Epidemiology of *Tilletia indica* Teliospores in Regulated Wheat Fields in Texas

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ABSTRACT

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To examine the epidemiology of *Tilletia indica* teliospores in naturally infested soils from wheat fields in both Karnal bunt–regulated regions in Texas, soil was grid sampled from fields that were bunted-kernel positive for Karnal bunt in 1997, 2001, both years, or never. Aliquots of soil from each point were pooled, and teliospores were extracted using a size-selective sieving–sucrose centrifugation method. Teliospores were enumerated microscopically, and low quantities (<8 per 25 g of soil) were identified in 14 of 15 fields sampled from the regulated regions of Texas, including fields that have never tested positive for bunted kernels, indicating a widespread distribution. No teliospores were isolated from the single field examined outside of the regulated regions. The percent clay was significantly, negatively correlated with the baseline teliospore number and the estimated (extrapolated) number of teliospores per sample, indicating a potential impact of soil composition on teliospore survival. The latter factor was also significantly, positively correlated to the number of times a field had tested positive.

Additional keywords: durum wheat, partial bunt, soil texture, spatial distribution, *Triticum aestivum*, *Triticum durum*, winter wheat

Karnal (partial) bunt of wheat (KB), caused by the fungus *Tilletia indica* M. Mitra, is currently a zero-tolerance regulated organism within the United States and under severe importation restriction in more than 40 countries (21). *T. indica* was considered by plant pathologists to be a minor pathogen of wheat (12), was found to have minimal impact on grain and flour quality (23), and had no adverse toxicological effects when bunted grain was used as feed (4). In diseased crops, reduction in yield and seed viability was found to be proportional to the severity of infection (3). Therefore, losses due to KB are overwhelmingly considered to be the result of the regulatory status of the pathogen (7,21).

Except for a five-county region in northern Texas, KB is currently restricted to areas within Arizona, California, and central Texas that have relatively limited wheat production (10). The regulated counties in northern Texas are exceptional because they represent the southern boundary of the Great Plains Wheat Belt, and any northerly movement of KB from this region could severely disrupt the U.S. wheat export market and multiple sectors of the agricultural industry. In response to this risk, the United States Department of Agriculture is working to deregulate the pathogen and is developing a Pest Risk Assessment to support this process. Understanding the ecology and epidemiology of *T. indica* will allow for the development of a more complete assessment.

Teliospores serve as the source of primary inoculum and are the over-wintering stage of *T. indica* (15). They can be disseminated in bunted kernels and soil, on nondiseased grain, on equipment or vehicles (24), and may even become airborne (7). The environmental conditions required for teliospore germination (24), sporidia multiplication (25), and the infection process (8) have been well documented; however, teliospore survival in the soil is not well understood and is of particular interest epidemiologically. Survival in artificially infested field soils has been studied previously, and it was concluded that percent teliospore germination decreased faster when teliospores were buried at increasing depths than when they were left at the soil surface (18). The rates of teliospore recovery and germination were also found to be affected by soil texture or source (2); however, the specific soil factor(s) involved in the decline of viability was not identified.

The baseline teliospore density of *T. indica* in naturally infested wheat fields has been previously examined in Punjab, India, Northwest Mexico, and Arizona, where an estimated 133 to 6,733 (20), 1,190 to 4,025 (11), and 1 (1) teliospores per 25 g of field soil were quantified, respectively, assuming a soil density range of 0.75 to 1.5 g/cm^3. However, there was no information concerning teliospore density in wheat

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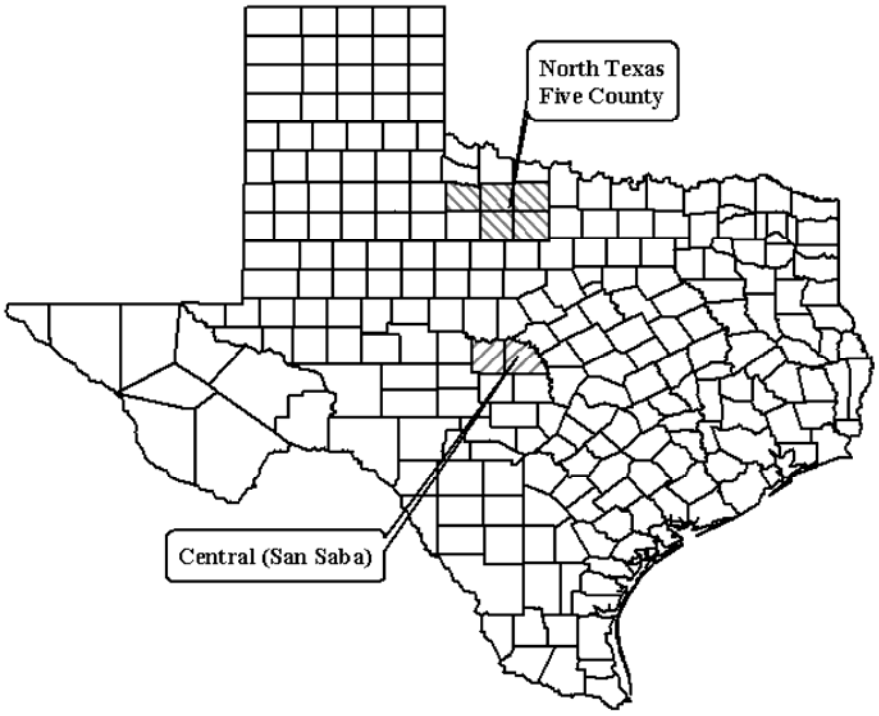

Fig. 1. Map of Texas showing Karnal bunt regulated counties.

field soils, nor the extent of pathogen introduction and/or establishment, within the regulated areas of Texas. With that in mind, the objective of this research was to study the epidemiology of *T. indica* teliospores in soil by examining (i) the presence and quantity of those in wheat fields within the KB regulated counties of Texas and (ii) any relationship(s) between number and extraction efficiency with soil texture, chemistry, and the number of years since, or times, a field tested bunted kernel positive.

MATERIALS AND METHODS

Soil sampling. Wheat fields in the two Karnal bunt regulated regions of Texas (Fig. 1) were grid sampled in June 2002 with a minimum 60 points per field (Table 1). A single wheat field in the Panhandle, at the Texas Agricultural Experiment Station in Bushland, TX (P1), was sampled as a nonregulated, negative control. Grid points were equally spaced, GPS-referenced, and the sampling area was generally dependent upon field size. At each sample point, ~500 g of soil from the top ~5 cm was collected and sealed in a plastic bag. Samples were transferred to the Texas Agricultural Experiment Station Karnal bunt Quarantine Lab in Bushland, air-dried in a Type IIA biological hood for 3 to 5 days, crushed with a rolling pin to break large clumps, and hand mixed to homogenize. The samples were double-bagged, and the rolling pin was rinsed off and dried if a bag ruptured and/or between samples from different fields. A 50-g aliquot from each sample point within a field was then combined into a pooled sample representing the entire field. This pooled sample was hand mixed, and six replicate 25-g aliquots per field were transferred into 50-ml screw-cap centrifuge tubes. Sample tubes were stored at room temperature in the dark until processing.

Characterization of soil texture, chemistry, and organic matter content. The percent sand, silt, and clay content was calculated using a particle-settling method as previously described by Gee and Bauder (14), and soil texture was determined based on USDA Soil Taxonomy nomenclature (16). To determine soil chemistry and organic matter content, ~300 g from each pooled sample was sent to the Texas A&M University Soil, Water, and Forage Testing Laboratory, College Station, for analysis. Samples were processed for organic matter content, pH, and parts per million (ppm) NO_3, P, K, Ca, Mg, Na, S, and salinity. All movement of soils occurred under the authorization of the USDA-APHIS and the Texas Department of Agriculture.

Extraction and enumeration of teliospores from soil samples. The extraction protocol used was a slight modification from that published by Babadoost and Mathre (1). Three 25-g soil replicates from each pooled field sample were rehydrated overnight at room temperature with 25 ml of a 0.1% polysorbate 20 (Tween 20; Acros Organics, Fair Lawn, NJ) in sterile deionized water (sdH_2O) solution. The soil was resuspended in the centrifuge tube by vortexing for 10 s (setting no. 9, Vortex Genie; Scientific Industries, Bohemia, NY), poured into a 600-ml glass beaker, and any material remaining in the tube was collected by rinsing with an additional 175 ml of the polysorbate solution. The soil suspension was then stirred rapidly with a magnetic stirrer for 5 min and poured onto a stacked, 53-µm-mesh top and 20-µm-mesh bottom, stainless steel sieve pair. The stacked sieve pairs were washed with dH_2O (approximately 3 to 5 liters) until the fraction ran clean. The fraction retained on the 20-µm sieve was collected into a 50-ml screw-cap centrifuge tube by tilting the sieve and spraying with a fine stream of dH_2O from a squirt-bottle. The suspension was then centrifuged for 5 min (2,500 × *g*) to pellet, and the supernatant decanted. The pellet was resuspended in 25 ml of a 1.6 M sucrose solution by vortexing for 10 s and centrifuged for 1 min (180 × *g*) to pellet the inorganic fraction. The supernatant was poured onto a 20-µm-mesh nylon sieve, and the resuspension and centrifugation steps were repeated four more times. The fraction on the 20-µm nylon sieve was washed with ~300 ml of dH_2O, collected into a 15-ml screw-cap centrifuge

Table 1. Field identification, year(s) a wheat crop was positive for Karnal bunt (KB), spatial sampling density, and soil characteristics and chemistry for each pooled field sample

Field ID	Year(s) positive[a]	Spacing (m)	Percentage[b] of soil				pH	Parts per million							
			Sand	Silt	Clay	OM		NO_3	P	K	Ca	Mg	Na	S	Salinity
Central Texas (San Saba County)															
C1	1997	64	92.8	3.9	2.1	1.2	6.3	53	31	135	593	87	250	29	514
C2	2001	64	56.4	25.1	16.6	1.9	6.6	57	20	483	2,678	207	293	36	447
C3	Never	64	49.1	26.4	21.8	2.7	7.3	8	24	571	4,551	226	272	36	326
C4	1997 & 2001	64	43.9	33.1	20.2	2.8	7.4	11	32	739	5,596	242	307	42	460
C5	1997 & 2001	64	52.4	25.1	20.1	2.4	6.7	10	38	821	3,588	289	309	43	279
C6	Never	64	46.7	25.3	23.9	4.1	7.5	12	135	985	6,622	328	354	57	339
C7	1997	64	48.7	35.3	14.4	1.6	5.6	6	23	368	1,020	156	328	42	241
C8	2001	7	23.4	50.1	23.5	3.0	8.0	12	219	655	57,691	690	324	83	252
C9	2001	64	66.3	22.2	10.1	1.4	5.7	11	25	269	2,294	160	297	34	180
		Mean	53.3	27.4	17.0	2.3	6.8	20	61	558	9,403	265	303	45	338
		Std. dev.	18.7	12.3	7.2	0.9	0.8	20	69	273	18,217	175	304	16	114
North Texas Five County Regulated Region															
N1	2001	12	55.9	33.6	8.7	1.8	6.2	12	59	612	1,238	198	293	39	236
N2	2001	12	50.8	34.2	13.3	1.7	5.5	22	63	589	889	241	257	35	295
N3	2001	10	48.9	34.3	15.2	1.6	6.0	7	34	381	1,439	230	271	37	249
N4	2001	12	49.7	30.5	18.6	1.2	5.9	15	50	431	1,280	251	230	31	250
N5	2001	12	76.6	16.6	6.5	0.3	6.2	5	19	179	622	161	319	25	85
N6	Never	10	60.3	22.2	16.5	1.0	6.1	21	46	440	1,239	277	295	42	247
		Mean	57.0	28.6	13.1	1.3	6.0	14	45	434	1,118	226	278	35	227
		Std. dev.	10.5	7.4	4.7	0.6	0.3	7	16	157	302	41	32	6	73
Texas Panhandle Negative Wheat Field (Bushland)															
P1[c]	Never	7	30.2	42.3	23.4	4.1	7.6	12	20	520	3,044	729	251	16	169

[a] All fields having a year positive were found to have a least one bunted kernel except C1. That field was regulated because it was planted with wheat seed that was infested with teliospores and associated with bunted-kernel positive seed lot. Field C1 was removed from regulation in 2004.

[b] Percent sand, silt, and clay calculated as described by Gee and Bauder (14). Percent organic matter (OM) was determined by dry weight and used to correct the clay fraction.

[c] This field is outside of the regulated areas, has never tested positive for KB, and was included as a nonregulated, negative control. Mean and standard deviation not applicable.

tube as previously described, and centrifuged for 5 min (2,500 × *g*) to pellet. The supernatant was pipetted off, and the pellet was resuspended in 700 µl of Shear's solution (17). The suspension was later transferred into a Sedgwick-Rafter counting chamber, and teliospore number was enumerated microscopically at ×100 or ×400 as required. Teliospores were counted only if they exhibited diagnostic characteristics of *T. indica*, i.e., they were the expected size and shape, were intact, and had diagnostic ornamentation (9). A visual appraisal of viability was not performed (2).

Determining teliospore extraction efficiency from each pooled field soil. To obtain teliospores for artificially infesting soil, 5 to 10 bunted wheat kernels obtained from a naturally infected wheat crop harvested in 2001 were placed into a 15-ml screw-cap centrifuge tube containing 5 ml of 0.1% polysorbate sdH_2O solution. The kernels were crushed with flame-sterilized forceps, vortexed at high speed for 10 s, and the contents poured onto a stacked pair of nylon sieves, as previously described. The sieves were washed with ~300 ml of dH_2O, and the fraction on the 20-µm sieve was transferred to a fresh 15-ml tube. The tube was centrifuged for 5 min (2,500 × *g*) to pellet, the supernatant decanted, and the pellet resuspended in 3 ml of a 0.1% agar solution (1.0 g/liter sdH_2O) by vortexing as before. Teliospore number was enumerated with a Fuchs-Rosenthal counting chamber (Hausser Scientific, Horsham, PA) using four independent, whole field counts. Broken and/or immature teliospores were not counted, and the entire process was repeated if insufficient teliospores were present. An aliquot of the teliospore stock suspension was then added to sdH_2O to make a final suspension of 1,000 teliospores per ml, for a total volume of 100 ml.

River sand purchased from a local garden center was thoroughly washed on a 53-µm-mesh stainless steel sieve, placed into a shallow baking dish that was then covered with aluminum foil, and autoclave-sterilized twice. The sand was left uncovered in a Type IIA biological hood until dry, while stirring regularly. Once dry, 100 g was measured aseptically and transferred into a sterile 500-ml beaker. To this, 100 ml of the 1,000 teliospore per ml suspension was added. The sand–teliospore combination was thoroughly and frequently mixed to homogenize, and then dried as previously described. Once dry and homogenized, 0.1 g of the teliospore-infested sand was added to each of three 25-g pooled field sample replicates. These were hand mixed to homogenize and stored dry at 20°C until processing. Teliospores were extracted and enumerated as previously described using a Sedgwick-Rafter counting chamber (Hausser Scientific). An infested, sand-only treatment was included as a control, and the means of the naturally infested teliospores for each sample were subtracted from the results prior to analysis.

Statistical analyses. First, the potential number of teliospores per 25-g replicate sample was estimated by dividing the baseline teliospore number of each replicate by the mean extraction efficiency for that pooled field sample. Then, to determine if field location and/or the number of times since last testing positive influenced the baseline teliospore number, extraction efficiency, or estimated number of teliospores, an analysis of variance (ANOVA) was calculated as a multi-factorial, completely randomized design using SAS (Proc MIXED; SAS Institute, Cary, NC) with the listed factors as the main effects. In addition, correlation analysis using Pearson's Product-Moment coefficient (Proc CORR in SAS) was used to examine the relationship between teliospore number or extraction efficiency, with field location, the number of years since, or times, testing positive, and the physical or chemical characteristics of the soils.

RESULTS

Characterization of soil texture, chemistry, and organic matter content. The percent sand, silt, clay, and organic matter in each pooled field sample varied greatly between fields, but was relatively similar between Texas KB regulated regions when averaged over location (Table 1). The soil textures, as calculated with an ASTM hydrometer, were primarily loam or loam-types, with a single field classified as a sandy soil (data not shown). Percent organic matter was below 5% for all fields, and pH ranged from 5.6 to 8.0 and 5.5 to 6.2 in San Saba County (SSC) and the North Texas Five County Regulated Region (NTFCRR), respectively. The only soil chemistry factor that had a standard deviation substantially larger than the mean within a region was Ca^{+2}, which ranged from 593 to 57,691 ppm in SSC (mean 9,403). The single nonregulated field (P1) had the highest percentage of clay and was classified as a clay loam.

Baseline and estimated teliospore number and the extraction efficiency for the pooled field grid samples. Teliospores of *T. indica* were extracted from soil collected from wheat fields in both KB regulated regions of Texas (Table 2); however, neither location nor the number of years since testing positive significantly affected teliospore number (Table 3). Teliospores were found in soil from fields that tested bunted kernel positive in 1997, 2001, both years, as well as neighboring fields that had never tested positive. Teliospores were absent from only one field that had previously tested positive (C2), and the average numbers of teliospores per 25-g pooled field sample were 1.6 (0.0 to 5.0) and 3.1 (1.0 to 7.7) for SSC and NTFCRR, respectively. Those that were extracted all had

Table 2. Field identification and last year with susceptible crop (wheat) with the mean number of *Tilletia indica* teliospores per 25 g in naturally infested field soils, and the extraction efficiency and estimated number of teliospores for these same soils

Field ID	Last suscept. crop tested[a]	Baseline extraction (#)		Extraction efficiency (%)[b]		Estimated #/ 25 g soil[c]
		Mean #	Std. dev.	Mean %	Std. dev.	
Central Texas (San Saba County)						
C1	1997	5.0	3.6	16.8	2.2	29.9
C2	2001	0.0	0.0	40.2	12.9	0.0
C3	2001	0.7	1.2	12.0	7.1	5.6
C4	2001	1.7	0.6	11.0	3.6	15.1
C5	2001	3.3	1.5	15.8	3.8	21.1
C6	1998	1.7	0.6	12.9	3.8	12.9
C7	1997	0.3	0.6	10.1	4.3	3.3
C8	2001	2.0	1.7	13.4	10.8	14.9
C9	2001	1.0	1.0	19.6	0.8	5.1
	Mean	1.7	n/a	16.9	n/a	12.0
North Texas Five County Regulated Region						
N1	2001	7.7	5.5	16.3	7.1	47.1
N2	2001	5.7	2.1	14.4	2.5	39.5
N3	2001	1.0	1.0	4.8	2.2	20.9
N4	2001	1.0	1.0	6.7	0.8	14.9
N5	2001	2.0	1.0	10.1	1.4	19.9
N6	2001	1.7	0.6	18.2	4.1	9.2
	Mean	3.2	n/a	11.7	n/a	25.3
Texas Panhandle Negative Wheat Field (Bushland)						
P1[d]	2001	0.0	n/a	1.4	1.4	0.0

[a] Year the field was last tested (prior to soil sampling) by the USDA-APHIS for the presence of bunted wheat kernels. All regulated fields planted with wheat and harvested are tested annually.

[b] Pooled field soils spiked with 100 teliospores in 0.1 g of infested sand and extracted as previously described and calculated as follows: [(# teliospores enumerated from spiked sample – mean of baseline extraction for field)/mean of sand-only control] * 100, e.g., [(18.0 - 5.0)/69.8] * 100 = 18.6.

[c] Extrapolation calculated as follows: (# of baseline teliospores extracted for sample/mean of extraction efficiency for field), e.g. (8.0 / 0.167) = 47.9.

[d] This field is outside of the regulated areas, has never tested positive for Karnal bunt, and was included as a nonregulated, negative control.

some level of aging and/or degradation (5), in that the gelatinous sheath was typically absent and spore color was much lighter than freshly harvested teliospores. Teliospores were not found in the single wheat field (P1) sampled that was outside of the KB regulated regions.

The pure sand-based inoculation control (no soil) had an extraction efficiency of 69.8%. Most fields sampled, regardless of location, had low teliospore extraction efficiency that typically ranged between 10 and 20% (mean 9.7%), with only three fields from the KB regulated regions outside of that range (Table 2). The location and location*year last testing positive interaction were significant ($P < 0.05$) for extraction efficiency (Table 3). The single field assessed from the Texas Panhandle (P1) had the lowest extraction efficiency (1.4%), whereas field C2 (SSC) had the highest, with 40.2%. The mean estimated numbers of teliospores per 25-g sample were 12.0 (0.0 to 29.9) and 25.3 (9.2 to 47.1) for SSC and NTFCRR, respectively. Fields N1 and N2 had the largest number of estimated teliospores per sample, with 47.1 and 39.5, respectively; however, none of the factors examined were significant for this variable (Table 3).

Statistical correlations. The percent sand experimental factor had a significant ($P < 0.01$), positive correlation (0.26) with baseline teliospore number, whereas percent clay (–0.41) was negatively correlated (Table 4). For the extraction efficiency study, the percent sand (0.27), ppm NO_3 (0.63), and salinity (0.39) had significant, positive correlations, whereas percent silt (–0.29) and ppm Mg (–0.29) had significant, negative correlations. For the estimated number of teliospores per soil sample, the number of times a field tested positive and percent clay were positively (0.25) and negatively (–0.37) correlated, respectively.

DISCUSSION

Teliospores of *T. indica* were extracted from pooled soil in all but one wheat field sampled in the regulated counties of Texas, including those that had never tested bunted kernel positive. Average teliospore density from the fields examined in this study was low (<8 baseline and <50 estimated, per 25 g) and very similar to that published by Babadoost and Mathre for a durum wheat field in Arizona (1). Because the majority of field soils contained teliospores, including those that have never tested positive, the authors hypothesize that *T. indica* has a widespread distribution in wheat fields within the regulated counties of Texas. If correct, large scale and/or multiple introductions probably occurred because *T. indica* is known to be a relatively poor pathogen (21) with low fecundity, has somewhat particular environmental requirements (12), and a putative minimum teliospore density threshold for infection and disease (13). If this pathogen were only introduced into a limited number of fields once, it might fail to become established unless environmental conditions were optimal for infection. A population study of *T. indica* in Texas using the appropriate genetic markers might offer additional information to help elucidate any pattern(s) of introduction.

The methods used in this study to extract and quantify *T. indica* teliospores from naturally and artificially infested field soils differed from those recently published, in that the extraction was from a larger quantity of soil (25 g instead of ≤10 g), the whole sucrose-float partition of the sample was examined (instead of subsamples), and fewer teliospores were spiked into the samples for the extraction efficiency assay (100 total instead of ≥1,000 per g of soil) (2). Because a previous study had quantified few teliospores in naturally infested soil (1), the extraction method modifications were designed to increase the probability of extracting and quantifying teliospores at low concentrations. These modifications resulted in a slight reduction in percent recovery compared with previously published results (1). Others using the nonmodified method have noted extreme variation between replicate extractions (6; M. Babadoost, *personal communication*). In comparison, these modifications resulted in a relatively consistent number of teliospores extracted between replicates, and even between fields with different soil textures. It has also been noted that the extraction efficiency using the nonmodified version of this method may be dependent upon the number of teliospores added to the soil (M. Peterson, *personal communication*). Thus, once the baseline teliospore density was determined, only 100 teliospores were introduced into the sample in order to keep a similar scale as the natural population for the extraction efficiency assay.

The modifications used in this study became problematic when examining samples with substantial amounts of silt and other material in the same size range as teliospores, and it was likely that some were successfully being extracted but not counted because they were obscured by other particles. Increasing the final volume of the sucrose-float partition would further

Table 3. The *P* value for the tests of fixed effects from the analysis of variance on the mean number of *Tilletia indica* teliospores per 25 g in naturally infested field soils, and the extraction efficiency and estimated number of teliospores for these same soils

	P value		
ANOVA: tests of fixed effect(s)	**Baseline extraction (#)**	**Extraction efficiency (%)**	**Estimated #/ 25 g soil**
Location[a]	0.430	0.029	0.327
Year last positive[b]	0.377	0.642	0.207
Location*YLP	0.511	0.020	0.176

[a] Location factor represents the three regions in Texas from which soils were sampled for this study: Central Texas (San Saba County), North Texas Five County Regulated Region, and a single nonregulated wheat field in the Panhandle.

[b] Fields that tested positive in both 1997 and 2001 were given duplicate entries into the dataset, one for each year. Those that never tested positive were given year values of "Never". Thus, this factor incorporates information concerning the number of times a field has tested positive.

Table 4. Experimental factor, Pearson's Product-Moment coefficient, and *P* value for the mean number of *Tilletia indica* teliospores per 25 g in naturally infested field soils, and the extraction efficiency and estimated number of teliospores for these same soils

Experimental factor	**Baseline extraction (#)**		**Extraction efficiency (%)**		**Estimated # / 25 g soil**	
	Coefficient	***P* value**	**Coefficient**	***P* value**	**Coefficient**	***P* value**
Yrs since positive[a]	–0.02[b]	0.921	0.07	0.691	–0.07	0.703
# Times positive[c]	0.21	0.151	0.11	0.443	0.25	0.089
% Sand	0.26	0.072	0.27	0.063	0.21	0.144
% Silt	–0.14	0.358	–0.29	0.043	–0.08	0.593
% Clay	–0.41	0.004	–0.17	0.247	–0.37	0.009
% Organic	–0.17	0.249	–0.17	0.236	–0.21	0.155
pH	–0.20	0.180	–0.10	0.513	–0.23	0.122
PPM NO_3	0.11	0.462	0.63	<0.001	0.02	0.912
PPM P	0.11	0.454	–0.04	0.777	0.11	0.473
PPM K	0.06	0.673	–0.01	0.924	0.04	0.798
PPM Ca	–0.05	0.744	–0.02	0.914	–0.05	0.719
PPM Mg	–0.20	0.175	–0.29	0.049	0.21	0.157
PPM Na	–0.09	0.539	0.16	0.269	0.05	0.744
PPM S	0.03	0.839	0.11	0.440	–0.13	0.390
PPM salinity	0.10	0.482	0.39	0.006	0.02	0.906

[a] Fields never testing positive were excluded from the analysis of year factor.

[b] Correlation coefficient value represents negatively correlated (<0) to positively correlated (>0) and essentially equals the *r* value from a simple linear regression.

[c] Number of times a field has tested or been regulated as Karnal bunt positive.

distribute the material and potentially allow more teliospores to be enumerated. However, it typically takes over 1 h for a trained observer to quantify teliospores from a sample, and one can only examine a limited number per day due to fatigue. Increasing the work required to analyze a sample could result in a diminishing return, especially if appropriate controls are included to reduce the impact of missed teliospores.

Unlike in previous studies using this methodology (1; M. Bonde, *personal communication*), a significant correlation between extraction efficiency and soil characteristics was noted. Specifically, the percent sand and silt were positively and negatively correlated ($P < 0.10$) with recovery, respectively. The reason for these correlations was probably based on particle size, in that sand particles are larger than 53 μm diameter, whereas silt particles are similar in size to *T. indica* teliospores. Thus, sand particles will remain on the first sieve, whereas silt particles will pass through and interfere with the extraction process during the sucrose centrifugation and/or quantification step by obscuring teliospores. The correlations between extraction efficiency and specific soil chemical factors are more difficult to explain and may be due to an interaction between the factor(s) in question and the dispersal agent and/or the soil particles themselves. Further investigation is required to confirm if such correlations are artifacts or truly affecting efficiency.

The initial artificial infestation method used to determine extraction efficiency consisted of a teliospore suspension (100 per ml) in a 0.1% water agar (1.0 g/liter) solution. A 1.0-ml aliquot of the suspension was added to each 25.0-g sample, the soil thoroughly homogenized by shaking, and incubated at 4°C until processing (up to 21 days later). Using this method, almost all of the teliospores failed to extract and the authors hypothesize that the loss was due to either teliospore germination, disintegration while in storage, or the binding and clumping of teliospores within the suspension and with soil particles. Regardless of the reason, artificially infesting soil with teliospores using a water-based inoculum, and not thoroughly drying the sample, may lead to results indicating artificially low extraction efficiency or a reduction in teliospore viability, depending on the study. Hence, the sand-based teliospore inoculum method was developed to address these issues and resulted in a more uniform distribution in the soil and a much-reduced risk of germination or decay during storage.

Previous research demonstrated that teliospores were found to survive near the soil surface for at least 32 months and survival was influenced by soil texture and/or source (2). A significant ($P < 0.10$) negative correlation (–0.37) between estimated teliospore number in pooled samples and the percentage of clay in the soil was noted in this study. While the original population of teliospores in each field is unknown, one can speculate that the fields with higher amounts of clay were less conducive for teliospore survival because they generally retain moisture more effectively than coarser soils. Because teliospores require prolonged periods of high soil moisture to germinate (19), those in high clay soils may be more likely to undergo germination in the absence of a susceptible host (suicidal-germination) than those in coarser soils (G. Peterson, *personal communication*). Such germination would result in a decline in teliospore density because the population was not replenished. The fact that wheat fields in the KB regulated counties of Texas are generally sandy in texture partially supports this hypothesis. Additionally, the number of times a field tested positive was significantly, positively (0.25) correlated with estimated teliospore number, indicating that the soil population may have increased following a diseased crop.

Field C1 was not planted with a susceptible crop since the assignment of regulated status in 1997, is geographically isolated from the rest of the regulated fields in San Saba County, and should not have accumulated any additional teliospores. The germination of teliospores from C1 was unsuccessfully attempted multiple times (data not shown), and teliospores artificially infested into soil have been noted to lose viability while maintaining some of their morphological characteristics (2). Therefore, the authors hypothesize that those found were probably from the original introduction in, or before, fall 1996.

During preliminary examination of the spatial distribution of teliospores from grid sample points in fields C1 and N1 (25% of the samples in each), two putative distribution patterns were found (data not shown). The samples examined from C1 all had a very low quantity of teliospores per 25 g (<8) with a uniform distribution. In contrast, field N1 had a distinct section with a comparatively high quantity of teliospores per sample (~50), suggesting an aggregate pattern. The two fields have different disease histories that may account for their teliospore densities. Specifically, field C1 was regulated because it was planted with teliospore-infested seed that was associated with a bunted kernel positive seed lot (G. Nash, *personal communication*), whereas N1 was regulated because it was found to have a bunted kernel positive crop (R. Smith, *personal communication*). The aggregated distribution in field N1 was possibly the result of a localized, high disease incidence in the crop with teliospore deposition from bunted kernels. A random, aggregated distribution was previously noted in durum wheat heads in Arizona (22). Statistical analysis on the remaining data and further studies into the relationship may help resolve questions concerning the teliospore density–infection threshold and other aspects of the epidemiology of *T. indica*.

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LITERATURE CITED

1. Babadoost, M., and Mathre, D. E. 1998. A method for extraction and enumeration of teliospores of *Tilletia indica, T. controversa*, and *T. barclayana* in soil. Plant Dis. 82:1357-1361.
2. Babadoost, M., Mathre, D. E., Johnston, R. H., and Bonde, M. R. 2004. Survival of teliospores of *Tilletia indica* in soil. Plant Dis. 88:56-62.
3. Bansal, R., Singh, D. V., and Joshi, L. M. 1984. Effect of Karnal-bunt pathogen [*Neovossia indica* (Mitra) Mundkur] on weight and viability of wheat seed. Indian J. Agric. Sci. 54:663-666.
4. Bhat, R. V., Rao, B., Roy, D. N., Vijayaraghavan, M., and Tulpule, P. G. 1983. Toxicological evaluation of Karnal bunt wheat. J. Food Safety 5:105-111.
5. Bonde, M. R., Berner, D. K., Nester, S. E., Peterson, G. L., Olsen, M. W., Cunfer, B. M., and Sim, T. 2004. Survival of *Tilletia indica* teliospores in different soils. Plant Dis. 88:316-324.
6. Bonde, M. R., Nester, S. E., Olsen, M. W., and Berner, D. K. 2004. Survival of teliospores of *Tilletia indica* in Arizona field soils. Plant Dis. 88:804-810.
7. Bonde, M. R., Peterson, G. L., Schaad, N. W., and Smilanick, J. L. 1997. Karnal bunt of wheat. Plant Dis. 81:1370-1377.
8. Bonde, M. R., and Smilanick, J. L. 1998. Life cycle and environmental requirements of *Tilletia indica*. Pages 137-147 in: Bunts and Smuts of Wheat: An International Symposium, Aug. 17-20, Raleigh, NC.
9. Castlebury, L. A. 1998. Morphological characterization of *Tilletia indica* and similar fungi. Pages 97-105 in: Bunts and Smuts of Wheat: An International Symposium, Aug. 17-20, Raleigh, NC.
10. Chapman, F. 2003. Agricultural statistics. National Agricultural Statistics Service, U.S. Dep. Agric.
11. Datnoff, L. E., Royer, M. H., Bonde, M. R., and Prescott, J. M. 1988. A quantitative method for estimation of teliospores of *Tilletia indica* in soil. Plant Dis. 72:209-212.
12. Fuentes-Davila, G. 1998. Karnal bunt of wheat. Pages 67-81 in: Bunts and Smuts of Wheat: An International Symposium, Aug. 17-20, Raleigh, NC.
13. Garrett, K. A., and Bowden, R. L. 2002. An allee effect reduces the invasive potential of *Tilletia indica*. Phytopathology 92:1152-1159.
14. Gee, G. W., and Bauder, J. W. 1986. Particle-size analysis. Pages 383-411 in: Methods of soil analysis. Part 1. Physical and mineralogical methods 2nd. A. Klute, ed. American Soci-

ety of Agronomy, Madison, WI.
15. Gill, K. S., Sharma, I., and Aujla, S. S. 1993. Karnal bunt and wheat production. 1st ed. Punjab Agricultural University. Ludhiana, PJ, India.
16. Golden, M. L. 1999. Soil taxonomy. Natural Resources Conservation Service, U.S. Dep. Agric.
17. Graham, S. 1959. The effects of various reagents, mounting media, and dyes on the teliospore walls of *Tilletia controversa*. Mycologia 51:477-491.
18. Krishna, A., and Singh, R. V. 1983. Longevity of teliospores of *Neovossia indica* causing karnal bunt of wheat. Indian J. Mycol. Plant Pathol. 13:97-98.
19. Rattan, G. S., and Aujla, S. S. 1992. Ecology of germination of the teliospore of Karnal bunt pathogen (*Neovossia indica*). Indian J. Agric. Sci. 62:228-231.
20. Rattan, G. S., Aujla, S. S., and Sharma, I. 1989. Estimation of teliospores of *Tilletia indica* from field soil. Plant Dis. 73:804.
21. Rush, C. M., Stein, J. M., Bowden, R. L., Riemenschneider, R., Boratynski, T., and Royer, M. H. 2005. Status of Karnal bunt of wheat in the United States: 1996 to 2004. Plant Dis. 89:212-223.
22. Sawyer, A. J., Huettel, R. N., and Meyer, R. J. 1997. Within-field spatial distribution of *Tilletia indica* (Karnal bunt): Implications for detection surveys. (Abstr.) Phytopathology 87:S85.
23. Sekhon, K. S., Singh, N., and Singh, R. P. 1992. Studies on the improvement of quality of wheat infected with Karnal bunt. I. Milling, rheological, and baking properties. Cereal Chem. 69:50-54.
24. Singh, A. 1994. Epidemiology and management of Karnal bunt disease of wheat. University of Agriculture and Technology Research Bull. No. 127. Pantnagar, UP, India.
25. Smilanick, J. L., Prescott, J. M., Hoffmann, J. A., Secrest, L. R., and Weise, K. 1989. Environmental effects on survival and growth of secondary sporidia and teliospores of *Tilletia indica*. Crop Prot. 8:86-90.