

**PART V**

**ROOTS**

**&**

**SOILS**

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# 16

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## Possible Role of Mycorrhizas in Resistance to Decline in *Arbutus menziesii*

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**Abstract:** A complex set of factors is causing decline of Pacific madrone (*Arbutus menziesii*) in the Puget Sound region; yet, some trees remain healthy. It is unclear why they do. We are investigating the hypothesis that symbiotic associations between the roots of madrone and soil-dwelling fungi make the trees less susceptible to disease. The effect could occur either directly or by providing physical links to nearby Douglas-fir (*Pseudotsuga menziesii*) trees through which carbon compounds are transferred to the mycorrhizal systems and, perhaps ultimately, to the madrones. In this initial study, our objectives are to determine whether: 1) madrones growing near Douglas-firs are healthier than madrones not growing near Douglas-firs; 2) healthy madrones have more and/or better developed mycorrhizal associations than declining madrones; and, 3) madrones associate with the same mycorrhizal fungi as nearby Douglas-firs.

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In the Puget Sound region, many Pacific madrone (*Arbutus menziesii*) trees are dying or in poor health. Usually health of the the affected trees declines over an extended period before they die, commonly from root-rot fungi such as *Armillaria*, heart-rot fungi, and wood-boring insects. Environmental stresses from a variety of causes such as over-watering, drought, high or low soil fertility and growing in exposed situations cause the trees to have low resistance to infections by fungi such as *Nattrassia mangiferae* (the cause of *Arbutus* canker) and *Fusicoccum aesculi* (the cause of branch dieback). Infection by these fungi reduces tree resistance and allows the root-rot fungi, heart-rot fungi and insects to become established. In general, reduced resistance seems to be associated with reduced capacity for photosynthesis (Elliott 1999). Despite the widespread occurrence of decline,

many madrones still appear healthy. This could be because they: 1) are genetically resistant to the disease organisms; 2) randomly escape infection; or, 3) grow with other organisms that help them to resist infection. In this study we are investigating aspects of the third point. In a related set of studies we are investigating the possible relationship between surrounding vegetation and madrone decline and the effect of nitrogen fertilization on disease resistance.

The roots of most plants form symbiotic associations with certain soil-dwelling fungi, and many plants cannot survive in nature without them. These associations are termed mycorrhiza (Greek for fungus and root). In most cases, mycorrhizal fungi greatly increase a plant's access to soil nutrients such as nitrogen and phosphorus. In return, these fungi receive photosynthate (carbon compounds produced in photosynthesis) from the plant. Many mycorrhizal fungi also provide plants with increased resistance to drought, soil acidity, heavy metals, and pathogens (Smith and Read 1997). In addition to such direct effects of mycorrhizal fungi on host plants, Simard, *et al.* (1997) showed that networks of mycorrhizal hyphae (the tube-like vegetative portions of a fungus) provide a system via which photosynthate can be moved from one tree to another. These sorts of effects of mycorrhizal associations could help explain the resistance of some madrones to decline.

The type of mycorrhiza formed by madrones is known as arbutoid. The fungi involved include many common woodland mushrooms (Plate 17-1). In arbutoid mycorrhizas, the fungal hyphae form sheaths around the root tips and also enter the cells of the roots in which they form spaghetti-like coils. Many other Pacific Northwest forest trees, including Douglas-fir (*Pseudotsuga menziesii*), western hemlock (*Tsuga heterophylla*), spruces (*Picea sitchensis* and *P. engelmannii*) and firs (*Abies* spp.) form a type of mycorrhiza called ectomycorrhiza. In these associations, the fungus forms a sheath around the fine roots just as it does in arbutoid mycorrhizas; however, unlike arbutoid mycorrhizas the fungal hyphae do not enter the cells of the root. Instead, they form a network surrounding the individual cells, much like the mortar in a brick wall. Many of the fungi that form arbutoid mycorrhizas also can form ectomycorrhizas with trees such as Douglas-fir (Molina and Trappe 1982). Arbutoid mycorrhizal and ectomycorrhizal roots tend to be short and stubby compared to non-mycorrhizal roots (Plates 17-2a and b). A third type of mycorrhiza, which is the most common type world-wide, is the arbuscular (or vesicular-arbuscular) mycorrhiza. In these associations, no fungal sheath is formed and the fungal hyphae



Plate 16-1 (above). Fruiting bodies (mushrooms) of *Amanita lanei* (also known as *A. calyprata*), a fungus which forms mycorrhizas with Pacific madrone. Magnification ~0.5X

Plate 16-2a (page 130, top). Mycorrhizal root tips of Pacific madrone showing the external morphology and fungal sheath. Note the similarity in morphology with the Douglas-fir mycorrhiza shown in Plate 16-2b. Magnification ~17X.

Plate 16-2b (page 130, bottom). Mycorrhizal root tips of Douglas-fir showing the external morphology and fungal sheath. Note the similarity in morphology with the madrone mycorrhiza shown in Plate 16-2a. Magnification ~17X.



enter the root cells, forming a variety of structures. The fungi that form arbuscular mycorrhizas are very different from those that form arbutoid mycorrhizas and ectomycorrhizas. Western redcedar (*Thuja plicata*) is the most common Pacific Northwest forest tree that forms arbuscular mycorrhizas.

It is possible that trees with decreased photosynthetic capacity are less able to support mycorrhizal fungi on their roots, thus decreasing their ability to obtain nutrients and protect themselves against pathogens and drought stress. Mycorrhizal connections with nearby trees such as Douglas-fir might provide a means by which madrones acquire additional photosynthate to support the mycorrhizal fungi as well as their own necessary physiologic functions. Simard *et al.* (1997) showed in British Columbia that carbon compounds could flow in both directions between Douglas-fir seedlings and birch seedlings planted adjacent to them. In this case shading one set of seedlings (in which case overall carbon flow was from the well-lit seedlings to those in the shade) influences the direction of net flow. Perhaps madrone and Douglas-fir can form a similar system in which carbon flows from the Douglas-firs to madrones allowing the madrones to be more resistant to disease.

The objectives of our study are to determine if: 1) madrones growing near Douglas-firs are healthier than madrones not growing near Douglas-firs; 2) healthy madrones have more and/or better developed mycorrhizal associations than declining madrones; and, 3) madrones associate with the same mycorrhizal fungi as nearby Douglas-firs.

## METHODS

Objective 1: In a separate study, we are documenting the vegetation (including Douglas-fir trees) surrounding 20 selected madrones. The madrones represent 4 experimental situations (with 5 trees in each): 1) healthy madrones growing near Douglas-firs; 2) unhealthy madrones growing near Douglas-firs; 3) healthy madrones not growing near Douglas-firs; and, 4) unhealthy madrones not growing near Douglas-firs. At each site we are quantifying madrone health and abundance of nearby Douglas-fir so that the hypothesis that madrones growing near Douglas-firs are healthier can be tested statistically.

Objective 2: We are collecting soil samples (including madrone roots) at each site. In the lab the samples are washed carefully to expose and separate out the fine roots. The roots are examined under a microscope to determine whether or not they are mycorrhizal. The

abundance of mycorrhizal roots in each sample is quantified in order to test the hypothesis that healthy madrones have more and better developed mycorrhizas than unhealthy madrones.

Objective 3: In the 10 plots with Douglas-firs, soil samples containing Douglas-fir roots are collected at the same time as the madrone root samples. Many of these samples contain roots of both madrone and Douglas-fir. After the fine roots are separated from the soil, they are examined under a microscope. The morphological features of any mycorrhizas present are described. Because different species of fungi form different-looking mycorrhizas, comparison of madrone and Douglas-fir mycorrhizas will provide us with one means to determine whether the 2 tree species share any of the same species of mycorrhizal fungi.

A second means we are using to determine whether madrones and Douglas-firs share the same species of mycorrhizal fungi is to compare the deoxyribonucleic acid (DNA) of the fungi. First, DNA is extracted from the fungal sheaths on mycorrhizal roots. Although only tiny amounts of DNA are obtained with this extraction, the extracts are amplified many times over using the polymerase chain-reaction (PCR) process. We then subject DNA from each sample to chemical procedures that fragment it. Each fungus's DNA yields a unique collection of fragments of different lengths; therefore, by comparing all of the patterns obtained from the root samples, we can determine whether the same fungi are associating with both madrone and Douglas-fir. In some cases we then can identify the fungi involved by matching these patterns with patterns obtained from mushrooms produced by mycorrhizal fungi, since patterns obtained from either root or mushroom fungal samples are the same if they are from the same species. At this initial stage, however, finding that the same species of fungi form mycorrhizas with both tree species is sufficient to suggest that the trees are connected via these mycorrhizal fungi whether or not we know their identity.

## **RESULTS**

The study is in its early stages and there are few results to report. It does appear, however, that: 1) the madrones in all of the study plots are surrounded predominantly by plants that do not form arbutoid mycorrhizas or ectomycorrhizas; 2) madrones growing near Douglas-firs associate with a greater diversity of mycorrhizal fungi than madrones not near Douglas-firs; and, 3) madrones and Douglas-firs living near each other do associate with some of the same mycorrhizal fungi.

We note, however, that the latter 2 findings are based on morphological characteristics only and are not yet supported by comparing DNA patterns.

## DISCUSSION

Plant ecologists have devoted much effort to studying the competition between plants for light, moisture and soil nutrients; however, other types of interaction must also be taken into consideration when attempting to determine how a plant community is functioning. Mycorrhizal associations and their potential for allowing cooperation among plants and with other organisms is one example of such an interaction. It is possible that plants with compatible mycorrhizas benefit each other either by transferring materials directly via their shared mycorrhizal links, or by some subsidizing the activities of others by providing a disproportionately large share of the resources needed to support the shared mycorrhizal network. Plants with non-compatible mycorrhizas (or no mycorrhizas) could not take part in such relationships.

It is possible, therefore, that madrones that grow in the midst of plants that do not form arbutoid mycorrhizas or ectomycorrhizas may be subjected to greater competitive interactions than those that grow near ectomycorrhizal plants such as Douglas-fir. Competition would reduce their ability to resist disease and the lack of compatible mycorrhizal plants would prevent them from realizing the possible benefits associated with mycorrhizal networks. Our study will provide information to assess whether the mycorrhizal status of the surrounding plants correlates with madrone health, whether the presence of Douglas-fir correlates with madrone health and whether the same mycorrhizal fungi are present in both madrone and Douglas-fir mycorrhizas.

If madrone and Douglas-fir are sharing the same fungal species, it allows the possibility (but does not prove) that photosynthate could be transferred from Douglas-fir to madrone through the mycorrhizal fungal network. This might prove important in sustaining madrone seedlings until they grow large enough to access sufficient light and other resources for photosynthesis. In addition, carbon sharing by trees in mixed forests might help to strengthen madrone trees during periodic times of stress (such as seasonal drought), allowing the trees to bolster their chemical defenses and reduce the likelihood of successful attack by fungi and other pathogens.



By evaluating the mycorrhizal status of madrone trees with and without Douglas-fir nearby, we also are examining the influence of Douglas-fir on madrone mycorrhizal diversity. Because it has been suggested that a large number of species of fungi can form mycorrhizas with Douglas-fir (perhaps 2000 or more), its presence could be especially important for maintaining a rich source of mycorrhizal inoculum in the soil. Because rapid inoculation could be critical for madrone seedling establishment, especially in disturbed soils, mixed planting of madrones and Douglas-firs might represent a means of reducing the regional decline of the madrones.

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