Karnal Bunt of Wheat

G. Fuentes Davila, CIMMYT, Mexico

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History

Karnal bunt (KB) or partial bunt is the most recently described smut disease of wheat (Mitra 1931, Mundkur 1943b, Bedi et al. 1949). It was first reported in 1931 in experimental wheats at the Botanical Station at Karnal, India (Mitra 1931) and was for many years known only in the plains of India and Pakistan. However, since 1974 it has been noted in many locations across northern India.

KB differs from other smuts of wheat in that the pathogen, *Tilletia indica*, infects during anthesis, unlike *T. caries*, *T.foetida*, *T. controversa*, and *Urocystis agropyri*, and it sporulates on the same generation of the host that it infects, unlike *Ustilago tritici*.

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Distribution and Importance

Besides India and Pakistan, KB is known to occur in Mexico (Durán 1972) and Nepal (Singh et al. 1989; Figure 1.3). Although it is reported from Iraq (Mathur 1968), this report has not been confirmed by field incidence. A similar situation occurs for Afghanistan and Lebanon where the pathogen was found in wheat samples imported into the USA and India, respectively (Locke and Watson 1955, Nath et al. 1981).

Kernels with small lesions produce normal seedlings, whereas those with severe infection have poor germination and produce weak, distorted seedlings (Rai and Singh 1978, Singh 1980, Bansal et al. 1984b). Despite the fact that increases in disease severity result in proportional decreases in seed weight (Singh 1980, Bedi et al. 1981), yield losses are generally light (Munjal 1975, Brennan et al. 1990). Even during years when epidemics were most severe in India (some seed samples had about 89% infected kernels), losses were only 0.3 to 0.5% of production (Joshi et al. 1983).

KB may also reduce flour quality. In view of the importance of color, odor, and palatability of whole meal and chapaties, 1-4% infected kernels may be sufficient to render wheat grain unacceptable for human consumption (Mehdi et al. 1973, Sekhon et al. 1980, Amaya 1982, Medina 1985, Hussain et al. 1988). At 5% infection, quality distinctly deteriorates (Sekhon et al. 1980). There is also a loss in flour recovery and chemical changes in composition of flour and gluten content cause poor dough strength (Gopal and Sekhon 1988). If grains are washed and steeped, wheat lots with 7-10% infected grains are acceptable for consumption (Sekhon et al. 1981, Medina 1985, Hussain et al. 1988).

In northwestern Mexico, lots of grain with more than 3% infected kernels are rejected by the milling industry. However, they can be used for animal feed (Anon. 1989b). Further, a federal quarantine prohibits growing bread wheat in fields that have more than 2% KB infection. The movement of wheat grain from affected counties to other parts of Mexico is prohibited unless fumigated with methyl bromide. Seed grown for certification has a 0% infection tolerance and must be treated with a fungicide. Trucks, combines, and agricultural machinery cannot leave the quarantined areas unless cleaned and treated as determined by the Department of Agriculture. Movement of germplasm, however, is allowed under certain, but strict quarantine rules (SARH 1987).

Quarantines for KB have been established by many countries to exclude the disease from entry. For example, the USA prohibits importation of all wheat from

Mexico, allowing entry only of experimental seed under permits requiring strict safeguards and handling procedures (Anon. 1983, Cooper 1983).

Approximate losses in northwestern Mexico due to KB, including losses in yield and quality, loss of seed and grain export markets, and costs associated with control measures, are estimated at about $US7 million annually (Brennan et al. 1990).
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Hosts

KB occurs naturally on bread wheat (*Triticum aestivum* ; Mitra 1931), durum wheat (*T .turgidum*), and triticale (X *Triticosecale* ; Agarwal et al. 1977). According to Aujla et al. (1985), *T. indica* also occurs on *Triticum shareonensis* , *T.variabilis* , *T. ovatum* , and *T.scerrit* . Although inoculation has infected accessions of the following species, it is not known whether they can be infected under natural conditions: *Oryzopsis miliacea* , *Bromus ciliatus* , *B. tectorum* , *Lolium multiflorum* , *L. perenne* , *Triticum monoccocum* , *T. timopheevi* , *T. tauschii* , *Aegilops triuncialis* , *Ae. mutica* , *Ae. columnaris* , *Ae. caudata* , *Ae.sharonensis* , *Ae. cylindrica* , *Ae.bicornis* , *Ae. comosa* , *Ae.searsii* , *Ae.tauschii* , and *Ae . triaristata* (Royer and Rytter 1988).
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Pathogen

Taxonomy

The KB fungus was originally classified as *Tilletia indica* (Mitra 1931) and was later placed in *Neovossia* on the basis of a long promycelium with a whorl of 32-128 nonfusing conidia at the apex (Mundkur 1940). However, others now consider *Tilletia* to be the appropriate genus (Fischer 1953, Durán and Fischer 1961, Waller and Mordue 1983).

Pathogenic Specialization Mitra (1935) reported two physiologic forms of *T. indica* on the basis of spore size. However, significant differences in teliospore size were not found in collections from five Indian states (Bansal et al. 1984a). Recently, three distinct pathotypes of *T. indica* , which differed in virulence, were present in Punjab (Dhiman 1982). Aujla et al. (1987) reported pathotypes K1, K2, K3, and K4 in 21 collections of *T.indica* from different regions of Punjab and Himachal Pradesh. Their pathotypes were single teliospore isolates from single kernels and were identified on the basis of pathogenicity to genotypes of *T. aestivum* , *T.durum* , X *Triticosecale* , and *Secale cereale* . The pathotypes were morphologically alike and all were virulent on the wheat cultivars used. Pairs of pathogenic monosporidial cultures, each isolated from different teliospores, generally infect greater numbers of spikes than pathogenic pairs from the same teliospore (Fuentes-Davila 1989). Crosses within monosporidial lines from India cause more diseased kernels and spikes than crosses between Mexican and Indian lines (Royer and Rytter 1985). More research is needed to confirm the existence of *T. indica* races and to determine their importance.

Teliospore Morphology, Germination, and Physiology Teliospores are brown to

dark brown, spherical or subspherical or oval; 22-42 x 2540 µm in diameter, average 35.5 µm; some may be 55 µm (Figure 3.1). They occasionally have an apiculus (Roberson and Lutrell 1987), papilla (Mitra 1931) or a vestige of attached mycelium (Durán and Fischer 1961). Teliospores have three wall layers (Khanna et al. 1966; Khanna and Payak 1968; Roberson and Lutrell 1987; Gardner et al. 1983a,b). The primary wall is continuous with the apiculus wall (Roberson and Lutrell 1987). Immature teliospores are sterile, numerous, yellowish or sub-hyaline, rounded, angular or lacrimiform, 10-28 µm in diameter, and have thin, laminated walls (Mitra 1931, Durán and Fischer 1961).

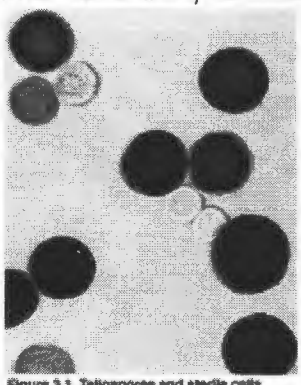

Figure 3.1. Teliospores and sterile cells *T. indica*. Mean diameter about 36 µm. Sterile cells lack pigment.

Though each teliospore generally produces one promycelium (Mitra 1931), several promycelia may arise from a single teliospore (Krishna and Singh 1981, Warham 1988). Promycelia vary in length up to 1500 µm (Mitra 1931, Holton 1949) and bear at the apex a whorl of 32 to 128 or more primary sporidia (Figure 3.2) (Mitra 1931, Holton 1949). The promycelium may branch (Mitra 1931, Krishna and Singh 1981). Variation in the enlargement of promycelial tips may occur. Primary sporidia are filiform, 64.4-78.8 µm long and 1.6-1.8 µm wide (Peterson et al. 1984).

Figure 3.2. Germinated teliospore of *T. indica* with promycelium and primary sporidia.

Germination. Primary sporidia germinate terminally or laterally to produce hyphae or sterigmata from which secondary falcate sporidia, 11.9-13 µm long and 22.03 µm wide (Peterson et al. 1984), are formed and forcibly discharged (Fuentes-Davila 1984). Falcate secondary sporidia produce hyphae or other sporidia by repetition. Hyphae that originate from primary or secondary sporidia produce large numbers of secondary, mononucleate falcate sporidia and somewhat fewer secondary filiform sporidia (Fuentes-Davila 1984). H-bodies are not formed by *T. indica* (Mitra 1931), although Holton (1949) reported seeing one H-body. Primary sporidia are initiated by the promycelium 6 to 44 hours after its extension, and production of secondary sporidia may require an additional 128 hours (Warham 1988).

During teliospore germination, meiosis occurs and the haploid nuclei migrate into the promycelium and primary sporidia, each of which receives one nucleus (Fuentes-Davila and Durán 1986). After one or two mitoses, most sporidia become septate forming two to four monokaryotic cells. Most secondary sporidia are mononucleate. Mycelial cells that originate from either type of sporidia are also mononucleate. After anastomosis, the dikaryotic sporogenous mycelium bears intercalated Y-shaped septa formed at the base of the probasidial initials. Nuclei migrate to the teliospore initials, which enlarge to form the teliospores, and the nuclei presumably fuse to form a diploid nucleus (Fuentes-Davila and Durán 1986, Roberson and Lutrell 1987).

T. indica is heterothallic (Durán and Cromarty 1977, Fuentes-Davila 1989). Heterothallism and pathogenicity are controlled by four alleles at one locus (Durán and Cromarty 1977, Fuentes-Davila 1989). Solopathogenic lines have not been found.

Variation in germination is common in reports from different researchers. Freshly collected teliospores are dormant, as indicated by failure of fresh teliospores to germinate (Mitra 1931, 1935). Others report low germination percentages of fresh

teliospores (Bansal et al. 1983, Smilanick et al. 1985b). The highest germination occurs with year-old teliospores (Mathur and Ram 1963, Kiryukhina and Shcherbakova 1976, Bansal et al. 1983).

Teliospores of *T. indica* germinate at 5-30 oC (Mitra 1935, Bansal et al. 1983, Krishna and Singh 1982a, Zhang et al. 1984, Smilanick et al. 1985b, Dupler et al. 1987). However, germination has been reported after 10 weeks at -18 oC (Zhang et al. 1984) or after 4-12 weeks at -30oC. Optimum temperature for teliospore germination is between 15 and 25oC (Mitra 1935, Mundkur 1943b, Holton 1949, Mathur and Ram 1963, Durán and Cromarty 1977, Krishna and Singh 1982a, Bansal et al. 1983, Zhang et al. 1984, Smilanick et al. 1985b). It is better in alternating light (Krishna and Singh 1982a, Zhang et al. 1984) than in darkness or near UV light, but germination in continuous light has been reported (Smilanick et al. 1985b).

Teliospores germinate between pH 4 and 11, the optimum being pH 6-9.5 (Krishna and Singh 1982a, Smilanick et al. 1985b). Under different osmotic and matric potentials, teliospore germination is delayed (Dupler et al. 1987) and both rate and percentage decrease with decreasing water potential. The highest percentage germination occurs at the highest potential tested, -1.4 bars.

Viability. Teliospores are viable in the laboratory for 5-7 years (Mathur and Ram 1963, Kiryukhina and Shcherbakova 1976, Zhang et al. 1984, Krishna and Singh 1983a). Teliospores in unbroken sori and buried 3 or 6 inches in field soil or left on the soil surface can remain viable for 27-45 months (Krishna and Singh 1982b).

Dissemination. Before harvest, dissemination of teliospores is limited, unless the sori break, which occurs infrequently. However, during harvest, sori may be broken and teliospores may contaminate healthy seed, soil, machinery, or vehicles and may be blown by the wind for long distances. Bonde et al. (1987) found viable teliospores up to 3000 m over burning wheat fields, suggesting the possibility of wind dissemination. Teliospores also germinate after ingestion by livestock and grasshoppers, providing another means of dissemination (Smilanick et al. 1986).

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Infection and Disease Cycle

The disease cycle of Karnal bunt is illustrated in Figure 1.7. Moderate temperatures, high relative humidity or free moisture, cloudiness, and rainfall during anthesis favor disease development (Mundkur 1943a, Bedi et al. 1949, Sattar and Hafiz 1952c, Agarwal et al. 1976, Aujla et al. 1977, Singh and Prasad 1978, Khetarpal et al. 1980, Krishna and Singh 1982c). Disease development was favored in soil at 17-21 oC (Aujla et al. 1977). High rates of nitrogen applications, as well as heavy manuring, may increase disease incidence (Bedi et al. 1949, Aujla et al. 1981, Ortiz-Monasterio et al. 1993). In India, cultivars sown late (November 25-December 15) tend to have greater KB incidence than when sown earlier (October 25-November 24; Singh and Prasad 1978, Aujla et al. 1981).

Symptoms

Not all spikes of a plant are affected by KB (Mitra 1935, Bedi et al. 1949), and usually only a few irregularly distributed kernels are bunted. Furthermore, infection of individual kernels varies from small points of infection to completely bunted kernels. Affected kernels are usually partially infected (Figure 3.3), and completely infected ones are rare (Mitra 1935, Bedi et al. 1949, Chona et al. 1961). The embryo is largely undamaged except when infection is severe. In infected spikelets, the glumes may be flared to expose bunted kernels (Figure 3.4), which reek of an odor similar to rotten fish caused by trimethylamine (Mitra 1935). The spikes of infected plants generally are reduced in length and in number of spikelets (Mitra 1937).

Figure 3.3. Healthy and affected kernels of wheat showing different levels of infection

Penetration The site of dikaryotization in *T. indica* is not known, although apparent hyphal anastomosis has been observed on glume surfaces. Conclusive evidence of the initiation of the dikaryotic phase will require elucidation of the nuclear condition of hyphae during the infection process (Goates 1988).

Sporidial germ tubes penetrate stomata in the rachis (Dhaliwal et al. 1989), glumes, lemma, and palea (Goates 1988, Salazar-Huerta et al. 1990). Growth of germ tubes towards stomata is common and, although germ tubes often penetrate beyond the stomatal ledges, they only occasionally pass into the substomatal chamber.

During early stages of infection, hyphae are intercellular among parenchyma and chlorenchyma cells in the distal to midportions of the glume, lemma, and palea, but not in basal portions. Later hyphae grow intercellularly toward the ovary, subovarian tissue, and rachis. After subovarian tissue is infected, the pathogen enters the pericarp through the funiculus. Hyphae have been seen in the rachis only during the later stages of infection. The epidermis of the ovary wall is not penetrated, even after prolonged contact with germinating secondary sporidia (Dhaliwal et al. 1983, Goates 1988).

Figure 3.4. Wheat spike infected by *T. indica*

During kernel development, intercellular hyphae proliferate in the middle layers of cells of the pericarp and form a compact, hymenium-like layer (Cashion and Luttrell 1988). These hyphae prevent fusion of the outer and inner layers of the pericarp and fusion with the seed coat. Their terminal cells give rise to the teliospores.

As the fungus grows, it ruptures the pericarp tissues in the bottom of the adaxial groove along the length of the developing kernel. This disrupts the flow of nutrients from the pericarp causing the endosperm to shrink and become cartilaginous. The embryo with attached endosperm may be easily removed and it germinates before or after removal. In the most severe infections, the kernel is reduced to a black membranous sorus and the embryo is killed.

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Control Strategies

Resistance Since the 1940s, cultivars of *T . aestivum* , *T.durum* , and *T. dicoccum* have been reported to be resistant to KB under field conditions in India (Anon. 1943; Bedi et al. 1949; Gautam et al. 1977; Singh et al. 1986, 1988; Singh and Srivastava 1990). X *Triticosecale* cultivars exhibited near immunity to KB in field trials (Meeta et al.1980). Inoculation indicated resistance among experimental lines and cultivars of *Triticum* , *Triticosecale* , and grass species (Aujla et al. 1980, Krishna and Singh 1983c, Warham et al. 1986, Gill and Aujla 1987, Royer and Rytter 1988, Singh et al. 1988, Singh and Srivastava 1990, Fuentes -Davila et al. 1992).

The reactions of lines or cultivars to KB are determined by percent infected kernels per spike (Fuentes-Davila and Rajaram 1994) or as coefficient of infection (Aujla et al. 1989). After a line or cultivar has been evaluated under inoculated and natural conditions in more than 12 tests, it is considered to be resistant if disease incidence averages 5% or less (Gill 1990, Fuentes-Davila and Rodriguez-Ramos 1993, Fuentes-Davila and Rajaram 1994).

In CIMMYT's Wheat Program, after five years of testing, 98 lines and cultivars of bread wheat have been identified as resistant, while at Punjab Agricultural University, 68 resistant lines have been identified. Most of these lines trace to germplasm from China, India, and Brazil. In 1992, KB resistant bread wheat cultivar Arivechi was released for commercial use by the Mexican national program for northwestern Mexico.

Inheritance. Studies on the mode of inheritance and allelic relationship among genes conferring KB resistance in bread wheat have indicated two partially recessive and four partially dominant genes (Fuentes-Davila et al. 1995). Other studies indicate polygenic and partially dominant genes; resistance genes are dispersed on chromosomes 1D, 2A, 3B, 3D, 5D, and 7A (Gill et al. 1993).

Impact of Cultivars

The importance and distribution of KB in India appear to be related to periodic widespread cultivation of susceptible or tolerant cultivars, although the impact of weather during these periods is also recognized (Gill et al. 1993). Prior to about 1968, indigenous tall wheats grown in the main wheat belt were KB susceptible, and the disease was often widespread, although usually not severe. The semidwarf wheats Kalyansona, PV18, and Sonalika (introduced in the late 1960s) were more resistant than indigenous wheats and KB decreased somewhat in importance. In 1975, high yielding but KB susceptible cultivars were released, and as their popularity grew, disease severity increased throughout northern India. After 1982, cultivars with KB tolerance were introduced and efforts were made to diversify the cultivars grown by farmers. These changes coincided with a reduction in disease incidence up to 1989. However, the situation changed again with the widespread use of KB susceptible cultivars.

Cultural Practices Cultural practices appear to have been of little practical value because most recommendations have been based on observation rather than long-term experiments (Table 3.1).

Warham and Flores (1988) surveyed farmers in the Yaqui Valley, Sonora, Mexico, from 1983 to 1985 to learn whether cultural practices to control KB were useful. They reported limited use involving soil type, land preparation, origin of seed, irrigation, nitrogen and other fertilizers, weed control, and crop rotation.

Rainfall or high humidity at flowering were more important than any of the cultural practices for conditioning KB. Adjustment of sowing dates did not appear to be beneficial because weather fluctuations at anthesis were probably more important. Limiting irrigation during flowering would have adverse effects on yield. A three-year rotation did not appear to be useful because it is too short to affect teliospores in the soil and because inoculum blew into the fields from adjacent areas.

In repeated experiments, Ortiz-Monasterio et al. (1993) found that disease incidence would increase with greater rates of nitrogen. Similar results were obtained with nitrogen applications during sowing compared to split applications. KB incidence was also greater when wheat cultivars were sown in flats instead of beds, and some cultivars showed a direct correlation between plant density and disease incidence

Table 3.1. Cultural practices suggested by various researchers for Karnal bunt control.

Cultural practice	Reference
Crop rotation	Mitra (1935), Padwick (1939), Singh and Mathur (1953), Singh et al. (1979)
Reduced irrigation and/or fertilizer	Bedi et al. (1949), Padwick (1939), Singh et al. (1979), Dhimann (1982), Ortiz-Monasterio et al. (1993)
Avoid continuous wheat	Singh et al. (1979)
Use disease-free clean seed, avoid late planting	Singh et al. (1979), Dhimann (1982), Lopez-Lugo (1986)
Use deep plowing, fallow after harvest	Dhimann (1982)
Reduce stand density	Lopez-Lugo, (1986), Ortiz-Monasterio et al. (1993)

Plant on light soils	Aujla.et al. (1986a)

Seed Treatment

Hot water and solar energy treatments have been applied to KB-infected seeds. However, they have had limited application. These treatments inhibit teliospore germination, but not as much as fungicide treatments (Mitra 1937). Fungicide seed treatments have been investigated for KB control since 1933 -34 (Mitra 1935). Although many fungicides have been tested for effectiveness (Table 3.2), results have not been satisfactory.

Table 3.2. Chemicals evaluated in wheat seed treatments for Karnal bunt control.

Aujla et al. **(1981)**
Ethylmercury chloride, Thiram

Aujla et al **. (1986b)**
Agrozim, Benomyl, Bitertanol, Butrizol, Captan, Carbendazim, Carboxin, ethylmercury chloride,Fenfuram, Fuberidazole, Furavax, Mancozeb, oxycarboxin, pentachloronitrobenzene, thiophanate-methyl, triadimefon, triadimenol

Figueroa-Lopez and Espinoza-Salazar **(1988)**
Chlorothalonil (EC), Chlorothalonil (WP)

Fuentes et al **. (1982)**
Benomyl, Carboxin, Corbel, 2 methoxyethyl mercury chloride, hexachlorobenzene, Mist-o-matic, oxycarboxin, phenylmercury acetate, propiconazole, RH 5871, thiabendazole

Fuentes et al. **(1983)**
Methylmercury guanadine, pentachloronitrobenzene
Mitra (1935, 1937)
Phenylmercury acetate, ethylmercury chloride, charcoal formaldehyde, copper carbonate, Formalin, Hortisan A, sulfur, Uspulum

Rai and Singh **(1979)**
Triphenyltin acetate, Brestanol, triphenyltin hydroxide, oxycarboxin, Butrizol

Salazar-Huerta et al **. (1986)**
Chlorothalonil, Mancozeb, Carbendazim + Mancozeb

Singh et al. **(1979)**
Phenylmercury acetate, ethylmercury chloride, improved ethylmercury chloride
Smilanick et al. (1985a)
Chlorine dioxide, ethanol 40%, formaldehyde, sodium hypochlorite, hot water (54oC), cupric acetate, Chloro-picrin, sulfur dioxide, methyl bromide

Smilanick et a **l. (1987)**

Triadimefon, Bitertanol, benomyl, Campogran, thiabendazole, propiconazole, Trimidol

***Valenzuela-Rodriguez* and** Navarro-Soto (1985)
Chlorothalonil (WP), Maneb, Carbendazim + Mancozeb
Carbendazim + Mancozeb, Maneb

***Warham and Prescott* (1989)**
Triphenyltin hydroxide, methoxyethylmercury acetate, ethylmercury chloride

Smilanick et al. (1987) applied seven systemic fungicides to seed (Benomyl, Bitertanol, Campogran, Trimidol, Propiconazole, Thiabendazole LSP, and Triadimefon), planted the seed, and inoculated spikes on some tillers. KB incidence was evaluated on inoculated and naturally infected spikes. None of the fungicides controlled development of the disease.

Some fungicides applied to infected kernels, which were then stored for various periods, inhibited teliospore germination, but others had little or no effect. Most of the fungicides reported to be effective inhibitors of teliospore germination have not been tested for germicidal properties. However, the fact that they inhibit teliospore germination after months of storage suggests they might be useful to control KB or to eradicate the pathogen from infected seed lots. For example, in one test, pentachloronitro-benzene in liquid or wettable formulations applied to wheat seeds inhibited teliospore germination up to two months

(Fuentes et al. 1983). Chlorothalonil, as emulsifiable concentrate and wettable powder applied to infected kernels, inhibited teliospore germination up to eight months (Figueroa-Lopez and Espinoza-Salazar 1988). Triphenyltin hydroxide, methoxyethylmercury acetate, and ethylmercury chloride inhibited teliospore germination for 18 months (Warham and Prescott 1989). Chorothalonil (powder), Mancozeb, and Carbendazim + Mancozeb on infected seed for 10 months resulted in about 97% inhibition of teliospore germination (Salazar-Huerta et al. 1986a,b).

On the other hand, Aujla et al. (1986b) treated infected seed with 17 protectant and systemic fungicides and buried the seed in soil for 16 days. None of these fungicides inhibited germination of teliospores that were recovered from the buried seed.

Fungicides that had little or no effect on teliospore germination have been reported in other experiments. Examples are: ethyl-mercury chloride, oxycarboxin, 4-butyl-1,2,4-triazole, chlorothalonil, and propiconazole, Mancozeb, tridimefon, and carboxin.

In vitro teliospore germination tests, in the absence of seeds, provide little information directly related to KB control, but they might be useful in preliminary identification of chemicals for later study. Such screenings were made by Krishna and Singh (1983b) with Carbendazim, copper oxychloride, thiophanate, Mancozeb M45, Mancozeb 78, triphenyltin hydroxide, edifenphos, Dinocap, Iprobenfos, Thiram, and carboxin. All of these fungicides inhibited teliospore germination to some extent.

Foliar Treatments

A number of experiments with foliar applications of fungicides have been reported since the late 1970s and all have had positive results **(Table 3.3).** Some studies reported low disease incidence in the checks (Singh and Prasad 1980, Quiñones-Leyva 1984), suggesting that future work on foliar applications should be done in conjunction with inoculations to assure adequate levels of disease in the tests.

Table 3.3. Fungicides applied to foliage for KB control.

Reference/ Fungicide	Percentage control
Krishna and Singh (1982b)	
Carboxin Carbendazim Oxycarboxin Triadimefon	82-87% control in greenhouse
Quiñones-Leyva (1984)	
Triadimenol Propiconazole	Reduced infection in field
Salazar-Huerta et al (1986a), Salazar-Huerta and Prescott (1986, 1987)	
Propiconazole	93-98% control in field
Singh et al. (1985a)	
Carbendazim Fentin hydroxide Mancozeb	Reduced infection in field
Singh and Prasad (1980)	
Benomyl Carbendazim Mancozeb Triphenyltin hydroxide	Reduced infection in field
Singh and Singh (1985)	
Triadimefon Triadimenol	Reduced disease incidence in field
Singh et al. (1985b)	
Bitertanol	64% control in field
Smilanick et al. (1987)	
Copper hydroxide Etaconazole Mancozeb Propiconazole	80% control in field

Soil Fumigation

Soil fumigation to control teliospore germination has been attempted with some success. In wet soil, methyl bromide reduced teliospore germination 98% when they were buried at depths up to 10 cm. Metam-sodium (Vapam) and formaldehyde were effective only on the soil surface. In dry soil, Vapam reduced germination 57-99% when spores were buried at depths up to 10 cm. Methyl bromide and formaldehyde were less effective (Smilanick and Prescott 1986). In other experiments, teliospore germination in samples buried at depths of 5 and 10 cm was 0, 0.2, and 2.4, and 0.6% in plots treated with methyl bromide and Dazomet, respectively, while in the untreated check soil, germination was 710% (Fuentes-Davila and Lawn 1992).

Combined Fungicide Application

During 1985 to 1987, the combination of soil fumigation with Brassicol (pentachloro-nitrobenzene), seed treatment with Bavistin (Carbendazim), and foliar sprays with propiconazole has shown a range of 50 to 80% control in experiments in India. Combinations of foliar sprays with seed treatment or with soil fumigation also reduce KB incidence (Gill et al. 1993).

Soil Drench

Applications of chlorothalonil (WP) and Mancozeb in irrigation water to 10 wheat cultivars did not significantly reduce KBincidence (Valenzuela-Rodriguez 1985).
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Techniques for Study

Culture Techniques

Teliospores of *T . indica* can be isolated by making a fine hole in an intact sorus and dusting the spores onto water agar in a petri plate. Alternatively, a teliospore suspension may be prepared by shaking sori in water containing a surfactant. Then sieve the suspension to remove debris and centrifuge to pellet spores, re-suspend the spores in water containing sodium hypochlorite (0.5%) and centrifuge; rinse in sterile water and centrifuge (repeat this step 2-3 times to remove disinfectant).

Plate teliospores on 1.5% water agar and incubate at room temperature (20-22 oC) and light; germination begins in 7-10 days. At this stage, single sporidia can be isolated by micromanipulation. Masses of sporidia from single teliospores can be isolated or pieces of agar bearing the fungus can be transferred to potato dextrose agar slants or petri plates. After 4-6 days, add sterile water and scrape the cultures into additional PDA test tubes or plates.

Inoculation Techniques

Moore's vacuum method. Wet the spikes during anthesis with a teliospore or sporidial suspension. Insert the stems through a rubber stopper cut along the radius and fit the stopper to the inferior end of a cylinder connected by a hose to a water suspension of inoculum. Pull the inoculum into the cylinder by vacuum. After spike exposure to the inoculum for about 1-2 minutes, release the vacuum by a valve located between the cylinder and the vacuum pump. The inoculum

suspension returns to the repository by gravity (Moore 1936). A range of 11100% infection has been obtained when inoculation is done with sporidial suspensions (Bedi et al. 1949, Chona et al. 1961). A number of factors may account for the range of infection, including: 1) viability of inoculum, 2)different cultivars, 3) varying environmental conditions, and 4) variation in efficient use of the inoculation technique.

Dropper method. Open the florets at anthesis and add a drop of sporidial suspension with an eye dropper. Chona et al. (1961) obtained 18 to 45% infection with this method.

Injection technique. Inject the inoculum with a hypodermic syringe into the boot just as awns emerge (Figure 3.5). High percentages of infection can be obtained with this technique (Chona et al. 1961, Durán and Cromarty 1977, Singh and Krishna 1982, Aujla et al. 1980). Mist-spraying inoculated plants also helps to obtain high percentages of infection (Aujla etal. 1982).

Go-go injection technique. Remove the central floret of an individual flower and clip off the awns with scissors. Then inject one or two drops of inoculum with a syringe into the remaining florets. This method gives satisfactory infection, but is more time consuming and less successful than the boot inoculation technique (Aujla et al. 1982, 1983).

Figure 3.5. Injection of T. indica inoculum into the boot of a wheat plant.

Other techniques that involve spraying spikes with an aqueous suspension of inoculum (Durán and Cromarty 1977) or applying sporidia with a small piece of cotton wool inside florets (Warham 1990) have given minimal infection.

Goates' inoculation method. Inoculate spikes inside an incubator. Lay pots and plants on their sides and place the spikes on fresh water agar in a plastic petri dish. Make a slit in the side of the petri dish to accommodate each stem. Then invert a petri plate containing the fungal colony over the spikes. Sporidia will shower from the culture onto the spikes. Inoculate the spikes in this fashion for 24-48 hours at 20 oC under continuous light (Goates 1988). This method is more suitable to screen for morphological resistance than the boot inoculation technique and the percentage of infection is higher than with the boot technique (Salazar-Huerta et al. 1990).

Disease Scoring

Disease scoring has been based on the number of healthy and infected spikes (Mitra 1935, 1937; Mundkur 1943a; Bedi et al. 1949; Chona et al. 1961). However, this classification gives equal weight to the presence of one infected kernel per head and to many infected kernels per head. Today, disease scoring is primarily based on the percentage of infected kernels (Singh and Krishna 1982, Aujla et al. 1982, Fuentes-Davila and Rajaram 1994).

Lines are considered to be resistant when kernel infection percentage is below 5% in 10 inoculated spikes after several tests (Fuentes-Davila and Rajaram 1994). Also used is a rating scale that considers the size of the lesion in the kernel, the number of kernels in each category, and the total number of kernels (Aujla et al. 1989).

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