# First-year survival of *Tsuga heterophylla* without mycorrhizae and subsequent ectomycorrhizal development on decaying logs and mineral soil

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Received August 20, 1981

CHRISTY, E. J., P. SOLLINS, and J. M. TRAPPE. 1982. First-year survival of *Tsuga heterophylla* without mycorrhizae and subsequent ectomycorrhizal development on decaying logs and mineral soil. Can. J. Bot. **60**: 1601-1605.

Roots of western hemlock (*Tsuga heterophylla*) seedlings 1-5+ years old that had established naturally on logs in three states of decay or on mineral soil were compared for numbers and kinds of ectomycorrhizae. Mycobionts colonizing root systems included *Cenococcum geophilum* Fr., *Piloderma croceum* (Bres.) Erikss. & Hjorts., and four unidentified fungi distinguished by color and morphology. About half the seedlings surviving the first growing season (2–7 months) were nonmycorrhizal. Nonmycorrhizal seedlings were most frequent on the least decayed logs. However, mycotrophy appeared to be advantageous to hemlock; 1st-year mycorrhizal seedlings had shoots 60% longer and roots 47% longer than 1st-year nonmycorrhizal seedlings. All 2nd-year and older seedlings were mycorrhizal. The ability of western hemlock to survive the first growing season without mycorrhizae may contribute to its success in colonizing decaying logs, which may contain microsites devoid of effective ectomycorrhizal inocula.

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Chez la pruche de l'ouest (*Tsuga heterophylla*), le nombre et les sortes d'ectomycorhizes ont été comparés dans les racines de plantules âgées de l à 5 ans, établies naturellement sur des morceaux de bois plus ou moins pourris (3 niveaux de pourriture) ou dans le sol minéral. Les mycobiontes colonisant les systèmes racinaires comprennent *Cenococcum geophilum* Fr., *Piloderma croceum* (Bres.) Erikss. & Hjorts. et quatre champignons non identifiés se distinguant par la couleur et la morphologie. Environ la moitié des plantules qui survivent durant la première saison de croissance (âge: 2 à 7 mois) ne sont pas mycorhizées. Les plantules non mycorhizées sont les plus fréquentes sur le bois le moins décomposé. Cependent, la mycotrophie semble avantageuse pour la pruche; les plantules mycorhizées de lère année ont des tiges 60% plus longues et des racines 47% plus longues que les plantules non mycorhizées de même âge. Toutes les plantules de deuxième année ou plus âgées sont mycorhizées. L'aptitude de la pruche à survivre durant la première saison de croissance sans mycorhizes peut en partie expliquer son succès à coloniser les morceaux de bois en décomposition où peuvent se rencontrer des microsites dépourvus d'inoculums ectomycorhiziens effectifs.

[Traduit par le journal]

#### Introduction

The importance of ectomycorrhizae to growth of conifer seedlings in nurseries has received considerable attention (Harley 1969; Mikola 1973; Trappe 1977). Naturally established seedlings have been studied less frequently, especially with regard to timing of colonization or effect of colonization on growth and survival. Of particular interest in this regard is naturally established western hemlock (*Tsuga heterophylla* (Raf.) Sarg.). An important timber species in northwestern North America and the climax tree species over most of its range (Franklin and Dyrness 1973), western hemlock is unusual in this region in preferentially establishing as an understory on partly decayed, large-diameter logs on

the forest floor (Minore 1972; Franklin *et al.* 1981). Rarely found on moss or litter, hemlock flourishes on rotten logs or mineral soil exposed on trails or on mounds and pits created by windthrown trees.

We hypothesized that hemlock might differ in mycorrhizal habit and growth rate depending on the substrate on which it established itself. To test this hypothesis, we examined the root systems of variously aged, naturally established hemlock seedlings collected from rotten wood and soil to determine proportions of ectomycorrhizal and nonmycorrhizal feeder roots and types of ectomycorrhizae. Shoot and root length was also recorded. For comparison, seedlings of Pacific silver fir (*Abies amabilis* (Dougl. ex Loud.) Forbes), an associ-

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CAN. J. BOT. VOL. 60, 1982



FIG. 1. Percentage of western hemlock seedlings colonized by each of six mycorrhizal fungi, collected from four substrates and segregated by age-class; N, number of seedlings samples. Nonmycorrhizal seedlings indicated by hatching for clarity.

ated species that establishes occasionally on rotten logs but usually on soil, were similarly collected and examined. Only a few *A. amabilis* seedlings were found in the study areas, however, owing to poor seed production by that species in the several years preceding our study.

#### Study area and methods

Seedlings were collected in August and September 1980 at sites between 500 and 1000 m in elevation on the H. J. Andrews Experimental Forest in the Oregon Cascade Mountains. Vegetation at all sites was dominated by old-growth Douglasfir (*Pseudotsuga menziesii* (Mirb.) Franco) ranging in age from 350–550 years. These old-growth stands contain hemlock and western redcedar (*Thuja plicata* Donn.) in the understory at lower elevations and hemlock and Pacific silver fir at higher elevations (Franklin and Dyrness 1973).

Most of the forest floor is deeply covered by moss, litter, and fallen tree boles (logs). Logs covered 10-30% of our study areas and accounted for 100-300 Mg (metric tonne)·ha<sup>-1</sup> of organic matter (Grier and Logan 1977; Franklin and Waring 1980). They are sufficiently important in nutrient cycling and wildlife relationships in old-growth forests that a system has been devised to classify them by stage of deterioration (see Triska and Cromack (1980) and Sollins (1982) for more detail): class I, bark and all wood intact, fine twigs attached; class II, bark and heartwood intact but sapwood partly soft, twigs absent; class III, bark loosened, sapwood decayed but still present, heartwood structurally sound; class IV, sapwood not structurally sound; class V, wood largely fragmented, an ill-defined, elongate mound on the forest floor.

With rare exception, western hemlock grew only on class III, IV, and V logs and on mineral soil. Consequently, only these four substrates were sampled. We attempted to collect 40 hemlock seedlings of each of four age-classes (1, 2, 3-4, and 5+ years old) from each substrate, although this many seedlings could not always be found within the time available for search. Actual numbers of seedlings collected are shown in Fig. 1. Age was determined by counting terminal bud scars, which can be done accurately during the first 5 years of seedling growth. Because rotten wood was friable and roots tended to grow in cracks, most root systems could be extracted intact with little difficulty. When roots were broken during excavation, the seedling was discarded.

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In the laboratory, seedlings were washed and length of shoot and longest root was measured. By stereomicroscopic examination we determined the percentage of all seedlings that were mycorrhizal and, of those, the percentage of feeder roots colonized by each of the six mycobionts: *Cenococcum geophilum* Fr., *Piloderma croceum* (Bres.) Erikss. & Hjorts., and four unidentified fungi termed tan, white mat, white crustose, and pale yellow. Mycobiont colonization classes were 1–5, 6–25, 26–50, 51–75, 76–95, and 96–100%. Initially, eight root tips were crushed and mounted in 5% KOH on microscope slides to check for Hartig-net formation; later, as similar forms of mycorrhizae recurred, this step was omitted. All tips enclosed in fungal mantles proved to be ectomycorrhizal; no ectendomycorrhizae were found. Translucent, white root tips invariably lacked mycobionts.

By contingency analysis, we first established percentages of nonmycorrhizal and mycorrhizal seedlings, then subdivided the latter category by fungal species. Next, one-way analysis of variance was used to test for significant differences by age and substrate in root length, shoot length, and percent colonization by each mycobiont. The Scheffé multiple range test served to establish significance of differences between means. All tests were performed with the SPSS package of computer programs (Nie *et al.* 1975).

## **Results and discussion**

Sixty-one percent of all 1st-year hemlock seedlings lacked mycorrhizae when sampled in August and September. For class III logs, 2 of 30 1st-year seedlings were mycorrhizal; for class IV logs, 5 of 6; for class V logs, 8 of 40; and for mineral soil, 15 of 21 (see Fig. 1). Of the 373 older seedlings collected, all contained at least one root tip with a mycelial mantle.

Western hemlock germinates in the study area between March and June (Gashwiler 1967, 1970); consequently, we concluded that the nonmycorrhizal 1st-year seedlings had survived 2-7 months. Other Pinaceae in the western Oregon Cascades (species of Abies, Pinus, and Pseudotsuga) routinely form mycorrhizae within 8 weeks of germination in forest soil (J. M. Trappe and W. Stein, unpublished data). For example, although we found only six 1st-year Pacific silver fir seedlings after extensive search, all were mycorrhizal (and all had established on mineral soil). The unusual ability of western hemlock to persist without mycorrhizae through the first growing season may contribute to its success in colonizing rotten logs, which may contain sites devoid of hyphae or other effective propagules of mycorrhizal fungi.1 If we assume that hemlock seedlings must eventually form mycorrhizae to survive and grow but can tolerate a delay of up to 7 months before doing so, then hemlock would be better able to colonize logs than other understory species that cannot tolerate such a delay.

Nonmycorrhizal, saprophytic fungi are common in rotten logs and probably antagonize many mycorrhizal fungi (Kropp 1982*a*). This antagonism could explain the frequent absence of mycorrhizae on hemlock rooted in rotten wood. Moreover, mycorrhizal fungi are at a disadvantage initially because they can colonize rotten wood only after receptive hemlock feeder roots are available as an energy source. It is unlikely that incompatibility between fungus and host accounts for the absence of mycorrhizae because western hemlock forms ectomycorrhizae with a broad range of mycobionts, including many that occur in rotten wood (Kropp and Trappe 1982).

In general, mycobiont species differed more by seedling age than by substrate. First-year mycorrhizal seedlings were colonized most commonly by the tan fungus, regardless of substrate (Fig. 1). Cenococcum

CLASS III CLASS IX CLASS X SOIL FIG. 2. Average percentage of western hemlock feeder otlets colonized by *Cenococcum geophilum* and an un-

% COLONIZED

FIG. 2. Average percentage of western hemlock feeder rootlets colonized by *Cenococcum geophilum* and an unidentified tan fungus. Seedlings collected from four substrates and segregated by age-class; numbers collected are shown in Fig. 1.

was less abundant, occurring only on 1st-year seedlings growing on class V logs and in mineral soil, and the other four mycobionts were even more infrequent. More than 85% of 2nd-year and older seedlings were colonized by the tan fungus, regardless of substrate (Fig. 1), but the other mycobionts occurred only in minor amounts. The 5th-year and older seedlings on mineral soil tended to have a more diverse mycorrhizal flora than younger seedlings or those on other substrates. Occasionally, the white-mat fungus formed a secondary mantle with the tan fungus, and vice versa.

Colonization by Cenococcum tended to increase with seedling age, regardless of whether colonization was expressed as presence or absence of Cenococcum (Fig. 1) or as the average percentage of feeder roots colonized by Cenococcum (Fig. 2). Such an increase is not surprising; Kropp (1982b) noted increased colonization by Cenococcum of outplanted hemlock seedlings over a single growing season. Abundant in rotten wood, Cenococcum slowly colonizes its habitat, mostly by vegetative growth; once established on a host root system, it spreads persistently to other rootlets (Trappe 1962). Its progress, unlike that of other mycobionts, is little inhibited by presence of nonmycorrhizal fungi; it tolerates temperatures, moisture conditions, and organic compounds that inhibit growth of many other mycorrhizal fungi (Trappe 1962).

The percentage of feeder roots colonized by *Ceno-coccum* was lower in class IV logs than in class III or V logs (Fig. 2). To explain this, one might hypothesize that, in class IV logs, *Cenococcum* growing in sapwood during the preceding class III stage would be largely removed as sapwood sloughs off and that other more rapidly growing spore-dispersed fungi would opportunistically colonize the remaining heartwood of class



<sup>&</sup>lt;sup>1</sup>R. S. Gill (personal communication) found that western hemlock rooted in sterile soil survived in the nursery up to 2 years but that growth ceased after the first 2-3 weeks.

с н'	Log decay class			
class, years	III	IV	v	Soil
	,	Shoot length, cm		
1	1.79a (0.07)	2.53b (0.16)	1.70a (0.12)	2.24b (0.09)
2	2.91a (0.09)	2.92a (0.11)	3.00a (0.12)	3.71b (0.15)
3-4	4.27 <i>a</i> (0.17)	4.90a (0.29)	5.02a (0.22)	5.03a (0.29)
5+	13.80 <i>a</i> (1.12)	15.77 <i>a</i> (1.52)	14.37 <i>a</i> (1.30)	15.77 <i>a</i> (1.84)
		Root length, cm		
1	2.76a (0.24)	3.17 <i>ab</i> (1.05)	3.30ab (0.74)	4.51 <i>b</i> (0.63)
2	4.36a (0.37)	3.83ab (0.32)	6.41c (0.45)	5.83bc (0.37)
3-4	5.85a (0.38)	4.94 <i>a</i> (0.35)	7.31 <i>b</i> (0.40)	4.70 <i>a</i> (0.36)
5+	16.19 <i>a</i> (1.64)	13.17a (1.18)	15.05 <i>a</i> (1.34)	13.86 <i>a</i> (3.18)

TABLE 1. Length of shoot and longest root of western hemlock seedlings, by age-c	lass and
substrate: standard error of estimate is shown in parentheses	

NOTE: Values in each horizontal line not followed by the same letter are significantly different (P < 0.05) according to ANOVA and the Scheffé multiple range test.

IV logs. In this case, considerable time might have to elapse before *Cenococcum* would regain by vegetative growth its level in class III logs.

Shoots of 1st-year seedlings growing on soil and class IV logs, the substrates with marked incidence of mycorrhizal seedlings, averaged significantly longer than those of seedlings growing on class III and V logs, the substrates with low incidence of mycorrhizal seedlings (Table 1). When data were averaged for all substrates, mycorrhizal 1st-year seedlings had shoots 60% longer and roots 47% longer than 1st-year non-mycorrhizal seedlings (P < 0.01).

Effects of substrate on rate of hemlock growth were more pronounced for 2nd-year seedlings. Those growing on mineral soil had significantly longer shoots than those growing on any log substrate, a difference due either to greater availability of nutrients in soil than wood (P. Sollins, unpublished data) or to earlier mycorrhizal formation on seedlings growing in soil. Differences in shoot growth disappeared among substrates for seedlings 3 years or older (Table 1).

Third- and 4th-year seedlings growing on class V logs had significantly longer roots than those growing on any other substrate (P < 0.0001). Among 1st- and 2nd-year seedlings, those growing on class V logs ranked either first or second with respect to root length (Table 1). Most likely, this phenomenon relates to moisture content and structural characteristics of this log class. Class V logs are markedly decayed, whereas class III and IV logs have large proportions of intact wood that roots cannot easily penetrate. Rotten wood, remaining moist after mineral soil has dried during summer drought, can be a superior substrate for root growth and mycorrhizal activity (Harvey *et al.* 1978). Although the roots of seedlings we collected from rotten logs did not extend into mineral soil, we have observed at these sites and elsewhere that roots of older hemlock saplings and trees extend into mineral soil from the rotten log or stump on which they originally established. After several hundred years, most of the original substrate has disappeared, leaving the tree perched above the ground atop large roots that had originally passed through the sapwood of the log or stump into the soil. The changes in mycorrhizal structure and function that accompany the shift from lignotrophic to lithotrophic nutrition merit further attention.

We destructively sampled seedlings only at the end of the growing season, so overall survival on different substrates cannot be inferred. A study of seedling demography by substrate is now completed and a manuscript is in preparation (E. J. Christy and R. N. Mack).

### Acknowledgments

This research was supported in part by grants from the National Science Foundation (DEB 7706075 and DEB 8004652). We thank Dr. G. Spycher for performing the statistical analyses and Drs. R. N. Mack, D. Minore, R. Molina, and J. N. Thompson for reviewing earlier drafts. This is paper No. 1584, Forest Research Laboratory, Oregon State University, Corvallis, OR.

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