The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay[1]

M. MEYER SCHOENBERGER AND D. A. PERRY

Forest Research Laboratory, Oregon State University, Corvallis, OR, U.S.A. 97331

Received August 10, 1981[2]

Accepted February 12, 1982

SCHOENBERGER, M. MEYER, and D. A. PERRY. 1982. The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. Can. J. For. Res. **12**: 343–353.

In a greenhouse bioassay of soils from the central Oregon Cascades, Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings had the most total and ectomycorrhizal root tips when grown in soil from an unburned clear-cut and the least when grown in soil from (*i*) a 20-year-old plantation that had been clear-cut and burned in the late 1950's and (*ii*) one old-growth forest. Tip formation was intermediate in soil from a second old-growth forest, a recently burned clear-cut, and a 40-year-old natural burn. Root weights followed the same trend, but top weights did not differ among the various soils. Ectomycorrhizal and total root tips of western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) were lowest in soils from the plantation and recently burned clear-cut. Unlike Douglas-fir, western hemlock's tip production was not greater in the unburned clear-cut than in the old-growth forest soils. In this species, both top and root weights varied according to soil, with the largest seedlings produced in soil from the unburned clear-cut. With both species, there was a significant interaction between ectomycorrhizal type and soil type. *Cenococcum geophilum* Fr. predominated on western hemlock and was reduced in soils from the burned clear-cut and plantation. In comparison with the mean for all soils, ectomycorrhizal types that predominated on Douglas-fir were enhanced in the unburned clear-cut soil and reduced in one old-growth soil, an effect apparently related to litter leachate.

SCHOENBERGER, M. MEYER, et D. A. PERRY. 1982. The effect of soil disturbance on growth and ectomycorrhizae of Douglas-fir and western hemlock seedlings: a greenhouse bioassay. Can. J. For. Res. **12**: 343–353.

Au cours d'un test biologique effectué sur des sols provenant du centre des Cascades en Orégon, des plantules de douglas taxifolié (*Pseudotsuga menziesii* (Mirb.) Franco) ont montré le plus grand nombre total de méristèmes apicaux et de méristèmes apicaux ectomycorhizés lorsqu'ils furent cultivés dans un sol provenant de coupe à blanc sans brûlis et le plus petit nombre lorsqu'ils furent cultivés dans des sols provenant (*i*) d'une plantation de 20 ans, qui avait été préalablement coupée à blanc et brûlée à la fin des années 1950 et (*ii*) d'une forêt surannée. La formation des méristèmes apicaux s'est avérée intermédiaire dans le sol venant d'une deuxième forêt surannée, d'une forêt récemment coupée à blanc et brûlée, et d'un brûlis naturel vieux de 40 ans. Les poids des racines ont suivi la même tendance mais les poids des parties aériennes ont montré peu de différence d'un sol à l'autre. Le total des méristèmes apicaux et les méristèmes apicaux ectomycorhizés chez la pruche occidentale (*Tsuga heterophylla* (Raf.) Sarg.) étaient moindres dans les sols provenant de plantations et de coupes à blanc suivies de brûlis. Contrairement au douglas taxifolié, la production de méristèmes apicaux racinaires n'était pas plus élevée dans les sols venant d'une coupe à blanc sans brûlis que de vieilles forêts. Chez cette espèce, les poids des tiges aussi bien que des racines varient selon le sol alors que les semis les plus lourds ont été obtenus sur le sol provenant d'une coupe à blanc sans brûlis. Chez les deux espèces, on note une interaction significative entre le type de mycorhize et le type de sol. Le *Cenococcum geophilum* Fr. dominait sur la pruche occidentale et était réduit dans des sols provenant de coupe à blanc avec brûlis. Comparativement avec la moyenne obtenue pour tous les sols, les types d'ectomycorhizes qui prédominaient sur le douglas taxifolié ont été stimulés sur les sols provenant de coupe à blanc avec brûlis et réduits dans le sol d'une des vieilles forêts; cet effet est apparemment lié au lessivage de la litière.

[Traduit par le journal]

Introduction

The importance of ectomycorrhizae to conifer growth and survival is well documented (Harley 1969; Meyer 1974; Marx *et al.* 1977). Nursery-formed ectomycorrhizae on outplanted seedlings are often replaced by fungi indigenous to outplanting sites (Beneke and Gobl 1974; Marx *et al.* 1977); the latter may benefit seedling growth as well as or better than ectomycorrhizae formed by nonnative fungi (Theodorou and Bowen 1970; Lamb and Richards 1971). How site disturbances affect indigenous ectomycorrhizal fungi is poorly understood. Greco (1978) found that bare-root seedlings planted in burned or scarified clear-cuts developed more ectomycorrhizae than did those planted in unburned and nonscarified clear-cuts; however, other studies have found that fire reduces ectomycorrhizal populations (Wright and Tarrant 1958; Mikola *et al.* 1964; Harvey *et al.* 1980*a*, 1980*b*). In a greenhouse bioassay of soils from southwestern Montana, Perry *et al.* (1982) found reduced ectomycorrhizal formation up to 16 years after both (*i*) clear-cutting and broadcast burning and (*ii*) clear-cutting and windrowing.

In this study we wished to assess both the immediate

[1]Paper No. 1555 of the Forest Research Laboratory.

[2]Revised manuscript received February 2, 1982.

0045-5067/82/020343-11$01.00/0

impact of disturbance on ectomycorrhizal formation and the length of time impacts might persist. To do so, we grew Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings in soils from contiguous disturbed and undisturbed sites of various ages in the Oregon Cascades. Bioassays were conducted in a greenhouse so that soil moisture, light, and temperature could be controlled, thereby allowing us to restrict between-site comparisons to the presence of fungi or other soil factors that might influence mycorrhizal formation.

Study area

We chose sites on which reforestation might be difficult: southern exposures, steep slopes, highly erodible soils. Under these conditions, the presence of ectomycorrhizal fungi may be critical to seedling survival and growth. All sites were within 2 km of each other on a steep southeast slope at 610 m elevation in the west-central Cascades near Blue River, Oregon (latitude 44°10′ N, longitude 122°20′ W), and all represented a dry Douglas-fir – western hemlock community (Dyrness *et al.* 1974). Despite reasonable uniformity in aspect and slope, there was some variation in Douglas-fir site class, which is an index of site quality (Table 1). The predisturbance forest consisted of overmature Douglas-fir (250+ years) with smaller components of western hemlock (100+ years) and western redcedar (*Thuja plicata* Donn) and a small component of bigleaf maple (*Acer macrophyllum* Pursh), golden chinkapin (*Castanopsis chrysophylla* (Dougl.) A. DC.), and Pacific madrone (*Arbutus menziesii* Pursh).

Mean precipitation in the area is 2300 mm/year. The area is characterized by cool, wet winters (mean temperature 2.3°C), during which the major portion of the precipitation falls, and hot dry summers (mean temperature 20.6°C) (Rothacher *et al.* 1967). Soils are shallow, rocky, unstable clay loams with low fertility and moisture-holding capacity. Parent material is colluvium and residuum derived from breccia and andesitic bedrock (Legard and Meyer 1973). Soils were sampled from the various sites in 1978. A description of each sampled site follows (sites are ordered from top to bottom of the sampled transect).

Revegetated natural burn

This site, which was burned by wildfire, supported a 36- to 40-year-old Douglas-fir overstory. The understory was composed of regenerating conifers of western hemlock and western redcedar, bigleaf maple, and chinkapin. California hazel (*Corylus cornuta* var. *californica* (A. DC.) Sharp), Oregon grape (*Berberis nervosa* Pursh), salal (*Gaultheria shallon* Pursh), Pacific rhododendron (*Rhododendron macrophyllum* G. Don), and vine maple (*Acer circinatum* Pursh) occurred under crown openings. There was a small to moderately developed herb layer consisting of Prince's pine (*Chimaphila umbellata* (L.) Bart.), modest whipplea (*Whipplea modesta* Torr.), twisted stalk (*Disporium smithii* (Hook.) Piper), and sword fern (*Polystichum munitum* (Kaulf.) Presl.). There was also a litter-moss layer and a buildup of aboveground woody residue.

TABLE 1. Characteristics of sampled sites

Site	Age since disturbance (years)	Slope[a] (degrees)	Site class[b]
Unburned clear-cut	1	76	IV
Natural burn	36–40	64	IV
Old growth (No. 1)	—	52	IV
Burned clear-cut	1	63	II, III
Young growth	20	52	III
Old growth (No. 2)	—	34	II, III

[a]All slopes had a southeastern aspect.
[b]I indicates high quality, IV indicates low (McArdle *et al.* 1961).

Old growth (No. 1)

This near-ridgetop site, one of two undisturbed plots (the other was at the opposite lower corner of the study area, had a different age distribution, and was less open), was a mature stand of Douglas-fir (250+ years old) and western hemlock (100+ years old). Some Douglas-fir, western hemlock, and western redcedar regeneration had occurred. There was a moderate to dense shrub layer dominated by rhododendron, vine maple, Oregon grape, and salal, as well as an herb layer dominated by beargrass (*Xerophyllum tenex*). Both above- and below-ground woody residue at various stages of decay and a litter-moss layer were present.

Unburned clear-cut

This site was clear-cut and "cablelogged" in March 1977. Postdisturbance vegetation consisted of scattered shrubs: vine maple, California hazel, Oregon grape, trailing blackberry (*Rubus ursinus* (Dougl.) Brown), and chinkapin. There was a sparse herb layer consisting mainly of assorted grasses, bedstraw (*Galium oreganum* Britt.), fireweed (*Epilobium angustifolium* L.), and modest whipplea. Because of the steepness of the slopes and the nature of the soils, local debris slides had caused incorporation of much of the organic residue into the mineral layer.

Burned clear-cut

This site was clear-cut in March 1977 (as was the other clear-cut site) and burned in March and April of 1978. By October 1978, vegetation was recolonizing the area. Vine maple, rhododendron, salal, and Oregon grape constitute the sparse shrub layer. A few herbs, dominated by thistle (*Cirsium arvense* (L.) Scop.), were scattered throughout the plot. No litter or moss layer had yet been established. The soil surface was covered with a dark ash layer. Charred woody debris was present.

Young-growth plantation

This site was logged in 1958, burned in 1959, and planted with 2-0 Douglas-fir in 1960. The unit was precommercially thinned in September 1978 to 500 trees per hectare. The overstory was pure Douglas-fir. A shrub layer of hazel, rhododendron, Oregon grape, and salal was evenly distributed throughout the stand, as was a weakly developed herb layer of beargrass, sword fern, bracken fern (*Pteridium aquilinum* Underw.), and modest whipplea. The litter layer was small to nonexistent. Slash from a recent thinning remained on the plot.

Old growth (No. 2)

This site was a mature stand of 150- to 250-year-old Douglas-fir and 100-year-old western hemlock. Western hemlock regeneration predominated more here than in the other old-growth site. Western redcedar was present to a lesser extent. There was a well-developed shrub stratum dominated by rhododendron and salal, a sparse herb layer of beargrass, and a few other species, and a well-developed litter and moss layer.

Methods

Soil analysis

At 10 randomly located sample points in the center of each site (5 points in each old-growth site), soil samples were collected once a month from April until October of 1978. At the same time, litter depths were determined and soil temperatures taken at 2 and 10 cm with a bimetal thermometer. October samples were analyzed for total nitrogen, water-soluble nitrate nitrogen, and total KCl-extracted ammonia nitrogen according to standard techniques (Black 1965). Mineralizable nitrogen was determined by the anaerobic-incubation technique of Keeney and Bremner (1966). Percentage of carbon was determined by a modified Walkley–Black method (Jackson 1958) after micronitric–perchloric acid digestion. Phosphorus was determined by the molybdate blue technique (Taras *et al.* 1971) and cations were determined by atomic absorption spectrophotometry. Soil pH was determined from a 1:2 mixture of soil and water. The percentage of light reaching the soil surface was determined by the Ozalid paper method of Friend (1961).

Greenhouse bioassay

During the 1st week of October 1978, four soil samples were collected to a depth of 10 cm around each sampling point. Litter and humus layers were not included. A 70% ethyl alcohol wash was used on all collecting tools to reduce contamination between points. Soil samples were transported to the laboratory and processed within 24 h of collection. The four samples from each point were combined and put through a 2-mm sieve to eliminate debris and rocks. As much of the material as possible was gently crumbled through the sieve. The sieve was sterilized with an ethyl alcohol dip between processing of composite samples to avoid contamination.

For the greenhouse bioassay, two parts of sieved forest soil were mixed with one part each of steam-sterilized peat and vermiculite. This mixture was necessary because the sieved soil tended to be compacted in the containers when watered. For each soil collection point, twelve 62.5-cm^3 leach tubes (top diameter = 2.5 cm, length = 12 cm) were filled with the soil mixture after sterilization in a weak sodium hypochlorite solution. Six tubes were planted with seeds of Douglas-fir and six with those of western hemlock. Tree seeds were selected from an appropriate seed zone and lot; before planting, they were surface sterilized for 15 min in a 30% hydrogen peroxide solution, then rinsed several times in sterile distilled water. A small cover of chicken grit was added to the top of each tube to prevent contamination by water splash. Several blanks, consisting only of the peat and vermiculite mixture, were planted to test for possible airborne or seed contamination by mycorrhizal fungi.

The tubes were located randomly within standard tube racks. These racks were randomly located on the greenhouse bench and rotated weekly to reduce environmental differences in the greenhouse. As seedlings emerged, the tubes were weeded to one seedling each.

Douglas-fir and western hemlock seedlings were grown for 4.5 and 6 months, respectively, and watered with an overhead mist system. Natural day lengths were extended to 16 h by high-intensity sodium vapor lamps.

We wished to harvest seedlings before significant tip-to-tip infection had occurred. To do so, we monitored tip development by lifting individual seedlings at various times after planting. As soon as roots had developed adequately to distinguish differences in inoculum potential, all seedlings were lifted. At harvest, the seedlings were removed from the tubes, the roots were gently cleaned under running water, and shoot and root lengths were measured. The entire root system of each seedling was then removed for root tip and mycorrhizal examination. Total root and mycorrhizal tips were counted with the aid of a stereomicroscope (×10 to ×70). Squash mounts and thin sections were used as needed to verify mycorrhizal status. All tips appeared active according to the criteria of Harvey *et al.* (1976). The root tips were further classified as to mycorrhizal type of the basis of visual morphological differences, i.e., color, texture, and branching patterns. Because of the short duration of this study, we did not isolate, culture, or identify the fungal symbionts.

Litter study

Litter and humus layers were collected from the five sampling points in each of the old-growth forest sites. The material was hand crumbled, and large sticks and debris were removed. Ray leach tubes were filled with two parts of forest soil and one part each of steam-sterilized peat and vermiculite. In order to investigate litter effects only, it was necessary to use the same growing media for all tests. We chose soils from the unburned clear-cut. The tubes were sown with seeds of Douglas-fir, as described previously. Treatments consisted of (*i*) placement of litter on top of one set of seedlings and (*ii*) application of a litter leachate on the other. The leachate was prepared by grinding litter material in a 20-mesh Wiley mill, inserting 25 mL of the ground material into a jar with 100 mL of sterile distilled water, putting the material on a shaker for 1 h, and then filtering. Each week, 5 mL of the fresh leachate was applied. Five Douglas-fir seedlings were planted per litter collection point. Control seedlings were planted in steam-sterilized peat and vermiculite to test for greenhouse contamination. The experiment was conducted in the greenhouse as previously described; the seedlings were harvested and processed at 4.5 months.

Statistical analysis

Data on seedlings were expressed as average values per seedling of each species for each soil source (site).

For each tree species, analyses of variance were made on shoot and root lengths and dry weights, total number of root tips, total number of ectomycorrhizal root tips, and numbers of ectomycorrhizal root tips by type. Differences

TABLE 2. Mean litter depth, soil characteristics, and percentage of light reaching the surface of each site

Site	Litter depth (cm)	Soil temperature[a] (°C) 2 cm	Soil temperature[a] (°C) 10 cm	Soil moisture[a] (%)	Soil pH	Light (%)
Unburned clear-cut	0.77	18.0	15.1	37.4	5.23	59.3
Natural burn	3.72	15.1	12.9	37.8	5.08	4.0
Old growth (No. 1)	2.33	16.3	13.2	31.7	5.42	2.9
Burned clear-cut	0.05	21.4	18.1	24.8	5.14	85.1
Young growth	0.72	17.4	15.2	24.4	5.20	24.4
Old growth (No. 2)	3.25	15.0	12.2	44.5	4.58	2.2

[a]Average value of data collected monthly from May to October 1979.

among means were evaluated with Fisher's protected LSD at $P = 0.05$ (Ott 1977). The relationship between soil and seedling variables was analyzed by regression (Steele and Torrie 1960).

Results

Environmental characteristics of sites

Harvested plots had the smallest litter layers and the highest average soil temperatures (Table 2). Soil moisture was lowest on the burned clear-cut and young-growth sites. Soil moisture of the unburned clear-cut was comparable to that of sites with developed overstories, perhaps because of incorporation of woody and other organic debris into surface soil layers (Harvey *et al.* 1979). Soil pH values did not differ significantly among the sites (average 5.11).

Soils on the unburned clear-cut had more total and mineralizable nitrogen (N) and more calcium (Ca) than those from all other units and more carbon (C) and phosphorus (P) than those on the burned clear-cut, young-growth, and first old-growth sites (Table 3). Nitrate nitrogen (NO_3) and potassium contents were greatest in soil from the burned clear-cut.

Ectomycorrhizae observed on Douglas-fir

The following types of ectomycorrhizae were observed on Douglas-fir.

D1

The sheath was black with black, stiff, hair-like hyphae extending from the club-like element. The symbiont was *Cenococcum geophilum* Fr.

D2

The sheath was black and white, with white predominating. White to buff-colored fibrous rhizomorphs were abundant. Individual elements were club-like, often occurring in clusters. In paraffin thin sections, the mantle was very conspicuous (50–107 μm) and dense, with an outer peridium-like layer sometimes present. The Hartig net was well-developed with no apparent intracellular penetrations. These characteristics plus the tendency for the sheath to turn pink when bruised suggested that these mycorrhizae were formed by a species of *Rhizopogon* (James M. Trappe, personal communication).

D3

The ectomycorrhizae were single, straight, slightly swollen, or pinnately branched structures. The sheath appeared smooth and was yellowish brown to tan under the stereomicroscope. Thin sections revealed a complete but thin mantle with a well-developed Hartig net.

D4

These appeared as unswollen to only slightly swollen, light brown root tips devoid of root hairs. Although this configuration is characteristic of uninfected Douglas-fir short roots (Bogar and Smith 1965), squash mounts revealed some tips to possess a discernible mantle and Hartig net. Thin sections varied from suberized roots with a collapsed cortical region to a tightly appressed mantle and a Hartig net one to three tiers of cells deep. As a majority of squash mounts showed some hyphae, root tips with this appearance were counted as mycorrhizal. Some evidence indicates, however, that these are senescent root tips, rather than a distinct ectomycorrhizal type.

D5

These resembled D3 but were darker and appeared more as alternating knobs projecting from a lateral root than as branched structures. Thin sections revealed a sparse to spotty mantle development with a well-developed Hartig net.

Mycorrhizae observed on western hemlock

The following types of ectomycorrhizae were observed on western hemlock (because each was visually distinct, no thin sections were made).

H1

These corresponded with the description of D1 and were formed by *Cenococcum geophilum*.

characteristics plus
n pink when bruised
were formed by a
1. Trappe, personal

le, straight, slightly
uctures. The sheath
1 brown to tan under
ns revealed a com-
eveloped Hartig net.

to only slightly
void of root hairs.
racteristic of unin-
r and Smith 1965).
o possess a discern-
ections varied from
ortical region to a
tig net one to three
of squash mounts
th this appearance
evidence indicates,
ot tips, rather than

rker and appeared
from a lateral root
sections revealed
nent with a well-

mlock
orrhizae were ob-
each was visually
).

ription of D1 and
lum.

TABLE 3. Soil chemical values (± standard error) for each site[a]

Site	N: Mineralizable (ppm)	N: Total (%)	NO_3 (ppm)	NH_3 (%)	C (%)	P (%)	Na (%)	K (%)	Ca (%)	Mg (%)	C:N
Unburned clear-cut	41.10a (±4.58)	0.36a (±0.05)	0.61b (±0.11)	22.80 (±2.13)	14.64a (±2.18)	0.13a (±0.01)	0.044 (±0.011)	0.094d (±0.007)	0.57a (±0.022)	0.056 (±0.050)	41:1
Natural burn	27.45b (±7.63)	0.25b (±0.06)	0.25b (±0.02)	19.60 (±5.12)	12.13ab (±4.83)	0.11ab (±0.01)	0.029 (±0.004)	0.084d (±0.005)	0.315b (±0.046)	0.400 (±0.044)	49:1
Old growth (No. 1)	12.78c (±3.36)	0.13bc (±0.01)	0.37b (±0.09)	14.67 (±0.67)	6.00bc (±0.81)	0.08bc (±0.01)	0.016 (±0.003)	0.063d (±0.002)	0.212c (±0.023)	0.397 (±0.036)	46:1
Burned clear-cut	8.47c (±0.75)	0.11c (±0.01)	2.00a (±0.74)	14.80 (±1.62)	3.67c (±0.41)	0.07c (±0.01)	0.027 (±0.003)	0.310a (±0.015)	0.174c (±0.014)	0.304 (±0.015)	33:1
Young growth	9.65c (±1.08)	0.12c (±0.01)	0.31b (±0.03)	14.60 (±2.11)	3.62 (±0.30)	0.08bc (±0.01)	0.029 (±0.002)	0.144c (±0.010)	0.229c (±0.021)	0.478 (±0.059)	30:1
Old growth (No. 2)	13.64bc (±3.04)	0.19bc (±0.02)	0.33b (±0.02)	17.00 (±4.00)	7.51abc (±0.81)	0.11abc (±0.02)	0.027 (±0.001)	0.244b (±0.020)	0.182c (±0.010)	0.406 (±0.008)	40:1

[a] In each column, values followed by the same letter are not significantly different ($P = 0.05$) as determined by a Fisher's protected LSD. Those columns without letters contain values not significantly different from each other.

H2

These were single, slightly clavate structures, usually 3–5 mm in length. No extending hyphae or rhizomorphs were present. The sheath was smooth and a consistent reddish brown. Hand sections revealed a well-developed Hartig net.

H3

These were single, slightly clavate, digitate structures somewhat like H2 but not as swollen. The sheath was light brown to beige and usually smooth. Squash mounts and hand cross sections revealed a small, tightly appressed mantle and the presence of a Hartig net.

H4

These were single or coralloid. The sheath was smooth with a pearly sheen ranging from white to beige to very light purple. Squash mounts and hand cross sections revealed a well-developed mantle and Hartig net. These characteristics suggested the fungus might be *Laccaria laccata* (Scop. ex Fr.) Berk & Br.

Root tip analysis

No ectomycorrhizae formed on control seedlings; thus, those formed on test seedlings were from forest soil inoculum and not from greenhouse, soil mixture, or seed contamination. Although soil from all sites produce seedlings with at least some ectomycorrhizae, the sites differed in quantity and quality present (Fig. 1) and in growth of the two species of seedlings.

Douglas-fir

For Douglas-fir, total root tips varied from 66.2 to 108.0 per seedling (Table 4). Seedlings grown in soil from the unburned clear-cut had a significantly greater number of tips ($P = 0.05$) than those grown in any other soil. The percentages of ectomycorrhizal tips ranged from 74.6 (old growth No. 1) to 49.4 (young growth). Soil from one old-growth stand produced a significantly lower percentage of ectomycorrhizal tips than soil from the other.

Differences in numbers of mycorrhizal tips among seedlings grown in soils from the various sites were accompanied by differences in total tip numbers (Fig. 1A) and overall percentages of each type of mycorrhizae. Contingency table analysis (Steele and Torrie 1960) showed significant interaction between mycorrhizal types and source of soil ($\chi^2 = 36.38$, $P = 0.025$).

Western hemlock

For western hemlock, soils from the first old-growth site produced seedlings with a significantly greater number of total root tips than did those from the naturally burned, burned clear-cut, young-growth, and second old-growth sites and a significantly greater number of mycorrhizal tips than soils from the burned clear-cut and young-growth sites (Table 5). The least amount of both total root and mycorrhizal tips were produced in

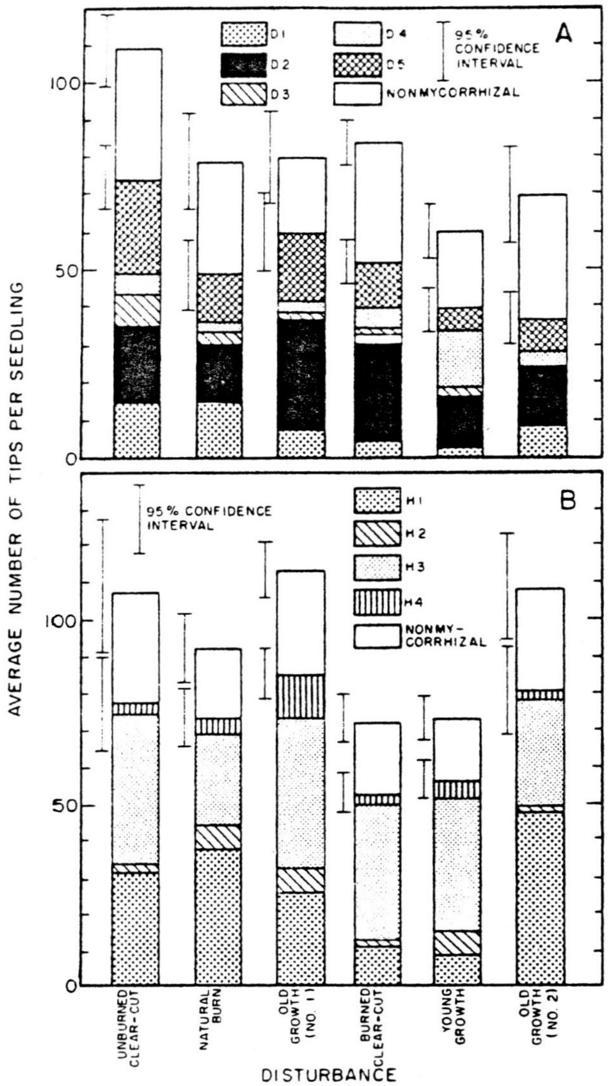

FIG. 1. Average number of total root and total ectomycorrhizal tips (by type) per seedling for Douglas-fir (A) and western hemlock (B) grown in soil from the various sites.

soils from the burned clear-cut, although the differences were not always significant. Percentages of mycorrhizal tips varied over a much smaller range than in Douglas-fir, but as in the latter, there was significant interaction between ectomycorrhizal type and source of soil (χ^2 = 65.66, P = 0.005). Much of this effect resulted because the proportions of *Cenococcum geophilum* (H1) tips in soils from plots that had been burned within the past 20 years, the burned clear-cut and young-growth sites, were lower than on other plots (Fig. 1B).

Seedling growth

Douglas-fir

For Douglas-fir, shoot dry weight did not differ significantly among seedlings grown in the various soils. Root dry weights ranged from 45.8 to 69.7 mg, with soils from the unburned clear-cut producing significantly greater root dry weights than did those from the naturally burned, young-growth, burned clear-cut, and second old-growth sites (Table 4). Consistently larger and heavier root systems were produced in soils from the unburned than from the burned clear-cut. This difference may have been due to factors other than burning, however, because soils from the first old-growth stand, which was adjacent to the unburned clear-cut, produced larger root systems than did those from the second old-growth stand, which was adjacent to the burned clear-cut. Root systems in soils from the burned clear-cut were larger than those in soils from both the adjacent old-growth and the adjacent young-growth stands. These differences paralleled those seen in total and ectomycorrhizal root tips.

Total root tips, ectomycorrhizal root tips, and percentage of ectomycorrhizal root tips were significantly but weakly correlated with root dry weight (Table 6). Differences in root weight explained 42% of the variation in total root tips and 34% of the variation in ectomycorrhizal tips. Tip numbers of all ectomycorrhizal types except one were positively correlated with root weight; however, for individual types no r^2 value was greater than 0.17.

Western hemlock

Western hemlock shoots and roots were heavier in soil from the unburned clear-cut than in that from any other site (Table 5). Unlike Douglas-fir, there was no difference in the size of hemlock seedlings grown in soils from the two old-growth stands, nor was there a difference in the root size of seedlings grown in soils from the burned clear-cut and the second old-growth site. Total and ectomycorrhizal root tips were significantly correlated with root weight, though root weight did not explain a large proportion of the variation (r^2 = 0.35 and 0.29, respectively) (Table 6). In contrast to Douglas-fir, correlation between ectomycorrhizal tips and root weight was due to only one of the recorded types, *Cenococcum geophilum*.

Correlation of seedling variables with site factors

Root dry weight of both species of seedlings was positively correlated with all soil nitrogen variables except nitrate and to soil carbon, calcium, and phosphorus (Table 7). Multiple regression with these variables did not materially improve their predictiveness. Controlled experimentation will be required to identify which, if any, of these factors are influencing root weight. Root weight of western hemlock was more closely correlated with nutrients than was that of Douglas-fir.

Number of mycorrhizal tips was correlated with roughly the same variables as was root weight. In this

TABLE 4.

Site
Unburned cle
Natural burn
Old growth (
Burned clear-
Young growt
Old growth (

"Data are ex
as determined b
"Statistical dif

TABLE 5.

Site
Unburned c
Natural burn
Old growth
Burned clea
Young grow
Old growth

"Data are e
as determined
"Statistical

case, however.
enced by nutri
calcium (Table
due to differer
species of myc
cies. *Cenococ*
clearly associa
more highly c
Douglas-fir th
suggesting tha

TABLE 4. Mean seedling values (± standard error) for Douglas-fir grown in soil from the various sites[a]

Site	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)	No. total root tips	No. mycorrhizal root tips	Mycorrhizal root tips[b] (%)
Unburned clear-cut	48.8a (±1.05)	175.5a (±1.54)	62.9 (±1.9)	69.7a (±2.3)	108.0a (±5.13)	73.9a (±4.12)	69.0ab (±2.24)
Natural burn	44.7b (±1.04)	164.0c (±2.37)	57.1 (±2.1)	52.4c (±2.4)	78.7bc (±6.20)	48.0bc (±4.69)	57.8c (±2.86)
Old growth (No. 1)	45.9ab (±1.40)	174.2a (±1.88)	63.4 (±3.2)	65.3ab (±2.5)	79.7bc (±5.93)	59.9b (±5.02)	74.6a (±1.80)
Burned clear-cut	45.8b (±0.91)	170.7ab (±1.29)	60.2 (±2.0)	59.8b (±1.9)	83.9b (±2.97)	52.3b (±2.72)	61.9bc (±2.58)
Young growth	45.2b (±0.92)	164.4c (±3.44)	60.0 (±2.3)	45.8d (±2.2)	66.2c (±3.51)	33.9d (±2.95)	49.4d (±3.53)
Old growth (No. 2)	44.0b (±1.39)	164.9bc (±2.97)	63.2 (±3.7)	50.4cd (±3.4)	70.2bc (±6.38)	36.5cd (±3.45)	54.8cd (±4.57)

[a]Data are expressed as average values per seedling. In each column, values followed by the same letter are not significantly different ($P = 0.05$) as determined by a Fisher's protected LSD. Those columns without letters contain values not significantly different from each other.

[b]Statistical differences determined on transformed data (arc sine $\sqrt{\%\ \text{mycorrhizal tips}}$). Values in tables are untransformed.

TABLE 5. Mean seedling values (± standard error) for western hemlock grown in soil from the various sites[a]

Site	Shoot length (mm)	Root length (mm)	Shoot dry weight (mg)	Root dry weight (mg)	No. total root tips	No. mycorrhizal root tips	Mycorrhizal root tips[b] (%)
Unburned clear-cut	36.7a (±1.52)	187.4a (±2.61)	29.5a (±2.3)	29.5a (±1.7)	105.6ab (±8.98)	74.8a (±5.87)	72.9c (±1.4)
Natural burn	28.8b (±1.19)	186.2a (±0.98)	19.0b (±1.2)	23.9b (±0.9)	90.2b (±4.71)	71.5a (±3.79)	79.5a (±1.0)
Old growth (No. 1)	26.0bc (±0.74)	181.7ab (±3.02)	17.7bc (±0.8)	22.5bc (±1.1)	111.2a (±3.40)	82.8a (±3.06)	74.4bc (±1.3)
Burned clear-cut	25.6c (±0.50)	177.4bc (±2.52)	16.1bc (±0.6)	22.4c (±0.6)	71.1c (±3.21)	51.2b (±2.37)	72.2c (±1.7)
Young growth	23.5c (±0.42)	172.8c (±2.43)	12.9c (±0.3)	17.1c (±0.5)	71.8c (±3.14)	55.1b (±2.25)	77.6ab (±1.1)
Old growth (No. 2)	24.0c (±0.95)	180.8ab (±4.03)	13.9c (±0.8)	19.4c (±1.0)	107.4b (±6.90)	79.1a (±5.61)	74.1bc (±1.9)

[a]Data are expressed as average values per seedling. In each column, values followed by the same letter are not significantly different ($P = 0.05$) as determined by a Fisher's protected LSD. Those columns without letters contain values not significantly different from each other.

[b]Statistical differences determined on transformed data (arc sine $\sqrt{\%\ \text{mycorrhizal tips}}$). Values in tables are untransformed.

case, however, Douglas-fir seemed to be more influenced by nutrients than was hemlock, especially by calcium (Table 7). This divergent behavior could be due to differences in tree species or to the particular species of mycorrhizae associated with each tree species. *Cenococcum geophilum*, the only fungal type clearly associated with both tree species, tended to be more highly correlated with soil nutrients when on Douglas-fir then when on western hemlock (Table 7), suggesting that the influence of soil factors on mycorrhizal formation is not solely due to direct effects on the fungus.

Effects of litter

Douglas-fir seedlings growing in soil to which either litter or litter leachate from the second old-growth site had been applied produced significantly fewer total and ectomycorrhizal tips than did those treated with litter from the first such site. Method of litter application made no difference, indicating that the effect was pro-

TABLE 6. Correlation matrix (correlation coefficient (r) of seedling growth measurements and root tip counts for Douglas-fir and western hemlock seedlings[a]

Species and variable	1	2	3	4	5	6	7	8	9	10	11	12
Douglas-fir[b]												
1. Shoot length (mm)												
2. Root length (mm)	NS											
3. Shoot dry weight (g)	0.55***	NS										
4. Root dry weight (g)	0.40***	0.26***	0.61***									
5. No. nonmycorrhizal tips	0.19**	NS	0.33***	0.30***								
6. No. ectomycorrhizal tips	0.22***	0.21***	0.19**	0.58***	NS							
7. % Ectomycorrhizal tips	NS	0.25***	NS	0.30***	−0.61***	0.67***						
8. No. D1 tips	NS	NS	NS	0.33***	0.27***	0.54***	0.13*					
9. No. D2 tips	0.24***	0.17**	0.28***	0.41***	−0.18**	0.40***	0.47***	NS				
10. No. D3 tips	0.17**	NS	NS	0.19**	0.13*	0.39***	0.13*	0.20**	−0.13*			
11. No. D4 tips	NS	NS	0.12	NS	0.15*	NS	NS	NS	−0.11	NS		
12. No. D5 tips	NS	0.15*	NS	0.16*	−0.22***	0.56***	0.46***	0.11	NS	NS	−0.24***	
13. No. total root tips	0.29**	0.14*	0.35***	0.65***	0.57***	0.81***	0.19**	0.60***	0.22***	0.40***	0.13*	0.34***
Western hemlock[c]												
1. Shoot length (mm)												
2. Root length (mm)	0.25***											
3. Shoot dry weight (g)	0.89***	0.29***										
4. Root dry weight (g)	0.77***	0.32***	0.87***									
5. No. nonmycorrhizal tips	0.48***	0.28***	0.62***	0.54***								
6. No. ectomycorrhizal tips	0.39	0.27***	0.55***	0.54***	0.61***							
7. % Ectomycorrhizal tips	−0.20***	−0.11	−0.21***	−0.13*	−0.63***	0.11						
8. No. H1 tips	0.37***	0.24***	0.52***	0.54***	0.58***	0.81***	NS					
9. No. H2 tips	NS	NS	0.11	NS	NS	0.13*	0.13*	NS				
10. No. H3 tips	NS	NS	NS	NS	NS	0.22***	NS	−0.29***	−0.15*			
11. No. H4 tips	NS	NS	NS	NS	NS	0.15*	0.20***	NS	NS	−0.13*		
12. No. total root tips	0.47***	0.30***	0.63***	0.59***	0.82***	0.95***	−0.17**	0.80***	NS	0.19***	NS	

[a]Significant at 0.1%, 0.5%*, 0.01%**, 0.001%*** level. NS = not significant.
[b]n = 246 seedlings.
[c]n = 284 seedlings.

TABLE 7. Linear correlations (correlation coefficient (r)[a]) between soil parameters and root dry weights, total ectomycorrhizal tips, and occurrence of *Cenococcum geophilum* on Douglas-fir and western hemlock seedlings

	Root dry weight		Total ectomycorrhizal tips		Occurrence of *C. geophilum*	
Soil parameter	Douglas-fir[b]	Western hemlock[c]	Douglas-fir[b]	Western hemlock[c]	Douglas-fir[b]	Western hemlock[c]
Mineralizable N	0.32***	0.55***	0.47***	0.28***	0.61***	0.43***
Total N	0.25**	0.47***	0.46***	0.24***	0.50***	0.40***
NH_3 N	0.15*	0.45***	0.35***	0.31**	0.58***	0.46***
NO_3 N	0.16*	NS	0.27**	NS	NS	NS
C	0.29***	0.49***	0.43***	0.30***	0.63***	0.49***
Na	NS	NS	0.17	−0.26**	NS	−0.15
K	NS	−0.19*	NS	−0.16	−0.20*	−0.18*
Ca	0.31***	0.50***	0.42***	0.15	0.38***	0.20*
Mg	NS	NS	NS	NS	−0.17	−0.24**
P	0.20*	0.36***	0.35***	0.24*	0.49***	0.36***
pH	NS	NS	NS	−0.17*	−0.32***	−0.39***
Litter layer depth	NS	NS	NS	0.14	NS	0.24**
Soil temperature (2 cm)	NS	NS	NS	−0.35***	−0.21*	−0.35***
Soil temperature (10 cm)	NS	NS	NS	−0.40***	−0.26**	−0.42***
Moisture	0.33***	0.47***	0.32***	0.49***	0.73***	0.67***

[a] Significant at the 0.1%, 0.05%*, 0.01%**, 0.001%*** level. NS = not significant.
[b] n = 126 seedlings.
[c] n = 141 seedlings.

duced by an unknown water-soluble substance. Table 8 shows results of application of litter as leachate. This reduction in ectomycorrhizae was primarily due to a reduction ($P = 0.01$) in type D2. These data correspond with the differences in tip formation between Douglas-fir seedlings grown without litter in soils from the two old-growth sites.

Litter leachate from the two stands also influenced other ectomycorrhizal types: types D3 and D5 were present on seedlings grown in soil from the unburned clear-cut to which no leachate had been added; but when leachate from litter of either old-growth stand was added, D3 was eliminated and D5 tips were greatly reduced. The two litter leachates did not differ in pH; both averaged 5.15.

Discussion

Our results suggest that changes in the soil environment after disturbance and during secondary succession significantly influence seedling growth and root-tip formation. The nature of the effect varies with type of disturbance (burn or no burn), ectomycorrhizal species, and because of differing affinities for particular ectomycorrhizal types, tree species.

Because at least some of the mycorrhizal types formed on these sites react differently to disturbance, mycorrhizal fungi, like tree species, may occupy different successional niches. *Cenococcum geophilum*, for example, seems especially sensitive to burning. Comparison of soils from the burned clear-cut, the plantation (burned 20 years ago), and the two undisturbed forests suggests that the effects of burning on *C. graniforme* persist for at least 20 years. In contrast, predominant ectomycorrhizal types on Douglas-fir were unaffected by burning. Negative effects of burning on ectomycorrhizae, also noted by Harvey *et al.* (1980*a*), have significance for reforestation practices.

The diversity of ectomycorrhizal types found in this study and their differential response to varying soils give these sites a certain capacity to buffer changes in soil environment. In contrast, the Montana site studied by Perry *et al.* (1982) had a single predominant type, and disturbance resulted in significant reductions in root-tip formation on three tree species. The diversity in ectomycorrhizal types does not mean, however, that all are equally available to all tree species. Douglas-fir and western hemlock grown in the same soils form different predominant ectomycorrhizal types. *Cenococcum geophilum*, for example, remains a minor symbiont on Douglas-fir and a major one on western hemlock in all soil types. Note that the late seral tree species, western hemlock, is associated with a fungus relatively sensitive to fire whereas the pioneer tree species, Douglas-fir, has the greatest affinity for ectomycorrhizal types that are unaffected by disturbance and that may be negatively affected by late-seral soils.

TABLE 8. Mean seedling values for Douglas-fir treated with litter from first and second old-growth sites

Seedling variables	Old-growth site No. 1	Old-growth site No. 2	Probability that means are drawn from same population
Shoot length (mm)	33.01	30.17	0.07
Root length (mm)	188.66	192.99	0.09
Shoot dry weight (g)	0.022	0.018	0.04
Root dry weight (g)	0.025	0.022	0.05
No. total root tips	69.48	55.24	0.01
No. ectomycorrhizal tips	38.94	30.56	0.03
No. D1 tips	0.85	1.01	0.76
No. D2 tips	28.77	19.63	0.01
No. D3 tips	—	—	—
No. D4 tips	7.14	6.73	0.73
No. D5 tips	2.18	3.18	0.44
Ectomycorrhizal tips (%)	54.8	54.1	0.84

It is likely that more than one factor caused the variable seedling size and root-tip formation seen in our study. Formation of ectomycorrhizal root tips is a function not only of the availability of inocula but of physical and chemical characteristics of the soil. The latter may directly influence formation of the symbioses (Slankis 1974) or indirectly influence them through effects on seedling root size and chemistry. The significant but minor effect of soil nutrients on mycorrhizal formation seen in our study is consistent with other work (Slankis 1974), as is the inhibitory effect of litter leachate (Mikola 1948; Olsen *et al.* 1971).

The high correlation between total and ectomycorrhizal root tips may indicate that the observed variation in the latter was caused by changes in infection sites (root tips), rather than in inocula. However, the number of root tips successfully formed may depend on the presence of ectomycorrhizal inocula (Wilcox 1967). Field variables such as soil temperature and moisture could not have directly influenced our greenhouse bioassay; thus, significant correlation between these and tip formation suggests that environmental conditions may alter the amount of inocula present. It seems unlikely that a simple causal relationship exists between the presence of ectomycorrhizal fungi and the formation of root tips.

Our results represent a bioassay conducted under controlled conditions on soils collected at a particular time of year. Their extrapolation to the field or to soil collected at another time may not be valid. Mycorrhizal formation can be affected by factors such as day length; therefore, the conditions under which our bioassay was carried out may have influenced our results. However, we did bioassay the same soils under varying conditions of light and moisture (F. H. Meyer, unpublished data) and found that while absolute measurements such as numbers of mycorrhizal tips changed, the relative performance of soils from the various disturbed sites was little affected. Thus, for purposes of comparison between types of disturbance, which was our primary objective, our conclusions appear valid.

Acknowledgments

Drs. Jim Trappe and Chet Youngberg provided guidance during all phases of this research. We also thank two anonymous reviewers, who made helpful comments on the manuscript.

BENEKE, U., and F. GOBL. 1974. The influence of different mycorrhizae on growth, nutrition, and gas exchange of *Pinus mugo* seedlings. Plant Soil, **40**: 21–32.

BLACK, C. A. (*Editor*). 1965. Methods of soil analyses. Part 2. American Society of Agronomy, Madison, WI.

BOGAR, G. E., and F. H. SMITH. 1965. Anatomy of seedling roots of *Pseudotsuga menziesii*. Am. J. Bot. **52**: 720–729.

DYRNESS, C. T., J. F. FRANKLIN, and W. H. MOIR. 1974. A preliminary classification of forest communities in the central portion of the western Cascades in Oregon. Coniferous Forest Biome, Ecosystems Analysis Studies, U.S. International Biological Program Bull. No. 4.

FRIEND, D. T. 1961. A simple method of measuring integrated light values in the field. Ecology, **42**: 577–580.

GRECO, A. R. 1978. Effect of root diseases and site preparation on the growth and nutrient status of bareroot and container grown seedlings. M.Sc. thesis, University of Washington, Seattle, WA.

HARLEY, J. L. 1969. The biology of mycorrhizae. 2nd ed. Leonard Hill, London.

HARVEY, A. E., M. J. LARSEN, and M. F. JURGENSEN. 1976. Distribution of ectomycorrhizae in a mature Douglas-fir/larch forest soil in western Montana. For. Sci. **22**: 393–398.

——— 1979. Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. For. Sci. **25**: 450–358.

——— 1980*a*. Partial cut harvesting and ectomycorrhizae: early effects in Douglas-fir–larch forests of western Montana. Can. J. For. Res. **10**: 436–440.

——— 1980*b*. Clearcut harvesting and ectomycorrhizae: survival of activity on residual roots and influence on a bordering forest stand in western Montana. Can. J. For. Res. **10**: 300–303.

JACKSON, M. L. 1958. Soil chemical analyses. Prentice Hall, Inc., Englewood Cliffs, NJ.

KEENEY, D. R., and J. M. BREMNER. 1966. Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron. J. **58**: 498–503.

LAMB, R. J., and B. N. RICHARDS. 1971. Effect of mycorrhizal fungi on growth and nutrient status of slash and radiata pine seedlings. Aust. For. **35**: 1–7.

LEGARD, H. A., and L. C. MEYER. 1973. Willamette National Forest soil resource inventory. U.S. For. Serv., Northwest Region.

MARX, D. H., W. C. BRYAN, and C. E. CORDELL. 1977. Survival and growth of pine seedlings with *Pisolithus* ectomycorrhizae after two years on reforestation sites in North Carolina and Florida. For. Sci. **23**: 363–373.

MCARDLE, R. E., W. H. MEYER, and D. BRUCE. 1961. The yield of Douglas-fir in the Pacific Northwest. U.S. Dep. Agric. Tech. Bull. No. 201 (revised).

MEYER, F. H. 1974. Physiology of mycorrhizae. Annu. Rev. Plant Physiol. **25**: 556–686.

MIKOLA, P. 1948. On the physiology and ecology of *Cenococcum graniforme*. Metsantutkimuslaitoksen Julk. **36**: 1–104.

MIKOLA, P., O. LAIHO, J. ERIKAINEN, and K. KUVAJA. 1964. The effect of slash burning on the commencement of mycorrhizal association. Acta For. Fenn. **77**: 1–11.

OLSEN, R. A., G. ODHAM, and G. LINDEBERG. 1971. Aromatic substances in leaves of *Populus tremula* as inhibitors of mycorrhizal fungi. Physiol. Plant. **25**: 122–129.

OTT, L. 1977. An introduction to statistical methods and data analysis. Duxbury Press, Scituate, MA.

PERRY, D. A., M. M. MEYER, D. EGELAND, S. L. ROSE, and D. PILZ. 1982. Logging and site preparation in a montane forest ecosystem: evidence for persistent nitrogen deficiency and reduced seedling root tip formation. For. Ecol. Manage. In press.

ROTHACHER, J., C. T. DYRNESS, and R. L. FREDRICKSEN. 1967. Hydrologic and related characteristics of three small watersheds in the Oregon Cascades. U.S. For. Serv., Pac. Northwest For. Range Exp. Stn.

SLANKIS, V. 1974. Soil factors influencing formation of mycorrhizae. Annu. Rev. Phytopathology. **12**: 437–457.

STEELE, R. G., and G. H. TORRIE. 1960. Principles and procedures of statistics. McGraw-Hill, Inc., New York, NY.

TARAS, M. J., A. E. GREENBERG, R. D. HOAK, and M. C. RAND. 1971. Standard methods for the examination of water and wastewater. 13th ed. American Public Health Association, Washington, D.C.

THEODOROU, C., and G. D. BOWEN. 1970. Mycorrhizal responses of radiata pine in experiments with different fungi. Aust. For. **34**: 183–191.

WILCOX, H. 1967. Seasonal patterns of root initiation and mycorrhizal development. *In Pinus resinosa* Ait. 14th IUFRO Conf., Section 24, München, Germany. pp. 28–39.

WRIGHT, E., and R. TARRANT. 1958. Occurrence of mycorrhizae after logging and slash burning in the Douglas-fir type. U.S. For. Serv. Pac. Northwest For. Range Exp. Stn. Res. Note No. 160.