EVALUATING CHEMICAL FINGERPRINTING AS A TOOL TO RAPIDLY SCREEN HYBRID CHESTNUT FOR DISEASE RESISTANCE

Summary
Screening chestnut hybrids for disease resistance is intensive because trees must be inoculated with either *Cryphonectria parasitica* (causal agent of chestnut blight) or *Phytophthora cinnamomi* (causal agent of *Phytophthora* root rot). Chemotyping (i.e. chemically phenotyping or fingerprinting) trees and identifying chemical markers associated with resistance is an alternative method that could be used to screen trees rapidly. The goals of this study are to chemotype inter-specific hybrids, to identify potential markers of disease resistance, and to develop and validate predictive models that can be used to prescreen hybrids for resistance prior to planting in seed orchards.

Principal Investigator
Anna O. Conrad, Forest Health Research and Education Center, Department of Forestry, University of Kentucky

Co-principal investigators
Albert G. Abbott, Forest Health Research and Education Center, Department of Forestry, University of Kentucky

C. Dana Nelson, Southern Institute of Forest Genetics, Southern Research Station, USDA Forest Service, and Forest Health Research and Education Center, University of Kentucky

Pierluigi Bonello, Department of Plant Pathology, The Ohio State University

Luis Rodgriguez-Saona, Department of Food Science and Technology, The Ohio State University

Collaborating Scientists
Jared Westbrook, The American Chestnut Foundation

Steven N. Jeffers, Department of Plant and Environmental Sciences, Clemson University

Tetyana Zhebentyayeva, Department of Genetics and Biochemistry, Genomics and Computational Biology Laboratory, Clemson University

Duration: 12 months

Total amount requested: $4,511

Short-term and long-term project goals
Short-term goals: (1) Chemotype inter-specific hybrid chestnut families. (2) Using chemometric analysis, identify potential chemical biomarkers of resistance for chestnut blight and *Phytophthora* root rot. (3) Develop and validate a marker-based statistical model to predict which trees will be resistant to each disease.
Long-term goals: (1) Use chemotyping to prescreen hybrids prior to planting in seed orchards.

Narrative

Rationale

Chestnut blight caused by the introduced pathogen *Cryphonectria parasitica* and *Phytophthora* root rot (PRR) caused by the invasive pathogen *Phytophthora cinnamomi*, are two diseases that continue to threaten native chestnut trees within their natural range in the United States. While chestnut is still found throughout its native range, continued disease pressure prohibits many trees from reaching the point in their life cycle where they are capable of sexual reproduction. As a result, chestnut restoration efforts have focused on breeding disease-resistant inter-specific hybrid chestnut trees that contain quintessential American characteristics but with introgressed disease resistance genes from Chinese chestnut.

Identifying disease resistant chestnut hybrids traditionally has relied on observing disease symptoms following natural or artificial inoculation and identifying only the most resistant individuals from those tests. This is an intensive process, since trees often must reach a certain age and size before they can be inoculated. However, there are other, more rapid methods for phenotyping trees that can detect more subtle differences between individuals and do not necessarily rely on artificial inoculation. One such method is based on identifying and distinguishing between chemotypes (chemical phenotype) of trees that vary in disease susceptibility. Each individual tree has a chemotype composed of plant specialized metabolites (PSMs)—e.g phenolics, terpenes, and alkaloids. Chemotyping is informative because PSMs are well known for their role in plant defense, and inter- and intra-specific differences in PSM composition and concentration, in addition to the timing of PSM accumulation, are all factors known to contribute to disease resistance in trees (Bennett and Wallsgrove, 1994; Kersten et al., 2013; Witzell and Martin, 2008). For these reasons, chemotyping has been used in other forest pathosystems to differentiate between and within species that vary in disease susceptibility. For example, chemotyping has been used to distinguish between *Quercus agrifolia* that were naturally resistant or susceptible to the non-native and invasive forest pathogen *Phytophthora ramorum*, the causal agent of sudden oak death (Conrad, 2015; Conrad et al., 2014; McPherson et al., 2014). Chemotyping combined with chemometric analysis (e.g. multivariate statistical analysis of metabolomic data) was also used to distinguish between elm species and clones that differed in susceptibility to *Ophiostoma novo-ulmi*, causal agent of Dutch elm disease (Martin et al., 2005, 2008). In this system, chemical fingerprinting was used to differentiate between resistant *Ulmus pumila,* susceptible *U. minor*, and resistant *U. minor* clones (Martin et al., 2008).

Chemotypic differences have been detected between *Castanea dentata* (American chestnut) and *C. mollissima* (Chinese chestnut), the two species most commonly used as parent species in American chestnut breeding programs (Cooper and Rieske, 2008). Additionally, results generated from this project in 2015 – 2016, indicate that chemotypic differences between hybrids exist and in some instances can be used to distinguish between hybrids that vary in disease susceptibility. However, additional evaluations, particularly of larger families and at earlier stages of intercrossing are needed to develop and validate more robust and reliable predictive models. A summary of our progress can be found in Appendix A.

The objectives of this study are to:

(1) Chemotype inter-specific hybrid chestnut families;
(2) Use chemometric analysis to identify chemotypic differences between individuals that vary in susceptibility to *Cryphonectria parasitica* (chestnut blight) and *Phytophthora cinnamomi* (PRR), respectively, and
(3) Develop and validate chemical marker-based statistical models to screen hybrids for resistance to chestnut blight and PRR.

Potential benefits to developing a chemotype-based screen include:

- Chemotypic based screening could identify the most susceptible individuals within hybrid families. These individuals could be removed, and then only those individuals with the highest probability of resistance could be subjected to more rigorous, inoculation-based screening procedures.
- The total amount of space and time needed to inoculate progeny/assess disease resistance would be reduced, since fewer total trees would need to be screened using inoculation-based methods.

Material and methods

Plant material

Chestnut blight assay
J. Westbrook will provide us with chestnut stem and foliar tissue from up to 40 individuals from each of 12 BC_3-F_3 families, including 6 Clapper and 6 Graves families. These individuals are being screened as part of a separate and ongoing experiment. J. Westbrook will also provide us with phenotypic data, such as blight rating and stem lesion length. Additionally, J. Westbrook will provide us stem tissue from ~150 living BC_3-F_2 mother trees that have been progeny tested for blight or PRR resistance (this tissue will also be used for the PRR assay).

Please see Appendix B for a letter of collaboration from J. Westbrook.

Phytophthora root rot assay
T. Zhebentyayeva provided us with foliar tissue and phenotypic data from BC_1 hybrid chestnut in two families, HB2 and NK4. This tissue was collected as part of a separate study in 2014, involving S. Jeffers and co-PIs Abbott and Nelson, funded by Foundation of the Carolinas to map *P. cinnamomi* root rot resistance in American/Chinese interspecific populations. For the present study, foliar tissue from HB and Nanking crosses will be re-analyzed using a modified extract and re-evaluated. An additional ~20 – 30 individuals, per family will be analyzed and used for a testing (i.e. validation) data set.

Plant material for blight assays and root rot assay will be analyzed separately according to the following procedure:

Chemotyping chestnut

Frozen plant tissue from each experiment will be finely ground in liquid nitrogen using a mortar and pestle. 100 mg ± 1 mg of finely ground plant tissue will then be extracted two times with 500 ml of HPLC-grade methanol, and pooled (Nagle et al., 2011). Aliquots of pooled extract will be concentrated (to 10X) using an Eppendorf Vacufuge running in alcohol mode at ambient temperature. Concentrated extracts will be stored at -80°C when not in use. Other extraction methods may be tested and used, if needed.

Extracts will be analyzed at Ohio State University using Fourier-transform infrared (FT-IR) spectroscopy, which can be used to rapidly chemotype extracts. FT-IR spectroscopy measures differences in molecular absorption of infrared radiation (Diem, 1993; Guillén and Cabo, 1997; reviewed in Rodriguez-Saona and Allendorf, 2011). This technique does not individually separate chemicals in plant extracts, but instead produces a chemical fingerprint (see **Figure 1** for an example output) based on levels of all the chemicals (i.e. chemical groups) present within an extract that can be detected over a specific spectral range (e.g. mid-infrared spectrum, 4000 to 700 cm^{-1}). A FT-IR spectrometer equipped with an attenuated total reflectance (ATR) accessory will be used to analyze samples based on the methods of Conrad et al. (2014), though other instrumentation, such as a handheld Raman spectrometer, which measures energy exchange after molecules are excited by a source such as a laser, may be used depending on preliminary results (reviewed in Conrad and Bonello, 2016). All instrumentation is available to us at Ohio State University. Two technical replicates will be analyzed for each biological replicate.

Statistical analysis

Data collected from FT-IR spectroscopy will be analyzed using the chemometrix software Pirouette (Infometrix Inc., Woodville, WA, USA). With Pirouette, data is easily organized, visualized, and mined; quantitative and qualitative analyses can be performed and complex signals can be deconvoluted (InfoMetrix, Inc., 2014). Soft independent modeling of class analogy (SIMCA) analysis combines principal components analysis with classification analysis, creating principal components models for each training group (e.g. resistant and susceptible trees). Partial least squares regression (PLSR) combines data reduction methods with regression allowing for the development of quantitative predictive models (reviewed in Conrad and Bonello, 2016). SIMCA and PLSR will be used to detect differences in chemotypes between groups (e.g. between hybrid chestnut that are resistant and susceptible to *P. cinnamomi*). SIMCA and PLSR will also generate models (based on the training data sets) that can be used to predict whether or not a tree will be resistant or susceptible based on regions of the chemical fingerprint that differ between groups. Data will be transformed and outliers trimmed as needed, based on preliminary analyses. Other statistical models will be tested and used if needed.

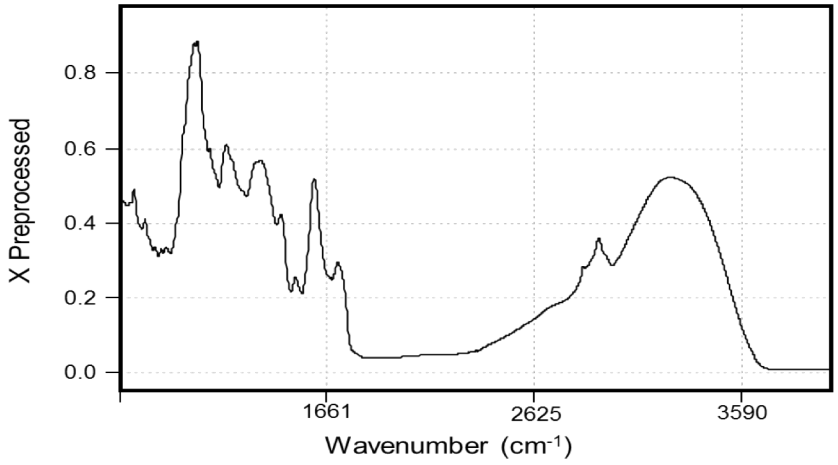

Figure 1. A representative chemical fingerprint collected from a methanol extract of hybrid chestnut stem tissue (figure from 2015 – 2016 analysis).

Literature cited

Bennett, R.N., Wallsgrove, R.M., 1994. Secondary Metabolites in Plant Defense-Mechanisms. New Phytol. 127, 617–633.

Conrad, A.O., 2015. Metabolomics of Quercus spp. to understand and predict resistance to Phytophthora ramorum. PhD Dissertation. The Ohio State University.

Conrad, A.O. and Bonello, P., 2016. Application of infrared and Raman spectroscopy for the identification of disease resistant trees. Front. Plant Sci. 6:1152. doi: 10.3389/fpls.2015.01152

Conrad, A.O., Rodriguez-Saona, L.E., McPherson, B.A., Wood, D.L., Bonello, P., 2014. Identification of Quercus agrifolia (coast live oak) resistant to the invasive pathogen Phytophthora ramorum in native stands using Fourier-transform infrared (FT-IR) spectroscopy. Front. Plant Sci. 5, 521.

Cooper, W.R., Rieske, L.K., 2008. Differential responses in American (Castanea dentata Marshall) and Chinese (C. mollissima Blume) chestnut (Falales: Fagaceae) to foliar application of jasmonic acid. Chemoecology 18, 121–127.

Diem, M., 1993. Introduction to Modern Vibrational Spectroscopy. Wiley, New York, NY.

Guillén, M.D., Cabo, N., 1997. Infrared spectroscopy in the study of edible oils and fats. J. Sci. Food Agric. 75, 1–11.

InfoMetrix, Inc. 2014. Pirouette: Comprehensive chemometrixs modeling software. http://infometrix.com/software/products/pirouette/.

Kersten, B., Ghirardo, A., Schnitzler, J.-P., Kanawati, B., Schmitt-Kopplin, P., Fladung, M., Schroeder, H., 2013. Integrated transcriptomics and metabolomics decipher differences in the resistance of pedunculate oak to the herbivore Tortrix viridana L. BMC Genomics 14, 737.

Martin, J., Solla, A., Woodward, S., Gil, L., 2005. Fourier transform-infrared spectroscopy as a new method for evaluating host resistance in the Dutch elm disease complex. TREE Physiol. 25, 1331–1338.

Martin, J.A., Solla, A., Coimbra, M.A., Gil, L., 2008. Metabolic fingerprinting allows discrimination between Ulmus pumila and U. minor , and between U. minor clones of different susceptibility to Dutch elm disease. For. Pathol. 38, 244–256.

McPherson, B.A., Mori, S.R., Opiyo, S.O., Conrad, A.O., Wood, D.L., Bonello, P., 2014. Association between resistance to an introduced invasive pathogen and phenolic compounds that may serve as biomarkers in native oaks. For. Ecol. Manage. 312, 154–160.

Nagle, A.M., McPherson, B.A., Wood, D.L., Garbelotto, M., Bonello, P., 2011. Relationship between field resistance to Phytophthora ramorum and constitutive phenolic chemistry of coast live oak. For. Pathol. 41, 464–469.

Rodriguez-Saona, L.E., Allendorf, M.E., 2011. Use of FTIR for rapid authentication and detection of adulteration of food. Annu. Rev. Food Sci. Technol. 2, 467–83.

Witzell, J., Martin, J.A., 2008. Phenolic metabolites in the resistance of northern forest trees to pathogens - past experiences and future prospects. Can. J. For. Res. Can. Rech. For. 38, 2711–2727.

Proposed timeline of research activities

Research activity	*3 mo.*	*6 mo.*	*9 mo.*	*12 mo.*
Chemotype American and Chinese chestnut parents and inter-specific hybrid families	X	X	X	
Perform chemometric analysis	X	X	X	
Optimize and validate predictive models		X	X	X
Prepare results for presentations and manuscript for peer-review publication			X	X

How results will be measured and reported

A final written research and financial report will be prepared for TACF. A project overview will be made available on the Forest Health Research and Education Center website (www.foresthealthcenter.org) and a general summary will be prepared for publication in the Journal of the American Chestnut Foundation. A manuscript reporting results of chemotyping and development of predictive models will also be prepared and submitted for peer-review publication. Finally, research results will be shared at scientific meetings (e.g. annual chestnut meeting, American Phytopathological Society annual meeting).

Breakdown of how and when funds will be spent

Expense	Number of units	Cost per unit	Total
Sample shipment			
Shipping samples from collaborators institutions for processing and FT-IR analysis. *Funds to be spent in the first 9 months of the project	~4 – 5	Variable	$250
FT-IR analysis			
Materials include, but are not limited to liquid nitrogen, methanol, microcentrifuge tubes, pipette tips, and freezer boxes for sample storage and shipment *Funds to be spent in in the first 9 months of the project.		Approximately, $250 for liquid nitrogen and $1000 for other lab disposables	$1,250
Support for undergraduate assistants			
Undergraduate assistants will be needed for sample preparation and analysis of samples by FT-IR. Approximately, 50 hours are needed to help prepare and analyze 100 samples. *Funds to be spent in the first 9 months of the project.	2 undergrad assistants (1 at UK and 1 at OSU)	Experienced undergraduate laboratory workers at UK & OSU, with an estimate of 100 hours total work for each assistant, 100 hours x $10/hour + 100 hours x $11/hour = $2100 + 7.65% FICA = $161	$2,261
Travel			
To meet with collaborators, analyze samples, and to present research results *Funds to be spent throughout the 12 month funding period	~3 times	Mileage reimbursement rate is 54 cents/mile. For example, ~463 miles round-trip between UK and OSU = ~$250/trip	$750
Total Amount Requested			**$4,511**

Budget Narrative: The proposed funds will pay for the transportation of tissue samples from collection sites or current sites of storage to the Forest Health Research and Education Center lab at the University of Kentucky for sample processing and preparation and to The Ohio State University for FT-IR analysis. Other funds requested will be used to purchase supplies and disposables needed for FT-IR sample preparation and analysis, and include but are not limited to liquid nitrogen for tissue grinding, methanol to extract chemicals from tissue, microcentrifuge tubes for sample storage and chemical extraction, and other materials needed to properly ship and store samples. Funds are requested to pay for mileage reimbursement for A. Conrad to travel between University of Kentucky and The Ohio State University to conduct FT-IR analysis, to meet with collaborators, and to present research results. Finally, funds are requested to support 2 undergraduate laboratory assistants who will help A. Conrad with sample preparation and analysis, since there are currently no automated methods available for processing and analyzing samples.

Brief C.V. for each Principal Investigator:

Anna O. Conrad – Biographical Sketch
Postdoctoral Scholar
Forest Health Research and Education Center
Department of Forestry
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Education
The Ohio State University, Columbus OH
Ph.D. in Plant Pathology, 2015
M.S. in Plant Pathology, 2013

SUNY College of Environmental Science and Forestry, Syracuse NY
B.S. in Environmental Biology, summa cum laude, 2010

Professional Experience
Postdoctoral Scholar, 2015 – present
Forest Health Research and Education Center
Department of Forestry, University of Kentucky, Lexington, KY

Graduate Research Associate, 2010 - 2015
Department of Plant Pathology, The Ohio State University, Columbus, OH

Teaching Experience
The Ohio State University, Columbus, OH
Graduate teaching assistant, Diseases of Forest and Shade Trees, 2012
Graduate teaching assistant, General Plant Pathology, 2011

Past Research Grants Received
"Evaluating chemical fingerprinting as a tool to rapidly screen hybrid chestnut for disease resistance" for $3000 (**Conrad**, Abbott, Nelson), 10/24/2015 – 10/24/2016, The American Chestnut Foundation
"Abiotic stress response and adaptive phenology in fruit trees" for ~$425,000 (PI: Abbott and co-PI's: Liu, Dardick, Zhebentyayeva, Staton, Nelson, **Conrad**), 12/03/2015 – 12/02/2018, AFRI NIFA
Graduate SEEDS grant for $5000 (2013), Ohio Agricultural and Research Development Center, "Transcript and metabolite profiling to understand mechanisms of oak resistance against the introduced pathogen, *Phytophthora ramorum*."

Refereed Publications

***Conrad, A.O.** and Bonello P. 2016. Application of infrared and Raman spectroscopy for the identification of disease resistant trees. Front. Plant Sci. 6: 1152. doi: 10.3389/fpls.2015.01152

Conrad, A.O., Rodriguez-Saona, L.E., McPherson, B.A., Wood, D.L., and Bonello, P. 2014. Identification of Quercus agrifolia (coast live oak) resistant to the invasive pathogen Phytophthora ramorum in native stands using Fourier-transform infrared (FT-IR) spectroscopy. Front. Plant Sci. 5: 521. doi: 10.3389/fpls.2014.00521

McPherson, B.A., Mori, S.R., Opiyo, S.O, **Conrad, A.O.**, Wood, D.L., and Bonello, P. 2014. Association between resistance to an introduced invasive pathogen and phenolic compounds that may serve as biomarkers in native oaks. Forest Ecol. Manage 312: 154-160.

Conrad, A.O., and Segraves, K.A. 2013. Mycorrhizal colonization of Palafoxia feayi (Asteraceae) in a pyrogenic ecosystem. Mycorrhiza 23(3): 243-249.

*Invited review paper.

Non-refereed Technical Publications

Conrad, A., McPherson, B., Wood, D., Opiyo, S., Mori, S., and Bonello, P. 2013. Metabolite profiling to predict resistance to Phytophthora ramorum in natural populations of coast live oak. In Proceedings of the Sudden Oak Death Fifth Science Symposium. PSW-GTR-243. 169pp.

McPherson, B.A., Wood, D.L., Mori, S.R., **Conrad, A.**, and Bonello, P. 2013. Phytophthora ramorum in coast live oak: search for resistance and mechanisms. In Proceedings of the Sudden Oak Death Fifth Science Symposium. PSW-GTR-243. 169pp.

Nagle, A.M., McPherson, B.A., Wood, D.L., Garbelotto, M., **Conrad, A.O.,** Opiyo, S., and Bonello, P. 2012. Relationship between field resistance to *Phytophthora ramorum* and constitutive phenolic chemistry of coast live oak. In Proceedings of the Fourth International Workshop on the Genetics of Host-Parasite Interactions in Forestry: Disease and Insect Resistance in Forest Trees. PSW-GTR-240. 372pp.

Albert G. Abbott – Biographical Sketch

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B. Professional Preparation:

1. University of Connecticut, Storrs, CT , Biological Sciences, B.S. 197 2-1976
2. Brown University, Providence, RI, Cell and Molecular Biology, Ph.D. 1980.
3. Plant Breeding Institute, Cambridge, England, Rockefeller Foundation Postdoctoral Fellow in Plant Molecular Biology (Jan. 1981-Jan. 1983). Worked with Dr. R. Flavell.
4. Brown University, NIH Postdoctoral Research Associate in Insect Molecular Biology with Dr. Susan Gerbi, (Jan. 1984-Aug. 1984).
5. Brown University, NSF Postdoctoral Research Associate in Plant Molecular Biology with Dr. S. Beale, (Aug. 1983-Jan. 1984)

C. Appointments:

1. Staff Scientist III, Dept. of Forestry, University of Kentucky. (2014-), Co-director of Forest Health Research and Education Center, UKY.
2. ANR Chaire d' Excellence, INRA Bordeaux France. (2012-2014)
3. Coker Chair in Plant Molecular Genetics, Dept of Genetics and Biochemistry, Clemson University (2011-2013),
4. Professor of Genetics and Biochemistry and Coker Chair in Plant Molecular Genetics, Dept of Genetics and Biochemistry, Clemson University (2001-2010)
5. Associate Professor of Biological Sciences, Dept. of Biological Sciences, Clemson University (1989-2000)
6. Assistant Professor of Biological Sciences, Department of Biological Sciences, Clemson University (Aug. 1984- 1989).

D. Closely related publications:

Jung S , Jiwan D, Cho I, Lee T, Abbott A, Sosinski B and Main D. 2009. Synteny of *Prunus* and other model plant species. BMC Genomics 10:76

Sook Jung, Alessandro Cestaro, Michela Troggio, Dorrie Main, Ping Zheng, Ilhyung Cho, Kevin M Folta, Bryon Sosinski, Albert Abbott, Jean-Marc Celton, Pere Arus, Vladimir Shulaev, Ignazio Verde, Michele Morgante, Daniel S Rokhsar, Riccardo Velasco and Daniel J Sargent. (2012). Whole genome comparisons of Fragaria, Prunus and Malus reveal different modes of evolution between Rosaceous subfamilies. BMC Genomics , **13**:129

Abdelali Barakat[1]*, Aditya Sriram[2], Joseph Park[3], Tetyana Zhebentyayeva[1], Dorrie Main[4], and Albert Abbott. 2012. Genome wide identification of chilling responsive microRNAs in Prunus persica *BMC Genomics*, 13:481

Abdelali Barakat, Meg Staton, Chun-Huai Cheng, Joseph Park, Norzawani BM Yassin, Stephen Ficklin, Chia-Chun Yeh, Fred Hebard, Kathleen Baier, William Powell, Stephan C Schuster, Nicholas Wheeler, Albert Abbott, John E Carlson, and Ronald Sederoff. 2012. Chestnut resistance to the blight disease: insights from transcriptome analysis. *BMC Plant Biology*, 12:38

E. Five other publications:

Guang-Chen Fang, Barbara P. Blackmon, Margaret E Staton, C. Dana Nelson, Thomas L. Kubisiak, Bode A. Olukolu, David Henry, Tatyana Zhebentyayeva, Christopher A. Saski , Chun-Huai Cheng, Megan Monsanto, Stephen Ficklin, Michael Atkins, Laura L. Georgi, Abdelali Barakat, Nicholas Wheeler, John E. Carlson,

Ronald Sederoff , Albert G. Abbott. 2012. A physical map of the Chinese chestnut (Castanea mollissima) genome, and its integration with the genetic map.Tree Genetics and Genomes 9:2, 525-537,

T.L. Kubisiak, C.D. Nelson, M.E. Staton, T. Zhebentyayeva, C. Smith, B.A. Olukolu, G.-C. Fang, F.V. Hebard, S. Anagnostakis, N. Wheeler, P.H. Sisco, A.G. Abbott, R.R. Sederoff 2013. A transcriptome-based genetic map of Chinese chestnut (*Castanea mollissima)*, and identification of regions of segmental homology with peach (*Prunus persica*). Tree Genetics & Genomes 9:557–571

Verde I, Abbott AG, Scalabrin S, Jung S, Shu S, Marroni F, Zhebentyayeva T, Dettori MT, Grimwood J et al. (2013) The high-quality draft genome of peach (Prunus persica) identifies unique patterns of genetic diversity, domestication and genome evolution. Nature Genetics, published on-line. doi:10.1038/ng.2586

Dardick C, Callahan A, Horn R, Carrasco K-R, Zhebentyayeva T, Hollender C, Whitaker M., Abbott A, Scorza R (2013) Identification of PpTAC1 as a functionally conserved regulator of axillary shoot growth angle in Prunus persica (peach) trees. Plant Journal, 75(4): 618–630.

Zhebentyayeva T., Fan S., Chandra A., Bielenberg D.G., Reighard G.L., Okie W.R., Abbott A.G. (2014) Dissection of chilling requirement and bloom date QTLs in peach using a whole genome sequencing of sibling trees from an F2 mapping population. Tree Genetics & Genomes, 10: 35-51.

F. Synergistic Activities:

1. Involved in teaching and designing summer workshops in genomics and genetics.
2. Service on National and International Rosaceae genomics research steering committees.
3. Serve on Ph.D. committees of students including Ph.D. granting institutions in other countries.
4. Host researchers from multiple countries for genomics and genetics research.
5. Contact person for the development of the Genome Database for Rosaceae as the central Web based databank for Rosaceae Genomics data worldwide.
6. Co-director of the Forest Health Research and Education Center, UKY

G. Collaborators and Other Affiliations:

1.Current Collaborators:
- Dr. P. Arus IRTA, Spain
- Dr. V. Baird, Horticulture, Clemson U.
- Dr. M.Badenes IVIA , Valencia Spain
- Bielenberg, Clemson U.
- Dr. A. Callahan, AFRS, WV.
- Dr. V.Decroocq INRA Bordeaux France
- Dr. D. Holland, ARO Israel
- Dr. R. Horn. IMPB, Rostock Germany
- Dr. S. Jung, Michigan
- Dr. D. Main Washington State University
- Dr. W. Okie, USDA/Byron GA
- Dr. G. L. Reighard.
- Dr. R. Scorza AFRS, WV
- Dr. B. Sosinski North Carolina State University
- Dr. I. Verde Fruit Institute, Rome
- Dr. T. Zhebentyayeva, Clemson U

2. Thesis Advisor: Dr. Susan Gerbi, Brown University
   Post doctoral adivsors: Dr. R.B. Flavell (John Innes)
   Dr. S. Beale, Brown University

3. Graduate Students
   - B. Levy MS.
   - J. Wang, MS
   - L. Medlin, MS, SLED, SC Dr. D. C. Zraket, MS
   - J. Qin, MS
   - S. Medel, MS, Fla.
   - D. Gupta, Ph.D
   - E. Hiatt Ph.D
   - P. Tate Ph.D., Clemson U
   - B. Sosinski Ph.D, NCSU
   - S. Jung Ph.D. WSU
   - F. Teule Ph.D. UWyo
   - D. Lalli Ph.D. AFRS/USDA
   - S. Hughes-Murphree, MS
   - B. Olukolu Ph.D. NCSU
   - F. Shenghua Ph.D. GGC

4. Postdoctoral associates
   - L. Georgi, Clemon
   - A. Lecouls. Montpellier Fr
   - S. Rajapakse, Clemson
   - K. Sossey, Buffalo, NY

C. Dana Nelson – Biographical Sketch

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a. Professional Preparation

Institution	*Major/Area*	*Degree*	*Year*
University of Minnesota	Forest Genetics	Ph.D.	1988
Oklahoma State University	Forest Genetics	M.S.	1984
Iowa State University	Forestry	B.S.	1982

b. Appointments (current in bold)

Jun 2016: Adjunct Professor, Department of Forestry, University of Kentucky
Oct 2013: Visiting Scientist, Department of Forestry, University of Kentucky
Mar 2008: Adjunct Faculty, Department of Ecosystem Management, Texas A&M University
Mar 2004: Adjunct Faculty, Department of Plant and Soil Sciences, Mississippi State University
Feb 2004: Project Leader, Southern Institute of Forest Genetics, USDA Forest Service
Oct 2002: Acting Project Leader, Southern Institute of Forest Genetics, USDA Forest Service
Nov 2001: Research Geneticist, Southern Institute of Forest Genetics, USDA Forest Service
Jan 1997: Adjunct Faculty, School of Forest Resources and Conservation, University of Florida
Aug 1994: Research Scientist/Project Leader, Forest Resources Division, International Paper Comp.
Oct 1993: Adjunct Faculty, School of Forestry, Wildlife and Fisheries, Louisiana State University
Apr 1989: Research Geneticist, Southern Institute of Forest Genetics, USDA Forest Service
1988-89: Postdoctoral Associate, Southern Institute of Forest Genetics, USDA Forest Service

c. Synergistic Activities

1. Dr. Nelson is Principal Investigator and co-Director for a new research partnership (Forest Health Research and Education Center, FHC) between the Southern Research Station (SRS) and the University of Kentucky. The FHC, developed in the spirit of the Forest Health Initiative (FHI, see below), is addressing biological and social aspects of the most pressing forest health challenges.
2. Dr. Nelson is the lead Principal Investigator for the FHI biological sciences team. The FHI is a multi-institutional research program designed to utilize biotechnology to produce a plantable American chestnut tree capable of resisting chestnut blight. Team accomplishments include: development and integration of and genome-wide genetic and physical maps for Chinese chestnut, identification of candidate blight resistance genes and their transformation into American chestnut, development of an early resistance screening method, and field testing transformed trees under APHIS permits.
3. Dr. Nelson is Supervisory Research Geneticist and Project Leader for an integrated genetics and physiology research unit within the SRS. This project, resulting from the merger of two projects (located in Mississippi and North Carolina), is integrating forest tree genetics and physiology research to an unprecedented degree.

4. Dr. Nelson developed high-throughput genetic mapping labs at both the SIFG (1992-94) and International Paper (IP, 1994-1996). The SIFG and IP labs have been successful in mapping forest trees and pathogens.

d. Publications

i. 5 related refereed publications

Dalgleish, HJ, **Nelson, CD**, Scrivani, JA, Jacobs, DF. 2016. Consequences of shifts in abundance and distribution of American chestnut for restoration of a foundation forest tree. Forests 7(1), 4. (9 pages)

Staton, ME, Zhebentyayeva, T, Olukolu, B, Fang, GC, **Nelson, CD**, Carlson, JE, Abbott, AG. 2015. Substantial genome synteny preservation among woody angiosperm species: comparative genomics of Chinese chestnut (*Castanea mollissima*) and plant reference genomes. BMC Genomics 16:744 (13 pages)

Nelson, C.D., W.A. Powell, S.A. Merkle, J.E. Carlson, F.V. Hebard, N. Islam-Faridi, M.E. Staton, L. Georgi. 2014. Chestnut. In: K. Ramawat, editor, *Tree Biotechnology, Chapter 1,* CRC Press, pp. 3-35.

Jacobs, D.F., H.J. Dalgleish, **C.D. Nelson**. 2013. A conceptual framework for restoration of threatened plants: the effective model of American chestnut (*Castanea dentata*) reintroduction. New Phytologist 197: 378-393.

Kubisiak, T.L., **C.D. Nelson**, M.E. Staton, T. Zhebentyayeva, C. Smith, B.A. Olukolu, G.-C. Fang, F.V. Hebard, S. Anagnostakis, N. Wheeler, P.H. Sisco, A.G. Abbott, R.R. Sederoff. 2012. A transcriptome-based genetic map of Chinese chestnut (*Castanea mollissima)*, and identification of regions of segmental homology with peach (*Prunus persica*). Tree Genetics and Genomes 9:557-571.

Fang, G-C., B.P. Blackmon, M.E. Staton, **C.D. Nelson**, T.L. Kubisiak, B.A. Olukolu, D. Henry, T. Zhebentyayeva, C.A. Saski, C-H. Cheng, M. Monsanto, S. Ficklin, M. Atkins, L.L. Georgi, A. Barakat, N. Wheeler, J.E. Carlson, R. Sederoff, A.G. Abbott. 2012. A physical map of the Chinese chestnut (*Castanea mollissima*) genome, and its integration with the genetic map. Tree Genetics and Genomes 9:525-537.

ii. 5 other refereed publications

Nelson, C.D., Powell, W.A., Maynard, C.A., Baier, K.M., Newhouse, A., Merkle, S.A., Nairn, C.J., Kong, L., Carlson, J.E., Addo-Quaye, C., Staton, M.E., Hebard, F.V., Georgi, L.L., Abbott, A.G., Olukolu, B.A., and Zhebentyayeva, T. (2013) The Forest Health Initiative, American chestnut (*Castanea dentata*) as a model for forest tree restoration: Biological Research Program. Acta Hort 1019:179-189.

Stewart JF, Will RE, Robertson KM, **Nelson CD**. 2015. Frequent fire protects shortleaf pine (*Pinus echinata*) from introgression by loblolly pine (*P. taeda*). Conservation Genetics 16: 491-495.

Pendleton, A.L., Smith, K.E., Feau, N., Martin, F.M., Grigoriev, I.V., Hamelin, R., **Nelson, C.D.**, Burleigh, J.G., Davis, J.M. 2014. Duplications and losses in gene families of rust pathogens highlight putative effectors. Frontiers in Plant Science 5:(299), 1-13.

Westbrook, J.W., M.F.R. Resende Jr., P. Munoz, A.R. Walker, D.B. Neale, J.L. Wegrzyn, **C.D. Nelson**, M. Kirst, D.A. Huber, S.A. Gezan, G.F. Peter, J.M. Davis. 2013. Association genetics of oleoresin flow in loblolly pine: discovering genes and predicting phenotype for improved resistance to bark beetles and bioenergy potential, New Phytologist 199:89-100..

Tauer, CG, JF Stewart, R Will, C Lilly, J Guldin, **CD Nelson**. 2012. Hybridization leads to loss of genetic stability in shortleaf pine: Unexpected consequences of pine management and fire suppression. Journal of Forestry (June):216-224.

Pierluigi Bonello – Biographical Sketch

Department of Plant Pathology
Center for Applied Plant Sciences and Translational Plant Sciences Graduate Program
Center for Microbe Interface Biology
Environmental Science Graduate Program
The Ohio State University

PROFESSIONAL PREPARATION

Undergraduate/Graduate/ Postdoctoral Institution	Location	Major/Area	Degree/Position, dates
University of Padova	Padova, Italy	Forest Sciences	"Laurea" (=MSc), 1987
University of Oxford	Oxford, UK	Forest Pathology	PhD, 1991
GSF - Forschungszentrum für Umwelt und Gesundheit	Munich, Germany	Air Pollution and Disease Resistance	Post-doc, 1991-1992
University of California	Berkeley, CA	Host-Pathogen-Insect Interactions; Mycorrhizal Ecology	Post-doc, 1994-1996
University of California	Davis, CA	Host-Pathogen-Insect Interactions	Post-doc, 1997-2000

APPOINTMENTS

2013-present Faculty member of the Doctoral Program, Department of Agricultural and Environmental Sciences, University of Florence, Italy

2012-present Adjunct Professor, Department of Biological Sciences, Wright State University

2010-present Professor, Department of Plant Pathology, The Ohio State University

2005-2010 Associate Professor, Department of Plant Pathology, The Ohio State University

2000-2005 Assistant Professor, Department of Plant Pathology, The Ohio State University

PRODUCTS

Five Products Most Closely Related:

1. Conrad AO, **Bonello P** (2016) (Invited article) Focused Review: Application of infrared and Raman spectroscopy for the identification of disease resistant trees. Frontiers in Plant Science 6 – DOI: 10.3389/fpls.2015.01152.
2. Conrad AO, Rodriguez-Saona LE, McPherson BA, Wood DL, **Bonello P** (2014) Identification of *Quercus agrifolia* (coast live oak) resistant to the invasive pathogen *Phytophthora ramorum* in native stands using Fourier-Transform Infrared (FT-IR) spectroscopy. Frontiers in Plant Science – Technical Advances in Plant Science 5: doi: 10.3389/fpls.2014.00521.
3. Sherwood P, Villari C, Capretti P, **Bonello P** (2015) Mechanisms of induced susceptibility to Diplodia tip blight in drought-stressed Austrian pine. Tree Physiology 35: 549-562, DOI:101093/treephys/tpv026.

4. **Bonello P**, Gordon TR, Herms DA, Wood DL, Erbilgin N (2006) Nature and ecological implications of pathogen-induced systemic resistance in conifers: A novel hypothesis. Physiological and Molecular Plant Pathology 68: 95-104, DOI: 101016/jpmpp200612002.
5. **Bonello P**, Blodgett JT (2003) *Pinus nigra-Sphaeropsis sapinea* as a model pathosystem to investigate local and systemic effects of fungal infection of pines. Physiological and Molecular Plant Pathology 63: 249-261, DOI: 101016/jpmpp200402002.

Five Other Significant Products:

6. Villari C, Herms DA, Whitehill JGA, Cipollini DF, **Bonello P** (2016) Invited Tansley Review: Progress and gaps in understanding mechanisms of ash resistance to emerald ash borer, a model for wood boring insects that kill angiosperm trees. New Phytologist 209: 63-79, DOI: 101111/nph13604. [Featured in the New York Times, Matter, August 27, 2015.]
7. Eyles A, **Bonello P**, Ganley R, Mohammed C (2010) Invited Tansley Review: Induced resistance to pests and pathogens in trees. New Phytologist 185: 893–908, DOI: 101111/j1469-8137200903127x.
8. Wallis CM, Eyles A, Chorbadjian R, McSpadden-Gardner BB, Hansen R, Cipollini DF, Herms DA, **Bonello P** (2008) Systemic induction of phloem secondary metabolism and its relationship to resistance to a canker pathogen in Austrian pine. New Phytologist 177: 767–778.
9. Blodgett JT, Eyles A, **Bonello P** (2007) Organ-dependent induction of systemic resistance and systemic susceptibility in *Pinus nigra* inoculated with *Sphaeropsis sapinea* and *Diplodia scrobiculata*. Tree Physiology 27: 511-517, DOI: 101093/treephys/274511.
10. **Bonello P**, Blodgett JT (2003) *Pinus nigra-Sphaeropsis sapinea* as a model pathosystem to investigate local and systemic effects of fungal infection of pines. Physiological and Molecular Plant Pathology 63: 249-261, DOI: 101016/jpmpp200402002.

SYNERGISTIC ACTIVITIES

1. Teaching, Advising, Training: Since 2000 I have advised 11 PhD and Masters students, 4 post-docs, and trained over 20 undergraduate student interns; taught and co-taught Advanced Fundal Biology, Diseases of Forest and Shade Trees, Ecology and Management of Insects and Pathogens Affecting Trees in Forest and Urban Environments, Molecular Bases of Plant Host-Pathogen Interactions, Plant Health Management, Mycology, Diagnostic Field Plant Pathology.
2. Section Editor for *Phytopathologia Mediterranea* (2009-2015); Editorial Board for *Physiological and Molecular Plant Pathology* (2007-present) and *Tree Physiology* (2007-present).
3. *Ad hoc* reviewer for 46 different journals since 2000. *Ad hoc* reviewer for proposals submitted to NSF, Canadian NSERC, Austrian Science Fund (FWF), The Netherlands' NWO Council for the Earth and Life Sciences (ALW), Research Council of Norway, USDA Forest Service, U.S. Civilian Research & Development Foundation, Collaboration in Basic Science and Engineering (COBASE) program, Ohio Agricultural Research and Development Center SEEDS program.
4. Past chair and official Ohio representative to Multistate Research Projects: W-2187, "Interactions Among Bark Beetles, Pathogens, and Conifers in North American Forests" (2000-2012) and NCERA-193, "IPM Strategies for Arthropod Pests and Diseases in Nurseries and Landscapes" (2000-present).
5. Member of the Scientific Advisory Board for "The Mountain Pine Beetle Epidemic Project: Using Genomics of the Interacting Bark Beetles, Fungal Pathogens and Host Pine Trees to Improve Forest Ecological Risk Models" between the provinces of British Columbia and Alberta in Canada (2008-2012).

Luis E. Rodriguez-Saona – Biographical Sketch

Education/Training

Institutions/Locations	Degrees	Year	Field of Study
Universidad Agraria La Molina Lima – Peru	B.S.	1989	Food Industry
Universidad Agraria La Molina Lima – Peru	Engineer	1991	Food Engineering
Oregon State University	M.S.	1993	Food Science and Technology
Oregon State University	Ph.D.	1998	Food Science and Technology

POSITIONS AND HONORS

Professor. Department of Food Science and Technology, The Ohio State University (9/2015- present).
Associate Professor. Department of Food Science and Technology, The Ohio State University (9/2009-8/2015).
Assistant Professor. Department of Food Science and Technology, The Ohio State University (12/03 – 8/2009).
Research Chemist, USDA Beltsville Human Nutrition and Research Center – Food Composition Laboratory (10/02 – 11/03). Research focused on chromatographic and infrared methods for determination of sulfur- and seleno-containing phytonutrients in vegetables.
Research Associate, Joint Institute of Food Safety and Applied Nutrition (JIFSAN) - University of Maryland and FDA (8/98 – 10/02). Grant sponsored by US Army TSWG Program: Combating Terrorism Technology Support Group.

HONORS AND AWARDS

- Winner of the 2016 North American Colleges and Teachers of Agriculture (NACTA) Educator Award at the Annual Conference held at the University of Hawaii, Manoa.
- Winner of the Ohio Agricultural Research and Development Center's (OARDC) 2014 Distinguished Junior Faculty Research Award.
- Winner of the 2012 CFAES Rodney F. Plimpton Outstanding Teacher Award
- Food Science Club Professor of the Year, 2007, 2010, 2012, 2013, 2015. Award selected by students from FST department.

PATENTS:

- U.S. Patent No 6,180,154 B1. Ronald E. Wrolstad and Luis E. Rodriguez-Saona. Natural Colorant from Potato Extract. Issued on Jan. 30, 2000
- Anand Subramanian, James W. Harper and Luis E. Rodriguez-Saona. Issued 2010. A Novel and Rapid Extraction Procedure for Analysis of Cheese Flavor using Infrared Spectroscopy. Patent no. 12/481,278. The Ohio State University.
- Charles A. Buffington, Daniel Emilio Rubio-Diaz, Luis E. Rodriguez-Saona and Judi L. Stella. Issued 2009. Rapid Diagnosis of a Disease Condition Using Infrared Spectroscopy. Patent no. 61/040,627. The Ohio State University.

SELECTED PEER-REVIEWED PUBLICATIONS (LAST 3 YEARS)

- Ayvaz, H, Bozdogan, A, Giusti, MM, Mortas, M, Gomez, R, Rodriguez-Saona, LE. **2016**. Improving the screening of potato breeding lines for specific nutritional traits using portable mid-infrared spectroscopy and multivariate analysis. Food Chem. 211:374-82.
- Ayvaz, H, Sierra-Cadavid, A, Aykas, DP, Mulqueeney, B, Sullivan, S, Rodriguez-Saona, LE. Monitoring multicomponent quality traits in tomato juice using portable mid-infrared (MIR) spectroscopy and multivariate analysis. Food Control. 66: 79-86.
- Ayvaz, H, Santos, AM, Rodriguez-Saona, LE. **2016**. Understanding Tomato Peelability. Compr Rev Food Sci Food Saf. 15(3): 619–632.
- Sikand V, Tong P S, Walker J, Wang T, Rodriguez-Saona L E. **2016**. Short communication: Effect of storage temperature on the solubility of milk protein concentrate 80 (MPC80) treated with NaCl or KCl. J Dairy Sci. 99(3): 1791-5.
- Aykas, DP and Rodriguez-Saona, LE. **2016**. Assessing Potato Chip Oil Quality using a Portable Infrared Spectrometer Combined with Pattern Recognition Analysis. *Anal Methods*. 8: 731 - 741.
- Wang, T; Tan, SY; Mutilangi, W; Plans, M; Rodriguez-Saona, LE. **2015**. Authentication of Whey Protein Powders by Portable Mid-Infrared Spectrometers Combined with Pattern Recognition Analysis. *J Food Sci*. 80(10): C2111-C2116.
- Plans, M; Wenstrup, MJ; Rodriguez-Saona, LE. **2015**. Application of Infrared Spectroscopy for Characterization of Dietary Omega-3 Oil Supplements. *J Am Oil Chem Soc*. 92(7): 957-966.
- Ayvaz, H; Santos, AM; Moyseenko, J; Kleinhenz M; Rodriguez-Saona, LE. **2015**. Application of a Portable Infrared Instrument for Simultaneous Analysis of Sugars, Asparagine and Glutamine Levels in Raw Potato Tubers. *Plant Foods Hum Nutr.* 70(2): 215-220.Ayvaz, H and Rodriguez-Saona, LE. 2015. Application of handheld and portable spectrometers for screening acrylamide content in commercial potato chips. *Food Chem*. 174:154-162.
- Ayvaz, H, Plans, M, Towers, BN, Auer, A, Rodriguez-Saona, LE. 2015. The use of infrared spectrometers to predict quality parameters of cornmeal (corn grits) and differentiate between organic and conventional practices. *J Cereal Sci*. 62: 22-30.
- Nangle, EJ, Gardner, DS, Metzger, JD, Rodriguez-Saona, L, Guisti, MM, Danneberger, TK, and Petrella, DP. 2015. Pigment Changes in Cool-Season Turfgrasses in Response to Ultraviolet-B Light Irradiance. *Agronomy J*. 107: 41-50.
- Conrad, AO, Rodriguez-Saona, LE, McPherson, BA, Wood, DL, Bonello, P. 2014. Identification of Quercus agrifolia (coast live oak) resistant to the invasive pathogen Phytophthora ramorum in native stands using Fourier-transform infrared (FT-IR) spectroscopy. *Front Plant Sci.* 5(521):1-9.
- Wenstrup, MJ, Plans, M, Rodriguez-Saona, LE. 2014. "Effect of a novel induction food-processing device in improving frying oil quality. *Intl J Food Sci Technol*. 49(10): 2223–2229.
- Lin, CA; Ayvaz, H.; Rodriguez-Saona, LE. 2014. Application of Portable and Handheld Infrared Spectrometers for Determination of Sucrose Levels in Infant Cereals. *Food Anal Methods*. 7 (7):1407-1414.
- Santos, AM; St-Pierre, NR; Francis, D., Rodriguez-Saona, LE. 2014. Feasibility of Predicting Ease of Peeling of Tomato Fruits by Using a Handheld Infrared Spectrometer. *J Food Process Preser*. 38(3): 1010-1017.
- Plans, M, Simó, J, Casañas, F, Romero del Castillo, R, Rodriguez-Saona, LE, José Sabaté. 2014. Estimating sensory properties of common beans (Phaseolus vulgaris L.) by near infrared spectroscopy. Food Res Intl. 56:55–62.

Appendix A. Progress report for "Evaluating chemical fingerprinting as a tool to rapidly screen hybrid chestnut for disease resistance"

This project was initiated in Fall 2015 with support from the American Chestnut Foundation (TACF) and in collaboration with researchers at University of Kentucky, The Ohio State University, Clemson University, USDA Forest Service Southern Research Station, and TACF.

The short-term goals of this study were to: (1) chemotype American and Chinese chestnut parents and inter-specific hybrid families; (2) use chemometric analysis to identify potential chemical biomarkers of resistance for chestnut blight and *Phytophthora* root rot, and (3) develop and validate a marker-based statistical model to predict which trees will be resistant to each disease.

Since the fall of 2015, we obtained chestnut tissue from collaborators at TACF and Clemson University that had been screened for blight resistance and PRR resistance. We collected chemical fingerprint data from ~90 BC_3F_3 hybrid chestnut from 21 families and ~19 American and Chinese chestnut seedlings that had been screened for blight resistance. We also collected chemical fingerprint data from ~104 BC_1 hybrid chestnut from 2 families (HB2 and NK4) that had been screened for *Phytophthora* root rot (PRR) resistance. We then evaluated whether chemical fingerprint data could be used to distinguish groups the varied in susceptibility to each disease.

There were clear chemotypic differences between American and Chinese chestnut (**Figure A1**). Chemical fingerprint data could be used to estimate blight lesion length, with a strong correlation between predicted and measured lesion length (r_{val} = 0.84) (**Figure A2**).

We were unable to find a clear association between blight susceptibility and chemical fingerprint data across all 21 BC_3F_3 families. However, when families were split into two groups based on the original BC_1 hybrid in which they were derived, i.e. Clapper vs. Graves, we were able to detect an association between chemical fingerprint data and lesion lengths for Clapper derived trees (**Figure A3**). We were unable to find a similar association for Grave derived trees, although our approach of including many families with a small number of individuals per family may have introduced too much variability into our models. Therefore, we plan to focus future analyses on fewer families with more individuals per family.

Similarly, we were unable to find a clear association between PRR susceptibility and chemical fingerprint data across both families (HB2 and NK4). However, when families were examined separately, there was some separation between PRR rating groups, particularly for samples from the HB2 family, which contained predominantly more susceptible individuals (i.e. PRR rating groups 2 and 3). Future work is planned to analyze additional individuals from HB2 and NK4 families, and also to analyze tissue collected from mother trees whose progeny has been screened for PRR resistance.

Overall, we believe that these results are promising; however, additional evaluations are needed to refine, optimize, and validate predictive models for both blight and PRR.

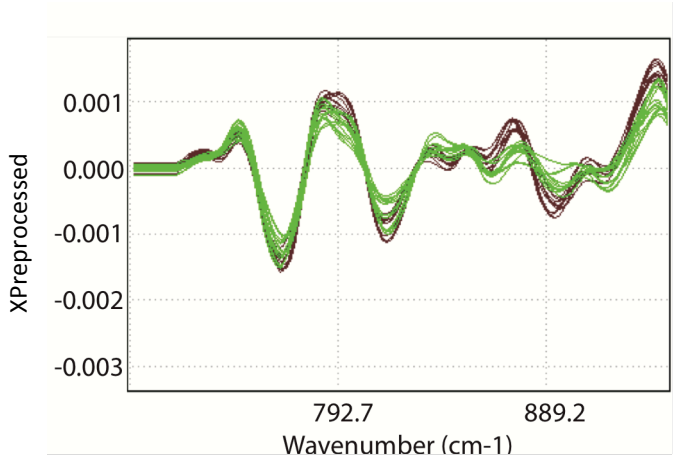

Figure A1. American (brown) and Chinese (green) second derivative transformed spectra, ranging from 698 – 947 cm^{-1}. Each line represents one spectrum (2 spectra were collected per individual). Chemotypic differences (areas of spectrum where American and Chinese chestnut differ) are evident, in particular around 792 and 889 cm^{-1}.

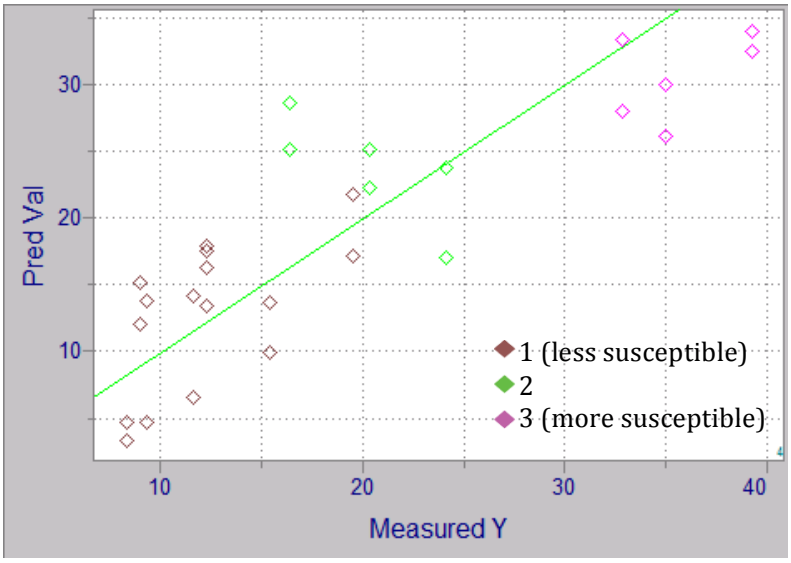

Figure A2. Results generated from 4-factor PLSR with leave one out cross validation. Relationship between measured and predicted blight lesion lengths (mm) for American and Chinese chestnut samples. Diamond colors represent different blight rating groups. N = 28 (including technical replicates with outliers and abnormal spectra removed). Chinese chestnut rated '1' (includes ratings of 1.5), while American chestnut rated '2' or '3'.

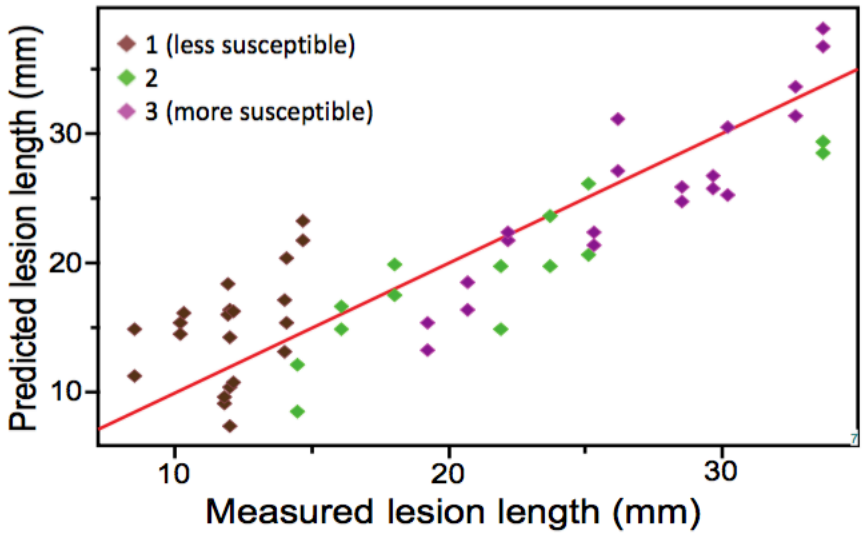

Figure A3. Results from a 7-factor PLSR with leave-one-out cross validation. There was a strong correlation, $r_{val} = 0.87$, between measured and predicted (based on chemical fingerprint data) lesion lengths (mm) caused by the pathogen *C. parasitica* on hybrid chestnut seedlings from Duncan farm descended from 'Clapper' BC_1 tree. Trimmed data set N= 55, including 2 technical replicates per biological replicate. Technical replicates were analyzed independently, and ~33% of technical replicates were removed based on preliminary PLSR analysis.

Appendix B.

THE AMERICAN CHESTNUT FOUNDATION®

August 9, 2016

Dear Dr. Conrad,

I am writing to express strong support of your proposal to TACF's external grants program for continued research into using Fourier transformed infrared (FT-IR) spectroscopy to predict chestnut seedlings' resistance to chestnut blight and *Phytophthora* root rot. Results from your work completed in the last funding cycle suggest that the FT-IR method is sufficiently sensitive to distinguish B3F3 seedlings at the extremes of the chestnut blight and PRR resistance spectrum. By screening American chestnut backcross hybrids for resistance to chestnut blight and *Phytophthora* root rot prior to planting, fewer trees with higher average resistance may be planted in seed orchards at wider spacing. Infrared spectroscopy has the advantage of being non-destructive to seedlings as compared with artificial inoculation.

To validate results from last season, you propose to develop FT-IR predictions of blight resistance using canker data from ~100 additional B3F3 individuals from the Clapper and Graves sources of resistance. Staff at TACF's Meadowview research farms have collected stem tissue and have completed inoculations of these seedlings in August of 2016. The second objective is to determine whether FT-IR can predict underlying genetic resistance of B3F2 mother trees whose B3F3 progeny have been screened for resistance to blight and PRR.

I am looking forward to continued collaboration with you on this project.

Jared Westbrook

Geneticist, Director of Science
The American Chestnut Foundation

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