Final Report:

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

Submitted to:

The American Chestnut Foundation (TACF)
50 North Merrimon Ave., Suite 115
Asheville, NC 28804

Submitted by:

Steven N. Jeffers, Ph.D.
Dept. of Plant and Environmental Sciences
105 Collings St., 214 BRC
Clemson University
Clemson, SC 29634-5702

12 August 2015

Project Title

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

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

Total Amount Awarded: $8,350

Original Project 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, Chinese, and backcross hybrid chestnut trees in the eastern USA to investigate the potential diversity of species of *Phytophthora* associated with this native tree species. We have isolated five species of *Phytophthora* from the roots of soil associated with American, Chinese, and backcross hybrid chestnut trees growing in field plots in four southeastern states, and we have evaluated the pathogenicity of these species to American and Chinese chestnut seedlings in the greenhouse. These results should affect how we select for resistance in American chestnut in the future.

Original Project 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

Summary of Project Results

This project was funded for one year and contributed to the Master of Science research project at Clemson University conducted my Ms. Suzette R. Sharpe. Ms. Sharpe is graduating 12 May 2017, and she has submitted a 130-page thesis to fulfill, in part, her degree requirements. The title of her thesis is:

Phytophthora Species Associated with American, Chinese, and Backcross Hybrid Chestnut Seedlings in Field Sites in the Southeastern United States

The objectives of this TACF research project are very similar to the objectives in Ms. Sharpe's thesis project with each objective being covered by one chapter in her thesis. Thesis chapters are titled:

Chapter 1: Isolation and Identification of *Phytophthora* Species Associated with American, Chinese, and Backcross Hybrid Chestnut Seedlings in the Southeastern United States

Chapter 2: Pathogenicity and Virulence of *Phytophthora* Species to American and Chinese Chestnut

Therefore, I am submitting Ms. Sharpe's thesis abstract as a final report for this TACF research project. I will provide a PDF copy of the thesis to TACF if it is requested.

Phytophthora root rot was first described on American chestnut (*Castanea dentata*) in a preliminary report in 1932 and the causal agent was tentatively and erroneously identified as *Phytophthora cambivora*. Soon after, the causal agent was correctly identified as *P. cinnamomi* and, since that time, *P. cinnamomi* has been the only species reported to cause Phytophthora root rot on American chestnut trees. In the early 1980s, The American Chestnut Foundation initiated a backcross breeding program to develop chestnut trees that had resistance to *Cryphonectria parasitica*, which causes chestnut blight. In the early 2000s, backcross hybrid chestnut seedlings ([American × Chinese] × American) were planted in field plots in the eastern United States to evaluate field performance of these seedlings. Between 2010 and 2014, 271 root and 353 soil samples associated with diseased American, Chinese, and mostly backcross hybrid chestnut seedlings were collected in eight field sites in four states in the southeastern United States, and these samples were assayed for presence of species of *Phytophthora*. *Phytophthora cinnamomi*, *P. cambivora*, *P. heveae*, *P. quercetorum*, and *P. cryptogea* were recovered by isolation from roots on PARPH-V8 selective medium and baiting soil with rhododendron and camellia leaf pieces. A total of 248 isolates were recovered and tentatively identified based on morphology; species identifications were confirmed by sequencing the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). *Phytophthora cinnamomi* was recovered most frequently—from 17% of root and 34% of soil samples. *Phytophthora cambivora* and *P. heveae* also were recovered—from 10% and 1% of root samples and 9% and 5% of soil samples, respectively. Nine of the isolates of *P. cambivora* were from roots of and

soils associated with backcross hybrid chestnut seedlings growing in in the field at a tree nursery in western VA. *Phytophthora quercetorum* was recovered from one soil sample, and *P. cryptogea* was isolated from roots of five backcross hybrid chestnut seedlings at a separate field site in northwestern South Carolina.

Isolates were characterized for mycelium growth habit, mating type, mefenoxam sensitivity, and sporangium production. Three mycelium growth habits were identified when isolates of *P. cinnamomi* were grown on PARPH-V8 selective medium. All 165 isolates of *P. cinnamomi* were mating type A2; but both A1 and A2 mating types were present in the population of isolates of *P. cambivora*. Isolates of all species recovered from forest sites and the site in South Carolina were sensitive to the fungicide mefenoxam at 100 ppm, but two isolates of *P. cambivora* from the nursery site were insensitive to this fungicide. The cytochrome oxidase subunit 1 (*cox1*) locus was sequenced for 52 isolates of *P. cambivora*, and all sequences were similar. A single nucleotide polymorphism was observed in sequences of the *rps10* gene for a small subset of *P. cinnamomi* isolates with a sparse mycelium growth habit.

Four species of *Phytophthora* were tested for pathogenicity to American and Chinese chestnuts—*P. cinnamomi, P. cambivora, P. heveae*, and *P. cryptogea*. To fulfill Koch's postulates, 3-month-old, open-pollinated American and Chinese chestnut seedlings were artificially inoculated by soil infestation using individual isolates of each species and periodic flooding. There was a significant amount of root rot caused by *P. cinnamomi, P. cambivora*, and *P. cryptogea*, and all species caused necrotic lesions on the main tap root of American chestnut seedlings. *P. cinnamomi* was the only species that caused significant root rot on Chinese chestnut seedlings, but *P. cambivora* and *P. cryptogea* occasionally caused necrotic lesions on the main tap root of Chinese chestnut. Virulence of *P. cinnamomi* and *P. cambivora* was compared on 2- and 3-year-old American chestnut seedlings using inoculum treatments composed of isolates from different geographical locations and substrates; each treatment was a mixture of two, three, or four isolates of one species. *Phytophthora cinnamomi* was more virulent than *P. cambivora* based on lesion height on the main stem and amount of root rot. No differences were observed among isolate treatments within each species. Three treatments of *P. cinnamomi* produced significant lesions on the main stems of seedlings. *Phytophthora cambivora* did not consistently produce symptoms under the experimental conditions used in the virulence experiment. This is the first study to report multiple species of *Phytophthora* as pathogenic to American and backcross hybrid chestnuts.