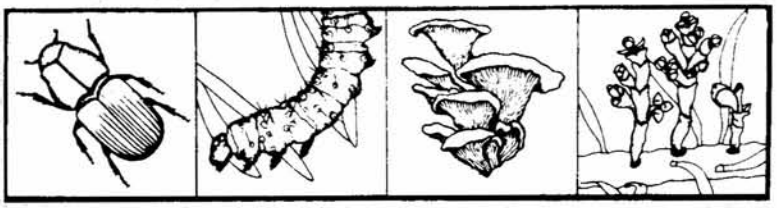
Insect and Disease Management



Report 95-9

3450
May 1995

INTERACTIONS BETWEEN COPPER-COATED CONTAINERS AND FUSARIUM ROOT DISEASE: A PRELIMINARY REPORT

R. K. Dumroese[1], R. L. James[2] and D. L. Wenny[1]

ABSTRACT

Using new copperblock and styroblock containers, three crops of Douglas-fir seedlings were grown at the University of Idaho Research Nursery, Moscow. After each growing season, healthy seedling and container colonization by *Fusarium* and *Cylindrocarpon* spp. was determined. Results indicated that copper treatment of containers reduced healthy seedling infection and colonization by *F. proliferatum*, but did not affect infection by other *Fusarium* spp. and *Cylindrocarpon* spp.

INTRODUCTION

Douglas-fir (*Pseudotsuga menziesii* var. *glauca* [Beissn.] Franco) is commonly grown in container nurseries in the northern Rocky Mountains. Seedling production is often hampered by Fusarium root disease (James and others 1987), and losses vary with seedlot and nursery. Of fusaria associated with this disease, *F. proliferatum* (Matsushima) Nirenberg is the most common species colonizing containers and seedlings (Dumroese and others 1993), and often the most virulent pathogen (unpublished). Besides *Fusarium*, recent work has found fungi in the closely related genus *Cylindrocarpon* may also be serious pathogens (James and others 1994). Although seed may be an important *Fusarium* inoculum source (James 1986), inoculum may also be carried from crop to crop on interior walls of reused containers (James and Gilligan 1988a; James and others 1988; Sturrock and Dennis 1988), being concentrated at cell bottoms (James 1989; James and Gilligan 1988b). Dipping containers in hot water (James 1989; James and Woollen 1989) or sodium metabisulfite solutions (Dumroese and others 1993) has reduced container-borne inoculum. Integrated pest management is the best way to control root disease in nurseries (James and others 1990). Using cultural practices to reduce inoculum build-up of potentially pathogenic fungi on containers would improve nursery managers' chances of controlling root disease.

[1] Research Associate and Professor/Manager, respectively, Forest Research Nursery, Department of Forest Resources, University of Idaho, Moscow.

[2] Plant Pathologist, USDA Forest Service, Northern Region, Forest Health Protection, Coeur d'Alene, Idaho.

United States Department of Agriculture

Forest Service

Northern Region

P.O. Box 7669
Missoula, Montana
59807

A second potential problem with container-grown seedlings is poor root form after planting (Burdett 1978, 1979a; Burdett and others 1986). Researchers have found a coating of copper on interior surfaces of containers changes root morphology at the nursery and improves root egress and form after outplanting (Burdett 1978; Burdett and Martin 1982; Burdett and others 1983; Dong and Burdett 1986; Wenny and others 1988; Wenny and Woollen 1989). Copper is a well-known fungicide, is the main ingredient in Bordeaux mixture, and is one of the oldest and most useful fungicides (Pirone 1978).

Our study objective was to determine how soon and to what degree containers became contaminated with *Fusarium* and *Cylindrocarpon* spp., if copper-coated containers could reduce fungal inoculum, and if reduction in container inoculum could be translated into reduction of seedling infection and colonization by these potentially pathogenic fungi.

MATERIALS AND METHODS

Copperblock 198/60 (styro 313A or 4A) and Styroblock 198/60 (styro 313A or 4A) containers were supplied by Beaver Plastics, Ltd. (Edmonton, Alberta). For spring crops in 1990, 1991 and 1992, Douglas-fir seed from seedlot Bovill 3, after being treated with bleach (Wenny and Dumroese 1987), were sown and grown under the growing regime of Wenny and Dumroese (1992). This seedlot had been assayed for *Fusarium* during an earlier study (James and others 1987). Between crops, containers were only rinsed with water to remove loose debris.

At the end of each growing season, 25 healthy-appearing seedlings were randomly selected from each container type and assayed for root colonization by *Fusarium* and *Cylindrocarpon* spp. Root systems of sampled seedlings were washed thoroughly and 10 root tips from each seedling were randomly removed and surface sterilized in a 1:10 bleach:water solution for 1 minute, rinsed in sterile water, and aseptically placed on a selective medium for *Fusarium* (Komada 1975). Plates were incubated under cool fluorescent light at 22° to 24°C for 7 days. Samples were assayed for infection; percentage colonization of infected seedlings was calculated by counting colonized root pieces.

After each growing season in 1990 and 1991, background inoculum levels of *Fusarium* were assayed on the containers. Two pieces of styrofoam, each about 0.5 cm^3, were removed from the bottom near the drainage hole of 20 randomly selected cells per container type, placed on a selective media for *Fusarium* (Komada 1975), and incubated as described above. Isolated fusaria from seedlings and containers were identified using the taxonomic methods of Nelson and others (1983).

After the 1992 growing season, 40 healthy-appearing seedlings were randomly selected and measured for height (from ground line to tip of terminal bud) and root collar diameter (RCD). Root volumes were determined by water displacement (Burdett 1979b). Oven-dry weights and biomass were determined after drying at 60°C for 24 hours.

Infection and colonization data and seedling morphological characteristics were statistically analyzed with a one-way analysis of variance (Snedecor and Cochran 1989). Means were converted to percentages after the analysis of variance and separated using Tukey's Honestly Significant Difference at $p = 0.05$.

RESULTS AND DISCUSSION

Container Infection

Fusarium spp. were isolated from about 14 percent of the seed used in this study (James and others 1987). After the 1990 growing season, copperblocks were colonized by *Fusarium* spp. less than regular styroblocks (Table 1). *Fusarium* levels were higher than what might be expected based on seed inoculant, although some additional infection may be due to inoculum within the growing medium (James 1985). Overall container infection levels were very low at the end of 1991, but for both years, combined infection by *Fusarium* spp., in general, and *F. proliferatum*, in particular, was significantly lower in the copperblocks. For both seasons, *Cylindrocarpon* infection was unaffected by the copper coating (data not shown).

Table 1. Colonization of copperblock and regular styroblock containers with *Fusarium* after production of Douglas-fir seedlings

	Percentage of Cells Infected	
Year & Container Type	*Fusarium* spp.	*Fusarium proliferatum*
1990		
Styroblock	65	35
Copperblock	20	10
p value[1]	0.0032	0.0606
1991		
Styroblock	5	5
Copperblock	10	0
p value[1]	0.5602	0.3236
Both Years		
Styroblock	35	20
Copperblock	15	5
p value[1]	0.0393	0.0431

[1] Comparisons of percentages from regular styroblock and copperblock containers.

Seedling Infection - Comparing Containers

After the first two growing seasons, we were unable to detect differences, for either year, in *Fusarium* spp., infection and colonization between seedlings grown in copperblocks and seedlings grown in styroblocks. However, combining results for all 3 years, copperblocks significantly reduced infection and colonization of *F. proliferatum* compared to styroblocks (Table 2). Combined results indicated that infection and colonization by other *Fusarium* spp. and *Cylindrocarpon* spp. was unaffected by treatment (Table 2).

Table 2. Infection and colonization of Douglas-fir seedlings grown in copperblock and regular styroblock containers wiht *Fusarium* and *Cylindrocarpon* spp.[1]

	Container Type		
Fungi	Styroblock	Copperblock	p Value[2]
Fusarium spp.			
Infection percent	80.0	76.7	0.5897
Colonization percent	51.1	54.6	0.4828
Fusarium proliferatum			
Infection percent	77.8	55.6	0.0014
Colonization percent	37.8	23.6	0.0022
Cylindrocarpon spp.			
Infection percent	54.5	45.6	0.2354
Colonization percent	25.0	17.0	0.1384

[1] Infection percentages based on number of sampled seedlings with roots infected with the particular fungus; colonization percentages based on number of root pieces infected (10 sampled per seedling). Data combines all years of study.

[2] Compares percentages from regular styroblock and copperblock containers.

Seedling Infection - Comparing Years

In general, seedling infection by *Fusarium* spp. and *F. proliferatum* increased each year regardless of container type (Table 3). Conversely, *Cylindrocarpon* infection and colonization decreased over the same time period. The third crop of seedlings grown in styroblocks had significantly more *Fusarium* and *F. proliferatum* than the first crop, and significantly less *Cylindrocarpon*. The same trend was observed in copperblocks, except the infection level by *F. proliferatum* was not significantly increased. James (1989) found *Fusarium* propagules increased in containers with repeated use, so increasing seedling infection each year can probably be attributed to increasing inoculum. Since both *Cylindrocarpon* and *Fusarium* inhabit similar ecological niches on container seedlings, *Cylindrocarpon* may not be as successful in colonizing seedling roots as *Fusarium*.

Seedling Morphology

Seedlings grown during 1992 in both copperblocks and styroblocks were statistically similar, except for height and root dry weight (Table 4). Seedlings from copper-treated containers averaged 23.8 cm and were 1.9 cm shorter ($p=0.0210$) than seedlings grown in regular styroblocks. However, seedlings from copper-treated containers averaged 0.60 g dry root weight, 0.14 g heavier than seedlings from regular styroblocks ($p=0.0117$). This reduction in height growth is inconsistent with Wenny and Woollen (1989) who found the copper treatment had no effect on Douglas-fir height.

Table 3. Infection of Douglas-fir seedlings grown in copperblock and regular styroblock containers with *Fusarium* and *Cylindrocarpon* spp. after successive seedling crops[1]

	Percentage of Seedlings Infected		
Container Type	*Fusarium*	*F. proliferatum*	*Cylindrocarpon*
Styroblock			
1 crop	60 A[2]	56 A	91 A
2 crops	80 A	68 A	100 A
3 crops	100 B	97 B	3 B
Copperblock			
1 crop	56 A	48 A	88 A
2 crops	60 A	44 A	76 A
3 crops	100 B	67 A	0 B
Combined containers			
1 crop	58 A	52 A	90 A
2 crops	64 A	56 A	88 A
3 crops	100 B	82 B	12 B

[1] Containers were rinsed with water to remove loose debris between seedling crops.
[2] For each container type, values in each column followed by the same capital letter are not significantly different (P=0.05).

Table 4. Effects of container type on selected morphological characteristics of Douglas-fir seedlings grown during 1992

	Container Type		
Growth Parameter	Copperblock	Sytroblock	p Value[1]
Height (cm)	23.8	25.7	0.0210
Root Collar Diameter (mm)	2.75	2.61	0.2227
Shoot Dry Weight (g)	1.13	1.13	0.9344
Root Dry Weight (g)	0.604	0.468	0.0117
Biomass (g)	1.73	1.60	0.3825
Root Volume (ml)	3.56	3.41	0.6778

[1] Comparisons of values from regular styroblock and copperblock containers.

Conclusions

This preliminary evaluation of copper-treatment of styroblock containers on incidence and severity of potentially pathogenic fungi infecting roots of Douglas-fir seedlings indicated copper may be effective in reducing occurrence of some *Fusarium* spp. on seedlings. Significant effects were especially noticed on incidence of *F. proliferatum*, a species which can be an aggressive incitant of disease. Effects on other surveyed fungi capable of causing seedling diseases were more subtle. Levels of these other fungi may not be high when *F. proliferatum* is present at high concentrations, because this latter fungus may out compete other fungi for infection sites on seedling roots. Since *F. proliferatum* is often the most important root-pathogenic fungus on container-grown Douglas-fir (Dumroese and others 1993), the positive effects of copper treatment of styroblock containers in reducing inoculum of this fungus is especially noteworthy. In addition, copper treatment also increased root dry weight, although seedling height was slightly reduced.

Based on this preliminary evaluation, we suspect using copper-treated styroblock containers will help reduce *F. proliferatum* inoculum resulting in lower seedling root infection and subsequent disease. A more comprehensive experiment is planned to evaluate alternative types of copper treatment on incidence of *Fusarium* and *Cylindrocarpon* and to determine treatment effects on root disease severity.

LITERATURE CITED

Burdett, A. N. 1978. Control of root morphogenesis for improved mechanical stability in container-grown lodgepole pine. Canadian Journal of Forest Research 8:483-486.

Burdett, A. N. 1979a Juvenile instability in planted pines. Irish Forestry 36(1):36-47.

Burdett, A. N. 1979b. A non-destructive method for measuring the volume of intact plant parts. Canadian Journal of Forest Research 9:120-122.

Burdett, A. N. and P. A. F. Martin. 1982. Chemical root pruning of coniferous seedlings. HortScience 17(4):622-624.

Burdett, A. N, D. G. Simpson and C. F. Thompson. 1983. Root development and plantation establishment success. Plant and Soil 71:103-110.

Burdett, A. N., H. Coates, R. Eremko and P. A. F. Martin. 1986. Toppling in British Columbia's lodgepole pine plantations: significance, cause and prevention. The Forestry Chronicle 62(105):433-439.

Dong, H. and A. N. Burdett. 1986. Chemical root pruning of Chinese pine seedlings raised in cupric sulfide impregnated paper containers. New Forests 1:67-73.

Dumroese, R. K., R. L. James and D. L. Wenny. 1993. Sodium metabisulfite reduces fungal inoculum in containers used for conifer nursery crops. Tree Planters' Notes 44(4):161-165.

James, R. L. 1985. Diseases associated with with containerized seedling soil mixes. Tree Planters' Notes 36(2):3-5.

James, R. L. 1986. Diseases of conifer seedlings caused by seed-borne Fusarium species. In: Shearer, R.C., compiler. Proceedings of the Symposium on Conifer Tree Seed in the Inland Mountain West; 1985 August 5-6; Missoula, Montana. General Technical Report 203. Ogden, UT: USDA Forest Service Intermountain Research Station. pp. 267-271.

James, R. L. 1989. Spatial distribution of fungi colonizing Leach pine cell containers - USDA Forest Service Nursery, Coeur d'Alene, Idaho. Report 90-3. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.

James, R. L. and C. J. Gilligan. 1988a. Fungal colonization of styroblock containrers - Plum Creek Nursery, Pablo, Montana. Report 88-1. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.

James, R. L. and C. J. Gilliagn. 1988b. Occurrence of Fusarium on Leach Pine cells from the USDA Forest Service Nursery, Coeur d'Alene, Idaho. Report 88-8. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.

James, R. L. and R. L. Woollen. 1989. An evaluation of the efficacy of hot water - chemical treatments to clean styroblock containers - Champion Timberlands Nursery, Plains, Montana. Report 89-5. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.

James, R. L, R. K. Dumroese, D. L. Wenny, J. F. Myers and C. J. Gilligan. 1987. Epidemiology of Fusarium on containerized Douglas-fir seedlings. (1) Seed and seedling infection, symptom production, and disease progression. Report 87-13. Missoula, MT: USDA Forest Service, Timber, Cooperative Forestry and Pest Management Northern Region.

James, R. L., R. K. Dumorese and D. L. Wenny. 1988. Occurrence and persistence of Fusarium within styroblock and Ray Leach containers. In: Landis, TD, tech. coord. Proceedings, Combined Meeting of the Western Forest Nursery Associations; 1988 August 8-11; Vernon, British Columbia. General Technical Report 167. Ft. Collins, CO: USDA Forest Service Rocky Mountain Forest and Range Experiment Station. pp. 145-148.

James, R. L., R. K. Dumroese and D. L. Wenny. 1990. Approaches to integrated pest management of Fusarium root disease in container-grown seedlings. In: Rose R, Campbell SJ, Landis TD, eds. Target Seedling Symposium: Proceedings, Combined Meeting of the Western Forest Nursery Associations; 1990 August 13-17; Roseburg, Oregon. General Technical Report 200. Ft. Collins, CO: USDA Forest Service Rocky Mountain Forest and Range Experiment Station. pp. 240-246.

James, R. L., R. K. Dumroese and D. L. Wenny. 1994. Observations on the association of *Cylindrocarpon* spp. with diseases of container-grown conifer seedlings in the inland Pacific Northwest of the United States. In: Perrin R, Sutherland JR. (eds.) Diseases and Insects in Forest Nurseries; 1993 October 3-10; Dijon, France. Paris, France: Institut National de la Recherche Agronomique, Les Colloques No. 68: 65-78

Komada, H. 1975. Development of a selective medium for quantitative isolation of Fusarium oxysporum from natural soil. Review of Plant Protection Research (Japan) 8:114-125.

Nelson, P. E., T. A. Toussoun and W. F. O. Marasas. 1983. *Fusarium* species: An illustrated manual for identification. University Park, PA: Pennsylvania State University Press. 193 p.

Pirone, P. P. 1978. Diseases and pests of ornamental plants, 5th ed. New York: John Wiley & Sons. 566 p.

Snedecor, G. W. and W. G. Cochrane. 1989. Statistical methods. 8th ed. The Iowa State University Press, Ames.

Sturrock, R. N. and J. J. Dennis. 1988. Styroblock sanitization: Results of laboratory assays from trials at several British Columbia nurseries. In: Landis, TD, tech. coord. Proceedings, Combined Meeting of the Western Forest Nursery Associations; 1988 August 8-11; Vernon, British Columbia. General Technical Report 167. Ft. Collins, CO: USDA Forest Service Rocky Mountain Forest and Range Experiment Station. pp. 149-154.

Wenny, D. L. and R. K. Dumroese. 1987. Germination of conifer seeds surface sterilized with bleach. Tree Planters' Notes 38(3):18-21.

Wenny, D. L. and R. K. Dumroese. 1992. A growing regime for container-grown Douglas-fir seedlings. Idaho Forest, Wildlife and Range Experiment Station Bulletin 49. Moscow, ID: University of Idaho.

Wenny, D. L. and R. L. Woollen. 1989. Chemical root pruning improves the root system morphology of containerized seedlings. Western Journal of Applied Forestry 4(1):15-17.

Wenny, D. L., Y. Liu, R. K. Dumroese, and H. L. Osborne. 1988. First-year growth of chemically root-pruned containerized seedlings. New Forests 2:111-118.