Sodium Metabisulfite Reduces Fungal Inoculum in Containers Used for Conifer Nursery Crops

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Containers from eight nurseries in the northern Rocky Mountains were treated with a 5% (w/v) solution of sodium metabisulfite. In general, after being used for at least one crop, Styroblock containers were more contaminated than pine cells before and after treatment. Treatment significantly reduced levels of both Fusarium *and other groups of fungi. Seedling disease levels were usually reduced in crops grown in treated containers; Douglas-fir (*Pseudotsuga menziesii *var.* glauca *[Beissn.] Franco) seedling growth was otherwise unaffected by the treatment.* Tree Planters' Notes **44(4)**: 161–165; 1993.

Fusarium root disease is a serious problem in the production of container-grown seedlings, especially Douglas-fir (*Pseudotsuga menziesii* var. *glauca* (Beissn.) Franco) (James 1986). Losses to disease vary with seed source and individual nursery, ranging from a few percent to over 70%. Control of this disease is best realized through integrated pest management (James and others 1990). Reducing exposure to *Fusarium* inoculum is paramount to reducing infection and subsequent disease (James and others 1991). Inoculum has been associated with seeds, growing media, containers, and weeds (James and others 1987, James and others 1991). After several crops, Styrofoam®—and to a lesser degree, hard plastic containers—develop cracks and holes in which root pieces and organic matter collect. Seedling roots often penetrate the inner walls of Styroblock containers and remain embedded when the seedlings are extracted. This residue, especially at the bottom of cells, harbors *Fusarium* inoculum (James and others 1988a, James and Woollen 1989) and is often the largest inoculum source for new crops of seedlings (James and others 1988b).

Sodium metabisulfite ($Na_2S_2O_5$) is used as an antifermenting agent in beer and winemaking and as a preservative for fruits and vegetables (Sturrock and Dennis 1988). When mixed with water, sodium metabisulfite (SMBS) releases sulfur dioxide (SO_2), which is toxic to many fungi (Hibben 1966). Therefore, treatment with this chemical may reduce inoculum of pathogenic fungi within growing containers.

Our main objective in this study was to determine whether SMBS could effectively reduce levels of inoculum of potential root-pathogenic fungi without adversely affecting seedling growth.

Materials and Methods

Uncleaned containers, previously used to grow conifer seedlings, were provided by eight forest seedling nurseries in the northern Rocky Mountains. Four nurseries each provided eight trays of Ray Leach Pine Cells® (200 cells per tray) while the other four nurseries each provided eight Styroblock 4's (160 cells per block). Before treatment, all containers were assayed for *Fusarium*, *Cylindrocarpon*, *Trichoderma*, and other fungi by aseptically removing two pieces of container, each about 10 mm long, 5 mm wide, and 1 mm thick, from near the bottom drainage hole of 10 randomly selected cells per container. Pieces were placed on a selective medium for *Fusarium* and closely related fungi (Komada 1975) and incubated under cool fluorescent light at 22 to 24 °C for 7 to 10 days. Selected isolates of *Fusarium* were single-spored onto both carnation leaf (Fisher and others 1982) and potato dextrose agar for identification using the taxonomic methods of Nelson and others (1983).

Four pine cell trays and four Styroblocks were completely immersed for 30 sec in a 5% (w/v) solution of SMBS and allowed to dry uncovered because the growers who requested this work were interested in using this chemical as a dip, without the additional expense of tarps and tarping. Once dry, the containers were again assayed using the above procedure; the same cells previously sampled were once again evaluated for the presence or absence of fungi.

To test effects of container treatment with SMBS on seedling growth, two seedlots of Douglas-fir from northern Idaho (8002 and Bovill) were surface-sterlized in a solution of 2 parts laundry bleach (5.25% sodium hypochlorite) with 3 parts water for 10 min (Wenny and Dumroese 1987), rinsed in running tap water for 48 h, and stratified for 28 days at

3 °C. After stratification, the seeds were rinsed for 24 h in running tap water and sown into treated and untreated containers filled with a 50:50 peat–vermiculite growing medium commonly used by container nurseries in the northern Rocky Mountains. Each treated and untreated container had half of its cells sown with the Bovill seed source and the other half with 8002. Containers were placed on tables in a randomized block design. Seedlings were grown under the regime of Wenny and Dumroese (1992). One month after planting, seedlings were fertilized and irrigated twice weekly with Peter's Conifer Starter® at 42 ppm N for 4 weeks, followed by twice weekly applications of Peter's Conifer Grower® at 120 ppm N alternated with liquid calcium ammonium nitrate at 81 ppm N for 6 weeks. Seedlings were then fertilized only when irrigation was necessary and received Peter's Conifer Finisher® at 24 ppm N alternated with liquid calcium ammonium nitrate at 161 ppm N until seedlings were extracted for cold storage.

Seedling emergence (based on germination of hand-sown seed within individual container cells) was calculated after germination was deemed complete. Post-emergence damping-off and root disease were periodically monitored throughout the growth cycle. Diseased seedlings were collected and isolations made from their roots to identify associated fungi. Thirty randomly selected seedlings from each treatment were measured for height and root collar diameter (caliper) at the end of the growth cycle, about 7 months after sowing. These same seedlings were assayed for root colonization by potentially pathogenic fungi. Isolations were made from randomly selected, surface-sterilized root tips onto Komada's medium and incubated as described above. Selected *Fusarium* isolates from roots were identified. Biomass was determined on a subsample of 15 seedlings after drying for 24 h at 60 °C.

Cell colonization before and after treatment was compared using the non-parametric test of Kruskal-Wallis (Ott 1984). Means were converted to percentages after the analysis of variance. Diseased and non-diseased seedlings and seedling morphological characteristics were statistically tested with a one-way analysis of variance (Snedecor and Cochran 1980) and means separated using Tukey's honestly significant different test at $P = 0.05$.

Results and Discussion

Immersing containers in SMBS significantly increased the percentage of clean cells (those not colonized by fungi) in both Styroblock and plastic containers. Styroblock containers had fewer clean cells before and after treatment when compared to pine cells (table 1). However, Styroblocks also had the highest levels of *Trichoderma*, a known *Fusarium* antagonist (Papavizas 1985), before and after the treatment. Percentage of cells colonized by *Fusarium* spp. was significantly decreased by the treatment for both container types. However, the inoculum level remaining in containers was higher than that reported by Sturrock and Dennis (1988). Their lower levels of inoculum may have been a function of enclosing the containers after treatment. Peterson (1990), using a 5% solution

Table 1—*Percentage of cells in each container type colonized with* Fusarium, Cylindrocarpon, Trichoderma, *and other fungi before and after immersion in sodium metabisulfite (5%)*

Container type and treatment	*Fusarium* (%)	*Cylindrocarpon* (%)	*Trichoderma* (%)	Other fungi (%)	Clean cells* (%)
Ray Leach Pine Cells					
Before	17.9 a	4.0 a	34 a	67 a	39 a
After	7.5 b	0.0 a	31 a	29 b	68 b
Styroblocks					
Before	41.2 a	13.0 a	55 a	35 a	2 a
After	20.8 b	15.0 a	56 a	32 a	35 b
All containers					
Before	29.8 a	8.7 a	45 a	51 a	20 a
After	14.2 b	7.5 a	43 a	30 b	51 b
Pine Cells vs. Styroblocks					
Pine Cells before	17.9 a	4.0 a	34 a	67 a	39 a
Styroblocks before	41.2 b	13.0 b	55 b	35 b	2 b
Pine Cells after	7.5 a	0.0 a	31 a	29 a	68 a
Styroblocks after	20.8 b	15.0 b	56 b	32 a	35 b

Means were converted to percentages after being compared with the Kruskal-Wallis test. For each container type, values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

*Cells without fungal or bacterial growth.

of SMBS and a 10-sec dip, effectively removed *Fusarium* spp. and other fungi by promptly enclosing containers with plastic. Containment of treated containers probably allows for a longer and more concentrated fumigation effect. The most frequently isolated species of *Fusarium* from containers was *F. proliferatum* (Matsushima) Nirenberg. Other isolated species included *F. oxysporum* Schlechtend.:Fr., *F. acuminatum* Ellis & Everh., *F. sporotrichioides* Sherb., and *F. tricinctum* (Corda) Sacc.

The percentage of cells colonized by other fungi was also significantly decreased by treatment with SMBS, indicating that the chemical was toxic to several different groups of fungi. Levels of *Trichoderma* and *Cylindrocarpon* on containers were unaffected. Species of *Trichoderma* were unidentified but most isolates of *Cylindrocarpon* were *C. destructans* (Zinssmeister) Scholten.

Table 2 shows the variation between inoculum levels in containers from different nurseries and subsequent inoculum reduction by the treatment. There was extensive disparity in container cleanliness among the nurseries sampled. Much of this disparity reflected container age—i.e., those containers that were older and had been used to grow several seedling crops (evident because of wear and deterioration of the cells) were colonized with all types of fungi at much higher levels than newer containers reused fewer times.

Seedling emergence was unaffected by treatment (data not shown), and levels of post-emergence disease were very low for both treatments (table 3). Significant reductions in number of diseased seedlings were observed in the Douglas-fir crops grown in SMBS-treated pine cells. Similar trends were noticed in Styroblock containers, with $P = 0.12$. Although the number of diseased seedlings were similar in treated and control containers, initial container inoculum was significantly reduced. When data from all containers were pooled, the number of diseased seedlings were significantly reduced in SMBS-treated containers.

Table 3—*Effect of treating containers with sodium metabisulfite (5%) on the number of post-emergent diseased Douglas-fir seedlings throughout the growth cycle*

Container type and treatment	Diseased seedlings	
	n	%
Ray Leach Pine Cells		
Treated	10 a	1.2
Untreated	16 b	2.0
Styroblocks		
Treated	24 a	3.8
Untreated	32 a	5.0
All Containers		
Treated	34 a	2.4
Untreated a	48 b	3.3

Because of low disease levels within each tray or block, data from each treatment/container-type combination were pooled prior to analysis. For each container type, values in each column followed by the same letter are not significantly different in $P \leq 0.05$.

Table 2—*Percentage of cells colonized with* Fusarium, Cylindrocarpon, *and* Trichoderma *spp. in containers from 8 nurseries before and after treatment with sodium metabisulfite (5%)*

Container type and nursery	Treatment	*Fusarium* (%)	*Cylindrocarpon* (%)	*Trichoderma* (%)	Clean cells* (%)
Ray Leach Pine Cells					
1	Before	0 a	5 a	55 a	60 a
	After	0 a	0 a	40 a	70 a
2	Before	8 a	2 a	3 a	43 a
	After	0 a	0 a	3 a	97 b
3	Before	0 a	7 a	23 a	52 a
	After	0 a	0 a	10 a	97 b
4	Before	64 a	3 a	55 a	0 a
	After	30 b	0 a	70 a	7 b
Styroblocks					
5	Before	77 a	2 a	55 a	0 a
	After	33 b	10 a	80 b	10 b
6	Before	13 a	5 a	35 a	3 a
	After	3 a	3 a	17 a	87 b
7	Before	3 a	40 a	88 a	3 a
	After	7 a	40 a	73 a	20 b
8	Before	73 a	7 a	42 a	0 a
	After	40 b	7 a	53 a	23 b

Means were converted to percentages after being compared with the Kruskal-Wallis test. For each nursery, values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

*Cells without fungal or bacterial growth.

Root colonization of non-diseased seedlings by selected fungi is summarized in table 4. Significant differences between seedlings grown in SMBS-treated containers and those grown in untreated containers were not apparent for root colonization by *Fusarium*, *Cylindrocarpon*, and *Trichoderma* spp. Most sampled seedlings were colonized by *Fusarium* spp., whereas about one-third were colonized by *Cylindrocarpon* spp. and nearly one-half by *Trichoderma* spp. The percentage of infected seedlings was probably influenced and confounded by inoculum from other sources than the containers, including seed and airborne additions. For both treatments and seedlots, about 50% of the root tips sampled were infected with *Fusarium* (data not shown). The most common *Fusarium* and *Cylindrocarpon* species colonizing roots were the same recovered from containers: *F. proliferatum* and *C. destructans*. *Fusarium proliferatum* was also the most commonly isolated fungus from diseased seedlings. We believe this species is capable of extreme virulence on Douglas-fir seedlings (unpublished data) and may also commonly colonize seedling roots without eliciting disease symptoms. It is readily adapted to greenhouse environments and is a common colonizer of roots of many different conifer species. Unlike several other species of *Fusarium*, *F. proliferatum* does not produce resistant chlamydospores or sclerotia (Nelson and others 1983), which aid in carrying the fungus between crops of susceptible seedlings. However, it (along with most *Fusarium* species) is able to remain viable for extended periods on both Styroblock and pine cell containers, as verified by this and other investigations (James and others 1988a, Sturrock and Dennis 1988).

Table 4—*Effect of container treatment with sodium metabisulfite (5%) on percentage of non-diseased seedlings with* Fusarium, Cylindrocarpon, *or* Trichoderma *spp. colonizing their root systems*

Treatment	*Fusarium* (%)	*Cylindrocarpon* (%)	*Trichoderma* (%)
Treated	95 a	37 a	40 a
Untreated	92 a	35 a	42 a

Percentages compiled for both pine cell and Styroblock containers at all nurseries. Values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

Seedling height, caliper, and ovendry weight were unaffected by SMBS treatment (table 5). However, seedlings from the Bovill seedlot in SMBS-treated containers were significantly taller than those in untreated containers. Apparently, residual SMBS on the containers was non-phytotoxic to Douglas-fir seedlings. This confirms work by Peterson (1990), who did not find phytotoxicity on sensitive lettuce plants in treated containers.

Table 5—*Effects of container treatment with sodium metabisulfite (5%) on mean height, caliper, and ovendry weight at lifting of Douglas-fir seedlings*

Seedlot and treatment	Height (cm)	Caliper (mm)	Ovendry weight (g)
Bovill			
Treated	18.3 a	2.25 a	1.33 a
Untreated	17.1 b	2.16 a	1.25 a
8002			
Treated	16.6 a	2.12 a	1.26 a
Untreated	16.4 a	2.18 a	1.22 a
Seedlots combined			
Treated	17.5 a	2.19 a	1.30 a
Untreated	16.7 a	2.17 a	1.23 a

For each seedlot, values in each column followed by the same letter are not significantly different at $P \leq 0.05$.

Management Implications

Our results indicate that SMBS was effective in reducing levels of several different groups of fungi, including potential pathogens in the genus *Fusarium*, on both pine cells and Styroblock containers. However, *Fusarium* propagules were not completely eliminated from the containers, indicating that this treatment is less effective in removing inoculum than immersing containers in hot water (James and Woollen 1989). Residual levels of pathogenic fungi following SMBS treatment were sufficient to cause some disease and seedling root infection during the crop cycle. However, effects on seedling growth as measured by height, caliper, and biomass production were usually undiscernible. We found that Douglas-fir seedlings with low levels (10 to 40%) of *Fusarium* infection on their roots grow and survive as well as uninfected seedlings after outplanting on a forest site (Dumroese and others 1993). Other concentrations and submersion times may improve the efficacy of SMBS treatments.

The major disadvantages of using SMBS commercially in container nurseries involve potential health risks to workers and environmental concerns involved in disposal after use. Workers should avoid skin exposure to SMBS by using chemical goggles and rubber boots, gloves, and aprons. Even with such protective clothing, some workers may show hypersensitivity to the sulfites produced in SMBS solutions. Because of the extreme corrosiveness of SMBS solution, it should only be used in stainless steel, plastic, or fiberglass tanks. Disposal problems can be reduced by adjusting the pH of the solution to neutral, separating any insoluble liquids or solids for hazardous-waste disposal, and flushing the aqueous solution down the drain with plenty of fresh water (Peterson 1991).

We conclude that SMBS is effective in reducing levels of potentially pathogenic fungi on Styroblock and plastic containers used in conifer seedling nurseries. Treatment with SMBS may especially be desirable if equipment is unavailable for immersing large numbers of containers in hot water solutions. Tarping treated containers immediately after dipping may enhance the fumigation effect. Whichever system is used, it is important that containers used to grow several crops of seedlings are sterlized between crops.

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