Relationship Between Soilborne and Seedborne Inoculum Density and the Incidence of Dwarf Bunt of Wheat

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ABSTRACT

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The incidence of dwarf bunt of wheat as a function of inoculum density was studied in a susceptible and a partially resistant cultivar at three disease-conducive locations for three seasons. Prior to seeding, plots were fumigated with methyl bromide to eliminate residual inoculum. Each cultivar was seeded into two 1.2-m rows in four replicates. The soil surface was inoculated with 0, 16×10^2 , 16×10^3 , 16×10^4 , 16×10^5 , and 16×10^6 teliospores of *Tilletia controversa* per row, or seed was inoculated with 0, 2×10^2 , 2×10^3 , 2×10^4 , 2×10^5 , and 2×10^6 teliospores per gram. To determine maximum possible infection, two 3.1-m rows of each cultivar were soil-surface inoculated at $10\times$ the highest treatment rate. In the soil-inoculated plots, a minimum of 16×10^3 teliospores/row was needed to cause trace amounts of disease (0.6% maximum), even when the positive indicator treatment had up to 88% incidence. Only trace amounts or no disease occurred below the 16×10^5 rate. In the seed-inoculated plots, infection was rare and occurred only at inoculation rates of 2×10^5 teliospores/g or higher; the highest incidence was 0.4%.

Additional keywords: epidemiology, smut

Dwarf bunt is a smut disease of winter wheat caused by the fungus *Tilletia controversa* Kühn in Rabenh. Infection occurs during the winter (17), when soilborne teliospores near the soil surface germinate and produce hyphae that penetrate seedlings after emergence, and become established in the apical meristem of tiller initials (3). Hyphae persist in the meristem during plant development and eventually enter the nascent caryopsis, where the fungus proliferates and sporulates forming teliospores in endosperm tissue. Typically, the entire grain is transformed into a generally spherical sorus termed a "bunt ball" that consists of a dark-colored mass of teliospores enveloped within a thin, papery remnant of the pericarp. Sori rupture during harvest or after becoming

saturated with water, releasing teliospores which contaminate the soil. Infected tillers are typically shorter than healthy tillers.

In the United States and world wide, dwarf bunt is restricted to areas that commonly have persistent snow cover and relatively warm temperatures over the winter (21). Dwarf bunt has the greatest potential to develop when deep, persistent snow occurs before the soil becomes frozen, which provides a long period of stable, cool, humid conditions that are optimal for teliospore germination and infection. The disease has not been recorded in areas with very cold winter climates, such as the Northern Great Plains of the United States and the Southern Canadian Prairies. Soils in these areas typically freeze during late fall to early winter, and it is believed that the soil temperatures over the winter are too low to support proper conditions for development of dwarf bunt, even though snow cover may last for an adequate period of time (D. Gaudet, *personal communication*). Low amounts of dwarf bunt have been observed or induced in the absence of snow cover when high levels of soil inoculum coincide with a long period of cool, cloudy, humid weather (25; B. J. Goates, *unpublished*). However, under natural conditions in commercial fields, dwarf bunt is an economic problem only where sufficient inoculum from a previously infected crop is present in conjunction with persistent snow cover.

Historically, dwarf bunt has caused significant yield and quality losses (11), but the disease is currently under excellent control in the United States through the use of resistant cultivars (7,9,18) and use of the highly effective fungicide seed treatment chemical, difenoconazole (6,14,19). Currently, the disease is of concern most often because of phytosanitary issues that influence international markets (15). In particular, the People's Republic of China (PRC), one of the major importers of U.S. wheat, has a quarantine against *T. controversa*. The PRC restricts the import of grain from the northwestern United States, where wheat is commonly contaminated with teliospores of the fungus in at least trace amounts that can be easily detected with non-sophisticated techniques (10).

Previously, dwarf bunt was thought to be caused by a race or biotype of the common bunt fungus *T. tritici* (Bjerk.) Winter (24). Part of the reason for the eventual distinction of *T. controversa* as a separate species was that common bunt is easily induced with seedborne teliospores, whereas dwarf bunt is not (13,23). In dwarf bunt epidemiology, seedborne inoculum is inconsequential to the development of disease in the resulting crop, but limited amounts of disease have been induced with very high levels of seedborne inoculum (10). The important consequence of planting teliospore-infested seed is the contamination of soils with long-lived teliospores that could effect future crops. Teliospores in soil under field conditions have been shown to survive in at least low levels for 10 years (22).

This study was conducted to determine the relationship between levels of teliospore inoculum and disease incidence of a susceptible and a partially resistant cultivar in varying environments. This information would allow better assessment of the risk of introducing dwarf bunt into new areas via teliospore-contaminated grain; in particular, to address international phytosanitary concerns (4). Teliospore contamination on seed or grain for domestic use in the United States is of no regulatory concern because dwarf bunt has apparently attained its ecological boundary. The primary objective of the study was to determine the level of teliospore inoculum required to cause infection in disease-conducive field environments when inoculum is placed on the soil surface. A

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secondary objective was to assess disease caused by seedborne teliospores in disease-conducive fields that are free of viable teliospores. A previous study (10), conducted in numerous locations, addressed the seedborne aspect of the epidemiology of dwarf bunt in fields that were usually naturally contaminated with teliospores, but statistical methods were mostly relied upon to differentiate the effects of teliospores applied to seed from natural infection in non-inoculated controls.

MATERIALS AND METHODS

Field locations. Experiments were conducted during the 1993 to 1994, 1994 to 1995, and 1995 to 1996 seasons at three locations that commonly have disease-conducive weather. The locations were a rain-fed irrigated farm at the Montana State University Research Center near Kalispell, Montana; an irrigated farm operated by the Utah State University Experiment Station at Logan, Utah; and a dryland farm located at the base of a mountain range just east of Logan called the Green Canyon Nursery Site, where dwarf bunt screening nurseries have been conducted for many years.

Fumigation. At 4 to 10 days before seeding, plots were fumigated with methyl bromide at a rate of 0.91 kg/9.3 m² to kill teliospores naturally present in the soil. Each location had two adjacent plots (8.8 × 26.8 m) that were each covered with a single sheet of 6-mil thick plastic. The plastic tarps were put in place 1 to 7 days prior to the fumigation. One plot was used for the seedborne inoculum study and the other for the soilborne inoculum study. Methyl bromide was injected beneath the plastic tarp at a minimum of 12 places around the perimeter of each plot. The tarp was removed immediately prior to seeding. Because fumigation under high humidity favors teliospore mortality (20), fields were irrigated, if necessary, prior to placing the tarp at Kalispell and Logan to insure high soil moisture, but irrigation was not available at Green Canyon.

The efficacy of the fumigation on loose teliospores and on teliospores contained within intact sori (bunt balls) was tested at each field site, with samples placed at 0, 5, 10, and 20 cm beneath the soil surface at three locations under the tarp, and one location outside the plot as a control. The samples were prepared by mixing teliospores or intact sori with soil from the respective sites. Teliospore-contaminated soil (5 g), and seven intact sori in 5 g of soil, were placed in bags (4 by 7 cm) made of nylon mesh with a 20-μm pore size (Spectrum Medical Industries, Inc., Houston, TX). After fumigation, teliospores in soil were recovered using a modified method of Datnoff and coworkers (2) which included mixing the 5 g of soil and 20 ml of 50% aqueous glycerol with a magnetic stir bar for 5 min in a 100-ml

beaker. After settling for 10 min, the supernatant glycerol solution contained suspended teliospores and fine soil particles. The teliospore suspension was aspirated from the beaker and centrifuged to separate the teliospores, which were then rinsed by resuspending them in water twice for 5 min. Sori were removed from the soil in bags, then crushed in water to suspend the teliospores. The suspensions were filtered through cheese cloth and then resuspended in 10 ml of water. The suspensions were left at ambient room temperature for 24 h to allow germination or hydration of contaminating fungi present in the soil, making them easier to kill in the following treatment. Standard methods were used to surface sterilize the teliospores for 1 min in 0.25% NaOCl and plate them on 2% soil extract agar in two 9-cm plastic Petri dishes per sample (8). Teliospores were incubated at 5°C with 12 h of daily illumination for up to 160 days and were examined periodically for germinating teliospores. An estimated minimum of 40,000 spores/replicate at each depth and each field site was examined.

Field plots. The experiments were conducted in separate seed-inoculated and soil-inoculated plots as previously described. Seed was planted approximately 2 cm deep by hand during late September to early October. Planting at this time typically produces seedlings in the most susceptible 1- to 3-leaf stage as they enter dormancy in early winter (12). Furrows were opened with a hand plow and closed with a garden rake after placing seed from individual packets into the furrows. Each inoculum level was tested in four-replicate four-row plots arranged in randomized blocks. Each replicate four-row plot contained two 1.2-m rows of the highly susceptible cultivar Cheyenne and two 1.2-m rows of the moderately susceptible cultivar Meridian, seeded at a rate of 4 g of seed/row. The cultivar Cheyenne is a hard red wheat typically used as a susceptible check in screening nurseries and fungicide efficacy tests, whereas cultivar Meridian is a moderately susceptible hard red wheat developed for irrigated areas where dwarf bunt is not a problem. To assure that no viable teliospores were present before the treatments were applied, all seed was pretreated with 1.6% aqueous NaOCl for 10 min, then rinsed and air dried. The four-row test plots were placed a minimum of 2.4 m inside the edge of the fumigated area and were surrounded with border rows of cultivar Cheyenne planted within the fumigated area. To avoid contaminating plots with non-fumigated soil on shoes and tools during planting and inoculation, movement in and out of the plots was minimized and, in 1994 to 1995 and 1995 to 1996, when work outside the plots was required, as much work as possible was conducted on the fumigated surface of the plastic tarps after they were removed from the plots.

Inoculation. Teliospores used for inoculum at Kalispell and Logan originated from naturally occurring diseased spikes collected near the experimental sites. The inoculum for Green Canyon consisted of a composite of pathogenic races and isolates from throughout the western United States that is used for dwarf bunt screening nurseries at the site. The approximate number of teliospores per unit of weight was determined by diluting 0.100 g of teliospores in water until a reasonable number of teliospores could be counted with an improved Neubauer corpuscle counting chamber (Hausser Scientific, Blue Bell, PA). The mean of 10 counts was used to determine that there are approximately 4×10^8 teliospores/g.

To prepare the teliospore inoculum, sori from 20 spikes were crushed and the teliospores were sifted through a 100-μm screen. For the seed-inoculated plots, 1.00 g of teliospores was suspended in water, diluted to the proper concentration, and then 5.0 ml of suspension was sprayed onto 100 g of seed placed in a single layer in a siliconized Pyrex baking dish (20 by 20 by 5 cm; Corning, New York) at the rate of 2×10^2 , 2×10^3 , 2×10^4 , 2×10^5 , and 2×10^6 teliospores/g of seed. Based on separate analyses, about 10% of the inoculum remained on the dish. Seed was air dried for 24 h, then placed into individual seed packets for each row. For the soil-inoculated plots, a suspension of teliospores was diluted to the proper concentration in 200 ml of water. A 3.8-liter garden sprayer was used to spray the 200 ml onto the soil surface as evenly as possible in a 15-cm band over the four rows (50 ml/row) of each four-row plot at the rate of 16×10^2 , 16×10^3 , 16×10^4 , 16×10^5 , and 16×10^6 teliospores/row. The teliospores were applied within 24 h after planting. The seed and soil inoculation tests each contained four non-inoculated control plots. Prior to applying teliospores to the soil, a 0.6-m-high, 1.8 × 1.2 m fence made of 0.96-cm-thick plywood and painted with white latex paint was placed around each four-row plot to prevent inoculum movement between plots during the fall and winter. The bottom of the fences was buried about 5 cm into the soil. The fences were removed in early April. Fences were not used in the seed-inoculated plots. To determine the maximum possible infection in a given environment, two 3.1-m rows of cultivars Cheyenne and Meridian were planted near the test plots and were soil-inoculated as described at a rate at least 10× the highest test rate. Previous experience has shown that inoculation rates higher than this do not significantly increase disease (B. J. Goates, unpublished). These plots were included only in 1994 to 1995 and 1995 to 1996 and are reported in the results as the positive indicator. The positive indicator plot at Logan in 1995 to 1996 was accidentally destroyed during routine culti-

vation. When plants were mature, the percent infected spikes in the two rows of each experimental unit was determined by examining individual spikes. Means and standard deviations were calculated from the four replicates of each variety, inoculum concentration, location, and year.

RESULTS

In general, plant stands were excellent in all plots in all years, and plants were at the most susceptible 1- to 3-leaf growth stage going into winter dormancy. The nurseries were in excellent condition in the spring, with the exception of slight damage from snow mold fungi at all locations in most years. The plots generally had excellent stands at maturity. In all years, at least one location showed high disease pressure evidenced by near maximum disease expression in the positive indicator plots and by the reaction of test plots. The fence that surrounded each soil-inoculated plot created a micro-climate that was warmer on the north side, which influenced plant emergence and growth. Plant rows were always oriented in a north-to-south direction, so the micro-climate influenced rows equally. Although plants at the north end of the rows were slightly further advanced during late fall and early spring, there was no noticeable difference in the incidence of disease at either end of the rows.

Fumigation with methyl bromide killed over 99.99% of teliospores (Table 1), enabling the introduction of known quantities of teliospores. In the teliospore viability assay, contaminating organisms did not grow in the Petri dishes prepared from the fumigated areas, and contamination in dishes from the control areas was absent or minimal and did not appear to influence teliospore germination. After the plastic tarps were put in place for the fumigation, water condensation appeared and remained on the underside of the tarps at Logan and Kalispell, indicating high humidity. After

placing tarps at Green Canyon, water condensation on the underside of the plastic was either not present or just barely detectable in the corners of the tarp. During all years at Green Canyon, the soil was quite dry and moisture was not visibly apparent in the soil at less than 20 cm below the surface. Actual soil moisture was not determined.

In two cases, the fumigation apparently partially failed in a portion of the plots, evidenced by a small area approximately 2 m square where an estimated 7 to 11% disease incidence occurred in the non-inoculated cultivar Cheyenne border rows located directly adjacent to the test plots. This occurred adjacent to one replicated block of the seed-inoculated plots at Logan during 1995 to 1996, and adjacent to one block of the soil-inoculated plots at Green Canyon in 1995 to 1996. Where the contamination occurred, it often appeared to influence a portion of the test plots, so data from the entire replicate block located adjacent to the area was excluded. Thus, final data for these two plots are based on three replicates rather than four. Failure of the fumigation did not appear to be a problem elsewhere.

At Green Canyon, the plots were located on a slope and, in 1994 to 1995 and 1995 to 1996, heavy rains caused teliospore-infested soil from the surrounding field to wash into the unfenced seed-inoculated plots as evidenced by soil erosion and infection in cultivar Cheyenne border rows. Also, infection occurred within the test plots where individual replicates of non-inoculated cultivar Cheyenne had from 0 to 13% infection in 1995 and from 0.2 to 3.2% infection in 1996. Therefore, data from these plots were excluded from the results. At Green Canyon in 1994 to 1995, two 24-m rows of cultivar Cheyenne planted in naturally infested soil near the experimental plots had approximately 80% infected spikes—demonstrating the highly

contaminated nature of the soil and the disease-conducive conditions. The seed-inoculated plot was always placed uphill from the soil-inoculated plot at Green Canyon, which helped to prevent washing of contaminated soil into the soil-inoculated plot. The fields at Kalispell and Logan were level, and movement of soil into these plots was minimal.

Border rows of cultivar Cheyenne adjacent to the outside edge of the fumigated areas were commonly diseased in at least trace amounts at all locations. In general, other than the problems at Green Canyon mentioned above, infection from outside contamination was confined to the rows close to the edge of the fumigated area and did not appear to interfere with the test plot results. However, it is possible that some of the infection within the test plots could have resulted from such contamination.

Disease incidence varied greatly among the locations and years (Tables 2 and 3). Overall, the Green Canyon plots produced the best conditions for infection and Kalispell the least. The Green Canyon site is highest in elevation and is situated on a northerly facing slope, where snow tends to collect earlier and remain longer than at the other locations. Disease in the cultivar Cheyenne positive indicator plots was theoretically the maximum amount of disease that could be produced in a given environment. Over the years, based on the reaction of the positive indicator plots, the relative disease pressure resulting from the environment appeared moderate to high at Green Canyon, low to moderate at Kalispell, and low to high at Logan. Although the 1995 to 1996 positive indicator plot at Logan was accidentally destroyed, the relatively high infection in the experimental plots indicated strong disease pressure. Similarly, the positive indicator plots were not planted in 1993 to 1994, but the infection potential of the

Table 1. Effects of fumigation with methyl bromide at 0.91 kg/9.3 m² on the viability of *Tilletia controversa* teliospores contained in bunt balls or existing free in the soil when placed at various levels in soil

Teliospores, depth (cm)	Number of teliospores germinated ^a			Germination (%) ^b		
	Green Canyon	Logan	Kalispell	Green Canyon	Logan	Kalispell
Free spores						
0	12	0	3	...	...	...
5	9	1	4	...	...	...
10	1	9	0	...	...	...
20	8	2	0	...	...	...
Non-fumigated						
All	...	...	...	89 ± 2.2	91 ± 3.7	93 ± 3.4
Bunt balls						
0	0	0	1	...	...	...
5	1	0	0	...	...	...
10	0	0	0	...	...	...
20	1	0	0	...	...	...
Non-fumigated						
All	...	...	...	93 ± 2.3	93 ± 4.5	94 ± 3.5

^a Based on the total number of teliospores germinated in three replicates of free teliospores or bunt balls. Estimated minimum of 120,000 teliospores examined per depth at each location.

^b Mean and standard deviation of percent germination of non-fumigated controls at all depths. Counts of 400 teliospores/depth.

respective environments can be estimated from the experimental results.

Among all years and locations in the soil-inoculated plots, it took a minimum of 16×10^3 teliospores/1.2-m row to cause trace amounts of disease in the highly susceptible cultivar Cheyenne. In the same tests, cv. Cheyenne inoculated at $10 \times$ this

rate still had only trace amounts or no disease. In one of the nine location-years, trace amounts of disease occurred in cultivar Meridian inoculated at the 16×10^3 rate, but Meridian usually was not diseased below the 16×10^5 rate. The 1994 to 1995 Green Canyon plots had the highest overall infection in the positive

indicator plots, but it took a minimum inoculum level of 16×10^5 spores/row to cause disease in cultivars Cheyenne and Meridian. In all tests, only trace amounts or no disease occurred in cultivars Cheyenne and Meridian inoculated below the 16×10^5 rate. In general, it took about $10 \times$ the inoculation rate of cultivar Cheyenne to produce a similar level of disease in cultivar Meridian.

Among all years and locations in the seed-inoculated plots, a trace of infection occurred in only one replicate of cultivar Cheyenne at the 2×10^5 teliospores/g inoculation rate, but otherwise it took a minimum of 2×10^6 teliospores/g to cause disease, and it occurred only in trace amounts, with the maximum infection of 0.4%. Cultivar Meridian was not diseased by seed inoculation, except for a trace of infection at the highest inoculation rate at Logan in 1995. Most often, seed inoculation produced no disease even at the highest inoculum level.

DISCUSSION

The results from these experiments clearly demonstrated the interrelationship of soil-surface inoculum levels with the environment to produce different levels of dwarf bunt, and how less inoculum is more effective under more-conducive conditions and more inoculum is required to produce disease under less-conducive conditions. In addition, the experiments showed the effects of disease pressure from inoculum concentration and the environment on the effectiveness of partial resistance. When each of the cultivars was under high disease pressure from both the inoculum and the environment, infection in cultivar Meridian compared to cultivar Cheyenne was much higher than with minimal pressure from either the environment or inoculum concentration. This result demonstrated that the effects of partial resistance are enhanced under limited disease pressure. The partial resistance of cultivar Meridian was adequate to completely prevent dwarf bunt in plots that had limited disease pressure.

Results from the seed-inoculation portion of this study generally confirm a previous study (10), in which statistical methods separated the effect of seed-applied inoculum from natural infection in non-inoculated checks. Grey et. al. (10) estimated that there are 20,000 spores/seed at infestation levels of 1 g of teliospores/kg of seed, which is similar to the number of spores contained in a gram that was estimated in this study, given that there are approximately 20 seeds/g. Grey et. al. (10) showed that infection occurred after a minimum inoculation rate of 1 g of teliospores/kg of seed. According to our estimates, this is equivalent to approximately 4×10^5 spores/g of seed. In the current study, a trace of infection (0.13%) occurred in one year at one location in the highly

Table 2. Percent bunted spikes in winter wheat cultivars Cheyenne (C) and Meridian (M) after inoculating soil with teliospores of *Tilletia controversa*

		Spores/row						
Site, year	Cv.	0	16×10^2	16×10^3	16×10^4	16×10^5	16×10^6	Indicator ^a
Green Canyon								
1994 ^b	C	0	0	0.6 ± 1.3	0	2.3 ± 2.6	14.9 ± 5.2	...
	M	0	0	0	0	0	1.3 ± 2.3	...
1995 ^b	C	0	0	0	0	3.4 ± 2.0	31.7 ± 17.6	88
	M	0	0	0	0	0.1 ± 0.1	4.3 ± 0.8	43
1996 ^c	C	0	0	0.3 ± 0.2	0.3 ± 0.5	19.1 ± 5.8	47.2 ± 15.7	87.2
	M	0	0	0.07 ± 0.1	0.3 ± 0.6	1.5 ± 1.3	5.4 ± 3.1	18.3
Logan								
1994 ^b	C	0	0	0	0	0.1 ± 0.2	1.7 ± 1.5	...
	M	0	0	0	0	0	0	...
1995 ^b	C	0	0	0	0	0.2 ± 0.4	7.6 ± 5.7	13.4
	M	0	0	0	0	0.1 ± 0.1	1.9 ± 0.5	1.2
1996 ^b	C	0	0	0	0.4 ± 0.2	3.2 ± 2.2	32.7 ± 11.5	ND ^d
	M	0	0	0	0	0.2 ± 0.4	2.8 ± 1.7	ND
KalisPELL								
1994 ^b	C	0	0	0.1 ± 0.2	0.03 ± 0.5	1.5 ± 1.0	12.3 ± 9.8	...
	M	0	0	0	0	0	0.5 ± 0.5	...
1995 ^b	C	0	0	0	0	0	0.8 ± 0.1	3.6
	M	0	0	0	0	0	0	0.1
1996 ^b	C	0	0	0	0	0	0	0.2
	M	0	0	0	0	0	0	0

^a Positive indicator based on soil inoculation of two 3.1-m rows at $10 \times$ the highest test rate. Included only in 1995 and 1996.

^b Mean and standard deviation of four replicates of two 1.2-m rows.

^c Mean and standard deviation of three replicates of two 1.2-m rows.

^d ND = rows accidentally plowed.

Table 3. Percent bunted spikes in winter wheat cultivars Cheyenne (C) and Meridian (M) after inoculating seed with teliospores of *Tilletia controversa*

		Spores/g						
Site, year	Cv.	0	2×10^2	2×10^3	2×10^4	2×10^5	2×10^6	Indicator ^a
Green Canyon								
1994 ^b	C	0	0	0	0	0.13 ± 0.3	0	...
	M	0	0	0	0	0	0	...
1995 ^c	C	...	...	...	...	...	...	...
	M	...	...	...	...	...	...	...
1996 ^c	C	...	...	...	...	...	...	...
	M	...	...	...	...	...	...	...
Logan								
1994 ^b	C	0	0	0	0	0	0.03 ± 0.05	...
	M	0	0	0	0	0	0	...
1995 ^b	C	0	0	0	0	0	0.4 ± 0.9	13.4
	M	0	0	0	0	0	0.2 ± 0.2	1.2
1996 ^d	C	0	0	0	0	0	0	ND ^e
	M	0	0	0	0	0	0	ND
KalisPELL								
1994 ^b	C	0	0	0	0	0	0	...
	M	0	0	0	0	0	0	...
1995 ^b	C	0	0	0	0	0	0	3.6
	M	0	0	0	0	0	0	0.1
1996 ^b	C	0	0	0	0	0	0	0.2
	M	0	0	0	0	0	0	0

^a Positive indicator based on soil inoculation of two 3.1-m rows at $10 \times$ the highest test rate. Included only in 1995 and 1996.

^b Mean and standard deviation of four replicates of two 1.2-m rows.

^c Data excluded because of plot contamination.

^d Mean and standard deviation of three replicates of two 1.2-m rows.

^e ND = rows accidentally plowed.

susceptible cultivar Cheyenne inoculated at 2×10^5 spores/g of seed, but other than this one instance, 2×10^6 spores/g was required to cause dwarf bunt by seed inoculation. The infection level never exceeded 0.4% in the seed-inoculated test, and most often no infection occurred.

Inducing dwarf bunt with seedborne teliospores has been noted previously (10,13,23), so it was not surprising to confirm those results here. It has been hypothesized that infection from teliospores on seed results from the removal of spores during planting, which then land near the soil surface and cause infection in the normal manner (10). However, it seems unlikely that careful planting by hand would result in significant removal and proper placement of teliospores on the soil surface in sufficient concentration to cause disease.

Experiments with fumigation of grain infested with *T. controversa* teliospores have shown that methyl bromide is a highly effective sporicide for *T. controversa* at high dosage, and particularly under humid conditions (18). In addition, methyl bromide is effective against teliospores of the Karnal bunt pathogen *T. indica* under field conditions (5). It was unknown if methyl bromide also was effective for *T. controversa* teliospores under field conditions; however, the results here demonstrate that fumigation at registered rates killed nearly 100% of loose teliospores in soil and teliospores contained within intact sori at all depths tested. This high efficacy was essential to the success of these experiments and allowed the introduction of known quantities of inoculum into fields where *T. controversa* teliospores naturally contaminate the soil. Methyl bromide is degraded or volatilized almost completely within 4 days of application (1), so it is unlikely that the fumigation influenced infection. In an attempt to enhance the activity of methyl bromide, plots were irrigated if necessary at Kalispell and Logan prior to fumigation to produce high soil moisture. Irrigation was not available at the dryland Green Canyon site but, despite low soil moisture there during all years, the fumigation was still very effective, as demonstrated by the efficacy tests and the absence of infection in non-inoculated control plots that were protected with the fence.

Some teliospore contamination into at least the edges of the fumigated areas, caused by fall or early winter rains washing surrounding non-fumigated soil into the plots, was expected. Border rows of cultivar Cheyenne that were planted to monitor such contamination showed that it was generally restricted to the outer border rows and did not seem to interfere with the test plots except in two of the three seed-inoculated tests at Green Canyon. The soil at Green Canyon is highly infested with teliospores from conducting screening nurseries there for many years, which was

evidenced by high disease incidence in nearby non-inoculated plants. The other two instances, where apparent contamination caused one replicate to be eliminated at both Green Canyon and Logan in 1996, seemed to be caused by the failure of methyl bromide rather than inoculum movement from outside the fumigated areas, because the contamination occurred in an isolated spot. In addition, at Green Canyon, the test plot that appeared to be influenced was surrounded with the 1.2-m high plywood fence, which eliminated washing into the plot.

The fences used to separate teliospore concentrations for the soil-inoculation experiments appeared to effectively contain the teliospores within test plots. This technique, when performed in conjunction with additional safeguards, could be used to test the disease-conducive nature of areas where the environmental suitability for dwarf bunt is questionable.

Dwarf bunt was not induced at soil inoculum levels below 16×10^3 teliospores/1.2-m row, but we cannot conclude that infection is impossible below this level. The disease likely could have been detected at inoculum levels lower than those found in these experiments if the plots were sufficiently large. Also, it is possible that dwarf bunt could have been detected at lower inoculum levels in environments that are even more conducive than those encountered during the course of this study. Under ideal environmental conditions that rarely occur in highly inoculated experimental screening nurseries, it is not uncommon for cv. Cheyenne to have more than 95% infected spikes and cv. Meridian to have up to 60% infected spikes (B. J. Goates, *unpublished*). Regardless, it appears that the inoculum level required to induce dwarf bunt under field conditions is fairly high.

Winter wheat plants are more susceptible to dwarf bunt when planted at the shallow seeding depths that were used in this study, as compared with deeper seeding (12,16,22). Also, plants are more susceptible at early developmental stages when they become dormant as they go into winter (12). In commercial farming operations, plants typically are well beyond the most susceptible 1- to 3-leaf stage during winter. If the plants in these experiments were planted at normal commercial seeding depths, and/or were beyond the most susceptible stage of growth that was tested here, it is likely that the disease reactions would have been less severe.

Theoretically, only a single teliospore is necessary to cause infection, but the chance of this occurring seems extremely remote. The results from these experiments aid the understanding of what lies between the theoretical single spore and actual number of teliospores required to cause detectable levels of disease in conducive environments. The experiments also dem-

onstrate the inoculum concentration required to cause slight to damaging levels of disease in highly conducive environments when plants are managed to be in a susceptible state. This information should aid assessment of the risk of introducing dwarf bunt into new areas by way of international grain shipments destined for milling.

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