

# Survival of Common and Dwarf Bunt Teliospores and Intact Sori After Fumigation of High and Low Moisture Content Winter Wheat

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## ABSTRACT

Smilanick, J. L., Hartsell, P. L., Denis-Arrue, R., Henson, D. J., McKinney, J. D., Tebbets, J. C., and Goates, B. J. 1992. Survival of common and dwarf bunt teliospores and intact sori after fumigation of high and low moisture content winter wheat. *Plant Dis.* 76:293-296.

Teliospores and sori of *Tilletia tritici* and *T. controversa* were mixed with wheat seed (cvs. Daws and Itana) and fumigated with methyl bromide. The fumigant was applied one to four times at atmospheric pressure or under vacuum. After fumigation, teliospore germination and wheat seed germination were determined. Applications of methyl bromide (240 g/m<sup>3</sup> for 24 hr) at atmospheric pressure repeated four times prevented teliospore germination of *T. controversa* in low moisture content wheat seed (10.2% moisture). Sori did not influence the sensitivity of teliospores to methyl bromide. Teliospores infesting moist wheat (14.7% moisture) were about six times more sensitive to methyl bromide fumigation than those in low moisture content wheat seed (10.2-12.4% moisture). *T. tritici* was more sensitive to all of the fumigants than was *T. controversa*. Fumigant doses that reduced teliospore germination were high and caused a marked reduction in wheat seed germination.

*Tilletia controversa* Kühn in Rabenh. is the causal agent of dwarf bunt of wheat, a disease occurring primarily in winter wheat growing regions having prolonged snow cover (12,29). Dwarf bunt has not been reported in the People's Republic of China (7,14). In 1973, the People's Republic of China required that imported wheat be free from *T. controversa* teliospores. An economically feasible method to decontaminate wheat containing teliospores of *T. controversa* could be a solution to this problem. Furthermore, an efficacious fumigant could be employed to contain accidental introductions of this pathogen into new areas. Because there has been no prior evaluation of fumigants for the post-harvest control of *T. controversa*, our objective was to investigate fumigants for this purpose. In preliminary tests with *T. controversa*, chloropicrin fumigation with 240 g/m<sup>3</sup> for 24 hr had no influence on teliospore germination. Propylene oxide applied under vacuum (50 mm Hg) at rates up to 2 ml/L for 24 hr did not reduce teliospore germination to less than 2%. Fumigation with methyl bromide at 25 g/m<sup>3</sup> for 24 hr at 2-wk intervals for 8 wk reduced teliospore germination irregularly. This rate is used for insect control in stored grains (3). Because methyl bromide is registered for

use on wheat and showed some activity in these tests, we chose to thoroughly evaluate this fumigant. Common bunt, caused by *T. tritici* (Bjerk.) G. Wint. in Rabenh. (syn. *T. caries* (DC.) Tul. & C. Tul.) and *T. laevis* Kühn in Rabenh. (syn. *T. foetida* (Wallr.) Liro), occurs in wheat-growing areas worldwide, is not associated with prolonged snow cover, and is of no quarantine significance (12). We included *T. tritici* in these tests because of its taxonomic and morphological similarity to *T. controversa* (16). This organism could provide a more rapid indicator of fumigant efficacy; teliospores of *T. tritici* germinate in only 1 wk instead of six or more weeks required for germination of teliospores of *T. controversa* (12,29).

## MATERIALS AND METHODS

Teliospores and sori of *T. tritici* and *T. controversa* were obtained from field plots in Logan, UT. The *T. tritici* collection was race T-23, whereas the *T. controversa* collection was a mixture of pathogenic types. Sori were removed from infected heads and placed with wheat seed or gently crushed and passed through a 100- $\mu$ m pore-size sieve. Either sori or teliospores (50 g) were mixed with wheat seed (150 g) of known moisture content. Seed samples were sealed in airtight containers for at least 2 wk at room temperature to ensure a homogeneous distribution of moisture. Wheat seed of the hard red winter cultivar Itana and soft white winter cultivar Daws were used. Moisture content was estimated by the air-oven method with five replicates per determination (13). Seeds were

ground to pass through a 0.5-mm mesh and about 2 g was distributed to predried, preweighed petri dishes. The dishes containing the wheat flour were weighed, dried for 1 hr at 130 C, and reweighed to calculate the moisture content. To raise the moisture content to 14-15%, water was added, and the moisture content was determined again after 2 wk. The moisture content ( $\pm$ SD) of Itana and Daws seed before adjustment was 10.2% ( $\pm$ 0.5) and 12.6% ( $\pm$ 0.4), respectively. After water was added, the moisture content of the Itana and Daws seed was 14.7% ( $\pm$ 0.6) and 14.7% ( $\pm$ 0.2), respectively.

Immediately before fumigation, 50 seeds with surface-borne teliospores and five intact sori were placed in a cheesecloth bag. When the influence of fumigation on seed germination also was assessed, this bag was enclosed inside a larger bag containing 400 clean seeds of the same moisture content and closed. The bags were placed inside 28-L fiberglass chambers equipped with air circulation fans. The load factor did not exceed 1%. All tests were conducted at 25 C.

Calculated dosages of the fumigants were dispensed into the chambers with a syringe. Controls were treated identically except no fumigant was injected. The lowest dosages of methyl bromide applied, 24 g/m<sup>3</sup>, are those recommended for commercial fumigation of cereals for insect control (3). Each chamber had a vacuum gauge to verify vacuum was sustained during vacuum fumigations. To determine fumigant sorption during fumigation and to calculate the concentration multiplied by time product (3) for each fumigation, methyl bromide concentrations were measured by gas chromatography (26). During fumigation at atmospheric pressure, samples were withdrawn 2-5 min and 0.5, 1.0, 2.0, 4.0, 8.0, and 24 hr after fumigation began. When tests were conducted under vacuum, only the initial dosages of methyl bromide were recorded. After all fumigations, the chambers were flushed with fresh air for 2 hr before the teliospore and seed samples were removed. For repeated fumigant application tests, the samples were refumigated at 2-wk intervals up to four times.

To determine the percentage of seed germination, bags containing the sori

Accepted for publication 23 October 1991.

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and surface-infested seed were separated from those containing clean seed. Four replicates of 100 seed each were prepared by placing clean seed on moist paper sheets. The percent germination was recorded after 4 days at 20 C with a 12-hr light/dark cycle. Sori were removed from bags containing the sori and surface-infested seed and gently crushed with a glass rod and suspended in sterile water to assess teliospore germination. Teliospores were recovered by rinsing surface-infested seed with a small volume of sterile water. Teliospores from both sori and seed were centrifuged briefly at 14,000 g, suspended in 0.26% (v/v) NaOCl (pH 10–11) for 1 min, recentrifuged, rinsed once more in sterile water, and resuspended in a small volume of sterile water. About 5,000 teliospores were placed on each of four replicate water agar plates, a density low enough to avoid self-inhibition of germination (28). Plates were incubated in transparent plastic humid chambers. Teliospores of *T. controversa* were incubated at 5 C with a 12-hr light/dark cycle (cool-white fluorescent, 20–80  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) and examined after 6 wk for germination. Teliospores of *T. tritici* were incubated at 20 C in the dark and examined after 1 wk. A teliospore was considered germinated if any germination product (pro-mycelium, primary sporidia, or secondary sporidia) was observed.

Any plates containing teliospores of *T. controversa* with no or very little germination were reexamined after an additional 6-wk incubation. On each of four replicate plates, an estimated 2,000 teliospores were examined. Any plates containing teliospores of *T. tritici* with no or very little germination were reexamined after an additional 2-wk incubation. Therefore, any plates reported as having no germination were incubated for a total of 12 and 3 wk for *T. con-*

*troversa* and *T. tritici*, respectively. Effective doses to prevent germination of 95% of the teliospores ( $\text{ED}_{95}$ ) were determined from the data using probit analysis (8). Analysis of covariance (ANCOVA) was applied to compare responses of teliospores in sori or borne on seed surfaces, on high or low moisture content wheat, or the influence of vacuum. The experiments were repeated twice.

## RESULTS

Germination of *T. controversa* teliospores after the control treatments was relatively high (overall mean  $\pm$  SD = 68.1  $\pm$  11.7%, range 49.2–80.0%) but variable. The germination of *T. tritici* after the control treatments was lower (overall mean  $\pm$  SD = 56.0  $\pm$  6.2%, range 43.5–65.8%) but less variable.

Methyl bromide concentration multiplied by time products ( $\pm$ SD) averaged over three 24-hr fumigations after initial dosages of 24, 48, 96, 144, 192, and 240  $\text{g}/\text{m}^3$  were 494 ( $\pm$ 24), 1,080 ( $\pm$ 55), 2,271 ( $\pm$ 294), 3,604 ( $\pm$ 519), 4,728 ( $\pm$ 370), and 5,917 ( $\pm$ 464) gram-hours (g-hr) per cubic meter, respectively. Mean sorption of the applied dosage was 12.9%.

The toxicity of methyl bromide to teliospores residing on the surface of seed or inside intact sori was not significantly different (Figs. 1 and 2). Increased moisture enhanced methyl bromide activity against teliospores residing in sori or on the seed surfaces of both cultivars. Probit analysis solutions of  $\text{ED}_{95}$  ( $\pm$ 95% CI) methyl bromide dosages on wheat seed of low moisture content were 161  $\text{g}/\text{m}^3$  (127, 238) and 320  $\text{g}/\text{m}^3$  (279, 473) for *T. tritici* (Fig. 1) and *T. controversa* (Fig. 2), respectively. On high moisture content wheat  $\text{ED}_{95}$  ( $\pm$ 95% CI), methyl bromide dosages were 25  $\text{g}/\text{m}^3$  (20, 30) and 58  $\text{g}/\text{m}^3$  (46, 111), respectively. Methyl bromide dosages of 48  $\text{g}/\text{m}^3$  or

more reduced seed germination to 35% or less (Fig. 3).

The presence of vacuum (50 or 100 mm Hg absolute pressure) did not significantly enhance methyl bromide inhibition of the germination of teliospores of *T. tritici* (Fig. 4) or *T. controversa* (Fig. 5). Seed germination of cv. Daws exceeded 95% after fumigation with or without vacuum with 24  $\text{g}/\text{m}^3$  on both high (14.7%) and low (10.2%) moisture content wheat seed. Germination of high and low moisture seed was 20.5–25.6 and 83.0–96.0%, respectively, after methyl bromide fumigation with 144 and 240  $\text{g}/\text{m}^3$ , with or without vacuum.

Methyl bromide fumigation repeated four times at 2-wk intervals under atmospheric pressure affected a progressive inhibition of germination of *T. tritici* and *T. controversa* teliospores (Fig. 6). *T. tritici* was more sensitive to methyl bromide than was *T. controversa*. On low moisture content seed, four applications of 144  $\text{g}/\text{m}^3$  or two applications of 240  $\text{g}/\text{m}^3$  completely inhibited germination of teliospores of *T. tritici*, and four applications of 240  $\text{g}/\text{m}^3$  completely inhibited germination of teliospores of *T. controversa*. On high moisture content seed, two applications of 144 or 240  $\text{g}/\text{m}^3$  completely inhibited germination of teliospores of *T. controversa*, although a few germinated teliospores were observed on one plate fumigated four times with 144  $\text{g}/\text{m}^3$ .

## DISCUSSION

Under conditions optimal for germination, the germination of bunt teliospores is often irregular and occurs over a prolonged period compared with other fungal spores (12). In the present study, the variation in the percentage of germination was high, especially among teliospores originating from different sori. This high variability could be attributed to differences in the maturity or parentage of the sori. More consistent germination was observed among teliospores taken from seed surfaces, because we applied a homogeneous mixture of teliospores from many sori to the seed in those tests.

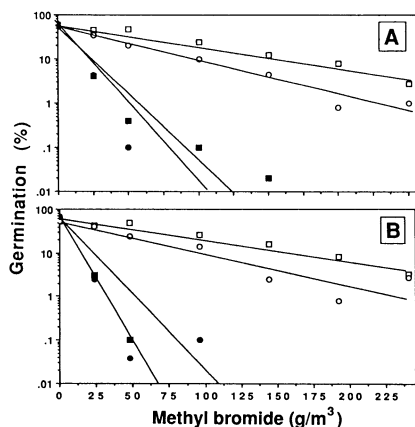


Fig. 1. Germination of *Tilletia tritici* teliospores from (A) seed surfaces and (B) sori after methyl bromide fumigation for 24 hr at 25 C. The teliospores infested cv. Itana wheat seed of moisture content 10.7% (○) or 14.7% (●) or cv. Daws wheat seed of moisture content 12.4% (□) or 14.7% (■).

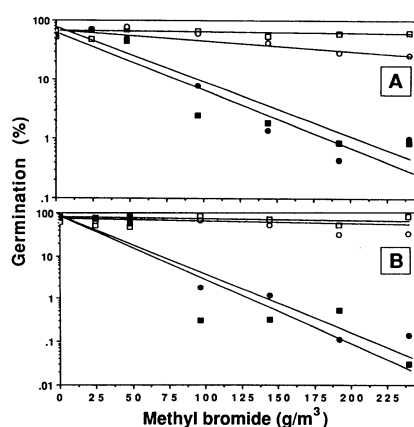


Fig. 2. Germination of *Tilletia controversa* teliospores from (A) seed surfaces and (B) sori after methyl bromide fumigation for 24 hr at 25 C. The teliospores infested cv. Itana wheat seed of moisture content 10.7% (○) or 14.7% (●) or cv. Daws wheat seed of moisture content 12.4% (□) or 14.7% (■).

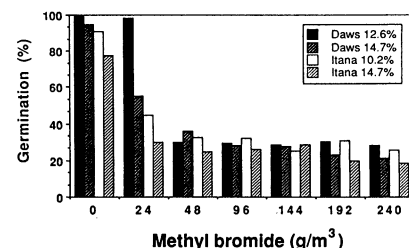


Fig. 3. Germination of wheat seed of cvs. Daws and Itana of low moisture content (12.6 and 10.2%, respectively) and high moisture content (14.7%) after one 24-hr fumigation with methyl bromide at 25 C.

Although methyl bromide could completely prevent teliospore germination, its use for the postharvest control of *T. controversa* is not promising for commercial or quarantine purposes, principally because the dosages required were excessively high. Wheat seed mortality caused by the high dosages is not acceptable if the objective of fumigation was decontamination of wheat germ plasm for international distribution. Repeated applications of 144 g/m<sup>3</sup> (approximately 10,000 g-hr/m<sup>3</sup>) or 240 g/m<sup>3</sup> (approximately 20,000 g-hr/m<sup>3</sup>) were required to stop germination of teliospores infesting the high and low moisture content wheat seed, respectively. These dosages exceed many times those required for insect control (25–200 g-hr/m<sup>3</sup>) that comprise the basis for current commercial practice (3). Methyl bromide dosages exceeding 48 g/m<sup>3</sup> for 24 hr (concentration multiplied by time products exceeding 1,000 g-hr/m<sup>3</sup>) can impart off-odors to flour (27), reduce bread-making quality (17), decrease the germinability of wheat seed and the subsequent vigor of wheat seedlings (15,17,25), leave residues above tolerances (27), and are prohibitively expensive. Adjusting the moisture content up-

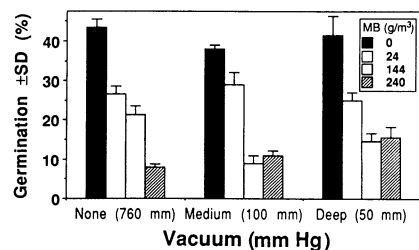


Fig. 4. Germination of teliospores of *Tilletia tritici* from sori after methyl bromide (MB) fumigation at 25 C under atmospheric pressure or vacuum of infested cv. Daws wheat seed at 10.2% moisture content.

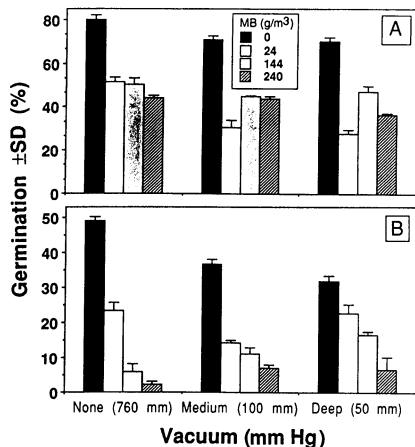


Fig. 5. Germination of teliospores of *Tilletia controversa* from sori after methyl bromide (MB) fumigation at 25 C under atmospheric pressure or vacuum of infested cv. Daws wheat seed at (A) 10.2% or (B) 14.7% moisture content.

ward to enhance the fumigant's activity can cause problems because above 14% moisture content, wheat becomes susceptible to decay, deleterious biochemical changes, and an accelerated loss of germinability during storage (6,21).

The teliospores of *Tilletia* spp. are remarkably resistant to fumigants. Smilanick et al (23) reported 20% or more of the teliospores of a related bunt fungus, *Tilletia indica* Mitra, germinated after 24-hr fumigations with methyl bromide, chloropicrin, or sulfur dioxide, although concentration multiplied by time products for each exceeded 7,500 g-hr/m<sup>3</sup>. Most bacteria or fungi die after exposure to a methyl bromide concentration multiplied by time product of less than 100 g-hr/m<sup>3</sup>, and few survive methyl bromide fumigation beyond 2,000 g-hr/m<sup>3</sup>, including resistant spores or sclerotia (9,18–20,22,32). The resistance of the teliospores to chloropicrin and propylene oxide in the unreported preliminary tests also was extremely high compared with other fungi. Little or no reduction in teliospore germination was observed after exposure to 240 g/m<sup>3</sup> chloropicrin for 24 hr, a concentration multiplied by time product of approximately 20,000 g-hr/m<sup>3</sup>. Munnecke et al (19) reported that only about 300 g-hr/m<sup>3</sup> of chloropicrin was needed to reduce the germinability of sclerotia of *Sclerotium rolfsii* Sacc. and *Verticillium albo-atrum* Reinke & Berthier by more than 90%. The propylene oxide resistance of the teliospores was extremely high; they survived a 4-hr vacuum fumigation with propylene oxide at 2 ml/L. Many bacteria, including spore-forming *Bacillus* spp., and fungi do not survive 2-hr fumigations at atmospheric pressure with propylene oxide at 1.25 ml/L (2,4,11).

The resistance of *Tilletia* spp. teliospores to fumigants probably is a result of their dry, dormant state and thick,

resistant walls. Lower moisture content usually renders fungal propagules more resistant to methyl bromide (18), chloropicrin (19), and propylene oxide (5,31). The teliospore morphology of *T. tritici* and *T. controversa* are similar; both contain thick secondary and tertiary inner walls separated by a partition layer (1,10). However, they do differ in some aspects of teliospore morphology. Most teliospores of *T. controversa* have reticulations 1.5–3.0 μm deep, the reticulations autofluoresce in blue light, and the entire teliospore is enclosed in a gelatinous sheath 1.5–5.0 μm in thickness. Most teliospores of *T. tritici* have reticulations 0.5–1.5 μm deep, the reticulations do not autofluoresce in blue light, and the teliospore sheath is only 1.5 μm in thickness (24,30,33). The greater resistance of teliospores of *T. controversa* to the fumigants in this study and their longer viability under field conditions is possibly a consequence of the thicker sheath (12).

#### ACKNOWLEDGMENTS

We thank W. Martinez, E. Civerolo, and T. Murray for technical advice. We also thank J. Litster for help in the preparation of this manuscript.

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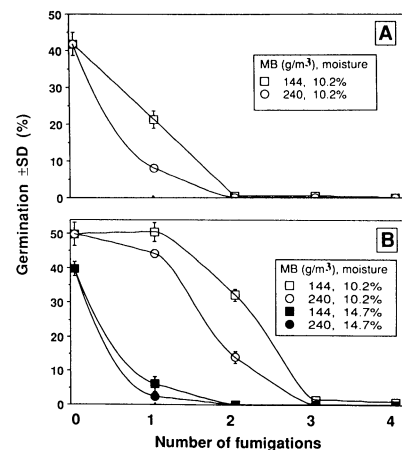


Fig. 6. Germination of teliospores from sori after repeated methyl bromide (MB) fumigation at atmospheric pressure at 25 C of infested cv. Daws wheat seed at 10.2 or 14.7% moisture content containing (A) *Tilletia tritici* or (B) *T. controversa*.

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