

From Forest Nursery Notes, Summer 2013

100. The use of prothioconazole to control forest nursery diseases of *Pinus* spp.
Starkey, T. E. and Enebak, S. A. Proceedings of the 7th Meeting of IUFRO Working Party 7.03.04, p. 92-103. USDA Forest Service, Southern Region, Forest Health Protection Report 10-01-01. 2010.

THE USE OF PROTHIOCONAZOLE TO CONTROL FOREST NURSERY DISEASES OF *PINUS* SPP

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ABSTRACT

Laboratory, greenhouse and field trials have shown Proline[®] to be efficacious against three fungal pathogens that cause damage and seedling mortality in forest-tree nurseries. Disease control using Proline[®] has been obtained at 365 ml/ha (5 fl oz/acre) for the control of fusiform rust (*Cronartium quercuum* f.sp. *fusiforme*) on loblolly pine (*Pinus taeda*) in both greenhouse and field trials. In greenhouse trials, a biweekly application, 365 ml/ha, (5 fl oz/acre) controlled *Fusarium circinatum* (Pitch Canker) on longleaf pine (*Pinus palustris*) and resulted in an 11% increase in seedling production over non-treated seedlings. *In vitro* studies using Proline[®] amended agar media resulted in 100% fungicidal control against *Fusarium circinatum* at all 5 rates used: 0.0625x, 0.125x, 0.25x, 0.5x and 1x the recommended label rate. A biweekly application of Proline[®], 402 ml/ha (5.5 fl oz/ac), in nursery field tests significantly reduced *Rhizoctonia* foliar blight on loblolly pine when compared to applications of azoxystrobin and the non-treated control. The monetary loss per hectare due to *Rhizoctonia* foliage blight was \$10,864, \$4,198 and \$44 for non-treated, azoxystrobin and Proline[®] respectively. In addition to disease control, Proline[®] treated seedlings were significantly larger and appeared greener than non-treated seedlings.

INTRODUCTION

The availability of fungicides to control specific forest seedling nursery diseases is either nonexistent, limited or faces possible loss of US label registration. Of the many insects and diseases that occur in forest-tree nurseries, three fungal pathogens stand out as problematic in southern US nurseries. These diseases include Fusiform Rust, Pitch Canker, and *Rhizoctonia* Foliar Blight. The most important disease of loblolly (*Pinus taeda*) and slash (*Pinus elliotti*) pine seedlings is fusiform rust caused by *Cronartium quercuum* f.sp. *fusiforme*. Since 1980, formulations of Bayleton[®] (triadimefon) have been the primary chemical used to control this disease (Carey and Kelley 1993) and has consistently provided excellent cost-effective control as both a seed treatment and foliar spray (Snow and others 1979, Carey and Kelley 1993, Carey 2004).

In July 2007, Bayer CropScience received US Environmental Protection Agency's (EPA) cancellation order for Bayleton[®]. While most of the food and non-food crops such as apples, pears, grapes and raspberries were removed from the US label, its use on pine seed and seedlings was still allowed. However, the availability and formulation remain unsettled, resulting in nurseries having difficulty locating and obtaining the product; an alternative is needed.

Pitch canker, caused by the fungus *Fusarium circinatum*, can cause significant seed and seedling mortality in nurseries and later after outplanting in the field (Carey and Kelley 1994, Dwinell 1978, Barrows-Broadus and Dwinell 1984, Blakeslee and Rockwood 1984, Lowerts and others 1985, Kelley and Williams 1982, Dwinell and Barrows-Broadus 1981). In the southern US, infection and seedling losses have been reported on loblolly, slash, longleaf (*Pinus palustris*), shortleaf (*Pinus echinata*) and Virginia (*Pinus virginiana*) pine. The fungus is also considered one of the most threatening diseases in many areas of the world, particularly the South African nurseries (Storer and others 1998, Viljoen and Wingfield 1994). Unlike fusiform rust, there are no fungicides registered for the control of pitch canker on either seed or seedlings and nursery growers are forced to use either bleach or hydrogen peroxide to disinfect seed.

Longleaf and loblolly pines are particularly susceptible to *Rhizoctonia* foliar blight. The disease is caused by a species of *Rhizoctonia* spp. or binculeate forms of sexual states belonging to the genera *Thanatephorus* or *Ceratobasidium*. *Rhizoctonia* foliar blight can cause significant pine mortality in nursery beds and typically occurs in late July when the seedling canopy closes in (Carey and McQuage, 2003). Symptoms of dead and dying needles and seedling mortality appear in patches within the bed where moisture and temperature favor infection. Many times the disease is not observed until seedlings are top-clipped to maintain seedling shoot:root ratios and heights. Varying degrees of resistance among seedling families can be found, with US gulf coastal seedlots more susceptible than Piedmont sources, and the disease is rarely observed on slash pine (McQuage, 2009 personal communication). *Rhizoctonia* foliar blight is not distributed uniformly throughout a nursery and is generally limited to isolated foci and the disease is also more severe in second crop fields. While there are fungicides registered for *Rhizoctonia* foliar blight, they are not always efficacious (Carey and McQuage 2004).

In an attempt to find an alternative for the control of fusiform rust, trials examining numerous fungicides by have been underway since 2004. In 2008, Proline[®] 480 SC (41% prothioconazole, Bayer CropScience) was examined as it had a broad spectrum systemic control of ascomycetes, basidiomycetes, and deuteromycetes on numerous field crops. Prothioconazole belongs to the new chemical class of triazolinthiones (Mauler-Machnik and others 2002) and inhibits the demethylation process at position 14 of lanosterol or 24-methylene dihydrolanosterol, which are precursors of sterols in fungi. Prothioconazole efficiently stops many steps of the fungal infection chain like appressoria and haustoria formation, mycelial growth as well as spore formation. Currently Proline[®] is registered in the US for food crops including peanuts, barley, wheat, sugar beets and soybeans.

Although Proline[®] is not currently registered for commercial use in US forest-tree nurseries, these studies examined Proline[®] in laboratory, greenhouse and field trials to determine if the fungicide was efficacious against the three fungal pathogens that are capable of causing significant damage and seedling mortality in forest-tree nurseries. Data collected from such studies will be used in an attempt at obtaining a full-use label from Bayer CropScience and US EPA for disease control in forest-tree nurseries in the southern US.

METHODS

Fusiform Rust Greenhouse Trials

Seed treatments. In 2006, 2007 and 2008 loblolly pine seed were stratified for 4 weeks, after which they were treated with fungicides prior to sowing (Table 1). For dry formulation fungicides, seed was first moistened in a seed tumbler, and the fungicide was added at the rate of 25 g/10 kg (2 oz/50 lbs) of seed. For liquid fungicides approximately 26 ml (2 fl oz) of the product was used per 10 kg (50 lbs) seed which was slowly added to pine seed in a tumbler. The fungicide and seed was tumbled until dry. All treated seed, as well as non-treated seed for both positive and negative controls, were double sown to Ray-Leach[®] containers and then thinned to one seedling per cell as they germinated.

Table 1. Fungicide rates, actual product per unit, used in 2006, 2007 and 2008.

Treatments	Active Ingredient	Foliar Treatment ¹		Seed Treatment
		1X	2X	1X
Check (water)				
Bayleton [®]	tridimefon 50%	560 ml/ha (8 oz/a)		25 g/10 kg seed (2 oz /50 lb seed)
Folicur [®]	tebuconazole 38.7%	292 ml/ha (4 fl oz/a)	584 ml/ha (8 fl oz/a)	
Provost [®] 433 SC	prothioconazole 12.9%	621 ml/ha	1.24 l/ha	25 g/10 kg
	tebuconazole 25.8%	(8.5 fl oz/a)	(17 fl oz/a)	(2 fl oz 50 lb)
Proline [®] 480	prothioconazole 41%	365 ml/ha (5 fl oz/a)		25 g/10 kg (2 fl oz 50 lb)

¹ Based upon 280 l/ha (30 gal of water/acre)

Foliar treatments. Loblolly pine seed were stratified for 40 days and then double sown to Ray-Leach[®] containers. Following germination, containers were thinned to one seedling per container and then randomly assigned fungicidal treatments. Seven weeks post-sowing, seedlings were treated at the Auburn University's Pesticide Research Facility. A Bayleton[®] and a water check were included for both positive and negative controls, respectively. Application rates for each fungicide included a 1x and 2x rate (except Bayleton[®] which only had a 1x rate) as listed in Table 1. Proline[®] was only tested in 2008 at the 1x rate. After spraying, seedlings were returned to the greenhouse to dry.

Inoculations. One day following the foliar fungicide application, the seedlings were transported to the USDA Rust Screening Laboratory in Asheville, North Carolina. Seedlings were allowed to acclimate to the new growing conditions for 5-7 days and then challenged with 20,000 basidiospores/ml of *Cronartium quercum f.sp. fusiforme* (collected from Zone 7 inoculum area) using the laboratory's standard inoculation protocols. Seedlings remained under the care of the USDA Rust Laboratory for the duration of the growing season. At 3 and 6 months post-inoculation, seedlings were evaluated for swellings along the main stem, an indication of basidiospore infection.

Fusiform Rust Field Trials

In 2008, two nurseries (South Carolina Forestry Commission Nursery in Trenton, SC and Arborgen Nursery in Shellman, GA) participated in testing Proline[®] operationally on several nursery blocks. Proline[®], Provost[®] and Bayleton[®] were compared to a non-treated control. At each nursery a randomized complete block design was used with treatments replicated 3 times at one nursery (SC) and 5 times at the other (GA); 0.24 ha (0.6 acre) and 0.405 ha (1.0 acre), respectively. Each replication/treatment was applied to either 3 adjacent nursery beds or a 9-bed nursery section using standard nursery spray equipment. Proline[®] and Provost[®] were applied 365 ml/ha (5 fl oz/acre) and 621 ml/ha (8.5 fl oz/acre), respectively, as well as the standard Bayleton[®] application. At the end of the growing season (December 2008), seedlings were collected from each treatment plot and examined for rust infection and measured for seedling quality. In addition, seedlings were collected from the nursery in February 2009 and outplanted at a site near Auburn, AL to monitor for any long-term effects of the fungicide treatments on seedling survival.

Pitch Canker Laboratory Trials

Laboratory fungal growth studies were conducted to determine if *Fusarium circinatum* was able to grow on agar media amended with varying concentrations of Proline[®] and Pageant[®] - BASF (Table 5). Potato Dextrose Agar (Difco[®] PDA) was amended with each fungicide after autoclaving and just before pouring the plates. Twenty plates of each fungicide concentration and 20 non-amended PDA plates as a control were used. A #4 cork-borer (8 mm) plug of *Fusarium circinatum*, taken from a 2-wk-old culture, was placed at the center of each plate. The radial growth of the fungus was measured over a period of 11 days. To determine if the treatments were either fungicidal (killed the fungus) or fungistatic (stopped fungal growth), 11 days after placing onto the amended media, the agar plugs within each treatment were removed and plated onto non-amended media. Fungal growth on the non-amended media was recorded for another 5 days.

Pitch Canker Greenhouse Trials

Longleaf seed known to be infested with *Fusarium circinatum* was stratified for 10 days and sown to Ray Leach[®] containers in the greenhouse in May 2008. To ensure disease and increase fungal pressure, an 8 mm agar plug from a 2-wk-old stock culture of *Fusarium circinatum* was added to ½ of the container cavities at the time of sowing. After sowing longleaf seed, all cavities were covered with a thin layer of coarse perlite and misted. In addition to the fungal plug of *Fusarium circinatum*, ½ of the containers were sprayed with Proline[®] at sowing and every 2 weeks throughout the study. There were 20 container sets sown to longleaf pine, each container set had 20 cavities for each treatment as follows: Trmt #1 = *F. circinatum* & no Proline[®] spray, Trmt #2 = *F. circinatum* & Proline[®] spray, Trmt #3 = No *F. circinatum* & no Proline[®] spray, Trmt #4 = No *F. circinatum* & Proline[®] spray. Following germination, seedling counts were measured weekly for 4 weeks and then once per month until October 2008. Samples of dead seedlings were later assayed to confirm the presence of *Fusarium circinatum*.

Rhizoctonia Foliar Blight Laboratory Trials

Laboratory fungal growth studies were conducted to determine if *Rhizoctonia solani* was able to grow on agar media amended with Proline[®] at 1x, 0.25x and 0.0625x the label rate of 365 ml/ha (5 fl oz/ac). Potato Dextrose Agar (Difco[®] PDA) was amended with Proline[®] after autoclaving and just prior to pouring the plates. There were 20 PDA plates of each fungicide concentration and 20 non-amended PDA plates used as a control. A #4 cork-borer (8 mm) plug of *Rhizoctonia solani* taken from a 12-day old culture was placed at the center of each plate. The radial fungal growth was measured over a period of 7 days. To determine if the treatments were fungicidal (killed the fungus) or fungistatic (stopped fungal growth), 7 days after placing the plugs onto the media, the agar plugs were removed from the amended agar media and placed onto a non-amended agar plate. Fungal growth on the non-amended agar plate was recorded for another 5 days.

Rhizoctonia Foliar Blight Field Trials

In 2008 a nursery in Mississippi tested Proline[®] 402 ml/ha (5.5 fl oz/ac), and Heritage[®] (50% azoxystrobin – 1.68 kg/ha (24 oz/acre)) operationally for the control of *Rhizoctonia foliar blight*. A randomized block design with four replications was used in a nursery section growing its second seedling crop following soil fumigation. Each replication plot was 12 m x 18 m wide with a non-treated plot (6 m x 18 m) left in the middle of the field as the disease control. Fungicides were applied on a two week interval beginning July 15, 2008 using a Hardee 1532 liter sprayer with a 9-bed spray boom with nozzles on 0.5 m centers. A total of 8 applications of both fungicides were made. Temperature and relative humidity 25.4 cm above the seed bed were recorded using a HOBO[®] data logger.

In early December 2008, seedling densities, disease incidence, severity and seedling loss were calculated in 2 subplots within each treatment plot. From each subplot, 30 seedlings were hand-lifted and later measured to determine seedling quality, root collar diameter, height, dry weight and root morphology for each treatment.

RESULTS

Fusiform Rust Greenhouse Trials

The Southern Forest Nursery Management Cooperative has tested many fungicides over the years looking for an efficacious alternative for Bayleton[®] (Carey 2004, Starkey and Enebak 2008). The first fungicide tested that provided disease control equal to or better than Bayleton[®] was Provost[®] (Fig. 1). Provost[®] is made up of prothioconazole and tebuconazole (Table 1) however, when Folicur[®] (tebuconazole) was tested, 50% of the seedlings formed fusiform rust galls and it was determined that disease control achieved with Provost[®] was due to the prothioconazole portion within that compound. When it came time to re-examine Provost[®], a technical representative suggested testing a new fungicide, Proline[®] (prothioconazole) which was first registered in the US in 2007. In subsequent greenhouse trials, Proline[®] provided control of fusiform rust on loblolly pine equal to or greater than Bayleton[®] as a foliar spray (Fig. 1, Table 3). In addition, when tested as a seed treatment, there was no reduction in seed germination and Proline[®] had disease control equal to that obtained with the current standard Bayleton[®] (Table 2).

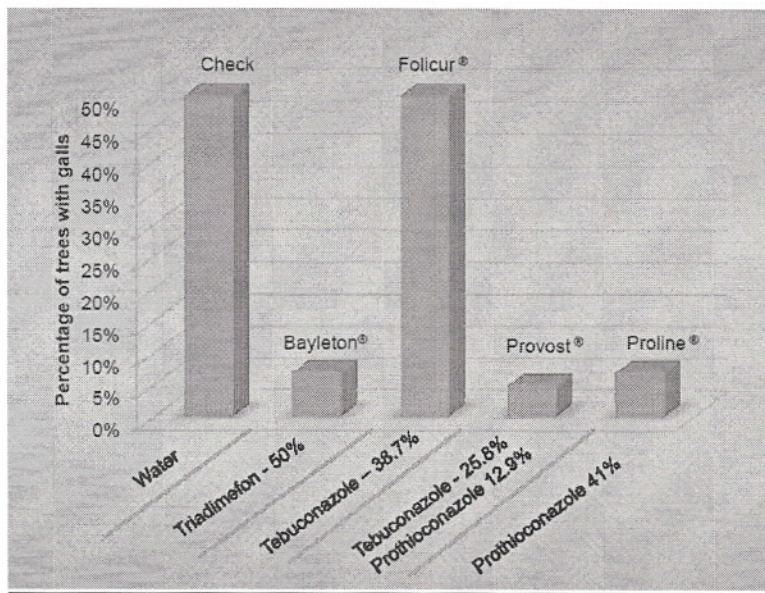


Figure 1. Three year average fusiform rust control on loblolly pine using foliar applications of fungicides.

Table 2. Seed treatment rates, germination and mean percent fusiform rust infection – 2008.

Seed Treatment Fungicides	% Germination	% Infection
Bayleton®	92%	0.0% a
Provost® 433 SC	96%	0.0% a
Proline® 480 SC	96%	1.0% a
USFS Check Seedlings		45%

Table 3. Foliar treatment rates and mean percent fusiform rust infection – 2008.

Foliar Treatment Fungicides	Foliar Rate ¹	% Infection
Bayleton®	560 g/ha (8 oz/a)	7.1% a
Provost® 433 SC	621 ml/ha (8.5 fl oz/a)	2.5% a
Proline® 480 SC	365 ml/ha (5 fl oz/a)	6.9% a
USFS Check Seedlings		45%

¹Based upon 280 l/ha (30 gal of water/acre)

Fusiform Rust Field Trials

At the South Carolina Forestry Commission's Nursery in Trenton, SC, the level of rust infection in the control plots was zero so the ability of Proline® to control fusiform rust infection in the field at that location could not be properly evaluated. However, at the Arborgen Nursery in Shellman, GA, 54% of the seedlings in the control plots were infected and had developed the characteristic symptom of stem swellings and galls by the end of the growing season in December 2008. No stem swellings or galls were detected on seedlings in any of the Proline®, Provost® or Bayleton® treated plots. There were no differences in the seedling quality (RCD, biomass) among the treatments except for seedling height in the control plots. Seedlings in the control plots were significantly taller than the three fungicidal treatment plots; this was most likely due to the seedlings in the control plots not getting top-clipped at the end of the season (the nursery was not going to sell the non-treated seedlings). Proline®-treated seedlings had significantly longer roots and a larger number of root tips than seedlings in the non-sprayed control plots (Table 4). Six months after outplanting, Proline®-treated and non-treated seedlings were similar in height and survival.

Table 4. Root length, average root diameter, root volume and number of root tips for each fungicide treatment¹.

	Total Root Length (cm)	Average Diameter (mm)	Root Volume (cm ³)	# of Root Tips
Proline®	320.7 a	0.59 a	0.89 a	854.1 a
Provost®	304.3 a	0.61 a	0.88 a	827.3 a
Bayleton®	287.8 ab	0.60 a	0.82 a	798.1 a
Control	241.4 b	0.63 a	0.76 a	683.6 b
lsd.05	53.4	0.04	0.21	105

¹Within column means followed by same letter do not differ at 0.05 level using Duncan's Multiple range Test

Table 5. Fungicide, active ingredient and rate used in *Fusarium circinatum* amended media trial.

Fungicide	Active Ingredient	Rate	ppm
Proline® 480 SC	prothioconazole – 41%	1x = 365 ml/ha (5 fl oz/a) ¹	1300
		0.5x = 183 ml/ha (2.5 fl oz/a)	650
		0.25x = 91 ml/ha (1.25 fl oz/a)	325
		0.125x = 46 ml/ha (0.625 fl oz/a)	162
		0.0625x = 23 ml/ha (0.321 fl oz/a)	81
Pageant®	pyraclostrobin 12.8% boscalid 25.2%	1x = 104.8 g/100 l (14 oz/100 gal)	1100
		0.5x = 52.4 g/100 l (7 oz/100 gal)	550
		0.25x = 26.2 g/100 l (3.5 oz/100 gal)	225

¹Based upon 280 l/ha (30 gal of water/acre)

Pitch Canker Laboratory Trials

In vitro fungal growth on agar media amended with Proline® resulted in 100% fungicidal control against *Fusarium circinatum* as fungal growth did not occur on any of the Proline®-amended PDA plates for any concentration examined for the 11-day experiment (Fig. 2). All

six rates of Proline[®] are at 0 mm on the Y-axis in Figure 2. On some Proline[®]-amended plates, the fungus grew from the original 8 mm plug for several mm, but never touched the agar surface. The appearance was that of a mushroom cap suspended over the soil. *Fusarium circinatum*, while somewhat inhibited on Pageant[®]-amended agar, was able to grow on all concentrations of Pageant[®] tested. There were no differences among the 3 concentrations of Pageant[®] tested. *Fusarium circinatum* growth on the non-amended control plates was significantly greater than either Pageant[®] - or Proline[®]-amended plates.

After 11 days, the plugs were removed from the amended media and put onto non-amended agar media. None of the agar plugs from the Proline[®] amended plates resumed fungal growth when returned to non-amended agar indicating that Proline[®] was fungicidal to *Fusarium circinatum*. However, agar plugs from the Pageant[®] amended media did resume growth on the non-amended agar indicating that Pageant[®] was fungistatic to *F. circinatum*.

Pitch Canker Greenhouse Trials

A biweekly application at 365 ml/ha (5 fl oz/a) on longleaf pine to control pitch canker (*Fusarium circinatum*) resulted in an 11% increase in seedling production over non-treated seedlings with no fungal plug added (Table 6). The percentage of seedlings obtained for no fungal plug and no Proline[®] (Trmt #3) is what a nursery sowing this seed would expect to obtain without fungicidal control. The same relationship held true with cavities that had a fungal plug added (increased disease pressure), for example, cavities with *F. circinatum* added to the cavity and no Proline[®] sprays had 62% fill at week 11. This was significantly less than cavities with no fungal plug and Proline[®]. Cavities with a fungal plug and Proline[®] had 17% greater fill percentage than without Proline[®]. Dead seedlings from Trmt #2 and #4 tested positive for *Fusarium circinatum*. Longleaf seedlings receiving Proline[®] sprays were significantly larger (height, root collar diameter and shoot dry weight) than non-Proline[®] treated seedlings.

Rhizoctonia Blight Laboratory trials

Agar media amended with Proline[®] resulted in 100% control against *Rhizoctonia solani* as fungal growth did not occur on any of the Proline[®]-amended PDA plates for any concentration used for the 7 day experiment (Fig. 3). All three rates of Proline[®] are at 0 mm on the Y-axis in Figure 3. After 7 days, the plugs were removed from the amended media and placed onto non-amended agar media. The agar plugs from each rate of the Proline[®] amended media resumed growth on the non-amended agar indicating that Proline[®] was fungistatic to *Rhizoctonia solani*.

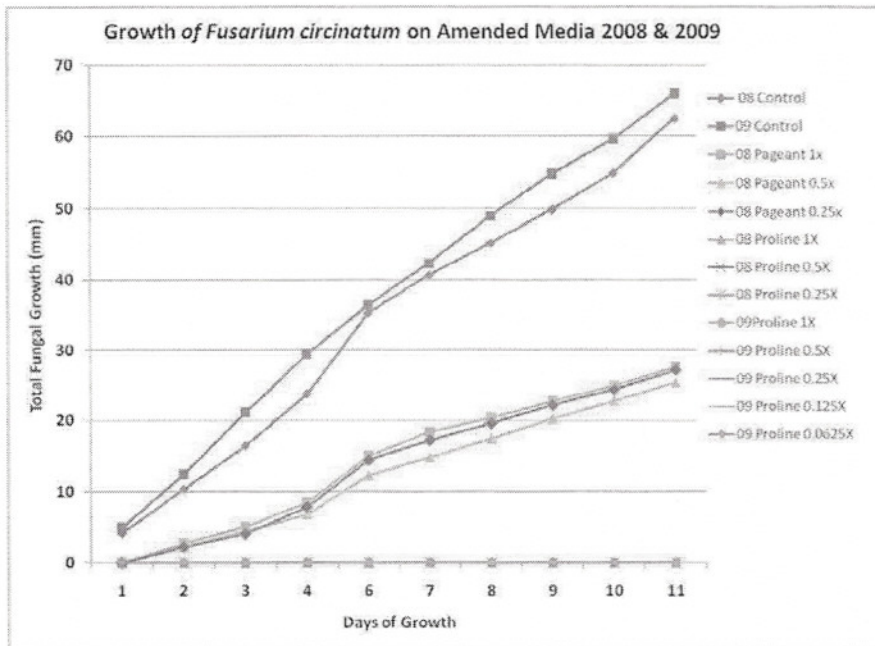


Figure 2. Radial growth of *Fusarium circinatum* on fungicide-amended and non-amended agar.

Table 6. Fill percentage and longleaf seedling quality in greenhouse pitch canker study¹.

Trmt #	Treatments	Proportion of Cavities Filled	Height (cm)	RCD (mm)	Top Dry Weight (g)
1	Fungal Plug + Proline [®]	0.79 a	32.0 a	4.6 a	1.40 a
2	Fungal Plug No Proline [®]	0.62 c	28.2 b	4.7 a	1.23 b
3	No Fungal Plug + Proline [®]	0.80 a	31.8 a	4.7 a	1.42 a
4	No Fungal Plug No Proline [®]	0.69 b	28.9 b	4.3 b	1.22 b
	lsd	0.07	0.5	0.2	0.11

¹Within column means followed by same letter do not differ at 0.05 level using Duncan's Multiple Range Test

Rhizoctonia Blight Field Trials

Disease incidence, severity and number of seedlings lost in the Proline[®]-treated plots was significantly lower than in the Heritage[®] and non-treated control plots (Table 7). An estimate of the potential loss (assuming similar incidence and severity throughout the acre area) indicated that losses from Proline[®] were negligible (0.03%). There were no significant differences in either seedling quality or root morphology, although the controls had numerically fewer seedlings (Table 7). The potential monetary loss in Table 7 reflects the seedling loss in the test plot, not the whole nursery as Rhizoctonia foliage blight tends to occur in isolated foci in susceptible seedlots. This particular nursery reported that within these susceptible seedlots, total loss to the disease would be less than 0.5%. Proline[®] was effective in reducing seedling loss due to Rhizoctonia that normally would occur. In years when the environmental parameters do not favor spread of the fungus through the seedling beds, Heritage[®] may provide a suitable level of control.

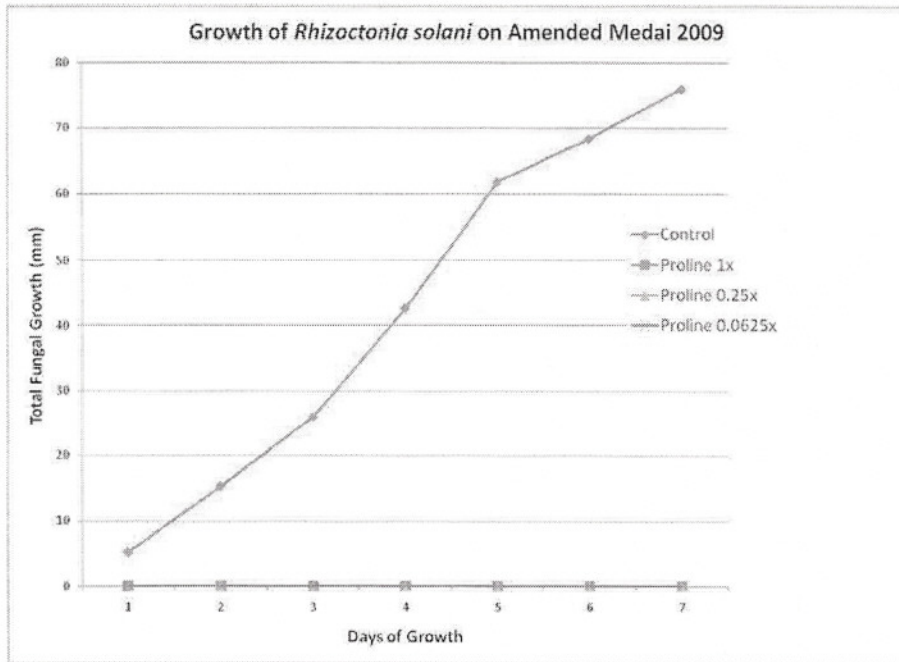


Figure 3. Radial growth of *Rhizoctonia solani* on fungicide-amended and non-amended media.

Table 7. Seedling density and disease loss as measured by incidence, severity and seedling loss per m² and potential loss per hectare caused by *Rhizoctonia* foliage blight.

Trmt	Seedling Density per m ²	Disease Incidence ¹	Disease Severity ²	Seedling loss per m ² ³	Potential Loss per ha
Control	246	0.354	0.182	32	\$10,864
Heritage [®]	254	0.162	0.083	13	\$4,198
Proline [®]	255	0.003	0.001	0.1	\$44
<i>Prob > F</i>	0.7762	0.0004	0.0004	0.0031	-

¹ Incidence = proportion of bed feet within a 1x4' frame with *Rhizoctonia* Foliar Blight

² Severity = proportion of tissue affected by *Rhizoctonia* Foliar Blight

³ Seedlings loss = # trees/drill x incidence/drill x severity /drill x seedling density

⁴ Controls were not included in the statistical analysis due to lack of replication among blocks.

DISCUSSION

Laboratory, greenhouse and field trials have shown Proline[®] to be efficacious against three fungal pathogens that cause damage and seedling mortality in forest-tree nurseries. Disease control of all three fungi using Proline[®] was obtained using rate of 365 ml/ha (5 fl oz/a) which is within the current Proline[®] range of 183 to 416 ml/ha (2.5 – 5.7 fl oz/ac) for registered crops. There is also an annual maximum use rate for each crop and these

laboratory studies show that Proline[®] is capable of controlling fungi *in vitro* at rates much lower than 365 ml/ha (5 fl oz/a). The key to any fungicide application is to apply the minimum rate necessary to control the disease and caution should be used when applying laboratory results to field or greenhouse studies. Small trials testing this product under the different environmental conditions that occur in nurseries are warranted prior to becoming operational.

Proline[®] is fungicidal to *Fusarium circinatum*, but is fungistatic on *Rhizoctonia* spp. Therefore, the timing of consecutive applications of Proline[®] would be important for the efficacious control of *Rhizoctonia* foliar blight in nurseries. Preliminary studies have shown that seed germination is not inhibited in loblolly, longleaf, slash or shortleaf pine. However, the minimum rate and method of application still must be examined as well as the minimum number of applications necessary to control pitch canker. Pitch canker losses occur either from external seed borne fungi or later in the season from seed infected internally and there may be a difference in seed treatment or foliar applications to control both of these modes of infection.

As part of the Southern Forest Nursery Management Cooperative's mission to bring new chemistry to its members, in early 2009, as a result of various experiments over the past three years, and in cooperation with Bayer CropScience, an application was filed with the US EPA in 6 southern US states for a Proline[®] 24(c) label. The intended Proline[®] label was for the control of pitch canker and *Rhizoctonia* foliar blight in loblolly and longleaf pine. Approval had been received in 5 of the 6 states when in March, 2009, US EPA requested Bayer CropScience pull the approved 24(c) labels. The US EPA determined that the forest nursery use is a new non-food use that requires a separate ecological risk assessment, and the existing data on file only supports food crops. In response to US EPA's denial, Bayer CropScience provided a response to support the continued use under the Section 24(c). In their response, Bayer's support of the 24(c) was based on several reasons including; 1) the minor acreage involved, 2) the use pattern is only for nursery and not forestry, 3) the proposed use pattern has a similar application method and exposure as the already registered crop use, and 4) the proposed use pattern poses no greater risk (or lower risk) compared to the currently registered uses. However, in the end, the US EPA did not change their ruling and Proline[®] is not yet available for forest-tree nurseries. Several other labeling efforts (IR4 and Section 18) were explored but found not feasible. The Southern Forest Nursery Management Cooperative is now pursuing requirements with US EPA and Bayer CropScience for a full product registration.

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Forest Service

Southern Region

Forest Health Protection
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2010

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Diseases and Insects in Forest Nurseries

Hilo, Hawai'i USA
July 13 to 17, 2009

Edited by
Michelle M. Cram

Forest Health Protection
United States Department of Agriculture
Forestry Service, Southern Region
Forest Health Protection Report 10-01-01

2010