Wet Seed Treatment with Peroxyacetic Acid for the Control of Bacterial Fruit Blotch and Other Seedborne Diseases of Watermelon

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ABSTRACT

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Prevention of seed transmission of *Acidovorax avenae* subsp. *citrulli* into the transplant house or field is the most effective control of bacterial fruit blotch of watermelon currently available. Peroxyacetic acid was evaluated as a disinfectant that might efficaciously eradicate *A. avenae* subsp. *citrulli* from contaminated seed and also be efficacious against other seed-transmitted diseases of watermelon. Peroxyacetic acid at low concentrations eliminated *A. avenae* subsp. *citrulli*, *Fusarium oxysporum*, and *Didymella bryoniae* from microbial suspensions. Treatments of seed contaminated with *A. avenae* subsp. *citrulli* and *D. bryoniae* with peroxyacetic acid at 1,600 µg/ml and higher for 30 min were effective in preventing seed transmission of bacterial fruit blotch and gummy stem blight. Hydrochloric acid treatments at 10,000 µg/ml, while effective in eliminating seed transmission to watermelon seedlings, can adversely affect seed germination, especially with triploid seed. Efficacious dosages of peroxyacetic acid can be applied safely to freshly harvested triploid watermelon seed without concerns for reduction in seed quality. A most effective wet seed treatment protocol involved a 30-min treatment with peroxyacetic acid at 1,600 µg/ml followed by seed drying at low humidity in a 40°C drying oven for 48 h.

Bacterial fruit blotch (BFB) of watermelon (*Citrullus lanatus*), caused by *Acidovorax avenae* subsp. *citrulli* (formerly *Pseudomonas pseudoalcaligenes* subsp. *citrulli*), was first observed in commercial production areas in the United States in the spring of 1989 (11,15), causing losses of more than 90% of the marketable fruit in some fields. BFB of watermelon has occurred in one or more watermelon-producing states in the United States every year since 1989 (13). A severe epidemic developed throughout the eastern United States in 1994, where thousands of hectares of watermelon in at least 10 states were affected (5).

The causal agent of BFB can be seedborne in watermelon (8,14,17). Contamination of watermelon seedlots is one way in which the bacterium may be introduced into a watermelon field or transplant house, where, under favorable environmental conditions, it can spread rapidly throughout the greenhouse (12) or field (4). Because of the pending lawsuits and the risk of

future litigation, the sale of seed in the United States was suspended temporarily in the fall of 1994 by most of the major watermelon seed companies, thus threatening the very existence of a watermelon industry in 1995 (10).

The most effective control of BFB of watermelon currently available is the exclusion of the bacterium from the field (9). The intensive efforts of the watermelon seed industry and the transplant industry to produce seed and transplants free of *A. avenae* subsp. *citrulli* have reduced the incidence of BFB significantly over the last five to six seasons (D. L. Hopkins, unpublished). These efforts include improved production practices by the seed producer, fermentation and acid treatment of diploid seed (5), use of seed from seed lots that have assayed negative for the presence of *A. avenae* subsp. *citrulli* by grow-out of at least 10,000 seedlings or polymerase chain reaction assay (16), and careful inspection of seedlings for symptoms by the transplant producer. In spite of these efforts, the bacterium still appears in a few fields every year. Currently, the only control option for fruit blotch once it gets into a field is multiple applications of a copper-containing bactericide/fungicide (3).

Although fermentation and hydrochloric acid (HCl) treatments are effective in removing the bacterium from seed, they can, in some cases, adversely affect seed quality. Thus, there is still a need for disinfectants that can efficaciously eradicate seed-

borne contaminants at the time of seed harvest and not reduce any measurable parameters of seed quality. Characteristics of peroxyacetic acid (PA) as a disinfectant indicate that it may be suitable for the elimination of *A. avenae* subsp. *citrulli* from contaminated watermelon seed (1). The objectives of this study were to evaluate the efficacy of PA as a disinfectant for the elimination of *A. avenae* subsp. *citrulli* from contaminated watermelon seed, to assess the significance of source of inoculum and seed drying conditions on the efficacy of disinfectants, and to evaluate the efficacy of PA against seed-transmitted *Fusarium oxysporum* and *Didymella bryoniae*.

MATERIALS AND METHODS

Pathogens. A strain of *A. avenae* subsp. *citrulli* (WFB89-1) isolated in 1989 in Florida from a commercial watermelon cultivar was used throughout these tests. WFB89-1 was stored at -70°C in nutrient broth or glycerol and was grown on nutrient agar for preparation of inoculum. This strain has given reproducible, severe symptoms on commercial varieties (7). An *F. oxysporum* strain (Fo96-3) originally isolated from wilted watermelon on the Leesburg research center in 1996 was used in the suspension culture experiments. A gummy stem blight (GSB) isolate 98-2B, also obtained from watermelon on the Leesburg research center in 1998, was used throughout these studies.

Suspension culture experiments. The rate at which PA killed the plant-pathogenic microorganisms was measured by inoculating use-solutions of each antimicrobial agent and then quantifying survivors after various exposure times. For these tests, WFB89-1 was grown on nutrient agar for 48 h and washed from the agar surface with sterile, deionized water. The bacterial suspension was adjusted to absorbance at 600 nm (A_{600nm}) = 0.25 with a spectrophotometer (10^6 to 10^7 CFU/ml). Conidial suspensions of Fo96-3 were prepared from 5-day-old liquid cultures in potato dextrose broth. Spores were collected by centrifugation and resuspended in water to a concentration of 10^5 conidia/ml as determined with a hemacytometer. GSB98-2B was grown on V8 juice agar for 3 weeks and spores were washed from the agar surface with sterile water. The concentration was adjusted to 10^5 spores/ml using the hemacytometer to count spores.

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For the suspension culture tests, disinfectant solutions included PA at 8, 80, and 800 µg/ml and HCl at 8, 80, 800, 1,000, and 10,000 µg/ml. The test solution (49.5 ml) was transferred to a flask and allowed to equilibrate to 25°C. The liquid in the flask was swirled vigorously in a rapid circular motion and 0.5 ml of a microbial suspension was added. After 30 s and 2, 5, and 10 min, 1-ml quantities of the disinfectant-microbial solution were transferred to 9 ml of a solution consisting of 0.1% sodium thiosulfate that inactivated PA. Aliquots and dilutions consisting of 0.1 ml of inactivating solution were spread-plated onto the appropriate agar media. In the case of *A. avenae* subsp. *citrulli*, 0.1 ml of inactivating solution and 0.1 ml of a 100-fold dilution were spread-plated onto a diagnostic medium (2). This diagnostic media consisted of peptone (5 g/liter), CaCl₂ (0.25 g/liter), Tween 80 (10 ml/liter), and agar (15 g/liter). Cycloheximide (50 mg/liter) and carbenicillin (50 mg/liter) were added after autoclaving.

With *F. oxysporum*, 0.1-ml aliquots of inactivating solution were spread-plated onto potato-dextrose agar. With *D. bryoniae*, 0.1-ml aliquots of inactivating solution were spread-plated onto V8 agar.

Production of seed contaminated with *A. avenae* subsp. *citrulli* and *D. bryoniae*. Contaminated seed for the first evaluation of PA concentrations and length of seed treatment (Table 1) were produced in the spring of 1999. 'Crimson Sweet' watermelon plants were grown on the research farm at Leesburg and were naturally infected with BFB and GSB. Using a commercial seed harvester, seed were collected from mostly symptomless fruit, although there were some BFB symptoms on a few fruit. After removal of the watermelon pulp, the seed were mixed back into watermelon juice. To assure seed contamination, *A. avenae* subsp. *citrulli* was added to the suspension of seed in watermelon juice diluted with water to give a bacterial concentration of 10⁵ to 10⁶ CFU/ml. *D. bryoniae* was added to a seed suspension at a

concentration of 3.6 × 10⁶ conidia/ml. The seed were incubated in the bacteria or spore suspension for 2 h with mixing every 30 min. After treatment, seed were rinsed briefly and separated for treatments, based on visual estimation of seed volume. A grow-out of the Crimson Sweet seed not incubated with bacteria or spores demonstrated that the seed were "naturally" infected with seed-transmissible BFB and GSB; therefore, these seed were both naturally contaminated and inoculated.

Seed for the second evaluations of PA concentrations and length of seed treatment (Table 2) were produced in the spring of 2000. 'Charleston Gray' watermelon plants were grown on the research farm at Leesburg and were naturally infected with BFB. Seed were collected by hand from fruit with symptoms, and symptomatic rind tissue was added to the watermelon juice slurry. BFB-free 'Saticoy' cantaloupe seed also were grown and collected by hand. Seed were inoculated with *A. avenae* subsp. *citrulli* as described above for watermelon in 1999. Watermelon seed were naturally contaminated and inoculated; cantaloupe seed were inoculated with *A. avenae* subsp. *citrulli*.

For the evaluation of the effect of seed drying conditions on treatment with PA, 'Sangria' watermelon fruit free of BFB were obtained from a commercial farm in November 2000. Seed were collected by hand and inoculated as described above. These seed were inoculated after harvest.

Wet seed treatment efficacy tests. Multiple experiments were conducted over 3 years to test efficacy of PA-based disinfectant Tsunami 100 (15% PA and 11% hydrogen peroxide; Ecolab Inc., Mendota Heights, MN). Seed were treated with disinfectant treatments within 1 h of the completion of contamination with *A. avenae* subsp. *citrulli* or *D. bryoniae*. Disinfectant treatments were conducted in 5-liter plastic buckets on the greenhouse bench. Seed were covered with approximately 1.5 liters of disinfectant solution. The solutions were stirred periodically throughout the treatments. After treatment, seed either were rinsed gently in a volume of tap water equal to that of the seed or not rinsed at all.

PA efficacy against seed transmission of BFB and GSB as a wet seed treatment was evaluated at concentrations ranging from 1,800 to 16,800 µg/ml with treatment exposure times of 15 and 30 min and was compared with HCl at 10,000 µg/ml (1%). Concentrations of PA and HCl in the treatments were calculated from the concentration of active ingredients in Tsunami 100 and concentrated HCl on a wt/vol basis. After treatment, seed were dried on screens on the greenhouse bench for 48 h at temperatures ranging from 26°C at night to 40°C in the afternoon. PA concentrations ranging from 900 to 1,800 µg/ml were evaluated for effect on seed transmission of BFB in Charleston Gray watermelon seed

Table 1. Effect of peroxyacetic acid concentration and duration of seed treatment on seed transmission of bacterial fruit blotch (BFB) and gummy stem blight (GSB) of watermelon

Treatment ^y	Concentration (µg/ml)	Exposure time (min)	Germination (%) ^z	BFB (%) ^z	GSB (%) ^z
Untreated	...	15	97 a	42 a	13 a
Peroxyacetic acid	1,800	15	96 a	0 b	2 bc
Peroxyacetic acid	5,600	15	96 a	0 b	0 c
Peroxyacetic acid	16,800	15	99 a	0 b	0 c
Hydrochloric acid	10,000	15	97 a	0 b	2 bc
Untreated	...	30	92 a	44 a	15 a
Peroxyacetic acid	1,800	30	97 a	0 b	0 c
Peroxyacetic acid	5,600	30	98 a	0 b	0 c
Peroxyacetic acid	16,800	30	98 a	0 b	0 c
Hydrochloric acid	10,000	30	94 a	0 b	5 b

^y BFB- and GSB-contaminated seed were treated with the peroxyacetic acid or hydrochloric acid solutions in 5-liter buckets, with stirring. After treatment, seed were collected on wire screens and dried for 48 h on a greenhouse bench at 25 to 35°C.

^z Values are the means of four replications of 200 seed each. Means followed by different letters within a column are significantly different by Duncan's new multiple range test, *P* = 0.05 level. The percent seed transmission of BFB was determined by a greenhouse grow-out of the seedlings and is the percentage of seedlings (germinated seed) that were symptomatic.

Table 2. Effect of peroxyacetic acid concentration and duration of seed treatment on seed transmission of bacterial fruit blotch (BFB) of watermelon and cantaloupe

Treatment ^z	Concentration (µg/ml)	Exposure time (min)	Watermelon test ^y		Cantaloupe test ^y	
			Germ. (%)	BFB (%)	Germ. (%)	BFB (%)
Untreated	...	15	90 a	1.0 b	87 a	3.0 a
PA	1,800	15	92 a	0.3 bc	87 a	0 c
PA	1,200	15	93 a	0.3 bc	86 a	0.3 bc
PA	900	15	87 ab	0.3 bc	81 a	0.3 bc
HCl	10,000	15	81 b	0 c	70 b	0 c
Untreated	...	30	91 a	3.0 a	85 a	1 b
PA	1,800	30	92 a	0 c	87 a	0 c
PA	1,200	30	92 a	0.5 bc	88 a	0 c
PA	900	30	92 a	0.3 bc	86 a	0.3 bc
HCl	10,000	30	68 c	0 c	82 a	0 c

^y Germ. = germination. Values are the means of four replications of 100 seed each. Means followed by different letters within a column are significantly different by Duncan's new multiple range test, *P* = 0.05 level. The percent seed transmission of BFB was determined by a greenhouse grow-out of the seedlings and is the percentage of seedlings (germinated seed) that were symptomatic.

^z BFB-contaminated seed were treated with the peroxyacetic acid (PA) or hydrochloric acid (HCl) solutions in 5-liter buckets, with stirring. After treatment, seed were collected on wire screens and dried for 48 h on a greenhouse bench at 25 to 35°C.

and Saticoy cantaloupe seed treated for 15 and 30 min.

Thirty-minute treatments of contaminated Sangria seed with PA at 320 and 1,600 µg/ml were used in studies comparing the effect of different drying conditions on efficacy of the disinfectant. The 48-h drying conditions were (i) greenhouse bench, where temperatures ranged from 24°C at night to 35°C in the afternoon; (ii) 40°C drying oven; (iii) 30°C drying oven; and (iv) 25°C drying oven for 24 h and a greenhouse bench for 24 h.

For the greenhouse assay of seed transmission of BFB or GSB to seedlings, seed were planted in 28-by-52-cm plastic trays filled with commercial potting mix. Two rows of 25 seed were planted in each tray. In the PA rate and treatment exposure time tests with Charleston Gray watermelon and Saticoy cantaloupe, four replications of 100 seed each were planted; all other tests consisted of four replications of 200 seed. When plants emerged, the seedling trays in a treatment replication were covered with plastic domes for two nights. Seedling trays were watered carefully on the surface of the potting mix with a hose, avoiding contact with the seedlings. Greenhouse temperatures ranged from 24 to 40°C. Disease incidence evaluations were made 10 days after planting. Percent seed transmission was calculated based on the number of emerged seedlings.

Seed safety evaluations. Triploid watermelon seed (cv. Tri-X-313), free of BFB, were used in this test because they are known to be most sensitive to disinfectants. Approximately 300 g of freshly harvested seed per treatment were submerged in mesh bags in disinfectant solution at room temperature for 15 min with periodic agitation. PA was evaluated at 3,200, 6,400, and 9,600 µg/ml and HCl was evaluated at 10,000 µg/ml. Following exposure to the disinfectants, seed either were rinsed in a stream of tap water for 5 min or immediately placed to dry. Seed were air dried in a stationary seed dryer. Germination was assessed by planting eight replications of 50 seed each into greenhouse substrate (Sunshine Mix #1) and counting the number of seedlings after 14 days. For dry weight determinations, seedlings were cut off at the base, placed in paper bags, dried in a convection oven for 4 days at 60°C, and weighed.

RESULTS

Suspension culture experiments. All three seedborne pathogens of watermelon that were evaluated were sensitive to PA at 80 µg/ml. PA at 80 µg/ml eliminated *A. avenae* subsp. *citrulli* from water suspensions in less than 30 s (Table 3). Approximately a 10-fold higher concentration of HCl achieved a similar level of efficacy. The fungal pathogens *D. bryoniae* and *F. oxysporum* were less sensitive to both PA and HCl than was *A. avenae* subsp. *citrulli*.

PA at 80 µg/ml eliminated *D. bryoniae* from suspensions in less than 2 min of exposure and *F. oxysporum* in less than 5 min; whereas HCl at 800 or 1,000 µg/ml did not kill either fungus with 10 min of exposure. HCl at 10,000 µg/ml did eliminate both fungi from suspensions.

Wet seed treatment efficacy tests. PA treatments at concentrations of 1,800 µg/ml and higher for 15 or 30 min completely eliminated seed transmission of BFB in contaminated watermelon seed without affecting seed viability (Table 1). There was 42 and 44% transmission of BFB to seedlings in the untreated seed. HCl treatment at 10,000 µg/ml (1%) also prevented seed transmission of BFB without affecting seed viability. Seed transmission of GSB was completely eliminated by a 30-min treatment with PA at 1,800 µg/ml, but not by the 15-min treatment (Table 1). After 15 or 30 min of exposure of seed to HCl at 10,000 µg/ml, seed transmission of GSB was reduced but not eliminated. There was 13 and 15% seed transmission of GSB in untreated seed.

With contaminated Charleston Gray watermelon seed, PA concentrations of 1,200 and 900 µg/ml reduced the seed transmission of BFB to <1% (1 or 2 seed transmissions per 400 seed), but did not eliminate it (Table 2). This seed lot had a relatively low seed transmission rate in the untreated seed of 1 and 3%. A 30-min treatment with PA at 1,800 µg/ml eliminated seed transmission of BFB but a 15-min treatment did not. HCl was effective, but reduced seed germination in the 30-min treatment. In a seedlot of Saticoy cantaloupe with 3 and 1% seed transmission of BFB in the un-

treated seed, PA at 1,800 µg/ml for 15 or 30 min of exposure and at 1,200 µg/ml for 30 min completely eliminated seed transmission of BFB (Table 2). At 900 µg/ml, PA reduced but did not eliminate seed transmission in cantaloupe. HCl at 10,000 µg/ml eliminated seed transmission, but appeared to reduce germination in the 15-min treatment.

The incidence of seed-transmissible BFB was different after aliquots from the same batch of noninfected seed were dried under different conditions (Table 4). Seed dried in the drying oven at 40°C or in the 25°C drying oven for 24 h and a greenhouse bench for 24 h yielded a significantly higher level of seed-transmissible BFB compared with seed dried in the 30°C drying oven or on the greenhouse bench, where temperatures ranged from 24°C at night to 35°C in the afternoon. However, when these contaminated seed were disinfected with PA at 1,600 µg/ml and dried under the same four conditions, only seed that were dried in the drying oven at 40°C had no seed transmission of BFB in either test (Table 4). Seed transmission was observed in only one of the two tests with seed dried in the 30°C drying oven or on the greenhouse bench. PA at 320 µg/ml did not eliminate seed transmission under any of the drying conditions.

Seed safety evaluations. Exposure to 15-min treatments of PA at concentrations of 3,200 and 6,400 µg/ml did not significantly reduce either germination or vigor (seedling weight) of triploid watermelon seed even if the seed were not rinsed after the treatment (Table 5). PA at 9,600 µg/ml and HCl at 10,000 µg/ml did not affect

Table 3. Rate of kill of seedborne watermelon pathogens in water suspension cultures by hydrochloric acid (HCl) and peroxyacetic acid (PA)

Treatment ^z	Concentration (µg/ml)	No. of surviving pathogens ^y			
		0.5	2.0	5.0	10.0
<i>Acidovorax avenae</i>					
subsp. <i>citrulli</i>					
Untreated	...	>10 ³	>10 ³	>10 ³	>10 ³
HCl	10,000	ND	ND	ND	ND
HCl	800	ND	ND	ND	ND
HCl	80	>10 ³	>10 ³	>10 ³	>10 ³
Peroxyacetic acid	80	ND	ND	ND	ND
Peroxyacetic acid	8	>10 ³	>10 ³	29	ND
<i>Didymella bryoniae</i>					
Untreated	...	4	4	5	6
HCl	10,000	ND	ND	ND	ND
HCl	1,000	6	6	5	5
PA	800	ND	ND	ND	ND
PA	80	2	ND	ND	ND
<i>Fusarium oxysporum</i>					
Untreated	...	>100	>100	>100	>100
PA	80	>100	20	ND	ND
HCl	10,000	ND	ND	ND	ND
HCl	800	>100	>100	>100	>100

^a Surviving pathogen populations per 0.01 ml of disinfectant-microbial solution after exposure to the treatments for 0.5, 2.0, 5.0, or 10.0 min. ND = not detectable.

^b Microbial suspensions (0.5 ml) were added to 49.5 ml of the treatment solutions at 25°C. The liquids in the flask were swirled vigorously in a rapid circular motion. After 30 s, 2, 5, and 10 minutes, 1 ml quantities of the treatments were transferred to 9 ml of a 0.1% sodium thiosulfate solution to deactivate peroxyacetic acid. Aliquots and dilutions consisting of 0.1 ml of disinfectant/microbial solutions were spread-plated onto the appropriate agar media.

germination or vigor if the seed were rinsed, but reduced germination and seedling vigor if there was no seed rinsing.

DISCUSSION

There are two functions that a seed disinfectant has to perform in order to satisfy the requirements for application to freshly harvested seed of cucurbit crops. The first function is to inactivate pathogens which are present in the solution containing the seed to prevent cross contamination of seed. The second is to eradicate pathogens which are already established on or in the seed. PA was most effective in the first function, eliminating *A. avenae* subsp. *citrulli* from suspensions when used at very low concentrations. PA also appears to be effective at low concentrations in suspensions against other seedborne cucurbit pathogens, including fungi such as *D.*

bryoniae and *F. oxysporum*. Approximately 10- to 100-fold higher concentrations of HCl were needed to achieve similar levels of efficacy.

In this study, PA disinfectant treatments of seed contaminated with *A. avenae* subsp. *citrulli* and *D. bryoniae* was effective in preventing seed transmission of BFB and GSB to watermelon seedlings. Treatment concentrations of 1,600 µg/ml and higher with 30 min of exposure of the seed to disinfectant were most effective. In previous studies, calcium hypochlorite and sodium hypochlorite were relatively ineffective as wet seed treatments to prevent seed transmission of BFB to watermelon seedlings, but HCl at 10,000 µg/ml was efficacious (5,14). In this study, the PA at 1,600 µg/ml treatment was as effective against BFB as was the HCl at 10,000 µg/ml treatment. Seed transmission of *A.*

avenae subsp. *citrulli* was demonstrated in nine different cucurbits, even when there were no fruit symptoms, illustrating that all cucurbit crops must be included in the control strategies for BFB (6). Any cucurbit crop being grown in the same transplant house or field is a potential source of the bacterium for infection of watermelon or other cucurbits on which disease loss can occur, such as muskmelon and honeydew. In this study, PA was as effective, or more effective, in preventing seed transmission of BFB to cantaloupe seedlings as it was with watermelon. Thus, PA could be used to prevent seed transmission of BFB on various cucurbit crops.

The percent seed transmission of BFB in untreated "naturally" contaminated seed and untreated seed that was inoculated after harvest was very similar (Table 1 compared with Table 4). The lower seed transmission in the experiments in Table 2 seemed to be unrelated to method of seed contamination, because the watermelon seed were contaminated naturally and the cantaloupe seed were inoculated. Efficacy of PA in preventing seed-transmissible BFB was consistent in all experiments, suggesting that it was equally effective on naturally contaminated seed and on inoculated seed.

Seed drying temperatures affected both the amount of seed transmission of BFB in nondisinfected seed and the effectiveness of PA in eliminating seed transmission of BFB. However, due to the variation of other parameters (e.g., relative humidity within the incubator) which affected the rate of drying, it is difficult to conclude which exact parameter (temperature or rate of drying) was the main factor in determining level of efficacy. It is interesting to note that one of the drying conditions (drying oven at 40°C) that resulted in the most seed transmission of BFB in untreated seed also appeared to be a good drying condition for efficacy of PA. Although drying conditions did not appear to have much effect on efficacy of PA, the 40°C drying oven treatment was the only one that had no seed transmission in either test in Table 4. The reason for this apparent paradox is unknown, but perhaps the temperature at which PA is most active is also the drying condition most favorable for bacterial survival on the untreated seed.

Fermentation and HCl treatments, while effective in eliminating seed transmission to watermelon seedlings, can adversely affect seed quality. This is especially a problem with triploid seed. Efficacious dosages of PA can be applied safely to freshly harvested triploid watermelon seed without concerns for reduction in seed quality.

Our results indicate that PA-based disinfectants can be used to control seed transmission of BFB and other seedborne diseases of cucurbits. An effective wet seed treatment protocol included a 30-min treatment with PA at 1,600 µg/ml followed by

Table 4. Effect of seed drying temperature and peroxyacetic acid wet seed treatment on seed transmission of bacterial fruit blotch (BFB) of watermelon

Treatment ^y	Temp (°C) ^z	Test 1 ^x		Test 2 ^x	
		Germination (%)	BFB (%)	Germination (%)	BFB (%)
Untreated	GH	91 bc	47 b	96 a	68 b
320 µg/ml	GH	94 ab	2 c	98 a	1 d
1600 µg/ml	GH	96 a	0 c	99 a	1 d
Untreated	40	86 c	85 a	94 a	86 a
320 µg/ml	40	95 ab	0.5 c	99 a	4 c
1600 µg/ml	40	94 ab	0 c	98 a	0 d
Untreated	30	93 ab	48 b	99 a	66 b
320 µg/ml	30	96 a	2 c	100 a	3 c
1600 µg/ml	30	97 a	0.3 c	99 a	0 d
Untreated	25	86 c	80 a	97 a	90 a
320 µg/ml	25	96 a	2 c	97 a	4 c
1600 µg/ml	25	96 a	0.8 c	98 a	0.3 d

^x Values are the means of four replications of 200 seed each. Means followed by different letters within a column are significantly different by Duncan's new multiple range test, *P* = 0.05 level. The percent seed transmission of BFB was determined by a greenhouse grow out of the seedlings and is the percentage of seedlings (germinated seed) that were symptomatic.

^y BFB-contaminated seed were treated with the peroxyacetic acid solutions for 30 min, with stirring.

^z Drying temperature. After treatment, seed were collected on wire screens and dried for 48 h on a greenhouse bench at 25 to 35°C (GH), or at 25, 30, or 40°C.

Table 5. Effect of peroxyacetic acid (PA) and hydrochloric acid (HCl) on seed germination and seedling dry weight accumulation for triploid watermelon (cv. Tri-X-313)^w

Disinfectant	Concentration (µg/ml)	Rinsing ^x	Germination (%) ^y	Seedling weight (g) ^z
PA	3,200	Yes	99 a	5.80 a
PA	3,200	No	97 a	5.15 a
PA	6,400	Yes	98 a	5.10 a
PA	6,400	No	97 a	4.95 a
PA	9,600	Yes	98 a	5.16 a
PA	9,600	No	69 b	3.08 b
HCl	10,000	Yes	98 a	5.13 a
HCl	10,000	No	4 c	0.12 c
Water	...	Yes	98 a	5.34 a
Water	...	No	95 a	5.20 a

^w Approximately 300 g of freshly harvested seed (per treatment) contained in mesh bags were submerged in disinfectant solution at room temperature for 15 min with periodic agitation.

^x Following exposure to the disinfectants, seed either were rinsed in a stream of tap water for 5 min (Yes) or immediately placed to dry (No).

^y Germination was assessed by planting eight replications of 50 seed each into greenhouse substrate (Sunshine Mix #1) and evaluated number of seedlings after 14 days. Means followed by different letters within a column are significantly different by Duncan's new multiple range test, *P* = 0.05 level.

^z Seedlings were cut off at the base, placed in paper bags, and dried in a convection oven for 4 days at 60°C. Means followed by different letters within a column are significantly different by Duncan's new multiple range test, *P* = 0.05 level.

seed drying at low humidity in a 40°C drying oven for 48 h. PA disinfectant could become an important tool in the management of seedborne BFB by the watermelon industry.

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