MOLECULAR IDENTIFICATION OF ECTOMYCORRHIZAL FUNGI ASSOCIATED WITH THE AMERICAN CHESTNUT

Shiv Hiremath and Kirsten Lehtoma

U.S. Forest Service, Northern Research Station
359 Main Rd., Delaware, OH 43015

ABSTRACT

We plan to use American chestnut trees for reforesting reclaimed mine sites in southeastern Ohio. The American chestnut was once common to this region but was displaced because of the blight-causing invasive fungus *Cryphonectria parasitica*. However, with increased efforts to generate blight-resistant varieties of the American chestnut through breeding programs as well as use of hypovirulent strains of the fungus that can provide resistance to the trees, restoration of the American chestnut now appears feasible. Our approach to using chestnut seedlings for reforestation addresses both issues: (1) reclamation of mined lands and (2) restoration of the American chestnut.

Mycorrhizal fungi can significantly aid in reforestation efforts because the fungi can offer many benefits for the survival and healthy growth of the planted seedlings in the nutrient-poor mined sites. While there have been many reports of association of mycorrhizal fungi with the American chestnut, systematic studies to identify species that form functional symbiotic interactions with chestnut tree roots and their utility in chestnut restoration efforts have not been carried out. The traditional methods of identifying the associated fungi using morphological techniques are often inconclusive or not precise. We are using molecular detection methods coupled with transmission electron microscope (TEM) analyses to identify functional associations of ectomycorrhizal fungi with the roots of chestnut seedlings.

The seedlings were inoculated under semi-sterile conditions in the laboratory, and mycorrhizal formation was allowed to progress in the greenhouse. After 3 months, roots were examined under a dissecting microscope for the presence of the micorrhiza. The mycorrhizal tissues were also subjected to TEM analysis to confirm symbiotic interaction. The tissue was then used for DNA extraction and for analyses. Identification of ectomycorrhizal fungal species was achieved through comparison of ribosomal DNA internal transcribed spacer (ITS) sequences with those deposited in public databases. Among the several pairs of primers suggested for these analyses, the following pairs were used: (ITS-1 – LR21) and (ITS-1 – ITS-4). We were able to identify and confirm that the following inoculated fungi can successfully form associations with American chestnut seedlings. They were *Cenococcum geophilum*, *Amanita rubescens*, *Laccaria bicolor*, *Pisolithus tinctorius*, and *Laccaria laccata*. In addition, we also identified two fungi that were not used in inoculation experiments. These were probably associated with the chestnut seeds before their germination. The molecular technique identified them as *Thelephora terrestris* and *Tometella* sp. We are generating and planting seedlings inoculated with these fungi in reclaimed regions and monitoring their survival and growth.