Ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga menziesii* establishment

Thomas R. Horton, Thomas D. Bruns, and V. Thomas Parker

Abstract: Chaparral on the central coast of California can occur as relatively stable patches of ectomycorrhizal *Arctostaphylos* directly adjacent to arbuscular mycorrhizal *Adenostoma*. Vegetation surveys and seedling survival assays show that *Pseudotsuga* establishes only in *Arctostaphylos*. We found no significant differences between *Arctostaphylos* and *Adenostoma* in allelopathy; light; temperature; or soil NH_4^+, NO_3^-, or K. *Arctostaphylos* soils tended to be higher in phosphate and were lower in pH, Ca, Mg, Ni, and Cr than those from *Adenostoma*. After 1 year of growth of *Pseudotsuga* seedlings in an *Arctostaphylos* patch, 17 species of fungi colonized both *Pseudotsuga* and *Arctostaphylos*. Fifty-six of 66 seedlings were colonized by fungi that also colonized *Arctostaphylos* within the same soil core. Forty-nine percent of the *Pseudotsuga* ectomycorrhizal biomass was colonized by fungi that were also associated with *Arctostaphylos* within the same core. Another 12% was colonized by fungi known to associate with *Arctostaphylos* from different cores. After 4 months of growth, *Pseudotsuga* seedlings in four of five *Arctostaphylos* plots were ectomycorrhizal and colonized by fungi in Russulaceae, Thelephoraceae, and Amanitaceae. *Pseudotsuga* seedlings in two of five *Adenostoma* plots were ectomycorrhizal but colonized by only two species of fungi in Thelephoraceae. These results provide compelling evidence that ectomycorrhizal fungi associated with *Arctostaphylos* contribute to *Pseudotsuga* seedling establishment.

Key words: arbutoid, Douglas-fir, ectomycorrhizae, manzanita, RFLP, PCR.

Résumé : Sur la côte centrale de la Californie, le chaparral peut se présenter sous forme de plages relativement stables d'*Arctostaphylos* ectomycorhizien directement adjacent à de l'*Adenostoma* mycorhizien arbusculaire. Les observations sur la végétation et des essais de survie de plantules montrent que le *Pseudotsuga* ne s'établit que dans les plages d'*Arctostaphylos*. Les auteurs n'ont perçu aucune différence significative entre l'*Arctostaphylos* et l'*Adenostoma* quant à l'allélopathie, la lumière, la température ou les NH_4^+, NO_3^- ou K du sol. Les sols sous *Arctostaphylos* ont tendance à contenir plus de phosphate, ont des pH plus bas et contiennent moins de Ca, Mg, Ni et Cr que ceux sous l'*Adenostoma*. Après 1 année de croissance des plantules de *Pseudotsuga* dans une plage d'*Arctostaphylos*, on retrouve 17 espèces de champignons colonisant à la fois le *Pseudotsuga* et l'*Arctostaphylos*. Sur 66 plantules, 56 sont colonisées par un champignon qui colonise également l'*Arctostaphylos* dans la même carotte de sol. Quarant-neuf pourcent de la biomasse ectomycorhizienne du *Pseudotsuga* est colonisée par des champignons qui sont également associés avec l'*Arctostaphylos* dans la même carotte de sol. Un autre 12% est colonisé par des champignons aptes à coloniser l'*Arctostaphylos* tel qu'observé dans d'autres carottes. Après 4 mois de croissance, les plantules de *Pseudotsuga* provenant de quatre des cinq parcelles d'*Arctostaphylos* portent des ectomycorhizes formées par des champignons appartenant aux Russulaceae, Thelephoraceae et Amanitaceae. Dans deux parcelles sur cinq de l'*Adenostoma* les plantules du *Pseudotsuga* ont des ectomycorhizes, mais seulement avec deux espèces de Thelephoraceae. Ces résultats démontrent clairement que les champignons ectomycorhiziens associés à l'*Arctostaphylos* contribuent à l'établissement des plantules du *Pseudotsuga*.

Mots clés : arbutoïdes, sapin Douglas, ectomycorhizes, manzanita, RFLP, PCR.

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Introduction

Chaparral is a major vegetation type in California and typically consists of a dense thicket of shrubs whose species are adapted to repeated cycles of drought and fire (Barbour and Major 1988). Aerial photographs taken over the last 50 years document forest succession in chaparral sites in Marin County, California. Field surveys suggest that *Pseudotsuga menziesii* (Mirb.) Franco (Douglas-fir) differentially establishes only in chaparral dominated by *Arctostaphylos* spp. (manzanita) and not in chaparral dominated by *Adenostoma fasciculatum* Hook. & Arn. (chamise), even

though these two species occur as adjacent pure patches within the chaparral (Parker 1991; Sparling 1994). Some ectomycorrhizal fungi can associate with *Pseudotsuga* and *Arctostaphylos* spp. (Trappe 1962; Zak 1976*a*, 1976*b*; Molina and Trappe 1982*a*, 1982*b*). In contrast, *Adenostoma* forms arbuscular mycorrhizae (Allen 1991) and presumably does not support ectomycorrhizal networks for *Pseudotsuga*. These mycorrhizal relationships suggest that *Pseudotsuga* establishment in our area may be influenced by mycorrhizal fungi associated with *Arctostaphylos*.

The sharing of ectomycorrhizal fungi between *Pseudotsuga* and *Arbutus* or *Arctostaphylos* spp. may play a major role in plant community dynamics (Molina and Trappe 1982*a*; Perry et al. 1989). Unidentified microorganisms in soil associated with *Arctostaphylos* and *Arbutus* may enhance *Pseudotsuga* seedling growth, survival, mycorrhizal root formation, and nitrogenase activity (Amaranthus and Perry 1989; Amaranthus et al. 1990). Perry et al. (1989) present a theoretical model in which soil microorganisms, *Pseudotsuga*, and arbutoid members of Ericaceae maintain one another through disturbance cycles. In this model, fungal networks of some species remain relatively intact through disturbance events because they form associations with plant members of both the post-disturbance chaparral and the subsequent forest.

Pure culture synthesis experiments demonstrate that some fungal species that form ectomycorrhizae with *Pseudotsuga* will form arbutoid mycorrhizae with *Arctostaphylos* spp. under controlled conditions (Zak 1976*a*, 1976*b*; Molina and Trappe 1982*a*). The arbutoid mycorrhizal morphology is found in species of *Arbutus* and *Arctostaphylos* and is characterized by the combined presence of a mantle, Hartig net, and in particular, intracellular penetration of the epidermal cells by fungal hyphae (Smith and Read 1997). Although the specificity of mycorrhizal associations under field conditions is influenced by many ecological factors not found in laboratory manipulations (Harley and Smith 1983; Molina et al. 1992), field collections of fruit bodies in pure stands also indicate that some ectomycorrhizal fungal species will associate with both *Pseudotsuga* and *Arctostaphylos* spp. (Trappe 1962). Recently, Simard et al. (1997) demonstrated carbon flow between *P. menziesii* and *Betula papyrifera* Marsh. (white birch), suggesting the two plants were connected by common mycelia. Horton and Bruns (1998) showed that most fungi in a mixed stand of *Pseudotsuga menziesii* and *Pinus muricata* D. Don colonized roots of both plant species when their roots intermingled.

Several factors must be considered to determine whether mycorrhizal fungi influence seedling establishment in one vegetation patch compared with another. First, environmental variables that influence plant establishment such as light, moisture, allelopathic inhibition, and soil nutrients should not explain differential establishment. Second, mycorrhizal inoculum should vary between patches and differential establishment of plant species should be correlated. A related study showed that *Arctostaphylos* patches had higher soil moisture levels than *Adenostoma* patches (Dunn 1994). In this study, we document the establishment pattern of *Pseudotsuga* in chaparral based on vegetation surveys and survival of *Pseudotsuga* seedlings in chaparral. We also analyze sites where *Pseudotsuga* does and does not establish (*Arctostaphylos* and *Adenostoma* patches, respectively) for differences in temperature extremes, light incidence, allelopathic inhibition, soil nutrients, and ectomycorrhizal inoculum.

Methods

Site characteristics

The research was conducted on the central coast of California within the 9000-ha Marin Municipal Water District watershed and contains a mosaic of forest, chaparral, and grassland communities. The watershed experiences great heterogeneity in microclimate because of rain shadows of three mountain ridge systems (Howell 1970). Mean annual precipitation ranges widely from less than 450 to over 1000 mm and occurs principally in winter. Mean annual temperatures range from 12 to 14°C.

In this part of California, both the mixed evergreen forest and chaparral communities are stable aspects of the vegetation landscape because of the Mediterranean climate, topography, soil type, and fire history. Depending on current climatic trends and fire history, however, vegetation boundaries are dynamic and mixtures of species from both communities occur in some locations.

The forest and chaparral communities include species that are locally dominant depending on microclimatic and topographic features of the watershed. Our study focused on forests consisting principally of *Lithocarpus densiflora* (Hook. & Arn.) Rehd. (tanbark oak), *Pseudotsuga menziesii*, *Arbutus menziesii* Pursh (Pacific madrone), *Umbellularia californica* (Hook. & Arn.) Nutt. (California bay laurel), and *Quercus wislizenii* A. DC. (interior live oak) (nomenclature after Hickman 1993). The chaparral community included *Arctostaphylos glandulosa* Eastw. ssp. *glandulosa* Eastw. and *Adenostoma fasciculatum* dominating as a mosaic of patches with other species scattered throughout including *Ceanothus cuneatus* (Hook.) Nutt. and *Q. wislizenii*. Both *Arctostaphylos* and *Adenostoma* resprout from a burl and may maintain active mycorrhizal associations after fire (Molina and Trappe 1982*a*; Amaranthus and Perry 1989).

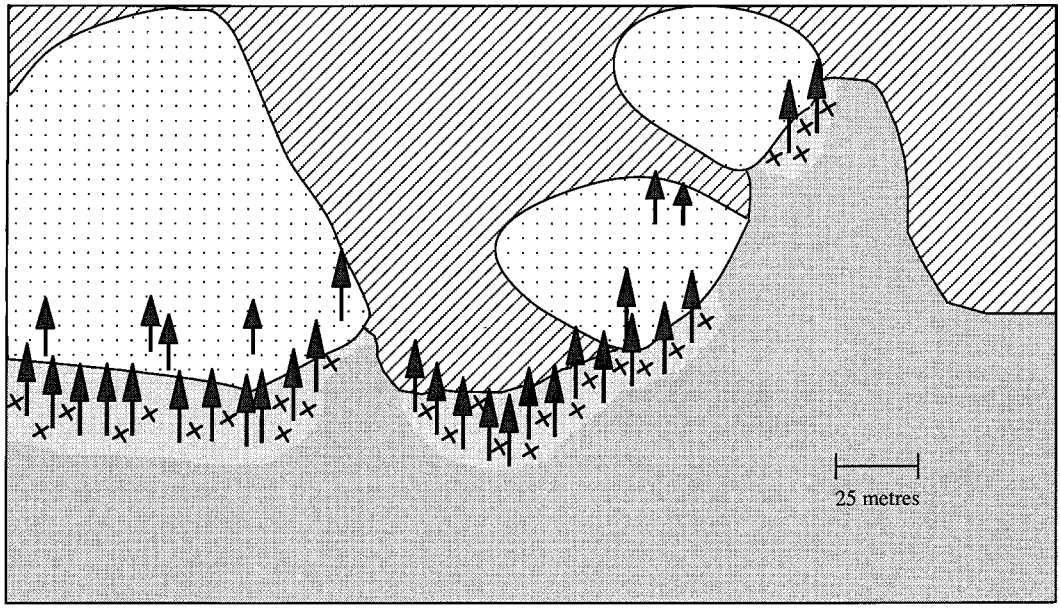
Vegetation surveys

Vegetation in the study area was divided into three zones and surveyed in January, 1991 (Fig. 1). The forest zone is described above. The mixed zone contained trees under 50 years old and included dead chaparral as an understory (Sparling 1994). In both of these zones, tree composition and dominance was determined along six randomly chosen 50-m transects. The point quarter method was used for the forest survey (Mueller-Dombois and Ellenberg 1974). Relative density, relative basal area, and relative frequency for each tree species was summed for an importance value (IV) (Mueller-Dombois and Ellenberg 1974). The third zone was the chaparral zone and is described above. Live chaparral with established *Pseudotsuga* trees was avoided for all aspects of the study; however, these areas were dominated by *Arctostaphylos*. The chaparral and mixed zones were sampled for shrub density with 2 × 2 m plots at 10 random points along each of six randomly placed 30-m transects. Identifying the species of dead chaparral was based on growth habit characteristics of the wood and burls. Chaparral relative cover was estimated using the percent cover of species in the quadrats (Barbour et al. 1987). All of the remaining experiments were conducted in the chaparral zone.

Seedling survival

Seeds of *Pseudotsuga menziesii* obtained from tree seed zone 096 (Schopmeyer 1974) were planted between late January and late February 1992. Three replicate locations were chosen for

Fig. 1. Diagram of a typical research location (not to scale). In general, the vegetation is made up of forest and chaparral communities. The forest consists of a heterogeneous mixture of several tree species and the chaparral consists of a mosaic of *Arctostaphylos* and *Adenostoma* patches. At the forest edge, dead chaparral is encountered with an overstory of *Pseudotsuga*. Beyond the forest edge, individual trees have established only in patches of live *Arctostaphylos*. For this study, three zones of vegetation were defined: forest, chaparral, and mixed. ⁘, chaparral zone with *Arctostaphylos*; ▨, chaparral zone with *Adenostoma*; ☒, mixed zone; □, forest zone; ↑ *Pseudotsuga*; ✗, dead chaparral.



planting. At each location, adjacent patches of *Arctostaphylos* and *Adenostoma* were present. All patches included occasional *Q. wislizenii* and *C. cuneatus*. At each location, 10 plots were dispersed in an area of approximately 10 m^2 within one patch of each vegetation type. Care was taken to choose large patches and not to plant near the edges. No *Pseudotsuga* were within 100 m of any planting location. Plots were not placed in animal trails. Each plot was a 10 × 30 cm area planted with 15 seeds. The seeds were surface sterilized in 5% bleach for 5 min, rinsed, and soaked in distilled water for 2 h. Before planting seeds, the soil was turned and mixed to a depth of approximately 10 cm. Plots were covered with 0.625 cm wire mesh screens to prevent herbivory. Field plantings were monitored for germination and survival. Several plots were severely disturbed by deer during the germination period and were replanted. Seedling survival data are shown as the percent of germinants alive per location (10 plots pooled per location, $n = 3$).

Environmental conditions

Temperature extremes were measured at approximately weekly intervals from May 3 through July 19, 1992, beneath the canopy of *Arctostaphylos* or *Adenostoma* patches. Two thermometers were used. One thermometer was placed near a seedling plot of each of the two vegetation types (seedling survival experiment) and rotated after each reading to a new location within that vegetation type. A total of seven readings were taken for each vegetation type. Thermometers were placed on level ground beneath open-ended aluminum foil shields to avoid heating by direct sunlight.

Light penetration beneath the canopies of *Arctostaphylos* or *Adenostoma* was measured on January 7, 1992, between 11:00 and 12:00. Data were collected at 27 locations within both *Arctostaphylos* and *Adenostoma* patches where *Pseudotsuga* seedlings were planted for the seedling survival experiment. Data were collected on a cloudy day to reduce variation from solar flecks. We alternated between vegetation patches every three readings. A LI-COR 185B quantum photometer with a LI-COR 190SB quantum sensor was placed at ground level to measure light levels. Because both plant species are evergreen, seasonal light differences were considered negligible. Light data are presented as a percentage of full sunlight.

Allelopathy

Branch and leaf material was collected within a 1-m^2 area of *Arctostaphylos* or *Adenostoma* following the protocol of Chou and Muller (1972). Collection occurred in April 1992, at least 2 weeks after any rain and during the period of seedling growth. Leachate was collected by spraying 3 L of distilled water over a 3 h period on branch and leaf material placed in a 1-m^2 funnel. The leachate was then vacuum filtered through a rinsed Whatman No. 5 filter paper. *Pseudotsuga* seeds were soaked for 2 h in a leachate or distilled water (control) and placed between two similarly soaked Fisher heavy-duty germination papers. Germination papers were presterilized by boiling them in distilled water for 30 min and then dried at 65°C for 20 min. For each vegetation type, 9 replicates were set up with 10 seeds each. Seeds and papers were enclosed in a sterile Petri dish, sealed with Parafilm, and placed in a controlled growth chamber (22°C, 12 h light : 12 h dark photoperiod). Germinants were harvested after 6 days of growth and the radicle length measured.

Soil nutrients

Soil was collected in five replicate patches each of *Arctostaphylos* and *Adenostoma* vegetation in December 1996. For each replicate, four 2.5 cm diameter × 20 cm deep soil cores were pooled. The soil samples were air-dried for 72 h in a ventilated 60°C oven and passed through a 2-mm soil sieve. The samples were then sent to the DANR (Division of Agriculture and Natural Resources) Analytical Laboratory, University of California, Davis, for analysis. The soils were analyzed for pH, NH_4-N, NO_3-N, Bray-P, exchangeable K, exchangeable Ca, exchangeable Mg, and total Ni and Cr.

Ectomycorrhizal sporocarps

Sporocarp sampling occurred from 1989 through 1996 while conducting various experiments in the area. Sampling was not systematic. From January to July 1992, patches that contained planted *Pseudotsuga* seedlings were sampled weekly. In this area, the peak fruiting season is January through April, and this period was surveyed for sporocarps more intensely throughout the study.

Field bioassay 1

This bioassay was conducted to investigate if fungi present as mycorrhizae on *Arctostaphylos* would colonize *Pseudotsuga* seedlings during their first year of growth. In February 1996, three 25-m transects were laid out in one *Arctostaphylos* patch. Transects were about 10 m apart and generally parallel except for the difficulty of maneuvering in the chaparral. No *Pseudotsuga* trees were closer than 100 m from any point along the transects. Every 5 m along the transects, 15 seeds were planted in four 10 cm diameter circles. The diameter of the circles was chosen to facilitate harvest with a 10 cm diameter soil corer. Seeds were otherwise treated and sown as described under seedling survival experiment. In November 1996, seedlings from the circle that had the greatest survival at points along the transect were harvested by driving a 10 cm diameter soil corer down 40 cm. Of the five possible points along each transect, the four with the greatest seedling survival were selected for harvest. This was necessary as two of the transects had one point in which all seedlings had died; on the third transect, the point with the fewest surviving seedlings was thrown out. In total, 12 soil cores were taken, bagged in the field, and brought back to laboratory for processing. All *Pseudotsuga* and *Arctostaphylos* roots were collected from the soil cores for this experiment. Some *Quercus* ectomycorrhizae were later identified using molecular methods (see section on Identification of fungi and plants from mycorrhizae); these data are not included.

Field bioassay 2

This bioassay was conducted to investigate if there was a difference between *Arctostaphylos* and *Adenostoma* patches in terms of the presence of ectomycorrhizal species capable of colonizing *Pseudotsuga* seedlings. It was known that all *Pseudotsuga* seedlings would likely die in the *Adenostoma* plots by the end of the summer (Horton 1992), so these seedlings were harvested after 4 months of growth. In February 1996, *Pseudotsuga* seed was planted in one patch each of *Arctostaphylos* and *Adenostoma* vegetation. No *Pseudotsuga* trees were closer than 100 m from any plots. Seeds were treated and sown as described for the seedling survival experiment. Seed was planted in five plots in each vegetation type, and 15 seeds were sown in each plot within a 10 cm diameter circle. In June 1996, seedlings were harvested from all plots to assess ectomycorrhizal colonization. Only ectomycorrhizae attached to the seedlings were collected for this experiment. We use the plot cores as the experimental unit and did not quantify ectomycorrhizal colonization for each seedling.

Processing of soil cores and seedlings

The soil cores were individually soaked overnight in water. *Pseudotsuga* seedlings were carefully removed and their ectomycorrhizae excised. Mycorrhizae in the remaining soils (Field bioassay 1) were collected by rinsing the soils through a No. 35 soil sieve (0.5 mm mesh size) before sorting by morphology under a dissecting microscope.

Most *Pseudotsuga* ectomycorrhizae were attached to the seedlings and plant identification was obvious. Mycorrhizae from the bulk soil were first sorted based on their distinct branching morphologies (robust–pinnate and fine–arbutoid, *Pseudotsuga* and *Arctostaphylos*, respectively). The plant species of unbranched, single root tips were later identified using molecular methods. Criteria for sorting mycorrhizas into fungal species included color, features of the mantle such as cystidia, branching pattern, and characteristics of rhizomorphs following Agerer (1994). One mycorrhizal root tip from each morphotype was sectioned to confirm the presence of a Hartig net. Mycorrhizal root samples were lyophilized in preparation for biomass measurements, molecular analyses, and storage. All samples were processed through the lyophilization step within 2 weeks after removal from the field. To quantify the mycorrhizas we measured the dry weight of each morphotype for each core and each plant species. We intentionally split the mycorrhizae from each core into more categories than was necessary and combined the biomass of like types only after molecular identifications were complete. This way, we avoided combining different species with similar morphotypes. This was particularly important for morphotypes within a family group (Russulaceae, Thelephoraceae, etc.), which often had subtle differences that were quickly recognized only after experience or considerable analysis of morphology. This approach allowed for a rapid sorting of root tips so as to preserve DNA for the molecular identification of the symbionts.

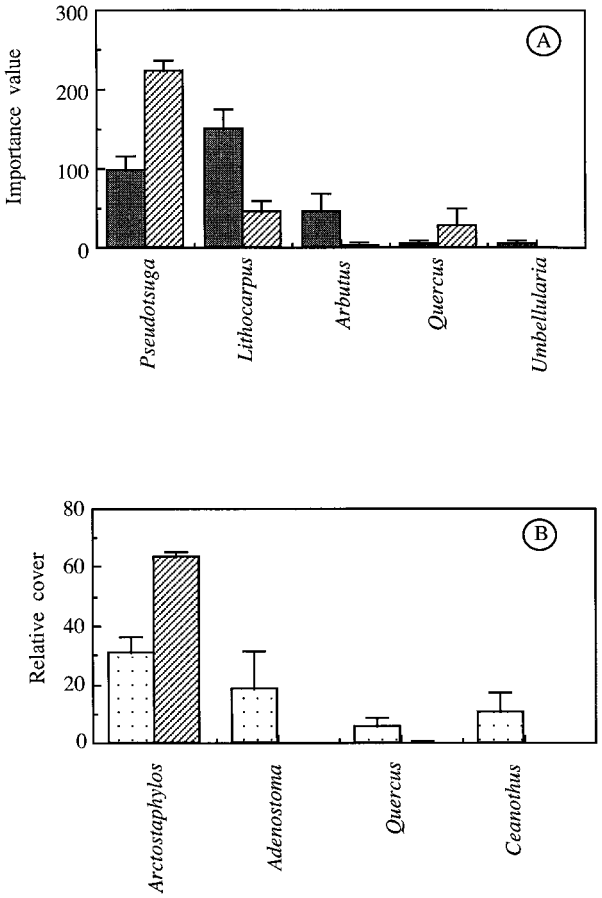
Identification of fungi and plants from mycorrhizae

DNA was extracted individually from one to three root tips as described in Gardes and Bruns (1993). Several tips were extracted together only when part of a single clump. When available, two replicates from each morphotype in each core were extracted. DNA was also extracted from small pieces of voucher sporocarps (from the hymenium) by the same method.

Species level characterizations of the ectomycorrhizal fungal symbionts were based on PCR amplification of the internal transcribed spacer (ITS) region of the rRNA gene using ITS-1F and ITS-4B, or ITS-1F and ITS-4 as primer pairs (White et al. 1990; Gardes and Bruns 1993). Both primer pairs preferentially amplify specific fragments of fungal DNA from mixtures of plant and fungal DNA. We used reagents, protocols, and cycling parameters described previously (Gardes and Bruns 1996). We characterized the ITS region by restriction fragment length polymorphism (RFLP) analysis, which was used to match mycorrhizae to one another and to sporocarps of voucher collections. Species-level identification was determined by identical RFLP matches with digests of three enzymes, *Alu*I, and *Hin*fI, and *Dpn*II. The plant-specific primer pair 28KJ and TW14 was used to amplify a portion of the 28S gene in the nuclear rRNA repeat from the mycorrhizal root extracts (Cullings 1992). The two plant species can be unambiguously differentiated with RFLP patterns generated when this region is digested with the restriction enzyme *Dpn*II.

Basidiomycete family identification from morphotypes whose fungal RFLPs were not matched to sporocarps was conducted using a working version of the mitochondrial large subunit rRNA gene database (Bruns et al. 1998) (PCR amplification with primer pair ML-5 and ML-6). Determination of the fungal division (Ascomycota or Basidiomycota) was accomplished with a database of the 5.8S nuclear rRNA gene (Cullings and Vogler 1998). Sequencing was done by the cyclic reaction termination method using fluorescence labeled dideoxyribonucleotide triphosphates. The processing of samples for sequencing were performed following the instructions for the sequencing kit (PRISM Ready Reaction Diedioxy Terminator Cycle sequencing Kit, Perkin-Elmer Corp.). Electrophoresis and data collection were done on an ABI Model 377 DNA sequencer (Perkin-Elmer Corp.). DNA sequencing Analysis (version 2.01) and Sequence Navigator software were used to process the raw data. Sequences were aligned by visual estimation using matrices created in PAUP 3.1.1 (Swofford 1993). Identifications were based on phylogenetic analysis with PAUP 3.1.1 and the heuristic search option. If no ITS–RFLP match was found for basidiomycetes identified to family, we labeled the fungus by its

Fig. 2. Mean importance value (IV) and relative cover for tree and shrub species, respectively. (A) Tree species found in the forest and mixed zones (mean ± SE, n = 6). IV = relative density + basal area + frequency of each species. The forest zone consists of a number of tree species. (B) Shrub species in chaparral and mixed zones (mean ± SE, n = 6). The chaparral zone consists of several species. All chaparral species in the mixed zone were dead. The mixed zone consists primarily of dead *Arctostaphylos* with an overstory of *Pseudotsuga*. □, forest zone; ▨, mixed zone; ⁘, chaparral zone.



family group name after replacing the ending with -oid followed by a number for that group (i.e., if the first unknown ITS–RFLP type is in the Russulaceae it becomes russuloid 1; if the fifth unknown ITS–RFLP type is a member of the Thelephoraceae, it becomes thelephoroid 5). Agaricoid refers to fungal sequences whose placement in the database was in an unresolved region that includes members of the Cortinariaceae, Tricholomataceae, Nematolomaceae, and Strophariaceae.

Statistical analyses

Percentages were arcsine transformed for statistical analyses and back transformed for graphical presentation. Student's t tests were used to test for differences between vegetation types in temperature and light. ANOVA was used to test for differences in allelopathic inhibition of *Arctostaphylos* and *Adenostoma* leaf leachates and sterile water on *Pseudotsuga* root growth; Petri dish means were used as replicates. Soil pH and nutrient level data deviated from a normal distribution (Kolmogorov–Smirnoff test for goodness of fit) and the Mann–Whitney U test was used to test for differences between *Arctostaphylos* and *Adenostoma* for these data. Statistical tests were conducted with a critical value of $\alpha = 0.05$.

Fig. 3. *Pseudotsuga* seedling survival in the chaparral zone for the 1992 field season. Seedlings were planted in either *Arctostaphylos* or *Adenostoma* patches. Data represent post-germination survival of planted seeds (mean ± SE, n = 3). The area without shading represents the summer drought period. □, *Archtostaphylos* patches; ■, *Adenostoma* patches.

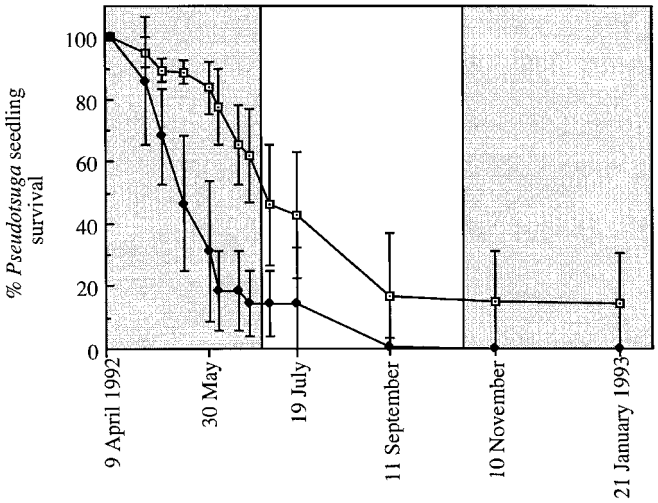


Table 1. Weekly high and low temperatures (°C) for *Arctostaphylos* and *Adenostoma* patches from May 3 through July 19, 1992 (n = 7).

		Range		
		Lowest	Highest	Mean ± SE
Arctostaphylos	Low	7.5	12.0	9.6±0.5*a*
	High	29.0	35.0	31.9±0.7*b*
Adenostoma	Low	8.0	13.5	9.8±0.7*a*
	High	28.0	36.5	32.6±1.0*b*

Note: Values in each temperature category followed by the same letter are not significantly different.

Results

Vegetation survey and seedling survival

Pseudotsuga menziesii was codominant with *Lithocarpus* and *Arbutus* in the forest zone, but was dominant in the mixed zone (Fig. 2A). In the chaparral zone, *Arctostaphylos* shared dominance with *Adenostoma*, *Ceanothus*, and *Quercus* (Fig. 2B). The live chaparral was essentially a mosaic of adjacent patches dominated by either *Arctostaphylos* or *Adenostoma*. However, in patches where *Pseudotsuga* was established (mixed zone), the dead understory of chaparral was dominated by *Arctostaphylos* (Fig. 2B). Mortality of experimental seedlings had slowed considerably in *Arctostaphylos* patches at least a month before the summer drought ended, but all seedlings died in *Adenostoma* patches (Fig. 3).

Environmental conditions

There were with no differences between *Arctostaphylos* and *Adenostoma* for weekly high and low temperature ex-

Table 2. Soil pH and nutrient levels in *Arctostaphylos* and *Adenostoma* patches (n = 5 patches, 4 pooled samples each).

Soil parameter	Mean (SE) *Arctostaphylos*	Mean (SE) *Adenostoma*	Mann–Whitney P value
pH	4.5 (0.1)	5.0 (0.1)	0.02
NH_4^+ (ppm)	22.6 (3.2)	30.2 (5.6)	0.40
NO_3^- (ppm)	1.2 (0.2)	1.2 (0.7)	0.53
Bray-P (ppm)	158.8 (39.0)	61.2 (13.1)	0.12
Exchangeable K (mequiv./100 g)	0.62 (0.12)	0.54 (0.1)	0.75
Exchangeable Ca (mequiv./100 g)	3.32 (1.01)	7.94 (1.45)	0.08
Exchangeable Mg (mequiv./100 g)	2.6 (0.2)	9.4 (0.9)	<0.01
Total Cr (ppm)	236.8 (66.1)	458.4 (77.5)	0.05
Total Ni (ppm)	217.0 (25.2)	469.4 (63.9)	0.02

Table 3. Fungal fruit bodies collected beneath patches of *Arctostaphylos* and *Adenostoma*.

Species and author	Voucher collection No.
Arctostaphylos	
Amanita gemmata (Fr.) Bertillon[a,b]	trh 122
Cortinarius sp. 1	trh 116
Inocybe geophila (Sow. ex Fr.) Kummer	trh 244
Inocybe whitei s.l. (B. & Br.) Sacc.	trh 246
Laccaria amythesteo-occidentalis Mueller[d]	trh 127
Lactarius loculentus Burlingham	trh 253
Lactarius xanthogalactus Peck	trh 333
Leccinum manzanitae Thiers[c]	trh 68
Melanogaster ambiguus (Vitt.) Tulasne[d]	trh 22
Russula cremoricolor Earle	trh 66
Russula silvicola Shaffer	trh 70
Russula splacita s.l. Burlingham	hdt 54451
Russula brevipes Peck	trh 237
Tricholoma flavovirens (Pers. Ex Fr.)	trh 132
Tricholoma manzanitae Baroni & Ovrebo	trh 197
Tricholoma saponaceum (Fr.) Kummer	trh 245
Xerocomus zelleri Murr.	trh 234
Xerocomus spadiceus Fr.	trh 235
Adenostoma	
Cortinarius sp. 2	trh 338
Laccaria amethysteo-occidentalis[d]	trh 172
Lactarius loculentus	trh 179
Lactarius xanthogalactus	trh 174
Rhizopogon mengei Allen, Trappe, & Horton (sp.nov.)	trh 201

Note: Footnotes show literature that identifies a species can associate with both *Pseudotsuga* and *Arctostaphylos* or otherwise has a broad host range. ITS–RFLP patterns of all species were produced for comparison to ectomycorrhizal samples.

[a]Trappe 1962.
[b]Largent et al. 1980.
[c]Molina and Trappe 1982*a*.
[d]Molina et al. 1992.

tremes (Table 1). Although the canopy of *Arctostaphylos* tended to reduce the amount of light reaching the ground more than that of *Adenostoma* (28.5 ± 2.5 and 34.9 ± 2.2% full sunlight, respectively; n = 27, mean ± SE), no significant difference was detected (P > 0.10).

Allelopathy

None of the treatments in the allelopathy experiment (leachates of *Arctostaphylos*, *Adenostoma*, or the water control) were significantly different in their effect on radical growth (mm) of *Pseudotsuga* (P > 0.10). Leachate treatments did not affect the germination of *Pseudotsuga menziesii*.

Soil nutrients

There was no difference detected between the two vegetation types for soil ammonium, nitrate, phosphate, and K (Table 2), although the variance in phosphate was quite high in the soils. The soils in *Arctostaphylos* patches were more acidic than those in *Adenostoma* patches. There were lower levels of Ca, Mg, Cr, and Ni in the *Arctostaphylos* soils than in the *Adenostoma* soils.

Ectomycorrhizal sporocarps

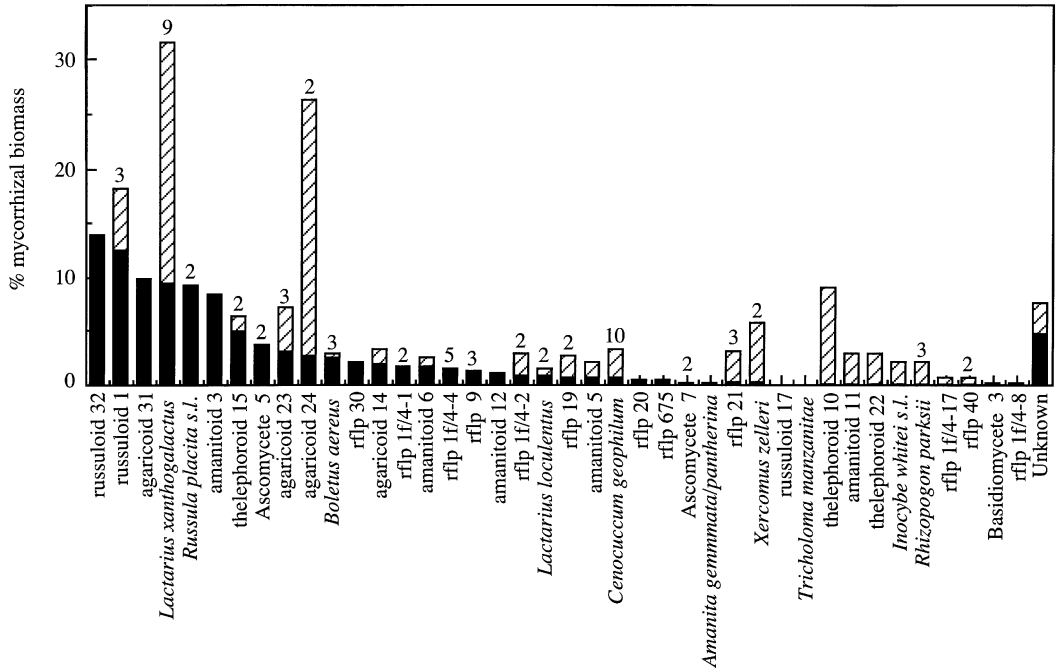
Sporocarps of more species of ectomycorrhizal fungi were collected beneath *Arctostaphylos* than *Adenostoma* (Table 3). Fewer fruit bodies occurred in the *Adenostoma* than in the *Arctostaphylos* (data not shown).

Field bioassay 1

We identified a total of 40 mycorrhizal fungi associated with either *Arctostaphylos*, *Pseudotsuga*, or both (Fig. 4, Table 4). All cores contained mycorrhizae of both plant species. Of the 31 ITS–RFLP types associated with *Arctostaphylos*, 17 were also associated with *Pseudotsuga*. Nine species were unique to Pseudotsuga. Members of the Russulaceae were frequent and abundant on the roots of both *Arctostaphylos* and *Pseudotsuga*. *Lactarius xanthogalactus* Peck was a frequent and abundant ectomycorrhizal type on *Pseudotsuga*. Agaricoid 24 was relatively abundant on *Pseudotsuga* but occurred in only two cores. Some fungi appeared specific to *Arctostaphylos*, abundantly colonizing only that plant when they occurred in a core: russuloid 32, agaricoid 31, *Russula placita* s.l., amanitoid 3, and ascomycete 5.

Fifty-six of the 66 *Pseudotsuga* seedlings (85%) that survived to the harvest date were colonized by fungi that also colonized *Arctostaphylos* in the same core. Forty-nine percent of the total *Pseudotsuga* ectomycorrhizal biomass was colonized by fungi that colonized *Arctostaphylos* within the same core, and another 23% was colonized by fungi known

Fig. 4. Mean percentage of ectomycorrhizal biomass each fungus species contributed to each plant's total. Fungal species are arranged according to *Arctostaphylos* abundance except those that occurred only on *Pseudotsuga*. *Pseudotsuga* associated with 17 of the 35 fungi that were observed on the *Arctostaphylos* roots. Ascomycete 5 and Ascomycete 7 colonized *Pseudotsuga* in minor amounts. Values above bars represent the number of cores (from a total of 12) in which that fungus occurred if greater than one. ▨, *Pseudotsuga*; ■, *Arctostaphylos*.



to colonize *Arctostaphylos*, but from a different core. Only 27% of the total *Pseudotsuga* ectomycorrhizal biomass was colonized by fungi that were unique to *Pseudotsuga*.

Field bioassay 2

We harvested the *Pseudotsuga* seedlings for this experiment in early June and some seedlings were already showing signs of stress in the *Adenostoma*. Six ectomycorrhizal species in at least four families colonized the seedlings in *Arctostaphylos*: *Amanita gemmata* (Fr.) Bertillon or *Amanita pantherina* (DC Per. Fr.) Krombh., amanitoid 5, *Inocybe whitei* (B. & Br.) Sacc., *L. xanthogalactus*, russuloid 1, and thelephoroid 22. Only two species in the Thelephoraceae, thelephoroid 1033 and 1040 (Table 4), colonized seedlings in *Adenostoma*. All of the fungi from the *Arctostaphylos* plots and none of the fungi from the *Adenostoma* plots were observed in field bioassay 1 (compare species in Fig. 4). Active ectomycorrhizal inoculum was detected in four of five *Arctostaphylos* plots but in only two of five *Adenostoma* plots. For the plots in which active inoculum was detected, 69.1 ± 0.06% and 67.0 ± 0.002% of the seedlings were colonized by ectomycorrhizal fungi in the *Arctostaphylos* and *Adenostoma*, respectively.

Discussion

Results from the vegetation survey and seedling survival experiments both support that *Pseudotsuga* seedlings survive in *Arctostaphylos* patches but not in *Adenostoma* patches. Although the seedling survival curves in the two vegetation types were similar, survival was higher in *Arctostaphylos*. These results, along with those of Sparling (1994) strongly suggest that conditions for *Pseudotsuga* establishment are met in the *Arctostaphylos* but not in the *Adenostoma* patches.

There were no differences detected between *Arctostaphylos* and *Adenostoma* in terms of soil surface temperatures, light levels, and allelopathic inhibition. Our allelopathy results agree with Horton (1992) who assessed the allelopathic effect of the same plant species on *Cucumis sativus* L. seeds and with Tinnin and Kirkpatrick (1985), who assessed the allelopathic affects of *Arctostaphylos patula* Greene, *Arctostaphylos viscida* Parry, and *Ceanothus velutinus* Dougl. on *Pseudotsuga menziesii*. Most of the soil nutrients were also not different between the two vegetation types. Although, our sample sizes for the nutrient analyses were low, ammonium and Ca tended to be higher in *Adenostoma* soils, while phosphate tended to be higher in the *Arctostaphylos* soils.

The variables that differed the greatest between *Arctostaphylos* and *Adenostoma* were pH, Mg, Ni, Cr, and presence of ectomycorrhizal fungus inoculum in the soils. *Pseudotsuga* seedlings grow optimally at pH 5.5 but can tolerate a relatively wide range that covers values from both vegetation types (Landis et al. 1994). High levels of Mg, Ni, and Cr indicate a serpentine effect in *Adenostoma* soils (Barbour et al. 1987). White (1971) reported that *Pseudotsuga* does not occur in serpentine soils in Oregon. However, in the Wenatchee mountains of Washington state, *Pseudotsuga* is an important coniferous tree in serpentine

Table 4. Restriction fragment band sizes (base pairs) for mycorrhizal morphotypes.

Fungus species and ITS–RFLP type	*Alu*I	*Hin*fI	*Dpn*II
Agaricoid 14	489/(399)/306/88	336/175/119/100	582/290
Agaricoid 23	689/84	339/300/120	511/203/95
Agaricoid 24	268/211/1941	369/342/253	871/262
Agaricoid 31	274/199/159/141	405/107	350/274/225/144
Amanita gemmata trh 122	410/251/106/91	372/121/98	641/240
Amanitoid 3	768/84	394/337/118	556/262
Amanitoid 5	336/246/212/91	399/358/121/98	565/268
Amanitoid 6	384/(230)/115	349/119	237/200/151/110
Amanitoid 11	406/223/103/87	373/236/129/118	345/240
Amanitoid 12[a]	463/137/86	332/113	543/197
Boletus aereus Fr. trh 282	506/326/86	250/230/178	355/293/234
Cenococcum geophilum[a]	376/183	149/109	312/153
Inocybe whitei s.l. trh 246	606/182	334/304/105	535/293
Lactarius loculentus trh 253	521/375/89	411/258/122/111	335/260/130/110
Lactarius xanthogalactus trh 333	540/142/134/92	415/264/121/110	353/274/135/111
rflp 1f/4-40[a]	246/116/97	382	280/201/192
rflp 1f/4-17[a]	234/225/190	249/175/104	331/222
rflp 1f/4-1[a]	367/292/212	495/205/109	414/194/133/102
rflp 1f/4-2[a]	234/225/190	249/175/104	331/222
rflp 1f/4-4[a]	363/301/223	310/(288)/196/171/120	419/201/135
Ascomycete 5[a,b]	422/196	268/209/131	318/213
Ascomycete 7[a,b]	500/105	316/257	301/201
rflp 1f/4-8[a]	761	316/274/127	423/287
Basidiomycete 3[a,b]	575/92	337/182/127	382/203
rflp 9	458/134/83	323/123	541/190
rflp 19	390/206/95	369/123	269/227/129/99
rflp 20	480/155/85	320/200/125	500/200
rflp 21	385/211/96	362/113	272/227/131/103
rflp 30	500/138/113/86	323/273/82	209/195/138
rflp 675	538/193/89	354/283/112/93	572/257
Rhizopogon parksii Smith trh 125	732/88	235/122/113/80	319/260/244
Russula placita s.l. hdt 54451	510/(370)/274/84	348/238/155/119	625/209
Russuloid 1	515/(347)/261/92	374/125/101	318/205/173/96
Russuloid 17	542/133/92	417/266/114	349/275/137/103
Russuloid 32	520/161/89	361/269/119/94	303/259/200
Thelephoroid 10	459/114/90	362/116	225/194/146
Thelephoroid 15	495/130/104/90	336/241/118/99	371/224/193/146
Thelephoroid 22	462/124/97	369/188/127/103	532/291
Thelephoroid 1033	349/207/101	(566)/380/236/184/102	553/161/141/98
Thelephoroid 1040	365/121/98	384/97	235/197/146
Tricholoma manzanitae trh 197	301/286/118/98/81	378/189/130/119	578/150/96
Xerocomus zelleri trh 234	542/188/162/93	380/247/100	370/293/135

Note: Voucher collections are noted for sporocarps that matched the mycorrhizae. Band sizes in parentheses are submolar, while those that are underlined are double bands of roughly the same size. Sporocarps of *Boletus aerues* and *Rhizopogon parksii* were found in the local forest; authorities for these species are given here.

[a]Fungal DNA was amplified with ITS-1f and ITS-4.

[b]Type was identified as a Basidiomycete or Ascomycete by phylogenetic analysis of the 5.8S gene.

soils (Franklin and Dyrness 1973). Indeed, in our area *Pseudotsuga* establishes frequently in serpentine soils, often associated with the serpentine endemic *Arctostaphylos hookeri* spp. *montana* (Eastw.) P. Wells (Parker 1991). Apparently, serpentine soils do not act as a critical factor controlling *Pseudotsuga* establishment in our study area.

Of the factors investigated here and by Dunn (1994), soil moisture, P, and fungal inoculum best explain *Pseudotsuga* establishment. Decoupling these factors is problematic since mycorrhizal fungi provide plants with greater access to soil moisture and P as well as other nutrients. However, we were particularly interested in whether ectomycorrhizal fungi associated with *Arctostaphylos* but not *Adenostoma* contributed to *Pseudotsuga* establishment and we turn to these data now.

It appears that *Adenostoma* patches did not offer adequate ectomycorrhizal inoculum for *Pseudotsuga* establishment. The second most common plant species in *Adenostoma* patches was *Ceanothus cuneatus*. *Ceanothus* spp. are thought to only associate with arbuscular mycorrhizal fungi

(Rose 1980; Rose and Trappe 1980; Rose and Youngberg 1980). At this point, the mycorrhizal status of *Adenostoma* is unclear. Although intracellular fungal hyphae are usually present, including some labyrinthine hyphal structures, we have never observed clear ectomycorrhizal or arbuscular mycorrhizal morphologies. Still, some sporocarps of ectomycorrhizal fungi were collected in *Adenostoma* patches. These fungi may have been associated with small *Arctostaphylos* or *Quercus* plants that occurred at distances of about 10 m from the sporocarps. *Adenostoma* has been reported to be both an ectomycorrhizal (Cooper 1922) and arbuscular mycorrhizal (Allen 1991) plant. Like other woody plants in the Rosaceae (Smith and Read 1997), *Adenostoma* may be capable of associating with ectomycorrhizal fungi. Even if *Adenostoma* is capable of associating with ectomycorrhizal fungi, we suggest that the ectomycorrhizal condition is not a major aspect of its life history when compared with plants in Ericaceae (*Arbutus*, *Arctostaphylos*), Fagaceae, and Pinaceae.

In contrast to the low ectomycorrhizal potential of *Adenostoma* patches, several lines of evidence show that *Arctostaphylos* patches contain fungi that will colonize *Pseudotsuga* seedlings. Species in most of the genera collected as sporocarps in *Arctostaphylos* patches can associate with *Pseudotsuga* and *Arctostaphylos* spp. (Trappe 1962; Largent et al. 1980; Acsai and Largent 1983; Molina and Trappe 1982*a*; Molina et al. 1992). In this study, many of the species of fungi found on the *Pseudotsuga* seedlings were also associated with *Arctostaphylos* within the same soil volume. We suggest that these fungi connected the two plant hosts when their roots intermingled. Since the seedlings were growing beneath the canopy of the *Arctostaphylos*, the conditions were conducive for carbon flow from *Arctostaphylos* to the seedlings. A similar interaction was reported by Simard et al. (1997) who demonstrated a net transfer of carbon between *Betula papyrifera* and shaded seedlings of *Pseudotsuga menziesii*.

During primary succession, mycorrhizal plant establishment is affected not only by the presence or absence of nonmycotrophic plants but by the dispersal pattern of fungal spores or other propagules (Allen 1988; Gemma and Koske 1990). Dispersal of propagules may occur by wind, water, or animal vectors, which have different consequences on the pattern of plant establishment (Allen 1988). In general, mycorrhiza-dependent plants establish only where available inoculum exists to facilitate survival. In the absence of fungal inoculum, nonmycotrophic plants are more successful than mycorrhizal plants (Janos 1980).

Rather than primary succession, our site is undergoing secondary succession, and mycelial networks are potentially well established within patches of both burl-forming plant species. Spores were probably a source of inoculum for some of the species observed on the seedlings in the *Arctostaphylos* and a likely source for the two thelephoroid species observed in the *Adenostoma* patches. However, the most important source of inoculum was probably the established mycorrhizal networks associated with *Arctostaphylos*. Although the seedlings may have benefited directly from fungal linkages to *Arctostaphylos*, mycelial networks are beneficial to newly establishing seedlings even in the absence of direct linkages and nutrient transfer between plant species. Seedlings that associate with an established fungal mycelium may not need to allocate any resources to produce fungal biomass (Newman 1988). Seedlings in the *Adenostoma* patches may have been colonized by fungi that were themselves attempting to establish and may have required substantial carbon inputs to generate fungal biomass. Seedlings in the *Arctostaphylos* patches were associated with existing fungal networks and survival was greatly increased even though other conditions such as light and nutrients were not particularly favorable.

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