Artificial Inoculation of Wheat with *Tilletia indica* from Mexico and India

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ABSTRACT

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Spring wheat cultivars Alex, Butte, Chris, Olaf, and Waldron were inoculated with sporidia obtained from germinating *Tilletia indica* teliospores from Mexico and India. Inoculations were performed by injecting sporidial suspensions into the boot and by drenching or atomizing the spikes before anthesis. Inoculations at the boot stage resulted in 35% spike infection; inoculations at heading, in 29% spike infection. Inoculations at the boot stage resulted in 65% of the kernels in an infected spike showing signs of infection; inoculations at heading, in 52% of the kernels showing infection, on the average. All cultivars inoculated were susceptible to one or both of the teliospore collections. Paired inoculations with monosporidial lines obtained from single primary sporidia indicated that at least three mating types were present in the lines isolated from the Indian and Mexican teliospores and that certain Indian isolates were compatible with certain Mexican isolates. There were no apparent differences in the degree of infection caused by sporidia obtained from germinated teliospores of the different collections. Crosses between Indian monosporidial lines appeared to cause higher degrees of infection than crosses between Indian and Mexican lines.

Karnal bunt, caused by *Tilletia indica* Mitra (= *Neovossia indica* (Mitra) Mundkur), has also been referred to as partial bunt, or new bunt. The disease and pathogen were first described in 1930 by Mitra (8), who observed the disease on wheat (*Triticum aestivum* L.) in experimental field plots at Karnal (Haryana) in the Punjab province of India. The disease was considered of minor importance until 1968 and was confined to northwestern India.

In 1965, India first imported semidwarf cultivars of wheat from Mexico. These were widely accepted and in great demand. Many Indian researchers (6,10) believed these exotic cultivars were more susceptible to *Tilletia indica* than those used previously and considered them a major cause of the increase in incidence of Karnal bunt in India between 1969 and 1975. Many Indian farmers continued to replant harvested seed regardless of whether it was diseased and therefore perpetuated the pathogen in the soil.

The disease was first recognized in Sonora, Mexico, by N. Borlaug and Mexican pathologists (J. Hoffmann, *personal communication*) and was reported by Durán and Cromarty in 1972 (4).

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Pathologists at CIMMYT (International Center for the Improvement of Maize and Wheat) have monitored Karnal bunt since 1971–1972. In their report to the USDA Plant Protection and Quarantine (PPQ) division of the Animal and Plant Health Inspection Service (APHIS), Phoenix, AZ, on 12 April 1983, they noted that the incidence remained at less than 0.02% on the basis of percent infected grains in a sample. In that report, they also noted that during 1979–1981 the disease had increased to a maximum of 17% infected grains for the cultivar Pima in the Mayo Valley of Sonora. APHIS intercepted Karnal bunt-infected wheat seed from Mexico on 9 July 1981 at the Plant Inspection Station at Laredo, TX. This prompted APHIS to adopt emergency regulatory measures prohibiting entry of all shipments of wheat and triticale from Mexico to the United States.

Several researchers (6,10,11) have reported that the disease was most severe in India when temperatures were cool, relative humidity was high, and rainfall was abundant at the time of wheat anthesis. Therefore, it appears that the disease could become established in the summer rainfall and irrigated wheat areas of the United States, such as the Pacific northwestern states.

There are no wheat cultivars known to be immune to infection by *T. indica*, either in India or Mexico; however, cultivars may vary in the number of spikes and number of kernels that become infected as well as in the proportion of the kernel that is converted into a sorus.

An inoculation technique is needed for screening germ plasm for resistance to *T. indica*. Techniques that have been used include injection of sporidia into the boot (1,2,4,12), injection of teliospores into the boot (2), atomization of sporidia onto the spike (4,12), dropping sporidial or teliospore suspensions into the florets without clipping the awns (2), dropping sporidial suspensions into the florets by clipping the awns to expose the stigma (1), vacuum infiltration of sporidial suspensions (2,9), and vacuum infiltration of teliospore suspensions (2). Inoculum sources varied from unspecified teliospore collections to monosporidial lines that were mixed and inoculated together.

The purpose of this investigation was to elucidate methods for artificial inoculation and to determine whether collections from Mexico and India would infect certain spring wheat cultivars commonly grown in the United States.

MATERIALS AND METHODS

Seed infected with *T. indica* collected from the Yaqui and Mayo valleys, Sonora, Mexico, in 1981 was obtained from J. A. Hoffmann (USDA-ARS, Utah State University). Infected seed of cultivar WL711 or WL1562 collected from Sangrur, Patiala, and Amritsar, India, in 1983 was obtained from L. M. Joshi (Indian Agricultural Research Institute, New Delhi, India). All infected seed samples were collected under permits issued by PPQ-APHIS. All experiments were performed in the containment laboratory and greenhouse facilities of the Plant Disease Research Laboratory at Fort Detrick, Frederick, MD, under permission of the Maryland Department of Agriculture and PPQ-APHIS.

Teliospores were removed from seed by placing several infected kernels in distilled water in sterile plastic 15-ml centrifuge tubes with screw caps and agitating them for 5–10 min. The teliospore suspension was then filtered through a 60-μm nylon mesh screen to remove larger debris. A few drops (0.1 ml) of a 0.5% solution of sodium hypochlorite were added to 15 ml of the filtrate immediately before centrifugation to prevent the spores from adhering to the wall of the centrifuge tube. The filtrate, containing teliospores, starch grains, and other small particles, was centrifuged for 2 min to pellet the teliospores. The teliospore pellet, containing a few starch grains, was resuspended in 0.5% sodium hypochlorite for 2 min to surface-sterilize the teliospores. The teliospore suspension was then rinsed twice in sterile distilled

Table 1. Results of artificial inoculation of U.S. spring wheat cultivars with sporidia of *Tilletia indica* from Mexico and India

Sporidial inoculum[a]	Cultivar	Incidence of infection: Spikes[b] Boot inoc.[d]	Spikes[b] Heading inoc.[e]	Kernels[c] Boot inoc.	Kernels[c] Heading inoc.
MEX81	Olaf	30/55	36/148	275/331	296/422
MEX81	Olaf	29/68	27/151	128/139	151/258
MEX81	Olaf	9/38	16/89	26/59	52/164
IND83	Olaf	4/19	18/72	11/42	53/88
IND83	Olaf	20/62	23/78	113/172	117/198
IND83	Butte	2/8	1/4	8/8	0/4
MEX81[f]	Waldron	0/0	1/8	0/0	2/2
MEX81[f]	Olaf	2/13	0/0	6/8	0/0
IND83[f]	Chris	0/0	1/13	0/0	1/1
IND83[f]	Alex	1/7	0/0	1/1	0/0
IND83[f]	Olaf	2/7	1/7	2/4	5/12
IND83-374	Olaf	3/23	2/19	12/21	8/24
MEX81-374	Olaf	12/21	12/17	40/45	79/161

[a] MEX81 = obtained from teliospores collected in Mexico in 1981, IND83 = obtained from teliospores collected in India in 1983, MEX81-374 = obtained from teliospores resulting from inoculation with MEX81, and IND83-374 = obtained from an IND83 inoculation.
[b] Number of infected spikes/number of inoculated spikes.
[c] Number of infected kernels/number of harvested kernels from infected spikes.
[d] Boot = growth stages 10–10.1 (7).
[e] Heading = growth stages 10.2–10.5.1.
[f] Inoculated by atomization of sporidia; all other inoculations were performed by injecting sporidia into the boot or drenching emerged spikes with sporidia.

Table 2. Infection results from inoculations with paired monosporidial lines of *Tilletia indica* from Mexico and India and postulated compatibility alleles

Monosporidial lines[a] (postulated alleles)	Monosporidial lines (postulated alleles): 18 (a_1)	29 (a_2)	72 (a_2)
18 (a_1)	0/62[b]	11/29	17/69
29 (a_2)	11/29	0/80	0/35
39 (a_3)	6/39	32/44	8/48
41 (a_3)	17/46	40/43	28/30
44 (a_2)	6/58	0/50	0/66
47 (a_2)	11/53	...[c]	0/73
57 (a_3)	3/81	...	2/79
72 (a_2)	17/69	0/35	0/60

[a] Lines 18 and 72 were isolated from teliospores collected in Sonora, Mexico, in 1981 and 1983, respectively. The remaining isolates were isolated from teliospores collected in different locations in India in 1983: 29 is from Sangrur; 57 is from Patiala; and 39, 41, 44, and 47 are from Amritsar. All lines except lines 44 and 47 were derived from different teliospores.
[b] No. of infected spikes/no. of inoculated spikes.
[c] Not tested.

water.

Teliospore suspensions were plated onto 5% water agar in petri plates (35 × 10 mm) at the rate of five to 10 teliospores per square centimeter. The plates were then incubated at 15 C in the dark. The surface of the agar was flooded with distilled water 10–15 days later after germination occurred, and the primary and secondary sporidia were dislodged with a rubber policeman. The sporidial suspension was filtered through a 60-μm nylon mesh screen to remove teliospores with attached mycelia and germinated sporidia. The inoculum was calibrated with a hemacytometer at ×400 to contain 4.1×10^4 to 2.8×10^5 total sporidia per milliliter. The percentage of infected spikes and infected kernels within a spike from concentrations at 10^3–10^5 were not significantly different as determined by prior experimentation.

Six wheat kernels were sown per 12-cm-diameter clay pot in a soil mix containing silty clay loam, sand, peat, perlite, 10:10:10 fertilizer, and hydrated lime (220:110:77:51:1:1). Plants were grown in a glasshouse at 18 ± 3 C until most of the tillers in a pot were between growth stages 10 and 10.5.1 (7), then all tillers within these growth stages were selected for inoculation and labeled. The date, inoculum identity, concentration of inoculum, pot number, plant number, and growth stage (10–10.1 = boot, 10.2–10.5.1 = heading) were recorded.

The sporidial inoculum was injected into the boot with a 3-cc syringe by inserting a 22-gauge needle into the space between the top of the spike and the enclosing boot sheath. About 1 ml was injected into the smaller boots of secondary tillers and 2 cc was injected into the boots of primary tillers. Spikes already emerged from the boot were drenched with inoculum from the syringe by dropping the sporidial suspension onto the florets until runoff. In one experiment, sporidial suspensions were atomized onto the emerged spikes at about 0.2 atm until runoff. Inoculated plants were placed in a misting tent for 3–4 days at 18 ± 3 C to provide constant free moisture. The plants were then removed to greenhouse benches and maintained at the same temperature for 3–5 wk until they had reached maturity (growth stages 11.3–11.4) and could be assessed for infection.

Koch's postulates were tested by recovering teliospores from spikes inoculated with sporidia from the teliospores collected in Mexico and India. The same procedure was used to induce teliospore germination and to inoculate wheat plants to verify that the causal agent of the symptoms was *T. indica*.

Monosporidial lines were established on potato-dextrose agar from single primary sporidia isolated from germinated Mexican and Indian teliospores. Inoculum was prepared by transferring 1-cm^2 blocks of agar from cultures of the lines to the lids of petri plates, then inverting the lids over 5% water agar and incubating the plates for 10–15 days. Sporidia that were released onto the surface of the agar were harvested and inoculum was quantified as described previously. Inoculum from single lines, quantified to 10^3–10^5 sporidia per milliliter, was mixed in pairs and inoculated onto wheat as before to determine if lines from Mexico would cross with lines from India and if any mating types were common between the two collections.

RESULTS

Results from inoculations with sporidia from germinating teliospores are presented in Table 1. Both the original collection of teliospores from Mexico and India and the teliospores recovered from inoculations with them produced infectious sporidia, thus verifying Koch's postulates. There were no apparent differences in the percentage of infected plants or percentage of infected kernels caused by the different collections, although this was not tested statistically because the experiments were conducted once. Further replication is in progress.

An average infection of 35 ± 17% of the spikes and 65 ± 25% of the kernels in an infected spike resulted when plants were inoculated by injection into the boot. An average infection of 29 ± 22% of the spikes and 52 ± 14% of the kernels in an infected spike resulted when plants were inoculated by drenching at the heading stage. To decrease the inaccuracies associated with small sample sizes, these percentages were calculated with samples in which more than 13 plants were inoculated. All cultivars inoculated were susceptible to both collections of *T. indica*.

Several monosporidial lines isolated from teliospores collected in Mexico were compatible with lines isolated from teliospores collected in India (Table 2).

DISCUSSION

Although injection of inoculum into the boot resulted in a higher level of infection than did inoculation of emerged spikes, it is not analogous to the natural mode of infection. If germ plasm is to be screened under conditions that simulate natural conditions, inoculations should

be performed by application of sporidia onto the plant surface. For example, injection of sporidia into the boot resulted in infection of triticale even though it is seldom infected under natural conditions (J. M. Prescott and K. S. Gill, *personal communications*). Cultivars with tight glumes may present more of a physical barrier to infection than the "loose glume" cultivars. For example, resistance of rice to the kernel smut of rice pathogen (*Neovossia horrida*), which also infects the plant before or near anthesis, has been suggested to be due to the distance to which the florets open and allow sporidia to enter (13).

There may be physiological resistance to *T. indica* in addition to the exclusion of sporidia by "tight glumes" according to some researchers (5), since cultivars had an equal range of susceptibility within long and short flowering periods. Additionally, Dhaliwal et al (3) found that cultivars differ in the number of primary infection sites, and the pathogen spreads in a localized, systemic fashion to other florets in the spike.

Singh and Krishna (12) obtained infection by inoculating wheat plants with sporidia at the boot to early flowering stages. They obtained 50, 72, and 21% bunted spikes, and 75, 84, and 42% bunted grains from inoculations at the boot, awns-emerging, and heading stages, respectively. Therefore, the awns-emerging stage appeared to be most favorable for obtaining high levels of infection. Duran and Cromarty (4) found that inoculation by injection at the boot stage was superior to atomization of sporidia at later stages; they obtained 36–62% bunted spikes by inoculations with paired, compatible monosporidial lines. Aujla et al (1) obtained 70–85% and Chona et al (2) obtained 59–86% bunted grains from inoculations of sporidia into the boot. Our results confirm that a higher percentage of infected grains results from injecting inoculum into the boot compared with applying sporidia at heading. The differences in levels of infection in our report compared with certain other studies may be due to the use of different wheat cultivars, isolates, methods of obtaining inoculum, temperature at inoculation, duration of dew period, and postinoculation conditions.

Six of the monosporidial lines isolated from teliospores from India were compatible with line 18 isolated from a teliospore from Mexico. The two lines isolated from the teliospores from Mexico were compatible, indicating the presence of two compatibility alleles according to the hypothesis of bipolar compatibility (4). Several arbitrary compatibility allele designations may be assigned to each line: a_1 to 18; a_2 to 29, 44, 47, and 72; and a_3 to 39, 41, and 57.

More bunted kernels per bunted spikes and more bunted spikes appeared to result from crosses within the Indian lines than from crosses between the Mexican and Indian lines. This was not tested statistically because of the small sample sizes.

The similarity between *T. indica* from Mexico and India can be best explained by movement of infected grain between the two countries. There is no evidence that *T. indica* evolved by parallel evolution in the two countries. If seed movement is responsible for international dissemination of the pathogen, then the presence of the disease in certain countries but not others poses particular questions to plant protection and quarantine officials throughout the world. For countries that exchange wheat germ plasm, issues arise concerning the cost of exclusion via quarantine (or entry for experimental purposes under special permission after the seed has been inspected) versus the impact of allowing infected grain to enter and potentially disrupt their export market. The geographical distribution of the pathogen, the paucity of resistant wheat varieties, the efficacy and cost of chemical control, and the perpetuation of the pathogen in the soil (6,10) pose challenging problems. It will be interesting to see how we respond to this potential pathogenic threat by putting to test modern plant protection techniques.

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