PROPOSAL TITLE FORM

Phytophthora Root Rot of Chestnut Trees: Research Projects at Clemson University in 2022-2023

Potential Funding Agency:
The American Chestnut Foundation (TACF)
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Submitted by:
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01 August 2022

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A. Project Title

Phytophthora Root Rot of Chestnut Trees: Research Projects at Clemson University in 2022-2023

B. Summary

We will conduct research projects and collaborate with colleagues to better understand and manage Phytophthora root rot on American and hybrid chestnut trees. I anticipate having carry-over funds from 2021-22, so I am only requesting funds to help with labor costs. In 2022-23, we will:

(1) Continue to assay soils in the eastern United States for *Phytophthora* spp. and collect and store representative isolates in a permanent collection
(2) Work with colleagues to complete an RNA sequencing project initiated in 2021-22
(3) Collaborate with TACF and the USDA Forest Service to conduct the annual *P. cinnamomi* resistance screening experiment.

C. Principal Investigator and Institutional Affiliation

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D. Duration of Project: 01 November 2022 – 31 October 2023 = 12 months

E. Total Amount Requested: $5,354
$2,811 = 52.5% Matching funds will be provided by Clemson University

F. Short- and Long-term Goals of Project

Short-term Goals

1. To continue to assay plant and soil samples, provided by TACF members, for species of *Phytophthora*, so we can identify where these plant pathogens are located in chestnut-growing and potential chestnut-growing sites in the eastern U.S.; then, culture and maintain representative isolates of *Phytophthora* spp. for future studies
2. In 2021, we began a project to identify genes involved in resistance to *P. cinnamomi* by inoculating both American and Chinese chestnut seedlings with zoospores of *P. cinnamomi* and then removing seedlings at four time points after inoculation: 3, 6, 12, and 24 h. We also isolated from seedling roots and fixed roots for microscopic examination to determine

pathogen ingress at these same time points. Data still need to be collected, analyzed, and summarized, so we will work with our collaborators to complete this project.

3. Identify and supply isolates recovered and stored in Goal 1 (above) for use in the annual TACF *P. cinnamomi* resistance screening experiment at the Resistance Screening Center (RSC) at USDA Forest Service Bent Creek Experimental Forest in Asheville, NC; then, assist with inoculating ad scoring hybrid chestnut seedlings in spring and fall, respectively, during the year.

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. In addition, 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—e.g., see Goal 3 below. This information is essential to breed chestnuts with resistance to all species of *Phytophthora* capable of causing Phytophthora root rot.
2. We have been collaborating with colleagues at the Pennsylvania State University (Tatyana Zhebentyayeva), the University of Kentucky (Bert Abbott and Dana Nelson), and TACF (Jared Westbrook) to identify the genes involved in resistance to *P. cinnamomi*. In 2021, we identified when zoospores infected roots and established a pathogenic relationship with chestnut seedlings. Therefore, we were able to conduct the RNA sequencing experiment in Spring 2022. Inoculated roots currently are being sequenced, so our colleagues then can identify which genes are involved in the resistance processes in *Castanea* spp. In addition, we isolated from inoculated roots to identify when roots became infected, and fixed root segments are waiting to be examined microscopically to visualize the infection process at the various time points.
3. By staying actively involved in the annual TACF effort to screen hybrid chestnut seedlings for resistance to *P. cinnamomi*, we can maintain continuity and consistency in this effort, which our lab initiated in cooperation with Dr. Joe James in 2004. Next year, 2022 will be the 19th year of this resistance screening effort.

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 of 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 only requesting financial support to cover labor costs for three ongoing research projects 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 19 years, we have processed hundreds of samples and have recovered *Phytophthora* spp. from 11 states—primarily in the southeastern and mid-Atlantic regions but also from a chestnut planting in southwestern Pennsylvania and recently from two sites in Missouri. 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. Based on our isolation efforts, we have confirmed PRR on chestnut seedlings 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. 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 prevent 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. Ove the years, we have identified five species of *Phytophthora* associated with chestnut trees, seedlings and soils: *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 currently have several hundred isolates already in our collection and will continue collecting new isolates over the coming years. Eventually, we would like 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.

RNA sequencing/root infection project

We collaborated with chestnut colleagues Tatyana Zhebentyayeva, Bert Abbott, Dana Nelson, and Jared Westbrook as well as with Clemson colleagues Julia Kerrigan and Linus Schmitz to study the initial infection process on chestnut roots. In 2021, funds were made available to fund

an RNA sequencing project, which was conducted in April 2022. This involved infecting both American and Chinese chestnut seedlings with *P. cinnamomi* by immersing seedlings in a suspension of zoospores in 19-liter buckets. Seedlings then were removed of at four time points—3, 6, 12, and 24 h after initial exposure—and roots were processed immediately after removal. The root system on each seedling was divided into three parts for different assays: one part was frozen in liquid nitrogen for RNA sequencing to identify genes involved in resistance, one part was surface disinfested and used for direct isolation to determine when roots became infected, and the third part was chemically fixed for microscopic examination to visualize how zoospores enter root tissue. The team is now collecting data. These data will be analyzed, and the three aspects will be combined into a cohesive picture of infection of chestnut roots by *P. cinnamomi* zoospores.

Annual TACF effort to screen hybrid chestnut seedlings for resistance to *P. cinnamomi*

In cooperation with Dr. Joe James, my lab at Clemson University initiated a project to screen American, Chinese, and hybrid chestnut seedlings for resistance to *P. cinnamomi* in 2004. That project has continued annually ever since, and now is conducted in cooperation with the USDA Forest Service at the RSC at Bent Creek Experimental Forest in Asheville, NC. Each year, the site where surviving seedlings from the annual trial will be planted in the field is selected. This site is based on soil samples collected from potential sites in the Mid-Atlantic and Southeastern states. Soil samples are processed in our lab to identify ones that contain *P. cinnamomi*. Isolates of this pathogen are subcultured and maintained in axenic culture in a permanent collection. Once the field site for out-planting surviving seedlings is identified, we provide appropriate isolates of *P. cinnamomi* for inoculum production by Dr. Katie McKeever at the RSC. Staff from my lab then participate in inoculation of seedlings in the spring and scoring seedlings for root rot severity in the fall. Our involvement helps maintain continuity and consistency in how seedlings are inoculated and rated in this annual evaluation.

Literature Cited

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

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

Ferguson, A. J., and Jeffers, S. N. 1999. Detecting multiple species of *Phytophthora* in container mixes from ornamental crop nurseries. Plant Disease 83:1129-1136.

Jeffers, S. N., and Martin, S. B. 1986. Comparison of two media selective for *Phytophthora* and *Pythium* species. Plant Disease 70:1038 1043.

Jeffers, S. N., Sturdivant, M. R., and Schmitz, L. T. 2020. Managing Phytophthora root rot on American chestnut with fungicides, 2019. Plant Disease Management Reports 14:PF004.

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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.

Westbrook, J. W., James, J. B., Sisco, P. H., Frampton, J., Lucas, S., and Jeffers, S. N. 2019. Resistance to *Phytophthora cinnamomi* in American chestnut (*Castanea dentata*) backcross populations that descended from two Chinese chestnut (*Castanea mollissima*) sources of resistance. Plant Disease 103:1631-1641.

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 109:1594-1604.

H. Timeline (Nov 2022 – Oct 2023)

Research Project	**Nov-Jan**	**Feb-Apr**	**May-Jul**	**Aug-Oct**
Assaying plant and soil samples and maintaining isolates of *Phytophthora* spp.	X	X	X	X
RNA sequencing experiment	X	X	X	
Annual TACF resistance screening effort	X		X	

I. Measurement and Reporting of Results

Projects will be conducted based on the timeline above. Data will be collected as it becomes available—i.e., after samples are received and assayed and at the ends of trials. Likewise, these 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 NE-1833 Meetings. Once a project has been completed and if data are based on replicated trials, results will be published in the *Chestnut* or other suitable peer-reviewed journal.

J. Budget: One Year (Nov 2022 – Oct 2023)

Expense	**TACF: Requested ($)**	**Clemson: Matching ($)**
10% of salary for Lab Manager/Research Technician	3,744	
Fringe Benefits: 43% for full-time employees	1,610	
Subtotal	5,354	
Unrecovered overhead = F&A @ 52.5% = $2,811	0	2,811
TOTAL	***5,354***	***2,811***

Budget Justification

Amount Requested from TACF

Funds are requested to cover 10% of the salary and fringe benefits for a Lab Manager and Research Associate who will manage and work on these projects. He or she will be paid $18.00/hour and work 40 hours/week ($37,440 annual salary).

Carry-over funds from the 2021 project will be used for expendable supplies and travel. 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 ($2,811) is used as matching funds from Clemson University.

K. Brief CV for each of 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-present: Professor – Dept. of ESPS; School of Agriculture, Forestry, and Environmental Sciences; Dept. of Agricultural and Environmental Sciences; Dept. Plant and Environmental Sciences (currently)
2001-2007: Associate Professor – Depts. PP&P; 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: 2014-Present
Oliveira, S. A., Dlugos, D. M., Agudelo, P., and Jeffers, S. N. 2021. First report of *Meloidogyne javanica* pathogenic on hybrid lavender (*Lavandula* ×*intermedia*) in the United States. Plant Disease 105:335.

Bell, N. L., Jeffers, S. N., Hitchcock, D. R., and White, S. A. 2021. Potential susceptibility of six aquatic plant species to infection by five species of *Phytophthora*. Plant Disease 105:4074-4083.

Krasnow, C. S., Rechcigl, N. A., Olson, J. D., Schmitz, L. T., and Jeffers, S. N. 2021. First report of stem and foliage blight of chrysanthemum caused by *Phytophthora drechsleri* in the United States. Plant Disease 105:3765 (PD Note).

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 29:736-744.

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 109:1594-1604.

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Gitto, A. J., Jeffers, S. N., Graney, L. S., Loyd, A. L., and Bechtel, C. N. 2018. First report of *Phytophthora occultans* causing root rot on American boxwood planted in residential landscapes in the eastern United States. Plant Disease 102: *in press.*

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

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.

L. Conflict of Interest or Commitment Statement

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