Ectomycorrhizal diversity associated with tanoak (*Lithocarpus densiflorus*)

Nicholas C. Kordesch, Sarah E. Bergemann, Matteo Garbelotto

Department of Environmental Science, Policy & Management, University of California, Berkeley, CA 94720

Abstract

***Phytophthora ramorum* has caused widespread mortality of tanoaks (*Lithocarpus densiflorus*) along California's coastal regions. Since tanoaks form ectomycorrhizal associations, local extinction may impact its ectomycorrhizal symbionts. The goals of this study were to in identify the primary ectomycorrhizal lineages and predominant fungi from tanoak stands. Five blocks divided into 3 plots were established in tanoak stands near the town of Whitethorn, California. Root tips were sorted from soil core sections of equal volume, bulked and amplified using general primers that target the ITS region and a more conserved region of the 28s rDNA. To rapidly identify unique ITS signatures, real-time PCR using melt temperature curves were analyzed from 80 PCR products cloned from each plot. PCR products with unique melt temperature profiles were sequenced to estimate taxonomic diversity and identify the predominant ascomycete and basidiomycete lineages associated with tanoak. Commonly occurring fungi include ascomycetes (*Cenococcum geophilum, Phialophora*) and basidiomycetes (*Boletus, Lactarius, Melanogaster, Ramaria, Russula, Tomentella, 'Thelephora-like,' Tricholoma*). Information collected from this study can be used as baseline information for future studies to investigate the impacts of carbon reduction by *Phytophthora ramorum* on the ectomycorrhizal community of tanoak.**

Introduction

Phytophthora ramorum has caused widespread mortality of tanoaks in many coastal regions of California. Because tanoaks form symbiotic associations with ectomycorrhizal fungi, local extinction of tanoaks may dramatically impact local populations of ectomycorrhizal associates. The goals of this study were to identify the predominant ectomycorrhizal lineages on tanoak and determine the commonly sampled ectomycorrhizal fungi.

Materials and methods

1. Environmental Sampling

Five, 1200 m^2 blocks were established near the town of Whitethorn, CA in stands dominated by tanoak (Fig. 1). These plots were divided into 3, 400 m^2 plots of equal size. A 15 x 15 grid in the center of plots was used to randomly position starting points for transects. Three soil cores were taken along 2, 6 m transects (Fig. 2). Root tips were sorted from 2 sections of equal volume from each core.

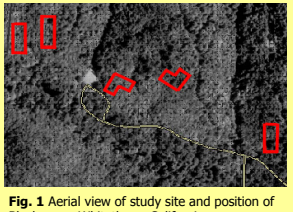

Fig. 1 Aerial view of study site and position of Blocks near Whitethorn, California.

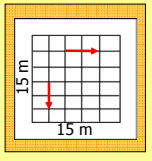

Fig. 2 Positions of transects.

Materials and methods

2. Identifying Taxa

A. Root tips were separated from soil.
B. DNA from bulked root tips were extracted and purified using a MoBio soil DNA extraction kit.
C. Equal volumes of extracted DNA were combined from each plot and amplified using fungal-specific primers (ITS1F, TW13).
D. PCR products from plots were cloned in plasmids and transformed in DHα1 strain of *E. coli* (Invitrogen, Carlsbad, CA).
E. To rapidly identify unique ITS signatures, real-time PCR amplification was performed using of eukaryote-specific ITS primers (ITS3, ITS4) with *Sybr* green fluorescent dye on the Bio-Rad Icycler (Fig. 4).
F. The ITS and 28s regions of the rDNA were sequenced on the ABI 3100.
G. Sequences were blasted against accessions in GenBank.

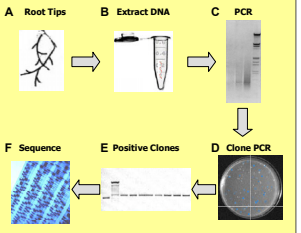

Fig 3. Methods used for molecular identification of ectomycorrhizal fungi associated with tanoak roots.

Results

In total, 128 ectomycorrhizal taxa were detected from 5 blocks. Forty-four ectomycorrhizal fungi were encountered on more than 1 plot (Fig. 5). Common ectomycorrhizal fungi included Ascomycetes (*Cenococcum geophilum, Phialophora*) and Basidiomycetes (*Boletus, Lactarius, Macowanites, Melanogaster, Ramaria*, and *Sebacina* spp.). Several ectomycorrhizal lineages were well represented including Cortinariod (*Cortinarius, Inocybe* - 10 spp.), Russuloid (*Lactarius, Macowanites, Russula* – 9 spp.) Thelephoroid (*Tomentella*, '*Thelephora*-like' – 10 spp.), and Tricholomatoid (*Tricholoma* – 7 spp.).

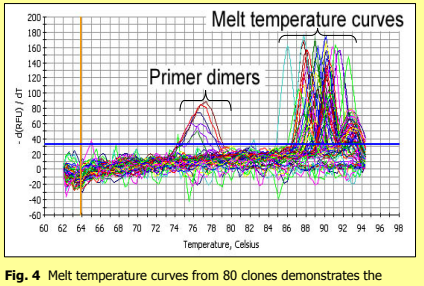

Fig. 4 Melt temperature curves from 80 clones demonstrates the diversity of ITS melt temperature signatures.

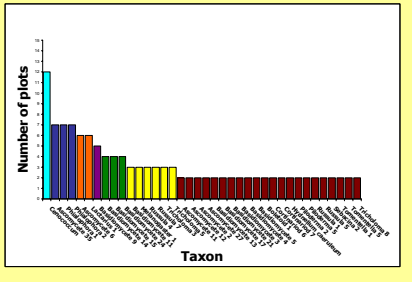

Fig. 5 The frequency of occurrence of ectomycorrhizal taxa detected from 15 plots.

'Crustlike' fruitbody of *Sebacina* sp.

Basidiocarp of *Tricholoma magnivelare*

Basidiocarp of *Macowanites* sp. (secotiod *Russula*)

Cenococcum geophilum ectomycorrhizal root tip

Conclusions

The ITS region is often employed in studies for molecular characterization of ectomycorrhizal communities to identify distantly related species or to differentiate closely related taxa (Horton *et al.* 2001). Although the ITS may fail to distinguish between sibling species, the rapid accumulation of sequence data in publicly accessible domains (e.g. GenBank) may significantly aid in identification when compared to traditional morphotyping methods (Horton *et al.* 2001, Buscot *et al.* 2000).

In this study, ITS and 28s rDNA sequences aided in identification of 128 ectomycorrhizal fungi. Compared with other studies of ectomycorrhizal diversity in stands dominated by one or more hosts, taxonomic diversity of ectomycorrhizal fungi associated with tanoak is similar (Horton *et al.* 2001). Several genera of ectomycorrhizal fungi recovered in this study including *Cenococcum, Phialophora, Sebacina,* and unidentified Thelephoroid spp. were previously identified on tanoak seedlings (Kennedy *et al.* 2003); however, the taxonomic diversity of *Tricholoma* was unexpected.

The data collected in this study can be used as a baseline comparison for future studies designed to investigate the impacts of *P. ramorum* on the biomass of ectomycorrhizal fungi associated with tanoak.

Literature cited

Buscot F, Munch JC, Charcosset JY, Nehls U, Hampp R. 2000. Recent advances in exploring physiology and biodiversity of ectomycorrhizas highlight the functioning of these symbioses in ecosystems. *FEMS Microbiology Reviews* 24:601-614.

Horton TR, Bruns TD. 2001. The molecular revolution in ectomycorrhizal ecology: peeking into the black-box. *Molecular Ecology* 10:1855-1871.

Kennedy PG, Izzo AD, Bruns TD. 2003. There is high potential for the formation of common mycorrhizal networks between understorey and canopy trees in a mixed evergreen forest. *Journal of Ecology* 91:1071-1080.

Acknowledgments

We would like to thank Timothy Metz for the use of his private land. Also, thanks to Katy Hayden, Rachel Linzer, Will VanSant, Steven Swain, and Lori Miles for assistance with field work. Special thanks to Matt Meshriy for photographs and assistance with several aspects of this project. Funding for this project was provided by the Gordon and Betty Moore Foundation.

For further information:

Please contact *sbergemann@nature.berkeley.edu*. More information on this and related Sudden Oak Death projects can be obtained at: http://nature.berkeley.edu/garbelotto/