Eur J Plant Pathol (2009) 125:497–507
DOI 10.1007/s10658-009-9498-z

Reaction of selected winter wheat cultivars from Europe and United States to Karnal bunt

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Received: 30 January 2009 / Accepted: 28 May 2009 / Published online: 23 June 2009

Abstract A study was conducted to determine the susceptibility of popular United States (U.S.) and European winter wheat cultivars to the fungal pathogen *Tilletia indica*. Historically, the disease has been limited to autumn-sown spring-habit wheat areas and not associated with winter wheat. In 1997, Karnal bunt was observed on winter wheat in limited regions of Texas. This region marks the southern end of the contiguous U.S. central winter wheat belt, which extends north into Canada. The aim of this study was to assess the levels of disease resistance in winter wheat. Fifty U.S. and European winter wheat cultivars were tested using two different greenhouse inoculation procedures. For each cultivar, 12 spikes in boot were inoculated by boot-injection with a sporidial suspension (1.0 ml/boot, 10,000 spores ml^{-1}), and 12 other emerged spikes were spray-inoculated with the same concentration. The experiment was repeated for three seasons. Among cultivars, mean seed infection ranged from 2.1 to 87.2% and 0 to 15.6% for boot-injected and spray-inoculated treatments, respectively. Results showed that the majority of winter wheat cultivars tested were susceptible to Karnal bunt.

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Keywords Disease incursion · Germplasm evaluation · Partial bunt

Introduction

Tilletia indica the causal agent of Karnal bunt (KB) of wheat, is a pathogen of international regulatory concern. Although KB has little effect on wheat yield, it may result in reduced exports due to international phytosanitary trade barriers. Many countries, including members of the European Union and the United States (U.S.), require phytosanitary certification when importing wheat from countries where KB is known to occur (Bonde et al. 1996; Sansford 1998). Until 1996, the disease had been reported in India, Pakistan, Nepal, Iraq (Warham 1986), Iran (Torarbi et al. 1996), Mexico (Duran 1972) and Brazil (Da Luz et al. 1993). In 1996–1997, KB was first reported from the southwestern U.S. in Arizona, southern California and Texas (Ykema et al. 1996). More recently, the disease was reported in South Africa (Crous et al. 2001).

Many aspects about the physiological or epidemiological factors that determined KB spread and establishment potential are unknown (Jones 2009; Peterson et al. 2006). Unlike other wheat-infecting *Tilletia* species (Hoffmann 1982) (*T. contraversa, T tritici,* and *T. laevis*), *T. indica* is not a systemic pathogen. Infection is initiated during heading when the exposed glumes are penetrated by haploid allantoid secondary sporidia arising from germinating basidiospores which originate from teliospores germinating on the soil surface. The formation of dikaryotic hypae is assumed to occur following establishment of infection, within the colonised rachis (Dhaliwal and Singh 1988; Goates 1988). It is believed that the disease cycle requires sufficient surface soil moisture to support teliospore germination and a high level of humidity and free moisture to support the infection process. The highest levels of disease are usually observed when cool temperatures, cloud cover and several days of rain occur during flowering (Joshi et al. 1983). Historically, the disease has only been reported on autumn-sown, spring wheat. Furthermore, the major distribution of the disease is confined primarily to irrigated agriculture in arid or semi-arid climates with well-defined dry and rainy seasons (Jones 2009). Prior to 1996, KB had not been reported in autumn-sown winter wheat or spring-sown cultivars. These observations suggest there are epidemiological limits to the spread of this disease.

However in 1997, KB was discovered on winter wheat grown under rain-fed field conditions in central Texas and 4 years later in northern Texas (Rush et al. 2005) indicating that KB was not limited to warmer wheat environments. The discovery in northern Texas raises additional concerns about the potential spread of KB since the northern Texas locations comprise the southern boundary of the U.S. Great Plains wheat production area that is contiguous through the central U.S. to Canada (Rush et al. 2005).

The unexpected establishment outside an irrigated fall-planted spring wheat production region on winter wheat raises questions about the susceptibility of this type of wheat to KB. Spring wheat nurseries in India and Mexico, have identified sources of resistance, and field-resistant cultivars have been released (Fuentes-Davila and Rajaram 1994; Singh and Dhaliwal 1989), but there has been little work on relative susceptibility of winter wheat to KB. This lack of data may be due to the earlier absence of KB from winter wheat production areas. To better understand host resistance and the potential for KB to pread further into winter wheat production areas, we report here results from artificial inoculations of 50 commonly-grown U.S. and European winter wheat cultivars with allantoid sporidia of *T. indica.*

Materials and methods

Growing plants

In October, 15 commonly-grown winter wheat cultivars from the Northwest U.S. (NW), 17 from the Central U.S. (CEN), and 18 from Europe (EU) (Table 1) were planted in 18-l plastic pots, 25 seeds per pot, two pots per cultivar. The spring wheat cv. WL-711 was used as a susceptible check. Plants were placed outside on a large asphalt pad and maintained under natural Frederick, Maryland climatic conditions for vernalisation. In April, general purpose N-P-K 20-20-20 (J.R. Peters, Inc; Allentown, PA) fertiliser was applied (3.8 g l^{-1}) to each pot every 2 weeks. The systemic insecticide, 1% Marathon (Olympic Horticultural Products Inc, Mainland, PA, USA), was applied once to the soil at a rate of 0.08 g (a.i.) per pot. Sets of WL-711 were planted at two-week intervals in three 18-l and 20 7.5-l pots, 25 and five seeds per pot, respectively, and grown in the greenhouse to serve as a comparative, highly susceptible control. As each winter wheat cultivar reached flag leaf stage (Feekes growth stage (GS) 9) (Large 1954), plants were moved into the greenhouses of the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) Biosafety Level-3 (BSL-3) Plant Disease Containment Facility (Melching et al. 1983) for observation. Greenhouse temperatures were maintained within a range of 18 to 28°C with an average of 23°C.

Inoculum production

Earlier studies comparing isolates of *T. indica* from India, Mexico and Pakistan did not reveal the existence of races among field populations (Bonde et al. 1996). The isolate of *T. indica* used in this study originated from infected wheat kernels obtained from a quarantined grain elevator in Blythe, California in 1996. Because freshly harvested teliospores often exhibit a period of dormancy and low germination (Aujla et al. 1986), teliospores to be used in this study were produced using the boot inoculation technique described later (Bonde et al. 1996) to inoculate wheat cv. WL-711 in the ARS BSL-3 containment facility 1 year prior to the initiation of this study. Production of allantoid sporidia followed procedures described by Bonde et al. (1996).

Table 1 Infection averages (data pooled from three replicated studies) of physiological resistance to Karnal bunt of wheat for 50 winter wheat cultivars grown in the United States and Europe

Winter wheat cultivar	Infected spikes/ total spikes[a]	Infected seed/ total seed[b]	% infected seed[c]	Confidence interval for % infected seed [d]	Bunted seed rating category[e]				COI[f]	Disease rating[g]
Western U.S.					0.25	0.50	0.75	1.00		
Bighorn	10.3/12.0	154.3/297.0	52.0	28.6	8.1	4.2	7.6	11.1	24.2	HS
Blizzard	10.0/12.0	122.7/247.3	49.6	2.3	11.5	9.3	9.5	6.8	32.3	HS
Eltan	2.0/11.3	8.3/390.0	2.1	2.6	0.3	0.1	1.0	0.3	0.9	R
Hill 81	8.3/12.0	83.0/353.3	23.5	21.8	2.5	2.9	4.6	5.0	9.5	MS
Judith	10.7/12.0	177.7/314.7	56.5	14.9	3.6	16.2	33.3	11.7	34.8	HS
Lewjain	7.7/12.3	74.7/369.3	20.2	23.5	6.7	10.5	9.0	2.3	12.6	S
Malcolm	9.3/12.0	155.7/351.7	44.3	18.4	10.7	14.8	25.5	3.9	27.1	HS
Manning	10.3/12.0	251.3/320.0	78.5	16.6	6.9	15.3	29.1	24.3	40.6	HS
McGuire	9.0/12.0	150.7/280.7	53.7	51.0	6.2	8.5	14.7	15.2	29.6	HS
Neeley	8.3/12.0	142.7/290.7	49.1	48.9	11.0	9.4	13.8	8.2	28.6	HS
Rampart	4.7/12.0	50.3/228.3	22.0	31.3	4.1	4.7	4.3	3.8	15.4	S
Rocky	8.3/12.0	112.0/264.0	42.4	30.2	3.3	2.5	3.5	18.4	19.6	S
Rough Rider	5.3/11.7	96.0/255.0	37.6	45.6	4.7	3.7	2.0	17.1	18.9	S
Stephens	8.0/11.3	83.7/314.3	26.6	24.4	5.0	2.5	1.3	8.2	11.3	S
Vanguard	6.7/12.0	57.0/196.3	29.0	29.8	6.2	4.8	8.8	3.0	24.8	HS
Central U.S.										
2137	8.7/12.7	135.0/341.0	39.6	30.2	7.3	6.7	16.0	15.9	20.4	HS
2163	10.3/12.7	189.7/331.3	57.2	25.5	11.2	6.8	22.9	13.7	29.6	HS
Arapahoe	9.3/12.0	227.7/356.7	63.8	35.6	5.7	8.9	18.8	39.1	27.3	HS
Betty	10.0/12.0	193.3/346.3	55.8	13.6	12.2	11.0	23.1	11.6	29.5	HS
Custer	9.7/11.3	195.7/278.0	70.4	22.3	4.1	8.7	21.1	29.3	37.5	HS
Halt	9.3/11.7	141.7/270.7	52.3	28.6	3.0	9.7	19.6	10.7	31.3	HS
Heyne	9.0/12.0	188.3/310.7	60.6	43.0	8.4	9.9	16.7	20.4	30.8	HS
Ike	10.7/12.0	241.0/318.3	75.7	30.0	6.6	15.1	39.6	17.1	43.0	HS
Jagger	9.0/11.7	139.3/334.7	41.6	39.6	8.6	8.7	12.4	7.4	21.3	HS
Karl 92	8.3/11.0	122.3/241.7	50.6	12.5	7.1	6.7	13.3	4.8	32.2	HS
Nekota	11.0/12.0	135.0/256.7	52.6	37.2	5.5	10.5	23.5	11.7	35.9	HS
TAM 107	8.7/10.7	86.3/179.3	48.1	55.2	1.9	2.0	1.9	9.6	30.0	HS
TAM 200	10.7/12.0	173.3/226.7	76.5	9.4	3.6	6.2	13.8	28.7	44.1	HS
TAM 202	10.7/12.0	191.3/267.3	71.6	22.9	6.8	10.0	17.0	18.3	39.4	HS
TAM 301	12.0/13.7	220.7/311.7	70.8	17.0	6.4	10.8	30.5	18.2	38.0	HS
Tomahawk	11.0/12.3	271.3/393.0	69.0	11.2	9.5	14.7	15.7	28.5	27.7	HS
Yumar	11.3/12.3	192.7/221.0	87.2	9.1	2.3	6.9	17.0	16.4	51.3	HS
European Union										
Batis	8.7/12.0	99.0/252.0	39.3	29.2	7.2	8.6	12.5	8.8	26.8	HS
Brigadier	2.7/10.3	24.7/252.7	9.8	24.8	1.3	1.3	0.5	13.0	6.4	MS
Charger	7.3/10.7	60.7/277.3	21.9	30.0	4.3	5.8	5.5	5.5	13.5	S
Consort	8.0/11.3	62.3/402.3	15.5	18.9	2.4	5.7	9.5	3.8	8.2	MS
Contra	8.0/11.3	74.3/215.7	34.5	31.4	5.2	6.2	7.8	7.5	24.8	HS
Greif	9.0/11.3	61.7/230.7	26.7	23.2	4.1	7.2	10.8	4.0	21.1	HS
Haven	8.7/12.0	90.0/239.0	37.7	18.5	4.4	3.5	5.8	12.3	21.5	HS

Table 1 (continued)

Winter wheat cultivar	Infected spikes/ total spikes[a]	Infected seed/ total seed[b]	% infected seed[c]	Confidence interval for % infected seed [d]	Bunted seed rating category[e]				COI[f]	Disease rating[g]
European Union					0.25	0.50	0.75	1.00		
Hereward	7.7/12.0	83.7/288.0	29.1	14.8	1.9	7.9	16.8	6.2	19.3	S
Napier	5.7/12.0	28.7/262.0	10.9	8.6	2.8	2.0	2.0	1.4	6.8	MS
Reaper	6.3/12.0	36.7/244.3	15.0	10.8	2.6	2.4	2.0	4.1	9.0	S
Rialto	7.7/11.7	128.0/270.3	47.3	33.4	4.8	10.7	17.3	10.8	29.6	HS
Riband	6.3/12.0	34.0/301.0	11.3	8.2	2.1	4.7	4.3	1.3	7.4	S
Ritmo	7.7/12.0	70.0/172.7	40.5	37.4	3.5	7.1	9.3	7.2	35.0	HS
Savannah	5.0/12.3	68.7/330.7	20.8	35.2	4.8	6.2	16.3	4.1	14.5	S
Shango	10.0/12.0	113.7/278.3	40.8	12.0	3.4	6.9	19.8	8.8	25.5	HS
Soissons	8.7/12.0	142.3/335.0	42.5	33.3	6.8	17.6	22.3	11.5	26.6	HS
Tremie	8.3/12.0	114.7/253.3	45.3	31.6	5.7	12.7	22.5	5.2	34.0	HS
Vivant	6.7/12.0	58.3/318.7	18.3	4.6	1.5	8.1	8.3	5.6	11.4	S

[a] Mean number of infected wheat spikes and total spikes determined from the three replicated experiments.

[b] Mean number of infected seeds and total seeds from the three replicated experiments.

[c] Percentage of infected seed derived from the mean infected seed data.

[d] 95% confidence interval for the mean of % infected seed.

[e] Derived from the mean number of bunted seeds in each infection category multiplied by the category value (0.25, 0.5. 0.75 or 1.00)

[f] Coefficient of Infection (COI) = [(0.25×mean no. seeds in infection category 0.25)+(0.5×mean no. of seeds in infection category 0.5) = (0.75×mean no. seeds in infection category 0.75)+(1.0×mean no. seeds in infection category 1.00)]×100/mean total grains from the three experiments.

[g] Disease Resistance is based on COI: Highly Resistant (HS) = 0; Resistant (R) = 0.1 to 5.0; Moderately Susceptible (MS) = 5.1 to 10; Susceptible (S) = 10.1 to 20; and Highly Susceptible (HS) >20.

Physiological resistance testing

The most common method used for screening wheat cultivars for physiological resistance to KB is by injecting a suspension of allantoid sporidia, at a known concentration, into the boot cavity of the wheat plant at Feekes GS 9. The method has been standardised in breeding programme in India and Mexico (Aujla et al. 1980). Although this method gives fairly reliable results because the environment within the closed boot is highly favourable for infection, it also by-passes morphological barriers to infection that may be important factors in field resistance (Warham 1986, 1988; Riccioni et al. 2008).

When wheat plants reached boot-stage (Feekes GS 9), a 1.0 ml of sporidial suspension was injected by means of a 2-cm^3 Cornwall syringe with 20-guage needle into each of 12 boots per cultivar (Fig. 1). During inoculation of each cultivar 12 wheat spikes of the susceptible WL-711 were injected with sporidia, and a second pot with five wheat spikes was injected with 1.0 ml of water. Not all cultivars reached the stage suitable for inoculation on the same day so inoculations were performed over a period of several weeks. Inoculated plants were placed in 18 m^2 greenhouse cubicals fitted with a controlled overhead misting system (Mist-o-matic, E.C. Geiger Inc. Harleysville, PA, USA) and a 70% overhead shade screen. The greenhouse was maintained between 18 and 22°C and a relative humidity (RH) of 85 to 97%. After 72 h, plants were moved into another greenhouse (21 to 25°C) until plants matured. The experiment was repeated three times.

Morphological (field) resistance testing

In contrast to the more aggressive boot-injection inoculation method, a second method of inoculation was used concurrently in a parallel study. This method of inoculation would be likely to show disease levels more reflective of natural field resistance than boot-injection (Warham 1986, 1988; Riccioni et al. 2008). Spray inoculations were per-

Fig. 1 Boot injection of wheat test plants (Feekes GS 9) with a suspension of *Tilletia indica* allantoid sporidia

formed as described by Singh and Krishna (1982). As the wheat spikes emerged from the plants (Feekes GS 10.1 to 10.3), 12 spikes from each cultivar were inoculated by spraying a suspension of 1.0×10^4 allantoid sporidia ml^{-1}, 1.0 ml per wheat spike, with an atomiser (air pressure=0.35 kg cm^{-2}) (Devilbliss 163, Micromedics, St. Paul, MN). As with the boot-injection protocol, pots of WL-711 were inoculated and treated with water as a control each time cultivars were inoculated. Inoculated plants were misted and monitored as described. The experiment was repeated three times.

Disease assessment

At maturity, individual spikes from each cultivar/ inoculation method were harvested and hand threshed. The number of infected plants and the number of infected grains in each wheat spike were recorded. For each inoculation protocol and cultivar tested, the mean infection level per cultivar was determined and data pooled to determine the mean infection percentage and confidence interval for all three experimental years. For each inoculation method, analysis of variance (ANOVA) among cultivars grouped by region was conducted for percent infected seed and infected plants with the means separated using ANOVA (Statview ver. 5.0.1; SAS Inst.; Cary, NC; USA) ($P<0.05$). For each inoculation method, the percentage of infected seed in WL-711 controls was correlated with the corresponding cultivar inoculation date. Data for each cultivar were pooled across the three studies to determine the mean percentage and confidence interval to compare percentage infection observed in test cultivars with the percentage in the WL-711 susceptible check utilising ANOVA.

The severity of the infection was rated by the method of Aujla et al. (1989) and used to calculate the coefficient of infection (COI). The rating system was based on assigning each seed from an individual wheat spike into one of five infection categories: 0, 0.25, 0.50, 0.75, or 1.00, where 0 represented a healthy seed; $0.25=0\%<x\leq25\%$ of the seed colonised; $0.50=25\%<x\leq50\%$ colonised; 0.75=if $50\%<x\leq75\%$ colonised; 1.00=if colonisation >75% (Fig. 2) COI was determined by multiplying the number of seeds in each category by the infection category, sum of the products of each category, determined by multiplying by 100 then dividing by the total number seeds in the group. In this study, for each cultivar, the COI was derived by pooling the data from all three experiments to determine the mean number of seeds in each infection category.

Fig. 2 *Tilletia indica* bunted winter wheat seeds representing the four infection categories (0.25, 0.50, 0.75 and 1.00) used to determine the COI (Aujla et al. 1989) as a measure of host resistance

The level of susceptibility was determined based on COI, where 0 = Highly Resistant (HR); 0.1 to 5.0 = Resistant (R); 5.1 to 10 = Moderately Susceptible (MS); 10.1 to 20.0 = Susceptible (S), and >20.0 = Highly Susceptible (HS).

Results

Physiological resistance

KB was observed in all winter wheat cultivars tested when inoculated by boot injection. Only cv. Eltan (NW), was scored as resistant in all three experiments. None of the uninoculated, highly susceptible, WL-711 control plants became infected. The average seed infection observed in NW, CEN and EU cultivars over the three experiments ranged from 2.1 to 78.5%, 39.6 to 87.2% and 9.8 to 47.3%, respectively (Table 1). The mean percent infection (SD) for all cultivars by region was from 39.1 (18.9) 61.4 (13.2), and 28.2 (13.0), for NW, CEN, and EU, respectively (Table 1). ANOVA among cultivars injected at boot stage found significant differences between regions for both percent infected plants (P=0.0007) and percent infected seeds (P=<0.0001).

Table 2 Average percentage of infected seed and confidence interval (CI) for each cultivar inoculated by boot-injection of spikes with allantoid sporidia at Feekes GS 9, and the average percent of infection observed in the Karnal bunt susceptible spring wheat cv. WL-711, inoculated as control plants along with each test cultivar

Wheat cultivar	Mean % Infected seed[a] (CI)[b]	% Infected WL711 seed[c] (CI)	Wheat cultivar	Mean % Infected seed (CI)	% Infected WL711 seed[c] (CI)
2137	39.6 (30.2)	64.4 (48.4)	Manning	78.5 (16.6)	79.7 (7.6)
2163	57.2 (25.5)	81.7 (23.0)	McGuire	53.7 (51.0)	80.4 (8.9)
Arapahoe	63.8 (35.6)	71.3 (3.8)	Napier	10.9 (8.6)	40.7 (35.0)
Batis	39.3 (29.2)	59.9 (42.9)	Neeley	49.1 (48.9)	81.6 (14.2)
Betty	55.8 (13.6)	80.3 (9.0)	Nekota	52.6 (37.2)	50.7 (49.9)
Bighorn	52.0 (28.6)	62.4 (9.9)	Rampart	22.0 (31.3)	48.2 (35.3)
Blizzard	49.6 (2.3)	63.8 (10.5)	Reaper	15.0 (10.8)	70.3 (17.5)
Brigadier	9.8 (24.8)	65.2 (18.6)	Rialto	47.3 (33.4)	57.7 (24.6)
Charger	21.9 (30.0)	75.1 (16.2)	Riband	11.3 (8.2)	53.5 (23.7)
Consort	15.5 (18.9)	74.3 (27.0)	Ritmo	40.5 (37.4)	56.4 (23.7)
Contra	34.5 (31.4)	62.5 (33.0)	Rocky	42.4 (30.2)	59.5 (42.6)
Custer	70.4 (22.3)	83.8 (4.4)	Rough Rider	37.6 (45.6)	61.7 (28.5)
Eltan	2.1 (2.6)	42.2 (32.2)	Savannah	20.8 (35.2)	52.2 (46.1)
Greif	26.7 (23.2)	62.4 (9.9)	Shango	40.8 (12.0)	73.2 (11.1)
Halt	52.3 (28.6)	59.0 (58.7)	Soissons	42.5 (33.3)	56.4 (38.8)
Haven	37.7 (18.5)	70.3 (17.5)	Stephens	26.6 (24.4)	66.7 (17,7)
Hereward	29.1 (14.8)	70.3 (17.5)	TAM 107	48.1 (55.2)	54.8 (48.2)
Heyne	60.6 (43.0)	88.7 (10.1)	TAM 200	77.6.(9.4)	88.3 (10.3)
Hill 81	23.5 (21.8)	69.2 (16.0)	TAM 202	71.6 (22.9)	56.2 (55.2)
Ike	75.7 (30.0)	83.1 (13.3)	TAM 301	70.8 (17.0)	88.3 (10.3)
Jagger	41.6 (39.6)	82.7 (95.3)	Tomahawk	69.0 (11.2)	81.0 (16.4)
Judith	56.5 (14.9)	75.6 (3.8)	Tremie	45.3 (31.6)	56.6 (39.0)
Karl 92	50.6 (12.5)	85.7 (5.1)	Vanguard	29.0 (29.8)	58.5 (42.2)
Lewjain	20.2 (23.5)	54.8 (46.7)	Vivant	18.3 (4.6)	47.2 (14.2)
Malcolm	44.3 (18.4)	71.4 (19.0)	Yumar	87.2 (9.1)	87.2 (10.6)

[a] Average percent infected Karnal bunt infected seed for each test cultivar in three study replications.

[b] 95% Confidence interval for mean percent seed infection.

[c] Average percent seed infection obtained in Karnal bunt susceptible bread wheat cv., WL 711, when inoculated in conjunction with each test cultivar, for all three experiments.

Table 3 Infection averages (data pooled from three replicated studies) of morphological resistance to Karnal bunt of wheat for 50 winter wheat cultivars grown in the United States and Europe

Winter wheat cultivar	Infected spikes/ total spikes[a]	Infected seed/ total seed[b]	% infected seed[c]	Confidence interval for % infected seed [d]	Bunted seed rating category[e]				COI[f]	Disease rating[g]
Western U.S.					0.25	0.50	0.75	1.00		
Bighorn	2.0/12.0	5.7/261.3	2.5	4.8	1.5	1.5	5.0	2.9	4.1	R
Blizzard	2.0/12.3	9.7/293.0	3.30	6.5	2.8	2.0	8.3	3.8	5.8	MS
Eltan	0.3/12.0	0.7/435.3	0.20	0.4	0.0	1.0	0.9	0.3	0.5	R
Hill 81	1.7/11.7	7.3/501.7	1.71	3.4	2.3	2.5	6.0	1.9	2.5	R
Judith	2.7/12.0	22.0/361.3	5.90	11.6	5.0	2.5	21.1	7.6	10.0	MS
Lewjain	0.7/12.0	10.0/389.3	2.82	5.5	3.7	1.0	6.7	2.5	4.1	R
Malcolm	1.0/12.0	5.7/293.0	5.50	10.8	1.2	1.5	6.4	2.1	5.3	MS
Manning	2.0/12.0	10.7/420.0	2.55	3.2	2.5	1.0	6.6	2.1	2.9	R
McGuire	0.3/12.3	2.0/279.7	0.88	1.7	0.3	0.3	0.8	0.4	0.6	R
Neeley	1.3/12.0	5.7/382.0	1.56	3.1	1.3	1.5	5.4	2.0	2.7	R
Rampart	1.0/12.0	3.7/320.0	1.18	2.3	1.2	0.0	2.8	1.2	1.6	R
Rocky	3.0/12.0	17.7/300.7	10.29	19.7	5.3	2.5	14.2	11.2	11.0	S
Rough Rider	3.3/12.0	15.3/294.0	4.78	6.9	4.9	0.0	9.5	4.6	6.5	MS
Stephens	2.0/12.3	14.7/378.0	3.71	7.3	5.7	1.5	9.2	3.1	5.1	MS
Vanguard	1.0/11.3	3.3/226.0	1.76	2.4	0.5	1.0	2.5	1.7	2.5	R
Central U.S.										
2137	1.0/11.7	2.0/305.0	0.8	1.5	0.3	0.0	1.5	0.7	0.8	R
2163	1.3/12.3	6.0/385.3	1.5	0.8	0.9	0.2	0.5	2.0	0.9	R
Arapahoe	1.3/12.0	10.3/285.7	3.3	3.7	1.8	1.3	1.5	1.0	2.0	R
Betty	0.3/12.0	4.7/340.7	1.2	2.4	0.6	1.2	0.0	0.0	0.5	RR
Custer	2.7/12.0	26.3/272.3	6.7	13.1	2.5	3.5	3.5	5.0	5.3	R
Halt	1.0/11.7	4.7/291.3	1.7	2.2	2.6	1.3	2.5	13.3	6.8	MS
Heyne	1.7/11.7	7.0/309.7	1.7	2.7	0.0	0.0	0.0	1.3	0.4	MS
Ike	1.7/10.3	11.7/193.7	7.4	7.6	1.0	0.7	1.0	0.7	1.7	R
Jagger	0.3/13.0	5.3/347.0	1.5	5.4	0.4	1.8	0.0	0.0	0.7	R
Karl 92	2.3/11.7	13.0/246.7	9.6	2.9	3.0	1.7	2.0	4.7	4.6	R
Nekota	4.3/12.0	25.0/310.0	8.5	5.2	3.5	3.5	8.5	8.7	7.8	MS
TAM 107	2.3/12.0	14.0/131.0	8.1	9.6	0.4	0.5	0.0	0.3	1.0	R
TAM 200	4.3/12.3	38.0/286.0	15.6	19.3	4.3	6.2	10.0	14.7	12.3	S
TAM 202	1.3/12.0	9.0/298.3	3.6	5.2	1.8	1.3	0.5	0.3	1.3	MS
TAM 301	3.3/12.7	10.7/293.7	4.1	5.5	3.3	1.0	2.3	8.0	4.9	R
Tomahawk	2.0/12.0	13.7/293.0	4.9	4.8	2.9	1.0	1.8	3.0	3.0	R
Yumar	3.0/11.3	34.0/336.0	9.6	17.3	3.5	8.7	14.0	16.0	12.5	S
European Union										
Batis	1.7/11.0	6.7/383.0	2.1	4.1	1.1	0.5	0.5	0.7	0.7	R
Brigadier	2.0/11.3	25.3/404.3	6.5	12.8	3.9	2.0	1.5	2.3	2.3	R
Charger	1.3/7.7	7.7/291.7	2.3	4.4	2.8	0.9	1.8	2.7	2.7	R
Consort	3.0/12.3	33.7/588.7	6.4	12.6	5.0	3.3	5.0	3.4	3.4	R
Contra	1.0/12.0	4.3/481.3	1.0	1.9	0.7	0.7	0.3	0.4	0.4	R
Greif	0.0/12.0	0.0/425.3	0.0	0	0.0	0.0	0.0	0.0	0.0	HR
Haven	1.7/11.0	6.7/364.3	2.2	2.7	0.8	0.2	1.2	1.0	1.0	R

Table 3 (continued)

Winter wheat cultivar	Infected spikes/ total spikes[a]	Infected seed/ total seed[b]	% infected seed[c]	Confidence interval for % infected seed [d]	Bunted seed rating category[e]				COI[f]	Disease rating[g]
European Union					0.25	0.50	0.75	1.00		
Hereward	2.3/12.0	12.3/388.3	3.7	7.3	4.2	1.8	6.6	6.3	6.3	MS
Napier	2.3/12.0	12.7/423.0	2.9	5.0	2.2	1.3	0.3	1.0	1.0	R
Reaper	0.3/12.0	0.3/270.3	0.2	0.4	0.8	0.7	3.3	3.0	3.0	R
Rialto	1.0/11.7	4.7/410.3	1.3	1.6	0.5	0.4	0.9	0.8	0.8	R
Riband	2.3/11.0	19.0/318.0	5.9	0.8	2.0	0.9	1.1	1.5	1.5	R
Ritmo	0.3/12.0	1.0/357.3	0.3	0.5	0.3	0.9	0.3	0.7	0.7	R
Savannah	1.0/12.7	1.7/439.3	0.4	0.7	0.4	0.0	0.0	0.1	0.1	R
Shango	0.3/11.7	6.0/475.7	1.4	2.7	0.8	0.9	1.0	0.8	0.8	R
Soissons	1.3/13.0	9.0/255.3	12.0	22.8	1.9	0.0	0.0	0.5	0.5	R
Tremie	2.0/12.0	12.7/416.7	4.0	7.8	2.4	1.6	0.3	1.1	1.1	R
Vivant	2.0/12.0	8.3/397.7	3.5	6.9	1.3	0.2	1.0	1.0	1.0	R

[a] Mean number of infected wheat spikes and total spikes determined from the three replicated experiments.

[b] Mean number of infected seeds and total seeds from three replicated experiments.

[c] Percentage of infected seed derived from the mean infected seed data.

[d] 95% confidence interval for the mean of % infected seed.

[e] Derived from the mean number of bunted seeds in each infection category multiplied by the category value (0.25, 0.5. 0.75 or 1.00)

[f] Coefficient of Infection (COI) = [(0.25×mean no. seeds in infection category 0.25)+(0.5×mean no. of seeds in infection category 0.5) = (0.75×mean no. seeds in infection category 0.75)+(1.0×mean no. seeds in infection category 1.00)]×100/mean total grains from the three experiments.

[g] Disease Resistance is based on COI: Highly Resistant (HS) = 0; Resistant (R) = 0.1 to 5.0; Moderately Susceptible (MS) = 5.1 to 10; Susceptible (S) = 10.1 to 20; and Highly Susceptible (HS) >20.

The average number of infected seeds and the corresponding level of infection observed in the susceptible spring wheat cv. WL-711 for each inoculation are presented in Table 2. On average, the level of bunt infection obtained from the inoculated test cultivars was 37.4% less than that for WL-711. ANOVA between infection in test cultivars and WL-711 was significant at $P=1.8\times10^{-10}$.

Morphological resistance

The percent seed infection observed in NW, CEN and EU cultivars over the three experiments ranged from 0.2 to 10.3%, 0.8 to 15.6%, and 0 to 12.0%, respectively (Table 3). SD for all cultivars by region was 3.2 (2.56), 5.3 (4.91) and 3.1 (3.0) for NW, CEN, and EU, respectively (Table 3). Results of ANOVA for the three regions indicate there were no significant differences in infection level between cultivars in the CEN vs EU ($P=0.059$), CEN vs NW ($P=0.088$), and EU vs NW ($P=0.920$) comparisons. No regional differences were observed for either percent infected plants ($P=0.9151$) or percent infected seed ($P=0.5249$) for plants inoculated by the spray method.

The average number of infected seeds and the corresponding level of infection observed in WL-711 for each inoculation are presented in Table 4. On average the level of bunt infection obtained from the inoculated test cultivars was 47.2% less than that observed in WL-711. ANOVA between the test cultivars and WL-711 was significant ($P=9.8\times10^{-5}$).

Boot injection vs spray inoculation

As expected, the levels of infection resulting from the boot-injection method were much higher than those obtained by the sporidial spray method, thus affecting the classification of individual cultivars by susceptibility group. Sixty-six percent of the cultivars inoculated by sporidial-spray were rated as R. None of the cultivars

Table 4 Average percentage of infected seed (confidence interval) for each cultivar inoculated by spraying wheat spikes with allantoid sporidia at Feekes GS 10.1-3, and the average percent of infection observed in the Karnal bunt susceptible spring wheat cv. WL-711, inoculated as control plants along with each test cultivar

Wheat cultivar	Mean % Infected seed[a] (CI)[b]	% Infected WL711 seed[c] (CI)	Wheat cultivar	Mean % Infected seed (CI)	% Infected WL711 seed[c] (CI)
2137	0.8 (1.5)	18.1 (21.3)	Manning	2.5 (3.2)	8.9 (10.0)
2163	1.5 (0.8)	18.1 (21.3)	McGuire	0.9 (1.7)	2.9 (3.3)
Arapahoe	3.3 (3.7)	3.2 (3.7)	Napier	2.9 (5.0)	4.4 (7.6)
Batis	2.1 (4.1)	4.4 (5.0)	Neeley	1.5 (3.1)	6.3 (7.1)
Betty	1.2 (2.4)	2.9 (3.3)	Nekota	8.5 (5.2)	0.1 (0.2)
Bighorn	2.5 (4.8)	0	Rampart	1.2 (2.3)	2.0 (2.3)
Blizzard	3.3 (6.5)	10.5 (11.9)	Reaper	0.2 (0.4)	6.3 (7.1)
Brigadier	6.5 (12.8)	8.6 (9.7)	Rialto	1.3 (1.6)	6.0 (6.8)
Charger	2.3 (4.4)	4.4 (5.0)	Riband	5.9 (0.8)	4.4 (5.0)
Consort	6.4 (12.6)	8.6 (9.7)	Ritmo	0.3 (0.5)	4.4 (5.0)
Contra	1.0 (1.9)	8.8 (9.9)	Rocky	10.3 (19.7)	0.1 (0.2)
Custer	6.7 (13.1)	7.8 (8.8)	Rough Rider	4.8 (6.9)	6.0 (6.8)
Eltan	0.2 (0.4)	8.6 (9.7)	Savannah	0.4 (0.7)	4.4 (5.0)
Greif	0.0 (0)	4.4 (5.0)	Shango	1.4 (2.7)	7.0 (7.9)
Halt	1.7 (2.2)	18.8 (21.3)	Soissons	12.0 (22.8)	0.1 (0.2)
Haven	2.2 (2.7)	8.9 (10.1)	Stephens	3.7 (7.3)	8.6 (9.7)
Hereward	3.7 (7.3)	13.1 (14.8	TAM 107	8.1 (9.6)	32.3 (36.6)
Heyne	1.7 (2.7)	3.2 (3.7)	TAM 200	15.6 (19.3)	16.4 (18.6)
Hill 81	1.7 (3.4)	10.5 (11.9)	TAM 202	3.6 (5.2)	3.2 (7.0)
Ike	7.4 (7.6)	2.9 (3.3)	TAM 301	4.1 (5.5)	2.7 (3.1)
Jagger	1.5 (5.4)	10.4 (11.8)	Tomahawk	4.9 (4.8)	1.6 (1.8)
Judith	5.9 (11.6)	1.7 (2.0)	Tremie	4.0 (7.8)	0.1 (0.2)
Karl 92	9.6 (2.9)	19.2 (21.8)	Vanguard	1.7 (2.4)	1.7 (2.0)
Lewjain	2.8 (5.5)	8.6 (9.7)	Vivant	3.5 (6.9)	8.6 (9.7)
Malcolm	5.5 (10.8)	6.3 (7.1)	Yumar	9.6 (17.3)	16.6 (18.8)

[a] Average percent infected Karnal bunt infected seed for each test cultivar for three study replications.

[b] 95% Confidence interval for mean percent seed infection.

[c] Average percent seed infection obtained in Karnal bunt susceptible bread wheat cv. WL 711, when inoculated in conjunction with each test cultivar, for all three experiments

Table 5 Single factor ANOVA comparing the difference in the levels of Karnal bunt infection obtained in winter wheat test cultivars inoculated by boot-injection and sporidial spraying with allantoid sporidia

Summary					
Groups	Count	Sum	Average	Variance	
Boot-injected	50	2,110.0	42.20	454.74	
Sporidial-spray	50	194.4	3.88	11.59	
ANOVA					
Source of Variation	SS	df	MS	F	P-value
Between groups	3,669,823	1	36,695.23	157.379	4.22292E-22
Among groups	22,850.03	98	23.16		
Total	59,545.26	99			

inoculated by sporidial spray were rated as HS, while only cv. Eltan, was rated R after using either inoculation method. Results from the two inoculation methods were significantly different (Table 5).

Discussion

The previous absence of KB in winter wheat growing areas could be attributed to a lack of introduction opportunity; however the current situation does not appear to fully support this concept. As in Arizona, where trace-back investigations showed the presence of teliospores in seed reference samples dating back to 1992, it is likely that a similar scenario may have occurred in Texas (Rush et al. 2005) from *T. indica*-contaminated seed planted prior to 1996. According to the Texas Agricultural Statistics Service, in 1990 and 2000, 10.2 and 5.6% of the wheat grown in Texas respectively, included durum wheat cultivars. More specifically, 20% of the wheat grown in the San Saba region was spring and durum cultivars while approximately 8% of the wheat grown in the Throckmorton, Baylor, Archer and Young region was durum cultivars. In two studies (Stein et al. 2005; Allan et al. 2008), sampling soil from wheat fields within the KB affected areas of Texas found teliospores of *T. indica* in all fields tested, including fields where no disease had been detected in earlier tests. Despite the presence of teliospores, no disease has been detected in the state of Texas since 2002 (Workneh et al. 2008), suggesting that the sequence of environmental events needed to trigger teliospore germination and head infection do not occur frequently in the these wheat regions.

Data from this study suggest that the physiological resistance identified in common U.S. and European winter wheat cultivars would not limit the possible spread of KB to winter wheat regions; however the levels of field resistance would be likely to limit disease incidence and yield loss. Although the levels of infection obtained by boot injection in this study were higher than that observed in similar field studies with spring wheat in India, the variability in infection levels among cultivars and between experiments was similar (Gill 1992). In the recently published European growth chamber study (Riccioni et al. 2008), when spring, durum and winter wheat cultivars were screened for KB resistance, significant variation was detected among cultivars within each wheat type, but no significant difference in infection levels was detected between the three groups, most notably between spring and winter wheat cultivars.

In this study CEN cultivars inoculated by boot-injection were significantly more susceptible to KB as a group than wheat from the other two regions. In contrast, no regional differences were observed in comparisons following sporidial-spray inoculations. These results would suggest less resistance is expressed in CEN cultivars due either to an absence of genes imparting physiological infection barriers, or to the metabolic effects of growing CEN cultivars in a mid-Atlantic environment. Reactions obtained by sporidial-spray inoculations may more closely mimic the natural infection process in the field and may therefore be a better measure for predicting cultivar performance.

Only cv. Eltan exhibited a high level of resistance in all tests, suggesting that it may be a good source of disease resistance. Results showed that winter wheat cultivars, although susceptible to KB, were significantly less susceptible than WL-711. Results obtained using the spray inoculation method showed that all cultivars expressed some level of morphological resistance to KB, and in most cases showed disease levels less or similar to WL-711. This would suggest that even if inoculum of *T. indica* were to move further into the U.S. winter wheat belt, the disease is unlikely to become a significant production factor, even if favourable disease-conducive field conditions occur.

Acknowledgements The author would like to acknowledge the technical support of Richard Creager, Lillian Zuback, Kayscha Morris, Megan Reeher, Kelly Poltrok and Kathy Fronda of USDA ARS FDWSR, Fort Detrick, MD. I thank Don Mathre, Montana State University, Bozeman; Gina Brown-Guedira, ARS, Raleigh, NC; Guillermo Fuentes-Davila, INIFAP, Cd Obregon Sonora, Mexico, and Bill Hollins, Plant. Breeding International, Cambridge UK for recommending and providing wheat germplasm for testing.

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