Effect of *Tilletia indica* Infection on Viability, Germination, and Vigor of Wheat Seed

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ABSTRACT

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The effect of Karnal bunt (*Tilletia indica*) infection on viability, germination, and vigor of wheat seed was determined using the topographical tetrazolium viability test, the rolled paper towel germination test, and the accelerated aging vigor test. Infection had very little effect on seed viability, irrespective of the age of the seed, while germination of infected seed appeared to depend upon the wheat cultivar and age of the seed. In contrast, there was a significant reduction in vigor of infected seed. Infected seeds would therefore appear to have a lower survival rate in storage compared to healthy seeds of the same seed lot.

Karnal bunt, caused by *Tilletia indica* (Mitra) (synonym *Neovossia indica* (Mitra) Mundkur), is a floral-infecting organism that infects seed of bread wheat (14), durum wheat, and triticale (1). Not all of the spikes on a plant are infected and, within a spike, only a few spikelets are bunted (13,16). Infected plants generally show a reduction in the length of spikes and in the number of spikelets produced (15). In severely infected spikelets, the glumes may spread apart near maturity, exposing the bunted grains, but this is not a common symptom (10). Infected grains are irregularly distributed in the spike; some are completely infected but most are only partially infected (11). As the amount of grain infection increases, the weight of the infected grains decreases (5,6,19). The difference in weight between heavily infected grains and slightly infected grains can be as much as 50% (7).

Studies of the relationship between Karnal bunt infection and seed germination have shown that as infection increases, the percentage of seed germination decreases. Seeds with moderate to severe infections tend to produce a greater percentage of abnormal seedlings (2,3,18,20,21). Karnal bunt infection had no significant effect on seed germination in studies conducted at CIANO (Centro de Investigaciones Agricolas del Noroeste, Ciudad Obregon, Mexico) using the rolled paper towel germination test (C. Garcia, *personal communication*).

This study investigated the influence of Karnal bunt infection on viability (the potential of a seed to germinate), germination (germination under favorable conditions), and vigor (germination under unfavorable conditions) of wheat seed. Seed viability was measured using the topographical tetrazolium viability test; germination was measured using the rolled paper towel germination test; and vigor was measured using the accelerated aging vigor test.

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MATERIALS AND METHODS

Karnal bunt-infected wheat seed of the cultivars CIANO 79, Tesia, and Seri was obtained at harvest time from the Yaqui Valley, Sonora, Mexico, and stored at 20 C and 50% relative humidity (RH). Individual seeds for each cultivar were graded on a 0–5 scale (ranging from 0 for no infection to 5 for a completely bunted seed) according to the amount of infection present (Fig. 1).

Determination of seed viability, germination, and vigor. *Topographical tetrazolium viability test.* Four replicates of 100 seeds were selected from each infection grade. The seeds were soaked overnight (18 hr) in distilled water, drained, and then sectioned longitudinally through the embryo. One half of each seed was then immersed in 1% tetrazolium solution and incubated for 5 hr in the dark at 30 C. The seeds were rinsed twice after treatment and examined immediately. The 2,3,5-triphenyl-2H-tetrazolium chloride imbibed by living cells in the seed is reduced to form triphenyl formazan—a red, stable, and nondiffusible substance. Dead cells remain colorless, as there is no reaction of 2,3,5-triphenyl-2H-tetrazolium chloride; those cells with varying proportions of necrotic tissue are partially stained. Seeds were classified according to the parts of the embryo stained; classification was done using the International Seed Testing Association (ISTA) rules (12).

Rolled paper towel germination test. Four replicates of 100 seeds were taken at random from each infection grade and then placed, uniformly spaced, between moist paper towels. The towels were then rolled and placed in a plastic bag. The bags were kept at 20 C and given 12 hr of light per day. After 8 days, the number of normal seedlings, abnormal seedlings, and ungerminated seeds were determined according to the ISTA rules (12). To be classified as normal, a seedling must fall into one of the following categories: 1) intact seedlings, with all essential structures well developed, complete in proportion, and healthy; 2) seedlings with slight defects of their essential structures, provided they show an otherwise satisfactory and balanced development comparable to that of intact seedlings in the same test; 3) seedlings with secondary infection that would have fallen into categories 1 or 2 but for infection by fungi or bacteria from sources other than the parent seed.

Accelerated aging vigor test. One hundred seeds were placed into a wire-mesh basket in a glass jar containing enough water to maintain near-100% RH. The basket was supported by a galvanized mesh stand that held the seeds 6–8 cm above the water source. Four replicate jars were made for each seed sample and then placed in a water bath at 45 C for 48 hr (4). After accelerated aging, rolled paper towel germination tests were conducted as above. Those seeds producing normal seedlings according to ISTA rules (12) were considered vigorous. The decline in germination following accelerated aging is proportional to the initial physiological potential of the seed. For example, high-vigor seeds show only small decreases in germination following accelerated aging, while low-vigor seeds demonstrate marked decreases (8).

Initial seed germination studies were conducted with 2-mo-old seed of CIANO 79 and Tesia. The seed was put into storage at 20 C and 50% RH for 3 yr, and then another set of seed viability, germination, and vigor tests was conducted. The same tests were also performed on 2-mo-old Seri seed. For each test, the differences between grades of infection for individual cultivars were analyzed using Duncan's multiple range test (9).

RESULTS

Only completely bunted (grade 5) seeds showed a significant reduction in seed viability; however, they had at least 94% viability irrespective of the age of the seed (Table 1).

The influence of infection on seed germination appeared to depend upon the wheat cultivar and the age of the seed (Table 1). In the case of 2-mo-old seed, none of the infection grades of Seri and Tesia were significantly different at $P = 0.05$. For CIANO 79, however, germination of grade-5 seeds was significantly lower at $P = 0.05$ than that of other infection grades. After 3 yr in storage, germination of CIANO 79 seeds showed greater effects from infection. Seeds in grades 2–5 had significantly lower germination than did healthy (grade 0) and point-infected (grade 1) seeds. In the case of Tesia, there was more variability in seed germination across the different infection grades.

In contrast, infection had a significant effect upon the vigor of wheat seed. Among 2-mo-old Seri seeds, for example, completely bunted (grade 5) seeds had 30% vigor compared to 57% vigor in the healthy (grade 0) seeds. After 3 yr in storage, point-infected (grade 1) seeds of Tesia had 15.5% vigor compared to 42% vigor in healthy (grade 0) seeds. Three-year-old CIANO 79 seeds showed similar results.

DISCUSSION

Seed germination studies reported in the literature are unclear as to whether the embryos of wheat seed are infected by *T. indica.* Mitra (14) reported that the embryo is partially infected, while Munjal and Chatrath (17) stated that the embryo is not infected. Later, Rai and Singh (18) held that poor germination and production of abnormal seedlings indicate that the embryo as well as the endosperm may be damaged in severely infected seeds. This study used the topographical tetrazolium test to determine which embryos were alive. Test results showed that 94% of completely bunted seeds retain viable embryos, irrespective of seed age. These results indicate that Karnal bunt may not infect the embryo, but histological tests need to be conducted upon bunted seed to confirm this conclusion.

Completely bunted seeds have considerably less endosperm reserves for storage and germination (due to replacement of endosperm by the fungus) compared to healthy seeds of the same seed lot. Seed vigor tests showed that completely bunted seeds are less likely to germinate in unfavorable conditions, and that the longer infected seeds are stored, the lower their vigor when compared to healthy seeds. However, even completely bunted seeds can germinate if conditions are favorable.

Tests conducted on infected seed shortly after harvest show that seed germination is minimally affected. Completely bunted 2-mo-old seeds of some cultivars have slightly reduced germination, while those of other cultivars show no reduction at all. In 3-yr-old seed, though, there is a significant relationship between Karnal bunt infection and seed germination. These varying effects of infection on seed germination could explain the differences between results obtained in earlier seed germination studies conducted in Mexico (C. Garcia, *personal communication*) and those published in the literature. Although there is no mention of the exact ages of seeds tested in the published literature, the results of the various studies (2,3,18,20,21) seem to indicate that older seed was used. The results of seed germination tests using 3-yr-old seed in this study agree with results published in the literature. However, the seed germination of completely bunted seeds in this study is still higher than the values reported by other researchers. This difference could be due to the age of the seed tested, storage conditions under which the seed was kept, or the germination test methods used.

When assessing the effect of Karnal bunt infection on seed germination, a clear distinction must be made between seed germination (germination under favorable conditions) and seed vigor (germination under unfavorable conditions). If not, the results will have little meaning because of the significant difference between the two with regard to the influence of Karnal bunt infection.

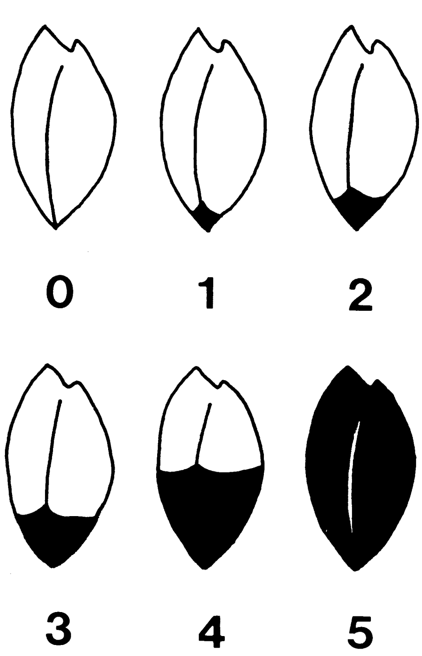

Fig. 1. Scale (0–5) of *Tilletia indica* infection severity in *Triticum aestivum.* Dark areas show the amount of bunting present in each infection grade.

Table 1. Effect of *Tilletia indica* on viability, germination, and vigor of wheat seed as determined by the topographical tetrazolium viability test, rolled paper towel germination test, and accelerated aging vigor test

Cultivar	Seed age	Infection grade	Viability (%)	Germination (%)[y] 1	2	3	Vigor (%) 1	2	3
CIANO 79	2 mo	0	...	99.3 a[z]	0.5	0.2	...	...	...
		1	...	98.0 a	2.0	0.0	...	...	...
		2	...	97.5 a	1.8	0.7	...	...	...
		3	...	97.0 a	2.5	0.5	...	...	...
		4	...	97.8 a	1.7	0.5	...	...	...
		5	...	88.5 b	4.0	7.5	...	...	...
Tesia	2 mo	0	...	94.0	3.3	2.7	...	...	...
		1	...	97.0	2.5	0.5	...	...	...
		2	...	96.8	2.5	0.7	...	...	...
		3	...	98.0	1.8	0.2	...	...	...
		4	...	95.0	4.0	1.0	...	...	...
		5	...	95.5	3.0	1.5	...	...	...
Seri	2 mo	0	98.5 ab	96.0	2.5	1.5	57.0 a	9.8	33.3
		1	99.3 a	97.8	1.7	0.5	53.5 a	12.3	34.3
		2	97.8 ab	95.0	2.3	2.7	41.5 ab	9.5	49.0
		3	98.0 ab	95.8	1.5	2.7	22.5 c	6.5	71.0
		4	97.0 b	94.0	2.8	3.2	28.5 bc	9.5	62.0
		5	95.0 c	94.3	2.5	3.2	29.5 bc	8.0	62.5
CIANO 79	3 yr	0	99.0 a	86.8 a	10.7	2.5	42.0 a	4.8	53.3
		1	98.5 a	88.5 a	10.0	1.5	22.0 b	2.3	75.8
		2	98.8 a	77.8 b	17.7	4.5	3.0 c	1.5	95.5
		3	98.0 a	78.8 b	17.0	4.2	9.0 bc	2.3	88.8
		4	97.8 a	75.5 b	15.3	9.2	8.5 bc	3.8	87.8
		5	93.8 b	74.8 b	15.7	9.5	11.8 bc	4.3	84.0
Tesia	3 yr	0	96.5 a	81.3 c	12.2	6.5	42.0 a	4.0	54.0
		1	97.0 a	91.0 ab	7.8	1.2	15.5 bc	3.0	81.5
		2	97.0 a	94.8 a	3.2	2.0	22.5 b	2.8	74.8
		3	97.0 a	86.2 b	10.8	3.0	17.8 bc	1.3	81.0
		4	97.5 a	94.0 a	4.3	1.7	10.0 bc	1.5	88.5
		5	94.5 b	90.3 ab	7.0	2.7	4.5 c	1.0	94.5

[y] 1 = Normal germination, 2 = abnormal germination, 3 = ungerminated seeds.
[z] Letters a–c denote significance ($P = 0.05$) using Duncan's new multiple range test; means with the same letter are not significantly different.

LITERATURE CITED

1. Agarwal, V. K., Verma, H. S., and Khetarpal, R. K. 1977. Occurrence of partial bunt on

Triticale. FAO Plant Prot. Bull. 25:210-211.
2. Aujla, S. S., Grewal, A. S., and Sharma, I. 1983. Yield and vigor components of wheat as affected by Karnal bunt pathogen (*Neovossia indica*). J. Res. Punjab Agric. Univ. 20:231-235.
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. Baskin, C.C. 1981. Accelerated aging test. In: Vigour Test Methods. D. A. Perry, ed. International Seed Testing Association, Zurich, Switzerland.
5. Bedi, P. S., and Meeta, M. 1981. Effect of Karnal bunt on weight and germination of wheat grains and subsequent metabolism of seedlings. Indian Phytopathol. 34:114.
6. Bedi, P. S., Meeta, M., and Dhiman, J. S. 1981. Effect of Karnal bunt of wheat on weight and quality of the grains. Indian Phytopathol. 34:330-333.
7. Bhat, R. V., Deosthale, Y. G., Roy, D. N., Vijayaraghavan, M., and Tulpule, P. G. 1980. Nutritional and toxicological evaluation of Karnal bunt affected wheat. Indian J. Exp. Biol. 18:1333-1335.
8. Delouche, J. C., and Baskin, C. C. 1973. Accelerated aging techniques for predicting the relative storability of seed lots. Seed Sci. Technol. 1:427-452.
9. Duncan, D. B. 1955. Multiple range and multiple *F* tests. Biometrics 11:1-42.
10. Gill, K. S. 1979. Present status and future strategy for development of wheat varieties resistant to Karnal bunt (*Neovossia indica*) disease. Seeds Farms. Oct. pp. 33-38.
11. Gill, K. S., Randhawa, A. S., Aujla, S. S., Dhaliwal, H. S., Grewal, A. S., and Sharma, I. 1981. Breeding wheat varieties resistant to Karnal bunt. Crop Improv. 8:73-80.
12. International Seed Testing Association. 1985. International rules for seed testing 1985. Seed Sci. Technol. 13:299-520.
13. McRae, W. 1934. Report of the imperial mycologist. Pages 134-160 in: Sci. Rep. Imp. Inst. Agric. Res. Pusa 1932-1933.
14. Mitra, M. 1931. A new bunt on wheat in India. Ann. Appl. Biol. 18:178-179.
15. Mitra, M. 1937. Studies on the stinking smut or bunt of wheat in India. Indian J. Agric. Sci. 7:459-478.
16. Mundkur, B. B. 1940. A second contribution towards a knowledge of Indian Ustilaginales. Trans. Br. Mycol. Soc. 24:312-336.
17. Munjal, R. L., and Chatrath, M. S. 1976. Studies on mode of infection of *Neovossia indica* incitant of Karnal bunt of wheat: J. Nucl. Agric. Biol. 5:40-41.
18. Rai, R. C., and Singh, A. 1978. A note on the viability of wheat seeds infected with Karnal bunt. Seed Res. 6:188-190.
19. Rai, R. C., and Singh, A. 1982. Estimation of loss of wheat grain weight due to Karnal bunt infection. Indian J. Mycol. Plant Pathol. 12:102-103.
20. Singh, D. 1980. A note on the effect of Karnal bunt infection on the vigour of wheat seed. Seed Res. 8:81-82.
21. Singh, R. A., and Krishna, A. 1982. Susceptible stage for inoculation and effect of Karnal bunt on viability of wheat seed. Indian Phytopathol. 35:54-56.