

# The Use of Proline<sup>®</sup> (Prothioconazole) to Control Pitch Canker, *Rhizoctonia* Foliar Blight, and Fusiform Rust in Forest Seedling Nurseries and Efforts to Acquire Registration

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Starkey TE, Enebak SA. 2011. The use of Proline<sup>®</sup> (prothioconazole) to control pitch canker, *Rhizoctonia* foliage blight, and fusiform rust in forest seedling nurseries and efforts to acquire registration. In: Riley LE, Haase DL, Pinto JR, technical coordinators. National Proceedings: Forest and Conservation Nursery Associations—2010. Proc. RMRS-P-65. Fort Collins, CO: USDA Forest Service, Rocky Mountain Research Station: 49-57. Available at: [http://www.fs.fed.us/rm/pubs/rmrs\\_p065.html](http://www.fs.fed.us/rm/pubs/rmrs_p065.html)

**Abstract:** Laboratory, greenhouse, and field trials have shown Proline<sup>®</sup> to be efficacious against three fungal pathogens that cause damage and seedling mortality in forest seedling nurseries. Disease control using Proline<sup>®</sup> has been obtained at 365 ml/ha (5 fl oz/ac) 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 at 365 ml/ha (5 fl oz/ac) controlled pitch canker (*Fusarium circinatum*) on longleaf pine (*P. palustris*) and shortleaf pine (*P. echinata*), and resulted in a significant increase in seedling production over non-treated seedlings. *In vitro* studies using Proline<sup>®</sup>-amended agar 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<sup>®</sup> at 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 US\$ 1735, 373, and 0 for non-treated, azoxystrobin, and Proline<sup>®</sup>, respectively. A second trial was conducted applying Proline<sup>®</sup> every 3 weeks. The monetary loss per acre was US\$ 2142, 1235, and 1 for non-treated, azoxystrobin, and Proline<sup>®</sup>, respectively. In addition to disease control, Proline<sup>®</sup>-treated seedlings were significantly larger and appeared greener than non-treated seedlings. Proline<sup>®</sup> did not affect longleaf, loblolly, slash (*P. elliotii*), or shortleaf pine seed germination.

**Keywords:** loblolly pine, longleaf pine, slash pine, shortleaf pine, chemical control, fusiform rust, pitch canker, *Rhizoctonia* foliar blight

## 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 seedling 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 (*P. elliotii*) pine seedlings is fusiform rust caused by *Cronartium quercuum* f. sp. *fusiforme*. Since 1980, formulations of Bayleton<sup>®</sup> (triadimefon) have been the primary chemical used to control this disease (Carey and Kelley 1993) and have consistently provided excellent cost-effective control as both seed treatments and foliar sprays (Snow and others 1979; Carey and Kelley 1993; Carey 2004).

In July 2007, Bayer CropScience received US Environmental Protection Agency (EPA) cancellation order for Bayleton<sup>®</sup>. 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 seeds and seedlings was still allowed. However, the availability of Bayleton<sup>®</sup> remains unsettled, resulting in nurseries having difficulty locating and obtaining the product; an alternative is needed.

Pitch canker, caused by the fungus *Fusarium circinatum* (= *Fusarium subglutinans*), can cause significant seed and seedling mortality in nurseries and later after outplanting in the field (Dwinell 1978; Dwinell and Barrows-Broadus 1981; Kelley and Williams 1982; Barrows-Broadus and Dwinell 1984; Blakeslee and Rockwood 1984; Lowerts and others 1985; Carey and Kelley 1994). In the southern US, infection and seedling losses have been reported on loblolly, slash, longleaf (*P. palustris*),

shortleaf (*P. echinata*), and Virginia (*P. virginiana*) pines. The fungus is also considered one of the most threatening diseases in many areas of the world, particularly the South African nurseries (Viljoen and Wingfield 1994; Storer and others 1998). Unlike fusiform rust, there are no fungicides registered for the control of pitch canker on either seeds or seedlings, and nursery growers are forced to use either bleach or hydrogen peroxide to disinfect seeds. Many of the fungicides registered for use in forest seedling nurseries indicate that they control fungi in the genus *Fusarium*. However, the degree of control of *F. circinatum* was insufficient to justify the cost of application (Runion and others 1993).

Longleaf and loblolly pines are particularly susceptible to *Rhizoctonia* foliar blight. The disease is caused by a species of *Rhizoctonia*, 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 (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. The disease is often not observed until seedlings are top-clipped to maintain seedling shoot-to-root ratios and heights. Varying degrees of resistance among seedling families can be found, with US Gulf Coast seedlots more susceptible than Piedmont sources, and the disease is rarely observed on slash pine (McQuage 2009). *Rhizoctonia* foliar blight generally is not distributed uniformly throughout a nursery and is limited to foci within nursery beds. The disease is also more severe in second crop, post-soil fumigated 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 have been underway at the Southern Forest Nursery Management Cooperative (SFNMC) 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, that 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 44 countries and in the US for food crops including peanuts, barley, wheat, sugar beets, beans, soybeans, and rapeseed.

Although Proline® is not currently registered for commercial use in US forest seedling 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 seedling nurseries. Data collected from such studies has been used in an attempt to obtain a full-use label from Bayer CropScience and US EPA for disease control in forest seedling nurseries in the southern US.

## Methods

### Fusiform Rust Greenhouse Trials

**Seed Treatments**— In 2006, 2007, 2008, and 2009, loblolly pine seeds were stratified for 4 weeks, and then treated with fungicides prior to sowing (Table 1). For dry formulation fungicides, seeds were first moistened in a seed tumbler, and the fungicide was added at the rate of 25 g/10 kg (2 oz/50 lbs) of seeds. For liquid fungicides, approximately 26 ml (2 fl oz) of the product were used per 10 kg (50 lbs) of seeds, and slowly added to pine seeds in a tumbler. The fungicide and seeds were tumbled until dry. All treated seeds, as well as non-treated seeds for both positive and negative controls, were double sown in Ray Leach containers (164 ml [10 in<sup>3</sup>], Stuewe and Sons, Tangent, OR) 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 <sup>1</sup>		Seed treatment
		1X	2X	1X
Check (water)				
Bayleton®	tridimefon 50%	560 ml/ha (8 oz/ac)		25 g/10 kg seeds (2 oz/50 lb seeds)
Folicur®	tebuconazole 38.7%	292 ml/ha (4 fl oz/ac)	584 ml/ha (8 fl oz/ac)	
Provost® 433 SC	prothioconazole 12.9% tebuconazole 25.8%	621 ml/ha (8.5 fl oz/ac)	1.24 l/ha (17 fl oz/ac)	25 g/10 kg seeds (2 oz/50 lb seeds)
Proline® 480	prothioconazole 41%	365 ml/ha (5 fl oz/ac)		25 g/10 kg seeds (2 oz/50 lb seeds)

<sup>1</sup> Based upon 280 l water/ha (30 gal water/ac)

**Foliar Treatments**—Loblolly pine seeds were stratified for 40 days and then double sown to Ray Leach containers. Following germination, seedlings were thinned to one seedling per container and then randomly assigned fungicidal treatments. Seven weeks post-sowing, seedlings were treated at the Auburn University Pesticide Research Facility (Auburn, AL). A Bayleton® and a water check were included for both positive and negative controls, respectively. Application rates for each fungicide included a 1x as listed in Table 1. Provost® was tested in 2007 and 2008. Proline® was only tested in 2008 and 2009. 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 Forest Service Rust Screening Laboratory in Asheville, NC. Seedlings were allowed to acclimate to the new growing conditions for 5 to 7 days, and then challenged with 20,000 basidiospores/ml of *Cronartium quercum f.sp. fusiforme* (collected from Zone 7 inoculum area) using standard inoculation protocols for the laboratory. Seedlings remained under the care of the 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), with plot sizes of 0.24 ha (0.6 ac) and 0.405 ha

(1.0 ac), 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 at a rate of 365 ml/ha (5 fl oz/ac) and 621 ml/ha (8.5 fl oz/ac), 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 fungicides on seedling survival.

In 2009, Proline® and Bayleton® were operationally field tested at the Arborgen Nursery in Shellman, GA. Experimental design, rates, and application methods were similar to those described above.

## Pitch Canker Laboratory Trials

Fungal growth studies were conducted in the laboratory to determine if *F. circinatum* was able to grow on agar media amended with Proline® and Pageant® - BASF (Table 2). 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 *F. circinatum*, taken from a 2-week-old culture, was placed at the center of each plate. The radial growth of the fungus was measured over a period of 11 days and recorded. 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.

**Table 2.** 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/ac) <sup>1</sup>	1300
		0.5x = 183 ml/ha (2.5 fl oz/ac)	650
		0.25x = 91 ml/ha (1.25 fl oz/ac)	325
		0.125x = 46 ml/ha (0.625 fl oz/ac)	162
		0.0625x = 23 ml/ha (0.321 fl oz/ac)	81
Pageant®	pyraclostrobin 12.8%	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
	boscalid 25.2%		

<sup>1</sup> Based upon 280 l water/ha (30 gal water/acre)

## Pitch Canker Greenhouse Trials

In 2008 and 2009, longleaf pine seeds were stratified for 10 days and sown to Ray Leach containers in the greenhouse in May. In 2009, slash, loblolly, and shortleaf seeds were stratified for 21, 40, and 45 days, respectively, and sown into Ray Leach containers in the greenhouse in May. Only the loblolly and shortleaf seeds were confirmed to be infested with *F. circinatum*. To increase fungal pressure, an 8 mm agar plug from a 2-week-old stock culture of *F. circinatum* was added to half of the container cavities at the time of sowing. After sowing longleaf pine seeds, all cavities were covered with a thin layer of coarse perlite and misted. In addition to the fungal plug of *F. circinatum*, half 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: treatment 1 = *F. circinatum* and no Proline® spray; treatment 2 = *F. circinatum* and Proline® spray; treatment 3 = no *F. circinatum* and no Proline® spray; treatment 4 = no *F. circinatum* and Proline® spray. Following germination, seedling counts were measured weekly for 4 weeks and then once per month until October. Dead seedlings were later assayed to establish the cause of death.

## *Rhizoctonia* Foliar Blight Laboratory Trials

Fungal growth studies were conducted in the laboratory 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 and recorded. To determine if Proline® was 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 forest seedling nursery tested Proline® at a rate of 402 ml/ha (5.5 fl oz/ac) and Heritage® (50% azoxystrobin) at a rate of 1.68 kg/ha (24 oz/ac) 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 x 18 m (39 x 59 ft) with a non-treated plot (6 m x 18 m [20 x 59 ft]) left as the disease control. Fungicides were applied on a 2-week interval beginning 15 July 2008 using a Hardee 400-gal (1532-L) sprayer (EVH Manufacturing Company, Loris, SC) with a 9-bed spray boom with nozzles on 0.5 m (1.6 ft) centers. A total of eight applications of both

fungicides were made. Temperature and relative humidity 25.4 cm (10 in) above the seed bed were recorded using a HOBO data logger (Onset®, Bourne, MA).

In early December 2008, seedling densities, disease incidence, severity, and seedling loss were calculated in two 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.

In 2009, the identical study was established at the same nursery using the same experimental design and application methods. However, the fungicides were applied every 3 weeks instead of every 2 weeks to determine the minimal spraying time interval for disease control.

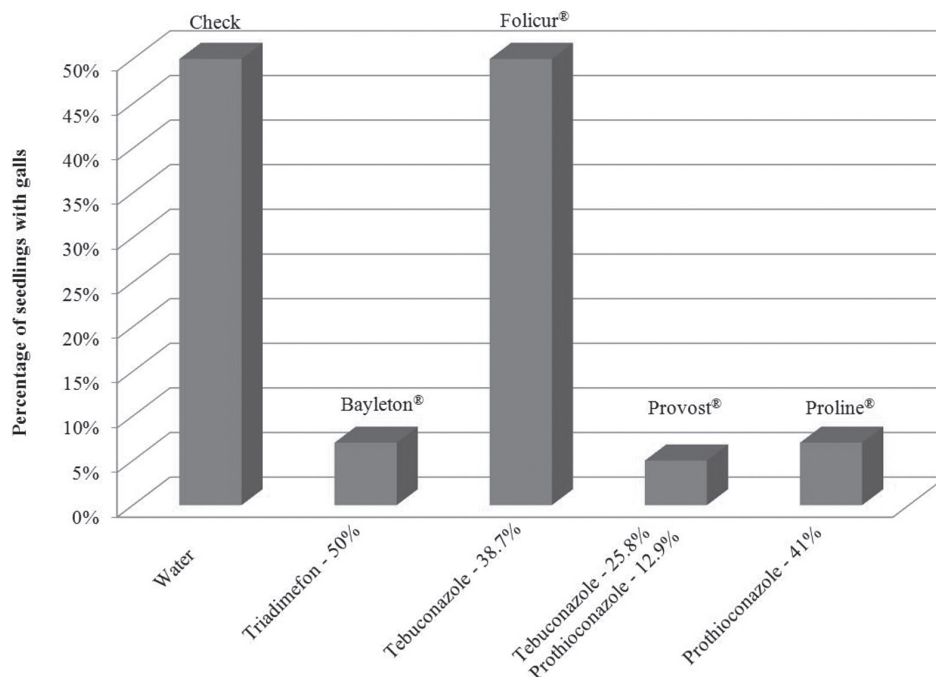
## Results and Discussion

### Fusiform Rust Greenhouse Trials

The SFNMC has tested many fungicides for an efficacious alternative for Bayleton® (Carey 2004; Starkey and Enebak 2008). One fungicide that provided disease control equal to or better than Bayleton® was Provost® (Figure 1), which contains prothioconazole and tebuconazole (Table 1). However, when Folicur® (containing only tebuconazole) was tested, 50% of the seedlings developed fusiform rust galls. It was later determined that disease control achieved with Provost® was due to the prothioconazole portion within that fungicide. A technical representative suggested testing Proline® (prothioconazole), which was 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 (Figure 1, Table 3). In addition, when tested as a seed treatment prior to sowing for disease control, there was no affect on seed germination, and Proline® had disease control equal to that obtained with the current standard Bayleton® (Table 4). If registered for forest seedlings, Proline® will provide a second efficacious fungicide for the control of fusiform rust in the southern US.

### Fusiform Rust Field Trials

At the South Carolina Forestry Commission Nursery, there was no rust infection in the control plots, so Proline® could not be evaluated. By the end of the growing season in December 2008 at the Arborgen Nursery, 54% of the seedlings in the control plots had developed main stem swellings or galls. In contrast, no stem swellings or galls were observed 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 heights and root mass. Seedlings in the control plots were significantly taller than seedlings grown in the three fungicidal treatment plots. This was due to control plots not getting top-clipped, because the nursery was not going to sell non-treated, infected seedlings. Proline®-treated seedlings had significantly longer roots and a larger number of root tips than seedlings in the non-sprayed control plots (Table 5).



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

**Table 3.** Foliar treatment rates and mean percentage of fusiform rust infection in 2008<sup>2</sup>.

Foliar treatment fungicides	Foliar rate <sup>1</sup>	% Infection
Bayleton®	560 g/ha (8 oz/ac)	7.1% <sup>a</sup>
Provost® 433 SC	621 ml/ha (8.5 fl oz/ac)	2.5% <sup>a</sup>
Proline® 480 SC	365 ml/ha (5 fl oz/ac)	6.9% <sup>a</sup>
USFS Check Seedlings		45%

<sup>1</sup> Based upon 280 l water/ha (30 gal water/ac)

<sup>2</sup> Within column means followed by same letter do not differ at  $\alpha=0.05$  using Duncan's Multiple Range Test.

**Table 4.** Seed treatment rates, germination, and mean percentage of fusiform rust infection in 2008<sup>1</sup>.

Seed treatment fungicides	% Germination	% Infection
Bayleton®	92%	0.0% <sup>a</sup>
Provost® 433 SC	96%	0.0% <sup>a</sup>
Proline® 480 SC	96%	1.0% <sup>a</sup>
USFS Check Seedlings		45%

<sup>1</sup> Within column means followed by same letter do not differ at  $\alpha=0.05$  using Duncan's Multiple Range Test.

To determine if treatments had any long-term affect on seedling growth and/or survival, seedlings from the Proline® and Bayleton® plots were outplanted following the 2008 growing season in a randomized complete block design in an area north of Auburn, AL. During the 2-year evaluation, there was no difference between Proline®- and Bayleton®-treated seedlings with respect to seedling height and survival.

**Table 5.** Root length, average root diameter, root volume, and number of root tips for each fungicide treatment<sup>1</sup>.

	Total root length (cm)	Average diameter (mm)	Root volume (cm <sup>3</sup> )	Number of root tips
Proline®	320.7 <sup>a</sup>	0.59 <sup>a</sup>	0.89 <sup>a</sup>	854.1 <sup>a</sup>
Provost®	304.3 <sup>a</sup>	0.61 <sup>a</sup>	0.88 <sup>a</sup>	827.3 <sup>a</sup>
Bayleton®	287.8 <sup>ab</sup>	0.60 <sup>a</sup>	0.82 <sup>a</sup>	798.1 <sup>a</sup>
Control	241.4 <sup>b</sup>	0.63 <sup>a</sup>	0.76 <sup>a</sup>	683.6 <sup>b</sup>
<i>Isd</i>	53.4	0.04	0.21	105

<sup>1</sup> Within column means followed by same letter do not differ at  $\alpha=0.05$  using Duncan's Multiple Range Test.

### Pitch Canker Laboratory Trials

*In vitro* fungal growth on agar amended with Proline® resulted in 100% fungicidal control against *F. circinatum*. Fungal growth did not occur on any of the Proline®-amended PDA plates for any concentration examined for the 11-day experiment (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. *F. circinatum*, while somewhat inhibited on Pageant®-amended agar, grew on all concentrations tested. *F. circinatum* growth on the non-amended control plates was significantly greater than either Pageant®- or Proline®-amended plates.

After 11 days, the agar plugs containing *F. circinatum* were removed from each of the amended media and put onto non-amended agar. Mycelia of *F. circinatum* did not resume growth when returned to non-amended agar. The lack of growth on non-amended media indicates that Proline® was fungicidal to *F. circinatum*. However, agar plugs from the Pageant®-amended medium did resume growth on the non-amended agar, indicating that Pageant® was fungistatic to *F. circinatum*.

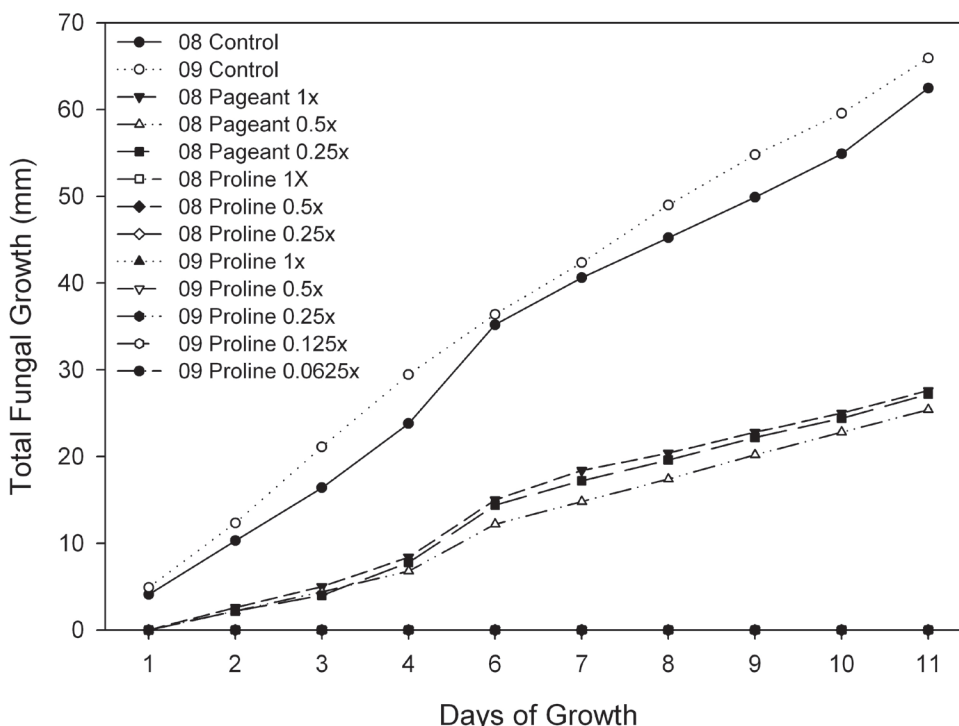


Figure 2. Radial growth of *Fusarium circinatum* on fungicide-amended and non-amended agar.

The fungicidal activity of Proline® on *F. circinatum* indicates that repeated applications throughout the season in a nursery may not be needed. Once the initial source inoculum has been controlled, repeated applications of Proline® may not be needed. Pitch canker losses occur either from external seed-borne fungi (early season) or later in the season from seeds infected internally. Further research needs to determine if several applications of Proline® early in the season will also control late season mortality.

### Pitch Canker Greenhouse Trials

A biweekly application at 365 ml/ha (5 fl oz/ac) on longleaf and shortleaf pine to control pitch canker (*Fusarium circinatum*) resulted in an 17% and 50% increase in seedling production over non-treated seedlings, respectively (Table 6). Most of the mortality in longleaf pine occurred early in the season, whereas, the greatest losses with shortleaf pine were later in the season. Not all shortleaf pine mortality was attributed to pitch canker. *Rhizoctonia* spp. was also isolated from dead shortleaf pine and slash pine late in the season. The application of Proline® to these pine species was effective in controlling both fungal pathogens in this study. The percentage (Table 6) of seedlings produced in treatments that did not get additional disease pressure (fungal plug) and no Proline® applied is what a nursery sowing these same seedlots would expect to obtain without any fungicidal control. Longleaf and shortleaf seedlings receiving Proline® and no fungal plug had significantly smaller root collar diameter that was due to seedling density. Seedling size generally increases with a decrease in seedling density (Landis 1990). The use of Proline® resulted in significantly greater seedling biomass for longleaf, shortleaf, and slash pine.

### *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 (Figure 3). After 7 days, the plugs were removed from the amended media and placed onto non-amended agar media and the mycelia of *R. solani* resumed growth, indicating that Proline® was fungistatic.

The fungistatic activity of Proline® on *R. solani* indicates that repeated applications throughout the period of peak infection in a nursery may be needed. New sources of inoculum can continually be reintroduced into a nursery bed through wind and soil/debris movement on machinery.

### *Rhizoctonia* Blight Field Trials

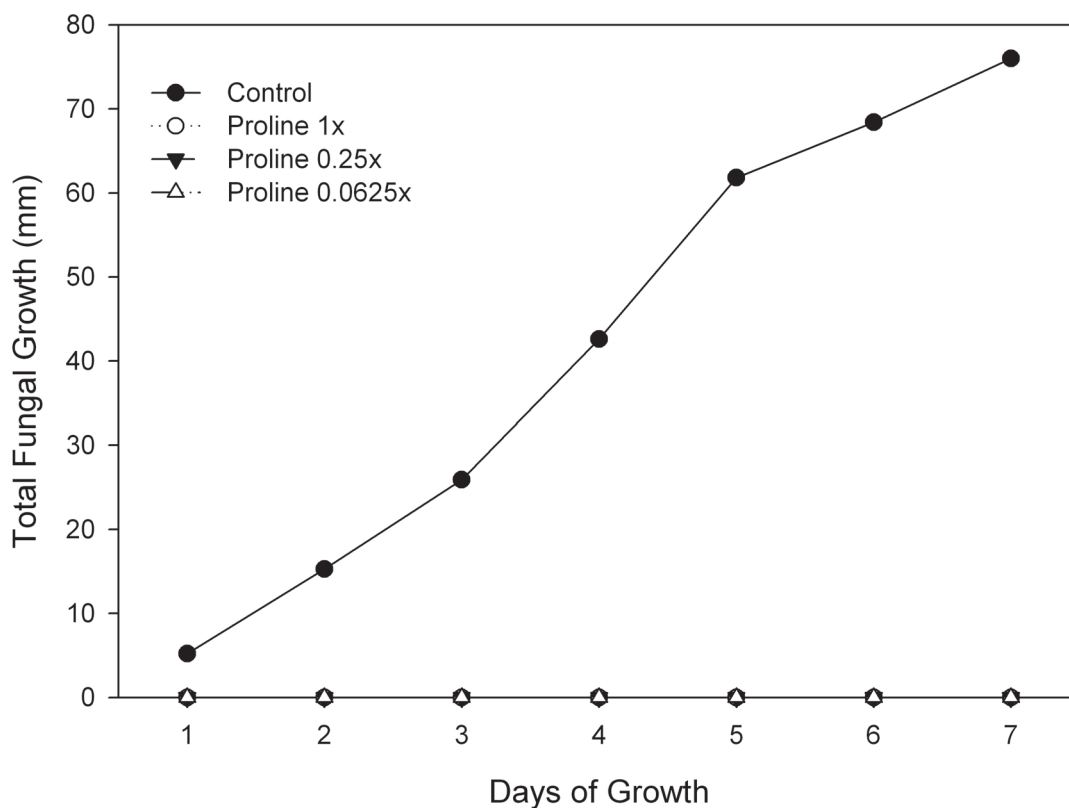
When Proline® and Heritage® were sprayed at label rates at 2-week intervals, disease incidence, severity, and number of seedlings lost in the Proline®-treated plots was significantly lower than in the Heritage® and non-treated control plots (Table 7). An estimate of the potential loss (assuming similar incidence and severity throughout the acre area) indicated that losses from Proline® were negligible (0%). There were no significant differences in either seedling quality or root morphology between fungicides tested, although the controls had numerically fewer seedlings. The potential monetary loss in Table 7 reflects the seedling loss in the test plot, not the whole nursery, because *Rhizoctonia* foliage blight tends to occur in isolated foci in susceptible seedlots.

**Table 6.** Fill percentage and longleaf, shortleaf, slash, and loblolly pine seedling quality in greenhouse pitch canker study<sup>1</sup>.

Pine species	Treatment	Percentage fill		Final		
		Week 5	Week 17	RCD (mm)	HT (cm) <sup>2</sup>	Biomass (gm/ft <sup>2</sup> )
Longleaf	Proline® + No Fungal Plug	88.3 <sup>a</sup>	88.3 <sup>a</sup>	4.8 <sup>c</sup>	14.1 <sup>ab</sup>	80.6 <sup>a</sup>
	No Proline® + No Fungal Plug	83.1 <sup>a</sup>	71.7 <sup>b</sup>	5.7 <sup>a</sup>	14.4 <sup>a</sup>	60.9 <sup>b</sup>
	Proline® + Fungal Plug	85.8 <sup>a</sup>	85.3 <sup>a</sup>	5.3 <sup>ab</sup>	13.6 <sup>b</sup>	77.7 <sup>a</sup>
	No Proline® + Fungal Plug	74.4 <sup>b</sup>	66.1 <sup>b</sup>	5.2 <sup>b</sup>	14.5 <sup>a</sup>	57.7 <sup>b</sup>
	<i>lsd</i>	6.1	7.2	0.4	0.5	6.7
Shortleaf	Proline® + No Fungal Plug	93.9 <sup>a</sup>	93.6 <sup>a</sup>	2.9 <sup>b</sup>	23.0 <sup>a</sup>	54.7 <sup>a</sup>
	No Proline® + No Fungal Plug	84.2 <sup>b</sup>	43.3 <sup>c</sup>	3.1 <sup>a</sup>	21.0 <sup>b</sup>	20.1 <sup>c</sup>
	Proline® + Fungal Plug	93.1 <sup>a</sup>	92.8 <sup>a</sup>	3.0 <sup>ab</sup>	22.9 <sup>a</sup>	58.3 <sup>a</sup>
	No Proline® + Fungal Plug	87.8 <sup>b</sup>	60.6 <sup>b</sup>	3.1 <sup>a</sup>	21.4 <sup>b</sup>	38.6 <sup>b</sup>
	<i>lsd</i>	4.6	10.4	0.1	1.2	6.7
Slash	Proline® + No Fungal Plug	91.9 <sup>a</sup>	91.7 <sup>a</sup>	3.7 <sup>ab</sup>	26.8 <sup>a</sup>	92.3 <sup>a</sup>
	No Proline® + No Fungal Plug	86.4 <sup>a</sup>	72.5 <sup>b</sup>	3.6 <sup>b</sup>	24.4 <sup>b</sup>	64.0 <sup>b</sup>
	Proline® + Fungal Plug	91.1 <sup>a</sup>	91.1 <sup>a</sup>	3.7 <sup>ab</sup>	25.5 <sup>ab</sup>	84.6 <sup>a</sup>
	No Proline® + Fungal Plug	83.3 <sup>b</sup>	74.4 <sup>b</sup>	3.8 <sup>a</sup>	25.6 <sup>ab</sup>	66.3 <sup>b</sup>
	<i>lsd</i>	5.9	8.2	0.1	1.4	8.5
Loblolly	Proline® + No Fungal Plug	91.4 <sup>a</sup>	91.4 <sup>a</sup>	3.1 <sup>b</sup>	25.4 <sup>c</sup>	77.7 <sup>a</sup>
	No Proline® + No Fungal Plug	90.6 <sup>a</sup>	88.3 <sup>a</sup>	3.4 <sup>a</sup>	29.3 <sup>ab</sup>	75.8 <sup>a</sup>
	Proline® + Fungal Plug	93.6 <sup>a</sup>	93.6 <sup>a</sup>	3.1 <sup>b</sup>	30.2 <sup>a</sup>	78.2 <sup>a</sup>
	No Proline® + Fungal Plug	91.6 <sup>a</sup>	90.3 <sup>a</sup>	3.3 <sup>a</sup>	28.5 <sup>b</sup>	78.9 <sup>a</sup>
	<i>lsd</i>	4.6	5.9	0.1	1.0	7.0

<sup>1</sup> Within column and within species means followed by same letter do not differ at α=0.05 using Duncan's Multiple Range Test.

<sup>2</sup> 1 cm = 0.4 in



**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<sup>1</sup>, severity<sup>2</sup>, and seedling loss<sup>3</sup> per ft<sup>2</sup> and potential loss per hectare caused by *Rhizoctonia* foliage blight (1 ft<sup>2</sup> = 0.09 m<sup>2</sup>).

TRT	Seedling density/ ft <sup>2</sup>	Disease incidence	Disease severity	Seedling loss/ft <sup>2</sup>	Potential loss/Ac (\$US)
Control <sup>4</sup>	22.9	0.354	0.182	3	\$1,735
Heritage	23.6	0.162	0.083	1.2	\$373
Proline	23.7	0.003	0.001	0.01	\$0
<i>Prob &gt; F</i>	0.7762	0.0004	0.0004	0.0031	—
Control <sup>4</sup>	16.8	0.509	0.213	3	\$2,142
Heritage	20.5	0.344	0.149	2.6	\$1,235
Proline	19.7	0.01	0.005	0.05	\$1
<i>Prob &gt; F</i>	0.51	0.0008	0.007	0.0013	—

<sup>1</sup> Incidence = proportion of bed area within a 0.4 m<sup>2</sup> (4 ft<sup>2</sup>) frame with *Rhizoctonia* foliar blight.

<sup>2</sup> Severity = proportion of tissue affected by *Rhizoctonia* foliar blight.

<sup>3</sup> Seedlings loss= seedling density x incidence/drill x severity /drill.

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

When the interval between fungicide sprays was increased to 3 weeks, Heritage® had a disease incidence of 34% compared to 1% for the Proline®. When comparing the two studies, the disease intensity more than doubled for the Heritage® applications, and the potential loss per acre increased by greater than three times when applied every two weeks rather than three weeks. When using Heritage® for *Rhizoctonia* foliage blight control, the interval between spray applications should be kept to the minimum as recommended on the label. This study suggests that the time interval between Proline® sprays using suggested label rates is not as critical as with Heritage®. It is possible that maintaining a 2-week spray schedule with a reduced level of Proline® may achieve the same economic level of control.

This particular nursery reported that within these susceptible seedlots, total seedling mortality to the disease would be less than 0.5%. Proline® was effective in reducing seedling mortality 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.

In summary, laboratory, greenhouse, and field trials have shown Proline® to be efficacious against three important fungal pathogens that cause damage and seedling mortality in forest seedling nurseries. Disease control of all three fungi using Proline® was obtained using rate of 365 ml/ha (5 fl oz/ac), that is within the current Proline® range of 183 to 416 ml/ha (2.5 to 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/ac). 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.

## Label Registration Efforts

Over 1 billion hardwood and conifer seedlings are produced in southern US forest seedling nurseries each year (Enebak

2009) on approximately 1012 ha (2500 ac). Despite the large number of seedlings produced, most chemical companies consider forest seedlings to be a low profit, minor crop and tend to avoid marketing products for such a small acreage. When the cost of discovery, development, and registration of a new pesticide exceeds US\$ 180 million (Whitford and others 2006), it is easy to understand why chemical companies focus their marketing efforts on commodities such as wheat, soybeans, and peanuts that will insure a profit from sales.

In the 1970s and 1980s, the SFNMC tested the efficacy of both registered pesticides and numbered compounds as provided and requested by chemical companies. Over time, due to the increased scrutiny by state and federal agencies, the cooperative found the registration of numbered compounds increasingly difficult to obtain and ceased testing compounds that were not currently registered for use in the United States. Currently, only registered pesticides are tested by the SFNMC with the hope of obtaining the necessary registration for use in forest seedling nurseries.

Part of the SFNMC mission statement is to bring new pesticide chemistry to its members. One of those new chemistries was prothioconazole, the active ingredient in Proline®. In early 2009, as a result of various experiments during several years, and in cooperation with Bayer CropScience, an application was filed with the US EPA in six southern US states for a Proline® 24(c) label. The intended special use label was for the control of pitch canker and *Rhizoctonia* foliar blight in loblolly and longleaf pine. Approval for its use had been received in five of the six states when in March 2009, US EPA requested Bayer CropScience pull the approved 24(c) labels. The US EPA determined that forest seedling nursery use is a “new non-food use” that requires a separate ecological risk assessment, and the existing data on file for Proline® only supports food crops. In response to US EPA request to pull the approved labels, Bayer CropScience requested the continued use under the Section 24(c) based on: 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 seedling nurseries. Several other labeling efforts (for example, IR4) were explored but found not feasible with a non-food crop.

In November 2009, after a number of conversations with both US EPA and Bayer CropScience, we were informed that our registration request for Proline® in forest seedling nurseries could be considered under the Pesticide Registration Improvement Renewal Act (PRIA) of 2007 under the category of “additional use, non-food; outdoor” (PRIA code R230). Bayer CropScience agreed to allow the request to go forward if the SFNMC were to pay the PRIA fee of US\$ 22,827.

In late December 2009, the US EPA acknowledged the Proline® registration package from Bayer CropScience for an additional use. The examination of Proline® for this additional, non-food, outdoor use is expected to take EPA about 15 months. Once this process has been completed, we anticipate a full label for Proline® to be registered for use on nursery seeds and seedlings of shortleaf, loblolly, slash, longleaf, and other pines, and other conifers and hardwoods. Until this is complete, nurseries are allowed under FIFRA rules to test a pesticide on areas less than 4 ha (10 ac) as long as they are collecting data for future use. Therefore, small trials testing this product under the different environmental conditions that occur in nurseries are warranted (and encouraged) prior to becoming operational.

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