to cause yield reductions in maize (Kumar 1988; Kumar and Asino 1994). Stalk damage due to stem borers has also been used to select maize genotypes resistant to second-generation European corn borer, *Ostrinia nubilalis* Hübner (Guthrie and Russell 1989). However, for other damage parameters to be useful in the selection of maize resistant to stem borers, their role in determining grain yield of the plant will have to be demonstrated.

Table 4. Correlation matrices of damage by *D. grandiosella* on two hybrids, infestation on three leaves at anthesis.

		Correlation coefficients				
Genotype	Site of infestation	Ear sheath vs stalk	-Ear sheath vs stalk	+ Ear sheath vs stalk	Husk vs stalk	Ear vs stalk
Ki3 x CML131	Ear leaf	-0.04	-0.13	0.14	0.14	-0.22
	- Ear leaf	0.04	0.28	0.28	0.35	0.41
	+ Ear leaf	-0.06	0.09	-0.13	0.16	-0.27
CML67 x CML135	Ear leaf	0.14	0.09	0.12	0.43	-0.21
	- Ear leaf	0.24	0.24	0.24	0.11	0.032
	+ Ear leaf	-0.09	0.07	0.52	0.16	0.20

Table 5. ANOVA for ear damage and stalk damage by *D. saccharalis* on two hybrids, infestation at three silking stages and three different times of day.

Source	df	Ear damage	Stalk damage
Genotype (A)	1	32.12**	1089.97**
Silking stage at infestation (B)	2	0.03NS	179.26*
Time of day (C)	2	0.35NS	97.29
ABC	4	0.85NS	32.03NS
Error	85	2.28	42.31

Table 6. Correlation matrix of ear damage and stalk damage by *D. saccharalis*.

Genotype	Silking stage	r	Significance	n
Ki3 x CML131	Pre-silk	0.42	**	45
	Green-silk	0.25	*	73
	Brown silk	0.49	**	84
CML67 x CML135	Pre-silk	0.29	*	71
	Green silk	0.24	*	88
	Brown silk	0.57	**	66

Table 7. ANOVA for damage by *D. grandiosella* to maize infestation at three silking stages, different times of the day.

		Mean squares for damage			
Source	df	Sheath	Husk	Ear	Stalk
Genotype (A)	1	0.02NS	2.85*	8.80**	596.67**
Silking stage at infestation (B)	2	8.97**	19.65**	0.37*	116.90*
Time of the day (C)	2	0.24**	0.48NS	0.14NS	4.92NS
A x B x C	4	0.23NS	0.09NS	0.10NS	31.22NS
Error	34	0.145	0.31	0.13	18.30

Breeding for resistance to second-generation stem borers

When the genetically diverse germplasm, with known resistance to first-generation stem borers and good agronomic traits, was infested with SCB at Poza Rica, 259 S_1 lines were selected based on a low number of internodes damaged by the borers (Fig. 3). The S_1 lines were planted at Poza Rica and infested at anthesis with SCB. At harvest, on the basis of a low number of internodes tunneled by the borers, 314 S_2 lines were selected. These S_2 lines were then planted at Poza Rica and Tlaltizapan and were infested with SCB and SWCB, respectively. At Poza Rica 369 and at Tlaltizapan 360 S_3 lines were selected on the basis of low number of internodes tunneled by the borers. These S_3 lines, at both locations, were planted in two replicates and infested with neonate larvae at anthesis. In the first replicate, random crosses were made among the selected lines and in the second replicate, the plants were selfed to generate S_4 lines. Almost 30 randomly selected S_3 lines were also sampled at each location and were evaluated for sheath damage, ear damage and stalk damage. Correlations were then calculated between sheath damage and stalk damage, and ear damage and stalk damage for the

plants infested with SCB. These data could not be collected in the plants infested with SWCB due to poor grain formation in the ears. There were significant differences among the S_3 lines in sheath damage, stalk damage and ear damage on plants infested with SCB and SWCB (Figs. 4 and 5). The correlations of leaf sheath vs. stalk damage, husk vs. stalk damage were generally not significant, but the correlation between ear damage and stalk damage were significant in some S_3 lines, but non-significant in the other lines. (Table 8). These data again showed that damage caused by stem borers to different reproductive parts of maize is independent of damage to others and observed relationships are highly genotype-specific.

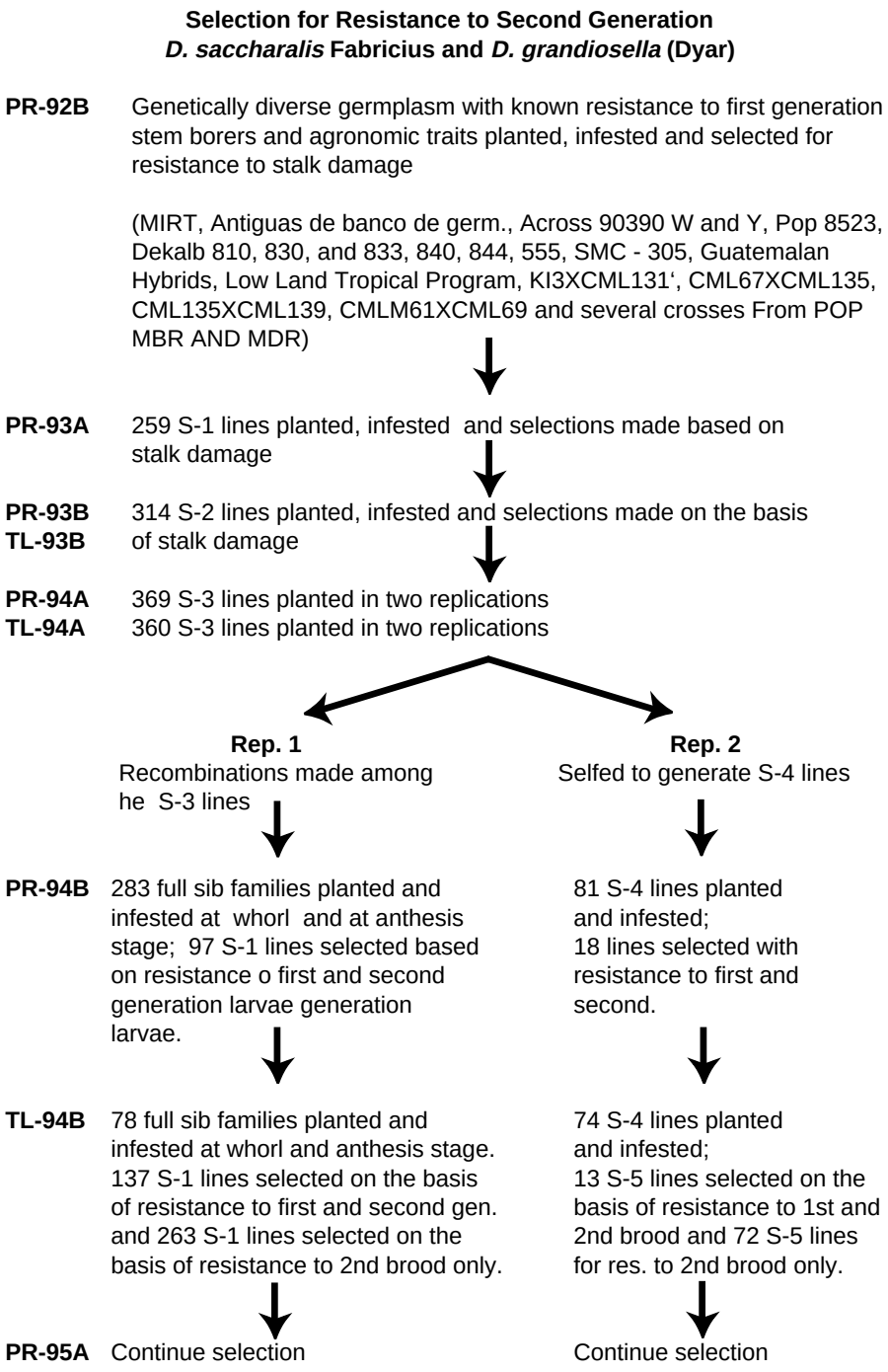

Figure 3. Schematic diagram showing the operations and breeding methodology used in developing populations and inbred lines for resistance to second-generation stem borers.

In Poza Rica, 283 full sib families and 81 S_4 lines were harvested, while at Tlaltizapan, 78 full sib families and 74 S_4 lines were generated. In the summer planting cycle of 1994, 283 full sib families and 81 S_4 lines were planted at Poza Rica station and infested with SCB at the whorl stage (6-7 leaf stage) and at anthesis in separate trials. Based on leaf feeding damage by the first-generation stem borers and stalk damage by the second-generation stem borers, 97 S_1 lines and 18 S_5 lines were selected having resistance to both generations of SCB. Also, 283 S_1 lines were selected with resistance to only second-generation SCB. In Tlaltizapan, 78 full-sib families and 74 S_4 lines were planted and infested with SWCB at whorl and anthesis stage maize. 137 S_1 lines and 13 S_5 lines were selected on the basis of resistance to first-and second-generation stem borers. Also, 263 S_1 lines and 72 S_5 lines were also selected for resistance to second-generation SWCB. Thus, of the large amount of germplasm with known levels of resistance to first-generation stem borers, a very low number of lines continue to show resistance to first-generation stem borer. In the process of selection for resistance to second-generation stem borer attacks, it seems that a large pool of genes was eliminated and that entirely different types of genes seem to control resistance to the two generations of the stem borers.

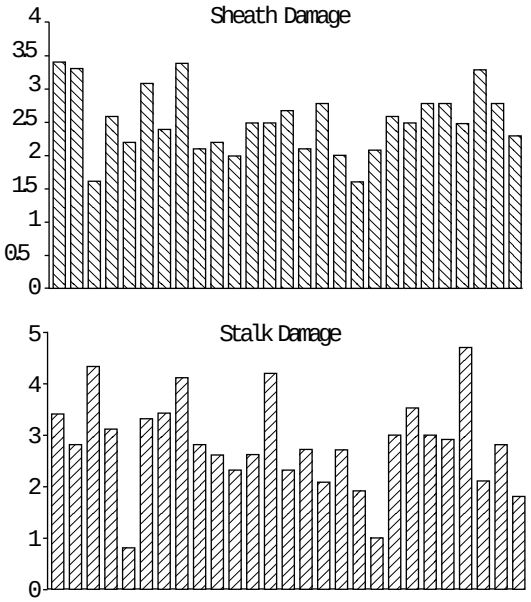

Figure 4. Sheath and stalk damage by SWCB to selected S3 lines of Population 391.

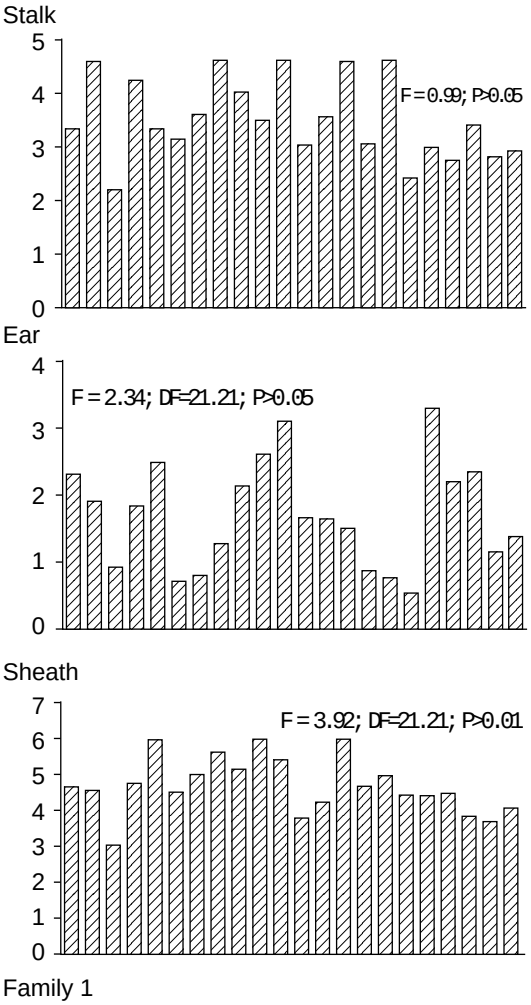

Figure 5. Sheath, ear and stalk damage by SCB on selected S_3 lines of Population 391.

Conclusions

In view of the highly variable correlations among the different damage parameters, the selections in maize for resistance to second-generation stem borers will continue to be made on the basis of stalk damage by the stem borers. The infestation of maize with SCB revealed adequate establishment of the larvae in the leaf sheath, ear husks and stems as indicated by the damage to these parts of the plants .The establishment of the SWCB larvae in the leaf sheaths and ear husks was low, but damage to the stems of the plants was moderate, thus facilitating the separation of the resistant and susceptible genotypes. It seems that the infestation level of SWCB on plants at Tlaltizapan will have to be high (> 60 larvae/plant) to get adequate establishment of the larvae in the leaf sheaths and ear husks.

Thus, using stalk damage by the stem borers as a selection parameter, two populations of maize have been synthesized with genes resistant to second-generation SCB and SWCB, respectively. Preliminary results also show that we are in the process of developing inbreds and populations which have high gene frequencies for both types of resistance.

Acknowledgments

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Table 8. Correlation matrices of damage by *D. saccharalis* on selected S_3 lines of population 391 at Poza Rica.

Family	n	Sheath vs. stalk	Husk vs. stalk	Ear vs. stalk
1	18	-0.178	0.308	0.468*
10	10	-0.352	-0.401	0.532NS
20	18	0.229	0.40	0.48*
30	20	-0.099	-0.134	0.203
40	20	0.513*	0.414	0.509*
49	20	0.086	-0.011	0.281
70	20	0.395	0.44	0.304
80	20	0.29	0.102	-0.055
89	20	0.44	0.48	0.63**
120	17	0.23	0.25	0.56*
130	16	0.12	0.25	0.06
140	20	-0.29	-0.13	0.43
150	20	0.106	-0.090	0.27
180	17	0.47	0.65	0.63
200	20	0.27	0.24	0.100
210	18	0.40	0.23	0.51*
223	20	0.13	-0.035	0.166
230	20	-0.129	0.35	0.61*
250	19	0.197	0.145	0.57*
260	20	-0.075	0.381	0.37
271	20	0.30	0.20	0.25
280	17	-0.021	0.172	0.184
291	10	-0.063	-0.61	-0.185
299	14	-0.158	0.00	-0.104

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Advances in Rating and Phytochemical Screening for Corn Rootworm Resistance

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Abstract

Evaluating and identifying sources of resistance to the corn rootworm, Diabrotica spp., continues to be a challenge due to subterranean feeding by the larvae and the destructive sampling to evaluate resistance. With the development of artificial infestation techniques, screening for resistance has progressed rapidly. However, evaluation of resistance continues to be labor intensive, with the most accurate rating system requiring root extraction, cleaning, and visual assessment of damage. Because field sampling and evaluation is costly, new evaluation techniques are constantly being evaluated. Refinement of field evaluation techniques using vertical root pulling resistance has increased the amount of corn germplasm that can be evaluated. In addition, consistent preliminary evaluations in the greenhouse and laboratory can reduce the amount of material screened in more costly field evaluations. Greenhouse evaluations have been used successfully to screen both maize germplasm and Tripsacum dactyloides L. for corn rootworm resistance. With the identification of DIMBOA as an antibiosis resistance mechanism, screening for elevated levels of DIMBOA in the roots can now be done on a large scale. Using a hydroponic system, over 100 genotypes a day can be evaluated for hydroxamic acid content and root mass. Genotypes with good root growth and high DIMBOA levels have shown field resistance to both artificial and natural infestations of Diabrotica spp. in sandy-loam and clay soil types. Bioassay systems are presently being developed to further large-scale screening efforts as well as our understanding of resistance mechanisms and feeding behavior of Diabrotica spp.

Introduction

The New World genus *Diabrotica* contains some of the world's most damaging agricultural insect pests. Among the ten known pest species in the genus, the western corn rootworm (WCRW), *D. virgifera virgifera* LeConte, and the northern corn rootworm (NCRW), *D. barberi* Smith and Lawrence, are the most important insect pests affecting maize, *Zea mays* L., production in the United States Corn Belt. Metcalf (1986) calculated that these two corn rootworms (CRW) cost US farmers US$1 billion annually in treatment expenses and crop losses. Other species, such as the southern corn rootworm, *D. undecimpunctata howardi* Barber and the banded cucumber beetle, *D. balteata* LeConte, are also pests of several crops in addition to maize.

Diabrotica beetles are most damaging in the immature stage. Larvae feed on the root system of the maize plant. Their feeding activity reduces maize yield by interfering with water and nutrient uptake. In addition, severe feeding damage often results in root lodging which can hinder mechanical harvesting, further reducing yield.

Pest *Diabrotica* in the US Corn Belt are generally controlled by crop rotation or soil insecticides. Because NCRW and WCRW larvae feed only on maize, crop rotation has traditionally been an effective control strategy. Females only lay eggs near maize, thus, maize planted following a rotation crop will avoid larval feeding damage. However, populations of NCRW have developed extended diapause in areas where a maize-soybean rotation is prevalent (Krysan et al. 1986; Steffey et al. 1992). In these populations, the eggs do not hatch in the first spring following overwintering. Instead, they hatch after two winters, thus damaging first year maize. This trait is becoming more widespread, making crop rotation a less useful control strategy.

In situations where it is not economical for farmers to rotate crops, insecticides

are widely used for CRW control. In some years, soil insecticides are applied to 50-60% of the total US maize acreage (Metcalf 1986). These insecticide treatments have generally been effective in protecting maize roots from feeding damage; however, a growing number of field reports suggest inconsistent performance of soil insecticides. Problems with variable insecticide degradation (Felsot 1989), and insecticide resistance in CRW (Chio et al. 1978), coupled with increasing safety and environmental concerns of these soil insecticides, point to a need to reduce soil insecticide use. To make this possible, host plant resistance will need to be more predominant in CRW management strategies.

Traditionally, host plant resistance has not played an important role in CRW management (Levine and Oloumi-Sadeghi 1991), despite 40 years of effort to select for CRW resistance. Melhus et al. (1954) conducted one of the first evaluations of CRW resistance and found resistance in Guatemalan maize strains. This resistance was found to be heritable and transmittable to a susceptible US hybrid. Welch (1977) described a recurrent selection program that enhanced CRW resistance by selecting for low damage ratings. Kahler et al. (1985) released a rootworm resistant synthetic selected using row vertical root pulling resistance. Unfortunately, the high costs of conducting a selection program for CRW resistance, inconsistent CRW infestations, difficulties in separating antibiosis from tolerance, and polygenic modes of inheritance have all kept CRW resistance from reaching the marketplace.

Currently, tolerance, in the form of large root systems and root regrowth after feeding damage has occurred, is the only mode of CRW resistance found in commercial maize germplasm. Evaluating maize germplasm for resistance to the CRW complex continues to be a challenge due to the subterranean feeding of the larvae and the destructive sampling methods necessary for evaluations. The development of techniques to artificially infest field plots (Sutter and Branson 1986) have enhanced CRW research considerably; however, evaluations for host plant resistance continue to be labor intensive and costly. Because of this, easier and more consistent field techniques are continually being developed and refined. The most reliable evaluations of CRW damage entails digging plants from the soil, washing soil off of the root system, and visually assessing damage using a rating system. These techniques are widely used, however, because of the labor and expense involved, they limit the amount of germplasm that can be evaluated in a growing season. Vertical root pulling strength, yield, and other methods of evaluation can potentially increase the output of a CRW resistance screening program. Corresponding with a field selection program, consistent laboratory and greenhouse techniques can be used to reduce the amount of material that is screened in more costly field evaluations. The ability to rapidly and consistently evaluate maize germplasm before initiating field evaluations can greatly increase the amount of material that can be evaluated. The following techniques, recently developed or refined at the USDA-ARS Plant Genetics Research Unit and the Agriculture Canada Plant Research Center, have been used to screen and select maize and maize relatives for host plant resistance to the WCRW in the field, greenhouse, and laboratory.

Field Evaluations

Vertical root pulling strength has long been used to evaluate maize for CRW resistance (Ortman et al. 1968). Several researchers have modified the technique to increase the consistency of the scores and reduce the amount of labor involved (Beck et al. 1987; Donovan et al. 1982; Penny 1981). Using hydraulic power, cable pullers, and hand-held computers, the Plant Genetics Research Unit has taken vertical root pulling strength on up to 3,000 plants in one day. Vertical root pulling strength can be used to measure maize resistance to CRW feeding; however, alone it does not differentiate between antibiosis, non-preference or tolerance. Moellenbeck et al. (1994) evaluated using differences in vertical root pulling strength in infested rows compared to the strength in uninfested rows to attempt to separate tolerance from antibiosis and non-preference. In that study, two commercial maize hybrids, Pioneer Brand 3377 and Pioneer Brand 3184; two inbred lines, CI31A and SC41R; and a B84/Iowa Stiff Stalk Synthetic breeding population selected for high vertical root pulling resistance, B84R, were tested using paired row vertical root pulling strength evaluations.

An artificial infester based on the model described by Sutter and Branson (1986) was used to distribute the eggs in the plots. Several slight modifications were made to their infester. First, two modified anhydrous fertilizer knives spaced 25.4 cm apart were used to 'knife' the egg/agar suspension into the soil. Flow to each knife was controlled by an individual solenoid that could be activated by the operator. A rotary flow indicator was placed in the solution line immediately above each knife to monitor solution

flow. A radar speed detector was also added to accurately monitor ground speed.

Ideally, infestations are made when plants reach the four-leaf stage to ensure adequate food supply for the hatching larvae (Branson and Sutter 1986); however, it is best to begin infesting when the plants are in the two-leaf stage to ensure infestations are completed by the four-leaf stage. Infestations later than the four-leaf stage often result in the plants having a large root system before the larvae reach the more damaging late instars, reducing the amount of damage inflicted on the maize plant. One row of each two-row plot was infested with 600 or 1,200 eggs per 30.5 cm. The 1,200 eggs per 30.5 cm rate was implemented by infesting 600 eggs per 30.5 cm on each side of the row. For 600 eggs per 30.5 cm, only one knife was activated. The second row of the plot was used as an uninfested control.

Root damage was evaluated using the vertical root pulling resistance (load-kg per plant) method described by Beck et al. (1987); however, cable pullers have replaced the clamp to reduce stalk breakage. Ten competitive plants within each row were pulled where possible. Noncompetitive plants or plants adjacent to previously uprooted plants were not used.

Cultivar resistance to rootworm damage was evaluated by taking the mean of the ten vertical root pulling resistance observations within an infested row and subtracting it from the mean of the adjacent uninfested row. Percent root pulling resistance differences were calculated by dividing the difference by the root pulling resistance of the uninfested row.

Wet conditions throughout July delayed root pulling until the maize plants reached the milk stage. Penney (1981) found that vertical root pulling strength differences are greatest when maize is at the milk stage; however, during pulling at both locations, heavy adult rootworm populations were noted. Kuhlman et al. (1970) found that the WCRW pupal stage lasts approximately 10.5 d at 22 °C. Thus, assuming the WCRW population was well synchronized, the cultivars had at least 10 d to recover from any root damage that had occurred. Differences in root pulling strength reductions among the cultivars may have been caused by differing levels of initial damage, recovery (regrowth), or both.

Combined vertical root pulling resistance averaged 217.7 ± 7.1, 181.1 ± 9.5, and 163.9 ± 8.3 load-kg per plant for 0, 600, and 1,200 eggs per 30.5 cm, respectively. WCRW infestations reduced vertical root pulling resistance at both locations. The interaction between infestation rate and cultivar was not significant at either location. The differences between uninfested rows and rows infested with 600 eggs per 30.5 cm, and the lack of interactions between cultivar and infestation rate, indicate that the lower infestation rate is adequate for evaluations. However, further studies (unpublished data) revealed that depending on climatic and soil conditions, 600 eggs per 30.5 cm may not be adequate. The higher infestation rate is now recommended to ensure adequate feeding pressure. In this test, cultivar ranks were similar at both infestation rates.

The five cultivars differed in vertical root pulling resistance at both locations. Vertical root pulling resistance differences (Table 1) between these cultivars were expected, because of the inclusion of commercial hybrids, inbreds, and a root-strength selected population. At both locations, inbred lines SC41R and CI31A had lower measurements than the other cultivars.

Calculating the reduction of vertical root pulling resistance of the infested row from the control row assesses cultivar response to CRW infestations, accounting for differences in their initial vertical root pulling resistance. Across all cultivars, rows infested with 1,200 eggs per 30.5 cm had significantly greater vertical rootpull resistance reductions than rows infested with 600 eggs per 30.5 cm. The cultivars did not differ in root pulling strength reduction (Table 1). In terms of percent reduction, the cultivars varied with inbred lines SC41R and CI31A having larger percent reductions than B84R and Pioneer Brand 3184. These differences are probably due

Table 1. Vertical root pulling resistance (load-kg per plant) for cultivars at two Missouri locations and combined vertical root pulling resistance reduction (load-kg per plant) due to corn rootworm infestations (from Moellenbeck et al. 1994).

Cultivar	Location 1	Location 2	Combined reduction [a]
B84R	275.7 a	197.0 b	45.7 a
CI31A	143.8 c	72.6 d	40.3 a
Pion. 3184 [b]	281.8 a	193.7 b	34.7 a
Pion. 3377 [b]	269.2 a	213.0 a	51.0 a
SC41R	190.5	113.7	54.1 a

Means (n=24) within a column followed by the same letter are not significantly different ($P = 0.05$)

[a] Vertical root pulling resistance of the control row - infested row. Values shown are combined across locations and infestation rates.

[b] Pioneer Brand

to the level of initial root strength and do not correspond to differences in WCRW feeding. Thus, differences in tolerance, based on vertical root pulling resistance of uninfested plants, were found in these cultivars; however, differences in antibiotic or antixenotic resistance were not found. The selected breeding population, B84R, and Pioneer Brand 3184 showed the greatest tolerance of the cultivars tested.

The lack of interactions between location and cultivar for root pulling resistance reduction indicates that cultivar differences are repeatable. This agrees with the findings of Rogers et al. (1976) who showed repeatability across different environments. The LSD for percent root strength reduction was found to be 10.2%. Thus, the infestation and root pulling strength measurement procedures used in the study can detect small differences among cultivars.

Paired-row evaluations for resistance to the CRW based on vertical root pulling resistance differences could greatly increase the number of cultivars that can be evaluated in a growing season. Cultivars selected based upon paired-row evaluations could then be more closely evaluated using root damage ratings. Because larval movement into the control rows could reduce the differences between infested and uninfested rows, Sutter and Branson (1986) recommended planting buffer rows between infested and uninfested rows to account for larval movement. Even in plots infested with 1200 eggs per 30.5 cm; however, significant root pulling strength reductions were found, indicating buffer rows may not be necessary. The artificial infestation methods and paired-row evaluations should be adequate for preliminary evaluations of maize germplasm for WCRW resistance.

To combine root rating data with vertical root pulling strength, it is possible to take root damage ratings (Hill and Peters 1971; Welch 1977) and secondary root developments ratings (Rogers et al. 1977) from the pulled plants. This allows the researcher to determine if higher vertical root pulling strength is caused by less feeding damage (antibiosis), larger root systems (tolerance) or by root regrowth (tolerance). Selections can then be based on both favorable root rating scores and low root pulling strength reductions.

Greenhouse and Growth Chamber Evaluations

Evaluations of CRW resistance in greenhouses and growth chambers can decrease the cost of a CRW breeding program. Preliminary evaluations can be conducted to cull susceptible material before it is planted in costly and labor intensive field plots. Greenhouse and growth chamber evaluations have been used extensively by the USDA-ARS Plant Genetics Research Unit to evaluate maize and maize relatives for CRW resistance. The following evaluation of *Tripsacum dactyloides* is an example of using a growth chamber to conduct initial evaluations.

T. dactyloides has shown antibiosis or extreme non-preference to the WCRW as mature plants and cuttings from mature plants (Branson 1971). If WCRW resistance from *T. dactyloides* is to be transferred into maize, and be useful, it must be present in maize seedlings. In order to locate resistance in seedlings Moellenbeck et al. (submitted to *J. Econ. Entomol.*) evaluated 50-day old *T. dactyloides* seedlings for resistance to the WCRW.

Stratified *T. dactyloides* seed (c.v. PMK 24) was obtained from Shepherd Farms of Clifton Hills, MO, and caryopses were germinated based on procedures described by Kindiger (1994). Emerging seedlings were transplanted into 10 cm clay pots containing a sand:silt (1:1) mix and maintained in a greenhouse at 25 ± 3 °C with a photoperiod of 14:10 (L:D) h prior to use in two separate evaluations.

One day prior to each infestation, WCRW eggs were suspended in glass centrifuge tubes containing 3 ml of a 1.5% agar solution. Each tube contained 50 counted WCRW eggs. Egg hatch was estimated at 80% prior to the evaluations. Pots containing 50-d old *T. dactyloides* seedlings (n=40) were infested with the egg/agar suspension using a pipetter on 4 May 1993. The suspension was placed 2.5 cm from the plant and 5.0 cm deep. Maize plants, planted and infested on the same day, were used as susceptible checks. The evaluation was conducted in a Conviron E15 growth chamber at 25 °C day and 20 °C night under a photoperiod of 14:10 (L:D) h. All plants were fertilized until soil saturation with a 250 ppm solution of 20-10-20 (N-P-K) fertilizer every 14 d.

A subset of plants was destructively sampled 3, 4, 5, and 6 wk post-infestation (larval hatch occurred from 14-18 d post-infestation). Ten *T. dactyloides* plants and five maize plants were evaluated at each sample date. The number of live larvae and mean larval weight were recorded for each plant by removing the plants and soil from the pots and placing them in containers of water. After hand mixing, larvae that floated to the top were collected. The use of a sand:silt mixture void of organic matter instead of a commercial growth mixture allows for

easier collection of the floating larvae. All of the larvae found in a single pot were weighed collectively. Mean larval weight per plant was then calculated by dividing the total weight by the number of larvae.

The number of larvae found on *T. dactyloides* and maize plants was not significantly different. The number of larvae recovered peaked on the maize plants 4 wk after infestation when 8.2 ± 2.1 (mean ± SE) larvae per plant were recovered. Larval populations on maize dropped to 3.4 ± 0.7 per plant six weeks after infestation. Larval populations on *T. dactyloides* reached 3.2 ± 0.4 at that date. The decrease in the number of larvae on maize most likely was caused by larval competition. Infested maize plants were heavily damaged at the final two sample dates and crowding in the small pots may have increased the competition for available feeding sites.

Larval weights on *T. dactyloides* were significantly less than larval weights on maize four, five, and six weeks after infestation (Table 2). Three weeks after infestation, the larvae were still first-instars and probably had not fed enough on either plant type to see any difference in weight. Six weeks after infestation, the larvae were 3 times heavier on maize than on *T. dactyloides*. This difference in weight is consistent with antibiosis or non-preference in the *T. dactyloides* seedlings.

Resistance found in young *T. dactyloides* plants may be more useful for transfer into maize. The mechanism of resistance in the seedlings has not been determined. A small percentage of the *T. dactyloides* seedlings did sustain larval growth, indicating either variation in *T. dactyloides* resistance to the WCRW or variation in the rootworms' susceptibility to the resistance factor(s). The *T. dactyloides* cultivar 'PMK 24' is not a homozygous breeding variety. Thus, the variation in the ability of some larvae to survive on these seedlings may be due to genetic variation among the seedlings. Because of this variation, breeding programs designed to transfer WCRW resistance from *T. dactyloides* into maize are advised to first evaluate the *T. dactyloides*.

Laboratory and Biochemical Evaluations

Recent studies on resistance mechanisms of maize to CRW have identified hydroxamic acids as resistance factors (Xie et al. 1990; Arnason these Proceedings). The major secondary compounds in maize roots include 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), 2,4-dihydroxy-7,8-dimethoxy-1,4-benzoxazin-3(4H)-one (DIM_2BOA), one lactam, 2-hydroxy-7-methoxy-1,4-benzoxazin-3(4H)-one (HMBOA) and one benzoxazolinone, 6-methoxybenzoxazolinone (MBOA). Screening roots for elevated levels of hydroxamic acids may provide a method for reducing the number of genotypes to be field evaluated. This approach has already been successfully applied to leaf tissue for European corn borer resistance screening (Russell et al. 1975). Once genotypes with elevated levels of hydroxamic acids in the roots have been identified, field evaluations can then be conducted to confirm resistance.

Germplasm used for this study included CRW resistant landraces (Aguascalientes 6, Chiapas 41, Durango 25, Guanajuato 69, Guatemala 166, Guatemala 189, Guatemala 196, Guatemala 489, Guatemala 633, Guatemala 757, Nayarit 203, Puebla 103, and San Luis Potosi 24) identified by field evaluation at CIMMYT (Mihm personal communication). This germplasm was crossed onto Agriculture Canada inbred lines (CO251, CO255, CO267, CO272, and CO289) with good agronomic traits. Crosses were selfed to obtain approximately 600 S_1 individuals which were phytochemically screened using the hydroponic technique described below. Seed from individual ears with extremely high or extremely low DIMBOA levels in the root were advanced and the S_2 generation was again evaluated for root DIMBOA content. Genotypes with extreme DIMBOA levels were considered for field evaluation.

Table 2. Mean weights of western corn rootworm larvae from corn (breeding population MoSQA) and *T. dactyloides* plants 3, 4, 5, and 6 weeks after infestation.

Plant	Weeks after infestation	Number of larvae[a]	Mean larval weight (mg)[a]
T. dactyloides	3	2.3 ± 0.6a	0.1 ± 0.1a
Maize (MoSQA)	3	5.4 ± 0.8a	0.3 ± 0.1a
T. dactyloides	4	6.9 ± 1.3a	0.5 ± 0.2b
Maize (MoSQA)	4	8.2 ± 2.1a	1.1 ± 0.2a
T. dactyloides	5	4.8 ± 1.2a	0.5 ± 0.2b
Maize (MoSQA)	5	3.8 ± 0.6a	1.6 ± 0.0a
T. dactyloides	6	3.2 ± 0.7a	2.3 ± 0.5b
Maize (MoSQA)	6	3.4 ± 0.4a	7.4 ± 1.0a

[a] Means ± SE (N = 10 for *T. dactyloides* and N = 5 for maize) within a sample date followed by the same letter are not significantly different ($P > 0.05$)

Approximately 15 seeds from each genotype were germinated on wet filter paper at 25 to 30 °C for 3 days until the radicle was approximately 2 cm long. Ten seedlings were then pinned to a Styrofoam block as illustrated in Figure 1. The pin did not penetrate the seed, but supported the seed firmly against the wall of the Styrofoam block to hold the seed at the water line. Each block held 50 seeds, allowing 5 genotypes to be tested per block, with each row labeled to identify the genotype. After pinning, the block was immersed into nursery flats that were half full of Hoagland's solution (Table 3). The trays were grown under optimal growing conditions (>80% RH, >25 °C, 16:8 (L:D)). After 14 d, the plants reached the 6 leaf stage and were removed from the trays. Tissue was stored at -20 °C for phytochemical analysis or used fresh for bioassays.

Frozen root tissue was removed from the freezer and allowed to thaw for 5 minutes so individual roots could be handled easily. Individual roots were weighed with a good sample size being approximately 0.5 g wet weight, but samples as low as 0.05 g could be analyzed. After recording the weight, the root sample was placed in a mortar and 3 ml of acidified 80% ethanol was added. Preparation of extraction solutions and colorimetric reagents is listed in Table 4. Root tissue was easily ground by mortar and pedicel so that no large sections of root tissue were left intact. After homogenizing, the supernatant was decanted off into centrifuge tubes. An additional 2 ml of acidified 80% ethanol was added to further grind the remaining pulp and rinse the mortar. The second volume was combined with the first, and the sample was centrifuged at 500 x *g* for 5 min. to provide a clear supernatant. Two ml of the supernatant was added to a spectrophotometer cuvette and an absorbance reading was taken at 520 or 590 nm. By taking the reading at 520 nm there is less interference by other root components that chelate with Fe^{3+}. For leaf tissue, absorbance readings at 590 nm are preferred due to chlorophyll interference. After recording the background reading, 50 ml of the dilute $FeCl_3$ solution was added and the solution mixed by pipette. Immediately after mixing, the second absorbance reading was taken. The absorbance drops rapidly over time so readings should be taken immediately after the addition of $FeCl_3$. The difference in absorbance before and after the addition of $FeCl_3$ is calculated, multiplied by the 5 ml extraction volume, and divided by the weight of root tissue to give a concentration in Abs520 per g wet tissue weight. A standard curve using authentic DIMBOA was generated to convert Abs520 into mg DIMBOA:

Table 3. Ingredients for Hoagland's solution for growing maize seedlings hydroponically.

Ingredients	Grams per 100 L of water
1) Magnesium sulphate ($MgSO_4$)	49.3
2) Potassium phosphate (KH_2PO_4)	13.6
3) Calcium nitrate $(Ca(NO_3)_2)•24H_2O$	118.1
4) Fe Chelate 13%	0.11
5) Potassium nitrate (KNO_3)	50.6
6) Minor Elements Solution	100 ml
Minor Element Solution	**Grams per 10 L of water**
$MnCl_2•4H_2O$	18.1
H_3BO_3	28.6
$CuSO_4•5H_2O$	0.8
$ZnSO_4•7H_2O$	2.2
$H_2MoO_4•H_2O$	0.2
KCl	63.0

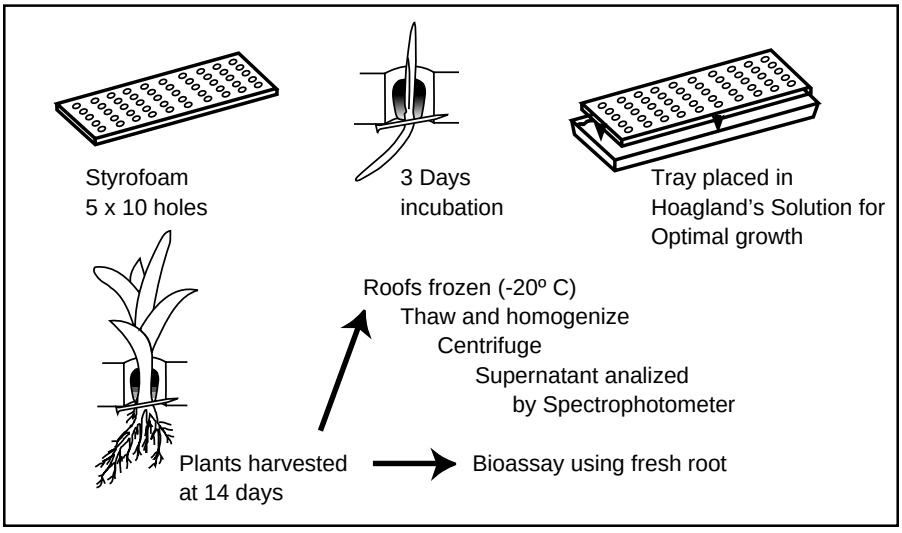

Figure 1. Phytochemical screening protocol for root tissue. Styrofoam trays are made from Stryofoam sheets cut to measure 25 x 50 cm (Dow SM, Dow Chemical Canada Inc., Weston, Ont. M9N 2M2). 50 1.2cm-holes were drilled using a high speed drill. Stryrofoam trays with seedlings pinned into holes were placed into heavy duty plastic nursery trays measuring 26 x 51 x 6 cm (model K10-20, Kord Inc., Toronto, Ont.).

Table 4. Preparation of solutions for $FeCl_3$ screening for DIMBOA.

1. $FeCl_3$ stock solution - store at <4 ∞C
   50 g $FeCl_3$ 6 H_20 in 495 ml H_2O and 5 ml of 11 N HCL, final pH of 2.
2. $FeCl_3$ screening solution - prepare as needed.
   Take 5 ml of $FeCl_3$ stock solution and add 45 ml distilled water.
3. 0.1N HCl in 80% ethanol
   Add 50 ml of 1N HCl to 450 ml of 95% ethanol.

mg DIMBOA / ml = 0.1183 x (Abs520 with $FeCl_3$ - Abs520 without $FeCl_3$)

For screening germplasm, only relative levels are required but the above equation should provide a reasonable estimate of DIMBOA levels in the tissue. Confirmation of DIMBOA levels should be done using a water based (Xie et al. 1991) or methanol based (Bergvinson et al. 1994) extraction method for quantification by high-performance liquid chromatography (HPLC). Genotypes with the highest relative levels of DIMBOA and large healthy roots should be considered for field evaluation using standard field screening techniques (these Proceedings; Branson and Sutter 1989).

For the present field study, three genotypes were selected for each of four categories based on DIMBOA level (high/low) and root mass at the 6 leaf stage (large/small). Plants had been evaluated at the S_2 stage using the above hydroponic system and seed from the same ear was used for field evaluation. Field trials were conducted in a clay soil with a high natural population of both NCRW and WCRW which had been maintained by planting sweet maize and grain maize of different maturities for four consecutive years. A complete randomized block design was used with three replicates and 12 plants per replicate. Ten weeks after planting, the plants were rated for lodging and the roots dug up, washed, and rated on a 9 point scale outlined by Branson and Sutter (1989). After rating, the roots were dried and weighed.

Field verification of the $FeCl_3$ screening method indicated that DIMBOA content in root tissue is an important component in host plant resistance to the CRW (Table 5). Genotypes with elevated levels of DIMBOA had less root pruning than low DIMBOA genotypes, which is consistent with earlier work (Xie et al. 1990). A recent survey of DIMBOA content in root tissue of commercial hybrids had demonstrated the low level of DIMBOA in the majority of hybrids, which may in part explain the susceptibility of commercial hybrids to root pruning by CRW larvae (Assabgui et al. 1993).

During the course of DIMBOA screening, root mass was also considered as an important component in root tolerance to CRW pruning and was included in the selection process. Despite the 10-fold difference observed in root mass at the 6 leaf stage in the hydroponic system, field grown plants did not differ considerably in root mass at the time of field assessment (Table 5). It appears that poor root establishment early in plant development is compensated for during the growing season in the genotypes tested. Reduced root growth early in plant development may be an avoidance mechanism, as these plants had the lowest root damage rating (Table 5). Given the nature of the damage rating scale, plants with a small root system early in development may have lower ratings due to a lower probability of root pruning given the reduced number of roots available for feeding. Despite the higher damage ratings for plants with large, densely branched root systems, this phenotype is often able to regenerate roots readily, a reaction that is considered an important component in resistance (Jenison et al. 1981). These observations may provide an explanation for the poor correlation between root lodging and the root damage rating ($r = 0.3$, $P>0.1$), as genotypes with large root systems early in development tended not to lodge.

Screening root tissue for elevated levels of DIMBOA has enabled resistant genotypes to be identified and can accelerate the development of resistant inbred lines as plants from both winter and summer nurseries can be evaluated in the laboratory. Using the $FeCl_3$ screening technique, one person can process 150 samples per day. With this processing capability, germplasm can be assessed after harvest and desirable ears identified before the next nursery for advancing another generation.

The potential danger of this screening method is only one phytochemical component is being assessed. Given the incomplete knowledge of root biochemistry as it relates to CRW resistance, other resistance mechanisms

Table 5. Field evaluation of S_2 genotypes selected by the iron chloride screening technique.

Plant Attributes	Background	Damage rating[a]	Root mass dry wt. (g)[a]
High DIMBOA	Durango 25 x CO255	4.9 e	93 abc
Large root mass	S. Luis Potosi 24 x CO289	5.0 e	97 ab
	Guanajuato 69 x CO251	5.1 e	109 a
Low DIMBOA	Durango 25 x CO255	8.4 a	75 cd
Large root mass	Durango 25 x CO255	6.5 cd	96 ab
	bxbx mutant (low DIMBOA)	5.7 de	86 bc
High DIMBOA	Guatemala 757 x CO289	3.3 f	87 bc
Small root mass	Guanajuato 69 x CO251	5.3 ed	101 ab
	Guanajuato 69 x CO251	3.7 f	92 abc
Low DIMBOA	MBR622 Lines developed	8.4 a	86 bc
Small root mass	MBR105 from MBR synthetic	7.6 ab	85 bc

[a] Means followed by the same letter are not significantly different, Student-Neuman-Kuels test ($P = 0.05$).

would not be detected using this screening protocol. For this reason, further work is needed to better understand the biochemical mechanisms of host plant resistance to the CRW. Work is also needed on identifying the changes that occur in root chemistry for both resistant and susceptible genotypes. Having identified germplasm with a range of DIMBOA levels and root mass, we can now address questions regarding the relative importance of antibiosis and tolerance. By understanding the traits most desirable for host plant resistance and at what stage in plant development these resistance mechanisms are most important will accelerate the development of resistant inbred lines.

Host plant resistance must play a more important role in future CRW management. As we learn more about mechanisms of CRW resistance, selection programs can continue to be refined. Evaluation of CRW resistance must always include field evaluations of feeding damage; however, techniques described here can reduce the amount of material that needs to evaluated in costly field testing by removing susceptible materials early in the screening process. Biotechnology and marker-assisted selection offer the opportunity to develop new ways to incorporate host plant resistance into commercial maize germplasm. Selection programs must continue to be refined in order to use these techniques efficiently.

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Factors Affecting a Laboratory Bioassay for Antibiosis: Influences of Maize Silks on the Corn Earworm and Fall Armyworm Larvae

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Abstract

A useful laboratory bioassay has been developed to screen for resistance to lepidopterous insects attacking maize, Zea mays L., and for use in studying the antibiotic mechanism and bases of resistance to these insects. The bioassay may be used to detect minor as well as major differences between the resistant and susceptible maize cultivars. The bioassay has been used to study the influence of: the environment; pollinated vs. nonpollinated silks; ear position; age and type of silk; and callus tissue on expressions of antibiosis against the corn earworm (CEW), Helicoverpa zea (Boddie), or fall armyworm (FAW), Spodoptera frugiperda (J. E. Smith), larvae. Studies on some of the factors, such as temperature, diet and diet ingredients, and insect feeding responses, revealed interactions with the expressions of antibiosis. The bioassay has also been used in studies on the relationship between low larval weight with maysin content and the genetic and chemical bases of resistance in maize to CEW and FAW larvae.

Introduction

Effective techniques are essential for the identification of sources of plant resistance to insect pests and, especially, to characterize the mechanisms and the chemical and genetic bases of resistance. Wiseman et al. (1984) evaluated a series of substandard (incomplete) diets modified from the regular pinto bean diet (Perkins 1979). Two diets were acceptable for plant allelochemical investigations: the regular pinto bean diet and the substandard pinto bean diet without yeast. Since then the pinto bean diet bioassay has been modified and has replaced the substandard diet and is now used to evaluate maize, *Zea mays* L., and sorghum, *Sorghum bicolor* (L.) Moench, for resistance to insects. Various forms and amounts of maize silks (Wilson et al. 1984; Wiseman and Widstrom 1986; Wiseman and Wilson 1987) have been incorporated into the pinto bean diet to characterize several factors of antibiosis to the corn earworm (CEW), *Helicoverpa zea* (Boddie) and the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith).

Much of the earlier work used large (several grams) amounts of plant material in 300-400 ml dilute pinto bean diet. Wilson et al. (1984) used 10 to 80 g of fresh maize silks in 300 ml of diet and 2 to 16 g of lyophilized silks in 300 ml of diet to evaluate against the CEW. Wiseman and Widstrom (1986) used 10 to 80 g of fresh silk in 300 ml diet to test against the FAW. Wiseman and Wilson (1987) were the first to use oven-dried silks in meridic diets against the CEW. Then Wiseman and Isenhour (1988) showed the importance of consistent handling of silks during the harvesting and drying process. They reported that silks harvested and immediately dried produced more consistent results than lyophilized or fresh silks in bioassay diets.

Diets of 400-500 ml quantities with 20-25 g of dry silks were generally made using a standard household blender and dispensed into 30 ml plastic diet cups at a rate of 10 ml/cup even though frequently the amount of the silk/diet mixture was expressed as 50 mg/ml diet (Wiseman and Isenhour (1989). Later, Wiseman et al. (1986) developed a microassay that used 20 ml pinto bean diet, 10 ml distilled water and as little as 2 g of fresh, dried, or equivalent extracted plant material (Wiseman and Isenhour 1991) blended in a 37 ml mini-blender and then aspirated into plastic soda straws. The final refinement came when Wiseman and Isenhour (1991) described a microtechnique for evaluating antibiosis against the CEW. The technique they developed used samples (0-100 mg dry weight) of silks from individual ears. Since then, the standard amount of silks/pipette bulb was increased to 150 mg. Dry silks

were placed into a detached bulb of a 7.5 ml disposable pipette in which 2 cc of dilute pinto bean diet (3:2 pinto bean diet:H_2O) was mixed, at first using a 3/8" reversible drill at 500-600 RPM. Later, the mixing of the silks into the dilute diet was accomplished by a "Biovortexer" or a modified "Tooth Polisher" (Fig. 1). The "Biovortexer" cost about $56 compared to $5.95 for the "Tooth Polisher". The remaining portion of this review will address the influence of the plant and insect affecting this laboratory bioassay and the expressions of maize silk antibiosis against the CEW larvae.

Influences of the Plant

Planting date

Silks grown at two locations (Tifton, GA and Ames, IA) and on two planting dates per location were fed in diets to CEW larvae (Wiseman and Wilson 1987). Weight of larvae from test locations showed significant differences between planting dates for the silks produced in Georgia, but not for those produced in Iowa. The differences between cultivars occurred irrespective of test location. Larvae fed Iowa-produced 'Stowell's Evergreen' (SEG) sweet maize silks weighed significantly more for each planting date than those fed SEG silks originating in Georgia. The reverse was true for larvae which were fed 'Zapalote Chico 2451 # P (C3)' (Z. Chico) silks. Larvae fed on Z. Chico silk-diets weighed significantly less than those fed on SEG silk-diets in every case, even though the differences in weight between larvae on Z. Chico versus SEG ranged from 181 to 723 mg on the 5 g silk-diets and 36 to 728 on the 10 g silk-diets. The CEW larvae tested at Tifton were generally larger than those tested at Ames probably because of the heterogeneity of the Tifton colony.

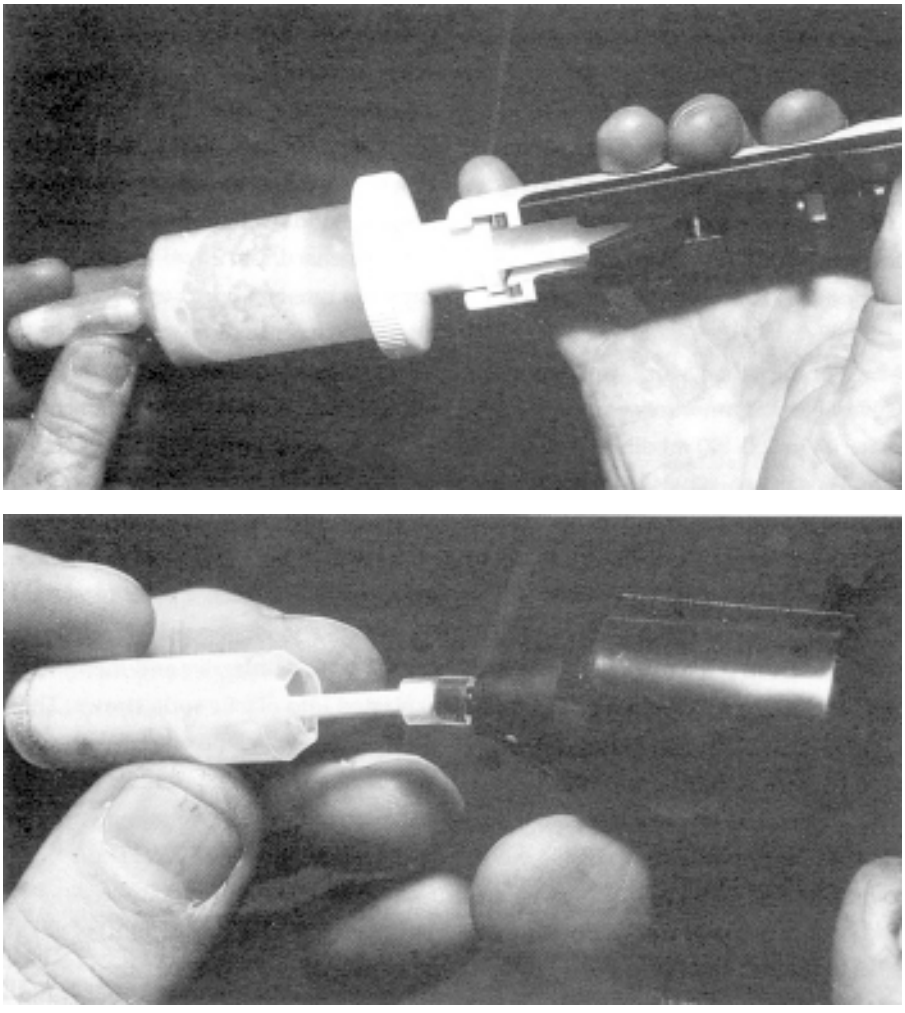

Figure 1. An Eppendorf repeater pipette was used to dispense 2 ml of dilute pinto bean-silk diet into a 7.5 ml detached bulb of a disposable pipette (top). The silk-diet mixture was mixed using a modified "Tooth Polisher" (bottom).

Wiseman and Isenhour (1992) studied environmental influences on silks resistant to the CEW. Environment had a greater influence on the response of CEW larvae fed silk-diets from an intermediate resistant or susceptible maize line, but had little influence on the feeding response of larvae on the highly resistant silk-diet of Z. Chico. In 8 of 12 tests using Z. Chico and 7 of 12 tests of 471-U6 X 81-1, no significant differences were found between planting dates for six characteristics of resistance. None of the intermediate or susceptible entries approached this level of consistency.

Pollinated silks vs. nonpollinated silks

Pollinated and nonpollinated silks from SEG and Z. Chico were incorporated, fresh and dried, in meridic diets and evaluated for their effects on the development of CEW larvae (Wiseman and Wilson 1987). Larvae weighed significantly less when fed fresh, pollinated silk-diets than when they were fed fresh, nonpollinated silk-diets. Differences between pollinated vs nonpollinated silks were not detected for other insect developmental characters when either fed as fresh or

dried silks in diets. The larvae that were fed fresh or dried Z. Chico silk-diets were significantly different for each developmental character than those fed on SEG silk-diets.

First ear vs. second ear

Silks from first or second ears and silks regrown for one or two days after cutting were evaluated for antibiotic responses to CEW larvae (Wiseman et al. 1993). Neonate CEW fed silk-diets from first ears weighed significantly less than larvae fed silk-diets from second ears. Silks regrown for one or two days after initial cutting and incorporated into diet produced larger larvae after eight days than those fed on silk-diets from the initial cutting. Weights of larvae were consistent among genotypes, whether the silks were from first or second ears. This was especially true for silks of PI340856, which had a high level of antibiosis. Larvae were quite small on silk-diets of both first and second ears of PI340856. It was concluded that silks could be sampled for chemical analysis and the regrowth used to bioassay larvae without risk of erroneous results, providing that silks are used from the same ear location.

Fresh vs oven dry silks

Wiseman et al. (1995) evaluated silks of fifty field corn inbreds in four separate bioassays (fresh silks, 2 g and 4 g oven-dried silks/100 g diet and maysin equivalent to 20 g fresh silks deposited on celufil and incorporated in 100 g diet) for growth responses of CEW larvae. Assays for maysin, isomaysin and apimaysin plus 3'-methoxymaysin content of silks were also made. Significant differences in growth of larvae were found among the silks of the fifty inbreds within each of the four bioassays. The correlation coefficients for weight of larvae that were fed fresh silk-diets or the 2 g and 4 g oven-dried silk-diets were similar (Table 1). Lower correlation coefficients occurred between bioassay results for larvae that were fed maysin deposited on celufil diets. This lower correlation coefficient was probably the result of having only one chemical responsible for the silk resistance when the chemical was applied on the celufil, whereas the silk-diets, fresh or dried, had all phytochemicals present. The 4 g oven-dried silk-diets of Ab616, Ab618, GE37, 8940C and 91201Y produced larvae with much smaller weights than any other type of diets tested. By adding the additional 2 g of oven-dried silk to the diets, a threshold was probably reached for the expression of antibiosis (Wiseman et al. 1992b). However, this did not affect the rankings of the inbreds in each test, hence the high correlation's between the flavones and weight of larvae among the four test diets.

Biological activity against CEW larvae with dry silks in diets appears to be enhanced over that of fresh silks in diets. The percent flavones (maysin) found in the fresh silk is based on the wet weight of the silks as compared to those found in the dried silks (maysin, isomaysin, apimaysin and apimaysin plus 3'-methoxymaysin) which were calculated on a dry weight basis; hence the higher amounts of flavones are noted for the dried silks. But, in fact the amounts are much less; i.e., the mg/g of maysin in fresh silks of Ab616 was 2.54 mg based on a wet weight basis, whereas the oven-dried silks of Ab616 had 8.94 mg/g based on a dry weight basis. If the fresh weight were calculated on a dry weight basis there would be ca. 25.4 mg maysin/g of silk. The percent moisture for each inbred silk would need to be calculated. If the silks of Ab616 are assumed to be 90% water, then a loss of 16.46 mg/g of maysin to undetected compounds is present in the oven-dried silks. Even with the addition of the isomaysin, there is still ca. 10.9 mg/g of maysin or breakdown products undetected in the dry silks. However, the biological activity was not lessened in the dry silks, but was enhanced. If the amount of dry silks is doubled in the diets — from 2 g to 4 g oven dry silk/ 100 g of diet — then the amounts of isomaysin and maysin available in the fresh silks are more than accounted for and the activity against the larvae appears to be enhanced. Isomaysin and apimaysin plus methoxymaysin were only detected in the oven-dried silks.

A highly significant ($\underline{P} \leq 0.01$) negative relationship was found between weight of larvae within each of the four diets and maysin in fresh silks or maysin and isomaysin in dried silks (Table 2). No significant correlation was found between weight of larvae and apimaysin plus methoxymaysin. When isomaysin

Table 1. Pearson correlation coefficients (r) and levels of significance among eight-day weights of corn earworm larvae fed diets of fresh and oven-dried silks and maysin deposited on celufil.

	Correlation coefficients[1]		
	2 g dry silks	4 g dry silks	maysin + celufil
Fresh silk	0.9193*	0.9202*	0.8058*
2 g dry silk	——-	0.9528*	0.7990*
4 g dry silk	——-	——-	0.7783*

[1] Ho: Rho = 0. n = 52. * = significance at 0.0001. (From Wiseman et al. 1996)

was added to maysin, the level of the relationship was only slightly enhanced for larvae that fed on the oven-dried silk-diets and the maysin on celufil diet, but not for those fed the fresh silk-diets. The highest correlation was found when these two flavones were combined with weight of larvae that fed on the maysin on celufil diets. However, when isomaysin, apimaysin, and methoxymaysin found in the dry silks were added to maysin, the relationship between weight of larvae and the flavones was enhanced significantly over maysin alone, i.e., 32.9% for the fresh silks and 43.7% for the oven-dried silks (4 g). However, the lowest correlation (-0.8542) in this group (all flavones combined) was found between all the flavones combined and the weight of larvae that fed on the maysin only in the celufil diet.

Although the results of the four bioassays compared favorably, those based on fresh silks or maysin deposited on celufil have limitations. In an evaluation, silks from inbreds or germplasm are not produced at the same time but mature over an extended time period, making it extremely difficult to use silks of the same age. Fresh silk in the quantity necessary to achieve large differences among weights of larvae are difficult to mix and/or dispense. Likewise, maysin deposited on celufil omits other flavones or unidentified chemicals from the bioassay. Thus, bioassays with oven-dried silks permits the use of larger amounts, (4 g instead of 2 g of material), which should enhance the antibiotic effects. Similarly, germplasm of varying maturities can be assayed when the oven-dried silk bioassay is employed.

Age of silk

The effects of age of Zapalote chico and Stowell's Evergreen silk on developmental characters of neonate CEW were studied by Wiseman and Snook (1995). Consistent significant differences between cultivars for each age group of silk (nonpollinated, two, five, and ten day pollinated), except ten day pollinated silks, were found among insect biological parameters measured. It appears that as age of silk increases, maysin content decreases and growth of CEW larvae often increases. It is not known if this phenomenon occurs in other cultivars, or if resistance decreases in cultivars with chemicals other than maysin as the basis of resistance.

Callus tissue

The use of callus tissue to screen for insect resistance has been suggested by some as a substitute for plant tissue (Williams et al. 1987; Isenhour and Wiseman 1988). Callus is an undifferentiated mass of living cells that can be grown on an agar-based nutrient medium under sterile conditions. Callus growth is initiated by placing a piece of plant tissue (explant) on nutrient medium, with both the explant type and nutrient medium specific for a given plant species. Williams et al. (1985, 1987) proposed the use of fresh callus tissue as a method for screening maize genotypes for resistance to lepidopterous larvae. Isenhour and Wiseman (1988) tested fresh callus tissue incorporated into meridic diets and compared biological differences of the FAW and CEW after feeding on calli-diets from resistant and susceptible genotypes. Callus-diet mixtures failed to confer the degree of resistance that foliage-diet mixtures did. In cases where antibiotic resistance factors were present in the silk, the callus-diet mixtures failed to exhibit any evidence of resistance.

Insect Influences

Temperature

Isenhour et al. (1985) studied the effects of varying temperature on bioassay results of resistant versus susceptible plants. They found no differences in

Table 2. Pearson correlation coefficients (r) and significance levels among eight-day weights of corn earworm larvae and percent maysin, isomaysin, apimaysin plus methoxymaysin, maysin plus isomaysin and maysin plus isomaysin plus apimaysin plus methoxymaysin.

	Correlation coefficients for[1]			
	Silks		Maysin	
Flavone	fresh	2 g dried	4 g dried	on celufil
Fresh silks				
Prior maysin[2]	-0.5164*	-0.5537*	-0.5134*	-0.6471*
1992 maysin	-0.6361*	-0.7356*	-0.6671*	-0.8033*
Oven-dried silks				
Maysin	-0.5951*	-0.5999*	-0.5424*	-0.6529*
Isomaysin	-0.5835*	-0.6242*	-0.5701*	-0.6736*
Apimaysin + methoxymaysin	-0.0415	-0.0396	-0.0169	-0.0635
Maysin + isomaysin	-0.5965*	-0.6150*	-0.5576*	-0.6674*
Maysin + isomaysin + apimaysin + methoxymaysin	-0.9656*	-0.9762*	-0.9793*	-0.8542*

[1] Ho: Rho = 0. n = 52. * = significance at 0.0001. (From Wiseman et al. 1996).
[2] Prior maysin indicates the determinations of maysin made on the same inbred silks prior to 1992.

weight of FAW larvae fed excised leaves of susceptible and resistant genotypes at 25ºC, but differences were found between weight of larvae that fed on genotypes at 30ºC and a fluctuating temperature regime of 31/20ºC. This variation did not occur when comparisons were made between susceptible and resistant genotypes using a foliage-diet mixture. Wiseman and Isenhour (1989) studied the effects of interactions among temperature (20, 25, and 30ºC), resistant and susceptible genotypes, and concentration of silk/diet (0 and 18.75, 37.5 and 67.0 mg/ml diet) on CEW developmental parameters. Significant differences caused by the resistant silks compared with the susceptible silks, were measured consistently at 25ºC for all four insect biological parameters.

The meridic diet

Wiseman and Isenhour (1993) evaluated the effects of the addition of varying levels of resistant silks, formalin, ascorbic acid, and yeast to the corn-soy-milk (CSM) diet (Burton and Perkins 1989) or modified pinto bean diet on weight of CEW larvae. Interactions were found among weight of larvae that were fed on CSM or pinto bean diets with or without formalin, varying levels of resistance, and varying concentrations of ascorbic acid or yeast. In all cases larvae that were fed on regular diet with formalin weighed significantly more than those that fed on diets without formalin. The oxidative process (top of diet turns brown) of the resistant silks was enhanced in the silk-diets without formalin and delayed in silk diets as the concentration of ascorbic acid was increased in the silk-diets. However, tests revealed that formalin did not react with maysin. Therefore, formalin would not cause any breakdown or degradation of maysin in meridic diets. Increasing concentration of yeast promoted growth of larvae that fed on silk-diets. Diets, therefore, must be fully characterized (i.e., components identified) because small changes in diet components can affect the apparent levels of resistance. Comparisons of data over more than one experiment should always be carefully interpreted, especially if diet components vary among experiments.

Insect feeding

Wiseman and Isenhour (1993) and Wiseman and Hamm (1993) noted that young CEW larvae tended to bore directly through the diet surface when resistant silk-diets showed an increased oxidative process (turned dark brown), whereas larvae on susceptible diets tended to eat along the diet surface. Wiseman and Carpenter (1995) studied the growth inhibition factor of the antibiotic silks. They found using neonate, fourth and fifth instar CEW larvae that the antibiotic resistance was the result of an anti-nutritive factor that possibly binds the protein or that results in degradation of essential amino acids, causing the larvae to excrete large amounts of protein.

Effectiveness of the Laboratory Bioassay

The laboratory bioassay has been used effectively in a number of studies to separate resistant and susceptible genotypes, first ears from second ears, and initial silks vs silks regrown for one or two days (Wiseman et al. 1992a,b, 1993). Significant negative relationships have been established for weight of CEW larvae and concentration of maysin (r = -0.811 and -0.655) (Wiseman et al. 1992a,b). Regression analysis of weight of CEW larvae and maysin content was cubic ($r^2 = 0.893$). A concentration of ≥0.2% maysin reduced CEW larval growth to ≥50% of that of the control. Higher amounts of maysin, such as 0.4%, reduced weight of CEW larvae to >70% compared with the control. A stepwise multiple regression analysis has shown that maysin was the major factor associated with resistance in silks of maize to both CEW and FAW larvae (Wiseman et al. 1992b). The addition of apimaysin to the regression analysis only improved the r^2 by about 10%. Neither chlorogenic acid nor 3'-methoxymaysin appeared to improve the r^2. However, when isomaysin, apimaysin and methoxymaysin found in the dry silks (Table 2) were added to maysin, the relationship between weight of larvae and the flavones was enhanced significantly over maysin alone, i. e. 32.9% for the fresh silks (t = 6.29; P = 0.001; n = 52) and 43.7% for the oven-dried silks (4 g) (t = 8.279; P = 0.001; n = 52).

Summary and Conclusions

A useful laboratory bioassay has been developed for both routine screening for resistance to CEW and FAW larvae attacking maize and to evaluate the antibiotic mechanism of resistance. Evidence exists that the laboratory bioassay can detect large differences between the resistant and susceptible maize cultivars. The bioassay has been used to study the influence of: the environment, pollinated vs. nonpollinated silks, ear position, age of silk and callus tissue on expressions of antibiosis against the CEW or FAW larvae. Some of the factors affecting the bioassay results were temperature, diet and diet ingredients, and insect feeding responses. The bioassay has also been used to study the relationship between

low larval weight and maysin content (Wiseman et al. 1992a) and the genetic (Wiseman and Bondari 1992, 1995) and chemical (Snook et al. 1993) bases of resistance in maize to CEW and FAW larvae. Through technology transfer, the methodologies and procedures used in the laboratory bioassay have been imparted to a number of commercial companies as well as researchers in public institutions.

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Development of Germplasm with Resistance to the European Corn Borer

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Abstract

The European corn borer (ECB), Ostrinia nubilalis *(Hübner), is a primary economic pest of maize,* Zea mays *(L.), in the United States. It was introduced into this country from Europe prior to 1917 when it was first described as a maize pest. Host-plant resistance studies began in the United States during the 1920s. Considerable progress in developing maize cultivars with first-generation ECB resistance was made by the 1950s when several inbreds with resistance to first-generation ECB were available. Due to lack of domestic resistant germplasm and the intensive labor required for identification of second-generation ECB resistance, few resistant cultivars were identified. However, with more emphasis placed on second-generation ECB resistance, it has been successfully identified by Missouri and Iowa scientists and levels enhanced by recurrent selection. In Missouri, germplasms Mo-2ECB and Mo-2ECB-2 and inbreds Mo45, Mo46, and Mo47 have been released as sources of resistance to both generations of ECB.*

The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a significant economic pest of maize, *Zea mays* (L.), in the United States. Annual losses are estimated between 200 and 500 million dollars for the Corn Belt. The ECB was first described as a pest of maize in the United States in 1917 (Vinal 1917), but it probably entered the country about 1914 in hemp, *Cannabis sativa* (L.), or hops, *Humulus lupulus* (L.). In 1918, devastation of maize production by ECB in Medford, MA, occurred and was recorded by B.E. Hodgson (Fig. 1).

As early as the late 1920s, Huber (Huber et al. 1928) suggested plant resistance as a control method. Patch, Schlosberg, and Vance promoted the idea while working with sweet and field maize (personal communication, Orlo Vance 1994). During the 1930s and 1940s, initial techniques for host-plant resistance research were established, and some varietal resistance was identified (Patch and Pierce 1933; Patch 1947; Patch and Everly 1948). However, this was for first-generation ECB, and at this time, it was not realized that resistance for second-generation ECB was a different genetic trait. F.F. Dicke and W.D. Guthrie assisted in developing several inbred lines with the antibiosis type of resistance for first-generation ECB, but germplasm for second-generation ECB was not readily available in Corn Belt germplasm, and labor required for identification prevented screening

Figure 1. European corn borer damage to maize in 1918, shortly after ECB was introduced in the United States (photograph by B. E. Hodgson, Medford, MA).

many germplasm sources. Dicke (1954) suggested that the way to manage the second-generation of ECB was to develop tolerant plants, and to a large degree, this has been done by the maize breeders in their stalk strength improvement programs. Figure 2, illustrating results of selection for high and low stalk rind strength, indicates a mechanism by which tolerance may be achieved. Although Figure 2 shows the efficacy of selection for rind strength, the biological response by ECB is yet to be determined.

The Iowa State team of entomologists and breeders has successfully identified inbreds, such as B52 and B86, and other germplasm sources with second-generation ECB resistance. In 1975, a new team including the disciplines of entomology, plant pathology, and breeding was organized in Missouri. At Columbia, this team could work with longer-season maize germplasm, including some tropical material, which could not be done in Iowa.

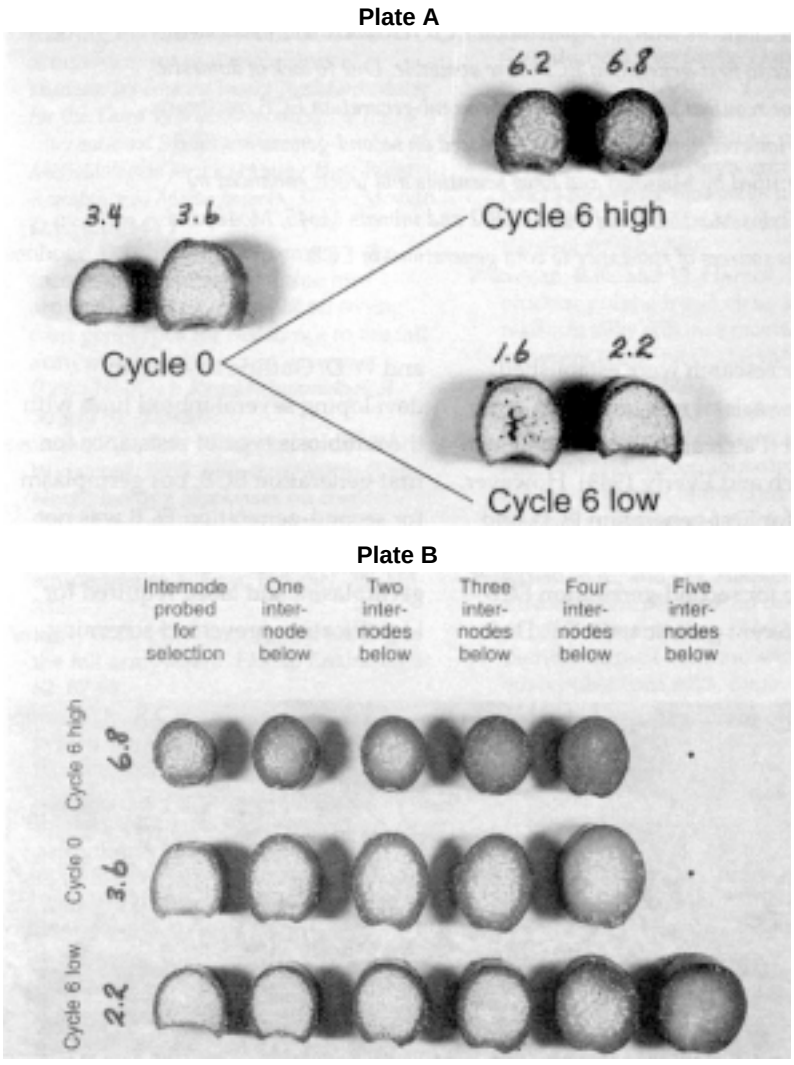

Figure 2. Cross sections of internodes below the top ear node from stalks of representative plants from cycle 0 and cycle 6 of bi-directional selection in the internode below the top ear node for rind penetrometer resistance in MoSCSSS and their respective rind penetrometer resistance readings (load-kg/plant) (Plate A). Cross sections of the internode used for selection and those below showing progressive changes in rind thickness, stalk diameter, and stalk morphology) (Plate B).

Because second-generation ECB resistant germplasm was not readily identified in the Corn Belt, it appeared that the logical place to seek new sources was the tropics. The first hint of a new source of resistance was in maize populations developed by Dr. M.S. Zuber, a USDA-ARS maize breeder at the University of Missouri, which he called PR-Mo2, PR-Mo2 x MoSQA and PR-Mo2 x MoSQB. The source of the resistance (PR-Mo2, released by USDA-ARS and the University of Missouri in 1975) was Nigeria Composite B, also a valuable source of resistance for *Puccinia polysora* (Underw.), *Bipolaris maydis* [(Nisik.) Shoem.], and *Ustilago maydis* [(DC.) Cda.]. Nigeria Composite B source material included Nigeria NS-1 (Caribbean origin); NS-5 (Local varieties, Mexico 5, EAAFRO 231, and Sicaragua); University of Ibadan Flint-Dent Composite; Pioneer Brand X301 and X306; Caribbean Composite; Jamaican Selected Yellow; Dahomy Jaune d'la Ina; EAAFRO 231 (Rocamex 520C); Mexico Hybrids H503, H504, and H507; Ivory Coast M.T.S.; Kenya Coast Composite (Local varieties, Caribbean, Mexican, and Colombian lowland germplasm); Nigeria Bida Yellow; South Africa Tsola; and selected Tuxpeño and Caribbean material from the International Maize and Wheat Improvement Center (CIMMYT).

By 1976, we had determined that PR-Mo2, PR-Mo2 x MoSQA, and PR-Mo2 x MoSQB were more resistant to second-generation ECB than an intermediately resistant hybrid, Pioneer Brand 3369A. These three populations had been selected for adaptation and, in MoSQA and MoSQB, for increased stalk crushing strength for several years by Dr. M.S. Zuber before we started our program. Our ECB breeding program

(USDA-ARS and University of Missouri) began in 1977 and has continued to the present time. Throughout the program, additional germplasm, principally from exotic sources, has been incorporated as it was identified.

In 1977, we planted 1000+ seeds of each of Zuber's populations and infested all plants with ECB egg masses and selfed about 400 of these plants. From 400 selfed plants in each population, about 200 were harvested and dissected to measure stalk tunneling, and 10% of these with the least amount of tunneling provided seed for genetic recombination in our Puerto Rican winter nursery. Selected ears from Puerto Rico were used for insect selection and selfing in Missouri for the next generation.

After five cycles of selection, Mo-2ECB (PR-Mo2 x MoSQB source) was released in 1983 (Barry and Zuber 1984), and following six cycles of selection, Mo-2ECB-2 (PR-Mo2 source) was released in 1984 (Barry et al. 1985). In order to determine if our modified recurrent selection program was making progress, evaluations were made after three cycles of selection (Tables 1 [includes evaluations for C4, C5, and C6], 2, and 3) (Barry 1989). The selection program was continued through cycle 6 for Mo-2ECB and Mo-2ECB-2, as well as PR-Mo2 x MoSQA (Table 1). Maize breeders had suggested that this would provide further improved populations with a more desirable level of ECB resistance.

We have also screened germplasm from the Regional Maize Disease and Insect Resistance Nurseries that originated from the North Carolina program. One of the early selections for second-generation ECB resistance from these materials was NC 4-275. It came from Dr. M.M. Goodman's collection PAG VI-A, race Moroti Guapi; and had been crossed with Dr. C.W. Stuber's "D-2" tester. This germplasm source has been included in an experimental maize population that we refer to as "Experiment 52." This population was primarily developed from domestic inbreds that demonstrated high yield potential and some resistance to first- and / or second-generation ECB (e.g., B52, SC13, SC13R, SC213R, NC33, Oh43, CI31A, B73, and Mo17).

Since then, we have identified several other resistant cultivars from these regional trials. We are working with 11 (list follows) of these which were crossed to a resistant (first- and second-generation ECB) hybrid, Pioneer Brand 3184, and the crosses were used to develop a composite breeding population that has been improved by using a modified recurrent selection program. Three inbreds, Mo45 (Negro de Tierra Caliente exotic source), Mo46 (Cravo Paulista exotic source), and Mo47 (Candela exotic source), have been released (Barry et al. 1995) as S_6 lines with resistance to first- and second-generation ECB. Evaluations of these inbreds for ECB resistance and yield in testcrosses (as S_3s and S_4s) were made at Columbia and Novelty, MO, during 1992. Results from these evaluations are presented in Table 3.

The 11 sources (race and collection given) currently undergoing selection include:

1. Cuban Tuscon, ECU 542
2. Early Caribbean, MAR 2
3. Nal-Tel A.T.B., GUA III
4. Negro de Tierra Caliente, GUA III
5. Moroti, PR II

Table 1. Mean stalk tunneling (cm) by larvae of second-generation ECB in three maize populations during six cycles of recurrent selection for resistance.

	Population			Control†	
Year / cycle	PR-Mo2	PR-Mo2 x MoSQA	PR-Mo2 x MoSQB	Inter-mediate	Resistant
1977 / C0	22.0	22.0	20.5	—	—
1978 / C1	22.6	25.3	20.3	22.6	15.3
1979 / C2‡	22.5	32.5	21.3	30.1	20.3
1980 / C3	9.9	13.9	11.8	15.4	10.4
1981 / C4	9.4	12.8	9.2	15.0	7.0
1982 / C5	7.3	8.4	6.1	10.6	6.2
1983 / C6	6.7	9.4	8.3	12.5	16.1

† The intermediately resistant control was Pioneer Brand 3369A, except for 1980, when a susceptible single cross (Wf9 x W182E) was used. The resistant control was Pioneer Brand 3184.

‡ Cycles of selection were conducted at two locations, Columbia and Portageville, MO, except in 1979, when drought destroyed the Portageville tests.

Table 2. Stalk tunnel length and least-squares estimates of gain from selection in three maize composite populations (PR-Mo2, PR-Mo2 x MoSQA, and PR-Mo2 x MoSQB).

	Mean stalk tunnel length (cm)†		
Type of meaná	PR-Mo2	PR-Mo2 x MoSQA	PR-Mo2 x MoSQB
C_0	12.9ab	16.4a	11.7b
C_1	12.3bc	13.9ab	10.2bc
C_2	11.4bc	14.4ab	8.6cd
C_3	10.0c	13.5b	7.3d
Resistant control	9.6c	9.6c	9.6bc
Susceptible control	15.2a	15.2ab	15.2a
Gain/cycle±SE	-0.96±0.41	-0.84±0.40	-1.48±0.34
Percent gain cycle, based on predicted value of C_0	7.3	5.3	12.7

† Means followed by the same letter within a column are not significantly different at the 0.05 probability level.

‡ Resistant control = Pioneer Brand 3184, susceptible control = Wf9 x W182E; checks were grown in common plots for all three populations.

6. Chandelle, CUB 54
7. Candela, ECU 344
8. Caingang, PR III
9. Cuban Flint, CUB 65
10. Avanti Moroti Mita, PAG 106
11. Cravo Paulista, SP II

The two composite maize populations and three inbred lines which have been released as ECB resistance sources should soon contribute to resistance in commercial hybrids. The hybrids developed should reduce yield loss caused by ECB and at the same time reduce the need for insecticide applications for control of ECB.

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Table 3. Mean ECB responses and testcross yields for Mo45, Mo46, and Mo47 evaluated at Columbia and Novelty, MO. (This table is from information provided with the original release notice for the three inbreds dated 22 February 1994).

Inbred and level of inbreeding	First-generation ECB rating[†] (1-9)	Second-generation tunneling (cm)	Tester			
			Mo17	MoSCSSS	Oh43	CI31A
			(t/ha)			
Mo45 as S_3	2.4	9.4	7.70	7.52		
Mo45 as S_4					7.86	8.41
Mo45 as S_6	1.9	3.9				
Mo46 as S_3	3.2	8.4	5.77	7.22		
Mo46 as S_4					7.20	8.05
Mo46 as S_6	1.9	2.6				
Mo47 as S_3	3.2	7.4	7.24	7.80		
Mo47 as S_4					6.64	7.77
Mo47 as S_6	1.8	1.8				
Rest. ck. for S_3 (Pioneer 3184)	4.0	4.6	7.64	9.17		
Rest. ck. for S_4 (Pioneer 3184)					9.88	8.73
Rest. ck. for S_6 (CI31A)	1.0	4.6				
Susc. ck. for S_3 (Wf9 x W182E)	6.0	16.5				
LSD 0.05			1.49	1.79	1.23	1.37

[†] The first-generation rating was based on a 1 to 9 scale in which 1 represented resistance and 9 represented susceptibility (Guthrie et al. 1960).

Variability for Resistance to Fall Armyworm in Guadeloupe among Maize Populations Improved for Resistance to Various Insects

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Abstract

Insect pests are one of the main constraints to the development and farming of maize in the Caribbean. INRA-CIRAD breeding efforts for well adapted maize populations with effective levels of resistance should contribute to the improvement of yield and yield stability. Screening of various insect resistant improved materials for resistance to fall armyworm, Spodoptera frugiperda *(J.E. Smith) and for other characters with agronomical interest was undertaken. Multiple resistance has been observed in introduced (MIRT, TZBR) and local (PopG, Spectral) populations. The results show the high level of resistance of MpSWCB4 and ANTIGUA Gpo2, but also their low productivity. Advanced cycles, obtained through a recurrent S_1 selection scheme, of a local improved population (PopG) show an intermediate level of resistance similar to FAWCC's, but are associated with high adaptability. A study of the variability within these populations and transfer of resistance to high yielding populations was initiated. The interest of this variability and its utilization in selection are discussed.*

Introduction

Insects pests are one of the main constraints to the development and farming of maize in the Caribbean. In Guadeloupe, joint breeding efforts of the French National Institute of Agricultural Research and the Center for International Cooperation in Agricultural Research for Development (INRA-CIRAD), France, for well adapted maize populations with effective levels of resistance to leaf-feeding by fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), should contribute to the improvement of yield and yield stability.

Initial breeding operations led to the creation in 1988 of a well-adapted variety, named 'Spectral', with medium susceptibility to insects. However, breeding for resistance to insects requires first an appropriate screening methodology (Mihm 1983) plus an assessment of the available variability (Painter 1951).

Our main objective was to identify and improve regional genotypes with host plant resistance to insects. The first step of the selection process was to introduce various insect resistant improved materials and to screen for FAW resistance under Caribbean conditions. Then, we screened the best adapted resistance sources. Studies of the variability within these populations and the transfer of resistance to high yielding populations were initiated. The results have highlighted the potential of some populations for use in a breeding program.

We report here the results on variability for insect resistance in breeding populations. These populations have shown different levels of adaptation to Caribbean conditions associated with their resistance level. In the future, agronomic characters such as vigor, plant height, ear productivity should be associated with insect resistance in a selection index (Overman 1989; Thome et al. 1994).

Materials and Methods

Since 1989, a wide diversity of germplasm has been screened for reaction to natural or artificial infestation by FAW and CEW, according to the artificial infestation methodology developed by Mihm (1983).

Previous host plant resistance results demonstrated that controlled, uniform, artificial infestations are needed to develop insect resistant germplasm (Williams 1978; Mihm 1989). Since 1993, we have developed, in association with French entomologists, efficient FAW mass rearing and screening methodologies. The mass rearing laboratory is based in France at Le Magneraud INRA Station (7000km from Guadeloupe). We have developed an efficient egg transfer from France, coordinating egg production and artificial infestations.

The first egg productions were used to screen various lines and populations for resistance to FAW. Nowadays, the laboratory produces 4 million eggs per year for the FAW resistance breeding program and for developing biological insecticides. Native strains of FAW are reintroduced into the mass rearing program every six generations in order to preserve insect diversity and vigor. Artificial infestations are made with 25 larvae per plant (5 leaves stage) and resistance evaluation is based on damage rating 14 days after infestation (DAI) using the Davis and Williams (1992) scale (0 to 9).

Plant materials chosen for studies were derived from populations improved by selection efforts in tropical and subtropical areas and introduced to Guadeloupe (Clavel et al. 1993). These included multiple resistance sources, like MIRT, MBR or Antigua populations developed by Mihm (Smith et al. 1989). We also screened more specific resistant germplasm from USDA such as FAWCC with resistance to FAW (Widstrom et al. 1992) and MpSWCB4 with resistance to SWCB (Scott et al. 1981), and TZBR populations improved for resistance to *Sesamia calamistis* (PSB) or to *Eldana sacharina* (ASCB) from IITA (Kling et al. 1994) (Fig. 1).

All these sources have been compared to local materials, such as PopG and pools of Guadeloupean ecotypes (Welcker 1993; Welcker et al. this review), and to INRA improved populations (Spectral, PopA, CR01) (Fig. 1). We have described this germplasm in Guadeloupean environments (particular climatic and soil conditions, under FAW pressure) for resistance parameters and other agronomical characters. The following results on resistance parameters are presented in chronological order, when breeding populations and screening methods were simultaneously enhanced.

Results and Discussion

Formation of FAW resistant composite

In 1989-90 advanced inbred lines from Antigua germplasm selected at CIMMYT for resistance to FAW and resistance to SWCB, plus full sib families of MBR selected for SWCB resistance, were tested (Clavel et al. 1993). Components of these populations were evaluated in 1989 for their *per se* value and, in 1990, for the best families from their S_1 progenies. We have selfed 98 plants within 24 selected families of Antigua-FAW. On the other hand, we have selected less families and plants of populations previously selected for SWCB. Our results have shown that available variability existed between and within these populations. According to relative population levels, more families and individuals from Antigua-FAW were kept in the formation of the

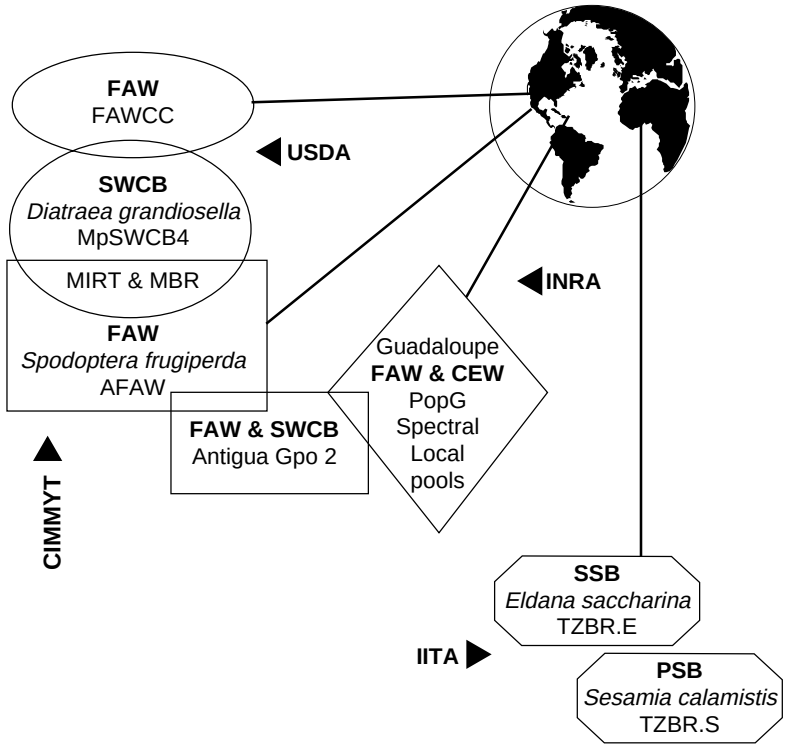

Figure 1. Maize germplasm with resistance to insects screened in Guadeloupe for fall armyworm resistance.

FAW resistant composite 'SPODO' (Fig. 2). The MBR population was not really well adapted to lowland tropics, affecting its resistance performance in our conditions. After two intercrossing generations, the composite 'SPODO' could be an interesting source of resistance to FAW.

Potential interest of MIRT

In 1991, 196 full sib families of MIRT were screened in an international testing trial proposed by John Mihm. Figure 3 illustrates the number of families classified as resistant, intermediate, and susceptible to FAW in Guadeloupe. Ratings were done on a scale of 1 (extremely resistant) to 5 (extremely susceptible). A susceptible check entry rated 3.8 and a resistant check entry rated 2.6. We selected ears from families rating 2.5 or less for utilization as resistant sources. Most families rated either as resistant or intermediate across sites (Smith et al. 1989). In Guadeloupe, the resistant category comprised no more than 10% of the families tested, but represents useful levels of resistance with good potential for adaptation to the area.

FAW resistance levels among various insect resistant populations

A wide range of germplasm was tested in a replicated trial in 1993 under artificial infestation with 25 fall armyworm larvae per plant. Materials included:

- MpSWCB4 from USDA, a population known for its high level of resistance to SWCB and FAW.
- GT populations with resistance to CEW from USDA.
- TZBR-E and TZBR-S populations introduced from IITA and improved respectively for resistance to ASCB and to PSB.
- Different breeding Antigua entries from CIMMYT.
- Main sources of resistance to CEW i.e. Zapalote and Maïa.
- Local germplasm such as native populations, pools of ecotypes and selected varieties (IRAT340 as a susceptible check) (Table 1).

The results underlined the good performance of MpSWCB4 and Antigua, an intermediate position of several populations including other Antigua materials and Guadeloupean materials (Fig. 4). PopG-C1 performed better than its pool of after two intercrossing generations and PopA — a result of selection between progenies of MpSWCB4, ETO and a recombined population of local material — presents an interesting level of insect resistance and lowland tropical adaptation. Populations improved for resistance to *Sesamia calamistis* in Africa perform better than those selected for *Eldana sacharina*. So, TZBR-S should present multiple resistance to PSB and FAW, although TZBR-E3, selected for *Eldana calamistis,* has shown a high level of resistance to CEW (Welcker, this review). Zapalote chico seems to be better than Zapalote grande and similar to our local early population Desirade.

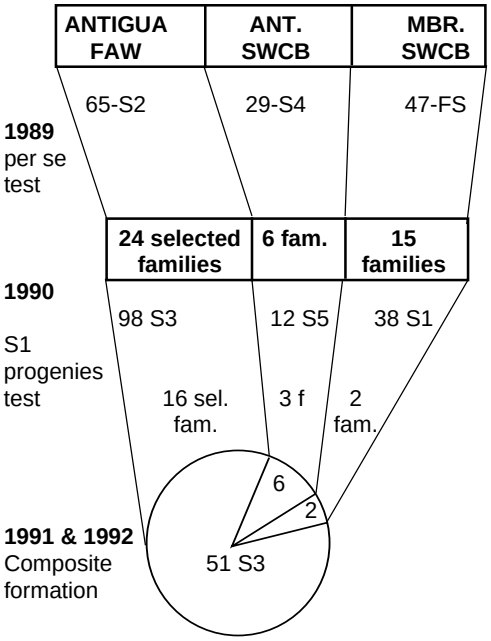

Figure 2. Formation of the fall armyworm resistant population 'composite SPODO'.

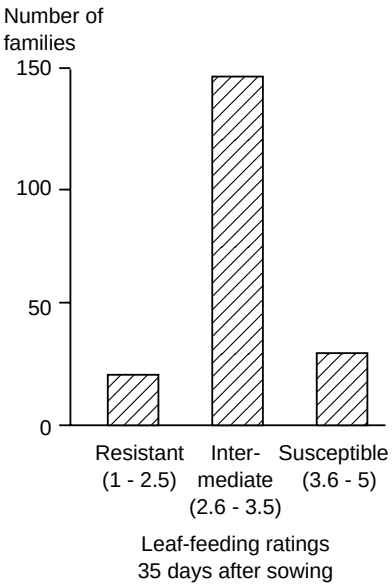

Figure 3. Damage ratings for fall armyworm on 196 full sib families of MIRT in Guadeloupe - 1991.

Table 1. Populations tested for resistance to insects (infested trial with fall armyworm) and adaptation in Guadeloupe in 1993.

Germplasm	Origin	Germplasm	Origin	Germplasm	Origin
1 MpSWCB4	USDA	9 Antigua gpo2	CIMMYT	17 Fond'or	INRA
2 GTRI4	USDA	10 Antigua 2D.118	CIMMYT	18 Desirade	INRA
3 GTRI9	USDA	11 A1-FAW-tux	CIMMYT	19 Pop1/2 P	INRA
4 TZBR-E1	IITA	12 A2-FAW-ntux	CIMMYT	20 Pop T	INRA
5 TZBR-E2	IITA	13 A3-FAWgca	CIMMYT	21 PopG-C1a	INRA
6 TZBR-E3	IITA	14 Zapalote Chico	CIMMYT	22 PopA	INRA
7 TZBR-S1	IITA	15 Zapalote Grande	CIMMYT	23 Spectral	INRA
8 TZBR-S3	IITA	16 Maïa XXIX	CIMMYT	24 IRAT 340	CIRAD

Evidence of genotype-by-environment interaction for FAW resistance

Based on these results, selected populations were tested in 1994 in different environments to determine the stability of their response and respective interest. MpSWCB4 was the resistant check and two high yielding varieties (FWIP136 and PioneerX304C) were used as susceptible checks. Figure 5 shows the variability of response to FAW of these populations in tests in four environments (i.e., different dates of sowing and differing intensities of FAW infestation).

Statistical analysis indicated the presence of significant genotype by environment (GxE) interactions. Extreme differences between resistant and susceptible checks appeared constant (Fig. 5). Response to FAW between other populations varied significantly from site to site, suggesting that the effect of environmental conditions on damage rating is generally high. Hence, it should be integrated as a main factor in selection (Smith et al. 1989; Widstrom et al. 1992). Stable performances of Spectral, selected in a multilocal trial for adaptation to environmental constraints of the area, could sustain this approach.

Genetic variation for FAW resistance within breeding populations

Although information on genetic variation between and within these populations can contribute to choosing an appropriate breeding strategy, plant to plant variation within some populations appears to be important, suggesting that potential variability remains in these populations. Figure 6 illustrates the results for five breeding populations, with observations on one hundred plants per population screened in the most discriminant environment of the latest multilocal experiment.

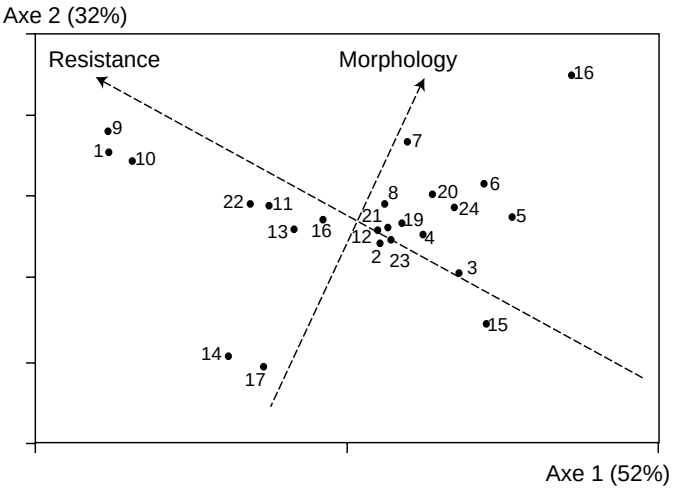

Figure 4. Variation among maize populations in Guadeloupe - 1993.

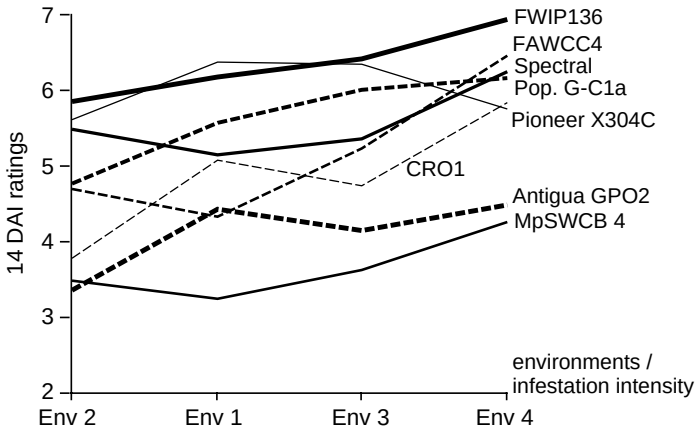

Figure 5. Fall armyworm damage ratings of breeding populations grown in four environments.

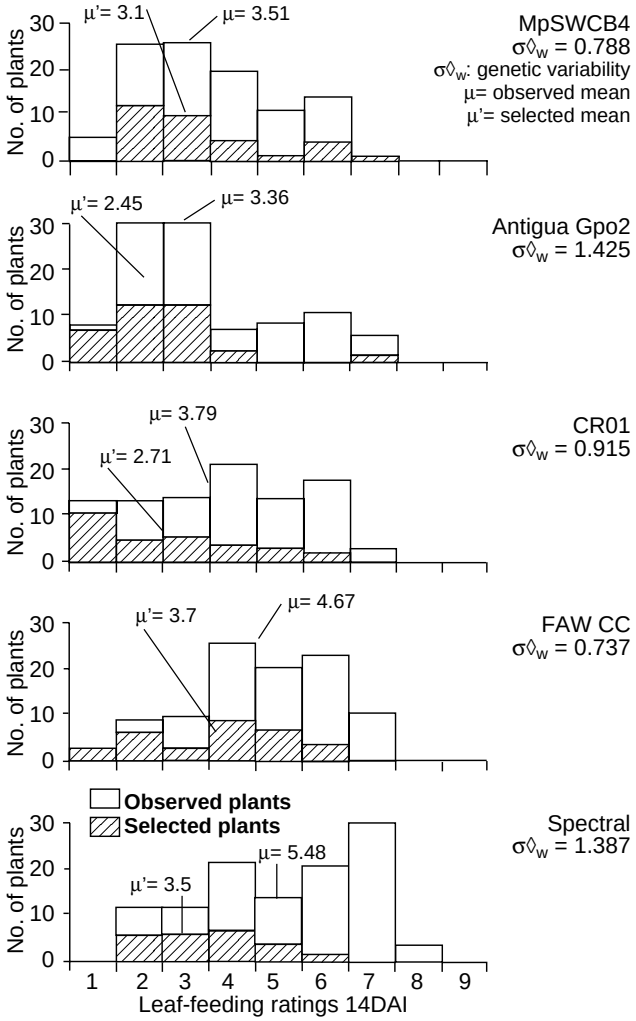

Figure 6. Genotypic variation within breeding populations for feeding damage by fall armyworm.

Compared to the mean value of MpSWCB4, CRO1 and Antigua Gpo2 show a good level of resistance. Nevertheless, there are differences between the damage rating distributions of the populations studied (Fig. 6). Within genetic variation was estimated from residual variance of the hybrid check and residual variance of the model for each population.

The results suggest that there remains sufficient variation within these populations to justify recurrent selection, especially in Antigua gpo2 (as J. Mihm proved), and in Spectral, the breeding population developed by INRA for adaptation to Caribbean conditions (Fig. 6).

The best plants were selfed, and S_1 progeny testing will provide useful information about genetic variability and expected selection response within each population.

Conclusion

The great variability and relatively good response observed in Antigua materials support their potential for use in a selection program and for crossing with other resistant sources and adapted populations to provide significant additive gain. The importance of GxE indicates the effectiveness of testing at more than one location and of enhancing international cooperation. Some attention will be given to agronomic characteristics in the future, while continuing to place the greatest emphasis on developing insect resistant source populations.

Acknowledgment

Thanks to John Mihm for his great and helpful collaboration.

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Maize Germplasm with Resistance to Southwestern Corn Borer and Fall Armyworm

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Abstract

Leaf feeding by the Southwestern corn borer (SWCB), Diatraea grandiosella *Dyar, and the fall armyworm (FAW),* Spodoptera frugiperda *(J.E. Smith), can result in substantial reductions in grain yield of maize,* Zea mays *L. Development and deployment of varieties and hybrids with resistance to these pests can greatly reduce these losses. Scientists working in Mississippi have developed and released nine maize germplasm lines and one population as sources of leaf feeding resistance to these pests. These lines were derived primarily from Antigua Gpo. 2 germplasm originally obtained from the International Maize and Wheat Improvement Center (CIMMYT). In developing the earlier released lines, selection was based entirely on visual ratings of leaf feeding damage; however, larval growth was also considered in the development and release of the newer lines. Analyses of diallel crosses among resistant and susceptible lines indicated that general combining ability was the primary source of variation in the inheritance of resistance to fall armyworm and southwestern corn borer whether resistance was measured as either reduced leaf feeding or reduced larval growth. In 1992, in cooperation with the United States Department of Agriculture, and Agricultural Research Service (USDA–ARS) scientists at Tifton, Georgia, GT–FAWCC(C5) maize germplasm population was released. This population was developed by five cycles of S_1 progeny selection for resistance to leaf feeding by fall armyworm.*

Introduction

Plant resistance is an attractive method of insect control. It provides farmers with a means of preventing or reducing yield losses while avoiding the costs and hazards associated with chemical insecticides. For plant resistance to be a viable alternative to chemical control of insects in maize, *Zea mays* L., sources of resistant germplasm must be identified, and agronomically acceptable hybrids and varieties deployed to farmers.

For almost 30 years, scientists with the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Corn Host Plant Resistance Research Unit at Mississippi State, Mississippi (USA) have conducted research on insect and disease pests of maize. The primary objectives of our research program have been:

- Identification of maize germplasm with resistance to fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), and southwestern corn borer (SWCB), *Diatraea grandiosella* Dyar.
- Development and release of breeding lines and populations to maize breeders with public or private institutions engaged in development of hybrids and varieties.

It is our expectation and desire that these breeders will use the germplasm we release to develop superior hybrids with resistance to FAW and SWCB, thereby ultimately making such hybrids available to farmers.

To successfully identify and develop maize with resistance to insects, a program such as ours must first have 1) a reliable source of insects for infesting plants; 2) techniques for evaluating damage; and 3) a source of resistant germplasm. At the first CIMMYT symposium on insect resistance, Frank Davis described our insect rearing program (Davis 1989) and the methods we use for evaluating germplasm for resistance to FAW and SWCB (Davis and Williams 1989).

When screening maize for resistance to leaf feeding by FAW and SWCB, we use similar procedures for the two insects. Most germplasm is evaluated for reaction to both insects. Although it depends somewhat on availability of seed and heterogeneity of the material to be evaluated, we most frequently evaluate breeding material in one row, 20 plant plots with two or three replications per insect. Plants in the 8–

to 10–leaf stage of growth are infested with 30 larvae / plant; leaf feeding is visually rated 14 days after infestation.

Breeding for Resistance

At the first CIMMYT symposium on insect resistance in maize, the breeding methods that we have used to develop maize germplasm lines with resistance to leaf feeding by FAW and SWCB were described (Williams and Davis 1989). The procedure that we have most frequently followed has been to self–pollinate plants in a source population; evaluate the S_1 progeny rows in replicated experiments; select those genotypes showing the least damage; self–pollinate plants in uninfested nursery rows; and repeat the process for approximately eight generations. At times, the procedures have been varied somewhat: plants in infested rows were self–pollinated, or remnant seed of selected rows was grown in a winter nursery.

Our breeding program has relied heavily on germplasm from CIMMYT. We have released and registered nine highly inbred germplasm lines and one heterogenous population as sources of resistance to leaf feeding by FAW and SWCB (Table 1): Mp496 (Scott and Davis 1981a); Mp701 and Mp702 (Scott et al. 1982); MpSWCB–4 population (Scott and Davis 1981b); Mp703 (Williams and Davis 1980); Mp704 (Williams and Davis 1982); Mp705, Mp706, and Mp707 (Williams and Davis 1984a); and Mp708 (Williams et al. 1990). All of these were derived from germplasm initially obtained from CIMMYT. It is also evident (Table 1) that Antigua Gpo. 1 and 2 and Republica Dominicana Gpo. 1 are the primary sources of this resistance. We have screened germplasm from other sources, but, unfortunately, we haven't found significant resistance in them.

The lines that we have released generally exhibit an intermediate level of resistance in our tests at Mississippi State (Table 2). Mp496, the first line released, frequently falls into the susceptible (7–9) rating category. The SWCB ratings in Table 2 are three–year averages. FAW damage was rather low in 1994, so those data were not combined with the 1992–93 data.

Table 1. Nine germplasms and one population with resistance to southwestern corn borer and fall armyworm developed and released[1] at Mississippi State, MS.

Germplasm	Year of release	Source	Grain color
Mp496	1974	Antigua Gpo. 2	Orange
MpSWCB–1 (Mp701)	1975	Antigua Gpo. 1,2	Yellow
MpSWCB–2 (Mp702)	1975	Antigua Gpo. 2, Republica Dominicana Gpo. 1	Yellow
MpSWCB–4 population	1976	Antigua Gpo. 1,2 Guadelupe Gpo. 1A, Republica Dominicana Gpo. 1A	Yellow–orange
Mp703	1979	Antigua Gpo. 1,2	
Mp704	1982	Mp496, Republica Dominicana Gpo. 1	Pale yellow
Mp705	1984	MpSWCB–4	Yellow
Mp706	1984	MpSWCB–4	Yellow
Mp707	1984	MpSWCB–4	Yellow
Mp708	1988	Mp704, Tx601	Yellow–orange

[1] Seed are available in limited quantities from the Department of Plant and Soil Sciences, Box 9555, Mississippi State, MS 39762 (USA).

We also cooperated with scientists in the USDA–ARS Insect Biology and Populations Management Research Laboratory in a joint release of GT–FAWCC(C5) maize germplasm population in 1992 (Widstrom et al. 1993). This population was developed by five cycles of recurrent S_1 progeny selection at Tifton, GA and Mississippi State, MS for resistance to FAW damage. The original breeding population was created by combining three broadbased breeding populations: a bulk of more than 60 Mexican and Caribbean collections, a bulk of six collections with Antigua background, and a bulk of 100 Brazilian collections.

Inheritance of Resistance

We have conducted only limited studies on the inheritance of leaf feeding resistance to either FAW or SWCB. The resistance is not simply inherited. Although visual ratings of leaf feeding are extremely useful in a breeding program when the primary focus is on eliminating susceptible germplasm as quickly and inexpensively as possible, they are less useful in differentiating among genotypes that vary only slightly in level of resistance. Regrettably, the latter situation is the one we usually find ourselves in when conducting genetic studies.

In an analysis of a diallel cross of nine inbred lines evaluated for FAW damage under natural infestation, both general and specific combining ability were found to be significant sources of variation in the inheritance of resistance to leaf feeding (Williams et al. 1978). In the analysis of a six–parent diallel evaluated for SWCB leaf feeding damage after infestation with 30 neonates per plant, general combining

ability was a significant source of variation, but specific combining ability was not (Williams and Davis 1985).

More recently, we evaluated an eight–parent diallel cross for both FAW and SCWB resistance (Williams et al. 1989). We selected parental inbred lines that had previously exhibited a range of leaf feeding damage by the two insects; however, we evaluated the diallel cross for larval growth and survival rather than using the more subjective leaf feeding ratings. The correlation between number of FAW and SWCB surviving on the different crosses was highly significant (r = 0.74) as was the correlation between larval weights of the two species (r = 0.81). General and specific combining abilities were significant sources of variation for both larval number and larval weight for both insects.

Effectiveness of Resistance

In our quest for maize germplasm with resistance to FAW and SWCB, we have developed several lines with leaf feeding resistance. The germplasm base for these lines is, unfortunately, rather narrow. We would very much like to identify additional sources of resistance. If other sources of resistance do not occur naturally, we may have to rely on genetic engineering approaches to provide them. Also, we have not yet identified germplasm that has resistance to SWCB during the reproductive stage of growth. This could be due to either unsatisfactory evaluation techniques or a lack of germplasm sources with resistance at this stage of growth, or both.

Although high resistance levels have not yet been obtained, the resistant germplasm that we have identified can reduce damage from these insects. In one experiment, leaf–feeding resistant hybrid, Mp496 x Mp701, and a leaf–feeding susceptible hybrid, Ab24E x Mp305, were infested with SWCB larvae while plants were in the whorl stage of growth (Williams and Davis 1984b). When infested with 40 larvae per plant, neither the height nor yield of the resistant hybrid was reduced. The height of the susceptible hybrid was reduced 18%, and yield was reduced 39%.

In Mississippi, FAW damage can be especially heavy on maize that is planted later than normal. Leaf–feeding resistant and susceptible maize hybrids were planted after wheat, *Triticum aestivum* L., was harvested to compare their yields in a double cropping system (Sanford et al. 1988). The maize hybrids were planted about two months later than usual for our area and were subjected to large naturally occurring FAW populations. The leaf feeding resistant hybrids yielded 62% more grain and 53% more silage than the susceptible hybrids.

Table 2. Mean ratings of leaf feeding damage sustained by released lines infested with southwestern corn borer (SWCB) and fall armyworm (FAW) larvae in 1992–1994 at Mississippi State, MS.

	SWCB[1]	FAW[1]	
Line	1992–94	1992–93	1994
Mp496	7.3	7.0	4.3
Mp701	6.0	6.4	2.7
Mp702	6.9	6.5	4.0
Mp703	5.3	6.3	3.3
Mp704	5.4	5.7	1.7
Mp705	6.6	6.3	2.7
Mp706	6.5	6.8	2.7
Mp707	5.8	6.0	3.0
Mp708	6.1	5.7	3.0
Ab24E[2]	7.9	7.8	7.0
Tx601[2]	7.9	8.4	6.0
LSD (0.05)	0.7	0.9	1.6

[1] Damage was visually rated 14 days after infestation with 30 neonates per plant on a scale of 0 (no damage) to 9 (extensive damage).

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Maintenance of, and Requests for, Maize Germplasm Having Resistance to Insect Pests

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Abstract

There are 33,766 maize accessions in the US National Plant Germplasm System (NPGS). Just over 13,000 are in the active collection maintained at the North Central Regional Plant Introduction Station at Ames, IA, USA. Through extensive evaluation, many of these accessions have been identified as containing genes for host-plant resistance to several maize insect pests. This presentation provides a general discussion of how the insect-resistant maize germplasm is regenerated and stored. Accessions from low latitudes present problems for seed regeneration in Iowa because of their photoperiod sensitivity. An increased frequency of requests for insect resistant germplasm usually follows the publication of evaluation results. These requests for seed are documented in the NPGS's Germplasm Resources Information Network (GRIN). Illustrations of the number of requests for insect resistant germplasm are presented. Requests, both foreign and domestic, originate mostly from private companies and public institutions. Researchers are asked at the time of seed request to provide the maize curator with a performance report of their evaluation results.

Introduction

The US National Plant Germplasm System (NPGS) includes a collection of 33,766 maize accessions. These accessions have been collected or donated from 127 countries around the world. When they are incorporated into the NPGS, the NPGS accepts the responsibility of maintaining them. The active, working collection of 13,000+ maize accessions is maintained at the USDA / ARS North Central Regional Plant Introduction Station (NCRPIS) at Ames, IA. Scientists needing maize germplasm for research should direct their requests to the NCRPIS in Ames.

The essence of this talk is a description of how the NCRPIS maize germplasm is stored, regenerated, and tested for germination. In addition, my progress with evaluating the collection for sources of host-plant resistance to insects will be reported. The requests for and the availability of insect resistant maize germplasm for researchers are discussed.

Maintenance of Maize Germplasm

The NCRPIS active maize collection is stored in clear plastic, one gallon (3.78 liter) jars at 4º C and a relative humidity of 25-40% (Clark 1989). The maize curator, Mark Millard, manages the collection. He decides which accessions need regeneration each year based on seed availability and germination percentage. If a particular accession is requested frequently (normal seed requests are for 100 kernels) and the supply of seed on hand is less than 2,500 kernels, then that accession will be regenerated. If routine germination tests indicate that fewer than 85% of the kernels of a particular accession germinate, then it will be regenerated.

Let's first look at germination testing. This test is performed at least every 5 years. Four replications of 50 seeds are placed on wet paper toweling, rolled into a tube, and placed in a germination chamber set at 20º C with 12 hours of darkness and 30º C during 12 hours of light. The number of seeds germinating is counted after 7 days and then again at 10 days. The total percent germination is calculated and entered into the computer records for that accession.

Regeneration of maize in the field is an important function performed routinely at the NCRPIS. When regenerating Corn Belt adapted maize germplasm, 200 plants are planted in blocks of four rows each and pair-crossed by hand. Shoot bags are placed on the developing ears before silks appear to prevent contamination by extraneous pollen. Larger bags are placed over the tassels to collect the

pollen. The pollen is collected from one plant and placed on the silks of another plant. Ideally, plants are used as either a male or a female parent but sometimes a plant may be used for both. This method helps maintain the genetic integrity for each accession.

Long-season, or day length sensitive maize lines, are regenerated in a winter nursery located near Isabela, Puerto Rico. Sometimes original accessions include few kernels and they must be increased in the greenhouse at Ames.

Uses of Maize Germplasm

One of the criticisms that has been directed to the NPGS is that there is not enough information available about its accessions. Chapman (1989) said "Until a collection has been evaluated and something is known about the material it contains, it has little practical value". Many plant scientists will not request maize accessions that are accompanied by little descriptive information. If a plant breeder or other scientist requests germplasm, they probably have a particular need in mind. For example, they may want maize with a certain maturity, or a particular height, or with host-plant resistance to a particular pest. Complete information is not available for all 13,000+ NPGS maize accessions. Much of the passport information (e.g. collection data, seed type, height, etc.) is available, but most accessions have not been evaluated for host-plant resistance to insects and pathogens.

As an entomologist in the NPGS, I evaluate NPGS accessions of maize and other species for new sources of host-plant resistance to insects. There are many domestic federal, state, and private scientists who cooperate with me in evaluating the large number of accessions maintained in the collection. Usually, when I find a new source of host-plant resistance to insects, these scientists will cooperate with me to confirm the resistance in other locations. The NCRPIS also receives requests for maize from scientists who are interested in evaluating the germplasm for new sources of host-plant resistance to insect pests which I am not able to evaluate.

Maize seed is sent to researchers at no cost. We ask that requesters send a progress report detailing results of their experiments. The information received can then be entered into the Germplasm Resources Information Network (GRIN) so that the evaluation data are available to all. Any scientist with a personal computer, a modem, and communication software can access GRIN. Login IDs can be obtained at no cost from the National Germplasm Resources Laboratory (Telephone No. 301-504-6235) in Beltsville, MD, USA.

Previously, I have evaluated maize germplasm for resistance to corn rootworms, *Diabrotica* spp., and black cutworm, *Agrotis ipsilon* (Hufnagel) (Wilson and Peters 1973; Wilson et al. 1983). At present, I evaluate maize for resistance to European corn borer (ECB), *Ostrinia nubilalis* (Hübner), and corn earworm (CEW), *Helicoverpa zea* (Boddie). There are many other important maize insect pests in the United States (e.g., southwestern corn borer, *Diatraea grandiosella* Dyar, corn rootworms, fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), etc.) for which resistant maize would be useful, especially in pest management and sustainable agriculture systems. Unfortunately, I do not have the financial resources to evaluate the NCRPIS collection for all of these important maize pests.

Evaluation of Maize at the NCRPIS for Host-Plant Resistance to Insects

European corn borer (ECB)

Evaluating for whorl leaf-feeding (in the United States, this would be the plant growth stage susceptible to 1st generation attacks) resistance involves a well established technique. Newly hatched ECB larvae (about 300) are placed in the whorl of six maize plants at the V4-V6 stage of development with the "bazooka" applicator (Mihm 1983). Three weeks after infestation, the plants are visually rated using the scale developed by Guthrie et al. (1960). Ratings of 1-3 are categorized as resistant, 4-6 are intermediate, and 7-9 are susceptible. Resistant inbred CI31A and susceptible inbred WF9 are also planted as checks. Using this technique, I can evaluate 700-1,000 accessions per year depending on the availability of land and the number of other projects in progress. Ratings obtained are entered into the GRIN system.

Evaluation for resistance to stalk boring (2nd generation) by the ECB requires a more labor-intensive method. During maize anthesis, newly hatched larvae (about 300) are placed in the leaf axils of 10 plants per accession. The plants are rated for damage 30 days after infesting by cutting them at soil level, splitting them with a band saw, and measuring the length of tunneling. At present, we evaluate about 300 accessions per year.

Corn earworm

Evaluation for silk-feeding resistance to CEW also requires a rather labor intensive technique. We collect fresh

silks (1-3 days old) from field-grown plants, then freeze dry and mill them in the laboratory. The milled silks are added to the standard laboratory diets used to rear CEW. A single, newly hatched, larva is placed into a 30 ml plastic cup containing about 10 ml of test diet. A paper lid is placed on the cup and, after 8 d, the larva is weighed. The test accessions are compared to results obtained from diets prepared with silks from a resistant check, 'Zapalote Chico', and a susceptible check, 'Stowell's Evergreen'. About 200 accessions are evaluated annually.

With so many maize accessions in the NPGS collection and the few that we can evaluate yearly, it is impossible to test them all (except perhaps for leaf-feeding by ECB). At 200-300 accessions per year, it will take from 45 to 65 years to evaluate the whole collection! And to further complicate the problem, the collection is growing at about 5% per year, with most of the accessions from low latitudes and, hence, difficult to manage in the Corn Belt.

The best way to approach the dilemma of too many accessions and too little time and resources is to be more selective in the material we evaluate. One approach is to define an "evaluation subset" that is genetically representative of the whole maize collection. Recently, such a maize subset of about 1,500 entries has been developed. It is heavily weighted with Latin American and North American maize with the intent of containing a maximum diversity of alleles. This evaluation subset can be requested from the NCRPIS in Ames (Telephone No. 515-294-6502).

Another aid for selecting germplasm to evaluate is selecting specific maize kernel types, e.g., popcorn, flour, dent, etc. An evaluation of all the popcorns in the NPGS collection identified several accessions with silk-feeding resistance to CEW and leaf-feeding resistance to ECB (Wilson et al. 1993).

Other criteria for selection might be specific races of maize or maize obtained from specific geographic areas of the world. For example, the 1,600 available NCRPIS accessions from Peru were evaluated for leaf-feeding resistance to ECB. Eleven accessions were found to have a unique leaf-feeding resistance that was not based on the chemical 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) (Abel 1993; Abel et al. 1995).

Requests for Resistant Maize Germplasm

Since 1987, the GRIN system has maintained a request history of germplasm orders. I thought it would be of interest to see how many requests for seed were received after information detaining the resistance to ECB, CEW, and FAW had been published. In 1987, I published a paper listing three PIs (PI 369361, PI 213705, and PI 340856) that had silk-feeding resistance to corn earworm (Wilson 1987). Since then, there have been 10 requests for PI 369361, 13 requests for PI 213705, and 43 requests for PI 340856. PI 340856 is part of a popcorn collection donated in 1960 to the NPGS by the late Dr. J. C. Eldredge, who was an Iowa State University plant breeder. This collection of 35 popcorns was evaluated for resistance to CEW, ECB, and FAW (Wilson et al. 1991). The number of requests for this germplasm since 1991 is listed in Table 1. The accessions that were noted in the publication as being resistant to these insects were requested more often than were the non-resistant accessions, with a few exceptions.

The entire popcorn collection, of 299 accessions, was evaluated for resistance to CEW and ECB between 1983 and 1990 (Wilson et al. 1993). This material has not been requested as much as the material from the earlier publications. For example, PI 245133 and PI 415283, rated as resistant to CEW, have been requested only 3 and 4 times, respectively.

Table 1. Number of requests for J. C. Eldredge collection since 1991.

Entry	No. requests	Resistant to
340835	1	
340836	8	
340837	7	
340839	11	
340840	18	CEW[a]
340841	9	
340842	7	
340843	6	
340844	13	CEW
340845	6	
340846	9	
340847	10	
340850	9	
340851	9	
340853	15	
340854	9	
340855	10	
340856	25	CEW
340857	13	ECB[b]
340858	5	
340859	16	CEW
340860	7	
340861	10	CEW
340862	7	
340863	11	
340865	11	FAW[c]
340866	16	CEW
340867	7	
340868	8	
340869	16	CEW
340870	14	CEW
340871	21	CEW
340872	7	
340873	13	CEW

[a] Corn earworm, *Helicoverpa zea* (Boddie)
[b] European corn borer, *Ostrinia nubilalis* (Hübner)
[c] Fall armyworm, *Spodoptera frugiperda* (J.E. Smith)

Since initiating this evaluation program at the NCRPIS in 1980, I have been evaluating maize for leaf-feeding resistance to ECB (1st generation). The ratings obtained each year were entered into GRIN. The number of accessions in the NCRPIS collection having a resistance rating of 3 or less is 217. Table 2 lists the number of requests for these 217 accessions. More than half (121) have been requested from one to five times. A few accessions were requested more than 30 times. Of course, not all of the germplasm requested was necessarily requested just for the ECB resistance. We are not always aware of the rationale for requesting germplasm from the NCRPIS. The maize may have been requested because it possesses other characteristics of interest.

There has been considerable interest in the Peruvian maize that Craig Abel evaluated for resistance to leaf-feeding by ECB as partial fulfillment of the requirements for his MSc degree (Abel 1993; Abel et al. 1995). I would anticipate that requests for this material will increase because the resistance apparently is not based on the chemical DIMBOA. At present, our resistant Corn Belt maize inbreds that possess resistance to ECB have DIMBOA based resistance. Testing is underway to determine the chemical(s) or other factors responsible for the resistance in the Peruvian maize.

Table 2. Number of requests for 217 maize accessions with European corn borer leaf-feeding resistance.

No. of requests	No. of accessions
1 - 5	121
6 - 10	36
11 - 15	19
16 - 20	12
21 - 25	15
26 - 30	6
31 - 35	4
36 - 40	3
41 - 45	0
46 - 50	1

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Recent Advances in the Development of Sources of Resistance to Pink Stalk Borer and African Sugarcane Borer

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Abstract

The lepidopterous stem borers Sesamia calamistis *Hampson (Noctuidae) and* Eldana saccharina *(Walker) (Pyralidae) are among the most important insect pests of maize in West Africa. Efforts to breed for resistance to these two borer species are an integral part of a project to develop control practices for maize pests at IITA. Since 1985, a wide diversity of maize germplasm has been evaluated for resistance to either* S. calamistis *or* E. saccharina. *Three populations with moderate resistance to* E. saccharina *(TZBR Eldana 1, 2, and 3) and two with moderate resistance to* S. calamistis *(TZBR Sesamia 1 and 3) were formed in the late 1980's and are being improved for adaptation and resistance levels primarily through* S_1 *family testing. The populations are intended as sources of resistance to be used by African national breeding programs, as well as by colleagues in other parts of the world. TZBR Eldana 3 was developed from elite, adapted populations and has performed well in multilocational yield trials in Nigeria and Cote d' Ivoire. TZBR Eldana 1 was derived from exotic germplasm and is less adapted to the lowland humid tropics. A selection index which combines agronomic characteristics and* E. saccharina *resistance, is used to improve the TZBR Eldana populations. Cycles of selection trials with these populations have shown continual progress in selecting for resistance to* E. saccharina. *Of the two Sesamia populations, TZBR Sesamia 3 appears to have higher levels of resistance than TZBR Sesamia 1. Future selection will be based on improved agronomic characteristics and disease resistance levels, concurrent with higher levels of resistance to* S. calamistis.

Introduction

Lepidopterous stem borers are among the most damaging insect pests of maize in Africa (Appert 1970). Four borer species are known to cause significant yield loss: the maize stalk borer, *Busseola fusca* Fuller (Noctuidae); the pink stalk borer, *Sesamia calamistis* Hampson (Noctuidae); the African sugar cane borer, *Eldana saccharina* Walker (Pyralidae), and the spotted stalk borer, *Chilo partellus* Swinhoe (Pyralidae) (Bowden 1954; Harris 1962; Appert 1970; Brenière 1971). The first three are African, and are present in most countries of sub-Saharan Africa, while *C. partellus* originated in Asia and was accidentally introduced to eastern Africa approximately 60 years ago. In West Africa, *E. saccharina* and *S. calamistis* are among the most damaging and widespread stem borer species of maize (Bosque-Pérez and Mareck 1990a; Shanower et al. 1991; Gounou et al. 1994).

Maize (*Zea mays* L.) is an exotic crop introduced to Africa in the 16th century by the Portuguese, from its native homeland in the Americas (Miracle 1966). The most important insect pests of maize in the field are indigenous to Africa and their natural hosts are indigenous grasses and sedges. Attempts to control indigenous insect pests must take into consideration the close relationship between their ecology and that of the native grasses (Bowden 1976; Shanower et al. 1993). Due to the complexity of these interactions, long-term control of stem borers can only be achieved through integration of various control practices such as biological and cultural methods, as well as host plant resistance. Breeding for resistance to stem borers at the International Institute of Tropical Agriculture (IITA), is part of a strategy to develop integrated control of maize pests.

Biology and Distribution of *S. calamistis*

S. calamistis is present in most countries of sub-Saharan Africa, Madagascar and the Comores. The host range of this pest is reported to be limited to the family Gramineae and includes cultivated crops such as maize, sorghum (*Sorghum bicolor* (L.) Moench) and millet (*Pennisetum americanum* (L.) K. Schum.), as well as wild grasses like *P. purpureum* Schum., *Panicum maximum* Jacq. and *Setaria* sp. (Harris 1962).

S. calamistis females lay their eggs between the leaf sheaths of the host plant. Under field conditions, eggs hatch in 5 to 6 days and most larvae penetrate the stem shortly after egg hatch. Larval feeding might result in the destruction of the growing point, typically referred to as "deadheart". At later stages, the tunneling and girdling activities of the larvae often result in stalk breakage. In the field, larval development takes 4 to 6 weeks and most larvae pupate within the stem or cobs. *S. calamistis* breeds throughout the year and has no resting stage (Harris 1962). However, densities of this pest are low during the dry season when its hosts are restricted to mature grasses and maize growing in hydromorphic soils.

Biology and Distribution of *E. saccharina*

E. saccharina was first described from Sierra Leone and has been known as a pest of graminaceous crops in West Africa for more than a century (Appert 1970). It probably occurs in all suitable areas of sub-Saharan Africa from approximately latitude 15° N to 30° S (Girling 1978). The most important hosts of this borer in West Africa are crop plants such as maize, sugarcane (*Saccharum officinarum* L), sorghum and millet. However, the original hosts of *E. saccharina* are sedges (*Cyperus* spp.) (Atkinson 1980).

Infestations of maize plants by *E. saccharina* usually start at anthesis (Carter 1985). Females lay eggs on debris on the soil (Atkinson 1980) or on the hairy margins of maize leaf sheaths (Cochereau 1985). Under field conditions, eggs hatch in 5 to 6 days and, after feeding on the leaf sheaths for a few days, larvae enter the stem where they continue to feed. Larvae may eventually move into the ears and feed on the grain. Pupation occurs inside the stem and the pupa is covered by a cocoon made of silk and plant debris. Adults emerge 7 to 14 days after pupation.

Although infestations by this stem borer occur relatively late in the development of the maize plants, damage as a result of their feeding can be severe, with yield losses of up to 20% (Bosque-Pérez and Mareck 1991). Damage caused by *E. saccharina* provides access into the stem and cobs for pathogens which cause rots. Infestations by this borer are associated with high levels of stalk lodging due to tunneling and the effect of stalk rots.

Formation of Stem Borer Resistant Populations

Since 1985 a wide diversity of germplasm has been screened at IITA for reaction to either *S. calamistis* or *E. saccharina*. This includes the BR (borer resistant) population of IITA (developed by screening for *S. calamistis* under natural infestations), and a wide range of germplasm from North and South America which has shown resistance to other species of stem borers (Mihm et al. 1988), including CIMMYT's MBR (multiple borer resistant) and MIRT (multiple borer resistant tropical) populations and a portion of the MIR (maize inbred resistant) lines from Hawaii. Sources of resistance to *S. calamistis* or *E. saccharina* were found among some of these germplasm groups.

Three TZBR (Tropical Zea Borer Resistant) populations with moderate resistance to *S. calamistis* were formed between 1987 and 1988 (Table 1). TZBR-Sesamia 1 was formed by recombining six introduced tropical inbred lines that had shown resistance to *S. calamistis* in our screening trials. TZBR Sesamia 2

Table 1. Genetic background of stem borer resistant populations[a]

Population	Genetic background
TZBR Eldana 1	14 test crosses[b] with hybrid 8338-1
TZBR Eldana 2	TZi 2, 10, 12, 15 and ICAL 27
TZBR Eldana 3	S_1 lines from DMR-LSRW (33 lines), La Posta (15 lines) and TZSR-W-1 (28 lines)
TZBR Sesamia 1	CM 116, INV 575, Cateto Grande Mil, Cateto Assis Brazil RGS x IV, Costeño Mag. 350 and Cubano Cateto Ecuador 339 crossed to TZi 4
TZBR Sesamia 3	29 lines, mostly from the CIMMYT MBR population, crossed to TZi 4.

[a] TZBR Eldana 3 has white grain; all others are of mixed grain color; all populations are late maturing (115-120 days).

[b] Fourteen entries used for test crosses: MP496 x VG-ECB-24X, MP702 x ECB PI 3, $PRMO_2$ x PRMOSQB 87-4-1, $PRMO_2$ (S_1) C6 88-3, $PRMO_2$ (S_1) C6 88-12, Pool 24 x (MP496 X MP706), $PRMO_2$ (S_1) C6 752X-2, $PRMO_2$ (S_1) C6 x (MP496 X MP701), $PRMO_2$ (S_1) C6 752-1, 100-5 x 44-6 (2), $PRMO_2$ (S_1) C6 752X-4, MP701, MP68, and MP704.

was formed after recombination of five IITA-developed inbred lines which showed some resistance to this pest. This population was eventually discontinued as it did not show adequate levels of resistance in subsequent trials. TZBR-Sesamia 3 was created by recombining 29 S_1 lines, derived mostly from the CIMMYT MBR population, crossed to the IITA inbred TZi 4.

Screening for resistance to *E. saccharina* has received major emphasis. After intensive screening from 1985 to 1987, three populations with moderate resistance to *E. saccharina* were formed between 1988 and 1989 (Table 1). In 1985, 102 accessions, most introduced from CIMMYT, were screened for resistance as test crosses with the hybrid 8338-1; superior materials were selected and backcrossed to their original introduction. TZBR Eldana 1 was formed from the best 14 of these backcrosses. Additionally, inbred lines developed at IITA were screened for resistance and the best five recombined to form the population TZBR Eldana 2. Tropically-adapted, early, intermediate and late-maturing open-pollinated populations were also screened for resistance in 1988-89 (Table 2). S_1 lines from the three most resistant late populations (La Posta, DMR-LSRW and TZSR-W-1) were screened and superior lines were selected and recombined to form the TZBR Eldana 3 population.

Improvement of Screening and Selection Methods

Sesamia calamistis

The development of screening methods and the selection of *Sesamia* resistant materials was enhanced by the identification of resistant (TZi 4) and susceptible (TZi 19 or TZi 25) inbred line checks (Mareck et al. 1989). To screen for resistance to *S. calamistis*, plants are infested with 25-30 eggs (black-head stage) obtained from a laboratory colony. Eggs are placed between the leaf sheaths at the base of the plant. For trials conducted in the screenhouse, plants are infested 3 weeks after planting, for those in the field, infestation takes place 2 weeks after planting. Damage ratings are taken 2 and 6 weeks after infestation using a 1-9 rating scale (Bosque-Pérez et al. 1989).

Resistance levels in the TZBR Sesamia populations are improved primarily through S_1 family testing. Plant vigor influences the plants' reaction to attack by *S. calamistis*. The possibility that differences in inbreeding depression among S_1 families could make it difficult to detect resistance that would be expressed in a non-inbred background was of concern. Experiments were thus conducted to simultaneously compare the resistance performance of S_1 families from TZBR Sesamia 1 Cycle 1, with test crosses derived from the same families (Kling and Bosque-Pérez 1995). There was no difference in damage ratings between 176 S_1 families and their test crosses, most likely because a highly susceptible inbred was used as the tester, in order to maximize expression of resistance among the test crosses. Highly significant differences in resistance levels were found among families, but the family x type (S_1 or test crosses) interaction was not significant. Analysis within types showed that genetic differences were significant among the S_1 families but not among the test crosses, implying more replication would be required to make comparable progress from selection based on evaluation of test crosses (Kling and Bosque-Pérez 1995). These results suggest that S_1 family selection for *S. calamistis* resistance will be more effective than selection using test crosses.

Table 2. Performance of elite, late and intermediate maize varieties under *E. saccharina* infestation, Ibadan, Nigeria, 1989.

Entry	Ear damage[a]	Frass rating[b]	Penetrometer reading[c]
8329-15	2.28	2.33	9.54
La Posta C8	2.39	1.67	11.87
DMR-LSRW	2.61	2.17	9.27
LB 8227	2.67	2.17	8.87
IK 83 TZSR-W-1	2.89	1.33	11.30
Ferke 8223	3.06	2.00	6.31
PR 8326	3.17	2.33	6.42
8338-1	3.28	1.33	11.72
EV 8725-SR	3.33	1.50	8.48
PR 8536	3.34	2.33	8.22
LB 8232	3.39	2.67	7.49
ACR 8224	3.86	2.00	8.38
LSD 5%	–	0.66	2.36
Prob. of F	0.139	0.001	<0.001
CV %	30.3	28.9	22.7

[a] 1-5 rating scale to assess percentage of grain consumed or damaged by the borer (1= 0-5; 2 = 6-25; 3 = 26-50; 4 = 51-75 and 5 = 76-100%).
[b] Amount of frass in the leaf axils where: 1= very little frass; 5 = abundant frass.
[c] Rind puncture determined as the force in kilograms required to penetrate the second internode above the ground. Readings taken at flowering; larger values indicate that greater force was required to penetrate the stem.

Eldana saccharina

To increase the number of breeding materials that can be screened for resistance to *E. saccharina*, an augmented natural field infestation method was developed (Bosque-Pérez and Mareck 1990b). Strips of a borer susceptible maize variety are planted one month prior to planting test materials to serve as spreader rows. Test materials are planted perpendicular to the strips using 3 m rows and 1 m alleys. Plants of the spreader rows are infested at silking with *E. saccharina* egg masses (65-75 eggs per plant) obtained from a laboratory colony. Adults which emerge from the spreader rows move to the test plants resulting in a 'natural' infestation. Test materials are checked regularly to ensure a uniform level of infestation has been achieved.

Improvement and Testing of TZBR Populations

Borer resistant populations are being improved for adaptation and resistance levels primarily through S_1 family testing. Mass selection for resistance to maize streak virus (MSV) and other diseases is carried out when individual plants are selfed to make new S_1 families. With the exception of TZBR Eldana 3, the TZBR populations that we have developed are intended as sources of resistance to be used by national breeding programs in Africa and collaborators elsewhere, rather than as final products. Selection for local adaptation will be required to fit the particular complex of biotic and abiotic stresses in any given location. The populations have been made available to collaborators in various countries including Cameroon, Ghana, Mali, Senegal, Zaire, Kenya (ICIPE), Uganda, and Guadeloupe. Feedback from our collaborators is used in further improvement of the populations.

TZBR Sesamia

In addition to the evaluation of 176 S_1 families and their test crosses from the TZBR Sesamia 1 population, 26 superior families were selected in 1993 for recombination to form the next cycle of selection. Recombination took place in 1994 and new S_1 families will be evaluated in the near future. Although we believe S_1 family selection for *S. calamistis* resistance will be more effective than selection using test crosses, one cycle of selection will be carried out separately for both types of families to determine actual progress that can be obtained from the two selection methods.

Evaluation of Cycle 0 of the TZBR Sesamia 3 population was conducted between 1991–92 by screening 204 S_1 families under artificial infestation in the screenhouse. Twenty families had better resistance ratings than the resistant check, TZi 4 (Fig. 1), and were selected for recombination to form the next cycle of selection. This population appears to have the greatest borer resistance of the two TZBR Sesamia populations presently under improvement. However, it is relatively susceptible to lowland rust, *Puccinia polysora*, probably due to its temperate background. Thus, more emphasis will be placed in the future to improving disease resistance as well as agronomic characteristics, while continuing to select for higher levels of resistance to *S. calamistis.*

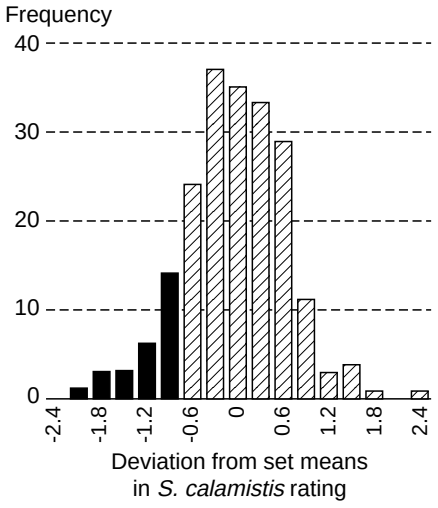

Figure 1. Distribution of deviations from set means in *Sesamia calamistis* resistance ratings of 204 S_1 families from TZBR Sesamia 3 C0. Overall mean = 2.8; susceptible check = 2.12; resistant check = -0.91. Probability of F = 0.002; LSD (5%) = 0.27.

TZBR Eldana

In screening for resistance to *E saccharina*, the following assessments are made: percentage of plants with broken stalks, plant aspect (plant and ear height, uniformity), ear aspect (size, uniformity), quality of husk cover and disease resistance (rust, blight, MSV), using 1-5 rating scales. Ear damage is assessed using a 1-5 scale that estimates the percentage of grain consumed or damaged by the borer (1= 0-5; 2 = 6-25; 3 = 26-50; 4 = 51-75 and 5 = 76-100%). Measurements on agronomic characteristics (days to silk, grain yield) are also taken. A selection index which takes into consideration agronomic characteristics and *E. saccharina* resistance is used to improve the TZBR Eldana populations (Kling and Bosque-Pérez 1995). The relative weights assigned to agronomic characteristics and *E. saccharina* resistance vary depending on the population and severity of infestation in a particular year.

To evaluate the progress achieved in selecting for resistance to *E. saccharina*, cycles of selection trials are periodically conducted. In 1991, Cycles 0 to 4 of TZBR-Eldana 1 and Cycles 0 to 2 of TZBR Eldana 2, along with a

susceptible check, were evaluated under artificial infestation. Ear damage ratings in later cycles were significantly lower ($P<0.05$) than on early ones, showing increased levels of resistance in these populations (Table 3). Results also showed that time to maturity increased in TZBR Eldana 1. The use of a selection index should prevent further inadvertent increases in maturity in the future.

Table 3. Ear damage ratings for TZBR Eldana cycles of selection, Ibadan, 1991.

Entry	Ear damage rating[a]
TZBR Eldana 1	
Cycle 0	2.3
Cycle 1	2.1
Cycle 2	1.8
Cycle 3	1.6
Cycle 4	1.3
TZBR Eldana 2	
Cycle 0	1.7
Cycle 1	1.7
Cycle 2	1.3
Susceptible check	2.1
Prob. of F	0.004
LSD 5%	0.54
CV %	27.2

[a] 1-5 rating scale to assess percentage of grain consumed or damaged by the borer (1= 0-5; 2 = 6-25; 3 = 26-50; 4 = 51-75 and 5 = 76-100%).

Table 4. Plant aspect ratings for TZBR Eldana cycles of selection, Ikenne, 1994.

Entry	Plant aspect rating[a]
TZBR Eldana 1	
Cycle 0	3.75
Cycle 1	4.00
Cycle 2	3.50
Cycle 3	3.67
Cycle 4	3.50
Cycle 5	3.17
TZBR Eldana 3	
Cycle 1	2.67
Cycle 2	2.42
Cycle 3	2.50
8338-1	3.25
Prob. of F	0.001
LSD 5%	0.38
CV %	12.42

[a] 1-5 rating scale, 1= good, 5 = poor.

New cycles of selection were evaluated during 1994. Ratings for plant aspect 3 months after planting showed that significant progress has been made in improving this character in TZBR Eldana 1, especially in the last cycle (Table 4). The use of a selection index which heavily weights agronomic characteristics has assisted us in ensuring that agronomic improvement is also made. The population TZBR Eldana 3 was developed from elite, adapted varieties, and plant aspect has always been superior in this population (Table 4).

Since TZBR Eldana 3 is adapted to the region, it may be more immediately transferred to NARS. Cycle 2 of this population performed well in multilocational yield trials in Nigeria and Cote d' Ivoire in 1993 (Table 5). It was included in IITA's International Variety Trials for the first time in 1994. TZBR Eldana 1 was derived from exotic germplasm and is less adapted to the lowland tropics. Because this population is intended for use as a source of *E. saccharina* resistance by national breeding programs, agronomic characteristics are given less weight in the selection index.

Mechanisms of Resistance

Studies on mechanisms of resistance to stem borers have been directed mainly towards *E. saccharina*, as more progress has been made in selecting for resistance to this pest. Recently tests have been initiated on *S. calamistis.*

Elevated plant silica content has been reported as a mechanism of resistance to various cereal stem borer species. This may be due to the important role of silica in strengthening plant cell walls (Painter 1951). For example, high larval mortality of *C. supressalis* Walker (Pyralidae) has been detected on rice varieties with high silica content (Djamin and Pathak 1967). In maize, resistance to the second generation European corn borer (ECB) (*Ostrinia nubilalis* Hübner) (Lepidoptera: Pyralidae) has been found to be significantly correlated with the silica content in the sheath and collar tissue (Rojanaridpiched et al. 1984).

To determine if increased levels of resistance in the TZBR Eldana populations are related to levels of silica, analysis of stem silica content was carried out for the various cycles of selection. Plant stem samples (three plants per plot, six replicates) were taken shortly after anthesis and oven

Table 5. Across site[a] performance of selected entries from the preliminary late variety trial, 1993.

Entry	Yield (t/ha)	Ear rot rating[b]	Husk cover rating[c]
8321-18	5.6	2.5	2.9
TZL Comp. 3 C1	5.1	2.5	2.9
TZL Comp. 4 C0	5.0	2.2	2.6
TZBR Eldana 3 C2	4.8	2.7	3.1
Acr 9022-SR	4.6	2.3	3.0
Acr 90 DMR-LSRW	4.4	2.9	2.1
Suwan 1-SR	4.1	3.1	3.1
Acr 9028-DMRSR	4.1	3.0	3.1

[a] Trials conducted in Ikenne, Mokwa, and Samaru, Nigeria and Sinemantiale, Cote d' Ivoire.
[b] Means for Ikenne only; 1-5 rating scale, 1= resistant, 5 = susceptible.
[c] Means for Mokwa only; 1-5 rating scale, 1= very good, 5 = poor.

dried at 65°C for 4 days. Stem pieces were then ground and silica content determined using an atomic absorption spectrophotometer after extraction with an acid mixture, using the method described by Novosamsky et al. (1984). No significant differences in stem silica content were detected among the cycles of selection (Table 6), suggesting that other mechanisms of resistance are probably involved.

Stalk strength has also been reported as a mechanism of resistance to stem borers. In our trials, stalk rind puncture is measured using a hand-held penetrometer with a spring resistance plunger (Thompson 1972) (Supplier: Cert Instrument Corporation, Oceanside, NY). Rind puncture is determined as the force in kilograms required to penetrate the second internode above the ground (Twumasi-Afriyie and Hunter 1982). Readings are taken at flowering; larger values indicate that greater force is required to penetrate the stem. Penetrometer readings were taken on a cycles of selection trial in 1994 and results showed that significant progress has been made in increasing stalk strength in the TZBR Eldana 1 population (Table 7). In contrast, no progress was observed in the TZBR Eldana 2 population (Table 8). This is consistent with the notion that greater genetic variability, and thus potential for progress in selection, exists in the former population.

Results of similar tests on cycles of selection of the TZBR Eldana 3 population were erratic. Stalk strength (as measured by penetrometer readings) increased significantly from Cycle 1 to 2, but no progress was made in the next cycle of selection (Table 8). Additional tests are required to clarify these findings.

Significant differences in penetrometer readings had earlier been detected in a trial to evaluate the performance of tropically adapted intermediate and late maize populations under *E. saccharina* infestations (Table 2). In this trial, the ability of the insect to feed and survive in the stem was indirectly measured by taking ratings of the amount of frass in the leaf axils using a 1 to 5 rating scale (1 = very little frass, 5 = abundant frass). A significant correlation between the penetrometer reading and frass rating ($r = -0.66$, $p<0.05$) was detected. Extent of ear damage was also recorded. While there was an indication of a possible relationship between ear damage and penetrometer reading ($r = -0.40$, ns), the estimate of the correlation between frass and ear damage rating was close to zero. This suggests that different mechanisms may be involved in determining *E. saccharina* resistance in the stalks and ears.

Table 6. Percentage silica content in stalks of stem borer resistant populations and selected checks, Ibadan, Nigeria, 1990.

Maize entry	Silica content (%)[a]
TZBR Eldana 1	
Cycle 0	0.53
Cycle 1	0.61
Cycle 2	0.47
Cycle 3	0.54
TZBR Eldana 2	
Cycle 0	0.43
Cycle 1	0.48
Susceptible synthetic	0.53
8338-1	0.51
8329-15	0.48
LSD 5%	0.117
Prob. of F	0.136
CV %	19.77

[a] Means of three plants per replication per treatment, 6 replications.

Table 7. Stalk penetrometer readings[a] on cycles of selection of the stem borer resistant population TZBR Eldana 1 and selected checks, Ibadan, Nigeria, 1994.

Maize entry	Penetrometer reading[b]
TZBR Eldana 1	
Cycle 0	6.64
Cycle 1	6.94
Cycle 2	6.93
Cycle 3	7.30
Cycle 4	8.92
Cycle 5	8.54
Susceptible synthetic	7.01
8338-1	7.21
8329-15	8.30
LSD 5%	1.467
Prob. of F	0.001
CV %	18.83

[a] Rind puncture determined as the force in kilograms required to penetrate the second internode above the ground. Readings taken at flowering; larger values indicate that greater force was required to penetrate the stem.

[b] Means of five plants per replication per treatment, 6 replications.

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Table 8. Stalk penetrometer readings[a] on cycles of selection of stem borer resistant populations and selected checks, Ibadan, Nigeria, 1994.

Maize entry	Penetrometer reading[b]
TZBR Eldana 2	
Cycle 0	9.24
Cycle 1	8.81
Cycle 2	7.47
Cycle 3	8.34
TZBR Eldana 3	
Cycle 1	8.71
Cycle 2	10.22
Cycle 3	9.02
Susceptible synthetic	7.01
8338-1	7.21
8329-15	8.30
LSD 5%	1.467
Prob. of F	0.001
CV %	18.83

[a] Rind puncture determined as the force in kilograms required to penetrate the second internode above the ground. Readings taken at flowering; larger values indicate that greater force was required to penetrate the stem.

[b] Means of five plants per replication per treatment, 6 replications.

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The Importance of Institutional Linkages for the Development of Multiple Borer Resistant Maize Hybrids

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Abstract

Stalk-boring and leaf feeding lepidoptera are major pests of maize worldwide. Improvement of plant resistance to these pests is an objective of public maize research groups at international, federal, and state institutions. These institutions have played important and unique roles in the development of insect rearing techniques, efficient methods for infesting and evaluating germplasm for resistance, screening germplasm to identify sources of resistance, and the release of resistant germplasm to the public. Commercial seed companies have become the primary institution for developing new lines and hybrids in the USA and Europe. Private seed companies, both international and domestic, are also becoming important seed suppliers for the rest of the world. For these reasons, the introgression of insect resistant sources into elite germplasm has required the transfer of knowledge and resistant sources from public to private institutions. The development of multiple borer resistant hybrids illustrates the value of good institutional linkages in the improvement of maize.

Introduction

Maize, *Zea mays*, L., ranks third in world production among the major food grains. The genetic improvement and protection of this crop is of national and international importance and the reliability of grain production globally is of concern to both exporting and importing countries. Public and private maize research institutions are established in many maize producing countries and have as their mandate the agronomic and / or genetic improvement of the crop for yield and control of maize pests.

Stalk-boring and leaf feeding lepidoptera are major maize pests in essentially all maize growing regions of the world. Development of plant resistance to these pests is an objective of maize research groups at CIMMYT, state and federal agencies in many countries, and various universities and colleges in the USA and internationally. These institutions have played and should continue to play important and unique roles in the development of insect rearing techniques, methods for infesting and evaluating material, training of personnel, screening of germplasm for pest resistance, and preserving maize germplasm and related species.

With the advent of hybrid maize, commercial seed companies evolved in the USA, Western Europe, Africa, and South America and have become the primary institutions for the development of new lines and hybrids. Private seed companies are also becoming important seed suppliers for the rest of the world. The introgression of insect resistance for the major maize pests is an objective of many seed companies and success in this venture has required the transfer of knowledge between the public (international, federal, and state agencies) and the private (seed companies) institutions.

Historical Perspective of the Multiple Borer Resistance Program at Dekalb Genetics Corporation

The evolution of the multiple borer resistance (MBR) program at DEKALB Genetics Corp. provides a historical perspective of the importance of institutional linkages and the unique contributions that international, federal, state, and private agencies make in the improvement of maize. DEKALB is an international seed company with that supplies improved maize hybrids for both US and international markets. In support of our domestic objectives, we have

established research stations in 16 states. The international markets are supported by research programs in 10 foreign countries.

Stalk-boring and leaf feeding lepidoptera are major pests of the maize plant and plant resistance is viewed as an opportunity to add value to those hybrids we market. Thus many of our breeding locations have selection for pest resistance as an objective. The European corn borer (ECB) *Ostrinia nubilalis* (Hübner), is the pest with the broadest distribution over our domestic, Canadian, and European markets and has received the most attention. However, many other species are of regional or international importance.

Early on investigators had observed genetic resistance to the ECB and USDA / ARS and university research groups were active in developing ECB mass rearing and screening techniques. Through the efforts of Dr. W.D. ("Bud") Guthrie and his staff at Ankeny, Iowa (USDA / ARS), this knowledge had been transferred to seed companies in the private seed industry and by the 1970s research was underway to commercialize resistance to ECB.

Since the late 1960s corn hybrids marketed in the southwest encountered the southwestern cornborer (SWCB) *Diatraea grandiosella* Dyar, which had moved in from Mexico up through Texas to southern Kansas and east as far as middle Tennessee. This pest was devastating to corn production, with losses coming from both physiological yield reduction and increased harvest losses from the insect's girdling habit.

The seed industry benefited from research begun in the mid-1960s by the USDA / ARS team at Mississippi State. By the mid-1970s the SWCB rearing methods developed by Dr. Frank Davis were being used by seed companies. DEKALB responded by establishing a facility at Union City, TN, in 1976, to work on SWCB and other insect problems for the southern USA.

By 1977 DEKALB had implemented Davis' SWCB rearing techniques and by 1979 had converted to using CIMMYT's bazooka device for infesting plants with 1st instar larvae. The development of the bazooka greatly increased the efficiency in infesting plants and allowed DEKALB to redesign its insect rearing lab to reduce labor and rearing costs.

From 1977 to 1980 DEKALB conducted extensive evaluations of maize germplasm and related species for resistance to SWCB (Table 1). For these evaluations the leaf feeding rating system to identify whorl stage resistance was used and no resistance was found in elite cornbelt lines, old open-pollinated varieties, Indian maize, cornbelt composites, southern US composites, teosinte, or tropical populations. Resistance was observed in *Tripsacum*, but not in tripsacoid maize. The absence of SWCB resistance in ECB resistant and high DIMBOA lines seemed to confirm Painter's axiom that resistance is species specific and not for an entire group of species such as the leaf feeding lepidoptera.

During this period SWCB resistance was observed in germplasm from the Davis, Williams, and Scott program (USDA / ARS). From 1974 to 1984 this program released one SWCB resistant population and eight resistant lines. This germplasm was subsequently shown not to be species specific. In 1974 Davis and Scott observed fall armyworm (FAW) *Spodoptera frugiperda*

Table 1. Germplasm evaluated for SWCB resistance

Germplasm	Source
Elite Inbreds	DEKALB Genetics Corporation
International Inbreds	"
Cornbelt Composites	"
ECB Resistant Lines	"
Southern Composites	"
Popcorn	"
Old Open-pollinated Varieties	"
Sweet Maize Collections	U.S. Plant Intro. Sta., Ames, Iowa
South American Maize Collection	"
Indian Maize Collection	"
South American Maize Collection	"
African Collection	"
Asian Collection	"
Popcorn Collection	"
European Collection	"
U.S. Varietal Collection	"
Trypsacum	"
Trypsacum Collection	D.H. Timothy, N.C. State Univ.
Trypsacoid Maize	J. Harlan, Univ. of Illinois
Trypsacoid Maize	V.E. Gracen, Cornell Univ.
Teosinte x Maize Crosses	"
CIMMYT x Temperate Maize	"
Teosinte	G.W. Beadle, Univ. of Chicago
SWCB Populations	Davis, Williams, & Scott (USDA/ARS)
SWCB Lines	"

(J.E. Smith) resistance in this germplasm and, in DEKALB trials at Union City, 1st brood ECB resistance was observed. Over the next several years this germplasm was evaluated against a broad range of lepidopterous species that feed in the maize whorl.

A cooperative study by Davis et al. (1988) showed multiple borer resistance (MBR) functioned against the sugarcane borer (SB), *Diatraea saccharalis* (Fabricius), in Mexico and Louisiana; ECB in Tennessee and Missouri; SWCB in Mexico; Missouri and Mississippi; and FAW in Georgia, Mississippi, and Mexico. Upon testing MBR germplasm, Ampofo et al. (1987) reported high levels of resistance to *Chilo partellus* in East Africa. Van Rensburg et al. (personal communication 1990) observed resistance to *Busseola fusca* in South Africa. Bato et al. (1983) in the Phillipines reported high levels of resistance to the Asian cornborer *Ostrinia furnacalis*. Bosque-Perez et al. (1987) observed resistance to *Eldana saccharina* in Nigerian tests. J. Reese (1987) noted black cutworm *Agrotis ipsilon* (Hufnagel) resistance. At this point there was little doubt that we were working with a defensive system with a broad spectrum of activity.

Advantages and Problems Associated with the Use of MBR

Multiple borer resistance provides breeding options that are not present with species specific sources of resistance, but also presents difficulties in introgressing it into elite germplasm. The following advantages are associated with MBR or can be inferred from the reaction of various pests to the MBR trait.

MBR is expressed from seedling to pretassel

Many cornbelt sources of 1st brood ECB resistance exhibit high levels of resistance only in the seedling and early whorl stages and only low levels as the plant approaches pretassel. Studies of MBR germplasm by the Davis and Williams group (USDA / ARS) under FAW infestation and by DEKALB using SWCB show increased levels of the resistance from seedling to pretassel. Resistance is highest at the late-whorl-to-pretassel stage, when the plant is most subject to physiological loss from insect tunneling.

Winter nurseries can be used to select for MBR

DEKALB has been conducting a recurrent selection program to incorporate MBR into elite lines (Overman 1987). No SWCB or FAW resistant segregates have been observed that are not also resistant to 1st brood ECB. In a cooperative study in 1993 with Dr. Meagher of Texas A&M, comparable levels of resistance to the sugarcane borer (SB) and SWCB were found in DEKALB's MBR hybrids. It is intuitive that both FAW and / or SB could be used for selecting for MBR in winter nurseries in south Florida, south Texas, Puerto Rico, Mexico, or Argentina.

Resistance can be developed to secondary or regional pests through surrogate selection

It is not economical or practical to incorporate species specific resistance into hybrids for all leaf feeding lepidoptera of maize. Many of these pests are regional in importance or affect only small maize markets. However, MBR provides an opportunity to improve resistance towards these pests through surrogate selection for resistance to other major pests. For example, we expect resistance developed for SWCB would be effective against the southern cornstalk borer *Diatraea crambidoides*, a problem in North Carolina and South Carolina.

MBR allows the breeder/ entomologist to use the species best adapted to their environment as the selective organism

Too often breeding locations attempt to select for pest resistance in environments that are not favorable for these evaluations, or a particular pest cannot be used because it is not endemic to the test region. For example, we often have difficulty in getting good 1st brood ECB establishment at Union City, but in 17 years of testing at that location the SWCB have never failed to achieve good survival. However, SWCB cannot be used at DEKALB's other US breeding locations where it is not endemic.

MBR is the only known source of resistance to many species

For many species the MBR system is the only resistant source available to the breeder. MBR hybrids could be deployed in the geographical areas listed in Table 2 for reducing the damage to a variety of pests.

The MBR system exhibits joint action with chemical controls

Larvae that survive on MBR plants grow at a slower rate and feed in the whorl for a longer period of time and are therefore more susceptible to pesticide control for a longer period of time.

Table 2. Geographical regions, and associated insect pests, where MBR hybrids could be deployed.

Species	Common Name	Region
Ostrinia furnicalis	Asiatic stalk-borer	China, Phillipines
O. nubilalis (Hubner)	European cornborer	U.S., Canada, Europe
Diatraea lineolata	Neotropical stalk-borer	Mexico, Central America
D. grandiosella Dyar	Southwestern cornborer	U.S., Mexico
D. saccharalis(Fabricius)	Sugarcane borer	Mexico, Argentina
D. crambidoides(Grote)	Southern cornstalk borer	U.S.
Chilo partellus	Asian maize borer	Africa, Asia
Bussiola fusca	African maize borer	Africa
Sesamia spp.	Pink stem borers	Africa, Middle East
Spodoptera spp.	Armyworms	The Americas

The MBR system can be used with other sources of ECB resistance

The MBR system can be selected independently from and used in combination with other ECB resistance genes to enhance the level and / or stability of pest resistance.

The MBR system is complementary with biological control

The slower growing larvae in the MBR plants are more subject to predation and parasitism.

Deployment of MBR system should reduce the population buildup of some migratory lepidoptera

The deployment of MBR hybrids in the southern USA, northern Mexico, and the Carribean should slow the development and size of migratory populations of FAW.

MBR hybrids are more likely to be compatible with other crops that are attacked by maize pests

FAW and / or ECB susceptible maize supports large populations of pests that can attack a wide variety of other crops.

The MBR system comes from a narrow germplasm base, has high ear placement, small ears and severe root lodging problems

This resistance is not simply inherited and some form of recurrent selection and usually several cycles of selection are needed to break linkages with unwanted genes.

The DEKALB/Union City MBR Program

Materials and methods

We introgressed MBR into both sides (F and M) of a heterotic pattern through recurrent selection (Overman 1987). Three inbreds from this program have been evaluated for MBR as lines and in hybrid combination for yield performance under ECB, SWCB, or FAW infestation. Whorl- or tassel-stage plants were infested by bazooka with laboratory reared neonate larvae.

Inbred test **-** Three DEKALB MBR lines (FMBR1, FMBR2, MMBR1), a CIMMYT MBR line (CML67), two USDA / ARS ECB resistant lines (Mo45, Mo47) and two elite checks (B73Ht, Mo17Ht) were evaluated against SWCB, FAW, and / or 1st and 2nd brood ECB. Leaf feeding ratings (1-9 scale) were determined 10-14 days following whorl stage. Infestations were made with 30 SWCB, 40 FAW, or 100 ECB larvae. Inbreds were grown in randomized complete block designs of two to four replicates. Entries were planted in single row plots four meters long and thinned to 15 plants. For 2nd brood ECB evaluations, each plant was infested at anthesis with 100 ECB larvae. The 2nd brood test was dissected 40 days after infestation and the length of tunneling / plant recorded.

SWCB and ECB hybrid yield trials **-** A DEKALB MBR hybrid (FMBR1 x FMBR2 / MMBR1) was compared for yield against the commercial hybrids DK683, DK714, and P3245 in non-infested and whorl stage infestations with 100 ECB or 30 SWCB larvae per plant. The hybrids were grown in 2-row plots 4 meters long and thinned to 30 plants / plot. The experimental design was a randomized split-block with whole plots as infestation treatment and hybrids as subplots.

FAW hybrid yield trial **-** The MBR hybrid and the commercial hybrid DK626 were tested in FAW infested and non-infested single row plots 4 meters long and thinned to 15 plants per row. Plants were infested at mid-whorl with 40 FAW larvae / plant and the leaf feeding rating taken 10 days later. Yields and moisture were recorded at harvest on the 1st 10 plants in each row.

Results and Discussion

DEKALB has utilized public MBR germplasm, insect rearing methodologies, and field infestation and evaluation techniques to develop a commercial program for introgressing MBR into elite germplasm. These improved lines have better agronomic

attributes while maintaining good levels of resistance to ECB, SWCB, and FAW (Table 3).

In the company's single-location yield trials, the MBR hybrid was competitive in non-infested plots with the most competitive commercial hybrids and showed a yield advantage under ECB, SWCB, or FAW infestation (Tables 4 and 5).

This progress would not have occurred without the work of public institutions in collecting and preserving germplasm, developing insect rearing methods, perfecting field methods and laboratory techniques to evaluate resistance, training scientists and technicians, and testing resistant germplasm products.

There is a need for the continued (if not expanded) involvement of public institutions to develop resistance in maize to arthropod pests. State, federal, and international institutions should be cautious about reducing support for pest resistance research on the assumption that private seed companies or other institutions can or will assume those responsibilities.

Table 3. Comparison of whorl stage resistance to ECB, SWCB, and FAW; and tassel stage ECB resistance in DEKALB MBR lines, elite public lines and resistant public lines.

Inbred	ECB Leaf Feeding	ECB Tunnel Index	SWCB Leaf Feeding	FAW Leaf Feeding
DEKALB Lines				
FMBR1	2	6	5	6
FMBR2	3	4	5	6
MMBR1	2	3	3	6
Elite Checks				
B73Ht	9	7	9	9
Mo17Ht	8	7	9	9
Resistant Checks				
CML67 (CIMMYT)	1	-	2	6
Mo45 (USDA/ARS)	2	6	-	-
Mo47 (USDA/ARS)	1	5	-	-
$LSD_{(.05)}$	0.9	3.1	1.5	2.1

(Rating of 1 = most resistant; rating of 9 = most susceptible)

Table 4. 1994 Yield comparison of MBR and commercial hybrids under late whorl stage infestations with SWCB, ECB, or no infestation.

Hybrid	Non-Infested t/ha	Non-Infested MST	SWCB t/ha	SWCB MST	ECB t/ha	ECB MST
FMBR1*FMBR2/MMBR1	11.9	29	11.8	27	11.4	27
DK683	12.2	25	11.1	24	10.1	23
DK714	11.8	27	10.9	25	11.0	25
P3245	11.9	22	11.2	22	9.8	20
LSD (.05)	1.0	1.0	1.0			

Table 5. 1994 Yield comparison of MBR and commercial hybrid under whorl stage infestation and non-infested FAW plots.

Hybrid	Non-Infested t/ha	Non-Infested MST	FAW Infested t/ha	FAW Infested MST	FAW Leaf Feeding
FMBR1 * FMBR2 / MMBR1	13.0	25	12.1	27	5
DK626	12.5	21	10.4	21	9

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Evaluation and Development of Maize Germplasm for Resistance to Spotted Stem Borer

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Abstract

Chilo partellus *(Swinhoe), commonly known as the spotted stem borer, is the most serious pest of maize (*Zea mays *L.) in India. The best approach to manage this pest is the development and use of maize cultivars having genetic resistance. In the cultivar development process, germplasm needs to be precisely evaluated on a large scale utilizing insect mass rearing techniques, synthetic diets, and artificial infestation of plants. Insect rearing laboratories have been set up and synthetic diets developed and improved. Extensive evaluation of germplasm by Punjab Agricultural University, Directorate of Maize Research and other institutes in India led to the identification of some relatively resistant materials. The more promising ones are populations Antigua Gr. 1, Arun, D 791, J 22, J 3022, Pool 27 and Tarun, and inbred lines CML 67, CML 71, CML 72, (Partap x Mo17.B57)-17(S6), Suwan 1(S) C6-40(S5) and Suwan 1(S) C6-53(S5). Further, MBR-SCB Res. EV (Y), MBR 86-Stars and Diamonds and Pop. 24 Bulk were identified to be resistant to* C. partellus *and* Ostrinia furnacalis *Guenee. Populations Parbhat and Navjot, and inbred lines CM110L, CM 201, J101(S2), J663(S7) and Vijay 444(S2) showed resistance to* C. partellus, *maydis leaf blight [*Drechslera maydis *(Drechsl.) Nisikado and Miyaki] and brown stripe downy mildew (*Sclerophthora rayssiae *var.* zeae *Payak and Renfro). Many of these materials have been used to develop open pollinated and hybrid cultivars and to derive inbred lines. In Ageti 76, Navjot and Kiran, two to three cycles of recurrent selection for resistance to* C. partellus *under natural conditions led to appreciable gains. In Ageti 76, selection was carried out only for insect resistance, whereas, in Navjot and Kiran, selection criteria were based on grain yield and other traits including insect resistance. In J 22, four cycles of recurrent selection for borer resistance under artificial infestation resulted in a significant improvement of this trait.*

Introduction

Maize is the third most important cereal crop, next to wheat and rice, in the world (FAO 1993). It is extensively used as food, feed and fodder, and in the production of starch, oil, liquor, dextrose, dyes, etc. The average world maize yield is 3.7 t/ha, whereas in India it is only 1.6 t/ha (FAO 1993), despite India ranking fifth in the world in terms of acreage. Maize is an important crop in the Indian State of Punjab, particularly in the rainy season, but it is also grown during winter and spring.

The number of insect and mite pests attacking maize exceeds 250 in India (Mathur 1991). About two dozen insects are known to cause moderate-to-heavy damage to this crop (Sekhon et al. 1993). Some of these pests are major constraints to maize cultivation, with the maize spotted stem borer, *C. partellus* being the most serious pest. The yield losses due to this pest were estimated to be 26.7 to 80.4% in different agro-climatic regions of the country (Sarup 1980).

Various methods of pest control — namely mechanical, cultural, biological and chemical — have been developed to check the damage due to different insects in maize. Historically, most emphasis was placed on chemical control. Chemical measures, however, are often not adopted by the farmers to the desired extent for various reasons. Furthermore, insecticide use has many ill effects, such as environmental pollution, residue problems and destruction of useful insects. Thus, the development and use of insect resistant cultivars by exploiting host plant resistance offers a better alternative. In resistant cultivars pest control is ensured, along with the seed, without incurring any extra expenditure. In addition, the control is non-polluting, stable and durable both through time and environments. Resistant cultivars can also be successfully incorporated into an integrated pest management strategy. In a resistance breeding program, a wide spectrum of

germplasm is evaluated for reaction to pests and the best is used in appropriate breeding programs to develop resistant cultivars possessing other desirable traits. This approach involves mass rearing of insects in the laboratory and germplasm evaluation under artificial infestation. This paper presents results of research during the last two decades on the standardization of mass insect rearing and germplasm evaluation techniques, and the identification and development of germplasm resistant to *C. partellus*.

Mass Rearing

The availability of many eggs or neonate larvae of *C. partellus* is a pre-requisite in investigations on host plant resistance. Since it is not possible to collect the required number of naturally occurring insects, these have to be reared on artificial diets. Thus, an insect rearing laboratory was established to provide congenial temperatures, relative humidities, light intensities, and improved artificial diets for extensive multiplication.

Initially, the artificial diet containing *rajmah* (*Phaseolus vulgaris* L.), developed by Siddiqui and Chatterji (1972) and Siddiqui et al. (1977), was used at different centers of the All India Coordinated Maize Improvement Programme. Then, Uma Kanta (1985) and Uma Kanta and Sajjan (1989, 1994) formulated 26 different diets. Some of these diets were based on the comparative nitrogen concentration of susceptible varieties, the diets under current use, and others on variable contributions of legumes, mainly *rajmah*, green gram (*Vigna radiata* L.), and sprouted legumes and cereals, namely green gram, maize and wheat (*Triticum aestivum* L.) (Tables 1 and 2). They observed that Diet I and Diet II of the nitrogen based diets gave rapid multiplication of *C. partellus* in comparison to the earlier diets developed by Siddiqui et al. (1977). Among the other diets, two green gram based diets, Diet III and Diet IV, proved to be better still. These diets reduced the period of insect development and increased the number of larvae per generation (Table 3). Hence, Diet III is now being used for the mass rearing of *C. partellus* at Punjab Agricultural University (PAU), Ludhiana.

Table 1. Artificial diets developed for the mass rearing of *C. partellus*.

	Siddiqui and Chatterji	Siddiqui et al. (1977)		Uma Kanta and Sajjan (1989)		Uma Kanta and Sajjan (1991)	
Treatment	(1977)	Diet I	Diet II	Diet I	Diet II	Diet III	Diet IV
Red rajmah powder (g)	74.8	75.0	-	90.0	105.0	-	-
Green gram powder (g)	-	-	75.0	-	-	90.0	75.0
Wheat powder (g)	20.0	20.0	20.0	20.0	20.0	20.0	-
Sprouted wheat powder (g)	-	-	-	-	-	-	20.0
Yeast (g)	4.0	5.0	5.0	5.0	5.0	5.0	5.0
Ascorbic acid (g)	1.3	1.7	1.7	1.7	1.7	1.7	1.7
Vitamin E (g)	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methyl Paraben (g)	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Sorbic Acid (g)	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Agar-Agar (g)	5.1	6.0	6.0	6.0	6.0	6.0	6.0
Formaldehyde 40% (ml)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Water (ml)	380.0	390.0	390.0	400.0	410.0	400.0	390.0
Total diet (g)	487.5	500.0	500.0	525.0	550.0	525.0	500.0

Table 2. Nitrogen concentration of plants of maize populations and of Rajmah diet.

	Nitrogen (%)					
	Whole plant/diet	Stem		Leaf		
Treatment	12 DAG[a]	24 DAG	36 DAG	24 DAG	36 DAG	Mean
Antigua Gr. 1	2.27	1.96	1.56	2.13	1.55	1.95
Ganga 5	2.45	2.03	1.76	2.10	1.02	1.95
JML 22	2.55	2.13	1.68	2.24	0.84	1.99
Vijay	2.97	2.10	1.12	2.20	0.77	2.02
Ageti 76	3.08	2.24	0.84	2.24	0.79	2.04
Basi Local	3.18	2.31	0.79	2.45	0.73	2.06
Makki Safed 1	3.15	2.31	0.80	2.24	0.73	2.06
Rajmah diet	2.66	-	-	-	-	-
C.D. (0.05)	0.21 (0.42)[b]	0.27	0.14	0.20	0.14	-

[a] DAG = days after germination.
[b] Includes Rajmah diet as a treatment for analyses.
Source: Uma Kanta and Sajjan (1989).

Artificial Infestation

Artificial infestation was carried out either by releasing ten larvae per plant-whorl 16 to 18 days after emergence, or by pinning tissue paper containing 25 to 30 black headed eggs onto each plant. The tissue papers were examined at random, a day after infestation, for hatching of eggs. A second release of eggs was carried out if infestation was low.

Grading Plant Damage

Insect damage was expressed as leaf scraping, small pin holes, or slit holes in the whorl leaves. Severe attack results in stunted growth, dead heart and stem breakage. A nine-class rating scale (1 = healthy, 9 = dead heart) was used. This was developed by Chatterji et al. (1970) and Sarup et al. (1974) by modifying the scale of Starks and Dogget (1970). Singh and Sajjan (1983) evaluated row grading methods, and found that recording a single observation on 5 to 10 plants in a row may be as efficient as the gradation of an individual plant in a row. They also compared different class rating scales, namely the 1-9 scale (Chatterji et al. 1970) and a 1-5 scale (Kandoria 1975), using leaf injury and dead heart, percent infestation, percent dead heart, tunnel length and number of borers, as their criteria. On the basis of a variance ratio test, coefficient of variation and relative ranking of genotype, the 1-9 scale was considered to be better than the others because it covers a wide range of leaf injury, including dead heart.

Identification of Resistant Germplasm

Extensive studies to evaluate exotic and indigenous germplasm under artificial

Table 3. Relative performance of artificial diets based on two generations of mass rearing of *C. partellus*.

Artificial diet[a]	Period of development[b] (days)	Moth emergence (%)	Pairs of moth (no.)	Progeny produced		Increase in number of larvae[c] (%)
				Eggs (no.)	Larvae (no.)	
Diets based on nitrogen concentration						
Diet I[d]	34.2	20.2	9	2340	2048	92
Diet II[d]	33.4	20.8	8	2240	1836	72
Diet I[e]	35.2	13.4	6	1296	1064	-
Diets based on green gram						
Diet III[f]	29.1	49.9	23	9039	6645	109
Diet IV[f]	29.7	51.9	24	8976	7524	136
Diet II[e]	29.5	45.6	19	5377	3185	-

[a] Details of the diet ingredients are given in Table 1.
[b] Period of development from larval hatching to adult formation.
[c] Increase over the diets developed by the Siddiqui et al. (1977).
[d] Uma Kanta and Sajjan (1989).
[e] Siddiqui et al. (1977).
[f] Uma Kanta and Sajjan (1991).

Table 4. Maize germplasm showing a relatively resistant reaction to *C. partellus*.

Germplasm	Location	Reference
Comp. A 53 (SA) x Comp A54 (EV) RU 21, EH 2230, EH 3136, J22, Opaque B-15	Ludhiana	Anonymous (1973)
(Dcota x GCC) br2-##	Pantnagar	Sharma and Singh (1975)
A6, A21, Amarillo Cristalino-1, Antigua Gr 1, Antigua Gr 2 Sel. Blanco, Antigua 7D, Antigua 8D, (Ant. x Cubans 157), British Virgin Island 117, Caribbean Flint Comp., Cuba 9, Cuba 12, Cuba 40, Dneproaskaja 200, Guatemala 257, M 512, MCPD(MS)6, Mezcla Amarilla Baja, Serie S3, R2CII, Thai DMR Comp. 17, V520CA, (Ver 181 x Ant GPO2) 02, CISTRON, EA1712 (late) FV 147 x BUP 116, FV147 x ZP 2077, K10 x 2 PR 588, LP 1712 x ZPR 588, MR 21 x R 588, MR 21 x SD 10, 0 118a x BUP 43, SD 10 x BUP 116 Syn 60J, T146 x BUP 116, T 146 x SD10, T166 x ZP 2077/54, T 116 x ZPR 588, T 169, T 341 x WF 9, U 221 x ZPR 588, VTR 116 x ZPR 588, YUZP 2077/54	New Delhi	Sarup et al. (1978)
Amber, Deccan 103, Sona, Vikram	Pantnagar	Sarup et al. (1979)
Antigua Gr 2, (CM 201) 5 br2#, IACP Comp.1, J22, Syn P 203 x Kisan)##	New Delhi and Ludhiana	
Harnampur Local, Kesari Local	New Delhi	Sarup et al. (1981)
BS20, Iowa Long Ear Syn, Honey June, NC 59663, Pool 15 Pool 16, Pool 17, Pool 19, Pool 24, Pool 25, Pool 26, Pool 27, Pool 28, Pool 29, Pool 30, Pool 32, Pool 33, Pool 33 QPM, Tuxperate x Tropical QPM (Dent)	Ludhiana	Anonymous (1984, 1985)
Tuxpeno QPM Antigua Gr 1, Mex 17	New Delhi	Durbey and Sarup (1985)

infestation have been conducted. The materials identified to be relatively resistant are listed in Table 4. These include indigenous collections from Punjab and Uttar Pradesh, indigenously developed hybrids, composites and synthetics, and exotic germplasm from the International Maize and Wheat Improvement Center (CIMMYT, Mexico), Caribbean Islands, Colombia, Guatemala, USA, Thailand and Pakistan. Some hybrids and composites released for commercial cultivation in India, namely Hybrids Deccan, Deccan 103, Ganga 2, Ganga 4, Ganga 5 and Sartaj, and composites Ageti 76 (J 603), Amber, Arun (A 68), Chandan, Dhawal, Hunius, Jawahar (A1 x Antigua Gr. 1), Kiran (J 660), Kisan, Kundan, Navjot (J 684), Parbhat (J 115), Partap (J 54), Sona, Tarun (Syn PK), Vijay and Vikram. Sartaj, Ganga 2, Ganga 5, Deccan 103, Parbhat, Vijay and Jawahar possess both high yield and wide adaptation, whereas Ageti 76, Arun, Kiran, Navjot and Tarun are early maturing, widely adapted and relatively good yielders. Sartaj, Parbhat and Navjot also possess resistance to one or more diseases.

Mean damage grade (m.d.g.) of some promising inbred lines and early maturing composites are presented in Tables 5 and 6, respectively. Three inbred lines showed a m.d.g. of 2.4 to 3.0 in comparison to 8.2 of the most susceptible inbred, CM 400 (Uma Kanta and Sekhon 1994). Five composites had a m.d.g. of 2.6 to 3.0 whereas the m.d.g. of susceptible material, D 741 EV 81 (Ranchi) was 4.8.

Table 4. cont'd

Germplasm	Location	Reference
Hunius, BS 7, BS 8, BS 14 Cooks Early Yellow Dent	New Delhi	Panwar and Sarup (1985)
Ganga 5, Antigua Gr 1, J 22, J 605	Ludhiana	Sekhon (1985)
Comp.217, Comp.218, Comp.219, Comp.222 Comp.223, Int. Comp.202, Int.Comp. 210, Int. Comp.214, Int. Comp.216, Int. Comp.217 AR 76, Comp.217, EVA 64-mst-80	New Delhi	Siddiqui et al. (1986)
Chandan, Deccan 103, Ganga 5, Jawahar (A1 x Antigua Gr. 1) Comp, Kundan Local Haryana-Hoshiarpur Local Gidderpindi, Lopon Yellow	New Delhi	Sarup et al. (1987)
CM 110L, CM 201, J 101(S2), J663(S6), J663(S7), Vijay (S3),	Ludhiana	Dey et al. (1987)
Ageti 76, Deccan Ganga 5, Ganga 2, Jawahar, Kisan, Tarun, Vijay, Vikram, Amarillo Pak, Caribbean Flint Comp, Cuba 11J, D 818, Golden Crystal, H 207, Hybrid Vanzyl, Mo x 117, Mo x 57, N 21, N 22, Pop. 31, PR 7921, Suwan 7528 Bulandshahar Local, Meerut Yellow Local, Saharanpur Local, Monghia Local, Gore Local and Dewarika Local	Pantnagar	Sharma (1987)
Ganga 4, Dhawal, Hunius, Jawahar x Thai	New Delhi	Singh (1988)
Comp 217	New Delhi	Siddiqui et al. (1988)
Comp. A-214, EA-82-4-87	New Delhi	Marwaha et al. (1990)
Arun	Chindwara	Sharma and Sharma (1992)
Ageti 76, J2012, J3022, Kiran, Navjot, Navjot (HS) C3, Parbhat, Sartaj	Ludhiana	Dey et al. (1993)
(J54xMo17.B57)-17-1-2-2-1-1-1#, Suwan 1(S) C6-40-1-1-1-2-1#, Suwan 1(S) C6-53-1-1-1-2-2#, Arun, D791, Kiran, Pool 17, Pool 27	Ludhiana	Uma Kanta and Sekhon (1994)
CML 67, CML 71, CML 72, MBR SCB Res.EV(Y), MBR 86 Stars and Diamond, Pop. 24 Bulk, Across 90390-W(IR), SCB(GCA) FAW (GCA), EEY DMR POOL (FS), EY Takfa (HS), Pop. 31 DMR C5 (S2 bulk)	Ludhiana	Uma Kanta et al. (Present publications)

Table 5. Reaction of promising inbred lines of maize to *C. partellus*.

Inbred	Damage grade (1-9)[a] 1985	1986	Mean
(J54 x Mo17.B57)-17-1-2-2-1-1-1#	3.9	2.1	3.0
Suwan 1 (S) C6-40-1-1-1-2-1#	2.6	2.2	2.4
Suwan 1 (S) C6-53-1-1-12-2#	3.9	2.0	3.0
CM 400 (Susceptible)	7.4	9.0	8.2

[a] 1 = healthy; 9 = dead heart.
Source: Uma Kanta and Sekhon (1994).

Table 6. Reaction of promising early maturing populations of maize to *C. partellus*.

Germplasm	Damage grade (1-9)[a] 1983	1984	Mean
Pool 17	2.6	3.4	3.0
Pool 27	2.6	2.7	2.6
Tarun	2.6	2.0	2.3
J 660 (Kiran)	2.2	3.3	2.8
A 68 (Arun)	2.6	2.8	2.7
D 791	2.8	2.3	2.6
D 741 EV81 (Ranchi) (Susc.)	4.2	5.5	4.8

[a] 1 = healthy; 9 = dead heart.
Source: Uma Kanta and Sekhon (1994).

Dey et al. (1987, 1993) evaluated 70 advanced inbred lines, 11 composites and 7 hybrids for multiple resistance to *C. partellus, D. maydis* and *S. rayssiae* var. zeae. The parameters of multiple resistance, namely mean and standard deviation were estimated following Dhillon et al. (1984). Low values of these parameters indicated uniform multiple resistance. Six inbred lines (Table 7), four composites and one hybrid (Table 8) showed multiple resistance. All five composites and one hybrid are released cultivars.

Utilization of Resistant Germplasm

The germplasm that has consistently shown resistance is Antigua Gr. 1 (CM 500), a Caribbean introduction. It has been used as a parent of the widely adapted, high yielding double top-cross hybrid, Ganga 5, and of the varietal hybrid used to develop Comp. Jawahar. Inbreeding in Antigua Gr. 1, however, did not yield good inbred lines. There are many other resistant germplasm sources that have been utilized in the development of promising composites and hybrids (Table 9).

Table 7. Parameters of multiple resistance of promising inbred lines of maize to *C. partellus, Drechslera maydis* and *Sclerophthora rayssiae* var. zeae.

Inbred	Multiple resistance	
	Mean (1-5)[a]	Standard deviation
CML 110 L	1.7	0.361
CM 201	2.0	0.874
J 101 (S2)[b]	1.9	0.513
J 663 (S7)	2.0	0.681
J 663 (S6)	1.8	0.577
Vijay (S2)	1.9	0.513

[a] 1 = healthy; 5 = susceptible.
[b] Generation of selfing.

Table 8. Parameters of multiple resistance to *C. partellus, D. maydis* and *S. rayssiae* var. zeae of some promising maize cultivars and local.

Genotype	Multiple resistance	
	Mean (1-5)[a]	Standard deviation
Hyb. Sartaj	2.5	0.374
Comp. Kiran	2.5	0.458
Comp. Navjot	2.4	0.600
Comp. Navjot (HSC3)	2.3	1.153
Comp. Parbhat	2.3	1.079
Local	4.2	0.200

[a] 1 = healthy; 5 = susceptible.

CIMMYT's Asian Regional Collaborative Project

Given the serious damage due to stem borers in South-East Asia, CIMMYT's Asian Region Maize Program initiated collaborative research on the evaluation and improvement of germplasm for resistance to *C. partellus* and *O. furnacalis* in 1990. Since then, inbred lines and multiple borer resistant (MBR), multiple insect resistant (MIR) and downy mildew (DMR) populations have been evaluated for reaction to *C. partellus* in India and *O. furnacalis* in the Philippines, and now efforts are being made to develop DMR-borer resistant germplasm.

Among the CIMMYT maize lines evaluated, three (CML 67, CML 71, CML 72) have shown a promising reaction to *C. partellus*. Their m.d.g. varied from 2.6 to 3.0 in comparison to 6.5 for the susceptible check (Table 10). However, these lines *per se*, as well as in cross combinations, did not show agronomically good performance under our conditions. We have planned to evaluate their heterotic relationships with our elite materials so as to utilize the inbred lines in second cycle breeding. The inbred lines CML 123, CML 126, CML 127 and CML 131 all showed susceptible reactions.

Table 9. Sources of resistant germplasm used in the development of promising composites and hybrids.

Source germplasm	Population or hybrid developed	Status
Population		
Antigua Gr. 1	Hyb. Ganga 5	Released at the national level
	Comp. Jawahar	Released at the national level
Arun and J 3022	Comp. Megha	Released at the national level
Tarun	Comp. Navjot	Released at the national level
Inbred(s) derived from		
Arun	EH 2420	Evaluation in FYT[a] in 1994
	EH 3021	Evaluation in FYT[a] in 1994
J 3022	EH 2420	As explained above
Suwan 1	EH 21058	Evaluated in FYT in 1993
Tarun	EH 3189	Evaluation in SYYT[a] in 1994
Vijay	EH 200174	Evaluation in FYT in 1979
J 3022 and Navjot	Indigenous early heterotic pool	
Tarun	Semi-exotic early heterotic pool	
Ageti 76, Arun	Makki Safed	
J 101, J660, J663, Kiran, Navjot, Partap, Tarun and Vijay	heterotic pool	
Cuba 11J and Suwan 1	Tuxpeno heterotic pool	

[a] FYT = final yield trial, SYYT = second year yield trial

Three MBR populations, MBR SCB Res. EV (Y), MBR Stars and Diamonds and Pop. 24 Bulk, and three MIR populations, Across 90390-W (IR), SCB (GCA) and FAW (GCA), showed relatively good reaction to *C. partellus* (Tables 11 and 12). The MBR populations were also evaluated for reaction to *O. furnacalis*. The three populations mentioned above showed a good level of resistance to this pest also (Table 13). Inbreeding was initiated in these three populations, but they showed intense depression for grain yield and agronomic traits. The MBR and MIR germplasm that showed a susceptible reaction to *C. partellus* included Phil. 05, Phil. DMR Comp. 1, TLY-DMR Pool C3 (HS), and Across 90390-Y(IR).

Table 10. Reaction of promising inbred lines of maize to *C. partellus*.

Inbred	Damage grade (1-9)[a] 1992	1993	1994	Mean
CML 67	2.5	2.2	3.1	2.6
CML 71	3.9	2.8	2.4	3.0
CML 72	3.3	2.4	3.2	3.0
Basi Local (Susc. check)	4.9	6.8	6.2	6.5

[a] 1 = healthy; 9 = dead heart.

Table 11. Reaction of promising multiple borer resistance (MBR) populations of maize to *C. partellus*.

Germplasm	Damage grade (1-9)[a] 1990	1991	1992[b]	Mean
MBR SCB Res. EV (Y)	3.8	5.0	4.0	4.3
MBR 86 Stars and Diamonds	3.8	5.2	3.8	4.3
Pop. 24 Bulk	3.3	3.9	3.4	3.5
Basi Local (Susc. check)	6.4	6.0	5.0	5.8

[a] 1 = healthy; 9 = dead heart.
[b] Based on S_1 lines, developed from resistant plants during 1991.
Source: Sekhon et al. (1992).

Table 12. Reaction of promising multiple insect resistance (MIR) populations of maize to *C. partellus*.

Population	Damage grade (1-9)[a] 1992	1993	Mean
Across 90390-W (IR)	4.1	3.3	3.7
SCB (GCA)	3.3	3.5	3.4
FAW (GCA)	3.8	4.5	4.2
Basi Local (Susc. check)	5.1	5.9	5.5

[a] 1 = healthy; 9 = dead heart.

Table 13. Reaction of multiple borer resistance populations of maize.

Germplasm	Damage grade (1-9)[a] *C. partellus* Ludhiana, India	*O. furnacalis* Los Banos, Philippines
MBR - SCB Res. EV(Y)	3.8	1.8
MBR 86- Stars and Diamonds	3.8	2.6
Pop. 24 Bulk	3.3	2.8
Susceptible Check[b]	6.4	5.2

[a] 1 = healthy; 9 = dead heart.
[b] Basi Local and Philippine Supersweet for *C. partellus* and *O. furnacallis*, respectively.

Table 14. Reaction of promising downy mildew resistance (DMR) populations of maize to *C. partellus*.

Pedigree	Damage Grade (1-9)[a] 1993	1994[b]	Mean
Early Yellow			
EEY DMR Pool (FS)	4.9	4.2	4.6
EY TakFa (HS)	3.6	3.9	3.8
Pop. 31 DMR C5 (S2 bulk)	5.5	3.4	4.4
Pop. 145 EY DMR Pool (S2 bulk)	5.5	5.8	5.7
Viemyt 49-Y (S2 bulk)	5.9	4.1	5.0
Early White			
EEY DMR Pool (FS)	5.1	5.1	5.1
Pop. 100 EW DMR (S2 bulk)	5.5	6.3	5.9
Late Yellow			
LY Takfa (HS)	4.3	5.7	5.0
Pop 28 EMR C6 (S2 bulk)	5.4	7.2	6.3
Pop. 345 LY DMR (S2 bulk)	5.7	7.1	6.4
Basi Local (Susc. Check)	5.9	5.9	5.9

[a] 1 = healthy; 9 = dead heart.
[b] based on S_1 lines developed from resistant plants identified during 1993.

In view of losses to downy mildew (*Sclerospora* spp. and *Sclerosphthora* spp.) in Asia, the collaborative project adopted DMR germplasm in 1993. Ten DMR populations were evaluated. The populations that showed a relatively resistant reaction were EY Takfa (HS), EEY DMR Pool (FS) and Pop. 31 DMR C5 (S2 Bulk) (Table 14). The plants resistant (m.d.f.> 4.0) to *C. partellus* at Ludhiana and Hyderabad in India and to *O. furnacalis* at Los Banos, the Philippines, were selfed to develop S_1 to S_3 lines in these materials and to constitute three populations, namely Early Yellow, Early White and Late Yellow. The materials developed at one center were exchanged with others. PAU was the primary location to form the Early Yellow population and contributed 110 selfed lines to the total of 231 lines used to develop this population. The number of S_1-S_3 lines contributed by different centers to develop the three populations are given in Table 15. As per the program of the collaborative project, resistant plants in resistant lines were recombined to reconstitute the population. These will be sent to various collaborators. In addition, we have continued selfing in selected lines.

Population Improvement

In the population improvement program for grain yield and other traits at PAU the families were also evaluated for resistance to *C. partellus*, *D. maydis*, and *S. rayssiae* var. zeae depending on the resources available. A number of composites, namely Ageti 76, Navjot, Parbhat, Partap, Vijay, Kiran and J 663 were subjected to population improvement for *C. partellus* under natural conditions using square planting. Depending on grain yield and other traits including pest and disease resistance, the populations

were reconstituted. The result was that most of the cultivars developed at PAU — namely Ageti 76, Navjot, Kiran, Parbhat, Partap and Sartaj during the late 1970s and 80s — combine high yield, wide adaptation and other desirable traits including disease and pest resistance.

The performance of Kiran (J 660) and Navjot (J 684) after two and three cycles of selection for various traits, including the reaction to *C. partellus*, is presented in Table 16. The selection was carried out under natural conditions, whereas the performance has been evaluated under artificial infestation. There was gain for resistance to *C. partellus* and *D. maydis*. However, no gain was observed in some other populations (Dey et al. 1988).

Recurrent selection for resistance only to *C. partellus* was carried out in two populations, Ageti 76 (J 603) and J 22. Ageti 76, an early maturing and high yielding cultivar, was subjected to two cycles of improvement under natural infestation by Singh et al. (1982). The first cycle was based on half-sib families and the second cycle on S_1 families. The reconstituted and original populations were evaluated under artificial infestation, wherein the former showed a lower m.d.g. than the later, indicating improvement for resistance to *C. partellus* (Table 17).

Four cycles of selection were carried out in Composite J 22 for resistance to *C. partellus* under artificial infestation (Dhillon et al. 1987). This population had high yield potential with good agronomic traits, resistance to *C. partellus* and *Atherigona* spp. and tolerance to zinc deficiency. The selection comprised one cycle of half-sib, one cycle of full-sib and two cycles of S_1 family selection, in that order. J 22 and the strains developed after second-to-fourth cycles of selection were evaluated under artificial infestation. The difference between J 22 C0 and the strains developed after four cycles of selection, J 22 C4, was significant (Table 18). The improved population is being used as a source germplasm to derive inbreds.

Table 15. Number of selfed lines developed in downy mildew resistant (DMR) populations of maize during 1993 and evaluated during 1994 in a collaborative program on multiple borer resistance.

	DMR lines developed at different locations (no.)				
	O. furnacallis	*C. partellus*			
Population	Los Banos Philippines	Hyderabad India	Ludhiana India	Total	Source germplasm
Early Yellow	90	31	110	231	EEY DMR Pool (FS), EY Takfa (HS), Pop. 31, DMR C5 (S2 bulk), Pop. 145 EY DMR Pool (S2 bulk) and Viemyt 49 Y (S2 bulk)
Early White	39	13	36	88	EEW DMR Pool (FS) and Pop. 100 EW DMR (S2 bulk)
Late Yellow	21	-	22	43	LY Takfa (HS), Pop. 28 EMR C6 (S2 bulk) and Pop. 345 LY DMR (S2 bulk)

Recurrent Selection and Hybrid Breeding

At PAU, Ludhiana, major research efforts are now being devoted to hybrid breeding. Therefore, we have initiated recurrent selection and inbred line development in two heterotic pools,

Table 16. Reaction of the original and improved versions of maize populations to *C. partellus* and *D. maydis*.

Population	*C. partellus* (1-9)[a]	*D. maydis* (1-5)[b]
J 660 C0	6.0	2.3
J 660 HS (MER) C2	5.0	2.0
J 660 HS C2	5.4	1.8
J 684 C0	5.9	2.5
J 684 HS (MER) C2	6.9	2.1
J 684 HSC3	4.8	1.9

[a] 1 = healthy; 9 = dead heart.
[b] 1 = healthy; 5 = susceptible.
Source: Dey et al. (1988).

Table 17. Reaction of original and improved versions of Composite J 603 after two cycles of selection for resistance to *C. partellus*.

Population	Damage grade (1-9)[a]	
	Natural infestation	Artificial infestation
J 603 C2 (B+W)	2.6	4.6
J 603 C2 (B+W)#	2.9	5.0
J 603 C2 (B)	2.7	4.9
J 603 C0	3.2	5.1

[a] 1 = healthy; 9 = dead heart.
[b] B = between family selection; W = within family selection.
Source: Singh et al. (1982).

Table 18. Reaction of original and improved versions of Comp. J 22 after two to four cycles of selection for resistance to *C. partellus*.

Population	Damage grade (1-9)[a]
J 22 C0	4.8
J 22 C2	5.0
J 22 C3	4.0
J 22 C4	3.5
Basi Local (Susc. check)	5.6

[a] 1 = healthy; 9 = dead heart.
Source: Dhillon et al. (1987).

Makki Safed and Tuxpeño developed by Khehra et al. (1986). Recurrent selection based on half-sib and selfed families (Dhillon et al. 1994) is being pursued, but selfing has been extended to the S_2 generation in view of the greater emphasis on hybrid breeding. In each pool 600 plants were artificially infested and the most promising 100 were selected. The S_1 lines of these plants were grown and subjected to among- and within-family selection. In the S_2 generation 91 lines of each pool were evaluated. Selected plants within selected S_2 families are being recombined and selfed to develop improved pools and inbred lines, respectively.

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Verification and Pre-Commercial Testing of European Corn Borer and *Gibberella* Ear Rot Resistant Varieties

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Abstract

*Adapted cultivars must have an acceptable level of tolerance or resistance to major insect and disease pests. The European corn borer, ECB, (*Ostrinia nubilalis, *Hübner) and ear molds (*Fusarium *spps. in particular* F. graminearum *Schwabe) are important pests throughout the Northern corn belt of North America. An understanding of the insect, disease and genetic mechanisms of tolerance or resistance have led to the useful development and application of new and established techniques for developing improved cultivars. Modes(s) of entry, mechanisms of tolerance or resistance, degree of reasonable tolerance vis a vis effects on yield, lodging, grain quality, and source of genetic variability are key long-term steps towards a satisfactory solution.*

Introduction

In Eastern Canada, agriculture is limited to the north by the Canadian Shield of rocks and forest. The soils range from glacial till, lake bottoms, forest podzols to beach sands; and farming is restricted to river valleys surrounded by forest and hardwood bluffs.

Agriculture includes cereals, forages and corn / soybean crops together with dairy, beef and some intensive pig / poultry enterprises. The studies reported occurred in the valleys of the Ottawa and St. Lawrence rivers, (Lat. N 44°43'- 45°40'; Long. W 75° 31'-76°45') similar perhaps to a region representing 55 RM to 80 RM using the Minnesota maturity rating system, or FAO 130-300 in Europe. The growing season begins after the last spring frosts in early May and is arrested by frost from mid-to-late September. Corn production is limited by the maturity of cultivars recommended in regional trials. The earliest cultivars approach 55 RM or FAO 130. The two major pests of maize are the European corn borer (ECB), *Ostrinia nubilalis* (Hubner), and ear rots caused by *Fusarium* spps. Building tolerance / resistance to both pests is a major goal of Canadian breeding programs.

European Corn Borer

The development of maize germplasm tolerant to the European corn borer has been in progress for many years, both nationally and worldwide. During the early history of corn breeding and the move toward early maturing hybrids, frequent devastation of farm fields and plant breeding nurseries occurred (Agr. Can. Ann. Reports 1923-27). In Canada, ECB continues to account for annual stalk breakage and loss of yield and quality.

Canadian research followed the work described by Dicke and Guthrie (1988) and Hudon et al. (1989), leading to the use of artificial infestation screening (1st generation leaf feeding damage, followed by 2nd generation tunneling below the ear). Generally there are three adult flights per year, with offspring reaching the mature larval stage by fall harvest.

Today, genotypic resistance to first generation (i.e. leaf feeding) pests is not as important an objective, because plant tolerance is sufficient. Second generation damage (i.e., tunneling), however, is certainly present, and plant dissection is the normal screening method. Limited resources require the development of an improved screening method for direct field evaluation in large plant breeding programs.

Cultural practices such as conventional ploughing and discing remain an effective control, but with the growing popularity of conservation tillage, other management alternatives are under investigation. This new environment has led to cool soil temperatures longer into the spring growing season, and necessitates a new look at corn borer behavior and methods of control.

The Canadian plant breeding effort does not use infestations as much as in the past. Certainly, ECB tolerance is observed and major companies have entomological input to complement development of stress tolerant inbred lines. Selection of tolerance at all stages of inbreeding is routinely practiced, and new line development evolves largely from elite commercial hybrids. Final evaluation of potential commercial hybrids occurs across many environments and the high natural population of ECB / stress provides a good measure of hybrid tolerance. In Canada, a new hybrid requires licensing through a provincial committee from data where the hybrid is adapted. The hybrid must demonstrate superior yield / moisture plus stalk quality at harvest. The average commercial life of a hybrid is less than 5 years. Host plant resistance requires continual research and has produced and continues to produce improved yields plus satisfactory tolerance to ECB.

Studies were conducted to investigate the present status of genetic tolerance to ECB. Further studies were made on the biology of the insect / plant behavior to develop a technique that would allow rapid monitoring of plant tolerance.

The study was conducted during the 1990 and 1991 growing seasons at two locations: Ottawa, 90RM zone, with artificial infestation; and Prescott, 95RM zone, with a natural population. Ten genotypes representing three maturity groups — early (inbreds CM7, CK44, and INRA synthetic SFP-1); medium (inbreds A619, DE811, hybrids Pickseed 4533 and Dekalb 435); and late (inbreds B73, CI31A and synthetic BS9 C0) — were selected for a wide genetic background and their differing susceptibility to ECB.

Genotype group (inbred, synthetic, hybrid) were the main plot units, and genotypes were randomized within blocks of the four replicate split plot design. Rows were 8m long and 0.9m wide, with approximately 50 plants per plot (55,000 / ha). Each experimental site was surrounded by four border rows of a susceptible commercial hybrid. Data on plant damage was obtained at grain harvest (i.e. late October). In each row, the four end plants were discarded and every third plant dissected.

ECB egg masses were produced at the Ridgetown College of Agricultural Technology RM90 zone, Ridgetown, Ontario using the rearing techniques of Guthrie (1989). Egg masses were sent to Ottawa, incubated till the black head stage and two masses deposited on each of two days (approx. 100 eggs / plant) at the whorl stage of maize development. Leaf feeding ratings were obtained at tassel elongation and tunneling measurements at grain harvest.

Both 1990 and 1991 were above average heat unit accumulation years. The maturity attained at Prescott, located on the St. Lawrence river, showed the more favorable environment *vis a vis* the maturity attained at Ottawa (90 km north) as measured by grain moisture (Tables 1 and 2). A larger population of corn borer was observed in the rural Prescott region, largely attributable to the cultural practices of minimum tillage and leaving abundant crop residues. In contrast, the fall management at Ottawa, where fields are located in an urban environment, together with flailing of the stubble and fall ploughing, reduced the natural population (Fig. 1).

Table 1. Average number of days to silking, grain moisture at harvest, and European corn borer leaf feeding, stalk damage and larval recovery for ten genotypes at Prescott (natural population) in 1990 and 1991.

Genotype	RM[1]	Tunnel[2] (cm)	Number of tunnels[2]	Number of larvae[2]	Leaf feeding[3]	Number of days silking	Grain moisture[4]
Inbreds							
Early							
CK44	60	863	133	71	1.7	71	7.8%
CM7	65	1218	184	108	1.7	65	11.8%
Medium							
A619	95	1087	157	75	1.4	78	15.8%
DE811	100	180	37	20	1.3	76	28.0%
Late							
B73	103	1165	177	113	2.3	82	21.1%
CI31A	110	500	90	63	1.0	89	32.1%
Hybrids							
4533	90	858	94	44	2.0	70	11.3%
DK435	95	286	40	16	1.1	71	12.1%
Synthetics							
SFP-1	65	642	86	26	1.3	67	9.6%
BS9C0	105	338	57	31	1.2	80	17.7%

[1] Relative maturity.
[2] Total for 40 plants.
[3] Average leaf feeding of 160 plants using Guthrie et al. (1960) rating.
[4] Average for 40 plants.

Table 2. Average number of days to silking, grain moisture at harvest, and European corn borer leaf feeding, stalk damage and larval recovery for ten genotypes at Ottawa (artificial infestation) in 1990 and 1991.

Genotype	RM[1]	Tunnel[2] (cm)	Number of tunnels[2]	Number of larvae[2]	Leaf feeding[3]	Number of days silking	Grain moisture[4]
Inbreds							
Early							
CK44	60	1153	179	106	1.1	63	11.7%
CM7	65	1373	183	68	1.2	63	17.0%
Medium							
A619	95	500	85	52	1.1	85	38.8%
DE811	100	224	38	10	1.3	91	45.9%
Late							
B73	103	1011	161	95	2.2	85	36.6%
CI31A	110	785	135	89	1.0	97	64.1%
Hybrids							
4533	90	1140	134	37	1.5	72	18.4%
DK435	95	844	103	23	1.2	74	16.2%
Synthetics							
SFP-1	65	894	120	22	1.6	69	15.6%
BS9C0	105	434	75	14	1.3	92	35.7%

[1] Relative maturity.
[2] Total for 40 plants.
[3] Average leaf feeding of 160 plants using Guthrie et al. (1960) rating.
[4] Average for 40 plants.

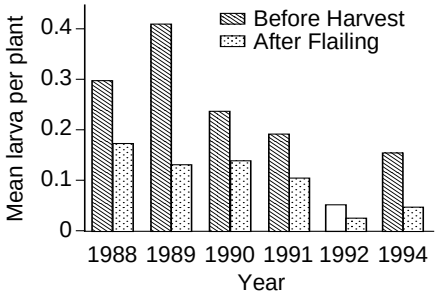

Figure 1. Average European corn borer larval population before harvest (0-30 cm) and after flailing the field (0-7.5 cm) at grain harvest from 1988-1994 (except 1993).

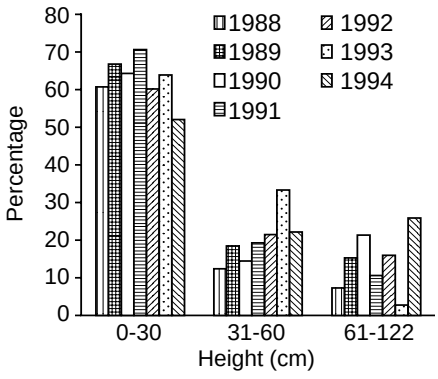

Figure 2. Percentage of European corn borer tunnel length below 30 cm, between 31-60 cm, and below the ear, from 1988 and 1994.

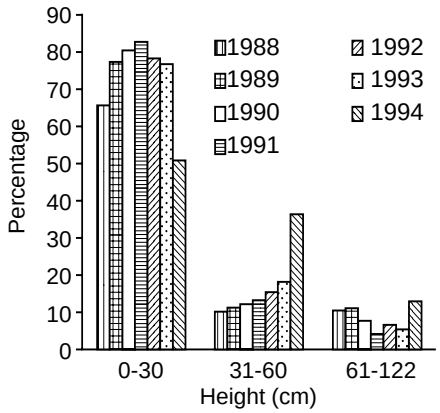

Figure 3. European corn borer larval recovery below 30 cm, between 30-60 cm and below the ear, from 1988 to 1994.

Leaf feeding

There was no significant difference in leaf feeding within locations and between years. All genotypes showed minimal leaf feeding damage. First generation resistance appeared to be satisfactory for this wide array of inbreds, hybrids and synthetics. However, these data contrast with studies at Ottawa which demonstrated a range in leaf feeding response under artificial infestation during 1991 and 1992 (Bergvinson et al. 1994). Nevertheless, little damage has been observed in the large nursery across many genotypes in this environment.

Genotype-ECB damage

There was a wide range in maturity as shown in Tables 1 and 2. Grain moisture ranged form 7.8 to 32.1% at Prescott compared with 11.7 to 64.1% in Ottawa. There was no significant difference in damage within locations between years. The early cultivars CM7 and CK44, used widely in the shortest season areas of Canada and Europe, are very susceptible. Similarly, B73, A619, Pickseed 4533 and the synthetic SFP-1 were also considered susceptible. However, in contrast to the early cultivars, medium and late inbreds DE811, CI31A, together with the resistant hybrid DK435 and SYN. BS9C0, showed good levels of tolerance.

A rapid screening technique

Since 1988, the vertical distribution of ECB within a plant has been monitored in several fields with corn hybrids of 70-90 RM maturity. Plants were dissected longitudinally at harvest (late October) and the presence/location of larvae and tunnel length was recorded. There were two important implications:

- Observations of tunnel length would be of most interest and most cost effective in the lower 30cm of the stalk since this is where more than 60% of tunnel damage occurs (Fig.2).
- Fall management of stubble to control ECB populations must include management of the lower stalk (Fig. 3).

Ear Rots

Fusarium graminearum Schwabe, the asexual state of *Gibberella zeae* (Schw.) Petch, is an important ear-rotting pathogen of corn in Canada, the US, Europe, and other countries (Sutton 1982). Infected host debris is believed to be the major source of inoculum, with inoculum being dispersed via wind, rain, insects and birds. Spore entry into corn ears can occur through wounds (e.g. insects or birds) or by growth of mycelium down the silks to the kernels and cob from spores germinating on the silks (Hesseltine and Bothast 1977; Koehler 1942; Sutton 1982). Mycelial growth on the kernels has a characteristic pinkish colour and cobs become soft and spongy with rot.

Although *F. graminearum* ear rot occurs sporadically, it can represent a serious problem due to mycotoxins with are produced by this pathogen (Vesonder et al. 1981). This is of considerable concern to livestock producers. Swine are the most sensitive to *F. graminearum* mycotoxins. Two major mycotoxins are produced by this pathogen: zearalenone and deoxynivalenol (DON, vomitoxin).

The most satisfactory solution to control the disease is the development of resistant corn hybrids. Due to the sporadic nature of the pathogen, artificial inoculation must be used to screen germplasm for resistance. Inoculation techniques are needed to test for resistance to both modes of fungal entry, i.e. growth down the silks vs. kernel wounding. We have found inbreds and hybrids with resistance to one, but not both modes of entry. Kernel resistance alone is not sufficient since earlier infections through the silk, when kernels are not yet fully developed, can result in extensive infection even in lines with high kernel resistance.

Screening for silk resistance usually involves one of three techniques:

- Insertion of a colonized substrate (e.g. toothpick or kernel) into the silk channel.
- Spraying a spore suspension on the exposed silks.
- Injection of a spore suspension into the silk channel.

Screening for kernel or wound resistance usually involves wounding through the husk, kernels, and cob followed by insertion of a colonized substrate (toothpick) or spores (saturated pipecleaner) into the wound. More recently, methods are being developed to avoid wounding the cob by just puncturing the husk and kernels followed by application of a spore suspension.

Screening techniques

We have developed a technique to screen for infection via the silk. This technique involves the injection of a spore suspension of *F. graminearum* into the silk channel, inside the husk and above the cob. A concentration of 5 x 10^5 spores / ml has been found to give maximum differentiation between genotypes (Reid et al. 1994). Higher concentrations significantly increase the amount of infection in more susceptible hybrids. Although no significant isolate effects have been found with the use of this technique (Reid et al. 1993), a mixture of two to three isolates is used. Two ml of inoculum (spore suspension) are injected into the silk channel of each primary ear using a self-refilling cattle vaccinator attached to a 2 L backpack (Fig. 4). Care must be taken to ensure that the needle is held horizontally (Fig. 5) so that inoculum is not forced down the silk channel onto the kernels. Higher volumes of inoculum significantly increase the amount of infection in more susceptible hybrids (Reid et al. 1994). A single individual can inoculate an average of 400-500

Figure 4. A self-refilling cattle vaccinator attached to a 2 L backpack is used to inoculate corn ears with 2 ml of *F. graminearum* spore suspension.

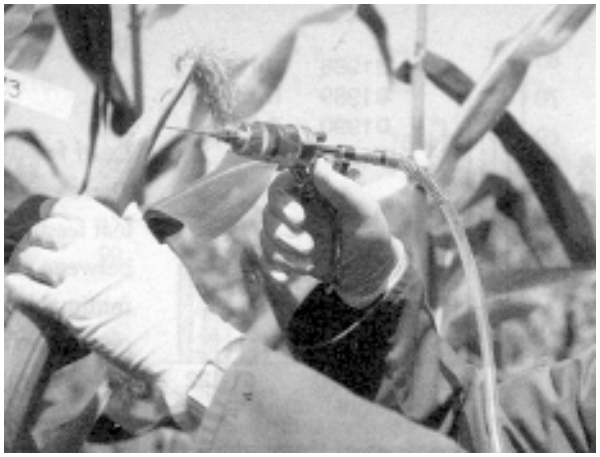

Figure 5. Injection of spore suspension into silk channel. Needle must be at right angles to ensure proper placement of inoculum.

ears per hour. Inoculations must be made 2-6 days post-silk emergence. Insufficient infection is obtained when inoculations are made later and incorrect assessments or no differentiation occurs (Reid et al. 1993). A humid environment should be maintained using irrigation, 2-5 mm daily, for the four-week period after inoculation. This technique has been used since 1987. It has allowed for good differentiation between inbreds and hybrids, ranging from very susceptible to highly resistant.

We have also been developing a technique to screen for kernel resistance, which involves wounding of the husk and kernels with four small (3 mm dia.) nails spaced in the four corners of a rectangle (7 mm long, 5 mm high). These nails have been driven into a 50 cm long wooden handle fabricated from a broomstick. Prior to wounding, the nails are dipped in a spore suspension. Inoculations are made 10-15 days post-silk emergence. We are currently investigating some of the parameters involved in this technique such as: time of inoculation, spore concentration, and position of wound.

For both techniques, a modified Bilay's liquid medium is used to produce inoculum: 2.0 g potassium dihydrogen phosphate; 2.0 g potassium nitrate; 1.0 g potassium chloride; 1.0 g magnesium sulphate; 0.0002 g / L each of the minor elements: ferric sulphate, manganese sulphate, and zinc sulphate; 1.0 L distilled water; 2.0 g soluble starch or sucrose or 1.0 g of dextrose. The medium is dispensed in 150 ml aliquots into 500 ml erlenmeyer flasks, autoclaved, then a 1 cm square piece of PDA with mycelium and spores is added. Cultures are shaken for 1 hr at 4 hr intervals under natural light supplemented with cool white fluorescent lights. Spore concentrations can reach 2 x 10^6 spores / ml in one week depending on isolate. Prepared inoculum can be stored at 2-4∞ C for a maximum of four weeks. Prior to inoculation, the mixture is diluted and filtered through two layers of cheese cloth.

A minimum of four replicates should be used for each genotype (approximately 40 treated plants). Each genotype can be planted in single row plots of 12-14 plants each, of which the primary ears of the center 10 plants are inoculated.

Ears are harvested at normal grain harvesting moisture in mid-late October. Visual rating scales (Fig. 6) have been developed for both techniques and correlated with actual numbers of infected kernels. The number of infected kernels have been correlated with toxin (DON) level in the grain.

Randomized complete block designs are usually used and data are analyzed and presented as a range in resistance. Relatively good reproduction of infection ratings has been obtained across years. Check hybrids for different levels of resistance have been identified and correlate well with natural infection from field observations.

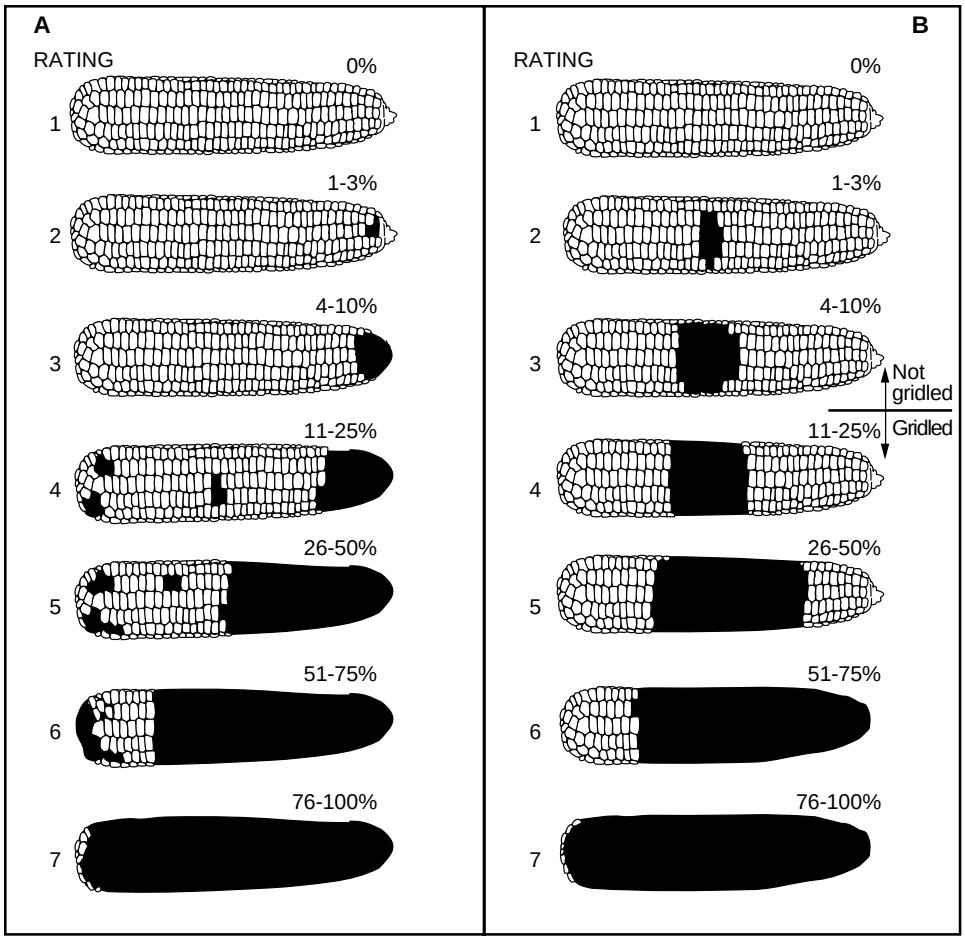

Figure 6. Disease severity rating scale for (A) silk channel inoculations and (B) kernel wound inoculations with *F. graminearum*.

Both of the techniques described above have been standardized and are suitable for routine use in breeding programs. A wide range in resistance ratings can be obtained, so that genotypic differences are easily observed (Fig. 7). We are currently testing the use of these techniques with other *Fusarium* species such as *F. moniliforme*, *F. subglutinans*, and *F. culmorum*.

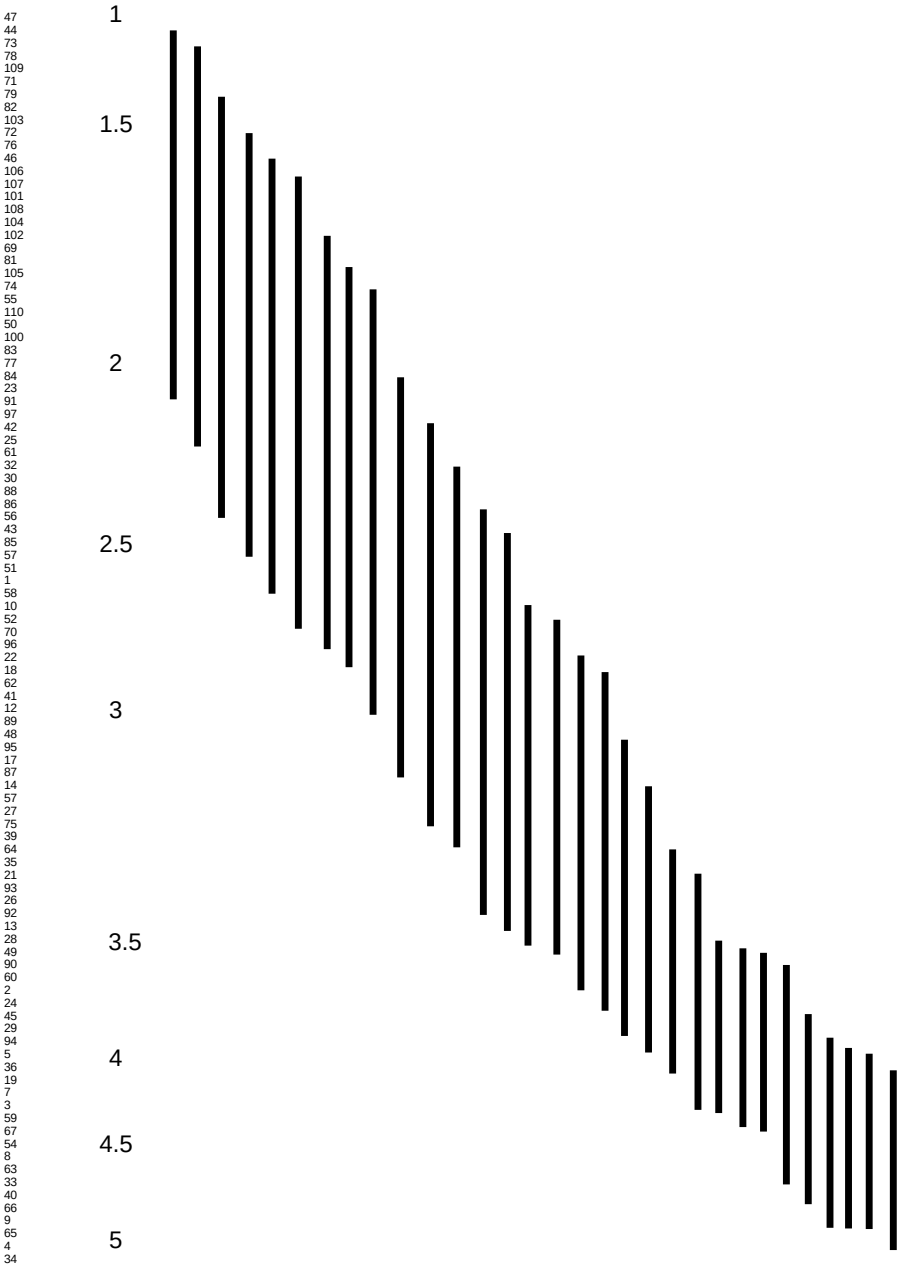

Figure 7. The range in resistance ratings for 98 Ontario hybrids inoculated with *F. graminearum* by silk channel injection (hybrids are coded to protect company confidentiality). Means followed by the same vertical bar are not significantly different at the 0.05 probability level by the Duncan's multiple range test.

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Introducing Unadapted, Insect–Resistant Maize Germplasm in Three–Way Hybrid Combinations for Resistance to the Maize Stalk Borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae)

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Abstract

The potential value of various levels of resistance to the maize stalk borer was evaluated by crossing three unadapted, resistant inbreds and three local elite inbreds in various combinations. The unadapted, resistant germplasm could be employed directly to introduce resistance, provided that undesirable traits inherent to the unadapted parents were sufficiently diminished by the genetic contribution of the adapted germplasm. The use of a single resistant parent in a three–way hybrid to increase the resistance level to 25% was sufficient to eliminate the need for chemical control at moderate levels of infestation. The use of two resistant parents to obtain a level of 50% resistance in the resultant three–way cross posed an unacceptable risk, due to an increased incidence of ear rot and lodging.

Introduction

Breeding for resistance to the African corn borer, *Busseola fusca* (Fuller), at the Grain Crops Institute was prompted when high levels of resistance to this species were observed in the Mississippi inbreds Mp705, Mp706 and Mp707 (Van Rensburg and Malan 1990). New sources of resistance have since been obtained in breeding material developed by CIMMYT, of which CML139 (yellow kernel type) and CML123 (white) proved to be particularly promising (Van Rensburg and Van den Berg 1995).

Antibiosis observed in the Mp–inbreds was shown to be 35% heritable. The gene action was largely additive, while the dominance and epistasis components of genetic variation were found to be negligible (Van Rensburg and Gevers 1993). As a result of the quantitative nature of the inheritance of resistance, the use of resistant exotic germplasm to introgress resistance into locally adapted materials is necessarily time consuming. Since useful genes found at low frequencies in the exotic source and absent in the adapted source are more likely to be lost when selecting in the backcross than in the cross (Crossa and Gardner 1987), several cycles of recurrent selection are required before backcrossing can be attempted. Furthermore, at least one backcross to the adapted parent would be required to ensure adaptation, adding to the time required to develop a resistant adapted population (Albrecht and Dudley 1987).

The question has arisen as to whether unadapted, resistant inbred lines can be utilized directly in hybrid development. Previous research of this nature dealt with methods and the possible consequences when exotic germplasm is used to increase the genetic diversity of adapted maize populations from which improved inbreds were to be extracted (Albrecht and Dudley 1987; Crossa and Gardner 1987; Michelini and Hallauer 1993). The direct use of unadapted inbred lines as parental sources in two–way hybrids was however not contemplated, since a delicate genetic balance for adaptability may easily be destroyed by genetic recombination in a two–parent cross between an adapted, insect–susceptible genotype and a non–adapted, insect–resistant genotype. But the introduction of unadapted breeding material can also be accomplished by employing three–way and four–way crosses (Gallun 1980). In this way a single resistant parent may serve to improve the resistance level in hybrids, whereas undesirable traits may be diminished by the contribution of the adapted parents. The viability of such a strategy to develop improved maize hybrids resistant to *B. fusca* was therefore

investigated, since local maize hybrids, until recently, were predominantly four–way crosses. Emphasis is now being placed on the development of three–way and modified single crosses, all of which involve more than two inbred parents.

The objective of the present investigation was to assess the levels of resistance obtained when utilizing one, two and three resistant inbreds in three–parent crosses, at the same time evaluating the direct use of exotic germplasm for other characteristics. It was deemed that the improvement of resistance in a hybrid combination to a level that would warrant an increase in the economic threshold for chemical control would be of considerable significance in practice, as opposed to striving for ultimate resistance levels.

Material and Methods

Two susceptible elite inbreds (S) and two resistant exotic inbreds (R) were crossed to obtain four single crosses SS, SR, RS and RR. These served as parents in crosses with two other inbred lines (one susceptible, one resistant) to obtain six three–way crosses ranging in susceptibility from SSS to RRR. The relative level of resistance of the combination RRR was assumed to be 100% and that of SSS to be nil. The hybrid combinations and their assumed resistance levels are provided in Table 1. These were evaluated in two field trials during 1993/94, conducted in the same field at Potchefstroom (26∞43'S, 27∞06'E), with a planting date of mid–November to avoid natural infestation.

In trial 1 the single and three–way crosses were evaluated in a randomized block design with six replications. The plot size was two rows of 10 m, with a row width of 1.5 m to avoid larval migration between rows. The trial was planted by hand using two seeds per hill and thinned one week after plant emergence to a uniform stand of 28 plants per 10 m. All plants in one row of each plot were artificially infested four weeks after emergence with 10 neonate larvae per plant, using techniques described for *B. fusca* (Van Rensburg and Van Rensburg 1993). Grain yield, number of damaged internodes in 20 stalks per row, percentage damaged ears, percentage lodging and percentage rotted ears *(Stenocarpella (Diplodia) maydis)* were determined at harvest. Yield data (converted to t/ha), percentage lodging and percentage diseased ears were subjected to factorial analyses, using genotypes as factor 1 and infestation (infested vs uninfested) as factor 2. Since no plant damage was recorded in the uninfested rows, data on ear and internode damage were subjected to analyses of variance aimed at genotype differences only. All percentage values were arcsin transformed before analyses.

Table 1. Experimental hybrid combinations derived from crosses between adapted, insect–susceptible (S) and unadapted, insect–resistant (R) inbred lines.

Genotype	Resistance designation	Assumed resistance level (%)
(F2834t x M37W) x KO315Y	(SS)S	0
(F2834t x M37W) x Mp706	(SS)R	50
M37W x (Mp706 x F2834t)	S(RS)	25
M37W x (Mp706 x Mp707)	S(RR)	50
(Mp706 x Mp707) x M37W	(RR)S	50
(Mp706 x Mp707) x CML139	(RR)R	100
M37W x F2834t	SS	0
M37W x Mp706	SR	50
Mp706 x M37W	RS	50
Mp706 x Mp707	RR	100

In trial 2 only the four crosses (SS)S, (SS)R, S(RR) and (RR)R were evaluated. The general trial procedure was similar to trial 1, but plot size was increased to six rows of 10 m per genotype. These served as sub–treatments in which different levels of artificial infestation were applied five weeks after plant emergence, namely 0, 3, 4, 6, 7 and 10 plants infested per 10 m. The same variables as in trial 1 were assessed at harvest. Yield data (square root transformed) and percentage damaged ears were regressed on levels of infestation as the independent variable, using the model $Y = aX^b$. A non–linear model Y = a + b * Hyptan (x – x) was applied to the number of damaged internodes per 20 plants. Data on lodged plants and rotted ears were subjected to analyses of variance.

Another experimental hybrid was developed for evaluation at the commercial level under conditions of natural infestation. The single cross P150 x Mp706, (SR) which previously proved to be drought tolerant (Van Rensburg and Gevers 1993), was crossed to the locally prominent inbred line I137TN (S) as a pollen parent. The three–way cross was tested during the 1993-94 season in commercial plantings at two sites in the Northwest Province, Rysmierbult (26º21'S, 27º08'E) and Ottosdal (26º52'S, 25º47'E). The seed was planted mechanically in 20 alternate rows with a different commercial hybrid as the standard treatment at each site. The row width was 1.5 and 2.2 m respectively, and within–row plant spacing equivalent to 20,000 and 18,000 plants per ha, in accordance with local practice. A late–November planting date resulted in both trials being subjected to natural infestation. No chemical control or irrigation was provided. Yield, damaged ears, damaged internodes

and rotted ears were determined at harvest. Plots of 20 adjacent plants were randomly taken from each row of the experimental hybrid, as well as from the commercial standard in 20 of the adjacent rows. Mean values for each variable were calculated over the 20 replicates per genotype and compared by means of confidence intervals.

Results and Discussion

Resistance assessments on single and three–way crosses (trial 1) are presented in Table 2. Yield responses to infestation were closely correlated with both the incidence of damaged ears (r = 0.83) and damaged internodes (r = 0.82). Yield losses due to infestation of all plants ranged from more than one t/ha in the susceptible three–way cross (SS)S to virtually no loss in the fully resistant three–way cross (RR)R. With the exception of the combination (SS)R, the use of one and two resistant parents in a three–way cross reduced yield losses in accordance with the assumed resistance level of the hybrid (approximately 25% reduction in loss for each resistant parent included). The use of one resistant inbred as pollen parent in the second cycle of producing a three–way cross (SS)R was more beneficial in enhancing resistance than when used as one parent in the preceding single cross S(RS).

Compared with those for three–way crosses, yield losses for the single crosses were less severe in the susceptible hybrid (SS) but more pronounced in the fully resistant hybrid (RR). This may be attributed to differences in crop vigor, since the single cross SS was more vigorous than the three–way counterpart (SS)S, whereas hybrid vigor was largely absent in the single cross RR due to the close genetic relationship between the inbreds Mp706 and Mp707. These differences are reflected in yield potential as indicated by the yields of the uninfected sub–plots.

Susceptibility to both ear rot and lodging in the unadapted, resistant parental lines is indicated by the results presented in Table 3. Lodging was largely diminished by the use of a single adapted parent in any hybrid combination, but susceptibility to ear rot seemed to be reduced significantly only when two adapted parents contributed 75% in a three–way cross S(RS). It is noteworthy that neither the incidence of ear rot nor lodging was affected significantly by stalk borer infestation, indicating both traits to be genetically inherent to the unadapted, resistant inbreds.

The resistance assessment of selected three–way crosses at various levels of infestation (trial 2) is provided in Figure 1. Regression analyses provided a significant fit for all hybrid combinations with regard to yield (R^2 values from 70.9 to 96.4) and stalk

Table 2. Evaluation of experimental single and three–way crosses for stalk borer resistance (Trial 1).

Resistance designation	Resistance level (%)	Yield (t/ha)		Yield loss (t/ha)	% Damaged ears	Damaged internodes /20 plants
		Infested	Uninfested			
(SS)S	0	5.288	6.387	1.099	12.5	36.7
(SS)R	50	6.728	6.910	0.182	2.8	13.8
S(RS)	25	5.883	6.699	0.816	9.1	23.3
S(RR)	50	6.214	6.750	0.536	6.5	10.5
(RR)S	50	6.270	6.842	0.572	4.8	11.6
(RR)R	100	5.893	5.895	0.002	1.0	6.7
SS	0	6.133	6.850	0.717	14.2	32.6
SR	50	6.448	6.802	0.354	8.2	18.4
RS	50	6.116	6.507	0.391	7.8	18.8
RR	100	1.246	1.465	0.219	0.4	3.7
Mean	5.622	6.111	0.489	5.5	17.6	

Significance for yield: Genotypes F = 119.3, P<0.001; Infestation F = 27.3, P<0.001; Interaction F = 1.2, P = 0.283.
Damaged ears F = 14.09, P<0.001, Damaged internodes F = 11.86, P<0.001.

Table 3. Evaluation of experimental single and three–way crosses for ear rot (*Stenocarpella maydis*) susceptibility and lodging. (Trial 1).

Resistance designation	% Diseased ears		% Lodging	
	Infested	Uninfested	Infested	Uninfested
(SS)S	0.3	1.3	1.0	3.7
(SS)R	3.9	1.9	3.0	2.3
S(RS)	0.1	0.2	2.3	0.9
S(RR)	4.3	3.4	6.5	9.1
(RR)S	4.1	4.3	4.3	3.0
(RR)R	5.5	3.0	32.1	36.6
SS	0.2	1.2	4.3	3.2
SR	5.1	5.0	4.3	1.7
RS	3.7	2.6	5.6	2.5
RR	7.7	6.8	26.7	23.4
Significance	F	P	F	P
Genotypes	11.08	<0.001	17.5	<0.001
Infestation	0.12	0.728	0.34	0.564
Interaction	0.92	0.515	0.53	0.835

damage (R^2 = 96.7 to 99.6). A significant fit for ear damage was only obtained for S(RR) (R^2 = 51.1) and (SS)S (R^2 = 97.7). All four hybrids displayed an initial reduction in yield at only three infested plants / 10 m, after which losses were less pronounced in all the hybrid combinations containing at least one resistant parent in the genetic composition (Fig. 1A). Based on the amount of yield reduction at increasing levels of infestation, the fully resistant hybrid (RR)R suffered notably less, and the susceptible hybrid (SS)S more yield loss than the other two hybrid combinations. The same result is also observed in the incidence of damaged internodes (Fig. 1B) and damaged ears (Fig. 1C). It is important to note stem damage by *B. fusca*. The accepted economic injury level of 10% infested plants (Van Rensburg et al. 1988) equates to three infested plants / 10 m in this study. At this level an average of less than one internode per plant was damaged, yet notable yield losses were observed in all hybrid combinations.

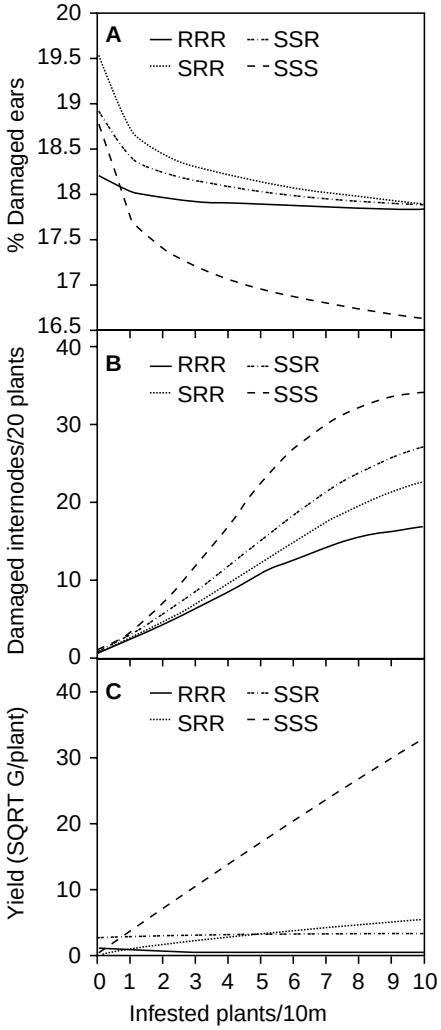

Figure 1. Yield responses and plant damage observed at increasing levels of infestation in three–way crosses with various levels of resistance.

The estimated yield losses derived by equation at the economic injury level were 5.8% (SS)S, 2.7% (SS)R, 4.0% S(RR) and 0.9% (RR)R. At a level of 35% infestation (10 infested plants / 10 m) the estimated yield losses were 11.5% (SS)S, 5.6% (SS)R, 8.3% S(RR) and 1.8% (RR)R. In spite of both (SS)R and S(RR) being 50% resistant, a greater level of resistance was achieved with the use of a single resistant parent than with two resistant parents in a three–way cross, illustrating the importance of the choice of parents in employing exotic, non–adapted germplasm in a hybrid combination (Gallun 1980).

The incidence of ear rot in trial 2 was 6.9% (RR)R, 4.5% S(RR), 2.9% (SS)R and 1.6% (SS)S. Lodging amounted to 41.4% (RR)R, 7.4% S(RR), 4.5% (SS)R and 4.3% (SS)S, confirming the susceptibility in insect resistant germplasm observed in trial 1.

The results obtained with an experimental three–way cross under commercial conditions are provided in Table 4. These results indicate the possible value that a level of only 25% resistance in a hybrid combination may have under practical conditions. At Rysmierbult the experimental hybrid suffered significantly less injury from stalk borer infestation than the commercial standard, resulting in a significant difference in yield of approximately 200 kg / ha. This yield difference is one which would normally justify the expense of chemical control of stalk borer. At Ottosdal the incidence of stalk and ear damage was significantly greater in the commercial standard than in the experimental hybrid, although yields did not differ significantly. The incidence of ear rot in the two hybrids was similar at Ottosdal, but significantly greater in the experimental hybrid at Rysmierbult. At both localities the level of infestation was moderate, whereas mid–summer drought conditions which often occur throughout the western production area were not experienced. Further testing under more typical conditions to assess agronomic acceptability of the experimental hybrid is therefore suggested. This also needs to be done in the absence of stalk borer infestation in order to ascertain comparative yield potential.

Table 4. Evaluation of an experimental three–way cross under commercial conditions at two localities. (Mean values followed by standard errors, n = 20 for all variables assessed).

Locality	Hybrid	Damaged internodes /plant	Damaged ears(%)	Diseased ears(%)	Yield (g/plant)
Rysmier	Experimental	0.84"0.17a	4.5"0.7a	17.0"2.4a	207.7"7.1a
bult	PAN 3614	2.10"0.30b	11.2"1.7b	10.2"1.1b	182.8"5.5b
Ottosdal	Experimental	0.72"0.13a	12.3"1.3a	3.6"0.6a	191.9"5.2a
	A 1650	2.08"0.23b	20.6"1.3b	3.5"0.5a	192.3"5.9a

Means within columns for each locality followed by different letters differed significantly at P=0.05 according to confidence intervals.

It can be concluded that unadapted germplasm may potentially be employed directly in three–way crosses in order to introduce resistance to *B. fusca*. This seems to be possible by using one resistant parent in the first cycle two–parent cross (RS). The expected increase in the resistance level of 25% of the resultant three–way cross (RS)S might be sufficient to eliminate the need for chemical control at moderate levels of infestation. This could be of considerable practical value, especially during years with reduced stalk borer populations. Since the seasonal abundance of stalk borers is linked to the rainfall cycle (Van Rensburg et al. 1987), comparatively low levels of infestation often occur over several years in a major part of the maize production area, resulting in significant yield losses but which cannot economically justify control by means of insecticides.

The use of either one or two unadapted parents to obtain 50% resistance in a three–way cross seems risky. In this study susceptibility to ear rot emerged at a low disease potential, suggesting an unacceptable risk of ear rot at higher disease potentials. This will also apply to other locally prominent diseases of which maize streak virus poses a particular hazard.

From the agronomic viewpoint ear prolificacy is a prerequisite of local maize hybrids. In this study the mean ear numbers per plant were recorded as 1.96 (RR)R, 1.81 S(RR), 1.76 (SS)R and 1.9 (SS)S, indicating prolificacy to be a positive trait of the unadapted germplasm used. Future evaluation is required, therefore, for other characteristics of local importance such as drought tolerance and kernel hardness.

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European Corn Borer Resistance: Evaluation of Commercial Maize Hybrids and Transgenic Maize Cultivars

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Abstract

Annual economic losses to producers because of European corn borer (ECB), Ostrinia nubilalis *(Hübner), damage to maize,* Zea mays *(L.), amount to several million dollars. This would be even greater if not for long-term host-plant resistance plant breeding programs in public and private organizations. To determine the degree of ECB resistance in commercial maize hybrids and the efficacy of transgenic plants to control ECB, experiments were conducted by manually infesting the plants in the research plots with neonate ECB larvae. Over a four-year period, 400 maize hybrids were evaluated. About 90% of the hybrids had some resistance to whorl-leaf feeding (first-generation ECB) and 75% had some resistance to sheath and sheath-collar feeding (second-generation ECB). In approximately two-thirds of these 400 hybrids, ECB resistance could be enhanced. Maize plants genetically transformed by using a gene(s) from* Bacillus thuringiensis *are effective in controlling the ECB throughout the life of the plant. As transgenic cultivars are developed and released, it will be necessary to have comparative, unbiased evaluations of performance from public institutions.*

If the contribution of host-plant resistance to crop production is recognized by producers and consumers, support for a practical, environmentally friendly means of control will be easier to obtain. The European corn borer (ECB), *Ostrinia nubilalis* (Hübner), is a major pest of maize, *Zea mays* (L.), throughout the maize growing areas of most of North America, Europe, and North Africa. In the US Corn Belt, estimated annual losses due to ECB range from $200-500 million. This loss would be much greater (Fig. 1, photo by B.E. Hodgson, 1918) if a significant proportion of commercial maize hybrids did not have some degree of resistance to ECB.

The ECB was introduced to the USA prior to 1917, when it was described as a pest of maize (Vinal 1917). Studies of plant resistance to ECB began in the USA as early as 1928 (Huber et al. 1928; Patch 1929, 1937, 1947; Patch et al. 1938, 1941). Many biological and ecological facts (Showers et al. 1989) were proven over the years, such as the existence of single or multiple generation strains of borer populations and of genetic differences among maize cultivars in susceptibility to ECB damage. Progress in developing first-generation, ECB-resistant inbreds was given a tremendous boost after artificial rearing techniques were developed (Beck et al. 1949; Bottger 1942; and Guthrie 1965). Two other significant contributions towards selection for ECB resistance were the development of a rating scale (Guthrie et al. 1960) and a manual infesting apparatus, the bazooka (Mihm 1983a, 1983b). For this symposium, we provide further explanation of maize plant resistance by using information of Barry and Darrah (1991):

Maize plant resistance to European corn borer embodies two distinct traits. One is resistance to whorl leaf feeding and the other is resistance to sheath collar feeding during flowering. These are quantitatively inherited traits, and if there are any common genes for resistance, they have not been identified. These traits, in the literature and in practice, are referred to as “first-generation European corn borer resistance,” which is associated with at least six genes (Scott et al. 1964, 1966) and “second-generation European corn borer resistance,” which is associated with at least seven genes (Onukogu et al. 1978). Generally, maize plants in early development (up to 25-30 cm tall for inbreds and 40-45 cm tall for hybrids) are naturally resistant to European corn borer. A chemical, 2-4 dihydroxy-7-methoxy-1,4-benzoxaxine-3-one, commonly known as DIMBOA,

which is in relatively high concentrations in young plants, can be the primary factor responsible for resistance to whorl leaf feeding. The whorl and flowering stages of plant development normally coincide with the spring emergence of adults and oviposition for first-generation adults, respectively; thus, the reasoning for the terms "first-generation European corn borer resistance." After borer adults emerge in the spring, approximately 45 d are required for the moths from the first generation to emerge as adults and by this time, maize plants are at the anthesis or flowering stage of development. This stage is favorable for the establishment of second-generation European corn borer. The plant provides a favorable oviposition site, and pollen grains are in abundance in the leaf axils for early larval feeding as the larvae migrate from the hatching site to the feeding site behind the leaf sheath.

The economic significance of European corn borer has been reduced by the identification and development of maize hybrids with genes for resistance to this insect. Sources of germplasm for whorl leaf feeding resistance have been identified within corn belt breeding material (Guthrie and Dicke 1972). Germplasm sources for sheath and sheath collar feeding resistance have been identified (Pesho et al. 1965; Guthrie et al. 1971; Onukogu et al. 1978; Russell and Guthrie 1979, 1982; Barry et al. 1983, 1985; Barry and Zuber 1984; Klenke et al. 1986a, b, c, 1987). Because they were not readily available in Corn Belt germplasm, and the identification process was much more laborious than for whorl leaf feeding resistance, the development of hybrids resistant to sheath and sheath collar feeding has lagged behind.

However, Barry et al. (1995) have released three inbreds, Mo45, Mo46, and Mo47 which have resistance to both generations of ECB. Commercial maize seed producers have been improving their hybrids by using information from public and private research to improve ECB resistance. In order to determine whether commercial maize hybrids were resistant to ECB, a four-year study was organized to evaluate 100 maize hybrids each year for four years (a total of 400 different maize hybrids were evaluated) (Barry et al. 1986, 1987, 1989). Because of drought in 1988, only whorl-leaf feeding data were taken and these were not publicly reported. A summary of the results of these evaluations are presented in Table 1 as adapted from Barry and Darrah (1991).

Hybrids that have been classified as susceptible in all years have a very small possibility of being misclassified for most environments, but hybrids classified as resistant or intermediate could possibly be more susceptible than indicated. This is because some plants may have been "escapes" or "partial escapes;" i.e., something may have happened to the manually infested insects other than the effects of any resistance factors in the hybrids.

The data for 1986, 1987, and 1989 show a higher degree of resistance to whorl-leaf feeding in commercial maize hybrids than for sheath and sheath-collar feeding. Means over years show 10% of the hybrids rating susceptible to whorl-leaf feeding and 25% for sheath and sheath-collar feeding. An explanation for this is that these are two distinct traits with different genes governing the expression of resistance. In Corn Belt germplasm, genes for resistance are present, and a technique is available to easily screen and rate whorl-leaf feeding resistance for a large number of genotypes. This is in contrast to the few genes identified as contributing to sheath and sheath-collar feeding resistance and the difficult, less precise techniques available for evaluating this damage.

The results of these evaluations, however, have shown that about 90% of the hybrids currently in production have some resistance to whorl-leaf feeding and about 75% have some resistance to sheath and sheath-collar feeding. Although not statistically comparable, similar whorl-leaf feeding data collected from 226 hybrids in Ohio in 1967 and 1968 (Barry 1969) indicate some resistance in 80% of the hybrids. For approximately two-thirds of the hybrids evaluated in Missouri, however, resistance levels could be further enhanced and susceptible hybrids could be improved with the introduction of additional genes for resistance.

Table 1. Distribution of commercial maize hybrids according to the level of resistance to whorl-leaf feeding and sheath and sheath-collar feeding (tunneling) by ECB. Adapted from Barry and Darrah (1991). Only whorl-leaf feeding ratings were obtained in 1988 because the plants were under extreme drought conditions, and those data are not included.

	ECB classification of hybrids tested		
Year	Percent Resistant	Percent Intermediate	Percent Susceptible
	Whorl-leaf feeding rating		
1986	25	67	8
1987	41	58	1
1989	26	54	20
Mean	31	60	10
	Sheath and sheath collar feeding		
1986	20	49	31
1987	44	50	6
1989	17	45	38
Mean	27	48	25

It has been estimated that the annual ECB damage to maize translates into a loss of several millions of dollars. If host-plant resistance selection were not a part of commercial maize breeding programs, the loss or increased cost of production would be much greater and might be sufficient to reduce maize production in some geographical areas.

After the ECB resistance for the various maize hybrids was determined (Barry and Darrah 1991), the question arose of would or how could the information could be used by producers. As it happened, two Illinois extension entomologists, Drs. M. Gray and K. Steffy, picked up on this and the following has been taken from their maize entomology recommendations:

We have gleaned the article (Barry and Darrah 1991) and have listed the corn hybrids that expressed the highest levels of resistance to both first- and second-generation corn borers in their trials. However, because tolerant hybrids were not identified, some corn hybrids that tolerate corn borer damage and produce yields at near-normal levels may not be listed. The hybrids are listed alphabetically; the order of the list suggests no preferences:

- Agrigene 7720
- Burrus 94
- Cargill 7877
- CFS 7615
- Crow's 688
- DeKalb Genetics 711
- Funk's G-4635
- Garst 8315
- Great Lakes GL-685
- McCurdy 7477
- Northrup King PX9581
- Pioneer Brand 3181
- Pioneer Brand 3184
- Pioneer Brand 3378
- Pioneer Brand 3471
- Taylor-Evans 7055
- Triumph 1990

Several of these varieties may share the same parentage as other popular varieties in Illinois. These hybrids may not be the highest yielding varieties, so you will have to weigh the importance of borer resistance against the importance of high yields in the absence of borers. If you are interested in more information about resistance of hybrids to borers, discuss this information with your seed dealer. It is important to note that the results of the evaluations in Missouri revealed that about 90% of the hybrids currently produced by the seed industry have some resistance to whorl leaf feeding and about 75% have some resistance to sheath and collar feeding.

Our strategies and method(s) of control for ECB are a continuous, on-going program of development in which we anticipate breeding for resistance with naturally-occurring genes to play a major part. We have a new tool from biotechnology, however, which we can use in pest management. It is called *Bt* (*Bacillus thuringiensis*) transgenic maize.

Transgenic maize plants are developed by bombardment of callus tissue with microprojectiles carrying *Bt*. By the genetic process of transformation, insecticidal crystal proteins (δ–endotoxins) are then able to be produced in maize plants. Some of the transgenic maize lines and hybrids developed from these efforts have proven to be very effective in controlling ECB. The insecticidal properties of these lines and hybrids are maintained throughout the growth of the plant. The concentrations of the δ–endotoxins in leaves, sheath, and sheath-collar sites, where young ECB larvae begin to feed, are effective in controlling both first and second generations of this insect. The larvae usually feed no more than enough to make a feeding scar (not even a hole) on the maize leaf or sheath. Most ECB larvae die within the day after attempting to feed and if any do not, they usually die shortly thereafter. Results of field evaluations of transgenic *Bt* cultivars clearly demonstrate the effectiveness of *Bt* plants as a tool for control of ECB (Table 2).

As with any management tool, use of *Bt* transgenic cultivars should be considered as part of the arsenal for controlling ECB. A significant concern is the development of resistance, over time, of pests to the insecticidal properties of *Bt* transgenic cultivars. Strategies are being developed in theory and practice to prevent or delay development of resistance in pests. Included are maintaining a population of ECB with a susceptible *Bt* transgenic cultivar (*refugia*), introducing more than one *Bt* transgenic source of resistance into the maize genome, and / or adding another effective non-*Bt* origin insecticidal protein to the genome.

Bacillus thuringiensis is a naturally occurring organism which is not harmful to higher animals. It has been registered as an insecticide (e.g., Bio-bit**,** Dipel) since 1961, and is considered one of the least hazardous insecticides ever developed.

The U. S. Environmental Protection Agency rules for the complete evaluation and use of these transgenic

Table 2. Effectiveness of *Bt* transgenic maize plants for control of ECB at Marshall, MO, 1994. Data are means from an evaluation done by D. Huckla and D. Barry (personal communication 1994).

Maize type	Insecticide treatment[†]	Manual infestation[‡]	Leaf-feeding rating[§]	No. of entry holes/plant	No. of larvae/plant	Tunnel length (cm/plant)	Harvestable ears[¶]	Yield (t/ha)
Non-*Bt*	None	ECB 1 & 2	2.8 ab[#]	1.7 a	0.3 bc	8.4 b	80.3 b-e	8.36 cd
	Pyrethroid weekly to post-anthesis	ECB 1	2.6 b	0.3 cd	0.0 d	1.3 ef	80.2 cde	8.71 bcd
	Pyrethroid weekly from V6 to V15	ECB 2	1.1 c	0.9 b	0.4 ab	5.6 c	81.7 b-e	9.41 ab
	None	None	1.1 c	1.6 a	0.5 a	10.9 a	77.0 e	7.85 d
	Dipel (SApp.)	ECB 1 & 2	2.6 b	1.0 b	0.1 cd	5.1 cd	79.2 a	8.38 cd
	Pyrethroid (SApp.)	ECB 1 & 2	2.8 a	0.5 c	0.1 cd	3.0 de	80.2 cde	8.59 cd
	Pyrethroid weekly from V6 to post-anthesis	None	1.1 c	0.0 d	0.0 d	0.3 f	82.5 a-d	9.97 a
Bt	None	ECB 1 & 2	1.0 c	0.0 d	0.0 d	0.0 f	85.2 ab	8.95 bc
	Pyrethroid weekly to post-anthesis	ECB 1	1.0 c	0.0 d	0.0 d	0.0 f	87.0 a	9.93 a
	Pyrethroid weekly from V6 to V15	ECB 2	1.0 c	0.0 d	0.0 d	0.3 f	85.0 abc	9.94 a
	None	None	1.0 c	0.1 d	0.0 d	0.3 f	83.8 a-d	9.17 abc
	Pyrethroid weekly from V6 to post-anthesis	None	1.0 c	0.0 d	0.0 d	0.0 f	84.8 abc	10.08 a

[†] Pyrethroid used was Pounce 3.2 EC. SApp. indicates a standard application done once 5d after manual infestation.
[‡] ECB 1 and 2 refer to first- and second-generation of ECB.
[§] Guthrie et al. (1960) 1-9 rating scale (1 = no damage, 9 = severe damage).
[¶] Mean number of harvestable ears in 18.3 m of row length.
[#] Means in a column with the same letter are not significantly different at the 0.05 probability level.

plants have not been completely formulated. As for conventional insecticides and resistant maize hybrids, these evaluations should be a part of public research programs.

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Use of CIMMYT's Multiple Borer Resistance Population for Developing Asian Corn Borer Resistance and Inbreds in China

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Abstract

After a brief background introduction on the importance of maize, Asian corn borer (ACB), Ostrinia furnacalis *(Guenée), and breeding and improving for host plant resistance (HPR) to ACB in China, we report on efforts to develop ACB resistant inbred lines for use in hybrids with CIMMYT's multiple borer resistance (MBR) populations. In 1986, ACB resistant inbred development with CIMMYT's MBR population was initiated. Several resistant inbreds, such as MC37, MC61, MC74, HM31 and HM67, with potential for use in hybrid crosses, were developed by selfing and selecting highly resistant types within each selfed generation after artificial infestation with ACB at whorl stage. On the basis of this work — together with additional support from CIMMYT in the form of highly resistant maize populations, financial contributions, vigorous efforts to promote cooperation between entomologists and breeders, and advanced training for young scientists — we began a new project to develop ACB-resistant inbreds using MBR-590 and the CIMMYT multiple insect resistance tropical population, MIRT-390. Finally , we describe a successful adaptation of "bazooka" technique in China.*

Introduction

Asian corn borer, (ACB), *Ostrinia furnacalis* (Guenée), is closely related to the European corn borer, *O. nubilalis* (Hübner), and is the most destructive insect pest of maize in China. From north to south, it has one to seven generations a year (Fig. 1). Throughout the vast territory of the country, however, for a particular crop of maize only one or two generation(s) occur. Generally, one generation attacks at the whorl stage and the other at the pollen-shedding stage. In a normal year, the annual loss caused by ACB is 10% in spring maize and 20-30% in summer maize, where no controls are used.

Many effective control methods, such as chemical treatment with extended residue granular insecticides, biological control with *Trichogramma,* and cultural practices, have been developed. Still, about 80% of the area in China's Corn Belt remains untreated for economic reasons and for lack of labor. Based on over 30 years experience in ACB research, Prof. Zhou concluded that components of an integrated management strategy for ACB must be inexpensive, have a high and stable controlling effect, be simple and easy to apply, and not pollute. With these criteria in mind, host plant resistance (HPR) in maize is considered the best and most basic way to minimize losses from ACB.

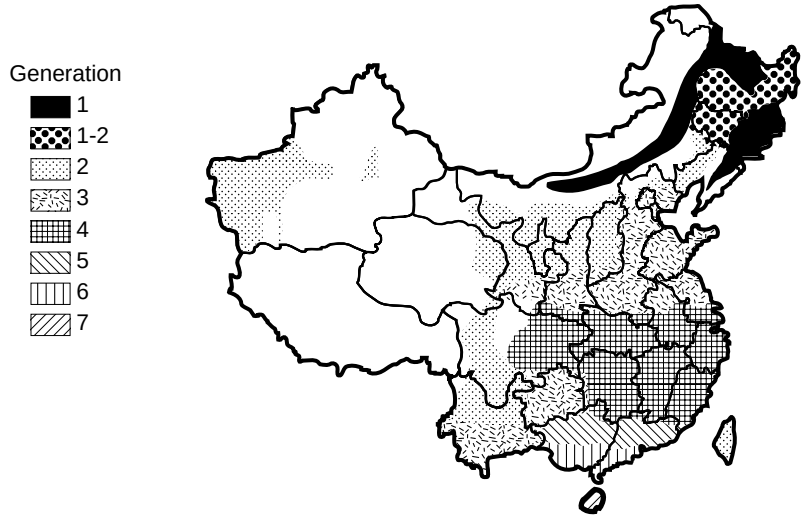

Figure 1. Approximate distribution of generation zones of the ACB in China.

Techniques for mass rearing ACB and evaluating resistance to ACB in maize, two essential elements for efficient screening and improving of HPR, have been developed successively in China (Zhou et al. 1980; Zhou 1982; Zhou and Chen 1989). As of 1982, more than 1,048 inbred lines and 485 varieties and synthetics were evaluated by the All China Corn Borer Research Group (ACCBRG). Although most of those lines, especially the elite ones, were found to be susceptible, a few resistant ones exist. Ji404 was an outstanding example. Later, certain promising lines with high resistance derived from Ji404 x elite line crosses were developed by using a method called second cycle selection. From this process, a single-cross hybrid, ZHIDAN NO.1, which could be used for efficient control of ACB at the whorl stage (Zhou et al. 1987), was released. Though the area planted to this hybrid was limited due to its substandard yield and the susceptibility to viral disease of the female parent, it still showed that the use of resistant hybrids is actually the best, most practical, most economical, and most effective means to minimize losses from ACB in China. Zhou et al. (1987) concluded that the availability of sufficient resistant germplasm and the application of modern and effective breeding techniques are the two most important factors in a successful program to develop ACB-resistant hybrids.

It is well known that heterosis is usually observed for crosses where the parent inbred lines are genetically diverse. Unlike correlation and visual selection, the genetic diversity of inbred lines used in crosses is generally recognized to be important. It is assumed that, to have a reasonable chance of success, one should make selections from exotic tropical and subtropical materials as sources of lines for use in hybrids and of genes for disease and insect resistance. In this paper, we describe the use of CIMMYT multiple borer resistance populations in resistance screening and the development of ACB-resistant inbred lines.

Materials and Methods

In 1986, 114 families of CIMMYT's Multiple Borer Resistant Population (MBR) were planted in Beijing. The evaluations of resistance to ACB were done by artificial infestation at whorl stage. Using these materials as an exotic source of resistant germplasm, efforts to develop ACB resistant inbred lines were initiated using the following two procedures.

1. Developing inbreds from the MBR population

Self-fertilization has been used primarily for inbreeding under artificial infestation with ACB at the whorl stage. In order to provide a broad base that permitted effective selection concurrently with inbreeding under diverse environmental conditions, the selection was conducted within-family in year 1. The S_1 seeds were bulked within-family to create the respective S_1 families. The following season, year 2, S_1 families were planted and infested again. Rows that appeared to be the most resistant were selected on the basis of ratings of leaf feeding damage. Within these rows the better plants were self-pollinated and progressed to S_2. The resulting ears from selfed plants were planted out ear-to-row in year 3. Selections and re-evaluations were made not only for ACB resistance, but also for other major diseases resistance, earlier maturity, short plant stature and tolerance to lodging among rows. Better plants were selfed within these rows. The process carried out in year 3 was repeated for three generations. These inbreds were then selfed and individually crossed onto local lines for yield trials under artificial infestation with ACB at the whorl stage. From the results, the potential single crosses and promising lines were predicted for experimental hybrids. At all times in line selection, detailed notes were taken on agronomic as well as resistance traits, as any new hybrid will have to be competitive against the released ones. The procedure we followed is outlined in Table 1.

2. Developing Inbreds from MBR X Local Lines

When MBR populations were evaluated and self-pollinated in 1986, some of their resistant families were also individually crossed as male parents onto several locally adapted lines, such as Zi330, Ji63, E28, 122 etc., which combined the two groups of the genetic bases (Table 2).

In case the MBR populations and their progenies would not be well adapted under all the diverse environmental conditions, these crosses would serve as the genetic base to permit further selection and modification of the desired traits. In the following season, the crosses were planted and infested with ACB at the whorl stage. Selections and self-pollination were made within rows. The resulting seeds were bulked and planted out next season, respectively. Additional selection and re-evaluation was carried out within the S_1s, which were then selfed to S_2. The following season, year 3, these S_2s were planted out ear-to-row, and those which appeared to be the most resistant were selected on the basis of ratings of leaf feeding damage under ACB infestation. Better plants were

Table 1. Procedure for developing ACB resistant lines from MBR population in Beijing.

Timescale	Processes
Year 1	
	Plant MBR population
	Artificially infest plants with ACB
	Evaluate for resistance
	Self-pollinate most resistant plants
	Bulk S_1 seed within-family
Year 2	
	Plant S_1 families
	Infest and evaluate
	Select most resistant S_1 families
	Self-pollinate better plants in selected rows
Year 3	
	Plant ears from selfed plants ear-to-row
	Infest and evaluate
	Select for ACB resistance, major diseases resistance, earlier maturity, short plant stature, tolerance to lodging and good plant aspect
	Self-pollinate better plants in selected rows
Following Years	
	Repeat the procedure described in year 3
	Self to inbred Cross onto local adapted lines
	Evaluate crosses for ACB resistance, yield performance and other agronomic traits
	Select the potential crosses and promising lines

Table 2. Procedure for developing ACB resistant lines from MBR x local lines in Beijing.

Timescale	Processes
Year 1	
	Form crosses between local lines and some resistant MBR families
Year 2	
	Plant the crosses
	Infest and evaluate
	Self-pollinate better plants in selected rows
	Bulk S_1 seeds, respectively
Year 3	
	Plant the S_1's
	Infest and evaluate
	Self-pollinate better plants within rows
Year 4	
	Plant S_2's ear-to-row
	Infest and evaluate
	Select for ACB resistance, major diseases resistance, earlier maturity, short plant stature, tolerance to lodging and good plant aspect
	Self-pollinate better plants in selected rows
Following Years	
	Repeat the procedure described in year 3
	Self to inbred
	Cross onto local lines
	Evaluate crosses for ACB resistance, yield performance and other agronomic traits
	Select the potential crosses and promising lines

selfed to S_3 within the rows. Then, the process of year 3 was repeated in the following seasons. Other processes and notes were taken as in procedure 1.

Results

A histogram showing the numbers of families classified as resistant, intermediate, and susceptible is presented in Figure 2. Ratings were done with a 1 to 9 scale, where 1 was the most resistant and 9 the most susceptible. The resistant class included families rated from 1 to 3, intermediate from 4 to 6, and susceptible from 7 to 9.

Most (85% or more) of the 114 MBR families tested were rated as resistant, and thus were comparable to the resistant check (122) which was one of a few best materials locally available for ACB resistance and showed no significant level of insect damage. One family rated intermediate, and 15 families(13.2%) susceptible. This indicates that MBR is an excellent source material of ACB-resistance.

Several highly resistant inbreds have been developed with the two procedures used by our program. Ratings of leaf feeding damage sustained by these inbreds and a local

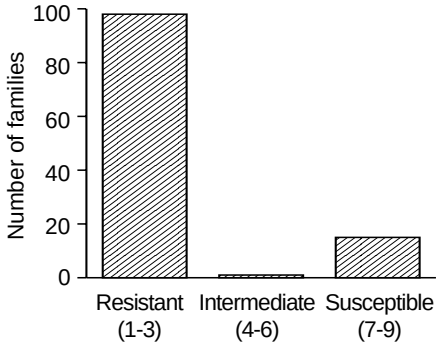

Figure 2. Asian corn borer damage ratings of 114 families of CIMMYT's MBR population planted in Beijing.

susceptible check, Zi330, in 1992 are given in Table 3. The inbreds MC37, MC61 and MC74 were derived from MBR, whereas the inbreds HM31, HM67 and HM15 were derived from local lines x MBR.

Table 4 shows the yield and ACB resistance performance of some potential crosses developed by our program under artificial infestation with ACB at the whorl stage. They were not only resistant to ACB, but also demonstrated their good yield potential, and promise in probable hybrid use.

Discussion

Although the MBR population was of tropical and subtropical adaptation and is considered to contain tremendous genetic diversity, compared with local temperate materials, all 114 families introduced were able to mature in spite of their relatively late maturity, high plant, big tassel and long and thick husk cover when grown under the temperate environment in Beijing. It was recognized that considerable potential existed for screening and developing highly adapted temperate ACB resistant lines from MBR. The two procedures used were effective in developing ACB resistant inbreds. However, certain deficiencies remain and still need to be improved for Chinese conditions. For instance, continuous self-fertilization seems to be too drastic, thus the MBR population traits were lost too quickly. A milder form of inbreeding that still permits effective selection should be used. In addition, yield and topcross testing should be done at an earlier stage.

Table 3. Ratings of leaf feeding damage of inbreds developed under artificial infestation with ACB at the whorl stage.

Inbred	Rating
MC37	1
MC61	1
MC74	2
HM31	1
HM67	1
HM15	2
Zi330	9

Table 4. Yield performance of the potential hybrid crosses developed

Hybrid Cross	Rating	Yield (g/plt.)
MC37 X YUANFU30	2	146.3
MC61 X HM31	1	132.3
MC74 X 525	3	117.6
SANTUAN4 X MC61	3	136.6
Zi330 X HM67	3	121.0
HM15 X YELLOW EARLY 4	2	127.8

Important contributions from CIMMYT to HPR study in China

Mihm (1985) stated that an interdisciplinary team having at least an entomologist and a breeder is desirable to carry out the HPR program. In China, however, most breeders pay no attention to HPR. They always consider that it is easy to control ACB by using insecticides, but breeding and improvement of HPR to insects is very difficult. So, until 1992 the research on HPR had been done mainly by the entomologists, who usually lack maize breeding skills. The situation, however, has been changed in certain aspects since Dr. Mihm's visit to Beijing in 1992. His viewpoint and outstanding work on HPR to borers made a very deep impression on Chinese breeders and resulted in a vigorous push towards cooperation between entomologists and breeders from major institution, although this process is just at the initial stage.

In 1993, fortunately, the senior author got a precious opportunity to attend the two training courses held at CIMMYT, i.e., Maize Breeding for Insect Resistance and The Maize Breeding Course. From these he obtained a lot of knowledge in the field of maize breeding and breeding for insect resistance. On the basis of work mentioned above and our situation, together with CIMMYT's further support in giving highly resistant maize populations and a financial contribution, a new project for developing ACB resistant inbreds with CIMMYT's populations MBR-590(temperate) and MIRT-390(tropics and subtropics) has been actively undertaken. One seasons results, histograms showing the contributions of numbers of families classified as highly resistant (HR), resistant(R), moderately resistant(MR), susceptible(S), and highly susceptible (HS), are presented in Figures 3 and 4.

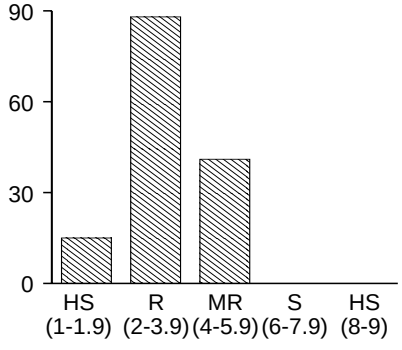

Figure 3. Asian corn borer damage ratings of CIMMYT's MBR-590 planted in Beijing.

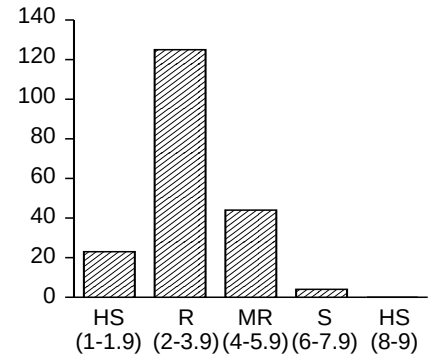

Figure 4. Asian corn borer damage ratings of CIMMYT's MIRT-390 planted in Beijing.

HR class included families rated from 1 to 1.9, R from 2 to 3.9, MR from 4 to 5.9, S from 6 to 7.9, and HS from 8 to 9. It indicated that MBR-590 and MBR-390 are excellent source materials of ACB resistance. We hope that new inbreds with resistance to ACB, and other major maize diseases, and with good yield performance can thus be developed and used in hybrid production by our new program.

Modification and adaptation of the bazooka method for efficient field infestation of ACB

Until 1993, artificial infestation with ACB in China had always been done by placing two egg masses or glass tubes containing 30 to 40 newly hatched larvae into maize plant whorls. These techniques can be used effectively for infestation, but they are very inefficient because of the many laborious steps, such as cutting egg masses, placing egg masses ready to hatch into glass tubes, and the slowness of field application. Although the bazooka method for larval infestation has been used to infest many species of lepidopterous insect pests (Mihm 1987), it had not been possible to adapt it to our situation, due to the fact that egg masses could not be removed from egg-mass sheets quickly. Hence, procedures were developed to overcome this problem.

In our laboratory, waxy paper(27 x 44 cm) sheets are placed on top of oviposition cages for oviposition. The sheets containing egg masses are removed and replaced with new ones every morning. The egg-mass sheets are then kept at 28ºC and >75% RH for about 2 days. When the egg masses become nearly ready to hatch, egg-mass sheets are dehumidified in a low humidity room for 30 to 60 min. and then cut into 3 strips (about 9 cm wide) along the long axis. After that, the same procedures described by Mihm(1989) are followed for removing and collecting egg masses, mixing the hatched larvae with corn cob grits, and infesting in the field.

In the other procedure, the egg-mass-sheets are slit into four equal-sized smaller pieces, and kept in total darkness at 28ºC. When egg masses reach the black-head stage, they are incubated at 15ºC until larvae hatch. At this relatively low temperature the egg masses can develop continually, but the newly hatched larvae are not active. For mixing the hatched larvae with corn cob grits, the larvae are transferred to the mixing bottle by snapping the back of the sheets with fingers. With such a procedure, the process of removing egg masses from the sheets can be omitted.

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Corn Borers Affecting Maize in Egypt

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Abstract

In Egypt, maize plants are severely attacked by different species of Lepidopteran pests, the most important being the corn borers: the pink borer or greater sugar cane borer, Sesamia cretica *Led (Noctuidae); the purple-lined borer or lesser sugar cane borer,* Chilo agamemnon *Bles. (Crambidae), which are the principal borers of sugar cane and rice in Egypt; and the European corn borer,* Ostrinia nubilalis *Hbn. (Pyraustidae). These borers are also considered the principal cause for the secondary infection of fungal and bacterial diseases.* Sesamia cretica *is considered the most serious of the borers. This species attacks maize plants shortly after emergence, devours the whorl leaves and may kill the growing point, causing dead hearts. It is also capable of damaging older plants and excavating tunnels into the stem, ears and/or cobs. This pest lays its eggs during March, so it causes complete death of small maize plants in April and May, leading to drastic yield losses. Chemical insecticides are commonly used to control* S. cretica, *but given the negative environmental side effects, associated with chemical control, development of maize cultivars with resistance to* S. cretica *offers a better alternative. The Egyptian national maize breeding program is concentrating its efforts to develop and release new white and yellow maize hybrids with high yielding ability, plus resistance to the major diseases such as late wilt, common smut, downy mildew and leaf blight, as well as resistance to insect pests.*

Introduction

Maize is considered one of the most important cereal crops in Egypt. The total cultivated area is about 0.84 million ha for early (May-June) and late (July-August) plantings. The total national production of maize is about 5.3 million tons. About 2.0 million tons of maize are imported annually as the total consumption has reached 7.0 million tons. The national yield average was 6.5 tons / ha in 1993, but this value is still below the expected yield potential (Abou El-Saad 1994). Our target is to reach an average yield of 8.5 tons / ha. This is a realistic possibility, because there is an increased tendency for farmers to use high yielding, disease and pest resistant single and three-way cross hybrids.

Control of *S. cretica* in maize fields is commonly done by the application of chemical insecticides, either as sprays or granules, directly to the whorl. Side effects of this chemical control on the agroecosystem include the destruction of natural enemies of pests, outbreaks of mite populations and environmental pollution. To avoid or at least minimize such side-effects, growing maize cultivars resistant to *S. cretica* is highly recommended (Simeada 1985). The Egyptian national maize breeding program is concentrating its efforts to develop and release new white and yellow maize hybrids with high yielding ability, plus resistance to the major diseases such as late wilt, common smut, downy mildew and leaf blight, as well as resistance to insect pests. A considerable number of new white and yellow inbred lines have been isolated and developed using different breeding techniques. Several genetic sources for higher yielding ability, better plant type as well as resistance to diseases and pests have been obtained.

Evaluation and Development of New Hybrids

New hybrids developed through the breeding program are evaluated and advanced in two stages, before release for commercial production. The first stage consists of four different on-station evaluation trials:

- A Trials. Top crosses are evaluated in 2-3 locations to estimate general combining ability (GCA) and specific combining ability (SCA), using the best local hybrids as checks. The promising hybrids are advanced to the AH Trials.
- AH Trials. For evaluating single, three-way and double crosses derived from the A Trials in three locations. The promising hybrids are advanced to B Trials.
- B Trials. Promising hybrids from the national maize program, as well as from local and foreign seed

companies, are tested at five research stations. Superior hybrids are advanced to C Trials.

- C Trials. Hybrids advanced from B Trials are tested in C Trials. These trials are conducted in a disease nursery at five research stations to evaluate hybrids for their resistance to the major diseases, late wilt, common smut, downy mildew and leaf blight. Promising hybrids are advanced to the verification trials in the farmers fields.

The second stage of the development process involves verification trials (D Trials), where superior hybrids derived from C Trials are evaluated in the farmers fields in trials conducted in at least 10 governorates in the Delta and Upper Egypt regions.

Progress Towards Host Plant Resistance in the Egyptian Maize Program

The greater sugarcane borer, *Sesamia cretica* Led., is the most important of the borers which affect maize in Egypt. Yet, despite the agricultural importance of this pest, very few studies exist in the published literature on the relative susceptibility of maize plants to *Sesamia cretica* Led. A review of the limited available knowledge on *Sesamia* indicated that several investigators had evaluated maize varieties commonly cultivated in Egypt, with respect to susceptibility to infestation by *S. cretica.* Unfortunately, most of these investigations were carried out on obsolete cultivars under natural infestations. Results obtained under these conditions do not usually reflect the real level of susceptibility or resistance in the cultivars screened.

Studies of the seasonal distribution of borers affecting maize, done about 20 years ago, revealed that fields planted before the beginning of May are severely infested by *Sesamia* (Fig. 1). They lay their eggs beneath the sheath of first or second leaves on maize plants 20 days after planting. After hatching the larvae feed on furled leaves causing leaf damage and dead hearts. Maize planted after the beginning of July is subject to high infestation with *Ostrinia,* which attacks maize 45 days after planting. Hence maize growers in Egypt are encouraged to plant their maize during the period from the beginning of May to mid-June, in order to escape severe infestation by the two borers. This recommendation decreases the need for intensive use of insecticides, so minimizing environmental pollution. Specific biological and ecological studies revealed that the main reason for this phenomenon was the environmental conditions occurring during summer in Egypt. The hot and dry conditions were found to be unsuitable for the adults to mate and lay fertile eggs. However, it was noted that a small proportion of the borer population became adapted to the summer conditions. This proportion is expected to increase gradually and threaten maize fields planted during the aforementioned "safe period". Hence, the Egyptian national maize breeding program has decided to attempt to develop, and use, maize with host plant resistance.

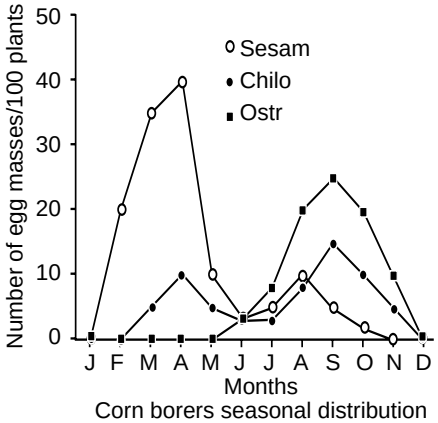

Figure 1. Corn borer seasonal distribution in Egypt.

Any program that is to be successful in developing maize varieties resistant to insect pests and with good agronomic characteristics has to have seven basic components (J.A. Mihm pers. comm.), these are:

- Maize germplasm, including some with genes for resistance.
- A supply of insects (a colony). Depending on the requirements and desires of the program, these may be reared on natural hosts, or on artificial diets in the laboratory.
- Capability to artificially infest.
- Capability to rapidly assess damage, or lack of it, after infestation. This usually means developing a rating scale that identifies the category and level of resistance (antibioses type), into high, intermediate, low or susceptible.
- Knowledge of the inheritance / heritability of the resistance.
- An interdisciplinary team, consisting of entomologists, breeders and pathologists.
- The resources to execute all steps of the program. This is basically the dedication, money and trained people.

Host plant resistance is based on the presence of genes for resistance. Hence, the first stage in our program has been to screen local materials for resistance. If these are found to be susceptible, then the second step is to screen exotic materials. We already have most of the most advanced and best materials, with known resistance to borers. Once genes for *Sesamia* resistance are identified they can be utilized.

There is no way to identify genes for resistance in maize plants without having insects on the plants at the proper stage. No program anywhere in the world has developed resistant varieties by selecting "undamaged" plants that were naturally infested (J.A. Mihm pers. comm.). In order to select plants with resistance genes, one has to see the amount and type of damage that occurs when insects are feeding on the plant. In order to achieve these goals we have just established a maize borer rearing laboratory.

Investigations into other non-chemical control methods, such as the effect of different plant densities, as well as, using different rates and combinations of nitrogen (N), phosphorus (P) and potassium (K) fertilizers on the infestation level of *Ostrinia,* have also been carried out (Awadallah, et al. 1980). The results indicated that the levels of N fertilizer, which increase grain yield without causing a significant effect on the infestation level, ranged between 144 and 216 kg / ha. Phosphorous and potassium applications did not affect the infestation level of this borer. For *Sesamia*, it was found that planting with 5-6 kernels / hill and removing the infested plants at thinning before the first irrigation resulted in the removal of about 80-85% of the insect population (Awadallah, et al. 1980). Other studies revealed that early maize can be intercropped in onion fields just before the last irrigation of onion. In this case, the onion's odor repels the *Sesamia* moth and consequently the infestation level with borer is greatly decreased, (Awadallah, et al. in press).

We hope to start up our breeding program for host plant resistance including artificial infestation for about 500 families (local and exotic) during the 1995 season.

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Search for Multiple Resistance in Maize to Stem-Borers Under Natural Infestation in Midaltitude Intermediate Maturity Areas in Kenya

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Abstract

The search for multiple borer resistance in maize, mainly against Chilo partellus *(Swinhoe) and* Busseola fusca *(Fuller), requires routine screening of a large number of germplasm sources. In the present investigation, the search for multiple borer resistance involved evaluation of inbreds, (local and exotic) synthetics, open pollinated materials and hybrids. The parameters that were used for evaluation were based on infestation level (larval and pupal density) and damage levels (foliar damage, stalk tunneling, borer exit/ entry holes). Preliminary results indicated significant (P=0.05) differences between cultivars/lines in the parameters that were used for evaluation. There was a positive and significant (P=0.05) relationship between foliar damage and tunnel length. As evaluations were done under natural infestation, results on yields as a measure of resistance were not considered. From the data presented, it can be concluded that some parameters, like foliar damage and tunnel length, may be used as possible selection characters in resistance breeding. However, controlled uniform artificial infestation is required to obtain consistent results.*

Introduction

Stem borers in maize are considered to be the most important pests of all graminaceous crops in the world (Jepson 1954; Hill 1975). These borers constitute one of the major constraints to efficient maize production in the developing world, where maize is considered as one of the most important subsistence crops (Scheltes 1978).

Studies in Kenya have showed that the stem borers *C. partellus*, *C. orichalcociliellus*, *B. fusca*, and *Sesamia calamistis* were the most important borers of maize and sorghum (Seshu Reddy 1983). *C. partellus* comprises 90% of all the borer species infesting maize in Kenya, causing yield losses of about 18% to 40%. Several stem borer control methods have been utilized, but the typical control method is insecticides (Warui and Kuria 1983). This is usually not an economic proposition and is often an ineffective approach in subsistence farming systems. This is because the current recommendations are only moderately effective, mainly due to the timing of application.

Host plant resistance (HPR) has been shown to offer the most effective, economical, stable and ecologically sound approach to reducing damage (Ampofo 1986). HPR is an innate quality that renders the plant unsuitable as food or shelter for insect pests.

Since most of the cultivars developed by Kenya's maize improvement program are susceptible to stem borers, it was necessary to look for ways of incorporating HPR into the currently recommended hybrids and open pollinated cultivars. However, successful breeding for multiple borer resistance (MBR) depends mainly on developing suitable procedures for screening and on identifying the physical traits responsible. The objectives of this study were to:

- Identify sources of resistance to stem borers.
- Develop procedures to be used in resistance screening in breeding programs.

Materials and Methods

This work was carried out at the Regional Research Center, Embu, during the 1992-94 cropping seasons. Twenty-three maize cultivars and inbred lines, obtained from the local breeding nursery and from the International Maize and Wheat Improvement Center (CIMMYT),

Mexico, were evaluated for resistance to stem borers. Two local commercial hybrids (H511 and H512) were included, together with two open pollinated cultivars (KCB and DLC 1). During the experimental period, inbred A was used as a susceptible check.

Each cultivar/line was planted in the field in triple row plots at a spacing of 90 x 30 cm between and within rows, respectively, in a randomized complete block design with three replications. This design was used to give all plants an equal opportunity of being selected by the ovipositing adult moths. The parameters that were tested as possible sources of resistance or susceptibility were:

- Foliar damage rating. This was done on 10 plants selected at random using a scale of 1 to 9, where 1 was regarded as no damage and 9 meant severe foliar damage (Guthrie et al. 1960).
- Stalk tunneling. Measurements were taken at harvesting from plants selected at random. The length of the tunnel above and below the ear was expressed as a percentage of plant height.
- Number of larvae and pupae of each species. This was determined at 3-week intervals from another random set of 10 plants per plot.
- Entry/exit holes. Below and above the ear from the plants that were used in (3) above. The holes were detected by the presence of frass deposits.

An analysis of variance was carried out for the various parameters used (damage and infestation levels), and multiple regression analysis was also done to test the relationship between these parameters.

Results

Most of the cultivars/lines that were screened under natural conditions for MBR showed significant (P=0.05) differences in their response to damage and infestation levels. CIMMYT-derived materials that were initially reported as borer resistant and the local composites showed lower levels of infestation and damage (Table 1). However, most of the inbreds derived from H511, E11 and E12 had significantly (P=0.05) higher infestation and damage levels for all the parameters that were tested when compared to susceptible check inbred A (Table 2). Foliar rating and tunnel length were significantly (P=0.05) higher in all those lines that showed higher means for all other parameters used. Similarly, these inbreds also had significantly (P=0.05) higher larval/pupal densities than those showing lower means. There were indications that lines extracted from H511, Embu 11 and 12 have a higher degree of borer susceptibility. This was the same in

Table 1. Levels of damage and infestation by the stem borer in different maize cultivars/lines.

Cultivar	Foliar damage rating	% tunnel length	Exit holes per plant	No. of larvae and pupae per plant
Inbred A	2.15a	2.20a	1.33	0.9b
H512	1.0b	1.34a	1.03	1.80a
E 11	0.95b	2.73ab	1.87	1.61a
PR 86 MBR	0.23b	1.67ab	1.07	1.09b
DLC1	0.68b	1.95b	1.29	1.37ab
KCB	0.50b	2.00ab	1.14	1.05b
PR 8523 SCB	0.73b	1.39ab	0.82	1.02b
H511	0.77b	1.83ab	1.05	1.13b
PR 86 CHICO	0.71b	1.33b	0.99	1.21ab
LSD	1.41	1.29	0.63	0.61

Table 2. Levels of damage and infestation by stem borers in different maize lines under natural infestation.

Cultivar	Foliar damage	Exit holes		Chilo spp.	Busseola spp.	Tunnels	
		Above ear	Below ear			Above ear	Below ear
E11 Syn1	1.08	0.93	0.98	0.73	0.76	1.03	0.99
E11 L.18	1.04	0.71	0.72	0.71	0.71	0.71	0.71
KCB	1.02	0.72	0.73	0.71	0.72	0.71	0.78
DLC 1	1.01	0.72	0.73	0.71	0.72	0.77	0.76
E12 L139	1.09	0.72	0.87	0.71	0.77	0.71	0.91
E12 L163	1.16	0.77	0.76	0.71	0.73	0.82	0.82
H511 L225	1.13	0.71	0.73	0.73	0.71	0.71	0.82
H511 L8	1.20	0.73	0.97	0.82	0.73	0.76	1.02
Popu X1	1.08	0.72	0.78	0.71	0.73	0.77	0.92
MUVC9014SR	1.07	0.89	0.77	0.78	0.71	0.94	0.77
E11 L133	1.13	0.79	0.77	0.74	0.71	0.78	0.87
H511 Syn1	1.09	0.78	0.87	0.73	0.71	0.73	1.00
E12 Syn1	1.00	0.74	0.81	0.71	0.74	0.78	0.87
Popu X2	1.02	0.73	0.77	0.71	0.71	0.72	0.82
E12 L3	1.06	0.79	0.94	0.73	0.75	0.81	1.30
E12 L210	1.06	0.72	0.86	0.71	0.76	0.77	1.29
Inbred A	1.13	0.71	0.95	0.71	0.72	0.71	1.02
H511 L196	1.09	0.72	0.82	0.73	0.72	0.72	0.84
H511 Comm	1.14	0.75	0.95	0.71	0.84	0.77	0.88
LSD	0.04	0.05	0.08	0.03	0.03	0.07	0.14
CV	12.50	19.33	28.71	9.36	9.78	26.01	41.84

varietal cross MUVC 9014 SR and double crosses that had the same parentage as H511. The most distinguishable parameters were the level of foliar damage, number of larvae and pupae, and stalk tunneling.

In multiple regressions to determine the correlation between parameters, all were positively correlated, with the correlation coefficient being highly significant (P=0.01) r =0.496. For example, there was a positive relationship (r=0.35) between foliar damage, tunnel length below the ear, and the larval/pupal density (Figs. 1 and 2). Regression analysis also clearly indicated that tunnel length increases considerably as rating increases.

Discussion

Locally grown open pollinated maize cultivars (composite) are more resistant to stem borers than the hybrids and inbred lines. Omolo (1983) had earlier attributed this to their early maturing nature, resulting in avoidance of second generation borers. This was also true for MBR materials from CIMMYT, which were early-to-medium in maturity. It is also evident that most of the inbreds derived from H511, E11 and E12 or their progenies have no resistance to borer damage. These lines, although of medium maturity, were attacked by second generation borers, as evidenced by data on the mean number of exit holes and mean tunnel length above the ear.

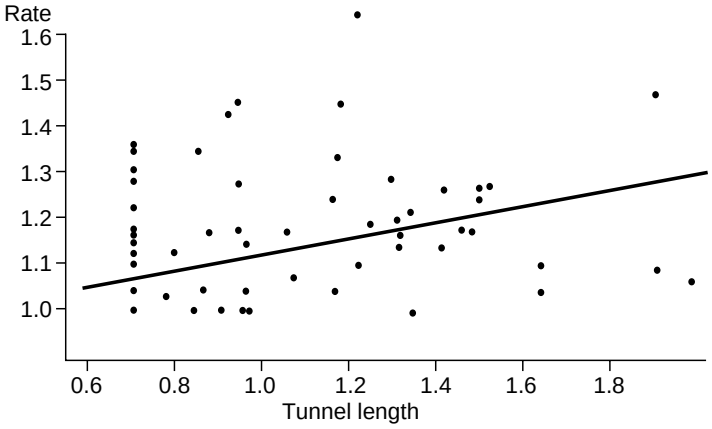

Figure 1. Relationship between foliar damage and tunnel length in maize under natural infestation with stem borers.

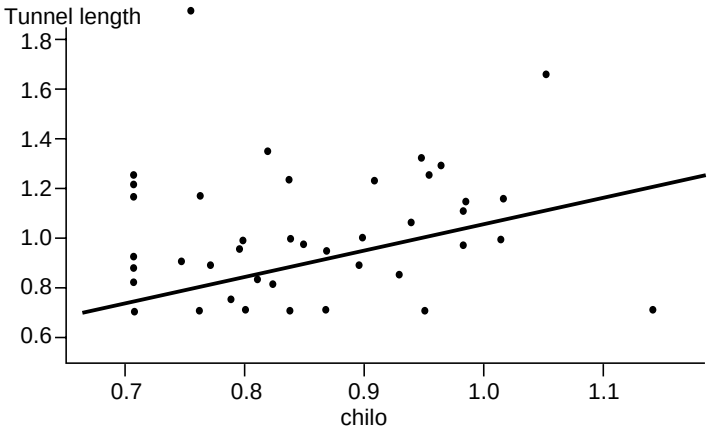

Figure 2. Relationship between tunnel length and borer numbers in maize under natural infestation with stem borers.

Similarly, synthetics that may be adapted to a wide range of environments showed high levels of susceptibility, as they were from the same parentage as the inbreds. However, some of the lines and crosses screened had lower values and hence may have good combining ability for specific characters. This is due to the fact that sources of resistance are diverse and have a different combination of resistance factors.

From this study it is clear that foliar damage and stalk tunneling are good indicators of resistance or susceptibility. Conversely, there are characters which, though singly of little importance, may contribute to reduce yields significantly when occurring in combination. For example when borer exit holes are coupled with stalk breakage due to weakened stems, there is a high reduction in yield due to reduced plant stand.

Thus, resistance sources are diverse, varying by maturity, morphology, and genetic traits, as reported by Sharma (1993). These sources can be adapted *per se* or used in maize improvement in different regions. This means that a breeding program focusing on different ecozones is advantageous. Those materials that are known to possess moderate levels of stem borer resistance could be used in breeding programs to generate better hybrids which are heterotically superior, removing those morphological and genetical characters contributing to susceptibility. However, these results need to be supported by challenging the materials with artificial infestation in the field.

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Developing Rootworm, *Diabrotica virgifera zeae* Krysan and Smith, Resistant Maize in México

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Abstract

The Mexican corn rootworm, (CRW) Diabrotica virgifera zeae, *is one of the most important insect pests of maize in the Mexican "Corn Belt" - the Bajio region of central Mexico. Field evaluations are presented for resistance characteristics of S_1, S_2 and S_3 maize lines derived from CIMMYT Population 593, selected for resistance to corn rootworms. The techniques used included: the use of a susceptible hybrid check planted at regular, repeated intervals throughout the screening nurseries; comparison of phenological development of maize in paired plots, with and without chemical protection against rootworms; degree and amount of root lodging; visual estimates of root pruning by CRW larvae; secondary root development; firmness of root anchoring, as measured by force required for vertical root pulling; and percentage of plants surviving CRW damage. Results are presented for two years of evaluation and selection for resistance. Lines selected in the 1993 summer screening nursery were planted for increase and improvement in a winter nursery. Of 16 materials selected for advancement, 8 were outstanding for rootworm resistance characteristics. In the 1994 summer nursery, advanced S_3 lines were screened at two locations, where 25 and 15 lines were selected, respectively. Considering the resources and techniques available for screening, the resistance mechanisms we are seeking are antibiosis and tolerance. In the coming winter nursery we are planning to make test crosses using selected resistant lines crossed onto a susceptible population tester, as well as to advance lines to another cycle of inbreeding.*

Introduction

Maize is grown in practically all farming areas of Mexico, with the greatest production in the states of México, Jalisco, Sinaloa, Tamaulipas, Puebla, Michoacan, Veracruz and Sonora. A considerable range of insect pests can cause maize production losses, but in the central part of the country, root pests are among the most important. Among the species which inflict root damage are: rootworms, *Diabrotica virgifera zeae* and *Diabrotica longicornis*; white grubs *Phyllophaga spp.*, *Anomala spp.* and *Cyclocephala spp.*; wireworms *Agriotes spp.*; cutworms *Agrotis spp.* and *Colaspis spp.* Of these, *D. virgifera zeae* is one of the principal root pests in 20 Mexican states, while *D. longicornis* has been reported in 6 states (Krysan and Smith 1987).

Few studies in Mexico have focused on host plant resistance to insect pests, and those have been conducted under the auspices of the National Institute for Research on Agriculture, Livestock and Forestry (INIFAP), the International Maize and Wheat Improvement Center (CIMMYT), and several universities.

Studies by Mexican scientists have looked at natural insect populations and have concentrated on maize, wheat, cotton, soybeans and other species. In the case of maize, research has been done on fall armyworm (Salazar 1991; Loera 1990), stem borers and leafhoppers. As for resistance to rootworms and specifically to *Diabrotica* no research has been reported, other than studies conducted in Jalisco by Pérez and Maya (1991).

Consequently, we present our current research on corn rootworm (CRW) resistant maize germplasm. Our objective is to identify sources of CRW resistance in maize and subsequently incorporate desirable resistance traits into advanced maize lines with high yield potential and good adaptation.

Materials and Methods

All tests were conducted under natural infestation, since facilities for mass-rearing CRW larvae were not available. Sites with high egg and larval infestations were selected.

1993

A screening nursery was established (June to December) in Zapotitan, in the municipality of Jocotepec, Jalisco, in a field with a history of very high CRW infestations. We screened 194 maize S_1 lines from CIMMYT's population 593, selected for rootworm resistance. Planting was done on June 23 in a plot having two 2.5 m rows, with two seeds from each line sown every 20 cm. Insecticide was applied to one row, while the other received no chemical treatment. The treated row received two insecticide applications: a dose equivalent to 15 kg/ha was applied at planting and again with the second fertilization. In all cases the insecticide treatment consisted of granulated 5% isozofos mixed with fertilizer. One out of every four test plots included a susceptible hybrid (H-355) as a replicated check. The hybrid was planted in the same manner as the test lines. A few squash plants (*Cucurbita pepo*) were sown, at the beginning of the cycle, in each plot to stimulate the development of rootworm populations for the following cycle. Experimental plots received adequate protection against weeds and leaf insect pests, and tillage operations were carried out periodically. Test materials were evaluated twice, once for comparative growth, root lodging and number of live plants at the 8-10 leaf stage and again at the milk stage for comparative growth, root damage, secondary root development and general appearance of the crop. For each variable, plants in treated rows were compared with those in untreated rows.

When the crop reached the hard dough stage, plants were tested for firmness of root anchoring, measured by the force required for vertical root pulling. At flowering, selected lines were selfed and pollinated and some crosses were done among the same materials. The resulting lines were advanced to S_3 in CIMMYT's winter nursery at Tlaltizapán, Morelos, under rootworm free conditions. All materials were selfed and some crosses were carried out at this location.

1994

The S_4 seeds resulting from selfing and crossing in Tlaltizapán were planted at two locations in Jalisco: Sabino, municipality of Tototlán, on June 24, and Jocotepec on July 6. One hundred twenty lines were evaluated in Jocotepec and 237 were evaluated in Tototlán. Trial design and management, as well as testing techniques, were similar to those used in the 1993 experiment. During both years, sampling was done in the test plots to gauge the size of *Diabrotica* larval populations.

Table 1. Maize lines screened for corn rootworm resistance in Jocotepec, Jalisco, México 1993.

	Root damage[1]		No. plants		Root lodging	
Pedigree	**With**	**Without**	**With**	**Without**	**With**	**Without**
Guat 166 x CO 289	2	4	9	7	0	3
Guat 189 *ff*3	4	5	8	10	0	1
200-6 x Guat 189	3	2	10	5	0	0
200-6 x Guat 189	4	4	8	8	0	2
200-6 x Guat 189	4	4	9	7	0	0
Guat 633 x CO 289	5	5	6	6	3	3
Agscal 6 x CO 272	5	5	4	5	3	1
Agscal 6 x CO 272	3	3	1	8	0	1
B68 Ht[2] x Guat 165	3	5	5	9	1	1
Guat 166 x B68	3	4	8	6	1	0
Guat 189 x B68	1	4	10	9	1	1
200-1 x Guat 189	3	3	3	10	0	0
200-1 x Guat 189	4	4	6	9	0	0
200-1 x Guat 633	3	3	2	7	0	1
200-7 x Maíz San Andrés	3	3	12	6	2	0
200-7 x Maíz San Andrés	3	3	7	9	2	0
200-7 x Maíz San Andrés	4	4	7	10	0	0
200-7 x Guat 189	4	4	8	10	3	0
200-6 x Guat 189	3	4	5	12	4	0
Agscal 6 x CO 289	3	3	8	6	1	2
Agscal 6 x CO 289	3	4	5	3	1	0
Guat 166 x CO 272	3	4	5	7	1	1
Guat 189 *ff*1	4	4	8	3	0	1
Guat 189 *ff*2	4	3	11	6	0	2
Guat 189 *ff*5	4	3	7	10	0	0
B68 Ht[2] x Guat 633	3	3	4	2	0	0
B68 Ht[2] x Guat 166	4	5	8	6	4	1
Guat 166 x B68	3	3	7	7	1	1
Guat 166 x B68	4	4	6	6	3	0
Guat 166 x B68	4	4	6	3	2	0
Guat 189 x B68	4	4	11	10	2	2
Check: H 355 [2]	4.0	5.4	8.5	8.1	0.8	2.2
[3]	0.2	0.2	2.2	2.2	1.31	2.2

Column headings refer to results from rows with and without pesticide treatments.
[1] Root damage evaluated on a 1-6 scale (Hills and Peters 1971).
[2] Average of 47 check plots.
[3] Standard deviation of check.

Results and Discussion

Rootworm damage was found in all materials planted in the three trials conducted to date. In 1993, 16 lines were selected, along with 15 others, that showed good traits for potential resistance (Table 1). Selection was based on the results of all tested variables. Lines selected in 1994 will be included in future tests. The level of corn rootworm infestation was, on average, 8.5 larvae per plant in the heaviest infestations, which allowed satisfactory evaluation.

In the 1994 cycle, 25 lines were selected in Totoltlán and 15 in Jocotepec (Tables 2 and 3) . Even when root damage was severe, as it was in some cases due to the intensity of the attack, resistance traits were observed. Most of these selected materials had been crossed with the S_2 lines from Tlaltizapán. Corn rootworm incidence in Totoltlán averaged 4 larvae per plant, compared with an average of 5 larvae per plant in Jocotepec during the period of heaviest infestation.

Inclusion of a susceptible hybrid as a replicated check allowed us to study the variation of pest populations distributed throughout the study area. Root damage assessments have shown that some selected materials have large, vigorous root systems with lots of secondary roots, while others have root systems that are not very large but develop abundant secondary roots after being damaged by rootworms. During the two years of trials, selected materials typically responded to rootworm damage by rapidly forming an abundance of new secondary roots.

Throughout the study, all variables and testing techniques utilized were given equal weight to ensure more reliable results, since evaluations were subject to the normally high variation in natural rootworm populations. Testing techniques were those proposed by Campbell (1989) and Branson and Sutter (1989).

Selected materials have advanced in the breeding process and are being crossed with CIMMYT's Population 390 MIRT to find potential sources of multiple insect resistance. However, since the data obtained so far do not indicate a definitive source of resistance, these results should be considered preliminary.

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Table 2. Maize lines screened for corn rootworm resistance in Totoltlán, Jalisco, México 1993.

	Root damage[1]		No. plants		Root lodging		Plant height	
Pedigree	**With**	**Without**	**With**	**Without**	**With**	**Without**	**With**	**Without**
200-7 x Maíz San Andrés	3	4	6	11	0	0	1.95	1.60
200-7 x Maíz San Andrés	3	3	7	6	0	1	1.90	1.85
200-7 x Maíz San Andrés	4	4	2	2	0	0	1.65	1.60
200-6 x Guat 189	3	5	14	10	0	0	2.00	1.95
200-6 x Guat 189	4	4	5	5	0	0	2.00	1.85
200-6 x Guat 189	5	5	7	5	0	0	2.00	2.05
200-6 x Guat 189	4	4	15	11	0	0	2.05	1.80
Guat-166 x B68	3	4	6	4	0	0	1.95	1.75
Guat-166 x B68	3	4	12	3	0	0	2.35	2.60
(200-1xGuat 189) x (68-3-1)	4	4	4	4	0	0	2.50	2.35
(200-3 x Guat-189) x (20 x 244-1)	3	4	12	11	0	0	2.15	2.25
(200-7 x Maíz San Andrés) x (68-3-1)	3	5	4	4	0	0	2.25	2.20
(200-7 x Maíz San Andrés) x (70-1-1)	3	2	7	6	0	0	2.65	2.45
(200-7 x Maíz San Andrés) x (51-2-1)	4	4	5	4	1	0	2.40	2.35
(200-7 x Maíz San Andrés) x (125-2-2)	4	3	6	4	0	1	2.10	1.90
(200-6 x Guat 189) x (408-3-1)	3	3	8	8	2	0	2.25	1.70
Check: H 355 [3]	3.6	4.8	16.2	13.9	0.2	0.3	2.21	2.18
[4]	0.23	0.26	2.66	2.7	0.94	0.83	0.25	0.26

Column headings refer to results from rows with and without pesticide treatments.
Plant height is in meters.
[1] Root damage evaluated on a 1-6 scale (Hills and Peters 1971).
[2] These were selected at the two 1994 test sites.
[3] Average of 57 check plots.
[4] Standard deviation of check.

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Table 3. Maize lines screened for corn rootworm resistance in Jocotepec, Jalisco, México 1994.

	Root damage[1]		No. plants		Root lodging		Plant height	
	With	Without	With	Without	With	Without	With	Without
200-3 x Guat 189	3	5	15	14	8	11	2.10	2.40
200-7 x Guat 633	5	4	4	6	0	0	2.10	1.90
200-7 x Maíz San Andrés	4	5	6	10	5	8	2.00	1.70
200-7 x Maíz San Andrés	4	4	4	5	0	0	1.85	1.85
200-6 x Guat 189	4	4	6	9	0	1	2.35	2.35
200-6 x Guat 189	3	4	12	11	9	6	2.00	1.95
200-6 x Guat 189	4	4	12	11	4	3	2.10	2.05
200-6 x Guat 189	2	3	13	13	0	0	1.80	2.00
200-6 x Guat 189	4	4	8	4	1	0	1.85	1.55
Agscal 6 x Co 272	4	6	9	8	0	1	1.60	1.45
(200-7x Guat 633) x (232-3-1)	4	4	2	3	0	0	2.35	1.90
(200-7x Guat 633) x (406-2-1)	3	3	12	10	1	2	2.30	2.35
(200-7x Guat 633) x (64-1-1)	5	4	5	5	0	3	2.65	2.60
(200-7x Guat 633) x (232-3-1)	5	4	13	12	5	2	2.35	2.05
(200-7x Guat 633) x (406-2-1)	3	4	18	16	3	3	2.65	2.85
(200-7x Maíz San Andrés) x (45-1-1)	5	5	12	8	8	3	2.65	2.65
(200-7x Maíz San Andrés) x (232-3-1)	4	3	12	13	0	3	2.50	2.10
(200-7x Maíz San Andrés) x (20x 217)-1	5	3	14	12	9	2	2.65	2.50
(200-7x Maíz San Andrés) x (70-1-1)	4	4	12	12	4	7	2.45	2.30
(200-7x Maíz San Andrés) x (70-2-1)	4	4	12	11	0	0	2.60	2.50
(200-7x Maíz San Andrés) x (51-2-1)	5	4	15	13	9	0	2.90	2.75
(200-7x Maíz San Andrés) x (45-1-1)	4	4	8	7	0	1	2.70	2.50
(200-7x Maíz San Andrés) x (70-1-1)	4	4	9	13	2	1	2.90	2.55
(Guat 189 x 1368) x (232-3-1)	4	4	12	6	7	0	2.45	2.50
Check: H 355 [2]	3.8	4.9	13.09	11.0	2.91	3.0	2.79	2.80
[3]	0.3	0.28	2.31	2.48	4.01	2.37	0.24	0.25

Column headings refer to results from rows with and without pesticide treatments.
[1] Root damage evaluated on a 1-6 scale (Hills and Peters 1971).
[2] Average of 47 check plots.
[3] Standard deviation of check.

Selection Methodology for Resistance to *Dalbulus maidis* and Fine Stripe Virus Disease in Maize in Peru

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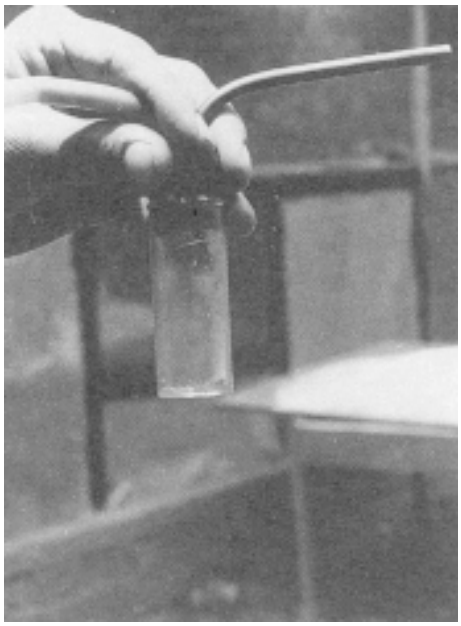
Abstract

This paper describes the methods used in the INIA Maize Research Program to obtain and maintain mass colonies of Dalbulus maidis, *and in the near future to improve resistance to Maize Fine Stripe Virus. The following steps were followed to achieve these objectives: 1) collection, identification and mass rearing of* D. maidis*; 2) greenhouse cultivation of a population of high-altitude maize (Peruvian Complexes), and subsequent inoculation with the virus; 3) transplanting into the field; 4) ELISA serological testing; 5) selfing of families showing tolerance and/or resistance to the virus; and 6) new potential sources of resistance in the Peruvian populations were identified through this approach.*

Introduction

Maize is one of the principal sources of food in Peru, grown on some 400,000 ha. However, yields are low (1.2 t/ha), due mainly to inadequate technology, diseases and pests. Maize fine stripe virus is one of the most serious diseases, transmitted by the leafhopper *D. maidis*, which is common to the Inter-Andean valleys of Peru (Fig. 1a) (Sarmiento et al. 1992). The INIA Maize Research Program (MRP) recognizes that the use of materials which are tolerant and/or resistant to the virus or its vector is an efficient method of controlling the disease. The release of resistant varieties is the best option which researchers can provide to farmers. Developing a workable method of mass-rearing the vector in captivity permitted us to make this alternative a reality.



Methodology

Mass rearing of the fine stripe vector in greenhouses

Collection and multiplication of the vector - Formulating a mass-rearing technique required the collection of the vector in valleys which experienced the greatest incidence of fine stripe virus in recent years. Using suction tubes and insect collection jars, the vector was captured from maize plants showing virus symptoms (Fig. 1b).

The collected insects were taken to the entomology laboratory at the National University of Cajamarca, where an average of 150 adult insects were identified and sexed. They were then taken to the MRP rearing laboratory and

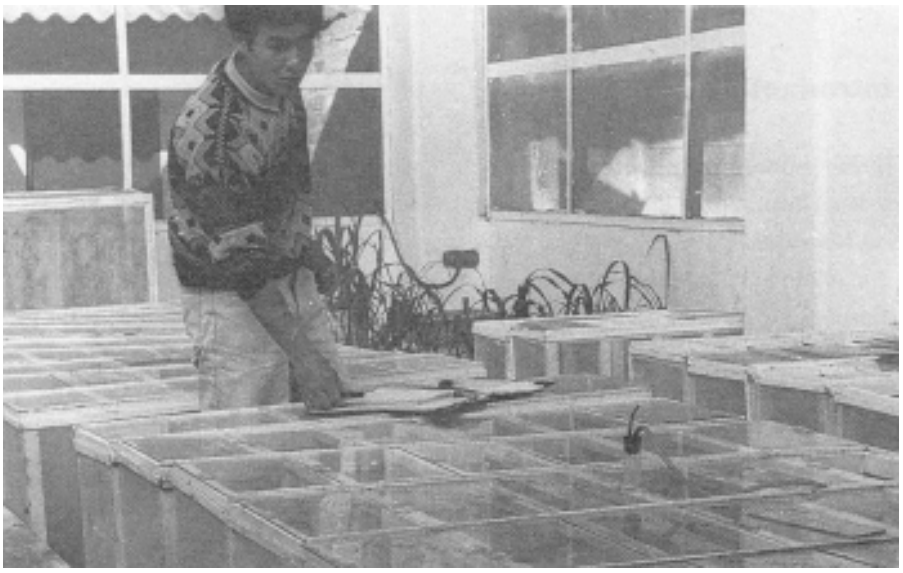
placed in wooden rearing boxes (1 x 0.5 x 0.5 m) lined with anti-aphid mesh screen (Fig. 2), thus providing adequate conditions for their development (Dabrowski 1989).

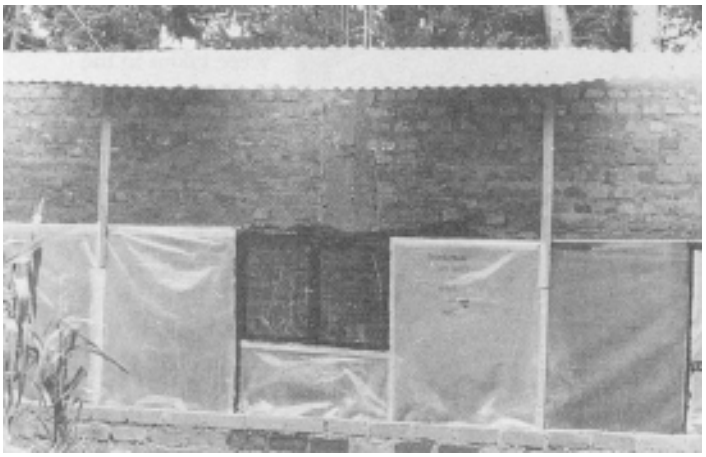
Maize plants of the susceptible variety Blanco Urubamba were placed in the boxes. The plants were sown in plastics pots containing a soil mixture of 2:1:1 earth:sand:moss. The first virus symptoms were observed 10 days after feeding by the *Dalbulus*, and were confirmed through ELISA testing.

The infected vectors were subsequently transferred to larger wooden cages (3.0 x 1.2 x 1.2 m) lined with heavy plastic and glass windows, containing maize varieties and soil similar to that described above. These larger boxes were maintained at 24-26ºC, and a relative humidity of 70%. The insects remained there for 40 days, the duration of the biological cycle of the insect. Asymptomatic plants were removed from the cages in order to obtain a high percentage of diseased plants and infected insects, and material which was biologically pure. This method guaranteed a population of approximately 20,000 insect vectors in each cycle, in cages of (3.0 x 1.2 x 1.2 m) (Fig. 3).

Greenhouse planting of materials - The MRP began planting 254 families of Population IV *canchero tardío* in greenhouses, sowing ten seeds per family in plastic bags containing 1 kg of soil (Fig. 4). Each family was placed in closed wooden boxes (1.2 x 0.5 x 0.35 m) with Saran screen mesh and glass. Planting was staggered over time to permit placement of insects in each box (30 insects per family).

When the plants reached an average height of 10 cm, they were infested with the insect vector for a period of 6 days, adequate time to ensure transmission of the virus (Fig. 6) Once





this was completed, the maize plants from each family were ready to be transplanted to the field (Fig. 7). At this point, it was important to apply a systematic insecticide to the inoculated material to eliminate the insects and propagate the virus, before replicating the plants in the experimental station fields.

Field stage

Once the fields were in optimal condition for plant development, the families were transferred to the field in plastic strips; the plastic was removed and the plants were carefully placed in the bottom of the furrow with a distance of 0.25 m between each plant (Figs. 8 and 9).

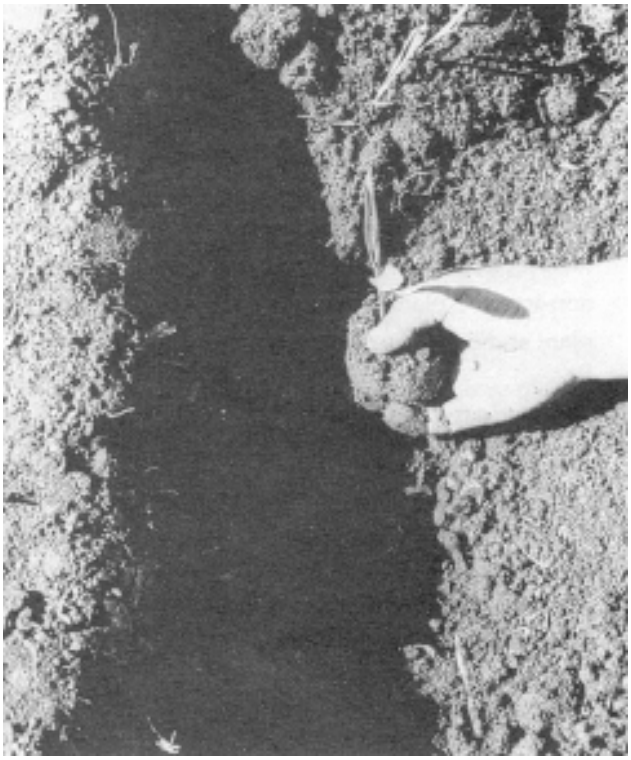
ELISA serological test

Asymptomatic materials, at the pre-flowering phase, were subjected to serological tests at the National University of Cajamarca. The best plants from the best families showing tolerance and/or resistance were self-pollinated and planted in the next cycle of selection.

Results and Discussion

The following results were achieved under the experimental conditions:

- Confirmation that the insect vector causing maize streak virus is *D. maidis*, common in the inter-Andean valleys of Peru.



- Symptoms develop 2 weeks after infestation, with young plants being the most affected.
- ELISA proved to be the most effective serological test for detection of maize fine stripe virus. Of the total number of experimental samples collected from asymptomatic plants, 84% were positive.
- A high percentage of serologically positive plants developed normally and produced ears. The susceptible families were heavily affected; most failed to achieve normal growth and did not produce ears.
- Eighty ears were harvested and identified from virus-infected and non-infected plants, one from each plant and up to four from a family.

Conclusions

The experiment resulted in the following conclusions:

- In the first cycle, 80 ears were selected from 56 families as being the most tolerant to the virus, as follows: (1992-93 entries, listed as family (row) number - plant number (within each row)) 1, 2, 3, 4-2, 5, 7, 8, 9, 9-1, 10, 11, 12, 13, 14, 15, 16, 17, 19, 23-1, 28-3, 30, 32, 33, 37, 40-1, 42, 43, 47, 50, 55, 63-6, 68, 73-2, 74, 75, 76, 78, 94, 98, 99-1, 99-2, 102-2, 104, 104-1, 107, 116-1, 116-2, 116-3, 122, 122-1, 124, 125-2, 125-4, 127, 182-1, 128-5, 130, 136-2, 140, 145, 148, 148-4, 148-5, 149, 150, 150-4, 165, 166-1, 173-1, 173-3, 173-6, 173-7, 182, 190, 195-1, 206, 227, 228, 243, and 253.
- In the second selection cycle, the ten most susceptible families were identified in the greenhouse, as follows: 9-1, 23-1, 28-3, 48, 104, 145, 149, 190, 206, and 227. These were confirmed by comparison with materials planted in the field, which failed to produce any ears.
- 27 families were selected which are currently being screened in the field: 1, 2, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 27, 30. 32, 33, 43, 47, 55, 65, 68, 74, and 76.

Acknowledgments

To Dr. John Mihm and to Luís Narro, CIMMYT Maize Program, for their constant support. To the staff of the Regional Maize Program, Peru, for their invaluable help in carrying out the present work, and to Ing. Gonzales Muñoz for his insightful suggestions.

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Mass Rearing of *Helicoverpa zeae* in Peru

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Abstract

The Maize Research Program (MRP), INIA-Peru, has successfully raised and maintained colonies of corn earworms, Helicoverpa zeae *(Boddie). With technical assistance from the International Maize and Wheat Improvement Center (CIMMYT), our laboratory currently can produce large quantities of this species to infest high-altitude maize varieties, facilitating the selection and breeding process for corn earworm (CEW) resistance.*

Introduction

The cosmopolitan species *Helicoverpa zeae* attacks more than 68 species of host plants (Vela and Quispe 1988), belonging to 26 different families (Paliz and Mendoza 1985). The insect species is distributed throughout all maize-growing regions, although it has a higher incidence in the inter-Andean valleys of Peru where the largest cultivation areas are found of the more susceptible sweet and waxy kernel maize varieties.

The most recent advances for combating CEW rely on integrated control measures, including the use of resistant varieties, to maintain insect populations below economic threshold levels. To confront this challenge, the MRP began mass-rearing of CEW under CIMMYT guidance, adapting the latest techniques and selection methods for maize breeding.

Methodology

The rearing and efficient field infestation techniques used are similar to CIMMYT's. These techniques were initiated last year. Previous efforts failed due to inadequate diets for *Helicoverpa*, and consequently a contamination of the samples. Once a reliable source of insects was obtained, artificial infestations with CEW were carried out on materials introduced from Mexico, which showed resistance there. These first infestations were done manually using camel-hair brushes, a method which has been used for more than 40 years (Blanchard et al. 1942), but which is extremely time- and labor-intensive. The innovation of using manual "bazookas" will simplify future infestations in Peruvian high-altitude maize populations.

Results

- Materials provided by CIMMYT (197 families) were infested, with encouraging results obtained in 32 of the families.
- Large populations of *Helicoverpa* can be raised using the meridic diet.

Discussion

- Thirty-two materials showed superior resistance (Table 1).
- In the current selection cycle, the best materials will be re-planted and selected.
- We will initiate CEW infestation with bazookas in the screening of high-altitude maize populations.

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Table 1. CIMMYT experimental maize varieties tested at the the La Victoria experiment station, National University of Cajamarca, Peru (1993-94).

Entry	Parentage	Pedigree Ba-92	Ear length (cm)	Damage (cm from tip)	Selection
2	(2501 X 2501) F4 (20 X 83) 4	283-4	10.00	4.00	I
11	F18 (60 X 127) - 3	297-3	8.00	5.00	I
12	F18 (60 X 127) - 5	297-5	9.00	5.00	I
14	F23 (9 5X 74) - 1	302-1	8.00	2.00	R
16	F27 (175 X 163) - 1	305-1	10.00	3.00	I
18	F267(175 X 163) -3	305-3	11.00	4.00	I
19	F27 (175 X 163) - 4	305-4	9.00	4.00	I
20	F27 (175 X 163) - 5	305-5	12.00	5.00	I
21	F27 (175 X 163) - 6	305-6	10.00	5.00	I
23	(2501 X 2517) F31 (21X102)-2	310-2	9.00	3.00	I
24	(2501 X 2517) F31 (21 X 102)-4	310-4	11.00	3.00	I
28	F40 (49 X 201)-1	319-1	7.00	3.00	I
30	F43 (54 X 100)-3	322-3	4.50	3.00	I
37	F49 (121 X 145)-3	328-3	8.00	4.00	I
77	(144 X 109)-1-2-1- #	41 #	9.50	3.00	I
85	F10	46X28	9.00	3.00	I
89	(2503 X 2503) F3 (100X1)-4	225-4	8.00	4.50	I
90	Linea S1	230-1	9.00	5.00	I
95	F26 (38 x 73)-1	248-1	8.00	4.00	I
99	F30 (57 X 105)-4	252-4	11.00	4.00	I
106	F46(228 X 87)-4	268-4	10.00	2.50	R
116	F16 (27 X 25)-1-1	201	5.00	3.00	I
131	F2	4X3	12.00	5.00	I
132	F3	10X5	10.00	5.00	I
133	F4	11X23	12.00	6.00	I
134	F6	33X27	11.00	4.00	I
135	F11	93X87	9.80	4.80	I
148	F35	170X163	9.30	4.50	I
176	(119 X 129)-5-2-3- #	77 #	11.10	3.90	I
182	(2504 X 2504) F1 cruzas	4X32	9.10	4.00	I
186	F27	69X80	8.70	4.10	I
194	F43	103X63	11.50	5.90	I

R = Resistant plants.

I = Plants of intermediate resistance.

Of the 197 families of CIMMYT maize, 32 showed superior resistance to Helicoverpa zea; the same families showed resistance in trials at CIMMYT, Mexico, 1992-93. (Blanchard et al. 1942).

Progress of Host Plant Resistance Research to the Asiatic Corn Borer in the Philippines

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Abstract

The Asiatic Corn Borer (ACB), Ostrinia furnacalis *(Guenee), remains the most serious insect pest of maize in the Philippines and parts of Tropical Asia. Advances in ACB resistance work have been obtained through an increase in information and materials, that have served as bases for future activities. Several hybrid varieties with resistance or tolerance to ACB were developed and released from 1992 to 1993. Possible genetic differentiation was identified in the local populations of ACB. Collaborative work with CIMMYT-ARMP was started in 1990 on the development of Asian Multiple Borer Resistant populations of maize.*

Introduction

The Asiatic Corn Borer (ACB), *Ostrinia furnacalis* (Guenee), remains the most serious insect pest of maize in the Philippines and some parts of the Tropical Asia. In commercial production, the use of chemicals to control this pest is recommended. However, this is seldom practiced by small-scale farmers due to the high cost of the pesticides and also because of their increasing awareness of the hazardous effect of these chemicals to human life, non-target organisms and the environment. In recent years, farmers are learning to appreciate and use crop varieties with built-in resistance to insect pests. In the Philippines, the establishment of the Institute of Plant Breeding (IPB) in 1975 helped advance the growing awareness of host plant resistance, as an approach to pest population regulation and management (Lit et al. 1987).

During the symposium held in March 1987 at CIMMYT, Mexico, with the theme "Towards Insect Resistant Maize for the Third World", Lit et al. (1989) presented the status of research activities on host plant resistance to ACB in the Philippines. This presentation covered the following areas:

- Biology of the ACB.
- Techniques for ACB mass rearing.
- Infestation and evaluation procedures.
- Sources of resistance.
- Breeding methodologies.
- Mechanisms of resistance.

At present, breeding for resistance to corn borer remains a high priority in the over-all maize breeding program of the IPB. This paper presents the progress of ACB resistance work in the Philippines since the last symposium. Most of the work was done at the Institute of Plant Breeding in collaboration with other Units/Institutions.

Information and Materials Generated

According to Salazar and Legacion (1991), past studies indicate that there is still genetic variation to be exploited in breeding for resistance to ACB damage. So, what is needed is a greater understanding of the mechanisms of resistance, coupled with more effective selection procedures.

The accomplishments achieved in the work for ACB resistance during the early 1990s, as summarized by Salazar and Legacion (1991), are in the form of information and genetic materials that serve as a foundation for future research activity:

- Information obtained
- Materials resistant to pre-tasseling corn borer damage are not resistant to post-tasseling damage.
- Antigua Grupo I is a reliable source of resistance to pre-tasseling corn borer damage.

- Heavy fertilization favors corn borer damage.
- ACB is more severe during the wet season, especially in late plantings.
- DIMBOA was positively correlated to pre-tasseling corn borer damage, but not to post-tasseling borer damage.
- Plants with erect leaves tend to exhibit less borer egg mass deposition.
- In a pre-tasseling corn borer resistant (CBR) composite population, significant additive genetic variance was found suggesting progress from recurrent selection.

Genetic materials available

- A CBR composite population, made up of 14 populations previously found to be resistant to pre-tasseling borer damage.
- Inbred lines which have undergone a general combining ability (GCA) test, extracted from superior families of CBR.
- Crosses of CBR populations with an elite breeding population.

Biological and Biochemical Studies on ACB Populations

A study on the biological and biochemical aspects of ACB populations was initiated by the group of Dr. Legacion to assess the performance of identified resistant materials against the three populations of corn borer: Laguna, VISCA and USM in Mindanao. Furthermore, it was aimed at determining whether local population differences existed.

Preliminary results from electrophoretic studies of population structure and population differentiation, within the Philippine corn borer species (Mendoza et al. 1992), showed that Laguna and USM populations had 5 alleles while VISCA had 6. Allele y was only observed in the VISCA population. Laguna and USM were more variable than VISCA, due to higher heterozygosity values. Significant heterogeneity was observed among the populations. However, when specific comparisons were made the Laguna population was significantly different from VISCA and USM population, but the latter two were not different (Table 1). The results suggest local genetic differentiation among the different populations of the borer. Of the three, the Laguna population seemed to be the most differentiated. However, the investigators believed that there were limitations to the results obtained and further studies are needed. If there is indeed local differentiation, the question is raised as to which ecological variable(s) is responsible for the population differentiation?

Table 1. Homogeneity tests among the gene frequencies between the three local populations of the corn borer, *Ostrinia furnacalis* (Guenee). [a]

Population /location	Los Baños Laguna	VISCA	USM Mindanao
Los Baños Laguna	-	13.34**	5.226*
VISCA		-	2.671ns
USM Mindanao			-

[a] From the report of Mendoza et al. (1992).

Varieties Developed

The progress and success of any breeding program is measured in terms of the final output - a variety. To fully appreciate the status and progress of host plant resistance activity to ACB in the Philippines, the list of corn varieties developed by IPB and approved by the Philippine Seed Board from 1990 to 1993 are presented in Table 2.

IPB Var 5 a varietal hybrid between IPB Var 1 x Suwan 2 was released in 1990. This was the first commercial varietal hybrid released by the public sector in the Philippines. Another varietal hybrid, IPB Var 4 (IPB Var 2 x Antigua GPo1) followed in 1991. No indication, however, was reported regarding their performance against pests, particularly the corn borer *O. furnacalis*. In 1992, a yellow corn hybrid named IPB 913 was developed with a moderately resistant reaction to ACB and earworm. Three

Table 2. Corn varieties developed at IPB and approved by the Philippine Seed Board from 1990 to 1993.

Variety name	Year released	Type	Yield (t/ha)	Reaction to pests
IPB Var 5 (IPB Var 1 x Suwan 2)	1990	Yellow Hybrid	-	-
Improved Macapuno	1991 (fresh)	Glutinous White	6.26	-
IPB Var 4 (IPB Var 2 x Antigua GPo 1)	1991	Yellow Hybrid	4.89	-
IPB 913	1992	Yellow Hybrid	6.58	moderately resistant to ACB and earworm
PSB Cn 93-49 (DLU Sweet)	1993	Glutimous White O.P	6.10	susceptible to ACB
IPB Var 7	1993	Yellow O.P	5.57	some resistance to DM
IPB 919	1993	Yellow Hybrid	6.35	tolerant to ACB, resistant to DM
IPB 921	1993	Yellow Hybrid	6.89	tolerant to ACB, resistant to DM
IPB 929	1993	Yellow Hybrid	7.01	tolerant to ACB, resistant to DM

more commercial hybrids: IPB 919, IPB 921 and IPB 929 were developed in 1993. Although these hybrids were not purposely developed for corn borer resistance, all turned out to have high level of tolerance to ACB. It is worth mentioning also, as shown Table 2, that yield level increased as new hybrids were developed. However, all these hybrid varieties bearing resistance or tolerance to ACB were for industrial purposes. We are yet to see a variety with resistance or tolerance to ACB that is utilized as "green corn".

Collaborative Work

The CIMMYT-Asian Regional Maize Program (ARMP), initiated in 1990, is a regional project for the development of maize populations resistant to downy mildew and tolerant to the species of borers most prevalent in Asia and Southeast Asia (Granados 1994). The Entomology Laboratory of the IPB, University of the Philippines, Los Baños was identified as one of the three original collaborators. This was due to the fact that *O. furnacalis* is being successfully reared at IPB (Rangdang 1971; Hirai and Legacion 1985) for artificial infestation of test materials.

In 1990, 25 cultivars were screened for ACB resistance. These included 11 DMR materials, 6 borer tolerant varieties from CIMMYT's MBR population, 5 borer tolerant varieties from the Philippines, 5 EV's from CIMMYT's population 28, 30, 32, and 36, and bulks of populations 24 and 26. The results showed that MBR-SCB Res. EV (yellow) had the lowest leaf feeding damage (1.8). The other resistant selections included Mbita 86 MBR Chilo (Yellow), MBR 86 Across borers, Across 8432, CBR-1, MBR 86 Stars and Diamonds and Pop. 24 bulk (Table 3). According to Granados (1994), MBR-SCB Res. EV (yellow), Population 24 and MBR 86 Stars and Diamonds were also found to be resistant to *Chilo partellus* in India. These three materials, however, are very susceptible to downy mildew.

Collaborative work in 1991-92 was concentrated on the evaluation of the derived EVs and inbred lines that CIMMYT's resident entomology program had generated from the MBR (Population 590) and MIRT (Population 390). A number of materials were identified as intermediate in their tolerance to *O. furnacalis* (Table 4).

At present, the focus of the collaborative work with CIMMYT-ARMP is on the development of Asian Multiple Corn Borer Tolerant, Downy Mildew Resistant (AMBT-DMR) Early

Table 3. Reaction of 25 corn materials artificially infested with larvae of *Ostrinia furnacalis* at IPB, Summer 1990.

Entry No.	Description	feeding damage [a]	Leaf % of check
1	CBR-1	2.6	50.0
2	Pop. 26 Bulk	3.4	65.3
3	Philippines 06	3.5	67.3
4	Philippines 17	3.0	57.6
5	MBR 86 Stars and Diamonds	2.6	50.0
6	MBR-SCB Res. EV (Yellow)	1.8	34.6
7	MBR 86 Across borers	2.4	46.1
8	Mbita 86 MBR Chilo (Yellow)	2.3	44.2
9	EY-DMR Pool C3 HS bulk	3.4	65.2
10	LY-DMR Pool C3 HS bulk	3.8	73.0
11	Across 8336	-	-
12	Poza Rica 8336	-	-
13	Phil. DMR Comp. 1	3.3	63.4
14	Pop. 28 DMR C3 HS bulk	3.0	57.6
15	Suwan 85 28	3.2	57.1
16	Pop. 24 Bulk	2.8	53.8
17	Pop. 31 DMR C4 HS bulk	2.9	55.7
18	Improved Tiniguib	4.7	90.3
19	Mbita 86 MBR Chilo (White)	2.7	51.9
20	MBR-SCB Res. EV (White)	2.9	55.7
21	EW-DMR Pool C3 HS	3.7	71.1
22	LW-DMR Pool C3 HS	3.1	59.6
23	Tiniguib Synthetic	3.6	69.2
24	Across 8432	2.5	48.0
25	Poza Rica 8530	4.1	78.0
	Phil. Super Sweet (suscept. Check)	5.2	100.0
	Mean	3.1	59.3

[a] Scale Rating: 1-9.

Table 4. Reaction of the materials from Pop. 590 (MBR) and Pop. 390 (MIRT) artificially infested with larvae of *Ostrinia furnacalis* (Guenee). IPB, Los Baños, 1991-92 trial.

Entry No.	Description	Leaf feeding damage [a]
Population 590 (MBR)		
1	Across 86590 (IR)	3.5
2	Across 86590-2 (ECB)	3.8
3	Poza Rica 86590 (SCB)	3.5
4	Mbita 86590 (Chilo)	4.1
5	Tlaltizapan 85590	4.1
6	CML 135/CML 139	3.9
7	CML 135/CML 67	3.8
8	Ki3/CML 131	5.2
9	MBR HT	4.2
	Local check (Susceptible)	4.8
Population 390 (MIRT)		
1	Across 90390-W (IR)	3.6
2	Across 90390-Y (IR)	3.6
3	SCB-GCA	3.6
4	FAW-GCA	3.8
5	Ki3/CML 139	3.8
6	CML 69/Ki3	4.0
	Local Check (Susceptible)	4.8

[a] Scale Rating: 1-9.

White, Early Yellow, and Late Yellow populations. The IPB, Los Baños, was designated the primary location for the development of Early White-DMR-Borer Resistant populations. Table 5 show the material composition of the three populations being developed.

In addition to the above, further evaluation of the IPB selected ACB resistant populations are continuing. The materials currently being advanced to develop better resistant lines, that may be of value to the breeders particularly for the hybrid program, are shown in Table 6. Materials from the breeding group are also being field evaluated for ACB resistance.

Looking to the Future

Despite the gains we have attained in the last few years through the release of varieties with built-in resistance or tolerance to ACB, there is no complacency in our efforts to effectively manage this pest. There are indications that the insect has differentiated into several populations, towards which the resistant varieties developed to date may behave differently.

Several years ago Lit et al. (1987) mentioned that, while efforts on field screening are modestly supported, funds for basic research have been very limited. The situation remains the same today or even worse. Despite this limitation, we recognize the need for a continuing effort to develop new varieties with a better and higher level of resistance to ACB. Likewise, there is a need to continue the work on determining the extent of population differentiation of the Philippine ACB. Further work must also be put in place to establish how the developed resistant varieties, and other resistant materials, will respond to these differentiated ACB populations if their existence is confirmed.

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Table 5. Composition of the three populations being developed for Asian Multiple Borer Tolerant-Downy Mildew Resistant (AMBT-DMR).

Population	Description	Material source description
1	AMBT-DMR Early White	a) Pop 100 EW-DMR S2 Bulk b) EEW-DMR Pool FS
2	AMBT-DMR Early Yellow	a) Pop 31 DMR S2 Bulk b) Viemyt 49 (Y) S2 Bulk c) Pop 145 EY-DMR Pool S2 Bulk d) EY TAK-FA HS e) EEY DMR Pool FS
3	AMBT-DMR Late Yellow LY TAK-FA HS Across 90390 W (IR) Across 86590 (IR) FAW - GCA	a) Pop 345 LY-DMR S2 Bulk b) Pop 28 DMR C6 S2 Bulk

Table 6. IPB selected populations continually evaluated for ACB resistance.[a]

Population	Number of lines	Generation
1. XV_3	132	S_6
2. Antigua Grupo I	120	S_5
3. IPB Var 1	84	S_6
4. S_3 (9PG-238)	52	S_5
5. S_4 (YOF-62	8	S_6
6. MIRT I	21	S_4
7. MIRT II	15	S_4
8. Other germplasm	36	S_2

[a] Materials available as of June 1994.

Two Experimental Maize Varieties Selected for Resistance to Fall Armyworm and Sugarcane Borer in Tabasco, Mexico

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Introduction

Stalk borers and fall armyworms (FAW), *Spodoptera frugiperda* (J.E. Smith), are the principal causes of maize crop damage, resulting in serious grain production problems. One option for reducing losses is the use of resistant varieties (Wiseman and Davis 1979).

The selection of genotypes with resistance to FAW began in 1956 in Brazil with *amargo*-type varieties, from which maize germplasm was identified with resistance to this pest (Wiseman and Davis 1979). The International Maize and Wheat Improvement Center (CIMMYT) has worked since 1986 to develop maize germplasm with host plant resistance (HPR) to multiple species of *Lepidoptera* identified as tropical maize pests. The CIMMYT materials demonstrate acceptable agronomic traits, beginning with Population 390 Multiple Insect Resistance Tropical (MIRT) selected under artificial infestation in Mexico. Subsequently, CIMMYT developed the experimental varieties Across 90390 (W) and Across 90390 (Y), which show resistance to *Diatraea grandiosella*, *D. saccharalis* (Sugarcane borer, SCB) and FAW (Mihm et al. 1991). However, the plants' resistance levels may vary if they are moved to a different environment (Wiseman and Davis 1979).

A heavy infestation of FAW was detected in subsistence maize crops in farmers' fields during the second half of May, 1994, in the municipality of Cardenas, Tabasco, Mexico, when the plants were at the 4-6 leaf stage. Chemical controls were not used in these plots. As a result, an evaluation was carried out of the damage to, and yields of, two varieties selected by CIMMYT for resistance to FAW and stalk borers, and a comparison with two varieties commonly grown in the region plus two hybrids — one identified by CIMMYT as susceptible and another as resistant — as checks on the infestation levels in the region. Finally, a comparison was done of the damage caused by FAW and SCB between plants with and without insecticide applications.

Materials and Methods

This research was conducted at the Colegio de Postgraduados' Tabasco Campus experiment station in Cárdenas, Tabasco. Planting took place in mid-July 1994 following the station's recommended agronomic practices. Seeds were treated with Furadan-thiram prior to planting to avoid damage by soil pests and according to the practices carried out at CIMMYT.

Planting was carried out using a divided-plot design. Eight furrows were sown with genotypes of the four varieties indicated in Table 1 (large plot); due to a lack of seed, only six furrows were planted with the hybrids. Furrows were 2.5 m long with ten plants per row, and the plot was divided in half. One half was treated with Methyl Parathion dust (3%) at the 8-10 leaf stage (small plots), and the other was left untreated. The harvested plot corresponded to the two central rows of each experimental unit. Four replications were done for each treatment.

The test variables consisted of: FAW foliar damage, first and second generations of borers, number of damaged stalks, internodes damaged in

Table 1. Genotypes, genetic composition, and origin of materials screened for damage by FAW and SCB in Cardenas, Tabasco, Mexico.

Genotype	Genetic composition	Origin
Across 90390 IRW	Variety resistant to FAW and SCB	CIMMYT
Across 90390 IRY	Variety resistant to FAW and SCB	CIMMYT
VS-536	Variety	Local commercial
Mejen	Variety	Local *criollo*
Ki3 x CML131	Susceptible hybrid	CIMMYT
CML135 x CML67	Resistant hybrid	CIMMYT

the first and second generation, and ear and grain yield adjusted to 11% humidity. The first two screenings were done 7 days after applying the insecticide and the last prior to flowering, using a foliar damage scale of 0-9 for FAW, and a damage scale of 1-9 for SCB, where 1 is resistant and 9 is susceptible (Mihm 1989). For the data on damage by FAW and borers only the average values were obtained, while for the number of damaged stalks and internodes data were analyzed under a divided-plot design and means were compared using a Tukey test. For the large plot (genotypes), an F-test was done using the mean squared of error of Gen*rep, to detect differences among them. Data for the number of damaged internodes was transformed before analysis due to the presence of zeros in the data (Steel and Torrie 1988). The means presented in the tables are not transformed.

Results and Discussion

Table 2 presents the average scores for FAW and borer damage prior to flowering. The low level of damage by FAW (1.5 and 1.8) and borers (1.0) seen in the susceptible check Ki3 x CLM131 indicates a low level of infestation, since the expected damage ratings would be 7-9 and 7-10, respectively. This response may be related to the seed treatment used prior to planting, since the product used is a systemic insecticide, but it normally persists only 10-12 days under tropical conditions. (Plots near the experiment and planted in the same period with VS-536, but without the seed treatment, showed natural FAW infestation levels of 18.5%+/- 3 of plants at the 6-8 leaf stage, corresponding to 16 days after planting.) The variety most affected by FAW was Mejen, with average damage scores of 2.1 and 3.2 with and without insecticide, respectively. The least affected was Across 90390 IRY, with damage scores of 1.5 under both treatments.

In the first borer generation, damage was quite low (1.0), an observation which was confirmed at harvest when an average of 0.22 damaged internodes were recorded (Table 2). In the second generation, damage scores ranged from 1.0 to 3.0 in all varieties and correlated to the number of damaged internodes, which averaged 0.558 (Table 2). In this case, one of the most affected genotypes was the susceptible check K13 x CML131, with damage scores of 2.0 and 1.3 with and without pesticides, respectively — but much below the expected score of 7-9, suggesting either that the borer population was low during this stage of plant development, or that the effect of the Furadan was still persisting.

Table 2 indicates that the variety VS-536 showed more foliar damage than the susceptible check, and that the least-affected genotypes were the resistant check and the variety Across 90390 IRY. The foliar damage caused by borers was greater during flowering, as it was observed at harvest that the majority of the damaged internodes were at the ear or the base of the ear, a location and phenological stage considered susceptible to the second generation of borers (Guthrie and Barry 1989; Chippendale 1978).

With regard to the level of borer damage in plants with and without protection (small plot), variance analysis of the factors damaged stalks (DS), internodes damaged in the first and second generation and in total, and maize and grain yields indicates no evidence of differences between plants with and without insecticide for any of the tested variables (Table 3). This may be related to the low level of damage (2-3) detected during plant development.

The genotype response study indicated that significant differences existed for damaged stalks, total number of damaged internodes, internodes damaged by second-generation borers, and ear and grain yield (Table 3). However, for the variable of damaged internodes, significant differences were also noted in the interaction of varieties and repetitions, hence the means and F tests for genotypes were not significant (Tables 3 and 4). This is reflected as well in the high coefficients of variation.

Table 2. Average scores of maize foliar damage by FAW and SCB, in Cárdenas, Tabasco.

	Armyworm damage[1]		Borer damage[2]	
Genotype	Treatment 1	Treatment 2	Treatment 1	Treatment 2
Across 90390 IRW	2.1	1.8	1.6	1.1
Across 90390 IRY	1.5	1.5	1.6	1.3
VS-536 (local)	2.7	2.0	2.2	2.0
Mejen (local)	3.2	2.1	1.7	1.7
Ki3 x CML131 (susceptible)	1.5	1.8	2.0	1.3
CML135 x CML 67 (resistant)	1.3	1.7	1.0	1.1

Note: Treatment 1 = no insecticide; treatment 2 = protected with 3% methyl parathion.
[1] Seven days after chemical protection.
[2] Pre-flowering.

It is possible to explain the variability in the damage response by irregularity in the distribution of the natural insect populations, except that it approximates a negative binomial distribution belonging to a contagious distribution family (Rojas 1970). Given the high response variability and low level of uniformity in natural infestations, Ortega et al. (1984) and Davis and Williams (1989) recorded limited efficiency for selection of resistant genotypes. Mihm (1989) considers this variability a limitation on the selection of insect-resistant genotypes, in that the natural insect populations are subject to uncontrollable environmental conditions with the result that usually they are neither uniform nor predictable over time, space, nor infestation level.

Nevertheless, the tendencies in the results show evidence of genotype response, even though they are not statistically different for the above-mentioned reasons. Table 5 shows the average values of the test variables. The following findings can be observed:

- The variety Across 90390 IRY showed the least stalk damage (at 3.375), and Mejen showed the most (at 5.875).
- In every case, internode damage in the first generation was less than 0.4, with the susceptible check Ki3 x CML131 showing the greatest damage (0.343) and Mejen the least affected (0.152).
- In the second generation, the least affected genotype was the resistant check (0.419), followed by Across 90390 IRY (0.424), with Mejen showing the most damage (0.824).
- This response is similar to that shown for total internode damage.

As for yields, the varieties with high ear and grain weights were the two hybrids (CML135 x CML67 with 901.44 g per harvested plot, and Ki3 x CML 131 with 868.82 g). Of the varieties, VS-536 had the highest ear yield (744.79 g) followed by Across 90390 IRY (735.95 g); Mejen was the lowest yielding, with an average of 582.88 g. However, in terms of grain yield, Across 90390 IRY and the two hybrids all exceeded the local varieties.

Table 3. Summary of F-values calculated for variance analysis of stalk damage, damaged internodes (first and second generations, and total), and ear and grain yield, for maize affected by FAW and SCB.

F-value	GL	Damaged stalks	Damaged internodes 1st gen.	Damaged internodes 2nd gen.	Total	Yield Ears	Yield Grain
Replications (Repl.)	3	2.08NS	24.37**	4.74NS	2.68	2.68NS	0.83NS
Genotypes (Gen.)	5	3.81*	2.02NS	7.57**	4.82**	11.84**	9.65**
Repl. x Gen.	15	2.59NS	1.90NS	5.65**	4.15**	1.02NS	1.10NS
Treatments (Trtmt.)	1	0.00NS	0.21NS	1.84NS	0.81	2.41NS	0.00NS
Gen. x Trtmt.	5	0.41NS	1.16NS	0.93NS	0.91	1.83NS	1.13NS
Variance coefficient		40.63	32.24	36.66	39.00	17.81	21.047
X		4.479	0.220	0.558	1.050	756.332	549.02

Table 4. F-values calculated to prove the varieties hypothesis for the variables: stalk damage, internode damage (first and second generations, and total), and ear and grain yield, for maize affected by FAW and SCB.

F-value	GL	Damaged stalks	Damaged internodes 1st gen.	Damaged internodes 2nd gen.	Total	Yield Ears	Yield Grain
Replications	3	0.80NS	12.85**	0.84NS	0.65NS	2.57NS	0.75NS
Genotypes	5	0.25NS	1.06NS	1.34NS	1.16NS	11.56**	8.75**

Table 5. Average values of the following variables: stalk damage, internode damage (first and second generations, and total), and ear and grain yield, for maize affected by FAW and SCB.

Genotypes	Damaged stalks	Damaged internodes 1st gen.	Damaged internodes 2nd gen.	Total	Yield Ears	Yield Grain
Across 90390 IRW	4.250A	0.189A	0.462A	1.000A	703.40CD	524.90AC
Across 90390 IRY	3.375A	0.170A	0.424A	0.965A	735.95BCD	636.16BC
VS 536	5.000A	0.207A	0.766A	1.129A	744.79ABC	467.74BC
Mejen	5.875A	0.152A	0.824A	1.143A	582.88D	431.93C
Ki3 x CML131	4.562A	0.343A	0.459A	1.059A	868.82AB	652.99A
CML135 x CML67	3.813A	0.256A	0.419A	1.010A	901.44A	580.42AB

Conclusion

Based on the results obtained in the present research, it is suggested that the scarcity of FAW and first generation borers in the experimental plot may be attributed to the lack of uniformity in natural infestations, and/or to the preventive seed treatment applied before planting. Therefore, in future research the seed treatment needs to be eliminated, to determine whether the plant response was due to *antixenosis* or the interference caused by the systemic insecticide. The low infestation levels detected prevented a clear demonstration of the antibiotic resistance of the materials in terms of the test variables. Future trials should be artificially infested, assuring results simulating what occurs when natural epidemics do exist in the region.

One hypothesis which emerges is that differences in the number of damaged internodes in the varieties might be related to stalk hardness. This variable was not evaluated in the current research, but differences were detected in the course of field observations, with the selected resistant varieties harder than the local ones. This trait seems to suggest the resistance mechanism which the plants develop against the insects.

With regard to damage and yield, it is clear that the local variety Mejen was the most affected by borers and had the lowest ear and grain yields. The selected resistant varieties suffered less damage and Across 90390 YRI also showed better yields, implying that it could compete with the commercial and criollo varieties planted in this region.

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Conclusion

Host Plant Resistance — Alleviating Poverty and Improving Environmental Stability

D.L. Winkelmann, Director General,
International Maize and Wheat Improvement Center
(CIMMYT), Mexico, 1985-1994

On behalf of the CIMMYT trustees, staff, and central management, I want to congratulate the participants in this symposium. Over the past week, you have worked through a marathon agenda comprising over 60 presentations on critical themes relating to insect resistant maize, including mechanisms and bases of resistance, advances in conventional techniques and the application of new biotechnology tools, and research to verify and utilize resistance. Certainly a rich and varied menu about insects.

And thanks will go to wild applause, maybe a WAVE, when these speculations are reflected in new varieties and hybrids that both resist insect pests and meet the other pressing needs of developing country farmers. Of particular relevance to CIMMYT, a center working for the benefit of the poor in developing countries, is that the products of your work can be delivered to farmers in that utterly traditional and convenient package—the seed.

The importance of helping poor farmers to improve their well-being can hardly be overstated. Like others involved in development, we at CIMMYT see poverty, environmental decline, and rapid population growth as the principal dilemmas affecting developing countries and motivating development assistance agencies. Poverty is the pivotal element in this triad of interacting problems. Poverty is toxic to the agricultural environment, as the poor press on fragile lands and forest margins to subsist. Poverty also increases the pace of population growth, which in itself aggravates environmental deterioration.

Inasmuch as poverty is the fulcrum of this nexus of problems, much of their solution then lies with raising the real incomes of the developing world's poor. How to raise incomes? For the poorest developing countries, achieving higher incomes will depend largely on improved productivity in agriculture. Agricultural productivity can serve as an engine of growth in poor economies, stimulating the demand for goods and services and leading to widening rounds of spending. Productivity gains in agriculture also lower the real price of food to consumers, further lubricating economic growth. Few poor societies have achieved increased incomes without having first improved productivity in agriculture.

Which brings us to the role of CIMMYT. The heart of our work is collaborative research to develop technologies that increase agricultural productivity while protecting soil, water, and forest resources, as well as crop biodiversity. Among other things, in concert with agricultural research institutions worldwide:

- We develop and disseminate improved varieties of maize and wheat that yield more while using available resources more efficiently;
- We contribute to the development of productivity increasing, resource conserving management technologies for maize- or wheat-based systems, as well as helping to formulate efficient approaches to research on such technologies; and
- We preserve, catalog, and utilize maize and wheat genetic resources, and assist others engaged in the same activities.

High yielding, insect resistant maize has enormous potential as a part of productivity enhancing, resource conserving maize farming. As mentioned throughout the symposium, insect pests cause enormous damage to maize crops worldwide, but their effects are especially acute in the tropical environments that predominate in developing countries. According to Dr. Mihm's recent estimates, the 19 leading maize producing nations of the developing

world could augment their harvests by approximately 4 million tons of grain annually — representing some US$400 million — if even *a fourth* of their farmers had access to insect resistant varieties and hybrids. Because these benefits are inherent in the seed, poorer farmers could obtain increased yields and yield stability without investing in pesticides or additional manual labor. As well, more prosperous farmers who normally protect their crops with chemicals would obtain additional savings in the form of reduced pesticide and labor costs. Farmers everywhere would find seed of genetically resistant maize easier and safer to use than knowledge-intensive IPM methods, such as tailoring pesticide use to quantitative estimates of pest and predator populations. It is a case where substituting chromosomes for chemicals has clear advantage.

Along with the productivity-enhancing features of insect resistant maize come significant environmental benefits. It is obvious that reducing pesticide use will lessen health hazards for the farmer and workers who apply such chemicals, for farm animals and wildlife that share the ecosystem, for consumers of farm products, and for ground water. We know that it is theoretically possible to develop highly resistant varieties which not only prevent damage losses but cause actual declines in pest populations, lessening the need for other control measures. Moreover, as specialists we know that once insecticides are removed from the cropping system, the natural dynamics between populations of insect predators and maize pests will come into play, helping regulate pests in a more sustainable fashion.

What is often not sufficiently appreciated are the indirect consequences of host plant resistance for the environment. By raising productivity on current maize lands, use of resistant seed will lessen the pressure to open more marginal lands and tropical forests to agriculture. This fact acquires special pertinence in view of recent predictions that, over the coming decade, demand for maize in developing countries will grow more than 4% each year.

So you see that your work in developing insect resistant maize ties directly into efforts to alleviate poverty and to reduce threats to the environment. Resistant varieties will make maize farming more productive and sustainable, while increasing the well-being of farmers and consumers. We value your collaboration. We pledge to continue to facilitate your research through the free exchange of germplasm and knowledge. Moreover, as your work proceeds, know that we will be open to new forms of collaboration that bring your talents closer to our needs.

I would like to acknowledge the special support of UNDP and the Rockefeller Foundation, as well as the private companies Mahyco, UpJohn, Pioneer, Cargill, and Dekalb, for this symposium. As well, I wish to join with you in congratulating Dr. H.C. Chiang, to whom the symposium is dedicated, for his pioneering research in host plant resistance and integrated pest management.

Finally, I want to bid an appreciative, respectful, and a fond farewell to John Mihm, who has played a pivotal role, a crucial role, in our progress in developing insect resistant tropical maize. In addition to the outstanding quality of his research during his 19 years at CIMMYT, John has become well-known for his individualistic fashion statements and his finely honed alertness during meetings and presentations. John is leaving CIMMYT as of January. CIMMYT will certainly miss his imposing presence and wishes him happiness and success in his new undertakings.

To all participants, may you have a safe trip home and continue your valuable research.

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