Proposal:

Diversity and Pathogenicity of Species of *Phytophthora* Associated with American Chestnut Trees

Submitted to:

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Project Title

Diversity and pathogenicity of species of *Phytophthora* associated with American chestnut trees

Summary

For over 80 years, the only species of *Phytophthora* associated with Phytophthora root rot on American chestnut has been *P. cinnamomi*. Since 2004, we have collected root and soil samples from American chestnut trees in the eastern USA to investigate the potential diversity of species of *Phytophthora* associated with this native tree species. To date, we have isolated at least four species of *Phytophthora* from the roots of American chestnut trees, and now we are determining if these species are capable of causing root rot. Our results will affect how we select for resistance in American chestnut in the future.

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Project Duration: 11/01/2015-10/31/2016 (one year)

Total Amount Requested: $10,730

Goals

Short-Term Goals

1. Determine if species of *Phytophthora* other than *P. cinnamomi* are associated with root rot of American chestnut trees
2. Determine if all species of *Phytophthora* recovered from diseased roots of American chestnut are capable of causing root rot on American and Chinese chestnut seedlings

Long-Term Goals

1. Determine if resistance mechanisms to all pathogenic species of *Phytophthora* are similar—i.e., are hybrid genotypes selected as resistant to *P. cinnamomi* also resistant to other species of *Phytophthora*?

2. If resistance mechanisms are not similar then our efforts to select hybrid American chestnut seedlings for resistance to *P. cinnamomi* will need to be broadened to select seedlings that are resistance to all species of *Phytophthora* capable of causing root rot
3. Ultimately, we would like to identify hybrid American chestnut genotypes that are resistant to all species of *Phytophthora* capable of causing disease so that trees planted in the forest or an orchard will survive for many years and will not succumb to Phytophthora root rot

Narrative

Introduction

Phytophthora root rot (PRR) or ink disease first was reported to be a disease affecting American chestnut (*Castanea dentata*) in a preliminary report published in 1932 (Milburn and Gravatt 1932). In that report, it is stated that the pathogen "appears to be the same as *Phytophthora cambivora*…the cause of the ink disease of European chestnut…" However, after a more thorough investigation of this disease at a number of different sites, the pathogen causing PRR on American chestnut was confirmed to be *P. cinnamomi* and not *P. cambivora* (Crandall et al. 1945, Hansen 2000). Since these initial studies on PRR, *P. cinnamomi* has been the only species of *Phytophthora* reported as causing root rot on American chestnut (Anagnostakis 2012, Hansen 2000, Zentmyer 1980).

Background and Significance

Records of *Castanea* spp. dying, presumably from PRR, in the southeastern USA date back to the 1820s or 1830s—long before chestnut blight came to this continent (Crandall et al. 1945, Milburn & Gravatt 1932, Freinkel 2007, Zentmyer 1980). Consequently, PRR was eliminating these forest giants from their southern range before chestnut blight starting taking its toll. Root rot is a much more serious disease than chestnut blight because PRR kills chestnut trees outright whereas chestnut blight only kills the above-ground parts of chestnut trees (Anagnostakis 2012, Freinkel 2007, Zentmyer 1980); therefore, the root system on blight-affected trees survives and can produce new shoots.

In Europe, where this disease also has been devastating, two species of *Phytophthora*, *P. cinnamomi* and *P. cambivora*, are the primary species of *Phytophthora* causing PRR on sweet chestnuts, *Castanea sativa* (Vettraino et al. 2001, Vettraino et al. 2005, Zentmyer 1980). However, six additional species have been found associated with diseased sweet chestnut trees in Europe: *P. cactorum, P. citricola, P. cryptogea, P. gonapodyides, P. megasperma,* and *P. syringae* (Vettraino et al. 2001, Vettraino et al. 2005). Since these studies were conducted and published, both *P. citricola* and *P. megasperma* have been determined to be species complexes, and each of these two species has been split into several genetically distinct species. Of the eight species of *Phytophthora* associated with PRR in Europe, only *P. cinnamomi*, *P. cambivora*, and *P. citricola* have been recovered from diseased roots; the other species were only found in soil associated with dead and dying chestnut trees (Vettraino et al. 2001, Vettraino et al. 2005). Therefore, there is a diverse population of *Phytophthora* spp. in Europe associated with chestnut trees affected by PRR. Consequently, we need to know if species of *Phytophthora*

other than *P. cinnamomi* are associated with and causing PRR on American chestnut trees in the USA. This could have a major impact on how we screen and select for resistance in chestnut breeding programs.

Approach and Procedures

In cooperation and collaboration with people from TACF and the USDA Forest Service, we have been assaying soil samples and chestnut roots for *Phytophthora* spp. since 2004. Samples have come from numerous locations in the eastern USA, but most samples have come from sites in the southeastern states of GA, NC, SC, TN, and VA. Since 2010, we have been collaborating with Dr. Stacy Clark, USDA Forest Service, to assay soil and root samples from plantings of TACF hybrid chestnut trees in research plots in national forests in NC, TN, and VA (Clark et al. 2014, Pinchot et al. 2014). All isolates that have been recovered over the years have been maintained in a permanent collection in our laboratory. To date, we have collected several hundred isolates of *Phytophthora* spp.—primarily isolates of *P. cinnamomi* but also isolates that we know are not *P. cinnamomi*.

Short-Term Goal 1—Phytophthora *spp. associated with American chestnut trees.* In 2014 with funds from the USDA Forest Service, a project was initiated to identify the diversity of species in a subset of isolates from our collection--i.e., those recovered primarily from Dr. Clark's samples. A graduate student (Ms. Suzette Sharpe) was hired to conduct this project, and she began in August 2014. So far, we have examined 215 isolates that were recovered from 357 root and soil samples. Initially, we have identified these isolates based on morphological features—including colony growth habit, type of sporangia, and presence or absence of oospores, chlamydospores, and hyphal swellings (Duan et al. 2008, Erwin and Ribeiro 1996). Mating type still needs to be determined for isolates in heterothallic species. To do this, each isolate will be grown with known A1 and A2 isolates on super clarified V8 agar in the laboratory (Duan et al. 2008, Erwin & Ribeiro 1996). Agar plates will be held at 20°C for 2-6 weeks, and plates will be examined microscopically for oospores once a week. Identification of representative isolates needs to be confirmed using molecular methods—i.e., PCR and sequencing specific DNA loci in the genome (e.g., *ITS, β-tub, cox1, cox2*) (Grünwald et al. 2011). The procedures described in the thesis of a recent graduate student in our lab will be followed (Schreier 2013). Based on tentative identifications, we have found at least four species of *Phytophthora* associated with diseased chestnut roots and soil around dead and dying trees: *P. cinnamomi*, *P. cambivora, P. drechsleri,* and *P. heveae*. In addition, we have several isolates that cannot be identified by morphological characters. This is the first time in over 80 years that species of *Phytophthora* other than *P. cinnamomi* have been found associated with American chestnut trees.

S*hort-Term Goal 2—Pathogenicity of* Phytophthora *spp. to American and Chinese chestnut*. Representative isolates of the species identified in Short-Term Goal 1 are being tested for pathogenicity to American and Chinese chestnut seedling. Seeds were planted in March 2015 in 1.6-liter Treepots containing Fafard 3B soil-less container mix and are growing in a greenhouse. Nine treatments are being evaluated: eight isolates from at least four *Phytophthora* species and a non-inoculated control. The eight isolates were selected as representative of those isolated from diseased chestnut roots: *P. cinnamomi*—1 isolate as a

known pathogen for comparison, *P. cambivora*—4 isolates from different geographical locations, *P. heveae*—1 isolate, *P. drechsleri*—1 isolate, and a species not yet identified—1 isolate. Inoculum was prepared following standard procedures by growing isolates on sterile vermiculite moistened with V8 Juice broth (Roiger & Jeffers 1991). In late June, all plants except controls were inoculated. For each inoculated plant, 5 ml of inoculum was sprinkled on the surface of the container mix, inoculum was gently worked into the top layer of the container mix by hand, and then the surface was covered with a 1-cm layer of fresh container mix to prevent desiccation. Ten seedlings were inoculated with each isolate—5 American and 5 Chinese—and two independent trials are being conducted. All seedlings are watered daily, fertilized weekly, and evaluated weekly for symptom development. Evaluations are in progress. Beginning at 7-8 weeks after inoculation, seedlings will be flooded once a week for a 24-hr period to enhance disease development.

Literature Cited

Anagnostakis, S. L. 2012. Chestnut breeding in the United States for disease and insect resistance. Plant Disease 96:1392-1403.

Clark, S. L., Schlarbaum, S. E., and Hebard, F. V. 2014. The first research plantings of third-generation, third-backcross American chestnut (*Castanea dentata*) in the southeastern United States. Acta Horticulturae 1019:39-44.

Crandall, B. S., Gravatt, G. F., and Ryan, M. M. 1945. Root disease of *Castanea* species and some coniferous and broadleaf nursery stocks, caused by *Phytophthora cinnamomi*. Phytopathology 35:162-180.

Duan, C.-h., Riley, M. B., and Jeffers, S. N. 2008. Characterization of *Phytophthora cinnamomi* populations from ornamental plants in South Carolina, USA. Archives of Phytopathology and Plant Protection 41:14-30.

Erwin, D. C., and Ribeiro, O. K. 1996. Phytophthora Disease Worldwide. The American Phytopathological Society, St. Paul, MN.

Freinkel, S. 2007. American Chestnut. The Life, Death, and Rebirth of a Perfect Tree. University of California Press. Berkeley, CA.

Grünwald, N. J., Martin, F. N., Larsen, M. M., Sullivan, C. M., Press, C. M., Coffey, M. D., Hansen, E. M., and Parke, J. L. 2011. Phytophthora-ID.org: A sequence-based *Phytophthora* identification tool. Plant Disease 95:337-342.

Hansen, E. M. 2000. *Phytophthora* in the Americas. Pages 23-27 in: Phytophthora Disease of Forest Trees. Proceedings from the First International Meeting on Phytophthoras in Forest and Wildland Ecosystems—IUFRO Working Party 7.02.09. E. M. Hansen and W. Sutton, eds. Oregon State University, Corvallis, OR.

Milburn, M., and Gravatt, G. F. 1932. Preliminary note on a Phytophthora root disease of chestnut. Phytopathology 22:977-978

Pinchot, C.C., Schlarbaum, S.E., Clark, S.L., Schweitzer, C.J., Saxton, A.M. and Hebard, F.V. (2014). Impact of silvicultural treatment on chestnut seedling growth and survival. Acta Horticulturae 1019:191-197.

Roiger, D. J., and Jeffers, S. N. 1991. Evaluation of *Trichoderma* species for biological control of Phytophthora crown and root rot of apple seedlings. Phytopathology 81:910 917.

Schreier, S. G. I. 2013. Diversity among isolates of *Phytophthora cinnamomi* from ornamental plants in South Carolina. MS Thesis. Clemson University, Clemson, SC.

Vettraino, A. M., Morel, O., Perlerou, C., Robin, C., Diamandis, S., and Vannini, A. 2005. Occurrence and distribution of *Phytophthora* species in European chestnut stands, and their association with Ink Disease and crown decline. European Journal of Plant Pathology 111: 169-180.

Vettraino, A. M., Natili, G., Anselmi, N., and Vannini, A. 2001. Recovery and pathogenicity of *Phytophthora* species associated with a resurgence of ink disease in *Castanea sativa* in Italy. Plant Pathology 50:90-96.

Zentmyer, G. A. 1980. *Phytophthora cinnamomi* and the diseases it causes. Monograph No. 10. The American Phytopathological Society, St. Paul, MN.\

Timeline: 01 November 2015 – 31 October 2016

Short-Term Goals	Nov-Jan	Feb-Apr	May-Jul	Aug-Oct
Goal 1: Mating type determination for all isolates	→			
Goal 1: Molecular Identification of representative isolates	→	→	→	
Goal 2: Pathogenicity of representative isolates of species	→			
Reports, thesis, and publications			→	→

Measurement and Reporting of Results

Short-Term Goal 1—*Phytophthora* spp. associated with American chestnut trees

Mating type of isolates of heterothallic species will be evaluated by microscopic observation. Molecular identification will involve PCR and DNA sequencing. Sequences will be analyzed aligned by appropriate computer software and then blasted in online databases.

Short-Term Goal 2—Pathogenicity of *Phytophthora* spp. to American and Chinese chestnut

Each week after inoculation, individual seedlings will be evaluated for symptom development (visible lesions or cankers on the lower stem and percentage of foliage exhibiting wilt and necrosis) and mortality (no. of days after inoculation a seedling lives). The height of a lesion above the container mix surface will be measured, and symptom severity on the foliage will be assessed using a scale of 0-5: 0 = healthy, no symptoms; 1 = 1-10% of the foliage wilted; 2 = 11-50% of the foliage wilted; 3 = 51-90% of the foliage wilted; 4 = 91-99% of the foliage wilted; and 5 = dead. At the end of the experiment disease progress curves will be plotted and areas under the disease progress curves will be calculated. Analyses of variance will be used to analyze all quantitative data using JMP statistical software. Treatment means will be compared in single-degree-of-freedom linear contrasts and by Fisher's least significant difference.

Reporting of Results

Results from this project will be reported at the annual meeting of the USDA regional project on chestnuts (NE-1333), at the annual meeting of the American Phytopathological Society, and to The American Chestnut Foundation. Ms. Sharpe will use these results in her MS thesis at Clemson University and to prepare a manuscript for publication in a peer-reviewed journal.

Budget: One Year (2015-16)

Expense	Amount
Graduate Student Stipend--partial	$10,000
Fringe benefits @ 7.3%	$730
Overhead / F&A – see blow	$0
TOTAL	*$10,730*

Budget Justification

All of the funds requested in this proposal will be used to cover 50% of one year of a graduate student stipend; the annual stipend for this MS graduate student is $20,000. The stipend will support the second year for Ms. Suzette Sharpe, the graduate student working on this project. All expenses for the first year of the project were covered by a grant from the USDA Forest Service. The other half of Ms. Sharpe's stipend for the second year and all other expenses for the project (e.g., supplies, travel, etc.) will be covered by other funds available to the PI.

Overhead/F&A Charges: TACF has a policy to not pay overhead (F&A – Facilities and Administration) charges since their grants are relatively small—see accompanying document and this link: http://www.acf.org/pdfs/ExternalGrants/TAC-RFP-2015.pdf

Please Note: Clemson University administration already has agreed to provide GAD support for this graduate student (Suzette R. Sharpe).

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
2007-2010: Professor, Dept. of ESPS/School of AFES/Dept. of Agric. & Environ. Sciences
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
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: 2011-Present

Drechsler, D. T., Jeffers, S. N., and Bridges, W. C. 2014. *Phytophthora nicotianae* can cause both crown rot and foliage blight on *Phlox paniculata* in South Carolina. Online. Plant Health Progress doi:10.1094/PHP-14-0020. [PHP 15:159-165]
https://www.plantmanagementnetwork.org/sub/php/volume15/number4/PHP-RS-14-0020.pdf

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.

Faulkenberry, M.S., Culin, J. D., Jeffers, S. N., Riley, M. B., and Bridges, W. C., Jr. 2012. Efficacy of imidacloprid and dinotefuran applied as soil drenches or trunk sprays for managing *Adelges tsugae* (Hemiptera: Adelgidae) on mature hemlock trees in a forest. Journal of Entomological Science 47(4):1-6.

Hwang, J., Oak, S. W. and Jeffers, S. N. 2011. Recovery of *Phytophthora* species from drainage points and tributaries within two forest stream networks: A preliminary report. NZ Journal of Forestry Science 41S:S83-S87.

Meadows, I. M., and Jeffers, S. N. 2011. Distribution and recovery of *Phytophthora cinnamomi* in soils of mixed hardwood-pine forests of the south-eastern USA. NZ Journal of Forestry Science 41S:S39-S47.

Meadows, I. M., Colburn, G. C., and Jeffers, S. N. 2011. Evaluation of a copper hydroxide-based algicide to eliminate propagules of *Phytophthora* spp. in naturally-infested streams in South Carolina, USA: A preliminary report. NZ Journal of Forestry Science 41S:S3-S5.

Meadows, I. M., Zwart, D. C., Jeffers, S. N., Waldrop, T. A., and Bridges, W. C., Jr. 2011. Effects of fuel reduction treatments on incidence of *Phytophthora* species in soil of a southern Appalachian Mountain forest. Plant Disease 95:811-820.

Selected Non-Peer Reviewed Conference Proceedings and Technical Reports

Zhebentyayeva, T., Chandra , A., Abbott, A. G., Staton, M. E., Olukolu, B. A., Hebard , F. V., Georgi, L. L., Jeffers, S. N., Sisco, P. H., James , J. B., and Nelson, C. D. 2014. Genetic and genomic resources for mapping resistance to *Phytophthora cinnamomi* in chestnut. Acta Horticulturae (ISHS) 1019:263-270.

Jeffers, S. N., Meadows, I. M., James, J. B., and Sisco, P. H. 2012. Resistance to *Phytophthora cinnamomi* among seedlings from backcross families of hybrid American chestnut. Pages 194-195 in: Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees. Sniezko, R. A., Yanchuk, A. D., Kliejunas, J. T., Palmieri, K. M., Alexander, J. M., and Frankel, S. J., tech. coords. Gen. Tech. Rep. PSW-GTR-240. US Dept. of Agric., Forest Service, Pacific Southwest Research Station. Albany, CA.