Effect of Temperature, pH, Light, and Desiccation on Teliospore Germination of *Tilletia indica*

J. L. Smilanick, J. A. Hoffmann, and M. H. Royer

Research associate and research plant pathologist, Crops Research Laboratory, U.S. Department of Agriculture-Agricultural Research Service, Logan, UT 84322, and research plant pathologist, Plant Disease Research Laboratory, USDA-ARS, Frederick, MD 21701.
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ABSTRACT

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Maximal germination (55–60%) of teliospores of *Tilletia indica* from both Mexico and India occurred after 3 wk of incubation at 15–20 C in continuous light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$, cool-white fluorescent) over a pH range of 6.0–9.5. Germination rate and maximum was similar on soil, soil extract agar, and water agar. The promycelia of teliospores germinating under 2 mm of soil or agar were incapable of reaching the surface; presumably, they could not release the wheat floret-infecting sporidia which are necessary to complete the life cycle. Teliospore germination resumed unhindered after a 1- or 3-wk interruption by freezing (−5 C) or desiccation. Teliospores collected from newly mature wheat spikes germinated poorly (<10%), but increased to 40–60% over a 4-mo period.

Karnal or partial bunt of wheat, caused by *Tilletia indica* Mitra [= *Neovossia indica* (Mitra) Mundkur], was first described by Mitra in 1931 (10). The disease occurs in India, Pakistan, Iraq, Afghanistan, and more recently, in Mexico (5). Florets of emerging spikes are infected by airborne sporidia produced by the germination of soilborne teliospores (5). To elucidate unknown aspects of the organism's life cycle and to evaluate the efficacy of chemical and cultural control measures, techniques are needed to obtain consistently high percentages of teliospore germination. Although research workers reporting previous studies (1,4–7,10,16,18) have described teliospore germination, the results are either contradictory or of an empirical nature; most of them report variable and low germination percentages.

The objective of this study was to make a quantitative evaluation of the cultural and environmental conditions conducive to maximal teliospore germination of *T. indica*.

MATERIALS AND METHODS

Teliospores. Wheat kernels naturally infected with *T. indica* were obtained from the Centro de Investigaciones Agricolas del Noroeste (CIANO), Cd. Obregon, Sonora, Mexico, and from Sangrur, India (provided by L. M. Joshi, Indian Agricultural Research Institute, New Delhi). The Mexican collection was used in all experiments, whereas the Indian collection was used only in a comparison of teliospore germination with the Mexican collection. Before being used in this study, both collections had been stored at room temperature (23–26 C) for 10–18 mo after harvest. To study teliospore dormancy from freshly harvested wheat, infected kernels were obtained from a resistance-screening trial at the CIANO station. The wheat was inoculated at the boot stage by injecting a sporidial suspension of *T. indica* (19) in January 1984. Infected kernels were removed from a bulk seed collection at harvest in April 1984. All of the collections of *T. indica* were imported into the United States under a permit from the United States Department of Agriculture, Animal Plant Health Inspection Service. The work was performed in approved containment laboratories in Logan, UT, and Frederick, MD.

Teliospores were obtained by agitating about 25 infected kernels in 10 ml of 0.01% Triton X-100 in water. The suspension was poured through two layers of cheesecloth and one layer of 60-μm-mesh nylon screen to remove the kernels and coarse debris. The teliospore suspension was then poured onto a 20-μm-mesh nylon screen, rinsed with deionized water (DW), and the teliospores were collected from the surface of the screen in a small volume of DW. This procedure removed most of the immature teliospores, sterile cells, and smaller debris. The teliospore suspension was centrifuged at low speed (1,000 *g*) and the supernatant was discarded. For plating on agar media, teliospores were surface sterilized by immersion for 1–2 min in a 5% (v/v) solution of commercial laundry bleach (0.26% $NaHClO_3$; pH 10) and recentrifugation at low speed. The teliospore pellet was then rinsed twice and resuspended in sterile DW to a concentration of 2,000–3,000 teliospores per milliliter. Teliospores were applied to agar media in 60-mm-diameter plates by pipeting 0.3-ml aliquots of suspension per plate. The teliospores were evenly dispersed over the agar surface with a glass rod. For plating on soil, the teliospore surface sterilization was omitted and the teliospore suspension was sprayed onto the soil surface in 60-mm-diameter plates with an airbrush. The inoculated agar or soil plates were placed in incubators in a randomized pattern. Teliospore germination was determined at intervals (2–7 days) during the incubation period by using light microscopy (×100) to count the number of teliospores with promycelia bearing primary sporidia. Each treatment was replicated three to five times, and 200–300 teliospores were counted in each replication.

Media. Teliospores were incubated on 2% water agar (WA), 2% washed water agar (WWA), soil extract agar (SEA), and unsterile soil. WWA was prepared by suspending 1 kg of agar in 3 L of deionized water at 5 C for 3 wk with thrice-weekly water changes. The washed agar suspension was dried for 48 hr at 50 C before use. SEA was prepared by extracting 75 g of soil (Green Canyon gravelly loam [lime, 0.4%; total nitrogen, 0.7%; Fe, 9.8 ppm; Zn, 8.6 ppm; K, 357 ppm; P, 33 ppm; total organic matter, 2.5%; and electrical conductivity, 0.8 mmhos/cm]) with 500 ml of boiling DW for 30 min. The extract was filtered to remove debris, 20 g of agar was added, and the volume was adjusted to 1L with DW before the mixture was autoclaved. Soil plates were prepared by

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pouring a water-saturated soil paste (Green Canyon gravelly loam; 37 ml DW + 100 g of soil) into 60-mm-diameter petri plates. The water content of cool, pre-dried (105 C for 24 hr) soil was adjusted from 2 to 35% (milliliters of DW per 100 g of soil), incubated in sealed containers for 2 days at 22 C, and the water potential was determined with a thermocouple psychrometer (Decagon Devices, Pullman, WA). The precent moisture and the logarithm of the water potentials were used to prepare a least-squares regression expression which was used as a standard curve relating soil water content and water potential ($\log Y = 4.099 - 2.737 \log X$, in which Y = water potential in bars and X = percent moisture; $r^2 = 0.97$). The unsterile soil plates were maintained in a moist condition (−0.1 to −0.3 bars) during incubation by the addition of DW. The pH of the WA, WWA, and SEA was 5.9 (±0.1). The pH of the soil plates was 8.0 (water-saturated paste method).

Temperature and light. Teliospores were incubated at 5, 10, 15, 20, and 25 C on SEA, WA, WWA, and soil for 3–6 wk to determine the optimum germination temperature. Teliospores were maintained in unlighted chambers and germination counts were made every 2–4 days. Duncan's multiple range test (DMRT) or Student's t-test were used to compare the maximal germination obtained on these media; a more rigorous statistical procedure was applied to comparisons of the germination of the Mexican and Indian teliospore collections on WA over a 5-wk period at 10, 15, and 20 C. Regression expressions describing germination initiation, rates, and maxima were developed. These quadratic regression solutions, which were developed for each incubation temperature, were tested for significant differences both by using a reduced quadratic equation with both Mexican and Indian collections together, and also by using a full model and considering them separately. Each analysis was followed by an F-test for significance (17).

The effect of light on germination was determined by incubating teliospores on WA at 5, 10, 15, and 20 C under continuous illumination (30 $\mu E \cdot m^{-2} \cdot s^{-1}$, cool-white fluorescent) or in ventilated light-proof boxes. Temperature differences of the agar surfaces in the light or dark when measured with a thermistor thermometer were less than 1 C.

Effect of pH. Teliospores were incubated 2 wk at 15 C under continuous light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$, cool-white fluorescent) on WA adjusted to pH values between 2.6 and 12.0 with 1N KOH or 1N HCl. Glycylglycine and sodium bicarbonate (each at 1.0 g/L) were added to buffer the medium.

Effect of burial, freezing, and desiccation. To determine if germinating teliospores could penetrate to the surface, teliospores were applied to SEA or soil and covered with an additional 2 mm of each medium, respectively. The plates were incubated at 15 C under a 12-hr light/12-hr dark cycle (30 $\mu E \cdot m^{-2} \cdot s^{-1}$, cool-white fluorescent) and observed periodically over a 6-wk period. To assess the effect of freezing, teliospores were applied to soil, exposed to the same light/dark cycle, and incubated at different time/temperature regimens (Table 1). Germination was determined before and after the freezing (−5 C) period. To assess the effect of desiccation, teliospores were applied to soil and incubated at different time/temperature regimens with desiccation periods provided by removing the plate lids and allowing the soil to dry. At the end of the drying period, the soil was remoistened and the lids were replaced. The water potential of the drying plates was estimated by periodic, gravimetric determination of the water content of similarly treated, uninoculated soil plates over a 3-wk period (Fig. 1). The temperatures and dry periods used are shown in Table 2. Germination was observed at 3–4 day intervals during the entire incubation period.

Dormancy. Infected kernels from newly matured, artifically inoculated wheat plants were stored after harvest at 23–26 C for 1, 5, 8, 11, 15, and 22 wk. At the end of the storage period, the teliospores were removed from the kernels, surface sterilized, and plated on WA. Teliospores stored for 1 wk after harvest were also plated without prior surface sterilization, by crushing infected wheat kernels over WA with sterile forceps. This was done to determine whether the teliospore removal and surface-sterilization procedure affected germination. All of the teliospores were incubated for 2 wk at 15 C under continuous light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$, cool-white fluorescent) and the percent germination was determined.

RESULTS

The maximal germination percentage (55–60%) on all media occurred at 15 C, although germination occurred in lesser amounts from 5 to 25 C (Fig. 2). The maximal germination percentage obtained by the surface-sterilized teliospores on agar media and surface-unsterilized teliospores on soil was not significantly different (DMRT, $P < 0.05$). Germination at 15, 20, and 25 C began about the same time, but the maxima were higher at 15 C than at the other temperatures (Fig. 2). Teliospore germination

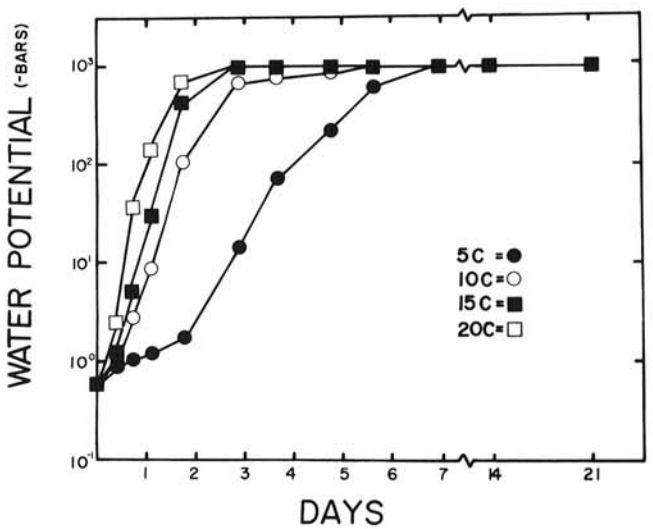

Fig. 1. Water potential of drying Green Canyon gravelly clay loam soil plates incubated at 5, 10, 15, and 20 C.

TABLE 1. Germination of teliospores of *Tilletia indica* on moist soil plates incubated at −5, 5, 10, or 15 C during 1-wk intervals for 6 wk

Treatment	Temperature (C) during incubation intervals (days) 0–7	8–14	15–21	22–28	29–35	36–42	Germination[a] (%)
1	15	−5	15	15	15	15	60
2	15	15	−5	−5	15	15	60
3	15	−5	−5	−5	15	15	62
4	10	10	10	−5	15	15	57
5	10	10	−5	15	15	15	57
6	10	−5	10	10	10	10	46
7	5	5	5	−5	15	15	52

[a] Each value is the average of two plates observed at day 42.

TABLE 2. Germination of teliospores of *Tilletia indica* on moist (M) or dry (D) soil plates incubated at 5, 10, 15, or 20 C during 1-wk intervals for 6 wk

Treatment	Temperature (C) and soil moisture[a] during incubation intervals (days) 0–7	8–14	15–21	22–28	29–35	36–42	Germination[b] (%)
1	20 M	20 D	20 M	20 M	20 M	20 M	49
2	20 M	20 D	20 D	20 D	15 M	15 M	50
3	15 M	15 D	15 D	15 D	15 M	15 M	55
4	15 M	15 D	15 M	15 M	15 M	15 M	62
5	10 M	10 M	10 D	10 D	10 D	15 M	60
6	10 M	10 M	10 D	10 M	10 M	10 M	46
7	5 M	5 M	5 M	5 M	15 M	15 M	62

[a] M = moist, −0.1 to −0.3 bars and D = dry; see Fig. 1.
[b] Each value is the average of two plates observed at day 42.

began after about 23, 9, 6, 5, and 5 days at 5, 10, 15, 20, and 25 C, respectively, and the maximal germination observed was not significantly different on SEA, WA, WWA, or soil (DMRT, $P<0.05$). The rate and percentage of the germination of the Mexican and Indian collections at 10, 15, and 20 C was not different ($P<0.01$) when quadratic regression solutions were compared.

Continuous low-intensity light increased the rate of germination at temperatures from 5 to 20 C (Fig. 3). The optimum pH for germination on WA was between 6.0 and 9.5, with inhibition (<5% germination) occurring below 4.5 and above 10.0 (Fig. 4). The pH of the medium did not change during the incubation period.

Teliospores that were covered with 2 mm of SEA germinated up to 60%, but promycelia or sporidia did not emerge from the agar surface. Likewise, there was no evidence of promycelia or sporidia at the soil surface from teliospores covered with 2 mm of soil over a period of 6 wk. Whether or not the teliospores beneath the soil surface had germinated was not determined.

Teliospore germination was arrested during the freezing (Table 1) or desiccation (Table 2) periods of 1 or 3 wk, but the treatments did not prevent the resumption of germination afterward. In all cases, teliospore germination achieved a maximum similar to that at the same temperatures without freezing or desiccation. The relationship between water potential of the desiccating soil and incubation period at 5, 10, 15, and 20 C is shown in Fig. 2. At 10, 15, and 20 C, the soil water potential achieved equilibrium at −1,000 bars in 3 days, whereas at 5 C, 1 wk was required to achieve the same equilibrium (Fig. 1).

Germination of teliopsores from freshly harvested kernels was low (6.7%), but increased with storage time (Table 3); after 4 mo of storage, germination was only 10% less than that of teliospores stored for 10–18 mo. Surface sterilization with $NaHClO_3$ did not significantly (Student's t-test; $P<0.05$) affect the germination of 1-wk-old teliospores; germination of teliospores with and without surface sterilization was 4.9 and 3.9%, respectively.

TABLE 3. Germination of teliospores of *Tilletia indica* after different postharvest storage periods[a]

Storage period (wk)	Germination[b] (%)
1	6.7 ±0.8
5	17.1 ±1.8
8	22.1 ±3.5
11	35.5 ±4.4
15	42.3 ±1.5
22	47.5 ±4.3

[a]Storage was at 23-26 C followed by incubation on water agar for 2 wk at 15 C under continuous cool-white fluorescent light (30 $\mu E \cdot m^{-2} \cdot s^{-1}$).
[b]Each value represents the average of four replicates ± one standard deviation.

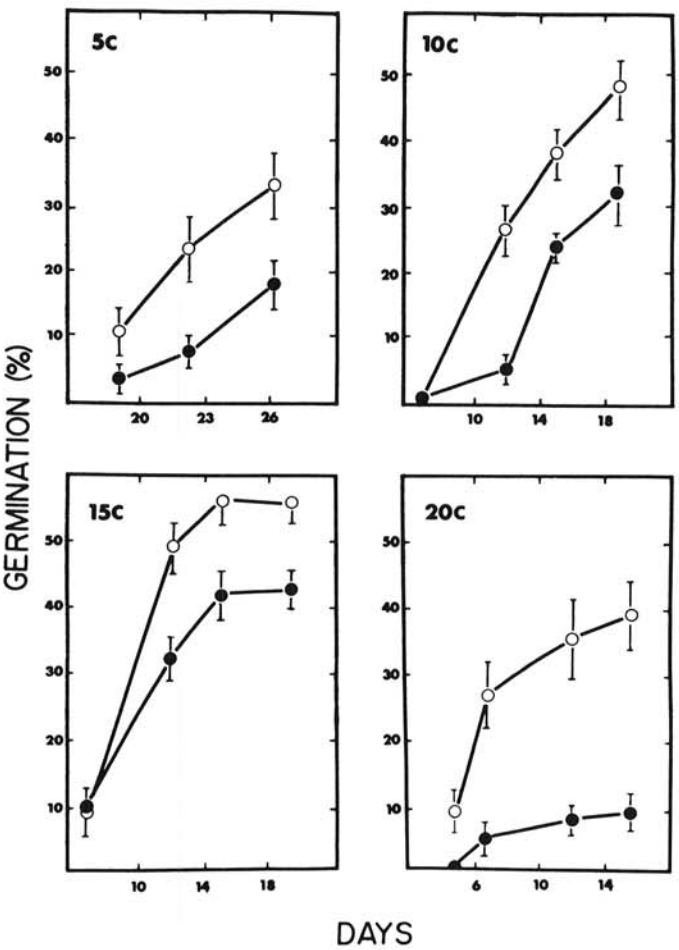

Fig. 3. Germination of teliospores of *Tilletia indica* at 5, 10, 15, or 20 C in darkness (●) or in light (O) (30 $\mu E \cdot m^{-2} \cdot s^{-1}$; cool-white fluorescent) after incubation on water agar. The vertical bars represent one standard deviation.

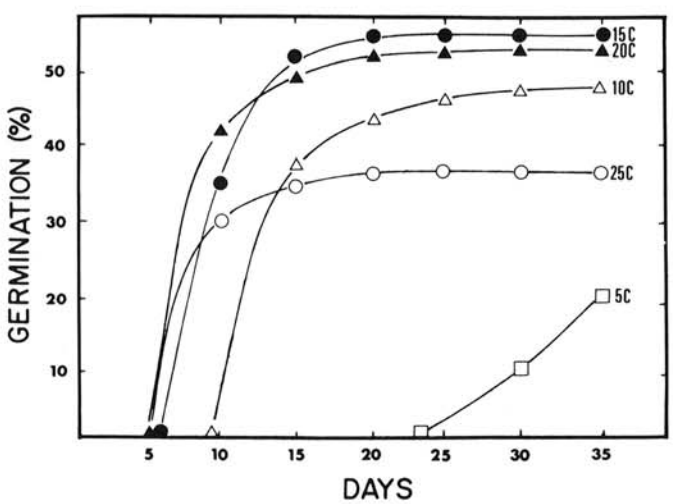

Fig. 2. Germination of teliospores of *Tilletia indica* at 5, 10, 15, 20, and 25 C on soil, water agar, washed water agar, or soil extract agar. Since the teliospore germination on these media was not significantly different, each value represents a combined mean obtained on these media.

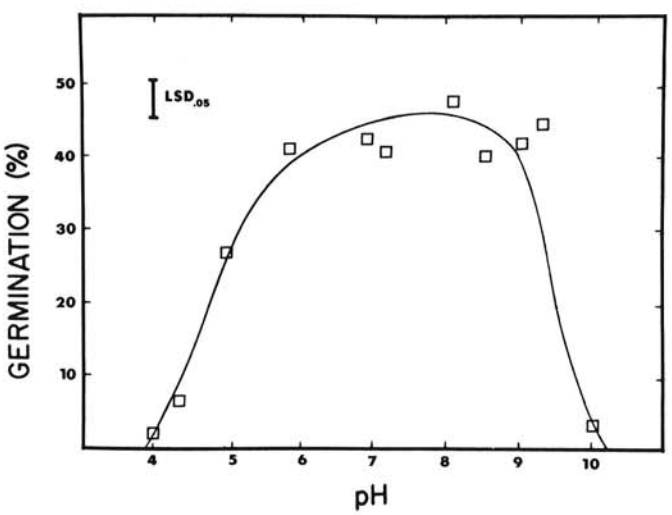

Fig. 4. Germination of teliospores of *Tilletia indica* at 15 C under continuous light after 2 wk on pH-adjusted water agar buffered with glycylglycine and sodium bicarbonate.

DISCUSSION

Teliospore germination of *T. indica* in earlier studies (4,[8], cited by Joshi et al, 5,11) was reported to be low and variable. However, more recent studies, using a variety of presoak treatments and media, have reported germination as high as 39% (1,[2] cited by Joshi et al, 5,6,7,14,16). The low germination rates observed may have resulted from incubation at excessively high temperature, the use of dormant teliospores, or the condensation of free water on the agar surface (a condition inhibitive to germination observed in our experiments). In this study, germination occurred equally well on WA, WWA, SEA, and nonsterile soil, indicating that the nutritional components of the media probably are not critically important. Optimal teliospore germination temperatures have been reported to be 25 C (7), 20 C ([12] cited by Joshi et al, 5), and 15–22 C (14,18). Interestingly, both the soil temperature range reported associated with high disease incidence, 17–21 C, and the air temperature associated with disease development in the field, 18–22 C (1), is similar to that found to be conducive to teliospore germination in this study.

Light was reported both to stimulate (7,14) and have no effect ([12] cited by Joshi et al, 5) on teliospore germination, although these studies reported neither the quality nor intensity of light used. In this study, cool-white fluorescent light of low intensity stimulated germination over a broad range of incubation temperatures (Fig. 3). The reduced germination of teliospores incubated in light-proof boxes, especially at 20 C, was not shown by teliospores incubated in unlighted chambers where a dark environment was not scrupulously maintained. It is probable that with *T. indica*, light stimulation of teliospore germination, like that of *T. controversa* (15), requires only a brief exposure to low-intensity light.

Mitra (11) and McRae ([8] cited by Joshi et al, 5), observed that freshly collected teliospores of *T. indica* would not germinate and required a "resting period" of 8–9 mo before germination occurred, although even then germination was "very poor." A resting period was also observed in this study, although of shorter duration (4 mo). Presumably this period conserves teliospores through the host-free period after harvest.

The optimum conditions for germination of teliospores of *T. indica* differ in several respects from those of other *Tilletia* species that infect wheat. Teliospores of *T. caries* and *T. foetida*, the causal agents of common bunt, germinate at high percentages (>90%) on a variety of media in 3–7 days at 15–17 C (20), are not stimulated by light (13,20), and exhibit no dormancy (3). Teliospores of *T. controversa* germinate equally well but require 3–8 wk of incubation at 5 C on soil or a soil extract substrate, and light is stimulatory (9). Dormancy is not exhibited in freshly harvested teliospores of *T. controversa* but it may be induced by protracted cool, moist conditions and broken by a subsequent period of warm, dry conditions (3).

The inability of teliospores of *T. indica* to produce promycelia or sporidia at the surface when covered with only 2 mm of soil or agar suggests that only teliospores located either at or very near the soil surface contribute to infection. Therefore, timely application of a fungicide to the soil surface to prevent teliospore germination might provide disease control.

Although teliospore germination was interrupted by periods of freezing or desiccation, the teliospores survived and continued to germinate unhindered when conditions were again favorable. This suggests that wheat-growing areas where freezing and dry periods typically occur are environments in which *T. indica* may survive. Although germination occurs in response to adequate moisture and favorable temperatures alone, with minor stimulation by light, the environmental conditions during heading and anthesis of the wheat plant affect infection directly and thus may be more important for disease establishment and perpetuation.

LITERATURE CITED

1. Aujla, S. S., Sharma, Y. R., Chand, K., and Sawhney, S. S. 1977. Influence of weather factors on the incidence and epidemiology of Karnal bunt disease in Punjab. Indian J. Ecol. 4:71-74.
2. Dhiman, J. S. 1982. Epidemiology of Karnal bunt of wheat in Punjab. Ph.D. dissertation. Punjab Agricultural University, Ludhiana, India. (cited by Joshi et al, 5).
3. Hoffmann, J. A. 1982. Bunt of wheat. Plant Dis. 66:979-986.
4. Holton, C. S. 1949. Observations on *Neovossia indica*. Indian Phytopathol. 2:1-5.
5. Joshi, L. M., Singh, D. V., Srivastava, K. D., and Wilcoxson, R. D. 1983. Karnal bunt: A minor disease that is now a threat to wheat. Bot. Rev. 49:309-330.
6. Krishna, A., and Singh, R. A. 1979. Effect of some organic compounds on the germination of teliospores of *Neovossia indica*. (Abstr.) Indian Phytopathol. 32:167.
7. Krishna, A., and Singh, R. A. 1982. Effect of physical factors and chemicals on the teliospore germination of *Neovossia indica*. Indian Phytopathol. 35:448-455.
8. McRae, W. 1932. Report of the imperial mycologist. Pages 73-86 in: Sci. Rep. Imperial Inst. Agric. Res. Pusa 1930-1932. (cited by Joshi et al, 5).
9. Meiners, J. P., and Waldher, J. T. 1959. Factors affecting spore germination of twelve species of *Tilletia* from cereals and grasses. Phytopathology 49:725-728.
10. Mitra, M. 1931. A new bunt of wheat in India. Ann. Appl. Biol. 18:178-179.
11. Mitra, M. 1935. Stinking smut (bunt) of wheat with special reference to *Tilletia indica* Mitra. Indian J. Agric. Sci. 5:51-74.
12. Munjal, R. L. 1975. Studies on spore germination and control of *Neovossia indica*, the causal agent of Karnal bunt of wheat. In Proc. INDO-USSR Symp. on Wheat Dis., 5–10 June 1975, Krasnadar, USSR. (as cited by Joshi et al, 5).
13. Purdy, L. H., and Kendrick, E. L. 1975. Influence of environmental factors on the development of wheat bunt in the Pacific Northwest. I. Effect of soil moisture and soil temperature on spore germination. Phytopathology 47:591-594.
14. Royer, M. H., and Rytter, J. L. 1984. A comparison of temperature and photoperiod requirements for *Tilletia indica* teliospore germination. (Abstr.) Phytopathology 74:758.
15. Schauz, K. 1977. Die Promyzel- und Sporidienbildung beim Zwergsteinbrand —*Tilletia controversa* Kühn—in Abhängigkeit von der Lichtquantität und -qualität. Phytopathol. Z. 89:135-145.
16. Singh, R. S., Singh, K. P., Pal, B. P., and Chaube, H. S. 1977. Note on effect of aldehydes and fatty acids on germination of chlamydospores of Karnal bunt fungus. Pantnagar J. Res. 2:238-240.
17. Steel, R. G., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill, New York. 481 pp.
18. Vasudeva, R. S. 1957. Report of the division of mycology and plant pathology. Sci. Res. Indian Agric. Res. Inst., New Delhi, India 1954-55:87-101.
19. Warham, E. J. 1984. A comparison of inoculation methods for Karnal bunt, *Neovossia indica*. (Abstr.) Phytopathology 74:856.
20. Zscheile, F. P., Jr. 1965. Germination of teliospores and type of growth of common and dwarf bunt fungi as affected by light, photoperiod, and temperature. Phytopathology 55:1286-1292.