Bacterial Inoculation of Lodgepole Pine, White Spruce, and Douglas-fir Grown in Containers[1]

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Chanway, C.P.; Radley, R.A.; Holl, F.B. 1989. Bacterial Inoculation of Lodgepole Pine, White Spruce, and Douglas-fir Grown in Containers. In: Landis, T.D., technical coordinator. Proceedings, Intermountain Forest Nursery Association; 1989 August 14-18; Bismarck, ND. General Technical Report RM-184. Fort Collins, CO: U.S. Department of Agriculture, Forest Service, Rocky Mountain Forest and Range Experiment Station: 93-97. Available at: http:www.fcnanet.org/proceedings/1989/chanway.pdf

Abstract -- Inoculation of lodgepole pine and Douglas-fir with two Bacillus strains was shown to promote seedling growth. Strain L6 caused significant increases in lodgepole pine shoot and root dry weight, root surface area, and root collar diameter after eight weeks growth from seed. When 1-0 containerized stock was inoculated, shoot growth was increased, but root weight increases were not significant. Strain L6 also increased the root surface area of Douglas-fir 12 weeks after inoculation. Strain L5 significantly increased the rate of spruce seedling emergence and root surface area of lodgepole pine after 12 weeks growth but dry weight gains were not significant.

INTRODUCTION

Microbial activity in the plant rhizosphere has substantial effects on plant productivity (Gaskins et al. 1985). Bacteria are the most abundant type of rhizosphere microorganism reaching populations of up to 3×10^9 cells per gram dry weight of soil (Rouatt and Katznelson 1961). There have been numerous investigations centered on the possibility of enhancing agricultural crop productivity by inoculating seed or seedlings with beneficial rhizobacteria. Substantial gains in plant biomass due to bacterial treatment have been demonstrated (Gaskins et al. 1985: Schroth and Weinhold 1986). The term plant growth promoting rhizobacteria (PGPR) has been used to describe soil bacteria that are able to colonize plant roots and stimulate plant growth when applied to seeds, tubers, or roots (Kloepper et al. 1980). Seed inoculation with bacteria also has been shown to stimulate seedling emergence (Roll et al. 1988). Strains belonging to several genera have been demonstrated to promote plant growth but those belonging to Pseudomonas, Azospirillum, Azotobacter, and Bacillus are most commonly encountered (Gaskins et al. 1985).

The mechanism by which PGPR exert such effects remains unknown but several possibilities have been examined. These include: (i) increased availability of some limiting nutrient, usually phosphorus, through secretion of phosphorus solubilizing compounds; (ii) suppression of pathogenic or deleterious bacteria in the rhizosphere of crop plants through antibiotic production or direct competition; (iii) bacterial production of plant growth substances such as cytokinins, gibberellins, or

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[1]Paper presented at the Intermountain Forest Nursery Association Annual Meeting, Bismarck, North Dakota, August 14-18, 1989

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auxins; and (iv) root-associated fixation of atmospheric nitrogen by diazotrophic microbes (Gaskins et al. 1985: Schroth and Weinhold 1986). Although not conclusively demonstrated, much experimental evidence suggests that bacterially-mediated phytohormone production is the most likely explanation for the activity of PGPR (Brown 1974; Tien et al. 1979; Holl et al. 1988).

Canada's productive forest land base of 251 million hectares currently contains 30 million hectares which are classified as not satisfactorily restocked (NSR) (Sutton 1985). Several factors contribute to seedling success in the field but the capacity for rapid and vigorous root growth after outplanting is essential. Inadequate root performance is a common characteristic of failing outplanted seedlings. Because PGPR have been shown to affect root characteristics such as branching, surface area, and biomass (Tien et al. 1979; Chanway et al. 1988a,b) they may be of value in current reforestation efforts.

The possibility of enhancing productivity of tree species by inoculation with bacteria has received little attention, however, growth promotion of deciduous species has been demonstrated (Gardner et al. 1984; Pandey et al. 1986: Caesar and Burr 1987). Only two studies with PGPR and coniferous species have been reported (Parker and Dangerfield, 1975; Pokojska-Burdziej, 1982). In both studies, positive effects due to inoculation were observed but neither study involved pure culture inoculation of seedlings in a standard seedling medium. Therefore, the objective of this study was to determine the effect of pure culture bacterial inoculation on emergence and growth of containerized lodgepole pine, white spruce, and Douglas-fir.

MATERIALS AND METHODS

Seed

Lodgepole pine (Pinus contorta Dougl.) was collected near Big Lake, British Columbia (52° 22' latitude, 121° 51' longitude) from an elevation of 945 m. Coastal Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) was collected from McCall N11, B.C., (52° 25' latitude, 126° 11' longitude) elevation 400 m. White spruce (Picea glauca Voss.) was collected near Quesnel, B.C. (53° 12' latitude, 122° 3' longitude) from an elevation of 1350 m. Douglas-fir and white spruce seed was stratified by in cold running water for 24 hours and storing at 4° C for 3 weeks after blotting dry. Lodgepole pine seed was used unstratified.

Microorganisms

Bacillus strains L5 and L6 were utilized as inoculants. These strains were isolated from the rhizosphere of a perennial ryegrass (Lolium perenne L.) - white clover (Trifolium repens L.) pasture and have been shown to stimulate the growth of various forage species (Roll et al., 1988: Chanway et al., 1988a).

Seedling growth in containers

Experiments were conducted in plastic cones (Pine Cell 64 cm^3, Ray Leach `Cone-Tainer' Nursery, Oregon, USA) filled with a peat/vermiculite nursery mixture (peat 60 L, vermiculite 20 L, dolomite 190 g, Osmocote 285 g, and micronutrients (Nutritrace, Plant Products Company Ltd., Brampton, Ontario, Canada) 38 g. Bacillus strains L5 and L6 were grown separately in nutrient broth for 4 days, harvested by centrifugation (10,000 x g for 15 min) and resuspended in 20 mM potassium phosphate buffer pH 7.0 to a concentration of ca. 10^7 cells per mL (OD_{420} = 0.15). Two (lodgepole pine and Douglas-fir) or 4 (white spruce) seeds were sown per cell. Cells were then inoculated with 1 mL of the appropriate bacterial suspension. Control cells received sterile phosphate buffer. Seed was then covered with ca. 10 mm granite grit (No. 1 Granite Grit, Imasco, Surrey, British Columbia, Canada) and cells were watered to saturation.

Seedlings were grown in a growth chamber (Conviron, Winnipeg, Man.) with a 16 h photoperiod, a day night temperature regime of 23:17° C, and photosynthetically active radiation at the canopy level of ca. 300 uE m^{-2}. Plants were watered to saturation every 3 days. Once a week, water was amended with .325 gL^{-1} of fertilizer (Plant Prod 20-8-20; Plant Products Company Ltd., Brampton, Ontario, Canada) supplemented with 15 mg $FeSO_4$. Each month, 10-15 plants per treatment were harvested. Roots were separated from shoots and shoot height and root collar diameter were measured. Plan material was then dried for 2 days (70 C) before root and shoot weights were recorded and root surface area was assessed using a video image analyzer similar to that described by Cunningham et al. (1989).

Seedling growth in pots

Containerized lodgepole pine seedlings were obtained from the UBC nursery in May and planted in 1 L pots containing greenhouse soil previously described by Holl et al. (1988). Bacillus strain L6 inoculum was prepared as described above. A 1 mL aliquot of bacterial suspension was applied to seedlings above the root system. Control seedlings received sterile potassium phosphate buffer. Plants were grown in the greenhouse under natural light. After 8 weeks growth, seedlings were destructively harvested. Roots were separated from shoots and shoot height, root length and root collar diameter were measured. Plant material was then dried for 2 days (70° C) before root and shoot weights were recorded.

Experimental design and statistical analysis

Experiments performed in containers were arranged in a completely random design and were repeated at least once. Data from experiments were pooled and analyzed as a single experiment with individual experimental trials as an additional factor. A latin square design was utilized in the single experiment involving strain L6 and 1-0 lodgepole pine seedlings. After ANOVA, treatment means were separated using Fisher's Protected LSD or orthogonal contrasts in all experiments.

RESULTS

Strain L6 had a stimulatory effect on seedling emergence of containerized lodgepole pine in one of two trials (data not shown). Five days after sowing, 57% ($p < 0.05$) more seedlings had emerged in L6-inoculated containers. The magnitude of the emergence stimulation decreased with time but when emergence was complete 11 days past sowing, L6-inoculated cavities had 12% ($p < 0.1$) more seedlings than controls.

After eight weeks growth, seedlings from seed inoculated with strain L6 also showed significant gains in root surface area (23%) and root (17%) and shoot (17%) biomass ($p < 0.05$) (table 1). Inoculation with strain L5 did not affect seedling biomass eight weeks after treatment. After 12 weeks growth, no effect of strain L6 could be detected. However, L5-inoculated seedlings had 16% ($p<0.05$) greater root surface area. Neither strain affected growth of lodgepole pine 16 weeks after seed inoculation.

TABLE 1. EFFECT OF BACTERIAL INOCULATION ON GROWTH OF CONTAINERIZED LODGEPOLE PINE

		ROOT DRY WEIGHT (mg)	SHOOT DRY WEIGHT (mg)	ROOT AREA (cm2)	SHOOT HEIGHT (cm)	ROOT COL. DIAMETER (mm)
8 WKS	CONTROL	18	82	9.8	8.7	0.84
	STRAIN L5	16 (-11)	73 (-11)	8.0 (-11)	9.2 (+6)	NA
	STRAIN L6	21** (+17)	96** (+17)	12.0** (+23)	9.3* (+7)	0.92** (+10)
12 WKS	CONTROL	52	219	21.9	11.9	1.3
	STRAIN L5	51 (-2)	206 (-6)	25.3** (+16)	11.5 (-3)	1.2 (-8)
	STRAIN L6	48 (-8)	224 (+2)	23.3 (+6)	12.4 (+4)	1.3 (0)

* p<0.10 ** p<0.05 ( ) = % OF CONTROL

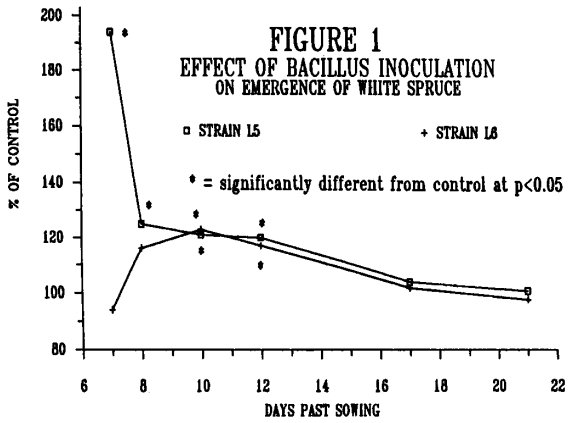
Bacillus strain L6 also stimulated growth of 1-0 bareroot lodgepole pine seedlings when tested in a pot assay in the greenhouse (table 2). Shoot dry weight was significantly higher in response to inoculation with Bacillus (17% $p<0.05$). Root dry weight and caliper also increased due to inoculation, but differences were not significant.

TABLE 2. EFFECT OF INOCULATION WITH STRAIN L6 ON GROWTH OF 1-0 CONTAINERIZED LODGEPOLE PINE

	ROOT DRY WEIGHT (mg)	SHOOT DRY WEIGHT (mg)	SHOOT HEIGHT (cm)	ROOT COL. DIAMETER (mm)
CONTROL	2.23	2.50	16.7	3.82
STRAIN L6	2.46 (+10)	2.93** (+17)	18.0 (+8)	4.01 (+5)

** p<0.05 ( ) = % OF CONTROL

Inoculation of white spruce with Bacillus strains L5 stimulated the rate at which seedlings emerged (figure 1). One week after sowing, L5-inoculated cavities had almost twice the seedlings (94%, $p<0.05$) when compared with controls. The magnitude of this effect decreased with time until 17 days past sowing when there was no difference between control and L5-treated containers. Strain L6 had no effect on the emergence of white spruce. Neither Bacillus strain affected the growth of white spruce.



Inoculation of Douglas-fir with Bacillus did not affect seedling emergence but significant growth effects were detected. Strain L5 stimulated a significant increase in stem diameter eight weeks after sowing but did not affect plant biomass or root surface area (table 3). Twelve weeks after sowing there were no detectable differences in the performance of L5 inoculated and control seedlings. Strain L6 did not affect seedling growth 8 weeks after inoculation, but a significant increase in root surface area (22%, $p<0.05$) was detected 12 weeks after treatment.

DISCUSSION

Our data confirm that bacterial inoculation of conifer species can stimulate seedling emergence or growth in containers. Uneven or protracted seedling emergence can result in additional costs to nursery operators due to oversowing and thinning operations. Seed inoculation with emergence-promoting bacteria would have obvious benefits in reducing costs associated with poor seedling emergence in the nursery. Bacillus strain L5 stimulated the rate of seedling emergence while strain L6 enhanced the number of lodgepole pine seedlings emerging in one of two trials. These bacterial strains were selected for study because they have shown growth promoting activity with agricultural crops (Chanway et al 1988a; Holl et al. 1988). Other bacterial strains specifically selected for conifer species may be more effective in promoting seedling emergence and require further study.

TABLE 3. EFFECT OF BACTERIAL INOCULATION ON GROWTH OF CONTAINERIZED DOUGLAS-FIR

		ROOT DRY WEIGHT (mg)	SHOOT DRY WEIGHT (mg)	ROOT AREA (cm2)	SHOOT HEIGHT (cm)	ROOT COL. DIAMETER (m)
8 WKS	CONTROL	28.1	135.5	4.1	9.2	0.84
	STRAIN L5	31.6 (+13)	145.3 (+7)	4.6 (+12)	9.1 (-1)	0.97** (+16)
	STRAIN L6	27.9 (-1)	136.8 (+1)	4.1 (0)	9.2 (0)	0.85 (-1)
12 WKS	CONTROL	91.9	363.0	9.2	17.4	1.45
	STRAIN L5	92.1 (0)	366.0 (+1)	9.2 (0)	18.0 (+3)	1.41 (-3)
	STRAIN L6	102.9 (+12)	390 (+7)	11.2** (+22)	17.1 (-2)	1.55 (+7)

** $p<0.05$ ( ) =% OF CONTROL

The most consistent effect of seed inoculation on plant growth was an increase in root dry weight and/or surface area. Grossnickle and Blake (1987) have demonstrated the importance of new root growth in establishment of outplanted pine and spruce seedlings. Therefore, bacterial inoculation of seedlings before outplanting may be a useful technique to increase seedling survival in the field.

It is interesting to note that the response of Douglas-fir to inoculation with strain L6 took 3 months to develop. Eight weeks after bacterial treatment no difference due to inoculation could be detected, but 12 weeks after sowing, root surface area was 22% greater than controls. Parker and Dangerfield (1975) also reported a delayed response to microbial inoculation in Douglas-fir. Five weeks after inoculation treated plants were reported to be slightly smaller and weakly chlorotic but 13 weeks after treatment inoculated seedlings were visibly larger. In contrast, the stimulatory effect of bacterial inoculation on the growth of lodgepole pine decreased with time. This may be due to poor inoculum survival or to the restricted area in which root systems developed. Root colonization studies with antibiotic resistant mutants of strain L6, reinoculation experiments, and experiments in larger size containers are underway to determine the cause of the time dependent decline in growth response. Results from our first re-inoculation experiment indicate that the growth response in pine can be maintained if seedlings are re-inoculated 8 weeks after sowing (data not shown). Further experiments with lodgepole pine, Douglas-fir, and Bacillus strain L6 are underway to determine the field response of seedlings to bacterial inoculation.

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