Greater Yellowstone Coordinating Committee

Progress Report
FY 2008

Unit: Cathy L. Cripps, MSU & Mary Hektner, Yellowstone National Park

Project Name: Soil Microbes that sustain YNP Whitebark Pine Forests: inventory of Native Mycorrhizal Fungi and their Preservation

Project Description: Whitebark pine trees/seedlings cannot survive in nature without the appropriate (native) mycorrhizal fungi. The overarching goal of this project is to discover the native mycorrhizal fungi (particularly suilloids) important to whitebark pine in YNP, and especially along Dunraven Pass. The second goal is to develop a method for long term preservation of viable DNA for these ecotypes. We seek to identify and preserve these fungi for future use and before they are lost.

GYCC Funding Received: $5,000
Partner Funding/In-Kind Received: 0 (but see notes below)

Status of the Project: <u>Continuing through August 2009. This is a progress report.</u>

Objectives and accomplishments to date:

1. Sporocarps of native ectomycorrhizal fungi were collected, identified, and recorded in a database during several field trips to YNP, particularly in whitebark pine forests along Dunraven Pass. These fungi, plus native mycorrhizal fungi with whitebark pine from the Greater Yellowstone Ecosystem are currently in vitro or in dried form at MSU. Ten of these are in culture and are being tested (see table). 2). Dry weather precluded fruiting of some fungi however our project continues through the field season and we will collect more species for testing at this time.

2) Methods for the long term storage of viable DNA of collected ecotypes of native ectomycorrhizal fungi from whitebark pine forests are underway at the MSU mycology lab for the following treatments:

Tissue culture of fungal sporocarps on agar media (MMN) stored in:

i. In Petri plates. ii. Slant tubes. iii. blocks in water. iv. cryofluid at -60^{0}C. v. on wood. vi. on paper.

Table of methods currently under investigation and ectomycorrhizal fungi being tested by each method.

	Long term preservation method					
	Agar plates	Agar slant tubes	Sterile water tubes	Cryo fluid tubes	Colonized toothpicks	Colonized filter paper
Cenococcum	✓	✓	✓	✓	✓	✓
GTL – *S. subalpinus*	✓	✓	✓	✓	✓	✓
Hysterangium	✓	✓	✓	✓	✓	✓
HYP 1 – *R. salebrosus*	✓	✓	✓	✓	✓	✓
2035 *Rhizopogon subpurpurascens*	✓	✓	✓	✓	✓	✓
2199 *Suillus* sp.	✓	✓	✓	✓	✓	✓
2341 *S. subalpinus*	✓	✓	✓	✓	✓	✓
2344 *S. variegatus*	✓	✓	✓	✓	✓	✓
2345b *S. subalpinus*	✓	✓	✓	✓	✓	✓
2347 *S. subalpinus*	✓	✓	✓	✓	✓	✓

<u>Methods</u>

<u>Agar plates:</u> All ectomycorrhizal fungi were grown on MMN agar plates. Subcultures (agar plugs with 0.5 mm diameter) of all fungi were taken from the active growing region and placed onto fresh MMN agar plates. Full grown plates will be stored at 4˚C.

Note: You may expand and reduce size of blocks.

Agar slant tubes: Standard glass culture tubes were partially filled with MMN agar and sterilized. The glass tubes were placed at an angle during cooling to give a large slanted surface for inoculation. One agar plug (5 mm in diameter) per agar slant tube of the tested actively growing ectomycorrhizal fungi was placed onto the middle of agar. The tubes were capped tightly and stored at room-temperature.

Sterile water tubes: Standard glass culture tubes were filled with 15 ml of bottled water and sterilized. Five agar plugs (5 mm in diameter) of all ectomycorrhizal fungi were added separately to the sterile water tubes, closed tightly and placed into a refrigerator.

Cryo fluid tubes: Cryo fluid consisting of 1 g tryptic soy broth (TSB), 10 ml glycerol, and 90 ml distilled water was sterilized. Two agar plugs (5 mm in diameter) of all ectomycorrhizal fungi were added separately to cryogenic vials (2 ml volume) and covered with 1.5 ml cryo fluid. The vials were closed tightly and placed into a freezer at – 80˚C.

Colonized toothpicks and filter paper: Standard Whatman filter paper was cut into 1 cm x 1 cm pieces and placed into glass jars. Regular toothpicks were also placed in separated glass jars and sterilized together with the filter paper pieces. One agar plug (5 mm in diameter) of actively growing ectomycorrhizal fungi was placed separately into the middle of regular MMN agar plates. Sterile toothpicks or filter paper were arrange around the fungal subculture to be colonized by the ectomycorrhizal fungus. Colonized toothpicks and filter paper will be dried and stored in sterile glass jars at 4˚C.

b. Viability of "stored fungi" will be tested at the end of the period by growing out fungi from storage.
c. Viability of fungal spores will also be tested (yet to be done).
d. We will also collect and test additional fungi during and after the next field season.

This project fits the GYCC priorities in terms of: Ecosystem Health (species of the brink) and protecting the integrity of the GYA Landscape. It also fits the NPS needs within the Rocky Mountains CESU in terms of several topics: Baseline data on Whitebark pine, Inventory Fungi, and Monitoring Whitebark pine.

Products that can be shared across the GYA: (GIS data layers, maps, new protocols and methods) An in depth report (separate from this document) with supporting data will be available to be shared across the GYA online. Products will be a) methods for the preservation of native mycorrhizal fungi with whitebark pine (before fungi are lost), b) a collection of viable native fungi valuable for restoration in the GYA, c) inventory of native mycorrhizal fungi important to whitebark pine in the GYA.

Project results: (Information worth sharing on methods, results, partnerships, etc)
This work is synergistic with our work funded as a Whitebark Pine Foundation project to inoculate nursery seedlings with native fungi ($7,800) The GYCC project has helped to provide native fungi for this other project and in turn the GYCC project has helped to develop a method for long term storage of these native fungi. We have had recent success producing nursery seedlings colonized with native fungi for reforestation! Our previous work along Dunraven Pass funded by GYCC suggested that not all planted seedlings of whitebark pine have timely colonization of mycorrhizal fungi and that it might be necessary to inoculate nursery seedlings in the future with non-exotic, not commercial fungi native to the region. We have also partnered with Cyndi Smith (Waterton Lakes National Park) and Bob Keane (Fire Ecology) in projects involving whitebark pine mycorrhizae. Our overarching goal is to discover these fungi, understand their ecology and physiology and to preserve them; they are a necessary component for the sustainability of whitebark pine forests into the future.

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Submit to Virginia Kelly**:** vkelly@fs.fed.us 406-587-6704. Contact Virginia with questions.