The Use of Plant Growth-Promoting Rhizobacteria in the Production of Loblolly, Slash and Longleaf Pine in Bareroot and Container Nurseries in the Southeastern United States

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Abstract
Approximately one billion pine seedlings are produced annually for reforestation throughout the southern United States. The majority (95%) of these seedlings are bareroot loblolly pine (*Pinus taeda*) with the remaining seedlings slash (*P. elliottii*) or longleaf (*P. palustris*) pine grown in either bareroot or containers. The short rotations (one growing season), small acreage (80 - 200 ha), high value ($45-$250/1000 seedlings), and intensive management make forest tree nurseries ideal for use of plant growth-promoting rhizobacteria. Numerous greenhouse and field studies have been conducted through the Auburn University Southern Nursery Management Cooperative on all three pine species to examine the effect of rhizobacteria on seedling emergence, seedling growth and disease reduction. Studies conducted in Alabama, Georgia, and South Carolina have shown that the use of rhizobacterial strains used as a seed treatment at the time of sowing increases the rate of emergence, root and shoot growth in all three species over non-treated control. Growth changes by rhizobacteria appear to be affected by pine species tested and half-sibling families within species. Additionally, increased emergence and growth was both dose (amount of bacteria) and bacterial strain dependent. These studies also indicated that increasing the amount of bacteria caused a significant reduction in the subsequent mycorrhizal colonization.

In addition to growth promotion and seedling emergence, it appears that some strains are capable of inducing resistance to fusiform rust, which is one of the most important and devastating diseases on loblolly pine. Under greenhouse conditions, loblolly pine seed treated with rhizobacteria have shown a significant reduction in infection by *Cronartium quercum* f.sp *fusiforme*. Commercial nursery and out-planting trials are currently underway in Alabama, Georgia, and Mississippi to examine ISR induced by rhizobacteria. In addition to rust protection, rhizobacteria have been shown to reduce the amount of foliar injury due to the air pollutant ozone and increase seedling biomass in both greenhouse and open-top chambers. As far as root disease control, bacterial-treated longleaf pine seed sown in artificially pathogen-infested soils did not result in an increase in the healthy stand of seedlings. The lack of differences between treatments may have had something to do with either the rhizobacterial strains used and/or the pathogen tested.

In addition to seed treatments, trials have been conducted that incorporate rhizobacteria into soil-less media for use in container-grown seedlings. Similar to seed treatments, pine seedling response to bacterial soil amendments were bacterial species specific. For example, loblolly pine sown with one bacterial strain significantly increased root growth, while two other strains increased shoot, but not root biomass. Slash pine was less affected by bacterial soil amendment with three strains increasing shoot length and none increasing root biomass. Longleaf pine, with its unique grass-stage growth characteristics, had average root diameters increased over non-treated controls. The potential for plant growth-promoting rhizobacteria in forestry are wide-ranging as bacterial treatments are inexpensive and easy to apply. It is evident

from these studies that the selection of rhizobacteria for the commercial production of southern pine should be carefully scrutinized and based on specific seedling parameters such as improved germination of seed lots, specific seedling growth desired (root collar diameter, shoot biomass) or disease reduction as one particular rhizobacterium will not do all things to all pine seedlings.

INTRODUCTION

The use of methyl bromide as a soil-fumigant prior to sowing is the most common disease control practice in forest-tree nurseries throughout the United States. However, because it has been classified as an ozone-depleting compound, this broad-spectrum disease, weed and insect control treatment will not be available after the year 2001. Approximately one billion pine seedlings are produced annually for reforestation throughout the southern United States. The majority (95%) of these seedlings are bareroot loblolly pine (*Pinus taeda*) with the remaining seedlings slash (*P. elliottii*) or longleaf (*P. palustris*) pine grown in either bareroot or containers. The short rotations (one growing season), small acreage (80 - 200 ha), high value ($45-$250/1000 seedlings), and intensive management make forest tree nurseries ideal for use of plant growth-promoting rhizobacteria. Over the past five years, we have examined the effects of using rhizobacteria as a seed treatment prior to sowing, as a root dip prior to planting in the field and as a soil amendment in the use of container-grown seedlings for the production of pine seedlings. The use of beneficial PGPR strains would be an important part of IPM in the southern United States and be a positive step in increasing the productivity of forest lands without a concurrent reduction in disease-free seedlings.

MATERIALS AND METHODS

Greenhouse screening assay – bacteria as seed treatment. Loblolly and slash pine seeds were sown in containers filled with soil-less media. The seed used represented second generation planting material and were collected as half-sibling families. The rhizobacteria used in these studies were from the culture collection of Dr. J.W. Kloepper and were used as a seed treatment. Inoculated seeds were covered with washed sand and watered to saturation. Seedling germination, final healthy stand, root respiration rate and total root indole-acetic acid (IAA) content were assessed. The experiment was repeated three times and the data pooled for each species.

Greenhouse assay – bacteteria as soil amendment. Loblolly, slash and longleaf pine seed were sown in containers that had been filled with a soil-less medium amended with one of nine biological preparations (endospores of two bacilli plus chitosan). Prior to sowing, pine seed was stratified at 4 C for 30 days and surface disinfested with 30% H_2O_2 for 5 minutes. The resultant seedlings were allowed to grow for 8 weeks post-sowing after which seedlings were harvested and root and shoot characteristics measured using a flatbed scanner and WinRhizo® software. In addition, stem caliper, height, biomass, foliar injury and root area were also measured.

Induced systemic resistance assays - greenhouse. Seeds from an open-pollinated, half-sibling family of loblolly pine were collected from a second generation seed orchard. Eight rhizobacterial strains were chosen because they had previously demonstrated either disease reduction or induced systemic resistance to various pathogens on agronomic crops. Stratified seeds were used for this experiment and the bacteria applied as seed treatment. One month after sowing, seedlings were sprayed with a basidiospore suspension of fusiform rust. Six months after inoculation, seedlings were examined for main-stem swellings or galls symptomatic of fusiform rust infection.

Bareroot seedling trials - field. Numerous field trials were conducted on loblolly and slash pine to examine the effect of rhizobacteria on seedling emergence, growth, mycorrhizal colonization and disease reduction. The seed were sown and maintained under commercial operating practices in the nursery and the number of treatments varied but included treated and untreated seed sown in either fumigated or non-fumigated soil. Bacteria were applied as seed treatments. Emergence of seedlings was assessed at 1, 2, 3, 4, & 5 wks after sowing. At the end of the growing season, seedlings were assessed for grade, stem caliper, height, biomass, mycorrhizal colonization and root surface area.

RESULTS

Greenhouse screening assay – bacteria as seed treatment. All bacterial strains tested significantly increased the speed of seedling emergence over a non-treated control. By 12 weeks, however, no differences in stand densities were observed between bacteria-treated and non-treated for either pine species. Post-emergence damping-off was reduced in loblolly pine by three of the 12 bacterial strains tested. There was no effect on post-emergence damping-off on slash pine seedlings by any of the strains. Loblolly pine shoot and root lengths, as well as the above and below-ground biomass, were significantly reduced by strains BS1 and BS2 (Table 1). In contrast, strains BS3, PM2, and INR7 significantly increased the below ground biomass of the seedling root systems. Slash pine seedlings had similar interactions with the bacterial strains. Strain BS1 significantly reduced shoot lengths, while strains 90-166, INR7 and SE49 increased shoot biomass. Strain INR7 decreased whole root system respiration by 22% and increased root biomass and root length compared to controls at 6 wk. Furthermore, bacterial strain INR7 produced 1.7 times the total root IAA concentration of controls at 6 wk, whereas LS211 had no effect (Table 2).

Table 1. Characteristics of slash and loblolly pine seedlings three months after treatment with plant growth-promoting rhizobacteria under greenhouse conditions.

Treatment	Slash pine				Loblolly pine			
	Shoot		Root		Shoot		Root	
	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)	Length (cm)	Weight (g)
90-166	16.3	0.67*	17.5	0.27	13.6	0.41†	17.4	0.17
BS1	14.7†	0.51	14.0†	0.19†	13.0†	0.43†	15.6†	0.15†
BS2	15.3	0.57	13.7†	0.18†	13.2†	0.47	15.9†	0.16†
BS3	16.0	0.58	17.2	0.22	14.6	0.51	17.8	0.24*
PM2	15.8	0.59	17.2	0.23	14.8	0.45	17.7	0.23*
INR7	16.1	0.69*	17.6	0.28	14.7	0.47	17.5	0.19
SE34	16.1	0.65	17.2	0.25	14.8	0.47	17.5	0.17
SE49	16.1	0.69*	16.8	0.25	14.6	0.45	17.5	0.16
T4	15.7	0.64	16.9	0.18*	13.3	0.44	17.4	0.18
Control	15.5	0.52	17.1	0.25	14.9	0.61	17.0	0.20

*Significant increase or †decrease in seedling parameter measured over non-treated controls.

Table 2. IAA concentration, root length, and root and shoot biomass of loblolly pine seedlings 6 and 12 weeks after treating with strains LS211 and INR7.

Harvest period	Treatment	IAA Concentration[1] (mol g^{-1} root tissue)	Total root length (cm)	Root biomass (mg)	Shoot biomass (mg)
6 weeks	Control	11,340b	18.6b	6.6b	29.1b
	LS211	15,590b	24.1b	9.2b	33.2b
	INR7	31,430a	42.7a	14.5a	46.9a
12 weeks	Control	35,760	428.7	129.8	394.8
	LS211	37,970	403.2	116.0	374.0
	INR7	35,600	384.1	126.3	409.1

[1]Indicates significant differences between means.

Greenhouse assay – bacteria as soil amendment. None of the soil amendments reduced the germination of pine species tested over the non-treated control blocks. However, seedling response to bacterial soil amendments was species specific. For example, loblolly pine sown in soil amended with LS256 significantly increased root growth, while LS255, LS257 increased shoot growth (Table 3). One treatment, LS254 reduced shoot length over controls. Slash pine growth was less affected by bacterial soil amendment with only three treatments; LS254, LS255 and LS213 increasing shoot length and no amendments affecting root growth (data not shown). Longleaf pine, with its unique grass-stage growth characteristics, was even less affected than slash pine. However, the average root diameter with seedlings sown in soil amended with LS256 was increased over non-treated controls (data not shown here).

Induced systemic resistance assay - greenhouse. Strains *Bacillus pumilus* (SE34) and *Serratia marcescens* (90-166) significantly ($P = 0.05$) reduced the number of fusiform rust galls compared to a nontreated control in both trials conducted in 1997 and 1998. Combined analysis from 1997 and 1998 data resulted in two additional strains, *B. pumilus* (INR7) and *B. pumilus* (SE52) significantly (P=0.05) reducing the number of galls. Averaged over both years, 31% of control seedlings were infected with fusiform rust while those seedlings treated with bacterial strains SE34, 90-166, INR7 and SE52, had 13, 14, 15 and 16% infection, respectively. These four PGPR strains appear to have induced systemic resistance to fusiform rust in loblolly pine resulting in less infection over non-treated control seedlings (Fig. 1).

Bareroot seedling production trials - field. Studies conducted in Alabama, Georgia, and South Carolina have shown that the use of rhizobacterial strains used as a seed treatment at the time of sowing increases the rate of emergence, root and shoot growth in all three species over non-treated control. Growth changes by rhizobacteria appear to be affected by pine species tested and half-sibling families within species. Additionally, increased emergence and growth was both dose (amount of bacteria) and bacterial strain dependent. Ectomycorrhizal colonization, estimated as the total number ectomycorrhizal

Table 3. Loblolly pine seedling root and shoot characteristics grown in bacterial amended soilless media 8 weeks after sowing under greenhouse conditions.

Treatment	Total root length (cm)	Total root area (cm^2)	Root surface area (cm^2)	Average root diameter (cm)	Total length volume (cm/m^3)	Total root volume (cm^3)	Shoot length (cm)
LS254	77.13	2.82	8.87	0.0367	76.99	0.830	6.08*
LS255	78.98	3.48	10.94	0.0444	78.91	0.121	7.13*
LS256	118.38*	5.26*	16.54*	0.0445*	18.19*	0.185*	6.31*
LS257	89.07	3.80	11.95	0.0430	88.77	0.128	6.10*
LS213	97.27	3.74	11.76	0.0383	97.18	0.114	6.23*
Control	83.44	3.35	10.54	0.0405	83.28	0.107	5.35

*Indicates a significant increase over a nontreated control according to Dunnet's mean separation test, $P = 0.05$, N = 40.

roots per seedling and averaging by gram root biomass, were not evident on any treatment at either 5 wk in the nursery or 4 wk in the greenhouse. However, by 8 wk after sowing, many treatments significantly (P<0.01) inhibited ectomycorrhizal root formation. Generally, increasing the rate of all bacteria applied in the greenhouse decreased ectomycorrhizal root formation. Conversely, strain LS212 at 10^{11} cfu resulted in an increase in ectomycorrhizal roots in the nursery. Increases in seedling root growth were also dependent on bacterial strain and rate, whereas shoot biomass was not effected. Decreases in ectomycorrhizae colonization accounted for 61% of the variation in increases in root biomass, suggesting high metabolic costs of mycorrhizal maintenance in the presence of some rhizobacteria. However, differences in shoot height and survival after outplanting could not be attributed to the mycorrhizal colonization effect (Fig. 2).

DISCUSSION

Our studies demonstrate a complex interaction between rhizobacteria and nursery or greenhouse grown pine seedlings. Depending upon the bacterial strain used, and genetics (family) of the tree species, one can expect increases, decreases or no effect on pine production. In many of the trials, seed treatment with bacteria increased the rate of emergence of seedlings. In some cases, this resulted in a significant increase in seedling densities at the end of the year, in other cases, no affect. Greenhouse studies suggest that IAA concentrations and root respiration rates are two physiological mechanisms correlated with rhizobacterial activity and growth promotion. The effects of rhizobacteria inoculation on seedling emergence and plant growth are independent and that the effects are species specific. Reasons for this variation across nurseries could be anything, but soil pH is the most likely factor determining growth promotion. Many of the bacterial strains used prefer higher pH's, while pine, cultivation requires soil pH's of less than 6.

It appears that some strains are capable of inducing resistance to fusiform rust, which is one of the most important and devastating diseases on loblolly pine. Under greenhouse conditions, loblolly pine seed treated with rhizobacteria have shown a significant reduction in infection by *Cronartium quercuum* f.sp *fusiforme*. Most of the $35 million annual loss attributed to fusiform rust results from rust associated mortality that is strongly correlated with infections before age 3-5 years. Based on seedling size, infection at the nursery and during the first three years after outplanting will almost invariably be close enough to reach the main stem, resulting in mortality. It therefore seems reasonable to predict that a small percentage reduction in infection during the first year after outplanting would produce proportionally larger impacts on final plantation volume and value at harvest. Thus, protecting seedlings for two years in the field can result in significant reduction in tree mortality over the life of the plantation. Therefore, commercial nursery and outplanting trials are currently underway in Alabama, Georgia, and Mississippi to further examine ISR induced by rhizobacteria reported above.

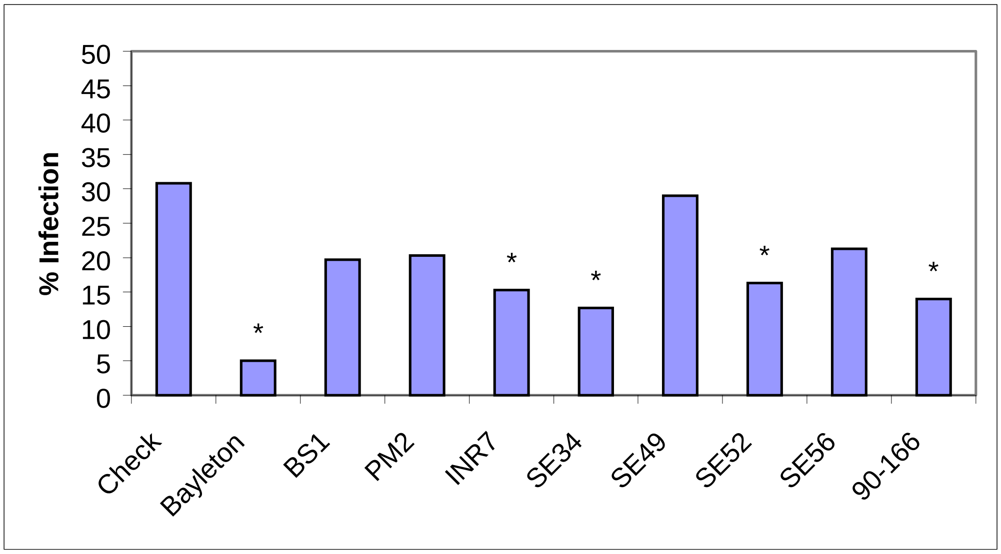

Fig. 1. Percent of loblolly pine seedlings with fusiform rust symptoms 6 months after sowing seed treated with PGPR strains. Letters above a column indicate a significant decrease in infection compared to a nontreated control. BSI = *Bacillus subtilis*, PM2 = *Peanibacillus macerans*, INR 7 = *B. pumilus*, SE34 = *B. pumilus*, SE49 = *B. pumilus*, SE52 = *B. pumilus*, SE56 = *B. sphaericus*, 90-166 = *Serratia marcescens*.

The potential for plant growth-promoting rhizobacteria in forestry are wide ranging as bacterial treatments are inexpensive and easy to apply. It is evident from these studies that the selection of rhizobacteria for the commercial production of southern pine should be carefully scrutinized and based on specific seedling parameters such as improved germination of seed lots, specific seedling growth desired (root collar diameter, shoot biomass) or disease reduction as one particular rhizobacterial strain will not do all things to all pine seedlings.

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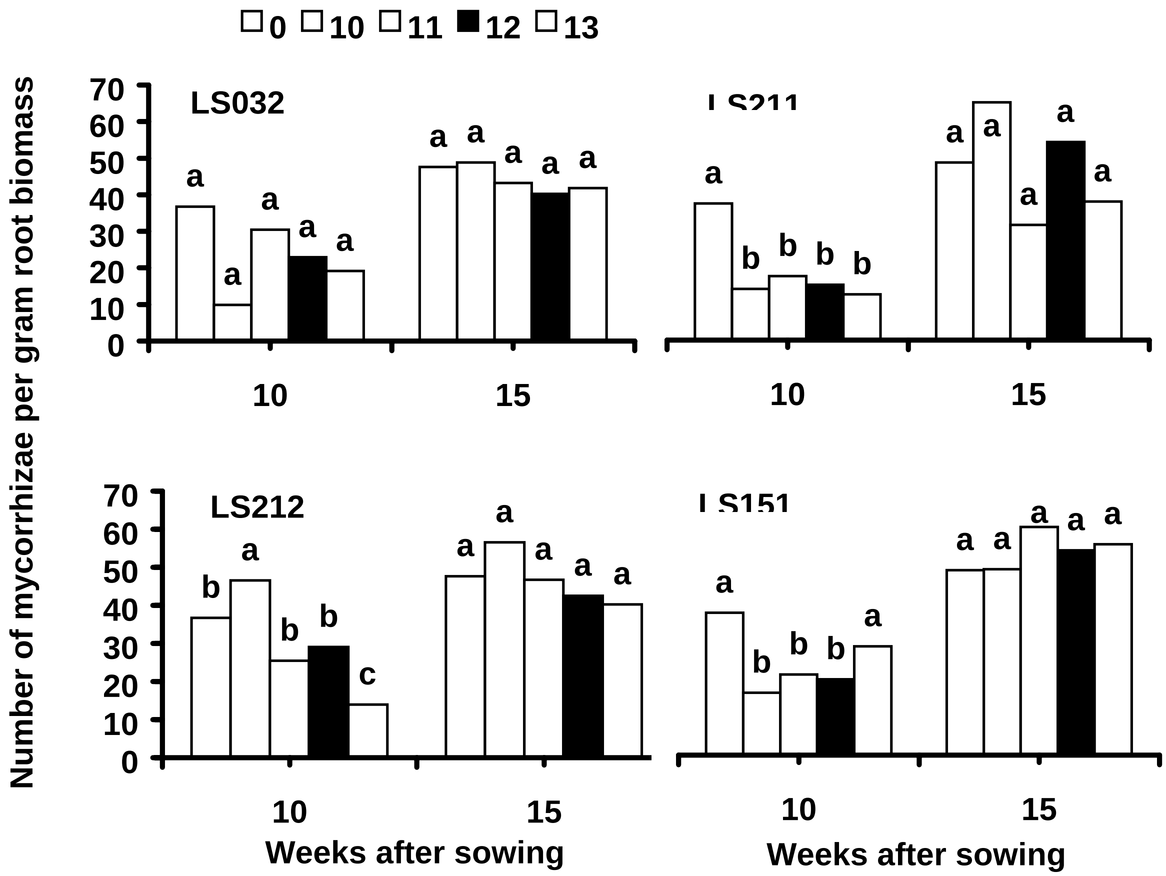

Fig. 2. Mycorrhizal roots per gram root biomass on loblolly pine seedlings in response to different inoculation rates (0, control; 10^{10}, 10^{11}, 10^{12}, 10^{13} cfu) of bacterial strains LS032, LS211, and LS151. Treatments denoted with the same letter are not significantly different at P = 0.05.