Hot Water and Copper Coatings in Reused Containers Decrease Inoculum of Fusarium and Cylindrocarpon and Increase Douglas Fir Seedling Growth

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Abstract. Inoculum of Douglas fir root diseases caused by the fungi Fusarium and Cylindrocarpon is carried from crop to crop in reused containers. Soaking containers for 90 seconds in 80 °C water reduced 99% of Fusarium and 100% of Cylindrocarpon inoculum between growing cycles. Overall seedling growth was also improved: seedlings grown in containers soaked between growing cycles were 10% taller and had 20% more biomass than seedlings grown in nonsoaked containers. We obtained a 15% increase in the number of deliverable seedlings from containers soaked in hot water between crops, from the use of copper coated containers, or from both practices combined.

Douglas fir [Pseudotsuga menziesii (Mirbel) Franco] is commonly grown for reforestation in the northern Rocky Mountains and Pacific Northwest. Seedlings are usually grown as a 1-year crop at high densities (up to 1076 seedlings/m²) in styrofoam or hard plastic containers and colonizing seedlings (Dumroese et al., 1993; James et al., 1997). Another root pathogen found in nursery environments is Cylindrocarpon (Beyer-Ericson et al., 1991), commonly found in the rhizosphere of container-grown conifer nursery stock (James et al., 1994). Often, infected plants lack aboveground symptoms (e.g., chlorotic or necrotic foliage) despite extensive root decay. Cylindrocarpon sp. may act in conjunction with other root pathogens to cause major disease problems (Bloomberg and Sutherland, 1971; Duda and Sierota, 1987).

Generally, root disease is difficult to control because root systems usually are extensively colonized before shoot symptoms appear (James et al., 1987), and chemical fungicide applications are generally ineffective (James, 1986a; James et al., 1988b). Therefore, integrated pest management is the best alternative to control root disease (James et al., 1990).

Reducing inoculum of pathogenic organisms is important for limiting seedling infection (James et al., 1990). Although seeds may be an important source of Fusarium (James, 1986b), inoculum may also be carried from crop to crop on interior walls of reused containers (James et al., 1988a; James and Gilligan, 1988a; Sturrock and Dennis, 1988), and it may be particularly concentrated at the bottom of containers (James, 1989; James and Gilligan, 1988b).

Immersion in hot water is a common method to sterilize plastic pots (Hartmann et al., 1990) and works well for both styrofoam and plastic containers used in reforestation nurseries (Sturrock and Dennis, 1988; James, 1989; James and Woollen, 1989). In forest nursery situations, hot water is preferable to chemical treatments such as sodium metabisulfite or bleach solutions due to reduced potential for worker exposure to irritating or toxic chemicals, problems of chemical disposal, and higher efficacy against pathogens (Dumroese et al., 1993; James and Sears, 1990; Peterson 1990, 1991).

Another potential problem with container-grown seedlings is poor root egress from the upper portions of the root plug after planting (Burgett, 1978, 1979a). New roots often fail to grow in a natural pattern (Balisky et al., 1995) that can affect seedling stability (Burgett, 1978) and may limit seedling access to nutrients, water, and mycorrhizal inoculum (Dumroese, 2000). Researchers have found a coating of copper on interior surfaces of containers changes Douglas fir root morphology at the nursery and improves root egress and form after outplanting (Burgett, 1978; Burgett and Martin, 1982; Wenny et al., 1988; Wenny and Woollen, 1989). This technique is also effective on many other horticultural plants (Appleton, 1995; Arnold, 1996; Brass et al., 1996).

In a pilot study, a copper-coating was also effective in reducing seedling infection and colonization by F. proliferatum (Dumroese et al., 1995). This was not surprising since copper is also one of the oldest and most effective fungicides (Pirone, 1978). Copper can be applied as sprays or dusts, protect plants against pathogens (Walker, 1969). Although toxic at high concentrations, copper is an essential nutrient for Fusarium sp. at low concentrations (Steinberg, 1950; Woltz and Jones, 1981).

Our objectives were to quantify the rate of fungal inoculum build-up on reused seedling containers, how that affected seedling growth, and compare the efficacy of hot water treatments with the copper-coated containers for controlling fungal inoculum in a commercial nursery setting.

Materials and Methods

Our experiment was a completely randomized two container type x two cleaning treatment factorial arrangement conducted for five growing cycles. The two container types were Styroblock and Copperblock containers that were identical (160 cavities per container aligned in 10 rows and 16 columns, 90-mL cavity volume, and 764 cavities per m²; Beaver Plastics, Edmonton, Alberta) except that Copperblocks have a proprietary copper oxychloride coating on cavity walls. Cleaning treatments were either no container cleaning (control) or submerging (soaking) containers in hot water (77 to 82 °C) for 90 s. We assigned three containers to each container type-soaking combination to serve as replicates and these containers remained within that treatment for five growing cycles. For each 10-month growing cycle, the same northern Idaho source of Douglas fir was sown in March and seedlings were harvested in December. We randomly placed all con-
From each seedling, 10 root tips, each and stem diameter at the root collar (RCD) were measured seedling root volume using washed to remove adhering medium. We deliverable or cull based on nursery criteria: mize edge effects. Seedlings were classified as the outer two rows and two columns to mini-
soaking combination), avoiding seedlings in container (60 seedlings per container type domly-selected starting point, we systemati-
target height.
arahana et al., 1997), and Trichoderma sp. (Mousseaux et al., 1998) as well.
Fungal identification involved obtaining single-spore isolates from colonies on Komada’s medium and culturing them on both carnation leaf agar (Fisher et al., 1982) and potato dextrose agar. Fusarium species were identified based on the presence or absence of chlamydospores, macroconidial morphology, and the production of microconidia on chains or in false heads borne on mono- or polyphialides (Nelson et al., 1983). Cylindro- cartonsp. were identified using Booth’s (1966) taxonomy; our identifications were based on macroconidial morphology, length, and sepa-
tables. Data were back transformed as necessary for tables.
Results
Seeding morphology. The interactions of cycle × container type and cycle × soak were both significant for height and RCD (Table 1) and the trend was the same: heights and RCDs were similar the first two cycles regardless of container type or soak, but seedlings the last three cycles were 5% taller with 12% thicker stems when grown in Copperblocks or 16% heavier shoots. Likewise, root volume was 8% lower for seedlings grown in Copperblocks the first two cycles but 8% higher the last three cycles when compared to Styroblocks.
The soak × container type interaction was
Table 1. Sources of variation and P values for Douglas fir seedling morphological characteristics and number of deliverable seedlings when new crops of seedlings were grown in each of five growing cycles in reused Styroblock or Copperblock containers either soaked or not soaked in hot water between cycles.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Height</th>
<th>RCD</th>
<th>Shoot wt</th>
<th>Root wt</th>
<th>Root volume</th>
<th>Deliverable seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Container</td>
<td>1</td>
<td>0.3</td>
<td>0.01</td>
<td>0.8</td>
<td>0.008</td>
<td>0.006</td>
<td>0.006</td>
</tr>
<tr>
<td>Soak</td>
<td>1</td>
<td>0.0005</td>
<td>0.003</td>
<td>0.001</td>
<td>0.0006</td>
<td>0.02</td>
<td>0.002</td>
</tr>
<tr>
<td>Container × soak</td>
<td>8</td>
<td>0.0007</td>
<td>0.02</td>
<td>0.002</td>
<td>0.008</td>
<td>0.03</td>
<td>0.006</td>
</tr>
<tr>
<td>Replicate (soak × container)</td>
<td>8</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0002</td>
<td>0.0001</td>
<td>0.003</td>
</tr>
<tr>
<td>Cycle</td>
<td>4</td>
<td>0.03</td>
<td>0.07</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.005</td>
</tr>
<tr>
<td>Cycle × soak</td>
<td>4</td>
<td>0.04</td>
<td>0.0008</td>
<td>0.04</td>
<td>0.3</td>
<td>0.01</td>
<td>0.2</td>
</tr>
<tr>
<td>Cycle × container</td>
<td>4</td>
<td>0.2</td>
<td>0.8</td>
<td>0.99</td>
<td>0.8</td>
<td>0.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The error term for main effects (container type and soaking) was the residual variation of these variables averaged over time (cycles).

Table 2. Means (±std) of Douglas fir seedling morphological characteristics and number of deliverable seedlings averaged over five growing cycles when new crops were grown each cycle in reused containers either soaked or not soaked in hot water between cycles.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Height (cm)</th>
<th>RCD (mm)</th>
<th>Shoot wt (g)</th>
<th>Root wt (g)</th>
<th>Root volume (cc)</th>
<th>Deliverable seedlings (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No soak</td>
<td>20.7 ± 0.6a</td>
<td>2.56 ± 0.43</td>
<td>0.74 ± 0.13</td>
<td>3.13 ± 0.77</td>
<td>110 ± 10</td>
<td></td>
</tr>
<tr>
<td>Soak</td>
<td>24.3 ± 1</td>
<td>2.86 ± 0.32</td>
<td>1.35 ± 0.30</td>
<td>3.82 ± 0.69</td>
<td>124 ± 4</td>
<td></td>
</tr>
<tr>
<td>No soak</td>
<td>22.5 ± 3.9</td>
<td>2.82 ± 0.24</td>
<td>0.81 ± 0.10</td>
<td>3.42 ± 0.46</td>
<td>125 ± 6</td>
<td></td>
</tr>
<tr>
<td>Soak</td>
<td>23.3 ± 3.8</td>
<td>2.88 ± 0.21</td>
<td>1.25 ± 0.19</td>
<td>3.48 ± 0.48</td>
<td>124 ± 7</td>
<td></td>
</tr>
</tbody>
</table>

The error term for main effects (container type and soaking) was the residual variation of these variables averaged over time (cycles). Statistical analysis. Percentage data were transformed using the arcsin of the square root of the value. We used a general linear model (PROC GLM; SAS Institute, 1989). The error term for main effects (container type and soaking) was the residual variation of these variables averaged over time (cycles). Treatment means were separated using Tukey’s honestly significant different test (HSD) when P ≤ 0.05. Data were back transformed as necessary for tables.
significant for all characteristics (Table 1). In general, Copperblocks yielded similar seedlings with or without soaking, whereas Styroblocks soaked in hot water yielded larger seedlings than nontreated Styroblocks (Table 2). For morphology characteristics, seedlings were significantly larger when grown in containers soaked in hot water between cycles (Table 3).

### Container infestation

We isolated seven *Fusarium* sp. from containers and seedlings: *F. acuminatum* Ell. & Ev., *F.avenaceum* (Fr.) Sacc., *F.graminearum* Schwabe., *F.oxysporum*, *F. proliferatum*, *F. sambucinum* Fuckel, and *F. sporotrichioides* Sherb. The three-way interaction of cycle, container type, and soak was significant for container infestation by *Fusarium* sp., but not for *F. proliferatum* (Table 4). Cycle interacted significantly with both container and soaking for *Fusarium* sp. and *F. proliferatum* (Table 4). Copperblocks had half the level of *Fusarium* sp. infestation for the first three growing cycles when compared to Styroblocks, but levels were similar the final two cycles. Conversely, soaked and nonsoaked containers had similar *Fusarium* sp. infestation after the first cycle but thereafter residual inoculum levels in soaked containers were about half those of nonsoaked containers. Growing cycle was significant (Table 4). The two-way interaction of container and soak was nonsignificant (Table 4), but overall, soaking was significant as nonsoaked containers were infested more (86%) than those soaked (59%).

For *Cylindrocarpon*, cycle interacted significantly with soak (Table 4): *Cylindrocarpon* was absent on cavity walls the first three growing cycles but 2× greater in the nonsoaked containers (~29% infection; ~8% colonization) as compared to the soaked containers (~64% infection; 24% colonization) than in the control; in the next three growing cycles, infection and colonization were similar and high (90% + infection; ~60% colonization) regardless of soaking treatment. After the final growing cycle, infection and colonization were very low but 2× greater in the nonsoaked containers (~26% infection; ~22% colonization) for both treatments. For *Cylindrocarpon* sp., the reductions in seedling growth during the first two growing cycles in Copperblocks were expected, as other studies at our nursery using Copperblocks have yielded similar results (Johnson et al., 1995; Skunkes et al., 2000). Generally, the reduced growth is thought to be either copper phytotoxicity or nutritional problems due to the copper ions, which can be avoided by modifying the fertilizer regime (Landis and van Steenis, 2000; van Steenis, 1994). We speculate the nutritional aspect is more likely than Hunt (1990) found significantly better height, stem diameter, and shoot weight when Douglas fir seedlings were grown in Copperblocks. Further, Copperblocks in this study were interspersed among a production lot being grown with a fertilizer regime specific for Styroblocks. Moreover, other studies at the Univ. of Idaho showed that Douglas fir seedlings treated with cupric carbonate had similar height, RCD, and biomass to controls (Wenny and Woollen, 1989; Woollen, 1987). Since copper oxychloride is more soluble than cupric carbonate, it is likely that medium chemistry, nutrient availability, and subsequent seedling growth would be impacted more by this form of copper. Despite this temporary growth reduction that did not reduce the number of deliverable seedlings and that could probably be ameliorated by adjustments to the fertilizer regime, copper oxychloride was not expected to affect the number of deliverable seedlings and that could probably be ameliorated by adjustments to the fertilizer regime.

### Discussion

The reductions in seedling growth during the first two growing cycles in Copperblocks were expected, as other studies at our nursery using Copperblocks have yielded similar results (Johnson et al., 1995; Skunkes et al., 2000). Generally, the reduced growth is thought to be either copper phytotoxicity or nutritional problems due to the copper ions, which can be avoided by modifying the fertilizer regime (Landis and van Steenis, 2000; van Steenis, 1994). We speculate the nutritional aspect is more likely than Hunt (1990) found significantly better height, stem diameter, and shoot weight when Douglas fir seedlings were grown in Copperblocks. Further, Copperblocks in this study were interspersed among a production lot being grown with a fertilizer regime specific for Styroblocks. Moreover, other studies at the Univ. of Idaho showed that Douglas fir seedlings treated with cupric carbonate had similar height, RCD, and biomass to controls (Wenny and Woollen, 1989; Woollen, 1987). Since copper oxychloride is more soluble than cupric carbonate, it is likely that medium chemistry, nutrient availability, and subsequent seedling growth would be impacted more by this form of copper. Despite this temporary growth reduction that did not reduce the number of deliverable seedlings and that could probably be ameliorated by adjustments to the fertilizer regime, copper oxychloride was not expected to affect the number of deliverable seedlings and that could probably be ameliorated by adjustments to the fertilizer regime.

### Table 3. Sources of variation and P values for container infestation and Douglas fir seedling infection and colonization by *Fusarium* sp., *F. proliferatum*, and *Cylindrocarpon* sp. when new crops of seedlings were grown in each of five growing cycles in reused Styroblock or Copperblock containers either soaked or not soaked in hot water between cycles.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th><em>Fusarium</em> sp.</th>
<th><em>F. proliferatum</em></th>
<th><em>Cylindrocarpon</em> sp.</th>
<th><em>Fusarium</em> sp.</th>
<th><em>F. proliferatum</em></th>
<th><em>Cylindrocarpon</em> sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection</td>
<td>Colonization</td>
<td>Infection</td>
<td>Colonization</td>
<td>Infection</td>
<td>Colonization</td>
</tr>
<tr>
<td>Container</td>
<td>0.07</td>
<td>0.16</td>
<td>0.16</td>
<td>0.3</td>
<td>0.2</td>
<td>0.16</td>
</tr>
<tr>
<td>Soak</td>
<td>0.04</td>
<td>0.6</td>
<td>0.006</td>
<td>0.99</td>
<td>0.4</td>
<td>0.04</td>
</tr>
<tr>
<td>Soak × container</td>
<td>0.2</td>
<td>0.3</td>
<td>0.6</td>
<td>0.5</td>
<td>0.3</td>
<td>0.9</td>
</tr>
<tr>
<td>Replicate (soak × container)</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cycle</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cycle × soak</td>
<td>0.0006</td>
<td>0.0005</td>
<td>0.0009</td>
<td>0.007</td>
<td>0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Cycle × container</td>
<td>0.001</td>
<td>0.02</td>
<td>0.2</td>
<td>0.3</td>
<td>0.16</td>
<td>0.7</td>
</tr>
<tr>
<td>Cycle × soak × container</td>
<td>0.03</td>
<td>0.14</td>
<td>0.3</td>
<td>0.13</td>
<td>0.2</td>
<td>0.3</td>
</tr>
<tr>
<td>Error</td>
<td>0.03</td>
<td></td>
<td></td>
<td>0.03</td>
<td>0.14</td>
<td></td>
</tr>
</tbody>
</table>

The error term for main effects (container type and soaking) was the residual variation of these variables averaged over time (cycles).
inoculum reductions, indicated that optimum soaking temperatures particularly with lum afforded by the copper. In the latter growing cycles was enhanced, this study) sell for erable.

Correlating organisms and populations and gard to container types and soaking treatments in re- 1973). Bloomberg and Lock (1972) concluded that several different diseases of conifer seedlings were probably caused by different strains of F. oxysporum. Further, virulent isolates may be collected from non-symptomatic seedlings whereas nonpathogenic virulent isolates may be collected from non- 


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Peterson, M. 1991. Guidelines for the sanitation of nursery seedling containers. BC Min. of Forests Supplement to FRDA 140, Victoria, BC.


