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Early colonization of red alder and Douglas fir by ectomycorrhizal fungi and *Frankia* in soils from the Oregon coast range

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Abstract. The potential for mycorrhizal formation and *Frankia* nodulation were studied in soils from six sites in the Pacific Northwest. The sites included young and old alder stands, a 1-year-old conifer clear-cut, a young conifer plantation, and rotation-aged and old-growth conifer stands. A bioassay procedure was used with both red alder and Douglas fir seedlings as hosts. After 6 weeks growth, seedlings of both hosts were harvested every 3 weeks for 21 weeks and numbers of nodules and ectomycorrhizal types estimated. Nodules formed on red alder and ectomycorrhizae formed on both alder and Douglas fir in soil from all sites. Nodulation potential was highest in soil from the alder stands and the conifer plantation. Seven morphologically distinct ectomycorrhizal types were recovered on Douglas fir and five on alder. Only *Thelephora terrestris*, a broad-host-range mycobiont, formed mycorrhizae on both hosts. New ectomycorrhizal types formed on both hosts throughout the bioassay. Ectomycorrhizal colonization of alder was greatest in the alder and clear-cut soils. Low ectomycorrhizal colonization on alder was found in soils from sites where conifers were actively growing. Ectomycorrhizal colonization of Douglas fir was highest in the young alder and conifer plantation soils and was low in the rotation-aged conifer soil. The highest diversity of ectomycorrhizal types was found on alder in the conifer clear-cut soil and on Douglas fir in the rotation-aged conifer soil. Effects of host specificity, nodulation and mycorrhiza-forming potential and nodule-mycorrhiza interactions on seedling establishment are discussed in relation to seral stage dynamics and attributes of pioneer ectomycorrhizal fungal species.

Key words: Ectomycorrhizae – *Frankia* – Propagules – Nodules – Succession

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Introduction

Red alder (*Alnus rubra* Bong.) is an early successional tree species that invades large patches after fire or clear-cutting in mesic regions of the Pacific Northwest. Forming a short-lived, transitional seral stage to conifers such as western hemlock [*Tsuga heterophylla* (Raf.) Sarg.], red alder has long been recognized for its contributions to soil fertility, particularly nitrogen accretion, in the Pacific Northwest (Tarrant and Trappe 1971). Red alder also suppresses regeneration of Douglas fir (*Pseudotsuga menziesii* Mirb. Franco), an economically important pioneering species, almost to its exclusion where the two species establish simultaneously on nitrogen-rich soils in the coast range (Newton et al. 1968; Binkley 1983, 1984; Binkley et al. 1984).

Red alder forms tripartite symbioses with a nitrogen-fixing actinomycete and mycorrhizal fungi (Trappe 1979). However, unlike other ectomycorrhizal tree species, relatively few mycobionts are associated with red alder (Miller et al. 1991), and many of these fungi appear be highly host-specific for alder (Molina 1979, 1981). Similarly, few of the many fungal species associated with Pacific Northwest conifers form ectomycorrhizae (EM) with red alder (Molina 1979, 1981), and alder-specific ectomycorrhizal fungi induce incompatible, hypersensitive responses on many conifer roots (Molina and Trappe 1982). Given that most conifer host-specific fungi are associated with pioneering tree species (Kropp and Trappe 1982; Molina and Trappe 1982), the specialized and often host-specific nature of alder ectomycorrhizal fungi may be essential to the successful establishment of red alder on disturbed sites.

This study entailed a soil bioassay procedure under controlled greenhouse conditions to evaluate nodulation and mycorrhiza-forming potentials of soils, and early colonization by ectomycorrhizal fungi over time from six sites representing a hypothetical successional sequence involving red alder and Douglas fir in the Pacific

Northwest. We also assessed the role that alder host specificity plays in the transition from one seral stage to another by determining which ectomycorrhizal fungi, if any, could form EM with both red alder and Douglas fir. A fourth objective was to observe *Frankia* nodule-mycorrhizae interactions in the context of seedling establishment and survival.

Materials and methods

Study area

Sites chosen for the study represented a successional sequence of young alder, old alder, conifer clear-cut, conifer plantation, rotation-aged conifer and old-growth conifer stands in the Coast Range of west central Oregon. The sites were approximately 10 km southwest of Corvallis, Oregon, in or near the city of Corvallis Watershed, or in the MacDonald Experimental Forest 10 km northwest of Corvallis, and were similar in elevation but differed in slope, aspect and proximity to the nearest alder populations.

Alder sites

The young and old alder stands were on private forest land (Starker Forest) near the Corvallis Watershed boundary and were nearly contiguous. Both alder sites were established on a flat bench within 25 m of a riparian alder zone along a small, seasonal drainage.

Young alder was a small (approximately 1 ha^2) 22-year-old, mesic, upland red alder stand resulting from natural seeding following logging. The stand was surrounded by 80- to 100-year-old western hemlock and Douglas fir forest.

Old alder was a large (approximately 10 ha^2) more than 70-year-old, mesic, upland red alder stand resulting from natural seeding following clear-cutting.

Conifer sites

The clear-cut and conifer plantation sites were contiguous on a moderate slope in the Corvallis Watershed approximately 50 m upslope from a riparian alder zone on Woods Creek.

Clear-cut was a 1-year-old clear-cut (approximately 50 ha^2) previously occupied by 120-to 150-year-old Douglas fir. The site was clear-cut and burned in preparation for planting back to Douglas fir.

Conifer plantation was an 18-year-old pure Douglas fir plantation (approximately 10 ha^2) established after clear-cutting 120- to 150-year-old Douglas fir.

Rotation-aged conifer was a 120- to 150-year-old Douglas fir/western hemlock stand in the Corvallis Watershed on a flat bench situated 0.5 km from the Corvallis City reservoir. Small, scattered populations of 5- to 10-year-old red alder saplings were present within 100 m of the site in mesic depressions.

Old-growth conifer was a more than 450-year-old Douglas fir/western hemlock stand (approximately 1 ha^2) located on a moderate slope outside the Corvallis Watershed boundary in MacDonald Forest, and was within 10 m of scattered alder saplings established near a road.

Soil analysis

Ten soil samples were taken from each of the field sites with a standard soil corer to a depth of 20 cm and analyzed for soil nutrient composition by the Soil Testing Laboratory at Oregon State University. Soil analyses are summarized in Table 1.

Table 1. Analysis of soil nutrients from alder, clear-cut and conifer sites from which bioassay soil was derived. Results are the means of seven individual random samples taken to a depth of 20 cm at each treatment site. Methods of analysis are those in standard use at the Oregon State University Soil Testing Laboratory. Numbers in parentheses are standard error terms for each soil. The same letter in each row indicates no significant difference between soils using Bonferroni/Dunn comparison of all means, $\alpha = 0.05$

	Young alder	Old alder	Conifer clear-cut	Conifer plantation	Rotation-aged conifer	Old-growth conifer
NH_4 (µg/g)	5.11 (±0.31) b	2.96 (±0.37) bc	1.35 (±0.25) bc	8.54 (±2.05) a	0.93 (±0.16) c	4.27 (±0.45) bc
NH_4 (incubated, µg/g)	91.47 (±2.79) b	92.85 (±6.76) b	68.67 (±12.40) b	136.10 (±16.68) a	55.01 (±3.93) b	90.98 (±2.57) b
NO_3 (µg/g)	6.22 (±0.48) b	11.40 (±0.46) a	3.32 (±1.13) bc	4.24 (±0.89) b	0.18 (±0.09) d	0.62 (±0.17) cd
Total N (%)	0.36 (±0.01) ab	0.39 (±0.02) a	0.27 (±0.04) bcd	0.33 (±0.03) abc	0.20 (±0.01) d	0.25 (±0.01) cd
Total C (%)	6.79 (±0.48) ab	7.20 (±0.43) ab	5.10 (±0.58) b	8.53 (±1.27) a	5.39 (±0.42) b	4.98 (±0.31) b
pH	4.97 (±0.07) b	4.94 (±0.13) b	5.94 (±0.06) a	5.90 (±0.05) a	5.64 (±0.07) a	5.91 (±0.03) a
Total P (%)	0.20 (±0.01) a	0.17 (±0.02) ab	0.12 (±0.01) bc	0.09 (±0.01) c	0.09 (±0.01) c	0.08 (±0.01) c
Na (mg/kg)	15.30 (±1.95) c	12.24 (±0.70) c	32.51 (±5.10) b	22.56 (±2.51) bc	24.73 (±1.30) bc	52.67 (±3.89) a
K (mg/kg)	475.14 (±57.04) ab	676.86 (±70.82) a	252.86 (±44.82) b	475.14 (±95.01) ab	216.14 (±21.30) b	423.57 (±44.32) ab
Ca (mg/kg)	2132.71 (±194.75) c	1793.14 (±197.22) c	3392.57 (±503.82) b	5345.57 (±204.33) a	1078.86 (±144.96) c	4279.43 (±139.25) ab
Mg (mg/kg)	538.29 (±38.80) b	427.86 (±44.46) b	1050.43 (±115.27) a	1281.70 (±136.83) a	306.57 (±23.86) b	1267.70 (±23.74) a

During early summer, ten samples of the top 20 cm of mineral soil from each of the six sites were taken with a shovel and placed in plastic bags. Sampling implements were sterilized with 10% bleach to prevent cross-contamination between soils from different sites. Immediately upon returning to the laboratory, samples from a given site were composited, screened through a sterilized 0.5-cm mesh soil sieve, and mixed 1:1 (v:v) with a peat moss:vermiculite potting mix. Half of each soil mixture was autoclaved for controls to monitor extraneous greenhouse contamination. Autoclaved and nonautoclaved soils from each site were filled into respective sterilized Leach seedling tubes (165-ml capacity). Seeds of red alder and Douglas fir from provenances in the coast range of west central Oregon were sown independently in 75 containers for each soil and autoclaving treatment.

The seedlings were grown in a non-climate-controlled greenhouse and mist-watered twice daily. Temperature, light intensity and photoperiod fluctuated throughout the study in the greenhouse in relation to ambient spring, summer and early fall conditions. No fertilization was provided. AT 6, 9, 12, 15, 18, and 21 weeks, ten seedlings from each treatment and controls were extracted, cleaned under running water, and stereomicroscopically examined for the number of *Frankia* nodules and ectomycorrhizal colonization. EM were described and identified according to Miller et al. (1991). Vesicular-arbuscular (VA) mycorrhizal colonization was evaluated by using the staining techniques of Phillips and Hayman (1970).

Results

Frankia nodules formed on red alder in all soils tested in the bioassay (Fig. 1). Numbers of nodules formed at each harvest date did not differ among most soils but the conifer plantation site had more nodules at week 9 than the other soils, and the rotation-aged conifer site developed the fewest nodules overall in the study. The young alder soil and the conifer plantation soil produced the greatest number of nodules by 21 weeks but did not differ from each other.

No VA mycorrhizae formed on either host in any soil during the 21-week harvest period. However, EM formed on both red alder and Douglas fir in all soils tested. Five distinct ectomycorrhizal types were observed on red alder during the study period and seven on Douglas fir (Tables 2–4). Only one mycobiont, *Thelephora terrestris* (Ehrh.) Fr., a possible greenhouse contaminant, formed EM on both red alder and Douglas fir.

Mycobionts of three of the red alder ectomycorrhizal types were identified as *T. terrestris*, *Alpova diplophloeus* (Zeller & Dodge) Trappe and Smith and *Lactarius obscuratus* (Lasch.) Fr. (Table 2). The remaining ectomycorrhizal types were readily recognizable, but the mycobionts could not be identified. Type 1 and *T. terrestris* EM appeared on red alder in the autoclaved control treatments at the 9-week harvest. Type 1, type 2, *T. terrestris* and *A. diplophloeus* EM were present on red alder at each harvest (Table 2). EM attributable to *L. obscuratus* were observed only during the final harvest in the conifer clear-cut soil. Type 1, *T. terrestris* and *A. diplophloeus* formed the most abundant EM on red alder and were present in soil from all sites (Tables 2, 4). Type 2 was present in most soils at low levels and reached greatest numbers by 18 and 21 weeks in the conifer plantation soil, where low levels of other ectomycorrhizal types were observed. Type 1 and *T. terrestris* EM were abundant early in the bioassay, especially in the young and old alder soils, but became less abundant by later harvests. *A. diplophloeus*, on the other hand, increased in abundance at each successive harvest.

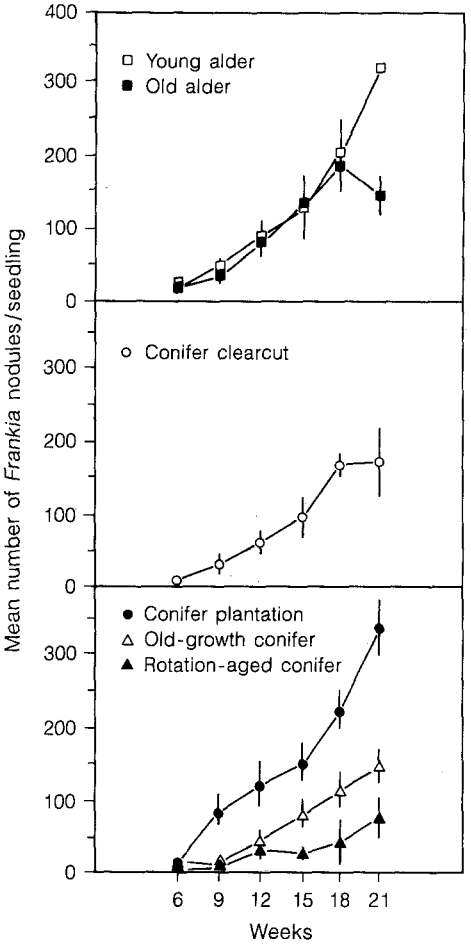

Fig. 1. Mean number of *Frankia* nodules on red alder in a greenhouse soil bioassay using soils from young and old alder stands, a 1-year-old conifer clear-cut, a young Douglas fir plantation, and a rotation-age and an old-growth Douglas fir stand at intervals after sowing red alder seeds. *Bars*, 95% confidence intervals for the means at each harvest

Descriptions of red alder ectomycorrhizal types are presented elsewhere (Miller et al. 1991) and will not be duplicated here. Salient features of Douglas fir ectomycorrhizal types are given below.

Thelephora terrestris type

Single to pinnately branched, straight, cylindrical to swollen structures. The mantle was smooth or silky with surface texture from uneven distribution of extramatrical hyphae, white, greyish-white to pale brownish-yellow, with scattered to abundant white, silky hyphal strands. Crushes revealed that the mantle was of variable thickness, composed of tightly or loosely interwoven, hyaline hyphae, with or without clamps, and with numerous blunted, finger-like hyphal end cells projecting from the surface.

Table 2. Mean estimated percentages of different ectomycorrhizal types (% of each ectomycorrhizal type present on colonized portions of red alder feeder roots) in greenhouse bioassay of soils from young and old alder stands, a 1-year-old conifer clear-cut, a young Douglas fir plantation, and a rotation-age and an old-growth Douglas fir stand at intervals after sowing red alder seeds ($n = 10$). 0, Indicates that no ectomycorrhizae of a particular type were found during a harvest; --, indicates that no ectomycorrhizae of any type were found during a harvest

Field site	Weeks after sowing					
	6	9	12	15	18	21
Young alder						
Type 1	49	49	20	25	22	17
Alpova diplophloeus type	0	0	30	37	59	61
Thelephora terrestris type	49	49	41	38	18	22
Type 2	2	2	1	0	0	0
Lactarius obscuratus type	0	0	0	0	0	0
Old alder						
Type 1	98	60	41	45	43	40
A. diplophloeus type	0	0	30	30	40	40
T. terrestris type	1	35	28	25	16	13
Type 2	1	5	1	0	1	5
L. obscuratus type	0	0	0	0	0	0
Conifer clear-cut						
Type 1	0	15	0	1	1	1
A. diplophloeus type	1	80	80	95	95	60
T. terrestris type	0	0	20	1	3	10
Type 2	1	5	0	0	1	8
L. obscuratus type	0	0	0	0	0	21
Conifer plantation						
Type 1	--	0	0	0	0	0
A. diplophloeus type	--	0	0	10	6	10
T. terrestris type	--	39	40	10	5	0
Type 2	--	1	1	2	30	25
L. obscuratus type	--	0	0	0	0	0
Rotation-aged conifer						
Type 1	--	--	0	5	1	1
A. diplophloeus type	--	--	15	20	37	21
T. terrestris type	--	--	15	5	5	2
Type 2	--	--	5	1	5	5
L. obscuratus type	--	--	0	0	0	0
Old-growth conifer						
Type 1	--	0	5	1	5	5
A. diplophloeus type	--	1	20	30	31	25
T. terrestris type	--	0	1	3	10	0
Type 2	--	0	0	0	0	6
L. obscuratus type	--	0	0	0	0	0

Table 3. Mean estimated percentages of different ectomycorrhizal types (% of each ectomycorrhizal type present on colonized portions of Douglas fir short roots) in a greenhouse bioassay of soils from young and old alder stands, a 1-year-old conifer clear-cut, a young Douglas fir plantation, and a rotation-age and an old-growth Douglas fir stand at intervals after sowing Douglas fir seeds. --, Indicates than no ectomycorrhizae of any type were found during a harvest; 0, indicates that no ectomycorrhizae of a particular type were found during a harvest

Field site	Weeks after sowing					
	6	9	12	15	18	21
Young alder						
Thelephora terrestris type	--	8	80	50	55	55
Rhizopogon sect. *villosuli* type	--	0	15	45	40	35
Cenococcum geophilum type	--	0	0	5	1	3
Type 3	--	0	0	0	0	0
Type 4	--	0	0	0	0	0
Type 5	--	0	0	0	0	0
Type 6	--	0	0	0	0	0
Old alder						
T. terrestris type	--	--	40	30	47	50
R. sect. *villosuli* type	--	--	20	20	35	30
C. geophilum type	--	--	5	5	3	5
Type 3	--	--	0	0	0	0
Type 4	--	--	0	0	0	0
Type 5	--	--	0	0	0	0
Type 6	--	--	0	0	0	0
Conifer clear-cut						
T. terrestris type	--	0	0	30	20	20
R. sect. *villosuli* type	--	5	5	25	60	60
C. geophilum type	--	0	0	5	15	15
Type 3	--	--	0	1	2	5
Type 4	--	--	0	0	0	0
Type 5	--	--	0	0	0	0
Type 6	--	--	0	0	0	0
Conifer plantation						
T. terrestris type	1	80	98	75	85	80
R. sect. *villosuli* type	0	0	0	15	2	5
C. geophilum type	0	0	0	5	10	10
Type 3	0	0	0	0	0	0
Type 4	0	0	0	0	0	0
Type 5	0	0	0	0	0	0
Type 6	0	0	0	0	0	0
Rotation-aged conifer						
T. terrestris type	--	0	0	3	22	30
R. sect. *villosuli* type	--	15	55	30	50	50
C. geophilum type	--	0	5	5	5	8
Type 3	--	--	0	0	0	0
Type 4	--	--	0	0	2	5
Type 5	--	--	0	0	1	3
Type 6	--	--	0	0	1	1
Old-growth conifer						
T. terrestris type	--	0	0	20	25	20
R. sect. *villosuli* type	--	15	80	30	65	70
C. geophilum type	--	0	5	0	5	5
Type 3	--	--	0	1	2	3
Type 4	--	--	0	0	0	0
Type 5	--	--	0	0	0	0
Type 6	--	--	0	0	0	0

Rhizopogon sect. villosuli type

Single at first then branching, pinnate or coralloid, cylindrical to swollen structures. The mantle was felted, thick, black at first then developing a white background with black crusty patches below and scattered to abundant, thick, black hyphae over the surface, and with thick, branching hyphal strands that were black or white with abundant, thick, black hyphae covering the surface, the white portions of mantle or hyphal strands often bruising pinkish red.

Table 4. Mean estimated total colonization (% of total red alder and Douglas fir roots colonized by each ectomycorrhizal type) in all soils at intervals after sowing seeds

Ectomycorrhizal type	Weeks after sowing					
	6	9	12	15	18	21
Red alder						
Type 1	25	21	11	12	12	11
Alpova diplophloeus type	<1	14	30	37	45	37
Thelephora terrestris type	8	21	24	14	10	8
Type 2	<1	2	1	<1	6	8
Lactarius obscuratus type	0	0	0	0	0	4
Douglas fir						
T. terrestris type	<1	14	37	35	42	41
Rhizopogon sect. *villosuli* type	0	6	29	28	42	41
C. geophilum type	0	0	3	4	7	8
Type 3	0	0	0	0	<1	<1
Type 4	0	0	0	<1	<1	<1
Type 5	0	0	0	0	<1	<1
Type 6	0	0	0	0	<1	<1

Cenococcum geophilum type

Single to rarely compound or pinnate, cylindrical or swollen structures. The mantle was crusted, greyish-black, or most commonly black, with abundant, stiff black bristles protruding only from the tip or overall.

Type 3. Single to compound and pinnate, cylindrical structures. The mantle was thick, smooth and succulent in appearance, bright orange to brownish-orange with scant extramatrical hyphae. This type was possibly EM of a *Lactarius* sp.

Type 4. Single to commonly pinnate, cylindrical or swollen structures. The mantle was thin, cottony or with silky sheen, white or pinkish-white. Clamps were present. This type was possibly EM of a *Laccaria* sp.

Type 5. Single to branching, cylindrical structures. The mantle was thick, velvety, dark-brown, and was composed of numerous dark-brown, thick-walled cystidia protruding from the surface.

Type 6. Single or barely compound, cylindrical structures. The mantle was opaque, cottony, white with abundant, thick, branching, powder-blue bristle hyphae scattered over the surface.

As with red alder, the three most abundant ectomycorrhizal types on Douglas fir were recovered in soil from all sites (Table 3). Two Douglas fir ectomycorrhizal types were identified as *T. terrestris* and *C. geophilum* Fr. Observation indicated that ectomycorrhizal formation by *C. geophilum* developed primarily from germinating sclerotia. *T. terrestris* EM appeared on Douglas fir seedlings in the autoclaved control soil by week 9. A third type was recognizable as an unknown species in the genus *Rhizopogon* sect. *villosuli.* These three were the most abundant on Douglas fir (Tables 3, 4). Douglas fir ectomycorrhizal types 3–6 appeared at successive harvests through the bioassay and were formed with unknown mycobionts (Table 3). These types were infrequent, appeared only in the rotation-aged conifer soil and colonized relatively few rootlets (Table 4).

The degree of ectomycorrhizal colonization of red alder and Douglas fir was different for each soil (Fig. 2). Red alder was heavily colonized in the young alder, old alder and conifer clear cut soils but only lightly to moderately colonized in the conifer plantation, rotation-aged conifer and old-growth conifer soils. Also, initial ectomycorrhizal formation was delayed on red alder in soils from sites with established conifer populations. The highest degree of colonization of Douglas fir was in the young alder and conifer plantation soils, being slightly less in the soils from conifer sites. Initial colonization of Douglas fir lagged behind that of red alder in all soils except those from the conifer plantation, rotation-aged conifer and old-growth conifer sites (Figs. 2, 3).

The number of ectomycorrhizal types that formed on red alder and Douglas fir also differed among soils (Fig. 3). The greatest number of red alder types occurred in the conifer clear-cut soil, followed by the rotation-aged conifer and young and old alder soils. The lowest ectomycorrhizal diversity on red alder was in the conifer plantation and old-growth conifer soil. The greatest diversity of types that appeared on Douglas fir was in soil from the rotation-aged conifer site. Diversity of types was lower for the conifer clear-cut and old-growth conifer sites but reached its maximum during later harvests. The least diversity in types an Douglas fir developed in the young and old alder and conifer plantation soil.

Discussion

The tripartite symbioses such as that occurring between red alder, *Frankia* and ectomycorrhizal fungi are found on only a few early successional vascular plant species. The effects of disturbance on survival and interaction among populations of these organisms and their contributions to succession or recovery from large-scale disturbance are unclear.

In the present study, formation of *Frankia* nodules on red alder seedlings planted in soils from each site indicated that *Frankia* is either able to exist as resistant propagules or in a free-living state or is regularly being reintroduced at each site. *Frankia* in air-dried nodules can survive laboratory storage at room temperature for up to 7 years (van Dijk 1979). Nodulation of *Ceanothus velutinus* in soils long devoid of that host led Wollum et al. (1968) to hypothesize that the nodule endophytes could survive in soil 100 years or more. Long-distance dispersal of *Frankia* has been shown to occur readily in streams (C. Koo, S. Miller and R. Molina, unpublished work), but it is not known whether dispersal can also occur by other means such as wind, mammals or invertebrates. Becking (1970) found that the nodulation potential of soil that had been free of *Alnus glutinosa* for 44 years was near zero. Since immigration of the endophyte from outside sources was impeded by brick walls surrounding the plots in his experiment, Becking concluded that continuous reintroduction of *Frankia* from

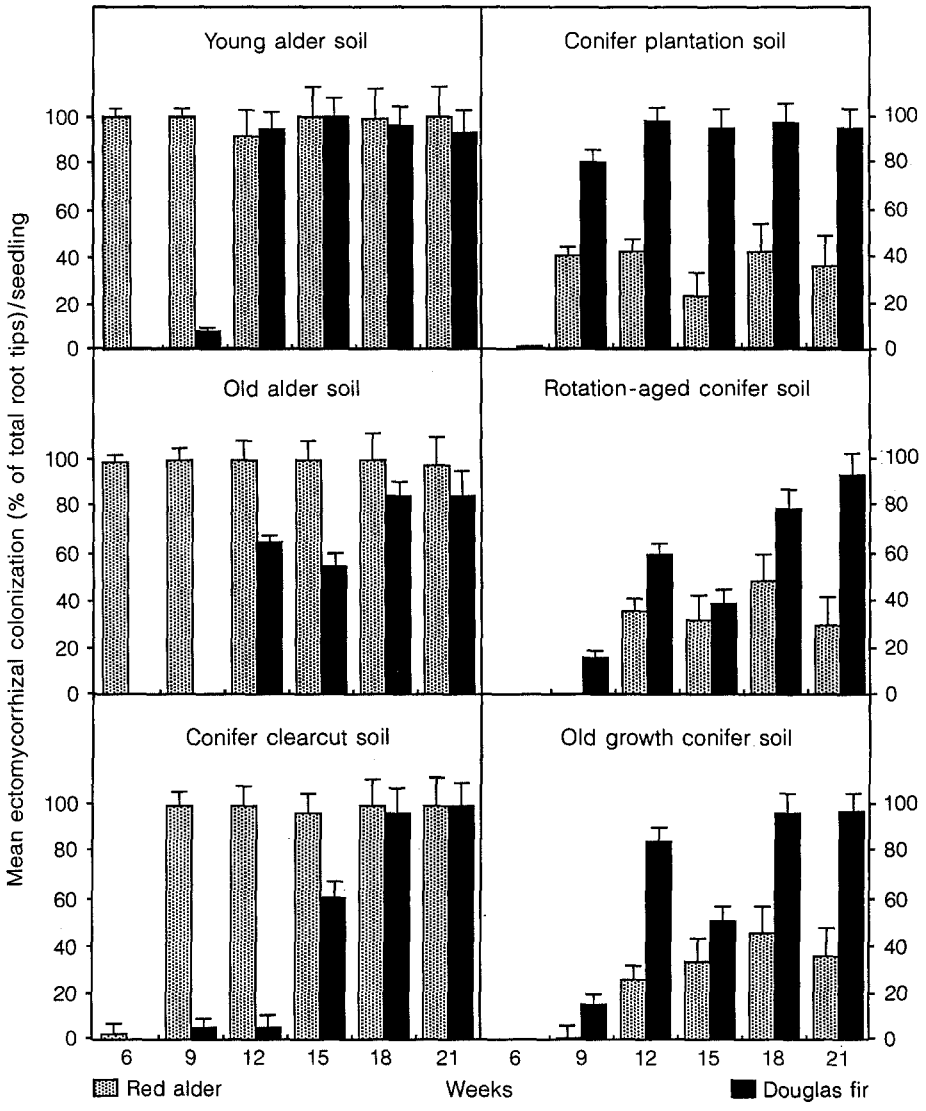

Fig. 2. Mean percentage of red alder and Douglas fir ectomycorrhizal colonization in a greenhouse soil bioassay using soils from young and old alder stands, a 1-year-old conifer clear-cut, a young Douglas fir plantation, and a rotation-age and an old-growth Douglas fir stand at intervals after sowing red alder seeds. *Bars*, 95% confidence intervals for the means at each harvest

outside sources is necessary for maintenance of nodulation potential once the host has disappeared.

Although nodules were formed in soil from all sites in this study, highest nodulation and rates of nodule formation occurred in the alder soils and the conifer plantation soil. This may be due to an nitrogen (N)-priming effect where rates of nitrogen fixation are initially higher in soils with high mineralizable nitrogen pools (Ingestad 1980), leading to more rapid growth of the host root system, increased root length and, therefore, increased rates of nodulation. Levels of mineralizable nitrogen and nitrate in the bioassay soils were indeed higher in soils from the alder and the conifer plantation sites, at least suggesting that an N-priming effect is involved in the nodulation rates observed in these soils. In addition, high nodule numbers possibly indicate sites where red alder has a high establishment potential. However, red alder establishes best on exposed mineral soil in disturbed sites; seeds germinate poorly and seedlings grow slowly in red alder stands or conifer forests with a heavy litter layer, low light levels and a predominance of far-red light on the forest floor (Fowells 1965; Bormann 1983).

Endophyte physiology and genetic composition also may have influenced nodulation potential. Two strains of *Frankia* are known to exist, one that commonly produces spores, Sp(+), and one that does not readily produce spores, Sp(−). Van Dijk (1979) reported that nodulation capacity of alder with Sp(+) *Frankia* strains is 100–1000 times that of Sp(−) strains. Van Dijk (1984) and Weber (1986) also found that Sp(−) nodules predominated in areas that had not supported growth of the host for some time. Holman and Schwintzer (1987) suggested that Sp(−) strains of *Frankia* are maintained by saprophytic vegetative growth in soil, whereas Sp(+) populations depend on the continuous presence of the host and maintain themselves by spore production within nodules. Nodulation potential of soil from alder sites, therefore, may be high due to predominance of Sp(+) *Frankia* populations and large numbers of spores. Low nodule numbers in the clear-cut, rotation-aged and old-growth conifer soils is possibly due to predominance of Sp(−) *Frankia* populations and low inoculum potential. Low nodule numbers in the rotation-aged conifer soil may also be expected since this is the site furthest from an outside source of *Frankia* inoculum.

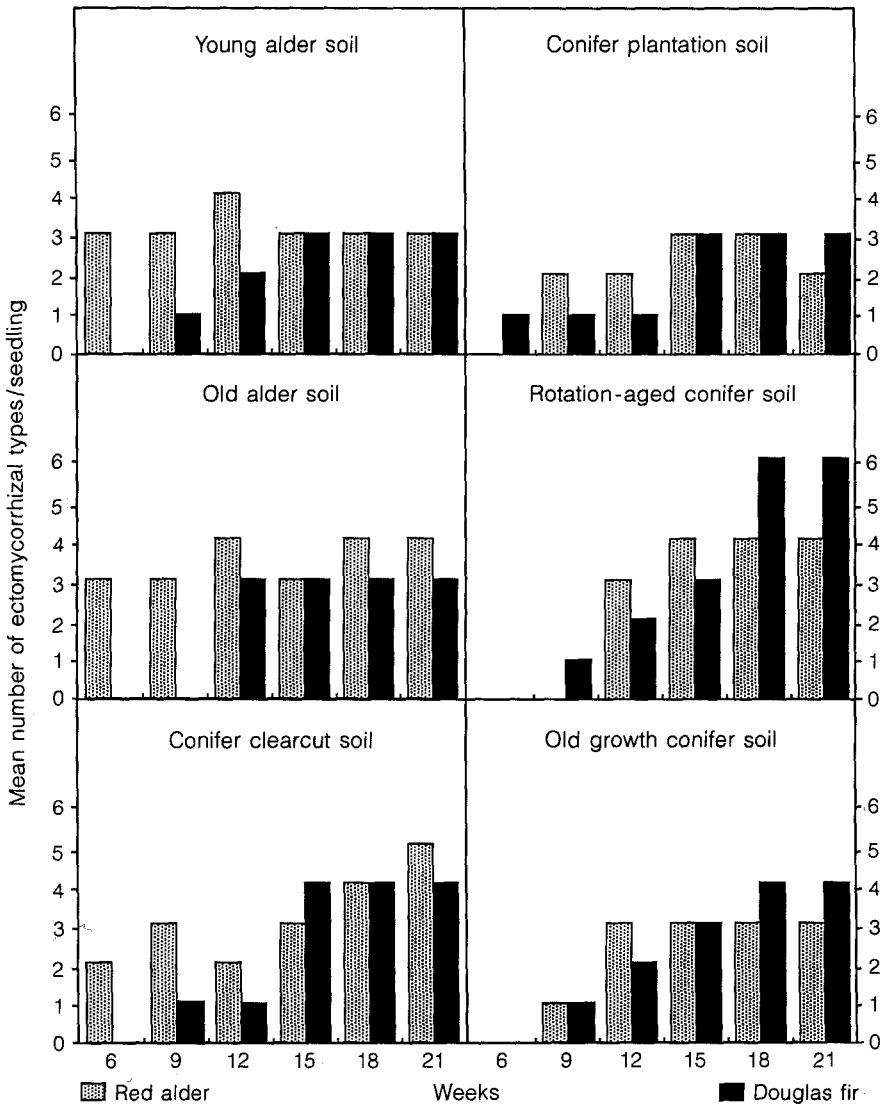

Fig. 3. Number of red alder and Douglas fir ectomycorrhizal types in a greenhouse soil bioassay using soils from young and old alder stands, a 1-year-old conifer clear-cut, a young Douglas fir plantation, and a rotation-age and an old-growth Douglas fir stand at intervals after sowing red alder seeds

The presence of ectomycorrhizal fungi may stimulate *Frankia* nodule formation in pure culture in the laboratory (R. Molina, unpublished work) and may have influenced nodulation potential in the bioassay. Accordingly, red alder seedlings planted in soils from the young and old alder sites showed both high ectomycorrhizal colonization and *Frankia* nodulation. However, soil from the conifer plantation, which also showed high nodulation, induced low ectomycorrhizal colonization of red alder. Conversely, the conifer clear-cut soil showed only moderate nodulation potential, yet induced colonization of EM on red alder as high as in soil from alder sites. The stimulatory effect of ectomycorrhizal fungi on nodule growth in the laboratory may be tempered by competition between *Frankia* and ectomycorrhizal fungi, differences in persistence of propagules in whole soil situations or differences in soil fertility.

Ectomycorrhizal colonization of red alder was greatest in soils from the red alder sites, and colonization of Douglas fir was high in soils from conifer (predominantly Douglas fir) soils. Ectomycorrhizal colonization of red alder was low in soils from sites where conifers were actively growing, yet was much higher in the conifer clear-cut soil only a year after clear-cutting. These results are similar to those reported by Pilz and Perry (1984) who found that more EM formed in soils from clear-cuts than in undisturbed forest, but differs from the results of Perry et al. (1982) and Parke et al. (1984), who found a reduced ectomycorrhizal soil inoculum potential in clear-cuts. In our study, the greatest diversity of ectomycorrhizal types on red alder was found in the conifer clear-cut soil.

The N-priming effect suggested for *Frankia* nodulation may also effect mycorrhiza forming potential. Schoenberger and Perry (1982) found more ectomycorrhizal root tips on Douglas fir grown in soil from an unburned clear-cut (41 ppm mineralizable N) than in soil from a burned clear-cut (8 ppm mineralizable N). In this study, the diversity of ectomycorrhizal types was the same for seedlings in both treatments.

It is also interesting that ectomycorrhizal colonization of Douglas fir growing in isolated soil from alder sites was high despite competitive interactions between the two hosts in the field (Newton et al. 1968). Although more information on actinorhizal and fungal propagule immigration and persistence must be gathered, it ap-

pears that mechanisms may be in place to increase ectomycorrhizal diversity and nodulation potential in transitional seral stages.

Recognizable mycobionts responsible for ectomycorrhizal types on Douglas fir and red alder were both host specifists and host generalists. *A. diplophloeus* and *L. obscuratus* on red alder are both host-specific to *Alnus* spp. (Froidevaux 1973; Molina 1979, 1981; Miller et al. 1991). Likewise, the undetermined species of *Rhizopogon* could be identified to sect. *villosuli*, most if not all species of which seem host-specific to Douglas fir. On the other hand, *C. geophilum* is known to be a broad-host-range species (Trappe 1962) on both hardwoods and conifers, although *C. geophilum* formed EM only on Douglas fir in this study. *T. terrestris*, the only mycobiont forming EM on both red alder and Douglas fir, is also a broad-host-range species. Although *T. terrestris* formed abundant EM on both red alder and Douglas fir, sporocarps of this fungus were most prolific on Douglas fir.

Because *T. terrestris* sporocarps were observed in several conifer field sites, the source of the *T. terrestris* contamination could have originated from within the bioassay soil, although exogenous contamination is a recurring problem in this greenhouse (Pilz and Perry 1984; Perry et al. 1989).

At least some mycobionts in transitional seral stages would likely be broad-host-range species to accommodate both incoming and outgoing hosts. Harley and Smith (1983) argued that mycorrhizal associations have evolved toward a lack of host-fungus specificity to increase the likelihood of rapidly establishing an ectomycorrhizal relationship in severely disturbed areas. Mikola (1970), on the other hand, suggested that given the strongly coevolved nature of many host-fungus associations, host-specific fungi are more specialized with respect to their hosts and so may be more effective than cosmopolitan fungi that can associate with many different hosts. Because the emphasis of this study was on early colonization by various ectomycorrhizal fungi, more thorough examination of soils from additional sites representing each seral stage is required before stronger patterns can be demonstrated.

Several of the ectomycorrhizal types, including *Cenococcum*, *T. terrestris*, *Rhizopogon* sp. and types 4 and 6 on Douglas fir, that appeared in our bioassay were also present in other greenhouse and field bioassays using Douglas fir as a host (Schoenberger and Perry 1982; Pilz and Perry 1984). It is remarkable that *A. diplophloeus* and the unknown *Rhizopogon* sp., two of the predominant mycobionts on red alder and Douglas fir, respectively, in all soils, form hypogeous sporocarps whose spores are not readily dispersed by wind. Likewise, *C. geophilum* reproduces effectively by sclerotia, and its method of dispersal is not known. The consistent appearance of EM of these fungi suggest that propagules produced solely below ground may be concentrated in the root zone in a fashion not typical of wind-dispersed species.

Temporal aspects of this study provide interesting information on dynamics of ectomycorrhizal colonization. New ectomycorrhizal types, especially on Douglas fir, continued to form throughout successive harvests in this experiment, suggesting that new propagules of ectomycorrhizal fungi may be stimulated to germinate by active growth of other fungi or that physiological changes stimulatory to ectomycorrhizal formation may occur in the host with changes in age. High diversity of Douglas fir ectomycorrhizal types in rotation-aged conifer soil coupled with relatively low colonization may result from competition for root space in the greenhouse containers. The fact that colonization of red alder by *A. diplophloeus* increased in many soils throughout the study, while type 1 EM decreased in the same soil, suggests that *A. diplophloeus* is an adept, early successional colonizer and competitor. Last et al. (1985) found that the ability to form EM in a variety of unsterile soils, to the near exclusion of EM attributable to other fungi, is a characteristic of early successional fungi. It is also possible that older trees, even seedlings harvested at later dates in this bioassay, could harbor different ectomycorrhizal fungi than younger seedlings. Miller et al. (1991) found that diversity of ectomycorrhizal types recovered in the bioassay was nearly double that observed at the sites where soil was collected. Several types seen in the field were not observed at all in the bioassay, and types determined as less prominent by the bioassay were much more abundant in the field. Fleming (1983, 1984) showed that birch seedlings raised in contact with root systems of mature parent birch trees developed ectomycorrhizal types characteristic of mature forests, whereas seedlings planted in the same soil isolated from parent tree roots developed EM attributable to early successional fungi. These findings suggest that more soil bioassays should include a temporal harvesting scheme. A single, early harvest may miss many minor types that have not yet developed, and a single, late harvest may show only the dominant ectomycorrhizal types.

The largest drawback of the bioassay procedure as it has been applied in the past is that resultant mycorrhizal formation is only an indirect measure of propagules present in the soil. In most if not all cases, the exact type and number of propagules forming a particular type of mycorrhiza are unknown. We can hypothesize that mycorrhizae appearing by the first harvest may be formed by active hyphae responding to the developing root systems, whereas mycorrhizae formed by subsequent harvests result from the slower processes of spore and sclerotia germination. Effects of different fertilization and watering regimes and growth conditions on different propagules can only be surmised, but such differences may amplify the early or late appearance of some mycorrhizae, and presumably the functioning of specific propagules. Therefore, it may not be appropriate to compare the results of one bioassay with those of another, or assays of one soil and those of another, without knowledge of specific soil preparation methods and growth conditions.

Although Rose (1980) encountered VA mycorrhizal colonization in her examination of red alder roots from the field, no colonization on red alder was observed in any soils tested in this study or in fact from field-col-

lected root material. This may have been due to disruption of soils during bioassay preparation, competitive exclusion under greenhouse conditions or the soil mixture that was used. It is not clear what role VA mycorrhizal fungi play in the early establishment of red alder.

High rates of juvenile growth of red alder may be responsible for failure of Douglas fir to maintain positions of dominance in mixed stands (Newton et al. 1968). Results presented here suggest that after clearcutting red alder may have a competitive advantage over Douglas fir because of faster initial ectomycorrhizal colonization. Nodulation and nitrogen fixation by *Frankia* probably also contribute to faster growth by red alder.

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