Auburn University Southern Forest Nursery Management Cooperative

RESEARCH REPORT 00-2

RHIZOBACTERIA STRAIN AND DOSE AFFECT THE ECTOMYCORRHIZAL COLONIZATION OF LOBLOLLY PINE SEEDLINGS

by
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INTRODUCTION

Mycorrhizae are regarded as an important factor in increasing nutrient uptake in plant roots and increasing outplanting survival. Mycorrhizal species of fungi that infect pine tree roots such as *Laccaria*, *Hebeloma*, *Rhizopogon*, *Pisolithus*, and *Suillus* are predominantly ectosymbiotic, a trait directly associating mycorrhizae on pines with inoculated rhizobacteria. While bacterial inoculates have repeatedly been shown to inhibit fungal pathogens, little documentation exists as to the effect PGPR have on mycorrhizal colonization of pine roots.

Ectomycorrhizae inoculation of pine seedlings has often been used to enhance survival in nutrient poor, acid-infertile, and pathogenic soils. Because mycorrhizae are often important in seedling establishment, any interactions between ectomycorrhizae and bacteria could have an effect on seedling survival and reforestation. An understanding of these interactions will determine if PGPR can be used as a viable technology for the production of loblolly pine in southern forest tree nurseries. For that reason, the objectives of this study were to determine what affect bacterial strain and dose as a seed treatment has on loblolly pine seedling mycorrhizal colonization, and what growth affects were evident due to mycorrhizal colonization.

METHODOLOGY

Loblolly pine (*Pinus taeda* L.) seed were obtained from Weyerhaeuser Corp. (Aiken, SC) and commercially treated by Gustafson, Inc. (Plano, TX) with four bacterial strains each at four rates. Seed were sown in Weyerhaeuser's Quail Ridge Nursery located in Aiken, South Carolina in

accordance with their standard operating procedures. Plots were fumigated with 158.8 kg/Ha (141.7 lbs/ac) methyl bromide/ chloropicrin (67/33) 14 months prior to sowing. Treatment plots were 9.144 meters (30 feet) in length and were established in five bedrows with a randomized complete block experimental design. Seed were mechanically sown at a target density of 275 seedlings per square meter (25/ft^2). Irrigation and fertigation was maintained by Weyerhaeuser in compliance with their standard operating procedures. The effect of bacterial strain and dose on loblolly pine seedling growth was also tested in the greenhouse, where single seeds were sown in Ray Leach conetainers randomized in complete blocks. Seedlings were allowed to grow in the greenhouse for twelve weeks, after which root growing volume was compromised. Greenhouse trials were replicated three times.

The effect of PGPR strain and dose on the rate of emergence was determined by counting the seedlings in permanent plots once a week for five weeks. Growth and ectomycorrhizal colonization was determined by sampling five seedlings from each treatment plot at 5, 10, 15 and 36 weeks (lifting stage) post-sowing in the nursery and at 4, 8, and 12 weeks post-sowing in the greenhouse. At each sampling date, loose soil was removed from roots, and seedlings were separated into roots and shoots. The number of ectomycorrhizal bifurcated roots were counted using a dissecting microscope. Treatment effect on seedling biomass was determined by drying roots and shoots at 70 C for 24 hours.

RESULTS

Nursery

No mycorrhizae were observed on seedling roots at 5 weeks. By 10 weeks., however, bacterial inoculum and dose within a strain had a significant impact on ectomycorrhizal root development in the nursery (Figure 1). Generally, increasing the applied rate of bacteria decreased mycorrhizae formation. Strain LS211 (10^{11}, 10^{12}, and 10^{13} cfu [colony forming units]) significantly (*alpha*=0.05) inhibited ectomycorrhizae at 10 weeks, yet mycorrhizal development recovered at different rates (See b. Figure 1). Strains LS032, LS212, and LS151 were also dose dependent in their affect on mycorrhizal colonization rate. However, at lifting, the percentage of ectomycorrhizal colonization did not differ between treated and control seedlings.

Mycorrhizal colonization and root biomass development in the nursery were inversely correlated. Decreases in ectomycorrhizal colonization accounted for 61% of the variation in increases in root biomass (Figure 2). Furthermore, mycorrhizae and root biomass reduction in the nursery did not decrease outplanting survival and growth. Shoot height after outplanting was not correlated to the mycorrhizal affect observed in the nursery (Figure 3).

Greenhouse

The inhibitory affects of bacteria on roots with ectomycorrhizae also occurred in the greenhouse. Ectomycorrhizae were not visible on roots four weeks post-sowing,. By 8 weeks, however, ectomycorrhizae were inhibited when compared to non-PGPR inoculated controls (Figure 4). The dependency of inoculum dosage on mycorrhizal inhibition varied by the bacterial strain used. The affects on mycorrhizae by two strains, LS032 and LS151, were not dose dependent, whereas affects by the other two strains, LS211 and LS212, were dose dependent. For strains LS211 and LS212, increasing PGPR inoculum decreased ectomycorrhizae.

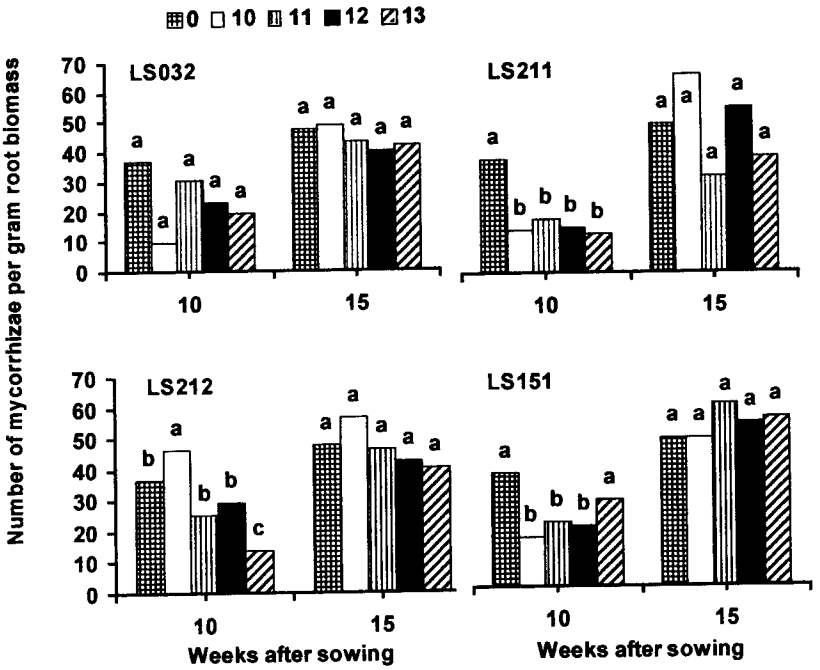

Figure 1. Number of mycorrhizal roots per gram root biomass on loblolly pine seedlings grown in a nursery in response to differing inoculation rates (0 (control), 10^{10}, 10^{11}, 10^{12}, 10^{13} cfu) of bacteria strains LS032, LS211, LS212, and LS151. Treatments denoted with the same letter are not significantly different (a = 0.05).

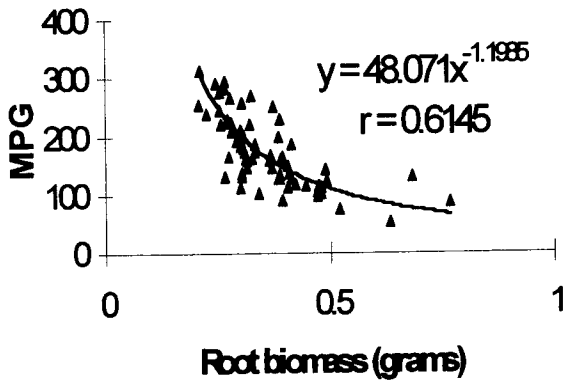

Figure 2. Relationship between amount of ectomycorrhizal roots per gram root biomass (MPG) and root biomass for seedlings grown in the nursery 15 wks post sowing.

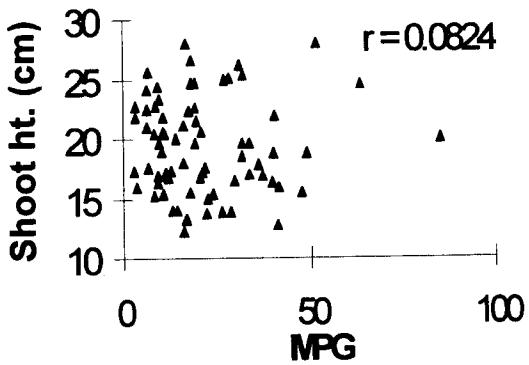

Figure 3. Relationship between amount of ectomycorrhizal roots per gram root biomass (MPG) in the nursery and shoot height (cm) six months after outplanting.

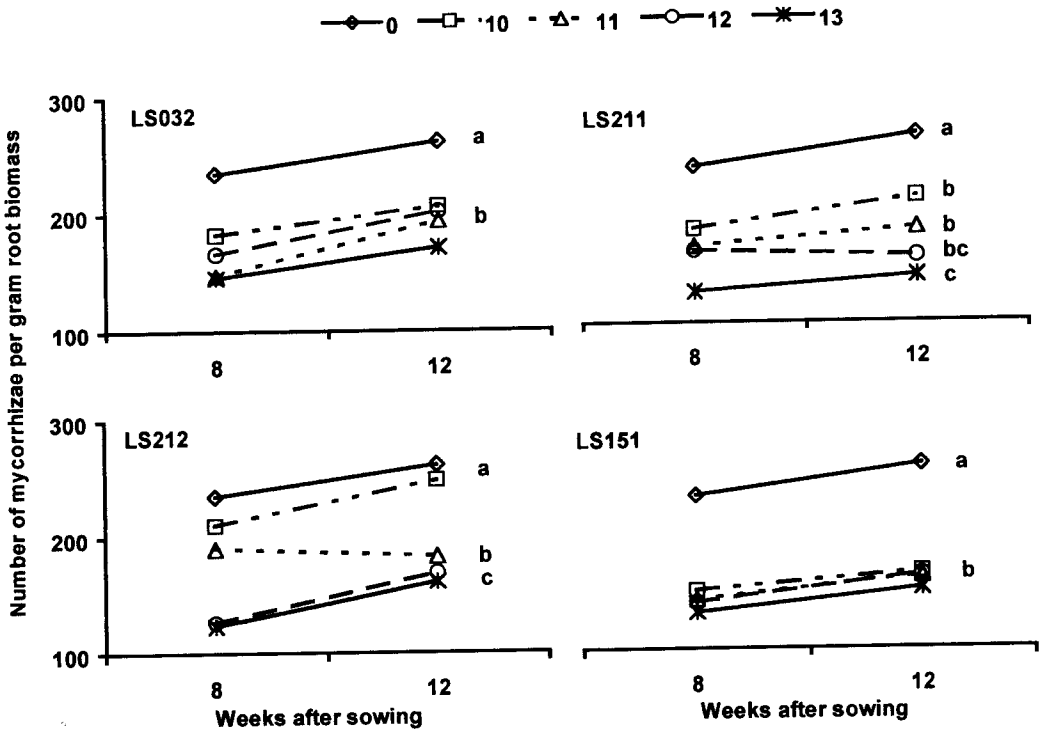

Figure 4. Number of mycorrhizal roots per gram root biomass on loblolly pine seedlings grown in a greenhouse in response to differing inoculation rates (0 (control), 10^{10}, 10^{11}, 10^{12}, 10^{13} cfu) of bacteria strains LS032, LS211, LS212, and LS151. Treatments denoted with different letters are significantly different (a=0.05).

Discussion

Bacterial treatments were shown to differentially affect ectomycorrhizal colonization, which was determined to be a function of inoculum dosage for the bacterial strains used on loblolly pine seedlings (Figure 1). Trials reported here illustrate direct interaction of ectomycorrhizae and bacterial inoculum for all bacterial strains. However, This dosage effect on ectomycorrhizal colonization was strain dependent. Therefore, it is apparent that complex interactions between soil microflora and inoculated bacteria in both nursery and greenhouse settings influence the formation of mycorrhizal symbioses and root growth potential.

Overall, the ectomycorrhizal fungi that infect loblolly pine in early stages of seedling growth were generally inhibited by the PGPR strains used (Figure 4). This is in direct contrast of documented studies investigating ectomycorrhizal colonization resulting from the inoculation of bacteria. Bacterial inhibition of ectomycorrhizae has been thought to result from a few factors which include: 1) bacterial niche exclusion on roots, 2) micro-scalar nutrient and water competition in the rhizosphere, and/or 3) the production of

antifungal metabolites by bacteria. The stimulation of mycorrhizal formation has been believed to be from the production of amino acids, vitamins and hormones by bacteria, however, no clear mechanism has been identified.

In our trials, fewer mycorrhizal roots correlated with increased seedling root mass (Figure 2). This is in stark contrast to many studies documenting the positive influence of mycorrhizal colonization on pine seedling growth. Fewer mycorrhizae have been shown to negatively affect the competitive ability of conifers against the saprophytic rhizosphere, commonly in terms of phosphorous and nitrogen competition. However, the negative affects of decreased mycorrhizal colonization can be overcome by the activity of potentially beneficial introduced bacteria.

Reduced mycorrhizae may not be desirable, as trees outplanted from nurseries must adapt to forest soils that may lack fertility regimes similar to those present in nurseries. This may pose a significant disadvantage to nursery trees with non-mycorrhizal roots. In these trials, however, mycorrhizal inhibition in the nursery had no affect on candle extension following bud break (Figure 3). Perhaps, the effect of increased root biomass in the nursery as a result of bacterial colonization or bacterial colonization itself may be sufficient for outplanted seedlings to overcome differences in nutrient availability from nursery to field.

At this stage in the development of PGPR for use in the forest tree nurseries, it is evident that the strain specific dose effect of bacterial inoculum can be a factor in seedling development. The growth effects demonstrated in these studies further underscore the importance of rhizosphere organisms to loblolly pine growth. The results with these treatments are indicative of complex bacterial interactions with plant and soil microflora that vary as bacterial inoculants mature in the rhizosphere which is influenced by all its constituent parts. More research into the effects of these relationships is needed.

MANAGEMENT IMPLICATIONS

The potential for PGPR in forestry are wide ranging, as bacterial treatments are inexpensive, easy to apply, and growth promotion with a number of strains has been demonstrated by previous research at AUSFNC. It is evident from these studies that the selection of rhizobacteria for the commercial production of loblolly pine should be based on specific seedling parameters, such as improved germination of seedlots, or root growth in non-mycorrhizal soils, which are unique to nursery soils.