Styroblock Sanitization: Results of Laboratory Assays from Trials at Several British Columbia Forest Nurseries[1]

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Abstract.—Moss and algae build-up on used styroblocks and an increase in root diseases of container-grown forest nursery seedlings in British Columbia prompted investigation of improved methods for sanitizing used styroblocks. Current block washing methods, pasteurization treatments, and several biocides were tested for their efficacy against algae and pathogenic fungi. Assays of treated styroblock pieces cultured on media in the laboratory indicate that pasteurization treatments reduced algae and virtually eliminated pathogenic fungi. Three biocides, i.e., captan, sodium metabisulfite, and methyl bromide were equally effective against pathogenic fungi. Additional testing of these and other sanitizing methods is needed to provide growers with a choice of block washing methods.

INTRODUCTION

In British Columbia forest nurseries conventional cleaning agents for used styroblocks include sodium hypochlorite (common household bleach) diluted with water and soaps specially formulated to kill moss and algae. Depending on when seedlings are lifted, styroblocks are either washed immediately after seedling removal or they are overwintered on the nursery site and washed before seed sowing. Some nurseries use sophisticated block washing machines; others use home-made dip tanks or heavy pressure hoses to wash blocks. Recent losses of seedlings to root diseases indicate that these cleaning procedures are only partially effective.

Molded from expanded polystyrene beads, styroblocks deteriorate over time, especially with heavy use. Cracks and holes which develop in the styrofoam accumulate root pieces and other organic debris which harbor fungi, algae, mosses, and insect eggs. This phenomenon cannot be overlooked in British Columbia for two important reasons: 1) enhanced production of container seedlings means prolonged use of styroblocks, e.g., blocks at some nurseries have been used for 7 to 10 seasons; and 2) an expanding 2+0 container seedling program means seedling roots are present in styroblock cavities for long periods.

Recognizing the need for an effective cleaning procedure for used styroblocks, British Columbia Ministry of Forests and Canadian Forestry Service extension personnel, and staff at both Ministry and private nurseries undertook cooperative styroblock sanitization trials which are described here. Although the trials were originally initiated because of concerns over moss and algae build-up on used styroblocks, laboratory assay results showed that inoculum of pathogenic fungi such as Fusarium survive even rigorous styroblock washing. The first two trials tested conventional washing techniques (i.e. bleach and soap washes) against pasteurization (i.e. using heated water or other solutions or steam for a few minutes at 100°C or less to reduce microbial populations). The remaining trials tested the efficacy of several biocides as sanitizing agents.

MATERIALS AND METHODS

Trial I: Conventional Washing Versus Pasteurization

Nine block washing treatments were tested for sanitizing used styroblocks (Table 1). The styroblocks treated were of uniform age and had been used several years. The stainless steel tank used for pasteurization treatments (3 to 7) accommodated one block at a time. Tank water was heated and the temperature was maintained by using an acetylene torch to heat air being circulated by a fan through a curved steel pipe inside the tank. Treated styroblocks were randomly sampled soon after treatment by cutting ten 60 x 10 mm pieces from each block with a hand saw. The saw was sterilized with 70% ethyl alcohol after each sample. To determine post-treatment survival of fungi, 80 pieces from each treatment were transferred to petri plates containing one of four culture media (20 pieces on each medium): a Pythium-selective medium (PA) (Hendrix and Kuhlman 1965) with the antibiotic Nystatin added (Eckert and Tsao 1962); a Pythium-selective V8 juice medium (PPA) (Peninsu-Lab, Kingston, WA 1984); a Fusarium- selective medium (KM) (Komada 1975); or a general medium, Difco (Difco Laboratories, Detroit, MI) acidified potato dextrose agar (APDA). To determine survival of algae, 10 block pieces per treatment were placed in test tubes containing either Bristol's Solution (BS) (Bold 1942), a

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Table 1.—Conventional washing versus pasteurization treatments of used styroblocks: Trial I.

Treatment	Description
1	Styroblocks washed[1] and dipped for 10 seconds in a tank containing a solution of Safer's DeMoss soap (1:19 v/v).
2	Styroblocks washed and dipped for 10 seconds in a tank containing a solution of sodium hypochlorite (6% household bleach) (1:11 v/v).
3	Styroblocks washed and dipped for 10 seconds in a tank containing a solution of Safer's DeMoss soap (1:19 v/v) at 30°C.
4	Styroblocks washed and dipped for 10 seconds in a tank containing a solution of Safer's DeMoss soap (1:19 v/v) at 50°C.
5	Styroblocks washed and dipped for 3 minutes in a tank containing water at 80°C.
6	Styroblocks washed and dipped for 1 minute in a tank containing water at 100°C.
7	Styroblocks washed and dipped for 3 minutes in a tank containing water at 100°C.
8	Styroblocks washed (control).
9	Styroblocks not washed (unwashed control).

[1]All styroblocks were washed with water in a block washing machine before subsequent treatment..

general medium for algae, or soil algae agar (SAA) (Poindexter 1971), a solid culture medium specific to fresh water and soil algae. Plates and tubes were incubated at room temperature under prevailing daylight for up to 8 weeks. Fungi growing from the styroblock pieces were identified as to genus and the colonies were counted. Algae colonies were not identified but were assessed as either present or absent.

Trial II: Conventional Washing Versus Pasteurization

Treatments 1, 5 and 9 from Trial I were repeated and five new pasteurization treatments were assessed (Table 2). The chamber used for treatments 5 through 7 was a standard greenhouse steam sterilizer approximately 1.07 m in diameter x 1.5 m in length. To prevent warping of the styroblocks, the steam was bled off intermittently to maintain the chamber temperature at 80 to 82°C. This meant that the styroblocks received a steam condensate treatment rather than a 100°C steam treatment. Treated styroblocks were sampled as in Trial I, but block pieces (20 per medium from each treatment) were plated on to only three culture media: KM, PA, and SAA. Numbers of fungi occurring on each styroblock piece were counted instead of counting individual fungus colonies, as in Trial I. Algae colonies were assessed as in Trial I.

Table 2.—Conventional washing versus pasteurization treatments of used styroblocks: Trial II.

Treatment	Description
1	Styroblocks washed[1] and dipped for 10 seconds in a tank containing a solution of Safer's DeMoss soap (1:19 v/v).
2	Styroblocks washed and dipped for 10 seconds in a tank containing a solution of Safer's DeMoss soap (1:19 v/v) at 80°C.
3	Styroblocks washed and dipped for 1 minute in a tank containing water at 80°C.
4	Styroblocks washed and dipped for 3 minutes in a tank containing water at 80°C.
5	Styroblocks washed and placed for 3 minutes in a steam chamber (80 to 82°C @ approximately 55 kPa).
6	Styroblocks washed and placed for 5 minutes in a steam chamber (80 to 82°C @ approximately 55 kPa).
7	Styroblocks washed and placed for 3 minutes in a steam chamber (80 to 82°C; no pressure).
8	Styroblocks not washed (unwashed control).

[1]All styroblocks were washed with water in a block washing machine before subsequent treatment.

Biocide Trials

From fall 1987 to spring 1988 growers tested several biocides for sanitizing used styroblocks. Test conditions were not consistent due to the operational aspect of these trials. Styroblocks tested had been used for at least two growing seasons. The more promising treatments are summarized in Table 3. These included dips in captan (treatment A) and in various concentrations of heated and non-heated solutions of sodium metabisulfite (treatments B, C1, C2, and C3, and D). Also known as anhydrous sodium metabisulfite (ABS) and sodium pyrosulfite, this free-flowing white, fine granular product is commonly used as (i) an anti-fermentative agent to kill naturally occurring yeasts in brewing and winemaking, (ii) a preservative for fruits and vegetables, and (iii) a dechlorinating agent in the production of colored paper. It is available in both technical and food grades. When mixed with water, ABS is mildly acidic and releases sulfur dioxide (SO_2) at a rate increasing with temperature. Potassium metabisulfite, which was tested in treatment A, is a product very similar to ABS. Testing continued with ABS because of its availability in commercial quantities.

Biocides tested which showed little or no effectiveness against pathogenic fungi included a detergent (G.H. Wood Detergent-

Table 3.—Summary of biocide treatments of used styroblocks.

Treatment		Description
A		Pieces of used, unwashed styroblocks dipped for 10 seconds in the following (all pieces air dried after treatment):
	1	a 0.016% solution of Captan 50WP.
	2	a 1.25% solution of potassium metabisulphite ($K_2S_2O_5$).
	3	water (control).
B		Pieces of used, unwashed styroblocks dipped for 6 seconds in the following solutions of anhydrous sodium metabisulfite (ABS) ($Na_2S_2O_5$), (a) immediately after solution was mixed or (b) 16 hours after mixing (b) (all pieces air dried after treatment):
	1a,b	ABS- 0.313% solution.
	2a,b	ABS- 0.625% solution.
	3a,b	ABS- 1.25% solution.
	4a,b	ABS- 2.5% solution.
	5a,b	ABS- 5% solution.
	6a,b	ABS- 10% solution.
	7	Pieces of used, unwashed styroblocks dipped for 6 seconds in water (control).
C		Pieces of used, unwashed styroblocks dipped for 6 seconds in the following solutions of ABS (a) immediately after solution was mixed or (b) 16 hours after mixing (all pieces air dried after treatment):
	1a,b	ABS- 2.5% solution.
	2a,b	ABS- 5% solution.
	3a,b	ABS- 10% solution.
	4	Styroblocks washed, placed under plastic tarp and fumigated with 100% methyl bromide at 0.6 kg/m^3.
	5	Pieces of used, unwashed styroblocks dipped for 6 seconds in water (control).
D		Styrdblocks washed and dipped for 20 seconds in the following solutions of ABS (all blocks rinsed with water then air dried after treatment):
	1a,b,c	ABS- 1.25% solution at 4, 40 & 70°C.
	2a,b,c	ABS- 2.5% solution at 4, 40 & 70°C.
	3a,b,c	ABS- 5% solution at 4, 40 & 70°C.
	4a,b,c	ABS- 10% solution at 4, 40 & 70°C.
	5a,b,c	ABS- water at 4, 40 & 70°C (controls).

Germicide 2004), Agribrom (a bromine based, oxidizing biocide; Tayama et al. 1986), Lysofume (a disinfectant containing formaldehyde), and two metalaxyl-benomyl (Ridomil-Benlate) solutions. Treated styroblocks were sampled as in Trials I and II but block pieces (approximately 10 per medium from each treatment) were plated on to only two culture media: KM and PA. Fungi were assessed as in Trial II. Presence or absence of algae was not determined.

RESULTS AND DISCUSSION

The results of laboratory isolations for fungi and algae from used styroblocks treated in Trial I are given in Table 4. In general, pasteurization (i.e. heat) treatments were more effective for sanitizing used stryroblocks than conventional cleaning treatments (i.e. soap and bleach). Although treatment 7 (3 min at 100°C) yielded the fewest colonies of pathogenic fungi and also reduced algae, this treatment is not considered practical because 100°C distorts styroblocks. The same is true for treatment 6 (1 min at 100°C). Thus, in terms of reducing algae and pathogenic fungi, treatment 5 (3 min at 80°C) is considered the best of the nine treatments, followed by the heated soap and bleach treatments 4, 3, and 2. These results suggest that heating water and soap solutions improves their ability to kill pathogenic fungi. High temperatures (e.g. 80 and 100°C) also appear to kill algae more effectively than moderate temperatures (e.g. 50°C), soap, and bleach treatments. Given that the mechanism of sterilization by heat involves protein denaturation (Davis et al. 1973), these results are not surprising. The 0.05% sodium hypochlorite solution (treatment 2) was clearly ineffective against algae, although it reduced pathogenic fungi. Because materials containing organic matter (e.g. used styroblocks) react rapidly with the Cl_2 component of a sodium hypochlorite solution, reducing its ability to disinfect (Davis et al. 1973), bleach may not be a wise choice for sanitizing used blocks. Using more concentrated bleach solutions will get around this problem somewhat but this may not be desirable to nursery personnel. Of the four media used to isolate fungi, KM and PA were the most selective for the pathogenic fungi of interest. The soil algae agar proved better than Bristol's solution for assessing algae survival.

Isolation results from styroblocks treated in Trial II are given in Table 5. The steam chamber treatments were generally more effective at sanitizing used styroblocks than the hot water dip and conventional treatments. Treatment 7 (3 min of steam chamber, no pressure) yielded no pathogenic fungi and most block pieces were completely clean. Treatment 5 (3 min of steam chamber) was also effective against pathogenic fungi with only small amounts of Fusarium and Phoma occurring. However, these two treatments differed in their

Table 4.—Results of trial I: conventional washing versus pasteurization.

Treatment[2]	Colonies[1] of pathogenic fungi				Percentage plates (SAA) or tubes (BS) yielding algae	
	Pythium	Fusarium	Cylindrocarpon	Phoma	SAA	BS
1	21	22	10	17	100	100
2	9	6	8	16	100	100
3	7	13	3	5	100	100
4	2	11	1	7	100	100
5	0	0	0	10	50	70
6	2	4	0	7	100	70
7	0	0	0	1	100	60
8	28	35	10	15	100	100
9	13	17	16	5	100	100

[1] Total number of colonies from four media (except for treatment 9 where three media were used) and 20 plates per medium (except for treatment 5 where 10 plates per medium were used).

[2] See table 1 for descriptions of treatments.

efficacy against algae. Treatment 5 was most effective against algae, followed by treatments 4 (3 min at 80°C), 3 (1 min at 80°C), and then 7. Reasons for these differences are difficult to explain. Treatments should be repeated and sample numbers increased to determine whether there was a real treatment difference. Interestingly, treatment 2 (soap at 80°C) yielded fewer pathogenic fungi than both treatments 3 (1 min at 80°C) and 1 (soap alone). These results also indicate that there is an additive cleaning effect when both soap and heat are combined. More stringent trials would determine the best combination of soap or water, exposure time, and temperature for sanitizing used styroblocks. Because laboratory assay results from treatment 6 (5 min of steam chamber) show it to be substantially less effective against fungi and algae than treatment 5 (3 min of steam chamber), it is suspected that a technical error occurred in treatment 6.

Results of laboratory assays for fungi from styroblocks treated in the Biocide Trials are given in Table 6. Pythium spp. are not included in this Table as none were isolated. Several biocide treatments were as effective as the two best pasteurization treatments in Trials I and II in that they drastically reduced or eliminated all pathogenic fungi. The captan solution (treatment A1) killed all fungi on blocks. Given that captan is a broad-spectrum protectant fungicide used on a wide range of pathogenic fungi, this result was not unexpected. While non-heated solutions of ABS ranging in concentration from 0.313 to 2.5% reduced but did not completely eliminate all fungi, concentrations of 5 and 10% generally did. Perhaps the acidity of these concentrated ABS solutions kills fungi on used blocks. Heating solutions of ABS from 4°C to 40°C and 70°C over several ranges of concentrations (i.e. 1.25 to 10%) did not appear to enhance their biocidal effect. This is in contrast to results from heated soap treatments in Trials I and II. Because the evolution of SO_2 from ABS solutions increases with temperature, it is possible that the material decomposes more rapidly at high temperatures, thus reducing its biocidal activity. Treating blocks approximately 1 day after mixing ABS solutions (treatments B and C) did not affect the material's performance. This might be important if block washing occurs over 1 or more days. Blocks treated with methyl bromide (treatment C4) also yielded no fungi in laboratory assays.

CONCLUSIONS

The results of these trials emphasize the importance of sanitizing used styroblocks and indicate that several suitable block washing techniques are available to growers. Before deciding on a particular scheme growers should consider several factors: (i) the history of disease and algae problems at the nursery; (ii) the costs of setting up a completely new or different system, or modifying their present system; and (iii) the pros and cons of the several treatments identified in these trials. For example, a pasteurization system will require some source of energy (e.g. gas, oil) for heating solutions or generating steam, plus specialized tanks and *temperature* gauges for maintaining and monitoring treatment conditions. While there are no hazards from toxic materials in such systems, high-temperature solutions and pressurized air must be handled carefully.

Biocide treatments are often a more practical alternative to pasteurization because they require relatively little specialized equip-

Table 5.—Results of trial II: conventional washing versus pasteurization

Treatment	Percentage[1] styroblock pieces yielding one or more pathogenic fungi				
	Pythium	Fusarium	Cylindrocarpon	Phoma	Percentage styroblock pieces yielding algae on SAA
1	7.5	82.5	27.5	47.5	100
2	0	0	7.5	0	87.5
3	0	25	5	17.5	80
4	0	2.5	0	12.5	56
5	0	2.5	0	5	55
6	5	37.5	20	40	100
7	0	0	0	0	81
8	17.5	100	62.5	50	100

[1]Percentages based on total fungi from two media and 20 plates per medium.

ment. However, these materials must be handled with extreme caution because of their potential toxic effects on nursery workers and seedlings. Product information on sodium metabisulfite warns that the material is irritating to the eyes, nose and throat and can be very irritating to the skin. Contact with skin and eyes and inhalation of dust and SO_2 should be avoided by wearing protective equipment such as rubber gloves, safety glasses or goggles and a proper respirator. Exposure to a concentration of SO_2 of 500 ppm by volume in air for a few minutes is very dangerous. A threshold limit value of 5 ppm for sulfur dioxide (concentrations in air to which nearly all workers may be repeatedly exposed during an 8-hour work day without adverse affects) was recommended by the 1968 American Conference of Governmental Industrial Hygienists (Baker and Mossman 1970). Experience with ABS in British Columbia has also shown that it can corrode block washing equipment. Materials such as methyl bromide, while a very effective biocide, are potentially very dangerous. Growers opting for this treatment must take all necessary precautions to ensure the safety of the nursery staff and, increasingly, their suburban neighbors.

To date, one small-scale trial with lettuce seeds sown into styroblocks treated with ABS concentrations of 2.5, 5, and 10% and one operational trial where several hundred blocks were washed with a 5% ABS solution, then rinsed and sown to conifers, suggest that at these concentrations, ABS is not phytotoxic.[3]

[3]Gates, W. 1988. Personal communication. British Columbia Ministry of Forests, Silviculture Branch Extension Services, Surrey Nursery, Surrey, B.C.

With nursery practices constantly changing (e.g. growing media, container types), the kinds and numbers of disease and other organisms may also change. These changes should be monitored and styroblock sanitization techniques properly tested and modified accordingly.

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Table 6.—Laboratory assay results of biocide trials.

Treatment[2]	Percentage[1] styroblock pieces yielding pathogenic fungi		
	Fusarium	Cylindrocarpon	Phoma
A1	0	0	0
A2	0	0	0
A3	40	5	0
B1a	10	20	5
B1b	25	85	30
B2a	0	50	5
B2b	0	45	5
B3a	5	45	15
B3b	0	25	30
B4a	0	20	5
B4b	0	0	0
B5a	0	0	0
B5b	0	0	0
B6a	0	0	0
B6b	0	0	0
C1a	0	0	0
C1b	0	0	0
C2a	0	0	0
C2b	0	0	0
C3a	0	0	0
C3b	0	0	0
C4	0	0	0
C5	45	20	10
D1a	0	15	25
D1b	10	20	15
D1c	10	5	0
D2a	0	5	40
D2b	0	20	35
D2c	35	25	10
D3a	0	0	0
D3b	0	5	10
D3c	0	0	0
D4a	0	0	0
D4b	0	0	15
D4c	0	0	0
D5a	25	65	25
D5b	15	90	15
D5c	5	90	0

[1]Percentages based on total fungi from two media and 10 plates per medium.
[2]See table 3 for descriptions of treatments.