

Effects of Triadimefon on Growth and Ectomycorrhizal Development of Loblolly and Slash Pines in Nurseries

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This paper reports research involving pesticides. It does not contain recommendations for their use, nor does it imply that the uses discussed here have been registered. All uses of pesticides must be registered by appropriate state and/or federal agencies before they can be recommended.

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ABSTRACT

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Three or four sprays with the systemic fungicide triadimefon (Bayleton) each at 0.56 kg a.i./ha applied in May and June to control fusiform rust significantly suppressed ectomycorrhizal development by artificially introduced *Pisolithus tinctorius* and naturally occurring fungi, by twofold to threefold on loblolly and slash pine seedlings throughout the growing season compared with seedlings sprayed (17-41 times) with the fungicide ferbam. The average indices of *P. tinctorius* in the three nurseries after triadimefon and ferbam sprays were 2 and 69, respectively. Loblolly pine seedling growth was not significantly affected by triadimefon in two of three nurseries. Basidiocarp production by ectomycorrhizal fungi in

triadimefon-treated plots occurred later in the growing season (September) and was 3-10 times less than in ferbam-treated plots. Residues of triadimefon and its metabolite triadimenol were detected in roots and tops of seedlings. These residues, especially triadimenol, were detected in one nursery up to 116 days after the last triadimefon spray in amounts sufficient to strongly inhibit growth of *P. tinctorius* and *Thelephora terrestris* in pure culture. The ED₅₀ of triadimefon on growth of *P. tinctorius* and *T. terrestris* was 0.98 and 1.66 mg/L, respectively; the ED₅₀ of triadimenol was 0.40 and 0.25 mg/L, respectively. Triadimenol residues ranging between 0.4 and 4.5 µg/g of root were detected up to 116 days after the last triadimefon spray.

Additional key words: *Pinus elliottii* var. *elliottii*, *Pinus taeda*, *Rhizopogon nigrescens*.

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, either 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 ectomycorrhizae of *P. tinctorius* improve seedling performance. Recently, Marx et al (9,13) reviewed the significance of ectomycorrhizae of *P. tinctorius* to nursery and field performance of tree seedlings. Since 1977, research has been conducted on the development of commercial sources of inocula of *P. tinctorius* for potential practical

application. In 1982, vegetative inoculum of *P. tinctorius* (MycorRhiz) produced commercially (Abbott Laboratories, Long Grove, IL) and vegetative inoculum produced by the Institute for Mycorrhizal Research and Development were compared on an operational level in 10 nurseries in the south on loblolly (*Pinus taeda* L.) and slash (*P. elliottii* var. *elliottii* Engelm.) pines. A midseason assessment of test seedlings in September showed no ectomycorrhizal development by *P. tinctorius* on seedlings in six of the 10 nurseries and less than expected in the other four, regardless of inoculum source. In previous years, inoculations in most of these nurseries resulted in abundant development of *P. tinctorius* when identical inoculum formulations were used (9).

The only cultural practice used in these 10 nurseries in 1982 different from previous years was use of the systemic fungicide triadimefon (Bayleton) instead of ferbam to control fusiform rust caused by *Cronartium quercuum* (Berk.) Miy. ex Shirai f. sp. *fusiforme* Burdsall and Snow.

During initial tests of triadimefon on southern pine seedlings, highly variable effects of this fungicide on naturally occurring ectomycorrhizae were observed. Snow et al (15) reported a significant reduction in ectomycorrhizae in midseason assessments in four nurseries after spraying seedlings four times with

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triadimefon (0.56 kg a.i./ha) but by January these differences were no longer evident, suggesting that the inhibitory effect was only temporary (15). Kelley (5) tested triadimefon at 0.28–0.56 kg a.i./ha rates on pine seedlings in nurseries and found no differences at midseason in ectomycorrhizal development, compared with nonsprayed seedlings. Rowan and Kelley (14) found that various rates (up to 1.68 kg a.i./ha) and multiple applications (up to four) of triadimefon did not significantly reduce ectomycorrhizal development on nursery seedlings assessed at lifting time in the fall. Kelley (5) reported ED₅₀ values of 3–20 mg of triadimefon per liter on mycelial growth of several ectomycorrhizal fungi in vitro. *P. tinctorius* was the most sensitive ectomycorrhizal fungus tested with an ED₅₀ of 1 mg of triadimefon per liter.

Triadimefon is highly effective in control of fusiform rust (4,5,14,15) and is cost effective compared with ferbam. However, before widespread application of the ectomycorrhizal fungus technology can be implemented, the effects of triadimefon on ectomycorrhizal development and seedling growth in nurseries must be understood. The objectives of our study were to determine the effects of triadimefon on development of ectomycorrhizae from vegetative inoculum of *P. tinctorius* applied before sowing or from naturally occurring ectomycorrhizal fungi, the effectiveness of midsummer application of basidiospore pellets of *P. tinctorius* (7) applied after completion of triadimefon spraying, the effects of benomyl on effectiveness of inoculum of *P. tinctorius* (12), and the effects of triadimefon on growth of loblolly and slash pine seedlings.

MATERIALS AND METHODS

An experiment was installed identically in April 1983 in three production forest tree nurseries. Unless otherwise stated, the following materials and procedures were used in each.

Inoculum production. *P. tinctorius* (isolate 288) was grown and processed by standardized procedures (11). Three different batches of vegetative inoculum prepared at the Institute for Mycorrhizal Research and Development were processed in early April 1983, mixed in uniform proportions, sampled for characterization, and divided into equal volumes for each of the three nursery locations. Characteristics of the vegetative inoculum were bulk density of 256 g/L, moisture content of 27.8%, pH of 4.9, and residual glucose content of 1.7 mg/g. Glucose analyses and all soil analyses were done by A&L Agricultural Laboratories, Inc., Memphis, TN. Inoculum was stored at 5 C for 3–9 days then transported to nurseries in sturdy ice chests containing artificial ice packs for cooling. Spore pellets were prepared with basidiospores of *P. tinctorius* (6) and stored in plastic vials at 5 C for 18–25 days before use.

Soil management and assay. Soil fumigation with methyl bromide, a prerequisite for effective development of *P. tinctorius* inoculum in bare-root nurseries (9), was done in the spring. Soil samples, a composite of five to seven subsamples from 0- to 15-cm depth from five locations in each test bed, were collected before and after soil fumigation and assayed (9) for pythiaceous fungi and nematodes. Comparisons of populations of these organisms before and after fumigation are good indicators of soil fumigation effectiveness (9). In these test nurseries, soil fumigation was effective since nematode spp. and *Pythium* spp. present in pre-fumigation soil samples were absent in all post-fumigation soil samples. Portions of post-fumigation soil samples also were air-dried for chemical and physical analyses.

Plot layout, design, and maintenance. After soil fumigation and a 5- to 7-day aeration with daily tillage, the soil was shaped into 1.22-m wide nursery beds. Five adjacent nursery sections, each containing six or nine beds, were used. Three adjacent beds per section were randomly selected for triadimefon 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. The first and third beds were used as buffers. All sprays were applied by a tractor-drawn hydraulic sprayer that straddled the test bed and sprayed three beds simultaneously. Eight 3.05-m-long plots, separated from each other by a 1.52-m buffer

strip and beginning 6.1 m from the end of each nursery bed, were laid out in each test bed.

The following eight treatments were randomly placed in plots in each of the five beds for triadimefon and five beds for ferbam sprays: vegetative inoculum only, vegetative inoculum plus 2.2 kg a.i. of benomyl per hectare applied as a drench at sowing and 3 and 6 wk after sowing, vegetative inoculum plus application of basidiospore pellets 3 wk after the last triadimefon spray, vegetative inoculum plus benomyl plus basidiospore pellets, no vegetative inoculum but soil disrupted with machine inoculator to standardize soil disturbance, no vegetative inoculum plus benomyl, no vegetative inoculum plus basidiospore pellets, no vegetative inoculum plus benomyl plus basidiospore pellets. Vegetative inoculum was machine injected (2) or applied by hand in furrows 2-cm deep in each of eight seed drill rows per bed at the rate of 1 L/2.29 linear meter of bed (2.79 m²). Basidiospore pellets (13 g per plot) were broadcast evenly by hand on each plot immediately before irrigation (9 mm) of all plots.

The experimental design was a split-plot with five blocks each of triadimefon- and ferbam-sprayed beds each containing the eight treatments. There were 80 plots per nursery.

After application of the vegetative inoculum, all beds were machine seeded. Seeds in the buffer strips between plots were removed by hand to maintain a 1.5-m seedling-free area for a buffer strip between plots to reduce chances for contamination between plots.

Triadimefon and triadimenol residue analyses. Two to 3 days after the last triadimefon spray and at scheduled dates thereafter, 12–15 seedlings were removed from each of 8–10 random locations in the buffer nursery bed next to irrigation risers and adjoining each of the 10 test beds. Seedlings were briefly rinsed in water, cut into tops and roots, transported to the laboratory in plastic bags in ice chests with artificial ice packs, and frozen within 72 hr of collection. The techniques of Specht (17) and Thornton (18) were used to quantitatively assay triadimefon and its metabolite triadimenol (Baytan) in the seedling tops and roots. Residue analyses were done by P. B. Bush, University of Georgia, Athens.

Periodic seedling sampling and assessment. Ten randomly selected seedlings from each of the 80 test plots per nursery were carefully removed from the beds, rinsed in water, wrapped in wet paper towels, placed in plastic bags, and transported in ice chests with artificial ice packs to the laboratory. Seedlings were sampled in mid-July, August, September, and November and assessed within 3 days of sampling. Each seedling was measured for height, root-collar diameter, number of first-order lateral roots (July sample only), and top and root fresh weights and was examined for fusiform rust galls. Ectomycorrhizae were visually assessed at ×5 magnification (8). Data on ectomycorrhizal development by *P. tinctorius* were transformed to a Pt index described by Marx et al (9).

Plots also were examined every 2 wk from mid-August until the end of each study for basidiocarps of *P. tinctorius* and other ectomycorrhizal fungi. Basidiocarps of *P. tinctorius* were recorded and removed to reduce chances of cross-contamination of control plots.

Final seedling assessments and analyses. The studies were terminated after seedling dormancy. After undercutting all beds to a 20-cm depth, three random subplots of seedlings, each 0.31 × 1.22-m wide, were removed by hand from each plot. Each subplot contained between 108 and 132 seedlings. Seedlings were counted, graded for size, and examined for rust galls. Seedlings less than 15 cm tall and having root-collar diameters of less than 2 mm or having forked tops and/or rust galls were considered culls and thus nonplantable. Twenty plantable-size seedlings per subplot were measured and visually assessed for ectomycorrhizal development and Pt indices were computed. Data obtained from each of the three subplots were averaged to represent the plot mean.

Dose-response tests in the laboratory. Solubility of triadimefon and triadimenol is 70 mg/L and 120 mg/L, respectively, in water at 20 C (Mobay Chemical Corporation, Kansas City, MO). In preparation of stock solutions for subsequent testing, 5 g of technical grade triadimefon or triadimenol was added to 600 ml of

deionized water and stirred thoroughly. The solutions were allowed to saturate at 20 C for 12 hr, then filtered through Whatman no. 42 filter paper to remove suspended particles.

Modified Melin-Norkrans (MMN) liquid medium was prepared (11) at 1.17 strength and volumes of 300 ml were placed in 500-ml Erlenmeyer flasks. Deionized water was added to the 1.17 × MMN medium in volumes needed to eventually have 1 × strength MMN and the desired test concentrations of triadimefon and/or triadimenol. The MMN media were autoclaved at 121 C and cooled. Required amounts of fungicides from the stock solutions were added aseptically to flasks of MMN medium by use of a syringe-filter (0.22 μ) unit. MMN medium, now at 1 × strength and containing the desired test concentrations of the fungicide, was aseptically dispensed by syringe in 50-ml volumes into sterile 125-ml Erlenmeyer flasks. Separate syringes were used for each fungicide concentration.

P. tinctorius (isolate 288) and *T. terrestris* (isolate 223) were grown on MMN agar medium for 4 wk at 25 C. Disks (5 mm in diameter) were removed from the outermost periphery of the mycelial colony, and a disk was placed in each of six flasks per fungus per test solution. Flasks were incubated at 25 C for the time required for each fungus to grow in fungicide-free MMN medium to cover the surface of the liquid medium. This was 30 days for *P. tinctorius* and 21 days for *T. terrestris*. Mycelial mats were collected on tared filter paper, dried at 70 C for 24 hr, and weighed. Weights were transformed to percentage of growth on the fungicide-free medium (control).

Data analysis. All data were subjected to analysis of variance,

and significant differences among means were identified with Duncan's new multiple range test at $P = 0.05$. Details specific to each nursery follow.

Buckeye Cellulose Corporation Nursery, Perry, FL. Previous cropping history of the test area was similar to that described earlier (9). Soil fumigation was done on 5 April 1983. On 14 April, vegetative inoculum of *P. tinctorius* was applied, untreated slash pine seeds (medium size from Buckeye Cellulose Corporation seed orchard) were sowed, and seedbeds were mulched with 1,120 kg/ha of commercial hydromulch. The status of soil fertility at study installation was similar in all beds. Total N, available P (Bray II), exchangeable K, Ca, and Mg, were 350, 56, 16, 165, and 65 μg/g, respectively. Organic matter was 1.45% and pH was 5.1. The soil type was a loamy sand and contained 88:5:7 sand, silt, and clay, respectively. Ferbam at 2.2 kg a.i./ha was sprayed 41 times at 1- to 5-day intervals from 28 April to 5 July. Triadimefon at 0.56 kg a.i./ha was sprayed on 28 April, 23 May, and 21 June. Ammonium nitrate at 56 kg/ha and KCl at 28 kg/ha were applied to all beds on 17 May, 7 July, and 14 July. Fertilizer (10-10-10) at 112 kg/ha was applied on 8 June. Seedling tops were mowed to a height of 20 cm in July and August. This study was terminated in December 1983.

Taylor Nursery, Trenton, SC. Previous cropping history in the test area was similar to that previously described (10). Soil fumigation was done on 5 April 1983. On 20 April, vegetative inoculum of *P. tinctorius* was applied by hand, thiram and latex sticker-treated seeds of loblolly pine (SC Piedmont improved seed orchard source) were sowed and seedbeds were mulched with a 1-cm layer of nonfumigated pine straw. The status of soil fertility at study installation was similar in all beds. Total N, available P (Bray II), exchangeable K, Ca, and Mg were 300, 68, 61, 164, and 66 μg/g, respectively. Organic matter was 1.21% and pH was 5.8. The soil type was a loamy sand containing 88:6:6 sand, silt, and clay, respectively. Ferbam at 2.2 kg a.i./ha was sprayed 17 times at 1- to 7-day intervals from 11 May to 4 July. Triadimefon at 0.56 kg a.i./ha was sprayed on 18 May, 1 June, and 28 June. Ammonium nitrate at 106 kg/ha was applied to all beds on 6 and 20 June, 11 July, and 8 August. KCl at 112 kg/ha was applied on 25 July. Oxyfluorfen (Goal) herbicide was applied monthly from late April to August at 2.5 L/ha. Seedling tops were mowed to a height of 20 cm in July and August. This study was terminated in December 1983.

International Paper Company Nursery, Bluff City, AR. Previous cropping history in the test area was similar to that at the Taylor Nursery. On 5 April 1983 the soil was fumigated. On 21 April, vegetative inoculum of *P. tinctorius* was applied by hand, thiram and latex sticker-treated seeds of loblolly pine (International Paper Company seed orchard) were sowed and covered with 1,200 kg/ha of commercial hydromulch. Status of soil fertility at study installation was similar in all beds. Total N, available P (Bray II), exchangeable K, Ca, and Mg were 240, 34, 82, 194, and 68 g/g, respectively. Organic matter was 0.85% and pH was 6.1. The soil type was a loamy sand containing 86:8:6 sand, silt, and clay, respectively. Ferbam at 1.84 kg a.i./ha was sprayed 17 times at 1- to 7-day intervals from 7 May to 24 June. Triadimefon at 0.56 kg a.i./ha was sprayed on 12 and 26 May and 9 and 16 June. Ammonium nitrate at 56 kg/ha was applied on 7 June, (NH₄)₂SO₄ at 112 kg/ha was applied on 7 and 19 July, and KCl at 112 kg/ha was applied on 24 August to all plots. Bifenox and oxyfluorfen herbicides were applied at recommended rates. Seedling tops were mowed to a height of 20 cm in July and August. This study was terminated in January 1984.

Statistical analyses of data obtained periodically from seedlings in all nurseries showed that none of the treatments with benomyl or the basidiospore pellets significantly affected either seedling growth or ectomycorrhizal development. The pellets contained too much moisture during storage since they were covered by mold fungi (*Penicillium* spp. and *Aspergillus* spp.) at application time. This contamination may have been responsible for inoculum failure. Benomyl drenches did not stimulate ectomycorrhizal development in these nurseries as reported earlier from a microplot study (12). Consequently, only data from the remaining four

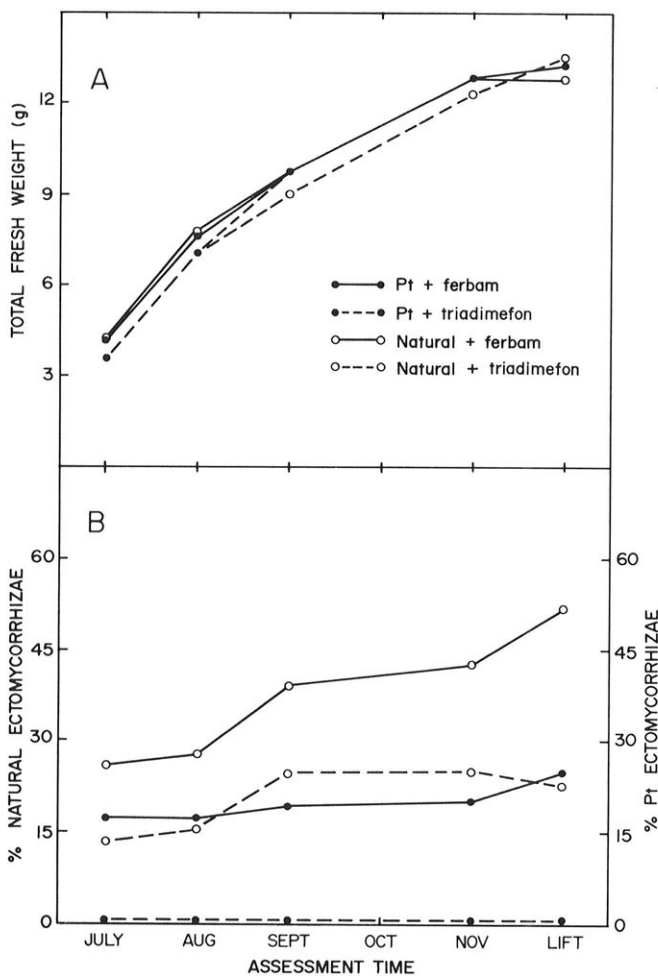


Fig. 1. A, Total fresh weights and **B,** development of *Pisolithus tinctorius* (Pt) and natural ectomycorrhizae on short roots of slash pine seedlings at different times during the growing season in plots sprayed with triadimefon or ferbam fungicides to control fusiform rust at the Buckeye Cellulose Corporation Nursery, Perry, FL.

nursery treatments, i.e., vegetative inoculum of *P. tinctorius* or no inoculum with triadimefon or ferbam sprays, are presented.

RESULTS

Buckeye Cellulose Corporation Nursery, FL. Seedling measurements obtained throughout the growing season were not significantly affected by any treatment (Fig. 1A). Also, at lifting date (December 1983), seedlings were not different in size (Table 1). However, both ectomycorrhizal development and basidiocarp production were significantly suppressed by triadimefon. Ectomycorrhizal development by *P. tinctorius* on triadimefon-sprayed seedlings did not exceed 1% (Fig. 1B, Table 1) at any time during the growing season and very few basidiocarps of *P. tinctorius* were produced in these plots (Table 1). Natural ectomycorrhizal development on triadimefon-sprayed seedlings averaged only 13–16% through August, increased to 25% in September, and did not change by lifting date. Basidiocarps of *T. terrestris* or *Rhizopogon nigrescens* sp. nov., the two main species of naturally occurring ectomycorrhizal fungi in this nursery, were first observed in early September, nearly 3 mo after the last triadimefon spray. Over 80% of these basidiocarps were produced in November and December.

Ectomycorrhizal development by *P. tinctorius* on ferbam-sprayed seedlings averaged approximately 16% from July through November. At lifting date, ectomycorrhizal development averaged 25% and the average Pt index was 54 (Fig. 1B, Table 1). The first basidiocarps of *P. tinctorius* were observed in late July in the ferbam-treated plots and increased monthly through November. More than 20 times more basidiocarps of this fungus were produced in ferbam-sprayed than in triadimefon-sprayed plots. Natural ectomycorrhizal development on ferbam-sprayed seedlings increased from an average of 27% in July to more than 50% at lifting date (Fig. 1B, Table 1). Basidiocarps of *T. terrestris* and *R. nigrescens* were first observed in early July in ferbam-

sprayed plots and were produced through November. More basidiocarps were produced in August than in any other month. These two fungi produced more than three times more basidiocarps in ferbam-sprayed than in triadimefon-sprayed plots (Table 1).

Total ectomycorrhizal development by *P. tinctorius* and naturally occurring fungi was the same on ferbam-sprayed seedlings but was more than twice the amount on seedlings sprayed with triadimefon.

Residue analyses showed that triadimefon was rapidly transformed to triadimenol in seedling tops and/or roots within 3 days after the last triadimefon spray (Table 2). Triadimenol accounted for 78 and 87% of the residue in seedling tops and roots, respectively, 3 days after the last triadimefon spray; it accounted for 100% of the residue in both tops and roots thereafter. Forty-two percent of the triadimenol detected 3 days after the last spray was still present in roots 64 days after the last spray. The significance of the earlier triadimefon sprays on residue concentrations in the seedlings cannot be determined since samples were only collected after the last spray.

Taylor Nursery, SC. Seedling size was significantly affected by the fungicides as early as July (Fig. 2A). The triadimefon-sprayed seedlings were significantly smaller in all growth measurements throughout the growing season than those sprayed with ferbam. At lifting date, seedlings sprayed with triadimefon in both ectomycorrhizal treatments had an average of 23% less total fresh weights than those sprayed with ferbam (Table 1). Ectomycorrhizal development by *P. tinctorius* significantly increased seedling top and root weights in the ferbam treatment and top weights in the triadimefon treatment. There were also significantly fewer cull seedlings in the plots of *P. tinctorius* sprayed with ferbam than in the triadimefon plots. Seedlings with ectomycorrhizae of *P. tinctorius* sprayed with triadimefon had an average Pt index of 4 at lifting date, whereas those sprayed with ferbam had an average Pt index of 83 (Fig. 2B). Development of naturally occurring

TABLE 1. Measurements of pine seedlings at lifting time with ectomycorrhizae formed by *Pisolithus tinctorius* from vegetative inoculum or formed by naturally occurring fungi after spraying with triadimefon or ferbam in three southern nurseries^x

Nursery treatments	Height (cm)	Root-collar diameter (cm)	Fresh wt (g)		Percent of short roots ectomycorrhizal with		Percent of seedlings with Pt	Pt index ^y	Cull seedlings (%)	(Total no. basidiocarps/m ²) ^z		
			Top	Root	Pt	All fungi				Pt	Tt	Rn
Buckeye Cellulose Corporation Nursery, FL; slash pine												
Triadimefon + vegetative inoc.	26.4 a	0.38 a	9.6 a	2.3 a	<1 b	20 b	4 b	<1 b	8.8 a	<1 b	2 b	<1 b
Ferbam + vegetative inoc.	28.1 a	0.43 a	10.8 a	3.1 a	25 a	47 a	94 a	54 a	11.4 a	19 a	6 a	2 a
Triadimefon + no inoculum	26.3 a	0.41 a	9.5 a	2.5 a	0	24 b	0	0	7.9 a	0	4 b	<1 b
Ferbam + no inoculum	27.5 a	0.41 a	10.7 a	3.0 a	0	52 a	0	0	12.4 a	0	7 a	4 a
Taylor Nursery, SC; loblolly pine												
Triadimefon + vegetative inoc.	17.7 b	0.37 bc	6.2 b	3.7 bc	6 b	32 b	18 b	4 b	30.5 ab	<1 b	2 c	0
Ferbam + vegetative inoc.	20.8 a	0.44 a	8.5 a	4.5 a	61 a	72 a	97 a	83 a	22.5 b	4 a	7 b	0
Triadimefon + no inoculum	17.2 b	0.35 c	5.5 c	3.0 c	0	24 b	0	0	37.5 a	0	2 c	0
Ferbam + no inoculum	20.1 a	0.40 b	6.9 b	3.9 b	3 b	63 a	9 b	<1 b	24.3 b	<1 b	21 a	0
International Paper Company Nursery, AR; loblolly pine												
Triadimefon + vegetative inoc.	26.0 a	0.36 a	9.0 b	2.0 c	<1 b	16 c	<1 b	1 b	8.9 a	2 b	4 b	0
Ferbam + vegetative inoc.	26.8 a	0.38 a	10.1 a	2.4 a	43 a	56 a	71 a	81 a	7.9 a	11 a	6 b	0
Triadimefon + no inoculum	26.0 a	0.36 a	9.3 b	2.1 bc	<1 b	18 c	<1 b	1 b	7.8 a	0	7 b	0
Ferbam + no inoculum	26.7 a	0.38 a	9.7 ab	2.2 b	<1 b	44 b	<1 b	1 b	8.0 a	0	27 a	0

^x Means in a column in a nursery followed by a common letter are not significantly different at $P = 0.05$ according to Duncan's multiple range test.

^y Pt index = $A \times (B/C)$ where A = % of seedlings with ectomycorrhizae formed by *P. tinctorius*, B = \bar{X} % of feeder roots with ectomycorrhizae formed by *P. tinctorius* (including 0% for those without *P. tinctorius*), and C = \bar{X} % of feeder roots with ectomycorrhizae formed by *P. tinctorius* and other fungi (9).

^z Pt = *Pisolithus tinctorius*, Tt = *Thelephora terrestris*, Rn = *Rhizopogon nigrescens*.

ectomycorrhizae on control seedlings sprayed with triadimefon did not exceed 25% throughout the growing season. Basidiocarp production by *T. terrestris*, the main naturally occurring ectomycorrhizal fungus in this nursery, was first observed in late August in triadimefon-sprayed plots. Control seedlings sprayed with ferbam had an average of more than 35% natural ectomycorrhizal development by August and more than 60% at lifting date. Basidiocarps of *P. tinctorius* were first observed in plots sprayed with ferbam in early August. Basidiocarps of *T. terrestris* first appeared in ferbam-sprayed plots in late July. By seedling lifting date (December 1983), more than seven times more basidiocarps of *P. tinctorius* and *T. terrestris* were produced in ferbam-sprayed plots than in triadimefon-sprayed plots.

The seedling residue analyses showed that within 3 days after the last spray the majority of the residues in the seedling tops (80%) and roots (71%) was triadimenol (Table 2). This percentage of triadimenol gradually increased with time until no triadimefon was detected 116 days after final spraying. Residues of both chemicals decreased with time at about 2.5 $\mu\text{g/g}$ per month. As mentioned earlier, the contribution of earlier triadimefon sprays to these concentrations cannot be determined.

International Paper Company Nursery, AR. Seedling size was not significantly affected by triadimefon treatment at any time during the growing season. Ectomycorrhizal development by *P. tinctorius* significantly increased total seedling fresh weights over the controls in ferbam-sprayed plots, but not in triadimefon plots, from August until the end of the study (Fig. 3A, Table 1). Seedling cull percentages were not affected by any of the treatments. Ectomycorrhizal development by *P. tinctorius* (Fig. 3B) and

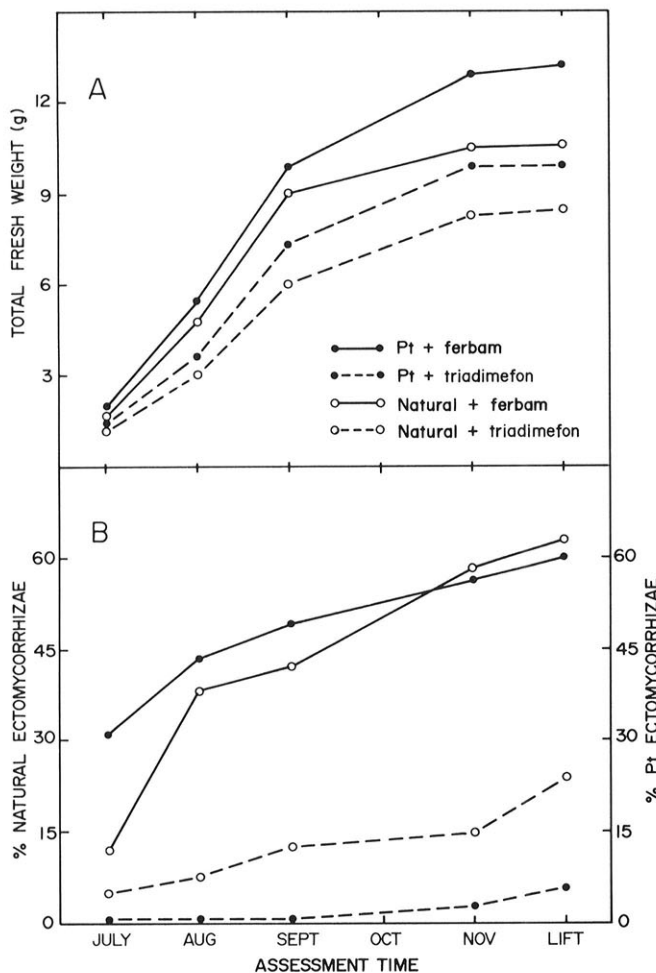


Fig. 2. A, Total fresh weights and B, development of *Pisolithus tinctorius* (Pt) and natural ectomycorrhizae on short roots of loblolly pine seedlings at different times during the growing season in plots sprayed with triadimefon or ferbam fungicides to control fusiform rust at the Taylor Nursery, Trenton, SC.

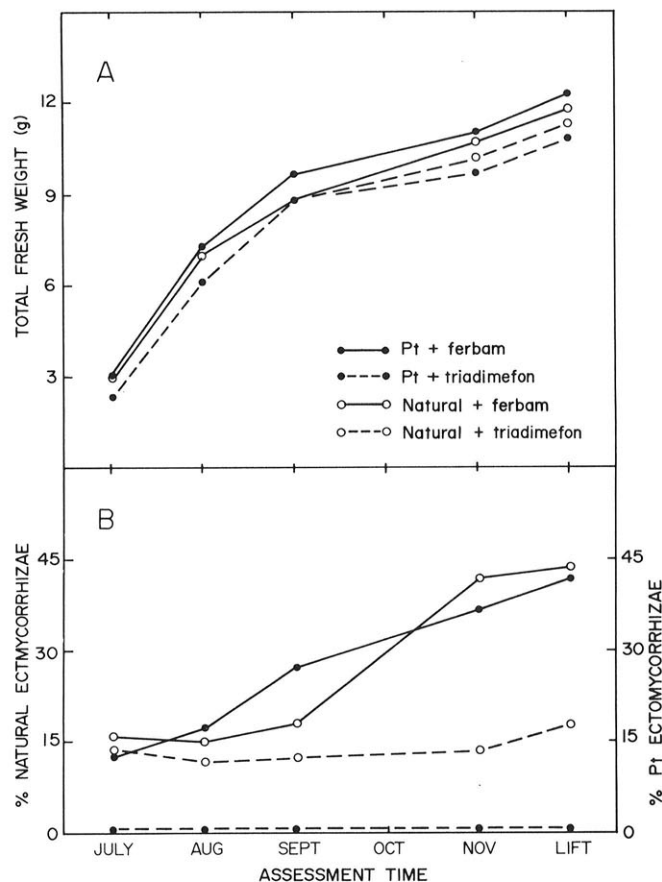


Fig. 3. A, Total fresh weights and B, development of *Pisolithus tinctorius* (Pt) and natural ectomycorrhizae on short roots of loblolly pine seedlings at different times during the growing season in plots sprayed with triadimefon or ferbam fungicides to control fusiform rust at the International Paper Company Nursery, Bluff City, AR.

TABLE 2. Concentrations ($\mu\text{g/g}$) of triadimefon and triadimenol in tops and roots of loblolly and slash pine seedlings at different times after spraying with triadimefon in three southern nurseries

Nursery and species	Days since last spray	Tops		Roots		Triadimenol (%)	
		Triadimefon	Triadimenol	Triadimefon	Triadimenol	Tops	Roots
Buckeye Cellulose, FL slash pine	3	1.8	6.3	0.7	4.5	78	87
	64	0.0	1.0	0.0	1.9	100	100
Taylor, SC loblolly pine	3	2.1	8.7	1.8	4.3	80	71
	22	1.8	6.5	0.6	1.9	78	76
	59	0.4	5.1	0.1	2.3	93	96
	116	0.0	2.5	0.0	0.4	100	100
International Paper, AR loblolly pine	2	2.7	2.0	0.9	4.2	43	82
	64	0.2	0.8	0.1	0.4	80	80

basidiocarp production (Table 1) were significantly suppressed in the triadimefon-treated plots as compared to ferbam. The Pt index for these seedlings averaged less than 1 at lifting date, and only two basidiocarps per square meter of soil surface were produced on these plots throughout the growing season. Natural ectomycorrhizal development on seedlings in the triadimefon-sprayed plots averaged between 12 and 18% throughout the growing season. Basidiocarp production by *T. terrestris* in these plots was eight per square meter of soil surface throughout the growing season, and most were produced between November and January. Ectomycorrhizal development by *P. tinctorius* on seedlings in the ferbam plots progressed steadily from an average of 13% in July to more than 40% in November. The Pt index at lifting date in these plots was 71 (Fig. 3B, Table 1). Basidiocarp production by *P. tinctorius* was more than five times greater in these plots than in triadimefon-sprayed plots. Natural ectomycorrhizal development on seedlings in ferbam-sprayed plots was about 14–18% from July through September and then it more than doubled to an average of more than 40% by November. Basidiocarp production by *T. terrestris*, the main naturally occurring ectomycorrhizal fungus in this nursery, was nearly four times greater in ferbam-sprayed than in triadimefon-sprayed plots. Most of these basidiocarps were produced by early November.

Triadimenol represented 43% of the fungicide residue in the seedling tops ($2.0 \mu\text{g/g}$) and 82% in the roots ($4.2 \mu\text{g/g}$) in seedling samples collected 2 days after the last spray (Table 2). Sixty-four days after the last triadimefon spray, the total fungicide residue in tops decreased nearly fivefold and in roots it decreased over tenfold from that present in seedling tops and roots 2 days after the last spray. Triadimenol represented 80% of the residues in tops ($0.8 \mu\text{g/g}$) and roots ($0.4 \mu\text{g/g}$) at this time; the remaining residue was

triadimefon.

Seedlings in the ferbam-sprayed treatment plots in all nurseries contained trace amounts ($0.01\text{--}0.03 \mu\text{g/g}$) of either triadimefon or triadimenol in tops and roots. These residues apparently originated from either triadimefon spray drift or from residual spray remaining in the sprayer. However, these low concentrations are considered insignificant and are not discussed.

Dose-response tests in the laboratory. Triadimefon and triadimenol inhibited mycelial growth of *P. tinctorius* and *T. terrestris* (Fig. 4). The effective dose at which 50% mycelial growth loss occurs (ED_{50}) was estimated using least squares techniques to model dose and log-dose probit relationships (1). Linear dose-probit models yielded satisfactory results with r^2 of 0.94, 0.92, 0.88, and 0.91 for *P. tinctorius* in triadimenol and triadimefon and for *T. terrestris* in triadimenol and triadimefon, respectively. The ED_{50} of triadimefon on growth of *P. tinctorius* and *T. terrestris* was 0.98 and 1.66 mg/L, respectively. The ED_{50} of triadimenol on growth of *P. tinctorius* and *T. terrestris* was 0.40 and 0.25 mg/L, respectively.

In mixed solutions of triadimefon and triadimenol, *P. tinctorius* was inhibited by at least 50% at all concentrations of triadimenol (0.1–0.5 mg/L) in mixture with 0.3 and 0.5 mg/L of triadimefon (Fig. 5). *T. terrestris* tolerated the test concentrations of triadimefon (0.1–0.5 mg/L) in mixture with 0.1 mg/L of triadimenol. At higher triadimenol concentrations with triadimefon, growth of *T. terrestris* was reduced but to a lesser extent than growth of *P. tinctorius*.

DISCUSSION

Three or four sprays of triadimefon each at 0.56 kg a.i./ha significantly suppressed ectomycorrhizal development either from

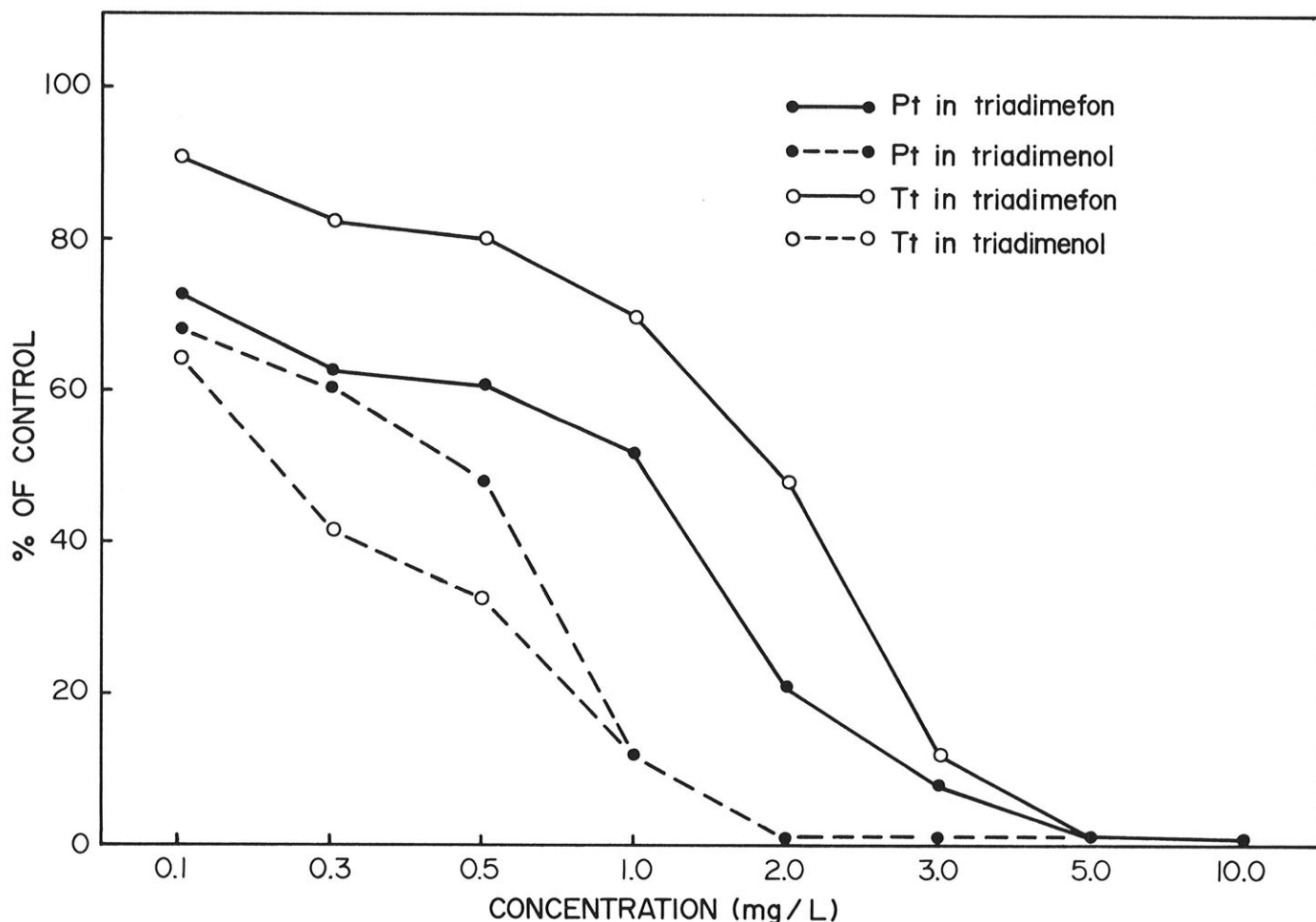


Fig. 4. Mycelial growth (dry weight, mg) of *Pisolithus tinctorius* (Pt) and *Thelephora terrestris* (Tt) in different concentrations of triadimefon or triadimenol expressed as percentage of growth of each fungus in control liquid medium.

artificially introduced vegetative inoculum of *P. tinctorius* or from naturally occurring fungi throughout the growing season in all nurseries. Correlated with this suppression was a marked decrease in basidiocarp production by the various ectomycorrhizal fungi. In one nursery, this reduced ectomycorrhizal development was also associated with a significant decrease in growth of loblolly pine seedlings. Also, associated with the suppression in ectomycorrhizae was the presence of residues of triadimefon and triadimenol fungicides in tops and roots of the seedlings. These residues, especially triadimenol, persisted in roots for nearly 4 mo (116 days) after the last triadimefon spray. Laboratory studies showed that both *P. tinctorius* and *T. terrestris* are significantly inhibited (ED_{50}) in mycelial growth by triadimenol concentrations as low as 0.40–0.25 mg/L and triadimefon concentrations of 0.98–1.66 mg/L. The observed effects of these two chemicals in various mixture ratios and concentrations in the laboratory suggest additive, rather than synergistic, effects on growth of these fungi. Concentrations of these fungicides that decreased growth of the ectomycorrhizal fungi in the laboratory were detected in equal or considerably higher concentrations in seedling roots from 2 to as long as 116 days after the last triadimefon spray in one nursery. Biologically significant concentrations of these fungicide residues, again primarily triadimenol, persisted in seedling roots for more than 2 mo (64 days) after the last triadimefon spray in the two other nurseries. Even though residues of these chemicals were determined in seedling tissues only after the last (third or fourth) spray, undoubtedly these chemicals also were present in significant concentrations in roots after sprays applied weeks earlier. At all sampling dates (2–116 days) after the last triadimefon spray, triadimenol residues in roots (0.4–4.5 $\mu\text{g/L}$) were higher than the concentrations that significantly inhibited mycelial growth of both

ectomycorrhizal fungi in the laboratory. Because triadimefon is reportedly absorbed and translocated systemically in the young growing tissues and is not readily translocated in older, woody tissues (3), its concentration and that of triadimenol detected in entire root systems (woody and primary tissues) in this study should be considered a conservative estimate of their actual concentration in younger short roots, the site of potential ectomycorrhizal development.

The twofold to threefold reduction of ectomycorrhizal development by naturally occurring fungi for much of the remaining growing season after triadimefon spray applications suggests that residues persisted in roots at high enough concentrations to depress root colonization by these fungi. This depression in ectomycorrhizal development also resulted in a general depression of basidiocarp production throughout the nursery. This decreased the amount of spore inoculum available later in the growing season for soil infestation and for new centers of ectomycorrhizal development.

The inhibitory effect of the triadimefon sprays on ectomycorrhizal development by *P. tinctorius* was dramatic. The average Pt index in all three nurseries after ferbam sprays was 69 and after triadimefon sprays was less than 2. Basidiocarp production by *P. tinctorius* was also significantly reduced tenfold. These results help to explain the problems encountered in the 1982 tests with MycoRhiz in 10 southern nurseries that used triadimefon to control fusiform rust (D. H. Marx, *unpublished*). The observed absence of late-season development of ectomycorrhizae of *P. tinctorius* can be explained on the basis of inoculum survival. Vegetative inoculum of *P. tinctorius* usually will not survive in fumigated soil beyond 6–8 wk in the absence of susceptible feeder roots with sufficient inoculum potential to compete with native

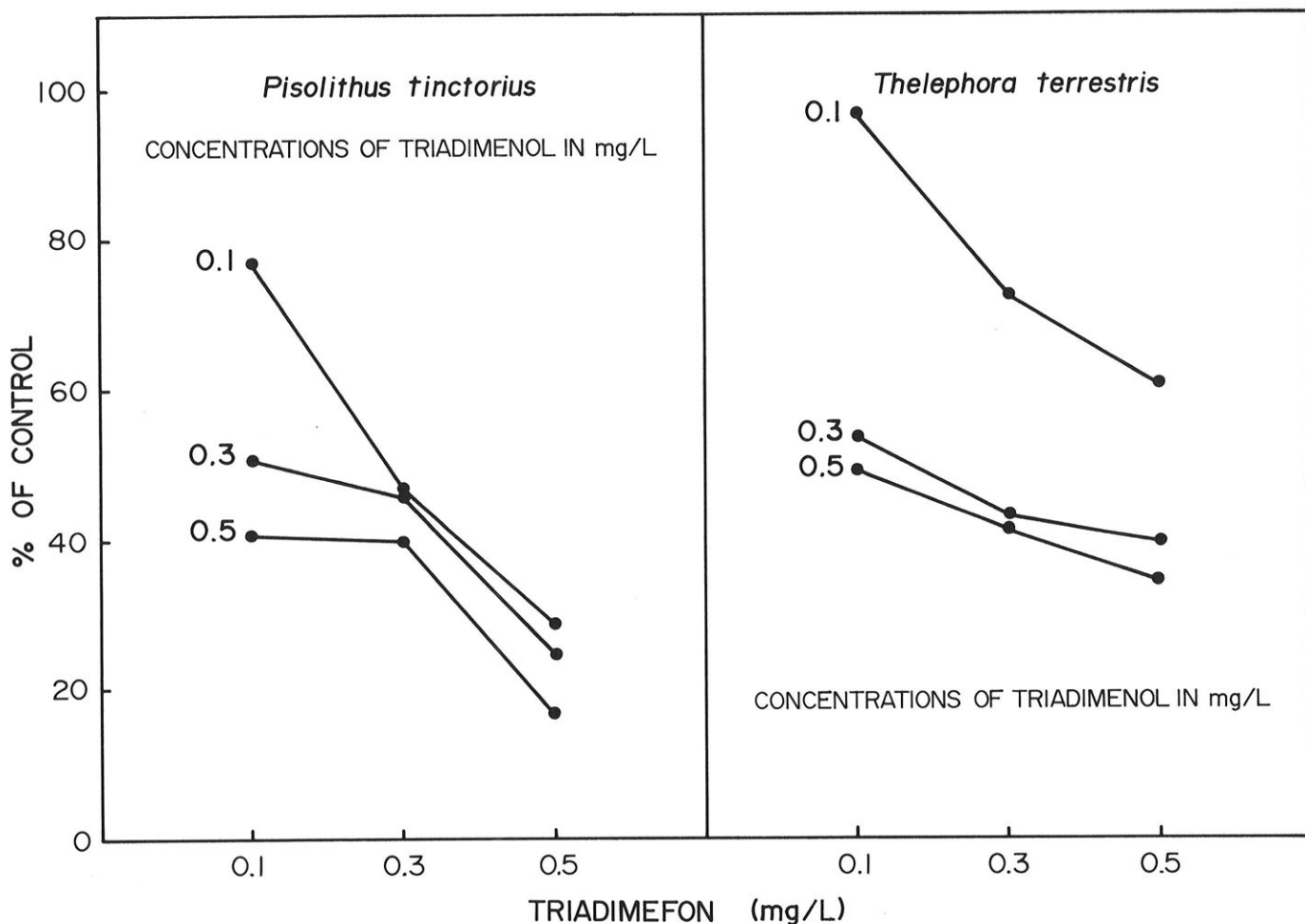


Fig. 5. Mycelial growth (dry weight, mg) of *Pisolithus tinctorius* (Pt) and *Thelephora terrestris* (Tt) in mixed solutions of triadimefon and triadimenol expressed as percentage of growth of each fungus in control liquid medium.

ectomycorrhizal fungi for feeder roots (D. H. Marx, *unpublished*).

Information is not available on the cumulative effects of triadimefon and triadimenol on pine seedling physiology other than that presented here. However, since the absorption, retention, and degradation of triadimefon and triadimenol occur for several weeks after the spray season, from a biological standpoint, the timing of sprays should fit the rust history of the nursery and not a fixed date for application. Perhaps lower rates and fewer sprays coupled with seed treatments with triadimefon (6) could effectively control fusiform rust without significantly affecting ectomycorrhizal development, especially that formed early in the growing season from artificially introduced inoculum.

The results of the studies reported here are somewhat in disagreement with earlier reports. It is not unusual for results from greenhouse studies (4,16) to differ from nursery tests because of different patterns of colonization of soil by naturally occurring ectomycorrhizal fungi in the two environments. The differences found among the various nursery studies may be explained, at least partially, by the timing of soil fumigation in the nurseries. In the present study, soil in all nurseries was fumigated just before inoculum introduction and seeding and therefore contained little inoculum of resident ectomycorrhizal fungi. In earlier nursery studies (14,15), many tests were installed in nursery soil that had never been fumigated or had not been fumigated for 1–2 yr before study installation. These soils would normally contain high inoculum potential of ectomycorrhizal fungi from earlier pine crops and from natural colonization. This high inoculum potential could be responsible for greater ectomycorrhizal development throughout the growing season than that reported in the present study. 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 triadimefon in the South Carolina nursery test but not 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 on seedlings (perhaps from inoculum present in the nonfumigated pine straw mulch) in ferbam-sprayed plots throughout the growing season, were grown in soil with initially higher N levels 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 triadimefon.

Research on the systemic action of triadimefon and triadimenol on various agricultural plants indicates that these fungal sterol-inhibiting triazoles are readily taken up by plant roots and translocated to transpiring leaves. With foliar application, however, these chemicals reportedly remain in the treated leaves. They are not usually redistributed in the plant after retention in leaves but may move to the leaf margins in dicotyledons or leaf tips in monocotyledons (3). The presence of triadimefon and triadimenol in roots of pine seedlings in this study suggests that these chemicals were either translocated downward from the needles after foliar sprays or were leached by water into the root zone and absorbed by roots. A considerable amount of triadimefon is sprayed on the soil surface during its application on small pine seedlings. If the latter occurs, then inoculum of the ectomycorrhizal fungi, both *P. tinctorius* and resident nursery fungi, may be directly suppressed by these fungicides in the soil. The main consequence of depressed ectomycorrhizal development on pine seedlings in the nursery is that the seedlings usually perform poorly in the field, especially on stressed sites and during drought years (10).

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