Phytophthora Root Rot on American and Hybrid Chestnut Trees: New and Ongoing Projects at Clemson University

Submitted to:

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12 August 2019
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Signing for

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A. Project Title

Phytophthora Root Rot on American and Hybrid Chestnut Trees: New and Ongoing Projects at Clemson University

B. Summary

Phytophthora species, primarily *P. cinnamomi*, cause Phytophthora root rot on American and hybrid chestnut trees. We will continue to assay soils from chestnut-growing areas of the eastern United States and to collect and store representative isolates in a permanent collection. Eventually, we will characterize the isolates and determine the geographic distribution of *Phytophthora* spp. in these chestnut-growing regions. We will determine the efficacy of commercially available fungicides to manage Phytophthora root rot on chestnut seedlings, so trees in Germplasm Conservation Orchards can be protected. We also will determine if isolates of *P. cinnamomi* vary in virulence to hybrid chestnut genotypes.

C. Principal Investigators and Institutional Affiliation

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D. Duration of Project: 01 November 2019 – 31 October 2020 = 12 months

E. Total Amount Requested: $9,979
Matching funds will be provided by Clemson University – see budget

F. Short- and Long-term Goals of Project

Short-term Goals

1. To continue to assay plant and soil samples provided by TACF for species of *Phytophthora*, so we can identify where these plant pathogens are located in chestnut-growing sites and potential chestnut-growing sites in the eastern U.S.
2. Collect and maintain representative isolates of *Phytophthora* spp. from chestnut-growing sites for future studies

3. Evaluate commercially available fungicides that target Oomycetes for efficacy against Phytophthora root rot on American chestnut seedlings
4. Determine if isolates of *P. cinnamomi* from different geographic locations vary in virulence to backcross hybrid chestnut seedlings developed by TACF

Long-term Goals

1. Identifying the location and distribution of *Phytophthora* spp., especially *P. cinnamomi*, is necessary, so TACF knows where it can and should not plant chestnut seedlings now and in the future. Germplasm Conservation Orchards (GCOs) should not be planted at sites where *P. cinnamomi* is present whereas field tests to evaluate hybrid seedlings for resistance to *P. cinnamomi* need to be planted in soils where this pathogen is present and active.
2. Isolates of *Phytophthora* spp. recovered from diseased chestnut trees and infested soils will be maintained in a permanent collection at Clemson University, so that we can use them in future research projects. Eventually, we will determine the diversity of *Phytophthora* spp. associated with chestnut trees in the eastern U.S., the pathogenicity of individual species, and the relative virulence of isolates within and among species. This information will be essential to breed chestnuts with resistance to all species of *Phytophthora* capable of causing Phytophthora root rot on the important host plant.
3. By identifying the most effective, commercially available fungicides for managing Phytophthora root rot on chestnut seedlings, we can work to get these products registered so they can be legally applied to chestnuts seedlings and trees.
4. TACF recently adopted a breeding strategy for evaluating resistance to *P. cinnamomi* that requires the use of different isolates each year—ones recovered from the location where surviving seedlings will be planted outside. Therefore, it is essential to determine if isolates from different geographic locations are virulent and capable of causing Phytophthora root rot so that hybrid seedling are adequately challenged each year.

G. Narrative

Introduction

Phytophthora root rot (PRR) is a lethal disease of the American chestnut tree (*Castanea dentata*) that was killing trees in the southern range of this native forest tree species long before chestnut blight was reported in North America (Anagnostakis 2012, Crandall et al. 1945). Currently, the American Chestnut Foundation (TACF) has an active breeding program to develop backcross hybrid American chestnut trees with resistance to both *Phytophthora cinnamomi*, which causes PRR, and to *Cryphonectria parasitica*, which causes chestnut blight. Since 2003, our lab at Clemson University has worked in collaboration with TACF to study PRR, but we have lacked the financial support to maintain a consistent research effort necessary to make significant impacts in the etiology and management of this important disease. Despite a minimum o financial support, we have made considerable progress, and our collaboration with TACF has been productive (Westbrook et al. 2019, Zhebentyayeva, et al. 2019). In this proposal, I am requesting partial financial support for the ongoing and new projects we are conducting that support TACF efforts to restore the American chestnut tree to our eastern forests.

Assaying plant and soil samples for species of *Phytophthora*

We began isolating *Phytophthora* spp. from chestnut trees and soils in 2003 and have continued to provide this service to TACF ever since. Isolates recovered from chestnut trees, seedlings, and soils are maintained in a permanent collection in our lab at Clemson University. Over 16 years, we have processed hundreds of samples and have recovered *Phytophthora* spp. from 10 states—primarily in the southeastern and mid-Atlantic regions but also from a chestnut planting in southwestern Pennsylvania. Therefore, we now have a better understanding of the distribution of *Phytophthora* spp. in the native chestnut growing regions of the eastern U.S. We have isolated *P. cinnamomi* primarily from these samples, but occasionally we isolate other species—see below. Based on our isolation efforts, we have confirmed PRR on or questioned the role of *P. cinnamomi* in seedling death of many plant samples sent to our lab for processing. We have confirmed the presence of *P. cinnamomi* in numerous chestnut planting sites or potential planting sites, which has prevented the death and loss of many valuable chestnut seedlings. Over the past winter and spring, we processed soil samples from eight Tennessee State Parks (TSP) that would like to host TACF chestnut plantings, and we expect to receive several more TSP samples this fall. To preserve American chestnut germplasm, TACF Chapters are trying to save representative seedlings and cuttings of surviving American chestnut trees. Often, these are preserved in GCOs located in states throughout the native range of the American chestnut. Before establishing a GCO, it is important to identify a site where *P. cinnamomi* is not present to avoid getting PRR on the planted material, which eventually will die if infected. Soil and plant samples are assayed using standard isolation protocols that have been developed in my lab over many years of studying *Phytophthora* spp. associated with fruit and ornamental crops (Ferguson and Jeffers 1999, Jeffers and Martin 1986).

Maintaining isolates of *Phytophthora* spp. in a permanent collection

Representative isolates of *Phytophthora* spp. recovered from plant and soil samples are maintained in a permanent collection in our lab, so they are available for futures studies and projects. For example, in May 2017, a graduate student working with me earned an MS degree studying *Phytophthora* spp. associated with American, Chinese, and hybrid chestnut seedlings planted in USDA Forest Service silviculture plots in Virginia, North Carolina, and Tennessee—maintained by Dr. Stacy Clark—and one plot at Dr. Joe James farm in South Carolina. Dr. Clark had a serious problem with PRR in several of her research plots and sent us approximately 300 samples to be assayed for *Phytophthora* spp. over a 3-year period (2009-2011). The graduate student worked with 248 isolates that had been preserved in out collection and identified five species of *Phytophthora* associated with plants in these plots: *P. cinnamomi*, *P. cambivora*, *P. cryptogea*, *P. heveae*, and *P. quercetorum* (Sharpe 2017). Before our study, only *P. cinnamomi* had been associated with American chestnut trees (Crandall et al. 1945, Westbrook et al. 2019). We then proved that three of these species (*P. cambivora*, *P. cryptogea*, and *P. heveae*), along with *P. cinnamomi*, were pathogenic to and capable of causing PRR on seedlings of American chestnut (Sharpe 2017). *P. quercetorum* was only found in one soil sample and has not been tested for pathogenicity, but additional isolates of *P. quercetorum* have since been found in soil samples from other states. These four additional species of *Phytophthora* were associated with seedlings in research plots in four southeastern states. How many species of *Phytophthora* are

present in forest soils throughout the American chestnut tree range? We currently have several hundred isolates already in our collection and will continue collecting new isolates over the coming years. Eventually, we need to characterize the diversity of the population of *Phytophthora* spp. associated with American chestnut. Eventually, the TACF breeding program will need to incorporate these other species into the annual screening procedure to be sure hybrid chestnut seedlings are resistant to all species of *Phytophthora* present in eastern forests.

Identifying the most effective fungicides for managing PRR on American chestnut seedlings

There are a number of commercially available fungicides that target *Phytophthora* spp. and other Oomycete plant pathogens. However, only products containing the active ingredient mono- and di-potassium salts of phosphorous acid are registered for applications to chestnut trees (e.g., Reliant, Fosphite). TACF Chapters are trying to collect and preserve diverse populations of surviving American chestnut trees in GCOs, but it is very likely these plants are susceptible to PRR. Therefore, if *P. cinnamomi* or another pathogenic species of *Phytophthora* is present in the soils where GCOs are located, PRR could attack and kill all the trees in the orchard. Consequently, we initiated a study in 2019 to evaluate the efficacy of eight commercially available fungicide products, each with a different active ingredient, that are registered to manage Phytophthora diseases. The first greenhouse project is in progress, and the products certainly vary in efficacy. This experiment will need to be repeated next year and then results confirmed in the field. Eventually, we will need to seek registrations for products with the most effective active ingredients, so these products can be used legally to protect chestnut trees growing in Phytophthora-infested soils.

Virulence of isolates of *P. cinnamomi* from different geographic locations

TACF recently adopted a new breeding strategy for evaluating resistance to *P. cinnamomi* that requires the use of different isolates each year—ones recovered from the location where surviving seedlings will be planted outside for field evaluation. Therefore, it is essential to determine if isolates from different geographic locations are virulent and capable of causing disease so that hybrid seedling are adequately challenged each year. Previously, we evaluated isolates of *P. cinnamomi* recovered from different chestnut substrates (roots, soil) and locations (NC, SC, TN, VA) for virulence to American chestnut seedlings (Sharpe 2017). There was no significant difference among the isolate treatments; all were equally virulent. However, other researchers have observed differences in virulence among isolates of *P. cinnamomi* from different host plants (e.g., Crandall et al. 1945). Therefore, we initiated an experiment in 2019 in conjunction with TACF to evaluate the virulence of the same *P. cinnamomi* isolates used by Sharpe (2017) but, this time, on hybrid chestnut seedlings with partial resistance to *P. cinnamomi*. This experiment is being conduced at the USDA Forest Service Resistance Screening Center in Asheville, NC as part of the annual screening of seedlings for resistance to *P. cinnamomi*. This experiment currently is in progress and needs to be repeated next year. Results from this experiment will tell us if isolates of *P. cinnamomi* vary significantly in virulence and, even if virulence varies, are all isolates capable of causing PRR on and mortality in hybrid chestnut seedlings. In the coming years as breeding for resistance to *P. cinnamomi* continues, we need to insure that seedlings are challenged each year with virulent isolates of the pathogen.

Literature Cited

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Sharpe, S. R. 2017. *Phytophthora* species Associated with American, Chinese, and Backcross Hybrid Chestnut Seedlings in Field Sites in the Southeastern United States. MS Thesis. Clemson University, Clemson, SC.

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Zhebentyayeva, T. N., Sisco, P. H., Georgi, L. L., Jeffers, S. N., Perkins, M. T., James, J. B., Hebard, F. B., Saski, C., Nelson, C. D., and Abbott, A. G. 2019. Dissecting resistance to *Phytophthora cinnamomi* in interspecific hybrid chestnut crosses using sequence-based genotyping and QTL mapping. Phytopathology 109: https://apsjournals.apsnet.org/doi/10.1094/PHYTO-11-18-0425-R

H. Timeline (Nov 2019 – Oct 2020)

Research Project	**Nov-Jan**	**Feb-Apr**	**May-Jul**	**Aug-Oct**
Assaying plant and soil samples	X	X	X	X
Maintaining isolates of *Phytophthora* spp.	X	X	X	X
Fungicide efficacy: Trial in 2020			X	X
Virulence of *P. cinnamomi* isolates	X		X	X

I. Measurement and Reporting of Results

All four projects are ongoing and will be continued. Data are collected whenever necessary—i.e., after samples are received and assayed and at the end of trials. Likewise, data will be summarized and analyzed when results are available. An annual report will be prepared in a timely manner based on results obtained in the 12-month period covered by the proposal. Progress and results to date will be presented at the NE-1833 Meeting in West Virginia in September 2019. Once a project has been completed, based on replicated trials, results will be published in the *Chestnut* or other suitable peer-reviewed journal.

J. Budget: One Year (Nov 2019 – Oct 2020)

Expense	**TACF: Amount Requested**
20% of salary for Lab Manager/Research Technician	$6,240
Fringe Benefits: 43.9% for full-time employees	2,739
Expendable lab supplies	1,000
Subtotal	9,979
Unrecovered overhead = F&A @ 52.5% = $5,239	0
TOTAL	*$9,979*

Budget Justification

Amount Requested from TACF

Most of the funds requested in this proposal will be used to cover 20% of the salary and fringe benefits for Mr. Linus Schmitz, the Lab Manager and Research Assistant who is managing and working on these projects. Mr. Schmitz makes $15.00/hour and works 40 hours/week. The remainder of the funds will be used to pay for expendable lab supplies.

Expendable lab supplies for this project will be primarily laboratory materials used for isolation from plants and soil, isolate identification, and culture storage—e.g., disposable petri dishes, disposable multi-well plates, agar media, chemicals, medium amendments, PCR primers, centrifuge tubes, glass storage vials, etc.

Matching funds provided by Clemson University

TACF has a policy to not pay overhead (F&A – Facilities and Administration) charges because their grants are relatively small—see accompanying document. Therefore, this amount ($5,239) is used as matching funds from Clemson University.

K. Brief CV for the Principal Investigator

Steven N. Jeffers, Ph.D. – Abbreviated CV

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Education
1985: Cornell University, Ithaca, NY: Ph.D. Plant Pathology (Soil Science minor)
1980: Cornell University, Ithaca, NY: M.S. Plant Pathology (Soil Science minor)
1976: University of California, Davis: B.S. (Highest Honors) Plant Science/Pomology

Employment
Clemson University, Clemson, SC
2007-2018: Professor, Dept. of ESPS/School of AFES/Dept. of AES/Dept. PES
2001-2007: Associate Professor, Depts. PP&P and Entomology, Soils, and Plant Sciences (ESPS)
1995-2001: Assistant Professor, Dept. of Plant Pathology and Physiology (PP&P)
EcoScience Corporation, Worcester, MA: 1992-1995; Senior Scientist
University of Wisconsin-Madison: 1985-1992: Assistant Professor, Dept. of Plant Pathology

Research Experience
1995-present: Development of integrated management strategies for diseases of ornamental crops and trees in South Carolina; biology and ecology of *Phytophthora* spp. in nurseries, greenhouses, landscapes, and natural ecosystems; management of rust diseases
1992-1995: Development of biological control products for postharvest diseases of fruit crops
1985-1992: Integrated management strategies for diseases of fruit crops grown in Wisconsin

Extension Experience
1995-present: Clemson University Extension Specialist, Diseases of Ornamental Crops & Trees
1985-1992: University of Wisconsin Extension Specialist, Diseases of Fruit Crops

Teaching Experience
Principles of Plant Pathology (PLPA 3100): 2013-present
Plant Diseases and People (PL PA 310): 2010-2012
Selected Topics/Introductory Plant Pathology for Graduate Students (PLPA 8020): 2010-present
Plant Disease Diagnosis (PLPA 4110/6110)—co-instructor: 2012-present

Peer-Reviewed Publications: 2013-Present
Ridge, G. A., Bell, N. L., Gitto, A. J., Jeffers, S. N., and White, S. A. 2019. Workshop: *Phytophthora* species associated with plants in constructed wetlands and vegetated channels at a commercial ornamental plant nursery over time. HortTechnology 29: https://doi.org/10.21273/HORTTECH04300-19.

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Canegallo, A., Martin, S.B., Camberato, J., and Jeffers, S. 2017. Seashore Paspalum cultivar susceptibility to large patch and fungicide evaluation for disease control in South Carolina. International Turfgrass Society Research Journal 13:185-190. doi:10.2134/itsrj2016.04.0265

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Ridge, G. A., Jeffers, S. N., Bridges, W. C., Jr., and White, S. A. 2014. In situ production of zoospores by five species of Phytophthora in aqueous environments for use as inocula. Plant Disease 98:551-558.

Valencia-Botín, A. J., Jeffers, S. N., Palmer, C. L., and Buck, J. W. 2013. Fungicides used alone, in combinations, and in rotations for managing gladiolus rust in Mexico. Plant Dis. 97:1491-1496

Dong, W., Jeffers, S. N., and Buck, J. W. 2013. Management of daylily rust with different fungicides and application methods. Plant Dis. 97: *accepted & in press.*

Scocco, E. A., Walcott, R. R., Jeffers, S. N., and Buck, J.W. 2013. Detection of *Puccinia pelargonii-zonalis*-infected geranium tissues and urediniospores. J. Phytopathology 161: *accepted & in press.*

Olson, H. A., Jeffers, S. N., Ivors, K. L., Steddom, K. C., Williams-Woodward, J. L., Mmbaga, M. T., Benson, D. M., and Hong, C. X. 2013. Diversity and mefenoxam sensitivity of *Phytophthora* spp. associated with the ornamental horticulture industry in the southeastern US. Plant Disease 97:86-92.

L. Conflict of Interest or Commitment Statement

There are no conflicts of interest for the above listed Principal Investigator regarding this project.