Secondary microorganisms in chestnut blight cankers: Can they reduce blight severity and be used as a biological control agents?

Investigators:
Andrew Jarosz, Michigan State University (MSU) – Departments of Plant Biology and Plant, Soil and Microbial Sciences
Matthew Kolp, MSU – Department of Plant Biology
Dennis Fulbright, MSU – Department of Plant, Soil and Microbial Sciences
William MacDonald, West Virginia University (WVU) – Division of Plant and Soil Sciences
Mark Double, WVU – Division of Plant and Soil Sciences

Duration of Project: 12 Months

Total amount requested: $10,000

Summary:
Antagonistic microorganisms have been implicated as potential biological control agents for chestnut blight in North America. However, potential antagonists have been isolated most often from soils or transferred from other plant-pathogen systems with limited success presumably because they do not survive and proliferate well within a chestnut blight canker. We propose to identify potentially antagonistic organisms by isolating them from non-girdling cankers on surviving American chestnut trees. These organisms will be evaluated for their ability to inhibit *Cryphonectria parasitica* growth. Our long-term goal is to develop protocols for their use as biological control agents.

Short and Long-term goals of project:
Short-term goals: Identify secondary fungi (Non-*Crypohonectria parasitica;* hereafter Non-CP) associated with non-girdling and girdling chestnut blight cankers on American chestnut trees in several regions of North America. Non-CP isolates from cankers will be evaluated for their ability to: i) inhibit *C. parasitica* growth under laboratory conditions; ii) reduce canker expansion on excised chestnut stems; and, iii) limit canker expansion on American chestnut trees. Long-term goal: Identify Non-CP species that can be used to manage chestnut blight either alone or in conjunction with hypovirus treatments.

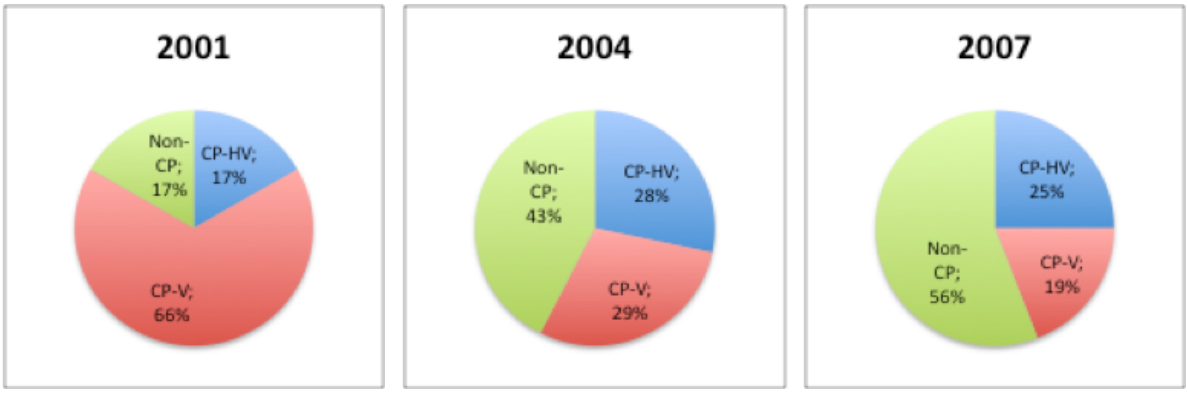
Background:
Chestnut blight, caused by the ascomycete fungus *Cryphonectria parasitica* (Murr.) Barr, is native to Asia but was introduced unintentionally to the North American and European continents. The epidemics that ensued devastated the American (*Castanea dentata* [Marsh.] Borkh.) and European (*C. sativa* [Mill.]) chestnut species, respectively (Griffin and Elkins 1986; Anagnostakis 1987). However, chestnuts in some areas of Europe have recovered from the epidemic due to the spread of hypoviruses. These virus-like agents can reduce pathogen virulence to the level that trees wall off and contain the infection, thereby enabling the tree to survive and grow (Heiniger and Rigling 1994). In North America, however, the spread of hypoviruses throughout pathogen populations in chestnut forests has been limited and as a result success comparable to that observed in Europe has not occurred (Milgroom and Cortesi 2004). There has been considerable speculation as to the reason hypoviruses have failed to regulate blight in North America. Anagnostakis *et al.* (1986) suggested that *C. parasitica* populations here are more diverse with respect to vegetative compatibility groups (*i.e.* genes governing transmission of hypovirus), which could impede the spread of hypoviruses within *C. parasitica* populations (Liu *et al.* 1996; Milgroom 1999). Hebard *et al.* (1984) suggested that the American chestnut is more susceptible to blight than European chestnut. The consequences of higher susceptibility in American chestnuts are not well known, but it may be due to the host's inability to wall off cankers by laying down lignified tissue around the perimeter of a canker. Hypovirus infection reduces the rate of canker expansion on infected trees (Van Alfen *et al.* 1975; Jaynes and Elliston 1980; Anagnostakis and Waggoner 1981) so that the tree has adequate time to successfully wall off a canker allowing them to recover from blight. If expansion rates on European chestnut are slower than on American chestnut, then it may allow more time for European chestnuts to acquire hypoviruses and it may explain why hypoviruses alone do not lead to general recovery in most American chestnut stands.

In the late 1980s, following the discovery of chestnut blight in an isolated American chestnut population in West Salem, Wisconsin, hypovirus was introduced annually (1992-1997 and again from 2004 to the present) to save the trees. As a corollary of monitoring hypovirus spread, it was found that cankers that do not girdle the stems display a pattern of accumulating secondary microorganisms (Non-CP) over time (Figure 1). The temporal pattern clearly showed that the percentage of *C. parasitica* isolates containing the EURO7 hypovirus remained relatively constant over time, and never exceeded 28% (CP-HV). In contrast, the percentage of virulent isolates of *C. parasitica* that do not contain hypovirus (CP-V) clearly decreased over time, while the percentage of Non-CP increased over the same period. We discovered that non-girdling, non-lethal cankers continue to harbor more Non-CP than girdling, lethal cankers that ultimately kill the tree they infect (Double *et al*. 2014). This pattern suggests that Non-CP may play a role in reducing the severity of chestnut blight.

The potential of Non-CP to inhibit *C. parasitica* and contribute to biological control has not been investigated thoroughly. Early studies involving the application of soil compresses directly to cankers reduced canker enlargement compared to autoclaved soils leading to the implication that microorganisms inhabiting the soil are antagonistic to *C. parasitica* (Weidlich 1978). Some of these soil inhabitants, as well as other microbes isolated directly from chestnut tissue were fungi in the genus *Trichoderma* and endophytic bacteria *Bacillus* spp. (Arisan-Atac *et al.* 1995; Tattar *et al.* 1996; Wilhelm *et al.* 1998; Groome *et al.* 2001; Akilli *et al.* 2011). These studies have

demonstrated inhibition of *C. parasitica* in laboratory culture and in chestnut tissue, and have provided a useful first step in our understanding of a potential biological control using secondary microorganisms.

Figure 1. Temporal pattern of isolate frequencies for 263 cankers that were first sampled in 2001. (MacDonald et al., unpublished data). Twelve samples were collected annually from each canker. CP-V represents the percentage of the 12 samples that contained virulent *C. parasitica* without hypovirus, CP-HV represents the percentage of isolates that were *C. parasitica* containing the hypovirus EURO7, and Non-PC represents the percentage of isolates that were not *C. parasitica*.



The preliminary laboratory studies did not address how Non-CP may influence the severity of blight on surviving American chestnut trees in the forest (Russin and Shain 1984). In 2010, identification of Non-CP recovered from cankers at the West Salem, WI chestnut stand began. DNA was extracted from the mycelium of the cultured isolates and the sequences were compared using the National Center for Biotechnology Information Basic Local Alignment Search Tool (NCBI-BLAST). Many of the recovered fungi were those investigated in the aforementioned studies, as well as closely related fungi associated with successful biological control scenarios found in other host-pathogen systems (*e.g.* Campanile *et al.* 2007; Card *et al.* 2009; Szwajkowska-Michalek *et al.* 2012). Our proposal will extend this work by comparing Non-CP found within girdling and non-girdling cankers from six geographical locations in North America, including the West Salem, Wisconsin site, four sites in Michigan and 20 American chestnut trees in Maryland. Two of the Michigan sites contain trees that are recovering from blight presumably due to the natural spread of hypoviruses. Trees at the other two Michigan sites are not recovering and hypoviruses are largely absent. Sampling in Maryland is included because trees at this site have "cruddy bark" (*i.e.,* swollen cankers with abundant callus) and display limited recovery. Our first goal is to sample cankers at each location to determine if particular species of Non-CP are associated with cankers that do not girdle trees as compared to isolates from girdling cankers. This first phase of the work will identify Non-CP that potentially may act to reduce blight severity. Funding requested by this proposal is for a one-year study, but the plan is to sample the same cankers annually to investigate how the Non-CP community within cankers may change over time. Our second goal will be to determine if the Non-CP associated with non-girdling cankers have the ability to inhibit growth of *C. parasitica*. The experimentation initially will be carried out in the lab, but later the most promising Non-CP isolates (*i.e.*, those displaying the best inhibition) will be tested on excised chestnut stems and

finally in the field on live American chestnut trees. Our long-term goal is to evaluate whether these Non-CPs can be utilized as agents of biological control either alone or in combination with hypovirus inoculations.

Methods:

1) What Non-CPs are associated with girdling and non-girdling cankers?

Canker sampling will be done at the West Salem, Wisconsin site with additional sampling of cankers on surviving American chestnut trees at four sites in Michigan and several trees Maryland. Michigan sites have been monitored for at least the last 17 years and have both recovering and non-recovering chestnut populations (Davelos and Jarosz 2004). As explained above, the Maryland trees have an unusual "cruddy bark" canker morphology and display limited recovery.

Twenty-four samples will be collected for each canker that we study by surface-sterilizing bark samples using a 10% Clorox® (NaClO) bleach and a squirt of Joy® dish soap solution in sterile water and plating them on potato dextrose agar (PDA) and recording as either a *C. parasitica* or Non-CP isolate. The 24 samples will obtained in a spatially explicit pattern within the canker, with 12 samples coming from the canker edge and the remaining 12 from the interior of the canker. For each isolate, we will record the population, tree, canker ID and position within the canker. From these data we can calculate the percentage of Non-CP within each canker and analyze patterns of occurrence for the Non-CPs with regard to canker type (i.e., girdling or non-girdling), tree type (i.e., recovering or non-recovering) and population.

Each Non-CP species will be identified initially based on colony and spore morphology. For those species that cannot be identified using only morphological traits, DNA will be extracted from fungal mycelium using the Qiagen DNeasy® plant extraction kit following the manufacturers' recommendations. We will process our fungal DNA by amplifying the internal transcribed space regions flanked by the large and small subunits of ribosomal DNA – a highly variable region of DNA used by most laboratories and considered the "fungal barcode". Forward and reverse primers (ITS1 and ITS2, respectively) will be used for sequencing (White *et al.* 1990). Analysis of the ITS sequence alignment will be conducted using the computer software package from DNA Star, Inc. and compared with sequences on NCBI's BLAST to identify our unknown isolates to genus and species. Other genes (*e.g.* beta-tubulin, elongation factor genes) will be sequenced as necessary to resolve species level identification.

2) Can Non-CPs inhibit *C. parasitica* in laboratory assays?

A. Dual culture testing in Petri dishes on culture media

Eight of the most common Non-CPs associated with non-girdling cankers and 5 common Non-CPs not associated with canker type will be chosen for this test. Each of these Non-CPs will be tested for their ability to inhibit a *C. parasitica* strain (LE221; an isolate of *C. parasitica* collected from Leelanau County, Michigan) with and without hypovirus. Petri

dishes (150 mm) containing 15 ml of sterile PDA will be inoculated with a 5 mm^2 plug of a one-week-old pure culture of LE221 followed by a plug of a potential antagonist Non-CP added two days later. The potential antagonist and an isolate of *C. parasitica* will be positioned 30 mm apart in each Petri dish. Each combination will be replicated four times. Negative controls will be inoculated with LE221 and paired with a sterile water agar plug. Colonies will be incubated at 25°C in the dark. The experiment will be repeated three times. Radial growth of the LE221 mycelium in dual culture will be recorded every other day by measuring colony area. Measurements will continue for ten days or until *C. parasitica* reaches the margin of the dish in the control plates. Inhibition due to antagonism by Non-CP will be expressed as a proportion of growth in the dual culture divided by growth of the negative control. After 2–5 days, when the two fungal colonies come into contact, the morphology of the hyphae and their interaction in the contact zone will be observed with a light microscope to determine if a Non-CP can inhibit *C. parasitica* via hyphal interactions (parasitism). Interactions between the fungi will be photographed using a scanning electron microscope.

B. Testing on excised American chestnut branches using different application methods

The three-to-five most promising species identified in 2A will be tested for their ability to inhibit canker expansion for infections with either a virulent strain (LE221) or a hypovirulent (LE221 containing a hypovirus) strain. Dormant American chestnut branches (diameter 4.0±1cm, length 95±15cm) will be cut and surface-sterilized using 10% Clorox® (NaClO) bleach before inoculation Six infections, three virulent and three hypovirulent, will be established on each branch. After approximately four weeks of canker expansion, one of four treatments (Table 1) will be applied to each branch. Two weeks later, canker expansion will be measured again, and recorded as t_1. We will continue measuring canker expansion at biweekly intervals until expansion stops or control treatments completely encircle the branch. Each treatment will be replicated on at least three branches giving nine replicates for each pathogen (virulent or hypovirulent) by treatment (Table 1) combination.

Table 1. Four application methods will be used to inoculate Non-CP species onto already established cankers.

i. Scratching bark near canker area with a knife; paint a pure, 'liquid' culture of Non-CP isolate grown in a 125mL flask of liquid media.
ii. Six plugs from an agar plate of single-spore culture of Non-CP isolate applied around the canker area using a core borer
iii. Six plugs of tree branch removed around the canker area as in treatment ii; 'liquid' culture applied as in treatment 'i'.
iv. Spore solution painted onto surface of branch directly at canker area from single-spore dilution in water [> 1.0 x 10^6 spores]

3) Can Non-CP inhibit canker expansion on living American chestnut stems?

The LE221 strain of *C. parasitica* used in part 2 above will be inoculated into living American chestnut stems (diameter at breast height > 4cm) to initiate cankers in the early

summer of 2015. Approximately one month later, cankers will be treated with a combination of the best performing Non-CP isolate(s) and the most effective application method identified in section 2B to evaluate the ability of Non-CP to inhibit canker exampansion. Each treatment combination of Non-CP isolate(s) and application method(s) will be replicated eight times on eight different stems during the growing season of 2015 and conclude with tree dormancy in Fall 2015.

References:

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Timeline:

	2014 Fall	**2015** Winter	Spring	Summer	Fall
1. Identify Non-CP isolates					
• Isolation of Non-CP from bark	----------				
• Morphological characterization		----------			
• Extract and sequence DNA		----------	----------		
2. Inhibition tests (in lab)					
a) Dual culture plating		----------	----------		
b) Excised chestnut stem			----------		
3. Inhibition tests (in field)					
• Canker expansion rates				----------	----------

How results will be measured and reported:
A descriptive report will be produced in the summer of 2015 that evaluates commonly isolated Non-CP for their ability to inhibit growth of *C. parasitica* in the lab on both agar plates and

excised wood. Reports on living trees will be presented in early 2016 after these experiments have been completed. Results from the laboratory work in section 2 and field experiment (section 3) will be reported at annual regional USDA chestnut meetings (NE 1333), at TACF meetings, and in scientific publications in journals such as Plant Disease and Phytopathology.

Budget:

	Cost
Travel to and lodging in Maryland, Wisconsin and northern Michigan chestnut populations to collect bark samples *(Fall 2014)*	$1,600
Laboratory supplies for isolation of fungi, laboratory inhibition experiments, and inoculation experiments in the field *(Fall, Winter, Spring, and Summer 2014-15)*	$4,000
Extraction of fungal DNA and amplification using PCR *(Winter and Spring 2015)*	$2,700
Clean up of DNA and sequencing *(Spring 2015)*	$1,700
Total:	$10,000

Note: Matt Kolp is a graduate student at MSU and is being co-advised by Drs. Jarosz and Fulbright. The work described above is a major part of Matt's thesis work. Matt is being funded as a teaching assistant at MSU. This proposal will provide research funds for him to carry out his work. Professor MacDonald and Mr. Mark Double have contributed both intellectually and technically to the current project. They will continue to cooperate with this study, especially with regard to ongoing work at the West Salem, WI site and in Maryland.

Andrew M. Jarosz
Associate Professor, Plant Biology and Plant, Soil, and Microbial Sciences
612 Wilson Road
Office: 517-432-2942
Email: amjarosz@msu.edu

Education and Positions Held

1976	B.S. Purdue University
1984	Ph.D. Purdue University
1985-88	Research Scientist, CSIRO, Australia
1988-89	Instructor, Emory University
1989-1991	Post-Doctoral Fellow, Duke University
1991 to present	Associate Professor, Michigan State University, Departments of Plant Biology and Plant, Soil, and Microbial Sciences.

Career Service

Associate Chair, Department of Plant Biology 2007 to present
Graduate Director, Department of Plant Biology. December 2005 to 2007
Senior Editor, Phytopathology. 1999 to December 2001.
Michigan Scientific Evolution Education Initiative (MSEEI) Advisory Committee, 1999 to 2002.
Associate Editor, Phytopathology, Jan 1997 to Dec 1999.
American Phytopathological Society Epidemiology Committee, 1994 to 1996.

Qualifications

I have worked on the epidemiology of plant diseases and their consequences for plant population dynamics for over 30 years, investigating rusts, mildews wheat scab, barley scald, white mold and chestnut blight. A major project in my lab investigates the epidemiology of chestnut blight on both native American chestnuts and on orchard chestnuts. A new lab focus is investigating the cause and epidemiology of spruce decline.

Recent Publications directly related to project

Davelos, AL, Jarosz, AM. 2004. Demography of American chestnut populations: effects of pathogen and a hyperparasite. Journal of Ecology 92:675-685.

Springer, JC, Davelos Baines, AL, Fulbright, DW, Chansler, MT & Jarosz, AM. 2013 Hyperparasites influence population structure of the chestnut blight pathogen, *Cryphonectria parasitica.* Phytopathology 103: 1280-1286.

Springer JC, Davelos Baines AL, Chansler MT, and AM Jarosz. 2013. Evaluating the long-term storage of *Cryphonectria parasitica*. Fungal Genetics Reports. 60:11-15.

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.

Davelos Baines, AL, DW Fulbright & AM Jarosz. 2014. Effects of Branch Size and Pathogen Virulence on Canker Development and Branch Mortalit. ISHA Acta Horticulture 1019: 23-29.

Medina Mora, C., AM Jarosz & DW Fulbright. 2014. SSR genotyping chestnut kernel from cross-pollination in Michigan. ISHA Acta Horticulture 1019: 173-178.

Jarosz, AM, JC Springer, and ML Double, DW Fulbright and WL MacDonald. 2014. Hypovirus influence on mortality and growth of American chestnuts at West Salem, Wisconsin, USA. ISHA Acta Horticulture 1019: 157-163.

Stevens, DL, K Soltau, A Davelos Baines, & AM Jarosz. 2014. American chestnut sprout dynamics. ISHA Acta Horticulture 1019: 223-227.

Double, ML, MR Kolp, AM Jarosz, A Davelos-Baines, DW Fulbright and WL MacDonald. 2014. Fungi associated with hypovirulent cankers of differing ages on American chestnut. Acta Hort. (*in press*).

Other recent publications and other relevant publications

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Matthew R. Kolp

kolpm@msu.edu
matkolp@gmail.com
(517)-285-4667

Education

PhD. student, Michigan State University Plant Biology (MSU); Ecology, Evolutionary Biology and Behavior. Expected graduation – 2017

B.S., Michigan State University Horticulture and Plant Science. Graduation – 2012.

Research Awards

2014	Paul Taylor Travel Award; MSU Plant Biology ($801) Graduate School Travel Award; College of Natural Science ($200) Ecology, Evolutionary Biology and Behavior Travel Award ($200)
2013	Paul Taylor Travel Award; MSU Plant Biology ($717) Graduate School Travel Award; College of Natural Science ($400) Ecology, Evolutionary Biology and Behavior Travel Award ($200)
2011	MSU College of Agriculture and Natural Resources ($1,500)
2010	MSU College of Agriculture and Natural Resources ($1,000)

Teaching

2014	"Plant Systematics" – Lab (Pant Biology 418)
2013	"Plants of Michigan "– Lab (Plant Biology 218)
2012-13	"Cell and Molecular Biology" Lab (Biological Science 171) and "Organisms and Populations" (Biological Science 172)

Scholarly Work

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).

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Rowe, D.B., Kolp, M. Getter, K. Duck, M. (2012). Comparison of water use efficiency of overhead, drip, and sub-irrigation for green roofs. Proc. of 10th North American Green Roof Conference: Cities Alive, Chicago, IL 17-20 October 2012.

Dennis F. Fulbright
1066 Plant & Soil Sciences Building | 517-819-1043 | fulbrig1@msu.edu
Department of Plant, Soil and Microbial Sciences
Michigan State University
East Lansing MI 48824

EXPERTISE

My laboratory played a critical role in discovering and understanding the hypovirulent nature of *Cryphonectria parasitica* strains in American chestnut stands in Michigan. Not only isolating and sequencing hypovirus CHV3-GH2, we also discovered and characterized hypovirulent strains caused by mitochondria mutations, insertions and plasmids. Work in my laboratory was pioneering in confirming the horizontal movement of both viral and mitochondrial cytoplasmic factors in the forest environment. Also, we have also performed research to find microbial inhibition products in chestnut tissues. In the past few years, we have helped growers establish a successful commercial chestnut industry in Michigan while using hypovirulent strains in the orchards to manage chestnut blight on blight-susceptible trees.

PERSONAL PREPARATION

Whittier College	A.B. Biology	1974
University of California-Riverside	Ph.D. Plant Pathology	1979

APPOINTMENTS

2001-present Professor, Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI (formerly Department of Plant Pathology 2001-12)

1979-2001 Assistant, Associate and Professor, Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI

PUBLICATIONS—*Related to Project*

Fulbright, D. W., W. H. Weidlich, K. Z. Haufler, C. S. Thomas and C. P. Paul. 1983. Chestnut blight and recovering American chestnut trees in Michigan. Can J Botany. 61: 3164-3171.

Garrod, S. W., D. W. Fulbright and A. V. Ravenscroft. 1985. Dissemination of virulent and hypovirulent forms of a marked strain of *Endothia parasitica* in Michigan. Phytopathology. 75: 533-538.

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Donis-González, I.R., Guyer, D.E., Fulbright, D.W., Pease, A. 2014. Postharvest noninvasive assessment of fresh chestnut (*Castanea* spp.) internal decay using computer tomography images. Post Harvest Biology and Technology 94: 14-25.

William L. MacDonald
1090 Agricultural Sciences | 304-293-8818 | macd@wvu.edu
Plant Pathology and Environmental Microbiology
West Virginia University
Morgantown, WV 26506-6108

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.

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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).

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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

Mark L. Double

1090 Agricultural Sciences, P.O. Box 6108 | 304-293-9277 | mdouble@wvu.edu
Plant Pathology and Environmental Microbiology
West Virginia University
Morgantown, WV 26506-6108

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).

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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