Evaluation of Chemical and Physical Treatments to Prevent Germination of *Tilletia indica* Teliospores

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ABSTRACT

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Mixtures of healthy wheat seeds and seeds infected with *Tilletia indica* were soaked in formaldehyde solution, ethanol, hot water (54 C), commercial bleach, chlorine dioxide, cupric acetate, quaternary ammonium solution, or mercuric chloride, exposed to dry heat, or fumigated with methyl bromide, sulfur dioxide, chloropicrin, or ethylene oxide. Teliospore germination was assessed after all treatments, and wheat seed germination was assessed after most treatments. No treatment was completely satisfactory as a seed treatment because teliospores could not be eradicated without a concomitant adverse effect on wheat seed germination. A 20- to 30-min soak in formaldehyde solution (5–10 mg/ml), 2% mercuric chloride, or 40% ethanol was initially promising, but the inability to eradicate deep-seated teliospores, transient inhibition of teliospore germination, and poor wheat seedling emergence and vigor in soil made these seed treatments unsatisfactory.

Tilletia indica Mitra (=*Neovossia indica* (Mitra) Mundkur) causes Karnal bunt (partial bunt) of wheat. The literature pertaining to this disease has been reviewed by Joshi et al (7) and Warham (19). Because of stringent

international quarantines established to prevent dispersal of this pathogen (1), treatments are needed to sanitize wheat seed intended for export and to decontaminate equipment moved from infected areas. Formaldehyde, hot water, cooper carbonate, and organic mercury seed treatments have controlled Karnal bunt in some tests (8,9), but sporicidal activity cannot be assessed by disease incidence alone because this disease is both seedborne and soilborne, and teliospores present in the soil from earlier seasons could interfere with tests (13,19). Some fungicide seed treatments have been shown to inhibit teliospore germination (2,11), but the inhibitory activity of these compounds is primarily fungistatic rather than fungicidal (J. A. Hoffmann, *unpublished*) and may not persist over the period of several years teliospores can survive in soil (19). The objective of this study was to find a seed treatment that eliminates viable teliospores of *T. indica* from wheat seed without a concomitant reduction in the germinability of the seed.
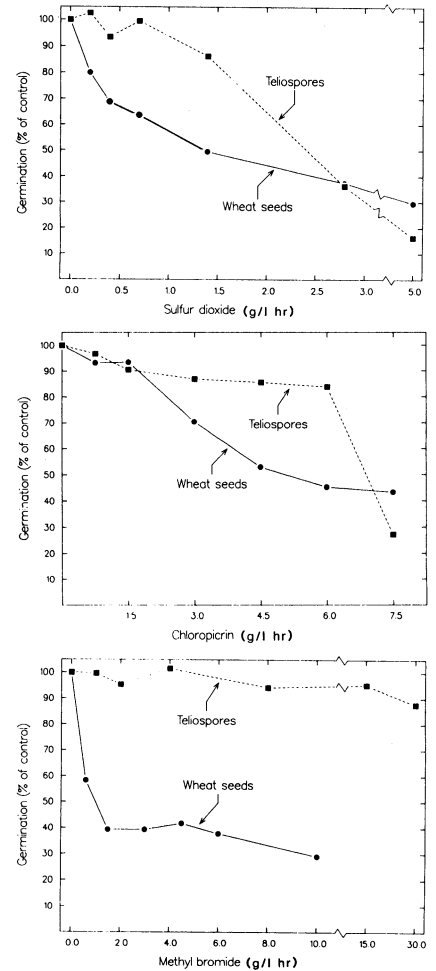

Fig. 1. Germination of teliospores of *Tilletia indica* and cultivar Wanser wheat seeds after a 24-hr fumigation with sulfur dioxide, chloropicrin, or methyl bromide. Germination of the unfumigated teliospore and seed controls was 43.2–56.3 and >95%, respectively.

Table 1. Germination of cultivar Wanser or Nugaines wheat seeds and teliospores of *Tilletia indica* after mixtures of healthy and infected kernels were immersed in formaldehyde solution, ethanol, 54 C water, hypochlorite bleach, or mercuric chloride and rinsed twice in deionized water

Treatment	Teliospore germination[a] (%)	Seed germination[b] (%)
Formaldehye solution (20-min soak) (mg/ml)		
0.0	56.9 ± 4.2	99[c]
0.25	32.2 ± 2.4	98
1.0	9.6 ± 1.7	99
1.75	2.5 ± 0.6	99
2.5	0.0	97
5.0	0.0	95
10.0	0.0	84
Ethanol (10-min soak) (%)		
0	54.3 ± 3.1	97[c]
5	54.8 ± 4.5	94
10	63.5 ± 5.0	92
20	49.9 ± 7.3	87
30	9.7 ± 2.6	86
40	0.3 ± 0.1	82
50	20.6 ± 2.9	77
70	49.2 ± 6.2	75
95	46.0 ± 4.9	66
Ethanol (10-min soak) (%)		
0	44.0 ± 3.7	99[d]
25	23.0 ± 1.4	98
30	3.5 ± 0.4	97
35	1.4 ± 0.5	97
40	0.0	98
45	1.8 ± 0.2	89
50	3.4 ± 0.7	89
55	27.0 ± 3.3	97
Hot water (54 C, soak) (min)		
0	31.3 ± 2.7	97[c]
1	23.4 ± 1.0	99
5	19.9 ± 0.6	99
10	10.9 ± 0.5	97
15	7.2 ± 1.3	93
20	23 ± 0.5	97
Hypochlorite (5% commercial bleach, 10-min soak) (pH)		
11	22.9 ± 2.3	−
10	9.8 ± 0.6	−
9	2.6 ± 0.9	−
8	0.5 ± 0.2	−
7	0.1 ± 0.06	−
6	0.0	−
Hypochlorite (pH 10.5, 10-min soak) (%, v/v)		
0.0	51.4 ± 1.5	95[c]
2.5	34.8 ± 2.7	93
5.0	24.3 ± 0.9	94
10.0	27.5 ± 1.8	92
20.0	7.1 ± 1.1	86
30.0	2.0 ± 0.8	83
50.0	35 ± 0.8	70
70.0	0.0	35
Mercuric chloride (0.2%, w/v, soak) (min)		
0	60.8 ± 4.2	98[d]
5	0.5 ± 0.2	98
10	0.1 ± 0.06	97
20	0.0	95
30	0.0	91

[a] Mean ± standard error of three to six replicates of 200–300 teliospores per plate.
[b] Mean of two replicates of 200 seeds.
[c] Cultivar Wanser.
[d] Cultivar Nugaines.

MATERIALS AND METHODS

Seed and teliospore origin and preparation. All experiments were conducted in federal- and state-approved containment facilities at Logan, UT. Each treatment was applied to a mixture of 25 wheat seeds naturally infected with *T. indica* and 300 noninfected wheat seeds. The ratio of seed volume to solution volume was 3 g/100 ml. Infected seeds were obtained from the Centro de Investigaciones Agrícolas de Noroeste, Cd. Obregon, Sonora, Mexico, and were a bulk seed collection of soft white spring cultivars from commercial wheat fields in the Yaqui and Mayo valleys. The sorus size on the bunted kernels was from a trace to about 20% of the seed (severity class 1–3 [20]). The noninfected seeds were 6- to 18-mo-old hard red winter wheat cultivar Wanser or soft white winter wheat (*Triticum aestivum* L. cv. Nugaines) grown and harvested at Logan. Infected and noninfected seeds contained 11.5–12.0% moisture as determined by the air-oven method (6). The seed mixtures were soaked in disinfectant solutions or hot water in glass tubes, heated in glass petri dishes one seed layer thick, or fumigated in 25-μm mesh nylon bags.

After treatment, infected seeds were removed and the noninfected seeds were tested for germination. Germination of seeds was determined after incubating two replicates of 200 seeds each on moist blotter paper at 23–26 C for 4 days. For more rigorous evaluation of the phytotoxicity of formaldehyde, ethanol, and mercuric chloride treatments, seed germination was assessed in soil flats. To prevent introducing teliospores into the greenhouse, seeds planted in soil flats were treated separately without *T. indica*-infected seeds. Ten blocks of 10 seeds were planted 4 cm deep in wooden flats containing moist nonsterile soil (Green Canyon gravelly loam [14]). Germination percentage and height of the seedlings were recorded after 7, 14, and 21 days at 14 ± 2 C.

Teliospores were removed from infected seeds by agitation in a solution of 0.01% Triton X-100 in deionized water (DW), strained through two layers of gauze, surface-sterilized by immersion for 1 min in a 5% (v/v) solution of commercial bleach (0.26% NaOCl, pH 10.0–10.5) in DW, and rinsed twice with sterile DW. Teliospores were applied to water agar in 60-mm-diameter plates by pipeting 0.3-ml aliquots containing

Table 2. Germination of cultivar Wanser wheat seeds and teliospores of *Tilletia indica* after mixtures of healthy and infected kernels were immersed in chlorine dioxide adjusted to pH 2.5 or 3.5, chlorine dioxide diluted to two concentrations, cupric acetate, or a quaternary ammonium solution and rinsed twice in deionized water

Treatment (min)	Teliospore germination[a] (%)		Seed germination[b] (%)	
Chlorine dioxide (16.7% Alcide Incyte)				
	pH 2.5	pH 3.5	pH 2.5	pH 3.5
0	50.7 ± 4.2	48.5 ± 1.9	98	99
5	38.4 ± 1.2	55.6 ± 3.4	90	97
15	24.2 ± 2.9	49.2 ± 1.0	89	95
30	26.6 ± 1.4	41.6 ± 3.1	88	93
45	11.3 ± 2.5	38.7 ± 2.1	82	91
60	7.1 ± 2.3	26.0 ± 2.7	80	93
90	2.1 ± 0.8	9.1 ± 1.2	68	82
Chlorine dioxide (Alcide LD pH 2.6)				
	8.3%	14.3%	8.3%	14.3%
0	40.3 ± 2.4	42.2 ± 4.7	98	98
5	35.6 ± 3.0	24.2 ± 3.2	96	75
15	31.1 ± 1.6	27.3 ± 1.5	93	56
30	7.3 ± 1.2	9.3 ± 1.6	82	45
45	6.8 ± 0.5	3.3 ± 0.9	81	44
60	4.3 ± 0.5	0.5 ± 0.2	74	34
90	0.5 ± 0.1	0.0	49	28
Cupric acetate (0.5/1.0% v/v)				
	0.5%	1.0%	0.5%	1.0%
0	51.8 ± 2.0	54.3 ± 3.6	98	99
5	43.3 ± 0.5	46.7 ± 4.1	84	84
15	56.6 ± 1.8	54.9 ± 1.4	79	70
30	53.1 ± 5.3	43.2 ± 3.1	74	61
60	37.6 ± 1.2	43.0 ± 4.4	66	53
90	39.7 ± 3.7	49.7 ± 1.8	57	42
Quaternary ammonium (0.5% v/v, soak)				
0	47.9 ± 2.8	···	···	···
10	58.1 ± 4.2	···	···	···
20	46.7 ± 7.3	···	···	···
30	57.9 ± 6.0	···	···	···

[a] Mean ± standard error of three to six replicates of 200–300 teliospores per plate. The teliospores were removed from the seeds by rinsing, surface-sterilized, and incubated on water agar 2 wk at 15 C.
[b] Mean of two replicates of 200 seeds. The seeds were incubated 4 days at 25 C on blotter paper.

2,000–3,000 teliospores onto the agar surface. The teliospores were evenly distributed with a sterile glass rod on the agar and incubated at 15 C under continuous illumination (30 μE m^{-2} s^{-1}, cool-white fluorescent). The inoculated agar plates were placed in incubators in a randomized pattern. Teliospore germination was determined after 14 days by light microscopy (100×). Teliospores were classified as germinated if promycelia had emerged. Maximum germination occurs within 12–16 days (14). All treatments were replicated three to six times with 200–300 teliospores counted in each replicate. Tests showing inhibition of teliospore germination with little seed phytotoxicity were repeated two to four times. Experiments were repeated if the germination of untreated teliospores was low (<30%) or the variance among replicates was high. Germination percentages reported are the means ± one standard error of several of representative experiments.

Teliospore survival was evaluated after formaldehyde, ethanol, and mercuric chloride treatment in a second series of experiments. To assess the germinability of teliospores that resided deep within infected seeds, the seeds were dried after treatment and ground with a hand mill to a coarse flour. Teliospores were separated from the flour by agitation in 0.1% Triton X-100, then the solution was filtered through gauze and nylon mesh and

prepared for incubation on water agar as previously described. To determine if inhibition of germination was transitory, incubation on water agar at 15 C was increased from 2 to 4 wk. To prevent agar from drying, the humidity was increased by placing pans of water in the incubator.

The effect of posttreatment storage on formaldehyde and ethanol toxicity to teliospores and seeds was assessed by evaluating germination immediately after treatment and again after storing the treated seeds for 30 days in open glass jars at 23–26 C. Seed germination was assessed on paper sheets and soil. The germination of teliospores recovered from flour was assessed on water agar.

Seed soaks. Commercial formaldehyde solution (37% [w/w] formaldehyde, 10% methanol, and 53% water; Fischer Scientific Co., Fairlawn, NJ) was diluted with DW to concentrations of 0.25, 1.0, 1.8, 2.5, 5.0, and 10.0 mg of formaldehyde per milliliter (pH 6.2, 5.5, 5.2, 5.0, 4.6, and 4.2, respectively). Seeds were immersed in these formaldehyde concentrations for either 10 or 20 min and rinsed twice with DW.

Seeds were immersed in solutions of ethanol diluted to 5, 10, 20, 25, 30, 35, 40, 45, 50, 55, 70, or 95% (v/v) at each concentration for 10 min, then rinsed twice with DW.

A water bath adjusted to 54 C was used to maintain a constant hot water treatment for seeds immersed for 1, 5, 10,

15, or 20 min. After hot water immersion, seeds were immediately cooled to room temperature in a water bath.

Commercial bleach (5.25% NaOCl, pH 10.0–10.5) containing 19.2 mg/ml free-chlorine and 25.4 mg/ml total chlorine as determined by the DPD method (4) was diluted to 5% (v/v, 0.263% NaOCl) in DW and adjusted with 1 N HCl and 1 N NaOH to pH 6, 7, 8, 9, 10, or 11. Seeds were immersed 10 min and rinsed twice with DW. In a second experiment, wheat seeds were immersed 10 min in pH 10.5 solutions containing 2.5, 5.0, 10.0, 20.0, 30.0, 50.0, or 70.0% bleach and rinsed twice with DW.

Mercuric chloride solutions were prepared by dissolving 0.2% mercuric chloride in DW (w/w). Seeds were immersed in this solution for 5, 10, 20, or 30 min and rinsed twice in DW.

Two commercial formulations of a chlorine dioxide-potentiated complex, Alcide LD and Alcide Incyte (Alcide Co., Westport, CT), were tested as seed soaks. Alcide LD solutions were prepared by mixing the base, activator, and DW in ratios of 1:1:10 and 2:2:10 (v/v) for low- and high-concentration treatments, respectively. The pH of these solutions was 2.6. Alcide Incyte solutions were prepared by mixing the base, activator, and DW in a ratio of 1:1:4 and adjusting the pH to 2.5 with 5 N HCl and to pH 3.5 with 5 N KOH for low and high treatments, respectively. Alcide solutions were stored 1 hr at 23–26 C before use. The chlorine dioxide concentration in these solutions at the time of use was determined spectrophotometrically (5). The 1:1:10 and 2:2:10 dilutions of Alcide LD contained 49.1 and 62.7 mg/L of chlorine dioxide, respectively. The 1:1:4 dilutions of Alcide Incyte adjusted to pH 2.5 and 3.5 contained 36.0 and 29.1 mg/L of chlorine dioxide, respectively. The seeds were immersed in the Alcide solutions for 5–90 min, then rinsed twice with DW.

Cupric acetate solutions were prepared by dissolving 0.5 or 1.0% cupric acetate in 5 mM acetic acid containing 0.1% Triton X-100 (w/v). The seeds were immersed in these solutions for 5–90 min, then rinsed twice with DW.

Seeds were immersed for 10, 20, or 30 min in a 0.5% (v/v) solution of quaternary ammonium (Cleansan 537; 20% methyldodecylbenzyl trimethyl ammonium chloride, 5% methyldodecylxylene bis[trimethyl ammonium chloride]; Brogdex Co., Pomona, CA) and rinsed twice with DW.

Fumigations. Mixtures of healthy and infected seed were fumigated at atmospheric pressure with sulfur dioxide, chloropicrin, or methyl bromide, using a static procedure modified from Vincent and Lindgren (18). This method does not replenish fumigant lost during treatment but will distinguish the relative sensitivity of cofumigated organisms. For fumigation

Table 3. Germination of teliospores of *Tilletia indica* obtained from flour prepared from infected wheat kernels immersed 30 min in formaldehyde solution, ethanol, or mercuric chloride and incubated on water agar at 15 C

Treatment	Teliospore germination (%)		
	14 Days	21 Days	30 Days
Water control	44.8 ± 3.5	37.9 ± 3.9	35.3 ± 3.4
5 mg/ml formaldehyde	6.2 ± 1.8	9.1 ± 2.5	11.3 ± 2.4
10 mg/ml formaldehyde	3.6 ± 1.0	9.8 ± 1.8	11.9 ± 3.2
40% EtOH	0.4 ± 0.4	6.4 ± 3.3	9.8 ± 1.9
Water control	29.5 ± 4.6	···	34.9 ± 1.7
2% HgCl$_2$	5.6 ± 2.6	···	4.3 ± 2.0

Table 4. Germination of cultivar Nugaines wheat seeds and teliospores of *Tilletia indica* after mixtures of healthy and infected kernels were immersed 30 min in formaldehyde solutions

Formaldehyde solution (mg/ml)	Seed germination[a] (%)	Teliospore germination[b] (%)	
		Rinsed	From flour
0	99	59.7 ± 2.0	32.7 ± 4.9
1	99	21.4 ± 6.1	22.6 ± 2.1
3	99	8.2 ± 1.3	18.5 ± 2.4
5	97	5.3 ± 2.6	12.7 ± 2.5
10	95	1.3 ± 0.2	3.9 ± 0.8
20	77	1.6 ± 0.3	2.5 ± 0.4
30	–	0.0	1.7 ± 0.5
40	–	0.0	0.6 ± 0.3
50	–	0.0	0.1 ± 0.02

[a] Mean of two replicates of 200 seeds. The seeds were incubated 4 days at 25 C on blotter paper.
[b] Mean ± standard error of four replicates of 200–300 teliospores per plate. The teliospores were removed by rinsing the surface of the seeds or by grinding the seeds into flour and sieving to recover the teliospores. The teliospores were incubated on water agar 4 wk at 15 C.

in sulfur dioxide, chloropicrin, and methyl bromide, seeds contained in nylon bags with a pore size of 25 μm were suspended in airtight 1.2-L flasks. The flasks were evacuated, the fumigant injected into the flasks, and the chambers purged to atmospheric pressure. The fumigant concentrations, expressed as a total dosage adjusted to 1 hr (17), were: sulfur dioxide, 0.18–5.60 g/hr; chloropicrin, 0.78–7.70 g/hr; and methyl bromide, 0.75–30.00 g/hr (Fig. 1). Seeds were incubated in flasks for 24 hr at 23–26 C with a 4.5-cm stir bar revolving at high speed to ensure uniform distribution of the gas. Flasks were then evacuated twice, purged with air, and the nylon bags removed. The relative humidity of the purge air was 30–40%. Seeds were fumigated with ethylene oxide in a commercial hospital sterilizer. Bags containing the seeds were fumigated 3 hr in 11% ethylene oxide and 89% carbon dioxide for 3 hr and flushed with sterile air for 15 hr inside the sterilizer. All bags containing fumigated seeds were stored an additional 2 days at 23–26 C in air to allow the residual fumigant to dissipate before germination was assessed. Ethylene oxide fumigation was preformed once, whereas the other fumigations were repeated two to five times.

Dry heat. To determine teliospore tolerance to dry heat, 25 infected seeds in 60-mm-diameter glass petri dishes were placed in a forced-air oven adjusted to 75, 90, 100, 110, or 125 C. Dishes were periodically removed, teliospores rinsed from the seeds and surface-sterilized as previously described, and percent germination determined after incubation for 4 wk at 15 C on water agar.

RESULTS

Wheat seed germination on blotter paper was 80% or greater after soaking in formaldehyde, ethanol, 54 C water, hypochlorite bleach, and mercuric chloride solutions, whereas teliospore germination was reduced to 5% or less when assessed after 2 wk by these treatments (Table 1). Hypochlorite inhibition of teliospore germination was enhanced below pH 10 (Table 1). Chlorine dioxide did not reduce teliospore germination without a concomitant reduction in wheat seed germination (Table 2). Cupric acetate and quaternary ammonium did not inhibit teliospore germination (Table 2).

Fumigation with sulfur dioxide reduced both seed and teliospore germination beginning at 0.25 and 1.50 g/hr, respectively. Chloropicrin fumigation reduced both seed and teliospore germination beginning at 3.00 and 7.50 g/hr, respectively (Fig. 1). Methyl bromide fumigation reduced seed germination at the lowest rate tested (0.75 g/hr), whereas teliospore germination was not affected even at the highest rate (Fig. 1). Ethylene oxide

reduced seed and teliospore germination from 99 and 54 ± 3.6% to 31 and 0.4 ± 0.1%, respectively.

When the germination percentage of teliospores and vigor of wheat seedlings was assessed by the more rigorous procedures, teliospore germination was higher and seedling emergence and vigor poorer than in the tests where teliospores were rinsed from treated seeds and when germination was assessed on moist paper sheets (Tables 3–6). Inhibited teliospores from seeds treated with formaldehyde and ethanol began to germinate when incubation periods were increased to 4 wk, although the teliospores from the control and mercuric chloride treatments did not increase when the incubation was increased (Table 3). Teliospores obtained from flour were often crushed or cracked. The germination of untreated teliospores from flour was 20–25% lower than those rinsed from infected seeds. However, after formaldehyde, ethanol, and mercuric chloride treatment, the teliospores from flour germinated significantly (pairwise t test, P = 0.01) more than those rinsed from infected seeds (Tables 5–7).

The emergence of seeds from soil after treatment with 5 or 10 mg/ml formaldehyde and 40% ethanol, either singly or in combination, was less than the germination of seeds obtained on blotter paper (Table 7). Furthermore, the ethanol

treatment significantly (P = 0.05) reduced the height of surviving seedlings (Table 7). When germination evaluations were conducted after a 30-day posttreatment storage period, seed germinability on blotter paper decreased whereas seedling emergence from soil flats increased up to 25% (Table 7). Teliospore germinability declined slightly after storage, but only the inhibition provided by treatment with 10 mg/ml formaldehyde solution and 40% ethanol combined was enhanced by the additional storage (Table 7).

Teliospore germination was reduced to less than 2% after 1, 12, and 88 hr at 125, 110, and 100 C, respectively (Table 8). Teliospore germination after treatment at 90 C declined to 8.4% after 120 hr (Table 8). The percentage of germination of teliospores after 240 hr at 75 C was 46.6% (Table 8).

DISCUSSION

No satisfactory eradicative seed treatment was found. Teliospores of *T. indica* were very resistant to chemical and physical treatments. None of the soak treatments could inhibit teliospore germination below 2—5% without becoming excessively phytotoxic. Mercuric chloride provided the most persistent, but still incomplete, inhibition of teliospore germination with minimal

Table 5. Germination of wheat seeds (cultivar Wanser) and teliospores of *Tilletia indica* after mixtures of healthy and infected kernels were immersed 10 min in 10–95% ethanol solutions

Ethanol (%)	Seed germination[a] (%)	Teliospore germination[b] (%)	
		Rinsed	From flour
0	97	64.7 ± 9.2	40.9 ± 3.7
10	93	34.7 ± 8.4	25.8 ± 1.5
20	92	58.6 ± 5.9	24.7 ± 3.9
30	92	10.4 ± 3.0	9.1 ± 2.4
40	87	0.8 ± 0.5	7.0 ± 1.7
50	78	9.7 ± 2.3	11.7 ± 2.3
70	76	48.0 ± 4.0	34.9 ± 9.1
95	75	61.7 ± 5.7	20.1 ± 4.3

[a] Mean of two replicates of 200 seeds. The seeds were incubated 4 days at 25 C on blotter paper.
[b] Mean ± standard error of four replicates of 200–300 teliospores per plate. The teliospores were removed by rinsing the surface of the seeds or by grinding the seeds into flour and sieving to recover the teliospores. The teliospores were incubated on water agar 4 wk at 15 C.

Table 6. Germination of wheat seeds (cultivar Nugaines) and teliospores of *Tilletia indica* after soaking in 2% mercuric chloride and rinsing the seed 24 hr in flowing tap water

Soak duration (min)	Seed germination (%)		Teliospore germination (%)[a]	
	Paper[b]	Soil[c]	Rinsed	From flour
0	98	97	56.6 ± 3.7	34.9 ± 1.3
5	98	95	1.2 ± 0.7	7.7 ± 1.2
10	97	97	0.1 ± 0.03	5.9 ± 1.1
20	95	96	0	7.6 ± 0.9
30	91	90	0	4.3 ± 2.0

[a] Mean ± standard error of four replicates of 200–300 teliospores per plate. The teliospores were removed by rinsing the surface of the seeds or by grinding the seeds into flour and sieving to recover the teliospores. The teliospores were incubated on water agar 4 wk at 15 C.
[b] Mean of two replicates of 200 seeds per blotter paper sheet. The seeds were incubated 4 days at 25 C on blotter paper.
[c] Mean of 10 blocks of 10 seeds each, planted in soil flats in a greenhouse after 3 wk.

Table 7. Percent germination of teliospores of *Tilletia indica* and cultivar Nugaines wheat seeds after mixtures of healthy and infected kernels were immersed 30 min in formaldehyde (F) or combined with ethanol (E)[a]

Treatment	Not stored				Stored 30 days		
	Seed germination (%)		Seedling growth[d]	Teliospore germination (%)[e]	Seed germination (%)		Teliospore germination (%)[e]
	Blotter[b]	Soil[c]			Blotter[b]	Soil[c]	
Control	99	92	1.07	31.5	99	97	35.3
5 mg/ml F	98	74	1.07	11.5	96	87	11.3
10 mg/ml F	99	79	1.04	12.0	96	82	11.9
5 mg/ml F + 40% E	93	6	0.66	13.1	80	26	10.3
10 mg/ml F + 40% E	88	7	0.43	6.7	70	12	0.2
40% E	92	5	0.82	10.5	70	30	9.8
LSD$_{0.05}$	···	8.5	0.14	8.3	···	13.1	4.5

[a] Germination was assessed immediately after treatment and after 30 days of storage at 23–27 C.
[b] Mean of two replicates of 200 seeds per blotter paper sheet. Seed germination was assessed on moist blotter paper sheets after 4 days at 25 C.
[c] Mean of 10 replicate blocks of 10 seeds each in greenhouse soil flats incubated 4 wk at 14 ± 2 C.
[d] Mean increase in seedling height (cm/day). Seedling height was determined by measuring the seedlings 7, 14, and 21 days after emergence.
[e] Mean of six to eight replicates of 200–300 teliospores from flour per plate. Teliospore germination was assessed after 1 mo of incubation on water agar at 15 C.

Table 8. Germination of teliospores of *Tilletia indica* from infected kernels exposed to dry heat

Exposure (hr)	Teliospore germination (%)[a]				
	75 C	90 C	100 C	110 C	125 C
0	45.8 ± 2.3	45.8 ± 2.3	56.6 ± 2.1	47.0 ± 2.2	52.9 ± 2.2
1	–	–	–	–	0.1 ± 0.06
5	–	–	–	21.0 ± 2.6	0.0
12	–	–	–	0.5 ± 0.2	0.0
17	–	–	–	0.0	–
24	–	39.1 ± 2.1	–	0.0	–
30	–	–	–	0.0	–
40	–	–	18.7 ± 1.4	–	–
48	48.5 ± 2.3	33.8 ± 1.0	12.9 ± 2.9	–	–
53	–	–	1.6 ± 0.5	–	–
65	–	–	1.8 ± 0.2	–	–
77	–	44.9 ± 3.2	10.0 ± 0.2	–	–
88	–	–	0.6 ± 0.3	–	–
96	54.3 ± 1.7	31.7 ± 4.2	–	–	–
120	–	8.4 ± 1.8	–	–	–
192	53.8 ± 4.3	–	–	–	–
240	46.6 ± 2.8	–	–	–	–

[a] Mean ± standard error of four replicates of 200–300 teliospores per plate. The teliospores were rinsed from the infected seeds after treatment and incubated on water agar 4 wk at 15 C.

seed phytotoxicity and merits further investigation. This compound eradicates the smut fungus *Sphacelotheca sorghi* on sorghum seed (10) and is the least phytotoxic of mercury compounds applied to wheat seed (3), but it is very dangerous to handle (8 mg/kg rodent LD$_{50}$ [16]). Neither formaldehyde nor ethanol was promising because of excessive seed phytotoxicity and transient inhibition of teliospore germination. Phytotoxicity of formaldehyde and ethanol treatments was underestimated when seed were germinated on moist paper sheets. Seeds were much more damaged by these treatments when the emergence and growth of seedlings were assessed in soil rather than on paper sheets.

Some teliospores within bunt sori probably escaped treatment. The sorus of *T. indica*-infected wheat is variable (20). In the commercially harvested and threshed wheat used in this study, a small percentage of infected seeds had bunt sori that replaced only the tip of the infected seed and were enclosed by the epidermis of the seed. In other seeds, the sori were broken open but extended deeply into the seed. These teliospores were presumably protected from the soak treatments, and they could only be recovered from the flour of the infected seeds. Improving the surface wetting of the soaks did not make them eradicative, because 2–5% of the teliospores recovered from flour germinated after 1 mo of incubation when 5% ethanol and 2% dimethyl sulfoxide was added to 40% ethanol, 5 μg/ml formaldehyde, or 2% mercuric chloride soaks (J. L. Smilanick, *unpublished*). We are not optimistic that a reliable eradicative seed soak or fumigation treatment can be developed for the international movement of wheat germ plasm if it demands that no viable teliospores remain after treatment.

Because the germination of untreated teliospores of *T. indica* in this study and others (14,19) was consistently low (30–65%), other methods of assessing the viability of treated teliospores should be developed. We found that the vital stains either do not penetrate or are obscured by the thick, dark teliospore wall (J. A. Hoffmann, *unpublished*). Fluorescence microscopy has been applied to other *Tilletia* spp. (15) and fungi (21) to assess spore viability and should be attempted with *T. indica*.

Several of the treatments potentially may be used as equipment-decontaminating agents. Commercial bleach (5.25% sodium hypochlorite), diluted to 5–10% (v/v) with DW and adjusted to pH 6, would be an inexpensive and convenient sanitizing agent, although the solution is unstable and should be used immediately (12). Because the teliospores were sensitive to hot water treatment, steam heat may also be an effective decontaminating agent. Decontamination by dry heat requires high temperatures and long treatment times. Ethylene oxide was the most effective fumigant treatment. Other commonly used disinfecting agents—commercial bleach (pH 10.0–11.0), chlorine dioxide, quaternary ammonium solution, and 70–95% ethanol—were not very sporicidal at the concentrations tested.

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