RESEARCH REPORT 10-11

CONTROL OF PITCH CANKER, *RHIZOCTONIA* FOLIAGE BLIGHT AND FUSIFORM RUST USING PROLINE® AND EFFORTS TO ACQUIRE REGISTRATION

by
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INTRODUCTION

The availability of fungicides to control specific forest-seedling nursery diseases is either nonexistent, limited, or faces possible loss of U.S. label registration. Of the many insects and diseases that occur in forest-tree nurseries, three fungal pathogens stand out as problematic in southern U.S. 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 et.al. 1979, Carey and Kelley 1993, Carey 2004). In July 2007, Bayer CropScience received U.S. 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 U.S. label, its use on pine seed and seedlings was still allowed. However, the availability of Bayleton® 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 (Carey and Kelley 1994, Dwinell 1978, Barrows-Broaddus and Dwinell 1984, Blakeslee and Rockwood 1984, Lowerts et. al. 1985, Kelley and Williams 1982, Dwinell and Barrows-Broaddus 1981). In the southern U.S., 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. 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 *Fusarium 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* spp. *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. 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 U.S. gulf coastal seedlots more susceptible than Piedmont sources, and the disease is rarely observed on slash pine (McQuage, 2009 personal communication). *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 Cooperative 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 et al. 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 44 countries and in the U.S. for food crops including peanuts, barley, wheat, sugar beets, beans, soybeans and rapeseed.

Although Proline® is not currently registered for commercial use in U.S. 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 has been used in an attempt at obtain a full-use label from Bayer CropScience and U.S. EPA for disease control in forest-tree nurseries in the southern U.S.

METHODOLOGY

Study 1 - Fusiform Rust Greenhouse Trials

Seed treatments. In 2006, 2007, 2008 and 2009 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.

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 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 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.

Study 2 - 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 (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/a) and 621 ml/ha (8.5 fl oz/a), 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.

Study 3 - Pitch Canker Laboratory Trials

Fungal growth studies were conducted in the laboratory to determine if *Fusarium circinatum* was able to grow on agar media amended with 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 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.

Study 4 - Pitch Canker Greenhouse Trials
In 2008 and 2009, longleaf seed was stratified for 10 days and sown to Ray Leach® containers in the greenhouse in May. In 2009, slash, loblolly and shortleaf seed were stratified for 21, 40 and 45 days respectively and sown to Ray Leach® containers in the greenhouse in May. Only the loblolly and shortleaf seed was confirmed to be infested with *Fusarium circinatum.* To 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*, 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: 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. Dead seedlings were later assayed to establish the cause of death.

Study 5 - 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.

Study 6 - Rhizoctonia Foliar Blight Field Trials
In 2008, a forest-tree nursery 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 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 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 three weeks, instead of every two weeks to determine the minimal spraying time interval for disease control.

RESULTS AND DISCUSSION

Study 1 - Fusiform Rust Greenhouse Trials

The Southern Forest Nursery Management Cooperative 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® (Fig. 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 U.S. 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 prior to sowing for disease control, there was no effect on seed germination; Proline® had disease control equal to that obtained with the current standard Bayleton® (Table 2). If registered for forest seedlings, Proline® will provide a second efficacious fungicide for the control of fusiform rust in the southern U.S.

Study 2 - Fusiform Rust Field Trials

At the South Carolina Forestry Commission's nursery in Trenton, SC, there was no rust infection in the control plots, so Proline® could not be evaluated. However, at the ArborGen nursery in Shellman, GA, by the end of the growing season in December 2008, 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 as 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 4).

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

Study 3 - Pitch Canker Laboratory Trials

In vitro fungal growth on agar amended with Proline® resulted in 100% fungicidal control against *Fusarium circinatum.* Fungal growth did not occur on any of the Proline®-amended PDA plates for any concentration examined for the 11-day experiment (Fig. 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, grew on all concentrations tested. *Fusarium 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 *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*.

Proline®'s fungicidal activity on *Fusarium 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 seed infected internally. Further research needs to determine if several applications of Proline® early in the season will also control late season mortality.

Study 4 - Pitch Canker Greenhouse Trials

A biweekly application at 365 ml/ha (5 fl oz/a) on longleaf and shortleaf pine to control pitch canker (*Fusarium circinatum*) resulted in a 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, with shortleaf pine, the greatest losses were later in the season. Not all shortleaf pine mortality was attributed to pitch canker. *Rhizoctonia sp.* 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.

Study 5 - 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). After 7 days, the plugs were removed from the amended media and placed onto non-amended agar media and the mycelia of *Rhizoctonia solani* resumed growth indicating that Proline® was fungistatic.

Proline®'s fungistatic activity on *Rhizoctonia 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.

Study 6 - Rhizoctonia Blight Field Trials
When Proline® and Heritage® where sprayed at label rates at two 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 - as *Rhizoctonia* foliage blight tends to occur in isolated foci in susceptible seedlots.

When the interval between fungicide sprays was increased to three 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 over three times that 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 two-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.

Label Registration Efforts
Over 1 billion hardwood and conifer seedlings are produced in southern U.S. forest-tree nurseries each year (Enebak, 2009) on approximately 1,012 ha (2,500 acres). 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 $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 Southern Forest Nursery Management Cooperative 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 Nursery 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 Southern Forest Nursery Management Cooperative with the hope of obtaining the necessary registration for use in forest-tree nurseries.

Part of the Southern Forest Nursery Management Cooperative's mission 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 over several years, and in cooperation with Bayer CropScience, an application was filed with the U.S. EPA in six southern 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 5 of the 6 states when, in March, 2009, U.S. EPA requested Bayer CropScience pull the approved 24(c) labels. The U.S. EPA determined that the forest-tree 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 U.S. EPA's 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, which is only for nursery and not forestry, 3) the proposed use pattern, which 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 U.S. EPA did not change their ruling and Proline® is not yet available for forest-tree nurseries. Several other labeling efforts (e.g. IR4) were explored but found not feasible with a non-food crop.

In November, 2009, after a number of conversations with both U.S. EPA and Bayer CropScience, we were informed that our registration request for Proline® in forest-tree 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 Southern Forest Nursery Management Cooperative were to pay the PRIA fee of $22,827.

In late December, 2009, the U.S. 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 & 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 10 acres 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.

MANAGEMENT IMPLICATIONS

For many years, the consistent use of methyl bromide by forest seedling nurseries has provided effective control of many soil-borne diseases. However, the implementation of the new EPA soil fumigant labeling and regulations and eventual loss of methyl bromide will impact nursery disease control. The uncertainty of the new fumigation plastics (VIF and TIF) and reduction in fumigant rates to achieve buffer zone requirements may result in an increase in disease problems in nurseries within the next few years.

When compared to food crops, forest seedling nurseries currently have relatively few registered fungicides at their disposal. Several of these fungicides such as captan and triadimefon have been used for many years (1952 and 1973, respectively) and their future registrations are at best uncertain.

Laboratory, greenhouse and field trials have shown Proline® to be efficacious against three important fungal pathogens that cause damage and seedling mortality in forest-tree nurseries. Disease control of all three fungi using Proline® was obtained using a 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.

Proline's® broad disease control reduces the need for multiple fungicides. This study was also the first to identify an efficacious fungicide for the control of pitch canker. The SFNMC will continue its fungicide testing program in order to identify effective chemistry that can be used in rotation with Proline®. Nurseries are encouraged to examine the Proline® label for other diseases that may be controlled in both conifers and hardwoods. Small trials with any new chemistry should be conducted before wide spread use is permitted.

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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% tebuconazole 25.8%	621 ml/ha 8.5 fl oz/a	1.24 l/ha 17 fl oz/a	25 g/10 kg 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)

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%

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

Table 4. Root length, average root diameter, root volume and number of root tips for each fungicide treatment[1].

	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

[1]Within 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] 0.5x = 183 ml/ha (2.5 fl oz/a) 0.25x = 91 ml/ha (1.25 fl oz/a) 0.125x = 46 ml/ha (0.625 fl oz/a) 0.0625x = 23 ml/ha (0.321 fl oz/a)	1300 650 325 162 81
Pageant®	pyraclostrobin 12.8% boscalid 25.2%	1x = 104.8 g/100 l (14 oz/100 gal) 0.5x = 52.4 g/100 l (7 oz/100 gal) 0.25x = 26.2 g/100 l (3.5 oz/100 gal)	1100 550 225

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

Table 6. Fill percentage and longleaf, shortleaf, slash and loblolly pine seedling quality in greenhouse pitch canker study[1].

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

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

Table 7. Seedling density and disease loss as measured by incidence[1], severity[2] and seedling loss[3] per m^2 and potential loss per hectare caused by *Rhizoctonia* foliage blight.

TRT	Seedling Density per sq. ft.	Disease Incidence	Disease Severity	Seedling loss per sq. ft.	Potential Loss per Acre
Control[4]	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
Prob > F	*0.7762*	*0.0004*	*0.0004*	*0.0031*	-
TRT	**Seedling Density per sq. ft.**	**Disease Incidence**	**Disease Severity**	**Seedling loss per sq. ft.**	**Potential Loss per Acre**
Control[4]	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
Prob > F	*0.51*	*0.0008*	*0.007*	*0.0013*	-

[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= seedling density x incidence/drill x severity /drill
[4]Controls were not included in the statistical analysis due to lack of replication among blocks.

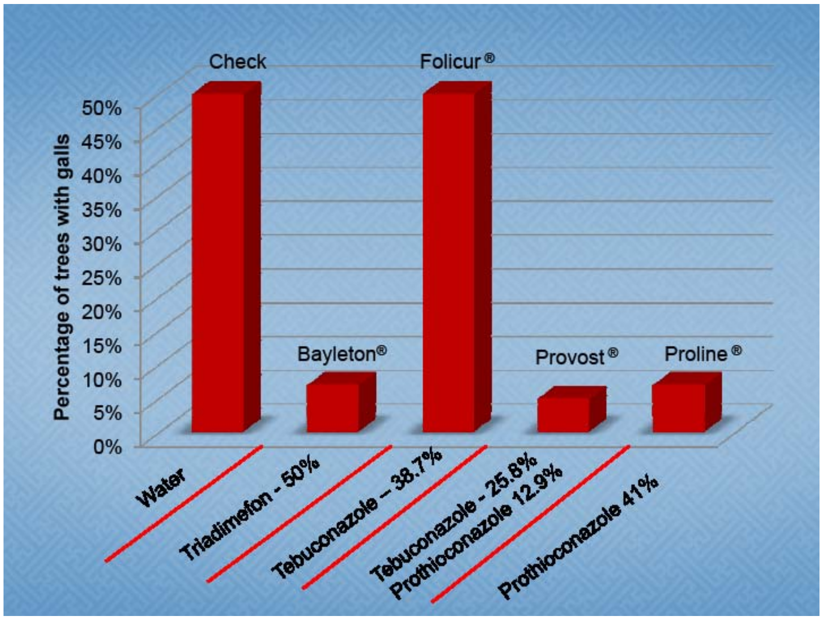

Figure 1. Three year average fusiform rust control on loblolly pine using foliar applications of fungicides.

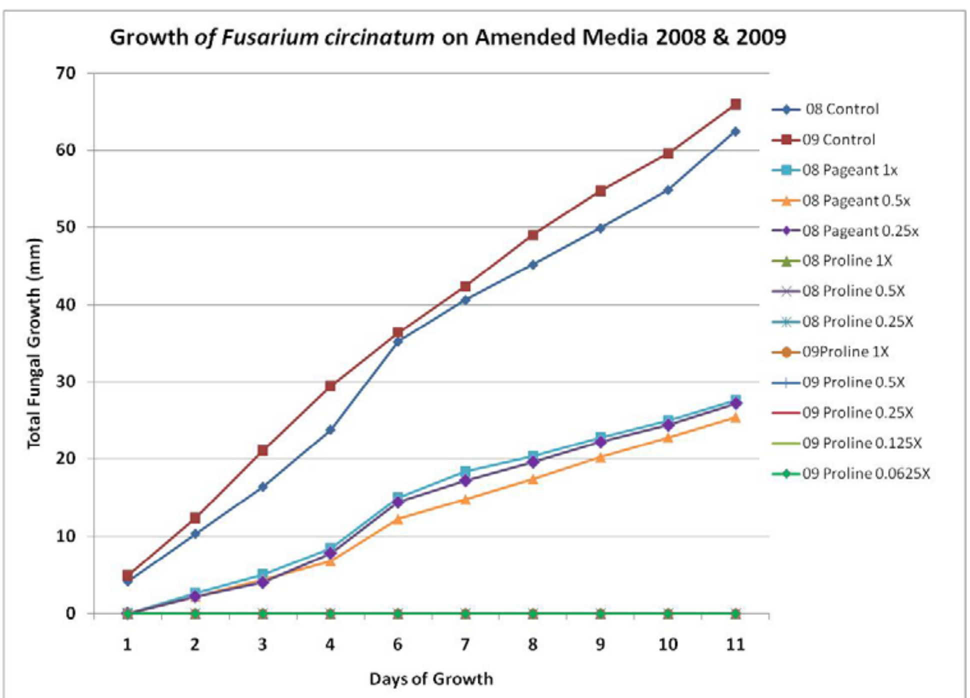

Figure 2. Radial growth of *Fusarium circinatum* on fungicide-amended and non-amended agar.

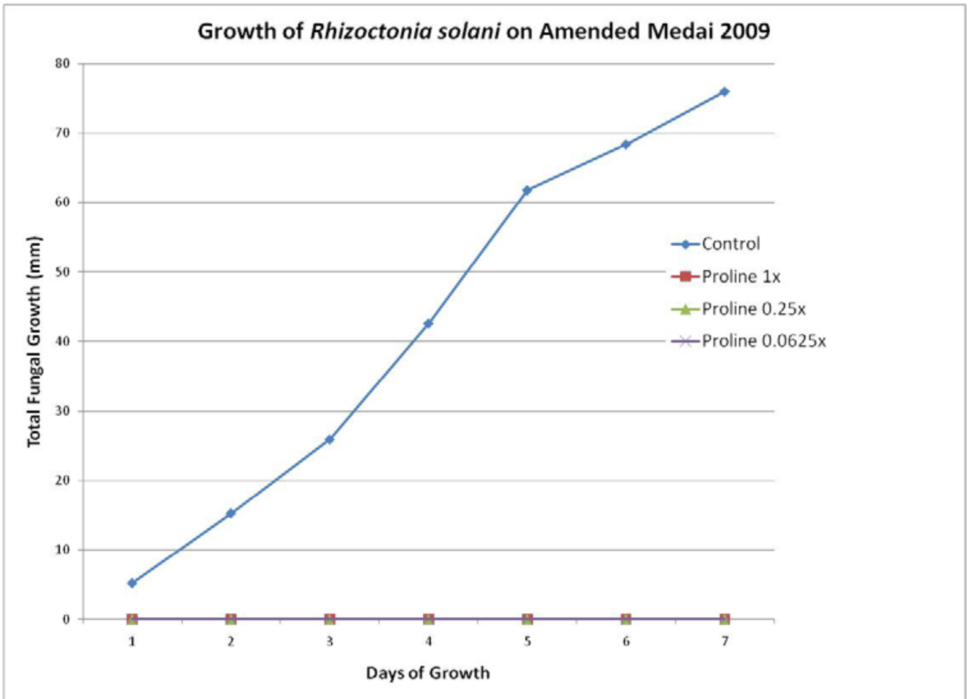

Figure 3. Radial growth of *Rhizoctonia solani* on fungicide-amended and non-amended media.