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Frankia in decaying fallen trees devoid of actinorhizal hosts and soil

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Abstract

Fallen trees, recruited by natural catastrophic events, are abundant on the forest floor of many natural forests. They pass through recognizable classes of decomposition, forming a major structural diversity with many ecological functions, and providing habitats for plants, animals, and microorganisms. In greenhouse studies, wood samples collected from within fallen trees decades-old, partially decomposed under both conifer and red alder stands induced effective nodule formation in *Alnus rubra,* indicating that the fallen trees, devoid of soil and actinorhizal host roots, contained infectious and effective *Frankia.* Nodule development, growth, and nitrogen fixation were greatest in wood from fallen trees in the alder stand. *Frankia* is apparently able to live saprophytically or exists in spores in partially decomposed wood.

Key words: *Frankia* – fallen trees – actinorhiza – decaying logs

Introduction

Dead, fallen trees, generated by natural catastrophic events, such as windstorm, fires, insects, diseases, suppression, and competition, are conspicuous features of the forest floor in forest ecosystems. They decay continuously, going through different stages of decomposition, and provide diverse structural habitats for plants, animals, and a variety of microorganisms, including asymbiotic nitrogen-fixing bacteria (Crawford *et al.* 1997; Larsen *et al.* 1978; Spano *et al.* 1982; Maser and Trappe 1984; Harmon *et al.* 1986). Maser and Trappe (1984) described five decay classes. The system was based on the characteristics of twigs, texture, shape and color of wood, position of logs on ground, and presence of invading roots. Tree species such as Sitka spruce (*Picea sitchensi* (Bong.) Carr.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), western redcedar (*Thuja plicata* Donn), and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) commonly grow on fallen trees; the actinorhizal red alder (*Alnus rubra* Bong.), common stands in the Coast Ranges of Oregon, however, rarely occurs on the dead, fallen trees (Harmon *et al.* 1986; Harmon, personal communication).

Frankia is a sporulating actinomycete that forms symbiotic nitrogen-fixing root nodules with twenty-four dicotyledonous plant genera, including alders (Baker and Schwintzer 1990). It is commonly found in soils devoid of actinorhizal hosts for decades, as evidenced by nodulation of host plants by these soils (Burleigh and Dawson 1994; Paschke and Dawson 1992; Smolander and Sarsa 1990), suggesting that *Frankia* can grow saprophytically in soil. However, some soils contain no *Frankia* (Akkermans and Houwers, 1979); low pH also reduces or inhibits nodulation (Ferguson and Bond 1953; Wheeler *et al.* 1981; Griffiths and McCormick 1984; Smolander *et al.* 1988).

In this paper, we present evidence that fallen trees in decay class III, devoid of actinorhizal host plants and lying on the forest floor after decades of decay, contain infectious *Frankia* populations.

Materials and methods

Wood substrate. Wood samples were collected from within downed, fallen trees of decay class III, 25 to 30 cm in diameter, located at two sites in the Oregon

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Coast Ranges. One site was located at 547 m elevation along the Woods Creek Road on Mary's Peak about 24 km southwest of Philomath. This old-growth stand consists of large 300 to 500 year old conifers, including western hemlock and several other tree species in the overstory and broad-leaved plant species in the understory. Another site was located on a harvested area where 30 to 60 year old red alders have been established. Ten dead, fallen trees from each site were randomly selected. Woody samples, 450 cm^3, were removed from within fallen trees of decay class III, which was characterized by presence of trace bark, absence of twigs, and round shape with large texture; tree was sagging near ground; wood was original color to faded; nonactinorhizal plant roots were invading in sapwood (Maser and Trappe 1984). Fallen trees with decay classes I, II, IV, and V were not selected for sampling. Wood in decay classes IV and V might have soil contamination because of advance decay; access to the interior of fallen trees with decay classes I and II was difficult because of their compact and hard texture. The wood samples collected in sterile bags were pulverized with a hammer, and the resulting samples were each passed through a sterile 2 mm screen.

Growth media and conditions. The potting substrate consisted of equal volumes of vermiculite and sphagnum peat moss and was pasteurized for 4 h with 90 °C steam. Leach tube containers, 165 ml capacity, were filled with the potting substrate and pulverized wood (at 1 : 1 ratio). Seeds of red alder were surface-sterilized in 30% hydrogen peroxide for 20 min and washed with sterile distilled water, and five seeds were sown in each leach tube. Seedlings were later thinned to one per tube. Seedlings in tubes containing a mixture of potting substrate and autoclaved woody samples served as control. Thus, the experiment consisted of four treatments: (1) 10 tubes from the conifer site, with one tube for each fallen tree, (2) 10 tubes from the alder-occupied harvested site, with one tube for each fallen trees, (3) 10 tubes from the conifer site, with one tube for each autoclaved woody material from fallen trees, and (4) 10 tubes from the alder-occupied harvested sites, with one tube for each autoclaved woody material from fallen trees. Seedlings were fertilized biweekly with N-free mineral solution (Pregent and Camire 1985) and grown for 4 mon in a greenhouse at 19 to 22 °C. The natural light was supplemented with sodium-vapor lamps at 11,000 Lx for a 16 h photoperiod.

Nitrogen fixation and growth assays. At the end of the experiment, 10 seedlings from each treatment were removed and placed in tubes for measuring nitrogen fixation by the acetylene reduction method. The top of the tube was sealed with a rubber stopper; 10% of the gas phase of the tube were replaced with acetylene, and the tubes were incubated for 2 h. Gaseous samples were withdrawn and analyzed for ethylene and acetylene on a Hewlett-Packard gas chromatograph equipped with a flame ionization detector and a 2.0 m × 2.1 mm column packed with Porapak R on 80 to 100 mesh chromosorb W. Stem height and caliper and dry weight of shoots, roots, and nodules also were measured.

Statistical analysis. Data analysis using t-test was performed (SAS Institute, Inc., Cary, North Carolina).

Results and discussion

Red alder grew and produced nitrogen-fixing nodules in a wood substrate of decay class III from fallen trees collected from both the conifer and the red alder stands. However, the wood substrate from the red alder stand had significantly more nodule growth, or nitrogen fixation than the wood substrate from the conifer stand, even though the pH values of the wood substrates from both stands were relatively similar (Table 1), suggesting that *Frankia* can occur and infect hosts in extremely acidic conditions. Red alder on sterilized wood substrates grew poorly and did not nodulate. Establishment of red alder on dead, fallen trees in forests of Oregon Coast Ranges rarely occurs. Decaying fallen trees contain many terrestrial invertebrates, vertebrates, and decay organisms (Harmon *et al.* 1986), and red alder seeds can be subjected to heavy losses from some of these organisms (Haeussler *et al.* 1995). The low rate of red alder emergence on dead, fallen trees in the forest understories also may be due to inhibition by light intensity and the transmission of predominately far-red wavelengths and because of rapid fluctuations in moisture content of downed, decaying trees (Maser and Trappe 1984). Moist condition with little fluctuations in temperature and evaporation are necessary for the successful establishment of red alder (Haeussler *et al.* 1995). In the harvested site, red alder seedlings on downed, decaying trees could be subjected to losses from heat and drought. They are also susceptible to smothering by debris on dead, fallen trees (Hauessler *et al.* 1995).

The presence of infective *Frankia* in dead, fallen trees lacking an actinorhizal host and soil suggests that *Frankia* occurs, if not live, as spores within partially decomposed fallen trees. We suggest that many terrestrial vertebrates in decaying fallen trees, using wood as food, protection, breeding, and reproduction, could be sources of biological vectors for dispersal of *Frankia* from soils to downed, decaying trees and be responsible for the presence of *Frankia* within the fallen trees. Birds (Paschke and Dawson 1992, 1993; Burleight and Dawson 1995; Turner and Vitousek 1987), earthworms (Reddell and Spain 1991; Paschke 1993), and possibly

Table 1. Growth and nitrogen fixation of *Alnus rubra* seedlings on wood substrates of decay class III fallen trees of conifer and red alder stands

Location of wood	Shoot height (cm)	Stem caliper (mm)	Shoot weight (mg)	Root weight (mg)	Nodule weight (mg)	Nitrogen fixation (µmoles C_2H_4 formed/plant/ 2 hr)	pH
Wood under *A. rubra*	13.9 a *(0.89)	2.0 a (0.18)	498.9 a (64.48)	213.6 a (31.50)	39.5 a (8.74)	1096.9 a (0.26)	3.6
Wood under conifers	6.3 b (0.52)	0.8 b (0.11)	181.2 b (33.00)	88.5 b (17.40)	9.5 b (1.33)	491.8 b (0.12)	3.7
Wood under *A. rubra* autoclaved**	0.5	0.1	1.0	4.0	0	0	3.3
Wood under conifers autoclaved**	0.5	0.1	1.0	4.0	0	0	3.3

Means followed by the different letters in the columns differ significantly ($P < 0.05$, T-test); *( ): standard error; ** Control seedlings grew poorly and were not included in the analysis.

stream water (Su and Lin 1989) can reportedly disperse this organism. The higher level of *Frankia* infectious capacity in the dead, fallen trees under red alder, as measured by nodule weight and nitrogen fixation, suggests that the decaying, fallen trees adjacent to red alder harbor *Frankia*.

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