ELSEVIER

Applied Soil Ecology 17 (2001) 141–149

Applied Soil Ecology

www.elsevier.com/locate/apsoil

Frankia and nodulation of red alder and snowbrush grown on soils from Douglas-fir forests in the H.J. Andrews experimental forest of Oregon☆

N.S. Rojas[a], C.Y. Li[b,*], D.A. Perry[c], L.M. Ganio[d]

[a] *87 Carrington Rd., Hendersonville, TN 37075, USA*
[b] *USDA Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 SW Jefferson Way, Corvallis, OR 97331, USA*
[c] *Box 8, Papa'au, HI 96755, USA*
[d] *Department of Forest Science, Oregon State University, Corvallis, OR 97331, USA*

Received 2 July 2000; accepted 31 January 2001

Abstract

This study examined colonization of *Frankia* on actinorhizal red alder (*Alnus rubra*) and snowbrush (*Ceanothus velutinus*) in soils from three stands located at the H.J. Andrews experimental forest in the western Cascade Range of Oregon: an 8-year-old clear-cut planted with Douglas-fir (*Pseudotsuga menziesii*), a young 20-year-old Douglas-fir plantation with an understory of snowbrush, and an old-growth Douglas-fir forest.

Ten soil samples were collected from within each of the three stands; in each of these soils, plants were grown in a mix of soil–vermiculite–perlite (2:1:1). Alder plants were grown for 6 months and snowbrush for a year. More alder than snowbrush survived and nodulated.

Of the plants that survived, 89% of the red alder nodulated; only 25% of the snowbrush produced nodules. More red alder plants nodulated when grown in clear-cut soils (100%) than in other soils, and more snowbrush nodulated (51%) when grown in soils from the 20-year-old plantation.

Red alder biomass and nodule weight were highest when plants were grown in clear-cut soils. Snowbrush biomass and nodule weight were highest on soils from the young stand. The biomass of snowbrush plants grown in clear-cut soils averaged higher in bottom slope soils than in soils from any other position within the clear-cut. Correlations between plant biomass, nodule weight, and acetylene reduction activity were positive and statistically significant for both species.

Differences in *Frankia* with regard to red alder and snowbrush were apparent in these study sites. The limited nodulation in snowbrush might have been due to an insufficient population of an effective *Frankia* population. Establishing snowbrush on this study site will require developing techniques for inoculating snowbrush seedlings with the relevant *Frankia* and then outplanting the inoculated seedlings on the sites.

Keywords: *Frankia*; Actinorhizal plants; Nitrogen fixation

1. Introduction

In the Pacific Northwest of North America, actinorhizal plants such as *Ceanothus* spp. and *Alnus* spp. are the primary sources of nitrogen for forest ecosystems

☆ This is Paper 3277 of the Forest Research Laboratory, Oregon State University, Corvallis, Oregon.

* Corresponding author. Tel.: +1-541-750-7386; fax: +1-541-758-7760.
E-mail address: cli@fs.fed.us (C.Y. Li).

0929-1393/01/$ – see front matter
PII: S0929-1393(01)00127-5

(Waring and Schlesinger, 1985; Perry, 1994). *Alnus* is found on mesic sites across a range of elevations; *Ceanothus* is commonly found on relatively dry sites at mid-elevations (Johnson, 1968; Franklin and Dyrness, 1973; Conard et al., 1985; Harrington et al., 1994). Both *Alnus* and *Ceanothus* are early colonizers after disturbances such as clear-cuts and fires. Of the *Alnus* species, red alder (*Alnus rubra* Bong.) has an important ecological value in forestry because of its nitrogen-fixing capability and its commercial value. Red alder, which grows fast and dominates sites quickly, covers 13% of the coastal commercial forest land of Oregon and Washington (Resch, 1988). Of eight species of *Ceanothus* in Oregon, snowbrush (*Ceanothus velutinus* Dougl.) is especially important in forest ecosystems because of its widespread occurrence and abundant regeneration after fire.

Red alder and snowbrush contribute to the nitrogen balance of forest ecosystems through a root symbiosis with a nitrogen-fixing endophyte *Frankia*, a sporulating, filamentous actinomycete. Four host-specificity groups for *Frankia* have been recognized by Baker (1987): (1) strains that nodulate *Alnus* and *Myrica*; (2) strains that nodulate *Casuarina* and *Myrica*; (3) strains that nodulate the Elaeagnaceae and *Myrica*, and (4) strains that nodulate only the Elaeagnaceae. Baker (1987) was not able to group the *Frankia* associated with *Ceanothus* because isolation from *Ceanothus* nodules has not been successful.

The presence of actinorhizal plants in Pacific Northwest forests is important because these forests are limited by nitrogen (Perry, 1994). Annual nitrogen fixation rates for red alder have been estimated at about 100 kg/ha/year (Bormann and DeBell, 1981); estimates for snowbrush average about 80 kg/ha/year (Cromack et al., 1979).

Actinorhizal plants in the Pacific Northwest are pioneers occupying sites that have not supported actinorhizal plants for hundreds of years, which raises the question of how long *Frankia* survives without actinorhizal hosts. Past studies in the Pacific Northwest generally have found abundant nodulation on alders but variable nodulation on snowbrush (Zavitkovski and Newton, 1967, 1968; Wollum et al., 1968; Youngberg and Wollum, 1976; Youngberg et al., 1979; Binkley, 1981). Wollum et al. (1968) found that snowbrush nodulated better when growing on sites formerly occupied by mid-aged conifer stands than on sites formerly occupied by old-growth; they hypothesized that *Frankia* declined over time in the absence of host plants.

This study was thus initiated to examine colonization of *Frankia* on red alder and snowbrush grown in soils from three Oregon sites: an 8-year-old clear-cut planted with Douglas-fir, a young (20-year-old) Douglas-fir plantation with an understory of snowbrush, and an old-growth Douglas-fir forest. The study was designed to determine whether the thick snowbrush cover present in the understory of the 20-year-old plantation would serve as a good source of *Frankia* inoculum for snowbrush, and whether some of the *Frankia* strains nodulating snowbrush would also nodulate red alder. The study would also answer whether the old-growth stand soil that had been devoid of actinorhizal plants for hundreds of years could induce nodule formation and nitrogen fixation for both red alder and snowbrush.

2. Materials and methods

2.1. Site description

We used soils from three sites on the H.J. Andrews experimental forest in the western Cascade Range, Oregon: (1) an 8-year-old clear-cut planted with Douglas-fir; (2) a young (20-year-old) Douglas-fir plantation with an understory of snowbrush; and (3) an old-growth stand dominated by Douglas-fir. All sites were within 1 km of each other in the Western Hemlock Zone of Franklin and Dyrness (1973). The H.J. Andrews experimental forest is located 80 km east of Eugene on the Blue River Ranger District of the Willamette National Forest (44°15′N, 122°10′W). Climate is mild, with dry summers that last about 3 months and wet winters. Precipitation averages 2400 mm a year, with most of it falling in the winter and early spring as rain or snow (Waring et al., 1978).

The clear-cut site is at 890 m above sea level. The site faces southeast on a slope averaging 22°. Formerly occupied by an old-growth forest of Douglas-fir, western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and western redcedar (*Thuja plicata* D. Don), the site was clear-cut in 1981, and logging slash was broadcast burned in 1982. Douglas-fir (900 seedlings per hectare) was planted 6 months after slash burning.

Although snowbrush normally regenerates abundantly following clearcutting and slash burning on such sites (F. Swanson, J.F. Franklin, D.A. Perry, and S.V. Gregory, unpublished), snowbrush cover on the study site remained quite low. At the time of soil collection (November 1990), snowbrush was unevenly distributed, with a few large plants growing on flats at slope bottoms and scattered small plants elsewhere. Spot surveys 8 years after broadcast burning showed that the larger plants at slope bottoms had nodulated, but the small plants growing on middle and upper slopes had not. Planted Douglas-fir has grown quite well on the site.

The 20-year-old Douglas-fir plantation with an understory of snowbrush, referred to as the "young stand", is at 976 m above sea level, approximately 1000 m from the other two stands. The site faces southwest, on a slope averaging 13°. There was abundant snowbrush cover in the understory of this site at the time of sampling.

The old-growth forest, directly adjacent to the upper boundary of the clear-cut, is at 1000 m above sea level and also faces southwest, on a slope averaging 5°. It is dominated by Douglas-fir trees up to several hundred years of age; also present are mature western hemlock and western redcedar. The understory consists of scattered shrubs, mostly vine maple (*Acer circinatum* Pursh), huckleberry (*Vaccinium* spp.), rhododendron (*Rhododendron macrophyllum* D. Don), wood sorrel (*Oxalis oregana* Nutt.), Pacific yew (*Taxus brevifolia* Nutt.), and mountain Oregon grape (*Berberis nervosa* Pursh).

2.2. Soil collection

Ten soil samples were collected from two transects in each site in early November of 1990. In the clear-cut, the first transect originated at a randomly chosen point on a flat at the base of a 22° slope and extended 60 m up-slope at right angles to the contour. The second transect was installed parallel to and 30 m distant from the first. Five sampling points were selected along each transect, one each at the bottom of the slope, lower mid-slope, mid-slope, upper mid-slope, and the top of the slope. At each sampling point, soils were collected at four spots surrounding the point and pooled to make a single sample. Samples were taken 1 m from the sample point in each of four compass directions. This sampling scheme was chosen so that samples reflected a range of slope positions within the clear-cut. The same procedure was used in the young stand and old-growth stand, except that the slopes in those stands were less steep than in the clear-cut.

All soil samples were obtained from mineral soil to a depth of 15 cm. Samples were labeled, covered with ice in a cooler, and taken to the laboratory on the day of sampling, where they were stored in a cold room at 4°C until further processing.

2.3. Plant culture

Two greenhouse bioassays were conducted, one with red alder and the other with snowbrush. Red alder seeds were surface sterilized with 30% hydrogen peroxide for 15 min and then rinsed with sterilized distilled water. Snowbrush seeds in a mesh tea infuser were immersed in boiling water for 5 min, then soaked overnight in tap water. They were surface sterilized with 30% hydrogen peroxide for 15 min, rinsed with sterilized distilled water, and partially immersed in potato dextrose agar in glass vials as described by Rose and Youngberg (1981); they were then left in a cold room (4°C) for 3 months for stratification.

Five surface sterilized red alder seeds were planted in 150 ml Ray Leach tubes containing one of the 30 soil samples in a mixture of soil–vermiculite–perlite (2:1:1); five germinating snowbrush seeds were planted the same way. At 4 weeks, tubes were thinned to one plant each. The alder bioassay was initiated with 360 seedlings (12 for each soil sample) and the snowbrush bioassay with 300 (10 for each sample).

While the plants were growing in the greenhouse, the temperature was kept at 21°C during the day and 16°C during the night; sodium-vapor lamps (11,000 lx) maintained a 14 h photoperiod. When needed, plants were watered twice daily; otherwise they were watered once a day. To avoid cross contamination from splash during irrigation, different treatments were separated by at least 20–30 cm. In order to minimize location effect, plants were rotated to different bench locations once or twice a week.

An N-free mineral solution (Pregent and Camire, 1985) was used to fertilize plants. From 6 weeks after planting until harvest, 10 ml of a 1:4 dilution of

full strength solution was used to fertilize each plant weekly.

2.4. Data collection

Alder plants were harvested after 24 weeks and snowbrush plants after 48 weeks. Four variables were measured for each species: total plant biomass, nodule weight, acetylene reduction activity per plant, and acetylene reduction activity per gram of nodule.

Prior to harvesting, we measured nitrogenase activity using the acetylene reduction technique described by Koo (1989) and Rojas et al. (1992), in which whole root systems of living plants are assayed. Each red alder plant was placed in a 525 ml plastic tube (PVC) so that the root systems were sealed off from the plant tops by means of a perforated rubber stopper. Snowbrush plants were also placed in PVC tubes, but the whole plant (roots plus tops) was contained within the tube. Each snowbrush tube was sealed with a regular non-perforated rubber stopper.

The sealed plastic tubes were injected with purified acetylene to 10% of the total gas volume of the tube. After 2 h of incubation at room temperature, a 0.1 ml gas sample was withdrawn from each tube and analyzed for acetylene and ethylene with a Hewlett-Packard 5830A gas chromatograph fitted with a 2.0 m × 2.1 mm stainless steel column filled with 80–100 mesh Porapak R. Oven temperature was adjusted to 70°C. Injection temperature and flame-ionization detector temperature were each adjusted to 100°C. Flow rate of the nitrogen carrier gas was adjusted to 40 ml per minute (Li and Castellano, 1987; Koo, 1989; Rojas et al., 1992).

At harvest, nodules were picked from roots and transferred to test tubes. Roots and tops (leaves and stems) were separated and stored in individual paper bags. Nodules, roots, and tops were oven dried for 3 days at 80°C prior to weighing.

2.5. Data analysis

Statistical analyses were performed with SAS for Windows, version 6.10 (SAS Institute, Inc., 1996). Data analysis involved using a model for a completely randomized design with subsampling (Petersen, 1985). In the model, species was nested within soil samples and soil samples were nested within stand locations (clear-cut, young stand, or old growth).

Because the design was unbalanced (unequal number of trees on the different soil samples), the regular F statistic (mean square for effect/mean square for error) does not have an F distribution. We used approximate F tests as described by Steel and Torrie (1980) and Littell et al. (1991) to test for location effect and for significant sources of variation.

Logarithmic transformation ($\log_{10}$) was not necessary for red alder. For snowbrush, logarithmic transformation was used for all four variables in order to satisfy the ANOVA assumptions of constant variance and normality. Pearson correlations between plant biomass, acetylene reduction, and nodule weight were also calculated.

To determine whether slope position within sites affected biomass and acetylene reduction, we used a one-way ANOVA. If the ANOVA indicated significant differences, we used the Bonferroni method as a mean separation technique ($\alpha = 0.005$). Because soils for this experiment were collected from two parallel transects within each site, there were two replications of each slope position.

3. Results

3.1. Differences in survival and nodulation percentage

3.1.1. Red alder

Of the 360 red alder seedlings planted in the three soils, 337 survived — 103 on clear-cut soils (86%), 115 on young stand soils (96%), and 119 on old-growth soils (99%). All surviving seedlings on clear-cut soils nodulated, 90% of the survivors nodulated in young stand soils, and 77% on old-growth soils.

3.1.2. Snowbrush

Of the 300 snowbrush plants, 237 survived — 93 on clear-cut soils (93%), 70 on young stand soils (70%), and 74 on old-growth soils (74%). Nodulation was low in soils from all sites; only 59 of the 237 surviving plants produced nodules — 21 in clear-cut soils (23%), 36 in young stand soils (51%), and 2 in old-growth soils (3%).

3.2. Differences among age classes

3.2.1. Red alder

Plant biomass was greatest for seedlings grown on the clear-cut soils ($P = 0.01$) (Fig. 1A), as was nodule weight ($P = 0.05$) (Fig. 1B). Acetylene reduction activity did not vary among soils ($P = 0.6549$) (Fig. 1C), but acetylene reduction activity per gram of nodule was lowest in clear-cut soils ($P = 0.05$) (Fig. 1D). Soils within locations were a significant ($P < 0.0001$) source of variation for alder biomass, nodule weight, and acetylation reduction per plant and per gram nodule (Table 1).

All tested correlations among alder response variables were statistically significant (statistically non-zero). Nodule weight correlated positively with plant biomass ($\rho = 0.60$, $P < 0.01$). Plant acetylene reduction correlated positively with plant biomass ($\rho = 0.86$, $P < 0.01$) and, more weakly, with nodule weight ($\rho = 0.41$, $P = 0.03$).

3.2.2. Snowbrush

As with red alder, all snowbrush variables except acetylene reduction activity per plant varied with soil source; however, specific responses differed (Fig. 2). Whereas biomass and nodule weight for alder were highest on clear-cut soils, these variables were highest for snowbrush on young stand soils. Acetylene reduction activity per gram of nodule was highest in old-growth soils. Soils within stands were a significant ($P < 0.0001$) source of variation for plant biomass, nodule weight, and acetylene reduction per plant and per gram nodule (Table 2).

All tested correlations among snowbrush variables were statistically significant (statistically non-zero). Plant biomass was strongly correlated with nodule weight ($\rho = 0.84$, $P < 0.01$) and with plant acetylene reduction ($\rho = 0.72$, $P < 0.01$). Nodule weight correlated positively with plant acetylene reduction ($\rho = 0.73$, $P < 0.01$).

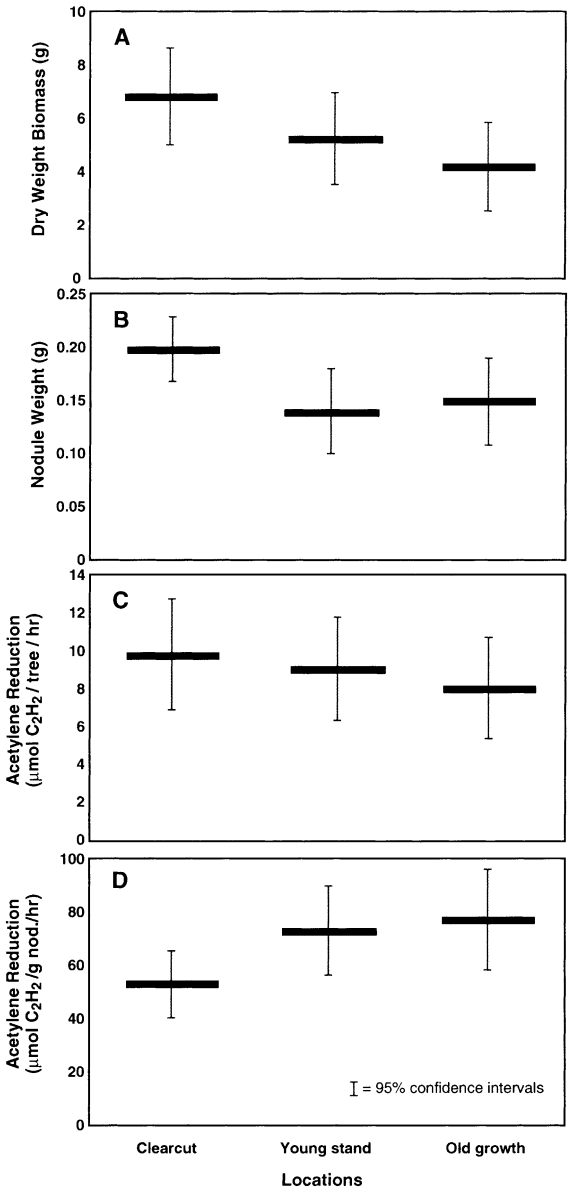

Fig. 1. Means and 95% confidence limits for (A) biomass (gram/tree); (B) nodule weight (gram/tree); (C) acetylene reduction (mmol C_2H_2/tree/hour), and (D) acetylene reduction per gram nodule (mmol C_2H_2/gram nodule/hour) of red alder seedlings grown on soils of the clear-cut, young, or old-growth stands.

3.3. Slope-related differences within age classes

3.3.1. Red alder

Patterns of within-stand variation (slope position) for red alder biomass and whole plant acetylene reduction activity showed no difference between means at the five positions (bottom slope, lower mid-slope, mid-slope, upper mid-slope, and top of the slope) along the two transects for any of the three stands.

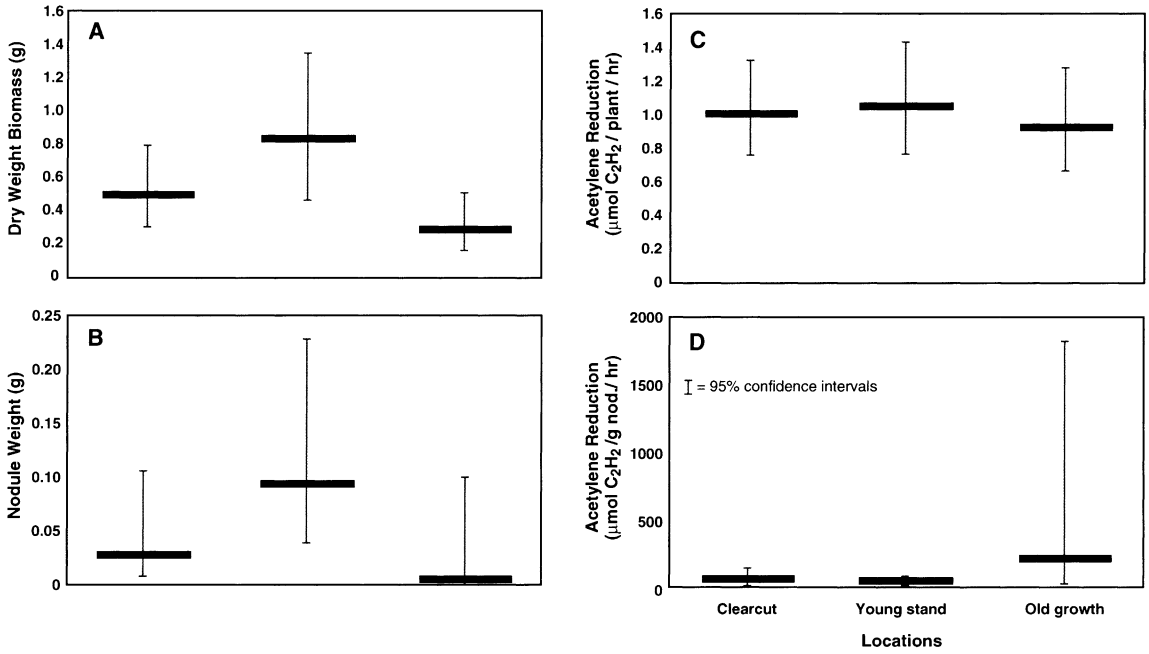

Fig. 2. Means and 95% confidence limits for (A) biomass (gram/plant); (B) nodule weight (gram/plant); (C) acetylene reduction (mmol C_2H_2/plant/hour), and (D) acetylene reduction per gram nodule (mmol C_2H_2/gram nodule/hour) of snowbrush plants grown on soils of the clear-cut, young, or old-growth stands.

3.3.2. *Snowbrush*

Snowbrush biomass and plant acetylene reduction in the young stand and the old growth showed no significant differences among slope positions. However, the one-way ANOVA for plant biomass in the clear-cut indicated that some position means differed ($P = 0.0049$). The biomass of plants grown in bottom slope soils was greater than those grown

Table 1
Analysis of variance for red alder[a]

Source	Biomass			Nodule wt. (g)			A.R. per plant			A.R. per gram nodule		
	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Locations	2	2.53	0.10	2	6.26	0.00	2	0.43	0.65	2	4.44	0.02
Soils within locations	27	27.47	<0.0001	26	3.09	<0.0001	27	15.67	<0.0001	26	3.29	<0.0001
Trees within soils within locations	302			270			307			270		

[a] F: approximate F statistic based on means square; A.R.: acetylene reduction activity.

Table 2
Analysis of variance for snowbrush $\log_{10}$[a]

Source	Biomass			Nodule wt. (g)			A.R. per plant			A.R. per gram nodule		
	df	F	*P*	df	F	*P*	df	F	*P*	df	F	*P*
Locations	2	3.72	0.04	2	5.28	0.02	2	0.16	0.85	2	7.27	0.01
Soils within locations	26	15.24	<0.0001	10	7.91	<0.0001	26	6.23	<0.0001	10	5.89	<0.0001
Plants within soils within locations	208			46			208			46		

[a] F: approximate F statistic based on means square, A.R.: acetylene reduction activity.

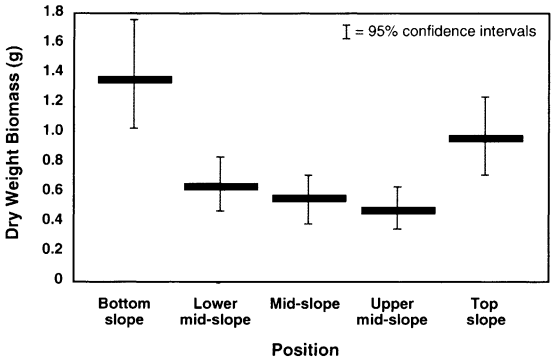

Fig. 3. Means and 95% confidence limits for the dry weight biomass (gram/plant) of snowbrush plants grown in clear-cut soils, by position.

in soils from any other position within the clear-cut (Fig. 3), and plants grown in top slope soils produced more biomass than those grown in upper mid-slope soils. Acetylene reduction in the clear-cut showed no significant differences among positions.

4. Discussion

When we account for differences in the number of plants nodulating, the sites and species are very different. Red alder plants nodulated well in soils from clear-cut, young, and old-growth stands. Snowbrush plants, on the other hand, nodulated poorly in soils from all three stands. Our hypothesis that nodule biomass and acetylene reduction for both red alder and snowbrush would be greatest in soils from the young stand and least in soils from the old-growth forest was not supported for red alder, but it was partially supported for snowbrush. Plant biomass, nodule weight (for all plants), and acetylene reduction per gram of nodule varied significantly among the soils from the three sites, but acetylene reduction per plant (nodulated and non-nodulated) did not. For both species, all four measurements were highly variable within stands.

Contrary to our expectations, red alder plants produced more nodules and biomass when grown in clear-cut soils than in soils from any other location (nodulation calculated using all plants). But red alder plants exhibited the lowest rate of acetylene reduction per gram of nodule when grown in clear-cut soils, probably because of the well-known inverse relation between nodule weight and nitrogenase activity per unit weight (Wheeler et al., 1981; Sempavalan et al., 1995). As expected, snowbrush plants produced more nodules and biomass when grown in young stand soils than in soils from the other locations (nodulation calculated using all plants). At the time of sampling, the young stand had a healthy and thick snowbrush cover that probably served as a good source of *Frankia* inoculum.

For red alder or snowbrush plants to grow, nodulate, and fix atmospheric nitrogen on soils from the clear-cut or the old-growth stand, *Frankia* populations in the soil must have either survived without hosts and stayed infectious until the establishment of actinorhizal plants, or dispersed onto the sites from elsewhere. The occurrence of *Frankia* in soils without hosts is due to the ability of this microorganism to survive saprophytically either in the soil (Smolander and Sundman, 1987) or by becoming dormant (Molina et al., 1994). It is also possible that *Frankia* spores were introduced into these stands by an unknown dispersal agent. Research conducted elsewhere has identified some vertebrates and invertebrates as being good dispersal agents of *Frankia* spores (Reddell and Spain, 1991; Paschke and Dawson, 1993; Burleigh and Dawson, 1995; Li et al., 1997).

If the nodulation potential for snowbrush in the old-growth stand that formerly occupied the clear-cut was similar to that in the present old-growth stand (where only 3% of plants nodulated), then either populations of snowbrush-nodulating *Frankia* must have increased since clearcutting or the nodulation potential of existing populations must have increased. The nodulation percentage differed among soils from different slope positions in the clear-cut: of the 21 snowbrush plants that nodulated in the clear-cut (23%), 12 were on soils from the bottom slope, 3 were from the lower mid-slope, 1 was from the upper mid-slope, and 5 were from the top of the slope. This tells us that *Frankia* populations at the bottom of the slope had increased — probably either because spores moved downslope with precipitation or because the population had been rebuilt via the presence of healthy snowbrush plants — and that the limited nodulation on the middle and upper slopes may have been due to insufficient snowbrush-nodulating *Frankia* strains.

The strain or strains of *Frankia* that induce root nodulation in red alder and snowbrush may be

plant-specific (i.e. unable to cross-inoculate other hosts); however, red alder nodulated well in soils from young stands and elsewhere where there was snowbrush but no alder.

The differences between red alder (which grew best on soils from the clear-cut) and snowbrush (which grew best on young stand soils) may have been related to the nutritional demands of either the plants or the *Frankia*, or it could have been due to changes in the physiological state of the *Frankia* population and the annual cycle of *Frankia* spore germination, growth, and sporulation (Myrold and Huss-Danell, 1994). The *Frankia* strains of these two actinorhizal plants are plant-specific; we were able to isolate and culture *Frankia* from red alder but not from snowbrush. Isolation and identification of the infective *Frankia* from *Ceanothus* has not been reported, and no pure-culture infective *Frankia* currently exists for *Ceanothus*. Clawson et al. (1998) reported that *Frankia* strains living in root nodules of *Ceanothus* did not share a common symbiotic origin with *Frankia* from other actinorhizal plants. Based on the extent of the restriction fragment length polymorphism from single nodules, Baker and Mullin (1994) reported a diversity of *Frankia* in *C. americanus*. The diversity of *Frankia*, however, may not accurately reflect the diversity of *Frankia* populations in the soil (Baker and Mullin, 1994).

N.S. Rojas, D.A. Perry, C.Y. Li, and L.M. Ganio (in preparation), working with soils collected from four locations along a slope transect from the same clear-cut in the H.J. Andrews experimental forest, also found strong differences in nodulation between red alder and snowbrush, with alder nodulating more readily on soils from middle and upper slopes and snowbrush on soils from the bottom of the slope; they speculate that the existence of strains with different adaptations may reflect a survival mechanism of the nitrogen-fixing endophyte *Frankia*.

Successful establishment of snowbrush on sites in the H.J. Andrews experimental forest will require developing techniques to inoculate *Frankia* specifically into snowbrush. Such inoculation will improve seedling growth in the nursery. Outplanting the inoculated nursery seedlings in planting sites devoid of snowbrush-nodulating *Frankia* will benefit seedling survival and growth.

Acknowledgements

We thank Jane Thomas for editorial assistance and advice on the manuscript, and Gretchen Bracher for preparation of the figures.

References

Baker, D.D., 1987. Relationships among pure cultured strains of *Frankia* based on host specificity. Physiol. Plant. 70, 245–248.

Baker, D.D., Mullin, B., 1994. Diversity of *Frankia* nodule endophytes of the actinorhizal shrub *Ceanothus* as assessed by RFLP patterns from single nodule lobes. Soil Biol. Biochem. 26, 547–552.

Binkley, D., 1981. Nodule biomass and acetylene reduction rates of red alder and Sitka alder on Vancouver Island, B.C. Can. J. For. Res. 11, 281–286.

Bormann, B.T., DeBell, D.S., 1981. Nitrogen content and other soil properties related to age of red alder stands. Soil Sci. Soc. Am. J. 45, 428–432.

Burleigh, S.H., Dawson, J.O., 1995. Spores of *Frankia* strain HFPCcI3 after passage through the digestive tracts of captive parakeets (*Melopsittacus undulatus*). Can. J. Bot. 73, 1527–1530.

Clawson, M.L., Carú, M., Benson, D.R., 1998. Diversity of *Frankia* strains in root nodules of plants from families Elaeagnaceae and Rhamnaceae. Appl. Environ. Microbiol. 64, 3539–3543.

Conard, S.G., Jaramillo, A.E., Cromack Jr., K., Rose, S., 1985. The role of the genus *Ceanothus* in western forest ecosystems. USDA For. Serv. Gen. Tech. Rep. PNW-182.

Cromack Jr., K., Delwiche, C.C., McNabb, D.H., 1979. Prospects and problems of nitrogen management using symbiotic nitrogen fixers. In: Gordon, J.C., et al. (Eds.), Symbiotic Nitrogen Fixation in the Management of Temperate Forests. Proceedings of the Workshop, 2–5 April 1979, Corvallis, OR. Forest Research Laboratory, Oregon State University, Corvallis, OR, pp. 210–223.

Franklin, J.F., Dyrness, C.T., 1973. Natural Vegetation of Oregon and Washington. Oregon State University Press, Corvallis, OR.

Harrington, C.A., Zasada, J.C., Allen, E.A., 1994. Biology of red alder (*Alnus rubra* Bong.). In: Hibbs, D.E., et al. (Eds.), The Biology and Management of Red Alder. Oregon State University Press, Corvallis, OR, pp. 3–22.

Johnson, F.D., 1968. Taxonomy and distribution of northwestern alders. In: Trappe, J.M., et al. (Eds.), Biology of Alder. USDA For. Serv. Pac. Northwest For. and Range Exp. Stn., Portland, OR, pp. 9–22.

Koo, C.D., 1989. Water stress, fertilization and light effects on the growth of nodulated mycorrhizal red alder seedlings. Ph.D. Thesis. Oregon State University, Corvallis, OR.

Li, C.Y., Castellano, M.A., 1987. *Azospirillum* isolated from within sporocarps of the mycorrhizal fungi *Hebeloma crustuliniforme*, *Laccaria laccata* and *Rhizopogon vinicolor*. Trans. Br. Mycol. Soc. 88, 563–565.

Li, C.Y., Crawford, R.H., Chang, T.T., 1997. *Frankia* in decaying fallen trees devoid of actinorhizal hosts and soil. Microbiol. Res. 152, 167–169.

Littell, R.C., Freund, R.J., Spector, P.C., 1991. SAS System for Linear Models. SAS Institute, Inc., Cary, NC.

Molina, R., Myrold, D.D., Li, C.Y., 1994. Root symbioses of red alder: technological opportunities for enhanced regeneration and soil improvement. In: Hibbs, D.E., et al. (Eds.), The Biology and Management of Red Alder. Oregon State University Press, Corvallis, OR, pp. 23–46.

Myrold, D.D., Huss-Danell, K., 1994. Population dynamics of *Alnus*-infective *Frankia* in a forest soil with and without host trees. Soil Biol. Biochem. 26, 533–540.

Paschke, M.W., Dawson, J.O., 1993. Avian dispersal of *Frankia*. Can. J. Bot. 71, 1128–1131.

Perry, D.A., 1994. Forest Ecosystems. The Johns Hopkins University Press, Baltimore, MD.

Petersen, R.G., 1985. Design and Analysis of Experiments. Marcel Dekker, New York.

Pregent, G., Camire, C., 1985. Mineral nutrition, dinitrogen fixation, and growth of *Alnus crispa* and *Alnus glutinosa*. Can. J. For. Res. 15, 855–861.

Reddell, P., Spain, A.V., 1991. Transmission of infective *Frankia* (Actinomycetales) propagules in casts of the endogeic earthworm *Pontoscolex corethrurus* (Oligochaeta: Glossoscolecidae). Soil Biol. Biochem. 23, 775–778.

Resch, H., 1988. Red alder: opportunities for better utilization of a resource. Oreg. State Univ. For. Res. Lab. Spec. Pub., p. 16.

Rojas, N.S., Perry, D.A., Li, C.Y., Friedman, J., 1992. Influence of actinomycetes on *Frankia* infection, nitrogenase activity and seedling growth of red alder. Soil Biol. Biochem. 24, 1043–1049.

Rose, S.L., Youngberg, C.T., 1981. Tripartite associations in snowbrush (*Ceanothus velutinus*): effect of vesicular-arbuscular mycorrhizae on growth, nodulation, and nitrogen fixation. Can. J. Bot. 59, 34–39.

SAS Institute, Inc., 1996. SAS/STAT Software: Changes and Enhancements Through Release 6.11. SAS Institute, Inc., Cary, NC.

Sempavalan, J., Wheeler, C.T., Hooker, J.E., 1995. Lack of competition between *Frankia* and *Glomus* for infection and colonization of roots of *Casuarina equisetifolia* L. New Phytol. 130, 429–436.

Smolander, A., Sundman, V., 1987. *Frankia* in acid soils of forests devoid of actinorhizal plants. Physiol. Plant. 70, 297–303.

Steel, R.G.D., Torrie, J.H., 1980. Principles and Procedures of Statistics. A Biometrical Approach. McGraw-Hill, New York.

Waring, R.H., Schlesinger, W.H., 1985. Forest Ecosystems: Concepts and Management. Academic Press, Orlando, FL.

Waring, R.H., Holbo, H.R., Bueb, R.P., Fredriksen, R.L., 1978. Documentation of Meteorological Data from the Coniferous Forest Biome Primary Station in Oregon. USDA For. Serv. Gen. Tech. Rep. PNW-73.

Wheeler, C.T., McLaughlin, M.E., Steele, P., 1981. A comparison of symbiotic nitrogen fixation in Scotland in *Alnus glutinosa* and *Alnus rubra*. Plant Soil 61, 169–188.

Wollum, A.G., Youngberg, C.T., Chichester, F.W., 1968. Relation of previous timber stand age to nodulations by *Ceanothus velutinus*. For. Sci. 14, 114–118.

Youngberg, C.T., Wollum, A.G., 1976. Nitrogen accretion in developing *Ceanothus velutinus* stands. Soil Sci. Soc. Am. J. 40, 109–112.

Youngberg, C.T., Wollum, A.G., Scott, W., 1979. *Ceanothus* in Douglas-fir clear-cuts: nitrogen accretion and impact on regeneration. In: Gordon, J.C., et al. (Eds.), Symbiotic Nitrogen Fixation in the Management of Temperate Forests. Proceedings of the Workshop, Corvallis, OR, 2–5 April 1979. Forest Research Laboratory, Oregon State University, Corvallis, pp. 224–233.

Zavitkovski, J., Newton, M., 1967. The role of snowbrush (*Ceanothus velutinus* Dougl.) and red alder (*Alnus rubra* Bong.) in forest regeneration in the Pacific Northwest. XIV IUFRO-Kongress 21, 429–440.

Zavitkovski, J., Newton, M., 1968. Ecological importance of snowbrush, *Ceanothus velutinus*, in the Oregon Cascades agricultural ecology. Ecol. 49, 1134–1145.