ENHANCEMENT OF INFECTION AND NODULATION IN ACTINORHIZAL PLANTS BY INOCULATION WITH *FRANKIA*-AMENDED SUPERABSORBENT POLYMERS[1]

by

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Abstract. Actinorhizal plants (non-leguminous nitrogen fixing tree species) are unique in forming a symbiosis with the actinomycete *Frankia*. They are economically and ecologically important due to their ability to colonize disturbed and nutrient-impoverished substrates. The degree of infection and nodulation in *Alnus glutinosa* and *Casuarina equisetifolia* were evaluated using a root dip consisting of a superabsorbent polymer slurry amended with various concentrations of *Frankia*. This delivery system markedly improved the degree of nodulation and growth of *Alnus* and *Casuarina* in both laboratory and field studies. Significantly greater nodulation and rate of growth were observed in plants treated with *Frankia*-polymer slurries compared to plants inoculated with the same amount of *Frankia* by standard techniques. Nodule number and nodule dry weight per plant were also observed to be two to three times greater in the polymer-*Frankia* treated plants. Unlike the plants treated with *Frankia* alone, nodules in the polymer-*Frankia* treated plants were distributed throughout the root system. When amended with polymer, plants inoculated with 5 to 10-fold lower titers of *Frankia* exhibited nodulation and growth equal to or greater than that of plants inoculated at standard titer by the standard methods. The mechanism of infection and nodulation enhancement appears to be related to the ability of the polymer delivery system to maintain the microorganisms in close contact with the rhizoplane of the developing root system. This is believed to be the first *Frankia* inoculum delivery system that enhances the nodulation of actinorhizal plants and also enables adequate nodulation with a 5 to 10-fold smaller inoculum. The system thereby improves the cost effectiveness of using *Frankia*-inoculated actinorhizal plants for mine-spoil reclamation. This delivery system is part of U.S. Patent #4,975,105.

Introduction

Actinorhizal plants are unique in establishing a nodule-forming symbiosis with the actinomycete *Frankia*. They are found in a wide variety of woody dicotyledonous plants including 8 families and 25 genera (Table 1). All are perennial dicots with the exception of *Datisca*, which has herbaceous roots (Tjepkema et al. 1986). These plants are noted for their ability to fix atmospheric nitrogen, thus enriching soil nitrogen levels. A number of actinorhizal species rival or exceed legumes in the amount of nitrogen that they have

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Table 1. Currently recognized actinorhizal families and genera previously used for reclamation purposes.[1]

Family	Genus[2]	# of Species
Betulaceae	*Alnus* *	47
Casuarinacea	*Allocasuarina* *	54
	Casuarina *	16
	Ceuthostma	2
	Gymonostoma	18
Coriariaceae	*Coraria* *	16
Daticacea	*Datisca*	2
Eleagnaceae	*Elaeagnus* *	38
	Hippophae *	2
	Shepherdia *	2
Myricacae	*Comptonia* *	1
	Myrica *	28
Rhamnaceae	*Ceanothus* *	31
	Colletia	4
	Discaria	5
	Kentrothamnus	1
	Retanilla	2
	Talguenea	1
	Trevoa	2
Rosaceae	*Cerocarpus* *	4
	Chamaebatia	1
	Cowania *	1
	Dryas *	3
	Purshia *	2

[1]Adapted from Baker 1988, and Baker and Schwintzer 1990.
[2]* Indicates previous use in land reclamation. From: Fessenden 1979, Hossner 1988, and Reddell et al. 1991.

been estimated to fix (Baker and Schwintzer 1990). For example, within the genus *Alnus*, estimated rates of N_2-fixation have ranged from 60-32 kg/ha/yr (Tarrant and Trappe 1971).

Actinorhizal plants are of economic and ecological importance partly due to their ability to colonize disturbed or nitrogen poor substrates (Benson and Hanna 1982). In addition to reclamation of disturbed lands, actinorhizal species are used for ornamental, wildlife, timber, fuelwood, pulpwood, nurse, and windbreak purposes (Dawson 1986). Besides improving the nitrogen status of soils, these species commonly have the ability to endure harsh environmental conditions while restoring the soil fertility of disturbed land by the addition of nitrogen rich organic matter (Visser et al. 1990). Not surprisingly, these plants have been highly recommended for land reclamation purposes and a wide variety have heen used for this purpose (Fessenden 1979, Hossner 1988, Dawson 1990). Still, the potential benefits of these species are not fully appreciated.

Just over a decade ago, *Frankia* was isolated in pure culture and its ability to fix nitrogen in association with its host plant was verified (Callaham et al. 1978). Little is known about the autecology of this microsymbiont (Baker 1988). In contrast to the use of *Rhizobium* with legumes, inoculation techniques using *Frankia* and actinorhizal plants have not been commercially developed.

The indigenous population of *Frankia* is often low or absent in disturbed substrates (Dawson et al. 1983, Visser et al. 1990). Thus, inoculation of actinorhizal plants with the appropriate microsymbiont is necessary to maximize the nitrogen fixing capabilities of these species. Standard inoculation techniques have included: aqueous suspensions of *Frankia* pure cultures at 0.01-0.05 ml packed cell volume (PCV) per plant; crushed nodule suspensions; and rhizospheric soil (Akkermans and Houwers 1979, Berry and Torrey 1985, Thomas 1986).

A. glutinosa and other *Alnus* spp. have been widely used for reclamation purposes in the United States and even more frequently in Europe, particularly on coal mine spoils (Fessenden 1979, Binns and Fourt 1980, Hielman and Ekuan 1982).

A. glutinosa is noted for tolerating a wide range of soil pH, which is a problem commonly associated with revegetating mine spoils (Funk 1965, Vogel 1981). *Casuarina* spp. are used in arid and semiarid locales in reclamation of disturbed land and to stabilize desert and coastal sand dunes. They have been widely planted in Egypt, other parts of Africa, India, China, and to some extent in the southeastern United States (Gerry 1983, Reddell et al. 1991). The Casuarinas are noted for their salt and drought tolerance, and they are excellent species for fuelwood production. The caloric value of the wood (5000 kcal/kg) is greater than almost all other tree species (National Research Council 1984, Reddell et al. 1991).

The quality and rate of land reclamation using actinorhizal species is dependent on exploitation of the microsymbiont (Wheeler and Miller 1990). Standard inoculation procedures have often proved to be ineffective in field, reclamation, and amenity plantings of actinorhizal species (Reddell et al. 1991). To date, no effective commercial *Frankia* inoculants are available (Benoit and Berry 1990).

Superabsorbent polymers have been proposed as a tool for mine reclamation due to their ability to absorb and retain hundreds of times their own weight in water, thus minimizing moisture stress in transplants. For a review of the use of non-amended superabsorbents in mine reclamation see Pritchard (1984). To our knowledge, ours is the first attempt to incorporate *Frankia* into superabsorbents as an inoculant for actinorhizal plants. The observed positive response with the two species evaluated in this study suggests that similar results might be obtained with other actinorhizals. The aim of this work was to evaluate the effectiveness of Frankia-amended superabsorbent polymer formulations under laboratory and field conditions compared to standard inoculation procedures.

Methods

Experiment I

In this experiment polymer-*Frankia* inoculum was compared to *Frankia* alone for inoculation of *A. glutinosa* under laboratory conditions. Seed of *A. glutinosa* was obtained from F.W. Schumacher, Inc., Sandwich, Mass. Seeds soaked in warm sterile water for 12 hours were subsequently transferred to a sterile mixture of moist sand and vermiculite and allowed to germinate. Rooted seedlings were transplanted to sterile perlite in 6" white plastic pots and pre-moistened with deionized water. While transplanting, plants were inspected for the absence of nodules. Cells of *Frankia* strain ArI4 (DDB01310210) were washed and homogenized, and 100:1 V/V dilutions were prepared by mixing 100 parts water or polymer slurry with one part *Frankia* packed cell volume (PCV). Polymer slurry consisted of 0.35 grams cross-linked potassium polyacrylate/polyacrylamide copolymer and 100 mls sterile water. The three treatments consisted of 1) control plants whose roots were dipped for 5 sec. in sterile water, 2) seedlings dipped in a sterile water suspension of *Frankia*, and 3) seedlings dipped in the polymer-*Frankia* slurry. There were nine seedlings per treatment, with three seedlings per pot. Within each treatment group, the remainder of the inoculum mixture not adsorbed onto the roots of the seedlings during dipping was dripped in equal aliquots at the base of each plant. The plants, grown in a large environmentally controlled growth room, were irrigated 5 times weekly with 250 ml of 1/4 strength Hoagland's nitrogen-free nutrient solution pH 5.5 supplemented with 0.05 mM KNO_3 (Hoagland and Arnone 1950). Twice weekly, plants were flushed with 400 ml deionized water. Plants were grown for a total of 8 weeks. The growth room was illuminated with 1000 Watt High Pressure Sodium and Metal Halide lamps in GE Duraglow fixtures in an alternating array. Photosynthetic flux density was determined to be 700-800 μmoles $m^{-2}s^{-1}$ with a

light dark cycle of 14:10 h and a day:night temperature cycle of 30:22°C. Treatments were randomized by pot in the growth room. At eight weeks, plants were harvested and their shoot length and nodule number were measured. Dry weights for each growth or nodulation parameter were determined after drying for 24 hr at 70°C.

Experiment II

A second experiment was performed with *A. glutinosa* to evaluate two different polymer formulations and the use of reduced *Frankia* titers in the polymer slurries. The same basic methods for propagation, inoculation, and environmental conditions used in the first experiment were also used in the second experiment. The seven treatments, with 9 seedlings per treatment, consisted of: a corn-starch based copolymer at the standard 100:1 *Frankia* titer (0.01 ml PCV/plant) and with a five-fold (5X) reduction in titer (0.002 ml PCV/plant); the same two inoculum titers with a potassium polyacrylate/polyacrylamide copolymer; standard and reduced titer *Frankia* inoculum without polymer; and uninoculated control plants treated in the same fashion as the treated seedlings. Seedlings of *A. glutinosa* were germinated as previously described and carefully inspected for the absence of nodules prior to transferring to the 6" pots. Plants were randomized, grown and maintained with the same nutrient regime as described in Experiment I. Plants were harvested at ten weeks post-inoculation and evaluated for growth and nodulation parameters as in Experiment I.

Experiment III

A field trial was designed in an attempt to translate the laboratory results to outplanted *A. glutinosa*. Seeds were germinated as previously described and transferred to 6" pots containing a sterile soil-perlite mixture. Plants were grown under the same temperature and light conditions as the other experiments. However, they were watered with tap water to field capacity when necessary. The plants were allowed to grow for 24 weeks and very carefully inspected for the absence of nodules prior to outplanting. The roots were washed free of any remaining substrate, randomized, and were transferred from the laboratory to the field in deionized water. They were outplanted in a randomized block in an old pasture dominated by *Agropyron repens* and *Andropogon* in an oak-savanna vegetation region within the Anoka Sand Plain. The area was located in Chisago County, South Sunrise Township, Minnesota (one hour north of Minneapolis-St. Paul). Climate of the area is characterized by short summers and long, cold winters. Annual precipitation in the area averages 660 mm, with a mean annual temperature of 22°C (Baker 1971, Grigal et al. 1974). The substrate was a well drained sandy-loam. Plant growth on these soils is commonly considered to be nitrogen-limited. The plants were grown for 4 months with no maintenance in the form of fertilization, watering, or weed control. Each treatment block was 5 x 5 meter ($25m^2$) in area with 8 to 9 plants per block. The four treatments consisted of a control group that received no pretreatment, plants that were treated only with a cross-linked potassium polyacrylate/polyacrylamide copolymer, a group receiving approximately 0.01 mls (PCV) of *Frankia* per plant and a treatment of the same inoculum titer with a cross-linked potassiumpolyacrylate/polyacrylamidecopolymer. All treated plants were root-dipped in the polymer, polymer-*Frankia* slurry, or *Frankia* alone as previously described. The seedlings were planted in mid-June and harvested in late October. The outplanting was periodically observed but no attempt was made to determine growth increments over the season. The plants were harvested by excavation and carefully washed to remove all rhizospheric soil. Growth and nodulation parameters were calculated using methods previously described.

Experiment IV

This experiment was designed to evaluate the

potential of the inoculant delivery system to enhance nodulation of *Casuarina equisetifolia* grown under laboratory conditions. Two different polymer formulations were evaluated with both a standard and a 10-fold (10X) reduction in *Frankia* inoculum (0.001 ml PCV/plant). Seeds from a plantation of *Casuarina* located in Hawaii were obtained courtesy of Dr,. Dwight D. Baker, Yale University, New Haven, CT. Rooted seedlings were transplanted to pre-moistened sterile perlite, with three plants per pot. Seedlings were inoculated (root-dipped) with either 1 ml each of a 100:1 dilution (PCV) or a further 10X dilution of washed and homogenized cells as described in Experiment I, using *Frankia* strain CCI3 (HFP020203). The 9 treatments, with 9 seedlings per treatment, consisted of: untreated control; polymer only (using both the potassium polyacrylate/polyacrylamide copolymer and corn starch-based copolymer individually); one treatment each of *Frankia* only at the standard titer (0.01 ml PCV/plant) and a 10X dilution; one treatment each with each polymer and the standard inoculum density and one treatment each with each polymer and the reduced inoculum density. Nutrient solution was used as in the other laboratory studies. However, at 8 weeks the application of the nutrient solution was reduced to once weekly for the remainder of the 12 week growth period. The rationale for the reduced nutrient application was based on the observation that *Casuarina* spp. are slower to nodulate than other actinorhizal species (Kohls and Baker 1989). This nutrient regime allowed for a more sensitive evaluation of the growth parameters of the nodulated plants. The plants were harvested at 12 weeks, and the various growth and nodulation parameters were evaluated as previously described.

Statistical analyses

Treatment grand means and associated standard errors were calculated for the plant growth and nodulation parameters. Data were analyzed for variance using ANOVA and Fisher's Least Squares Differences method to identify statistically significant ($P \leq 0.05$) treatment effects.

Results

Experiment I.

Plant growth. The growth parameters for the controls, inoculated plants, and polymer treated plants for Experiment I are presented in Tables 2 and 3. The controls appeared chlorotic, which is common for nitrogen deficient plants. Their growth was significantly stunted when contrasted with the two other treatments. No statistically significant differences were found between the plants treated with polymer-*Frankia* and those treated with *Frankia* alone with respect to the biomass of the plant components. However, there was a significant difference in the root/shoot ratios (Table 2).

Nodulation. Data for the nodulation parameters for Experiment I can be found in Table 3. As noted, there was no significant difference in shoot weight between plants inoculated with polymer-*Frankia* and *Frankia* alone. However, the number of nodules per plant (measure of infection), nodule weight per plant (measure of nodule development), and nodule dry weight as a percent of whole plant dry weight were all approximately 2-fold greater in the polymer-*Frankia* treatments than in the *Frankia* treatments without polymer.

Experiment II.

Plant growth. Plant growth increased dramatically as a result of polymer-*Frankia* treatments in Experiment II (Table 4). A 2 to 3 fold difference in plant shoot weight was observed between the controls and the polymer-*Frankia* treatment. Though not statistically significant in all cases, use of *Frankia* alone increased shoot, root, and whole plant dry weight. However, the root/shoot ratio was not significantly altered. With some exceptions in root dry weight, the use of standard

Table 2. Experiment I: Growth measurements for *Alnus glutinosa* grown indoors.

	Grams Dry Weight [Mean ± (std error)]				Mean ± (SE)
Treatment[1]	n	Shoot[2]	Root	Whole Plant	Root/Shoot
Controls	9	0.20 (0.01)a	0.22 (0.03)a	0.42 (0.03)a	1.13 (0.08)a
Frankia alone	9	0.30 (0.01)b	0.24 (0.02)a	0.54 (0.03)b	0.87 (0.06)b
Frankia + Polymer	9	0.33 (0.03)b	0.21 (0.01)b	0.54 (0.04)b	0.67 (0.07)c

[1]Definitions of treatments: Controls, no inoculum or polymer; *Frankia* alone, standard inoculum titer without polymer; *Frankia* + Polymer, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

Table 3. Experiment I: Nodulation data for *Alnus glutinosa* grown indoors.

Treatment[1]	n	Shoot length[2] (cm)	Nod. #/Plant	Nod. weight (gm)	% Nod.wt./Plant
Controls	9	10.4 (0.58)a	0	0	N/A
Frankia alone	9	12.2 (0.28)b	203.9 (11.4)a	0.07 (0.01)a	13.1 (1.26)a
Frankia + Polymer	9	13.1 (0.46)b	489.7 (33.4)b	0.13 (0.02)b	23.6 (1.93)b

[1]Definitions of treatments: Controls, no inoculum or polymer; *Frankia* alone, standard inoculum titer without polymer; *Frankia* + Polymer, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

and reduced titer polymer-*Frankia* resulted in significantly greater shoot, root, and whole plant dry weights than use of *Frankia* alone. Plants inoculated with the standard and 5X dilution of polymer-*Frankia* exhibited greater shoot and whole plant dry weight than plants exposed to both reduced and standard *Frankia* inoculants alone. In addition, lower root/shoot ratios were observed in polymer-*Frankia* treated plants compared to the other treatments (Table 4). Moreover, statistically significant differences were observed in mean shoot length between controls, *Frankia* alone, and polymer-*Frankia* treatments (Table 5).

Nodulation. Data for the various nodulation factors between treatments in Experiment II are presented in Table 5. Nodule number per plant was greatest in the polymer-*Frankia* standard concentration and lowest in the 5X dilution using *Frankia* alone. Within each titer, approximately 2-fold differences were observed for nodule

Table 4. Experiment II: Growth measurements for *Alnus glutinosa* grown indoors.

	Grams Dry Weight [Mean ± (std error)]				Mean ± (SE)
Treatment[1]	n	Shoot[2]	Root	Whole Plant	Root/Shoot
Controls	9	0.077 (0.004)a	0.058 (0.001)a	0.136 (0.011)a	0.74 (0.062)a
FB	9	0.111 (0.009)a	0.084 (0.001)b	0.195 (0.018)b	0.75 (0.059)a
FA	9	0.096 (0.010)a	0.062 (0.004)a	0.158 (0.012)c	0.68 (0.061)a
FB-010	9	0.134 (0.019)b	0.083 (0.014)b	0.217 (0.032)d	0.60 (0.024)a
FB-B204	9	0.207 (0.010)c	0.108 (0.011)c	0.315 (0.017)e	0.53 (0.046)b
FA-010	9	0.179 (0.018)c	0.095 (0.019)b	0.274 (0.031)e	0.52 (0.041)b
FA-B204	9	0.230 (0.018)c	0.066 (0.011)a	0.296 (0.028)e	0.37 (0.024)b

[1]Definitions of treatments: Controls, no inoculum or polymer; FB, 5X reduction in inoculum titer without polymer; FA, standard inoculum titer without polymer; FB-010, 5X reduction in inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FB-B204, 5X reduction in inoculum titer with starch-based polymer; FA-010, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FA-B204, standard inoculum titer with starch-based polymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

Table 5. Experiment II: Nodulation data for *Alnus glutinosa* grown indoors.

Treatment[1]	n	Shoot length[2] (cm)	Nod. #/Plant	Nod. weight (gm)	% Nod.wt./Plant
Controls	9	6.36 (0.18)a	0	0	N/A
FB	9	8.02 (0.29)b	43.0 (2.7)a	0.017 (0.001)a	9.9 (1.2)a
FA	9	8.61 (0.25)b	93.0 (3.0)b	0.022 (0.002)b	13.9 (0.8)b
FB-010	9	9.50 (0.27)c	110.0 (5.3)c	0.033 (0.002)c	17.9 (3.6)c
FB-B204	9	10.80 (0.40)c	129.0 (9.8)c	0.041 (0.003)d	13.2 (1.1)b
FA-010	9	10.20 (0.22)c	272.0 (15.8)d	0.058 (0.001)e	18.4 (2.0)c
FA-B204	9	10.50 (0.55)c	266.0 (13.3)d	0.060 (0.004)e	21.2 (2.3)c

[1]Definitions of treatments: Controls, no inoculum or polymer; FB, 5X reduction in inoculum titer without polymer; FA, standard inoculum titer without polymer; FB-010, 5X reduction in inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FB-B204, 5X reduction in inoculum titer with starch-based polymer; FA-010, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FA-B204, standard inoculum titer with starch-based polymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

Table 6. Experiment III: Growth measurements for *Alnus glutinosa* field trial.

	Grams Dry Weight [Mean ± (std error)]				Mean ± (SE)
Treatment[1]	n	Shoot[2]	Root	Whole Plant	Root/Shoot
Controls	8	1.31 (0.19)a	2.83 (0.46)a	4.14 (0.49)a	2.41 (0.50)a
Polymer alone	7	2.02 (0.36)b	3.22 (0.46)a	5.24 (0.53)a	1.96 (0.44)a
Frankia alone	9	2.35 (0.40)b	2.90 (0.58)a	5.25 (0.78)a	1.37 (0.30)a
Frankia + Polymer	9	2.90 (0.019)c	1.74 (0.21)b	4.64 (0.36)a	0.60 (0.07)b

[1]Definitions of treatments: Controls, no inoculum or polymer; Polymer alone, cross-linked potassium polyacrylamide/polyacrylate copolymer without inoculum; *Frankia* alone, standard inoculum titer without polymer; *Frankia* + Polymer, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

Table 7. Experiment III: Nodulation data for *Alnus glutinosa* field trial.

Treatment[1]	n	Shoot length[2] (cm)	Nod. #/Plant	Nod. weight (gm)	% Nod.wt./Plant
Controls	8	18.6 (1.30)a	7.0 (1.4)a	0.038 (0.001)a	0.96 (0.15)a
Polymer alone	7	21.3 (0.65)a	8.8 (1.7)a	0.046 (0.001)a	0.95 (0.22)a
Frankia alone	9	20.5 (0.83)a	46.0 (3.5)b	0.070 (0.001)b	1.20 (0.15)a
Frankia + Polymer	9	24.9 (0.93)b	131.0 (19.7)c	0.016 (0.002)c	3.82 (0.63)c

[1]Definitions of treatments: Controls, no inoculum or polymer; Polymer alone, cross-linked potassium polyacrylamide/polyacrylate copolymer without inoculum; *Frankia* alone, standard inoculum titer without polymer; *Frankia* + Polymer, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

number and nodule weight per plant between the polymer-*Frankia* treated plants and plants inoculated with *Frankia* alone. Irrespective of titer, the percent of nodule weight per plant was equal to or significantly greater with the polymer-*Frankia* treatments than with *Frankia* alone.

Experiment III.

Plant growth. Plant growth parameters for field inoculated *A. glutinosa* are shown in Tables 6 and 7. Following one growing season, there was a significant increase in shoot dry weight and shoot length with polymer-*Frankia* than with the other treatments. A corresponding decrease in the root dry weight accounts for the lack of a significant difference in whole plant dry weight. This resulted in a very significant decrease in root/shoot ratio with the polymer-*Frankia* treatment.

Table 8. Experiment IV: Growth measurements for *Casuarina equisetifolia* grown indoors.

	Grams Dry Weight [Mean + (std error)]				Mean ± (SE)
Treatment[1]	n	Shoot[2]	Root	Whole Plant	Root/Shoot
Controls	8	0.184 (0.022)a	0.306 (0.048)a	0.490 (0.068)a	1.68 (0.16)a
P-010	8	0.287 (0.025)b	0.369 (0.033)a	0.657 (0.052)b	1.23 (0.06)b
P-B204	9	0.241 (0.020)b	0.321 (0.027)a	0.563 (0.045)b	1.34 (0.06)c
FB	9	0.286 (0.032)b	0.395 (0.033)a	0.682 (0.046)b	1.57 (0.23)a
FB-010	8	0.368 (0.033)c	0.332 (0.034)a	0.700 (0.056)b	0.93 (0.09)d
FB-B204	8	0.391 (0.058)c	0.269 (0.042)b	0.661 (0.077)b	0.73 (0.13)e
FA	9	0.433 (0.036)c	0.502 (0.085)c	0.935 (0.109)c	1.15 (0.14)b
FA-010	9	0.783 (0.078)d	0.576 (0.059)c	1.359 (0.131)d	0.73 (0.06)e
FA-B204	9	0.666 (0.046)d	0.469 (0.031)c	1.135 (0.052)d	0.75 (0.05)e

[1]Definitions of treatments: Controls, no inoculum or polymer; P-010, cross-linked potassium polyacrylamide/polyacrylate copolymer without inoculum; P-B204, starch-based polymer without inoculum; FB, 10X reduction in inoculum titer without polymer; FB-010, 10X reduction in inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FB-B204, 10X reduction in inoculum titer with starch-based polymer; FA, standard inoculum titer without polymer; FA-010, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FA-B204, standard inoculum titer with starch-based polymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

Nodulation. The nodulation patterns for field inoculated *A. glutinosa* are shown in Table 7. The presence of nodules on the controls and plants treated with polymer alone indicate that an indigenous population of *Frankia* capable of nodulating this species was present at this location. The nodule number and weight formed by the indigenous microsymbiont were significantly lower when compared to the inoculated plants. After one growing season, plants treated with polymer-*Frankia* had significantly greater mean nodule number and nodule weight than in all other treatments. Furthermore, nodule weight as a percent of whole plant dry weight was increased more than 3-fold by using polymer-*Frankia* treatments.

Experiment IV.

Plant growth. Data for *C. equisetifolia* growth responses are presented in Tables 8 and 9. Dramatic growth differences were observed between the various treatments. Within each inoculum titer, shoot weight was significantly greater for the polymer-*Frankia* treatments than for *Frankia* alone. Root/shoot ratios for standard and reduced titer polymer-*Frankia* treatments were significantly lower than for the standard titer *Frankia* alone.

Nodulation. The nodulation results for the laboratory evaluation of *Casuarina equisetifolia* are presented in Table 9. Within each titer the polymer-*Frankia* treatments resulted in 2 to 3-fold greater response for all nodulation parameters than treatments with *Frankia* alone.

Table 9. Experiment IV: Nodulation data for *Casuarina equisetifolia* grown indoors.

Treatment[1]	n	Shoot length[2] (cm)	Nod. #/Plant	Nod. weight (gm)	% Nod.wt./Plant
Controls	8	12.2 (0.46)a	0	0	N/A
P-010	8	14.5 (0.50)b	0	0	N/A
P-B204	9	14.7 (0.65)b	0	0	N/A
FB	9	15.8 (0.69)b	3 (0.4)a	0.009 (0.0009)a	1.47 (0.12)a
FB-010	8	17.4 (0.83)c	8 (1.1)b	0.022 (0.0003)b	3.27 (0.38)b
FB-B204	8	17.2 (0.09)c	6 (1.4)b	0.020 (0.0004)b	3.28 (0.73)b
FA	9	17.6 (0.98)c	4 (1.1)a	0.019 (0.0005)b	1.88 (0.29)c
FA-010	9	22.2 (1.02)d	24 (2.6)c	0.051 (0.0003)c	3.96 (0.32)d
FA-B204	9	22.6 (0.61)d	28 (2.1)c	0.048 (0.0003)c	4.31 (0.28)d

[1]Definitions of treatments: Controls, no inoculum or polymer; P-010, cross-linked potassium polyacrylamide/polyacrylate copolymer without inoculum; P-B204, starch-based polymer without inoculum; FB, 10X reduction in inoculum titer without polymer; FB-010, 10X reduction in inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FB-B204, 10X reduction in inoculum titer with starch-based polymer; FA, standard inoculum titer without polymer; FA-010, standard inoculum titer with cross-linked potassium polyacrylamide/polyacrylate copolymer; FA-B204, standard inoculum titer with starch-based polymer.
[2]Values within a column are not significantly different ($P \leq 0.05$) if they share a lower case letter.

Nodule initiation and development

Inspection of the root systems in the laboratory studies showed distinct nodules 10-12 days post-inoculation for *Alnus*, and 21-28 days for *Casuarina*. We did not determine the time required for nodule initiation in the field trial, to avoid disturbing the plants in any way. In all experiments, the nodule distribution of the polymer-*Frankia* treated plants was observed to occupy the majority of the plant root system. In these treatments, the nodules found on the lower and distal regions of the tap and lateral roots appeared smaller than those confined to the upper portions of the root system. In contrast, the majority of nodules on plants inoculated with *Frankia* alone were confined to the upper sections of the tap and lateral roots; few if any nodules were present on the distal portions of these root systems. This observation was consistent for both plant species and all experimental conditions.

Discussion

The results indicate that superior growth and nodulation of actinorhizal plants are obtained by inoculating with polymer-*Frankia* slurries. The number of nodules per plant has been proposed as the best measure of microsymbiont infection (Streeter 1988). On this basis, our data strongly supports the premise that polymer-*Frankia* formulations promote increased infection and nodulation. Dry weight and nodule data support the concept that overall nodule weight corresponds to plant productivity (Hielman and Ekuan 1982). Lower root/shoot ratios for plants inoculated with the polymer-*Frankia* inoculum indicate a shift in dry weight allocation towards shoot growth. Polymer amended with reduced *Frankia* concentrations generally exhibited equal or greater nodulation and growth response than standard treatments. This supports our hypothesis that these formulations provide an environment for the

microsymbiont to persist, grow, and colonize new roots on the developing root system. The size of the nodules on the more distant portions of the polymer-*Frankia* treated root systems were consistently smaller. This suggests continued infection of the developing root system by the microsymbiont. It has also been observed that moisture deficits can adversely affect *Frankia* growth (Shipton and Burgraff 1982); this condition may have been circumvented by the water retaining capacity of the polymer. The optimal pH for *Frankia* growth is close to 6.0, and it is possible that the polymer stabilizes the pH of the rhizosphere, allowing for increased *Frankia* survival (Holman and Schwintzer 1987). We have observed that the polymer serves as an excellent buffer for in vitro ectomycorrhizal fungal growth (data not presented).

Though we cannot be certain why the *Alnus* inoculated in the first experiment did not exhibit the dramatic relative growth increases observed in the other studies, it is likely that relative increases in above ground biomass would have been observed if the plants were allowed to grow for longer periods as in subsequent experiments. Under similar conditions in Experiment II, polymer-*Frankia* treated plants yielded substantially increased biomass over the other treatments. These plants were allowed to grow 2 weeks longer than the plants in Experiment I. Another possible explanation is that the abundant nodulation observed in the first experiment caused a shift in the allocation of photosynthates to the developing nodules.

The data from Experiment III indicates that significant improvements in nodulation and growth responses can be obtained with the polymer-*Frankia* treatments under field conditions. The polymer-*Frankia* treated plants dramatically outperformed both the controls and plants treated with polymer or *Frankia* alone. The difference in shoot weights between treatments was statistically significant, though field observations suggest that results may have been slightly skewed by browsing or competition by associated grasses. It was surprising that an infective *Frankia* population existed at this site. To our knowledge, no actinorhizal species had ever been observed in this area. It has been suggested that the microsymbiont can persist in soils devoid of actinorhizal species (Smolander and Sundmund 1987). The observations at this site support these findings. Our observation of superior nodulation in inoculated plants at this site strongly suggests that polymer-*Frankia* inoculum was highly effective even in the presence of the indigenous *Frankia* population.

The relative contributions of the polymer-*Frankia* slurry to both nodulation and growth in *Casuarina* indicate that the formulation is superior to standard inoculation techniques in a controlled environment. A field trial using these same treatments would allow us to further assess the performance of this inoculum formulation. Our laboratory results are consistent with other studies using *Frankia* entrapped in alginate beads and supplied to *Casuarina* seedlings (Sougoufara et al. 1989). We believe that lower cost of superabsorbent polymer formulation, water retention capability, and adherence of the polymer to the root system offer significant advantages over the alginate inoculation technique.

Though the use of superabsorbent polymers has been proposed as a means for increasing transplant survival (Callaghan et al. 1988), we did not observe any significantly different rates of survival between plants with and without polymer treatment. However, plants in these experiments were not grown under high stress conditions. Plants treated with polymer alone in Experiments III and IV showed significantly greater shoot growth and correspondingly lower root/shoot ratios. This suggests that the polymer itself may serve to facilitate the uptake of water and available nutrients. We did not observe any marked differences between the effectiveness of the two different types of polymers used.

The development of these formulations could further the use of actinorhizal plants for the reclamation of disturbed land. Besides improving soil fertility, actinorhizal trees and shrubs can increase cation exchange capacity and availability of phosphorous and sulfur. They can be an important food source for wildlife (Thilenhus and Hungerford 1967, Binkley 1986, Johnson et al. 1986), and also facilitate the growth of adjacent plant species (Dawson 1990). The use of improved inoculation techniques would enhance these other factors along with survival and growth of the actinorhizal plants.

In summary, we have demonstrated that 1) infection, nodule development, and actinorhizal plant growth can be enhanced by the use of superabsorbent polymers amended with *Frankia*, 2) for *Alnus* and *Casuarina*, superabsorbent polymers amended with reduced titers of *Frankia* are as effective if not more effective than higher titers used with standard inoculation techniques, and 3) laboratory results with polymer-*Frankia* inoculation translate well to plants grown under field conditions.

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