A. Project Title

Phytophthora Root Rot of Chestnut Trees: Research Projects at Clemson University

B. Summary

We will continue to conduct independent research projects and collaborate with colleagues to better understand and manage Phytophthora root rot (PRR) on American and hybrid chestnut trees. In 2021, 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) Repeat an experiment to determine the efficacy of fungicides to manage PRR on American chestnut seedlings
(3) Determine when zoospores of *P. cinnamomi* infect American chestnut seedlings
(4) Collaborate with TACF and the USDA Forest Service to conduct the annual *P. cinnamomi* resistance screening experiment.

C. Principal Investigators and Institutional Affiliation

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D. Duration of Project: 01 November 2021 – 31 October 2022 = 12 months

E. Total Amount Requested: $9,958
$5,228= 52.5% Matching funds will be provided by Clemson University

F. Short- and Long-term Goals of Project

*Per Jared W, added $7000 RNA seq experiment—including histopathology work in collaboration with PSU Tatyana Z. Paid for with McQuaid Family Fdn grant issued for PSU.

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 sites and potential chestnut-growing sites in the eastern U.S.; then, culture and maintain representative isolates of *Phytophthora* spp. from chestnut-growing sites for future studies
2. Complete an evaluation of commercially available fungicides that target Oomycetes for efficacy against Phytophthora root rot on American chestnut seedlings
3. Determine when during the first 24 hours of exposure to zoospores of *P. cinnamomi* this pathogen establishes infection on American chestnut seedlings
4. 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 4 below. This information is essential to breed chestnuts with resistance to all species of *Phytophthora* capable of causing Phytophthora root rot.
2. 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. We conducted one trial of this experiment in 2019 and now need to repeat it to validate results. We tried to repeat the experiment in 2020, but COVID restrictions hampered our efforts.
3. We have been collaborating with colleagues at the Pennsylvania State University (Tatyana Zhebentyayeva) and the University of Kentucky (Bert Abbott and Dana Nelson) to identify the genes involved in resistance to *P. cinnamomi*. By identifying when zoospores infect roots and establish a pathogenic relationship with chestnut seedlings, we can pinpoint when roots of both American and Chinese chestnut seedlings need to be harvested for RNA sequencing—so our colleagues can identify which genes are involved in the resistance processes in *Castanea* spp.
4. 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 requesting partial financial support for research projects we plan to conduct 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.

Identifying effective fungicides for managing PRR on American chestnut seedlings

There are numerous 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, these pathogens could attack and kill all the trees in the orchard. Consequently, we conducted a greenhouse experiment in 2019 to evaluate the efficacy of eight commercially available fungicide products registered to manage Phytophthora diseases; each product contained a different active ingredient. Results from this experiment were very encouraging (Jeffers et al. 2019). Reliant, Aliette, and Subdue Maxx provided the best protection, but other products also were effective. This experiment needs to be repeated to validate and confirm these results. 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.

Determine when zoospores of *P. cinnamomi* infect chestnut seedlings

We will conduct an experiment to determine when American chestnut seedlings become infected during the first 24 hours of exposure to zoospores of *P. cinnamomi*. Seedlings will be grown in the greenhouse in Deepots (Stuewe and Sons, Inc., Tangent OR) containing soilless container mix (Fafard 3B: Sun Gro Horticulture, Agawam, MA) or particulate field conditioner (Turface MVP: Profile Products LLC, Buffalo Grove, IL). Seedlings will be removed from the pots, and the substrate will be washed from the roots. Seedlings then will be submerged in 50 liters of a zoospore suspension in 60-liter tubs (Fig. 1).

Figure 1. American chestnut seedlings suspended in a suspension of *P. cinnamomi* zoospores. Washed roots are submerged in the suspension.

Seedlings will be removed from the suspension at 3, 6, 12, and 24 hours after initial exposure, roots will be surface disinfested in a sanitizer solution for 30 sec and rinsed under running water, and seedling roots will be placed in clean tap water for up to 7 days for symptoms of PRR to develop. After 7 days in water, we will isolate from representative roots of each plant to determine the degree of infection. Rhododendron leaf disk baits also will be floated in the tubs and removed at each time point to verify zoospore activity throughout the experimental period.

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

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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.

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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 2021 – Oct 2022)

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
Fungicide efficacy: Trial in 2022		X	X	
Zoospore infection experiment	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 2021 – Oct 2022)

Expense	**TACF: Requested ($)**	**Clemson: Matching ($)**
15% of salary for Lab Manager/Research Technician	5,616	
Fringe Benefits: 41.7% for full-time employees	2,342	
Expendable lab supplies	2,000	
Subtotal	9,958	
Unrecovered overhead = F&A @ 52.5% = $5,228	0	5,228
TOTAL	***9,958***	***5,228***

Budget Justification

Amount Requested from TACF

Funds are requested to cover 15% of the salary and fringe benefits for Mr. Linus Schmitz, the Lab Manager and Research Associate who is managing and working on these projects. Mr. Schmitz makes $18.00/hour and works 40 hours/week ($37,440 annual salary). 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 ($5,228) is used as matching funds from Clemson University.

K. Brief CV for each of the Principal Investigators

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:xxxx (PD Note). Published online 27 July 2021.

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:zzzz-yyyy. Published online 11 June 2021.

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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.

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Education

2019: Clemson University, Clemson, SC: M.S. Plant and Environmental Sciences
2016: Bonn Rhein-Sieg University of Applied Sciences, Germany: B.S. Forensic Sciences

Employment

Clemson University, Clemson, SC
2019-present: Lab Manager and Research Associate, Dept. of PES
2017-2019: Graduate Research Assistant, Dept. of PES

Research Experience

2019-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

2017-2019: Investigation of pre-harvest disorders of peach fruit in South Carolina; efficacy of fungicides for management of brown rot of peach; fungicide resistance monitoring of *Botrytis cinerea* on strawberry

Teaching Experience

Principles of Plant Pathology (PLPA 3100/3101): 2020-present; teaching assistant
Selected Topics (PLPA 8020): 2020-present; teaching assistant

Recent Peer-Reviewed Publications and Technical Reports

Krasnow, C., Rechcigl, N., Olson, J., Schmitz, L., Jeffers, S.N. First report of stem and foliage blight of chrysanthemum caused by *Phytophthora drechsleri* in the United States. 2021. Plant Dis. doi: 10.1094/PDIS-03-21-0631-PDN.

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Schmitz, L. T., Jeffers, S. N., and Baker, R. B. 2020. Evaluation of fungicides for managing Fusarium root and crown rot on liriope, 2019. Plant Disease Management Reports 14:OT014

Schmitz, L. T. and Schnabel, G. 2019. Infrequent occurrence of peach skin streaking and the role of rainwater attributes on symptom development. Plant. Dis. 103: 2606-2611

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L. Conflict of Interest or Commitment Statement

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