Distribution and Recovery of *Tilletia indica* Teliospores from Regulated Wheat Fields in Texas

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ABSTRACT

Allen, T. W., Maples, H. W., Workneh, F., Stein, J. M., and Rush, C. M. 2008. Distribution and recovery of *Tilletia indica* teliospores from regulated wheat fields in Texas. *Plant Dis.* 92:344-350.

Eight wheat fields from the Karnal bunt-regulated regions within Texas were grid sampled to gain a better understanding of the ecology and epidemiology of teliospores produced by the causal agent, *Tilletia indica*. Teliospores from 25-g aliquots of soil from each grid point were extracted using a size-selective sieving sucrose-centrifugation procedure. Teliospores were recovered from all eight fields and, in some cases, from every grid point within a field. Total teliospore numbers ranged from 0 to 1,305 per 25 g of soil. Over 70% of the total grid sampled points contained one or more teliospores. The relation between soil chemical and physical characteristics and teliospore numbers from each field was evaluated. In general, no consistent, significant trend could be made between soil factors and teliospore numbers. Geostatistics were used to analyze data from grid points and create contour maps. Teliospore distribution was aggregated in four of the fields, random in three of the fields, and discontinuous (neither random nor aggregated) in a single field. This is the first report of widespread distribution and high teliospore numbers from wheat field soils in the United States.

Karnal bunt (also known as partial bunt) of wheat (Kb), caused by the fungus *Tilletia indica* Mitra (= *Neovossia indica* (Mitra) Mundk.), is a zero-tolerance, federally regulated disease within the United States (8,11,43). The disease causes a partial conversion of wheat kernels into sori filled with teliospores, which results in the loss of both yield and seed quality (11). Since 1931, Kb has been reported from Afghanistan, Brazil, Iran, Iraq, India, Mexico, Nepal, Pakistan, Northern Cape Province of South Africa, and the United States (7,16,21,36,41,43). Kb first was identified in the United States in Arizona in 1996 (54) and, since then, has been reported from California and Texas (8,43). Regulations imposed by the United States Department of Agriculture Animal and Plant Health Inspection Services (USDA-APHIS) on areas that tested positive for the disease included a 3-mile area surrounding each positive field (43). Restrictions on the export of wheat from regulated areas have led to significant economic

losses. In Texas, the estimated economic loss to producers from the northern regulated counties of Archer, Baylor, Knox, Throckmorton, and Young exceeded \$25 million in 2001 and 2002 (43).

T. indica produces teliospores that serve as long-lived, survival structures and the primary source of inoculum for infection and dispersal (33,46). Teliospores germinate and give rise to primary sporidia which, in turn, give rise to secondary sporidia, the infective spore stage. In soil, teliospores can survive and germinate on or near the surface (8,27,33) and, if the environment is conducive, secondary sporidia can infect wheat plants in the susceptible growth stage, which occurs from the time florets begin to emerge from the boot through anthesis (Feekes 10.0 to 10.53) (28,46). However, new research suggests that the infection window may go beyond anthesis and through the soft dough stage (Feekes 11.2) (29). Once teliospores are established in the soil, they can survive for 36 to 43 months (6,32,52) and have been reported to survive under laboratory conditions for at least 16 years (8). Teliospores can be naturally introduced into soil by infested or infected seed and debris at the time of harvest or threshing (18,19,32). The spread of teliospores to other fields can occur by the movement of soil, birds, equipment or vehicles, manure, and wind currents (8,11,47,48,52,53). Research conducted in Mexico reported that burning wheat stubble could send teliospores 3,000 m into the atmosphere, pre-

sumably by convection (9). The long-range dissemination of teliospores into new areas can occur by the transport of contaminated seed (8) or the movement of animals or animal waste (47). Boratynski et al. (10) reported finding *T. indica* teliospores and bunted kernels in rail cars moving from Mexico to California.

Soil teliospore density previously has been quantified on a limited basis (4,19,40,49). Soil samples from 50 wheat fields in Punjab, India were reported to contain 2,000 to 50,500 teliospores per 250 cm³ of field soil (approximately 133 to 6,733 per 25 g of soil, assuming a soil density range of 0.75 to 1.5 g/cm³) (40). Datnoff et al. (19) reported that naturally infested soil samples from northwest Mexico contained 476 to 1,617 teliospores per 10 g of field soil (1,190 to 4,042 per 25 g). However, the work conducted by Datnoff et al. (19) estimated the number of teliospores in field soil by creating a prediction equation and a resultant standard curve derived from artificially inoculating field soils rather than extracting teliospores from naturally infested field soil. In the United States, Babadoost and Mathre (4) found two *T. indica* teliospores in 35 soil samples from a single naturally infested Arizona wheat field. More recently, Stein et al. (49) reported 0 to 5 teliospores per 25 g of soil from bunted-kernel-positive wheat fields in Texas. However, they reported the average number of teliospores per field based on pooled soil aliquots and did not consider teliospore distribution in each of the 14 regulated fields that were sampled. In this study, we used geostatistics to describe the spatial distribution of teliospores in bunt-positive fields in Texas.

Geostatistics has been widely used in plant pathology in describing the spatial component of plant disease and pathogen distribution (5,13,17,35,45) and its use in conjunction with GIS has been reviewed recently (38). Geostatistics is a set of statistical methods that measure spatial patterns (42,50) and can detect spatial dependence by measuring variation between samples separated by the same distance (14) and determine if they are spatially correlated (15). Conventional statistical methods can be difficult to use to describe data that are spatially correlated. Spatial analyses enables one to predict values from unsampled locations.

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Accepted for publication 17 October 2007.

doi:10.1094/PDIS-92-3-0344
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To achieve a better understanding of the ecology and epidemiology of *T. indica* in wheat field soil, investigations were conducted with two primary objectives: (i) to quantify teliospore density and distribution within the regulated fields in Texas, and (ii) to determine the relationship between soil physical and chemical characteristics and the incidence and distribution of *T. indica* teliospores. This information could be important in making decisions in the eventual deregulation of Kb within the United States. Results from this research may provide information regarding the initial introduction of *T. indica* into Texas wheat fields.

MATERIALS AND METHODS

Soil sampling and soil sample preparation. Wheat fields in the two Kb-regulated regions of Texas, the northern five-county region (Archer, Baylor, Knox, Throckmorton, and Young) and the southern two-county region (McCulloch and San Saba), were grid sampled in April and May 2002, respectively. From the two regions, eight wheat fields (two in the south and six in the north) were selected based on the results of a previous study (49). The sizes of the fields ranged from 0.4 to 22 ha. Grids consisted of either a sample point every 10 by 10 m or 60 by 60 m (with the exception of one field where a 7-by-7-m grid was used) dictated by field size, with a minimum of 60 and a maximum of 72 grid points per field. The perimeter of each wheat field was mapped using a Hewlett Packard Jornada Pocket PC (Hewlett Packard Inc., Palo Alto, CA) with Farm Works Mate software (version 8.01; CTN Data Service Inc., Hamilton, IN). Field maps were created using perimeter data and by interpolating distances between grid points in ArcMap (version 8; ESRI, Redlands, CA).

At each sample point, approximately 500 g of soil from a 1-m² area representing the top 5 cm was collected and sealed in plastic bags. Samples were transported to the Texas Agricultural Experiment Station Karnal Bunt Quarantine Lab in Bushland, TX, air dried in a Type IIA Biological hood for 3 to 5 days, and crushed with a rolling pin to break large clumps. Soil in the individual bags was mixed thoroughly and a 25-g aliquot was removed and placed into a 50-ml screw-cap centrifuge tube. Sample tubes were stored at room temperature (approximately 22°C) until processing. All movement of soil from the regulated areas to the quarantine laboratory in Bushland was approved by a USDA-APHIS permit and the Texas Department of Agriculture prior to initiation of this study.

Characterization of soil texture, chemistry, and organic matter. The percentage of sand, silt, and clay from each soil sample was determined using a gravimetric method, as previously described

by Gee and Bauder (26). The soil textural class then was determined for each sample based on USDA Soil Taxonomical nomenclature (30). Organic matter content and basic soil chemical entities, which included pH, NO₃-N, P, K, Ca, and salinity (soluble salts), in 100 g of soil from each grid point was determined by a commercial laboratory (Servi-Tech Laboratories, Amarillo, TX).

Extraction and enumeration of teliospores from soil samples. The extraction protocol was a modification of that used by Babadoost and Mathre (4) and Stein et al. (49). Briefly, a 25-g aliquot of soil from each sampled point was hydrated overnight at room temperature (approximately 22°C) with 25 ml of 0.1% Tween 20 (Acros Organics, Fair Lawn, NJ) in sterile reverse osmosis (RO) water. Following hydration, samples were vortexed for 10 s (setting no. 9, Vortex Genie; Scientific Industries, Bohemia, NY) to resuspend the soil suspension, poured into 600-ml beakers containing a magnetic stir bar, and the remaining contents of the centrifuge tube were washed out with RO water. Three drops of Tween 20 were added, and the suspension was stirred rapidly on a stir plate for 5 min. The contents of the beaker were poured onto a stacked sieve pair, 20- μ m bottom with a 53- μ m top sieve. The top sieve then was placed over a second 20- μ m sieve, measuring 5 cm in height (VWR International, West Chester, PA), and the soil suspension was rinsed through the sieve with the remaining contents of the beaker and thoroughly rinsed with approximately 5 to 7 liters of tap water. The contents of the first 20- μ m bottom sieve were rinsed into the second 5-cm, 20- μ m sieve and the soil fraction was rinsed thoroughly until the remaining water that drained through the bottom was no longer cloudy. The remaining contents on the sieve were washed back into the 50-ml centrifuge tube and the contents centrifuged for 5 min (2,500 $\times$ g). The supernatant was poured off and 25 ml of a 1.6 M sucrose solution (800 g of granulated sugar in 1 liter of RO water) was added to the centrifuge tube. The suspension was vortexed for 10 s (setting number 9) and centrifuged for 1 min (180 $\times$ g), and the supernatant was poured through a 20- μ m-mesh nylon sieve. The sucrose procedure was repeated four additional times. The contents of the 20- μ m sieve were rinsed with approximately 1 liter of tap water and the fraction remaining on the sieve was washed into a 15-ml centrifuge tube. The contents of the tube were centrifuged for 5 min (2,500 $\times$ g) and the supernatant was poured off. The remaining pellet was resuspended in 700 μ l of Shear's solution (31). The suspension later was transferred to a Sedgwick-Rafter counting chamber (Kahl Scientific Instrument Corporation, El Cajon, CA) and teliospores were enumerated microscopically at $\times 100$ or $\times 400$ as necessary using

darkfield microscopy. Only intact teliospores with the correct size for *T. indica*, a diameter of 22 to 47 μ m (22), and exhibiting the characteristic ornamentation (12), including an epispodium adorned with thick, truncated projections, were recorded. Teliospore viability was not determined.

To determine teliospore extraction efficiency, 10 bunted kernels from a 2004 Arizona collection were placed in a 15-ml centrifuge tube and 20 ml of a 0.1% Tween 20 solution was added. Bunted kernels were crushed with a flame-sterilized glass rod and the suspension was vortexed for 10 s. The resulting suspension was poured through a stacked 53- μ m-mesh top and 20- μ m-mesh bottom sieve pair. The sieves were rinsed with 1 liter of tap water, and teliospores were collected into a 15-ml centrifuge tube and centrifuged for 5 min (2,500 $\times$ g). The supernatant was decanted and the resulting teliospore pellet was rinsed an additional three times with sterile RO water. Teliospore numbers were enumerated with a hemacytometer using four independent counts and calculating the average. A final suspension of 1,000 teliospores/ml was created using sterile RO water for a final volume of 100 ml. The final teliospore suspension was added to sterilized (20 min on two successive days) river sand. In a 500-ml beaker, 100 g of sterile sand and the 100-ml teliospore suspension were combined and allowed to dry in a type IIA biological hood for 5 to 7 days. Once completely dry, 1 g of the sand-teliospore suspension was added to six 25-g sandy loam soil samples in 50-ml centrifuge tubes. Three of the centrifuge tubes were washed using the methodology of Stein et al. (49) and the other three were washed using the modified methodology described above.

Correlation analysis. Pearson's correlation coefficients were calculated to examine the relationships between soil physical and chemical properties, elevation, and the number of teliospores from grid points within each field using PROC CORR in SAS 9.1 (SAS, Inc., Cary, NC). Digital elevation models (DEMs) were downloaded from the Texas Natural Resources Information System and United States Geological Survey (USGS) DEM Resources for quadrangles within each of the counties that contained wheat fields, and applied to georeferenced sample points in ArcMap by overlaying sample points onto the DEM to obtain the elevation (meters above sea level) for individual points. Use of either the Texas Natural Resources or USGS DEM Resources maps depended on quadrangle availability. The number of years and the last year each field tested bunted kernel positive also were examined for correlation to teliospore density.

Geostatistical analyses. Geostatistical analyses were conducted with GS+ (version 7; Gamma Design Software, Plain-

well, MI) to create semivariograms. Semivariograms measure the variation between samples in both distance and direction and were used here to determine teliospore distribution among grid points in wheat fields. Essentially, the variogram is a plot of the semivariance versus separation distance (24). The plot is derived from one-half of the mean squared difference (semivariance) of paired sample measurements and presents this as a function of the distance between samples (51). An increase in semivariance with a corresponding increase in the separation distance is indicative of spatial dependence or an aggregated pattern. However, constant values across all separation distances indicate randomness (20,42). Both linear and nonlinear variogram models (either linear, spherical, exponential, or Gaussian) were tested to determine their relative fit to data and the best one was chosen based on the lowest residual sums of squares (RSS) and highest coefficient of determination (R^2). The nugget variance (C_0), sill ($C + C_0$), and range (A) also were taken into account when choosing a model. Nugget effect represents the degree of dissimilarity between two sample points that are very close to one another. It is the error variance considered to be the magnitude of discontinuity at the origin or, more simply, the y-

intercept of the model. The sill represents spatially independent variance and is a constant value where the semivariance levels off (24). The range, or effective range, is the distance at which the semivariogram reaches the sill and is the distance beyond which the samples are not autocorrelated or spatially independent. Spatially dependent samples have a semivariance that begins small and increases with an increasing lag distance (23).

Contour map display. GS⁺ was used to create contour maps of fields and interpolate the values in nonsampled areas using the inverse distance weighting (IDW) feature. Interpolation estimates are based on values from nearby locations and are weighted by distance from the interpolation location (24). The measured distance between grid points was recorded for each field in ArcMap and used for map points on a Cartesian plane.

RESULTS

Extraction and enumeration of teliospores from soil samples. Teliospores were recovered from all sampled fields, but numbers were highly variable. The average teliospore density from the eight fields ranged from 1.0 to 51.7 teliospores (Table 1). On a field-by-field basis, teliospores were recovered from a minimum of

33% of the total grid points and a maximum of 100% of the grid points in two fields. Total teliospores recovered ranged from 0 to 1,305 per 25-g aliquot. Only two fields had teliospore numbers exceeding 1,000 spores per 25 g of soil, and both were in the northern region. Of the 540 total soil samples collected, at least one teliospore was recovered from 381 of them. In general, a greater average number of teliospores was extracted from fields in the northern counties, ranging from 1.0 to 51.7 per 25-g sample, compared with a range of 1.5 to 4.3 per 25-g sample in the central Texas fields (Table 1). Only one field in the northern region (field 29) had a lower average number of teliospores than the central-Texas fields.

Correlation analyses. There were significant positive correlations between percent sand and the number of extracted teliospores in three of the eight fields sampled (Table 2). Percent silt and clay were each significantly negatively correlated with teliospores in two different wheat fields. Parts per million P and Ca had significant, positive correlations at a single wheat field while salinity, NO₃-N, P, and K each were significant and positively correlated at the same site (Table 2), respectively. Only one field (field 29) had no positive or negative significant correlations

Table 1. Number of teliospores per sample, year field tested bunted kernel positive, elevation, soil texture, and number of bunted kernels from naturally infested wheat fields sampled in 2002 from regulated areas in either Central or North Texas

Field ID	Year positive ^b	Elevation ^c	Soil texture ^d	Teliospore range ^e	No. of soil samples within each numerical range of teliospores ^a										Bunted kernels ^f	
					0	1–5	6–10	11–20	21–40	41–60	61–100	101–120	121–160	>1,000		
Central Texas																
10	2001	365–366 (365.3)	Silt loam	0–40 (2.9)	21	29	9	...	1	...	...	...	...	...	...	1
11	1997	1,468–1,499 (1,487.3)	Sand	0–42 (4.3)	7	42	5	5	...	1	...	...	...	...	...	0 ^g
15	1997, 2001	376–378 (377.4)	Sandy loam	0–9 (1.5)	23	34	3	...	...	...	...	...	...	...	...	1
North Texas																
21	2001	1,129–130 (1,129.4)	Sandy loam	3–121 (45.8)	...	4	3	14	16	11	21	2	1	...	...	1
22	2001	1,127–1,128 (1,127.4)	Sandy loam	1–1,305 (51.7)	...	8	6	17	23	7	7	2	1	1	...	1
27	2001	401–402 (401.8)	Loamy sand	0–111 (6.1)	20	39	5	3	3	1	...	1	...	...	...	1
28	2001	400–402 (400.9)	Sandy loam	0–1,021 (16.2)	48	16	2	4	...	1	...	...	...	...	1	1
29	2001	1,232–1,237 (1,234.9)	Sandy loam	0–16 (1.0)	46	24	...	2	...	...	...	...	...	...	...	1
Total	...	...	...	...	165	196	33	45	43	21	28	5	2	2	...	...

^a Number of teliospores is based on the total number of spores removed from a 25-g aliquot of soil from points within each field (either 60 or 72 grid points). Teliospores were extracted from soil by a combined sieving and sucrose centrifugation technique.

^b Year positive refers to the first year that the field tested bunted kernel positive by the U.S. Department of Agriculture–Animal and Plant Health Inspection Services (USDA-APHIS).

^c Elevation is based on the distance above sea level in meters that each point exists based on downloaded digital elevation models (DEMs) from the Texas Natural Resources Information System or the U.S. Geological Survey DEM Resources depending on map quadrant availability. Files were downloaded and individually, geographically referenced points from each field were overlaid on the DEM file in ArcMap to determine elevation at each point. Elevations are presented as a numerical range across the field, with the average number in parentheses.

^d Soil texture is based on the percent sand, silt, and clay calculated using a particle-settling method as described by Gee and Bauder (26) and then classified into a textural class based on USDA Soil Taxonomy nomenclature (30).

^e Teliospore numbers are presented in ranges of the number of spores within each numerical range, with the average number in parentheses.

^f Number of bunted kernels typically was enumerated from a 1.81-kg sample harvested from a single location at the edge of each field and provided by the USDA-APHIS. In Central and North Texas, bunted kernel counts stopped when the first bunted kernel was found in each field.

^g A bunted kernel was not found during sampling of grain from this field. This field was included in the regulated area because it was part of a composite National Survey sample that included three other fields that were found to contain several bunted kernels. Intensive surveys of the farmer's storage and land did not lead to a bunted kernel being found. The field was released from regulation in 2004.

between teliospores and soil characteristics. Two fields had one variable significantly correlated, three fields had two variables significantly correlated, and three fields had three variables significantly correlated (Table 2). All other correlations between extracted teliospores and soil physical or chemical characteristics were not significant. Elevation was not significantly correlated to teliospore number. In addition, the number of years since each field tested positive was not significant, nor was the last year each field tested positive (either 1997 or 2001).

Geostatistical analysis. Diagrammatic representations of teliospore density and distribution within each wheat field are presented in Figure 1. In general, the spatial distribution of teliospores within the eight wheat fields can be grouped into three different categories: (i) aggregated (fields 10, 11, 22, and 27), with teliospore distribution best described by a Gaussian model; (ii) random (fields 21, 28, and 29), with teliospore distribution best described by the linear model; and (iii) neither random nor aggregated, considered to be discontinuous (field 15), with teliospore dis-

tribution best described by an exponential model. Analysis of the teliospore distribution for each field using the IDW technique (24) showed different levels of teliospore aggregation for each field. Because of this, contour maps and semivariograms for three fields which each represent a unique situation are presented (Figs. 2, 3, and 4). Field 10, tested bunted kernel positive in 2001, had one large teliospore mass (or aggregate) in approximately the center of the field (Fig. 2A), and the teliospore distribution across the field was aggregated ($R^2 = 0.95$; Fig. 2B) as indicated by in-

Table 2. Pearson's correlation coefficients (r) calculated between the numbers of teliospores per 25 g of soil from each sample point in each field ($n = 60$ or 72), and soil textural and chemical characteristics

Field ID	Correlation coefficient ^a									
	Soil (%) ^b					Parts per million				
	Sand	Silt	Clay	OM	pH	NO ₃	P	K	Ca	Salinity
Central Texas										
10	0.26*	-0.03	-0.21	-0.04	0.16	0.01	-0.05	-0.05	0.18	0.00
11	-0.06	0.03	0.06	0.09	0.16	0.19	0.04	-0.06	0.26*	0.08
15	0.02	0.04	-0.12	0.34**	0.10	0.06	0.32*	0.22	0.12	0.06
North Texas										
21	0.32**	-0.19	-0.27*	0.02	0.16	-0.02	-0.29*	-0.13	0.05	0.01
22	0.32**	-0.32**	-0.01	0.37**	0.13	-0.06	-0.13	0.08	-0.01	-0.03
27	-0.08	0.05	0.08	0.18	0.02	-0.09	0.37***	0.24*	0.01	-0.02
28	-0.15	0.16	0.03	0.08	-0.23*	0.49****	0.17	0.09	0.08	0.38**
29	0.06	-0.07	0.02	-0.03	-0.17	-0.07	-0.04	-0.04	-0.18	-0.10

^a Each coefficient was determined for either 60 or 72 points from each field location; *, **, ***, **** = coefficients significant at $P = 0.05, 0.01$, and 0.001 and $P < 0.0001$, respectively.

^b Percent sand, silt, and clay were calculated using a particle-settling method as described by Gee and Bauder (26) whereas percent organic matter (OM) was determined by dry weight.

Fig. 1. Teliospore densities from grid points within each of eight bunted-kernel-positive Texas wheat fields sampled in 2002. Densities are reported as the total number of teliospores extracted from 25-g aliquots of a bulk soil sample collected at each grid point. Fields 10, 11, and 15 were from central Texas regulated counties while all others were from north Texas counties. Fields 21, 22, 27, 28, and 29 were sampled on a 10-by-10-m grid, whereas fields 11 and 15 were sampled on a 60-by-60-m grid. Field 10 was sampled on a 7-by-7-m grid due to the small size of the field.

creasing variance with increasing distance up to a range of 16.9. Field 15 tested bunted kernel positive in 1997 and again in 2001; however, the pattern of teliospore

distribution throughout the field was discontinuous ($R^2 = 0.06$; Fig. 3A and B). Teliospores in field 21 were found throughout the entire field (Fig. 4A) and

their spatial pattern was near random ($R^2 = 0.81$; Fig. 4B). The nuggets were particularly high for fields 15 and 20 (Figs. 3 and 4), which indicates that there is great variability between teliospore data in each sample.

DISCUSSION

Over the past two decades, there have been numerous ecological and epidemiological Kb studies conducted (4,7,9,19,25,28,29,34,39,40,44,47–49). However, the spatial distribution of teliospores within soils under field conditions has not been investigated. Rush et al. (43) and Sawyer et al. (44) presented results on the distribution of bunted kernels within wheat fields but did not include data on soil teliospore incidence or distribution. In our study, teliospores were recovered from eight wheat fields in two separate, regulated wheat-growing regions in Texas. The average teliospore densities had a wide range across all fields and, in some cases, tended to be high (>45 per 25 g of soil). In two of the fields, over 1,000 teliospores (1,021 and 1,305) from a single point each were recovered. These are the highest recorded soilborne teliospore numbers from the United States to date. Recently, 704 soilborne teliospores were reported from an Arizona durum wheat field following two consecutive bunted-kernel-positive crops (1). Previous studies on the extraction of teliospores from wheat field soil in the United States (4,49), and elsewhere (19,40), have reported soilborne teliospore numbers ranging from approximately 1 to 6,733. The results of our work indicate that *T. indica* teliospores can be readily extracted from field soil and are widespread in bunt-positive Texas wheat fields.

In previous studies, correlations were made between teliospore extraction efficiency and soil characteristics (19,49). Babadoost and Mathre (4) artificially infested different soil types with teliospores, but did not determine extraction efficiency based on soil type. Extraction efficiency in this study was determined to be 18%, compared with 10% efficiency for the method used by Stein et al. (49). Even though some correlations between numbers of teliospores and soil physical and chemical characteristics were significant, there were no consistent, significant trends. Stein et al. (49) reported a significant negative correlation between percent clay content in soil and the estimated number of teliospores in a 25-g pooled soil sample across 15 sampled wheat fields. However, Stein et al. (49) only investigated the average number of teliospores from each pooled sample compared with soil characteristics. In our study, even though there were negative trends in some of the fields, there were no significant relationships between teliospore density and the percentage of clay except in one field. Datnoff et al. (19) suggested that there was

Fig. 2. A, Contour map and B, the corresponding semivariogram plot depicting the spatial pattern of *Tilletia indica* soilborne teliospores from field 10, where teliospore numbers ranged from 0 to 40 and the semivariogram is representative of a field with an aggregated teliospore distribution and best described by a Gaussian model ($R^2 = 0.95$). Teliospore numbers are based on the total number of teliospores extracted from a 25-g soil sample.

Fig. 3. A, Contour map and B, the corresponding semivariogram plot depicting the spatial pattern of *Tilletia indica* soilborne teliospores from field 15, where teliospore numbers ranged from 0 to 9 and the semivariogram is representative of a discontinuous pattern and best described by an exponential model ($R^2 = 0.06$). Teliospore numbers are based on the total number of teliospores extracted from a 25-g soil sample.

Fig. 4. A, Contour map and B, the corresponding semivariogram plot depicting the spatial pattern of *Tilletia indica* soilborne teliospores from field 21, where teliospore numbers ranged from 3 to 121 and the semivariogram is representative of a random teliospore distribution and best described by a linear model ($R^2 = 0.81$). Teliospore numbers are based on the total number of teliospores extracted from a 25-g soil sample.

a potential for teliospores to bind to soil particles; however, at present, research has not been conducted in this particular area. Previous studies (4,39) suggest that teliospores are more suitable to survive in loamy sand soil than in clay or sandy loam soils. However, no work has been conducted in the United States to determine the survivability of teliospores in soil maintained under field conditions in bunted-kernel-positive wheat fields. The previous studies (4,39) suggest that there is a direct correlation between recovery and survivability. However, correlations between percent soil particles and the recovery of teliospores were not computed. More in-depth studies on the long-term survival of teliospores from naturally infested soils are needed to understand the role that soil textural class could have on disease development and soil survivability.

Based on conservative estimates, a single wheat kernel converted entirely to a sorus filled with teliospores could contain approximately 1.0×10^6 to 1.4×10^6 teliospores (25). However, because wheat kernels have varying degrees of disease ranging from small point infections to expansion into the kernel groove (29), this estimate is high, and a range between 1.0×10^4 and 1.4×10^5 , based on 1 to 10% of a wheat kernel by weight becoming bunted, is more realistic. As stated by Murray and Sansford (37), numerous teliospores can be dislodged from a bunted kernel. This could create a localized concentration of soil-borne teliospores in approximately the same location where disease had occurred. As each successive year passed, and with susceptible wheat crops planted, a greater number of disease foci could occur and more teliospores accumulate in the soil and be dispersed across a larger area. Because teliospores were widely spread in some of the Texas fields, it is possible that *T. indica* teliospores have been in the United States since before Kb first was identified in 1996. A recent study on the spread of *T. indica* teliospores from an artificially infested area source supports this hypothesis (2). However, as widely speculated, introduction through infested seed can result in a distribution similar to what was detected in this study within a short period.

Prior studies in Arizona bunt-positive fields reported that Kb had a random, aggregated distribution within a durum wheat field (43) and a highly aggregated pattern with clusters of randomly distributed bunted kernels (44). However, both studies were conducted in only a single wheat field. Similar to previously published research, two types of distribution were found in our survey, one random and one aggregated. In general, a statement cannot be made regarding the structure of the distribution within Texas wheat fields as a whole because there were differences within and between fields from each of the regulated regions. Manure is not widely

used as a source of fertilizer in Texas, but fields often are grazed by cattle. The relationship between grazing and teliospore density and distribution needs investigation. Four fields, nos. 21, 22, 27, and 28, from the northern regulated region contained a greater number of teliospores and a wider teliospore distribution within the fields than the other fields. A greater number of teliospores may have been extracted from northern wheat fields because the fields tested bunted kernel positive in 2001 and only 11 months separated the harvesting of diseased wheat kernels and soil sampling. Fields 21 and 22 had the highest average number of teliospores and widely distributed teliospore masses, with teliospores recovered from every sample point. Widespread disease within these two wheat fields is an unlikely explanation for teliospore distribution because Kb has been reported to be a localized disease, typically occurring in random aggregates (43,44). Even during epidemic years in India and Mexico, Kb never was distributed throughout an entire wheat field, nor have there been reports of widespread disease or crop losses across entire regions (3,8,29,43). Even if there was a 3% level of infected grain within a single wheat field, a relatively low number of wheat heads would be infected. Fields with this level of disease have not been reported in the United States. However, the possibility exists that disease was present in fields 21 and 22 prior to 2001 and went unnoticed. Kb is difficult to diagnose at the field level because wheat kernels are attacked randomly and diseased heads are not distorted by infected kernels as in other bunt diseases (54). Unless teliospores are observed microscopically by a trained professional, the disease could be misidentified. Additionally, Kb and common bunt both produce a similar, characteristic fishy odor when large amounts of bunted kernels are present in grain (11,54). It is possible that wheat growers in the two regions were misdiagnosing grain with Kb as having common bunt instead and this is one of the reasons why it was not detected prior to 1997 or 2001. Although a fishy odor is a diagnostic characteristic for Kb, it should not be relied on as the sole criterion for diagnosis.

This research documents a widespread distribution of soilborne teliospores from eight bunted-kernel-positive wheat fields from regulated Texas counties. In the initial Texas bunted-kernel surveys, the USDA-APHIS counted a 1.8-kg grain sample from each field and stopped counting kernels when a single bunted kernel was found. However, in 1997 and 2001, disease incidence within each field was considered to be low. Even though disease incidence was low and because fields were reported to only contain a single bunted kernel, we cannot make a statement regarding a particular soil teliospore thresh-

old required for disease to occur. More in-depth studies on inoculum density and the resulting disease incidence relationship for *T. indica* are needed.

ACKNOWLEDGMENTS

This research was supported by funds from the Karnal Bunt Initiative/USDA-Agricultural Research Service (ARS) and a USDA-CSREES Critical Issues Grant under a specific cooperative agreement between Texas A&M University and USDA-ARS under project number 5430-21000-005-00D. We thank B. Annen, C. Childers, D. Harvey, D. Jones, N. Simpson, K. Steddom, B. Timmens, C. Timmens, H. Timmens, and E. Villanueva for experimental and technical assistance; B. Smith and R. Guerra of the USDA-APHIS for assistance in identifying regulated fields, contacting producers, and temporary storage of regulated material; D. Kostroun and S. Nilakhe of the Texas Department of Agriculture for their invaluable help during the permit application process; and G. H. Nash from the USDA-APHIS-PPQ (Austin, TX) for providing support as well as locating producers and fields that previously had tested bunted kernel positive.

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