# Control of Rhizoctonia Foliar Blight in Forest Seedling Nurseries: A 3-Year Study

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Abstract: Laboratory and field trials have shown Proline® (prothioconazole) to be efficacious against the causal agent of Rhizoctonia foliar blight on loblolly pine (Pinus taeda). A biweekly application of Proline® at 5 fl oz/ac in nursery field tests significantly reduced Rhizoctonia foliar blight on loblolly pine when compared to applications of Abound® azoxystrobin (24 fl oz/ac; 1 fl oz/ac = 73.1 ml/ hectare) and the non-treated control. The potential monetary loss due to Rhizoctonia foliar blight per acre was \$967 for non-treated, \$209 for azoxystrobin treated, and <\$1 per acre for prothioconazole treated. A second trial was conducted applying prothioconazole and azoxystrobin every 3 weeks. The potential monetary loss per acre was \$1,294 for non-treated, \$693 for azoxystrobin treated, and <\$1 per acre for prothioconazole treated. In addition to disease control, Proline®-treated seedlings were significantly larger and appeared greener than either non-treated seedlings or seedlings treated with azoxystrobin. In a third trial comparing 5 and 3 fl oz/ac of Proline®, loss of seedlings occurred at the lower rate after seedling canopy closure. Disease spread within test areas was not correlated with the prevailing winds indicating the inoculum source was within the test area. Forest seedling nurseries should change their initial fungicide application from canopy closure to 1-2 fungicide applications prior to canopy closure to reduce inoculum and disease pressure later in the growing season. Proline® is currently labeled for use in forest seedling nurseries.

Keywords: loblolly pine, longleaf pine, slash pine, chemical control

# 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. Nearly all nurseries in the southern US routinely apply fungicides to prevent fusiform rust on seedlings. *Rhizoctonia* foliar blight is the second most frequently occurring disease in this region.

Longleaf (*Pinus palustris*) and loblolly (*P. taeda*) pines are particularly susceptible to *Rhizoctonia* foliar blight (English and others 1986, Runion and Kelley 1993). 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. Often the disease is 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 Coastal seedlots more susceptible than Piedmont sources, and the disease has not been observed on slash pine (*P. elliotti*) (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 or third year crops post soil fumigation. While there are fungicides registered for *Rhizoctonia* foliar blight, they are not always efficacious (Carey and McQuage 2004) with azoxystrobin being the most commonly used fungicide (McQuage, 2009 personal communication). In most nurseries in the southern region, fungicide applications begin in mid-July when seedling canopy closure occurs.

In an attempt to find alternatives for the control nursery diseases, trials examining numerous fungicides by have been conducted by the Southern Forest Nursery Management Cooperative since 2004. In 2008, Proline<sup>®</sup> 480 SC (41% prothioconazole, Bayer CropScience) (Table 1), was examined as it had a broad spectrum systemic control of ascomycetes, basidiomycetes, and deuteromycetes on numerous field food 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. When these research studies were initiated, Proline® was only registered in the US for food crops including peanuts, barley, wheat, sugar beets, beans, soybeans, and rapeseed. Beginning in 2008, data collected from the studies reported in this paper and other studies by the Southern Forest Nursery Management Cooperative were used to obtain a full-use label in December 2011 for disease control in foresttree nurseries in the southern US.

Table 1. Fungicide rates,	active ingredient and manufacturer.
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Fungicide	Active Ingredient	Manufacturer	
Proline <sup>®</sup> 480 SC	prothioconazole 41%	Bayer CropScience	
Abound®	azoxystrobin 23%	Syngenta Crop Protection	

The creation and expansion of these programs had a profound effect on state nurseries. An examination of seedling production shows this effect. In 1939, the 17 state nurseries that were operating produced 77,205,000 seedlings, but by 2010, the 16 state nurseries still in operation produced 118,299,000 seedlings. This dramatic increase was the result of increased mechanization, improved seed quality, and an improved understanding of seedling growth and development. These improvements made it possible for production to reach 2.3 billion tree seedlings in one year at the peak of the CRP. At the same time, the numbers of state nurseries were also at their all-time high of 42. Correspondingly, as the CRP slowed down, the number of state nurseries also decreased.

## Methods 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<sup>®</sup> at 1x, 0.25x and 0.0625x the label rate of 5 fl oz/ac (1 fl oz/ac = 73.1 ml/hectare). Potato Dextrose Agar (Difco<sup>®</sup> PDA) was amended with Proline<sup>®</sup> 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 (0.31 in; 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.



Figure 1. Experimental plot design used in each study 2008, 2009, and 2011.

#### Rhizoctonia Foliar Bight Field Trials

In 2008, Proline<sup>®</sup>, at 5 fl oz/ac, and Abound<sup>®</sup> (23% azoxystrobin) at 24 fl oz/ac were tested at the Pearl River, MS nursery and was applied 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 40 ft x 60 ft (12.2 m x 18.3m) wide with a non-treated plot 20 ft x 60 ft (3.7m x 18.3m) left as the disease control (Figure 1). Seedling sample plots were assigned to bed-rows 2, 4, 6, and 8. Fungicides were applied on a two week interval beginning July 15, 2008 using a Hardee 1532 liter sprayer with a 9-bed spray boom; nozzles on 1.5 ft (0.5 m) centers. A total of 8 applications of both fungicides were made. Temperature and relative humidity were recorded just above the seed bed using a HOBO<sup>®</sup> 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, root collar diameter, height, dry weight, and root morphology measured to determine seedling quality 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, to determine the minimal spraying time interval for disease control beginning mid-July.

In 2011, Proline<sup>®</sup> was tested at, 5 fl oz/ac and 3 fl oz/ac. Fungicide applications were made every 2 weeks, beginning mid-July at canopy closure and applied operationally using the same experimental design described above. The purpose of this study was to determine if reduced rates of Proline<sup>®</sup> effectively controlled *Rhizoctonia* foliar blight. Seedling counts within each treatment were made at germination, prior to canopy closure and at prior to lifting to determine when seedling losses occurred.

# **Results and Discussion** *Rhizoctonia* Foliar Blight Laboratory Trials

Agar media amended with Proline<sup>®</sup> resulted in 100% control of *Rhizoctonia solani* as fungal growth did not occur on any of the Proline<sup>®</sup>-amended PDA plates for any concentration used for the 7 day experiment (Figure 2). This control of fungal growth with all concentrations of Proline<sup>®</sup> cannot be directly extrapolated to field use, but it does indicate that rates lower than recommended label rates may be effective.



Figure 2. Radial growth of *Rhizoctonia* solani on fungicide-amended and non-amended media.



Figure 3. Control plot photo taken late September showing *Rhizoctonia* foliar blight.

#### Rhizoctonia Foliar Blight Field Trials

In 2008, when Proline<sup>®</sup> and Abound<sup>®</sup> where sprayed at label rates at two week intervals, disease incidence, severity and number of seed-ling mortality in Proline<sup>®</sup> treated plots was significantly less than in the Abound<sup>®</sup> and non-treated control plots (Table 2). The potential monetary loss due to *Rhizoctonia* foliar blight was \$967, \$209 and <\$1, per acre for non-treated, azoxystrobin, and prothioconazole, respectively. The potential monetary loss in Table 2 reflects the seedling loss in the test plot, not the whole nursery as *Rhizoctonia* foliar blight tends to occur in isolated foci in susceptible seedlots. There were no significant differences in either seedling quality or root morphology between the two fungicides tested, although the control plots had numerically fewer seedlings.



**Figure 4.** Abound<sup>®</sup> plot photo taken late September showing *Rhizoc-tonia* foliar blight.



Figure 5. Proline<sup>®</sup> plot photo taken late September showing inhibited senescence and greening effect.

Seedlings sprayed with Proline<sup>®</sup> exhibited a "greening-up" effect that remained until the final application of Proline<sup>®</sup>. This "greening-up" effect had never been observed with any other fungicides at this nursery previously. Figures 3, 4 and 5 represent the control, Abound<sup>®</sup> and Proline<sup>®</sup> sprayed plots, respectively. The greening up effect has been observed on other crops treated with Proline<sup>®</sup> and it is reported that triazole fungicides can act to delay senescence by the generation of H<sub>2</sub>O<sub>2</sub> as an intermediate metabolite (Audenaert and others 2010).

When the interval between fungicide sprays was increased from 2 to 3 weeks, Abound<sup>®</sup> had a disease incidence of 8.6% compared to <1% for the Proline<sup>®</sup> (Table 2). When comparing the two studies, the disease intensity doubled for the Abound<sup>®</sup> applications and the potential loss per acre increased over 300% when applied every 2 weeks versus

Table 2. Seedling density and disease loss as measured by incidence, severity and seedling loss per ft<sup>2</sup> and potential loss per acre caused by Rhizoctonia foliar blight in 2008 and 2009.

			2008				
TRT	Seedlings per ft <sup>2</sup>	Disease Incidence*	Disease Severity <sup>‡</sup>	Seedling loss per ft <sup>2</sup>	Potential Loss per Acre		
Control <sup>†</sup>	22.9 (0.81)	0.089 (0.03)	0.3637 (0.14)	0.74	\$967		
Abound®	23.6	0.041	0.167	0.16	\$209		
Proline®	23.7	0.0008	0.003	0.00006	<\$1		
Prob > F	0.7762	0.0004	0.0004	-	-		
2009							
			2009				
TRT	Seedlings per ft <sup>2</sup>	Disease Incidence*	2009 Disease Severity <sup>‡</sup>	Seedling loss per ft	Potential Loss per Acre		
TRT Control	<b>Seedlings per ft<sup>2</sup></b> 16.8 (0.92)	Disease Incidence*	2009 Disease Severity <sup>‡</sup> 0.43 (0.11)	Seedling loss per ft 0.99	Potential Loss per Acre \$1,294		
TRT Control Abound <sup>®</sup>	<b>Seedlings per ft<sup>2</sup></b> 16.8 (0.92) 20.5	Disease Incidence* 0.13 (0.03) 0.086	2009 Disease Severity <sup>‡</sup> 0.43 (0.11) 0.30	Seedling loss per ft 0.99 0.53	Potential Loss per Acre \$1,294 \$693		
TRT Control Abound <sup>®</sup> Proline <sup>®</sup>	Seedlings per ft <sup>2</sup> 16.8 (0.92)           20.5           19.7	Disease Incidence*           0.13 (0.03)           0.086           0.003	2009 Disease Severity <sup>‡</sup> 0.43 (0.11) 0.30 0.001	Seedling loss per ft           0.99           0.53           0.00006	Potential Loss per Acre           \$1,294           \$693           <\$1		

\* Incidence = proportion of RFB infected seedling per square foot of bed.

*‡* Severity = proportion of tissue infected with RFB per square foot of bed.

† Controls were not included in the statistical analysis due to lack of replication among blocks. Number in parenthesis is standard error of the mean.

every 3 weeks. The potential monetary loss was 1,294, 693, and < per acre for non-treated, azoxystrobin, and prothioconazole, respectively. When using a fungicide other than Proline<sup>®</sup>, it may not be possible to increase the spray interval greater than what is specified on the label. This study suggests that the time interval between Proline<sup>®</sup> sprays using suggested label rates is not as critical as with Abound<sup>®</sup>.

The goal of the disease management programs at this nursery within these susceptible seedlots would be to keep seedling mortality to less than 0.5% (McQuage, 2009 personal communication). Proline<sup>®</sup> was effective in reducing seedling mortality due to Rhizoctonia sp. below the nursery disease threshold.

In 2011, the appearance of *Rhizoctonia* foliar blight in the nursery was limited to the control plots in our study and the field edges that received uneven fungicide applications due to stopping and starting of the sprayer. In addition, the appearance of *Rhizoctonia* foliar blight did not occur until early September which is 3-4 weeks later than previous growing seasons. When comparing Proline<sup>®</sup> sprayed at 5 and 3 fl oz/ac, more seedlings were lost at the 3 fl oz/ac than 5 fl oz/ac following canopy closure (Figure 6). After canopy closure the temperature and moisture conditions, along with seedling-to-seedling contact facilitated the aerial spread of *Rhizoctonia* foliar blight.

The appearance of *Rhizoctonia* foliar blight within the field plots in 2008 and 2009 was limited to the unsprayed control plot followed by plots treated with Abound<sup>®</sup>. There was little movement from either the control or Abound<sup>®</sup> plots into the Proline<sup>®</sup> plots. In 2011, *Rhizoctonia* foliar blight was limited to the control plots followed by the Proline<sup>®</sup> plots sprayed at 3 fl oz/ac. In each year the study-plot location was in a different area of the nursery. There was no correlation of disease spread and the prevailing wind direction during any year indicating that the inoculum within the field was the primary source for plot to plot and row to row spread.

*Rhizoctonia* foliar blight has 2 phases of disease development (Yang and others 1990). The first phase occurs before canopy closure, when the inoculum spreads within the field to seedlings by rain splash and irrigation. The second phase occurs after canopy closure when aerial spread of the fungus is facilitated by seedling-to-seedling contact in the presence of free moisture. Favorable fungal growth is reported to occur between 75° and 86° F (Copes and Scherm 2010; Frisina and Benson 1987) when there are 6 to 8



Figure 6. Loss of seedlings before canopy closure (June to August) and after canopy closure (August to December).

hours of relative humidity > 95% (Copes and Scherm 2010). There is minimal fungal growth when the temperatures exceed 90° F (Frisina and Benson 1987).

Figures 7-9 show the environmental conditions over the growing season within each trial and indicate (red star) the time of occurrence of *Rhizoctonia* in the nursery. The shaded boxes are the period when the temperature and free moisture fell within the bounds favorable for *Rhizoctonia* reported above. The earliest occurrence of *Rhizoctonia* occurred in 2008 and the latest was in 2011. In 2011, it is possible that the aerial spread on *Rhizoctonia* was inhibited due to the high temperatures that summer. The temperatures in 2011 were the 3rd hottest temperatures on record in 117 years. Temperatures exceeded 90° F 69% of the days from canopy closure to the first appearance of Rhizoctonia foliar blight.

In December 2011, Proline<sup>®</sup> was approved by EPA for use in forest-tree nurseries. Proline<sup>®</sup> can be used to control diseases on nursery seed and seedlings of shortleaf, loblolly, slash and longleaf pines in addition to other pines, conifers and hardwoods. Before using any new pesticide, it is always a safe practice to apply it to a test area before larger areas in the nursery. The Proline<sup>®</sup> label can be viewed at http://www.cdms.net/LDat/ld89K000.pdf.

### **Management Implications**

Laboratory studies have shown Proline<sup>®</sup> is capable of controlling fungi in vitro at rates much lower than the label rate of 5 fl oz/ ac. In field studies, Proline<sup>®</sup> effectively controlled the spread of *Rhizoctonia* using 5 fl oz/ac which is within the range of 2.5 - 5.7 fl oz/ac for registered crops. Proline<sup>®</sup> provided significantly better control than Abound<sup>®</sup> when applied at a spray interval of every 2 or 3 weeks. Increasing spray intervals with fungicides other than Proline<sup>®</sup> may not be feasible in nurseries prone to *Rhizoctonia* foliar blight.



Figure 7. 2008 environmental data showing temperature, free moisture, date first Rhizoctonia foliar blight observed (star), and period in which environmental parameters favored fungal growth (shaded boxes).



Figure 8. 2009 environmental data showing temperature, free moisture, date first Rhizoctonia foliar blight observed (star), and period in which environmental parameters favored fungal growth (shaded boxes).



Figure 9. 2011 environmental data showing temperature, free moisture, date first Rhizoctonia foliar blight observed (star), and period in which environmental parameters favored fungal growth (shaded boxes).

The current practice of initiating fungicide applications for the control of *Rhizoctonia* foliar blight at canopy closure needs to be changed. Nurseries should start applying 1-2 fungicide applications before canopy closure when it is easier to apply fungicides to the lower stem and needles of the seedling. This will help to reduce the initial amount of inoculum found within the nursery beds and decrease disease pressure later in the growing season. In soybeans, 60% of the primary infection of *Rhizoctonia* foliar blight occurred before canopy closure (Yang and others 1990).

Mulching may also help prevent initial infection within a nursery bed. In dry beans, (*Phaseolus vulgaris* L.), mulching was highly effective for the control of *Rhizoctonia* web blight (Galindo and others 1983). The mulched layer acted as a physical barrier to prevent or reduce the splashing of the inoculum up onto the bean tissue.

The aerial spread of the fungus depends upon free moisture in the canopy. Therefore, in nurseries where *Rhizoctonia* is a problem, irrigating early in the day so as to allow the foliar to dry before nightfall will reduce the available number of hours of free moisture.

*Rhizoctonia* foliar blight overwinters on seedling debris in the field. Use caution when sowing susceptible families on 2nd or 3rd year land following fumigation.

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