a. Title: A proposed study for the identification of differentially expressed genes associated with exposure to *Phytophthora cinnamomi* in *Castanea dentata* (susceptible) and *C. mollissima* (resistant)

b. Summary (100 words):

We will attempt to identify genes that are differentially expressed in the very early stages of *Phytophthora cinnamomi* infection by *Castanea dentata* (susceptible) and *C. mollissima* (resistant). The basic approach is analogous to previous studies in *C. sativa* (susceptible) and *C. crenata* (resistant); however, we plan to use Representational Difference Analysis (RDA), rather than whole transcriptome analysis, to identify genes of interest related to susceptibility/resistance. Our results should inform work by others intended to identify and employ genes for Pc-PRR resistance in chestnuts, and should assist in identifying the mechanisms by which such resistance is established in Asian *Castanea* species.

c. Principal Investigator(s) and Institutional Affiliation(s)

Martin Cipollini and Michael Morgan, Department of Biology, Berry College, Mount Berry, GA.

Pingsheng Ji, Department of Plant Pathology, University of Georgia, Tifton, GA.

d. Duration of project: (note, funding is for one year only, with resubmission allowed annually for up to five years):

In this proposal, we are requesting funding that will enable us to reach short-term goal #1 and #2, as described below. We intend to request another year's funding directed toward achieving short-term goal #3 and long-term goals #4-6, based upon the results obtained from initial TACF funding.

e. Total amount requested. Please list sources and amount of matching funding for the same project.

Total requested in TACF grant, year 1 = $5287

Matching contributions made by Berry College, year 1:

1) qPCR materials – $930 total. One 96-well plate analyzing 7 GOIs + endogenous control needs 1ml SYBR green =$86.00. Estimating to initially screen ~70 RDA products (ESTs) = 10 plates = $860. 96-well plate pkg of 10 plates = $70. These materials are presently on hand and are to be considered in-kind contributions.
2) Student work hours – $7.25/hr. 8hrs/week for 11 weeks/semester = $628/semester. Total = $1276/academic year.
3) Insofar as TACF does not allow grant funds to go toward indirect (overhead) costs, Berry College will provide $1295 as matching funds toward indirect costs representing (24.5% of total).

Total matching contributions, year 1 = $3501

f. Short and long-term goals of the project

Short-term goals:

1) Identify sets of genes (candidate biomarkers) differentially expressed in roots of *C. dentata* and *C. mollissima* upon exposure to Pc, since these species form the basis of TACF's backcross breeding program.

2) Determine if differentially expressed genes in roots of *C. dentata* and *C. mollissima* are similar in genes found in *C. sativa* vs. *C. crenata* previously identified as candidates for Pc-PRR resistance (Santos, et al., 2017a; Serrazina, et al., 2015).

3) Perform additional RDA hybridizations by cross-hybridizing cDNAs of American Pc-infected vs Chinese Pc-infected to isolate subsets of genes that are potentially Pc-PRR and/or Pc-susceptible.

Long-term goals:

4) Characterize responsiveness of candidate biomarkers across a spectrum of time courses to characterize host-pathogen interactions.

5) Determine if genes up-regulated in *C. mollissima* are located within QTLs found to correlate with Pc-PRR resistance in *C. mollissima*/*C. dentata* hybrid breeding populations (Staton, et al. 2015; Zbentyayeva et al., 2016).

6) Explore potential functions of differentially expressed genes as they relate to the host-pathogen interaction.

Together, our results may help inform work by others intended to identify and employ genes for Pc-PRR resistance in chestnuts, and may assist in identifying the mechanisms by which such resistance is established in Asian *Castanea* species.

g. Narrative (no more than five (5) pages)

Introduction:

Phytophthora root rot (PRR) is a soil-borne disease that is extremely damaging to a wide variety of plant species (Hardman and Blackman 2018; Sena et al., 2018). It is especially lethal to North American *Castanea*, including American chestnut [*C. dentata* (Marsh.) Borkh.], Allegheny chinquapin [*C. pumila* (L.) Mill. var. *pumila*], and Ozark chinquapin [*C. pumila* (L.) Mill. var. *ozarkensis* (Ashe) Tucker] (Crandall et al. 1945; Jeffers et al. 2009, 2012). This disease is also devastating to the economically important European chestnut, *C. sativa* Mill. (Santos et al. 2014). The principal cause of PRR is the oomycete *Phytophthora cinnamomi* Rands (Pc). Pc was likely introduced from southeastern Asia to the southeastern U.S. via ornamental plants in the early 1800s. Pc was associated with the dieback of *C. dentata* and *C. pumila* var. *pumila* in the southeastern Coastal Plain and Piedmont during that time frame (Anagnostakis, 2001; Crandall et al., 1945). Presently, Pc can be found in dying *Castanea* at a broad range of locations beyond the southeastern U.S., ranging northward to Pennsylvania and westward to Arkansas (Crandall et al., 1945; Fitzsimmons 2016; Jeffers et al., 2009). Spread is associated with movement of contaminated plant material and soils, and recent and projected northward spread may be related to climate change (Burgess et al., 2017).

Symptoms of PRR in chestnuts (root necrosis, leaf chlorosis and wilt, and branch dieback) generally kill susceptible trees within weeks or months after infection (Crandall et al., 1945; Jeffers et al., 2009). Symptoms may be controlled using phosphites or other chemicals (James, 2011a), but this is not a viable solution for control in natural settings. Asian *Castanea*, in particular Chinese chestnut (*C. mollissima* Blume) and Japanese chestnut (*C. crentata* Sieb. & Zucc.), are generally resistant to Pc-induced PRR (Pc-PRR) (Perkins et al., 2019; Santos et al. 2014). In addition to loses due to PRR, North American *Castanea* species have been devastated across their range by chestnut blight, caused by *Cryphonectria parasitica*

(Murr.) Barr (Alexander et al., 2005; Anagnostakis 2001; Paillet 1993, 2002). As with PRR, Asian *Castanea* species generally show resistance to chestnut blight (Burnham 1988; Hebard 2005).

Because *C. dentata* is susceptible to both Pc-PRR and chestnut blight, restoration of *C. dentata*-type trees across large portions of its original range requires restoration stock resistant to (or tolerant of) both pathogens. Because Asian *Castanea* species harbor blight- and Pc-PRR resistance, these species are of potential use in obtaining resistance to both diseases in *C. dentata* via breeding and biotechnological approaches. Since 1989, The American Chestnut Foundation (TACF) has used backcross methods to introgress blight resistance from *C. mollissima* into a *C. dentata* genetic background (Hebard 2005; Westbrook 2017). Steiner et al. (2017) affirmed that blight-tolerant trees with a *C. dentata* phenotype (Cipollini et al., 2017; Diskin et al., 2006) can be developed via introgression of extraspecific alleles for blight resistance. Very recently, Westbrook et al. (2019) reported that the level of blight resistance in TACF backcross lines will not likely meet the original expectations of the breeding program. Current plans involve the potential use of a *C. dentata* line transformed using an oxalic acid oxidase (OXO) gene from wheat that confers blight resistance (Westbrook 2016; Westbrook et al. 2019b; Zhang et al., 2013). The OXO transgenic chestnut carries no resistance to Pc-PRR and there are no *C. dentata* lines genetically modified for PRR resistance in the U.S. regulatory pipeline (J. Westbrook, TACF, personal communication).

Because it was only relatively recently recognized that PRR might seriously impede *C. dentata* restoration; TACF's breeding program initially focused solely on selection for blight resistance. PRR caused extensive losses in orchards and experimental plantings, in many cases before backcross trees could be evaluated for blight resistance (Clark et al., 2014, 2019; Jeffers et al., 2009, 2012; Sisco 2009). In 2004, James (2011a, 2011b) and Jeffers et al. (2009, 2012) initiated a screening program in South Carolina to detect Pc-PRR resistance, focused primarily on TACF's backcross trees. Via this program and a similar program established by Frampton (Bowles 2006) in North Carolina, Pc-PRR resistance has been detected in TACF backcross trees derived from the 'Graves' line, with somewhat lower frequencies among 'Clapper' lines (Jeffers et al., 2009; Westbrook et al., 2019c). Additionally, Pc-PRR resistance has been identified in TACF's 'Nanking' line (Zhebentyayeva et al., 2014; Zhebentyayeva et al., 2019) and in novel *C. mollissima* sources (Perkins et al., 2019).

Studies of quantitative trait loci (QTL) associated with resistance to Pc-PRR suggest control by a relatively small number of genomic regions. QTLs for Pc-PRR resistance have been found on linkage groups E and K of *C. crenata* (Santos et al., 2017b) and on the same linkage groups in *C. mollissima* (Zhebentyayeva et al., 2014; Zhebentyayeva et al., 2016). This suggests common mechanisms of Pc-PRR resistance in different species of Asian *Castanea* (Santos et al., 2017). These regions may be targets for marker-assisted selection (Santos et al., 2017b; Zhebentyayeva et al., 2014). It remains possible that Asian *Castanea* have additional alleles for Pc-PRR resistance, and that *C. mollissima* and *C. crenata* have different resistance alleles. The mechanisms of resistance to Pc-PRR in *Castanea* are relatively unexplored (Santos et al., 2017a).

Using transcriptome analysis wi*th C. sativa vs. C. crenata* exposed and unexposed to Pc*,* Serrazina et al. (2015) and Santos et al. (2017a) reported differential gene expression profiles and identified several candidate genes for Pc-PRR resistance. This subset of genes had a lower and later response in *C. sativa* (susceptible) relative to their expression in *C. crenata* (resistant). To date, candidate genes for Pc-PRR resistance have not been evaluated experimentally in *Castanea*, although some cis- and trans-genes

have been inserted into *C. dentata* and other species ((cf., Costa 2018, S. Merkle, University of Georgia, personal communication).

The challenge faced by using whole transcriptome analysis is the fact that a majority of the genes identified are not differentially expressed. Representational Difference Analysis (RDA), as developed by Hubank and Schatz (1999), is a powerful and sensitive tool for identifying differentially expressed genes of interest (GOIs) in one physiological, developmental, or diseased condition as compared to the presence/absence of the same transcripts in another alternative condition. RDA is a form of PCR-based subtractive hybridization that amplifies and subsequently detects rarer transcripts. Compared to cloning entire cDNA libraries, RDA is capable of selectively enriching the GOIs that may be differentially expressed within the populations being investigated. RDA has successfully been used to identify differentially expressed GOIs from a diverse suite of animal and human diseases ranging from Yellow Band disease in corals (Morgan et al., 2015), cervical cancer (Sgarlato et al., 2005), and tumor progression in prostate cancer (Takahashi et al., 2002). RDA has also successfully detected developmental genes (Cooper et al., 2000) and even viral candidate genes from brain tissues infected with Creutzfeldt-Jakob disease (Dron and Manuelidis 1996). The RDA approach is ideally suited for detecting critical differences in Pc resistance in *C. mollissima* compared to *C. dentata.* Zhebentyayeva et al. (2019) describes the *Castanea* genus as having weak reproductive barriers enabling development of hybrid populations. This fact suggests that an RDA approach might be an attractive technique for detecting a critical subset of molecular differences in Pc resistance in *C. mollissima* compared to *C. dentata*. Perkins et al. (2019) hypothesize that multiple alleles may encode pc-PRR resistance. Once again, the RDA approach offers the possibility of detecting alternative forms of GOIs. RDA represents an *a priori* approach to identifying GOIs that can be representative biomarkers of Pc-PRR.

Experimental approach:

C. mollissima and *C. dentata* seedlings were grown in pots started from seed in January 2019. Two seedling sources are being used in this study: *C. dentata* (susceptible) CAT277 X opAM obtained from P.H. Sisco (CC-TACF) and *C. mollissima* (resistant) GAFL1 X opCH. CAT277 was obtained from a tree in the American chestnut section of the Cataloochee Ranch orchard in North Carolina. GAFL1 is a source of both Pc and blight resistance in F1s and B1s in the GA-TACF chapter breeding program. The growing medium is 1:1 course vermiculite:PRO Mix BX, supplemented with small amounts of Osmocote. Watering is ad lib. Pots were 4 X 4 X 14 inch deep Stuewe Treepots.

On July 22, we exposed subsets of both species to a prepared inoculum of a Pc strain known to be pathogenic to *C. dentata*. A parallel set of seedlings of both species under otherwise identical growing and treatment conditions, but were sham inoculated using sterile inoculation medium.

The Pc culture used for this study was isolated from a naturally infested orchard in south Georgia. Pathogenicity of the isolate was verified by inoculating *C. dentata* seedlings under greenhouse conditions in Tifton, GA. Vermiculite inoculum of the Pc isolate was prepared using the method of Aghighi et al. (2016). Chestnut seedlings were grown in potting mix in 4 x 4 x 14 inch plastic pots in the greenhouse. Forty day-old seedlings were inoculated by making a 3-inch deep hole in the soil around the taproot and applying 7.5 ml vermiculite inoculum in the hole. The holes were covered by potting mix and the pots were kept in the greenhouse. Disease development was assayed weekly after inoculation. A similar inoculation method was used in this study. Inoculum was prepared as described above and 30 ml inoculum was used to inoculate each plant (the seedlings were bigger than those used in Tifton so we

used two 3 inch deep holes with 15 ml of inoculum in each hole). Both inoculated and sham inoculated seedlings were flooded with water periodically for 1 hr post-inoculation to ensure distribution of inoculum throughout the medium. The potting medium was kept moist via watering as needed thereafter. After 52 hrs, we took small root samples for RNA extraction from all four sets (*C. mollissima* exposed and unexposed, *C. dentata* exposed and unexposed). The 52 hr time course emulates the expression profiling time course for early responses to *P. cinnamomi* infection studied by Santos et al. (2017a; 24-48 hr exposure), Serrazina et al. (2015; 2-7 day exposure), and Fernandes et al., 2017 (3.5-72 hr exposure).

At the same time, we took a subset of roots from inoculated and sham inoculated samples to verify that Pc infection had taken place and to describe morphological conditions of the roots at that time frame. To do so, roots from 0-3 inch under the soil surface were used. Root samples were surface disinfested by 0.5% NaOCl, rinsed three times with sterile distilled water, and cultured on PARPH agar plates (Jeffers and Martin 1986). Fungus-like cultures grown from the roots were purified and identified by morphological characteristics and PCR analysis (Erwin and Ribeiro 1996; Kong et al., 2003). Disease symptoms, pathogen signs and root morphology were also observed under a microscope.

To investigate infection resistance responses in *Castanea* sp., tissues collected at the 52 hr post-inoculation point were placed in RNAlater, and subsamples were flash frozen in liquid N_2 and immediately placed in -80°C freezer. Total RNA will be extracted using RNeasy plant kit. Messenger RNA will be isolated from total RNA, DNase I digested, and reverse-transcribed into cDNAs (Qiagen, NEB, Invitrogen, respectively) from the four chestnut populations (*C. dentata*-uninfected, *C. mollissima*-uninfected, *C. dentata*-Pc infected, *C. mollissima*-Pc infected). The cDNAs from each population will be digested by Dpn II restriction enzyme. All digested fragments >100bp will be ligated with R-24 primer and PCR will generate amplicons for each chestnut population. Each amplicon will be digested a second time with Dpn II to remove the R-24 primer and create a pool of cut "drivers". A portion of each cut driver amplicon will also be used to generate a "tester" pool by subsequent ligation of J-24 primer. Drivers and Testers will be combined and allowed to hybridize in ratios of 100/1. Hybridizations will result when two identical cDNA transcripts anneal to each other. There will be three hybridization possibilities: driver/driver, driver/tester, or tester/tester. The driver/driver will not amplify because there are no primer binding sites. The driver/tester combination will amplify in a linear fashion (because only tester has primer site) and can be eliminated in the RDA protocol. The tester/tester combination will amplify exponentially and enrich the amplification of transcripts uniquely associated with the tester population. Table 1 provides an organizational outline of targeted hybridizations for year 1.

Hybridization	Driver	Tester	Anticipated results
1	Am cntl	Am Pc inf	Pc susceptible genes up-regulated or induced in Am Pc inf
2	Am Pc inf	Am cntl	Pc susceptible genes down-regulated or turn off in Am cntl
3	Ch cntl	Ch Pc Inf	Pc-PRR resistant genes up-regulated or induced in Ch Pc inf
4	Ch Pc inf	Ch cntl	Pc-PRR genes down-regulated or turned off in Ch cntl

Table 1. Driver/Tester hybridizations and predicted outcomes. Hybridizations 1-8 will be investigated in this proposal. Abbreviations: Ch=Chinese; Am=American; Pc inf= *P. cinnamomi* infected; cntl=control sample.

RDA Hybridizations products (ESTs) representing putative GOIs will be cloned and sequenced. Basic Local Alignment Search Tool (BLASTX or TBLASTX) searches will identify putative gene homologs. Primers will be developed for each EST and confirmation of differential expression will be determined using qPCR

and calculating the $2^{-\Delta\Delta Ct}$ relative expression. All four populations (Am cntl, Am Pc inf, Ch cntl, Ch Pc Inf) will be simultaneously screened by qPCR for each GOI. Actin 7 will be used as the endogenous control gene as it was in Serrazina et al. (2015). Other endogenous control genes might also be used. The Morgan lab has found ribosomal protein L11 (RPL11) has also proven to be a reliable endogenous control in other fungal-related qPCR projects (data unpublished). Zhebentyayeva et al. (2019) describes the *Castanea* genus as having weak reproductive barriers enabling development of hybrid populations. This fact suggests that an RDA approach might be ideally suited for detecting critical molecular differences in Pc resistance in *C. mollissima* compared to *C. dentata*. All qPCR assays will be performed using three replicate reactions for each GOI as well as the endogenous control (as previously identified). We anticipate screening approximately 70 GOIs and qPCR will confirm a subset as differentially expressed. Those GOIs with confirmed differential expression will subsequently be searched for in *Castanea* sp OTLs of interest.

h. Timeline:

Short term goal 1: Start date - Nov 1, 2019 for RNA extraction. RDA products generated by Dec 31, 2019. RDA products cloned and sequenced by Mar 1, 2020. Beginning Apr 1, putative candidate GOIs will screened by qPCR to investigate differentially expressed genes found in *C. sativa* vs. *C. crenata* exposed to Pc. Conclude qPCR by Jun 1, 2020.

Short term goal 2 completed at end of year 1: Bioinformatic analyses of GOIs will provide basis for comparing RDA products of *C. dentata* vs. *C. mollissima* exposed to Pc to the published transcriptome responses of *C. sativa* vs. *C. crenata* exposed to Pc. Submit proposal for second year of TACF funding by Aug 1.

Short term goal 3 completed after year 1: Perform additional RDA hybridizations by "cross-hybridizing" cDNAs of American Pc-infected vs Chinese Pc-infected to isolate an additional subset of genes that are potentially Pc-PRR and/or Pc-susceptible and distinctively different from genes isolated in goal 1. This RDA "cross-hybridization" represents a novel application of the technique.

The work accomplished in goal 1 will provide a set of candidate genes (aka biomarkers) that are putatively representative of early stage Pc-PRR and/or Pc-susceptible responses. Through bioinformatics analyses, candidate biomarkers (isolated in goal 1) can be characterized by annotations and gene ontology (GO) terms (goal 2) to offer insight into understanding gene functions (long term goal 6). The work in goal 1 will identify Pc-susceptible genes in Am Pc inf and Pc-PRR genes in Ch Pc inf. GOIs isolated in goal 1 will be used as a preliminary screening tool for identifying additional GOIs isolated by cross-hybs (goal 3) in subsequent year. GOIs isolated in goal 1 and 3 will be the basis for analyzing time course expression (long term goal 4) and potentially representative candidate genes located within *Castanea* sp. OTLs (long term goal 5).

i. How results will be measured and reported.

Data collection methods are described in the narrative above. In addition to reporting results in an article for the Chestnut, we plan to submit a manuscript to the journal New Forests or an equivalent peer-reviewed journal.

Breakdown of how and when funds will be spent

Items 1-4 will be purchased on Nov 1 and used by Dec 31, 2019. Items 5-7 will be purchased by Feb 1 and used Mar 1, 2020. Item 8 will be purchased by Apr 1 and used by Jun 1. Item 9 will be divided up between the shipment of items 1-8.

1. Total RNA isolation - RNeasy plant kit - $391
2. Oligotex mRNA enrichment - $352
3. Reverse transcription with Superscript IV - $200
4. RDA reagents (Taq, Dpn II, T4 DNA ligase, Glycoblue) (NEB) - $400
5. Cloning RDA products – Topo TA kit - $528
6. Plasmid purification using PureLink™ Pro Quick96 well vacuum purification kit. The costs is $2.60/plasmid which = $250/96 well plate. For 4 plates $1000
7. Sequencing of RDA products – service by GenScript. The cost is $5.25/rxn which = $504/96-well plate. For 4 plates = $2016
8. Primers for qPCR - $200 estimated
9. Estimated Shipping costs = $200

k. Brief Curriculum Vitae (CV) for each Principal Investigator, including recent publications and grants received. Please restrict each CV to two (2) pages.

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Education:

1991. Ph.D. Ecology. Rutgers University, New Brunswick, NJ.
1984. M.S. Biology. Indiana University of Pennsylvania, Indiana, PA.
1981. B.S. Biology major/Chemistry minor. Indiana University of Pennsylvania, Indiana, PA.

Current Professional Positions:

1995 – present. Dana Professor of Biology, Berry College, Department of Biology, Mount Berry, GA. Courses taught: Field Botany, Principles of Cell Biology, Principles of Microbiology and Botany, General Microbiology, Forest Ecology, Tropical Ecology and Conservation in Cuba and in Costa Rica, Biology Seminar.

2001 - present. Director of the Berry College Longleaf Pine Project, Department of Biology, Mount Berry, GA.

2002 - present. Research Collaborator: Plant Ecology Laboratory. Smithsonian Institution, Smithsonian Environmental Research Center, Edgewater, MD.

2006 – present. Director of the Berry College American Chestnut Project and undergraduate internship program (collaborative program with the Georgia Chapter of the American Chestnut Foundation [GA-TACF]; GA-TACF Science Coordinator and Treasurer for the state of Georgia).

2013 – present. Steering Committee, Talladega-Mountain Longleaf Pine Conservation Partnership (chair of the Northwest Georgia Working Group).

Relevant Peer-reviewed Publications (last five years):

Cipollini, M.L., N. Wessel, J.P. Moss, and N. Bailey. Seed and seedling characteristics of hybrid chestnuts (*Castanea* spp.) derived from a backcross blight-resistance breeding program. New Forests (accepted pending revision July 2019).

Ali, M.E., A. Hajihassani, S. Waliullah, M. Cipollini, P. Ji, and T.B. Brenneman. First report of *Meloidogyne javanica* infecting American chestnut trees (Castanea dentata) in Georgia. Plant Disease 22 May 2019 https://doi.org/10.1094/PDIS-03-19-0604-PDN

Perkins, M.T., A.C. Robinson, M.L. Cipollini, and J.H. Craddock. 2019. Identifying host resistance to *Phytophthora cinnamomi* in hybrid progeny of *Castanea dentata* and *Castanea mollissima*. HortScience 54(2):221–225. https://doi.org/10.21273/HORTSCI13657-18

Cipollini, M.L., N.R. Dingley, P. Felch, and C. Maddox. 2017. Evaluation of phenotypic traits and blight-resistance in an American chestnut backcross orchard in Georgia. Global Ecology and Conservation 10:1–8. http://dx.doi.org/10.1016/j.gecco.2017.01.004

Coughlin, E., M. Cipollini, and S. Watkins. 2013. Chestnut's newest enemy: Ambrosia beetles. The Journal of The American Chestnut Foundation 27(3):28-31. (reviewed by science cabinet)

Relevant Professional Presentations (last five years, exclusive of GA-TACF meetings and routine public outreach events):

Cipollini, M.L., A.M. Metaxas, J. Klaus, N. Klaus, JP. Moss, W. Walker, and N. Bailey. 2018. Georgia offers unique opportunities to incorporate greater genetic diversity in *Castanea* breeding programs. TACF Annual Meeting, Huntsville, AL. October 26-27, 2018

Cipollini, M.L. 2018. Restoration of American chestnut in the southeastern United States; strategies for the development of disease resistance and conservation of genetic diversity. GA-DNR Master Naturalist Program, Canton, GA. October 19, 2018.

Cipollini, M.L. 2018. Restoration of American chestnut in the southeastern United States; strategies for the development of disease resistance and conservation of genetic diversity. Dahlonega Science Café, Dahlonega, GA. July 16, 2018.

Cipollini, M.L. 2018. Restoring Mountain Longleaf Pine and American Chestnut in Georgia, Georgia Urban Forest Council Meeting, Rome, GA. April 25, 2015.

Cipollini, M.L. 2017. Insect pests of chestnuts, with emphasis on ambrosia beetles. TACF Annual Meeting, Portland, ME. October 5-7, 2017.

Cipollini, M.L., N. Wessel, J.P. Moss, N. Bailey, and D. Nicely. 2017. Seed and seedling characteristics of hybrid chestnuts (*Castanea* spp.) derived from a backcross blight-resistance breeding program in Georgia. TACF Annual Meeting, Portland, ME. October 5-7, 2017.

Cipollini, M.L. and N. Wessel. 2017. Seed and seedling characteristics of hybrid chestnuts (*Castanea* spp.) derived from a backcross blight-resistance breeding program in Georgia. Ecological Society of America Annual Meeting, Portland, OR. August 9, 2017.

Cipollini, M.L. 2017. Restoration of disease resistant American chestnuts: Role of TACF state chapters and "citizen science". Georgia Mountain Research and Education Center Community Council. Blairsville, GA, May 11, 2017.

Cipollini, M. L, N. R. Dingley, and P. Felch. 2016. Progress over the first ten years of The American Chestnut Foundation blight resistance breeding program in Georgia. Natural Areas Conference, Davis, CA. October 20, 2016.

Cipollini, M.L. 2016. Restoring the Mountain Longleaf Pine and American Chestnut. Association of Natural Resource Extension Professionals Annual Meeting and Professional Improvement Conference. Berry College, Mount Berry, GA. August 1, 2016.

Cipollini, M.L., N.R. Dingley, and P. Felch. 2015. Progress over the first ten years of the GATACF blight resistance breeding program. TACF Annual Meeting 2015 with Schatz Tree Genetics Colloquium. State College, PA. October 23-24, 2015. (Cipollini presenting).

Cipollini, M.L., Watkins, S., T. Kantelis, N.R. Dingley, and P. Felch 2014. The first tests of blight-resistance in backcross hybrid American chestnuts (*Castanea dentata*) in Georgia: an assessment of leaf, stem, and bud traits. The American Chestnut Foundation 2014 Annual Meeting. October 17-19, 2014. Front Royal, VA.

Watkins, S., T. Kantelis, N.R. Dingley, P. Felch, and M. Cipollini. 2014. The first tests of blight-resistance in backcross hybrid American chestnuts (*Castanea dentata*) in Georgia: an assessment of leaf, stem, and bud traits. 73rd Annual Association of Southeastern Biologists Meeting, April 2-5, 2014, Spartanburg, SC.

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Education:

Ph.D., Biology, Biochemistry Minor 2001, Georgia Institute of Technology, Atlanta, Georgia
M.A., Biology - Loma Linda University, Loma Linda, California 1992
B.S., Biology - Zoology emphasis - Pacific Union College, Angwin, California 1983

Professional Experience:

Professor of Biology 2019 to present
Associate Professor of Biology, Berry College 2009 to 2019
Affiliate Professor in Biochemistry, Berry College 2017 to present
Invited Visiting Professor, Rosario Beach Marine Laboratory Summers 2013 & 2017
Assistant Professor of Biology, Berry College 2003 to 2009
Postdoctoral Associate, Georgia State University 2001 to 2003
Postdoctoral Fellow, Georgia Institute of Technology 2001
Graduate Research Assistant, Georgia Institute of Technology 1995-2001
Secondary school science teacher, Rosemont Middle School 1994-1995
Secondary school science teacher, San Gabriel Academy 1992-1994
Secondary school science teacher, Loma Linda Academy 1990-1992
Graduate Teaching Assistant, Loma Linda University 1988-1990
Secondary school science teacher, Escondido Adventist Academy 1983-1988

Professional Memberships:

International Society of Reef Studies, Sigma Xi, Phi Kappa Phi, Society of Environmental Toxicology and Chemistry, The American Chestnut Foundation

Selected Publications:

Mowry, C. B., C. M. Keene. S. E. Prisland, B. D. Tyler, A. A. Montgomery, A. P. Mowry, R. A. Martin, S. Stevens, J. Ellwanger, M. B. Morgan. 2017. A Survey of *Batrachochytrium dendrobatidis* Occurrence in Amphibians of Walker and Floyd Counties, Georgia, USA. Herpetological Review 48 (4): 777-779

Morgan, M.B., S.E. Edge, A.A. Venn, R. Jones. 2017. Developing transcriptional profiles in *Orbicella franksi* exposed to copper: Characterizing responses associated with a spectrum of laboratory-controlled environmental conditions. Aquatic Toxicology 189: 60-76

Morgan, M.B., K. Goodner, J. Ross, A.Z. Poole, E. Stepp, C.H. Stuart, C. Wilbanks, E. Weil. 2015. Development and application of molecular biomarkers for characterizing Caribbean Yellow Band Disease in *Orbicella faveolata*. PeerJ 3:e1371.

Edge, S.E., T.L. Shearer, M.B. Morgan, T.W. Snell. 2013. Sub-lethal coral stress: Detecting molecular responses of coral populations to environmental conditions over space and time. Aquatic Toxicology 128-129:135-146

Morgan, M. B., C. C. Parker, J. W. Robinson, E. M. Pierce. 2012. Using Representational Difference Analysis to detect changes in transcript expression of *Aiptasia* genes after laboratory exposure to lindane. Aquatic Toxicology 110-111: 66-73.

Edge, S. E., M. B. Morgan, and T. W. Snell. 2008. Temporal analysis of gene expression in a field population of the Scleractinian coral *Montastraea faveolata*. Journal of Experimental Marine Biology and Ecology 355:114-124

Morgan, M. B., and T. W. Snell. 2006. Expression of a ceruloplasmin homolog in corals: An informative biomarker of stress. Proceedings of the 10th International Coral Reef Symposium. p.822-830

Morgan, M. B., S. E. Edge, and T. W. Snell. 2005. Profiling differential gene expression of corals along a transect of waters adjacent to the Bermuda municipal dump. Marine Pollution Bulletin. 51: 524-533

Edge, S. E., M. B. Morgan, and T. W. Snell. 2005. Development of a cDNA array to examine gene expression profiles in *Monstastrea faveolata* exposed to environmental stress. Marine Pollution Bulletin. 51: 507-523

Snell, T. W., S. E. Brogdon, and M. B. Morgan. 2003. Gene expression profiling in ecotoxicology. Ecotoxicology. 12: 475-483.

Morgan, M. B., and T. W. Snell. 2002. Characterizing stress gene expression in reef-building corals exposed to the mosquitoside dibrom. Marine Pollution Bulletin. 44(11): 1204-1216

Morgan, M. B., D. L. Vogelien, T. W. Snell. 2001. Assessing coral stress responses using molecular biomarkers of gene transcription. Environmental Toxicology and Chemistry 20(3): 537-543

Selected Presentations since 2016:

Morgan, M.B., J. Williams, D. Qualley. *Aiptasia* responses to benzyl butyl phthalate. International Conference on Plastics in the Marine Environment. Singapore. December 2018.

Morgan, M.B., J. Ross, J. Ellwanger, R. A. Martin, D. Qualley. 2017. Sterol transport, Hedgehog signaling, and endocrine disruption: Are there linkages? European Coral Reef Symposium, Oxford University, December 2017. Oxford, England.

Morgan, M.B. 2017. Estrogen, sewage, and sea anemones: How hormone pollution can impact anemones. Invited Speaker. E. O. Grundset Lecture series. Southern Adventist University, October 2017, Collegedale, Tennessee.

Morgan, M.B., J. Ellwanger, R. Martin, J. Ross. 2017. Laboratory induced estradiol exposure stimulates transcriptional responses in the sea anemone *Exaiptasia pallida*. Symbiofest, University of Georgia, April 2017. Athens, Georgia.

Youngblood, H., and M.B. Morgan. 2017. Fungal infections in *Montipora* sp. from American Samoa. Symbiofest, University of Georgia, April 2017. Athens, Georgia.

Morgan, M. B., J. Ross, and R. A. Martin. 2016. Laboratory induced estradiol exposure stimulates transcriptional responses in the sea anemone *Exaiptasia pallida*. 13th International Coral Reef Symposium, June 2016. Oahu, Hawaii.

Selected External Grants since 2016

University of Hawaii/NOAA. 2011. Molecular characterization of pigmentation response in Samoan corals. $8740.00 (contracted research)

National Science Foundation. 2018. Robert Noyce Teacher Scholarship Program, “STEMTeach at Berry” 2018-2023. $1,191,705

National Science Foundation. 2018. Major Instrumentation Research Program, “Berry College MRI: Acquisition of a Flex Real-Time PCR System for Undergraduate Research and Teaching” 2018-2021. $99,645

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Education:
Ph.D. in Plant Pathology, Auburn University, 1999.
M.S. in Plant Pathology, Beijing Agricultural University, China, 1988.

Positions:
2018 – present Professor, Plant Pathology, University of Georgia
2013 – 2018 Associate Professor, Plant Pathology, University of Georgia
2007 – 2013 Assistant Professor, Plant Pathology, University of Georgia
2003 – 2007 Research Associate, Plant Pathology, University of Florida
2000 – 2003 Research Associate, Soil Microbiology, Auburn University
1999 – 2000 Postdoctoral Researcher, Microbiology, University of California at Berkeley

Membership:
American Phytopathological Society (APS)
International Society for Molecular Plant-Microbe Interactions
International Society for Horticultural Science Georgia Association of Plant Pathologists
Sigma Xi, the Scientific Research Society Gamma Sigma Delta, Honor Society of Agriculture
The American Chestnut Foundation

Professional service:
Senior Editor, Plant Disease, 2016 to 2018.
Associate Editor, Plant Disease, 2013 to 2015.
Co-Organizer, Second International Soilborne Oomycete Conference, 2017 to 2018.
Co-Organizer, First International Soilborne Oomycete Conference, 2014 to 2015.
Co-Chairman, Editorial Board, Acta Horticulturae 695 (Proceedings of the First International Symposium on Tomato Diseases), 2004 to 2005.

Selected recent research grants:
USDA-NIFA SCRI program, 2014-2018. A systems approach to improve disease management and production of watermelon in the southeastern U.S. $1,741,071, PI.
USDA-NIFA AFRI program, 2015-2019. Enzyme based nanocomposites for highly selective detection of fungi induced volatiles. $496,196, co-PI.
USDA-NIFA MBT program, 2018-2021. Integrated management of Fusarium wilt, nematode and weed complex using methyl bromide alternatives in watermelon production system. $375,695, co-PI.
Georgia Commodity Commission for Vegetables, 2019. Detection of multiple pathogens on vegetables. $30,500, co-PI.
National Watermelon Association, 2019-2020. Assessment of sensitivity of *Fusarium oxysporum* f. sp. *niveum* to fungicides. $10,000, PI.

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Burgess, T.I., J.K. Scott, K.L. Mcdougall, M.J.C. Stukely, C. Crane, W.A. Dunstan, F. Brigg, V. Andjic D. White, T. Rudman, F. Arentz, N. Ota, G.E.St.J. Hardy. 2017. Current and projected global distribution of *Phytophthora cinnamomi*, one of the world's worst plant pathogens. Global Change Biology 23(4): 1661-1674. https://doi.org/10.1111/gcb.13492

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