PROCEEDINGS OF THE

FIFTH BIENNIAL SMUT WORKERS' WORKSHOP

April 28-30, 1986
Ciudad Obregon, Sonora, Mexico

CENTRO INTERNACIONAL DE MEJORAMIENTO DE MAIZ Y TRIGO
INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER
Lisboa 27 Apartado Postal 6-641 06600 México, D.F. México

PROCEEDINGS OF THE

FIFTH BIENNIAL SMUT WORKERS' WORKSHOP

April 28-30, 1986
Ciudad Obregon, Sonora, Mexico

Distributed by CIMMYT
The International Maize and Wheat Improvement Center
Lisboa 27 Apdo. Postal 6-641 06600 Mexico, D.F., Mexico

TABLE OF CONTENTS

Introduction

The Fifth Biennial Smut Workers' Workshop was held in Ciudad Obregon, Sonora, Mexico, on April 28-30, 1986. Just 15 minutes away from Ciudad Obregon, in the middle of Mexico's most important wheat-growing region, is the Northwest Agricultural Research Center (CIANO). Various CIANO staff members participated in the workshop, which was of particular interest both to CIANO and CIMMYT this year because it provided the opportunity to discuss research being conducted on Karnal bunt. The disease has become a problem to Mexico and CIMMYT due to the threat it poses to grain exportation and the distribution of international trials.

Workshop activities began with a short welcome address by Dr. Ernesto Samayoa, Assistant Director for Research, CIANO/INIFAP (Mexico's National Institute of Forestry, Agriculture, and Livestock Research) for the northern area. Researchers from Canada, India, Mexico, and the USA took part in the workshop. In addition to two days of conferences and discussions, the workshop agenda included a field day at CIANO to view Karnal bunt-infected grain, research installations, and fungicide validation experiments at several locations in the Yaqui and Mayo Valleys. At the end of the workshop, Dr. Arthur Klatt, Associate Director of CIMMYT's Wheat Program, briefly summarized the most important aspects discussed during the workshop and proposed that another such event be held soon, in a more formal setting.

This document contains brief abstracts of the papers presented at the workshop.

Evaluation of Treatments for Controlling Dwarf Bunt of Wheat

J.A. Hoffman

Bitertanol and thiabendazole seed treatments (1.5 g ai/kg) and PCNB soil treatment (2.67 kg ai/ha) were compared for control of dwarf bunt in Nugaines and Wanser winter wheat seeded on four dates. PCNB applied to the soil surface in late fall after emergence was the most effective treatment. It reduced disease incidence by 88-96% in both cultivars seeded on all four dates.

Bitertanol was about twice as effective as thiabendazole and reduced disease to less than 5.0% in both cultivars with the later seeding dates of 27 September and 11 October. Thiabendazole seed treatment provided control only in wheat seeded late, on 11 October. Neither seed treatment was effective with early seeded wheat and both reduced wheat yields. Dwarf bunt reduced yields about 0.8% for each percent of disease incidence. These results indicate a need for seed treatment effective for early seeded wheat.

Common Bunt Research in the US Pacific Northwest

R.J. Metzger

Common bunt is caused by the fungi Tilletia caries and T. foetida, and dwarf bunt is caused by T. controversa. Although dwarf bunt can still be a problem at the higher altitudes of the Pacific Northwest of the US, there has not been a common bunt problem in this region in many years due to widespread use of fungicides and the incorporation of effective resistant gene combinations in commercial cultivars.

In both bread and durum wheat, the same genes give resistence to T. caries, T. foetida, and T. controversa. However, common bunt races seldom have more than 3-4 genes for virulence, while dwarf bunt races often have as many as 7-8. Host differentials for the genes Bt1 to Bt12 have been established and more than 40 races identified.

More than 26,000 accessions have been screened for resistance to common and dwarf bunts. Adequate sources of resistance have been found in wheats obtained from eastern Turkey and adjacent areas in Russia, Iraq, and Iran. Several sources of resistance have also been obtained from the mountainous regions of Yugoslavia. Good resistance is found in Hohenheimer (Bt-5), P.I.178383 (Bt-8,9,10), P.I.166910 (Bt-9,11) and P.I.119333 (Bt-12). Triticum monococcum, T. uruartu, and Aegilops spp. are also good sources of bunt resistance, but can be difficult to work with. The "D" genome appears to be a poor source of bunt resistance.

Breeding for Resistance to Loose Smut

J. Nielsen

The most important prerequisites for successfully breeding for resistance to loose smut in wheat are a knowledge of the races of the pathogen, including its genes for virulence, and a pool of resistance sources.

Because the disease is seedborne, all genes for virulence must be considered in order to achieve durable resistance, even if certain races are not present in the area where the variety will be grown. Virulence by any of the 41 known races can be explained by the single or combined action of as many as 9 genes that have been identified to date, of which 5 are known to be recessive.

Over the years, about 150 common wheats and 60 durum wheats have been found to be resistant to all 41 races.

Two or three races carrying the most important genes for virulence are used to screen breeding material. The partial vacuum method is used to inoculate the spikes, and the reaction is based on the percentage of infected plants grown from inoculated seed.

The above test reveals both physiological and morphological resistance; the latter is apparently based on the plant's ability to keep spores out and would be the ideal form of resistance. However, no single morphological trait that is clearly correlated with this form of resistance has been identified. If such a trait is found and proves to be transferable, it will be very useful to breeders.

Ploidy Levels in Smut Fungi

R. Durán

Quantitative studies of deoxyribonucleic acid (DNA) in Ustilago hordei, Ustilago scitaminea, and Ustilago zeae indicate that ploidy levels of sporidia, dikaryons, and teliospores (as well as meiotic sites) can be determined by fluorometric techniques. Regression of fluorescence and DNA content was demonstrated in Saccharomyces cerevisiae and in diploid, tetraploid, and hexaploid species of Aegilops and Triticum species by staining the nuclei with pararosaniline. Saccharomyces cerevisiae, shown by denaturation studies to contain about 15×10^3 kilobase pairs of DNA, was used as an internal standard.

To calculate absolute DNA content of smut nuclei, the ratio of fluorescence intensity of haploid yeast cells (i.e., 15.4 fluorescence units) to 15×10^3 kilobase pairs was used as a constant, where

$$\frac{\text{Ifl yn}}{15 \times 10^3} = \frac{\text{Ifl sn}}{x}$$

and Ifl yn = fluorescence intensity of yeast nuclei, 15×10^3 = kilobase pairs of yeast DNA, Ifl sn = fluorescence intensity of smut nuclei, and x = kilobase pairs of smut nuclei.

Studies on *Tilletia controversa*

H.S. Fenwick

Studies initiated in 1982 at the University of Idaho on *Tilletia controversa* included the following:

1. Effects of fluctuating temperatures on teliospore germination.
2. Effect of different temperatures on the production of primary sporidia after promycelial formation.
3. Effect of freezing or desiccation on germination of teliospores preconditioned for 1-12 weeks in soil extract broth.
4. Effect of infection on the number of spikes produced per plant.
5. Contributions of nonsmutted, partially smutted, and totally smutted plants and spikes to total yield.

Results obtained in these studies are briefly summarized as follows:

1. Data suggest that the temperature and time requirements for teliospore germination of *Tilletia controversa* are cumulative.
2. After mycelial formation, primary sporidial production occurs rapidly. Incubation at 22 °C prevented further development of the germination process in over 50% of the identified teliospores.
3. It appears that teliospores can be preconditioned to germinate in liquid media for at least six weeks without deleterious effects from freezing and up to seven weeks without being affected by desiccation.
4. The means of total spikes per plant were consistently highest with partially smutted plants and lowest with totally smutted plants.
5. The mean number of smutted (partially and totally) plants was 22.3% for the period 1982-85. Of these, 94.3% contributed to yield. Data suggest that since partially smutted plants produce more spikes per plant than nonsmutted plants, a fairly high percent of the plants and spikes in a field may be smutted, but yield may not be correspondingly reduced.

Isozyme Analysis for Smut Identification

M.R. Bonde

Isozyme analysis has been used for many years on animals and plants, but not very extensively on plant pathogens. Generally, when applying this technique to plant pathogens, scientists have interpreted isozyme banding patterns in terms of the number of bands in common between isolates and the intensity of isozyme bands.

At the United States Department of Agriculture (USDA) in Frederick, Maryland, banding patterns are being interpreted using a genetic approach. There, the capability exists of staining for approximately 70 different enzymes, 40 of which are used routinely with fungal pathogens. Tilletia indica, causal agent of Karnal bunt, has been successfully differentiated from other smut pathogens, including morphologically similar Neovossia horrida, causal agent of kernel smut of rice. Nine of twelve enzymes tested unequivocably differentiated these two pathogens.

The equipment utilized in isozyme analysis is inexpensive and the techniques are not difficult. Because smut organisms are single-spored and have a haploid stage, their isozyme banding patterns are very easy to interpret.

Searching for *Tilletia indica* in Southwest USA

T. Matsumoto

In late 1983, Karnal bunt (*T. indica*) spores were found in railroad cars coming from Mexico into the United States. As a result, in 1984 a survey using 5,000 wheat samples was conducted in California, Arizona, and Oregon, but no Karnal bunt was discovered.

In 1985, the search continued in California, Arizona, and Mexico (Mexicali area). The California Department of Food and Agriculture (CDFA), the Arizona Commission of Agriculture and Horticulture (ACAH), USDA-APHIS, Mexico's Sanidad Vegetal, and the Agricultural Research Center for Northwest Mexico (CIANO) collected and processed approximately 1,200 wheat samples using a centrifuge wash technique. All samples were negative for Karnal bunt.

Although it is usually seedborne, there are several other ways in which *T. indica* spores may be spreading. Besides railroad cars, many trucks coming to the US from Mexico have been found to be carrying spores. Also, when stubble in an infected field is burned, the spores are swept up into the air and carried over long distances. Plans are under consideration for taking air samples from an airplane after a stubble burn. The spores thus collected would then be tested for viability.

A cooperative project involving CIMMYT, CIANO, Sanidad Vegetal, USDA-ARS, USDA-APHIS, ACAH, and CDFA studied various detection and laboratory methods at the CIMMYT-CIANO Research Station near Ciudad Obregon, Mexico, in 1986. As a result of this project, a promising new assay for detecting *T. indica* teliospores in soil was demonstrated during the present workshop by Dr. L.E. Datnoff (see page 21).

Smut Identification Using Exospores

In view of the general confusion regarding exospore descriptions for different species of smut, a program was set up to study exopores and develop a key for identifying them. As a result of these efforts, a laboratory guide for the identification of smut fungi of quarantine significance will soon be published by the CDFA. It may be obtained by writing to this author or to C. Krass at the California Department of Food and Agriculture, 1220 N Street, Sacramento, California, 95814.

Head Smut of Sorghum (*Sphacelotheca reiliana*)

J. Narro S.

Of the three smut species that attack sorghum in Mexico, only one, head smut (*S. reiliana*), causes serious damage on susceptible varieties. It was first reported in Mexico in 1968 by H. Angeles.

In 1979, a survey on the identification, distribution, and incidence of sorghum smuts was initiated. It confirmed these diseases were present in the States of Jalisco, Guanajuato, and Michoacan, with the highest incidence (20%) recorded in Michoacan.

In 1982, covered, loose, and head smuts were detected on 8.4%, 12.0%, and 16.3% of plants, respectively, in the Bajio region. Efforts to control the disease have concentrated on breeding resistant hybrids, such as BJ-83, BJ-84, and BJ-85, and on monitoring pathogen populations. In spite of these efforts, most resistant hybrids produced by government research programs and private seed companies are expected eventually to become susceptible, since their source of resistance has proven susceptible to a new pathogenic race in Texas.

Control of Head Smut of Maize in Central Jalisco, Mexico

H. Sánchez A.

Two species of smut, common smut (Ustilago maydis) and head smut (Sphacelotheca reiliana), attack maize in Mexico. While common smut is endemic and of minor importance, in 1959 head smut was found to reduce yields an average of 30% in the State of Guanajuato (López et al., 1959). Resistant hybrids were developed and disease incidence diminished significantly.

Head smut appeared again in 1977 on maize growing under residual moisture conditions in the State of Jalisco and more recently has been found in the Valley of Mexico. By 1981, head smut had spread to 1,800 ha in Central Jalisco, where incidence was 10-40%. Since 1982, the disease has become widespread throughout the area planted to maize under residual moisture conditions, though incidence is variable.

Factors favoring the disease are: 1) wide distribution of inoculum; 2) climatic conditions suitable for infection; and 3) use of susceptible varieties. Since 1982, the following measures to control the spread of infection and yield losses have been carried out:

a) Removal of susceptible varieties from the market;
b) Burning crop residues on contaminated fields;
c) Destroying diseased plants before harvest; and
e) Crop rotation.

In addition, work is currently under way to develop varieties resistant to head smut of maize.

Research project--Three kinds of control studies are being conducted at the Agricultural Experiment Station in Central Jalisco:

a) Chemical seed treatments using systemic fungicides;
b) Cultural methods, especially the effects of planting dates and fertilizer application; and
c) Use of resistant varieties.

Bioregulators Associated with Common and Dwarf Bunts of Wheat

E.J. Trione

It was hypothesized that two essential bioregulators produced by the wheat plant control the growth and sporulation of the bunt pathogenic hyphae. The dikaryon bioregulator is assumed to be present in the culm, whereas the sporulation factor is present only in the young ovary.

Symptoms of the bunt diseases suggest that the pathogens cause a hormonal imbalance in infected wheat plants. When grown in pure culture, monokaryons and dikaryons were found to produce high levels of three identified plant cytokinins (as well as five unidentified ones). Enhanced cytokinin production is thought to be implicated in the disease symptoms: increased tillering, increased cell division, increased number of infected kernels, and the darker green color of diseased plants.

It is also hypothesized that the bunt fungi produce three other important bioregulators associated with wheat plant development: a pollen gametocide, an inhibitor that stunts culm growth, and an apomictic (or parthenocarpic) factor that stimulates development of the unfertilized ovary.

Karnal Bunt in the Yaqui Valley

M. Lira

Sonora is Mexico's major wheat-growing state and produces 38% of the country's total wheat (1.5 million tons per year). Some of the certified seed produced in Sonora has been exported in the past, but now exports have diminished as a result of many countries becoming self-sufficient in seed production and because of Karnal bunt. Farmers have suffered losses of 1,400 million pesos due to reduced exportation of seed.

The principal wheat-producing areas in Sonora are the Yaqui and Mayo Valleys. In 1983-84, the Yaqui Valley was almost free of Karnal bunt and the Mayo Valley had a low incidence, but in 1984-85, the incidence of the disease in both valleys increased significantly. A total of 55,182 hectares of wheat were infected, and it was recommended that areas with the highest infection levels (levels above 2% are considered very heavy) be planted with durum wheat the following cycle, since it is not as susceptible to Karnal bunt. Farmers in the Yaqui Valley followed the recommendations, but most Mayo Valley farmers did not.

As a result, this year infection levels in the Yaqui Valley are far lower than in the Mayo Valley. So far, with only 15% of the wheat harvested, 1059 samples collected in Sonora had an average of 39.8 infected grains per kilo (0.16% infection), and only 6 samples showed more than 3% infection. Of the 654 samples taken from the Yaqui Valley, 50% had 0 infection, 41% showed very light infection, and only 2% were heavily infected. However, half of the disease-free grain is durum wheat.

Karnal Bunt Research in Mexico

J.M. Prescott

Karnal bunt is very important to CIMMYT because the distribution of germplasm throughout the world is an essential part of the Wheat Program's breeding efforts. Therefore, quarantine regulations that impose limitations on seed shipments seriously jeopardize the whole Program. For this reason, the Center now has a major research program directed at studying the disease. Research activities are being carried out in the field at CIANO (Agricultural Research Center for Northwest Mexico) and in the laboratory at El Batan.

Field efforts--One of the main field activities is screening germplasm under natural and artificial inoculation conditions to identify resistant or immune lines. Of the more than 23,000 lines tested so far, some have consistently shown very low or zero infection. Another aspect that is being studied is the effectiveness of various fungicides as seed treatments. Many of these fungicides have proved to be fungistatic, but none are fungitoxic. Systemic fungicide seed treatments have not been successful, as they do not persist long enough in the plant to prevent infection. Karnal bunt, though seedborne, is not systemic, as are the other bunt diseases caused by Tilletia spp., and infections of healthy plants occurs during flowering.

Other aspects of field work include studying the weather variables involved in infection using electronic data pods to gather information, conducting surveys on grain arriving at elevators, and taking spike samples before harvest to determine infection levels and trends. Teliospore and sporidia production and dispersion are also being studied; especially important is finding the different ways in which spores are carried over long distances. One method used for doing this is trapping airborne teliospores using an airplane while farmers are burning stubble. Teliospores have been found at various altitudes, but not in great quantities.

Laboratory studies--Ongoing laboratory efforts are directed at testing inoculation techniques, host range studies, testing the effect of Karnal bunt on germination, as well as its effects on animals that eat contaminated grain. Surveys are also being conducted to examine the role of different agronomic practices on Karnal bunt infection.

Histopathology of Karnal Bunt Infection of Wheat

B. Goates

Karnal bunt inoculation of wheat plants was performed by 1) spraying plants with a suspension of secondary sporidia (about 5,000 sporidia/ml) followed by 48 hours in a humid chamber, or 2) inverting, for 1 or 2 days at 20 °C, a Tilletia indica culture which was actively discharging secondary sporidia over spikes placed on a petri dish containing water agar.

Scanning electron micrographs of glumes fixed after 24 hours' incubation time showed hyphae from secondary sporidia penetrating the stomatal apertures. Hyphae either penetrated the stomata directly or were oriented parallel to the stomatal aperture and produced lateral branches which penetrated the aperture. Sections about 1.5-2.0 mm thick of florets embedded in epoxy resin showed hyphae going into the substomatal chamber of both the glume and the lemma 48 hours after inoculation. The presence of hyphae was confirmed with electron microscopy. Hyphae penetrated the stomata, but not directly through the cuticle of the epidermis.

Sections of florets fixed 3.5 days after a 2-day incubation period showed hyphae in the mesophyll of the glumes and lemmas. There were more hyphae in the glumes than in the lemmas, with the number of hyphae generally decreasing near the middle and basal portions of either tissue. Sections of the floret base (which included the glume, lemma, palea and basal portion of the ovary) of all samples showed no hyphae in this tissue. There were also no stomata in this region. Whether hyphae in the mesophyll eventually reach the ovary remains to be determined.

Laboratory and Greenhouse Studies on Karnal Bunt

E. Warham

Studies were carried out at CIMMYT headquarters in El Batan on aspects related to culturing the pathogen, inoculation techniques, resistance screening, and seed treatments.

The best medium for germinating teliospores was found to be water agar, while potato-dextrose agar gave the best results for secondary sporidia production. A temperature of 20 °C favors teliospore germination and secondary sporidia production, though they can occur throughout the 5-30 °C range; pH values between 3 and 9 have little effect on both processes.

Boot and spray inoculation were compared on susceptible cultivars to determine the most effective growth stages, inoculum concentrations and humidity requirements. The boot inoculation technique required low secondary sporidia concentrations and no humidity, and produced higher infection levels at awn emergence. In constrast, plants were infected at other growth stages using the spray inoculation technique, but high humidity and high secondary sporidia concentrations were necessary.

Both inoculation techniques were used to screen 400 lines of germplasm, including bread wheats, durums, triticales, Triticum carthlicum, ryes, and barleys. Infection percentages obtained with both techniques were comparable, but lines having 0 infection with one technique did not necessarily have 0 infection with the other. This indicates that the two techniques identify different types of resistance: the boot inoculation technique screens for physiological resistance while the spray inoculation technique tests morphological resistance and, to a lesser extent, physiological resistance.

Preliminary screenings of bread wheats indicated that those with either Alondra or Tzpp in their pedigrees were more resistant. Consequently, a greater number of lines containing Alondra or Tzpp was screened, but very few showed any resistance. Other materials screened included Triticum carthlicum, ryes, and wheats having the 1B/1R translocation, which all had lower levels of infection compared to the bread wheats.

Forty-six chemicals were tested to determine their efficacy as seed treatments for controlling Karnal bunt. None was found to be fungitoxic, but some were fungistatic for periods of 6 to 18 months. The chemical which consistently yielded the best results was triphenyltin hydroxide (Du-Ter).

Natural Field Infection and Agronomy

M. Osmanzai

A series of experiments were conducted in Yaqui Valley, Sonora, to evaluate Karnal bunt infection with different agronomic inputs. Results indicate that disease incidence was positively correlated to the presence of NH^{+}_{4} in the soil ($r = 0.92^{**}$). The application of N-Serve enhanced the effect of nitrogen and chicken manure by inhibiting the nitrification process in the soil to increase the concentration of NH^{+}_{4}.

Karnal bunt incidence with a given amount of organic manure was not as high as with the same amount of nitrogen derived from ammonium sulfate because organic manure is a slow release nitrogen fertilizer. Increasing the application of nitrogen as ammonium sulfate from 75 kg/ha to 260 kg/ha resulted in an even higher incidence of Karnal bunt.

Neither spacing nor seeding rate had significant effect on disease incidence, but it diminished considerably under water stress conditions. In conclusion, Karnal bunt incidence may be reduced by minimum irrigation at the critical time for infection and by the application of urea or nitrates.

Chemical Seed Treatments for Karnal Bunt

J.A. Hoffman

Chemicals used as seed treatments known to prevent teliospore germination of the Karnal bunt fungus were tested to determine whether their activity was fungistatic or fungicidal. Two days to one year following seed treatment, teliospores were removed from the seed and rinsed with appropriate solvents (chloroform, acetone or water) to remove the chemical treatment. The teliospores were then placed on agar and incubated under optimum conditions to assess their viability.

In repeated tests, all seed treatments were found to be fungistatic; a significant percentage of teliospores were viable after removing the seed treatment chemical. Mancozeb (Manzate 200) and carbendozine (DPx965-50) appeared to be the most effective in reducing teliospore viability. However, suitable solvents for these materials were not identified and their greater apparent activity may have been due to the incomplete removal of the chemicals from the teliospores.

Because most, though not all, seed treatments are fungistatic, fumigant gases and chemical seed soaks were tested as seed sanitizing agents. Formaldehyde solution and 40% ethanol showed high potential in preliminary studies, but failed in more rigorous testing.

Current studies indicate that mercuric chloride solution may be an effective sanitizing agent. Seed soaks employing this solution have reduced teliospore viability to less than 5% without diminishing seed viability significantly. Additional testing using mercuric chloride will be completed in the next four months.

Fungicide Studies in the Field

J.L. Smilanick

The recent occurrence in northwest Mexico of Karnal bunt resulted in the establishment of quarantines against importing wheat from this area. CIMMYT's winter-cycle field operations, which are conducted there, are much impeded by such measures. For these reasons, USDA/ARS, CIMMYT, and the Mexican National Institute of Forestry, Agriculture and Livestock Research (INIFAP) set up a cooperative project to describe the biology of Tilletia indica Mitra, the causal agent of Karnal bunt, and to develop techniques for its control. The specific objectives of the project include:

* conducting field studies in Mexico to evaluate fumigants and fungicides applied to soil, seeds, or foliage to control Karnal bunt;

* recording the crop environment throughout the growing season in Mexico, by means of electronic weather data loggers, to define ideal conditions for disease development;

* determining the efficacy and mode of action of chemical seed treatments which could kill contaminating teliospores without a concomitant adverse effect on seed viability;

* determining factors which affect teliospore and sporidia germination, dormancy, longevity, and dissemination, and

* elucidating, by means of light and electron microscopy, how and where infection occurs, and in what plant tissues the fungus is found.

A summary of 1985-86 as well as of the two previous years of work shows substantial progress has been made in achieving these objectives; the following conclusions are relatively certain:

1) Chemical control in Mexico:
   a) The most effective soil fumigant was methyl bromide, for it reduced teliospore germination by more than 99% to a depth of 10 cm when used in wet soil, and 85% to a depth of 10 cm in dry soil.

b) Three fungicides, propiconazole (Tilt), etaconazole (Vangard), and mancozeb (Manzate 200), when applied to wheat at spike emergence, have reduced Karnal bunt infection by 85% or more in repeated experiments in the 1984-85 growing cycle.

2) Weather influence on disease development in Mexico:
   a) Records suggest that rainy periods promote natural Karnal bunt infection. Artificial inoculation, however, resulted in high percentages of infected plants regardless of the weather. Further observation and analysis are needed before definite conclusions can be reached.

3) Teliospores and secondary sporidia:
   a) Teliospores are extremely resistant structures and will survive and germinate after: 1) ingestion by grasshoppers, chickens and, probably, cows; 2) temperatures up to 100 °C for 4 days when dry; and 3) burial in dry field soil for at least two years.

   b) Teliospores are relatively insensitive to pH, are mildly stimulated by light, and resume germination unimpeded after interruption by desiccation or extreme temperatures. They will not germinate without abundant moisture, however, and are more sensitive in this respect than most fungi. Teliospores must be on the soil surface to release sporidia after germination, for they were not able to penetrate 2 mm of soil to reach the surface.

   c) Secondary sporidia survived 2, 2, 7, 8, and 11 hours at 25, 50, 70, 85, and 90% relative humidity, respectively. Germination was most rapid at 15-25 °C, and greatly slowed at 5 °C and 30 °C.

4) Chemical seed treatments:
   a) No seed soak or fumigation procedure killed all teliospores recovered from infected seeds without severely reducing the seeds' ability to germinate.

   b) All seed treatments tested were fungistatic, not fungitoxic.

Isozyme Analysis with *Tilletia indica*

M.R. Bonde

The purpose of this paper is to report the results of studies that confirm Mendelian inheritance for isozymes coded by four loci in *Tilletia indica*. Demonstrating that isozymes are genetically inherited provides valuable credence to pathogen identification using isozymes in the future. Other possible uses of isozymes in *Tilletia indica* research will be discussed.

Parent basidiospore lines and five teliospore progeny for each of 15 separate crosses were compared side-by-side on starch gels. The gels were stained and each parent and progeny for four isozyme loci were scored. Each isozyme locus followed Mendelian inheritance and can be used as a genetic marker.

At least 12 additional polymorphic loci are potential genetic markers. These markers could be very valuable in learning about the genetics of the organism. Genetic markers could prove useful for many kinds of experiments, such as studying population genetics of the pathogen in the field. Isozyme analysis therefore can be useful for learning important aspects about the life cycle of an organism.

There is particular interest in using isozyme analysis for identifying fungal pathogens. Although most of the work to date has been with fungi, bacterial pathogens, plants, and insects are beginning to be studied at the USDA in Frederick, Maryland, in cooperation with scentists at other institutions.

Recovery of *Tilletia indica* Teliospores from Soil

L.E. Datnoff

A new method was described for recovering and enumerating *Tilletia indica* teliospores from soil. Two grams of infested soil containing between 600 and 5,000 teliospores/g of soil was placed into a 20 cm x 2 cm fitted disc glass column containing 16 ml of a bubbling 50% glycerol solution. The suspension was bubbled for 5 minutes and allowed to settle for 10 minutes.

The supernatant was then removed by suction 5 mm above the settled soil. New glycerol was added, bubbled and removed by suction as previously mentioned and repeated twice. Collected supernatant was poured through a 20-µm sieve. Concentrated teliospores were suspended in water and enumerated on a Hawksley eelworm cell. Between 73 to 92% of the teliospores were recovered using this method.

Sporidia Trapping Studies of Karnal Bunt

J.M. Prescott

A sporidia trapping study was initiated in February 1986 at CIANO in Sonora, Mexico. Its objectives were: 1) to determine when sporidia of the Karnal bunt fungus are released into the crop canopy atmosphere and 2) to determine the environmental factors necessary for these sporidia to be produced.

The spore trap utilized was a Burkhard seven-day volumetric spore trap manufactured in England. This trap includes an internal drum that revolves in front of an air intake aperture; plastic tape coated with a plastic base and an adhesive mixture is secured on the drum. Any spores present in the canopy atmosphere are impacted on this tape. Since the drum rotates 2.0 mm every hour, it allows pinpoint accuracy in determining when a particular spore was impacted. In addition, 10.0 liters of air per hour move over the impaction tape, thus making it possible to quantify the number of spores impacted. It was necessary to use relatively high power (>10x40X) on a compound microscope, plus a cotton blue/lacto-phenol stain, so as to observe the sporidia easily.

Preliminary analyses indicate that sporidia of the Karnal bunt fungus are released at night, between 1800 and 0800 hours, during periods of higher relative humidity and somewhat cooler air temperatures. The peak period of sporidia impaction was about 0300 hours. In the evening, when the dew point was reached, sporidia were caught in the trap, suggesting they are released during periods of higher relative humidity. In the early morning, after the dew-point was passed again, far fewer or essentially no sporidia were impacted. During the day, i.e., between 0800 and 1800 hours, very few sporidia were impacted on the tape. This pattern of nocturnal/high relative humidity sporidia release has been consistent for the three-week period already examined. The study will be continued and possibly expanded next year.

No teliospores of the Karnal bunt fungus were impacted on the tape for the time frame examined.

Pathogenicity and Environment: Karnal Bunt

M.H. Royer

No information is available in the literature or from communication with researchers in India pertaining to natural hosts of *T. indica*. Such information is necessary to complete an assessment of the risk of disseminating Karnal bunt by natural or man-assisted movement of infected grass hosts.

Eight grass tribes from India or Mexico were tested for susceptibility to *T. indica* by artificial inoculation. Several *Aegilops* species, as well as *Lolium multiforme*, *L. perenne*, and *Bromus ciliatus* were found to be susceptible.

Tilletia barclayana (*Neovossia horrida*), causal agent of kernel smut of rice, can sometimes be confused with teliospores of *T. indica* by inexperienced staff, or when only a few teliospores are present in a sample. Some researchers propose a similarity between *T. barclayana* and *T. indica* that goes beyond morphology and suggest an evolutionary link that may still exist and allow interspecific hybridization. Therefore, we have inoculated rice with single monosporidial lines of *T. barclayana* mixed with sporidia of *T. indica*, rice with sporidial mixtures of *T. indica*, and wheat with single monosporidial lines of *T. indica* mixed with sporidia of *T. barclayana*. Interspecific hybrids could not be produced by this technique; furthermore, *T. indica* would not infect rice nor would *T. barclayana* infect wheat.

Tilletia barclayana and *T. indica* were also compared to determine the length of dew period required for infection after inoculation of rice and wheat, respectively. Dew periods of 6 hours at 18 °C after atomizing sporidia of *T. indica* onto wheat spikes resulted in low levels of infection. Longer dew periods resulted in more bunted kernels per spike. Inoculation of rice with *T. barclayana* resulted in relatively more infected panicles at 20 °C for 6 hours than did the inoculation of wheat described above. Additional environmental studies are in progress to quantify the effect of dew period x temperature interaction on infection, and to investigate the influence of photoperiod on the quantity and periodicity of secondary sporidia production.

Statistical and practical considerations for sample sizes needed to detect Karnal bunt are presented to help cooperators understand the limitations and capabilities of survey and detection programs. This will stimulate discussion on how regulatory agencies can prioritize resources until models are constructed to predict the relationship between spore load, environment, host phenology, and Karnal bunt development.

Screening for Resistance to Karnal Bunt

R.J. Metzger

More than 16,000 selections of bread and durum wheats and triticales have been screened for resistance using the boot inoculation procedure.

Bread wheat, durum wheat, and triticale selections (1,469) that exhibited resistance in both the 1983-84 and 1984-85 cycles were retested in 1985-86. Results, though incomplete, clearly suggest adequate resistance, but not immunity, is present in bread and durum wheats and triticale. It is not clear whether resistance is conveyed by physiological or morphological factors, or a combination of both.

Approximately 60 of the 270 bread wheat selections that were retested have exhibited sufficient resistance to justify their release to breeders as potential sources of resistance. These lines will be planted at various sites throughout the Yaqui Valley in 1986-87. Adequate resistance is already present in the germplasm currently used by the durum and triticale breeders.

Karnal Bunt Research at Punjab Agricultural University

S.S. Aujla

Karnal bunt, also known as "new" or '"partial" bunt, was first collected by Mitra in 1930 from wheat grown at the Botanical Station in Karnal, India. Although it occurs sporadically, the disease is a serious problem which causes heavy losses in epidemic years, as, for instance, in 1978-79 and 1980-81 in northern India.

Information on the nature of the Karnal bunt pathogen, its mode of survival, alternate hosts, pathogenicity, and epidemiology are inadequate. Research on some of these aspects at the Punjab Agricultural University in Ludhiana began in 1975.

Experiments were conducted under laboratory as well as field conditions, and included a disease survey on farmers' fields. These investigations have shown that high humidity, frequent light rains, cloudy weather, and low temperatures promote rapid disease development. They also have established that high doses of nitrogen and excessive irrigation further increase its incidence.

Until recently, it was believed that each of the affected grains in a spike was the result of a separate infection by airborne sporidia at anthesis. However, Dhaliwal et al. (1983) found that the disease progressed from 1 to 4 primary infection centers per spike to other florets within the same spike and then to the spikes around it. Secondary spread from the primary infection center can take place at any phase of development from anthesis to the dough stage.

No effective chemical means of controlling Karnal bunt have been developed. The causal organism survives in the soil and on seed and infects florets of the wheat plant. This makes chemical control of the disease a complex problem. As yet there is no known method of erradicating the organism from the soil or the seed. Perhaps the most economical method of controlling the disease is developing varieties that are resistant to Karnal bunt.

Useful information on various aspects of the disease has been obtained lately, but additional research on the following problems must be undertaken:

1) Spore survival
2) Identification of specific propagules that cause infection and spread the disease
3) Alternate host identification
4) Heteroallelism and infectious stage of the pathogen
5) Physiological specialization and identification of patogenic races
6) Biochemical and morphological components of resistance
7) Identification of resistance genes

Breeding for Karnal Bunt Resistance

K.S. Gill

The most economical method of controlling Karnal bunt is through the development of varieties that have genetic resistance to the disease. However, knowledge of the genetics of Karnal bunt resistance is far from adequate. Punjab Agricultural University has recently initiated studies on the inheritance of resistance using four approaches:

1) A diallel set involving parents with various levels of resistance;
2) Use of aneuploid series;
3) Use of wheat-rye chromosome addition lines; and
4) Use of wheat-barley chromosome addition lines.

The University has also increased Karnal bunt resistance through intermating and selection cycles. Based on screening efforts of the last three years, wheat lines showing low levels of infection with artificial inoculation have been used in multiple crosses followed by intercrossing of resistant plants in segregating generations for pyramiding genes for Karnal bunt resistance.

In order to strengthen the breeding programs for Karnal bunt resistance, emphasis should be given to identifying and cataloguing the genes governing resistance. More experimentation is needed to identify the morphological and biochemical components of resistance. Efforts should also be directed at breeding varieties with multiple resistance.

Quarantine of Karnal Bunt in Canada

P.M. Martin

At present, there is not sufficient evidence as to whether Tilletia indica, causal agent of Karnal bunt, would survive Canada's cold winter conditions and short growing season. Therefore, Agriculture Canada has maintained a conservative and rather rigid position on the disease, in effect insisting on zero tolerance. Apart from the entry of the pathogen on the wheat seed itself, three problems are a source of concern to the agency:

a) the entry of the fungus on seed of nonhost crops, namely barley, oats, rye, sorghum and millet, which could be contaminated by being threshed at the same facilities as wheat;

b) the effective elimination of teliospores in all seeds through chemical treatments; and

c) the residual effects that seed treatments such as formalin may have on seed viability, though there does not appear to be any "hard" evidence that seed treatments are dangerous when used at recommended concentrations.

Agriculture Canada has tried to modify its position in the light of last year's survey of Field 710 at CIMMYT, though new regulations are not yet finalized. The main reason is that formalin treatments may not be relied on to eliminate the fungus entirely.

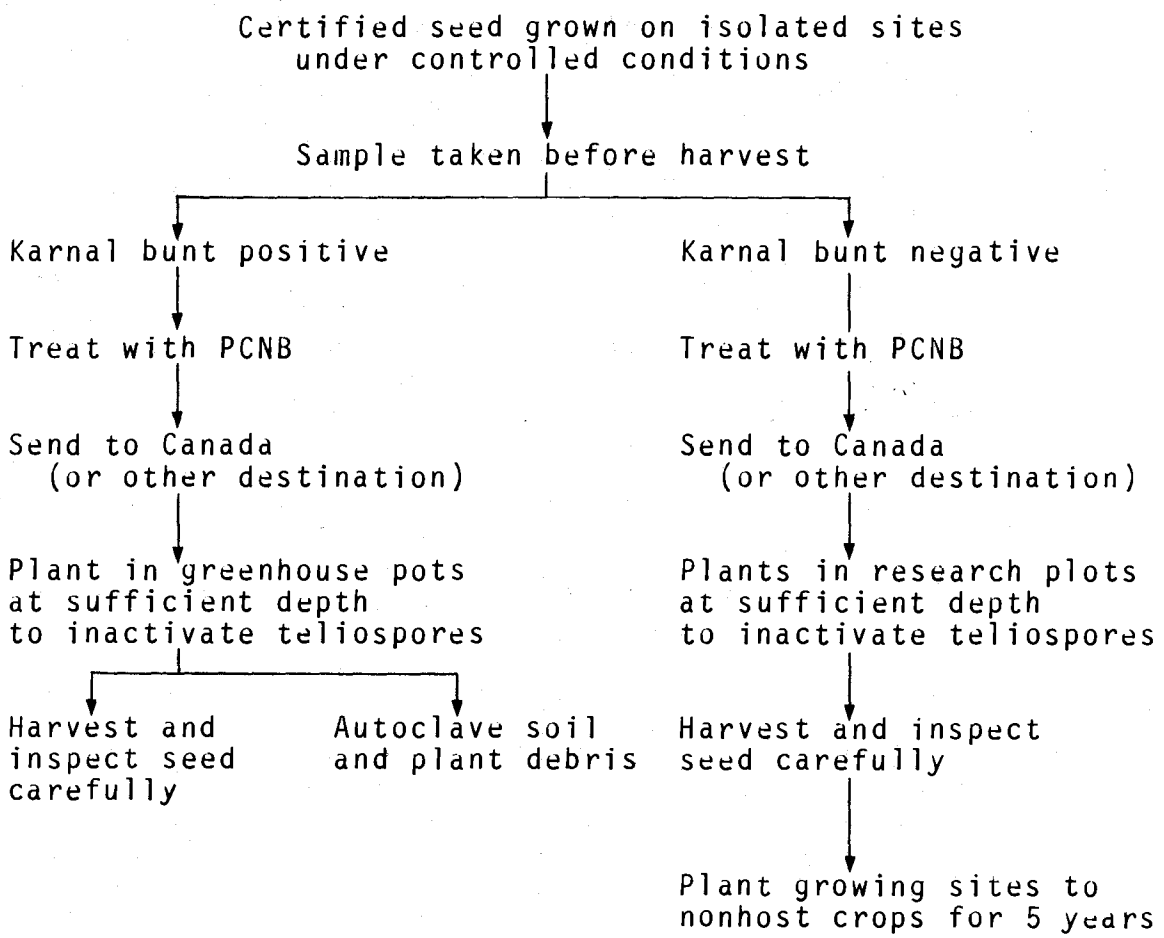
In 1985, a survey was carried out on 400 plots with 25-40 varieties each, comprising 260 bread wheat, 120 durum wheat, and 20 triticale plots. The plots were combined in pairs to give 130 samples of bread wheat, 60 samples of durum wheat, and 10 samples of triticale; the samples were divided almost evenly between the USA and Canada for examination.

The 96 Canadian samples yielded 29 samples that were positive for Karnal bunt; of these, 26 were detected by means of a seedwash, and 3 which were negative on the seedwash were detected through visual inspection of internally borne spores. The highest rate of infection was 22 seeds out of 5,000 (0.44%) and rates varied between 1 and 2 seeds per 5,000 (0.01-0.02%). A total of 82 positive infected seeds were found. Equal proportions of bread wheat and durum wheat cultivars were positive, though the highest incidence rate given above came from a durum wheat sample. No triticale samples were positive.

The results indicate considerable persistance on the part of the pathogen in spite of the extremely rigorous preventive measures used in Field 710. These included the use of PCNB in irrigation water at planting time and spray applications of manzate at the boot stage. There was concern about the 3 seeds with internally borne Karnal bunt because this demonstrated that seedwashing was not a completely effective means of detection. Evidence that formalin can penetrate such seeds and inactivate the spores without affecting germination has so far been equivocal.

The exporting agency or the Plant Health Laboratory should treat seeds with formalin, but the soaking and careful drying of tiny seed lots is a tedious process which takes up too much of the technician's time. Therefore, regulations that do not require the use of formalin on a large scale would be ideal.

A scheme for dealing with seeds coming from known infested areas might be as follows:



The nonhost material referred to above would be treated according to the right-hand column, since only surface contamination would occur.

Seed from areas not known to be infested could also be treated in this way, but the decision would be based on information given on the phytosanitary certificate. Seed treated with PCNB would be handled according to the right-hand column, but nontreated seed would have to be carefully examined first under the dissecting microscope. Small samples can be dealt with expeditiously, since the large black spores are conspicuous. Seed coming from an area which has been inspected and found to be free of the disease would be admitted without inspection, except for a certain number of audited samples.

Agriculture Canada thus is relying on the discovery that spores cannot germinate to form sporidia if buried beyond a certain depth (about 2 mm) beneath the soil. Exploiting the weaknesses of the pathogen is a valid way of building a quarantine procedure.

Karnal Bunt and Quarantine in the USA

B.P. Singh

One of the avenues available to plant quarantine officials consists of restricting plant pathogens through exclusion. Pathogens are placed on the US quarantine list according to certain criteria: 1) it does not occur in the US or has a limited presence only; 2) the affected crop is economically important; 3) there is a description of the nature of the pathogen (for example, to what extent it causes disease and under what conditions); 4) the pathogen is capable of establishing itself in the US; and 5) the pathogen affects aesthetic value (of wheat exports).

Wheat seed and wheat products from countries where Karnal bunt has been found (Mexico is one of them) are under quarantine, except for wheat flour and wheat seed for experimental purposes. All experimental wheat seed must have an APHIS (Animal and Plant Health Inspection Service) permit. If the seed comes from an infected area, it can be grown only in the greenhouse, unless APHIS finds it to be disease-free.

In the past few years, the seed in CIMMYT's international trials has been found to have Karnal bunt, and therefore its entry into the US has been restricted. In 1985-86, however, trials were grown near Hermosillo, a disease-free area in northwest Mexico. APHIS collected samples on site and gathered weather and survey data from the area in order to determine whether it will grant the required permit or not. Results are not in as yet.

Regulations regarding countries suspected of having Karnal bunt will be modified because many have not conducted surveys to detect the disease, as required by APHIS. Only if such surveys are conducted and no Karnal bunt is found will their seed be allowed into the US. At present, there is concern about Mediterranean countries, such as Iraq and Syria. Although each country's plant protection service is responsible for monitoring seed exports, all seed is inspected at US ports of entry as well.

Karnal bunt does not cause major yield losses, but it must be kept out of the US to avoid jeopardizing wheat exports to other countries.

Karnal Bunt and Quarantine in India

A.K. Lambat

Plant quarantine responsibilities in India have been divided among four agencies:

1) Directorate of Plant Protection, Quarantine and Storage for commercial consignments;

2) National Bureau of Plant Genetic Resources (NBPGR), New Delhi, for consignments of agricultural and horticultural crops for research purposes;

3) Forest Research Institute, Dehra Dun, for consignments of forestry interest; and

4) Botanical Survey of India for consignments of general botanical interest.

Wheat can only be imported for research purposes through the Director of the NBPGR and is grown in post-entry quarantine. Nearly all known smuts and bunts have been observed on seed coming into India. Though most of them have worldwide distribution, their entry is controlled in order to prevent the introduction of new races. New races are new pathogens, as far as plant quarantine is concerned.

In the last few years, NBPGR has repeatedly intercepted *Tilletia foetida*, *T. caries*, and *Neovossia indica* (causal agent of Karnal bunt) on wheat and barley imported from many countries. It is evident that proper and effective cooperation among agencies involved in the exchange of seed is urgently needed.

Summary and Comments

A. Klatt

On behalf of CIMMYT, CIANO, and INIFAP, I take this opportunity to thank the smut workers of North America for the time they spent discussing Karnal bunt during this workshop. While other smut diseases were discussed, Karnal bunt is of special interest to CIMMYT because of its potential threat to the Center's international trials and nurseries. My comments will therefore concentrate on this disease.

To review what was said about other bunts and smuts, Jim Hoffmann discussed seed treatments for dwarf bunt protection, which are effective only under certain conditions and are not a very promising means of control. However, there are sources of resistance to dwarf bunt, for example, in wild relatives of wheat and in P.I.178383, a selection from Turkey that contains at least three genes for resistance.

Dr. Nielsen has identified 41 races of loose smut for which there are numerous sources of resistance. He suggests it would not be difficult to incorporate genetic resistance if it is done in a systematic manner.

Other important topics that were touched upon were ways of identifying the dwarf bunt pathogen (e.g., isozyme analysis) and the factors which enhance or reduce levels of dwarf bunt infection. Also, a publication dealing with surveys of smuts and bunts in California will be available soon; I am sure it will be most helpful to cooperators the world over.

Presentations by Horacio Sánchez and Jesús Narro centered on head smut of sorghum and maize in Mexico, a problem that is especially severe in the Bajío region, and the means used to control it (e.g., removal of susceptible varieties from the market, crop rotation, and destroying diseased plants before harvest).

Ed Trione gave an interesting presentation on bioregulators in plants, which actually facilitate the infection process of smuts and bunts. He thinks it may be possible to breed for the lack of one or both of the bioregulators that he has identified, thereby incorporating immunity or good resistance into plants.

Karnal bunt is not economically significant to Mexico as an explosive disease problem, but it has proved to be very costly for CIMMYT, with expenditures approaching US$1 million in the last four years. This is out of proportion with the US$35,000-$50,000 loss the disease causes in the Mayo and Yaqui Valleys of northwest Mexico in a severe year. However, if restrictions on germplasm movement are imposed, the Center has no reason to exist, for it will not be able to carry out its mandate, i.e., the development and distribution of improved germplasm. Consequently, it is imperative that we convince quarantine organizations to create reasonable restrictions. It is particularly important that the US and Canada realize that many countries will adopt the recommendations and regulations they set.

To review some facts about Karnal bunt: we know that high levels of humidity and temperatures of 15-20 °C enhance infection. Sources of genetic resistance to Karnal bunt have been found in durum wheat, triticale and wild relatives of wheat, so that we now have workable levels of resistance to the disease; the next step is to incorporate this resistance into good agronomic phenotypes for areas where Karnal bunt is found.

Other significant work that has been done includes: a host range for the Karnal bunt fungus has been identified, inoculation techniques have been perfected in the laboratory and greenhouse, and the infection process has been found to begin through the glumes. Studies indicate that a 3-5% infection can affect quality, though how infected grain and fungicide residues affect human beings and animals is not yet known.

In the future, more work has to be done on agronomic factors that affect Karnal bunt infection. For example, we know that increased levels of nitrogen fertilization bring about increased levels of the disease, as does a high amount of humidity at or near flowering. What can be done agronomically to reduce infection? Obviously, the farmers of the Mayo and Yaqui Valleys are not going to reduce the amount of nitrogen they apply to their crops for the simple reason that they are not losing yield because of Karnal bunt, but would greatly diminish them if they used lower levels of nitrogen fertilizer.

As for the amount of moisture applied to crops, timing is an all-important factor. Is there a way to give crops enough moisture at the most critical times (especially during flowering) while keeping the soil surface sufficiently dry? Perhaps watering crops 10 days to 2 weeks before flowering to let the soil surface dry out would be the solution, since only the teliospores in the upper 2 mm of soil can germinate, producing sporidia which cause infection.

Several chemicals were mentioned that afford good control on seed, but they are all fungistatic. Systemic fungicides are not effective on seed because they do not persist in the plant long enough. Foliar fungicides applied 7 days after heading followed by an application of Tilt 14 days after heading give good control, according to Smilanick. In monetary terms this would cost approximately US$40/ha, with the advantage that fungicide application usually results in approximately a 10% increase in yield by controlling the rust and delaying maturity a few days. This would mean yields of 0.5 ton/ha with a 50% return, which might make it feasible for the farmers in the Mayo and Yaqui Valleys.

The following are subjects for future research which would be a tremendous aid to quarantine and regulatory agencies:

* How many teliospores in the soil are necessary to produce infection;

* How many teliospores on seed pose a threat of disease establishment;

* What factors must be present for sporidia to spread from the soil to the plant;

* How spores are dispersed (perhaps by wind);

* What environmental conditions are required for Karnal bunt to establish itself; and

* How the inoculum levels present in the soil can be reduced.

Determining what methodology is needed to grow disease-free seed in Karnal bunt infected areas would greatly aid CIMMYT's cause. We also require the assistance of countries such as the US, Canada, India, and Pakistan in conducting research to control the disease. More information will be obtained in the next year and it should be published as soon as possible. I propose that the best way to go about compiling this information would be to hold a Karnal bunt workshop with formal written presentations so that a proceedings could be published. CIMMYT will investigate the feasibility of hosting this workshop in 1987.

APPENDIX I: CONFERENCE PARTICIPANTS

S.S. Aujla	Punjab Agricultural University, India
A. Barreras	INIFAP-CIAPAN, Sinaloa, Mexico
G. Bekele	CIMMYT, Mexico, D.F., Mexico
M. Bonde	USDA-ARS, Maryland, USA
P. Brajcich	CIMMYT, Mexico, D.F., Mexico
L.E. Datnoff	USDA-ARS, Maryland, USA
M. Dickmann	ICARDA, Aleppo, Syria
R. Durán	Washington State University, USA
H.S. Fenwick	University of Idaho, USA
L. Gilchrist	CIMMYT, Mexico, D.F., Mexico
K.S. Gill	Punjab Agricultural University, India
B. Goates	USDA-ARS, Utah, USA
V. Goodfellow	CIMMYT, Mexico, D.F., Mexico
G.A. Granados	PRONASE, Mexico, D.F., Mexico
J. Hadad C.	Cd. Obregon, Sonora, Mexico
J.A. Hoffmann	USDA-ARS, Utah, USA
A. Klatt	CIMMYT, Mexico, D.F., Mexico
C. Krass	CDFA, California, USA
A.K. Lambat	NBPGR, New Delhi, India
M. Lira	SARH, Mexico
P.M. Martin	Agric. Canada, Ontario, Canada
J. Martínez S.	CIANO, Cd. Obregon, Sonora, Mexico
T. Matsumoto	CDFA, California, USA
J. McCarty	CDFA-USDA-ARS, California, USA
V.R. Mercado	Cd. Obregon, Sonora, Mexico
R.J. Metzger	Corvallis, Oregon, USA

L. Mikesell	USDA-ARS, Utah, USA
J. Nielsen	Agric. Canada, Winnipeg, Canada
M. Osmanzai	CIMMYT, Mexico, D.F., Mexico
J.M. Prescott	CIMMYT, Mexico, D.F., Mexico
S. Rajaram	CIMMYT, Mexico, D.F., Mexico
M. Razura M.	PRONASE, Cd. Obregon, Sonora, Mexico
M.H. Royer	USDA-ARS, Maryland, USA
E.E. Saari	CIMMYT, Mexico, D.F., Mexico
E. Samayoa	CIANO-INIFAP, Mexico
P. Sánchez	SNICS, SARH, Cd. Obregon, Sonora, Mexico
H. Sánchez A.	SARH-INIFAP, Jalisco, Mexico
A. Sánchez O.	DGSPAF, Mexico, D.F., Mexico
B.P. Singh	USDA-APHIS, Maryland, USA
R.P. Singh	CIMMYT, Mexico, D.F., Mexico
J. Smilanick	USDA-ARS, California, USA
C. Torres B.	CIANO, Cd. Obregon, Sonora, Mexico
E.J. Trione	USDA-ARS, Oregon, USA
E. Warham	CIMMYT, Mexico, D.F., Mexico
R. Ykema	ACAH, Arizona, USA
H. Zerón	CIBA-GEIGY, Mexico, D.F., Mexico

CENTRO INTERNACIONAL DE MEJORAMIENTO DE MAIZ Y TRIGO
INTERNATIONAL MAIZE AND WHEAT IMPROVEMENT CENTER
Lisboa 27 Apartado Postal 6-641 06600 México, D.F. México