

Hyphal mats formed by two ectomycorrhizal fungi and their association with Douglas-fir seedlings: A case study

ROBERT P. GRIFFITHS¹, MICHAEL A. CASTELLANO² and BRUCE A. CALDWELL¹

¹Department of Microbiology, Oregon State University, Corvallis, OR 97331, USA and ²United States Department of Agriculture, Forest Service, Pacific Northwest Research Station, Forestry Sciences Laboratory, 3200 Jefferson Way, Corvallis, OR 97331, USA

Received 24 April 1990. Revised December 1990

Key words: Douglas-fir seedlings, ectomycorrhizae, *Gautieria monticola*, hyphal mats, *Hysterangium setchellii*

Abstract

The ectomycorrhizal fungi *Gautieria monticola* and *Hysterangium setchellii* both form dense hyphal mats in coniferous forest soils of the Pacific Northwest. We recently observed that all Douglas-fir seedlings found under the canopy of a maturing 60–75 year stand were associated with mats formed by ectomycorrhizal fungi. The significance of these mat communities in relation to seedling establishment and survival is discussed.

Introduction

A number of ectomycorrhizal fungi form mat-like structures in the upper soil profile (Cromack et al., 1979; Fisher, 1972; Hintikka and Naykki, 1967). These hyphal mats are long-lived structures of the below-ground ecosystem and may influence site productivity. In the Pacific Northwest, two distinct mat-forming ectomycorrhizal fungi commonly occur in Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) forests; *Hysterangium setchellii* Fischer and *Gautieria monticola* Harkness (Hunt and Trappe, 1987). These mats are easily recognized with the unaided eye, can colonize up to 28.4% of the forest floor surface and 16.7% of the upper 10 cm of soil (Cromack et al., 1979). The genus *Hysterangium sensu lato* (50 species worldwide) is widely distributed with many species restricted in their ectomycorrhizal host association (Castellano, 1988). Many of these host-restricted *Hysterangium* species form similar mat-like structures (Table 1).

During our studies of *Hysterangium setchellii* and *Gautieria monticola* mats in Douglas-fir forests in western Oregon, we observed that Douglas-fir regeneration beneath the closed canopy occurred only within soils colonized by mat-forming fungi. Results from a survey to qualify this phenomenon are reported.

Methods and materials

Surveys of mat-seedling associations

A survey for Douglas-fir seedlings was conducted in a 60–75 year-old second growth, site class 2, Douglas-fir stand at approximately 460 m elevation with a gravelly loam soil derived from a colluvium of weathered basalt and sandstone. The site is located 30 km southwest of Corvallis, Oregon and has been extensively studied and is described in detail by Fogel (1976) and Cromack et al. (1979). Twenty random belt transects (Mueller-Dombois and Ellenberg, 1974) 25 m

Table 1. *Hysterangium* species that from mat-like structures in soil (taken from Castellano, 1988)

<i>Hysterangium</i> Species	Hosts	Distribution
<i>affine</i>	<i>Eucalyptus</i>	Tasmania
<i>aggregatum</i>	<i>Eucalyptus</i>	Queensland
<i>gardneri</i>	<i>Eucalyptus</i>	Tasmania, California
<i>inflatum</i>	<i>Eucalyptus</i>	Tasmania, California, France
<i>album</i>	<i>Quercus</i>	New York
<i>asperatum</i>	<i>Fagus</i>	North Carolina, Tennessee, Maine
<i>calcareum</i>	<i>Fagus</i> , <i>Betula</i>	Germany, Italy, Romania
<i>cistophilum</i>	<i>Quercus</i> , <i>Carpinus</i> , <i>Cistus</i>	France, Italy, Czechoslovakia
<i>nephriticum</i>	<i>Fagus</i> , <i>Quercus</i>	England, Hungary, Germany
<i>thwaitesii</i>	<i>Fagus</i> , <i>Quercus</i>	England
<i>americanum</i>	<i>Tsuga</i>	New York
<i>setchellii</i>	<i>Pseudotsuga</i>	Oregon, California
<i>coriaceum</i>	<i>Pseudotsuga</i>	Oregon
<i>crassirhachis</i>	<i>Pseudotsuga</i>	Oregon

long and 2 m wide were made to cover 0.1 ha. The species of mycorrhizal fungi forming mats in which the seedlings were located were identified by microscopic analysis of the sporocarps found fruiting within the mats.

In a second survey, all Douglas-fir seedlings found growing beneath the canopy of a 60–75 yr-old second growth stand (ca. 0.4 hectare) were examined for their occurrence within hyphal mats.

Specimen preparation for microscopy

Seedlings associated with *Hysterangium setchellii* and *Gautieria monticola* mats were prepared for stereomicroscopic examination by soaking roots in water for 2 hours and gently agitating them to remove most of the soil. A two-year-old Douglas-fir seedling growing within a well-developed *Hysterangium setchellii* mat was used to demonstrate the connection between the seedling and mat fungal hyphae using scanning electron microscopy. The washed seedling roots were prepared by critical-point drying and mounted onto a peg with double-sided sticky tape. Mycorrhizal root material was then coated with approximately 10 nm of gold-palladium alloy

(60:40), and photographed with an Amray 2000 scanning electron microscope on P/N 55 Polaroid film.

Results

Description of mats

Hysterangium setchellii

Ectomycorrhizae were eggshell white with occasional reddish brown stains on the mantle surface (Fig. 1). The mantle surface was coarsely textured, extremely tomentose with abundant multi-branched, white, stout rhizomorphs emanating from the surface (Fig. 1). Ectomycorrhizal root tips were prolifically branched in a pinnate, sometimes elongated, pattern with length usually exceeding 7 mm and a diameter of up to 1 mm. *Hysterangium setchellii* forms a profuse network of fungal material, both hyphal and rhizomorphic, throughout the nearby soil substrate. The fungal material is easily seen interconnecting various ectomycorrhizae and extensively exploring the soil. Colonized soil can become wet seasonally.

Gautieria monticola

Ectomycorrhizal roots were bright white to grayish white underlain by a brown base (Fig. 2). Ectomycorrhizae were elongate to simply pin-



Fig. 1. Photograph of *Hysterangium setchellii* mat rhizomorphs colonizing Douglas-fir roots.



Fig. 2. Photograph of Douglas-fir roots colonized by *Gautieria monticola*.

nate, with length exceeding 7 mm and a diameter up to 1 mm. The mantle surface was woolly tomentose without rhizomorphs, but with some mycelial strands. *Gautieria monticola* also forms a profuse network of fungal material throughout the soil substrate. Colonized soil is hydrophobic nearly year round.

Seedlings associated with mats

In our initial seedling survey, we found 4 Douglas-fir and 47 western hemlock seedlings within a sample area. Of the Douglas-fir seedlings, two were found in *Gautieria monticola* mats and two were in *Hysterangium setchellii* mats (Fig. 3). Of the 47 western hemlock seedlings found, 4 were in *Gautieria monticola* mats, 4 were in *Hysterangium setchellii* mats, and the remaining seedlings were associated with buried wood in advanced decay.

In addition to the random belt transect observations, we also recorded the relationship between Douglas-fir seedlings and mat communities within a contiguous area of approximately 0.4 ha. Within this additional 0.4 ha sample, we found that all 45 Douglas-fir seedlings were associated with *Gautieria monticola* or *Hysterangium setchellii* mats with none found in rotten wood. We observed the same phenomenon in Douglas-fir seedlings in younger stands



Fig. 3. Douglas-fir seedlings associated with a *Hysterangium setchellii* mat. The seedling is shown growing within soil extensively colonized by *H. setchellii* rhizomorphs.

(40–50 years) located at the H.J. Andrews Experimental Forest in the Cascade Mountains. At the same general location, one-to two-year-old seedlings were found growing in the deep shade of 15-year-old Douglas-fir trees naturally regenerated within a shelterwood. These seedlings were also located within *Hysterangium* mats.

A scanning electron micrograph of a Douglas-fir seedling removed from a *Hysterangium setchellii* mat clearly shows the colonization of the seedling roots by this mat-forming fungus (Fig. 4).

Discussion

Because less than 28% of the Douglas-fir forest floor surface is colonized by mat communities (Cromack et al., 1979), we would expect no more than approximately 28% of the surveyed seedlings would be found within these mat communities. In younger Douglas-fir forests in the Cascade Mountains where the incidence of mats is even lower, we discovered that all seedlings were within mats. These observations strongly suggest that mat communities can act as nursery sites for conifer seedling establishment.



Fig. 4. Scanning electron micrograph of seedling root surface showing interaction with *Hysterangium sechellii* hyphae at a magnification of 40 \times .

Within these mats, Douglas-fir seedlings are physically linked to mature host trees via a seedling-ectomycorrhizal-mat-root connection (Figs. 4 and 5). Although a physical, but not physiological connection between the roots of the overstory Douglas fir and the seedlings was established, the fact that both of these root systems were within the same mat strongly suggests that they are physiologically linked as well. Further work to confirm a physiological link is needed.

Cromack et al. (1988) found fungal mats of *Hysterangium sechellii* to have a significantly greater microbial biomass and also greater number of microarthropods than adjacent non-mat soil. These mats also had higher concentrations of soil carbon and soil nitrogen, higher respiration and higher levels of several enzymes than non-mat soil.

We want to emphasize that the non-mat soil is thoroughly colonized by plant roots and their associated microorganisms and will have a wide diversity of ectomycorrhizal fungi from one sample to another. The mat soil is characterized by a single strikingly dominant ectomycorrhizal fungus, i.e. *Hysterangium sechellii* and to a lesser degree *Gautieria monticola*, to the apparent exclusion of other ectomycorrhizal fungi.

There are several basic mechanisms that may allow these mats to act as nurseries for seedlings: (1) by providing a greater opportunity to quickly form ectomycorrhiza with an established mycorrhizal fungus network; (2) by providing the seedling with carbohydrates under stressful light-limiting conditions; and by (3) suppressing pathogen infection of the seedling. Of these alternatives, the one that seems most important is the role of these mats in transferring energy and possibly water and nutrients from the overstory tree to the seedlings. Since Douglas fir is relatively intolerant of shade (Isaac, 1943), it is likely that energy for seedling establishment must come, in large part, from an overstory tree in the form of carbohydrates. Considering the extent of fungal biomass, it is unlikely that a

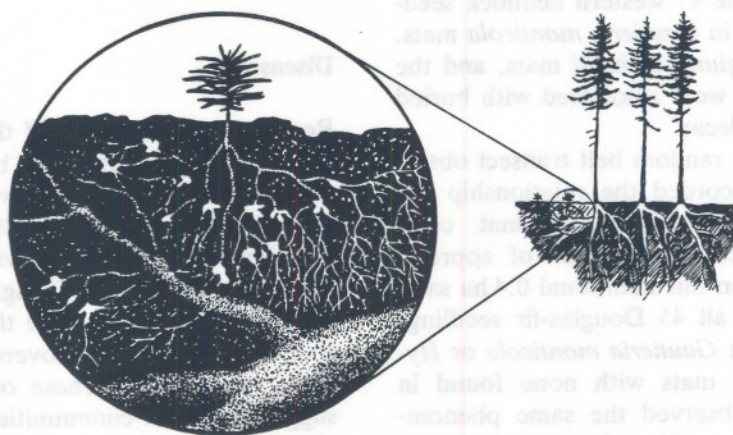


Fig. 5. Schematic drawing of connection between seedling and overstory tree via the mat.

seedling could support the mass of external hyphae with which it is associated. Based on both experimental evidence and previous field observations (Chippindale, 1932; Hutchinson, 1967; Mohmoud and Grime, 1974; Salisbury, 1930), Read and coworkers (1985) suggested that mycorrhizal fungi may act as a conduit for carbohydrate transfer from overstory plants to those shaded by these plants. This process would allow a shade-intolerant species to become established under conditions of deep shade. A subsequent forest disturbance such as windthrow or insect outbreaks leading to increased overstory mortality would permit such seedlings opportunity to grow.

Acknowledgements

The authors would like to thank A H Soeldner for doing the scanning electron microscopy and J R Leake for taking the photographs in Figs. 1 and 2. This research was supported in part by NSF grant BSR 8717849 and USDA Competitive Grant 86-FSTY-9-0224.

References

- Castellano M A 1988 The taxonomy of the genus *Hysterangium* (Basidiomycotina, Hysterangiaceae) with notes on its ecology. Ph.D. Thesis, Oregon State University, Corvallis, OR. 238 p.
- Chippindale H G 1932 The operation of interspecific competition in causing delayed growth of grasses. *Ann. Appl. Biol.* 19, 221–242.
- Cromack K Jr, Fitcher B L, Moldenke A M, Entry J A and Ingham E R 1988 Interactions between soil animals and ectomycorrhizal fungal mats. *Agric. Ecosys. Environ.* 24, 161–168.
- Cromack K Jr, Sollins P, Graustein W, Speidel K, Todd A, Spycher G, Li C Y and Todd R L 1979 Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassum*. *Soil Biol. Biochem.* 11, 463–468.
- Fogel R 1976 Ecological studies of hypogeous fungi: Sporocarp phenology in a western Oregon Douglas-fir stand. *Can. J. Bot.* 54, 1152–1162.
- Fisher R F 1972 Spodosol development and nutrient distribution under *Hydnaceae* fungal mats. *Soil Sci. Soc. Am. Proc.* 36, 492–495.
- Hintikka V and Naykki O 1967 Notes on the effects of the fungus *Hydnellum ferrungineum* (Fr.) Karst. on forest soil and vegetation. *Comm. Inst. Forest. Fenn.* 62, 1–23.
- Hunt G A and Trappe J M 1987 Seasonal hypogeous sporocarp production in a western Oregon Douglas-fir stand. *Can. J. Bot.* 65, 438–445.
- Hutchinson T C 1967 Comparative studies of the ability of species to withstand prolonged periods of darkness. *J. Ecol.* 55, 291–299.
- Isaac L A 1943 Reproductive Habits of Douglas-fir. Charles L. Pack Forestry Foundation, Washington, DC. 105 p.
- Mohmoud H and Grime P G 1974 A comparison of negative relative growth rates in shaded seedlings. *New Phytol.* 73, 1215–1219.
- Mueller-Dombois D and Ellenberg H 1974 Aims and Methods of Vegetation Ecology. Wiley, New York. 547 p.
- Read D J, Francis R and Finlay R D 1985 Mycorrhizal mycelia and nutrient cycling in plant communities. In *Ecological Interactions in Soil: Plants, Microbes and Animals*. Eds. A H Fitter, D Atkinson, D J Read and M B Usher, pp 193–217. Blackwell Scientific Publ., Oxford.
- Salisbury E J 1930 Mortality amongst plants and its bearing on natural selection. *Nature* 125, 817.

