Growth, Survival, and Ectomycorrhizal Development of Slash Pine Seedlings Inoculated with *Pisolithus tinctorius* and Sprayed with Ferbam and Bayleton Fungicides

by

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Abstract: Bayleton (triadimefon) severely stunted tree growth and inhibited ectomycorrhizal development when 8 ounces a.i. were applied per acre per application as four foliar sprays. Ectomycorrhizal spore inoculum prepared as vermiculite coated particles was used successfully when applied three weeks after the last Bayleton spray application. Early field survival (June) of the smaller, Bayleton sprayed seedlings with depressed ectomycorrhizae was significantly greater (86.1%) than with the larger, ferbam sprayed seedlings with abundant ectomycorrhizae (78.3%). Increasing quantities of Pt ectomycorrhizae did not increase the rate of survival. Reducing seedbed density increased seedling size, number of feeder roots, and the rate of field survival, but the percentage of ectomycorrhizal roots was not affected by seedbed density.

Although inoculation of nursery seedbeds with *Pisolithus tinctorius* (Pt) has produced seedlings with an abundance of ectomycorrhizae, their survival and growth in field plantings have not always exceeded those of noninoculated check seedlings (Marx, et. al., 1977; Powers and Rowan,

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1983; Leach and Gresham, 1983). The reasons are not clearly defined, but site quality may partially explain the discrepancy (Marx and Artman, 1979). Seedlings ectomycorrhizal with Pt have been reported to have the greatest advantage on adverse planting sites, whereas inoculation often has not influenced seedling performance on good sites (Marx, et. al., 1977).

An early report suggested that high dosages of Bayleton (8 oz. a.i./acre/application) inhibited growth and ectomycorrhizal development of pine seedlings (Snow, et. al., 1979). Because of this inhibition, the recommended dosage was first reduced to 6 oz. a.i./acre/application (Rowan and Kelley, 1980) and then to 4 oz. a.i./acre/application (Kelley, et. al., 1984) because it was found that this lower rate would control the disease (Kelley, 1984). In an attempt to further test the effects of Bayleton on seedling quality, a study was begun in 1983 to compare the effects of ferbam and Bayleton fungicides, quantity of Pt inoculum, nursery bed density, and date of soil incorporation of Pt inoculum on pine seedling quality at both the time of lifting and after outplanting.

Both vegetative and spore inocula of Pt were tested. Vegetative inoculum was produced (Marx and Bryan 1975) and applied at planting at both a low rate (one liter per five linear feet of bed) and a high rate (two liters per five linear feet of bed). The inoculum was applied by hand to 2-inch wide bands 1 inch beneath each drill. Spore inoculum was prepared by mixing the required weight of spores with 1 liter of vermiculite and then adding 200 milliters (ml) of water wetted with 0.2 ml of Tween 80. This preparation wetted the spores and caused them to adhere to the particles of vermiculite. This spore inoculum was applied at a low rate (633 mg spores/square foot of bed) and a high rate (6333 mg spores/square foot of bed) to 2-inch wide hands 1-inch beneath each drill at planting or to slits made beside each drill of seedlings to a 2-inch depth in mid-July (3 weeks after the last Bayleton spray). The slits were made with a spade so that the bottom of the slit was wider than the top to insure that the inoculum was nearly beneath the young seedlings. Only the spore inoculum was applied in July.

Slash pine seeds were sown on Bayleton-sprayed plots at a rate needed to produce 30 seedlings per square foot and on ferbam-sprayed plots at a rate needed to produce, after rogueing, 10, 20, 30, and 40 seedlings per square foot. Ferbam (75 WP) was applied three times each week at 3 lbs product/acre/application and Bayleton (50 WP) was applied four times during the rust hazard season (April to June 23) at 1 lb. product/acre/application. All plots were also sprayed four times with Benlate (50 WP) at 2 lb. product/acre/application to stimulate ectomycorrhizal development (Marx and Rowan, 1981).

Randomly selected seedlings (10 each) were lifted from ferbam and Bayleton plots at intervals (Table 1) during the year and monitored for growth and ectomycorrhizal development. Bayleton inhibited seedling growth and ectomycorrhizal development at the rate used, confirming the results reported earlier (Snow, et. al., 1979). Vegetative inoculum of Pt was ineffective, probably because insufficient leaching water was used, leaving too much sugar residue. No Pt mycorrhizae were produced in any Bayleton treated plot and only 5.5% to 6.0% in ferbam plots receiving vegetative inoculum (Table 2). Although the development of Pt mycorrhizae was slow, significant quantities were present on root systems in plots receiving spore inoculum by February 1984 (Table 2). Numerous fruiting bodies of the Pt fungus began appearing in late winter months, particularly in ferbam-sprayed plots.

Because of the appearance of Pt mycorrhizae on seedlings during winter, an outplanting was made in March to further test the effects of Bayleton and Pt ectomycorrhizae on the rate of survival and growth of seedlings in the plantation. The outplanting included 50 seedlings from each treatment plot planted in each of three replicate blocks for a total of 4650 seedlings. A planting site with a high fusiform rust hazard was chosen to determine if any increase in growth increment due to Pt inoculation or seedbed density is nullified by rust infections.

The following parameters were measured on 25 randomly selected trees from each plot at lifting: height, shoot weight, number of first-order lateral roots, fresh and dry weights of large (>5.6mm) and small roots, and the percentage of feeder roots ectomycorrhizal with Pt and with other symbionts. Seedbed density dramatically affected both seedling size and root biomass. The lower the seedbed density, the larger the seedling shoot and root biomass. While reductions in seedbed density increased root biomass (large and small root numbers), they did not increase the percentage of ectomycorrhizal feeder roots.

Since survival was observed in June of the year of planting, firm conclusions are impossible. However, based on numerous outplantings over 26 years of observations, early trends are seldom erroneous. Only two significant differences were found between the survival of seedlings sprayed with Bayleton versus those sprayed with ferbam: (1) Seedlings sprayed with ferbam and inoculated with the low rate of spore inoculum at planting (Table 3) did not survive as well as those receiving the same Pt inoculation treatment but sprayed with Bayleton. (2) Survival among seedlings sprayed with Bayleton was significantly better than among those sprayed with ferbam regardless of Pt inoculation treatment, amount of Pt ectomycorrhizae, or total quantity of ectomycorrhizae produced by all fungus symbionts (Tables 2 and 3). The rate of survival increased as seedbed density decreased indicating the importance of large roots and top/root ratio to the survival of outplanted seedlings.

These data indicate that heavy applications of Bayleton are detrimental to ectomycorrhizal development and growth of seedlings in the nursery. Although Bayleton sprayed seedlings were smaller and had fewer ectomycorrhizae than ferbam sprayed seedlings, their survival, nevertheless, was improved in this study. Because survival was increased by reducing seedbed density in spite of the good-wet-spring, use of this nursery treatment to improve the performance of seedlings in the plantation may be preferential to inoculations with mycorrhizal fungi. Use of Bayleton at the currently recommended rate (4 oz. a.i./acre/application) should, however, minimize the inhibition of seedling growth and mycorrhizal development.

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Table 1.--Growth and ectomycorrhizal development in slash pine seedlings after nursery inoculations with *Pisolithus tinctorius* and applications of ferbam and Bayleton fungicides

	7/20/83		10/9/83		2/25/84	
	Ferbam	Bayleton	Ferbam	Bayleton	Ferbam	Bayleton
Height (mm)	103.8	95.1	215.1	167.3	287.7 a[1/]	195.3 b
Top wt. (g)	2.9	2.2	11.3	5.0	19.9 a	7.5 b
Pt mycorrhizae (%)	-	-	T	-	37.7 a	5.2 b
Total mycorrhizae (%)	20	19	62	41	75 a	42 b

[1/] Means of each measured parameter followed by a common letter do not differ (P=0.05) according to Duncan's multiple range test.

Table 2.--Percentages of feeder roots ectomycorrhizal in slash pine seedlings inoculated with differing amounts of vegetative (Veg) and spore inoculum at different times (AP = at planting; J = July) in the nursery and after seedlings were sprayed with ferbam and Bayleton fungicides

Treatment	Fungicide	% Ectomycorrhizae	
		Pt	All fungi
Check	Ferbam	0.0	72.5
Low (Veg-AP)	Ferbam	5.5	73.4
High (Veg-AP)	Ferbam	6.0	75.5
Low (Spores-AP)	Ferbam	32.9	76.3
Low (Spores-AP+J)	Ferbam	42.5	79.6
Check	Bayleton	0.0	41.7
Low (Veg-AP)	Bayleton	0.0	41.7
High (Veg-AP)	Bayleton	0.0	41.7
Low (Spores-AP)	Bayleton	0.7	41.7
Low (Spores-J)	Bayleton	3.3	41.0
High (Spores-AP)	Bayleton	0.7	40.0
High (Spores-J)	Bayleton	8.3	42.3
Low-Low (Spores AP+J)	Bayleton	5.0	40.0
Low-High (Spores AP+J)	Bayleton	10.0	42.3
High-Low (Spores AP+J)	Bayleton	5.0	41.7
High-High (Spores AP+J)	Bayleton	8.3	45.0

Table 3.--Survival of slash pine seedlings with various amounts of *Pisolithus* and natural ectomycorrhizae resulting from nursery inoculations and application of ferbam and Bayleton fungicides

Pt inoculum treatment	% Survival	
	Ferbam	Bayleton
Check	75.8 a[2/]	94.2 a
Low-vegetative	72.5 a	75.8 a
High vegetative	80.1 a	84.2 a
Low spore (AP)[1/]	74.4 a	96.5 b
Low spore (AP+J)	88.6 a	79.7 a
Average	78.3 a	86.1 b

1/ Inoculum was applied at low and high rates at planting (AP) and in July (J).

2/ Within rows, means followed by a common lower case letter do not differ (P=0.05) according to Duncan's multiple range test.