EFFECTS OF FERTILIZATION AND ENDOPHYTE APPLICATION ON GROWTH OF PLANTED LONGLEAF PINE

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ABSTRACT

Longleaf pine (*Pinus palustris*) is an important economic and ecological species in the Southeast. The removal of fire from southeastern forests and a landowner preference for loblolly pine (*P. taeda*) and slash pine (*P. elliottii*) has resulted in a major decrease of the range of longleaf pine. This study investigates the potential of two fertilization methods and inoculation with diazotrophic endophytes to reduce time in the grass stage and improve diameter growth in longleaf pine. The study area was located in Clarke County, MS, on soils that consisted of a mix of fine sands and sandy clays. A total of four treatments were applied in a randomized complete block design to a recently clearcut stand. The site was chemically prepped prior to establishment. Treatments included control, broadcast fertilizer [nitrogen (N)-phosphorus (P)-potassium (K) 32-14-5] applied at a rate of 35 pounds per acre, and controlled release fertilizer bags (NPK 19-8-3) applied adjacent to each seedling. Diazotrophic endophytes were also applied in a separate treatment adjacent to each planted seedling 1 year following establishment. We measured root collar diameter (r.c.d.), survival, and the number of seedlings that had initiated height growth. Overall r.c.d. and grass stage emergence did not show a significant difference during second year measurements for both fertilization treatments and endophyte inoculation. Survival, however, was significantly lower in the broadcast fertilization treatment compared to the control. Increased competition from shrubs, grasses, and naturally regenerated loblolly pine in broadcast fertilization plots likely contributed to the low survival. Reducing time spent in the grass stage may help re-establish longleaf pine to its native range and increase ecological diversity across the Southeast.

INTRODUCTION

Longleaf pine (*Pinus palustris*) is an ecologically important species in the Southeast (Jose and others 2006). Longleaf pine has lost 95 percent of its historical range due to fire exclusion, its preference for use in naval store production, and landowner preference for loblolly pine (*P. taeda*) and slash pine (*P. elliottii*) due to their quick initial growth and flexibility of use across different site conditions. Longleaf pine exhibits slower initial growth due to the “grass stage,” an adaptation to fire where most resources are allocated to root development (Haywood 2000, Ramsey and others 2003). This stage can last from 1 to 15 years (Boyer 1990). Emergence from the grass stage is dependent on genetics, micro- and macro-site conditions, and competition. Grass stage seedlings generally emerge after reaching a root collar diameter (r.c.d.) of 0.5 inches in the absence of competition, though there is considerable variation in that threshold (Ramsey and others 2003). Following the grass stage, a period of rapid height growth and limited lateral expansion may occur.

Competition control is essential to successful establishment of longleaf pine. The use of herbicides alone to control competing vegetation can dramatically influence time in the grass stage (Haywood 2000, Ramsey and others 2003).

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Fertilization has the potential to reduce this time but only when competition is reduced or eliminated (Derr 1957, Haywood 2007). Broadcast spraying is the most widespread method of fertilizer application but often results in increased nontree competition (Powers and Ferrell 1996). Localized methods of fertilization, like controlled release point source fertilizers (hereafter "teabag") may improve seedling growth while limiting nutrient availability to nontarget vegetation (Thiffault and others 2005).

Inoculation with diazotrophic endophytes may be another method of reducing time in the grass stage. Endophytes are bacterial communities first derived from poplar (*Populus* spp.) and willow (*Salix* spp.) but later derived from Douglas-fir (*Pseudotsuga menziesii*) and western redcedar (*Thuja plicata*) in the Pacific Northwest. The role of endophytes in plant growth is unclear; however, endophyte inoculations have the potential to improve productivity (Aghai and others 2019, Chanway and Holl 1991) and stress tolerance (Khan and others 2016).

Reducing time spent in grass stage may provide opportunities to shorten rotation length and thus increase adoption of longleaf pine across the landscape. The objective of this study was to evaluate the influence of different fertilization treatments and endophyte inoculation on growth and survival. Specifically, our objectives were to 1) evaluate longleaf pine seedling response to broadcast and teabag fertilization application methods, and 2) quantify response to inoculation of diazotrophic endophytes.

METHODS

The study was located in Clarke County, MS. The property was owned by the Mississippi State Bulldog Forest Foundation. The site was clearcut in March 2020. Prior to harvest, the stand was composed of shortleaf (*Pinus echinata*), loblolly, and longleaf pines in mixtures of hardwoods. The site index was 85 feet (base age 50) with soils consisting of fine sands to silt loams (Lauderdale-Eustis complex) and Shubuta fine sandy loam. The site had slightly rolling topography that resulted in erosion, which formed sandy fans and deep cuts following harvest of some areas.

Chemical site preparation was applied using backpack sprayers in September 2020. Multiple waxy-leafed species were present on site including American holly (*Ilex opaca*) and yaupon (*I. vomitoria*) which are known to resist chemical application, warranting extra consideration for these species. Sprayers were filled with an herbicide mixture containing a solution of 4 pounds imazapyr (2.5 percent), glyphosate (5 percent), nonionic surfactant (0.25 percent), blue dye (0.4 percent), and water (92.1 percent). Herbicide was spot sprayed at a rate not exceeding labelled broadcast rates.

Containerized longleaf pine seedlings were planted by hand in April 2021 at a 10-foot by 10-foot spacing. Following planting, treatments were applied in plots containing 100 trees in a complete block design. Treatments were assigned randomly except for broadcast fertilizer treatments which were oriented to avoid precipitation runoff into other treatment blocks. Treatments were replicated four times in blocks oriented parallel to the slope contour. Treatments included applications of broadcast and controlled release teabag fertilizer, a fungal endophyte treatment, and a no treatment control.

Fertilizer treatments were applied following planting in April 2021. The first treatment utilized broadcast fertilizer applied at a rate of 35 pounds N per acre. (NPK 32-14-5). Broadcast fertilizers were weighed, bagged, and applied using a hand spreader and cart spreader such that the rate was distributed across the plot. The second treatment utilized Reforestation Technologies International (RTI) Silva Pak Worm Blend controlled release fertilizer bags (NPK 19-8-3). Teabags were inserted 2–3 inches deep on the uphill side and approximately 0.5 inches from the seedling using a dibble bar. Diazotrophic endophytes were applied in February 2022. Granular endophytes were applied top dressed in close proximity to each seedling, individually and manually at a rate of one-half teaspoon per seedling.

Trees in each treatment were measured in February 2022 and 2023. To avoid edge effect, only the inner 80 trees were measured for each plot. Measurements included r.c.d. and survival. Emergence from the grass stage was recorded in year 2. Analysis was performed using the

R statistical programming language (R Core Team 2023). A nested linear mixed effects model was developed to analyze r.c.d. To analyze both survival and emergence from the grass stage, a nested mixed effects logistic regression was developed using the lme4 package (Bates and others 2015). For both models, treatment was a fixed effect and block was a random effect.

RESULTS

Second-year survival ranged from 73.5 percent in the broadcast fertilizer treatment to 84.1 percent in the control and was significantly different between control and broadcast treatments (P <0.05, fig. 1). Root collar diameter was statistically similar between treatments (P >0.05, fig. 2) and ranged from 1.28 to 1.49 inches. The proportion of seedlings that had emerged from the grass stage 2 years posttreatment was statistically similar between treatments (mean = 10.6 percent, P >0.05, fig. 3). Teabag fertilization and endophyte inoculation had a considerable amount of variation compared to the control.

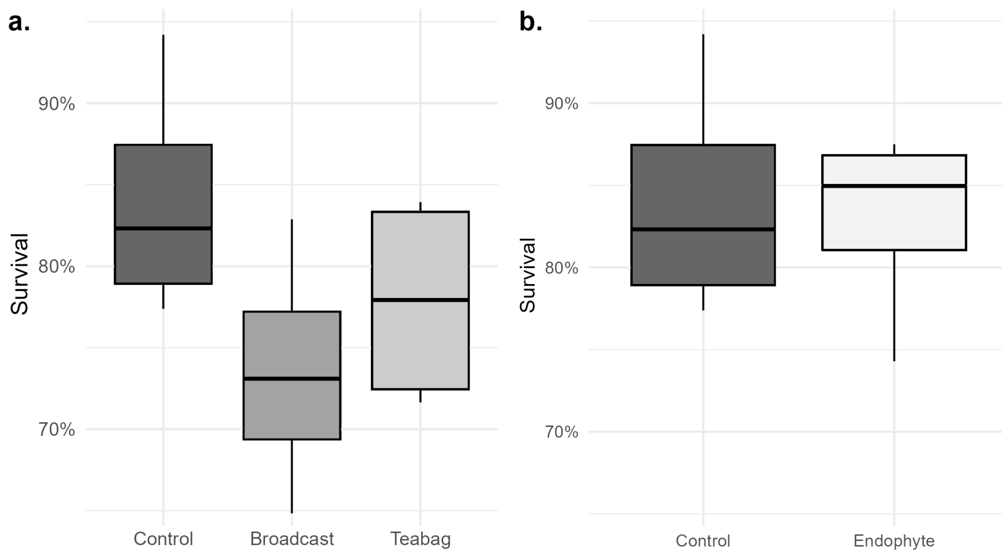

Figure 1—Precent survival of planted longleaf pine in central Mississippi (a) 2 years following treatment of fertilizer and (b) 1 year following endophyte inoculation.

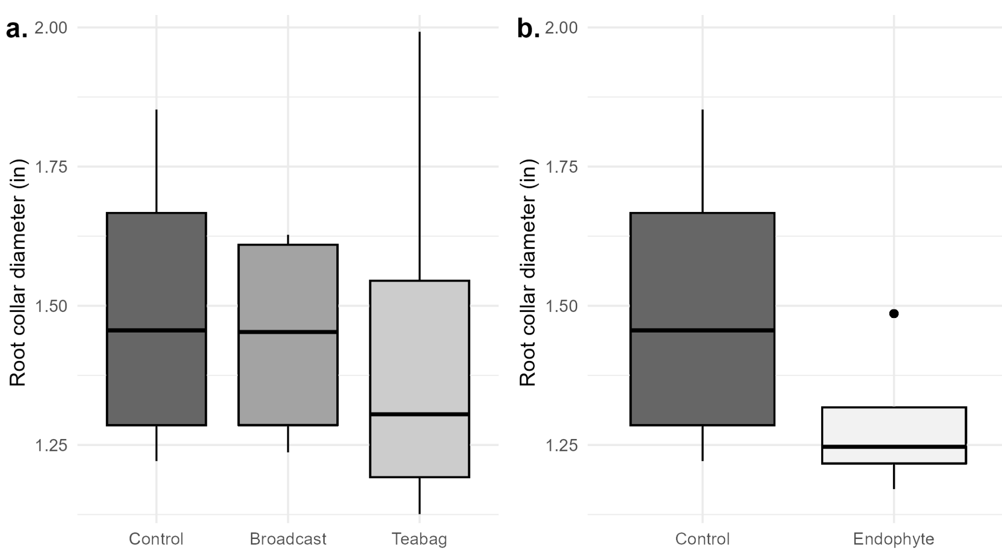

Figure 2—Root collar diameter (inches) of planted longleaf pine in central Mississippi (a) 2 years following treatment of fertilizer and (b) 1 year following endophyte inoculation.

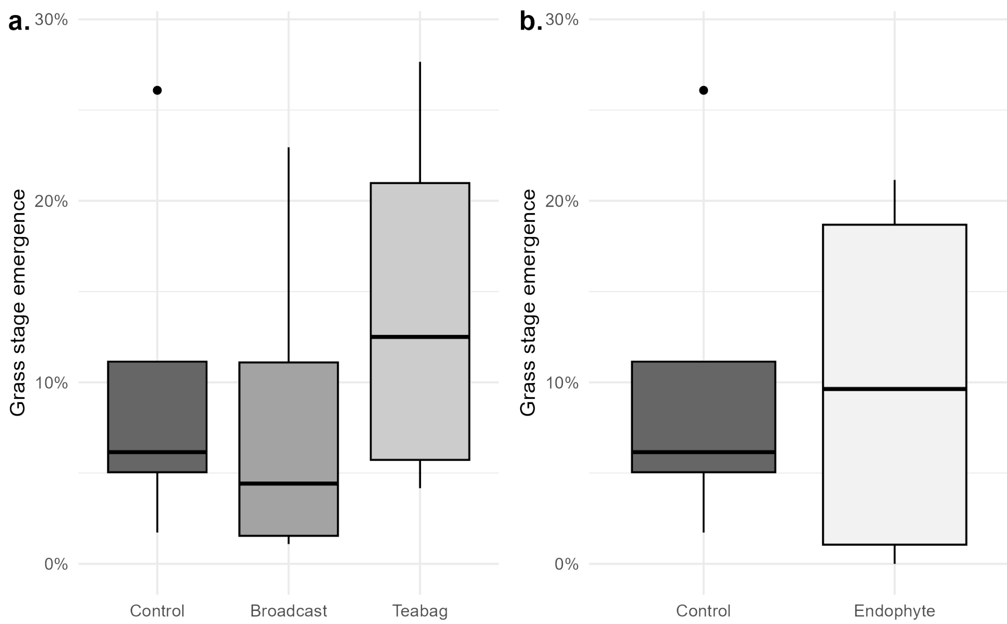

Figure 3—Proportion of grass stage emergence of planted longleaf pine in central Mississippi (a) 2 years following treatment of fertilizer and (b) 1 year following endophyte inoculation.

DISCUSSION

Overall, the results indicated that there was no significant difference between emergence from the grass stage and r.c.d. 2 years posttreatment among the different fertilizer treatments used in this study. However, broadcast fertilization resulted in a significant decrease in survival of seedlings which may have long-term implications for growth and stand development. Application of endophytes produced no growth or survival improvement 1 year following treatment.

While we did not detect significant trends in seedling growth associated with fertilization, seedling survival in the broadcast fertilizer treatment was significantly lower compared to the control. The decreased survival found in the broadcast fertilizer treatment may be associated with increased competition associated with fertilization, where neighboring vegetation likely capitalized on increased nutrient availability. We did not measure nontarget vegetation in this study. Earlier studies of fertilization and competition control in longleaf pine have demonstrated a neutral to negative interaction between fertilization and competing vegetation. For example, survival was significantly lower in fertilized plots and emergence from the grass stage was best predicted by absence of neighboring competition in the Florida panhandle (Ramsey and others 2003). In central Louisiana, a lack of response to fertilization was thought to be a result of increases in competing vegetation (Haywood 2007). In our study, application of chemical site preparation the same year as harvest likely increased competing vegetation as application did not maintain control of competing vegetation throughout the following growing season. Though we found no statistical evidence for growth differences to fertilization 2 years posttreatment, long-term growth and development of longleaf pine trees on plots treated with broadcast fertilizer may be significantly impacted, as competing vegetation can reduce growth and increase mortality.

Like many species, fertilization response in longleaf pine seedlings varies by site, application rate, and existing competition. Root collar diameter was unaffected by applications of fertilizer or endophytes. Ramsey and others (2003) hypothesized that limited response to fertilization in longleaf pine seedlings resulted from poor taproot development associated with lateral root growth stimulated by fertilization or that root development does not require nutrients at the same level of older trees that are putting on both primary and secondary growth. Young grass-stage seedlings do not turn over new needles as quickly as older seedlings and thus are likely not capitalizing as much as older species or species with more rapid initial height growth. The containerized seedlings used in this study were fertilized during nursery production which likely confounds our results, as fertilization during production can have lasting impacts on survival and growth. Competing vegetation capitalizing on increased nutrient levels may also negatively influence r.c.d. which may explain some of our results. Nutrient uptake in deficient stands can result in increased water use efficiency (Anderson and Johnsen 2009). Variation in soil quality caused by erosion on this site may result in greater heterogeneity in seedling performance, because soil texture is also related to nutrient availability, water holding capacity, and the presence of competing vegetation.

Height growth response to longleaf pine fertilization appears to be directly affected by the level of competition. Derr (1957) only found a fertilizer response in plots that received intensive vegetation control while Ramsey and others (2003) found no fertilizer effect on height growth. Though we did not measure height growth, the initiation of height growth (emergence from the grass stage) was unaffected by either treatment. It is notable that the teabag treatment, though not statistically significant, had a higher percentage of seedlings that had initiated height growth compared to all other treatments. Endophyte applications also appear to have a significant amount of variation relative to the control.

Logistical challenges prevented application of endophytes that coincided with the time of fertilizer application. Unsurprisingly, there was no statistical treatment effect just 1 year following treatment which is consistent with other studies (Aghai and others 2019). It is notable, however, that variability and mean of grass stage emergence was higher compared to broadcast and control treatments. Studies of endophyte inoculation have reported increases in survival, growth, and water use efficiency in western conifers (Aghai and others 2019; Khan and others 2015, 2016; Padda and others 2021; Renninger and others 2021). However, many questions remain as to the influence of endophyte inoculation on seedlings as well as nontarget vegetation.

Studies comparing the effect of broadcast applications to point source, controlled-release fertilizers like teabags are rare. Notably, teabag fertilization did not result in a decrease in survival in this study though mean survival was lower than the control. There is some indication that these targeted methods do result in more efficient uptake of available N (Hangs and others 2003). The differences in application rates between fertilization methods may confound some of our treatment results. As each teabag weighs 0.71 ounces, applying an equivalent broadcast amount would result in an application rate of 1.5 pounds per acre; a rate that is likely insufficient to produce a desired response based on preliminary soil analysis. Further studies will be necessary to evaluate appropriate application rates of targeted fertilizers compared to traditional methods.

CONCLUSIONS

Fertilizer did not improve growth rate or the proportion of seedlings that emerged from the grass stage. We also found no effect of endophyte inoculation 1 year posttreatment. Broadcast fertilization, however, significantly reduced seedling survival while point source teabag fertilizers were similar to the control. Competition from herbaceous vegetation and naturally regenerated loblolly pine likely influenced treatment outcomes in this study. Successful competition control is essential to establishing fast growing longleaf pine.

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