



RESEARCH REPORT 23-04

NURSERY TRIAL ASSESSING THE IMPACT OF FUNGICIDE APPLICATION ON MYCORRHIZAL COLONIZATION OF LOBLOLLY AND SLASH PINE

by
Annakay Newell and Nina Payne

INTRODUCTION

Cronartium quercuum f.sp. *fusiforme*, the fungus that causes fusiform rust, is a major disease in forest nurseries that produce Loblolly (*Pinus taeda*) and Slash (*Pinus elliottii*) pines. The use of fungicides is currently the most effective control strategy for management of the disease. Seed and foliar applications of the fungicide Proline® (prothioconazole) are used to reduce the incidence of the disease in southern forest nurseries to almost negligible. The testing of alternative chemistries for control of fusiform rust is an ongoing activity of the Southern Forest Nursery Management Cooperative, as Proline® is the only fungicide that is currently labelled for the disease in forest nurseries after removal of Bayleton® by the Environmental Protection Agency in 2012.

Mycorrhizae are fungi that have a symbiotic relationship with the roots of many plants. Mycorrhizae cannot photosynthesize and receive carbohydrates from host plants while increasing the absorption of nutrients from the soil for the plant. They are made up of root-like structures that increase the absorption surface area for nutrient acquisition. As a result, plants with abundant mycorrhizae typically have a greater biomass, increased resistance to foliar and root pathogens, and increased salt and drought tolerance. Mycorrhizal associations are important for the growth of various pine species. The application of fungicides to control harmful fungal species in nurseries, however, can also negatively impact colonization of the roots by mycorrhizae.

A tradeoff between seedling quality and disease control may exist. The aim of this study was therefore to assess whether mycorrhizae colonization and seedling growth is impacted by the application of fungicides for control of fusiform rust in loblolly and slash pines.

MATERIALS and METHODS

Trial layout

Studies were undertaken under normal operational procedures at the ArborGen Nursery in Shellman, Georgia. An untreated control, and treatments with Proline® or Protect™ DF (Mancozeb) were randomly applied to 40ft plots of loblolly and slash pine. There were five replications in each species.

Seedling treatment

The labelled rates of Proline® (5 fl oz/acre) or Protect™DF (36.3g/1gal) were applied to respective plots 21 days after sowing and in 5 14-day intervals after the initial application from April to June. The study was initiated 21-days post sowing because the activity of systemic fungicides such as Proline® in the plant typically lasts for 21 days. We could therefore ensure that there was no residual activity from seed treatment.

Measurements and analyses

At the end of the growing season in November, 5 seedlings were collected from each plot for a total of 25 seedlings per treatment. Roots were removed from seedlings at the root collar and soil removed with a gentle stream of water to conserve mycorrhizae. A visual scale from 1 (no visible mycorrhizal roots) to 5 (abundant mycorrhizal roots) was used to rate each plant.

Two 4 ft x 9 in counting frames were also used to collect seedlings in each plot of which the root collar diameter (RCD), height, shoot weight, root weight and root weight ratio (RWR) were measured. Analyses were carried out using the R ver 4.2.3 statistical software. The Tukey's multiple comparison test was used to determine if there were differences between the means of each treatment. A value at $p < 0.05$ was considered statistically significant.

RESULTS and DISCUSSION

There were no significant differences in seedling quality measurements in loblolly pine (Table 1). There was also no significant difference in mycorrhizal colonization although the ratings for those treated with Protect™DF were lower than the untreated control and treatment with Proline® (Figure 1). In slash pine, the Proline®-treated seedlings resulted in significantly smaller mean RCD and height when compared to that of the control seedlings (Table 2). There was also a significant difference in height between seedlings treated with Protect™DF and the control (Table 2). These differences, however, may be biologically insignificant. The smaller RCD and height of treated seedlings is correlated with the lower ratings of mycorrhizal colonization. The inter-quartile ranges were also lower than that of the control (Figure 2). Seedling density in nursery beds can have an effect on quality metrics but there were no significant differences in the seedling density of loblolly and slash pine plots between treatments. There are various factors that influence mycorrhizae abundance on plant roots, some of which are hard to measure. Fungicides may directly impact soil microbial communities and their symbiotic relationship with plants. For example, the presence of multiple species of mycorrhizae on a plant can yield more positive responses to fungicide stress than that of a single species. Some of these factors may explain why there were differences between slash and loblolly pine colonization. One interesting finding is the significantly higher RWR of slash pine seedlings treated with Proline® and Protect™DF when compared to the control (Table 2).

MANAGEMENT IMPLICATIONS

Fungicides used to treat pine seedlings for diseases in forest nurseries may decrease mycorrhizae on roots and negatively impact seedling quality. The control of fusiform rust and other economically significant diseases is likely worth this tradeoff.

ACKNOWLEDGEMENTS

We would like to thank the staff of the ArborGen Shellman, Georgia nursery for their assistance with this study.

REFERENCES

Hage-Ahmed K, Rosner K, Steinkellner S. Arbuscular mycorrhizal fungi and their response to pesticides. *Pest Manag Sci*. 2019 Mar;75(3):583-590.

Okiobe ST, Meidl P, Kothe T, Olschewsky D, Rillig MC and Lammel DR. Root colonization by arbuscular mycorrhizal fungi is reduced in tomato plants sprayed with fungicides. *Front. Agron*. 2022; 4:1028195

Pagano MC, Kyriakides M, Kuyper TW. Effects of Pesticides on the Arbuscular Mycorrhizal Symbiosis. *Agrochemicals*. 2023; 2(2):337-354.

Schreiner RP, Bethlenfalvay, GJ. Plant and soil response to single and mixed species of arbuscular mycorrhizal fungi under fungicide stress. *Applied Soil Ecology* 1997; 7:93-102.

Table 1. Seedling quality measurements of loblolly pine seedlings grown under operational conditions and treated with fungicides compared to an untreated control. (*indicates a significant difference at $p < 0.05$).

Treatment	RCD (mm)	Height (cm)	Shoot Weight (g)	Root Weight (g)	RWR (%)	Density ^a
Control	6.65 ± 0.083	42.97 ± 0.463	6.47 ± 0.303	1.26 ± 0.094	16.2	29
Proline®	6.55 ± 0.076	42.98 ± 0.355	6.39 ± 0.324	1.21 ± 0.074	15.9	29
Protect™DF	6.54 ± 0.079	43.06 ± 0.463	6.37 ± 0.378	1.20 ± 0.075	15.8	30

^aseedlings per ft²

Table 2. Seedling quality measurements of Slash pine seedlings grown under operational conditions and treated with fungicides compared to an untreated control. (*indicates a significant difference at $p < 0.05$).

Treatment	RCD (mm)	Height (cm)	Shoot Weight (g)	Root Weight (g)	RWR (%)	Density ^a
Control	6.34 ± 0.072	38.60 ± 0.338	6.26 ± 0.296	1.11 ± 0.040	15.1	29
Proline®	6.13 ± 0.064*	37.70 ± 0.312*	5.66 ± 0.179	1.09 ± 0.039	16.1*	30
Protect™DF	6.32 ± 0.070	37.00 ± 0.336*	5.89 ± 0.179	1.12 ± 0.040	16.0*	30

^aseedlings per ft²

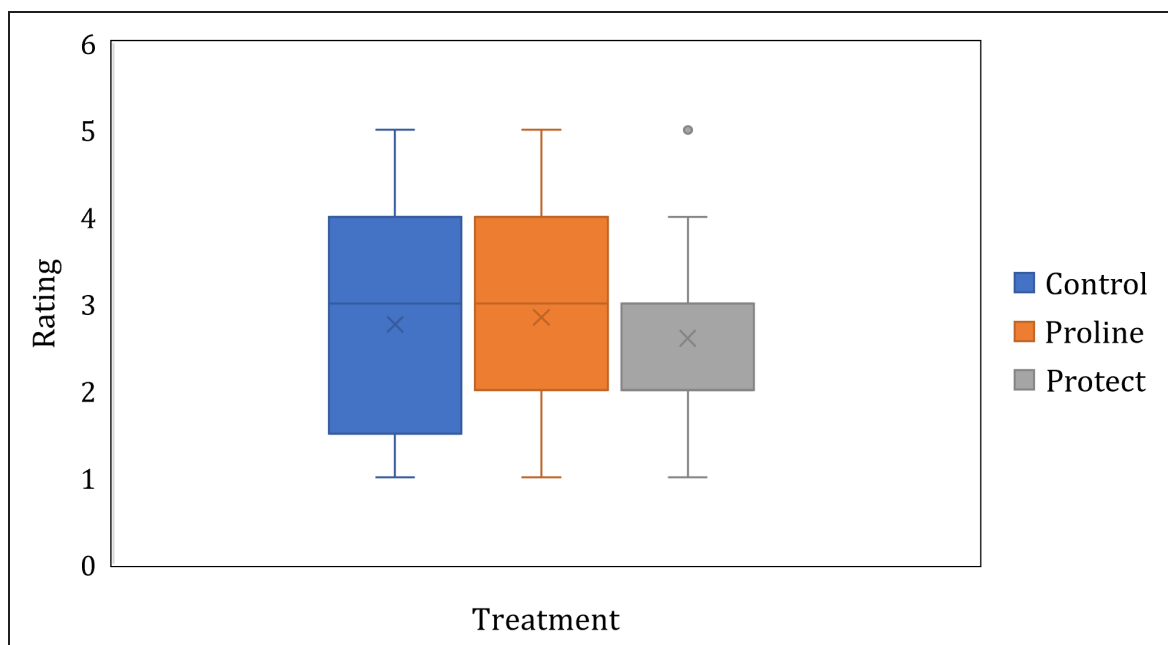


Figure 1. Mycorrhizal colonization of loblolly pine seedling roots grown under operational conditions and treated with fungicides compared to an untreated control. (Different letters on bars indicate significant differences at $p < 0.05$).

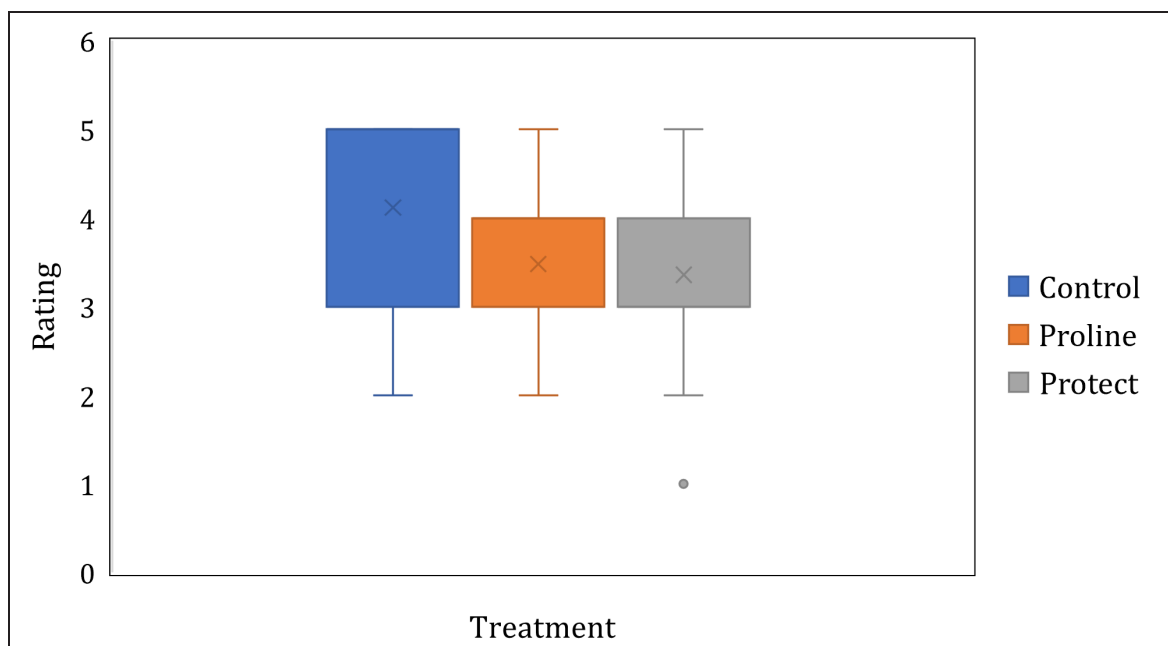


Figure 2. Mycorrhizal colonization of Slash pine seedling roots grown under operational conditions and treated with fungicides compared to an untreated control. (Different letters on bars indicate significant differences at $p < 0.05$).