BAYLETON EFFECTS ON PINE SEEDLING ECTOMYCORRHIZAL DEVELOPMENT

Donald H. Marx and Charles E. Cordell 1/

Abstract.--The systemic fungicide triadimefon (Bayleton®) provides highly effective and practical control for fusiform rust in southern nurseries. However, results obtained from three southern nurseries in 1983 revealed that this fungicide had significant negative effects on both artificially inoculated Pisolithus tinctorius (Pt) and naturally occurring Thelephora terrestris ectomycorrhizae in these nurseries as well as on quality of loblolly pine seedlings in one nursery. Bayleton and its chemically converted isomer, Baytan, persisted in the seedling roots at levels detrimental to ectomycorrhizal production for extended periods. In laboratory studies with pure culture fungus, Baytan significantly reduced the mycelial growth of both Pt and T. terrestris to levels considerably lower than those detected in nursery seedling roots for extended periods following the last Bayleton spray treatments. Supplemental Bayleton-ectomycorrhizal nursery studies are being conducted in two southern nurseries in 1984.

Additional keywords: Baytan, Pisolithus tinctorius, Thelephora terrestris, bare-root nurseries, Pinus taeda, Pinus elliottii.

Since 1977, research concerning the development of a commercial source of vegetative inoculum of Pisolithus tinctorius (Pers.) Coker & Couch (Pt) has been underway between the USDA Forest Service and Abbott Laboratories. A functional inoculum, MycoRhiz®, was formulated and thoroughly tested by the end of the 1980 nursery season (Marx and others 1982, 1984). In addition, a MycoRhiz inoculator - seed-sowing machine was developed to aid in the practical application of this inoculum in nurseries (Cordell and others 1981).

In the spring of 1982, 13 nurseries were involved in the operational (machine-applied inoculum) testing of MycoRhiz and the research inoculum of Pt produced by the Institute of Mycorrhizal Research and Development (IMRD). In September 1982, a midstudy assessment for ectomycorrhizae was made by IMRD and S&PF staff on the test seedlings. The results from the 10 southern nurseries were quite different from those obtained in past studies in these nurseries. Seedlings in 6 of the 10 nurseries had very little or no Pt ectomycorrhizal development even from IMRD inoculum. Results from past studies in five of these nurseries had been extremely good for Pt ectomycorrhizal development from

---

[13] Director, Institute for Mycorrhizal Research and Development, SEFES, Athens, Ga.; and National Mycorrhizae Applications Coordinator, Forest Pest Mgt., State & Private Forestry, Region 8. Both are with the USDA Forest Service.

IMRD inoculum. Seedlings in 4 of the 10 nurseries had erratic but acceptable quantities of Pt ectomycorrhizae from IMRD inoculum. The only cultural practice used in these 10 nurseries that was common to all but different from past years (when results from IMRD inoculum were good) was the use of 3 to 5 Bayleton® sprays instead of the usual 12 to 50 Ferbam® sprays used to control fusiform rust (Cronartium quercuum (Berk.) Miyabe ex. Shirai f. sp. fusiforme Burdsall & Snow). Some researchers have observed fewer naturally occurring ectomycorrhizae on pine seedlings in nurseries sprayed with Bayleton (Snow and others 1979, Kelley, 1980, 1982). Others have observed no appreciable change. Seedlings from nursery study plots receiving 2 or 4 times the recommended Bayleton rate were affected more than those receiving the recommended rate (6 oz a.i.). In 1982, certain nurseries--St. Regis in Florida (slash pine), Taylor in South Carolina (loblolly pine), and MacMillan-Bloedel in Alabama (loblolly pine)--Ferbam versus Bayleton-sprayed (recommended rate) seedlings were made available for examination at the IMRD. The incidence of naturally occurring ectomycorrhizae (by Pt and by Thelephora terrestris (Ehrh.) Fr.) was 20 to 50% (as elsewhere) less on Bayleton-treated seedlings than on those sprayed with Ferbam. The majority of the seedling samples were obtained in October or November which is several months following the last Bayleton spray (June) and a few months from lifting date.

These results suggested that Bayleton applied between April and June is absorbed into the seedling tissues and not only protects the succulent foliage and stems of young pine seedlings from the rust fungus through July (fusiform rust infection period in the South), but also may be translocated to the feeder roots where, in some nurseries, it apparently inhibits normal ectomycorrhizal development (either from natural or introduced inoculum). Unfortunately, it is during this same period of Bayleton-induced "host resistance" that Pt vegetative inoculum must infect seedling feeder roots. If the roots are not susceptible to Pt infection until after the effects of Bayleton have passed--probably July or later--then this form of Pt inoculum (succulent vegetative mycelium in vermiculite) may not endure extended survival in the soil until roots are present. If this process takes place, this could explain the problems encountered in our 1982 inoculation tests with various Pt inocula.

In the 1982 MycoRhiz study, the Reidsville, GA, nursery had better Pt ectomycorrhizal development on loblolly and slash pine seedlings than other southern nurseries. These seedlings had been sprayed three times with Bayleton but had also been drenched three times with 2.24 kg a.i./ha (2 lb a.i./a) of benomyl (Benlate®) (another systemic fungicide). Earlier studies by Marx and Rowan (1981) showed that benomyl stimulated Pt ectomycorrhizal development on loblolly pine seedlings. Perhaps benomyl applied at sowing and again early in the growing season could enhance the survival of Pt inoculum which could then be functional after the effects of Bayleton have passed.

Based on these observations, a study was designed in 1983 to determine (1) the possible combined effects of Bayleton and benomyl on Pt ectomycorrhizal development from vegetative inoculum applied at sowing, (2) the feasibility of inoculating seedlings with Pt spores after spraying with Bayleton, and (3) the correlation of Bayleton residues in tops and roots of seedlings with ectomycorrhizal development.

MATERIALS AND METHODS

The following treatments were applied at the Buckeye Cellulose Corp. Nursery, Perry, FL (slash pine), Taylor Nursery, Trenton, SC (loblolly pine), and International Paper Co. Nursery, Bluff City, AR (loblolly pine):

1. IMRD vegetative inoculum (1 liter/2.3 m (1 liter/7.5 ft) of bed, machine applied).

2. IMRD vegetative inoculum + benomyl at 2.24 kg a.i./ha (2 lb a.i./a) applied at sowing date and at 3 and 6 weeks following sowing.

3. IMRD vegetative inoculum + reinoculation with Pt spore pellets (13 g/3.7 m - 13 gm/40 ft') applied 3 weeks after the last Bayleton spray.

4. IMRD inoculum + benomyl + Pt pellets.

5. No inoculum.

6. No inoculum + benomyl.

7. No inoculum + Pt pellets.

8. No inoculum + benomyl + Pt pellets.

These eight treatments were applied at random in five nursery beds each for Ferbam and Bayleton sprays. Each treatment plot was 3 meters long (10 ft); each plot was separated by a 1.5-meter (5-linear-ft), seedling-free buffer strip. Seed were sown in April following standard nursery procedures, and treatments were applied according to schedule. Bayleton .56 kg a.i./ha (8 oz a.i./a) was applied three times (April 28, May 23, and June 21) at the Buckeye Cellulose Corp. Nursery, three times (April 28, May 23, and June 28) at the Taylor Nursery, and four times (May 5, May 25, June 8, and June 22) at the International Paper Co. Nursery. Ferbam plots were sprayed 17 to 41 times during the rust hazard period as needed in these nurseries.

Ten seedlings/plot/nursery were assessed for growth and ectomycorrhizal development in July, August, September, and November. At lifting date, 20 seedlings/plot/nursery were assessed. Seedlings for Bayleton residue analysis were collected from 1 to 116 days after the last Bayleton spray. Laboratory studies on the effects of Bayleton and Baytan on pure culture fungus growth of Pt and T. terrestris were also conducted.

---

1 Analyses performed by Dr. Parshall Push, Poultry Sciences Laboratory, Riverbend Research, University of Georgia, Athens, 30602.

RESULTS AND DISCUSSION

Neither the benomyl nor the Pt spore pellet treatments significantly affected either seedling growth or ectomycorrhizal development; thus, data from these treatments will not be presented. The results, by nursery, are briefly summarized as follows:

Buckeye Cellulose Corp. Nursery, Perry, FL (slash pine)

1. Seedling growth was not significantly affected by either Bayleton or Pt ectomycorrhizae.

2. Natural ectomycorrhizal development (formed by T. terrestris and Rhizopogon nigrescens N. sp.) was significantly reduced by Bayleton through the growing season. At lifting date (December 1983), Ferbam- and Bayleton-sprayed seedlings had 52 and 22% natural ectomycorrhizae, respectively.

3. Bayleton-sprayed and Pt-inoculated seedlings had a Pt index <1; those sprayed with Ferbam had a Pt index 54. Abundant Pt ectomycorrhizae were detected as early as July in the Ferbam-treated plots.

4. Over 20 times more Pt, 3 times more T. terrestris, and 4 times more R. nigrescens fruiting bodies were produced in Ferbam-sprayed plots than in Bayleton-sprayed plots.

5. Less than 0.1% of the seedlings had fusiform rust in either Ferbam- or Bayleton-sprayed plots (nonsprayed seedlings in a nearby nursery section had 22.5% rust).

Taylor Nursery, Trenton, SC (loblolly pine)

1. Seedling growth was significantly reduced by Bayleton throughout the growing season. At lifting date, seedling fresh weights were 9.2 g for the Bayleton treatments and 11.9 g for the Ferbam treatments.

2. Pt ectomycorrhizae in the Ferbam treatments significantly increased seedling growth. Fresh weights of seedlings with Pt ectomycorrhizae and natural ectomycorrhizae was 13.0 and 10.8 g, respectively, in the Ferbam treatment.

3. Natural ectomycorrhizal development (formed by T. terrestris) was significantly reduced by Bayleton during the growing season. At lifting date (December 1983), Ferbam-sprayed and Bayleton-sprayed seedlings had 63 and 24% natural ectomycorrhizae, respectively.

4. Bayleton-sprayed, Pt-inoculated seedlings had a Pt index 4; those sprayed with Ferbam had a Pt index 83. Abundant Pt ectomycorrhizae were detected as early as July in Ferbam-sprayed plots.

5. Over 6 times more Pt and 10 times more T. terrestris fruiting bodies were produced in Ferbam-sprayed plots than in Bayleton-sprayed plots.

6. Less than 0.3% of the seedlings had fusiform rust galls in either Ferbam- or Bayleton-sprayed plots.

International Paper Co. Nursery, Bluff City, AR (loblolly pine)

1. Seedling growth was not significantly affected by Bayleton but was significantly increased by the Pt ectomycorrhizal inoculations in the Ferbam treatment.

2. Natural ectomycorrhizae development (formed by T. terrestris) was significantly reduced by Bayleton during the growing season. At lifting date (January 1984), Ferbam-sprayed and Bayleton-sprayed seedlings had 44 and 18% natural ectomycorrhizae, respectively.

3. Bayleton-sprayed, Pt-inoculated seedlings had a Pt index <1; those sprayed with Ferbam had a Pt index 71. Abundant Pt ectomycorrhizae were detected as early as July in Ferbam-sprayed plots.

4. Over 5 times more Pt and T. terrestris fruiting bodies were produced in Ferbam-sprayed plots than in Bayleton-sprayed plots.

5. Sample seedlings assessed at lifting date were free of fusiform rust in both the Bayleton- and Ferbam-sprayed plots.

The results of the Bayleton residue analysis (ppm) of seedling tissues are summarized as follows:

Nursery and species	Days since last spray	Tops		Roots	
		Bayleton	Baytan	Bayleton	Baytan
Buckeye, FL	2	2.7	2.0	0.9	4.2
slash	64	0.2	0.8	0.1	0.4
Taylor, SC	3	1.8	6.3	0.7	4.5
loblolly	22	1.8	6.5	0.6	1.9
	59	0.4	5.1	0.1	2.3
	116	0.0	2.5	0.0	0.4
Int. Paper, AR	1	1.8	6.3	0.7	4.5
loblolly	64	0.0	1.0	0.0	1.9

It is obvious from the residue analysis that Bayleton is rapidly chemically converted to the isomer Baytan in the seedling tops and roots. Baytan also persists in relatively high concentrations in these tissues, especially roots, for several weeks.

Pure culture fungus studies in liquid culture revealed that Bayleton reduced mycelial growth of Pt and T. terrestris by 50% at about 2 ppm. Earlier, Kelley (1982) reported that 1 ppm of Bayleton reduced Pt mycelial growth on agar medium by 50% and that 5 ppm inhibited growth completely. Based on our seedling residue analysis, Bayleton in roots did not exceed 0.9 ppm. Our results with Baytan, however, were quite different. Baytan as low as 0.3 to

0.5 ppm reduced the growth of both Pt and T. terrestris by 50% in pure culture. The residue analysis showed that Baytan occurred at concentrations ranging from 0.4 to 4.5 ppm in seedling roots. In fact, 0.4 ppm of Baytan were detected in roots of loblolly pine 116 days after the last Bayleton spray (June 22) at the Taylor Nursery, SC. Since Bayleton and Baytan occur together in various ratios in seedling roots, laboratory growth studies were performed with several mixtures of the two chemicals. The results showed that 0.1 ppm of Bayleton together with 0.3 ppm of Baytan reduced the growth of Pt by 50% in pure culture. It required 3 ppm Baytan together with 0.3 ppm of Bayleton to reduce T. terrestris in a similar amount. These mixtures of Bayleton and Baytan at these concentrations or higher were found in seedling roots through early August in two of the three nurseries (Buckeye, FL and Taylor, SC).

CONCLUSIONS

1. Bayleton significantly reduced the effectiveness of artificially introduced vegetative inoculum of Pt. Ferbam-sprayed seedlings had an average Pt index of 69; those sprayed with Bayleton had an average Pt index of 1.5 for all three nurseries.

2. Bayleton also significantly reduced the development of natural ectomycorrhizae throughout the growing season. Seedlings sprayed with Ferbam had 2 to 3 times more natural ectomycorrhizae at lifting date. However, all seedlings sprayed with Bayleton had some ectomycorrhizae at lifting date (18 to 24%).

3. Fruiting body production (a reflection of the degree of ectomycorrhizal development) by Pt, T. terrestris, and R. nigrescens was decreased by 3 to 20 times in Bayleton-sprayed plots.

4. Seedling growth (loblolly pine) was significantly reduced by Bayleton only in the Taylor Nursery, SC.

5. Baytan, the chemically transformed isomer of Bayleton, was formed soon after each application of Bayleton and persisted in the roots for nearly 4 months following the last spray treatment.

6. Baytan significantly reduced the mycelia] growth of both Pt and T. terrestris in pure culture fungus growth studies at concentrations (0.3 to 0.5 ppm) which were considerably lower than those found in seedling root tissues (1.9 to 2.3 ppm) 59 to 64 days following the last Bayleton spray at two of the nurseries. These residue concentrations of Baytan were detected in the seedling roots at the same time that ectomycorrhizae (by Pt and naturally occurring fungi) were developing normally and rapidly on Ferbam-sprayed seedlings.

CURRENT RESEARCH ACTIVITIES

Supplemental nursery studies were established in 1984 on slash pine (Buckeye Nursery, FL) and loblolly pine (Taylor Nursery, SC) to determine the effects of Bayleton seed soaks at sowing date with Ferbam, and .14, .21, .28, and .42 kg a.i./ha (2, 3, 4, and 6 oz a.i./a) Bayleton (three sprays). The

objective is to determine a rate of Bayleton high enough to control fusiform rust yet low enough to have minimal negative effect either on artificially inoculated Pt or natural ectomycorrhizal development. Monthly seedling root assessments and Bayleton + Baytan residue analyses will be obtained.

LITERATURE CITED

Cordell, C. E., D. H. Marx, J. R. Lott and D. S. Kenney. 1981. The practical application of Pisolithus tinctorius ectomycorrhizae inoculum in forest tree nurseries. In Forest Regeneration, p. 38-42. Proc. Symp. Engineering Systems for Forest Regeneration Am. Soc. Agric. Engineers, St. Joseph, MI.

Kelley, W. D. 1980. Evaluation of systemic fungicides for control of Cronartium quercum f. sp. fusiforme on loblolly pine seedlings. Plant Dis. 64:773-775.

Kelley, W. D. 1982. Effect of triadimefon (Bayleton) on ectomycorrhizae of loblolly and slash pines in Alabama. For. Sci. 28:232-236.

Marx, D. H. and S. J. Rowan. 1981. Fungicides influence growth and development of specific ectomycorrhizae on loblolly pine seedlings. For. Sci. 27:167-176.

Marx, D. H., C. E. Cordell, D. S. Kenney, J. G. Mexal, J. D. Artman, J. W. Riffle, and R. J. Molina. 1984. Commercial vegetative inoculum of Pisolithus tinctorius and inoculation techniques for development of ectomycorrhizae on bare-root tree seedlings. For. Sci. Monogr. 25. 101 p. [In press].

Marx, D. H., J. L. Ruehle, D. S. Kenney, C. E. Cordell, and O. C. Goodwin. 1982. Commercial vegetative inoculum of Pisolithus tinctorius and inoculation techniques for development of ectomycorrhizae on container-grown tree seedlings. For. Sci. 28:373-400.

Snow, G. A., S. J. Rowan, J. P. Jones, W. D. Kelley, and J. G. Mexal. 1979. Using Bayleton (triadimefon) to control fusiform rust in pine tree nurseries. USDA For. Serv. Res. Note SO-253.