Southern Forest Nursery Management Cooperative

RESEARCH REPORT 21-01

NURSERY TRIALS ASSESSING THE EFFICACY OF TWO NEW SYNTHETIC FUNGICIDES FOR THE CONTROL OF FUSIFORM RUST OVER TWO YEARS OF SEEDLING PRODUCTION (2019 AND 2020)

by
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INTRODUCTION

Cronartium quercuum f.sp. *fusiforme*, the causal agent of fusiform rust is still of major concern to many Loblolly (*Pinus taeda*) and Slash (*Pinus elliottii*) seedling growers. Although both genetic and cultural control options are available to reduce the risk of this disease, the most effective control in nursery production is the use of fungicides. Seedling infections can be significantly reduced by applying registered fungicides either as a seed treatment before sowing or as a foliar spray following germination (Carey 2004, Starkey and Enebak 2008, 2010a).

One of the major accomplishments of the Southern Forest Nursery Management Cooperative (SFNMC) was in 1980 by providing assistance in the registration of Triadimefon (Bayleton®) for fusiform rust control (Carey and Kelley 1993). At that time it was estimated that the incidence of rust fell from 2.5% to 0.01% of all seedlings due to this chemistry. In addition the fungicide usage fell from 4 lbs/ac/yr to less than 1 lb/ac/yr due to the reduced number of applications required per season. The SFNMC continued to look for alternative chemistries to assist with the control of fusiform rust and was instrumental in obtaining a registration for the active ingredient Prothioconazole (Proline®) in 2011 as both a foliar spray and seed treatment (Starkey and Enebak 2010a).

In 2012, the Environmental Protection Agency (EPA), along with the registrant, voluntary removed Bayleton® from the market, reducing the availability of fungicides that are effective in controlling fusiform rust to just that of Proline®(Starkey and Enebak 2010b). To ensure that alternative chemistries are available to control fusiform rust, the SFMNC continues to test annually the effectiveness of viable chemistries in controlling this disease. With the identification of two new active ingredients following pine seedling treatment greenhouse trials undertaken in conjunction with the US Forest Service Rust Testing Laboratory in Asheville, NC (Nadel and Enebak 2018), field trials were needed. The aim of the studies reported in this research report was to test the infield effectiveness (under operational conditions) for the two fungicides identified to reduce the incidence of fusiform rust galls.

MATERIALS and METHODS

Trial layout

Greenhouse studies previously undertaken by the SFNMC identified two new fungicides to be effective in reducing the incidence of fusiform rust galls in controlled studies (Nadel and Enebak 2018). These two fungicides were tested operationally in the 2019 and again in the 2020 growing seasons, to determine their effectiveness in reducing the incidence of fusiform rust on pine seedlings.

Studies were undertaken at the ArborGen Nursery in Shellman, Georgia where the SFNMC tested the fungicides Compass® and STRATEGO® operationally on several nursery blocks. Compass®, STRATEGO® and the currently used Proline®, were compared to a non-treated control (Table 1). In 2019 a randomized complete block design was used with treatments replicated 3 times for 4 seedlots (2 seedlots being slash pine and 2 seedlots being loblolly pine) (Figure 1). In 2020, 1 seedlot of loblolly pine was tested in a randomized complete block design with each treatment replicated 6 times (Figure 2).

Seedling treatment

Pine seedlings were sown and grown under standard nursery operational conditions. Pine seedling treatments were applied via spraying fungicides on 5 separate occasions. For each spraying event, treatments and their replicates were applied using standard nursery spray equipment. Proline® was applied at 5 fl oz/acre, Compass® at 3oz/ acre and STRATEGO® at 10 fl oz/ acre (Table 1). The first spray occurred 21 days following seed sowing to ensure the impact of seed treated with Proline® would no longer be effective in protecting the seedling from rust infection, prior to commencing this study. Following the initial seedling spray, subsequent fungicide sprays occurred 14 days apart commencing in April and concluding at the end of June, when conditions namely that of temperature and relative humidity were no longer conducive for natural rust infection (infection occur when 24 hours of temperatures are between 60 - 80 °F and relative humidity between 97 - 100%) (Enebak et al. 2004).

Measurements

At the end of each growing season (December 2019 and 2020), seedlings were collected from each treatment plot and examined for the incidence of galls (indication of rust infection) in addition to undertaking several measurements of seedling quality that include root collar diameter (RCD), height, shoot weight, root weight and root weight ratio (RWR).

Analyses

Percent gall formation between treatments were ArcSine transformed before being analyzed as a generalized linear model (GLM) with complete block design, using SAS ver. 9.4 (SAS institute Inc.) statistical software. The mean differences between treatments were determined using a Dunnett's multiple comparison procedure test to compare each treatments with that of the control. Duncan's Multiple Range test was used to compare means between all treatments. Means were considered significant at $p < 0.05$.

RESULTS and DISCUSSION

Gall formation

For the 2019 growing season, the level of fusiform gall incidence was significantly higher for all the untreated control seedlings compared to seedlings sprayed with fungicides (Figures 3, 4, and 5). The incidence of fusiform rust galls was, however, not significantly different between the three fungicides tested although seedlings sprayed with Compass® show the lowest level of gall formation (Figures 3, 4, and 5).

Unfortunately, we were unable to assess the efficacy of the fungicide treatments applied to the Slash pine seedlot 2 due to animal browsing (squirrel and deer) that resulted in low seedling survival for all treatment and control plots and thus the inability to compare fungicides to that of the control in 2019.

For the 2020 growing season, for a single seedlot of loblolly pine, the level of fusiform gall incidence was highest for the untreated control seedlings and lowest for seedlings treated with Compass® (Figure 6). There was no significant difference with fusiform incidence between the fungicides tested (Figure 6). There was, however, a significantly lower fusiform gall incidence of Compass® and Proline® treated seedlings when compared to that of the control (Figure 6).

When comparing the 2019 and 2020 growing seasons, the level of infection based on gall incidence was highest for the 2019 growing season. Although there was a difference in the fusiform rust gall incidence between growing seasons, likely a result of environmental conditions needed for infection, nevertheless the same trends for fusiform rust infection occurred for both years with the untreated controls having significantly higher levels of gall formation for both years compared to the treatments. The fungicide Compass® was found to have the lowest level of rust incidence for all seedlots, species and production seasons (Figure 3-6).

Morphological measurements

For the 2019 production year, for loblolly pine seedlots, the Proline® treated seedlings resulted in significantly larger mean RCD, shoot weight and root weight when compared to that of the control seedlings (Table 2 and 3). These results were likely as a result of the Proline® treatments having grown at significantly lower seedling densities compared to that of the control. Growing at lower seedling densities results in larger seedlings with larger shoots and root weights (South 2000). The reason for the seedlings growing at lower densities is not completely known and may be as a result of animal browsing that occurred more so on Proline® plots as previous work has shown this fungicide to result in seedlings that are greener with higher levels of nitrogen.

For the 2020 production year, all treated seedlings were shown to be significantly taller than that of the untreated control (Table 5). Although these seedlings had been top-clipped, this difference may potentially be as a result of the seedling being able to put more resources into growth instead of seedling defense as would have occurred for the control seedlings that were had a significantly higher incidence (although levels were low) of fusiform galls.

From the two years of seedling production and the assessment of different seedlots. There is no evidence of phytotoxicity of the new fungicides tested and they are unlikely to reduce seedling quality if used to control fusiform rust. Results from this study further indicated that the fungicides tested gave significantly better results in reducing the incidence of rust galls compared to that of the untreated controls with results being similar to that of the currently registered used Proline®.

The fungicide Compass®, however, was also found to result in lower levels of fusiform rust infection than that of Proline® treated controls over both production years and seedlots tested.

MANAGEMENT IMPLICATIONS

- The two new fungicides tested as a seedling control were found to be effective in reducing the incidence of fusiform rust galls.
- The active ingredients Trifloxystrobin (Compass®) and Propiconazole + Trifloxystrobin (STRATEGO®) were found to be as effective as Prothioconazole (Proline®) in reducing the incidence of fusiform when used infield.
- These new fungicides (chemistries) show promise as potential alternatives as a fusiform rust seedling treatment after both successful greenhouse and field trials.
- The fungicide Compass® is already labelled as a broad-spectrum fungicide for the control of certain foliar, stem and root diseases of ornamentals grown in forest nurseries and field nursery plantings.
- STRATEGO® is currently not registered for use in forest nurseries and will, however, require registration for use against fusiform rust control prior to being used commercially.

ACKNOWLEDGEMENTS

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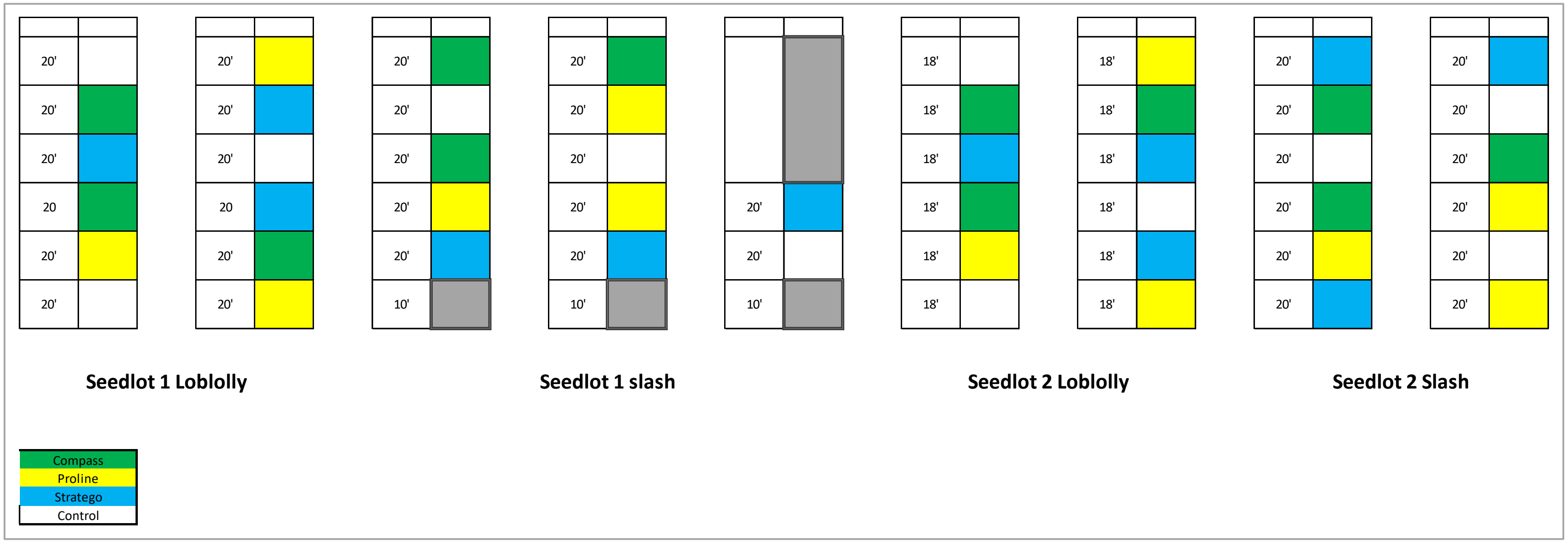

Figure 1. The 2019 trial Layout testing 3 active ingredients compared to an untreated control. Replicated 3 times for each of the 4 seedlots tested.

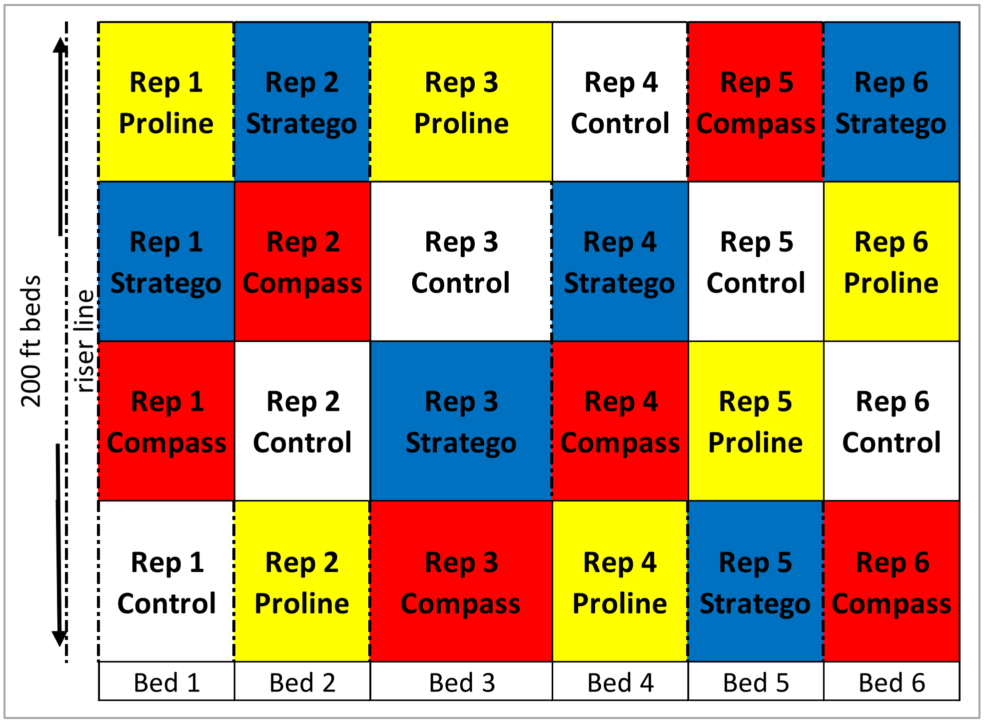

Figure 2. The 2020 trial layout testing 3 active ingredients compared to an untreated control. Replicated 6 times on a single loblolly seedlot.

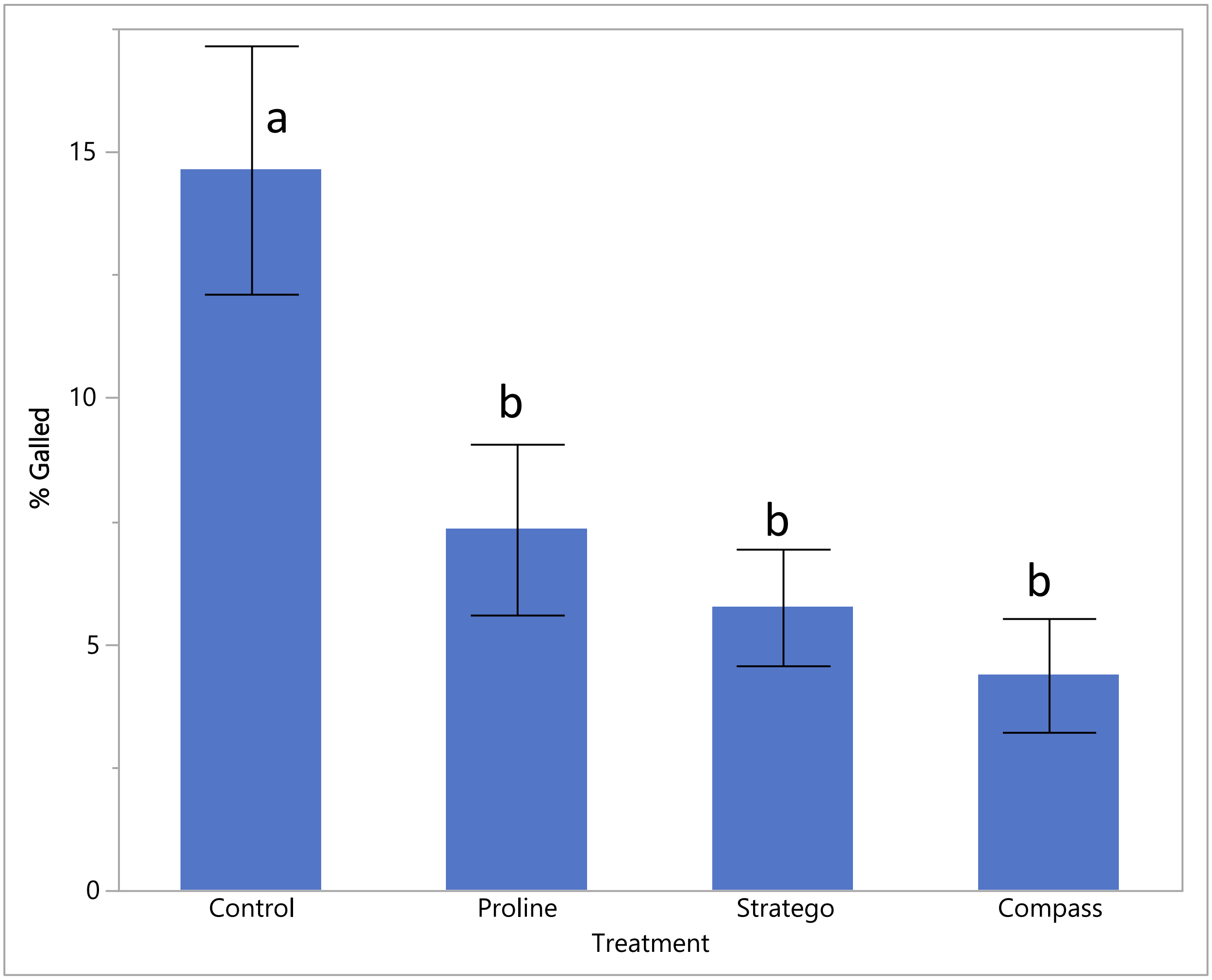

Figure 3. The 2019 growing season incidence of rust galls on Loblolly pine seedlot 1 seedlings grown under operational conditions and treated with fungicides compared to untreated seedling controls. (Different letters on bars indicate significant differences at $p < 0.05$)

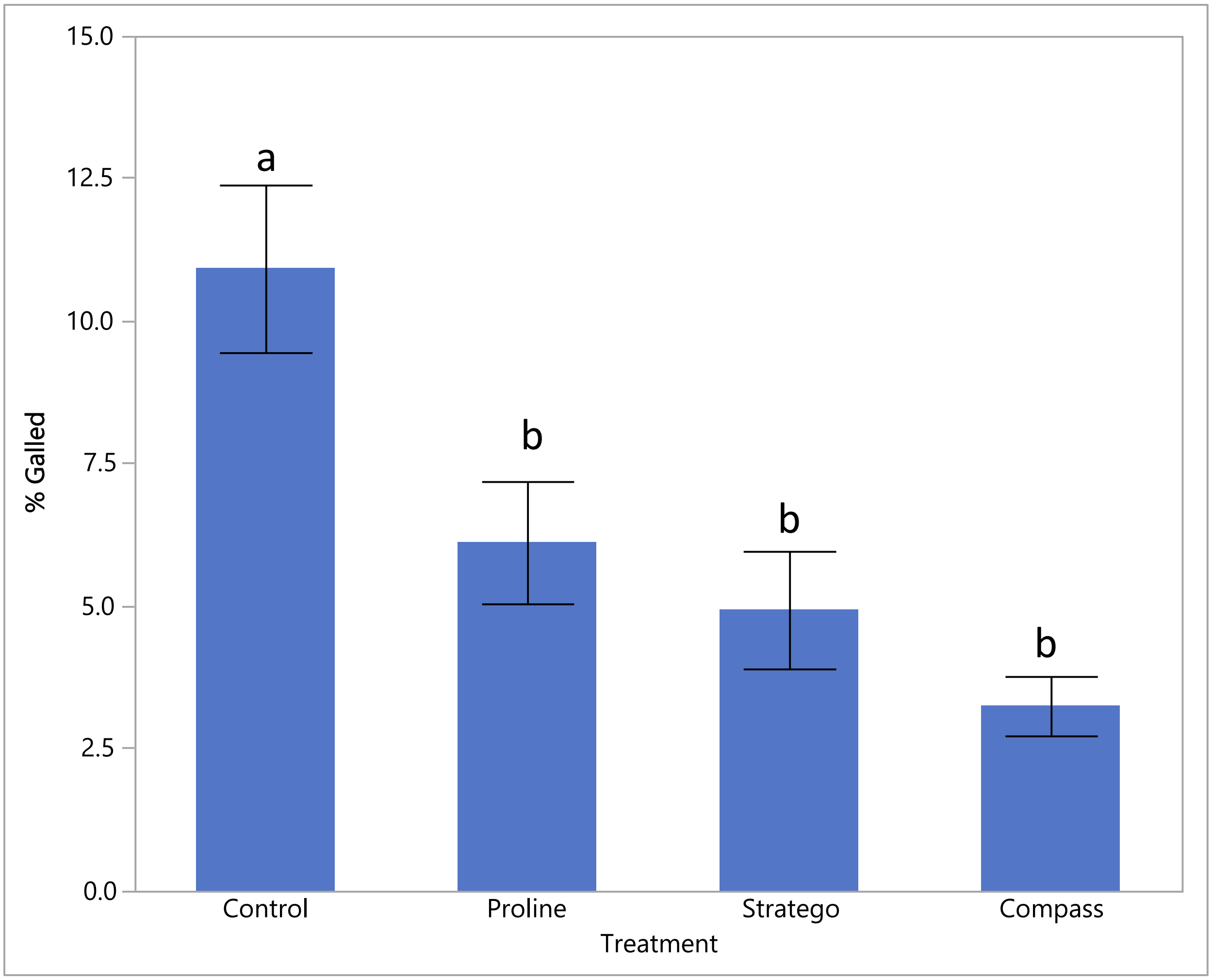

Figure 4. The 2019 growing season incidence of rust galls on Loblolly pine seedlot 2 seedlings grown under operational conditions and treated with fungicides compared to untreated seedling controls. (Different letters on bars indicate significant differences at $p < 0.05$)

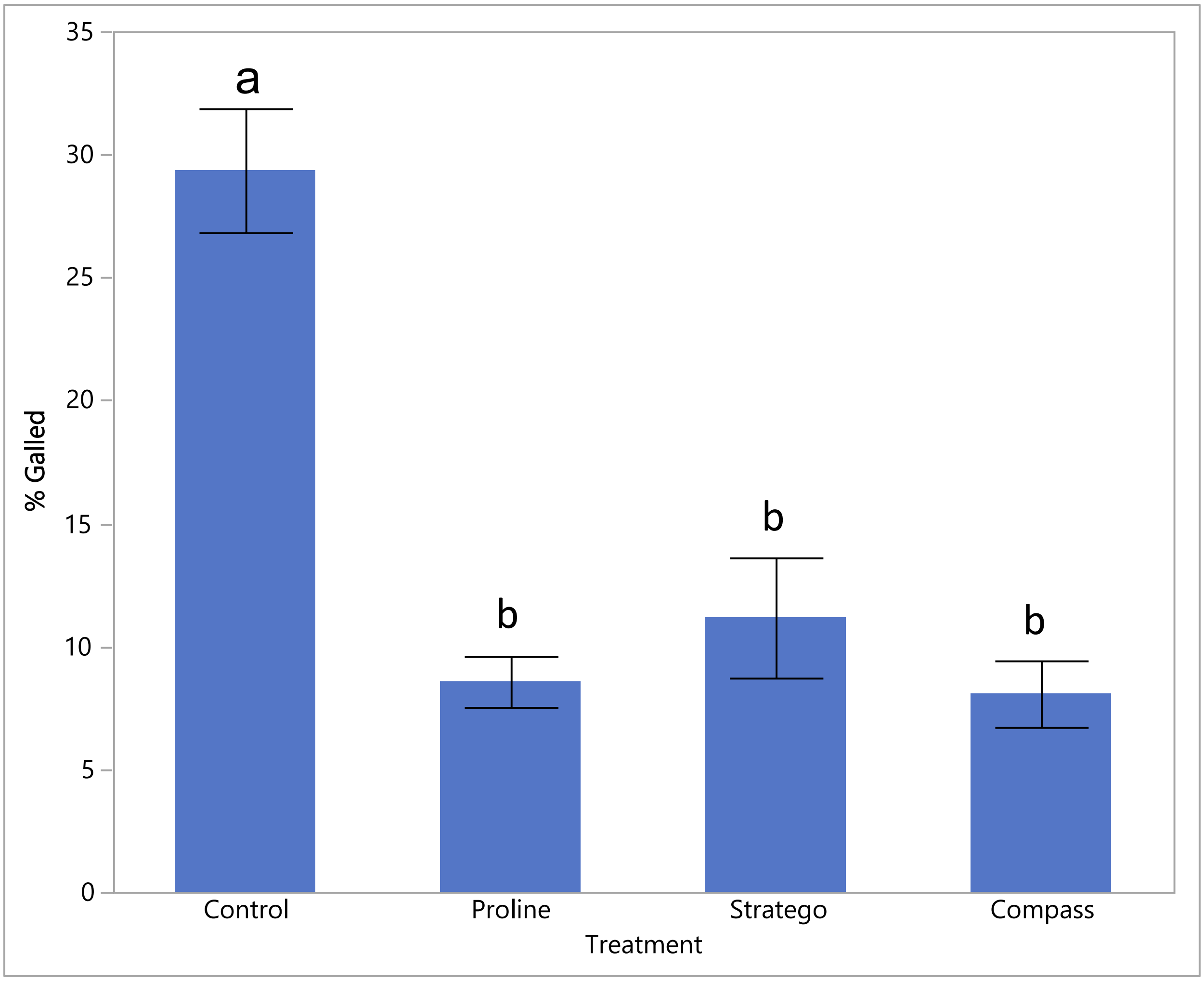

Figure 5. The 2019 growing season incidence of rust galls on slash pine seedlot 1 seedlings grown under operational conditions and treated with fungicides compared to untreated seedling controls. (Different letters on bars indicate significant differences at $p < 0.05$)

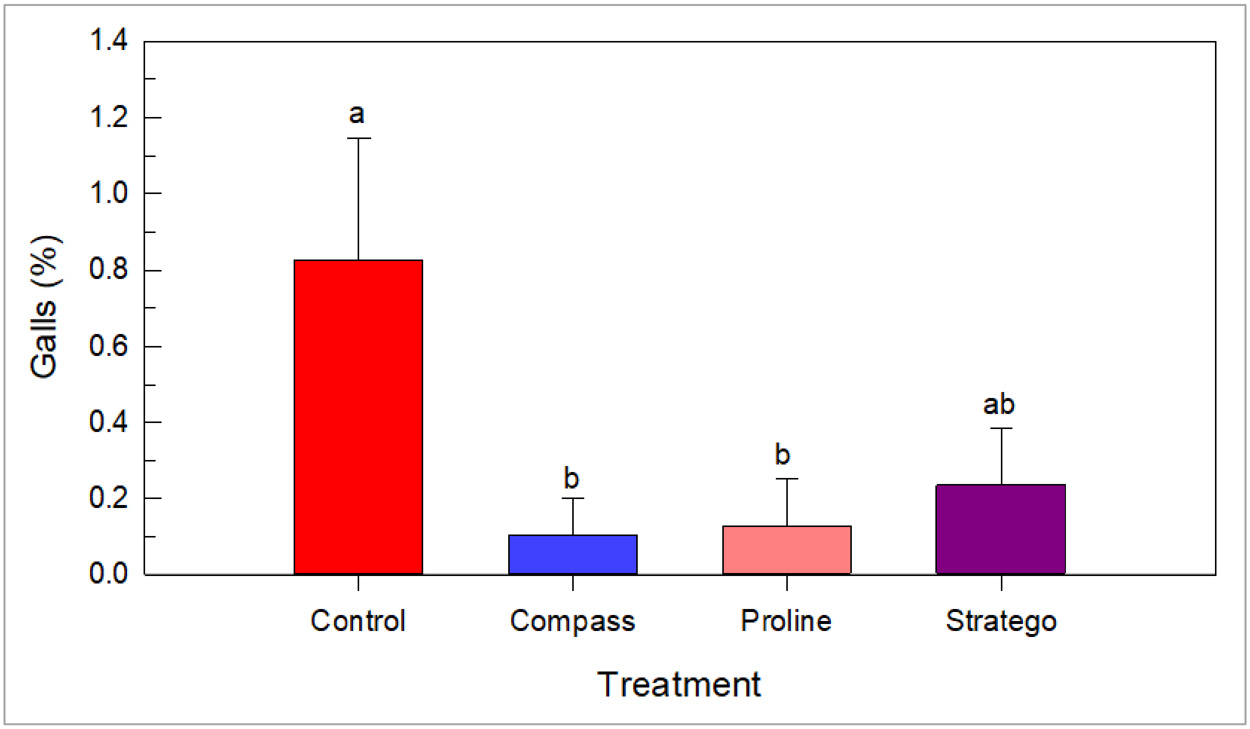

Figure 6. The 2020 growing season incidence of rust galls on Loblolly pine seedlings grown under operational conditions and treated with fungicides compared to untreated seedling controls. (Different letters on bars indicate significant differences at $p < 0.05$)

Table 1. Fungicides tested and their application rate on Loblolly and Slash seedlings grown infield and naturally infected by basidiospores of *Cronartium quercuum* f.sp. *fusiforme*

Fungicide	Manufacturer	Active Ingredient	Chemical Class	Rate Tested
Compass®	Bayer Cropscience	Trifloxystrobin – 50%	Oximino acetates	3 oz. per acre
STRATEGO® 250EC	Bayer Cropscience	Propiconazole – 11.4% Trifloxystrobin – 11.4%	Oximino acetates + Triazoles	10 fl oz. per acre
Proline®	Bayer Cropscience	Prothioconazole – 41%	Triazoles	5 fl oz. per acre

Table 2. Morphological measurements from the 2019 production season for Loblolly pine seedlot 1 seedlings treated with fungicides compared to untreated seedling control. (*indicates a significant differences at $p < 0.05$ when compared to that of the control)

Treatment	Height (cm)	RCD (mm)	Shoot Weight (g)	Root Weight (g)	RWR (%)	Density (seedlings per ft^2)
Control	37.38 ± 5.7	5.45 ± 1.20	4.7	0.96	17	23
Proline®	38.25 ± 4.56	5.92 ± 1.24*	5.41*	1.12*	17.2	19*
STRATEGO®	38.38 ± 4.54	5.65 ± 1.11	5.25	0.96	15.5*	23
Compass®	38.72 ± 5.25	5.71 ± 1.16	5.19	1.06	17	22

Table 3. Morphological measurements from the 2019 production season for Loblolly pine seedlot 2 seedlings treated with fungicides compared to untreated seedling control. (*indicates a significant differences at $p < 0.05$ when compared to that of the control)

Treatment	Height (cm)	RCD (mm)	Shoot Weight (g)	Root Weight (g)	RWR (%)	Density (seedlings per ft^2)
Control	38.24 ± 4.37	5.91 ± 1.12	4.95	0.83	14.5	19
Proline®	37.27 ± 5.06	6.20 ± 1.60	5.78	1.02	15	14*
STRATEGO®	38.83 ± 3.81	5.91 ± 1.36	5.33	0.85	13.9	20
Compass®	37.10 ± 3.6	5.82 ± 1.32	4.95	0.85	14.6	21

Table 4. Morphological measurements from the 2019 production season for Slash pine seedlot 1 seedlings treated with fungicides compared to untreated seedling control. (*indicates a significant differences at $p < 0.05$ when compared to that of the control)

Treatment	Height (cm)	RCD (mm)	Shoot Weight (g)	Root Weight (g)	RWR (%)	Density (seedlings per ft^2)
Control	34.09 ± 4.48	7.40 ± 1.65	6.82	1.33	16.3	13
Proline®	35.54 ± 3.96	7.84 ± 1.81*	7.54	1.48	16.3	13
STRATEGO®	34.83 ± 3.37	7.95 ± 2.1*	8.39*	1.72*	17	12
Compass®	34.51 ± 3.59	7.45 ± 1.66	7.11	1.32	15.7	14

Table 5. Morphological measurements from the 2020 production season for Loblolly pine seedlings treated with fungicides compared to untreated seedling control. (*indicates a significant differences at $p < 0.05$ when compared to that of the control)

Treatment	**Height (cm)**	**RCD (mm)**	**Shoot Weight (g)**	**Root Weight (g)**	**RWR (%)**	**Density (seedlings per ft²)**
Control	26.20 ± 2.03	5.72 ± 0.82	3.50	0.83	19.1	17
Proline®	27.34 ± 2.55*	5.75 ± 0.86	3.73	0.82	18.1*	17
STRATEGO®	27.34 ± 2.57*	5.90 ± 1.20	3.73	0.86	18.7	17
Compass®	27.11 ± 2.41*	5.76 ± 0.88	3.72	0.81	18.1*	17