BAYLETON (TRIADMEFON) AFFECTS ECTOMYCORRHIZAL DEVELOPMENT ON SLASH AND LOBLOLLY PINE SEEDLINGS IN NURSERIES

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Abstract.--With loblolly and slash pines in three nurseries in 1983 and in two nurseries in 1984, Bayleton sprayed three to four times (April - June) at 0.56 kg a.i./ha (1983) or three times at 0.14, 0.21, 0.28 or 0.42 kg a.i./ha (1984) inhibited ectomycorrhizal development by naturally occurring *Thelephora terrestris* (Tt) and other fungi, and by *Pisolithus tinctorius* (Pt) from introduced vegetative inoculum. At the high rate, Bayleton reduced Pt indices over 30-fold and natural ectomycorrhizal development 2- to 3-fold by lifting time in comparison to ferbam sprayed on seedlings. Fruit body production by the ectomycorrhizal fungi was also decreased 3- to 20-fold. Seedling size was significantly reduced in one of three tests at the high rate. Degree of ectomycorrhizal development by either Pt or naturally occurring fungi was related to rate of Bayleton application in 1984. Generally, 0.14 kg a.i./ha reduced all ectomycorrhizal measurements by half and 0.42 kg a.i./ha reduced them by two-thirds. Bayleton rates of 0.21 kg a.i./ha or higher gave better rust control than the lowest rate of Bayleton or ferbam sprays. Chemical residue analyses of tops and roots revealed that Bayleton was rapidly (1 to 3 days) changed metabolically to its isomer Baytan, which persisted in roots for up to 4 months after the last spray. Baytan reduced the mycelial growth of Pt and Tt in pure culture studies at 0.3 to 0.5 mg/l; these concentrations were considerably lower than 1.9 to 2.3 µg/g found in root tissue as long as 2 months after the last spray. Baytan was more inhibitory than Bayleton to mycelial growth. Bayleton sprays at 0.21, 0.28, and 0.42 kg a.i./ha reduced survival of Pt vegetative inoculum in soil and the two highest rates reduced susceptibility of roots to Pt colonization. Seed soaking with Bayleton prior to sowing in the nursery did not significantly affect ectomycorrhizal development or seedling growth. However, seed soaking reduced seedling density of loblolly and slash pines by over 16 percent and reduced the number of plantable seedlings by 11 to 13 percent in the 1984 tests.

In the last decade, there has been considerable research on the use of specific ectomycorrhizae on tree seedlings to improve reforestation and reclamation success in the United States and abroad. Numerous authors have reported that various species of pine and oak seedlings with abundant ectomycorrhizae formed by *Pisolithus tinctorius* (Pers.) Coker & Couch (Pt), either

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in container or bare-root nurseries, survive and grow better after outplanting than seedlings with naturally occurring ectomycorrhizae. Generally, the more adverse the site the more Pt ectomycorrhizae improve seedling performance. Recently, Marx et al. (1982, 1984a) reviewed the significance of Pt ectomycorrhizae to nursery and field performance of tree seedlings. Since 1977, research by the USDA Forest Service and the private sector has been conducted on the development of commercial sources of Pt inocula for potential practical application. Basic techniques have been developed to produce and apply basidiospore (Marx and Bell 1985, Marx et al. 1984b, Marx et al. 1979) and vegetative (Marx and Kenney 1982, Marx et al. 1984a) inocula of this fungus.

In spring 1982, vegetative inoculum of Pt produced by Abbott Laboratories (MycoRhiz®) was compared with that produced by the Institute for Mycorrhizal Research and Development (IMRD) in operational tests in 10 pine nurseries in the South on loblolly (*Pinus taeda* L.) and slash (*P. elliottii* var. *elliottii* Engelm.) pine seedlings. A midseason assessment showed no Pt development on seedlings in 6 of the 10 nurseries and less than expected in the other 4 regardless of inocula. In previous years, inoculations in these nurseries resulted in abundant Pt development when identical inoculum formulations were used. The only cultural practice in these 10 nurseries in 1982 that was different from previous years was the use of the newly introduced systemic fungicide triadimefon (Bayleton®) to control fusiform rust disease caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* Burdsall and Snow. Bayleton at 0.42 or 0.56 kg a.i./ha had been sprayed three to five times from April to July. In the past, 20 to 50 sprays of ferbam (Fermate®), a contact fungicide, were used in these and other southern nurseries to protect pine seedlings from rust infection.

During the initial tests of Bayleton for rust control, variable effects of this fungicide on naturally occurring ectomycorrhizae were observed. Snow et al. (1979) reported a significant reduction in ectomycorrhizae in August assessments in four nurseries after spraying seedlings with Bayleton (0.56 kg a.i./ha). However, by January these differences were no longer evident, suggesting that the inhibitory effect of Bayleton was temporary. In a greenhouse study, Kelley (1980) reported no reduction of fruit bodies of the ectomycorrhizal fungus *Thelephora terrestris* (Ehrh.) Fr. (Tt) on pine seedlings 36 weeks after various Bayleton sprays. Kelley (1982) also tested two to three sprays of Bayleton at 0.28 to 0.56 kg a.i./ha on loblolly and slash pine seedlings in nurseries and found no differences in ectomycorrhizal development on these seedlings in comparison to nonsprayed seedlings assessed in August and September. Rowan and Kelley (1983) found Bayleton sprayed at rates up to 1.68 kg a.i./ha up to four times did not significantly reduce ectomycorrhizal development on nursery-grown loblolly and slash pine seedlings assessed at the end of the growing season. In a greenhouse study, South and Kelley (1982) found incorporating 1 and 2 kg a.i./ha of Bayleton into the soil before planting decreased both short root production and the incidence of bifurcate roots on 7-month-old loblolly pine seedlings. In pure culture studies, Kelley (1982) reported variable effects of Bayleton on mycelial growth of seven ectomycorrhizal fungi on agar medium. Growth of some species was reduced by 50 percent at concentrations of 3 to 20 mg/l; growth of others ceased at 5 to 40 mg/l. Pt was the most sensitive fungus tested, with a 50 percent growth reduction at 1 mg/l and total growth reduction at 5 mg/l.

Bayleton is highly effective in controlling fusiform rust, requires fewer sprays and less effort than ferbam, and is cost effective. However, before widespread application of the ectomycorrhizal fungus technology can be implemented, the effects of Bayleton on ectomycorrhizal development and seedling growth in production nurseries must be resolved. This paper summarizes results of research done in 1983 and 1984 on the effects of Bayleton on growth and ectomycorrhizal development of slash and loblolly pines in nurseries.

1983 TESTS

Materials and Methods

Identical experiments were installed in three production tree nurseries in 1983. Pt (isolate 288) was grown vegetatively, processed, and characterized using standardized procedures (Marx et al. 1984a). Methyl bromide soil fumigation, a prerequisite for effective Pt inoculations in bare-root nurseries (Marx et al. 1984a), was done in each nursery during the spring. Five adjacent nursery sections, each containing six or nine beds, were used in each nursery. Three adjacent beds per section were randomly selected for Bayleton spray treatments and three other adjacent beds per section were selected for ferbam spray treatments. The middle bed of each set of three beds was used as a test bed. All sprays were applied by a tractor-drawn, hydraulic sprayer that straddled the test bed and sprayed three beds simultaneously. Plots, 3.05 m long, separated from each other by a 1.52 m buffer strip and starting 6.1 m from the end of the nursery bed, were laid out in each test bed. Several treatments were used in 1983 but the only ones of significance were Pt vs natural ectomycorrhizae in unsprayed plots and those sprayed with Bayleton or ferbam. Only these treatments will be presented and discussed here. IMRD vegetative inoculum was machine-injected (Cordell et al. 1981) or hand-applied 5 cm deep in each of eight seed drill rows per bed at the rate of 1 liter/2.29 linear m of bed (2.79 m^2). After hand applications, the furrows were closed with rakes after inoculation. Control beds were disrupted with the machine inoculator without inoculum to standardize soil disturbance.

After IMRD inoculum was applied all beds were seeded with local machinery. Seeds in the buffer strips between plots were removed by hand to maintain a 1.5 m seedling-free buffer strip between plots to reduce chances for contamination between plots. The experimental design was a split-plot with five blocks each of Bayleton- and ferbam-sprayed beds, each containing the two inoculation treatments.

Two to 3 days after the last Bayleton spray, and at scheduled dates thereafter, 12 to 15 seedlings were removed from each of 8 to 10 random locations in the buffer nursery bed next to the irrigation risers and adjoining each of the 10 test beds. Seedlings were briefly rinsed in water, separated into tops and roots, and frozen within 72 hours of collection. The techniques of Specht (1977) and Thornton (1977) were used to quantitatively assay Bayleton and its metabolite Baytan (triadimenol) in the seedling tops and roots.[2]

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[2] Bayleton and Baytan analyses were conducted by Parshall B. Bush, Poultry Sciences Laboratory, Riverbend Research, University of Georgia, Athens, Ga.

Ten randomly selected seedlings from each of the 20 test plots/nursery were removed in mid-July, August, September and November. Height and root collar diameter of each seedling was measured, tops and roots were weighed, and stems were examined for fusiform rust galls. Ectomycorrhizae were visually assessed (Marx and Bryan 1975) at 5X magnification. Data on Pt ectomycorrhizae were transformed to a Pt index (Marx et al. 1984a). Plots were also examined every 2 weeks from mid-August until the end of the growing season for fruit bodies of Pt and other ectomycorrhizal fungi.

The studies were terminated when seedlings became dormant. After undercutting all beds at a 20 cm depth, seedlings from three random subplots each 0.31 x 1.22 m wide were removed by hand from each plot. Subplots contained between 108 and 132 seedlings. Seedlings were counted, graded for size and examined for rust galls. Rust incidence was estimated by removing the needles from all seedlings in a subplot and examining the stems for rust galls. Seedlings with heights less than 15 cm tall, root-collar diameters less than 2 mm, forked tops, or rust galls were considered culls. Twenty plantable (noncull) seedlings per subplot were measured and assessed for ectomycorrhizae as previously described, and Pt indices were computed (Marx et al. 1984a). Data obtained from each of the three subplots were averaged to represent the plot mean.

For the pure culture laboratory tests, 5 g of technical grade Bayleton or Baytan were added to 600 ml of deionized H_2O and stirred thoroughly. The solutions were incubated at 20 °C for 12 hours and filtered through Whatman #42 filter paper to remove suspended particles. These stock solutions were diluted to desired concentrations with modified Melin-Norkrans (MMN) liquid medium and placed in culture flasks. Pt (isolate 288) and Tt (isolate 223) were grown on MMN agar medium for 4 weeks at 25 °C. A 5-mm-diameter disc of a fungus was removed from its colony and placed in each flask; six flasks per fungus were prepared per test solution. Flasks were incubated at 25 °C for the time required for growth of each fungus in fungicide-free MMN medium to cover the surface of the liquid medium--about 30 days for Pt and 21 days for Tt. Mycelial mats were dried at 70 °C for 24 hours, and weighed. Weights attained on medium with fungicide were expressed as percentages of that on the fungicide-free medium (control). All data were subjected to analysis of variance, and significant differences among means were identified with Duncan's new multiple range test at $\underline{P} = 0.05$.

Each nursery used its standard cultural practices (herbicides, fertilizer, top-pruning, etc.) throughout the growing season. Specific details are available but will not be presented here. However, certain details pertinent to the study are presented below.

After soil fumigation at the Buckeye Cellulose Corporation Nursery, Perry, Florida, vegetative inoculum of Pt was applied on April 14, untreated slash pine seeds were sown, and seedbeds were mulched with hydromulch. Soil fertility was 350 ppm total N, 56 ppm available P (Bray II), and 16, 165, and 65 ppm of exchangeable K, Ca, and Mg, respectively. Soil pH was 5.1, and organic matter content was 1.45%. The soil type was a loamy sand (88:5:7 percent sand, silt and clay). For the ferbam treatments, ferbam at 2.2 kg a.i./ha was sprayed 41 times at 1- to 5-day intervals from April 28 to July 5. For the

Bayleton treatments, Bayleton at 0.56 kg a.i./ha in Agri-dex adjuvant was sprayed on April 28, May 23, and June 21. Ammonium nitrate at 56 kg/ha and KCl at 28 kg/ha were applied to all beds on May 17, July 7, and July 14. In early June, 112 kg/ha of 10-10-10 fertilizer were applied. Seedling tops were mowed to a height of 20 cm in July and August. The study was terminated in December 1983. Successful inoculations of slash pine were made with MycoRhiz® or IMRD vegetative inoculum of Pt using ferbam to control rust in this nursery from 1977 thru 1981 (Marx et al. 1984a).

After soil fumigation at the Taylor State Nursery, Trenton, South Carolina, vegetative inoculum of Pt was applied by hand on April 20, treated seeds of loblolly pine were sown and seedbeds were mulched with unfumigated pine straw. Soil fertility was 300 ppm total N, 68 ppm available P (Bray II), and 61, 164, and 66 ppm of exchangeable K, Ca, and Mg, respectively. Soil pH was 5.8, and organic matter content was 1.21%. The soil type was a loamy sand (88:6:6 percent sand, silt and clay). For the ferbam treatments, ferbam at 2.2 kg a.i./ha was sprayed 17 times at 1- to 7-day intervals from May 11 to July 4. For the Bayleton treatments, Bayleton at 0.56 kg a.i./ha in Agri-dex adjuvant was sprayed on May 18, June 1, and June 28. On June 6 and 20, July 11, and August 8, 106 kg/ha of ammonium nitrate were applied to all beds; 112 kg/ha of KCl were applied on July 26. Seedling tops were mowed to a height of 20 cm in July and August. The study was terminated in December 1983. Successful inoculations of loblolly pine were made with MycoRhiz® and IMRD vegetative inoculum of Pt using ferbam to control rust in this nursery from 1979 thru 1981 (unpublished data).

After soil fumigation at the International Paper Company Nursery, Bluff City, Arkansas, vegetative inoculum of Pt was applied by hand on April 21, and treated seeds of loblolly pine were sown and covered with hydromulch. Soil fertility was 240 ppm total N, 34 ppm available P (Bray II), 82, 194, and 68 ppm of exchangeable K, Ca, and Mg, respectively. Soil pH was 6.1, and organic matter content was 0.85%. The soil type was a loamy sand (86:8:6 percent sand, silt and clay). For the ferbam treatments, ferbam at 1.84 kg/ha was sprayed 17 times at 1- to 7-day intervals from May 7 to June 24. For the Bayleton treatments, Bayleton at 0.56 kg a.i./ha in Agri-dex adjuvant was sprayed on May 12 and 26, and June 9 and 16. On June 7, 56 kg/ha of ammonium nitrate were applied, 112 kg/ha of $(NH_4)_2SO_4$ were applied on July 7 and 19, and 112 kg/ha of KCl were applied on August 24 to all plots. Seedling tops were mowed to a height of 20 cm in July and August. The study was terminated in January 1984. Successful inoculations of loblolly pine were made with IMRD vegetative inoculum of Pt using ferbam to control fusiform rust in this nursery in 1980 and 1981 (unpublished data).

Results

Buckeye Cellulose Corporation Nursery, Perry, FL

Growth of slash pine seedlings was not significantly affected by either Bayleton or Pt ectomycorrhizae. Natural ectomycorrhizal development (formed by Tt and *Rhizopogon nigrescens* N. sp.) was significantly reduced by Bayleton through the growing season. At lifting time, ferbam- and Bayleton-sprayed seedlings had 52 and 22% natural ectomycorrhizae, respectively. Bayleton-sprayed and Pt-inoculated seedlings had a Pt index <1; those sprayed with ferbam had a Pt index of 54. Abundant Pt ectomycorrhizae were detected as early as July in the ferbam-treated plots. Over 20 times more Pt, 3 times more Tt, and 4 times more R. nigrescens fruit bodies were produced in ferbam-sprayed plots than in Bayleton-sprayed plots. Less than 0.1% of the seedlings had fusiform rust in either ferbam- or Bayleton-sprayed plots. In a nearby nursery section 22.5% of unsprayed seedlings had rust.

Taylor Nursery, Trenton, SC

Loblolly pine 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. Pt ectomycorrhizae in the ferbam treatments significantly increased seedling growth. Fresh weights of seedlings with Pt ectomycorrhzae and natural ectomycorrhizae were 13.0 and 10.8 g, respectively, in the ferbam treatment.

Natural ectomycorrhizal development (formed by Tt) was significantly reduced by Bayleton during the growing season. At lifting time, ferbam-sprayed and Bayleton-sprayed seedlings had 63 and 24% natural ectomycorrhizae, respectively. Bayleton-sprayed, Pt-inoculated seedlings had a Pt index of 4; those sprayed with ferbam had a Pt index of 83. Abundant Pt ectomycorrhizae were detected as early as July in ferbam-sprayed plots. Over 6 times more Pt and 10 times more Tt fruit bodies were produced in ferbam-sprayed plots than in Bayleton-sprayed plots. Less than 0.3% of the seedlings had fusiform rust galls in either ferbam-or Bayleton-sprayed plots.

International Paper Company, Bluff City, AR

Loblolly pine seedling growth was not significantly affected by Bayleton but was significantly increased by Pt ectomycorrhizae in the ferbam treatment. Natural ectomycorrhizae development (formed by Tt) was significantly reduced by Bayleton during the growing season. At lifting time, ferbam-sprayed and Bayleton-sprayed seedlings had 44 and 18% natural ectomycorrhizae, respectively. Bayleton-sprayed, Pt-inoculated seedlings had a Pt index <1; those sprayed with ferbam had a Pt index of 71. Abundant Pt ectomycorrhizae were detected as early as July in ferbam-sprayed plots. Over five times more Pt and Tt fruit bodies were produced in ferbam-sprayed plots than in Bayleton-sprayed plots. Seedlings assessed were free of fusiform rust in both the ferbam- and Bayleton-sprayed plots.

The results of the Bayleton residue analysis (μg/g) of seedling tissues are summarized in Table 1. Bayleton was rapidly converted to its isomer Baytan in seedling tops and roots. Baytan also persisted in relatively high concentrations in these tissues, especially roots, for several weeks after the last Bayleton spray.

In the pure culture laboratory tests, Bayleton reduced mycelial growth of Pt by 50% at concentrations of about 1.0 mg/l and that of Tt at 2.0 mg/l. Bayleton concentrations above 5.0 mg/l completely inhibited growth of both fungi. Baytan inhibited Pt by 50% at approximately 0.5 mg/l and Tt between 0.1 and 0.3 mg/l; neither fungus grew at concentrations of 2.0 or more mg/l of

Baytan. In mixed solutions of Bayleton and Baytan, Pt was inhibited by 50% at all concentrations of Baytan (0.1 to 0.5 mg/l) in mixture with 0.3 and 0.5 mg/l of Bayleton. Tt tolerated the test concentrations of Bayleton (0.1 to 0.5 mg/l) in mixture with 0.1 mg/l of Baytan. At higher Baytan concentrations with Bayleton, growth of Tt was reduced but to a lesser extent than Pt.

Table 1.--Concentrations (μg/g) of Bayleton and Baytan in tops and roots of loblolly and slash pine seedlings at different times after spraying with Bayleton in three southern nurseries

Nursery and species	Days since last spray	Tops		Roots	
		Bayleton	Baytan	Bayleton	Baytan
Buckeye Cellulose, FL	3	1.8	6.3	0.7	4.5
slash pine	64	0.0	1.0	0.0	1.9
Taylor, SC	3	2.1	8.7	1.8	4.3
loblolly pine	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
International Paper, AR	2	2.7	2.0	0.9	4.2
loblolly pine	64	0.2	0.8	0.1	0.4

Conclusions

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 in the three nurseries.

Bayleton also significantly reduced the development of natural ectomycorrhizae throughout the growing season. Seedlings sprayed with Bayleton had between 18 and 24% ectomycorrhizal development by lifting time. Seedlings sprayed with ferbam had two to three times more natural ectomycorrhizae at lifting date.

Fruit body production by Pt, Tt, and *R. nigrescens* was decreased by 3 to 20 times in Bayleton-sprayed plots.

Seedling growth (loblolly pine) was significantly reduced by Bayleton only in the South Carolina nursery.

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

Baytan significantly reduced the mycelial growth of both Pt and Tt in pure culture studies at concentrations of 0.3 to 0.5 mg/l, which were considerably lower than those found in seedling root tissues (1.9 to 2.3 μg/g) 59 to 64 days after the last Bayleton spray at two of the nurseries. These residual 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.

1984 TEST

Kelley et al. (1984) recommended that the rate of Bayleton be reduced to 0.28 kg a.i./ha because of the inhibitory effects of higher rates on ectomycorrhizal development. Kelley (1985) and Rowan (1984) also recommended that pine seed be soaked in Bayleton prior to sowing to provide protection from rust throughout the seedling emergence period, because Bayleton sprays protect only the seedlings that have emerged before the spray is applied.

Our tests in 1984 were designed to determine the effects of lower rates of Bayleton sprays and the seed soaking treatment on rust control, seedling growth, and ectomycorrhizal development of slash and loblolly pines. Other tests were installed to determine the survival of Pt vegetative inoculum in soil after spraying with several concentrations of Bayleton and the susceptibility of loblolly pine seedlings to Pt after the various sprays. Seed and seedling tissues were also analyzed for residues of Bayleton and Baytan after seed treatment and spraying.

Materials and Methods

The experimental designs of the 1984 tests in the Buckeye Cellulose Nursery in Florida (slash pine) and the Taylor State Nursery in South Carolina (loblolly pine) were identical. Soil in each nursery was fumigated in the spring, and cultural practices and experimental procedures, unless otherwise stated, were the same as those used in 1983. Five test blocks of three adjacent nursery beds were selected. Seven 18.29-m-long plots were laid out in the middle bed of each block. Within each plot, two 4.57-m-long subplots were laid out. Each subplot began 3.05 m from the edge of the plot and was separated from other subplots by 3.05 m. One subplot was inoculated by machine with Pt vegetative inoculum, and soil in the other subplot was disrupted by the machine, but received no inoculum (control). The seven treatments were no spray, ferbam, ferbam + seed soak, and three sprays each of Bayleton at 0.14, 0.21, 0.28, or 0.42 kg a.i./ha + seed soak. After seeds were soaked in Bayleton and dried, they were sown at specified densities, which did not vary by seed treatment. Seedlings were sampled and assessed at various intervals between sprays during the growing season as in 1983.

To determine survival of Pt inoculum in soil after Bayleton spray treatments, nylon bags containing Pt vegetative inoculum were buried 10 cm deep in seedling-free areas of each plot in the South Carolina nursery and assessed for viability at different times by use of the inoculum slurry, root dip technique on nonmycorrhizal seedlings of loblolly pine (Marx et al. 1984a). Soil from above the buried inoculum was sampled when the inoculum was removed and assayed for Bayleton and Baytan residues. To determine susceptibility of seedling roots to Pt colonization, seedlings from plots in the South Carolina nursery were removed 2 weeks after the last Bayleton spray, exposed to fresh vegetative inoculum of Pt by the root dip technique, and incubated for 1 month in the special growth room at the IMRD.

Results

In the Florida nursery, the slash pine seedlings in inoculated subplots, without Bayleton spray, developed an average Pt index of 69 by lifting time. Control seedlings, without Bayleton spray, formed naturally occurring ectomycorrhizae on an average of 56% of feeder roots. Seedlings with Pt ectomycorrhizae in these treatments were larger than controls.

Top/root ratios increased with increasing rates of Bayleton application, and total seedling weights were less in the 0.42 kg a.i./ha treatment than in other treatments. Pt indices of slash pine seedlings sprayed with Bayleton at 0.14, 0.21, 0.28, and 0.42 kg a.i./ha were 15, 6, 7, and 4, respectively; natural ectomycorrhizal development on uninoculated seedlings was 35, 31, 21, and 24%, respectively.

Number of Pt fruit bodies averaged $16/m^2$ in subplots without Bayleton sprays and 5, 6, 2, and $3/m^2$ in subplots sprayed with 0.14, 0.21, 0.28, and 0.42 kg a.i./ha, respectively; fruit bodies of other ectomycorrhizal fungi averaged $24/m^2$ in subplots without Bayleton spray, and 10, 8, 12, and $7/m^2$, respectively, for the increasing Bayleton spray rates.

All spray and seed treatments significantly reduced fusiform rust disease on the slash pine seedlings (table 2). There was no difference in rust incidence in the ferbam alone, ferbam + seed soak, and the 0.14 kg a.i./ha of Bayleton with seed soak treatments. These treatments reduced rust incidence from 9.9% unsprayed plots to an average of 1.6%. The three other rates of Bayleton + seed soak did not differ in rust control but reduced the incidence to less than 0.8%.

All treatments that included seed soaking reduced seedling density by lifting time by an average of 16.4% and number of plantable seedlings by 11.2%, when compared to treatments without the seed soaking (table 2).

In the South Carolina nursery, the loblolly pine seedlings in inoculated subplots, without Bayleton spray, developed an average Pt index of 60 by lifting time. Control seedlings, without Bayleton spray, formed naturally occurring ectomycorrhizae on an average of 67% of feeder roots. Seedlings with Pt ectomycorrhizae in two of these three treatments were larger than controls.

Top/root ratios of seedlings at lifting time were not affected by any treatment. Pt indices of seedlings sprayed with various amounts of Bayleton were between 0 and 1. Natural ectomycorrhizal development on control loblolly pine seedlings was 54, 51, 46, and 37% of feeder roots for the 0.14, 0.21, 0.28, and 0.42 kg a.i./ha Bayleton treatment, respectively.

Table 2.--Effects of Bayleton seed soak and spray on control of fusiform rust disease and seedling density[1,2]

Treatment	Total seedlings lifted/m^2	Percent rust	Number plantable[3] seedlings/m^2
		Slash pine	
No spray	563a	9.9a	468a
Ferbam	525a	1.9b	476a
Seed soak + ferbam	438b	1.2b	404b
Seed soak + Bayleton (a.i.)			
@ 0.14 kg/ha	460b	1.7b	425b
0.21 kg/ha	478b	0.9c	435b
0.28 kg/ha	447b	0.9c	419b
0.42 kg/ha	452b	0.4c	412c
		Loblolly pine	
No spray	459a	4.7a	387a
Ferbam	465a	1.4b	390a
Seed soak + ferbam	404b	0.3bc	333b
Seed soak + Bayleton (a.i.)			
@ 0.14 kg/ha	362b	0.8b	316c
0.21 kg/ha	384b	0.0	338b
0.28 kg/ha	391b	0.0	344b
0.42 kg/ha	383b	0.1c	329bc

1 Subplot data from ectomycorrhizal treatments were averaged.

2 Numbers in columns of a pine species followed by the same letter are not significantly different at $P = 0.05$.

3 Rust minus other culled seedlings = No. of plantable seedlings

Number of Pt fruit bodies was 2 to 3/m^2 without Bayleton sprays and less than 1/m^2 with Bayleton sprays. Fruit bodies of other ectomycorrhizal fungi averaged 18/m^2 without Bayleton sprays and decreased from 13 to 6/m^2 with increasing rates of Bayleton.

All spray treatments significantly reduced fusiform rust disease on loblolly pine seedlings (table 2). There was no difference in rust incidence in the ferbam alone, ferbam + seed soak, and the 0.14 kg a.i./ha of Bayleton + seed soak treatments; these reduced rust from 4.7% (nonsprayed) to an average of 0.8%. The higher rates of Bayleton application furnished nearly 100% rust control.

All treatments with seed soaks reduced seedling density by lifting time by an average of 16.5% and the number of plantable seedlings by an average of 13.4% when compared to treatments without seed soaks (table 2).

Results of the buried Pt vegetative inoculum test indicated that by 4 weeks after the last Bayleton spray (July 11), viability of the inoculum decreased from an average of 58 to 28% of fresh inoculum in the 0.21 kg a.i./ha and higher Bayleton spray rates. This decrease in inoculum survival was associated with residues of Baytan in the soil of 0.07 to 0.19 µg/g; the amount of residue was proportional to the Bayleton application rate.

Among loblolly pine seedlings sprayed in the nursery, root susceptibility to fresh Pt vegetative inoculum decreased as the Bayleton spray rate increased. Pt indices averaged 17 for the 0.28 and 0.42 kg a.i./ha Bayleton spray rate and 39 for the other five treatments.

Bayleton residues >1.0 µg/g and Baytan residues >0.5 µg/g are considered biologically significant. Analyses of slash pine seed and seedling tissues at intervals after seed soaking in Bayleton showed that most of the Bayleton was retained in the seedcoats (table 3). Baytan was not detected in the endosperms of ungerminated seeds, but was found in all seedling parts during germination and early seedling development. Concentrations of both residues, but especially Bayleton, decreased in needles and roots after 28 days of seedling development.

Table 3.--Residues (µg/g) of Bayleton and Baytan in seed and seedling tissue of slash and loblolly pines after Bayleton seed soak

	Slash pine		Loblolly pine	
Tissue	Bayleton	Baytan	Bayleton	Baytan
		2 days after soak		
Seed coat	324.1	0	145.4	0
Endosperm	2.3	0	2.7	0
		14 days after soak		
Seed coat	11.9	0.8	4.6	0
Remnant endosperm	2.4	1.9	2.3	1.0
Primary needles	0.6	1.3	1.5	4.0
Radical	6.7	7.9	4.5	5.2
		28 days after soak[1]		
Needles	0.02	0.23	0.04	0.42
Roots	0.06	0.28	0.42	0.32

[1] Before first Bayleton spray.

Analyses of tops and roots of slash pine seedlings after the different Bayleton sprays showed that significant concentrations of Baytan were present in most samples until midsummer (table 4). Significant concentrations of

Bayleton were not detected in tops or roots at any sampling time. The Baytan concentrations reported here are the least amounts present in these tissues, since samples were collected from 10 to 47 days after spraying. Undoubtedly, higher concentrations would have been detected had samples been collected sooner after treatment. Baytan concentrations in tops and roots increased with increasing rates of Bayleton spray. Significant concentrations of Baytan were present at the last sample period and probably persisted for weeks after.

Table 4.--Baytan residues (μg/g) in slash and loblolly pine seedlings after Bayleton sprays[1]

Bayleton spray rate (kg a.i./ha)	Slash pine Tops	Slash pine Roots	Loblolly pine Tops	Loblolly pine Roots
		14 days after 1st spray		
0.14	1.09	0.44	1.19	0.26
0.21	1.36	0.75	1.42	0.39
0.28	1.50	1.33	1.51	0.61
0.42	1.58	1.49	2.46	0.73
		10 to 14 days after 2nd spray		
0.14	1.31	0.28	1.54	0.31
0.21	1.33	0.28	1.75	0.41
0.28	1.50	0.33	1.72	0.49
0.42	1.86	0.40	2.14	0.65
		18 to 25 days after 3rd spray		
0.14	0.92	0.35	1.15	0.47
0.21	0.77	0.82	2.27	0.68
0.28	1.01	0.73	2.33	1.42
0.42	2.86	2.66	4.66	3.82
		47 to 53 days after 3rd spray		
0.14	0.27	0.22	0.52	0.27
0.21	0.48	0.45	0.82	0.38
0.28	0.61	0.44	1.15	0.44
0.42	1.10	0.76	2.23	0.72

[1] Bayleton residues did not exceed 0.30 μg/g for loblolly pine or 0.23 μg/g for slash pine in any sample or tissue; these concentrations are considered biologically insignificant and are not presented.

Residue analyses of loblolly pine seed and seedling tissues at intervals after seed soaking of stratified seeds in Bayleton showed, as with slash pine, that most of the Bayleton was retained in the seedcoat (table 3). Baytan was not detected in the endosperm of nongerminated seeds, but was found in all seedling parts after germination. Both residues decreased in needles and roots after 28 days of seedling development.

Analyses of loblolly pine seedlings after the different Bayleton sprays showed that significant concentrations of Baytan were present in tops and roots in nearly all samples (table 4). As with slash pine, Baytan concentrations reported here are the least amounts present in tissues since samples were collected 14 to 53 days after spraying. Undoubtedly, higher concentrations would have been present had samples been collected sooner after treatment. Significant concentrations of Bayleton were not detected at any sampling period. Generally, tops and roots of loblolly pine had higher concentrations of Baytan than slash pine at the same time after treatment and for the same spray rate.

Conclusions

Soaking seeds in Bayleton did not significantly decrease the incidence of fusiform rust disease on loblolly and slash pine seedlings, but did significantly decrease seedling density and the number of plantable seedlings/m^2.

Bayleton sprayed at 0.14 kg a.i./ha + seed soaking was as effective as ferbam sprays with or without seed soaking in controlling fusiform rust on both pine species, but significantly depressed ectomycorrhizal development and fruit body production by Pt and naturally occurring fungi during the growing season. Bayleton sprays at the higher rates significantly decreased Pt and natural ectomycorrhizal development as well as fruit body production to a greater extent.

The higher rates of Bayleton (i.e., 0.21, 0.28, and 0.42 kg a.i./ha) spray decreased Pt inoculum survival in soil, and the two highest rates also decreased susceptibility of roots to Pt colonization. Biologically significant concentrations of Baytan were present in roots of slash and loblolly pine seedlings during the time when inoculum was losing viability. Thus, the inhibitory effect of Bayleton sprays on Pt ectomycorrhizal development are apparently due to (1) presence of significant concentrations of Baytan in roots, and (2) decreased survival of Pt vegetative inoculum in soil. Both of these factors may be responsible for the depression of natural ectomycorrhizal development as well.

DISCUSSION

There is little doubt that even the lowest rate of Bayleton spray needed to significantly control fusiform rust disease on slash and loblolly pine seedlings is still too high to allow significant development of Pt etomycorrhizae from introduced vegetative inoculum. Apparently, both reduced root susceptibility to Pt colonization and reduced Pt inoculum survival in soil are responsible for depressed Pt ectomycorrhizal development. Both of these suggestions are supported by the presence of significant amounts of Baytan residue in roots and soil--amounts that inhibited vegetative growth of Pt in the laboratory.

Although the data were not presented, all rates of Bayleton spray (0.14 to 0.56 kg a.i./ha) depressed natural ectomycorrhizal development as early as July and the effect persisted until lifting time. The degree of suppression was related to rate of Bayleton applied. Comprehensive field studies are needed to determine the significance of depressed ectomycorrhizal development to field performance of these pine species. Earlier work has demonstrated that pine seedlings with few ectomycorrhizae, either formed by Pt or nursery fungi, do not survive and grow as well as those with more ectomycorrhizae especially on stressed sites or during periods of drought (Marx and Hatchell, in press). On better sites and in years of adequate rainfall, seedlings with few ectomycorrhizae may perform as well as those with more ectomycorrhizae. Results from these field studies will furnish the evidence needed to decide whether the slight increase in control of fusiform rust disease furnished by Bayleton spray is worth, both economically and biologically, the loss of seedling quality inherent in depressed ectomycorrhizal development.

Other than that presented here, information is not available on the cumulative effects of Bayleton and Baytan on pine seedling physiology. However, since absorption, retention, and degradation of Bayleton and Baytan continue for several weeks after the last spray, it seems from a biological standpoint that the timing of sprays should fit the rust history of the nursery and not a fixed date for application.

The results of the studies reported herein are somewhat in disagreement with earlier reports. It is not unusual for results from greenhouse studies (Kelley 1980, Kelley and South 1982) to differ from nursery tests because of different patterns of colonization of soil by naturally occurring ectomycorrhizal fungi between the two conditions. The differences found between the various nursery studies may be explained, at least partially, by the timing of soil fumigation in the nurseries. In the studies reported herein, soil in all nurseries was fumigated just before study installation and, therefore, contained little inoculum of resident ectomycorrhizal fungi. In earlier nursery studies (Rowan and Kelley 1983, Snow et al. 1979), most tests were installed in soil that had never been fumigated or that had not been fumigated for 1 to 2 years before study installation. These soils could be expected to have unusually high ectomycorrhizal fungus inoculum potential. This high inoculum potential could be responsible for greater ectomycorrhizal development throughout the growing season than that reported in the present studies. Most forest tree nurseries in the South fumigate soil either in the fall or spring preceding sowing of pine seed in the spring.

Inhibition of growth of the loblolly pine seedlings by three sprays of 0.56 kg a.i./ha of Bayleton in the South Carolina nursery but not by four sprays in the other loblolly pine study in Arkansas cannot be explained at this time. However, there were many differences between these nurseries. Seedlings in the South Carolina nursery had more naturally occurring ectomycorrhizal development in ferbam sprayed plots throughout the growing season, were grown in soil with higher initial N concentrations and twice as much available P, and received nearly twice as much N during the growing season as did seedlings in the Arkansas nursery. Perhaps these ectomycorrhizal and fertility effects were responsible for the different reactions to Bayleton.

Research on the systemic action of Bayleton and Baytan on various agricultural plants indicates that these fungal sterol-inhibiting triazoles are readily taken up by plant roots and translocated to transpiring leaves. After foliar application, however, these chemicals are reported to remain in the sprayed portion of the plant. They are not usually redistributed in the plant following leaf absorption but may move to the leaf margins in dicotyledons or leaf tips in monocotyledons (Davidse and DeWaard 1984). The presence of Bayleton and Baytan in roots of pine seedlings in these studies and the results of the inoculum survival study suggest that translocation downward from the needles, leaching into the soil, and root absorption may occur. A considerable amount of Bayleton is sprayed on the soil surface during its application to small pine seedlings.

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