Current Investigations of Fungal Ectomycorrhizal Communities in the Sierra National Forest[1]

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Abstract

Progress on two main studies on fungal ectomycorrhizal communities in the Sierra National Forest is discussed. One study examined the short-term effects of ground fire on the ectomycorrhizal community and the other examined the ectomycorrhizal associates of snow plant (*Sarcodes sanguinea*). In the ground-fire study we found that a large initial reduction in ectomycorrhizal biomass is caused primarily by combustion of the upper organic layers; prefire dominants in the Russulaceae and Thelephoraceae are dramatically reduced; and species at greater depths appear to survive the fire. We speculate that fire causes a short-term increase in species evenness. In the field portion of the *Sarcodes* study, we found that *Sarcodes* is specialized on a single mycorrhizal associate, *Rhizopogon ellenae*; the presence of *Sarcodes* is associated with dense islands of *R. ellenae*; *R. ellenae* appears to be a minor below-ground component of the red fir (*Abies magnifica*) community in locations near but not immediately adjacent to *Sarcodes* plants; and the *Abies magnifica* ectomycorrhizal community is dominated by members of the Russulaceae and Thelephoraceae. From studies of *Sarcodes-R. ellenae* interactions under laboratory conditions, we report that seed germination is stimulated by isolates of *R. ellenae*, isolates of *R. ellenae* derived from *Sarcodes* and *Abies* roots are capable of forming mycorrhizae on pine (*Pinus*) roots, and we have achieved initial success in establishing a tripartite *Sarcodes-Rhizopogon-Pinus* association under laboratory conditions.

Ectomycorrhizae (EM) are dual organs composed of the fine roots of plants and fungal mycelia. They are the primary interface through which most temperate forest trees receive their mineral nutrients. All members of the Pinaceae require these mutualistic fungi for normal growth and survival (Smith and Read 1997).

The diversity of EM fungi is extremely high. Over 6,000 species have been described (Molina and others 1992) and, because of the current state of our taxonomic knowledge, this probably represents a gross underestimation of the total number. Even at a local scale, diversity is very high. In single-species pine stands of approximately 0.1 ha, 15 to 35 species of EM fungi are typically reported, single soil cores often contain several species (Eberhart and others 1996), and even adjacent root tips are frequently colonized by different fungi (Bruns 1995).

In spite of the importance of EM fungi, little is known about the structure of the complex communities that they form or the functional differences among the component species. Until recently, almost no quantitative descriptions of the abundance of EM species on roots were reported. This lack of information was largely caused by the difficulty in identifying fungal species in their vegetative states (that is, as mycorrhizae or mycelia). With the advent of the polymerase chain reaction (PCR), molecular-based methods now make identification much

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more feasible. As a result, many EM community studies are beginning to be reported (Erland 1995, Gardes and Bruns 1996a, Horton and Bruns 1998, Horton and others 1998, Horton and others [In press], Kårén and Nylund 1996).

The long-term goals of all our studies are to examine the structure of EM communities in diverse settings, to look for common themes in these structures, and to elucidate the functional differences among EM fungi. Our strategy has been to focus on a series of much smaller but more researchable questions and to assemble results of these studies into a general picture of fungal EM communities. Our prior work in coastal pine and Douglas-fir (*Pseudotsuga menziesii*) forests has shown that the species that dominate fruiting can be minor components of the below-ground community; mature communities are typically dominated by members of the Thelephoraceae and Russulaceae; where roots of Douglas-fir and pine or Douglas-fir and manzanita (*Arctostaphylos* spp.) intermingle, they usually are associated with the same fungal species; and a spore-bank guild, dominated by members of the Suillineae (primarily *Rhizopogon*) and members of the Ascomycota, is present in forest soils, and these species dominate pine seedlings in the first few years after stand-replacing fires (Baar and others 1999, Gardes and Bruns 1996a, Horton and Bruns 1998, Horton and others 1998, Horton and others in press, Taylor and Bruns 1999).

In the Sierra National Forest (SNF) we focused on two studies that complemented our earlier or ongoing studies elsewhere. In the first, we examined the short-term effects of ground fire on the EM community associated with ponderosa pine (Stendell and others 1999). The second study focused on EM associates of snow plant and on the EM community on red fir (*Abies magnifica*) surrounding snow plants. *Sarcodes* (*fig. 1*) is a nonphotosynthetic member of the Ericaceae, subfamily Monotropoideae. It is "epiparasitic" in that it obtains all of its carbon from surrounding trees via an EM fungus. Early studies in our lab on other nonphotosynthetic epiparasitic plants showed that, in contrast to photosynthetic plants, virtually all of the nonphotosynthetic plants that we studied had highly specific fungal associations. The only apparent exception was

Figure 1—*Sarcodes sanguinea.*

Sarcodes, and because it is a common plant in SNF, we decided to sample it more intensively and more locally than in our previous study, which included only 12 *Sarcodes* plants (Cullings and others 1996). In the course of sampling we found a site where *Sarcodes* was unusually abundant. This "high-density" *Sarcodes* site, situated in a red fir monoculture, enabled us to examine the EM community adjacent to *Sarcodes* and compare it with that in the surrounding red fir forest. It also provided an ideal setting for studying the sizes of fungal clones associated with *Sarcodes*.

Methods

Collection locations for fungi and *Sarcodes* were dispersed throughout the mixed-conifer and red fir zones of SNF. Global positioning system (GPS) coordinates were recorded for collection sites and plots. Collections of fungal fruitbodies were dried and retained in our herbarium, later to be deposited in the University of California Herbarium. Larger collections were split, with half of each deposited at Oregon State University. Most hypogeous collections were examined and identified by Dr. Jim Trappe of Oregon State University. In addition, *Rhizopogon* species in section Amylopogon were identified by either restriction fragment length polymorphism (RFLP) or sequence analysis of the internal transcribed spacer region (ITS).

Pine and fir roots were washed from soil cores of 4 X 40 cm. EM were sorted by shape and color, freeze-dried, weighed, and saved for molecular analysis. *Sarcodes* roots were collected by digging alongside flowering spikes until the rootball was encountered. Portions of the rootball were then removed, washed, and freeze-dried or used for isolation of the fungal symbiont into axenic culture. EM fungi were identified by molecular analysis as described previously (Bruns and others 1998, Gardes and Bruns 1993, Gardes and Bruns1996b).

Plots for the fire study were located at 36°58'48"N latitude and 119°8'13"W longitude. Six sampling plots were located in small, mature stands of ponderosa pine on a south-facing slope of approximately 11° on either side of the SNF service road 10S67. North of the road, in an area intended for a prescribed burn, three plots were established prior to the burn, and three control plots were established south of the road outside the designated burn area.

Studies of the EM community surrounding individual *Sarcodes* plants were done in a pure red fir stand near the junction of the SNF service roads 9S62 and 9S10 at 37°09'06" N latitude and 119°07'50" W longitude. Plants in this plot were counted along a 100-m transect, mapped within a 750-m^2 grid, and soil cores were taken along four 10-m transects centered on individual *Sarcodes* plants.

Results and Discussion

Results from the fire study (Stendell and others 1999) showed that the presence of all species was highly patchy in both the prefire samples and postfire control plots. Even two cores taken from a single m^2 plot often contained entirely different species. In spite of this, we observed that members of the Russulaceae and Thelephoraceae dominated the community—a pattern shared by all other undisturbed California pinaceous communities that we have studied (Gardes and Bruns 1996a, Horton and Bruns 1998, Horton and others [In press], Taylor and Bruns 1999). The short-term effect of this ground fire on the mycorrhizal community was an eightfold reduction in ectomycorrhizal biomass. The reduction was directly correlated with incineration of the litter layer and heating of the top few cm directly below it. These two regions accounted for the overwhelming majority of prefire mycorrhizae. There appeared to be a trend toward disproportionate reduction of the most abundant ectomycorrhizal fungi—members of the Russulaceae and Thelephoraceae. We speculate that this resulted

because these fungi are primarily dominant in the organic upper soil layers that were destroyed. There appears to be a trend for rare types to be more common at greater depths in all communities we have studied. Thus, one of the immediate effects of fire may be to increase species evenness in the ectomycorrhizal communities by destroying the organic layers where a few species dominate, while preserving the deeper layers where species richness is greatest.

During the summer of 1998, we returned to the fire site and resampled the ectomycorrhizal community to see how it had recovered in the third year after the fire. Although we will not have the answer to this question for several months, we did observe abundant fruiting of *Rhizopogon subcaerulescens* at the site that summer. This is interesting because we knew from our 1-year sample that it is one of the species that survived the fire. We also know from our studies of the Pt. Reyes wildfire that *R. subcaerulescens* is one of the most dominant and responsive members of the sporebank in fire-disturbed settings (Baar and others 1999, Horton and others 1998, Taylor and Bruns 1999). It would not be surprising, therefore, if its abundance increased as a result of the burn.
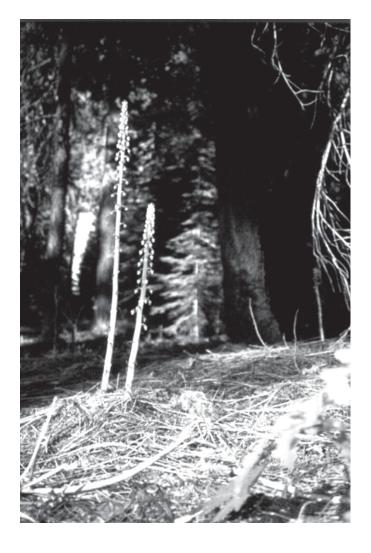
From our sample of 64 *Sarcodes*, we determined that the only mycorrhizal associate for *Sarcodes* within the SNF and the Tahoe basin is in the *Rhizopogon ellenae* species complex. Previously, we knew that the associate was close to *R. subcaerulescens,* but with a refined ITS sequence database, we discovered that the associate is distinct from *R. subcaerulescens* and closer to *R. ellenae*. This is interesting because it means that snow plant targets a different fungus from its close relative, pine drops (*Pterospora andromedea—fig. 2*); the latter is associated with the *R. subcaerulescens* complex. The fact that these two plants often grow within a few meters of each other demonstrates that the plant-fungal specificity is based on intrinsic factors of the interaction rather than habitat. It also shows that these two fungi, which are difficult to discriminate taxonomically, are not redundant; the absence of either would translate into the absence of either *Sarcodes* or *Pterospora*.

The identification of *R. ellenae* as the associate of *Sarcodes* also revealed the confusion within identified *Rhizopogon* collections. Initially, we thought that *R. ellenae* was rare in the SNF because it did not appear to be included in any of our collections from the area. After we applied ITS-RFLP analysis to our collections, however, and to many of those housed in the Thiers Herbarium at San Francisco State University, we discovered that the names *R. subcaerulescens* and *R. ellenae* have essentially been applied interchangeably, even though two distinct lineages can be identified easily by ITS sequences.

The soil cores taken along four transects and centered on individual snow plants have revealed two interesting results. First, we found that both the presence of *R. ellenae* on fir roots and the number of fir roots are orders of magnitude greater near the rootball of *Sarcodes* than in the surrounding forest. This suggests that *Sarcodes* is either finding dense islands of *R. ellenae-Abies* mycorrhizae, or it is creating them. If the latter is the case, then the relationship between snow plant and *Rhizopogon* does not look strictly parasitic, as we had thought, and it opens the question of how this nonphotosynthetic plant is capable of stimulating both its fungal associate and the roots of a photosynthetic host. Second, we found that *R. ellenae* is so rare on the roots of *Abies* 500 cm from *Sarcodes* plants that it is undetected in our sample, and the dominant fungi are members of the Russulaceae and Thelephoraceae. This result provides further evidence that their dominance is a general pattern in California pinaceous ecosystems.

With the knowledge that all *Sarcodes* plants were associated with *R. ellenae,* the flower spikes were used as a convenient way to locate sites where the fungus was present. We selected plants at the high-density site, mapped them, and cultured *R. ellenae* from their roots. We are now analyzing these isolates to determine which, if any, are genetically identical. Because *Rhizopogon* has no

Figure 2—*Pterospora andromedea.*

known asexual spore state, genetic identity can be achieved only by vegetative growth. Therefore, by relating identical genotypes back to their mapped locations in the forest, we will be able to tell the size of a given fungal individual. We did not know what to expect for a *Rhizopogon* species, but we did know that *Suillus* species, which are closely related to *Rhizopogon*, often have individuals that span 100 m² or more (Bonello and others 1998, Dahlberg 1997, Dahlberg and Stenlid 1994, Jacobson and others 1993). Our preliminary results from *R. ellenae* at the dense site suggest that the average individual is small and that more than one individual may colonize a single *Sarcodes* plant. If confirmed, this would suggest that the abundance of *R. ellenae* at this site is the result of recent establishment by spore. This may be related to the past history of this site, which was burned after thinning about 6 years prior to this study. We know from our other studies that spores of several *Rhizopogon* species respond positively to fire or soil disturbance (Baar and others 1999, Horton and others 1998, Taylor and Bruns 1999).

Recently we have been using cultures of *R. ellenae* and *R. subcaerulescens* that we isolated from snow plant and pine drops, respectively, to study the physiological ecology of the ectomycorrhizal interaction. Our preliminary results show that both species are capable of forming mycorrhizae with lodgepole pine (*Pinus contorta)* and bishop pine (*Pinus muricata*), which agrees with previous findings by Molina and Trappe (1994). We also have preliminary evidence that *R. ellenae* stimulates the germination of snow plant seeds. This is the first record of *in vitro* germination for this species; ongoing tests will determine whether germination is specific to *R. ellenae.* Very recently we have succeeded in growing

Sarcodes in the laboratory in a tripartite system with lodgepole pine and *R. ellenae*. This is the first time a member of the Monotropoideae has successfully been grown in the laboratory, and it offers us an opportunity to use this system to further study the physiology of these plants.

A general picture of EM communities is starting to come into focus. They are clearly diverse and highly patchy communities, but their structure, at least at the family level, appears to be similar across different types of California pinaceous forests. Understanding the autecology of individual species is a daunting task, but one that is necessary if we wish to understand how these organisms interact with plant and animal communities.

Acknowledgments

References

Baar, Jacqueline; Horton, Thomas R.; Kretzer, Annette; Bruns, Thomas D. 1999. **Mycorrhizal recolonization of *Pinus muricata* from resistant propagules after a catastrophic wildfire.** New Phytologist143: 409-418.

Bonello, Pierluigo; Bruns, Thomas D.; Gardes, Monique. 1998. **Genetic structure of a natural population of the ectomycorrhizal fungus *Suillus pungens*.** New Phytologist 138: 33-542.

Bruns, T. D. 1995. **Thoughts on the processes that maintain local species diversity of ectomycorrhizal fungi.** Plant and Soil 170: 63-73.

Bruns, T. D.; Szaro, T. M.; Gardes, M.; Cullings, K. W.; Pan, J.; Taylor, D. L.; Li, Y. 1998. **A sequence database for the identification of ectomycorrhizal fungi by phylogenetic analysis.** Molecular Ecology 7: 257-272.

Cullings, K. W.; Szaro, T. M; Bruns, T. D. 1996. **Evolution of extreme specialization within a lineage of ectomycorrhizal epiparasites.** Nature 379: 63-66.

Dahlberg, A. 1997. **Population ecology of *Suillus variegatus* in old Swedish Scots pine forests.** Mycological Research 101: 47-54.

Dahlberg, Anders; Stenlid, Jon. 1994. **Size, distribution and biomass of genets in populations of *Suillus bovinus* (l.: fr.) Roussel revealed by somatic incompatibility.** New Phytologist 128: 225-234.

Eberhart, J. L.; Luoma, D. L.; Amaranthus, M. P. 1996. **Response of ectomycorrhizal fungi to forest management treatments—a new method for quantifying morphotypes.** In: Azcon-Aguilar, C.; Barea, J. M., eds. Mycorrhizas in integrated systems: from genes to plant development. Luxembourg: Office for Official Publications of the European Communities; 96-99.

Erland, S. 1995. **Abundance of *Tylospora fibrillosa* ectomycorrhizas in a South Swedish spruce forest measured by RFLP analysis of the PCR-amplified rDNA ITS region.** Mycological Research 99: 1425-1428.

Gardes, M.; Bruns, T. D. 1993. **ITS primers with enhanced specificity for basidiomycetes— application to the identification of mycorrhizae and rusts.** Molecular Ecology 2: 113-118.

Gardes, M.; Bruns, T. D. 1996a. **Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: above- and below-ground views.** Canadian Journal of Botany 74: 1572-1583.

Gardes, M.; Bruns, T. D. 1996b. **ITS-RFLP matching for identification of fungi.** In: Clapp, J. P., ed. Methods in molecular biology, Vol. 50. Species diagnostics protocols: PCR and other nucleic acid methods. Totowa, NJ: Humana Press Inc.; 177-186.

Horton, Thomas R.; Bruns, Thomas D. 1998. **Multiple-host fungi are the most frequent and abundant ectomycorrhizal types in a mixed forest of Douglas-fir (*Pseudotsuga menziesii* D. Don) and bishop pine (*Pinus muricata* D. Don).** New Phytologist 139: 331-339.

Horton, Thomas R.; Bruns, Thomas D.; Parker, Thomas. [In press]. **Ectomycorrhizal fungi associated with *Arctostaphylos* facilitate the establishment of *Psuedotsuga menziesii* during succession.** Canadian Journal of Botany.

Horton, Thomas R.; Cázares, Efren; Bruns, Thomas D. 1998. **Ectomycorrhizal, vesicular-arbuscular and dark septat fungal colonization of bishop pine (*Pinus muricata*) seedlings in the first 5 months of growth after wildfire.** Mycorrhizae 8: 11-18.

Jacobson, K. M.; Miller, O. K., Jr.; Turner, B. J. 1993. **Randomly amplified polymorphic DNA markers are superior to somatic incompatibility tests for discriminating genotypes in natural populations of the ectomycorrhizal fungus *Suillus granulatus*.** Proceedings of the National Academy of Sciences (USA) 90: 9159-9163.

Kårén, O.; Nylund, J. E. 1996. **Effects of N-free fertilization on ectomycorrhiza community structure in Norway spruce stands in southern Sweden.** Plant and Soil 181: 295-305.

Molina, R.; Trappe, J. M. 1994. **Biology of the ectomycorrhizal genus *Rhizopogo*n. I. Host associations, host-specificity and pure culture syntheses.** New Phytologist 126: 653-675.

Molina, R.; Massicotte, H.; Trappe, J. M. 1992. **Specificity phenomena in mycorrhizal symbioses: community-ecological consequences and practical implications.** In: Allen, M. F., ed. Mycorrhizal functioning: an integrative plant-fungal process. New York, NY: Chapman and Hall, New York; 357-423.

Smith, S. E.; Read, D. J. 1997. **Mycorrhizal symbiosis.** 2nd ed. New York, NY: Academic Press; 605 p.

Stendell, E.; Horton, T. R.; Bruns, T. D. 1999. **Early effects of prescribed fire on the structure of the fungal ectomycorrhizal community in a Sierra Nevada ponderosa pine forest.** Mycological Research 103: 1,353-1,359.

Taylor, Donald L.; Bruns, Thomas D. 1999. **Community structure of ectomycorrhizal fungi in a *Pinus muricata* forest: resident colonizers and resistant propagules overlap in space but are dominated by different species.** Molecular Ecology 8:1,837-1,850.

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