Morris R. Bonde, Gary L. Peterson, and Norman W. Schaad
USDA-ARS-FDWSRU, Frederick, Maryland

Joseph L. Smilanick
USDA-ARS, Horticultural Crops Research Laboratory, Fresno, California

Karnal Bunt of Wheat

Karnal bunt of wheat (*Triticum aestivum* L.), caused by the smut fungus *Tilletia indica* Mitra (=*Neovossia indica* (Mitra) Mundkur), was first discovered in 1930 at the Botanical Research Station, Karnal, Haryana, in northwest India (29), and now is considered common in the Punjab region of India. The disease has been reported from Pakistan, Iraq, and Nepal, and is found in wheat from Afghanistan (6). It was first reported in Mexico in 1972 (16), and since then it has occurred sporadically in localized areas in the states of Sonora and Sinaloa, northwest Mexico. Because the disease was not known in major wheat-producing countries, trade of Karnal bunt–infested wheat grain became highly regulated internationally, and the Mexican government in 1984 placed an internal quarantine on Karnal bunt to prevent disease spread within the country (27).

On 8 March 1996, the U.S. Department of Agriculture (USDA) and the Arizona Department of Agriculture announced the discovery of Karnal bunt in Arizona (Release No. 0115.96, Ag News FAX; 56). Efforts were initiated to quarantine suspect wheat fields in Arizona because of the discovery of bunted seeds and the confirmation of *T. indica* infection by polymerase chain reaction (PCR) (17,48). Bunted seeds also were found in remnant samples of Arizona wheat seed remaining in Arizona after a portion of the lots had been planted in Arizona, Texas, and New Mexico. Fields in Texas and New Mexico planted with the seed were deep plowed as a precaution. On 21 March 1996, the Secretary of Agriculture announced a "Declaration of Extraordinary Emergency" to deal with the disease and set into motion the mechanism to compensate growers and handlers for losses due to quarantine actions (54). On 25 March, a federal quarantine for Karnal bunt was placed on the state of Arizona and parts of Texas and New Mexico where the Karnal bunt–contaminated wheat from Arizona had been planted (25). Later, the discovery of Karnal bunt–infected wheat in California extended the quarantine to portions of that state (Fig. 1), and by late summer a national Karnal bunt survey was underway. The efforts of hundreds of state and federal personnel in Arizona (Fig. 2) and California, and of many more workers in other states, and thousands of pages devoted to Karnal bunt on the Internet underscore the impact of the recent discovery of Karnal bunt in the United States.

The main effect of extensive Karnal bunt is to reduce yield (4) and impart a fishy odor and taste to wheat flour, thus reducing the quality of the flour (2). Yield

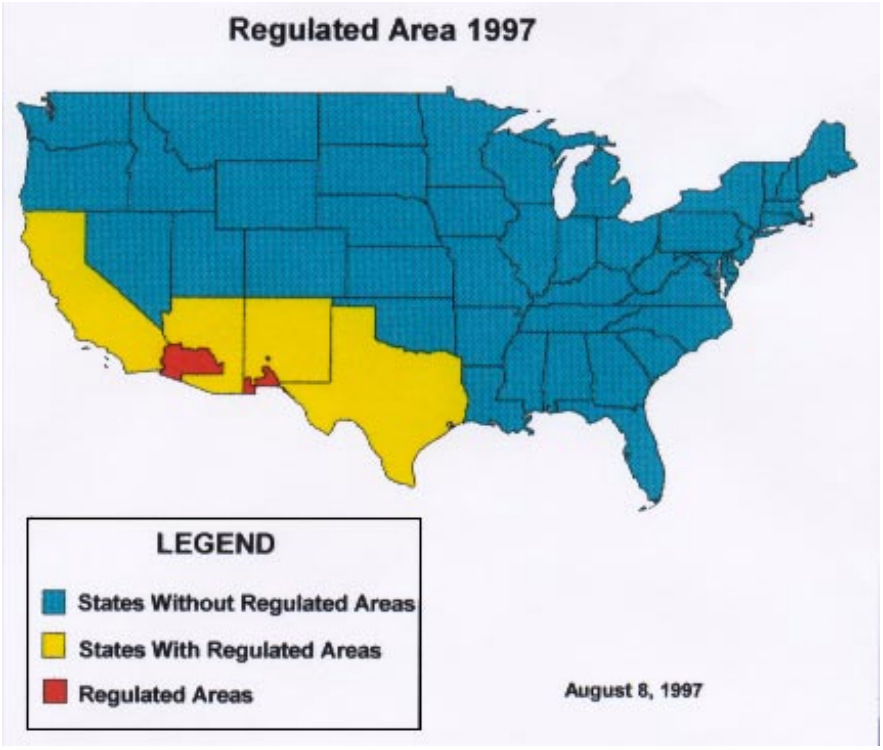

Fig. 1. Map of the continental United States showing Karnal bunt–regulated areas in August 1997.

Dr. Bonde's address is: USDA ARS FDWSRU, 1301 Ditto Avenue, Fort Detrick, MD 21702-5023
E-mail: bondem@ftdetrck-ccmail.army.mil

Publication no. D-1997-1024-01F

and quality losses are considered by many smut pathologists to be minor (11,51). However, since Karnal bunt is the subject of strict quarantines by several wheat-importing countries, *T. indica* can profoundly affect international trade of commercial grain and the movement of wheat germplasm (51). In 1983, the Animal and Plant Health Inspection Service (APHIS) placed restrictions on wheat coming from countries with Karnal bunt, recognizing that establishment of Karnal bunt in the United States could have major economic ramifications on U.S. wheat exports. The spread of Karnal bunt to the United States and its establishment therein could have placed the country at a marked disadvantage in the international wheat market as the first major wheat-exporting country to have Karnal bunt. As shown by responses following the discovery in March 1996 of Karnal bunt in Arizona, and later in California, the disease has generated considerable concern and debate both within and outside the country. The American Phytopathological Society took the position that Karnal bunt is of little agronomic significance and should not be regulated (1).

Since 1972, research on specific foreign plant pathogens of major threat to U.S. agriculture has been a primary objective of the USDA, Agricultural Research Service (ARS), Foreign Disease-Weed Science Research Unit at Fort Detrick, Frederick, Maryland. In 1992, the major objective of this program became the development of rapid molecular means of detecting and making accurate, timely identifications of foreign plant pathogens. Because of its regulatory significance, *T. indica* was a primary target.

The Frederick unit conducts research on foreign pathogens in a plant disease containment facility (Bldg. 374) leased from the Department of Defense (DOD) at Fort Detrick (28). The facility is a 12.5 × 53.7 m brick and concrete building with five attached 7.6 × 18.3 m glasshouses under negative air pressure. All waste water is decontaminated by DOD upon exiting the facility. Each glasshouse has double-layered glass panels supported by a steel superstructure. With permission of state and federal regulatory officials, plant pathogens and diseases from anywhere in the world can be investigated at Bldg. 374 (28).

ARS initiated Karnal bunt research at Frederick in 1982, after Karnal bunt appeared in northwestern Mexico. The Karnal bunt research program at Frederick was the first initiated in the United States and has continued for 15 years.

In 1983, ARS initiated cooperative research projects on Karnal bunt in India and Mexico, and established and maintained a containment laboratory in Logan, Utah, under the direction of James A. Hoffmann. He and his staff cooperated closely with J. Michael Prescott of the International Maize and Wheat Improvement Center (CIMMYT), who managed an extensive Karnal bunt research program in Mexico.

This presentation is an overview of Karnal bunt, its importance in international agriculture, and past and present research to better understand and control the disease and make rational decisions. Recent reviews include Gill et al. (20), Mathur and Cunfer (27), and Singh (36). A very comprehensive literature review was published by Warham (51) in 1986.

Symptoms of Disease and Life Cycle of Pathogen

The disease is difficult to detect under fields conditions, and generally only careful examination will reveal evidence of disease. Only a few kernels of some wheat heads are infected, and usually only a portion of an infected kernel is replaced with fungal sorus (Fig. 3). *T. indica* is a basidiomycetous pathogen belonging to the order Ustilaginales. Black, dusty-appearing teliospores give this group of organisms the name "smut." The life cycle of *T. indica* is depicted in Figure 4. The teliospores (Fig. 5) of *T. indica* are diploid (2N), thick walled, globose to subglobose, and average 35 µm in diameter (range 22 to 49 µm) when mature (27). They are very resistant to adverse environmental conditions, remaining viable for 2 to 5 years in contaminated soil (27). The pathogen is seedborne but is not transmitted directly from seed into plant (27). Teliospores of *T. indica* are considered dormant immediately after formation and have poor germination up to approximately 9 months (36).

After a period of dormancy and in the presence of moisture, teliospores at the soil surface germinate (45). During the germination process, the nucleus undergoes meiosis followed by several mitotic divisions. A promycelium (basidium) grows out from the spore, and as many as 180 haploid (1N) basidiospores (also known as primary sporidia) are produced at the tip (18,20,36) (Fig. 6). Normally, teliospores germinating under 2 mm of soil are incapable of reaching the surface (45). However, it is not known whether teliospores beneath the soil surface germinate. The primary sporidia mean lengths and widths among isolates range from 64 to 79 µm and from 1.6 to 1.8 µm, respectively. Sporidia germinate to produce mycelia, which in turn produce large numbers of secondary

Fig. 2. Arizona Department of Agriculture laboratory in 1997 testing wheat seed samples for the presence of *Tilletia indica* teliospores. In 1996, more than 4,700 wheat fields were preharvest and postharvest tested.

Fig. 3. Typical wheat head with Karnal bunt infection. Under natural field conditions, symptoms are not readily apparent. Surveying for Karnal bunt infection requires harvesting the seed and threshing the grain to expose the kernels.

sporidia with mean lengths for different isolates of 11.9 to 13.0 µm, and a mean width of 2.0 µm (31). At the time of flowering of the wheat plants, primary and secondary sporidia are presumably splashed and blown onto the surface of glumes enclosing developing wheat kernels. Dhaliwal and Singh (14) presented evidence that *T. indica* may travel in steps from the soil surface to susceptible heads. According to them, sporidia from the soil surface germinate on lower plant leaves, colonize the leaf surface, and produce further sporidia which are splashed or blown to higher leaves. In this manner, the fungus travels up the plant to reach developing heads. Here, the sporidia on the glumes germinate and penetrate the stomates if the plant is in the 2- to 3-week susceptible period at or near anthesis. Mycelia grow to the base of the glumes and up into the developing kernels (21). The fungus is restricted to the pericarp, where it is entirely intercellular (12,35). As the kernels mature, large numbers of teliospores are produced (Fig. 7). At harvest, they are redeposited on the soil surface to perpetuate the pathogen and disease. Teliospore numbers in a *T. indica*–contaminated wheat field in the Punjab in India were reported to vary from 2×10^3 to 50.5×10^3 spores per cm^3 of soil (20).

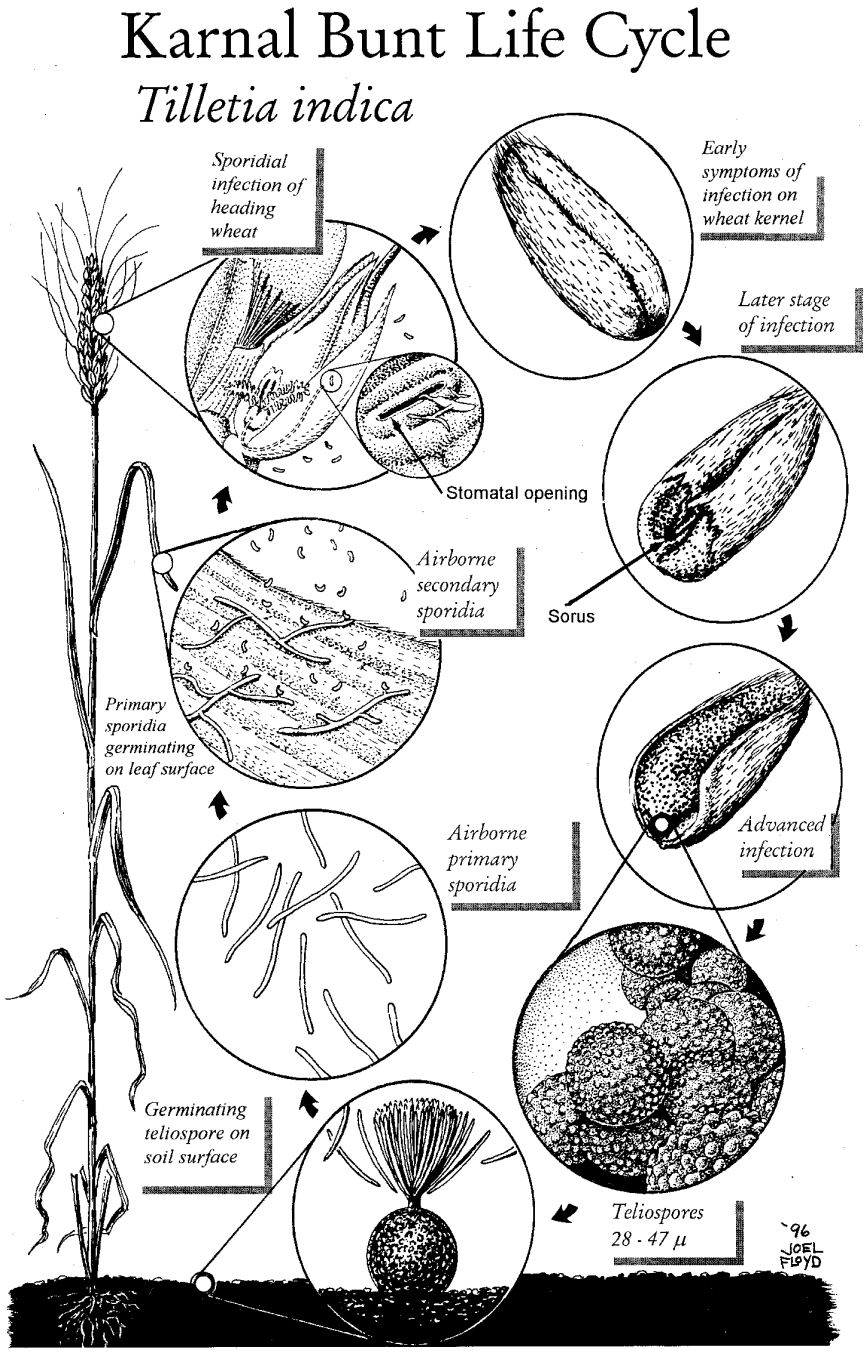

Fig. 4. Life cycle of *Tilletia indica.* Teliospores (diploid) germinate at the soil surface and produce haploid primary sporidia (equal basidiospores), which are blown or splashed to the surface of leaves. These germinate to produce mycelia, which produce secondary sporidia. The secondary sporidia are blown or splashed to higher leaves, germinate, and produce more secondary sporidia. In this way, the pathogen moves in steps up to the developing wheat head. On the head, secondary sporidia germinate, penetrate the glumes through stomates, and establish infection. Mycelia grow down to the base of glumes and up into the developing kernel. Eventually, diploid teliospores are produced, which are returned to the soil.

Epidemiology

Karnal bunt initiation and development is dependent on suitable weather conditions during the period wheat plants are flowering and most susceptible to infection (36). According to Singh (36), the optimum temperature range for teliospore germination is 15 to 25°C. Smilanick et al. (45) reported that the optimum after a 3-week incubation in continuous light was 15 to 20°C over a pH range of 6.0 to 9.5. Moisture is a critical element in determining whether there will be a disease outbreak (20,36,45). Teliospore germination requires at least 82% relative humidity

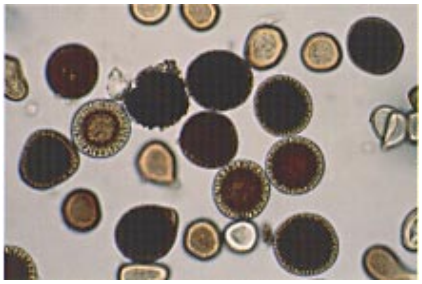

Fig. 5. Teliospores of *Tilletia indica* observed microscopically. Mature spores are dark brown, and immature spores are light brown. Teliospores average 35 µm in diameter and are easy to see in a water wash from contaminated wheat seed.

Fig. 6. Germinated teliospore with long slender primary sporidia (basidiospores) growing from the tip of a promycelium (basidium).

(RH) and preferably, free water (36). If high RH and/or several rainy days occur during the 2- to 3-week window at flowering, infection of wheat kernels is likely to occur (36). The longer the period of high humidity and rainy weather, the greater the number of seeds infected and the higher the extent of infection of individual seeds. Apparently, these conditions are rarely, if ever, encountered over large land areas, or the inoculum levels are not adequate, since Karnal bunt disease losses have never been reported to be large.

For Karnal bunt to establish and perpetuate in most of the major U.S. wheat production regions, teliospores of the pathogen must be able to survive freezing conditions. In laboratory tests, teliospores survived freezing over several months, although some workers reported germination was delayed (13) or reduced (57) by freezing. It is not known whether Karnal bunt can survive in northern states such as Montana or North Dakota. However, the Frederick unit has been conducting an overwintering temperature experiment in sealed containers in Maryland since 1992, with the appropriate state and APHIS permission. Preliminary results indicate that many Karnal bunt teliospores can survive Maryland winters in buried sealed containers for at least 3 years. Soil temperatures during the period dipped periodically to 2 to 3°C below freezing for a few weeks. Although *T. indica* spores can survive the mild winter conditions found in Maryland, little is known about survival under more extreme conditions further north and the survival of free spores. Moisture at the time of flowering may be the most critical factor in determining where in the U.S. Karnal bunt could become established. Predictions made from weather data and known requirements for infection by *T. indica* suggest that, under current climatic conditions, Karnal bunt will never cause major crop losses in the U.S. (15).

Dissemination of the Pathogen

Dry teliospores can survive for many years under laboratory conditions. In fact, spores used in experiments at Fort Detrick have been stored on laboratory shelves for up to 16 years. Long-range teliospore dissemination can occur by transport of infected and/or contaminated seed (36). However, teliospores can also be spread by air currents. Teliospores of *T. indica* were collected 3,000 m above fields being burned after harvest in Mexico (9). The evidence indicated that the updraft of air from burning fields carried teliospores to great heights and acted as a mechanism of dispersal. Vehicles also transport the pathogen. Boratynski et al. (10) demonstrated that *T. indica* teliospores were present on rail cars moving from Mexico into California. Animals and birds act as transporters of viable teliospores on their bodies and in their digestive systems (41); however, whether these spores result in disease is not documented.

Pathogen Variation

Isozyme analyses have established evidence of high genetic variation within *T. indica*. Bonde et al. (7,8) resolved the protein products of 36 presumed isozyme loci of 66 monoteliospore cultures of *T. indica*. Of these 36 isozyme loci, 15 (42%) were polymorphic (having allelic variation). The relatively low average coefficient of similarity (0.83) among cultures was in marked contrast to that of *T. foetida* and *T. caries*, causal agents of common bunt of wheat, in which intraspecific variation was nearly absent. The authors believed the greater variation in *T. indica* was due primarily to the high level of outcrossing in *T. indica*. In contrast, the common bunt pathogens have ineffective sexual cycles, and outcrossing is rare. Fusion of basidiospores of *T. foetida* and *T. caries* occurs almost totally between basidiospores formed on the same basidium. Forced outcrossing of *T. indica* probably promotes sexual recombination and genetic variation.

Variability was also observed in numbers and sizes of chromosomes in *T. indica* (49). At least 11 chromosomes were observed in *T. indica*, ranging in size from about 1 to 3.3 megabases. Many isolates contained unique karyotypes. Differences in karyotypes of teliospores and monosporidial lines derived from the same teliospore indicated that karyotype changes may occur through meiosis.

The existence of races of *T. indica* is controversial. Gill et al. (20) reported the identification of four races in India based on different levels of aggressiveness. Bonde et al. (6) recognized differences in aggressiveness among four teliospore collections but concluded that there was no evidence for races. Each collection (two from Mexico and one each from India and Pakistan) produced the same disease severity rankings on six wheat accessions that differed in susceptibility to *T. indica*. In nature, heterothallism of *T. indica* most likely causes a constant reassortment of genes, thus leading to the instability of races. Teliospore collections can thus be expected to be an assortment of spores of different genetic makeup.

Subsequent to discovery of Karnal bunt in Arizona and California in 1996, teliospores of a smut pathogen were found that tested positive for *T. indica* using PCR primers developed at Frederick to differentiate *T. indica* and *Tilletia horrida*, the rice kernel smut fungus sometimes referred to as *T. barclayana.* This smut was determined to be infecting ryegrass seeds in Oregon and the southeastern United States. Extensive sequencing of regions of mitochondrial and nuclear DNA is being conducted by ARS and APHIS scientists to better understand the molecular variation within *T. indica* and to determine the relationship of the ryegrass pathogen in Oregon and the southeastern United States to *T. indica.*

PCR for Rapid Identification of *T. indica*

Although there is considerable genetic variability within *T. indica*, the use of PCR primers selected from mitochondrial DNA sequences has proven extremely valuable for rapid presumptive identification of *T. indica*. The technique is especially useful for differentiating free teliospores of *T. indica* from those of *T. horrida* that are in the same size range. By using primers TI17M1/M2 and TI57M1/M2 (48), or primers Ti-1/Ti-4 (17), DNA extracted

Fig. 7. Bunted kernels removed from infected plants in the field. Most infected kernels are only partially infected.

from mycelia obtained from germinated teliospores washed from wheat seeds can be quickly tested. These primers tested positive with over 78 isolates of *T. indica* and negative with 69 isolates of *T. horrida* (48). Because the selected primers failed to differentiate between *T. indica* and isolates of the newly discovered ryegrass pathogen discussed above, primers are now being designed from sequences of the cloned 2,300-bp product from amplification with primers Ti-1/Ti-4. Also, inter-transcribed spacer (ITS) regions of nuclear fungal rDNAs have been sequenced by APHIS to gain more information on the relatedness between the ryegrass and wheat isolates (L. Levy, R. Meyer, and A. Tschanz; APHIS, PPQ, Beltsville, MD.; *personal communication*) and may be useful for identification.

Crop Losses

In the foothills of Himachal Pradesh, Jammu, and Kashmir states of India, Gill et al. (20) reported that the percentage of wheat samples that contained at least one infected kernel increased from 25% in 1977–78 to 86% for the 1982–83 crop year. Percentages of wheat seed samples with bunted seeds were 9, 34, and 17% in Haryana during the 1974–75, 1977–78, and 1978–79 cropping seasons, respectively, and 60% of samples had infected kernels in 1982 (38). In 1989–90, 1990–91, and 1991–92, 63, 94, and 62% of wheat samples, respectively, had bunted kernels (20). Taken collectively, these figures suggest that disease levels increased slowly over time. However, the actual percentage of infected seeds per sample is a much better measure of the effect of the disease on crop yield and quality.

A detailed analysis of wheat samples collected from 15 districts of eastern Uttar Pradesh in 1987, a year of particularly high Karnal bunt incidence in India, revealed that an average of 3.79% of seeds per sample were infected (32,33). In 1978–79, one field in Madhya Pradesh had 23% of the seed infected (40). However, data collected by survey teams in northwest India showed that even during the worst epidemic years, the loss was only 0.2 to 0.5% of total production (24). According to Singh (36), the state of Uttar Pradesh in northeast India experiences less than 1% loss even in the worst years. Yield losses in Mexico from 1982 to 1989 averaged 0.12% (11). The data from India and Mexico suggest that a few fields can have significant levels of disease in some years, but disease severity over large areas is never more than a few percent. In general, the percentage of bunted seeds reported in surveys is consistently low, although the percentage of positive samples (where Karnal bunt could be detected) can be high after seasons with particularly conducive weather.

In many ways, the epidemiology of Karnal bunt parallels that of dwarf bunt of wheat, caused by *T. controversa*, considered by many plant pathologists to be a minor disease of the U.S. Pacific Northwest, with high rates of infection in localized small areas within individual fields (26). The environmental requirements of the two diseases are different, but disease incidence for each is driven by very specific weather factors. Whereas dwarf bunt occurs only in autumn-sown wheat in areas where snow cover maintains temperatures near freezing for several weeks at the soil surface (26), Karnal bunt apparently occurs only where high moisture, preferably rain, occurs for several days at the time of flowering of wheat plants.

Control

Control of Karnal bunt is desirable at two levels: (i) to manage the disease where it occurs so that losses in yield and quality are minimized, and (ii) to contain the disease or contaminating teliospores for trade or regulatory purposes. Control measures suitable for regulatory purposes usually must be more stringent than those needed for management purposes. For example, treatments applied to comply with insect quarantines, so that potentially infested products can leave regulated areas and be accepted elsewhere, often must meet a minimum efficacy standard termed probit 9, which dictates not more than three pests should survive in a population of 100,000 treated individuals (34). Several attributes of the etiology of Karnal bunt and the teliospores of *T. indica* make control a very challenging problem. Cultural practices that reduce Karnal bunt incidence, such as delays in sowing date, reduced nitrogen fertilization, or reduced planting density, only affect modest reductions in Karnal bunt incidence and may themselves reduce yields (20,36,52). The teliospores, which are long-lived and very resistant to chemical and physical treatments (46,53), are borne within and protected by the sorus and remainder of the partially bunted seeds typical of the disease. Teliospores are produced in large numbers; one infected seed can contain >100,000 teliospores. Teliospores within the interior of sori are further protected by the surrounding mass of teliospores. Teliospores buried in soil persist longer than those on the soil surface and are more protected from physical and chemical treatments (47).

Seed and soil treatments applied for the control of Karnal bunt have been only partially successful. Fungicides applied to seeds do not kill the teliospores but inhibit their germination (23,53); some chemicals with fungistatic activity that will persist more than 6 months include carboxin, thiram, pentachloronitrobenzene, and chlorothalonil (53). Seed treatment fungicides do not protect wheat plants from infection when seeds are planted in teliospore-infested soil, and they do not persist long enough within the plant to inhibit the infection of florets (43). Seed treatment fungicides are of value only when infected or contaminated seed is planted in soil not infested with teliospores. Hot water treatments can reduce teliospore germinability without killing the seed (30,46), but water temperatures that kill all teliospores within infected seeds reduce seed germination (42). Nonselective heat treatments, such as water of 60°C or higher (42), burning wheat stubble (37), or soil solarization (36,37), dramatically reduce the viability of teliospores. Practical limitations to the utilization of these methods include the expense of hot water or solarization and, as previously cited, the dispersal of viable teliospores in the air above burning fields (9). Fungicides applied to soil at seeding have not reduced the disease (43), probably because the infections originated from airborne infectious sporidia from teliospores that germinated outside the test plots. Among the fumigants tested to control teliospores, only methyl bromide killed teliospores within the unbroken sori of infected seeds when used at high doses on moist soil (47) or on moist grain after harvest (J. L. Smilanick and M. R. Bonde, *unpublished*). Seed fumigation tests with ethylene oxide were promising, although both it and methyl bromide reduced seed germination dramatically when applied at effective doses (46; M. R. Bonde, *unpublished*).

Foliar fungicide applications have achieved significant control of Karnal bunt. Two or more applications of propiconazole at or after spike emergence reduced the incidence of infected seeds by 95% (3,37,43,44). In many locations, the cost of propiconazole use can be partially recouped by increases in yield or quality that often occur by the control of diseases other than Karnal bunt. However, when propiconazole is applied to emerged spikes, as was done in some reports where good control of Karnal bunt occurred, residues of the fungicide may occur in the grain and pose a regulatory issue. In contrast to results in Mexico (43,44), Singh and coworkers (37) reported that an application of propiconazole that preceded spike emergence by 2 days, eliminating or minimizing grain residues, reduced Karnal bunt incidence by more than 95%. More studies of fungicide timing are needed, particularly with natural infection, but the sparse and irregular incidence of Karnal bunt makes this work difficult and time-consuming to conduct.

Genetic resistance can provide excellent control of Karnal bunt. Workers in Mexico and India have identified resistant lines whose ancestry was traced to China, India, or Brazil (19,20,39). Tolerant wheat cultivars, such as WL 1562 in India and Arivechi and Guamuchil in Mexico, have been released to growers; genes involved in resistance have been identified; and

immune selections, based on resistance originating from goat grass (*Triticum tauschii*), are under development (50).

Treatment of Karnal Bunt–Contaminated Wheat

Steam-flake milling. Following the discovery of Karnal bunt in Arizona and California, there was a major effort to test wheat in fields, grain elevators, and rail cars for the presence of teliospores of *T. indica*. As a result of these assays, large quantities of grain testing positive for Karnal bunt were left in storage facilities throughout the region. It was recognized by USDA and state officials in Arizona and California that a method was needed within a few weeks to treat the contaminated grain so it could be moved safely from these storage facilities and preferably still maintain some economic value. One such method tested, found highly successful and adopted, was steam-flake milling. This milling procedure is used to generate feed for huge livestock feed lot operations, is readily available in the region, and was shown effective in destroying Karnal bunt teliospores with no modification to its standard operational specifications (Fig. 8).

In a large cooperative test conducted by ARS (G. L. Peterson), APHIS (T. Boratynski), Arizona Department of Agriculture (D. Harder), and California Department of Food and Agriculture (K. Kosta), four truckloads of Karnal bunt–contaminated durum wheat were treated by steam-flake milling. In the process, grain was loaded via closed system conveyer belt into 7.6-m-high steam cabinet towers and heated 30 min to 109°C, then passed through rollers that compressed the steamed grain into flakes. Grain samples were taken from the trucks prior to treatment, then sampled every 15 min as grain moved through the mill. Subsequent teliospore germination tests showed that all spores were killed by the process, and steam-flake milling was adopted for large-scale use within the quarantine areas.

Holo-Flite Thermal Processor. It generally is believed that flour milled from contaminated grain poses no phytosanitary risk. However, the untreated mill feed byproduct does pose a minimal risk because of the potential introduction of viable teliospores into a field via animal waste. In a cooperative effort between Bay State Milling (R. Hampel, D. Reinig, R. Strewsbury), ARS (G. L. Peterson), and CDFA (K. Kosta), tests were conducted to evaluate the effectiveness of heat treating mill feed using a system known as a Holo-Flite Thermal Processor**.** The system consists of a hollow, jacketed tube containing two hollow twin augers. Temperature is regulated by heated oil, which is pumped through the hollow augers and jacket, evenly transferring the heat into the commodity. Temperature of the product is regulated by oil temperature, auger speed, and length of tube. The Holo-Flite Thermal Processor, or similar dry heat processor, is more economical to purchase and operate than methods such as pelletization or extrusion, other methods that potentially destroy Karnal bunt spores.

The study was conducted in a Karnal bunt–contaminated shed belonging to Arizona Grain, Inc., in Casa Grande, using a small scale Holo-Flite test model. Clean mill feed was artificially infested with teliospores of *T. indica*. The Holo-Flite was operated at a range of temperatures and speeds, and three replicated samples were taken at each time/temperature setting. The temperature of the treated mill feed was recorded as it left the machine. Spore viability was determined by extraction of teliospores from the product and germination testing. Results indicated that teliospores in mill feed can be killed with dry heat if the product reaches temperatures of 84, 101, or 110°C for 12, 5, or 2 min, respectively (G. L. Peterson, T. Boratynski, D. Harder, and K. Kostas, *unpublished*).

Present Problems and Future Research

One of the main reasons for the mammoth effort to deal with Karnal bunt in the United States is an economic consideration. The United States sells about $5 billion worth of wheat per year in the foreign market. In order to maintain these sales, phytosanitary certificates are required indicating that the wheat comes from regions where Karnal bunt is not known to occur. In the United States, as wheat moves along the transportation pipeline, much of it making its way to the major ports for exporting, it is commingled with other wheat lots. As long as foreign customers are concerned about Karnal bunt and have regulations preventing entry of wheat with *T. indica* teliospores, it is imperative this pipeline be kept free of Karnal bunt contamination. For this reason, quarantines were put into effect in the United States.

Karnal bunt likely has little direct effect on wheat yield or quality except perhaps for localized small areas of high infection. Indeed, the quality effects from these hot spots can be easily diluted by mixing infected seed with seed lots with no disease. However, in spite of the minor direct effects on crop yield and quality, the potential for economic losses to the U.S. wheat industry is real because of possible export reductions.

APHIS, ARS, several state departments of agriculture, and international organizations such as CIMMYT (Mexico) are working cooperatively to answer questions pertaining to the Karnal bunt problem. Immediately following discovery of Karnal bunt in the United States, research at APHIS and ARS centered on improving techniques to detect and identify the teliospores of *T. indica*, which are necessary to conduct the massive Karnal bunt survey (4,700 fields were pre- and postharvest tested in Arizona alone) and developing feasible methods to decontaminate harvested wheat, facilities, and equipment. However, during the first year of the National Karnal Bunt Survey, it became apparent that other smut pathogens, morphologically similar to *T. indica*, were also present and complicated identifications. During the summer of 1996, *T. indica*–like teliospores were detected as free spores in wheat in the southeastern United States. In the fall of 1996, similar teliospores were detected in ryegrass seed lots in Oregon. This was particularly significant because as much as 80% of the world's grass seed is produced in Oregon, and as much as 60% of the ryegrass seed lots tested were contaminated with a pathogen that could not be reliably distinguished from *T. indica* at that time by either PCR or spore morphology (M. R. Bonde, M. Palm, G. L. Peterson, L. Levy, and R. Meyer, *unpublished*). However, despite the fact that ryegrass and wheat are both grown in Oregon (often adjacent to each other), and teliospore numbers were sometimes high in ryegrass samples, no spores of *T. indica* morphology could be detected in wheat samples from the same areas. This suggested that in Oregon the pathogen infected ryegrass and not wheat, and therefore was an organism different from *T. indica*.

In January 1997, R. Ykema, Arizona Department of Agriculture, found an infected ryegrass seed in an Oregon seed lot (*personal communication*), and in February, infected ryegrass seeds were discovered by G. Peterson in wheat samples from

Fig. 8. Steam cabinet towers in Arizona used for steam-flake milling to decontaminate wheat infected and/or infested with Karnal bunt teliospores.

southeast United States (G. L. Peterson, *unpublished*).

In spring 1997, the Arizona Department of Agriculture in Phoenix and the USDA, ARS in Frederick independently demonstrated that the "ryegrass pathogen" could infect wheat after injections of sporidia into the boot cavity under optimum greenhouse conditions and high inoculum concentrations. However, virulence tests performed under highly conducive artificial conditions can result in erroneous conclusions. For example, it is well recognized that some bacterial pathogens only infect certain plant species under artificial conditions (22). Some fungal pathogens, such as *Peronosclerospora sorghi* and *P. sacchari*, have been shown to infect a broader range of plant species when large numbers of spores are used under optimum greenhouse conditions but are not known to infect those same species in the field (5). As pointed out by Whitney (55), the inappropriate reduction to synonymy of the rice smut pathogen, *T. horrida*, with *Neovossia barclayana* probably resulted from erroneous conclusions made from infection studies when two species of *Pennisetum* were inoculated and incorrectly described as infected by *T. horrida*. Presently, evidence is not conclusive as to whether *T. indica* and the ryegrass pathogen are the same organism. Field studies are being initiated to determine if the ryegrass pathogen will infect wheat under natural field conditions.

Research is underway on the taxonomy of *T. indica* and similar species, and on the identity of the ryegrass pathogen. In order to answer some of the most pressing questions about Karnal bunt, an expanded research effort is being mounted by the USDA and some states in order to better understand the relationship of *T. indica* to other smut organisms.

In addition to resolving taxonomic questions, further research is required in the areas of ecology and epidemiology, especially on the eventual geographical limits of the disease caused by environmental restrictions. Although present research information suggests that Karnal bunt is limited by very specific moisture conditions, further research is required to develop more accurate disease models to predict Karnal bunt outbreaks. Additional research also is required to improve detection and identification of *T. indica*; improve decontamination of wheat seeds, facilities, and equipment; develop Karnal bunt–resistant wheat cultivars; and refine chemical control strategies.

Karnal bunt is an important disease because of its influence on global trade of grain. A solution to the present situation will require continued cooperative efforts of scientists and regulators at an international level.

Acknowledgments

We thank Robert Nave, USDA, APHIS, National Coordinator, Karnal Bunt Program, for fruitful discussions and suggestions, and for providing the map of the 1997 Karnal bunt regulated area and the photograph of the steam-flake milling facility; and Joel Floyd, APHIS, PPQ, for drawing and providing the Karnal bunt life cycle. Thanks also are expressed to Roy Gingery, Wilda Martinez, Mary Palm, Arnold Tschanz, and Ron Ykema for critical reviews of the manuscript, and to Pat Freaner and Gail Hoover for typing the manuscript, and Susan Nester for taking photographs and preparing figures.

Literature Cited

1. American Phytopathological Society. 1996. Position statement of: The American Phytopathological Society: The use of quarantines for wheat Karnal bunt. APSnet. On-line: Karnal Bunt Symposium.
2. Aujla, S. S., Grewal, A. S., Gill, K. S., and Sharma, I. 1980. Effect of Karnal bunt on chappati making properties of wheat grains. Crop Improve. 7:147-149.
3. Aujla, S. S., Sharma, I., Singh, P., Singh, G., Dhaliwal, H. S., and Gill, K. S. 1989. Propiconazole - a promising fungicide against Karnal bunt of wheat. Pesticides 23:35-38.
4. Bansal, R., Singh, D. V., and Joshi, L. M. 1984. Effect of Karnal bunt pathogen (*Neovossia indica* [Mitra] Mundkur) on weight and viability of wheat seed. Indian J. Agric. Sci. 54:663-666.
5. Bonde, M. R., and Peterson, G. L. 1983. Comparison of host ranges of *Peronosclerospora philippinensis* and *P. sachari*. Phytopathology 73:875-878.
6. Bonde, M. R., Peterson, G. L., Fuentes-Davila, G., Aujla, S. S., Nanda, G. S., and Phillips, J. G. 1996. Comparison of the virulence of isolates of *Tilletia indica*, causal agent of Karnal bunt of wheat, from India, Pakistan, and Mexico. Plant Dis. 80:1071-1074.
7. Bonde, M. R., Peterson, G. L., and Matsumoto, T. T. 1989. The use of isozymes to identify teliospores of *Tilletia indica*. Phytopathology 79:596-599.
8. Bonde, M. R., Peterson, G. L., and Royer, M. H. 1988. Inheritance of isozymes in the smut pathogen *Tilletia indica*. Phytopathology 78:1276-1279.
9. Bonde, M. R., Prescott, J. M., Matsumoto, T. T., and Peterson, G. L. 1987. Possible dissemination of teliospores of *Tilletia indica* by the practice of burning wheat stubble. (Abstr.) Phytopathology 77:639.
10. Boratynski, T. N., Matsumoto, T. T., and Bonde, M. R. 1985. Interceptions of *Tilletia indica* at the California-Mexico border in Mexican railroad boxcars. (Abstr.) Phytopathology 75:1339.
11. Butler, L. 1990. Karnal bunt, quarantine and the international shipment of CIMMYT wheat seed. Proc. Bien. Workshop Smut Fungi, 7th.
12. Cashion, N. L., and Luttrell, E. S. 1988. Host-parasite relationship in Karnal bunt of wheat. Phytopathology 78:75-84.
13. Chahal, S. S., and Mathur, S. B. 1992. Germination of deep-frozen *Tilletia indica* and *Tilletia barclayana* teliospores. FAO Plant Prot. Bull. 40:31-35.
14. Dhaliwal, H. S., and Singh, D. V. 1988. Up-to-date life cycle of *Neovossia indica* (Mitra) Mundkur. Curr. Sci. 57:675-677.
15. Diekmann, M. 1993. Epidemiology and geophytopathology of selected seed-borne diseases. Int. Center Agric. Res. Dry Areas (ICARDA).
16. Duran, R. 1972. Further aspects of teliospore germination in North American smut fungi. Can. J. Bot. 50:2569-2573.
17. Ferreira, M. A. S. V., Tooley, P. W., Hatziloukas, E., Castro, C., and Schaad, N. W. 1996. Isolation of a species-specific mitochondrial DNA sequence for identification of *Tilletia indica*, the Karnal bunt of wheat fungus. Appl. Environ. Microbiol. 62:87-93.
18. Fuentes-Davila, G., and Duran, R. 1986. *Tilletia indica*: Cytology and teliospore formation in-vitro and in immature kernels. Can. J. Bot. 8:1712-1719.
19. Fuentes-Davila, G., Rajaram, S., and Singh, G. 1995. Inheritance of resistance to Karnal bunt (*Tilletia indica* Mitra) in bread wheat (*Triticum aestivum* L.). Plant Breed. 114:250-252.
20. Gill, K. S., Sharma, I., and Aujla, S. S. 1993. Karnal Bunt and Wheat Production. Punjab Agricultural University, Ludhiana.
21. Goates, B. J. 1988. Histology of infection of wheat by *Tilletia indica*, the Karnal bunt pathogen. Phytopathology 78:1434-1441.
22. Hildebrand, D. C., Scroth, M. N., and Sands, D. S. 1988. Laboratory Guide for Identification of Plant Pathogenic Bacteria, 2nd ed. N. W. Schaad, ed. American Phytopathological Society, St. Paul, MN. pp. 60-80.
23. Hoffmann, J. A. 1986. Chemical seed treatments for Karnal bunt. Proc. Bien. Smut Worker's Workshop, 3rd. Ciudad Oregon, Mexico.
24. Josh, L. M., Singh, D. V., and Srivastava, K. D. 1980. Wheat Disease Survey - I. Karnal Bunt 1975-1980. Wheat Pathology Series No. 7. Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi.
25. Mark, L. 1996. Karnal bunt - A strange-sounding wheat disease with serious implications. APHIS News Feature. On-line: Press Release, August 8.
26. Mathur, S. B., and Cunfer, B. M. 1993. Dwarf bunt. Pages 23-29 in: Seed-borne Diseases and Seed Health Testing of Wheat. Jordbrugsforlaget, Frederiksberg, Denmark.
27. Mathur, S. B., and Cunfer, B. M. 1993. Karnal bunt. Pages 31-43 in: Seed-borne Diseases and Seed Health Testing of Wheat. Jordbrugsforlaget, Frederiksberg, Denmark.
28. Melching, J. S., Bromfield, K. R., and Kingsolver, C. H. 1983. The plant pathogen containment facility at Frederick, Maryland. Plant Dis. 67:717-722.
29. Mitra, M. 1931. A new bunt of wheat in India. Ann. Appl. Biol. 18:178-179.
30. Mitra, M. 1937. Studies on the stinking smut or bunt of wheat in India. J. Agric. Sci. 7:459-478.
31. Peterson, G. L., Bonde, M. R., Dowler, W. M., and Royer, M. H. 1984. Morphological comparisons of *Tilletia indica* Mitra from India and Mexico. (Abstr.) Phytopathology 74:757.
32. Rai, R. C., and Singh, A. 1989. Karnal bunt of wheat. A threat to wheat seed production in eastern Uttar Pradesh. Narendra Deva J. Agric. Res. 4:27-31.
33. Rai, R. C., Singh, A., and Singh, R. V. 1988. Status of Karnal bunt of wheat in eastern Uttar Pradesh. Narendra Deva J. Agric. Res. 3:183-185.
34. Robertson, J. L., Preisler, H. K., Frampton, E. R., and Armstrong, J. W. 1994. Statistical analyses to estimate efficacy of disinfestation treatments. Pages 47 to 65 in: Quarantine Treatments for Pests of Food Plants. J. L. Sharp and G. J. Hallman, eds. Westview Press, San Francisco.
35. Roberson, R. W., and Luttrell, E. S. 1987. Ultrastructure of teliospore ontogeny in *Tilletia indica*. Mycologia 79:753-763.
36. Singh, A. 1994. Epidemiology and Management of Karnal Bunt Disease of Wheat. Research Bulletin No. 127, Directorate of Experiment Station, G. B. Pant University of Agriculture and Technology, Pantnagar, India.
37. Singh, B. B., Aujla, S. S., and Sharma, I. 1993. Integrated management of wheat Karnal bunt. Int. J. Pest Manage. 39:431-434.
38. Singh, D.V., Josh, L. M., and Srivastava, K. D. 1986. Varietal susceptibility and spread of Karnal bunt of wheat in India. Rachis Barley Wheat Newsl. ICARDA 4:10-16.
39. Singh, G., Rajaram, S., Fuentes-Davila, G.,

and Morgunov, A. 1995. Genetic basis of resistance to Karnal bunt in bread wheat. Bien. Workshop Smut Fungi, 9th. CIMMYT, El Batan, Mexico.
40. Singh, S. 1980. Report on Coordinated Experiments (Wheat Pathology) 1978-79. Wheat Project Directorate, Indian Agric. Res. Institute, New Delhi.
41. Smilanick, J. L., Dupler, M., Goates, B. J., Hoffmann, J. A., Clark, D., and Dobson, D. 1986. Germination of teliospores of Karnal, dwarf, and common bunt fungi after ingestion by animals. Plant Dis. 70:242-244.
42. Smilanick, J. L., Hershberger, W., Bonde, M. R., and Nester, S. E. 1997. Germinability of teliospores of *Tilletia indica* after hot water and sodium hypochlorite treatment. Plant Dis. 81:932-935.
43. Smilanick, J. L., Hoffmann, J. A., Cashion, N. L., and Prescott, J. M. 1987. Evaluation of seed and foliar fungicides for the control of Karnal bunt of wheat. Plant Dis. 71:94-96.
44. Smilanick, J. L., Hoffmann, J. A., and Prescott, J. M. 1987. Control of Karnal bunt with foliar fungicides, 1986. Fungic. Nematicide Tests 42:96.
45. Smilanick, J. L., Hoffmann, J. A., and Royer, M. H. 1985. Effect of temperature, pH, light, and desiccation on teliospore germination of *Tilletia indica*. Phytopathology 75:1428-1431.
46. Smilanick, J. L., Hoffmann, J. A., Secrest, L. R., and Wiese, K. 1988. Evaluation of chemical and physical treatments to prevent germination of *Tilletia indica* teliospores. Plant Dis. 72:46-51.
47. Smilanick, J. L., Prescott, J. M., Hoffmann, J. A., Secrest, L. R., and Wiese, K. 1989. Environmental effects on survival and growth of secondary sporidia and teliospores of *Tilletia indica*. Crop Prot. 8:86-90.
48. Smith, O. P., Peterson, G. L., Beck, R. J., Schaad, N. W., and Bonde, M. R. 1996. Development of a PCR-based method for identification of *Tilletia indica*, causal agent of Karnal bunt of wheat. Phytopathology 86:115-122.
49. Tooley, P. W., Carras, M. M., Beck, R., Peterson, G., and Bonde, M. R. 1995. Separation of *Tilletia indica* chromosomes using CHEF gel electrophoresis. Mycologia 81:61-67.
50. Villareal, R. L., Mujeeb-Kazi, A., Davila, G. F., and Rajaram, S. 1996. Registration of four synthetic hexaploid wheat germplasm lines derived from *Triticum turgidum* × *T. tauschii* crosses and resistant to Karnal bunt. Crop Sci. 36:218.
51. Warham, E. J. 1986. Karnal bunt disease of wheat: A literature review. Trop. Pest Manage. 32:229-242.
52. Warham, E. J., and Flores, D. 1988. Farmer surveys on the relation of agronomic practices to Karnal bunt disease of wheat in the Yaqui Valley, Mexico. Trop. Pest Manage. 34:373-381.
53. Warham, E. J., Prescott, J. M., and Griffiths, E. 1989. Effectiveness of chemical seed treatments in controlling Karnal bunt disease of wheat. Plant Dis. 73:585-588.
54. Washington Post. 1996. Declaration of extraordinary emergency. March 22. p. A23.
55. Whitney, N. G. 1989. Taxonomy of the fungus causing kernel smut of rice. Mycologia 8:468-471.
56. Ykema, R. E., Floyd, J. P., Palm, M. E., and Peterson, G. L. 1996. First report of Karnal bunt of wheat in the United States. Plant Dis. 80:1207.
57. Zhang, Z., Lange, L., and Mathur, S. B. 1984. Teliospore survival and plant quarantine significance of *Tilletia indica* (causal agent of Karnal bunt) particularly in relation to China. EPPO Bull. 14:119-128.

Morris R. Bonde

Dr. Bonde graduated with a B.S. degree in botany from the University of Maine in 1967 and from Cornell University with M.S. and Ph.D. degrees in plant pathology in 1969 and 1974, respectively. Since 1974, he has been employed by the USDA-ARS Foreign Disease-Weed Science Research. He has conducted research to determine the threat of foreign plant pathogens to major U.S. crops. Among the diseases are Karnal, dwarf, and common bunts of wheat; downy mildews of maize, sorghum, and sugarcane; soybean rust; and sorghum ergot. He began his research program on Karnal bunt in 1982 and has had extensive cooperative research projects in India and Mexico.

Gary L. Peterson

Mr. Peterson is a biologist who received his B.S. degree from St. Mary's College of Maryland in 1977. Since 1978, he has been with the USDA-ARS Foreign Disease-Weed Science Research Laboratory and currently is assigned to research on new and emerging plant diseases that affect international trade. In addition to work on Karnal bunt, he has conducted research on graminaceous downy mildews, soybean rust, citrus canker, sorghum ergot, and kernel smut of rice. His research and professional activities since 1982 have focused on detection, identification, epidemiology, and trade issues associated with Karnal bunt and dwarf bunt of wheat.

Norman W. Schaad

Dr. Schaad received his B.S., M.S., and Ph.D. degrees from the University of California, Davis, in 1964, 1966, and 1969, respectively. He then studied the characterization of bacterial ribosomes for 2 years under the direction of C. I. Kado. Dr. Schaad was a professor of plant pathology at the University of Georgia in Griffin from 1971 until 1982, specializing in identification, detection, and ecology of bacteria. He taught seed pathology and headed a research program in detection and control of seedborne bacteria at the University of Idaho from 1982 until 1986. In 1986, he took a position as Director of Biotechnology of Harris Moran Seed Company in Gilroy, California. Since 1992, he has been Research Leader and Bacteriology CRIS Leader of the USDA-ARS Foreign Disease-Weed Science Research Unit in Frederick, Maryland, where he specializes in developing improved molecular techniques for detecting seedborne pathogens.

Joseph L. Smilanick

Dr. Smilanick is a research plant pathologist at the Horticultural Crops Research Laboratory of the USDA-ARS facility in Fresno. He received a B.S. from the University of California, Davis, in plant science in 1977, an M.S. in plant pathology in 1980 from Colorado State University, and a Ph.D. in plant pathology in 1984 from the University of California, Riverside. Prior to moving to Fresno in 1986, he studied the biology and control of Karnal bunt in a cooperative project with CIMMYT as a research associate of James A. Hoffmann of the USDA-ARS in Logan, Utah. Subsequently, he investigated postharvest treatments employing biological control, fumigants, heat treatments, and fungicides for small grains and horticultural crops to reduce postharvest decay losses or to affect containment of pathogens for quarantine purposes. His present research activities are focused primarily on the use of biological control and heat treatments for citrus fruit to control postharvest decay.