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Roles of Woody Root-Associated Fungi in Forest Ecosystem Processes: Recent Advances in Fungal Identification

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Abstract

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Interactions between fungi and woody roots may be critical factors that influence diverse forest ecosystems processes, such as wood decay (nutrient recycling); root diseases and their biological control; and endophytic, epiphytic, and mycorrhizal symbioses. However, few studies have characterized the diversity and the spatial and temporal distribution of woody root-associated fungi in forest ecosystems. Molecular genetic techniques that facilitate fungal identification are now available to help investigate complex and dynamic interactions of these fungi.

Key Words: Ascomycetes, Basidiomycetes, fungi, endophyte, polymerase chain reaction, root rot, Zygomycetes.

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Root Fungi: Neglected Biological Components of Forest Ecosystems

In recent years, ecologists have increased their efforts to understand belowground, biological interactions in forest ecosystems. Interactions that occur among plant roots, animals, and microorganisms are dynamic and substantially influence ecosystem processes. Ecologists have just begun to study the biota contributing to these interactions (Copely 2000).

Among belowground biota are the fungi, which are tremendously diverse and play wide-ranging roles in forest ecosystem processes (Rossman and others 1998). A primary role of fungi is the degradation of plant and animal matter, which allows recycling of resources (Rossman and others 1998). Fungi are aided in this role by their ability to obtain nutrients through absorption. Their capacities for growth on various substrates depend on their abilities to: (1) degrade complex nutrient sources extracellularly; (2) transport simple nutrients across cell membranes for metabolic processes (nutrition through absorption); and (3) tolerate or deactivate compounds that inhibit fungal growth.

Studies of fungal biodiversity in forest ecosystems can provide baseline information for determining interrelationships among organisms and indicate potential roles of fungi in forest ecosystem dynamics. Understanding the role of fungi in forest ecosystem processes is key to characterizing stability and succession of biological components (for example, trees), while information on fungal biodiversity can provide insight on sustaining fungi as beneficial resources (Rossman and others 1998).

Due to the widespread distribution of fungi and their association with diverse organic and inorganic substrates, the actual number of fungal species in existence is difficult to assess (Rossman and others 1998). Fungi isolated from wood include such diverse groups as mucoraceous species (zygomycetes), ascomycetes, basidiomycetes, and deuteromycetes (asexual fungi). Fungi

associated with woody roots have not been well studied, in part because roots are not readily observed without excavation. In addition, root substrates vary temporally and spatially within the soil profile over the life span of a tree. This paper addresses the potential diversity and ecological functions of fungi associated with woody roots of forest trees.

Wood Decay Fungi

Wood decay fungi are commonly associated with woody roots. In the forest, decay fungi play an important role in carbon and nitrogen cycling, while helping to convert organic debris into humus. Some fungi attack living trees; others invade dead or down timber and slash on the forest floor. Wood-decaying basidiomycetes colonize and degrade wood using enzymatic and mechanical processes. Brown-rot fungi preferentially attack and rapidly depolymerize structural carbohydrates (celluloses and hemicelluloses) in the cell wall leaving the modified lignin behind. White-rot fungi can progressively utilize all major cell wall components, including both carbohydrates and lignin (Jasalavich and others 2000).

Although wood decay fungi are primarily basidiomycetous, some ascomycetous fungi cause rapid white-rot of various hardwood trees (Nilsson and others 1989). A comprehensive review of decay fungi in trees has been presented by Gilbertson (1980). Species surveys of fungi in coniferous forests are usually based on the distribution of fruiting bodies (sporocarps) (fig. 1). Because fruiting bodies only develop under specific environmental conditions, such inventories may span several years but still not accurately reflect the abundance of fungal species (Johannesson and Stenlid 1999).

Pathogenic Fungi

Pathogenic fungi are also common associates of woody roots. Various pathogenic heart-, butt-, and root-rot fungi kill living trees by attacking functional



Figure 1. Fruiting body of *Boletus mirabilis*, a mycorrhizal fungus that is frequently associated with decomposing woody debris (photo by L.M. Carris).

vascular and cambium tissues. They enter roots by direct penetration or by stump- or bole-to-root colonization. Although some pathogenic fungi are not predictably distributed within a forest, others (for example, root- and butt-rot fungi) can kill trees in slowly expanding mortality centers that create gaps in the forest canopy. Well-known examples of root pathogens include some *Armillaria* species (basidiomycetous fungi that can cause “shoestring rot”; fig. 2), *Phellinus sulphurascens* and *P. weirii* (causal agents of laminated root rots), and *Heterobasidion annosum* (causal agent of annosus root rot). Infection centers of root pathogens can be widespread and locally abundant, and the centers persist from centuries to millennia. Root- and butt-rot pathogens are among the most influential agents of forest disturbance in some conifer ecosystems, and they shape the structure and composition of natural and managed forests (Hansen and Goheen 2000). *H. annosum* is a complex of biological species specialized to pine, spruce, and fir hosts. Colonization of stumps and wounds initiates infection centers, but tree-to-tree spread along roots expands centers of infection and mortality on the appropriate host (Garbelotto and others 1997; Stenlid and Redfern 1998). Like *Heterobasidion*, the genus *Armillaria* also contains a number of poorly differentiated species, and more studies are needed to fully understand the ecological role of many such species.

Endophytic Fungi

A fungal endophyte lives asymptotically within plant parts (intercellularly or intracellularly) for at



Figure 2. *Armillaria ostoyae* causes root and butt rot on a diverse host tree species. (A) mycelial fan beneath the bark is a sign of Armillaria disease on Douglas-fir (photo by J.D. Rogers). (B) Fruiting bodies help to identify the species of *Armillaria*, but fruiting is typically sporadic in nature (photo by G.I. McDonald).

least part of its life cycle (Saikkonen and others 1998). Endophytic fungi usually occur in aboveground plant tissues but are also found in roots. Unlike mycorrhizal fungi, fungal endophytes of roots lack extraradical (outside the root) hyphal networks and mantles (sheaths around the roots).

While much information is available on pathogens and mycorrhizal fungi, little is known about symptomless (possibly neutral or mutualistic) endophytes of plants. Endophytic fungi are often divided into two groups: the “clavicipitaceous system” (ascomycete family Clavicipitaceae), which contains endophytes of grass that often confer resistance to herbivory and pathogens, and endophytes representing all other fungal groups on diverse herbaceous and woody hosts (Petrini 1997). Fungal endophytes have been found in all woody plants studied to date. Trees and shrubs usually contain numerous fungal species; however, there has been little attempt to correlate ecological and evolutionary aspects of fungal endophytes of grasses versus woody plants (Saikkonen and others 1998). Based on the endophytic fungi of grasses, some endophytic fungi of woody roots may have potential for biological control of pathogens and/or insects.

Epiphytic Fungi

The external plant surface provides an environment for fungal growth that varies spatially and temporally and can also be unique to the host. New surfaces become progressively available for colonization as the

plant grows (Andrews and Harris 2000), and plant organs differentiate and undergo developmental processes. Each aspect of plant growth and development can affect microbial flora on the surface. Many researchers have noted seasonal patterns in the composition of phyllosphere (leaf surface) microbial communities. Epiphytic fungi of woody roots are not well documented and warrant further study. It is also not known if root epiphytes may also occur as endophytes.

Ectomycorrhizal Fungi

Ectomycorrhizal (EM) fungi are typically associated with feeder roots; however, the potential interactions of these fungi with woody roots is unknown. EM symbioses are important on a global scale because most trees throughout the world's boreal, temperate, subtropical, and tropical forests are ectomycorrhizal. For host trees, these fungi gather nutrients and scavenge nitrogen, phosphorus, and trace elements from both inorganic and organic pools in the soil, while extracting fixed carbon from the tree (Horton and Bruns 2001). These mutualistic fungi also provide host plants with increased access to water and protect plants from pathogens, chemical extremes (for example, high pH), and heavy-metal contamination (Cullings and others 2000). Until the mid-1990s, knowledge of EM fungal community structure and the degree to which species assemblages show predictable colonization patterns in space and time was almost exclusively based on sporocarp surveys (Dahlberg 2001). EM fungi are generally basidiomycetes (for example, species of *Russula*, *Cortinarius*, *Chroogomphus*, *Lactarius*, *Suillus*, *Paxillus*) (Cullings and others 2000; Horton and Bruns 2001).

Mycoparasitic Fungi

Mycoparasitic fungi obtain at least part of their nutrients from other fungi. Some are being investigated for their roles in the biocontrol of plant pathogens in the rhizosphere (root surface) and spermosphere (seed surface) (Whipps 2001). Mycoparasites are extremely common in soil, where they can act as natural regulators of fungal populations. They can be biotrophic on their host fungus, with the host fungus showing few symptoms other than reduced growth rate, or necrotrophic, with host cells killed under some conditions. Commercial formulations of some mycoparasitic fungi (for example, *Trichoderma* spp.) are available for use against certain plant pathogens including root rot pathogens, such as *Pythium* spp. and *H. annosum*.

Identification, Species Concepts, and Ecological Roles of Fungi

Morphological identification of the diverse fungi isolated from wood is difficult and tedious. Moreover, many fungal species that grow in wood may not be culturable under laboratory conditions (Johannesson and Stenlid 1999). Of those that are culturable, the wood-rotting fungi present an additional problem: Petri plate cultures of most basidiomycetes do not produce fruiting bodies that supply critical characters for identifying genera, families, and species (Nobles 1948).

The species concept is central to biology, and yet a universally useful definition of species is elusive. Though much of the discussion of variability within species has centered on animals and plants, surveys of fungal diversity must also consider the species concept. Fungal species definitions have been based on overall phenotypic similarity, ecological distributions, reproductive isolation versus cohesion, evolutionary divergence, and combinations of the above (reviewed by Harrington and Rizzo 1999). In practice, fungal species concepts have evolved from strictly morphological descriptions, to biological (reproductive) species concepts, to phylogenetically (deoxyribonucleic acid, DNA, divergence) based species concepts. Morphological, ecological, and pathological species are all defined from phenetic (observable) characters, most of which relate directly to structures and functions (Harrington and Rizzo 1999). For example, some ecological species have been based on adaptation to a particular niche, or on disease symptoms and host association for some plant pathogens.

To facilitate fungal identification and examine relationships among fungal groups, a molecular tool called the Polymerase Chain Reaction (PCR) can be used to amplify (produce numerous copies of) specific fragments of DNA. Such fragments must contain conserved (less variable) sequences at either end of the fragment that correspond to short, artificially constructed "primer" sequences that "prime" the process of generating new copies of the target region by a DNA polymerase enzyme. DNA fragments are analyzed by comparing differences in sequences, which allows analyses of relationships within and among phenetically defined species (Bridge and others 1998). Sequence analysis may reinforce or invalidate phenetic species definitions while providing valuable insights into their evolutionary history. DNA-based analysis can be especially important for species complexes where major defining characters relate to environmental adaptation, as is the case for

ecological species (Bridge and others 1998). Molecular techniques are currently used to identify fungi from plants for diverse ecological studies (examples include Horton and Bruns 2001; Johannesson and Stenlid 1999; Vandenkoornhuyse and others 2002) and have also revealed evolutionary relationships among major groups of fungi (Klich and Mullaney 1992).

In general, conserved DNA regions are used to resolve broad evolutionary relationships among fungi (for example, at the family, order, and class levels), but more variable DNA regions are used to resolve relationships among or within species. Ribosomal DNA (rDNA) is well suited for both purposes. The fungal genome (a haploid set of all DNA within the cell nucleus) contains roughly 200 tandem and nearly identical repeats of the rDNA region (Berbee and Taylor 2001). This rDNA region typically contains genes for the Small Subunit (= SSU, 17S), 5S, 5.8S, and Large Subunit (= LSU, 25S) ribosomal subunits, Internal Transcribed Spacers (ITS), and Intergenic Spacers (IGS) (Horton and Bruns 2001). The high proportion of rDNA in the genome usually enables amplification of rDNA targets from even low-quality DNA preparations (Berbee and Taylor 2001).

The ITS region is well suited for identification because unknown organisms can usually be identified to the species or species group level by comparing ITS sequence data from unknown isolates to the extensive ITS fungal data currently deposited in electronic databases (for example, GenBank, EMBL, and DDBJ). Because ITS sequences typically vary with species, closely related taxa should show close sequence similarity (Horton and Bruns 2001). The major limitations to this approach are the inability of a researcher to be certain that a fungus whose sequence has been entered in the database has been accurately identified, and the low representation of fungi from some substrates.

For the purposes of assessing fungal diversity associated with woody roots, utilizing ITS data can contribute to a more comprehensive assessment than surveys based solely on morphology, by allowing identification of fungal species that are nonsporulating and nonsporocarp forming. PCR and DNA sequencing, combined with morphological verification can greatly facilitate surveys of fungal diversity by validating taxonomic identification (fig. 3 and 4). One such study on endophytes of woody roots has found 27 fungal genera associated with Douglas-fir [*Pseudotsuga menziesii* (Mirb.) Franco] and ponderosa pine (*Pinus ponderosa* Douglas ex Lawson & C. Lawson) (Hoff 2002). An unexpected finding from that study was that the two predominant groups of isolated fungi corresponded to an ascomycetous genus of heat-resistant fungi (*Byssoschlamys*) that may be active in

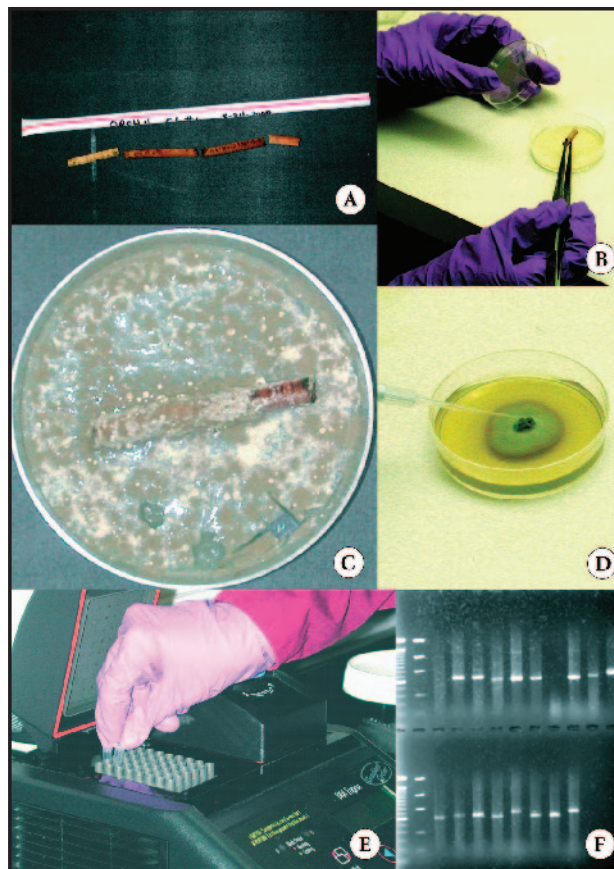


Figure 3. Fungal isolation, culture, and DNA amplification using PCR. (A) Root increment core. (B) Isolation of fungi by growth on a selective culture medium. (C) Fungal culture initiating from a root core. (D) A pipette tip gently scraped across a fungal culture provides suitable template DNA for PCR. (E) Reaction tubes are placed in a temperature cycler for PCR. (F) Electrophoresis gel showing DNA products produced by PCR.

bioprotection, and a zygomycetous genus (*Umbelopsis*) that is a common soil fungus not previously detected in living roots of these tree species. Whether frequency and intensity of wildfires or prescribed burns affect levels of such root fungi is currently unknown, but the implications for fire and host succession effects on disease through these or other previously overlooked fungi are of great interest. Future studies will determine if prescribed burning could be used to favor beneficial, root-associated fungi that are heat resistant, while discriminating against fungal pathogens that are less heat tolerant.

Implications

The diversity of fungi occupying ecological niches on and in woody roots of forest trees is largely unexplored.

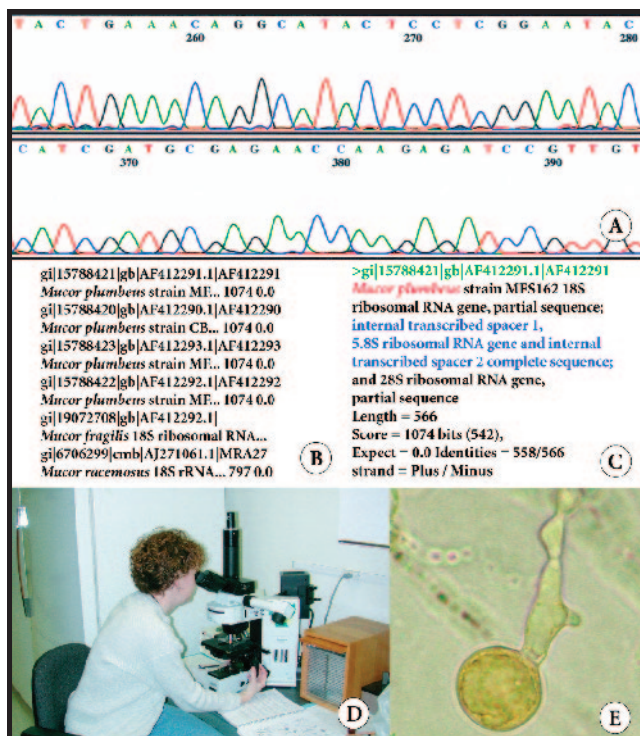


Figure 4. DNA sequencing of PCR products, comparisons with sequence data bases, and morphological characterization for species identification. (A) Example of a DNA sequence chromatogram showing portions of the DNA sequence of a PCR product. (B) Results of a GenBank Blast search identifying fungal DNA sequences with the most similarity to a sequence from an unknown fungal isolate. (C) Additional information from GenBank Blast showing percent identity of sequences from the unknown fungal isolate with the database sequence that it most closely matches. (D) Microscopic observations are used to verify genus and species identification. (E) Example of microscopic structure used for morphological verification of sequence-assisted species identification.

Unexpected results from recent studies (Hoff 2002) indicate a great need to further explore the diversity and ecological roles of fungi associated with woody roots of various forest trees in diverse ecosystems. As the role of fungal diversity in ecosystem processes is better understood, information should become available to improve sustainable forest practices.

For more information on terminology used in mycology, the reader is referred to: Ulloa, M.; Hanlin, R.T. 2000. Illustrated dictionary of mycology. APS Press, St. Paul, MN. 448 pp.

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