3) A mechanical container treatment (slots in the containers) was not useful. The growing medium tended to dry out in a normal greenhouse growing regime and seedlings were stunted or died.

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Effect of $CuCO_3$ Container Wall Treatment and Mycorrhizae Fungi Inoculation of Growing Medium on Pine Seedling Growth and Root Development[1]

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Abstract

Coating the inside surfaces of containers with cupric carbonate ($CuCO_3$) stopped lateral root growth of ponderosa pine (*Pinus ponderosa* Laws.) and lodgepole pine (*Pinus contorta* Dougl.) at the container wall. Subsequent higher order laterals proliferated and were similarly arrested. The $CuCO_3$ treatment was compatible with inoculation of the growing medium with the mycorrhizae fungi *Pisolithus tinctorius* (Pers.) Coker and Couch and *Suillus granulatus* (L. ex. Fr.) O. Kuntze. Combined inhibitor—inoculum treatments resulted in pine seedlings that were bigger, had more lateral roots, and greater mycorrhizal infection rates than untreated seedlings.

Index words: Container, mycorrhizae, ponderosa pine, lodgepole pine, inoculum, inhibitor

Introduction

Cupric carbonate ($CuCO_3$) applied to container walls to control root development of conifer seedlings has resulted in increased lateral root development (2, 5, 6). Ponderosa pine (*Pinus ponderosa* Laws.) and lodgepole pine (*Pinus contorta* Dougl.) root growth is arrested when root tips encounter a container wall painted with $CuCO_3$ (50 to 100 g/l of exterior latex paint). Subsequent higher order lateral roots then proliferate from the inhibited primary laterals and are similarly arrested, but resume growth upon removal from the container. Consequently, tree seedlings treated in this manner should develop greater wind firmness soon after planting.

Standard procedures for rearing containerized ponderosa pine seedlings (7) includes the addition of 3% pine duff by volume to the growing medium for mycor-

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rhizae fungus inoculations. Hatch and Doak (3) theorized that mycorrhizal fungi can only successfully attack unlignified roots. Since $CuCO_3$ treatment of containers caused more lateral root development, more unlignified lateral root initials should be available to mycorrhizal infection. Short roots are the principal site of mycorrhizal infection (1, 8) and occur in variable numbers on long roots. There is a stimulation of long root generation on seedlings grown in $CuCO_3$-treated containers, so there should also be more short roots available for mycorrhizal development.

Since it is desirable that seedlings become mycorrhizal, the object of this study was to determine the effect of the $CuCO_3$ treatment on formation of mycorrhizae.

Materials and Methods

Two mycorrhizae fungi, *Suillus granulatus* (L. ex Fr.) Kuntze (L. Gillman, Colorado isolate 75-20) and *Pisolithus tinctorius* (Pers.) Coker and Couch (D. Marx, Georgia isolate 133), were prepared as fungal inoculum in a sterile peat-vermiculite-nutrient medium using the technique of Marx and Bryan (4). Two to three months were required for complete permeation of the medium by fungal mycelium. Since *P. tinctorius* grew faster than *S. granulatus,* the containers were placed in a cold room at 5 °C (41 °F) as the fungi totally permeated the substrate in each container. After mycelium completely perneated the culture medium of all the jars, the inoculum of both species was removed and rinsed. It was then incorporated into a growing medium (at a rate of 1 part inoculum to 9 parts medium) composed of vermiculite (horticulture grade 2) and sphagnum peat (1:1 by vol).

Spencer-LeMaire Rootrainer® containers (7) of about 492 cm^3 (30 in^3) capacity were used. These containers are polystyrene thermo-formed book-type tree seedling containers each having four cavities. Each cavity is about 5 by 5 cm (2x2 in) at the top and 20 cm (8 in) deep. Each container tapers to approximately 3 by 3 cm (1.2 x 1.2 in) at the bottom and has a 1x2 cm (0.25 x 0.5 in) drain (root egress) hole. One-half (240) of the containers had their interior surfaces coated with a mixture of white acrylic exterior latex paint and $CuCO_3$ (50 gm/l of paint). The mixture was applied to the open containers with a brush and air dried. Another 240 containers were not painted. One-third of the painted and unpainted containers were each then filled with growing medium that was, respectively, (1) uninoculated, (2) inoculated with *Suillus granulatus,* or (3) inoculated with *Pisolithus tinctorius.* One-half of each container growing medium treatment was sown with ponderosa pine seed; the other half with lodgepole pine seed. The ponderosa pine seed source was 822-NS from near Roundup, Montana. The lodgepole pine seed source was DR-4010-5-74 (Alberta, Canada). Both these seed sources germinate promptly without stratification so the seed was sown unstratified.

Seeds were sown in the filled containers on June 4, 1980, and covered with a 4 mm (0.16 in) layer of perlite sieved so that no particle was smaller than 2 mm (0.08 in) in diameter. Containers were placed in wire racks, each holding 10 books of 4 cavities each, or a complete species-inoculation-container treatment group per rack. Racks were randomly placed on greenhouse benches and the trees grown according to standard procedures described in Tinus and McDonald (7).

By November 28, 1980, the root plugs could easily be removed intact from the containers. Ten trees of each treatment were randomly selected and the following parameters measured: height, stem diameter at the root collar, top and root dry weight, number of roots air pruned at the bottom of the root plug, number of roots deflected at the container wall, and the number of short roots infected and not infected with mycorrhizae fungi. Mycorrhizal infection was identified by visual characteristics including color, short root branching pattern, and the presence of a fungal sheath. Nine primary lateral roots from each seedling were randomly selected. The seedlings' root systems were separated into upper, middle, and lower sections, with 3 primary laterals randomly selected from each location. This analysis procedure resulted in examination of 35% to 70% of the short roots for each seedling. Percent mycorrhizal infection was determined by dividing the total number of mycorrhizal short root apices by the total short root apices and multiplying by 100.

Mean separation of measured parameters was tested using Duncan's Multiple Range test for significance. Also stem height, root collar diameter, number of secondary needles, length of secondary needles, % mycorrhizal roots, number of short roots, number of roots deflected at the container wall, number of roots air pruned, were analyzed by standard analysis of variance procedures for a factorial design. Species was included in the analysis, along with container treatment and fungal inoculation, as a main effect variable and interactions between variables were tested.

Results and Discussion

Seedlings of both species generally grew faster (stem height, root collar diameter, shoot and root dry weight) when subjected to $CuCO_3$ or a mycorrhizae fungus inoculation or a combination of the 2 (Tables 1 and 2). Seedlings treated with $CuCO_3$ on the average were 1.2 times taller and had 1.1 times larger root collar diameters than untreated trees (Table 3). Similarly, seedlings treated with mycorrhizae fungus inoculum were 1.4 times taller and had 1.3 times larger root collar diameters than non-inoculated trees.

Lodgepole pine seedlings inoculated with Pisolithus, but given no $CuCO_3$ treatment exhibited no more mycorrhizae than control seedlings (Table 2). The same was true of ponderosa pine seedlings treated with Suillus (Table 1), so there were some species-host interactions. In all other treatments in both tree species, the degree of mycorrhizal root infection exceeded that for control seedlings. Analysis of pooled data showed all seedlings treated with $CuCO_3$ had 1.4 times more infected roots than those receiving no treatment (Table 3). Similarly, seedlings treated with fungal inoculum had 1.3 times as many infected roots as untreated trees. Both $CuCO_3$ and inoculation treatments significantly increased the number of mycorrhizal roots. Note that some mycorrhizal infection took place in the control seedlings (Tables 1 and 2). This was attributable to air-borne spore inoculation of the containers in the ventilated greenhouses the

Table 1. Ponderosa pine seedling morphological parameters as affected by $CuCO_3$ container treatment and growing medium inoculation with mycorrhizae fungi.

Parameter Measured	Inoculum					
	Suillus granulatus		Pisolithus tinctorius		None	
	Container Treatment^z					
	$CuCO_3$	none	$CuCO_3$	None	$CuCO_3$	None
Stem Ht (cm)	10.7 a^y	7.9 a	10.8 a	8.8 a	6.9 a	6.5 b
Root collar diam (mm)	2.7 a	2.4 a	2.6 a	2.2 a	2.2 a	1.8 b
% Mycorrhizal infection	33.8 a	15.8 b	38.7 a	20.2 a	24.4 a	18.4 b
No. short roots	733.3 ab	595.5 bc	1089.9 a	355.9 c	698.1 b	458.1 bc
No. roots deflected	15.6 c	61.8 a	14.5 c	38.2 b	9.7 c	41.8 b
No. roots air pruned	15.9 c	35.5 a	24.7 b	26.8 b	14.6 c	30.3 ab
Short dry wt (gm)	1.5 a	1.3 a	1.6 a	1.6 a	1.4 a	0.8 b
Root dry wt (gm)	0.7 a	0.6 a	0.7 a	0.6 a	0.5 b	0.5 b

^{z}Container interior walls treated with a solution containing 50 gm/l of $CuCO_3$ in exterior latex paint.

^{y}Mean separation within rows followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

Table 2. Lodgepole pine seedling morphological parameters as affected by $CuCO_3$ container treatment and growing medium inoculation with mycorrhizae fungi.

Parameter Measured	Inoculum					
	Suillus granulatus		Pisolithus tinctorius		None	
	Container Treatment^z					
	$CuCO_3$	none	$CuCO_3$	None	$CuCO_3$	None
Stem ht (cm)	14.4 a^y	12.8 a	13.8 a	14.1 a	10.0 a	8.7 b
Root collar diam (mm)	2.6 a	2.5 a	2.6 a	2.7 a	2.1 a	1.9 b
% Mycorrhizal infection	35.6 ab	42.2 a	48.8 a	25.0 bc	36.2 ab	18.9 c
No. short roots	764.8 bc	1217.4 a	813.5 bc	875.2 b	893.2 b	651.5 c
No. roots deflected	12.3 a	33.1 b	9.5 a	28.1 b	8.1 a	23.3 b
No. roots air pruned	14.7 a	30.9 b	19.1 a	30.9 b	7.8 a	22.8 b
Shoot dry wt (gm)	1.3 ab	1.5 a	1.3 ab	1.4 ab	1.1 b	0.7 c
Root dry wt (gm)	0.8 a	0.9 a	0.8 a	0.9 a	0.6 b	0.6 b

^{z}Container interior walls treated with a solution containing 50 gm/l of $CuCO_3$ in exterior latex paint.

^{y}Mean separation within rows followed by the same letter are not significantly different at the 5% level using Duncan's Multiple Range Test.

Table 3. Ratios of the means of fungus, tree species, and cupric carbonate container treatments (ratios are shown only where mean difference was significant by F test).

Parameter Measured	Ratio of Means			Three-way Interactions
	Tree species effect	$CuCO_3$ effect	Fungus effect	
	LPP:PP	Cu:None	Fungus:None	
Stem ht	1.40	1.16	1.43	ns
Root collar needles	ns^z	1.13	1.26	ns
No. secondary needles	ns	ns	1.77	ns
Length secondary needles	0.65	ns	1.36	—^{y}
% mycorrhizal roots	1.22	1.36	1.25	ns
No. roots deflected	0.62	0.30	1.43^v	—^{x}
No. roots air pruned	0.85	0.54	1.31	—^{w}

^{z}Not significant.

^{y}Lodgepole pine was affected more than Ponderosa pine in tree species/fungus interation.

^{x}(1) $CuCO_3$ reduced root deflection more with Ponderosa pine than Lodgepole pine.
(2) $CuCO_3$ reduced root deflection less with *Pisolithus tinctorius* than with *Suillus granulatus* or no inoculum.
(3) $CuCO_3$ and fungi, in combination, reduced root deflection on both species compared to no treatment.

^{w}In the presence of $CuCO_3$, the number of roots air pruned was less when *Suillus granulatus* or no inoculum was used. *Pisolithus tinctorius* made little difference on either tree species.

^{v}Significant effect using *Suillus granulatus* only.

tests were conducted in. Treated and untreated containers were both exposed to the same degree of contamination. Consequently we assumed differences between treatments and controls were attributable to treatment effects.

For both tree species the $CuCO_3$ treatment reduced the number of roots deflected downward at the container wall, as well as the number of roots air pruned at the bottom of the container (Tables 1 and 2). The $CuCO_3$ treatment was effective whether or not combined with mycorrhizal inoculation in almost every instance. Cupric carbonate treatments, in combination with a mycorrhizae fungus inoculation, generally increased numbers of short roots on ponderosa pine (Table 1), but not lodgepole pine (Table 2). On the other hand, treatments with inoculum alone increased short root numbers on lodgepole pine, but not on ponderosa pine.

Inoculation of growing medium with mycorrhizae fungi significantly affected vigor of the tree seedlings as expressed in numbers of secondary needles developed and their length (Table 3). The effect of this expression of vigorous development on subsequent growth or survival rates was not tested, but, we assume, would generally be positive.

Significance to the Nursery Industry

The study clearly showed the $CuCO_3$ treatment enhances seedling growth and stops the growth of many lateral roots at the root-tip container wall interface, corroborating earlier work (6). This observation may have important connotations for control of root development of woody plants in containers. However, the new fact coming from this study is that the $CuCO_3$ treatment does not inhibit the formation of mycorrhizae on the roots of lodgepole and ponderosa pine seedlings. Indeed $CuCO_3$ treatment appears to have a positive effect. This means nurserymen can employ the $CuCO_3$ treatment to acquire the associated root control and possible post-planting benefits without compromising mycorrhizae development in the containers. Mycorrhizae development will actually be enhanced in most cases, depending on fungus-tree species compatibility.

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