Forest Sci., Vol. 28, No. 2, 1982, pp. 232–236

Effect of Triadimefon (Bayleton) on Ectomycorrhizae of Loblolly and Slash Pines in Alabama

W. D. Kelley

ABSTRACT. Effect of the systemic fungicide triadimefon (Bayleton®) on growth and development of selected ectomycorrhizal fungi of loblolly and slash pines was determined in laboratory and field studies. In the laboratory triadimefon exhibited activity against all of the test fungi on culture media; however, the concentrations of triadimefon necessary to restrict colony areas by 50 percent or more generally were higher than would be expected to occur in soil following recommended rates of application. A possible exception to this was the symbiont *Pisolithus tinctorius*, where a triadimefon concentration of only 1 μg/ml restricted colony area by 50 percent and no growth occurred at a concentration of 5 μg/ml. In field studies, no effect on mycorrhizal roots was observed on seedlings collected in August and September from plots that had received triadimefon treatments in May and June. Triadimefon also did not affect seedling height or the distribution of *P. tinctorius* and *Thelephora terrestris* basidiocarps among the plots. FOREST SCI. 28:232–236.

ADDITIONAL KEY WORDS. *Pisolithus tinctorius, Thelophora terrestris, Pinus taeda, Pinus elliottii,* forest tree nurseries, systemic fungicide, Bayleton®.

FOR SOUTHERN FOREST NURSERY MANAGERS, two of the most important factors in the production of southern pine seedlings for outplanting are development of mycorrhizal roots and control of fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Chirae f. sp. *fusiforme* Burdsall & Snow). The importance of mycorrhizae to growth and survival of southern pines is well documented (Berry and Marx 1978, Marx and others 1976, Marx and others 1978). Control of fusiform rust has been accomplished in forest nurseries for many years with ferbam (ferric dimethyldithiocarbamate); however, ferbam lacks systemic activity, and numerous applications (20 to 50) often are required to protect the young seedlings during the spore-flight period (Czabator 1971).

In recent years, several systemic fungicides have been tested as possible alternatives to ferbam for controlling fusiform rust (Hare 1973, Hare and Snow 1976, Kelley 1978, Kelley 1979, Mexal and Snow 1978, Snow 1978, Snow and other 1979). The most promising of these is triadimefon (Bayleton®, 1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)-2-butanone) (Kelley 1979, Mexal and Snow 1978, Snow 1978, Snow and others 1979). Although effective against fusiform rust, the effect of triadimefon on mycorrhizal fungi of southern pines is not known. The purpose of this study was to determine whether triadimefon affects these symbionts.

MATERIALS AND METHODS

Laboratory Tests.—Cultures of mycorrhizal fungi were obtained from D. H. Marx, Director, USDA Forest Service Institute for Mycorrhizal Research and Development, Athens, GA 30601. Fungi tested were: *Pisolithus tinctorius* (Pers.) Coker & Couch, *Thelephora terrestris* (Ehrh.) Fr., *Suillus luteus* (L. ex Fr.) S. F. Gray, *S. cothurnatus* Singer, *S. hirtellus* (Pk.) Kuntze, two isolates of *Laccaria laccata* (Scop. ex Fr.) Berk. & Br., and *Cenococcum graniforme* (Sow.) Ferd. & Winge. Cultures were maintained on modified Melin-Norkrans (MMN) agar medium (Marx 1969) at 26°C in darkness.

Triadimefon was tested as a wettable powder containing 50 percent active ingredients (Bayleton 50 WP). The compound was sterilized dry in a Cryotherm gas sterilizing oven (American Sterilizer Co., Model 62108) in an atmosphere of 12 percent ethylene oxide : 88

The author is an Associate Professor, Botany, Plant Pathology, and Microbiology Department, Auburn University Agricultural Experiment Station, Auburn University, AL 36849. Manuscript received 4 August 1980.

TABLE 1. Concentrations of triadimefon in modified Melin-Norkrans agar necessary to restrict colony area of various mycorrhizal fungi by 50 percent or more and to result in no growth by the fungi after 3-week incubation at 26°C.

Mycorrhizal fungus	Concentration of triadimefon (μg ai/ml) (PPM) restricting colony area by 50 percent or more	Concentration of triadimefon (μg ai/ml) (PPM) resulting in no growth by the fungus
Cenococcum graniforme	20	40
Suillus hirtellus	5	20
Suillus cothurnatus	3	10
Suillus luteus	20	40
Thelephora terrestris	5	40
Pisolithus tinctorius	1	5
Laccaria laccata	3	40
Laccaria laccata	3	20

percent dichlorodifluoro methane (w/w) at 2.1092 kg/cm² and 100°C for 4 h. Preliminary tests revealed that this procedure killed contaminating fungal spores without affecting the fungicide.

A suspension of triadimefon containing 4,000 μg active ingredient (ai)/ml was prepared in sterilized water in a 500-ml volumetric flask. This was serially diluted in 100-ml volumetric flasks to provide final concentrations of 0, 1, 3, 5, 10, 20, 40, 60, 80, and 100 μg ai/ml of medium when 10 ml of the appropriate stock was added to 390 ml of MMN agar medium. The fungicide was added to molten MMN agar (50°C) and thoroughly mixed immediately prior to pouring into petri dishes. Each dish received ca. 15 ml of the medium, and for each fungus isolate, five replicates (dishes) were prepared for each fungicide concentration and nonfungicide control. Each dish of agar was inoculated in the center with a 6-mm-diam disk of the test fungus cut with a flame-sterilized cork borer from the periphery of an actively growing colony; disks were positioned mycelium-side down on the agar surface of the test dishes. The cultures were incubated in darkness at room temperature (26° ± 2°C). The diameter of each colony was measured and recorded after 1, 2, and 3 weeks of incubation (see Table 1). All tests were repeated at least one time.

Field Tests.—Field tests were established at the Miller State Nursery near Autaugaville, Ala., and at the Hauss State Nursery near Atmore, Ala. Soil at the Miller Nursery was a Ruston sandy loam containing 67 percent sand, 13 percent silt, and 20 percent clay; soil at the Hauss Nursery was a Greenville sandy loam containing 55 percent sand, 20 percent silt, and 25 percent clay. Plots were laid out in randomized complete blocks with 8 replications per treatment; plot size was 1.2 × 9.1 m (4 × 30 ft). Treatments were: (a) untreated control; (b) triadimefon seed soak (seeds soaked for 24 h in aqueous suspension containing 800 mg ai triadimefon/liter) (Mexal and Snow 1978); (c–f) triadimefon seed soak + foliar applications of triadimefon at rates of 0.28 and 0.42 kg ai/ha (4.0 oz and 6.0 oz ai/acre) at 2- and 3-week intervals; (g–h) triadimefon seed soak + foliar applications of triadimefon at a rate of 0.56 kg ai/ha (8.0 oz ai/acre) at 3- and 4-week intervals; and (i–j) preplant, soil-incorporated triadimefon at rates of 0.56 and 1.12 kg ai/ha. Plots at the Miller State Nursery were planted with loblolly (*Pinus taeda* L.) pine seed (State loblolly seedlot) on April 17, 1979; foliar applications of triadimefon were begun on May 9 and were terminated on June 20. Plots at the Hauss State Nursery were planted with slash (*P. elliottii* Engelm.) pine seed (State slash seedlot) on May 8, 1979; foliar applications of triadimefon were begun on May 21 and were terminated on June 19. The number of applications of triadimefon at the various rates are shown in Table 2 (Miller Nursery) and Table 3 (Hauss Nursery). All foliar applications were at volume of 341 liters/ha and included 437 ml of Agridex® surfactant-oil blend per hectare volume of spray.

During August (Miller Nursery) and September (Hauss Nursery), randomly selected seedlings from each plot were lifted with a spade and transported to the laboratory for mycorrhizal evaluations. Ten seedlings from each plot were evaluated individually using the Velcro tape method described by Anderson and Cordell (1979). Data recorded included

TABLE 2. Effect of field applications of triadimefon at the Miller State Nursery on production of short feeder roots by loblolly pine seedlings, percentage of short roots with mycorrhizae, and seedling height.

Treatment	Rate	Spray interval	Applications	Short feeder roots /10 cm of lateral	Short roots with mycorrhizae	Seedling height
	kg/ha (oz/ac)		*number*	*number*	*percent*	*cm*
Control	—	—	—	50.4[x]a[y]	35.2 a	17.2 a
Seed soak (triadimefon 800 mg/l for 24 h)	—	—	—	51.5 a	32.1 a	13.7 a
Triadimefon foliar spray + seed soak	0.28 (4 oz)	2-week	3	47.7 a	32.0 a	15.8 a
Triadimefon foliar spray + seed soak	0.28	3-week	2	51.9 a	35.2 a	15.7 a
Triadimefon foliar spray + seed soak	0.42 (6 oz)	2-week	3	50.0 a	28.8 a	16.2 a
Triadimefon foliar spray + seed soak	0.42	3-week	2	51.8 a	35.4 a	15.6 a
Triadimefon foliar spray + seed soak	0.56[z] (8 oz)	3-week	2[z]	41.7 a	24.8 a[z]	15.3 a
Triadimefon foliar spray + seed soak	0.56	4-week	2	48.0 a	30.4 a	16.2 a
Triadimefon preplant soil incorporated	0.56	—	1	47.9 a	35.9 a	14.4 a
Triadimefon preplant soil incorporated	1.12 (16 oz)	—	1	47.1 a	34.8 a	14.5 a

[x] Average of 10 seedlings from each of 8 replicate plots.

[y] Means within a column followed by the same letter do not differ ($P = 0.01$) according to Duncan's multiple range test.

[z] 3–4 applications of the high (8 oz ai/acre) rate at shorter spray intervals may have provided a reduction in mycorrhizae occurrence. Southern nurserymen are now using 4 applications of Bayleton at the 8 oz ai/acre rate in several nurseries.

number of feeder roots per 10 cm of lateral root and the percentage of feeder roots with mycorrhizae. In addition, the height of each seedling above the root collar was measured and recorded.

At the Hauss Nursery, each plot was examined during September for the presence of basidiocarps of the mycorrhizal symbionts *Thelephora terrestris* and *Pisolithus tinctorius*. No attempt was made to count the basidiocarps.

All data were subjected to analysis of variance and, where appropriate, means were compared for significant differences by Duncan's multiple range test.

Results

Laboratory Tests.—Triadimefon exhibited activity against all mycorrhizal fungi tested (Table 1); however, considerable variation in sensitivity to the chemical was observed among the test fungi. *Pisolithus tinctorius* was most sensitive, with a 50 percent decrease in colony area (cm^2) occurring at a triadimefon concentration of 1 μg/ml of medium and no growth by the fungus at 5 μg/ml. The fungi most tolerant to triadimefon were *Cenococcum graniforme* and *Suillus luteus*, where a 50 percent decrease in colony area occurred at a concentration of 20 μg/ml and no growth was recorded at 40 μg/ml.

Field Tests.—Results from tests with loblolly pine at the Miller Nursery (Table 2) indicate that triadimefon had no significant effect on the number of short feeder roots present, the

TABLE 3. Effect of field applications of triadimefon at the Hauss State Nursery on production of short feeder roots by slash pine seedlings, percentage of short roots with mycorrhizae, and seedling height.

Treatment	Rate	Spray interval	Applications	Short feeder roots /10 cm of lateral	Short roots with mycorrhizae	Seedling height
	kg/ha (oz/ac)		*number*	*number*	*percent*	*cm*
Control	—	—	—	60.7[x]a[y]	52.7 a	30.5 a
Seed soak (triadimefon 800 mg/l for 24 h)	—	—	—	43.4 b	49.0 a	29.0 a
Triadimefon foliar spray + seed soak	0.28 (4 oz)	2-week	4	57.9 a	45.4 a	31.0 a
Triadimefon foliar spray + seed soak	0.28	3-week	3	56.8 a	49.0 a	32.5 a
Triadimefon foliar spray + seed soak	0.42 (6 oz)	2-week	4	59.1 a	43.5 a	31.7 a
Triadimefon foliar spray + seed soak	0.42	3-week	3	60.9 a	39.2 a	32.0 a
Triadimefon foliar spray + seed soak	0.56 (8 oz)	3-week	3	58.0 a	43.7 a	31.0 a
Triadimefon foliar spray + seed soak	0.56	4-week	2	59.1 a	44.1 a	31.5 a
Triadimefon preplant soil incorporated	0.56	—	1	50.7 a	44.0 a	28.4 a
Triadimefon preplant soil incorporated	1.12 (16 oz)	—	1	45.2 b	37.7 a	27.4 a

[x] Average of 10 seedlings from each of 8 plots.

[y] Means within a column followed by the same letter do not differ ($P = 0.01$) according to Duncan's multiple range test.

percentage of short roots with mycorrhizae, or seedling height. With slash pine at the Hauss Nursery (Table 3), triadimefon did not affect the percentage of short roots with mycorrhizae or seedling height; however, significant decreases in the number of short feeder roots were observed for the triadimefon seed soak treatment and for the highest rate of triadimefon applied preplant soil incorporated.

Basidiocarps of *T. terrestris* were present in all of the plots at the Hauss Nursery in September (*data not shown*). Basidiocarps of *P. tinctorius* were present in only 51 of the 80 study plots; however, there was no discernible pattern to the distribution of *P. tinctorius* basidiocarps among the plots.

Discussion

Results from the laboratory portion of this study showed that triadimefon was active against the ectomycorrhizal fungi tested, and that there was considerable variation in sensitivity to triadimefon among the fungi. However, the concentrations of triadimefon necessary to stop growth of the fungi in cultures were considerably higher than would be expected to occur in soil following recommended rates of application, provided activity of the fungicide toward these fungi in soil is analagous to that in the culture medium used in the tests. Concentrations of triadimefon well above field rates were included in these tests in order to determine what rates are necessary to significantly inhibit growth of the test fungi.

Results from the field studies showed that triadimefon had little effect on development of short roots, on seedling height, or on development of mycorrhizal roots. These results contrast somewhat those reported by Snow and others (1979). In their study, significant

decreases in the percentage of mycorrhizal roots were observed in two Georgia and two Florida nurseries in August. However, when the seedlings were lifted in January, significant effects by triadimefon were observed in only two of the four nurseries; one nursery in Georgia had a decrease in percent mycorrhizal roots, but only at the highest rate tested, and one nursery in Florida had increases in percent mycorrhizal roots at all rates of triadimefon tested. Snow and others (1979) also reported that triadimefon significantly decreased seedling height at the same four nurseries. Their results and the results of the present study suggest that soil type or soil factors may affect the activity of triadimefon on development of mycorrhizal roots. Further tests will be necessary to answer this question.

The significant decrease in the production of short roots for the seed soak treatment at the Hauss Nursery cannot be due to triadimefon alone. All of the plots receiving foliar applications of triadimefon were planted with seed that had been similarly treated with triadimefon. It is unlikely that subsequent foliar applications of triadimefon would circumvent a detrimental effect due to seed treatment. Also, a similar decrease in production of short roots was not observed at the Miller Nursery for the triadimefon seed-soak treatment.

Results of this study show that the use of triadimefon to control fusiform rust at the Miller and Hauss nurseries did not result in significant detrimental effects to the mycorrhizal fungi. The presence of basidiocarps of *P. tinctorius* (the symbiont most sensitive to triadimefon in the laboratory study) in field plots treated with triadimefon at the Hauss Nursery makes it doubtful that this fungicide affected any of the mycorrhizal symbionts.

Literature Cited

Anderson, R. L., and C. E. Cordell. 1979. How to recognize and quantify ectomycorrhizae on conifers. USDA Forest Serv For Bull SA-FB/P 8, 9 p.

Berry, C. R., and D. H. Marx. 1978. Effects of *Pisolithus tinctorius* ectomycorrhizae on growth of loblolly and Virginia pines in the Tennessee copper basin. USDA Forest Serv Res Note SE-264, 6 p.

Czabator, F. J. 1971. Fusiform rust of southern pines—a critical review. USDA Forest Serv Res Pap SO-65, 39 p.

Hare, R. C. 1973. Soil applications of systemics for prevention and eradication of fusiform rust. Plant Dis Rep 57:776–780.

Hare, R. C., and G. A. Snow. 1976. Two systemic fungicides show promise for control of fusiform rust. Plant Dis Rep 60:530–531.

Kelley, W. D. 1978. Control of *Cronartium fusiforme* on loblolly pine seedlings with the experimental fungicide, Benodanil. Plant Dis Rep 62:595–598.

Kelley, W. D. 1979. Control of fusiform rust on loblolly pine seedlings with the systemic fungicide, Bayleton. Phytopathology 69(1):1-A5-1A-6.

Marx, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections: I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. Phytopathology 59:153–163.

Marx, D. H., W. C. Bryan, and C. E. Cordell. 1976. Growth and ectomycorrhizal development of pine seedlings in nursery soils infested with the fungal symbiont *Pisolithus tinctorius*. Forest Sci 22:91–100.

Marx, D. H., W. G. Morris, and J. G. Mexal. 1978. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated and nonfumigated nursery soil infested with different fungal symbionts. Forest Sci 24:193–203.

Mexal, J. G., and G. A. Snow. 1978. Seed treatment with systemic fungicides for control of fusiform rust in loblolly pine. USDA Forest Serv Res Note SO-238, 4 p.

Snow, G. A. 1978. Reduction of fusiform rust on slash pine seedlings with foliar sprays of Bayleton. Phytopathol News 12(9):197.

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 Forest Serv Res Note SO-253, 5 p.