Cyproconazole for Control of Fusiform Rust on Loblolly Pine Seedlings

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ABSTRACT. *The efficacy of cyproconazole seed treatments and foliar sprays for control of fusiform rust on loblolly pine seedlings was evaluated in greenhouse and nursery trials. At the nursery, the percentage of untreated seedlings that were galled at lifting was high (54%). No seedlings receiving cyproconazole at the lowest dosage foliar spray (4 sprays at 123.25 g ai/ha/application) without the seed treatment, and only 0.1% of seedlings receiving only the seed treatment (1.25 g ai/kg seed), were galled. Laboratory inoculations indicated that seed treatments with cyproconazole at 1.25 and 2.5 g ai/kg seed were effective for at least 20 and 30 days, respectively, and that foliar sprays of 185.0 or 246.5 g ai/ha were effective for 14 but not 21 days. Eleven months after sowing, mycorrhizal development in the nursery was similar among seedlings receiving approximately equal dosages of either cyproconazole or triadimefon. Seedling growth was not significantly different between control seedlings and those receiving the various dosages of cyproconazole. South J. Appl. For. 18(3): 101–104.*

Fusiform rust, caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme,* is the most important nursery disease of loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm.) pine seedlings throughout the southeastern United States. Since 1982, triadimefon (Bayleton®) has been the predominant fungicide used to prevent rust infections in regions where the hazard for infection is highest (Carey and Kelley 1993). Utilization of a triadimefon seed treatment and three foliar sprays (Kelley and Runion 1991) has reduced seedling infections in nurseries across the region from an average of 2.5%, when prophylactic fungicides were relied on (Rowan 1977), to less than 0.25% over the last decade (Carey and Kelley 1993).

Effects of a fungicide on both target and nontarget organisms are important. In addition to phytotoxic effects on host plants, effects of a fungicide on mycorrhizal development on nursery seedlings also are important. Although triadimefon leaves little to be desired as a rust preventative, its effects on development of ectomycorrhizal fungi in nurseries have long concerned researchers (Kelley 1982). The benefit of good mycorrhizal development in nursery beds on seedling performance after outplanting is most likely a function of reducing postoutplanting stress (Marx 1987). Differences in ectomycorrhizal development due to triadimefon treatment in nurseries either have (Marx 1987) or have not (Rowan and Kelley 1986) correlated with seedling development after outplanting. However, the greater effectiveness of triadimefon compared to alternative fungicides for rust control has in most instances outweighed any decrease in mycorrhizal development.

The utility of fungicides can be affected both by biological adaptation of target pests and by disfavor from regulatory agencies. With this in mind, Auburn University Southern Forest Nursery Management Cooperative scientists routinely assess new fungicides for rust control. In 1991, cyproconazole (SAN 619F, Sandoz, Ltd., Basel, Switzerland) was subjected to preliminary tests and demonstrated potential as an alternative for triadimefon. A field trial and a more extensive greenhouse trial were completed in 1992. These trials confirmed that both foliar sprays and seed treatments with cyproconazole controlled fusiform rust and that cyproconazole may be an alternative to triadimefon if it is awarded an EPA registration.

Materials And Methods

Greenhouse Study

The USDA Forest Service Rust Resistance Screening Center (RRSC) near Asheville, NC, provided seed of a susceptible loblolly family [lot 10-8(3)] and performed standardized inoculations on the resultant seedlings on July 9, 1992, using basidiospores of *C. quercuum* f. sp. *fusiforme*

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produced at the Center (Knighten et al 1988) To determine the period of time for which treatment remained effective, seeds were sown and fungicide treatments were applied in their various combinations both before and after inoculation.

Seed were stratified beginning April 6, 1992. Seed for all foliar-spray treatments were sown 61 days before inoculation (May 9, after 33 days stratification). Seed treatments were applied 4 days before ending stratification and the seed were sown 30 or 20 days before inoculation (June 9 or 19, respectively, after 64 or 74 days stratification). To control effects of seedling age/size on inoculation efficiency, seed for untreated (control) seedlings were also planted 60 and 20 days before inoculation.

Seedlings were grown as specified by the RRSC in 1.5 × 8 in. conical pots (Cone-tainers®, Cone-tainer Nursery, Canby, OR) containing a commercial soil mix (Premier Pro-Mix BX). Two seeds were sown per pot and thinned to one seedling per pot before cotyledons expanded. Twenty single seedling pots in a 25.4 × 16.5 cm rectangular rack comprised a replicate, and 5 replicates were used for each of the 22 treatments.

Foliar sprays of cyproconazole (SAN 619F 100 SL, Sandoz) were applied in a spray chamber with spray-volume equivalent of 411 L/ha. Spray volume remained constant among the treatments, differing only in amounts of active ingredient applied. Three rates (123.5, 185.0, or 246.5 g ai/ha) applied either at 7, 14, or 21 days before or 5 or 10 days after inoculation resulted in the 15 treatment combinations. The standard seed treatment of triadimefon (Baytan® 30 FL) applied at a rate of 1.25 mg ai/g seed was compared to cyproconazole (SAN 619F 40 WG, Sandoz) at rates of 1.25 or 2.5 mg ai/g seed. Seedlings from triadimefon-treated seed were inoculated 20 days after sowing; seedlings from cyproconazole-treated seed were inoculated 20 or 30 days after sowing.

Seedlings were maintained in a greenhouse on the Auburn University campus before inoculation and at the RRSC greenhouse at Asheville, NC, following inoculation and until evaluated. Seven months after inoculation, numbers of seedlings with rust galls were recorded, and aboveground seedling weights were determined.

Field Study

The Union Camp Corp. Nursery near Union Springs, AL, was selected for the field trial based on its perennially high incidence of rust infections among nontreated seedlings. Five replicate plots per treatment were established in randomized complete blocks with eight treatments per block. Blocked by bed, each of five, 122 m long nursery beds was divided into eight, 12.2 m treatment plots with 3 m buffers between plots.

Stratified seed to sow the study area were obtained from the Union Camp Corp. Nursery. Half of the seed were treated with Gustafson® 42-S (Gustafson, Inc., Plano, TX) as a bird repellant/fungicide at a rate of 83.3 ml/kg seed (35 g thiram/kg seed). The remaining seed received the same rate of Gustafson® 42-S plus cyproconazole at a rate of 1.25 g ai/kg seed. Study plots and adjoining nursery beds were sown on April 25. Seed sown outside the study area were treated with triadimefon at 1.25 g ai/kg seed.

Foliar treatments were 0 0, 123.25, 185.0, or 246.5 g ai/ha/application of cyproconazole. Plots sown with seed not treated with cyproconazole were sprayed 15 days after sowing (May 10) and three additional times at 14 day intervals (May 25, June 10, and June 24). Plots sown with seed treated with cyproconazole received three sprays beginning 29 days after sowing and coinciding with the dates of the last three sprays for nontreated seed. Except for rust control, normal nursery culture (fertilization, root pruning, etc.) was carried out by Union Camp personnel according to their schedule

Numbers of galled and healthy seedlings within two 30 5 cm wide × 1.22 m long counting frames per plot were determined January 5, 1993. On January 22, approximately 50 seedlings were removed from each of the 40 plots and from 5 randomly selected sites in the nursery outside the study area. Seedlings were lifted carefully using a shovel in order to recover as much of their root systems as possible. Half the seedlings from each location were sent to the USDA Forest Service Institute of Tree Root Biology (Carleton Street, Athens, GA) for an assessment of mycorrhizal development, the other seedlings were brought to the laboratory where height, diameter, and biomass were determined. Treatment effects were compared using analysis of variance (SAS, Cary, NC).

Results and Discussion

In the greenhouse study, foliar sprays of cyproconazole provided significant protection for at least 14 days (Table 1) Although it is not possible to compare these data directly to results of infections under field conditions, this is roughly equivalent to the protection afforded by triadimefon applied

Table 1. Efficacy of cyproconazole treatments for preventing infection of loblolly pine by basidiospores of *C. quercuum* f sp. *fusiforme* under greenhouse conditions.

Treatment	Application[1]	Rate[2]	Age[3]	Interval[4]	Galls[5]
None	NA	NA	60	NA	68
None	NA	NA	20	NA	44
Cyproconazole	Foliage	123.5	60	21	30
		185.0	60	21	26
		246.5	60	21	11
		123.5	60	14	5
		185.0	60	14	1
		246.5	60	14	3
		123.5	60	7	0
		185.0	60	7	1
		246.5	60	7	0
		123.5	60	-10	63
		185.0	60	-10	35
		246.5	60	-10	33
		123.5	60	-5	24
		185.0	60	-5	14
		246.5	60	-5	8
	Seed	1.25	20	20	0
		2.50	20	20	0
		1.25	30	30	11
		2.50	30	30	1
Baytan 30		1.25	20	20	0

1 NA = not applicable.
2 Foliage rates g ai/ha, seed rates are g ai/kg seed.
3 Number of days between sowing and inoculation.
4 Number of days between treatment and inoculation (number preceded by (-) indicates days after inoculation and before treatment.
5 Number of galls for 100 seedlings.

at 280 g ai/ha. The postinoculation sprays had insufficient therapeutic activity to warrant further investigation. Average dry weight of control seedlings sown on May 9 was 2.19 g/seedling. This did not differ significantly (data not shown) from the greatest mean (2.65 g/seedling), recorded for the 246 5 g ai/ha foliar spray applied 14 days pre-inoculation or the smallest mean (1.84 g/seedling) recorded for the 185.0 g ai/ha foliar spray applied 21 days pre-inoculation.

In the field study, the lowest levels of cyproconazole tested provided adequate protection (Table 2). Both the cyproconazole seed treatment (1.25 g ai/kg seed) without foliar sprays and the lowest rate of cyproconazole foliar spray without seed treatment prevented rust infection. Although optimal periods for infection certainly occurred, it cannot be determined when they occurred; they may have been in the very early spring. At the RRSC, the effectiveness of the 1.25 g ai/kg of seed rate of cyproconazole diminished between 20 and 30 days after sowing, but the 2.5 g ai/kg rate was effective for at least 30 days. Because the RRSC utilizes larger dosages of basidiospores than probably occur naturally, and because temperature and moisture conditions are kept optimum for infection, it is safe to assume that an infection percentage recorded at the RRSC for a particular treatment would be less under field conditions. Thus, the high rate of cyproconazole seed treatment (2.5 g ai/kg seed), would eliminate the need for two foliar sprays of cyproconazole or triadimefon; in the case of ferbam, at least 10 sprays could be avoided.

The mycorrhizae morphology ratings presented in Table 2 have been described by Marx (1990). This rating system partitions mycorrhizal roots by percentages with various numbers of root tips. Compared among plots, the correlation between mycorrhizal root percentages and morphology ratings was positive (r = 0.91) and highly significant (P = 0.0001). Neither estimate of mycorrhizal development differed significantly among treatments, but each generally decreased as fungicide rate increased. The fact that mycorrhizal development was similar between seedlings receiving the high rate of cyproconazole and those treated with triadimefon is important. In a recently published European study (Desprez-Loustau et al. 1992), the mycorrhizae species tested were more inhibited by cyproconazole than by triadimefon. The European study did not include either *Thelephora terrestris* (Ehr.), the most abundant natural mycorrhiza at the Union Camp nursery, or *Pisolithus tinctorius* (Pers.) Cok. & Couch, the most frequently used mycorrhizal amendment in the southeastern United States. Thus, differences between species of mycorrhizal fungi in the European study and in our study may account for the differences observed in sensitivity to cyproconazole.

Contracts for seedlings enhanced with amendments of *P. tinctorius* sometimes specify that ferbam be used for rust control instead of triadimefon. If cyproconazole seed treatment alone or a combination of a seed treatment and late-season sprays with ferbam could reliably control rust without reducing mycorrhizal development, most of the up to 20 applications of ferbam required for rust control could be omitted. Further testing with cyproconazole is underway. At present, cyproconazole is not registered for use in forest nurseries.

No significant differences were found among treatments for seedling diameters, weights, or numbers per counting frame (Table 3). Number of seedlings per counting frame were recorded to determine the percentage of galled seedlings. Such counts reasonably estimate seedlings per unit area (seedling density) where nursery bed structure is uniform. Frequently, tractor traffic and surface water flow result in deterioration in the ends of nursery beds, and this occurred in the research area. Seven of the ten bed-end plots contained cyproconazole-treated seed, and three of these were from the treatment with the fewest seedlings recorded per frame. Although significant differences (P = 0.002) for seedling counts between bed-end and interior plots were greater than for cyproconazole seed treatment (P = 0.13), the economic impacts of even small differences in seed efficiency make it prudent to determine the impacts of cyproconazole seed treatment more precisely. Numbers of seedlings per counting frame negatively correlated with seedling weight (r = -0.62, P = 0.0001). This is a normal relationship between seedling growth and bed density (South et al. 1990), and indicates that the seed treatments did not reduce growth.

Table 2. Effect of fungicide treatments on rust infection of and mycorrhizae development on 9-month-old loblolly pines in an Alabama bareroot nursery.

Product				Mycorrhizae	
Seed[1]	Foliar[2]		Galls[3] (%)	Percent[4]	Morphology[5]
Cyproconazole	None		0.1	39.7	3.89
|	Cyproconazole	3 @ 123.5	0.0	36.5	3.38
|	|	3 @ 185.0	0.0	38.4	3.45
|	|	3 @ 246.5	0.0	31.6	2.90
None	None		54.4	42.2	3.97
|	Cyproconazole	4 @ 123.5	0.0	34.2	3.97
|	|	4 @ 185.0	0.0	37.7	3.39
|	|	4 @ 246.5	0.0	38.4	3.19
Baytan	Bayleton®	3 @ 280.0	0.0	31.0	3.07

1 Both seed treatments are 1.25 g ai/kg seed.
2 Fungicides, number of applications and g ai/ha.
3 Percentage of galled seedlings within two 30.5 m wide counting frames (counts averaged 93.5 seedlings per frame) for each of five replicates plots per treatment.
4 Treatment averages are for five 25-seedling replicates and within a column do not differ significantly (SAS Duncan's α = 0.05).
5 Ratings range is 1 to 10 for seedlings with all simple short roots to those with all multiple corraloid short roots.

Table 3. Effect of fungicide treatment on bed densities, diameters, and dry weights of 9-month-old loblolly pines in a bareroot nursery.

Treatment				
Seed[1]	Foliar sprays[2]	Counts[3]	Diameter[4]	Weight[5]
Yes	None	96.5 a[6]	4.96 a	4.78 a
Yes	3 @ 123.5	96.4 a	4.90 a	4.52 a
Yes	3 @ 185.0	88.4 ab	5.02 a	4.82 a
Yes	3 @ 246.5	77.1 b	5.34 a	5.83 a
No	None	100.2 a	4.85 a	4.28 a
No	4 @ 123.5	97.7 a	5.14 a	5.10 a
No	4 @ 185.0	88.5 ab	5.31 a	5.35 a
No	4 @ 246.5	103.8 a	4.84 a	4.37 a

1 1.25 g ai cyproconazole per kg seed.
2 Number of applications at rate in grams ai per ha of cyproconazole.
3 Average number of seedlings within approximately 0.37 m for two samples in each of five treatment replicates.
4 Average ground line stem diameter (mm) for five 25-seedling samples.
5 Average ovendry weight (g) per seedling for five 25-seedling samples.
6 Treatment averages within a column followed by the same letter are not significantly different (SAS Duncan's $\alpha = 0.05$).

Conclusions

Fusiform rust can be controlled in loblolly pine nursery beds with the systemic fungicide cyproconazole. Seed treatments and foliar sprays with cyproconazole both were effective. In addition, mycorrhizal development on seedlings from cyproconazole-treated plots, as assessed 11 months after treatment, did not differ from that of seedlings from triadimefon-treated plots. Cyproconazole may be an alternative to triadimefon for control of fusiform rust in forest nurseries if it is awarded an EPA registration.

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