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Benomyl Stimulates Ectomycorrhizal Development by Pisolithus Tinctorius on Shortleaf Pine Grown in Containers[1]

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SUMMARY

Container-grown shortleaf pine (*Pinus* echinata Mill.) seedlings inoculated with *Pisolithus tinctorius* and drenched with benomyl formed more mycorrhizal roots than undrenched seedlings. Seedlings were drenched (2.5, 5, and 10 mg ai in 15 ml of water per individual) prior to sowing and at either 2-, 4-, or 8-week intervals. *Pisolithus* formed best at the highest benomyl level, 10 mg every 2 weeks. Benomyl application increased seedling diameter, height, and weight. Highest benomyl dosages produced the largest seedlings.

Additional keywords: *Pinus echinata,* containerized seedlings, seedling size and weight, growing medium.

INTRODUCTION

A recent study (Pawuk et al. 1980) evaluated the effects of several fungicides on mycorrhizal formation by *Pisolithus tinctorius* [(Pers.) Coker and Couch] on container-grown longleaf pine (*Pinus palustris* Mill.) in milled pine bark. Benomyl 50 WP, methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate, applied at 5 mg ai per seedling every 4 weeks, significantly increased mycorrhizal formation over the control. Modifications of dosage levels and drenching schedules might theoretically identify treatments to further enhance mycorrhizal development.

Although the original study was done using a pine bark medium, peat-vermiculite mixes are more commonly used in container culture (Tinus and McDonald 1979). *Pisolithus* develops well in a peat-vermiculite medium (Marx and Barnett 1974, Ruehle and Marx 1977), but the effect of benomyl on mycorrhizal development is untested in this medium.

This experiment was initiated to test the effect of benomyl in stimulating mycorrhizal development on shortleaf pine (*Pinus echinata* Mill.) in a peat-vermiculite medium and to identify effective drench rates and schedules.

METHODS

This study was designed as a randomized complete-block design with four replications. Stratified seeds of shortleaf pine were sown into Spencer-Lemaire Rootrainers (164 cm^2 per cavity). Cavities contained equal parts of peat and vermiculite with vegetative inoculum of *Pisolithus tinctorius* (Isolate 138), prepared according to Marx and Bryan (1975), added at 1 to 8 parts of medium. Seedlings were thinned to one per cavity 3 weeks after sowing and extra seedlings were then transplanted to containers

[1] Mention of trade names is for information only and does not constitute endorsement by the USDA Forest Service.

having germination failures. Sixteen seedlings were grown for each treatment replication.

Total treatment combinations consisted of three dosage levels and three time intervals (nine treatment combinations) and an untreated control. Seedlings were drenched with 2.5, 5.0, and 10.0 mg of benomyl per seedling before sowing and at either 2-, 4-, or 8-week intervals. Seedlings receiving no benomyl were grown as a control. All were fertilized at 3-week intervals after transplanting with a 2500 ppm solution of N-P-K fertilizer (20-19-18) which was applied to saturation.

Ten seedlings were randomly selected from each treatment-replication and the percentage of *Pisolithus*-infected short roots was estimated for each seedling 18 weeks after sowing. At that time, seedling diameter, height, and average dry weights were also determined.

Data were analyzed by ANOV. Mean separation of major effects was made using single degree of freedom tests while mean separation of minor effects was made with Duncan's multiple range tests. The 0.05 level was used to evaluate statistical significance throughout the study.

RESULTS

Mycorrhizal Development. **—** ***Pisolithus tinctorius*** formed mycorrhizae in all treatments. Control seedlings, with 8 percent, had least infection. Seedlings drenched with high rates of benomyl and seedlings receiving frequent drenchings generally formed the most mycorrhizae (table 1). However, a dosage rate x drench frequency interaction was present (table 2).

Seedlings drenched with 10 mg every 8 weeks did not form more mycorrhizae than seedlings drenched with 2.5 or 5.0 mg every 2 weeks. Furthermore, no difference occurred between the 5- and 10-mg rate at the 4-week drench. At 5 mg, more mycorrhizae formed at the 4-week schedule than at the 2-week schedule, and mycorrhizal development did not differ for the 4- and 8-week drenches at 2.5 mg.

During the study, seedlings received 5, 10, 20, 40, or 80 mg benomyl depending on drench schedule and dosage rate. With one exception (table 3), mycorrhizal development increased with increased amounts of benomyl. Regression analysis compared total amount of benomyl applied (X) to mycorrhizal development (Y). The calculated F equals 39.99, r^2 equals 0.54, and Y equals 21.35 + 0.34X with percent mycorrhizal development expressed in transformed arc sine values.

Table 1 .—*Mycorrhizal* development by Pisoiithus tinctorius end *growth of shortleaf pine seedlings drenched with benomyl (main effects table)*

Benomyl	P.t.	Diameter	Height	Dry weight
dosage rate mglseedllng	*% infection*	*mm*	*cm*	*mg*
2.5	17.67'	2.06 a[2]	13.54 a	462 b
5.0	24.00	2.04 a	13.26 a	476 b
10.0	36.67	2.12 a	13.60 a	506 a
Drench frequency wk				
2	34.33'	2.19 a	14.44 a	531 a
4	27.00	2.06 b	13.92 a	402 b
S	17.00	1.96 b	12.24 b	435 c
Control	8.00	1.94	11.90	406

[1]The dosage rate x drench frequency interaction was significant. See table 2 for mean separation.
[2]Means followed by the same letter within columns are not significantly different at the 0.05 Level.

Seedling Growth. -Diameter and height, not influenced by dosage rate, were affected by drench frequency. Although actual differences were small, less than 0.2 mm, seedlings drenched every 2 weeks had larger diameters than those drenched at 4 or 8 weeks (table 1). Differences between 4 and 8 weeks were not significant. Height growth, consistent between the seedlings in the 2- or 4-week treatments, was 16 percent taller than in seedlings drenched every 8 weeks.

Seedling dry weights were affected by both dosage rate and drench frequency. Seedlings drenched with 10 mg benomyl were heavier than those drenched with 2.5 or 5 mg. Differences were not significant

Table 2.—*Mycorrhizal development by* Pisolithus tinctorius *as affected by benomyl dosage and drench frequency (interaction table)*[1]

Drench frequency	Dosage rate (mg) 2.5	5.0	10.0
wk	-----------% *infection*	----------	
2	25 c	24 c	54 a
4	16 c	31 b	34 b
8	12 e	17 d	22 c

[1]Means followed by the same letter are not significantly different at the 0.05 level.

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between the 2.5- and 5-mg treatments. Dry weights also significantly increased with increased drench frequency. Increasing frequency of application from 8- to P-week intervals increased seedling dry weights by 22 percent.

Table 3.—*Mycorrhizal development by* Pisolithus tinctorius *on shortleaf pine seedlings as influenced by total amount of benomyl applied*

Dosage rate	Number drenches[1]	Total benomyl	Mycorrhizal development
mg		*mg*	*percent*
10	8	80	54
10	4	40	34
5	4	20	31
2.5	S	20	25
10	2	20	22
5	8	40	24
5	2	10	17
2.5	4	10	16
2.5	2	5	12
0	0	0	8

[1]Seedlings drenched every 2 weeks = 8, 4 weeks = 4, and 8 weeks = 2.

DISCUSSION

This study supports previous findings (Pawuk et al. 1980) that *P. tinctorius* forms more mycorrhizae with southern pine seedlings when benomyl is present in the soil or potting media than when it is absent. A possible explanation is that benomyl inhibits the growth of antagonistic soil fungi. Soil fungi such as *Trichoderma* and *Pennicillium,* antagonistic to many fungi, are inhibited by low concentrations of benomyl (Edgington et al. 1971).

Pisolithus appears to have a high tolerance to benomyl and develops best when benomyl concentrations are high. Root pathogens such as *Fusarium* are common in container media growing southern pine seedlings (Pawuk 1978, Pawuk and Barnett 1974). *Fusarium* reduces root development and growth and may kill seedlings. Healthy seedlings, free of *Fusarium,* have vigorous root systems and provide more sites for ectomycorrhizal infection.

Benomyl stimulated both mycorrhizal formation and seedling growth. The growth response is probably due to control of soil fungi that reduce seedling growth or to effect of increased mycorrhizal development on benomyl-treated seedlings. In all cases, benomyl-treated seedlings were increasingly larger when fungicide concentrations increased.

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