Germinability of *Tilletia* spp. Teliospores After Hydrogen Peroxide Treatment

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ABSTRACT

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Three methods of hydrogen peroxide treatment tested to control germination of teliospores of *Tilletia* spp. contaminating wheat or barley seeds were: 1) immersion in heated aqueous solutions of hydrogen peroxide, 2) exposure to water-saturated hydrogen peroxide vapors produced by bubbling air through hydrogen peroxide solutions, and 3) treatment in a chemosterilizer that produces hydrogen peroxide vapor by a pulse-injection system (VHP) that includes control of vapor moisture content. Teliospores of *T. controversa* and *T. tritici* would not germinate after immersion for 6 min in 1.0 M (3.5%, w/v) hydrogen peroxide solution at 45 or 50 C. Teliospores would germinate after treatment with water-saturated hydrogen peroxide vapors for as long as 15 min. Teliospores on the surface of wheat seed would not germinate after treatment for 5 min in the VHP system at 46–48 C. Germination was reduced 95–99% by treatment for 1 min in the VHP system. The system was equally effective when applied with deep (38 mm Hg) or shallow (680 mm Hg) vacuum. The treatment did not wet wheat or barley seeds or influence their germinability even if applied for more than 30 min. Barley malting quality was not influenced by VHP system treatment. When intact sori were fumigated, however, most teliospores within the sori of both fungi survived. Similar results were obtained with *T. fusca* var. *guyotiana* and *T. f. bromi-tectorum*. Although the VHP system has insufficient activity for quarantine purposes if sori are present, it may be a practical seed-surface disinfestation process for nonhost seeds, such as barley, where contaminating teliospores from grain handling equipment are borne superficially on seed and sori are rarely or not present. The potency of hydrogen peroxide vapors from the VHP system was equivalent to brief immersion in heated hydrogen peroxide or sodium hypochlorite solutions but superior to water-saturated hydrogen peroxide vapor and to fumigants such as methyl bromide, propylene oxide, and chloropicrin.

Tilletia controversa Kühn in Rabenh., the causal agent of dwarf bunt of wheat (*Triticum aestivum* L.), occurs in winter wheat-growing regions that regularly have prolonged snow cover (12,35). The bunt fungi grow inside wheat plants and replace seeds with sori consisting of dark masses of teliospores surrounded by a thin remnant of the seed periderm. The thick-walled, long-lived teliospores of *Tilletia* spp. are among the most resistant of all microbial spores. Dwarf bunt has not been reported in the People's Republic of China (9,15), and in 1973, the People's Republic of China required that imported wheat be free from *T. controversa* teliospores. An economically feasible method to decontaminate grain containing teliospores of *T. controversa* could be a solution to this problem. We recently completed an evaluation of the fumigants methyl bromide, chloropicrin, and propylene oxide for postharvest control of *T. controversa* (29). None of these gases had sufficient activity to employ for this purpose. Therefore, we have extended our evaluation to other treatments in the present work.

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. It is not associated with prolonged snow cover and is of no quarantine significance (12,35). However, we included *T. tritici* in these tests because of its similarity to *T. controversa*. Since teliospores of *T. tritici* germinate in only 1 wk instead of the 3 wk or more required for germination of teliospores of *T. controversa*, treatment efficacy can be assessed more quickly.

Malting barley (*Hordeum vulgare* L.) is a candidate commodity for export to the People's Republic of China from the United States. *T. controversa* has never been observed on spring-sown barley and is extremely rare on spring wheat. However, the surface of barley seed often becomes contaminated with *T. controversa* teliospores because wheat and barley share harvest equipment and postharvest facilities. Furthermore, wild grasses occurring within barley and wheat fields are hosts of bunts of the *T. fusca* (Ellis & Everh.) group, such as *T. f.* var. *guyotiana* and *T. f. bromi-tectorum*, whose teliospores are morphologically similar to those of *T. controversa* (8). Because of this similarity, their presence can be problematic for phytosanitary certification purposes.

New methods to decontaminate seed infested with *Tilletia* spp. would be of value in the management of these pathogens for export markets. Among many decontaminating treatments evaluated, sodium hypochlorite solutions showed promise, especially if heated (5) or applied at low pH (30), while many other sanitizers and fumigants did not (29,30). Hydrogen peroxide, classified as "generally recognized as safe" by the Food and Drug Administration, occurs naturally in many foods, such as honey and

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dairy products, and is used in other foods as a bleach or disinfectant (2). It has fewer dietary residues or environmental issues associated with its use than other biocides, such as sodium hypochlorite, ethylene oxide, or formaldehyde. Hydrogen peroxide has been used to disinfect seeds and to improve their germination (26). Kisser and Portheim (17) reported that immersion in 10% or more hydrogen peroxide for 20 min at room temperature prevented germination of teliospores of *T. tritici*. They reported that similar treatments could not be recommended because they did not completely prevent teliospore germination, reduced wheat seed germination slightly, and did not reduce the incidence of common bunt when treated seed were planted. However, many workers have reported that solutions of hydrogen peroxide are effective in killing bacterial cells and fungal spores, especially when they combined elevated temperatures with hydrogen peroxide (6,11,13,32,37,39). In 1979, Moore and Perkinson (21) reported that when vaporized, hydrogen peroxide was 10 times more potent than as a liquid, and they patented a process to produce vapors. In an adaptation of this process, Klapes and Vesley (18) reported potent sporicidal activity when hydrogen peroxide vapors were produced by a novel vapor-pulse injection process that controlled the moisture content of the vapor. At 4 C, resistant spores of *Bacillus subtilis* and *B. stearothermophilus* were killed by an 8-min treatment. Edwards (10) demonstrated an adaptation of the process by the pharmaceutical industry to sterilize vial filling lines.

Our objectives were to evaluate grain decontamination by three methods: 1) immersion in heated hydrogen peroxide or hypochlorite solutions, 2) treatment with water-saturated hydrogen peroxide vapor, and 3) treatment with low-moisture content hydrogen peroxide vapor by a vapor-pulse injection process with a prototype sterilizer (VHP system, American Sterilizer Co., Apex, NC).

MATERIALS AND METHODS

Teliospores. Teliospores and sori of *T. tritici* and *T. controversa* were obtained from field plots in Logan, Utah. The *T. tritici* collection was race T-23, whereas the *T. controversa* collection was a mixture of pathogenic types. Sori of *T. f. guyotiana* were obtained from infected wild *Bromus japonicus* Thunb. ex J.A. Murray and *B. brizaeformis* Fisch. & C.A. Mey. grasses in Logan, Utah. Sori of *T. f. bromi-tectorum* were obtained from infected wild *B. tectorum* in the same location. All sori were carefully removed from infected heads and either treated intact or gently crushed with a mortar and pestle and passed through a 100-μm pore-size sieve before treatment.

Hydrogen peroxide and hypochlorite treatments. Solutions for the immersion treatments were prepared by dilution of 35% hydrogen peroxide or 5.25% NaOCl (pH 10.5) in distilled water daily before use. The solution temperature was adjusted to 23, 45, or 50 C with a hot plate/stirrer and was monitored constantly during treatment with a thermocouple thermometer. Approximately 50 mg of teliospores or 300 wheat seed were placed in 10 or 25 ml, respectively, of the test solutions at the beginning of the treatment period. At the end of this period, seeds were rinsed for 2 min in ice-cold distilled water. Teliospore treatment was terminated by removing about four drops of teliospore suspension from the test solution, adding this to 2 ml of distilled water, centrifuging the teliospore suspension at 14,000 *g* for 30 sec, and resuspending the teliospore pellet in distilled water.

Water-saturated hydrogen peroxide vapor or water vapor was produced by passage of air through a copper-coil heat exchanger to maintain a 40 C temperature, then the air was bubbled via air stones at a rate of 3 L/min through two 1-L jars, each containing 250 ml of 30–35% hydrogen peroxide or water (11). Jars were maintained in a 40 C water bath to assure good temperature control. The resulting mist contained about 1.1 μg/ml of hydrogen peroxide based on the saturation concentration of hydrogen peroxide in air bubbled through a 35% hydrogen peroxide solution at 40 C. To apply water-saturated hydrogen peroxide vapor treatment, dry teliospores were placed on glass slides and the slides were placed in a stream of hydrogen peroxide vapor for the treatment period, then were removed from the stream and washed with sterile distilled water. Washings were centrifuged at 14,000 *g* for 30 sec, and teliospores were collected and prepared for germination.

Controlled-moisture content vapor phase hydrogen peroxide treatment was conducted with the self-contained prototype sterilizer, the VHP system. Rickloff (25) described the development and operation of this instrument. It vaporizes hydrogen peroxide from 35% hydrogen peroxide solution and injects it into a 10 × 10 cm chamber. Hydrogen peroxide within the chamber is approximately at a concentration of 3–4 μg/ml. Since the volume of hydrogen peroxide solution vaporized and the chamber moisture content are controlled, the presence of free water is minimized. We used two operating cycles; cycle 1 employed a deep vacuum (approximately 38 mm Hg) and cycle 2, a shallow vacuum (680 mm Hg) to inject the hydrogen peroxide vapor. Both cycles were aerated for 5 min by deep vacuum evacuation of the chamber to remove residual hydrogen peroxide vapor, followed by introduction of fresh air. The exhaust vapor passed through a catalytic cell that oxidized the hydrogen peroxide to water and oxygen. The chamber temperature was 46–48 C during a sterilizing cycle. Seeds contaminated with teliospores, teliospores on glass slides, or sori were placed inside the fumigation chamber of the vapor-phase hydrogen peroxide generator. At the end of the fumigation period, the chamber was vacuum-aerated. The moisture content of the wheat seeds was evaluated and found to be unchanged by the treatment.

Teliospore germination assessment. After treatment, teliospores from both sori and seed were suspended in a small volume of water and centrifuged briefly at 14,000 *g*, resuspended in 0.26% (w/v) NaOCl (pH 10–11) for 30 sec, recentrifuged, rinsed once more in sterile water, then resuspended in a small volume of sterile water. This brief surface sterilization with NaOCl does not reduce teliospore germinability, although there may be some interaction of this procedure on germination when it is combined with other treatments. Approximately 5,000 teliospores were placed on each 9-cm-diameter water agar plate. This density is low enough to avoid self-inhibition of germination (34). The conditions and duration of incubation for each species were selected to optimize germination. Two replicate plates were prepared for *T. tritici* and four for *T. controversa*. Plates were incubated in transparent plastic humid chambers with a 12-hr light/dark cycle (cool-white fluorescent, 250–1,000 lux). Teliospores of *T. controversa* were incubated at 5 C and examined after 6 wk for germination. Teliospores of *T. tritici* were incubated at 20 C and examined after 1 wk and 3 wk, respectively. Four replicate plates of teliospores of *T. f. guyotiana* and *T. f. bromi-tectorum* were incubated at 10 C and examined after 6 wk for germination. A teliospore was considered germinated if any germination product (promycelia, primary sporidia, secondary sporidia) was observed. On each plate, 150–200 teliospores were examined initially. All experiments were repeated twice, except one test where sori of *T. f. guyotiana* and *T. f. bromi-tectorum* were treated independently with the VHP system.

Any plates containing teliospores of *T. controversa* with no or very little germination were reexamined after an additional 6 wk of incubation. Any plates containing teliospores of *T. tritici* with no or very little germination were reexamined after an additional 2 wk of incubation. On each plate, about 2,000 teliospores were examined.

Seed. Wheat seed of the hard red winter cultivar Sprague and barley seed of the cultivar Klages were used. Moisture content of the wheat seed was 9.2% ± 0.2 by the air-oven method, with five replicates per determination. Seeds were ground to pass a 0.5-mm mesh, and about 2 g were distributed to predried, preweighed petri dishes. Dishes contain-

ing the wheat flour were weighed, dried 1 hr at 130 C, and reweighed to calculate the moisture content. To determine seed germinability and vigor after the treatments, three replicates of 100 seed each were prepared by placing clean seed on moist paper sheets. The percent germination and radicle lengths were recorded after incubation for 4 days at 20 C with a 12-hr light/dark cycle. Seeds were infested with teliospores of *T. f. guyotiana, T. f. bromi-tectorum, T. controversa*, or *T. tritici* by mixing 50 mg of teliospores in 150 g of seed. Before VHP system treatment, samples were sealed in airtight containers for 1 wk at room temperature to ensure a homogeneous distribution of moisture.

RESULTS

Hydrogen peroxide immersion treatments. Heat greatly increased the activity of all immersion treatments. Water alone heated to the temperatures we used was not sporicidal. Both 0.5 M (1.7%, w/v) hydrogen peroxide (Table 1) and 0.25% sodium hypochlorite (Table 2) solutions at 50 C for 6 min or more prevented germination of teliospores of both bunt fungi. Germination of seed of wheat cv. Sprague exceeded 95% after all treatments (*data not shown*).

Hydrogen peroxide vapor treatments. Water-saturated hydrogen peroxide vapor applied at 40 C had no influence on subsequent germinability of *T. tritici* teliospores. Germination of teliospores after treatment with water vapor or water-saturated hydrogen peroxide vapor for 15 min was 81.7 and 78.5%, respectively, and was not significantly different (*t* test, $P = 0.05$).

Controlled-moisture content hydrogen peroxide vapor system treatments. Seed of wheat cv. Sprague was included in each test. Seed germination exceeded 98%, and growth of treated seeds did not differ from that of untreated seeds (*data not shown*). When water instead of hydrogen peroxide was applied with the VHP system sterilizer for up to 30 min, the germination of teliospores and wheat seeds was not affected. Hydrogen peroxide applied with the VHP system sterilizer rapidly killed teliospores but not wheat seeds (Table 3). Treatments exceeding 1–3 min prevented subsequent germination of teliospores of *T. tritici* and *T. controversa*. Results with deep (38 mm Hg) and shallow (680 mm Hg) vacuum were similar (Tables 3 and 4). Teliospores on glass slides (Table 3) or infesting the surface of wheat seedd (Table 4) were equally sensitive to the treatments. Teliospores obtained from treated sori, however, could germinate even after 30 min of treatment, although some treatments significantly reduced germination (Table 3).

Germination of teliospores from the two collections of *T. f. guyotiana* and one collection of *T. f. bromi-tectorum*

Table 1. Germination of *Tilletia tritici* and *T. controversa* teliospores after immersion in water or hydrogen peroxide solutions at 23, 45, or 50 C

Time (min)	Teliospore germination (%)[x]								
	Water			0.5 M (1.7%) H_2O_2			1.0 M (3.5%) H_2O_2		
	23 C	45 C	50 C	23 C	45 C	50 C	23 C	45 C	50 C
T. tritici[y]									
Control	81.7 a	86.8 a	80.5 a	...	...	...	...	...	...
3	72.7 ab	75.1 a	73.4 ab	74.5 a	9.8 e	10.3 e	51.3 bc	0.3 f	0.0 f
6	65.1 a	81.4 a	71.6 ab	58.5 b	0.3 f	0.0 f	55.2 bc	0.0 f	0.0 f
9	76.0 a	76.2 a	74.4 a	73.2 ab	0.0 f	0.0 f	57.8 bc	0.0 f	0.0 f
18	66.1 ab	67.0 ab	70.1 ab	55.6 bc	0.0 f	0.0 f	34.3 d	0.0 f	0.0 f
T. controversa[z]									
Control	...	...	73.5 a	...	...	...	...	...	...
3	...	...	70.0 a	...	0.0 b	0.1 b	...	0.0 b	0.0 b
6	...	...	74.7 a	...	0.0 b	0.0 b	...	0.0 b	0.0 b
9	...	...	75.3 a	...	0.0 b	0.0 b	...	0.0 b	0.0 b
18	...	...	71.3 a	...	0.0 b	0.0 b	...	0.0 b	0.0 b

[x]Values followed by unlike letters are significantly different at the 5% level by Duncan's new multiple range test applied to arcsine transformed data. Actual values are shown.
[y]Means of one experiment with two replicates.
[z]Means of two experiments with four replicates each.

Table 2. Germination of teliospores of *Tilletia tritici* and *T. controversa* after immersion in water or sodium hypochlorite solutions at 23, 45, or 50 C

Time (min)	Teliospore germination (%)[x]								
	Water			NaOCl 0.25%			NaOCl 0.50%		
	23 C	45 C	50 C	23 C	45 C	50 C	23 C	45 C	50 C
T. tritici[y]									
Control	81.7 a	86.8 a	80.5 a	...	...	...	...	...	...
3	72.7 ab	75.1 ab	73.4 ab	25.7 cd	0.0 e	10.3 d	4.3 de	0.0 e	0.0 e
6	65.1 b	81.4 a	71.6 ab	12.7 cd	0.0 e	0.0 e	0.2 e	0.0 e	0.0 e
9	76.0 ab	76.2 ab	74.4 ab	0.2 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
18	66.1 b	67.0 b	70.1 ab	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e	0.0 e
T. controversa[z]									
Control	...	...	73.5 a	...	...	...	...	...	...
3	...	...	70.0 a	...	0.0 b	0.1 b	...	0.0 b	0.0 b
6	...	...	74.7 a	...	0.0 b	0.0 b	...	0.0 b	0.0 b
9	...	...	75.3 a	...	0.0 b	0.0 b	...	0.0 b	0.0 b
18	...	...	71.3 a	...	0.0 b	0.0 b	...	0.0 b	0.0 b

[x]Means within each *Tilletia* sp. followed by unlike letters are significantly different at the 5% level by Duncan's new multiple range test applied to arcsine transformed data. Actual values are shown.
[y]Means of one experiment with two replicates.
[z]Means of two experiments with four replicates each.

Table 3. Germination of *Tilletia tritici* and *T. controversa* teliospores after fumigation in the VHP system hydrogen peroxide sterilizer

Treatment	Time (min)	H_2O_2[y] (ml)	Teliospore germination (%)[z]			
			T. tritici		*T. controversa*	
			Naked	From sori	Naked	From sori
None	0	0	81.0 a	81.0 a	69.5 a	67.9 a
Deep vacuum	3:15	1.1	0.1 b	68.2 ab	0.0 b	49.2 ab
	8:15	2.3	0.0 c	63.3 ab	0.0 b	74.1 a
	16:30	5.2	0.0 c	52.0 b	0.0 b	74.0 a
	21:00	6.6	0.0 c	76.0 a	0.0 b	33.3 b
	30:00	9.8	0.0 c	74.3 a	0.0 b	35.7 b
Shallow vacuum	1:00	0.3	0.0 c	71.3 ab	0.0 b	71.2 a
	5:00	2.1	0.0 c	74.4 a	0.0 b	65.5 a
	10:00	4.6	0.0 c	55.1 b	0.0 b	51.7 ab
	21:00	7.9	0.0 c	77.8 a	0.0 b	67.7 a
	29:00	11.0	0.0 c	35.4 c	0.0 b	41.5 ab

[y]Volume of hydrogen peroxide consumed by sterilizer during operation.
[z]Teliospores were fumigated on glass slides (naked) or within intact bunt sori. Values are the means of one experiment with four replicates. Means in columns followed by unlike letters are significantly different at the 5% level by Duncan's new multiple range test applied to arcsine transformed data. Actual values are shown.

on barley seed surfaces was reduced more than 95% by 3 min of treatment with the VHP system and completely inhibited after 17 min of treatment (Table 5). Germination of teliospores from these collections within sori was not controlled by this treatment.

DISCUSSION

The germinability of *T. tritici* and *T. controversa* teliospores was similarly affected by treatments applied in most tests and showed that the use of the faster germinating *T. tritici* can facilitate the development of sporicidal treatments.

Heat greatly increased the activity of all immersion treatments. Water alone heated to the temperatures employed was not sporicidal. Both 0.25% sodium hypochlorite and 1.7% hydrogen peroxide solutions at 45 and 50 C applied for 6 min had excellent activity against teliospores of both bunt fungi. The treatments had no influence on wheat seed germinability, even when the solution concentrations were doubled and applied three times longer than the sporicidial treatment. These results with heated sodium hypochlorite corroborate the work of Chastain (5). Mild heating enhanced the hydrogen peroxide potency dramatically in our tests. Sori were not included in these tests because, although sori are hygroscopic, they float and wet slowly. Therefore, the mechanical removal or rupture of sori is required for implementation of an immersion treatment. Also, wetting of the seed and posttreatment drying under noncontaminating conditions would be required (22). Wetting decreases seed storage life by acceleration of physiological deterioration of the seed and increases opportunities for the growth of storage fungi.

Water-saturated hydrogen peroxide vapor applied at 40 C had no influence on subsequent germinability of teliospores of *T. tritici*. It is unknown why this treatment has less potency than the drier VHP system. Wang and Toledo (39) stated that a major problem in utilizing water-saturated hydrogen peroxide vapor, in addition to the vapor being of lower hydrogen peroxide content (1.1 vs. 3–4 μg/ml), is its tendency to condense, especially when liquid droplets are present.

The principal mechanism of hydrogen peroxide action proposed by Jonas et al (16) is the production of hydroxyl radicals within cells by the Fenton reaction with ferrous ions. Hydrogen peroxide is more effective at killing bacteria and fungi at elevated temperatures (3,6,11,13,32,37,39). Hydrogen peroxide, like most biocides, increases its potency twofold to threefold for each 10 C increase in temperature (19,24). The components of the influence of heat on biocide potency are a summation of the effect of temperature on respiration, rate of thermal death, transport across membranes, and rate of reaction of the toxicant with target sites (19). Detoxification mechanisms may be inactivated by heat and render cells more susceptible to a toxicant. In the respiration of eucaryotes, enzymatic antioxidants, such as the peroxidases, catalase, and superoxide dismutase, together with nonenzymatic antioxidants such as ascorbate and glutathione, are regarded as detoxifying agents for oxygen radicals, such as hydrogen peroxide and superoxide anion, that occur as consequences of respiration (28). Conceivably, heat could reduce the activity of protective enzymes and render microorganisms more sensitive to hydrogen peroxide. The protection of *Salmonella typhimurium* at ambient temperature from hydrogen peroxide is afforded by catalase on the surfaces of eggs the bacteria colonize; this protection was reduced by mild heating (40 C) to temperatures above that tolerated by the protective egg catalase (36). Similarly, Toledo and coworkers (33) reported that a nonlethal, 20-min heat shock at 80 C prior to hydrogen peroxide treatment greatly enhanced mortality of *B. subtilis*.

In addition to combination with heat, hydrogen peroxide can provide potent antimicrobial action under other conditions that potentiate its activity. We and others have reported potent disinfectant activity of hydrogen peroxide when it was vaporized in air under conditions of controlled moisture (18,21,25). Hydrogen peroxide potency was also enhanced when it was combined with peracetic acid (1,20), ultraviolet light (4,27), ozone (40), transition metals (23,38), or radiofrequency energy (14).

The VHP system is a rapid process for disinfestation of dry seed surfaces. It may prove useful for eliminating other persistent, troublesome microbial contaminants of cereals (22), all of which should be less resistant to hydrogen peroxide than the *Tilletia* spp. The VHP system has promising activity for the sanitation of nonhost seeds, such as barley, where contaminating teliospores are borne superficially on seed and sori are rarely or not present. Unfortunately, even if applied under vacuum, the hydrogen peroxide vapors did not penetrate sori. As with wet treatments, mechanical removal or rupture of sori is required for hydrogen peroxide treatment to succeed. Methyl bromide gas penetrated the sorus readily but had insufficient potency to eradicate teliospores for phytosanitary purposes (29). The periderm of the sorus was not a barrier to methyl bromide (29), although

Table 4. Germination of teliospores of *Tilletia tritici* and *T. controversa* from the surface of wheat seed (cv. Sprague) after fumigation in the VHP system hydrogen peroxide sterilizer

Treatment	Time (min)	H_2O_2[y] (ml)	Teliospore germination (%)[z]	
			T. tritici	*T. controversa*
None	0	0	76.8 a	77.5 a
Deep vacuum	3:15	1.0	0.0 d	1.6 c
	8:15	2.4	0.0 d	0.0 d
	16:30	5.2	0.0 d	0.0 d
Shallow vacuum	1:00	0.4	26.4 b	10.7 b
	3:25	1.2	1.1 c	0.9 c
	5:00	2.5	0.0 d	0.0 d
	9:50	3.3	0.0 d	0.0 d

[y] Volume of hydrogen peroxide consumed by sterilizer during operation.
[z] Wheat seed was included in each test and germination exceeded 98% after all tests. Values are the means of two experiments with four replicates each. Means in columns followed by unlike letters are significantly different at the 5% level by Duncan's new multiple range test applied to arcsine transformed data. Actual values are shown.

Table 5. Germination of teliospores of *Tilletia fusca* var. *guyotiana* from *Bromus japonicus* (host no. 1) or *B. brizaeformis* (host no. 2) and *T. bromi-tectorum* from *B. tectorum* after treatment under shallow vacuum of sori alone or teliospores on the surface of barley seed (cv. Klages) in the VHP system hydrogen peroxide sterilizer

Time (min)	H_2O_2[w] (ml)	Teliospore germination (%)[x]			
		From seed surfaces[y]	From sori[z]		
			T. f. guyotiana		
		Mix of all	Host no. 1	Host no. 2	*T. f. bromi-tectorum*
0	0	36.5 a	22.3 a	85 a	20 a
1:09	0.2	28.2 a	41.9 a	75 a	80 b
3:00	1.1	1.1 b	...	...	...
17:00	7.0	0.0 b	24.3 a	50 b	80 b

[w] Volume of hydrogen peroxide consumed by sterilizer during operation.
[x] Means in columns followed by unlike letters are significantly different at the 5% level by Duncan's new multiple range test applied to arcsine transformed data. Actual values are shown.
[y] Means of two experiments with four replicates each.
[z] Means of one experiment with four replicates.

T. controversa was consistently more difficult to kill than *T. tritici* with that fumigant. Premoistening or predrying sori, which influenced their susceptiblity to methyl bromide (29,31), did not influence susceptibility of teliospores to hydrogen peroxide vapors in this study.

Malting quality of barley seed after VHP system treatment also was assessed. Two replicates of 450 g each of barley seed were treated with the VHP system or water under shallow vacuum for 2.5 or 9 min. Control treatments also included untreated barley seed. Malting quality was assessed by determination of kernel protein content, weight, plumpness, color, percent extractable malt, wort color and clarity, β-glucan content, wort protein content, percent soluble protein, and units of α-amylase activity. No quality determinant was influenced by treatment with the VHP system. Prior workers reported that soaking barley seed in hydrogen peroxide solutions up to 1.5% for 24 hr improved germination for malting purposes, and a patent was issued for this application (26).

Hydrogen peroxide warrants thorough investigation for plant disease control applications. It has fewer dietary residue or environmental issues associated with its use than many other pesticides or disinfectants, caused little or no seed injury in our tests or those of others, and with the use of synergists, can be a potent sanitizer of seeds and plant surfaces for many applications.

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