Chemical Control of *Rhizoctonia* Seedling Blight of Longleaf Pine

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ABSTRACT. *Benomyl, chlorothalonil, benodanil, triadimefon, diniconazole, terbuconazole, SN-84364 (Nor-Am experimental) and RH-3486 (Rohm & Haas experimental) effectively inhibited growth of* Rhizoctonia *sp. isolates from longleaf pine (*Pinus palustris *Mill.) seedlings when incorporated in agar medium. All fungicides were effective at concentrations as low as 1 μg ai/ml. However, disease incidence was not affected by diniconazole, benodanil or SN-84364 at concentrations of 140 or 280 g ai/ha in a preliminary field trial, and diniconazole exhibited phytotoxic effects on longleaf pine seedlings. Triadimefon at 280 g ai/ha was not effective in controlling longleaf pine seedling blight in an operational field study. SN-84364 at 560 g ai/ha applied every 2 wk reduced disease incidence compared to an untreated control in spring-sown longleaf pine at one nursery, but was not significantly different from the nursery's standard disease management practice of benomyl and chlorothalonil. The nursery where longleaf seed were spring-sown had a significantly greater incidence of longleaf pine seedling blight than nurseries where seed were fall-sown. At one nursery, disease incidence was significantly lower in a bed of fall-sown longleaf pine seedlings than in an adjacent bed of spring-sown seedlings. South. J. Appl. For. 18(1):5–9.*

Davis (1941) first reported the role of *Rhizoctonia* in damping-off of longleaf pine (*Pinus palustris* Mill.) seedlings. He reported that, although damping-off becomes negligible 4–6 wk after emergence for most pine species, losses from *Rhizoctonia* damping-off of longleaf pine are sometimes as great in 4-mo-old seedlings as during emergence. This "late" damping-off was termed "longleaf pine seedling blight" and was initially attributed to *Rhizoctonia solani* Kühn (Barnard 1979). The disease is now known to be caused by a binucleate *Rhizoctonia solani*-like fungus in anastomosis group CAG-3 (English et al. 1986).

Longleaf pine seedling blight has become a serious problem in southern forest tree nurseries during the last decade. During this time, a renewed interest in longleaf pine for reforestation has developed in the southeastern United States resulting in an increased demand for longleaf pine planting stock (Gilly et al. 1985). In Florida, the disease was responsible for the loss of 120,000 seedlings in one nursery in 1979 (English and Barnard 1981) and a loss of 20% of the longleaf pine seedling crop in one nursery in 1983 (Gilly et al. 1985). Losses of 20%–50% were observed in two Alabama nurseries in 1986 (Kelley, unpubl.), and a loss of 18% was observed in one Alabama nursery in 1989 (Runion and Kelley, unpubl.).

Reduced incidence of longleaf pine seedling blight has been achieved with several mulches (Davis 1941, Gilly et al. 1985), most likely due to a reduction in the amount of sand splash in mulched seedbeds. However, the type and application of mulches varies greatly among southern forest nurseries due to availability (Barnard 1979), cost, and other factors.

Many fungicides have activity against *Rhizoctonia* spp. (Thomson 1991) and several effectively reduce disease losses in forest tree nurseries (Barnard 1979, Gilly et al. 1985). Until 1991, when the manufacturer removed foliar applications of benomyl (Benlate 50WP) on pines and ornamentals from the product label, benomyl was used to control longleaf seedling blight in most nurseries. Benomyl required numerous applications and some nurseries still experienced disease losses. *Rhizoctonia* spp. are known to develop resistance to repeated use of fungicides (Elsaid and Sinclair 1964, Shatla and Sinclair 1963), and several fungi have developed resistance to benomyl (Delp 1979, Smith 1988).

Longleaf pine seedling blight has become a serious and widespread problem in southern forest nurseries. Therefore, it is necessary to develop a control strategy which may necessitate an effective fungicidal spray program. The availability of several effective fungicides is warranted to prevent or delay the development of resistance (Delp 1988) in the fungus and to offer alternatives in the event a product is removed from the market. The goal of this study was to

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identify fungicides effective in controlling longleaf pine seedling blight for possible inclusion in southern forest nursery disease management practices.

Materials And Methods

In vitro Study

Isolates of binucleate *Rhizoctonia* sp. were collected from infected longleaf pine seedlings at Hauss nursery near Atmore, AL, Andrews nursery in Chiefland, FL, and Munson nursery in Munson, FL. All isolations were made on potato dextrose agar (PDA). Isolates used in the study were derived from hyphal tip cultures and were maintained on PDA prior to use in the experiment.

Eight fungicides were selected based on known activity against *Rhizoctonia* spp. (Thomson 1991):

1. benomyl (Benlate 50WP)
2. chlorothalonil (Bravo 500)
3. benodanil (Benefit 50WP)
4. triadimefon (Bayleton 50WP)
5. diniconazole (Spotless 25WP)
6. terbuconazole (Folicur 25WP)
7. SN-84364 (Nor-Am experimental, 50WP)
8. RH-3486 (Rohm & Haas experimental, 50WP).

Fungicides were sterilized prior to use by exposing 160 mg ai of each fungicide, in 50 ml volumetric flasks, to a propylene oxide saturated environment for 72 hr. Stock solutions of each fungicide (4000 µg ai/ml) were prepared by adding 40 ml of twice autoclaved (1 hr, 17 psi, 121 °C) water to each flask. Fungicide concentrations of 1, 10 and 100 µg ai/ml were obtained by adding 0.1, 1.0, or 10 ml stock solution to a prescription bottle containing 400 ml of PDA. Each amended medium was dispensed (20 ml) into each of twenty 100 mm diameter petri dishes. Controls were not amended with fungicide.

A 7 mm diameter mycelial plug, cut from the margin of a 2-day-old culture, was placed in the center of each of five dishes containing each fungicide concentration for each fungal isolate tested. Fungal colony diameters were measured in two directions, perpendicular to each other, after 48 hr incubation at room temperature. Percent radial growth inhibition was determined for each isolate at each concentration based on the appropriate nonamended check. The experiment was conducted twice.

Preliminary Field Study

Benodanil, diniconazole, and SN-84364 were selected for use in the preliminary field study. Each fungicide was applied at concentrations of 140 or 280 g ai/ha and compared to a nonsprayed control. Studies were conducted at Florida's Division of Forestry nursery at Munson, FL, and at Container Corporation of America's Rock Creek nursery near Brewton, AL. At the nursery in Munson, longleaf pine seed were fall-sown and at the nursery in Brewton, seed were spring-sown. Studies at each nursery contained four randomized complete blocks Each plot was 7.6 m along one nursery bed (1.2 m wide). Fungicides were applied once every 3 wk from installation of the studies in July through October 1988 using a bicycle-type, CO_2 pressurized spraying apparatus of our own design.

Disease incidence was assessed as the number of symptomatic seedlings within the interior 6.1 m length of each test plot. Disease data were collected just prior to the initial fungicide application (July 1988) and every 6 wk thereafter until seedlings were lifted (December 1988). Five to ten arbitrarily selected symptomatic seedlings were collected from each nursery at each evaluation date for confirmation of infection by *Rhizoctonia* sp. All seedlings within three randomly selected 0.3 m long sections of each measurement plot were harvested just prior to lifting, and groundline diameters and seedling dry weights were measured to assess phytotoxic effects of the treatments.

Operational Field Study

Treatments consisted of the experimental compound SN-84364 at 560 g ai/ha applied every 2 wk, triadimefon at 280 g ai/ha applied every 4 wk, and an operational treatment consistent with standard *Rhizoctonia* control practices of each nursery, plus nonsprayed controls. Studies were conducted at the same two nurseries as the preliminary field test plus Florida's Division of Forestry nursery at Chiefland, FL. Operational treatments consisted of: Brewton, AL = tank-mix of benomyl (1.1 kg ai ha^{-1}) and chlorothalonil (3.3 kg ai ha^{-1}) every other week from May through November; Munson, FL = alternating applications of benomyl (0.6 kg ai ha^{-1}) and chlorothalonil (1.2 kg ai ha^{-1}) every other week from April through October; Chiefland, FL applied no fungicides. At the nurseries in Munson and Chiefland, longleaf pine seed were fall-sown and at the nursery in Brewton, seed were spring-sown. Studies at each nursery contained four randomized complete blocks. Test plots were 15.2 m long and three nursery beds wide. Three measurement plots (each 3.1 m long) were systematically arranged within the interior portion of the middle bed of each test plot.

Disease incidence was assessed as the number of symptomatic seedlings within each measurement plot. Disease data were collected just prior to the initial fungicide application and every 6 wk thereafter until lifting (December 1989) Five to ten arbitrarily selected symptomatic seedlings were collected from each nursery at each evaluation date for confirmation of infection by *Rhizoctonia* sp. All seedlings within one randomly selected, 0.3 m long section of each measurement plot were harvested just prior to lifting, and dry weights were determined to assess potential phytotoxic effects of the treatments.

Sowing Date Study

At Hauss nursery near Atmore, AL, longleaf pine seedling beds, differing only in date of sowing (fall vs. spring), had been established adjacent to one another. Just prior to lifting in 1989, incidence of longleaf pine seedling blight was recorded. A counting frame (0.3 × 1.2 m) was placed at 20, randomly assigned locations along one bed of fall-sown and one bed of spring-sown longleaf pine seedlings. Numbers of symptomatic and healthy seedlings were recorded. All seedlings within the counting frame were then lifted, and dry weights were determined.

Data analyses for all experiments were conducted using the General Linear Models procedure of the Statistical Analysis Systems (SAS Institute Inc. 1982).

Results And Discussion

In vitro Study

There were no significant differences in response of longleaf pine *Rhizoctonia* isolates to the fungicides tested, and data were averaged over the three isolates. All fungicides restricted growth of all *Rhizoctonia* isolates, even at the lowest concentrations tested (Table 1). The goal of the *in vitro* study was to select fungicides for a preliminary field test and, based on relative performance in the laboratory study (Table 1), SN-84364, benodanil and diniconazole were chosen. Diniconazole was included because it was effective against *Rhizoctonia* diseases of peanuts in field tests (Sumner and Littrell 1989). Benomyl ranked fifth in the *in vitro* test, but was not tested further given its previous role as the standard fungicide used for control of longleaf pine seedling blight. However, because benomyl is immobilized in soil (Aharonson and Kafkafi 1975) and as the fungus resides in and spreads through the soil (Barnard 1979), other fungicides would likely provide better disease control.

Preliminary Field Study

The primary purpose of this study was to determine fungicides, concentrations, and application frequencies to be used in the operational field test. However, few differences in disease incidence were observed among treatments (Table 2), which was most likely due to the fact that the study was begun in July, after most infection had occurred. As slight increases in disease incidence were observed after the initial (July) evaluation, it is also possible that the concentrations of the compounds tested (particularly the 140 g ai/ha concentration) and/or the frequency of application were not sufficient to control disease.

Table 1. Percent radial growth inhibition[1] for the various concentrations of each fungicide in the *in vitro* study.

	Concentration (µg ai/ml)			
Fungicide	1	10	100	Ave.
SN-84364	88.4 a[2]	98.6 a	99.6 a	95.5 a
Diniconazole	82.1 b	95.9 a	98.2 a	92.1 a
Benodanil	53.7 c	95.4 ab	99.8 a	82.9 b
RH-3486	77.0 b	84.4 c	87.2 c	82.9 b
Benomyl	42.9 de	91.7 b	100.0 a	78.2 bc
Terbuconazole	48.5 cd	77.7 d	93.6 b	73.3 cd
Triadimefon	37.4 e	79.2 d	93.2 b	70.0 de
Chlorothalonil	50.2 c	68.6 e	82.8 d	67.2 e

[1] Data represent the mean response of three *Rhizoctonia* isolates from longleaf pine. There were five plates for each isolate in each of two runs of the experiment.

[2] Within a concentration, means followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's New Multiple Range Test.

There were no significant differences among treatments at either nursery for the number of seedlings per linear meter of bed or for average dry weights of seedlings. Average groundline diameter was significantly lower and the corresponding percentage of cull seedlings was significantly higher for seedlings receiving either concentration of diniconazole than for seedlings receiving other treatments (data not shown). Diniconazole also has been reported to reduce heights and dry weights of peanuts plants (Sumner and Littrell 1989). Given these apparent phytotoxic effects, diniconazole was not considered for further testing.

Operational Field Study

Benodanil was not included in the operational field test because it was no longer commercially available and was replaced with triadimefon, even though it ranked low in the *in vitro* test (Table 1), due to availability and familiarity of nursery personnel with the compound. Triadimefon is routinely used for fusiform rust (*Cronartium quercuum* (Berk.) Miyabi ex Shirai f. sp. *fusiforme* Burdsall and Snow) control in southern pine nurseries. To facilitate the possible incorporation of triadimefon into a longleaf pine seedling blight control program, the concentration and frequency of application recommended for fusiform rust control were tested. The concentration of SN-84364 was increased following the preliminary field trial due to its lack of efficacy in that study; applications continued to be at 2-wk intervals.

Disease incidence at the Brewton nursery was significantly lower for the operational treatment and the SN-84364 treatments compared to the control (Table 3). SN-84364 controlled the disease as well as 11 applications of benomyl tank mixed with chlorothalonil. SN-84364 resulted in the lowest disease incidence for all treatments at the Munson and Chiefland nurseries; however, because of the low overall incidence of disease at these nurseries, differences among treatments were not statistically significant. SN-84364 appears to be a viable compound for inclusion in longleaf pine seedling blight control strategies. It may be possible to reduce concentration or application frequency of SN-84364 from those of the operational field study and still achieve adequate disease control, and this possibility deserves further study. Triadimefon had no effect on disease incidence at the concentration and application schedule tested (Table 3).

There also were significantly more seedlings per linear meter of bed in plots treated with SN-84364 compared to controls at the Brewton nursery (data not shown). It is presumed that this increase in the number of seedlings was due to reductions in disease incidence, but it is not known why a similar significant increase in seedling number was not also observed for the operational treatment.

Disease incidence at the Brewton nursery, which used spring-sown seed, was significantly greater than at the two nurseries where seed were fall-sown. However, since fall and spring sowing took place at different nurseries, differences in cultural practices (i.e., type and amount of mulch used, sowing density, fertilization practices, etc.) may contribute to differences between the nurseries.

Table 2. Disease incidence[1] for each evaluation date at each nursery in the preliminary field study.

Nursery	Fungicide	Concentration (g ai/ha)	Evaluation date: July	Aug.	Sept.	Dec.	Total[2]
Brewton	Control	0	2.3	22.0	7.8	1.3	31.0
	Benodanil	140	1.5	8.3	9.5	3.0	20.8
	Benodanil	280	0.8	13.3	11.3	3.0	27.5
	SN-84364	140	1.5	17.8	5.3	3.5	26.5
	SN-84364	280	1.5	19.5	6.8	3.0	29.3
	Diniconazole	140	1.5	19.5	6.8	4.3*[3]	30.5
	Diniconazole	280	2.3	19.5	4.5	2.5	26.5
Munson	Control	0	11.3	0.5	0.5	1.5	2.5
	Benodanil	140	14.3	2.3*	1.3	2.8	6.3*
	Benodanil	280	17.0	1.5	0.8	2.0	4.3
	SN-84364	140	17.0	0.8	2.3	3.3	6.3*
	SN-84364	280	16.5	1.3	2.0	1.3	4.5
	Diniconazole	140	15.0	1.0	1.3	2.8	5.0
	Diniconazole	280	14.3	0.8	1.5	2.0	4.3

[1] Disease incidence defined as the number of seedlings symptomatic of *Rhizoctonia* infection within the interior 6.1 m of each test plot.
[2] Total is the combined incidence from all evaluations occurring after the initial fungicide application, i.e., does not include the pretreatment (July) evaluation.
[3] A single asterisk (*) indicates significance (compared to controls) at $P = 0.05$ according to contrasts conducted under GLM of SAS.

Sowing Date Study

Data from the preliminary and operational field tests inferred a substantial effect of sowing date on disease incidence, and the objective of this study was to provide preliminary data demonstrating this effect in a similar environment and under similar management practices. The benefit of fall sowing longleaf pine seed for reducing incidence of longleaf pine seedling blight was first noted by Davis (1941). This reduction in disease incidence, noted by Davis and in this study, could be due to the fact that fall-sown seedlings are larger and possibly less succulent during the period of infection and are able to inhibit fungal penetration. It is also possible that the seedling canopy architecture of fall-sown seedlings reduces sand splash, a means of fungus dissemination (Davis 1941).

Beds of fall-sown longleaf pine seedlings had signifi-

Table 3. Disease incidence[1] for each evaluation date at each nursery in the operational field study.

Nursery	Treatment	Evaluation date: Mar.	May	June	July	Sept.	Oct.	Dec.	Total[2]
Brewton	Triadimefon	---	1.3	21.4	36.8	23.0	15.4	5.5	102.1
	SN-84364	---	1.3	11.8	6.8**[3]	1.1**	1.2**	0.3**	21.1**
	Operational	---	0.8	9.1	4.6**	2.1**	0.8**	0.4**	17.0**
	Control	---	0.8	16.2	43.7	23.3	15.2	8.5	106.8
Munson	Triadimefon	1.3	1.1	1.1	0.3	1.3	0.9	0.7	5.3
	SN-84364	0.7	0.9	0.5	0.4	1.0	1.0	0.5	4.3
	Operational	1.5	1.6	1.4	1.4	1.8	0.9	1.2	8.3
	Control	1.0	1.1	0.8	0.5	1.3	1.6	1.3	6.7
Chiefland	Triadimefon	0.8	0.3	2.5	4.2	3.3	2.3	1.3	13.8
	SN-84364	0.3	0.3	1.2	0.5	0.4	0.4	0.3	3.1
	Operational	1.1	0.6	2.7	1.2	2.3	2.1	1.3	10.1
	Control	2.5	0.5	2.4	1.3	2.3	1.1	1.7	9.3

[1] Disease incidence is the number of seedlings symptomatic of *Rhizoctonia* infection within a 3.1 m measurement plot and is the average of three measurement plots in each of four blocks at each nursery.
[2] Total is the combined incidence from all evaluations occurring after the initial fungicide application, i.e. does not include the pretreatment (March for Munson and Chiefland or May for Brewton) evaluation.
[3] A double asterisk (**) indicates significance (compared to controls) at $P = 0.01$ according to contrasts conducted under GLM of SAS.

cantly more healthy seedlings per linear meter of nursery bed, more total seedlings per linear meter of bed, and greater average dry weight per seedling than adjacent beds of spring-sown seedlings in the same nursery (Table 4). Numbers of symptomatic seedlings and percent disease were significantly lower in fall-sown longleaf pine seedling beds compared to beds of spring-sown seedlings.

Since this study examined incidence and seedling numbers only at the end of the growing season, fewer total seedlings in the spring-sown beds cannot be positively associated with disease losses. However, since both beds were sown at the same density and received similar cultural treatments after emergence, differences in disease incidence are a logical explanation for the difference in number of seedlings per linear meter. Further testing is required to explicitly determine the benefits of using fall-sown seed for control of longleaf pine seedling blight.

Sowing seed in the fall, monitoring disease incidence levels, and timely application of fungicides should adequately control longleaf pine seedling blight in southern nurseries. Inclusion of the experimental compound SN-84364, should it become registered, as a part of this control strategy may aid control and prevent development of resistance from frequent and exclusive use of other fungicides.

Table 4. Healthy and diseased seedlings, percent disease and seedling dry weight of fall- and spring-sown longleaf pine at Hauss nursery in the sowing date study.

Sowing date	Healthy trees[1]	Diseased trees[2]	Percent disease[3]	Ave. wt[4]
Fall	49.0	1.0	2.0	15.1
Spring	36.4**[5]	3.2**	8.2**	12.9**

[1] Healthy trees is the average number of asymptomatic seedlings within the 0.3-linear meter plots ($n = 20$).
[2] Diseased trees is the average number of seedlings symptomatic of *Rhizoctonia* infection within the 0.3-linear meter plots ($n = 20$).
[3] Percent disease is the number of seedlings symptomatic of *Rhizoctonia* infection divided by the total number of seedlings (diseased trees plus healthy trees) × 100, within the 0.3-linear meter plots ($n = 20$).
[4] Ave. wt = average dry weight of seedlings in grams.
[5] A double asterisk (**) indicates significance between sowing dates at $P = 0.01$ according to contrasts conducted under GLM of SAS.

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