

MAINTAINING A BACKCROSS ORCHARD PLANTING TO ASSESS THE INTEGRATION OF HOST RESISTANCE AND HYPOVIRULENCE

A research proposal submitted to:

The American Chestnut Foundation

7 March 2012

Principal Investigators:

William MacDonald and Mark Double, West Virginia University

In cooperation with

Sara Fitzsimmons and Fred Hebard, The American Chestnut Foundation

Duration of Project: May 1, 2012 to December 31, 2012

Total Amount Requested: \$ 2,150

This proposal mirrors one that was funded in 2011 and provided critical support for this project.

Rationale:

The goal of The American Chestnut Foundation is to restore the American chestnut tree to the forests of the eastern United States. The Foundation has focused its efforts on the development of a blight resistant tree that combines the genes from resistant Chinese chestnut and susceptible American chestnut through a backcross breeding program. A second approach to the control of chestnut blight is the utilization of the phenomenon of hypovirulence whereby a virus reduces the ability of the blight fungus to produce lethal cankers on American chestnut. Numerous studies in the Appalachians have employed hypovirulent strains for biological control, but in most instances, virulent infections by *Cryphonectia parasitica* eventually have resulted in tree mortality because of the extreme susceptibility of the American chestnut to blight.

The backcross breeding program should yield progeny that possess genes to enhance their resistance to chestnut blight. However, at this time, no long-term tests of these “blight resistant” trees have been conducted. Their success in Appalachian forests may depend on the varied levels of resistance they possess as well as other environmental, genetic and cultural factors. One component that may aid in the successful establishment of the backcross trees is the contribution that can be made by the utilization of hypovirulent strains of *C. parasitica*. We believe hypovirulent strains can play a more significant role if they are able to persist longer on populations of moderately resistant backcross trees. The long-term objective of this project, therefore, is to evaluate the enhanced control of chestnut blight that results when trees with increased levels of blight resistance are combined with hypovirulent strains of *C. parasitica*. An experiment that couples these two technologies is underway at the West Virginia University Plant and Soil Sciences Farm in Morgantown.

Project Background Information:

This project was initiated in April 2005 in cooperation with Sara Fitzsimmons and Fred Hebard of The American Chestnut Foundation. The experimental design and planting were facilitated collaboratively. Plantings were established that year and in subsequent years with the invaluable help of the Meadowview Farm crew. The planting consists of six replicate plots each containing 150 trees. Backcross lines (B₂F₂ and B₂F₃), obtained from The American Chestnut Foundation, along with pure American, Chinese and European chestnuts were included in each of the plots. Animal predation of the seed initially was a problem, destroying most of the planting in 2005. In other years, drought has been a problem. Plots have been replanted each year from 2006-2011. Many of the trees in the six plots now range in size from 1-to-3 meters, although some are still seedling size (Fig.1). As of May 2011 survival was as follows: American lines (87%); Backcross lines (88%); Chinese lines (95%); and, European lines (45%).



Figure 1. A row of trees in Plot 1 photographed in May 2011.

Purpose of this funding request:

This project continues to be labor intensive since its initiation in 2005. Each summer, we have made every effort to maintain the plantings at the level necessary to maximize tree growth. This situation has improved during the past three summers as a result of the funding TACF has provided for maintenance. These monies have enabled us to provide partial funding for undergraduate forestry/horticulture students who can assist us on a part-time basis. In the absence of this assistance, the various maintenance tasks fall largely to Double/MacDonald. The College of Agriculture, Natural Resources and Design does not have an adequate number of farm workers to assist with planting and maintenance efforts. This year, maximizing tree growth is particularly important given that we will be introducing hypovirulent inoculum in the near future.

Project goals:

Short-term goal: The short-term goal is to optimize the level of orchard maintenance to enhance growth.

Long-term goal: The ultimate goal is to evaluate the control of chestnut blight that occurs when trees with various levels of blight resistance are combined with diminished virulence of *C. parasitica* resulting from hypovirus infection.

Procedures:

Several problems are associated with the growth and survival of the chestnut trees at the West Virginia University Plant and Soil Sciences Farm. Animal issues that plagued us in the past have been minimized, but competing vegetation is the most significant problem affecting tree growth. When planting in old orchard or farm sites, competition from grass and other vegetation can limit growth and survival. The orchard is still young so maintenance remains a critical issue to the success of the project.

Planting and maintenance strategies, provided by Sara Fitzsimmons, include:

- Multiple applications of herbicide to control vegetation
- Insecticide sprays for Ambrosia beetle control
- The application of high nitrogen fertilizer (slow release) in the spring and possibly a second application later in the season
- Watering when drought conditions develop
- Mulching to help retain soil moisture and control weeds

Timeline:

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| Spring 2012 | Inventory plots for growth and survival of existing seedlings, replant as necessary. Fertilize and mulch existing trees, initiate weed and insect control efforts, mow planting. |
| Summer 2012 | Maintain weed and insect control; begin watering program, continue to monitor inventory, mow planting. |
| Fall 2012 | Continue weed control program and watering regime, apply fall fertilizer treatments, mow planting, supplement mulching as needed. Complete 2012 seedling inventory. |

Budget Dialogue:

Monies requested would be used to support part-time undergraduate students to provide the majority of the maintenance of the planting from May-October. The students would work variable hours depending on the need for weed and insect control, mowing, mulching, watering, and would assist Double/MacDonald with inventory and record keeping. Additional monies are included for mulch, fertilizer and herbicides.

Budget:

May 1, 2012-Dec. 31, 2012

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| Undergraduate student labor (May-Oct.) approx. 8 hr/wk @ \$9.50/hr..... | \$1800.00 |
| Supplies (mulch, fertilizer, insecticide, herbicide) | \$350.00 |
| Total Requested..... | \$2150.00 |

CURRICULUM VITAE

Name: William L. MacDonald
Date of Birth: March 25, 1943
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West Virginia University
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EDUCATION

B.A. Miami University, Oxford, Ohio
Ph.D. Iowa State University, Ames, Iowa

POSITIONS HELD

1970-71 Post-Doctoral Fellowship; Department of Plant Pathology, University of Wisconsin, Madison
1971- Assistant Professor (1971-76); Associate Professor (1977-82); Professor (1983-present), Division of Plant and Soil Sciences, West Virginia University

RESEARCH INTERESTS

General area of training has been in forest pathology, particularly fungus diseases of hardwoods. Doctoral and post-doctoral research dealt with vascular wilt diseases (Dutch elm disease and oak wilt). Initial and continuing research at West Virginia University is with oak wilt including study of the factors influencing disease spread (e.g. root grafting) host-parasite interactions and resistance. Major research emphasis since 1978 has been with the biological control of chestnut blight using transmissible hypovirulence. Research has been to study the biology of virulent and hypovirulent strains in forest settings.

PROFESSIONAL MEMBERSHIPS

The American Phytopathological Society
Phi Kappa Phi
The American Chestnut Foundation

RECENT PROFESSIONAL ACTIVITIES AND HONORS

Member of the Board of Directors, The American Chestnut Foundation
Benedum Distinguished Scholar Award (1998)

SELECTED CHESTNUT-RELATED PUBLICATIONS

Liu, Y.-C., P. Cortesi, M.L. Double, W.L. MacDonald, and M.G. Milgroom. 1996.
Diversity and multilocus genetic structure in populations of *Cryphonectria parasitica*.
Phytopathology 86:1344-1351.

- Liu, Y.-C., M.L. Double, W.L. MacDonald, and M.G. Milgroom. 2002. Persistence of *Cryphonectria* hypoviruses after their release for biological control of chestnut blight in West Virginia forests. *Forest Pathology* 32:345-356.
- MacDonald, W.L. and M.L. Double. 2006. Hypovirulence: use and limitations as a chestnut blight biological control. Pages 87-95 in: Steiner K.C. and J.E. Carlson, eds. *Restoration of American Chestnut to Forest Lands-Proceedings of a Conference and Workshop*. May 4-6, 2004, The North Carolina Arboretum, Natural Resources Report NPS/NCR/CUE/NRR-2006/001, National Park Service, Washington, DC.
- MacDonald, W.L., M.L. Double and D.L. Nuss. 1998. Variation in growth and sporulation of *Cryphonectria parasitica* isolates as influenced by hypovirus infection. *Proceedings of the Second International Chestnut Symposium*. Bordeaux, France. October 19-23, 1998.
- McGuire, I.C., J.E. Davis, M.L. Double, W.L. MacDonald, J.T. Rauscher, S. McCawley and M.G. Milgroom. 2005. Heterokaryon formation and parasexual recombination between vegetatively incompatible lineages in a population of the chestnut blight fungus, *Cryphonectria parasitica*. *Mol. Ecol.* 14: 3657-3669.
- Root, C., C.J. Balbalian, R. Beirman, L.M. Geletka, S.L. Anagnostakis, W.L. MacDonald, M.L. Double and D.L. Nuss. 2005. Multiseasonal field release and spermatization trials of transgenic hypovirulent strains of *Cryphonectria parasitica* containing cDNA copies of hypovirus CHV1-EP713. *Forest Pathology* 35:277-297.

Recent Theses and Dissertations:

- Eggers, Jordan E. 2009. Variation among *Phytophthora cinnamomi* isolates from oak forest soils in the eastern United States. M.S. Thesis, West Virginia University.
- Jakobi, Steven R. 2005. The Effects of hypovirulent *Cryphonectria parasitica* inoculum, developmental stages of cankers, and time of year of wounding on the survival of the American chestnut, Ph.D. Dissertation, West Virginia University.
- Rittenour, W.R. 2005. The biological control potential of *Cryphonectria parasitica* strains containing an infectious cDNA copy of the hypovirus CHV1-Euro7. M.S. Thesis, West Virginia University.

Recent Grants:

- Evaluation of the effect of *Cryphonectria parasitica* epidemic and hypovirus introduction at the West Salem, WI American chestnut stand. The American Chestnut Foundation, \$5,500 (2003); \$2,500 (2006); \$5,000; \$5,500 (2008)
- Evaluation of select chestnut sites in the Great Smoky Mountains National Park for putatively hypovirulent isolates of *Cryphonectria parasitica*, \$18,000 (2010-2012)
- Ecological factors influencing incidence and severity of Beech bark disease. USDA-Forest Service, \$69,500 (2010-2012)
- *Phytophthora* species associated with potential *Phytophthora ramorum* in the central and eastern U.S. USDA-Forest Service, \$50,000 (2004-2006)
- Release of transgenic strain of *Cryphonectria parasitica* for a biological control of chestnut blight. USDA-Forest Service, \$54,700 (2004-2007)
- Evaluating soil-borne organisms associated with white oak decline in southern Ohio. USDA-Forest Service, \$26,000 (2006-2008)