Importance of Seedborne *Tilletia controversa* for Infection of Winter Wheat and Its Relationship to International Commerce

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ABSTRACT

Grey, W. E., Mathre, D. E., Hoffmann, J. A., Powelson, R. L., and Fernández, J. A. 1986. Importance of seedborne *Tilletia controversa* for infection of winter wheat and its relationship to international commerce. Plant Disease 70:122-125.

Samples of wheat being exported from Pacific Northwest ports in 1983 were examined for the presence of the dwarf bunt fungus, *Tilletia controversa*. Of 552 samples examined, 141 were free of dwarf bunt. The remaining samples, representing all classes of wheat, contained dwarf bunt teliospores, but none of these seed lots were heavily infested. Field tests were conducted in five states over 2 yr to determine if seedborne teliospores of *T. controversa* could induce disease. Bunted spikes resulted only when heavily infested seed (≥1 g of teliospores per kilogram of seed) was planted in disease-conducive locations. None of the 552 samples examined in 1983 had dwarf bunt infestation levels ≥1 g of teliospores per kilogram of seed (equivalent to 20,000 teliospores per seed). Therefore, in areas where this disease is not known to occur, there seems to be minimal risk that the importation of grain with low levels of infestation will result in significant development of dwarf bunt.

Additional key words: *Triticum aestivum*

The international movement of agricultural products, including seed, is of major importance to the economies of many nations. Grain exports are particularly important to the grain producers of the United States who must market a large portion of their crops overseas. One factor that can hamper the movement of plant products is the implementation by importing countries of quarantines against various pests. Currently, the People's Republic of China has a quarantine against importation of grain containing teliospores of the dwarf bunt fungus, *Tilletia controversa* Kühn. Of concern is whether inoculum of such a pathogen on grain is sufficient to establish infection and perpetuate the disease such that a quarantine based on zero tolerance is justifiable.

Historically, the bunt fungi (*Tilletia* spp.) have caused major losses in grain production and quality wherever wheat has been grown (2). The common bunt fungi (*T. foetida* (Wallr.) Liro and *T. caries* (DC.) Tul.) have been effectively controlled with chemical seed treatments (5). Dwarf bunt, however, found only in localized regions of the world, has resisted control by these same chemicals (3). The failure to control dwarf bunt (as opposed to common bunt) by seed treatment can be attributed to the delayed time of infection in winter wheat (4). In the Pacific Northwest, infection of the plant typically occurs during January through March (7) in association with a continuous snow cover of 60–90 days. Presumably, snow cover provides the high moisture and cool temperatures conducive to the germination of teliospores on the soil surface and subsequent infection by the germination products (9). Although seedborne teliospores of *T. controversa* are not believed to serve as the primary inoculum (8), the question of whether seedborne inoculum can establish the disease in a new location has been the subject of discussions with the People's Republic of China in attempts to modify the quarantine against wheat containing dwarf bunt teliospores (9).

This study was conducted to determine the significance of seedborne inoculum of dwarf bunt in disease development. In particular, we wanted to determine the relationship, if any, between the level of teliospore inoculum on seed and percent infection and whether grain produced in and being exported from the Pacific Northwest has an infestation level sufficient to cause dwarf bunt infection if the seed were planted in a new environment.

Research supported in part by USDA-CSRS-Special Research Grant and the wheat commissions of Idaho, Oregon, Montana, and Washington.

Contribution from the Montana Agricultural Experiment Station. Journal Series Paper 1695.

Accepted for publication 2 July 1985.

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MATERIALS AND METHODS

Seedborne inoculum and infection. A slurry of *T. controversa* teliospores and 1% carboxymethylcellulose (CMC) solution was added to seed at a rate of 5% (v/w) and allowed to dry before being packaged. The inoculum was a composite of dwarf bunt teliospores collected from different cultivars and geographical areas to comprise a broad range of germination rates, virulence genes, and adaptability to environment. Seeds with CMC, but without dwarf bunt teliospores, served as an uninoculated check.

In the crop year 1980–1981, field studies were conducted in 11 locations either known to be conducive to dwarf bunt disease or without a history of this disease in Oregon, Utah, Montana, and Idaho. Seed of susceptible winter wheat (*Triticum aestivum* L. 'Wanser' and 'Nugaines') was inoculated with dwarf bunt teliospores at levels of 10, 1, 0.1, 0.01, and 0.001 g/kg of seed. Seeds were planted mechanically or by hand in machine-opened furrows at a rate of 6–7 g/3-m row, either shallow (1.3 cm) or deep (5.0 cm) in 1980 and shallow (1.3 cm) in 1981. Seeding dates ranged from 1 to 27 October in 1980 and 1981. The number of infected spikes per 3-m row was recorded the following summer, or when heavy infection occurred, the number of bunted spikes per 100 wheat spikes 1 m from the end of the row was recorded. The field plot design in 1980–1981 was a split plot (where seeding depth was the main plot and cultivar by level of inoculum was the subplot) with four replicates.

Field trials in 1981–1982 were conducted at 10 locations in Idaho, Montana, Utah, Washington, and Wyoming. Seed of the susceptible winter wheat cultivars Nugaines and Cheyenne was inoculated with dwarf bunt teliospores at levels of 10, 1, and 0.1 g/kg of seed. Emerged seedlings in one-half of the plot were covered with vermiculite to simulate a snow cover from about 1 December 1981 to 15 March 1982 unless there was adequate snow cover early in the winter.

A vermiculite covering was applied at Cavendish, ID, Bozeman and Ft. Ellis, MT, Pullman, WA, and Laramie, WY. At Laramie, the wind blew the vermiculite off the plots by mid-January. The cultivar and inoculum treatments were randomized in a complete block design with four replicates.

Grain sampling and *T. controversa* teliospore monitoring procedures. The presence of *T. controversa* teliospores on grain shipments from Pacific Northwest ports was determined on grain samples collected by the Federal Grain Inspection Service (FGIS) between 20 January and 31 October 1983. Samples representing the three major market classes of wheat, hard red spring (HRS), white wheat (WW), and hard red winter (HRW), were collected from 10 elevators in Portland, OR, and Longview, Tacoma, Vancouver, Kalama, and Seattle, WA. Collected as a random sample as it was being loaded onto ships, the grain represented a 900-g subsample of that normally collected by FGIS for grading purposes.

A 50-g sample of seed was removed from the grain sample and added to 100 ml of distilled water plus one drop of Tween 20 (Difco) in a clean 250-ml Erlenmeyer flask. The seed and water were agitated on a rotary shaker for 5 min. The liquid was filtered through three layers of cheesecloth and collected in two centrifuge tubes. The volume in each tube was brought to 45 ml with water and centrifuged at 1,500 *g* for 2 min. The supernatant was decanted and the precipitate in both tubes combined and brought to 1 ml with water. An equal volume of methylene blue (1 mg/ml, 89% a.i.) was mixed with the precipitate. A drop of the suspension was examined for dwarf bunt teliospores in a No. 820 Howard mold-counting chamber (Hausser Scientific, Blue Bell, PA) at ×100. Any teliospore with a reticulate exospore was examined at ×430 for the presence of a sheath or capsule to avoid possible misidentification with *T. caries*. Identification of teliospores was based on their morphology as described by Duran and Fischer (1) and Trione and Krygier (10).

RESULTS

Infection. In the 1980–1981 and 1981–1982 field tests, Nugaines had 29 and 25% fewer smutted spikes than Wanser and Cheyenne, respectively. There were no significant interactions between cultivars and treatments in either year, suggesting that the cultivars reacted in a similar fashion to the treatments. However, because Nugaines had less disease incidence, the number of dwarf-bunted spikes in either Wanser or Cheyenne was used in the analysis. In 1980–1981, only the number of bunted spikes per treatment, totaled over four replicates, was tabulated because of the low infection at most locations. Therefore, for statistical analysis, the locations were handled as replicates.

Dwarf-bunted spikes occurred in rows planted with inoculated seed and in rows planted with uninoculated seed. Only rows planted with seed inoculated at the highest level (10 g/kg of seed) had significantly higher percentages of bunted spikes than occurred with lower levels of inoculum (Table 1). Higher numbers of infected spikes developed in shallow-seeded rows than in deep-seeded rows (Table 1), confirming previous studies (4,8).

In 1980–1981, of the seven locations with a history of dwarf bunt, all but one (Kalispell, MT) had low to moderate incidences of dwarf bunt in plots planted with infested seed. Kalispell experienced a winter season without snow and had no dwarf bunt. With the exception of Bozeman, MT, all other locations also had dwarf bunt in the plots planted with uninoculated seed that probably resulted from natural soilborne inoculum. At Logan, UT, there was a tendency toward a higher incidence of dwarf-bunted spikes at the 1-g/kg of seed inoculum level compared with this level at the other locations (Table 1). At the four locations where dwarf bunt had not been reported previously (Hyslop, Pendleton, and Moro, OR, and Conrad, MT), no dwarf bunt developed in the uninoculated plots. At Pendleton and Moro, however, there was a trace (0.1% smutted heads) of infection in the plots planted with infested seed. Obviously, the environment played an important role in disease development; only at those locations with an environment known to be conducive for dwarf bunt development was there a significantly larger number of bunted spikes at the highest level of inoculum (10 g/kg of seed) compared with the check plots sown with uninoculated seed (Table 1).

In 1981–1982, only three of the 10 locations, Bozeman and Ft. Ellis, MT, and Cavendish, ID, had a significantly greater percentage of dwarf-bunted spikes that developed from inoculated seed (1 and 10 g/kg of seed) than from uninoculated seed (Table 2). Bozeman was the only site where significant levels of dwarf bunt developed from 1- and 10-g/kg of seed inoculum rates compared with lower inoculum rates, and this occurred only in the vermiculite-covered plot (Table 2). Very little residual soil inoculum existed at Bozeman and Ft. Ellis, MT, and Laramie, WY, because the check plots had either a trace of infection or none. In comparison, the check plots at seven of the other locations had infection levels higher than those in the inoculated plots at Bozeman, Ft. Ellis, or Laramie (Table 2). This also suggests the greater importance of soilborne inoculum compared with seedborne inoculum for dwarf bunt infection.

In one (Bozeman, MT) of five locations in 1981–1982, the vermiculite covering resulted in a greater number of dwarf-bunted spikes (Table 2). However, the use of the vermiculite did not increase disease

Table 1. Percentage of dwarf-bunted spikes per 3-m row of Wanser winter wheat resulting from infested seed planted in 1980–1981 at two depths in locations with a history of dwarf bunt

	Inoculum level (g teliospores/kg seed)						
Location	**Check[a]**	**0.001**	**0.01**	**0.1**	**1**	**10**	**Mean**
Franklin County, ID							
Shallow[b]	5	5	4	2	1	26	7.2
Deep	3	2	0	3	5	2	2.5
Bozeman, MT							
Shallow	0	0	0	0	2	4	1.0
Deep	0	0	0	0	0	0	0.0
Ft. Ellis, MT							
Shallow	1	0	0	1	0	2	0.7
Deep	0	0	0	0	0	0	0.0
Kalispell, MT							
Shallow	0	0	0	0	0	0	0.0
Deep	0	0	0	0	0	0	0.0
Flora, OR							
Shallow	0	3	0	0	4	6	2.2
Deep	2	2	0	0	15	0	3.2
Blue Creek, UT							
Shallow	2	4	3	3	2	12	4.3
Deep	1	0	3	5	4	1	2.3
Logan, UT							
Shallow	2	2	9	5	46	65	21.5
Deep	0	0	5	0.	0	17	3.7
Overall mean[c]							
Shallow	1.7	2.3	2.7	1.8	9.2	19.8	6.2
Deep	1.0	0.7	1.3	1.3	4.0	3.3	2.0

[a] Check value is the mean of two uninoculated plots.

[b] Seeds were planted shallow or deep (1.3 and 5.0 cm, respectively).

[c] LSD ($P \leq 0.05$) = 9.01 for row means, and LSD ($P \leq 0.05$) = 3.41 for column means.

incidence above that which occurred under a continuous snow cover during the same time period. For example, at Bozeman, where snow cover was often interrupted during the winter by warm winds, the percentage of infected spikes in the vermiculite-covered plots was similar to that in plots at Ft. Ellis, where the snow cover remained throughout the winter. Two other locations (Cavendish, ID, and Pullman, WA) had no increase in infection with vermiculite, presumably because snow cover was of adequate duration at these locations (Table 2). Therefore, even heavily infested seed did not result in significant amounts of dwarf bunt unless it was planted in an environment where the conditions of high moisture and cool temperatures, such as provided by a cover of snow or vermiculite, were uninterrupted for 60–90 days.

Grain sampling. A total of 552 grain samples were examined for *T. controversa* teliospores between 20 January and 31 October 1983. The number of grain samples received was nearly the same each month, with slight increases in early spring and late fall. Each of the three classes of wheat (HRS, WW, and HRW) were represented in each month of the survey. Eight of the 10 elevators accounted for 98% of the grain samples and handled each of the three classes of wheat in about equal proportions. In 141 of the 552 grain samples (26%), there were no detectable *T. controversa* teliospores (Table 3). Sixty-four (35%), 43 (20%), and 34 (22%) of the HRS, WW, and HRW grain samples, respectively, had no detectable dwarf bunt teliospores. Because spring wheat is not affected by dwarf bunt, it is of interest that the grain from such a crop is carrying any *T. controversa* teliospores. However, the percentage of HRS grain samples with no detectable teliospores was higher than that of the winter wheat grain samples. There were 350, 54, and 7 grain samples with dwarf bunt teliospore infestation levels of 0.001, 0.01, and 0.1 g/kg of seed, respectively (Table 3). No grain samples contained infestation levels corresponding to either 1 or 10 g/kg of seed, which were the minimum seedborne inoculum levels that resulted in dwarf bunt in either the 1980–1981 or 1981–1982 field trials (Tables 1 and 2).

DISCUSSION

The data collected in 1980–1981 and 1981–1982 indicated that dwarf bunt infection from seedborne teliospores could occur but was very rare. At all test locations, the minimum seedborne inoculum level for infection was 1 g/kg of seed (about 20,000 teliospores per seed) (Table 1). None of the 552 grain samples examined during the 1983 survey contained this minimum level of infestation (Table 3). In a related study conducted by the Pacific Northwest Grain Quality Council during 1975–1977 and 1981, 3,786 grain samples from inland elevators were examined for *T. controversa* teliospores by the Oregon Department of Agriculture (K. Packard, *unpublished*). Eighteen percent of these grain samples had detectable dwarf bunt compared with 74% of those collected from Pacific Northwest ports in the 1983 survey. Five of the 3,786 grain samples from inland elevators had infestation levels corresponding to a dwarf bunt inoculum level of 1 g/kg of seed. Although not directly comparable, this suggests that by the time grain reaches the export elevators, the infestation level is reduced. The frequent mixing and handling of grain during transport probably reduce the infestation level but also contaminate otherwise clean grain and crops not affected by dwarf bunt, such as spring wheat (6). Therefore, the possibility of keeping any one lot of grain completely free of dwarf bunt seems remote.

This research has established that current-season dwarf bunt can occur from seedborne inoculum, although the incidence is low and the seed must be heavily infested. In theory, only one teliospore is necessary for infection, but the results of these studies indicate that an inoculum level of 1 g of teliospores per kilogram of seed (about 20,000 teliospores per seed) is necessary to cause significant infection. These studies do not prove that infection originated directly from seedborne spores, with penetration and infection occurring at the time of seed germination, as with common bunt. If dwarf bunt infection did occur directly from seedborne spores, one would expect dwarf bunt incidence in plants from deep-sown seeds to be as high (or higher) as in those from shallow-sown seed. It is our hypothesis that dwarf bunt spores on seed do not infect directly but rather are rubbed off the seed and contaminate the soil during planting. The spores, thus being soilborne, cause infection in the usual manner, i.e., at a later stage of seedling growth.

In our survey, the levels of *T. controversa* teliospores in grain shipped from Pacific Northwest ports were not sufficient to result in "significant" disease development in the initial crop if such grain had been planted. The probability of dwarf bunt would be further decreased by practices or conditions that reduce the efficiency of the inoculum, such as deep planting, late planting, and planting at sites not conducive for survival of inoculum and infection. Nonetheless, the introduction of the dwarf bunt fungus into a location with no history of the disease could possibly result from the "escape" of teliospores from infested

Table 2. Percentage of dwarf-bunted spikes per 3-m row of Cheyenne winter wheat resulting from infested seed planted in 1981–1982, where seedlings were either covered or not covered with vermiculite

	Inoculum level (g teliospores/kg seed)					
Location	Check[a]	0.1	1	10	Mean	LSD[b]
Cavendish, ID	36.2	45.2	45.0	47.1	41.9	10.5
Franklin County, ID						
Location 1	63.5	64.3	71.5	59.6	64.5	16.4
Location 2	71.1	67.3	69.0	66.5	69.0	13.1
Bozeman, MT						
Vermiculite[c]	0.7	0.8	4.0	7.8	2.8	2.4
Control	0.2	0.5	0.8	1.8	0.7	···
Ft. Ellis, MT	1.5	1.0	2.6	8.4	2.8	2.7
Kalispell, MT	18.6	8.9	7.4	7.5	9.5	7.2
Blue Creek, UT	85.6	90.5	93.4	82.1	87.4	10.8
Logan, UT	23.2	19.6	19.4	35.5	24.2	20.0
Pullman, WA	14.0	16.4	15.0	15.8	15.0	4.2
Laramie, WY	0.0	0.0	0.1	0.0	0.1	0.5

[a] Check value is the mean of two uninoculated plots.
[b] LSD ($P \leq 0.05$), df = 28 for row means.
[c] Seedlings were covered with vermiculite from December through March 1982 to simulate snow cover.

Table 3. Level of *Tilletia controversa* teliospore infestation in wheat grain samples obtained from Pacific Northwest ports from January to October 1983

	No. of grain samples at each infestation level (g teliospores/kg seed)						
Wheat class[a]	ND[b]	0.001	0.01	0.1	1	10	Total
HRS	64	110	7	0	0	0	181
WW	43	153	13	5	0	0	214
HRW	34	87	34	2	0	0	157
Total	141	350	54	7	0	0	552

[a] HRS = hard red spring, WW = white wheat, and HRW = hard red winter wheat.
[b] ND = No detectable *T. controversa* teliospores. Minimum detection level was one spore = 14,500 teliospores per kilogram of seed.

grain during transport and in milling effluents. However, such introduction of the dwarf bunt fungus probably would not lead to establishment and disease development. A low incidence of dwarf bunt can occur without continuous and prolonged snow cover, especially when the inoculum potential is high and/or when favorable conditions of moderate temperatures and frequent precipitation are provided without snow. On the other hand, the incidences sufficient to cause economic losses in the Pacific Northwest have only been provided by a persistent snow cover. Furthermore, the sites favorable for establishment and epidemic development are small and rarely influence crop production. Therefore, for grain not intended for planting and where the effluent from the milling process is directed into areas not used for wheat production and into bodies of water not used for irrigation, we suggest that a permissible low level of dwarf bunt contamination in grain may be an acceptable compromise to the current zero-tolerance requirement.

ACKNOWLEDGMENTS

We thank Zhang Zhiyong, Institute of Plant Quarantine, Ministry of Agriculture, Animal Husbandry and Fishery, Beijing, People's Republic of China; J. Horton, Division of Plant Science, University of Wyoming; R. J. Johnston, Research Associate, Department of Plant Pathology, Montana State University; V. Stewart, Agronomist, Northwestern Agricultural Research Station, Kalispell, MT; and J. T. Waldher, USDA (retired), Pullman, WA, for assistance in field tests. We also thank K. Packard, Pacific Northwest Grain Quality Council, and members of the Federal Grain Inspection Service for assistance in collecting grain samples and Ms. J. Jennings for assistance in examination of grain samples.

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