

Cryogenic Preservation of Fungi on Barley Grains

Melodie Moss, Dr. William Jacobi, and Ronda Koski
Department of Bioagricultural Sciences and Pest Management

Introduction

The Forest and Shade Tree Health Laboratory of the Bioagricultural Sciences and Pest Management Department of Colorado State University has a fungal collection with over 350 specimens. Maintenance of the collection is a time intensive process, and storage of the collection requires two full-size refrigerators.

A procedure developed at North Dakota State University allows for the long-term storage of fungal pathogens on sterilized barley grains at -80°C thus minimizing both the time and space requirements. The information on the new procedures suggested that fungi could be transferred to barley and stored in a -80°C freezer for an extended period of time and still remain viable.

Presently, the isolates are maintained on potato dextrose agar (PDA) slants in test tubes and stored in the refrigerators. Periodically, each isolate in the collection has been transferred to a petri dish to assure viability then transferred back to a PDA slant for storage.

Research Objective

The objective of this research is to test each of the specimens in the fungal collection of the Forest and Shade Tree Health Laboratory to verify that it can withstand the -80°C freeze treatment and still remain viable.

Methods

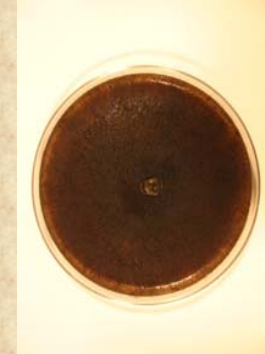
- Fungal tissue from each PDA slant was placed in a 100 x 15 mm petri dish containing approximately 20 mL of PDA.
- Each petri dish was sealed with wax film.
- Fungal tissues were allowed to grow.
- Pearl barley was sterilized in an autoclave and stored in a freezer until use.
- Two sterilized barley grains were placed in each petri dish.
- Each petri dish was again sealed with wax film.
- The barley grains stayed on the mycelium until they appeared to be infested with the fungal isolate.
- Each barley grain was removed, placed in a vial, and stored at -80°C .
- Barley grains were stored in the freezer for a minimum of two weeks.
- After removal from the freezer, each barley grain was placed in a petri dish containing approximately 20 mL of PDA and allowed to grow.

Results

Fungi successfully recovered from infested barley grains stored at -80°C include: *Thyronectria austro-americana*, *Leptographium wageneri*, *Armillaria* species, *Fomes nigrolimitatus*, *Stereum sanguinolentum*, and *Nectria cinnabarina*. We have had limited success with various *Cytospora* species.



Thyronectria austro-americana



Leptographium wageneri



Armillaria sp.

Conclusion

Although we have only completed about one-fourth of our research project, it appears that the -80°C freeze treatment will be a more efficient way to store the fungal collection of the Forest and Shade Tree Health Laboratory.

Future Research

Further research needs to be conducted to determine the effects of subjection of plant pathogenic fungi to -80°C . Thus the following questions are worthy of future scientific investigation:

- Does the subjection of plant pathogenic fungi to -80°C cause certain fungi to become stressed enough to change from asexual to sexual stages?
- Does the subjection of plant pathogenic fungi to -80°C cause genetic mutations that alter host specificity?
- Does the subjection of plant pathogenic fungi to -80°C affect the virulence of the fungal pathogens?
- Can all plant pathogenic fungi be stored on infested barley grains for long periods of time at -80°C ?