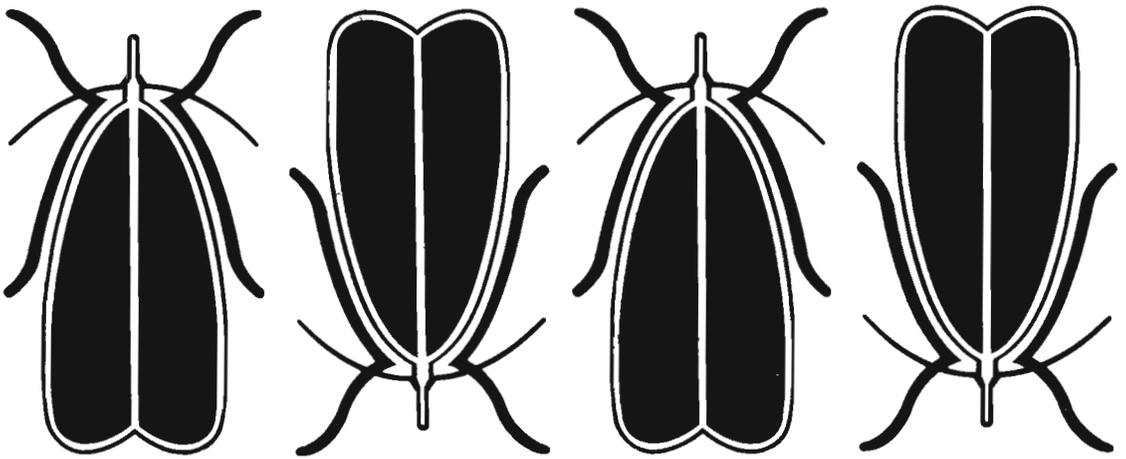


**Efficient Mass Rearing and Infestation  
Techniques to Screen for Host Plant  
Resistance to Maize Stem Borers,  
*Diatraea Sp.***

John A. Mihm



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A *Diatraea saccharalis* larva feeding on the ear shoot of a susceptible maize plant.

## summary

The practice of growing varieties, lines, or hybrids resistant to attack by insects, and their subsequent effectiveness in reducing pest populations and corresponding crop losses, is well documented for several agricultural crops and pest species.

The development of many of these resistant cultivars has resulted from or been facilitated by (1) many years of study of the insect pests, (2) the development of techniques to mass rear the insects, artificially infest the crop species, and screen the germplasm of the species (or their wild relatives) for resistance, and (3) the successful application of appropriate breeding procedures for improvement of the resistance characteristic over succeeding cycles or generations of population improvement (Guthrie, 1974, 1980).

The basic components necessary to identify or develop germplasm with resistance, or with higher levels of resistance than present cultivars utilized by farmer/producers, include:

- (1) A colony of the insect species, which exhibits the vigor and vitality of the damaging pest population within the geographical area that is affected.
- (2) The capability to efficiently mass culture the species, including the rearing facility, trained personnel, natural, meridic, or defined diets, and rearing procedures and containers.

- (3) Germplasm resources that are representative of the genetic variation within the crop and/or its closely related species.
- (4) Methods for uniform artificial infestation.
- (5) Methods for assessing resultant damage, or lack of damage, to the plants subjected to deliberate infestation (rating scales to determine classes or categories of resistance or susceptibility).
- (6) Screening to determine whether adequate levels of resistance exist within suitable agronomic types (equivalent or better than currently grown cultivars), and an effective selection/breeding scheme established to improve either the resistance levels or agronomic characteristics of the "improved" materials.

## introduction

This bulletin presents the techniques developed and experience accumulated at CIMMYT over the past six years for efficient mass rearing and infestation with three maize stem borers: *Diatraea saccharalis* Fabricius, sugarcane borer (SCB), *D. grandiosella* Dyar, southwestern corn borer (SWCB), and *D. lineolata* Walker, neotropical corn borer, (NCB), for screening and improving host plant resistance in maize and sorghum. The *Diatraea* sp. complex is the most important group of stem borers that attacks maize, sorghum, and sugarcane in Mexico and Central and South America. Some of the techniques described have been adopted by entomologists working with other pest and crop species in screening and breeding initiatives in other parts of the world. Other techniques show promise of increasing the efficiency of mass rearing and infestation.

These techniques include the establishment and maintenance of the insect colony and the requirements for efficient mass rearing. The latter details the rearing facility, diets, containers, and procedures for handling the various life stages, illustrated in Figures 1 to 3.

A method of efficient, uniform field infestation is presented along with a description of the rating scales and procedures used to evaluate resultant damage and aid in the identification of resistant genotypes.

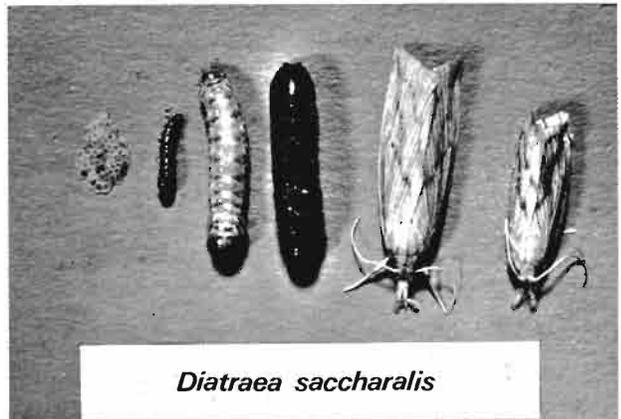


Figure 1. Life stages of *Diatraea saccharalis* Fabricius.

## establishment of the colonies

Guidelines established and recommended by entomologists who have developed maize cultivars with resistance to maize stem borers (Davis, 1976; Guthrie, *et al.*, 1982), that also have been proven by experience under CIMMYT conditions, are followed to establish and maintain healthy, vigorous *Diatraea* sp. maize stem borer colonies.

Colony maintenance can be accomplished by periodic complete replacement of the colony, selecting for field hardiness by subjecting insects to a generation on plants every five or six generations, or crossing laboratory colony adults to feral adults (Guthrie and Carter, 1972; Guthrie *et al.*, 1982). The CIMMYT borer colonies are maintained in a vigorous condition by one or more of these procedures at least every 10 generations.

## efficient mass rearing

**Rearing facility.** The CIMMYT rearing facility is a simple, inexpensive structure which satisfies the basic requirements for insect rearing. It has separate rooms or areas for infesting diet, larval rearing, adult emergence, oviposition, and egg incubation, where temperature, relative humidity, and photoperiod are controlled. There are also areas for diet preparation, dish washing, and storage of supplies and equipment. In addition, there are large refrigerators and freezers for storing diet ingredients and a small workshop for making, modifying, or maintaining the necessary rearing equipment.

**The diet.** Singh (1977) lists eight diets that have been successfully used for

rearing *Diatraea* sp. borers: two for *D. grandiosella* and six for *D. saccharalis*. Davis (1976) gives a diet that has been used successfully for mass rearing SWCB for over 15 years. In fact, this diet was the basis for the SWCB commercial diets that are presently available from Bioserv, Inc.

Two diets are used for rearing *Diatraea* sp. at CIMMYT (See Page 19). Under the conditions in our rearing facility, these have given higher insect production than the commercially available diets or others reported in the literature. The basic differences between our diet and other diets are (1) that we need to use more microbial inhibitors to keep the diet in good condition for the duration of the larval cycle, and (2) that we add sterilized maize tassel powder (green tassels collected before pollen shed, dried, ground and autoclaved). The addition of the tassel powder enhances larval establishment, shortens larval period, and results in larger, heavier pupae. Guthrie *et al.* (1969) obtained similar responses to pollen as food when studying the importance of maize pollen to *Ostrinia nubilalis* survival.

**Rearing containers.** Containers used for rearing *Diatraea* sp. and other borer species include glass vials or jars (Guthrie *et al.*, 1965; Chatterji *et al.*, 1968), plastic "jelly cups" (Brewer and Martin, 1976; Davis, 1976), and round plastic dishes of various dimensions (Guthrie *et al.*, 1965; Reed *et al.*, 1972).

All of these containers may be utilized efficiently in a mass rearing

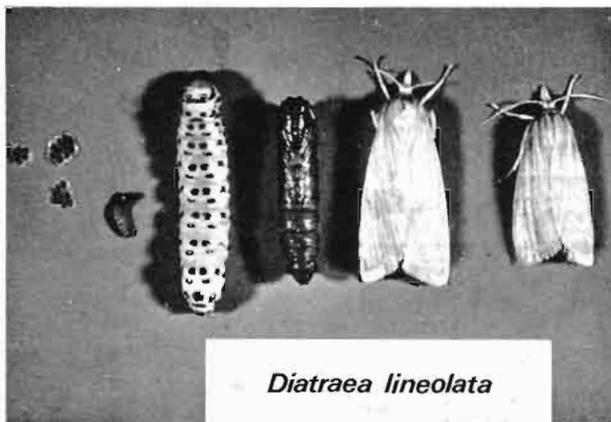


Figure 2. Life stages of *Diatraea lineolata* Walker.

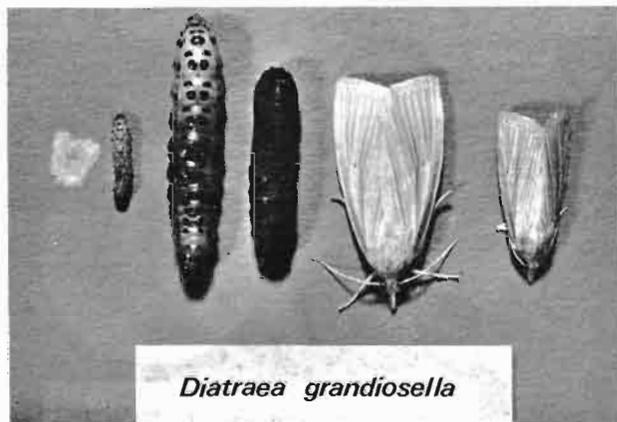
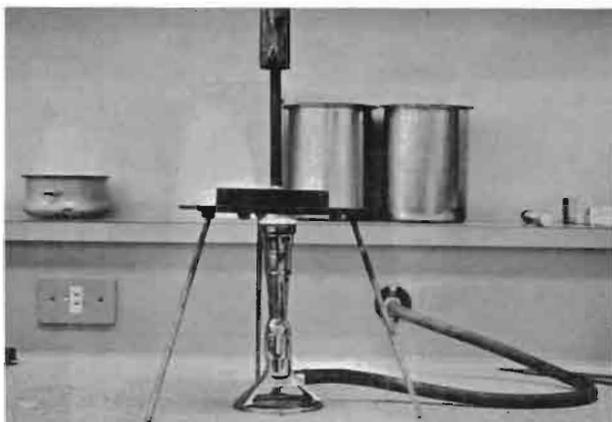
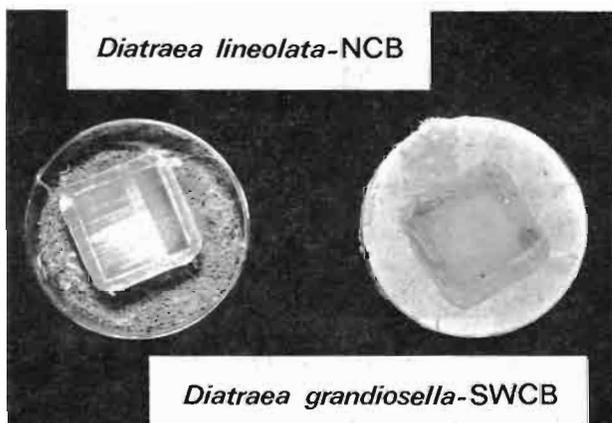


Figure 3. Life stages of *Diatraea grandiosella* Dyar.



**Figure 4.** A hot branding iron is used to perforate the rearing dish lid. A soldering iron is used to melt the into the lids.



**Figure 5.** Top view of dishes used for rearing NCB and SWCB.

program. Container type is influenced by the size of the rearing operation, cost and amount of available labor, cost, availability, reusability, and durability of a given container, and the biology of the borer species being reared. For example, experience has shown that jelly cups are unsuitable for rearing *Diatraea* sp. in Mexico and Surinam (Van Dinther and Goossens, 1970), and *Ostrinia furnacalis* in the Philippines because the larvae perforate the cups and escape from them.

The most widely utilized containers for rearing maize stem borers are round plastic dishes with tight-fitting lids, of dimensions commonly 15-30 cm in diameter and 7-10 cm in depth (Guthrie *et al.*, 1965; Reed *et al.*, 1972). Usually the lid is perforated and fitted with a fine mesh (60 or finer) brass screen (Reed *et al.*, 1972). At CIMMYT, we found that the easiest and best way of affixing the screen was to perforate the lid with a "branding iron" (Figure 4). Then, using a soldering iron, the screen is melted into the plastic lid, providing a permanent seal. The surface area of the screened section of the lid must be fixed in accordance with the species being reared and rearing conditions (amount of diet per dish, temperature and R.H. maintained in the rearing rooms). For *Diatraea grandiosella* larvae, which tend to enter diapause if the diet dessicates too much, we use lids with 64 cm<sup>2</sup> of screen. For *D. saccharalis*, diapause is not a problem, but mold growth on the diet is; hence a less humid environment within the box is desirable and we use lids with 121 cm<sup>2</sup> screen. Rearing dishes with larvae are illustrated in Figures 5 and 6.

The dishes and lids are disinfected by washing in a Mikro-Quat solution (470 ppm a.i. quaternary disinfectant) followed by a 10-minute UV light exposure before the hot diet is poured into them.

**Adult stages.** Adults are transferred daily from emergence cages (Figure 7) to oviposition cages (Figure 8). These consist of wire mesh cylinders (No. 5 mesh for SWCB, No. 4 mesh for SCB, which lay larger egg masses) whose walls are covered with 8 mil plastic bag tubing as oviposition substrate. The cage is placed over a plastic sheet with 1 mm perforations that cover a pad of cotton moistened with tap water. Adults can drink the water through the perforations, yet cannot oviposit through them on the moistened cotton pad. *Diatraea* sp. have a marked tendency to oviposit heavily on the moist cotton pad if not prevented from doing so; such masses are wasted as it is difficult to remove and utilize them. A screen of 20 to 30 mesh could serve the same function and be reusable if it were made of non-oxidizable metal or fiber.

The moist cotton pad also serves to maintain high humidity within the oviposition cage. This seems critical for maximum oviposition with *Diatraea* sp., especially for SCB. Conditions maintained within the oviposition rooms for best egg production are 95 plus percent R.H. and fluctuating temperatures (controlled by time clocks) of 25/20°C (day/night) for SCB and NCB, and 28/23°C for SWCB. Frequent power interruptions in Mexico prohibit the maintenance of regular and constant photoperiods. Our studies indicated that total oviposition is not

## rearing procedures and colony handing

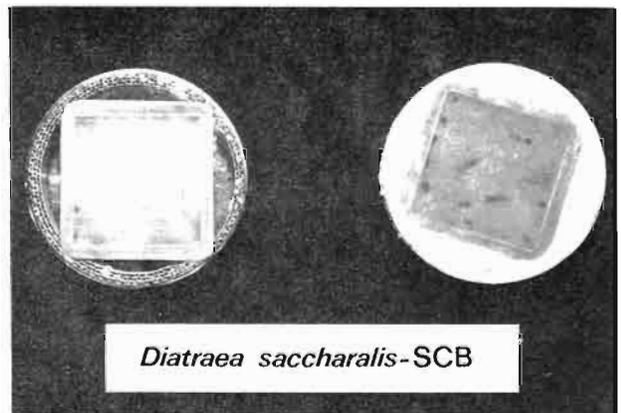


Figure 6. Top view of dishes used for rearing sugarcane borer.

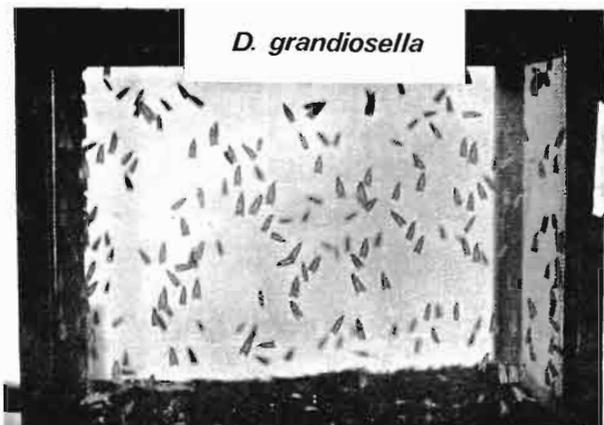


Figure 7. SWCB adults in an emergence cage.



Figure 8. Oviposition cage with one day's production of egg masses.

affected by photoperiod, so lights are shut off in the oviposition rooms when no one is working in them.

The cages and oviposition substrate were chosen because they are simple, inexpensive, and easy to construct and change. The plastic bag tubing is purchased in large rolls to fit three basic cage sizes: 35 x 90, 25 x 90, and 15 x 40 centimeters. Sections are simply cut off the roll to the length appropriate to the cage height. The roller device illustrated in Figure 9 facilitates cutting the tube to the appropriate length. These are slipped over the wire mesh cylinders, folded, and held tight against the screen with a few pieces of masking tape. Adults hang on the wire mesh and oviposit on the plastic through the spaces of the mesh. The number of adults placed in a cage is calculated on the basis of 1 moth per 10 cm<sup>2</sup> surface area. This allows sufficient space for female moths to oviposit with a minimum of interference from other moths.

The cages are changed daily (Figure 10) by removing the egg-laden plastic cylinder and replacing it with a new one. If the lights are turned on 15-20 minutes before entering the oviposition room, the moths settle down and become sedentary. This aids in minimizing adult escape while changing the cages.

An alternative oviposition cage and adult handling technique for SWCB has been developed recently by Davis (1982). Its advantages are less handling of adults and ease of oviposition sheet removal.

**Egg stages.** Egg-laden plastic cylinders are slit open to form a sheet and then cut into strips about 10 cm wide. Egg masses are separated from the plastic substrate by pulling the strips in a back and forth motion over a metal plate with a semi-sharp edge (Figure 11). It is essential that the egg masses are on the outside surface of the plastic strip or they will be crushed instead of popped off. The metal sheet is mounted in a frame surrounded by a Plexiglas enclosure which funnels the egg masses into a collection dish below. This procedure is useful only if infestations are made with newly hatched larvae. Egg mass removal is not essential, as larvae will hatch from egg masses not removed from the substrate (Davis, 1982). However, it does offer the following advantages:

a) Specially processed oviposition substrate need not be purchased; most rearing programs that use waxed paper purchase 30/45 wet waxed, bleached kraft paper (Guthrie *et al.*, 1965; Davis, 1982). Reasons for using specially processed waxed paper were for punching disks when egg mass infestations used to be standard, and for better hatchability of larvae from egg masses. In Mexico, we have observed reduced hatchability of egg masses when commercial waxed paper was used as oviposition substrate. This has not been a problem since switching to plastic.

b) Pure egg masses are obtained which facilitate incubation and handling of newly hatched larvae in the preparation of the larval mixture for field infestation. The removal of egg masses from heavy, bulky oviposition substrate increases in importance with increasing



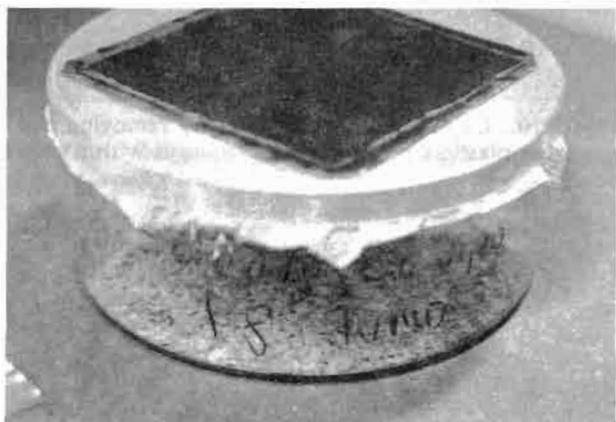
**Figure 9.** The roller device facilitates cutting tubes for borer oviposition cages.



**Figure 10.** Cages are changed daily by removing the egg-laden plastic cylinder and replacing it with a new one.



**Figure 11.** Removal of egg masses from the plastic substrate. Strips are pulled back and forth over the semi-sharp edge.



**Figure 12.** Egg masses are placed in a plastic dish for incubation to black head stage.

distance of the rearing site from field infestation site, and increased size of the rearing operation. Hundreds of thousands of pure insect eggs can be transported or mailed in a very small box or dish.

c) Compared to punching discs or cutting egg masses from oviposition sheets, popping them from the plastic is at least 10 times faster and easier.

Egg masses are placed in plastic dishes (Figure 12) and incubated to black head stage. At this stage, they can be stored in a refrigerator (at 10°C) for up to five days without affecting hatching, or they can be allowed to hatch for infesting diet or plants in the field.

**Larvae.** At CIMMYT, newly hatched *Diatraea* sp. larvae are used for infesting the diet to maintain or increase the laboratory colony. Using a camel's hair brush, larvae are transferred from the hatching dish. Approximately 250 are placed in each dish, which contains about 1.3 kg diet.

Davis and Owalt (1979) use a SWCB larval-corn-cob-grits mixture dispensed by machine to infest diet-filled jelly cups before they are capped. We also use a larval-grits mixture for infesting diets with corn earworm (Mihm, 1982) and fall armyworm (Mihm, 1983). However, since reduced levels of microbial inhibitors are necessary in the diets we use for *Diatraea* sp. borers, increased problems with microbial contamination were noted when diet was infested with borer larval-grits mixtures.

Infested boxes are held in rearing rooms with temperatures from 20-30°C and 60-70 percent R.H., depending on

how quickly the larvae are needed for field infestations. At 30°C first pupae are obtained in 20 days, at 20°C in 35 days. If necessary, nearly mature larvae can be stored at 15°C for several weeks, stopping their development.

**Pupal stage.** For several years, pupation modules of corrugated paper similar to those described by Reed *et al.* (1972) were used for SCB production at CIMMYT. Because corrugated paper with fewer than three convolutions per inch was not available, and SWCB and NCB larvae are too large to fit in the module chambers, they were not used for these two species. Eventually, non-availability of the corrugated paper prompted testing of SCB pupal production with and without pupation modules. As results indicated no significant difference, their use was discontinued for SCB; this also resulted in less microbial contamination of the rearing dishes.

Within three to four days of first pupation, pupae are extracted manually from the boxes and diet plugs, Figure 13. This procedure is repeated once or twice until 80-90 percent of the potential pupae are harvested. Pupal extraction remains one of the major labor-intensive parts of our rearing efforts but seems to be dictated by the biology of the tropical *Diatraea* sp. borers. In six years of rearing, the borers have always shown asynchronous pupation occurring over a period of 10 or more days. Entomologists who rear temperate borer species are spared this extra effort, as pupation is more synchronous.

After extraction, the pupae are placed in dishes or pans which are put in



Figure 13. *D. grandiosella* pupae are manually extracted from the rearing boxes and diet plugs.

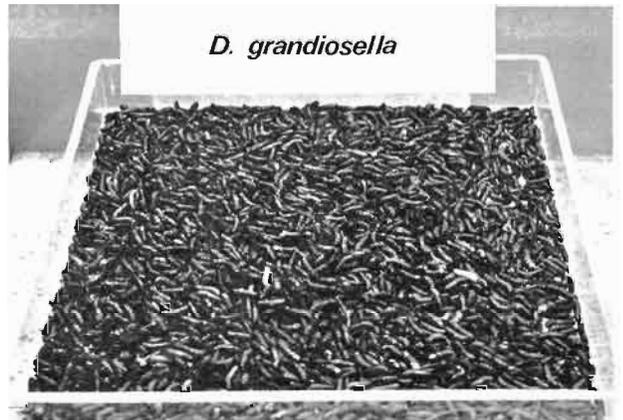


Figure 14. Pupae are placed in dishes or pans in the bottom of adult emergence cages.

the bottom of adult emergence cages (Figure 14). Each day, the pan with pupae is removed, emerged adults are shaken into oviposition cages, and the pan with pupae is replaced. Temperatures for pupal development range from 20-30°C, depending on how quickly adult emergence is desired. Relative humidity is always maintained at 95 percent or higher, simulating the moist conditions of the natural pupation site within the stem of a growing maize plant.

### efficient field infestations



Figure 15. *D. saccharalis* larvae are ready to mix with grits.

Artificial infestations with maize stem borers were done using egg masses (Davis, 1976; Guthrie, 1982) for a half-century until Mihm and colleagues at CIMMYT developed the bazooka and larval infestation technique in 1976 (CIMMYT Review, 1977). The use of this technique and its advantages for use with at least 11 species of lepidopterous insect pests have been described in detail by Ortega *et al.*, 1980; Wiseman *et al.*, 1980; Mihm, 1982, and Davis, 1982.

Borer egg masses ready to hatch are transported to the field for mixing and infestation. For SCB and NCB the egg masses are placed inside a small plastic box, held in place by small pieces of styrofoam within the standard plastic rearing dish. As larvae of these species are strongly positively phototactic, it is important that they be held in the dark during final incubation and larval hatching. If exposed to a light source they tend to aggregate, spin silk, and form clumps (illustrated in Figure 15) which

are hard to disperse when trying to achieve a uniform larval-grits mixture.

With SWCB, the egg masses are placed in a plastic box which has a mesh screen fitted in the bottom, Figure 16. These larvae are not strongly positively phototactic, but hydrotactic, and this characteristic is used to separate the larvae from the layer of unhatched egg masses. A moistened paper towel is placed in the bottom of the outer box, and the larvae crawl through the screen toward the moisture source. If this is not done, larvae tend to remain in the layer of unhatched egg masses.

Eggs which have not hatched can be separated from the larvae by quickly removing the inner box and brushing off the few larvae still crawling on it before setting it aside or placing it in an empty rounded plastic dish for further incubation. If some larvae have not dispersed from the egg mass layer, they are separated from it by dumping the masses on a paper towel. After a few minutes, the masses are poured onto another towel, and the larvae crawling on the towel can be transferred to the large mixing bottle by gently brushing or tapping.

A small amount of corn cob grits is added to the round plastic dish and the larvae are mixed by gently rotating the box. The mixture from several hatching boxes is accumulated in a large plastic bottle (2-20 liter), depending on the number of larvae and plants to be infested. After thorough mixing, the concentration is checked by counting several sample deliveries from the bazooka. If the quantity of grits is recorded, a series of dilutions is easily



Figure 16. Container of egg masses in black head stage, ready for field infestation.



Figure 17. Plants are infested with *D. grandiosella*.



Figure 18. Infestation at anthesis.

made until the desired number of larvae per delivery is attained. An alternate method for preparing the larval-grits mixture if egg masses are not removed from the oviposition papers is described by Davis and Williams (1980).

Routinely at CIMMYT, we infest all the borer species with 30-40 larvae per plant, applied in 3-4 successive passes of about 10 larvae per delivery. Our data (Table 1) indicate three shots per plant gives acceptable uniformity. Greater uniformity is possible, but not worth the additional time and effort required for additional passes.

**Table 1. Plant-to-plant variation in number of sugarcane borers with successive applications of larvae using bazooka**

	Number of Applications						
	1	2	3	4	5	6	7
Mean	8	17	28	36	43	50	57
S.D.	5	5	6	7	8	9	10
C.V.	55	31	22	19	18	19	17

To minimize variation which may be caused by the person doing the infestation, different people go over a section of the nursery on successive passes.

At CIMMYT, for infesting maize to simulate the attack of a first brood (Figure 17), plants in the six to eight fully expanded leaf stage are infested by dispensing the larval-grits mixture into the whorl. To simulate the attack of a

second brood, maize plants are infested at anthesis (Figure 18); the leaf axils of the ear leaf and one above and below the ear are infested; for sorghum, this can be accomplished by dispensing onto the panicle. When doing second brood infestations, care must be taken not to bump the plants for a few minutes, so that larvae have time to attach themselves to the plant.

Evidence of the utility and adaptability of this technique comes from its rapid and widespread adoption for several lepidopterous pest species in several crop species (Davis and Oswalt, 1979; Davis and Williams, 1980; Hall *et al.*, 1980, Wiseman, *et al.*, 1980).

The rating scales and quantification of damage used to categorize the performance of plants (resistant or susceptible) after infestation in the field or greenhouse are listed in Table 2. Plants ranging from susceptible to intermediately resistant are illustrated in Fig. 19.

For evaluating resistance to leaf feeding (done by the early instars of most borer species), standard scales of one to nine are used by most entomologists in the world (Guthrie *et al.*, 1965; 1960; Davis, 1980; Dolinka *et al.*, 1973, Ortega *et al.*, 1980).

Resistance to sheath and collar feeding (usually done by early instar second brood borer larvae) is also evaluated using a one to nine class scale (Guthrie, 1982).

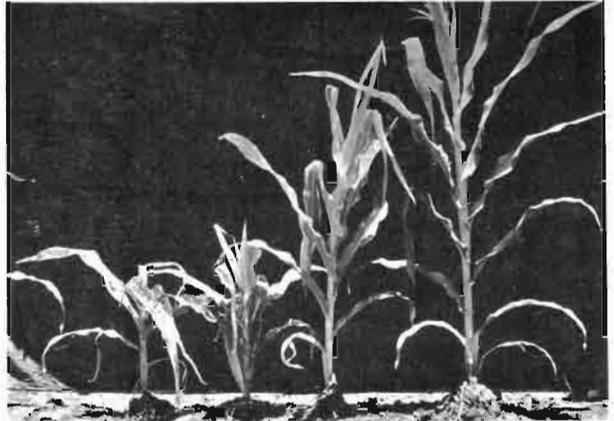


Figure 19. Plants showing susceptible reaction (left) to SWCB to intermediately resistant reaction (right).

## damage evaluation

Table 2. Commonly used damage rating scales for evaluation and development of resistance to maize borer pest species\*

Categories of Resistance or Susceptibility Indicated by the Rating Classes

Least Damaged .....Most Damaged  
 Excellent .....Very Good .....Good .....Fair .....Poor .....Unacceptable  
 Highly Resistant .....Resistant .....Intermediately Resistant .....Susceptible

Insect Group	Plant Part Damaged	Reference**
Leaf feeders/ stalk borers ( <i>Ostrinia</i> , <i>Chilo</i> , <i>Diatraea</i> )	Leaf	12
	(1) No damage or few pinholes	(7) Long lesions common on half of the leaves
	(2) Few shot holes on few leaves	(8) Long lesions common on 1/2 to 2/3 of leaves
	(3) Shot holes on several leaves	(9) Most leaves with long lesions
	(4) Several leaves with shot holes and few long lesions	(7) Holes bigger than match head size rare or sporadic
	(5) Several leaves with long lesions	(8) Intermediate
Leaf	(6) Several leaves with lesions > 2.5 cm	(9) Many holes bigger than match head
	(4) Match-head sized holes rare or sporadic	(7) Holes bigger than match head size rare or sporadic
	(5) Match-head holes intermediate	(8) Intermediate
Sheath & Collar (1 lesion per plant)	(6) Many match-head holes	(9) Many holes bigger than match head
	(1) Pinholes rare, sporadic	(7) Holes bigger than match head size rare or sporadic
Stalk	(2) Pinholes intermediate	(8) Intermediate
	(3) Many pinholes	(9) Many holes bigger than match head
Cumulative number of lesions where a lesion = feeding of 1/2 distance around stalk (10 lesions per plant)		16
Cumulative number of "cavities," where a cavity = 2.5 cm of tunneling in stalk		17

\* Adapted from ch.16, Breeding plants resistant to insects. Maxwell, F.G. and P.R. Jennings (Eds.) John Wiley and Sons, NY. 683 pp.

\*\* 12, Guthrie *et al.*, 1960; 13, Starks and Doggett, 1970; 14, Davis *et al.*, 1973; 15, Mihm *et al.*, unpublished; 16, Dolinka *et al.*, 1973; 17, Guthrie, 1974.

Resistance to stalk boring is evaluated by counting cavities (where one inch of damage to the stalk equals a cavity) (Guthrie, 1982), the number of internodes damaged, or number of exit holes per plant (Peairs, 1977; Hershey, 1978). Counting broken tassels and/or lodged plants resulting from borer damage to the maize stem also is occasionally done (Chiang and Hudon, 1976).

Resistance to ear damage is evaluated by scoring amount of kernel damage, cob tunneling, and shank damage (Galt, 1977; Grier and Davis, 1980). Occasionally, percent ear droppage is enumerated and is of importance, especially where maize is harvested mechanically.

The techniques and experience described in this bulletin for efficient mass rearing and infestation have shown to be adaptable to other pest and crop species and to screening and breeding initiatives in other parts of the world. The final objective in the application of these techniques to any program of efficient mass rearing and infestation is to identify resistant genotypes for immediate use or to identify the most resistant genotypes (plants) for use in a breeding program. Varieties with improved resistance can serve as one of the major components in the effort to manage *Diatraea* sp. or other pest species populations.

## conclusion



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**Correct Citation:** Mihm, John A., 1983. Efficient Mass Rearing and Infestation Techniques to Screen for Host Plant Resistance to Maize Stem Borers, *Diatraea sp.* Centro Internacional de Mejoramiento de Maíz y Trigo. El Batán, México.

CIMMYT. The International Maize and Wheat Improvement Center (CIMMYT) receives support from government agencies of Australia, Canada, Denmark, France, Federal Republic of Germany, Japan, Mexico, The Netherlands, Norway, The Philippines, Spain, Switzerland, United Kingdom, and USA; and from the Ford Foundation, Inter-American Development Bank, OPEC Fund for International Development, Patronato de Sonora (PIEAES), Rockefeller Foundation, United Nations Development Programme, and the World Bank. Responsibility for this publication rests solely with CIMMYT.



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