

INTEGRATION OF HOST RESISTANCE AND HYPOVIRULENCE

A research proposal submitted to:
The American Chestnut Foundation

1 August 2014

Project Summary:

TACF's backcross breeding program should yield offspring with enhanced resistance to chestnut blight. 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*. Such strains may play a more significant role in controlling blight if they can persist longer on populations of moderately resistant trees. The long-term objective of this project remains to evaluate whether the use of hypovirulent strains can complement the enhanced level of resistance provided by the backcross trees. An experiment that couples these two technologies has been underway at the West Virginia University Plant and Soil Sciences Farm in Morgantown since 2005. The monies requested by this proposal largely will be used to maintain the plantings.

Principal Investigators:

William MacDonald and Mark Double, West Virginia University

collaborating with

Sara Fitzsimmons and Fred Hebard, The American Chestnut Foundation

Duration of Project: January 1, 2015 to December 31, 2015

Total Amount Requested: \$ 3150.00

The maintenance portion of this proposal reflects previously funded TACF proposals. A summary of the research component for which the orchards are being developed has been added.

Project goals:

Short-term goal: The short-term goal remains to optimize the level of orchard maintenance thus enhancing tree growth.

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

Narrative:

The American Chestnut Foundation has undertaken the goal of restoring 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 blight resistant Asiatic chestnut and susceptible American chestnut via 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 this biological control approach, 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.

Background Information:

This project was initiated in April 2005 in collaboration with colleagues from The American Chestnut Foundation. Plantings were established that year with the invaluable help from the Meadowview Farm crew. The research planting consists of six replicate plots each containing 150 trees. Backcross lines (B₂F₂, B₃F₂ and B₂F₃), obtained from the Meadowview Farm are included in the research plots along with are pure American, Chinese and European chestnuts. Unfortunately, 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 2006-2012. Many of the trees in the six plots now range in height from 1-to-9 meters, although some are still seedling size (Fig.1). As of May 2014 survival was as follows: American lines (63%); Backcross lines (71%); Chinese lines (93%); and, European lines (44%). An ancillary aspect of this project is the experimentation will provide a comparative test of the resistance/susceptibility of the three chestnut species and the various backcross hybrids included in the plantings.

Purpose of this Funding Request:

Maintenance: This project continues to be labor intensive since its initiation in 2005. Each growing season, we have made every effort to care for the plantings at the level necessary to maximize tree growth. Maintenance has improved during the past five summers as a result of the funding TACF has provided. These monies have enabled us to provide partial funding for undergraduate forestry/horticulture students who can assist us on a part-time basis. Unfortunately, the College of Agriculture does not have an adequate number of farm workers to help with the planting.

Research Component: As of 2013, many of the trees had reached the size where their growth was adequate to begin the experiment.

Procedures:

Maintenance: Competing vegetation is the most significant problem affecting tree growth. The plantings are on an old farm site, so competition from grass and other weedy vegetation remains a significant problem. Because the orchards are still young, a high degree of maintenance is critical. Included in the list of maintenance issues are:

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

Research Component: The steps used in 2013 to begin evaluating the hypovirus-genotype interaction have been continued in 2014 and are now part of this 2015 funding request:

- Each season, different sets of the largest trees of each species or hybrid group in each plot will be inoculated with a virulent strain (Weekly) of *C. parasitica*
- As wild-type cankers arise in the hypovirulent plots, they will be treated with a mixture of isolates infected by two CHV1 hypoviruses (Euro 7 and WK2) and two CHV3 hypoviruses (GH2 and County Line); cankers that arise in the virulent plots will be treated with water agar
- A late-season sampling of all infections will be made to verify the infecting strain and to determine whether they have acquired hypovirus.
- Canker morphology (appearance-virulent or hypovirulent) will be scored using a subjective rating scale
- Annually, the diameter, height, infection status and survival of all trees in the three virulent (control) and three hypovirulent plots will be recorded.

Timeline:

Spring 2015	Fertilize and mulch trees, continue weed and insect control as needed, mow planting examine the trees for new infections, treat infections
Summer 2015	Continue canker treatment as new infections are discovered.
Fall 2015	Apply plot maintenance procedures. Continue canker treatment. Sample all infections to verify the infecting strain and evaluate hypovirus acquisition. Evaluate canker morphology.

Analysis and Reporting:

Growth and survival measurements taken at the end of each growing season will be used as one measure of the effect of the virulent and hypovirulent infections on each of the species or TACF hybrids. Additionally, the development of infections that arise from inoculations as well as those that develop naturally will be assessed by measuring their size, evaluating their morphology and by culturing the infecting strains to determine whether they are virulent or hypovirulent. As the experiment progresses periodic reports will be made to TACF and the USDA-regional chestnut research project (NE-1333). Because the project is long-term, final results will not be published for 3-4 years.

Budget Dialogue:

Monies requested would be used to support part-time undergraduate students to provide the majority of the maintenance of the planting from April-November. The students would work variable hours depending on the need for weed and insect control, mowing, mulching, watering, They also would assist Double/MacDonald with canker sampling, plot inventory and record keeping. Additional monies are included for mulch, fertilizer and herbicides. WVU will contribute to the salaries of the principal investigators and the field and laboratory facilities to conduct the experimentation.

Budget: January 1, 2015-December 31, 2105

Undergraduate student labor (April-Nov.) approx. 8 hr/wk @ \$9.00/hr	\$2800.00
Supplies (mulch, fertilizer, insecticide, herbicide)	\$350.00
Total Requested.....	\$3150.00



Figure 1. Trees in Plot 1 in July, 2014.

CURRICULUM VITAE

William L. MacDonald

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EXPERTISE

Area of training has been in forest pathology, particularly in fungus diseases of hardwoods. Major research emphasis 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. In the past six years, other studies have included the roles species of *Phytophthora* play in oak forest health relative to root disease and the ecological factors that influence the incidence of Beech Bark Disease.

PERSONAL PREPARATION

Miami University, Oxford, OH	Botany	B.A.	1965
Iowa State University, Ames, IA	Plant Pathology	Ph.D.	1970
University of Wisconsin, Madison, WI	Post-Doctoral Fellowship		1970

APPOINTMENTS

1983-present	Professor, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV
1977-1982	Associate Professor, Division of Plant and Soil Sciences, West Virginia University
1971-1976	Assistant Professor, Division of Plant and Soil Sciences, West Virginia University
1970-1971	Post-Doctoral Fellowship; Department of Plant Pathology, University of Wisconsin, Madison

PUBLICATIONS

Most Closely Related to Project

- Hebard, F.V., M.L. Double and W.L. MacDonald. 2007. A Pathogen Without Rival. In: *Mighty Giants, An American Chestnut Anthology*, Pages 171-177. C. Bolgiano and G. Novak, eds. American Chestnut Foundation, Bennington, VT.
- 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.

- 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.

Other Significant Publications

- Balci, Y., R. Long, M. Mansfield, D. Balser and W. MacDonald. 2010. Involvement of *Phytophthora* species in white oak (*Q. alba*) decline in southern Ohio. *Forest Pathology* 40:430-442.
- Double, M.L., W.L. MacDonald and G. Taylor. 2013. Evaluation of *Cryphonectria parasitica* isolates collected from the Great Smoky Mountains National Park. 2013. In: Proceedings of the Fifth International Chestnut Symposium, Sept 4-8, 2012, Shepherdstown, WV, ISHS Press, Leuven, Belgium (in press).
- Double, M.L., W.L. MacDonald, A.M. Jarosz, D.W. Fulbright, J. Cummings Carlson, S. Dahir and A. Davelos Baines. 2013. Recapping twenty years of biological control efforts in a stand of American chestnut in western Wisconsin. *J. American Chest. Found.* 27:19-23.
- Eggers, J., Y. Balci and W.L. MacDonald. 2012. Variation in *Phytophthora cinnamomi* isolates from oak forests in the eastern United States. *Plant Disease* 96:1608-1618.
- Juzwik, J., D. Appel, W. MacDonald and S. Burke. 2011. Challenges and successes in managing oak wilt. *Plant Dis.* 95:888-900.

SYNERGISTIC ACTIVITIES

- Member of the Board of Directors, The American Chestnut Foundation
- Member, USDA Board of Invasive Species
- Member, Forest Pathology Committee, The American Phytopathological Society
- Member, National Academy of Science Committee on Predicting Invasives of Indigenous Plants and Pests
- Senior Editor, *Plant Disease*

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EDUCATION

West Virginia University, Morgantown, WV	B.A., Biology	1974
West Virginia University, Morgantown, WV	M.S., Environmental Microbiology	1977

APPOINTMENTS

1977-present Research Associate and Chemical Hygiene Officer
Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV

SELECT PUBLICATIONS

- Double, M.L., W.L. MacDonald, A.M. Jarosz, D.W. Fulbright, J. Cummings Carlson and S. Dahir. 2013. Recapping twenty years of biological control efforts in a stand of American chestnut in western Wisconsin. *J. Amer. Chest. Found.* 27:19-23.
- Double, M.L., W.L. MacDonald and G. Taylor. 2014. Evaluation of *Cryphonectria parasitica* isolates collected from the Great Smoky Mountains National Park. *Acta Hort.* 1019:85-89.
- Double, M.L. and M. Marshall. 2014. Strawberry amendment to potato dextrose agar to increase conidiation in *Cryphonectria parasitica*. *Acta Hort.* 1019:81-84.
- Double, M.L. and W.L. MacDonald, eds. 2014. Proceedings of the Fifth International Chestnut Symposium, 4-8 Sep 2012, Shepherdstown, WV, 270 pp.
- Double, M.L., M.R. Kolp, A.M. Jarosz, A. Davelos Baines, D.W. Fulbright and W.L. MacDonald. 2014. Fungi associated with hypovirulent cankers of differing ages on American chestnut. Second European Congress on Chestnut, 9-12 Oct 2013, Debrecen, Hungary (in press).
- Hebard, F.V., M.L. Double and W.L. MacDonald. 2007. A Pathogen Without Rival. In: *Mighty Giants, An American Chestnut Anthology*, Pages 171-177. C. Bolgiano and G. Novak, eds. American Chestnut Foundation, Bennington, VT.
- Jarosz, A.M., J.C. Springer, D.W. Fulbright, M.L. Double and W.L. MacDonald. 2014. Hypovirus influence on survivorship and growth of American chestnut at West Salem, Wisconsin, USA. 2013. *Acta Hort.* 1019:157-163.
- Kenaly, S.C., M.L. Double and W.L. MacDonald. 2014. Effect of spore concentration on the establishment of cytoplasmic hypovirulent (hv), transgenic hv and virulent isolates of *Cryphonectria parasitica*, the chestnut blight fungus. *Acta Hort.* 1019: 165-171.
- 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.

McGuire, I.C., J.E. Davis, M.L. Double, W.L. MacDonald, T. Raushcer, 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. Bierman, 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. For. Path.35:277-297.

UNIVERSITY COMMITTEES

- ❖ Davis College of Agriculture, Forestry and Consumer Sciences Staff Council Executive Committee, 2007-2012; Chair 2008-2009
- ❖ South Agriculture Sciences Safety Committee, Chair, 2006-2014
- ❖ CERT (Campus Emergency Response Team) Training, 20 hour course, 2009
- ❖ Davis College Communication Team, 2011-2012
- ❖ Plant and Soil Sciences Division Director Search Committee, 2014