Benomyl in Acetone Eradicates *Fusarium moniliforme* and *F. oxysporum* from Asparagus Seed

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ABSTRACT

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Treating asparagus seed with 10% chlorine bleach solution, 2,000 ppm benomyl in water, or hot water at 50–55 C significantly reduced but did not eliminate *Fusarium moniliforme* and *F. oxysporum*. Hot-water treatments drastically reduced germination. Both fusaria were eradicated from asparagus seeds and germination was stimulated when seeds were soaked in 25,000 ppm benomyl in acetone for 24 hr. A reliable method for producing *Fusarium*-free asparagus seedlings was developed.

Fusarium moniliforme Sheldon and *F. oxysporum* Schlecht. cause root, stem, and crown rots of asparagus (*Asparagus officinalis* L.) (2,6). These diseases result in the asparagus decline and replant problem that is gradually eliminating asparagus culture in Massachusetts.

Both fusaria are known to be associated with asparagus seed (3,5,8). In Washington State, up to 9.3% of seed carried *F. oxysporum* and *F. moniliforme* (5). Conidia were lodged in deep crevices in the seed coat and at asparagus beetle feeding sites. In Massachusetts, we have found both *F. moniliforme* and *F. oxysporum* associated with asparagus seed in every commercially obtained seed lot we have sampled (8).

Treating asparagus seed with chlorine bleach solutions (10–25%) before planting is often recommended to eradicate fusaria. The systemic fungicide benomyl has been shown to be effective against both fusaria associated with asparagus (7) and other seedborne fungi (4). Acetone can be used as a nontoxic carrier to infuse benomyl and other fungicides into seeds to eradicate infections (4,9,10). Hot-water treatment is also a proven method for eradicating seedborne pathogens (1).

The close association of *F. moniliforme* and *F. oxysporum* with asparagus seed has made it impossible to produce large numbers of disease-free seedlings for use in the laboratory and greenhouse or to develop disease management strategies in the field. To solve this critical problem, we compared the efficacy of several benomyl and hot-water treatments for eradication of *F. moniliforme* and *F. oxysporum* from asparagus seed.

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MATERIALS AND METHODS

Two lots (A and B) of commercially obtained seed of asparagus cultivar Mary Washington were used. Seeds were plated on potato-carrot agar (PCA) acidified with lactic acid to pH 4.0 (PCAL) to determine the presence of *F. moniliforme* and *F. oxysporum*. Five replicates of 100 seeds each were treated and plated for each treatment. All plates were incubated at 24 C for 3 wk. Percentage seed germination and incidence of fusaria were recorded. Fusaria were subcultured and identified according to the methods and scheme of Toussoun and Nelson (11). All data were analyzed with a one-way analysis of variance and Duncan's multiple range test ($P = 0.05$).

Seeds given hot-water treatments were divided into two sublots. One sublot was presoaked in sterile, distilled water for 24 hr; the other was not. Each sublot was then divided in half again. One half was placed in 50-C water for 25 min and the other half was placed in 53-C water for 15 min. Untreated seeds were used as controls.

The effectiveness of benomyl in water or acetone was compared with a Clorox treatment on seed from lot A. Seeds were treated in 15-g lots in 100 ml of solution in 250-ml flasks and aerated on a rotary shaker for 24 hr. Treatments consisted of 2,000 ppm benomyl in water; 6,250, 12,500, and 25,000 ppm benomyl in acetone; acetone alone; and 10 min in 10% Clorox solution. Presoaked, untreated seeds were used as controls. All treated seeds were repeatedly washed in sterile, distilled water after treatment to remove benomyl residues. All seeds were then soaked for 24 hr in sterile, distilled water and plated on PCAL. Additional randomly selected seeds were plated on PCA seeded with spores of *F. moniliforme* and *F. oxysporum* to detect any remaining benomyl residues in seeds and seedlings. This experiment was repeated

Table 1. Effectiveness of treatments for eliminating *Fusarium moniliforme* and *F. oxysporum* from asparagus seed

Treatment	Colonies from seed[x,y] (no.)		Seed germination[y,z] (%)
	F. moniliforme	*F. oxysporum*	
		Seed lot A	
Control (none)	1.4 a	2.2 a	79.6 ab
10% Clorox (10 min)	0.6 bc	0.8 b	78.8 ab
Acetone (24 hr)	0.8 ab	1.8 a	78.0 ab
Benomyl (2,000 ppm) in water (24 hr)	0 c	1.0 b	76.4 a
Benomyl (6,250 ppm) in acetone (24 hr)	0 c	0.2 b	82.6 b
Benomyl (12,500 ppm) in acetone (24 hr)	0 c	0.2 b	83.6 b
Benomyl (25,000 ppm) in acetone (24 hr)	0 c	0 b	84.0 b
		Seed lot B	
Control (none)	9.8 a	7.2 a	70.0 ab
10% Clorox (10 min)	1.2 b	0.8 b	70.6 ab
Acetone (24 hr)	0.6 b	1.4 b	65.4 a
Benomyl (2,000 ppm) in water (24 hr)	0.4 b	0.4 b	67.2 a
Benomyl (25,000 ppm) in acetone (24 hr)	0 b	0 b	74.8 b

[x] Average number of *Fusarium* colonies per 100 seed (five replicates, 100 seed per replicate).
[y] Means in a column for each seed lot followed by the same letter are not significantly different ($P = 0.05$), according to Duncan's multiple range test.
[z] Seed soaked in sterile, distilled water for 24 hr after treatment and then incubated on acidified potato-carrot agar for 21 days.

on seed from lot B with benomyl in acetone at only the 25,000 ppm rate.

RESULTS

All hot-water treatments significantly reduced the number of colonies of *F. moniliforme* and *F. oxysporum* associated with asparagus seed by 40–60%. Germination rates, however, were reduced as much as 37%, making this an undesirable procedure.

Clorox reduced but did not eliminate fusaria from seeds (Table 1). Benomyl in water and at three concentrations in acetone eliminated *F. moniliforme*; however, only benomyl in acetone at 25,000 ppm eliminated *F. oxysporum*. All benomyl in acetone treatments increased percentage germination (Table 1).

Washing seeds after benomyl treatment effectively removed fungicide residues. Seeds plated on PCA seeded with spores of both fusaria germinated and died. Zones of spore germination inhibition around plated, washed seeds or resulting seedlings were not observed.

DISCUSSION

Acetone is known to be an effective vehicle for infusing seed coats and seeds with fungicides (9,10). Benomyl in acetone apparently can penetrate the deep crevices in asparagus seed coats where *Fusarium* spores lodge (5) much more effectively than benomyl in water (9,10).

Repeated washing of seeds treated with benomyl in acetone to remove fungicide residues is an important step. We have noticed toxicity symptoms (reduced germination, stunting of seedlings) when benomyl is applied to seed as a dust. When washed seed was used, none of these adverse effects were observed. Germination is increased and healthy, vigorous seedlings result. This increase in germination may be caused by the leaching of inhibitors or a softening of seed coats during the treatment.

We routinely use the 25,000 ppm benomyl in acetone seed treatment in our laboratory as a reliable method for producing large numbers of asparagus seedlings free of *F. moniliforme* and *F. oxysporum*. The benomyl in acetone treatment assures germination in steamed soil, soilless growing media, sand, or nutrient solutions without introducing *F. moniliforme* or *F. oxysporum* on the seed.

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