5-2018

Characterization of Black Walnut Genotypes for Resistance to Thousand Cankers Disease, Frost Hardiness and Other Desirable Horticultural Traits

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CHARACTERIZATION OF BLACK WALNUT GENOTYPES FOR RESISTANCE TO THOUSAND CANKERS DISEASE, FROST HARDINESS AND OTHER DESIRABLE HORTICULTURAL TRAITS

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Biology

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UTAH STATE UNIVERSITY
Logan, UT

2018

ABSTRACT

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by

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Utah State University, 2018

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The black walnut, *Juglans nigra* L., is an important native tree species in the United States of America. In the latter part of the 20th century, the decline and then death of black walnut was observed in many western states and subsequently the disease complex known as Thousand Cankers Disease (TCD) was discovered. Thousand cankers disease is the result of a symbiotic vector-pathogen relationship between the walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman., and the fungus, *Geosmitha morbida* Kolařik. Thousands of WTB will swarm and enter a black walnut tree effectively vectoring the pathogen at each entry point where subsequently a canker develops, expands and coalesces, girdling the branch or stem and killing the tree in 3-5 years. Concern for industries related to black walnut has led to management research of the WTB, but there are currently no management practices for TCD. The development of resistant black walnut cultivars is an important aspect of disease management and incorporating resistant germplasm into current breeding programs could alleviate disease pressure in black walnut.

Over the course of three growing seasons (2015-2017), researchers from Utah State University evaluated black walnut (*J. nigra*), black walnut hybrids (*J. nigra* x *J. regia*) and other *Juglans* trees at the Cyril Reed Funk Research Farm in Richmond, UT and Dayton, ID for resistance to TCD through direct inoculation with the pathogen *G. morbida*. Of the approximately 336 trees inoculated each year, only one tree consistently exhibited traits associated with resistance throughout the project. An additional 14 trees exhibited resistance to the pathogen for two growing seasons.

Differential Thermal Analysis (DTA) of bud tissue took place over two winter seasons (December, 2015-March, 2016 and December, 2016-March, 2017) and yielded unsatisfactory results. This suggests that the protocol used for DTA of black walnut bud tissue may not be effective in determining cold hardiness of *J. nigra*. Resistant trees were evaluated for traits valuable to foresters, nut producers, nurserymen, breeders, urban foresters and homeowners such as form, nut production and growth habit.

(55 pages)

PUBLIC ABSTRACT

Characterization of Black Walnut Genotypes for Resistance to Thousand Cankers Disease, Frost Hardiness and Other Desirable Horticultural Traits

J. Elisa Lauritzen

The black walnut, *Juglans nigra* L., is native to the United States (USA) and is a valuable timber and nut tree. Just before the beginning of the 21st century, several western states observed a decline in the health and, later, death of black walnut trees. The pathogen-vector complex now known as thousand cankers disease (TCD) was shown to be the cause. The disease, caused by *Geosmithia morbida* Kolařik, is vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman. Thousands of WTB will swarm and enter a tree vectoring the fungus at each entry point where cankers then develop, quickly expand, coalesce and kill the branch or stem. The disease has been confirmed across the USA and in parts of Europe. The research and development of resistant cultivars is important to maintain native populations and livelihoods. The purpose of this project was to evaluate black walnut and hybrid trees for resistance to TCD through direct inoculation with the pathogen *G. morbida*. Inoculation of limbs took place in early summer of 2015, 2016 and 2017 at the Cyril Reed Funk Research Farm in Richmond, UT and Dayton, ID. Inoculated limbs were removed from the tree after senescence and canker size measured. An average of 336 trees were inoculated. One tree consistently exhibited resistance to TCD indicated by no canker staining. An additional 14 trees exhibited resistance for two of the three years. The results of this project indicate that breeding for resistance to TCD could be a management option for the disease.

ACKNOWLEDGMENTS

I would like to thank my graduate advisor, Dr. Claudia Nischwitz, for her support, guidance, and patience over the course of this project. I would also like to thank my other committee members, Drs. Brad Kropp and Larry Rupp, for their time and valuable feedback.

This acknowledgment would be incomplete without thanking James Frisby for patiently walking me through the process of DTA, Carol Peterson for her kindness and information about IPPFBE, and the team of undergraduate researchers in the Plant Pathology Lab who enthusiastically assisted with this project.

Finally, words fail to convey the depth of gratitude I feel for my incredible husband and three amazing daughters.

J. Elisa Lauritzen

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CHAPTER 1

INTRODUCTION

Native Range and Physical Description of *J. nigra* L.

Black walnut, *Juglans nigra* L., (Juglandaceae) is an important nut and timber crop in the United States of America (USA). A tree native to the eastern USA, it is estimated that some 306 million black walnut trees are an intrinsic part of native forest ranges (Randolph et al., 2013). It is one of six native *Juglans* species in the eastern and central USA with the native range extending from the East coast to Texas and from Massachusetts in the north to Florida in the south (Baughman & Vogt, 1996).

The black walnut is a cold hardy (United States Department of Agriculture (USDA) Plant Hardiness Zone 4-9) deciduous tree that is long-lived (150-250 years) and can reach mature heights in excess of 30 meters (100 feet) with an equal spread in its round to oval crown. The bark is furrowed and dark gray to black with tight grained wood. Leaves are compound and arranged alternately on 30-60 centimeter (12-24 inch) long pubescent stems with 15-23 serrated leaflets that are each 5-12 centimeters (2-5 inches) long and 2-5 centimeters (0.75-2 inches) wide. Leaves are fragrant when damaged and at senescence a shield shaped leaf scar remains on the branch (Dirr, 1977). The monoecious tree produces catkins (staminate/male flowers) on the previous year's growth and pistillate (female) flowers on current year's growth in clusters of 2-5 just after leaf emergence (late May-June). Small to medium sized nuts ripen in mid-to-late fall and are surround by fleshy green husks that turn brown-black when they fall from the tree.

The husk is bound tightly to the hard, ridged shell and makes kernel extraction difficult and messy due to the pigments and tannins it contains. The tree prefers well-drained soils, about 90 centimeters (35 inches) of annual precipitation and is generally found in riparian zones but can survive outside of this optimal range (Franch, 2010).

Economic and Historical Importance

Historically, the tree was used as an important food item for native and early Americans as both a primary food source and as a feed source for wild and domesticated animals. The high-quality wood was used for flooring, furniture, fine veneers and gunstocks (Woodroof, 1979). Black walnut continues to be a valuable source of superior quality wood for consumers and for timber revenue. An estimate by the USDA Animal and Plant Health Inspection Service (USDA APHIS) in 2009 stated that the "value for the black walnut growing stock in the United States is estimated to be over half a trillion dollars" (Fowler et al., 2009). The state of Missouri is home to the largest processing operation of the nutmeat where 23 million pounds of nuts were processed in 2015, most of which were foraged from native stands (Gounley, 2016). Black walnut products are sold nationally and internationally with China being the largest importer of nut products from the USA (McNeil, 2013).

Due to the trees highly valuable wood and nuts, it has been planted outside of its native range both in urban forests and in commercial settings within the USA (Tisserat et al., 2009) including locations in Hawaii. Because of changes in environmental conditions and hybridization with other *Juglans* species, the black walnut range now includes isolated locations in Mexico and Canada where the tree is heavily utilized, especially for veneer (Anonymous A, 2014). In the 17th century the tree was introduced to Europe for cultivation primarily as a timber source and has since become a naturalized fixture in many European forests where it is utilized for its timber and nuts (Nicolescu, 1998). The black walnut is also an economically important tree in Asia as tree parts are used medicinally, timber is used for fine wood products including veneers, and the nuts are used in cuisine.

Production of black walnut byproducts are not limited to timber and nuts. The tree produces an organic compound called juglone that has herbicidal properties for use in agriculture but is most commonly used in dyes, inks and cosmetics (Duke, J., 1983). Juglone has been used historically in Chinese medicine for its antimicrobial properties and is still a major economic driver for the walnut market in China. Investigative studies have produced several juglone derivatives for use in medicine and include current research studying the anticancer properties of the compound (Fang et al., 2015). The shell of the nut, historically regarded as a waste product, is now used for water filtration, as a principle component of clean-up in the oil industry, as a filler product in paints, wood products, gun powder and beauty products, and as an abrasive cleaner for machinery (Anonymous B, n.d.).

In addition to the black walnut's many uses, it is also an attractive tree and its demand as a landscape specimen is increasing. Current landscape trends include plants that are native to geographical locations, adapted to urban soils, water conservative and a potential food source for wildlife and people. The tree's relative low water use, nut production, and adaptability to many soil conditions, including high pH, are attractive for many property managers and homeowners looking for plant material that will work in dual-purpose landscapes.

Pests and Diseases of *J. nigra*

With the exception of the thousand cankers disease (TCD) complex, the black walnut is relatively free of serious pests and diseases. Some of the nuisance pests include the walnut husk fly (*Rhagoletis completa*) (Childers et al., 1995), the walnut weevil (*Conotrachelus tetentus*) (Warmund, n.d.), the walnut caterpillar (*Datana intergerrima*) (Woodroof, 1979), and aphids with the latter being the most serious of those listed but seldom warranting treatment (Wiman & Bell, 2017). The walnut husk fly larvae feed on the husk and can make harvesting the nut difficult (Childers et al., 1995). Walnut weevil or black walnut curculio can affect kernel development and cause early nut drop (Warmund, n.d.), while aphids feed on sap reducing overall vigor of the tree and increasing secondary pathogen development. In poorly drained soils the tree is susceptible to Nectria canker (*Neonectria ditissima*, formerly *Nectria galligena*) (Woodruff, 1979). Annual canker (*Fusarium solani*) will contribute to the failure of the tree (Tisserat et al., 2009) while anthracnose (*Gnomonia leptostyla)* can cause leaf spot, early defoliation and an overall reduction in vigor (Siegle, 2007). These diseases are less important that the aforementioned thousand cankers disease.

Thousand Cankers Disease

Shortly after the beginning of the 21st century, a decline in the health and increase in mortality of black walnut trees was observed in several western states and subsequently the pathogen-vector complex now known as thousand cankers disease (TCD) was discovered. Thousand cankers disease, caused by the fungus *Geosmithia morbida* Kolařik, is vectored by the walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman, that aggressively feeds on the tree. There is currently little information on the pathogen-vector relationship between the WTB and *G. morbida,* but several studies are currently underway in an attempt to better understand the relationship. The WTB is native to southwestern parts of the USA and is thought to have co-evolved with its native plant-host, the Arizona walnut, *Juglans major* Torr., which is not fatally damaged by the pest, and *G. morbida* (Tisserat et al, 2011). The pathogen is introduced by the WTB that creates small pin-prick sized entry holes in the branches and trunk of the tree as they burrow into the vascular cambium to feed, overwinter and lay eggs. Cankers then develop at each entry point and quickly expand, coalesce and effectively girdle the branch or stem causing tissue death. While the disease is often observed in trees exhibiting symptoms of stress, it does affect trees that appear healthy (Tisserat et al., 2009). The disease name is derived from the overwhelming number of cankers that develop at each entry point from swarms of WTB that attack the tree.

While canker development can be found on all *Juglans* species, black walnut and its hybrids are most susceptible to the disease, often succumbing in 3-5 years (Marshall et al., 2011). There are currently no management practices for TCD (Frank & Bambara, 2010). Pheromone trapping is being used in some areas to determine whether WTB is present in a given area and wood products are being quarantined in some states (Thousand Cankers Disease, 2014). Currently, TCD has been confirmed in nine western states and six eastern states with quarantines issued in 18 states that restrict the movement of untreated wood products (Thousand Cankers Disease, 2014) (Fig. 1). Additionally, in 2013, TCD was found in northeastern Italy (Montecchio et al., 2014).

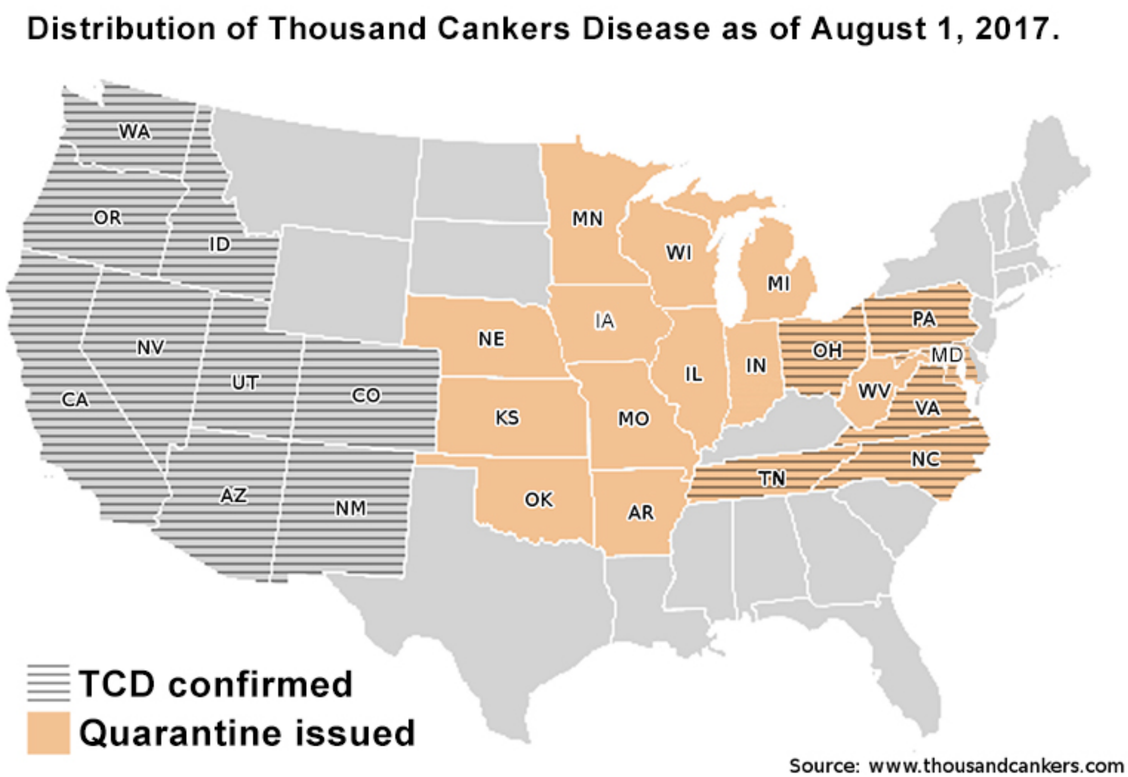

Figure 1. Distribution of confirmed TCD infections and black walnut wood product quarantines in the United States.

Potential damage to forest ecosystems, wood industries, nut producers, and nurserymen are huge with worldwide implications. The state of Missouri is estimated to lose some $36 million in annual timber production and an additional $4-6 million per annum in nuts sales if the disease were to infect trees in the state (Moltzan, 2011). Twenty-year projected economic losses could reach $851 million for the state (Missouri Department of Conservation, 2013). Current research on TCD focuses on reporting but very little is being done to discover potential methods of control. The research and development of resistant cultivars is important to maintain sensitive ecosystems and livelihoods of forest managers, timber and nut producers, and growers.

The purpose of this project was to evaluate black walnut, black walnut hybrids, and other *Juglans* species at two Utah State University research farms for natural resistance to TCD and for horticultural traits that may make black walnuts desirable for urban landscapes. Material from identified TCD resistant trees can then be made available to black walnut breeders across the country.

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CHAPTER 2

RESISTANCE TO THOUSAND CANKERS DISEASE

Introduction

Thousand Cankers Disease (TCD) is a significant threat to native, naturalized and cultivated *Juglans* species across the USA and worldwide (Montecchio et al., 2014). The disease was first described in 2008 (Tisserat et al., 2009) but is suspected to have impacted regions in the western USA as recently as the early 1990's (Fowler et al., 2009). The disease is the result of a symbiotic relationship between the walnut twig beetle (WTB), *Pityophthorus juglandis* Blackman (Coleoptera, Scolytinae), and the pathogenic fungus, *Geosmithia morbida* Kolařik (Ascomycota, Hypocreales). The WTB is a minute wood-boring beetle that aggregates, colonizes, feeds on and overwinters in both healthy and stressed *Juglans*. Tens of thousands of WTB will swarm a tree and vector the pathogen at each entry point (Anonymous C, 2014). The overwhelming number of wounds that the WTB inflict on the tree is indicated in the disease name. Inky dark brown to black cankers will develop in the phloem at each point of infection, then enlarge until several cankers coalesce creating a region of dead tissue that no longer supports the tree. Outward symptoms of the disease are often associated with drought stress and include yellowing of leaves, dieback of a single branch or section of the tree, and finally death. Trees that are attacked by the WTB can succumb to the disease in a very short period of time, usually 3-5 years after symptoms are first observed (Marshall et al., 2011).

Social and ecological impacts of TCD on *Juglans nigra* L. are currently unknown but the implications are huge. Ecologically the tree serves as a valuable food source for wildlife, stabilizes sensitive soils, works as a carbon sink, and is a valuable component on every ecosystem scale from “micro” to “biome (Anonymous D, n.d.). Social impacts are equally as difficult to project but concern for loss in public, private and urban forest settings could affect property values (Treiman et al., 2010) and public interaction with outdoor spaces could affect land use designations (Clay & Daniel, 2000) and public health (Donovan et al., 2013). Economic impacts in the USA are estimated based on an approximation of the standing volume, or growing stock, of *J. nigra* in its native range only and subsequently does not account for the far reaching implications TCD may have on other industries that rely on the tree (Daniels et al., 2016). Estimated losses will exceed $500 billion and do not include the value-added industries or costs related to the diagnosis, treatment and subsequent removal of declining or dead trees.

Thousand cankers disease has been confirmed in nine western states and six eastern state with quarantines issued in 18 states (Anonymous E, 2017). In 2013, TCD was reported for the first time outside of the USA in the European country of Italy (Montecchio et al., 2014). Currently, management practices for TCD revolve around reducing the spread of the disease through quarantines of wood products and monitoring for the WTB. Post-harvest treatment of timber and vector management are currently the focus of several studies and include research on semiochemicals as repellants, attractants and detractants (Chen & Seybold, 2014). Efficacy of insecticides, both contact and systemic, in controlling WTB attacks is unknown. While the use of the systemic insecticide imidacloprid is currently not approved for use in black walnut due to residual amounts of the insecticide found in the nutmeat, recent studies indicate that the use of some systemic measures such a dinotefuran may be effective in reducing WTB strikes (Nix, 2013). However, concern for both the known and the unintended consequences of conventional methods of insect control warrants the utilization of trees that are naturally resistant to TCD in breeding programs. This method of disease control can contribute to a sustainable system wherein management of TCD is achieved and leads to the improvement of the species (Sniezko & Koch, 2017).

The investigation of genetic resistance is an invaluable aspect of disease reduction in any economically important crop. Emerging technologies can help to expedite the development of resistant breeding stock but initial steps include determining the range of natural resistance through controlled artificial inoculation (Sniezko & Koch, 2017). The purpose of this project was to determine if natural resistance to the pathogenic fungus, *Geosmithia morbida*, causal agent of TCD, exists within a maintained collection of black walnut, black walnut hybrids and other native walnut species in two Utah State University owned collections.

Materials and Methods

Inoculation with *Geosmithia morbida*

Black walnut, black walnut hybrids and other *Juglans* tree branches at the Utah State University Cyril Reed Funk Research Farms in Richmond, Utah (UT) and Dayton, Idaho (ID) were inoculated during late spring for three seasons (June 1, 2015-June 12, 2015 - 341 trees total; May 30, 2016-June 24, 2016 - 377 trees total; May 23, 2017-June 06, 2017 - 291 trees total) with the pathogen *Geosmithia morbida*. The same trees were inoculated each year of the project unless limb size was smaller than 1.5cm diameter, tree health was at risk, or the tree had died. The *G. morbida* inoculum was isolated from the Richmond, UT (isolate 1223) location in 2008 (Zerillo et al., 2014) and maintained on ½ strength potato dextrose agar (PDA; BD Difco, Becton, Dickinson and Company, Sparks, MD). A #4 cork borer (8mm) was used to remove two small portions of outer bark to the vascular cambium approximately 15cm apart on a single branch (1.5cm diameter or larger) (Utley, et al., 2013). The inoculum was placed in the wound furthest away from the main stem and a control of an inoculum free ½ strength PDA plug was placed in the wound closest to the main stem. A strip of parafilm (Bemis Co., Oshkosh, WI) was then wrapped around each wound site to ensure that the sample did not dry too quickly or fall out (Utley, et al., 2013). Then a section of duct tape (3M Duct Tape) was wrapped around the parafilm to secure the inoculum and PDA control in place for the remainder of the growing season (Fig. 2). Inoculated limbs were harvested by removing it from the tree at leaf senescence in mid to late fall of each year (October 13, 2015-October 27, 2015; October 19, 2016-November 9, 2016; October 16, 2017-November 3, 2017). In the field, the bark layer was carefully removed to the vascular cambium using a folding work knife (Columbia River Knife and Tool's M21-04G) from the branch at each wound site and cankers were evaluated for size (length, width and bark swelling). Measurements of length and width were taken with a fabric measure tape and recorded. Resistance was defined as having no canker development (no staining beyond the margins of the wound).

In 2015, 40 of the trees across both locations (20 trees in Richmond, UT and 20 trees in Dayton, ID) were randomly selected to retain the inoculated limb for an additional season. This was done to determine the effects an additional growing season would have on the inoculated branches.

Figure 2. Inoculation protocol utilized; a wound was made with a #4 cork borer (1) then a plug of inoculum or inoculum free PDA was placed in the wound (2), wrapped in parafilm (3) and duct tape (4), and then left on the tree for the remainder of the growing season (5).

Pure Culture Maintenance of *Geosmithia morbida*

The *Geosmithia morbida* isolate was maintained on ½ strength potato dextrose agar (PDA) and transferred to new ½ PDA plates every 6-8 weeks for the duration of the project. A small section of *G. morbida* was placed on a fresh plate of ½ PDA, wrapped in parafilm and permitted to develop at room temperature (23 °C) under normal lab lighting for 7-14 days, after which the new culture was placed in a 4 °C incubator until sequencing. DNA sequencing was used every 8-12 weeks for the duration of the project to ensure the culture was preserved. A DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract the DNA from small sections of the isolate before being transferred to new plates. PCR was completed using Invitrogen AccuPrime Pfx Supermix (Thermo Fisher Scientific, Carlsbad, CA), ITS 4 (100pmol/microliter) and ITS 5 (100pmol/microliter) (White et al., 1990) and underwent 35 cycles (initial denaturation at

95 °C for 5 minutes; 35 cycles: 95 °C for 15 seconds, 52 °C for 30 seconds, 68 °C for 1 minute; finish extension 72 °C for 10 minutes) in at Techne TC-4000 thermocycler (Model FTC4/05; Cole-Parmer Ltd, Vernon Hills, IL). PCR products were visualized using gel electrophoresis (1% agarose stained with ethidium bromide) and bands at 750bp were cut and extracted from the gel and sequenced using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Samples were then sent for sequencing to Eton Bio Science in San Diego, CA. Sequences were then compared in a BLAST (Basic Local Alignment Search Tool) search to *Geosmithia morbida* sequences in the NCBI (National Center for Biotechnology Information) GenBank database.

Fusarium Isolation, Identification and Inoculation

During data collection in the fall of 2016, a second type of canker was observed on inoculated branches. Woodchip cultures were used to isolate and identify the cause. Layers of bark on the affected branch were carefully removed to reveal the canker and then small sections (approximately 0.5cm x 0.5cm) of the exposed cambium were excised and placed in ½ strength PDA with the antibiotic, streptomycin (0.125g per 500mL). These cultures were allowed to incubate for 1-5 days depending on growth rate or until the development of removable structures occurred. The culture was then transferred to ½ PDA until a pure culture was obtained. Basic morphology of the culture was noted and then DNA sequencing was utilized to determine species.

The DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) was used to extract the DNA from small sections of the isolate before being transferred to new plates. PCR was done using AccuPrime Pfx Supermix (Thermo Fisher Scientific, Carlsbad, CA), ITS 4

(100pmol/microliter) and ITS 5 (100pmol/microliter) (White et al., 1990), and underwent 35 cycles (initial denaturation at 95 °C for 5 minutes; 35 cycles: 95 °C for 15 seconds, 52 °C for 30 seconds, 68 °C for 1 minute; finish extension 72 °C for 10 minutes) in at Techne TC-4000 thermocycler (Model FTC4/05; Cole-Parmer Ltd, Vernon Hills, IL). PCR products were visualized using gel electrophoresis (1% agarose stained with ethidium bromide) and bands at 750bp were cut and extracted from the gel and sequenced using the QIAquick Gel Extraction Kit (Qiagen, Hilden, Germany). Samples were sent for sequencing to Eton Bio Science in San Diego, CA. Sequences were then compared to other *Fusariums* in a BLAST search in the NCBI GenBank database and matched *F. tricinctum* and *F. proliferatum*.

To determine the independent effects of the *Fusariums* on the walnut trees, 40 trees in 2017 were inoculated with both *F. tricinctum* (F2) and *F. proliferatum* (F3) in conjunction with the annual inoculation of the trees with *G. morbida*. Branches for this purpose were selected independently of the branches utilized for the annual inoculation but the protocol remained the same. A #4 cork borer (8mm) was used to remove three small portions of outer bark to the vascular cambium approximately 15cm apart on a single branch (1.5cm diameter or larger). The two inoculums were placed in the wounds furthest away (F2 placed closest and F3 furthest) from the main stem and a control of an inoculum free ½ strength PDA plug was placed in the wound closest to the main stem. A strip of parafilm (Bemis Co., Oshkosh, WI) was then wrapped around each wound site followed by a section of duct tape (3M Duct Tape) to secure the inoculums and control in place for the remainder of the growing season. Inoculated limbs were harvested by removing them from the tree at leaf senescence in mid to late fall of 2017 (October 16, 2017-November 3, 2017). The bark layer was carefully removed to the vascular cambium using a folding work knife (Columbia River Knife and Tool's M21-04G) from the branch at each wound site and, if present, cankers were evaluated for size (length, width, and color). Measurements of length and width were taken with a fabric measure tape and recorded.

Results

Culture maintenance of *G. morbida* was regularly checked through molecular analysis to ensure a pure isolate consistently provided a viable inoculum. The 750bp sequences obtained from the *G. morbida* culture throughout the project best matched (99%) NCBI GenBank BLAST accession numbers KJ664795.1 and KF808301.1.

For the purpose of this project, resistance is defined as the absence of any canker staining beyond the margins of the original wound site (Fig. 3). Two predominate phenotypes existed within the collection and trees were categorized based on these characteristics either as "black walnut type" (for trees with features that most closely resembled *J. nigra*) or "Persian walnut type" (for trees with features that most closely resembled *J. regia*). Trees exhibited resistance each year of the project, but rates of resistance varied from year to year. In 2015, 341 trees were inoculated and 59 trees were rated as resistant (17%). In 2016, 377 trees were inoculated and nine trees were rated as resistant (2%). In 2017, 291 trees were inoculated and 26 trees were rated as resistant (9%). One tree consistently exhibited resistance throughout the project with 14 additional trees exhibiting resistance for two of the three seasons (Table 1). Resistance was equally distributed across phenotypes; seven black walnut type trees were rated as resistant for two years, six Persian walnut type trees were rated for resistance for two years, while one Persian type walnut consistently exhibited resistance all three years. All but one of the resistant trees was located in the Richmond, UT plot.

Figure 3. No canker development in response to control wound ("C", left) and inoculation wound ("I", right). Persian walnut type tree, Richmond, UT (Spring Hill), row one, tree 18, 2017 season.

Table 1.

Trees Resistant to Thousand Cankers Disease

Location	Row	Tree	Phenotype	Control Area 2015	Inoculum Area 2015	Control Area 2016	Inoculum Area 2016	Control Area 2017	Inoculum Area 2017
SHN	1	18	PW	2.25	4.48	0	0	0	0
SHN	5	12	PW	0	0	0	4.42	0	0
SHN	**7**	**21**	**PW**	**0**	**0**	**0**	**0**	**0**	**0**
SHN	8	4	PW	0	0	0	0	x	x
SHN	8	14	BW	0	0	2.4	3.6	0	0
SHN	10	8	PW	0	0	3.57	6.38	0	0
SHN	11	10	PW	0	0	0	5.7	0	0
SHN	11	12	PW	0	0	0	5.46	0	0
SHN	12	6	BW	0	0	49.2	72	0	0
SHN	12	9	BW	0	0	7.77	5.72	0	0
SHN	14	8	PW	0	0	3.99	10.08	0	0
SHN	15	3	BW	0	3.96	0	0	0	0
SHN	15	10	BW	0	0	x	x	0	0
SHN	16	9	BW	0	8.4	0	0	0	0
DN	27	137	BW	0	0	5.75	3.23	0	0

Note: List of trees that exhibited resistance for at least two of the three of the project. One tree exhibited resistance all three years of the project (SHN, Row 7, Tree 21, boldfaced). Canker size (cm^2) is included for trees that developed cankers for a single year of the project. Data points lost due to breakage are delineated with an "x" for that year.

Trees permitted to retain inoculated branches for two growing seasons had their branches removed in the fall of 2016. Canker sizes from 2015 inoculated wounds were two to four times larger when allowed to develop for two growing seasons than the inoculated wounds from 2016 allowed only to develop for a single growing season. Of the 40 trees that retained an inoculated branch for two seasons, only one exhibited resistance for the 2015 inoculated wound but the tree developed a canker on the 2016 inoculated wound.

Cankers associated with TCD are distinctive with dark brown to black staining and smooth margins (Fig. 4). Canker size area around the inoculated wound was consistently larger on trees that exhibited physical characteristics more commonly associated with *J. nigra* than those associate with *J. regia* (Fig. 5). On average, wounds that were inoculated with *G. morbida*, had staining that was two and half times larger than the control wound (Fig. 6). The range of the wound responses varied from no response to massive button-like calluses (Fig. 7). The presence of a wound response (woundwood or callus) at the original wound sites did not affect whether a canker was present in the inoculated wound but did reduce the frequency of canker development in the control wound.

Figure 4. Typical canker development in response to inoculation wound ("I", left) and control wound ("C", right). Black walnut type tree, Richmond, UT (Spring Hill), row four, tree 15, 2017 season.

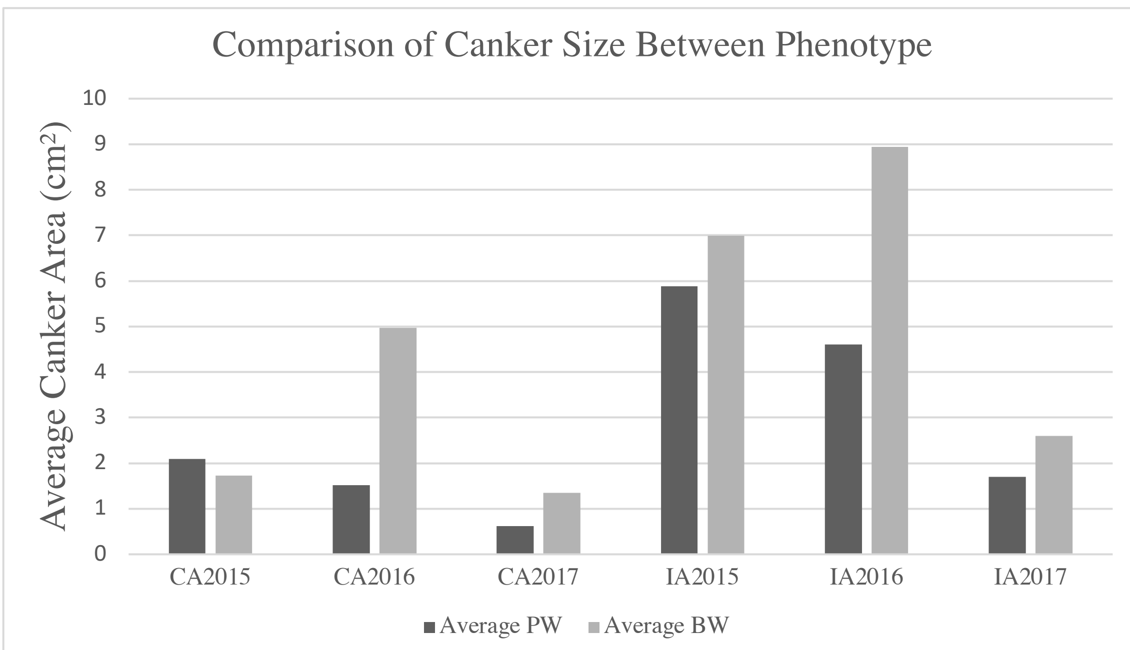

Figure 5. Comparison between phenotypes of the total average canker size (cm^2) of the control area (CA) and the inoculated area (IA) from 2015-2017. Canker size of trees with predominately black walnut characteristics is represented by light gray (BW) with canker size of trees exhibiting primarily Persian walnut characteristics is represented by dark gray (PW).

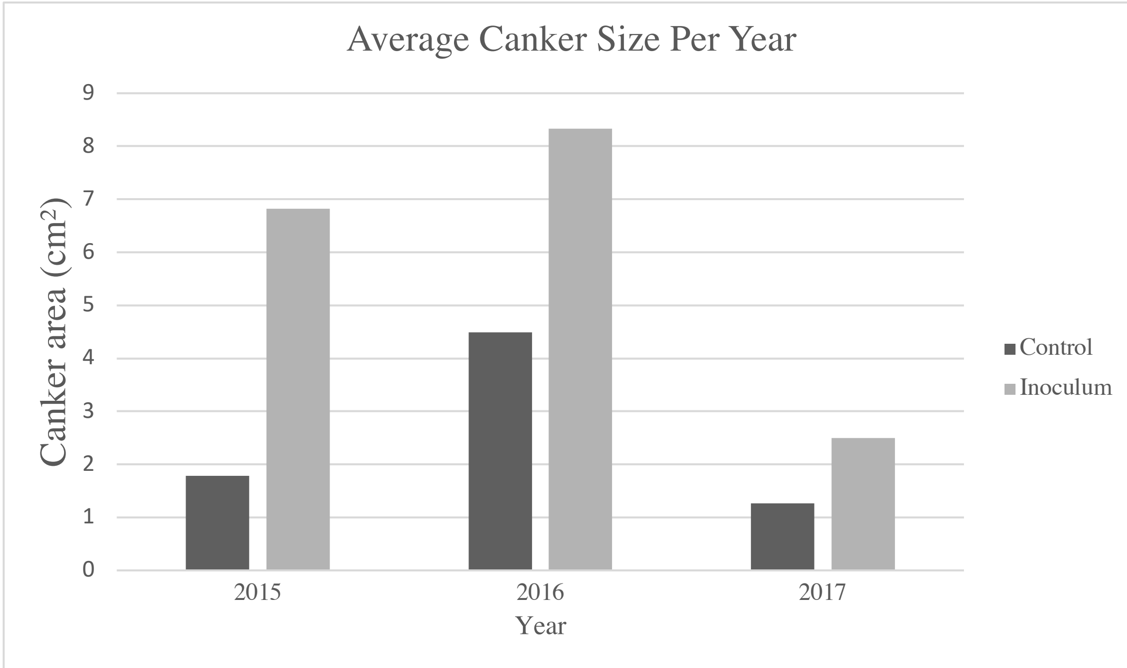

Figure 6. Comparison of the average control (dark gray) and inoculum (light gray) canker size (cm²) of all trees for all three years of the project (2015-2017). Inoculum cankers averaged 2.5 times larger than cankers that developed at the control wound.

Figure 7. A range of wound and callus responses to the injury incurred for inoculation from large calluses on the left to no woundwood on the right.

In 2016, irregular cankers were observed and cultured from trees that had been inoculated in 2015 and permitted to overwinter and grow a second season with the inoculum, as well as regularly inoculated trees from spring 2016. This second canker type was lighter in color and considerably larger than the canker most often associated with *G. morbida*, often exceeding the length of the removed branch section and encompassing the entire circumference (Fig. 8). Two species of *Fusarium*, *F. tricinctum* and *F. proliferatum*, were subsequently isolated and used in the 2017 inoculation of 40 trees. Eight of the trees that were inoculated with *F. tricinctum* and *F. proliferatum* developed *Fusarium* cankers at each of the points of inoculation. Woodchip cultures were isolated from the *Fusarium* cankers and confirmed to be the inoculums *F. tricinctum* and *F. proliferatum*.

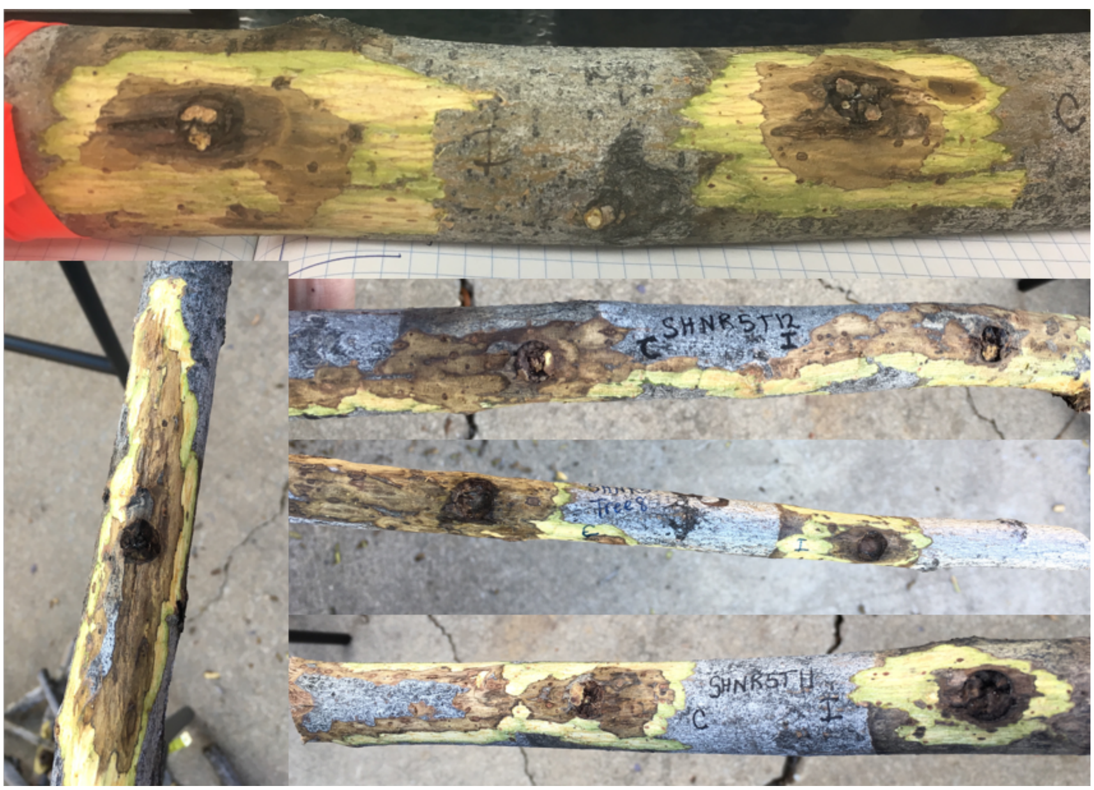

Figure 8. Cankers associated with *F. tricinctum* and *F. proliferatum* were larger and lighter than the canker associated with *G. morbida*. The bottom right wound (SHNR5T11) is the typical canker shape, color and size of *G. morbida* while canker development on the left side of the same branch is typical of the *Fusarium* cankers.

Discussion

The 2016 data saw a significant decrease (2%) in rates of resistance compared to 2015 (17%) and 2017 (9%). It is interesting to note that the trees were unintentionally subjected to herbicidal drift (2,4-D and dicamba) and subsequent injury early in the growing season in both 2015 (June 20, 2015 symptoms observed) and 2017 (June 24, 2017 symptoms observed). Some research suggests that herbicide-induced resistance, especially from auxin-based formulations, can reduce disease. Velini et al. (2010) discussed the plant defenses that can be triggered when herbicide is applied, specifically the effects of auxin-based herbicides as plant growth inhibitors. Cohen et al. (1986) observed that resistance to *Fusarium* wilts was reduced in seedlings that had been treated with dinitramine. Of the 15 trees that exhibited resistance to TCD, nine of them developed *G. morbida* cankers only in 2016 when the trees were free from herbicide damage suggesting a potential association between herbicide application and TCD resistance.

Opportunistic fungi, like *Fusarium* species, could play a role in *G. morbida* canker development. The first report of TCD by Tisserat et al. (2009) reported that *F. solani* was associated with a second canker type in conjunction with *G. morbida*. Many trees in 2016 developed a lighter and larger canker than the canker associated with *G. morbida,* including several of the 40 trees permitted to retain the inoculated limb for two growing seasons (inoculated 2015, harvested fall 2016). Secondary canker development often exceeded 10cm in length and usually extended across the entire circumference of the branch. Coalescing cankers from both wound sites would expand beyond the length of the cut section of branch. These stains were morphologically different from those associated with *G. morbida* canker exhibiting lighter and larger stains and irregular margins. *Geosmithia morbida* cankers seldom exceeded 5cm, had dark brown to black stains and maintained smooth, near oval margins. Woodchip sections from the secondary cankers were cultured on ½ PDA+ (potato dextrose agar with streptomycin) to determine the cause of the additional staining and regularly came back as *Fusarium trisinctum* and *F. proliferatum*. In 2017, 40 trees were inoculated in the same manner as *G. morbida* with both *F. trisinctum* and *F. proliferatum* to determine if symptoms would develop on limbs in the absence of *G. morbida*.

The development of a second canker type caused by *Fusarium* may have been reduced during years that the trees were subjected to herbicide injury as these cankers were observed only in 2016. The Cohen et al. (1986) studies in herbicide-induced resistance specific to *Fusarium* may support this. In addition, of the 40 trees inoculated with *Fusarium* only eight exhibited any staining, indicating a potential relationship with *G. morbida,* but further investigation is needed. All of the *Fusarium* affected trees also developed a canker at the *G. morbida* inoculation site.

The discovery of 15 trees that exhibited resistance to TCD for at least two seasons support further investigation of naturally resistant trees as a potential means of control to TCD through breeding programs. The determination of whether the resistance is categorized as complete or incomplete will need to be determined later in the evaluation processes (Sniezko & Koch, 2017). Additionally, evaluations to determine whether trees are exhibiting true resistance or tolerance will need to be undertaken. Incorporating resistant trees into breeding programs is an important step to ensure the continued success of black walnut as a native part of delicate ecosystems as well as a valuable asset in the timber and nut producing industries.

Due to the inoculation of unique black walnut and black walnut hybrids, statistical analysis was not possible as the trees are considered pseudo-replicates.

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CHAPTER 3

HORTICULTURAL TRAITS AND COLD HARDINESS

Introduction

The black walnut is a valuable and attractive multi-use native tree. It is grown primarily as a timber tree and demand in wood-based industries continues to drive its cultivation both within and outside of its native range (Reid et al., 2009). In spite of the trees persistent husk, the tree is being grown with increased frequency for its nuts (Reid et al., 2009). The nut is regaining favor culinarily due to its excellent flavor. Demand for native landscape plants (Moran, 2014) that prove adaptive to a variety of conditions as well as meeting expectations of landscape managers and homeowners has generated an interest in the tree. While the high value wood products, nuts, and wildlife habitat remain the primary reason the tree is grown, several alternative uses for the tree are gaining favor publicly, including oil pressing, tapping for syrup production, and natural dye (Duke, 1983). Nut collection is primarily a foraging activity but improvements in cultivar selection is proving to be valuable to those who want to grow trees in traditionally cultivated nut groves. Breeders, nurserymen and hobbyist have worked to develop superior cultivars to meet the needs of each respective industry (Victory et al, 2004) and more than 700 named cultivars of both timber type and nut type trees are thought to exist. However, little has been done to develop cultivars that meet multiple criteria such as improved yield in combination with cold hardiness and improved form. Trees that are able to meet the multifaceted needs of growers, nurserymen and other consumers will ensure the trees continued economic and environmental value.

Current breeding programs are working to fine-tune existing cultivars and develop new ones that improve kernel quality, yield, cold tolerance, vigor and timber quality. Differential thermal analysis (DTA) was analyzed as a tool to determine cold hardiness of the trees. DTA is a technique that is used to measure phase changes in a substance that emits or absorbs heat. In plant material, DTA can be used to determine the point at which intracellular water freezes and lethally damages plant tissue. A major limiting factor of the cultivation range of black walnut is cold temperatures just before dormancy (early fall frosts/freezes) and just after the tree breaks dormancy (late spring frosts/freezes). During dormancy the formation of intracellular ice is limited due to a process known as supercooling. This process allows plants to maintain turgor within the cell during dormancy and reduces water loss in the plant. Cold injury occurs when intracellular water freezes and water crystals form in the apoplast drawing water out of the symplast (ice nucleation) (Ritchie & Landis, 2003). This results in dehydration of cell contents and, ultimately cell death (lysis). DTA of plant tissue is a measure of exothermic energy that tissue will release as it freezes and is a particularly valuable tool in fruit production as it can aid in the determination of the range in temperatures that fruit and nut producing plant tissue can withstand. DTA has been effectively used in several horticulturally important crops including *Prunus*, *Malus*, *Rubus*, and *Vitis* species to understand the mechanisms involved in the freezing process (Quamme, 1991).

Weather data collection is an important aspect of effectively determining resistance in a population to both the vector and pathogen, especially when plant material is grown in ranges that exceed the preferred growing conditions. Instances of resistance or susceptibility to a disease or pest, including the survivability of the pest or pathogen, can be altered due to weather extremes (Juroszek & Von Tiedemann, 2011).

Through the collaborative effort of Dr. C. Reed Funk and other research organizations around the world, a great deal of genetic variety exists within the black walnut collection at the Cyril Reed Funk Research Farms and may contain several valuable trees with traits that are useful. Only a small percentage of the overall available *Juglans* trees were evaluated for horticultural traits associated with TCD.

Recent average temperature changes across the USA are believed to have contributed to the change and increase in the geographical range of the WTB and spread of TCD to the black walnut (Hefty et al., 2017). Management practices for TCD will need to adjust for potential increases in the range of both the pest and its symbiotic pathogen. Climate conditions are the focus of much discussion but influences on physiological changes that occur within in a plant and potentially increase vulnerability should be addressed (Juroszek & Von Tiedemann, 2011). In order to do this, information on the current range of climate conditions and hardiness of plant tissue can be addressed through the utilization of analytical tools already in place such as DTA.

The objective for this portion of the project was to determine if trees that exhibit traits in combination with resistance to TCD at the research farms may be valuable to breeding programs and the overall improvement of the tree. Some of these horticultural traits include morphological characteristics, susceptibility or resistance to extreme environmental conditions and basic measures to determine vigor, all of which are important to ensure the health of the trees, especially those that will be cultivated outside of its native range.

Materials and Methods

Horticulture Trait Data Collection

The evaluation of observable horticultural traits of all trees took place in early summer and mid-fall 2016 and 2017. Trees that were found to exhibit resistance were evaluated each year for morphological traits including phenotype (black walnut types, Persian walnut types), canopy shape (form), branch angles (60°-45°=wide; <45°=narrow), bark type (smooth, rough, mixed) and trunk shape (straight, irregular). Trees were measured for diameter at breast height (DBH=tree circumference cm/3.14159) approximately 1.3m (4.5ft) above ground on the leeway side if a slope existed) and inoculated branch diameter was measured. Environmental damage was noted when warranted. Nut development was noted when nuts were present. No evaluation of nut size, count or quality was undertaken. Trees that did not exhibit resistance, but that were otherwise noted as excellent, also had the same data collected.

Differential Thermal Analysis

A ceramic thermal electric plate (design and protocol developed by D. Gibeaut and T. Einhorn, WSU) (Fig. 9) was used to generate exothermic data through differential thermal analysis (DTA) of bud tissue. Collection of plant material took place from November to March for 2015-2016 and again for 2016-2017 every four weeks. Eleven trees from each location (Richmond, UT and Dayton, ID) were randomly selected and vigorous branches with no signs of damage were harvested from a single branch from each tree. Leaf and flower buds were removed and sorted. Bud quantity varied (3-13) from tree to tree but generally averaged around 10 buds per branch. Apical buds were tested separately due to their size. Buds from each tree were placed and secured (by folding) in a small section of aluminum foil (8cm x 8cm) and then placed in a holding cell on top of a thermo-electric module (TEM) imbedded on a metallic plate (12 cells per plate: 11 samples and one “control”). Each filled plate was placed in a programmable thermal chamber which then reduced the internal temperature by 4 °C every hour until -25 °C was reached. Exothermic readings were taken for each cell every five seconds and recorded with a data logger (CR1000, Campbell Scientific, Logan, UT). In order to allow for multiple readings to take place simultaneously, a multiplexor was used in conjunction with the data logger (25-Channel Solid State Thermocoupler Multiplexor, model AM25T, Campbell Scientific, Logan, UT).

Figure 9. Thermal chamber (top two photographs) with one of three ceramic thermal electric plates (bottom photograph) for use in DTA. Plant tissue from individual black walnut trees would be placed in every cell, except cell seven (control), on a ceramic thermal electric plate and then placed in the thermal chamber for the analaysis. Design developed by D. Gibeaut and T. Einhorn, (WSU) for DTA.

Weather Data Collection – Winter/Spring 2017

On March 2, 2017, a Remote Automated Weather Station, Fire Weather (RAWS-F; Campbell Scientific, Logan, UT; https://www.campbellsci.com/raws-f) was deployed in Dayton, ID in collaboration with Utah Climate Center at Utah State University. This was done to determine the reliability with which data from a permanent weather station, located in Preston, ID, 11.25km (7 miles) east of Dayton, ID, could be extrapolated as a tool to explain how weather may be affecting trees in Dayton, ID. Weather data from Dayton, ID was collected from March 4, 2017-April 5, 2017. Air temperature, wind speed and direction, precipitation, humidity, barometric pressure and solar radiation data were collected every 15 minutes and transmitted via the Campbell Scientific GOES satellite transmitter. Weather data for Richmond, UT and Preston, ID (Network UAGRIMET) was retrieved from the Utah Climate Center website. Weather data from Preston, ID was collected every 15 minutes with air temperature, relative humidity, precipitation, wind speed and solar radiation measurements collected. Data retrieved from the Richmond, UT station reflects daily averages and rates of precipitation and temperature. Data from all three locations was compared visually using an Excel (Microsoft, 2010) comma separated value (CSV) spreadsheet and basic statistics were employed to determine the range of weather condition the trees were exposed to for part of the season.

Results

Horticultural Traits

A total of 15 trees were found to be resistant to TCD for at least two years of the project (Table 2) with eight exhibiting traits more commonly associated with Persian type trees (smooth bark, oval leaflets) and seven of the tree appearing to be black walnut (rough, dark bark with more lancet shaped leaves). Canopy shape is predominately round (10 trees total of the 15 counted) with the remaining exhibiting an oval shaped canopy. In 2016 DBH measures ranged from 5.3cm DBH to 10.7cm DBH and by the end of the season in 2017 DBH measures ranged from 5.6cm DBH to 12.9cm DBH. Three of the trees are multi-stemmed and the remaining have single stems. Branch circumference was smaller in 2017 (average 6.5cm) than in 2016 (average 7cm) and all branch angles were considered wide (>45). Three trees consistently produced a nut crop. Herbicide injury was the most common injury the trees sustained (100%) followed by southwest winter injury (33%), animal damage including bird strike (13%), and mechanical damage (13%).

Table 2.

Horticultural Traits of Trees Resistant to TCD

Location	Row	Tree	Phenotype	Canopy Shape	Branch Angle	2016 Branch Diameter (cm)	2017 Branch Diameter (cm)	2017 Spring DBH (cm)	2017 Fall DBH (cm)
SHN	1	18	PW	R	W	1.88	2.36	10.68	11
SHN	5	12	PW	O	W	1.88	2.83	5.81	5.97
SHN	**7**	**21**	**PW**	**R**	**W**	**2.51**	**x**	**6.6**	**6.3**
SHN	8	4	PW	S	W	2.2	1.57	x	x
SHN	8	14	BW	O	W	2.2	2.51	5.34	5.65
SHN	10	8	PW	R	W	2.2	2.83	12.57	12.88
SHN	11	10	PW	R	W	2.51	2.36	9.74	9.74
SHN	11	12	PW	R	W	1.88	1.73	x	10.37
SHN	12	6	BW	O	W	2.04	1.57	9.4	11
SHN	12	9	BW	R	W	2.83	x	2.2	3.14
SHN	14	8	PW	S	W	2.51	1.73	4.08	6.6
SHN	15	3	BW	S	W	x	1.88	6.6	7.54
SHN	15	10	BW	R	W	x	1.41	7.54	7.85
SHN	16	9	BW	O	W	1.88	1.88	6.6	7.54
DN	27	137	BW	R	W	2.2	2.04	5.97	7.85

Note: Valuable horticultural traits of resistant trees including phenotype (PW = Persian walnut, BW = black walnut), canopy shape (R = round, O = oval, S = shrubby), branch angle (W = wide > 45°, N = narrow < 45°), and ABH (diameter of trunk at breast height). The Richmond, UT plot is identified as SHN (Spring Hill North) and the Dayton, ID plot is identified as DN (Dayton North).

Planting records indicate the tree that consistently exhibited TCD resistance (SHNR7T21; Spring Hill North, Row 7, Tree 21) is a small Persian walnut, *J. regia* ‘Combe’, that had been grafted in 2006 on to a black walnut cultivar, *J. nigra* ‘Davidson’ (Fig. 10). It is located at the Richmond, UT plot, is susceptible to late spring frosts (new growth and small twig dieback), has a round canopy, and did not produce nuts. The only tree from the Dayton, ID location to exhibit resistance to TCD for at least two years is a black walnut simply identified as a “Purdue Timber Type 263” and was planted in 2010. The tree has an irregular trunk, a round canopy, is susceptible to late spring frosts (new growth dieback) and produces a large nut crop.

Figure 10. A selection of trees that exhibit resistance to TCD. From left to right: SHNR7T21 (Richmond, UT, row 7, tree 21), a grafted Persian walnut resistant all three years; SHNR1T18, a large hybrid Persian type tree that did not produce nuts; SHNR8T14, a hybrid black walnut with an open canopy; and DNR27T137, a timber type black walnut that produced a large nut crop.

Differential Thermal Analysis

Analysis of the DTA data yielded no significant results. Successful DTA of fruit or nut producing tissue generally results in two to three distinct exothermic peaks as temperatures drop to lethal levels and energy is released from various tissue types (xylem, phloem, flower, etc…). Exothermic profiles of the individual black walnut trees generated a small range of outcomes, but most trees exhibited no exothermic peaks and no reliable data was generated (Fig. 11). Figure 11 demonstrates the variability of the results and shows a selection of exothermic readings from four samples collected in March, 2016. The data from trees one and two (FP_Exo01 and FP_Exo02) show distinct peaks that indicate the point at which leaf buds froze and released exothermic energy. However, results from trees three and four (FP_Exo03 and FP_Exo04) were typical of most of the tree samples collected for DTA and indicate no recorded change in the tissue as temperatures dropped to -30 ºC.

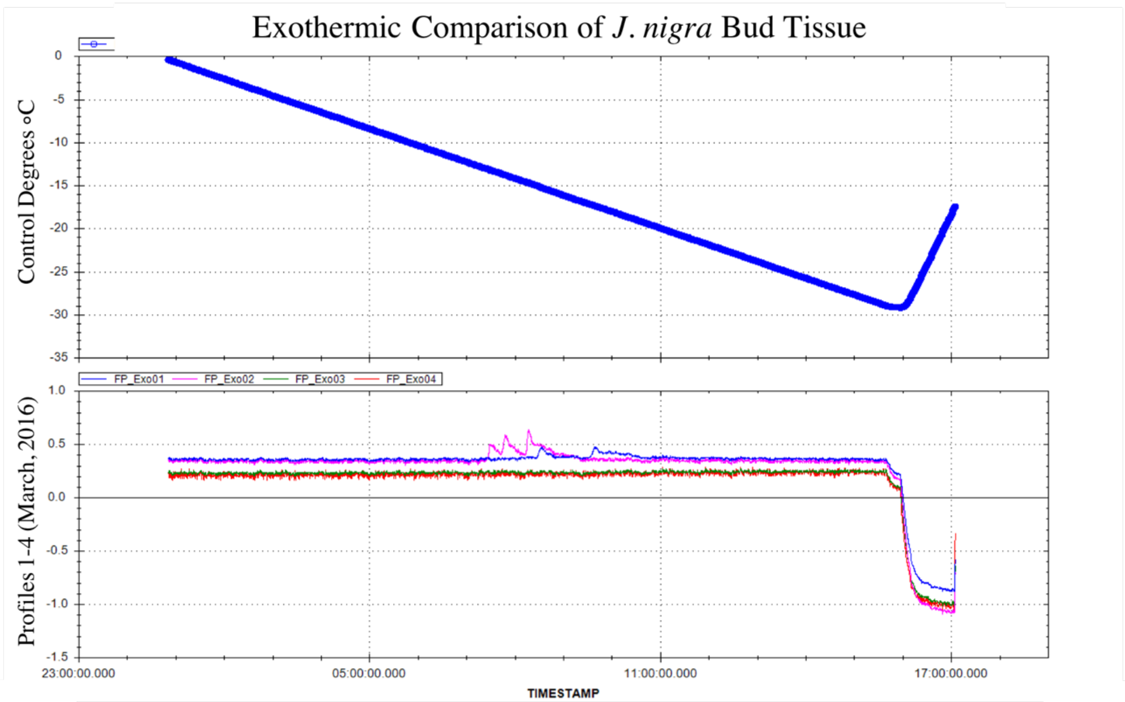

Figure 11. A comparison of DTA exothermic readings from four different *J. nigra* from Richmond, UT in March, 2016. The top graph is a visualization of the controlled reduction in temperature over time as recorded by the control cell (0-30 ºC). The bottom graph is a representation of the temperatures over time as recorded by cells one through four which all contained bud tissue.

Weather Data Collection

Weather data was collected from March 4, 2017-April 4, 2017 to aid in the determination of tree health and whether the weather station in Preston, ID could be extrapolated reliably for the Dayton research farm. Based on the data comparison of the two locations, Preston, ID weather information can be effectively used as an indicator of weather conditions in Dayton, ID (Fig. 12). Average maximum and minimum daily temperatures fluctuated slightly between the two locations, but overall temperatures were not significantly different. When temperatures between the two research farm locations were compared, Richmond, UT exhibited temperatures both slightly higher and slightly lower than Dayton, ID (Fig. 13).

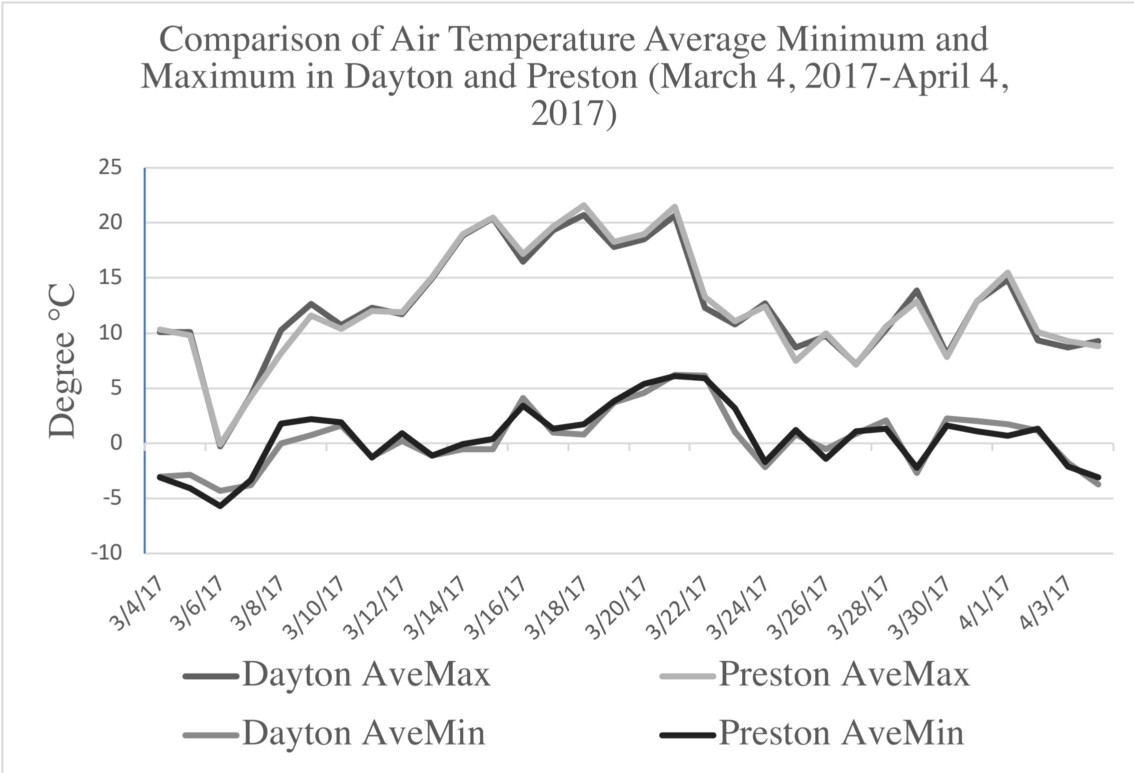

Figure 12. Comparison of the average daily minimum and maximum temperatures (°C) from Dayton and Preston, ID.

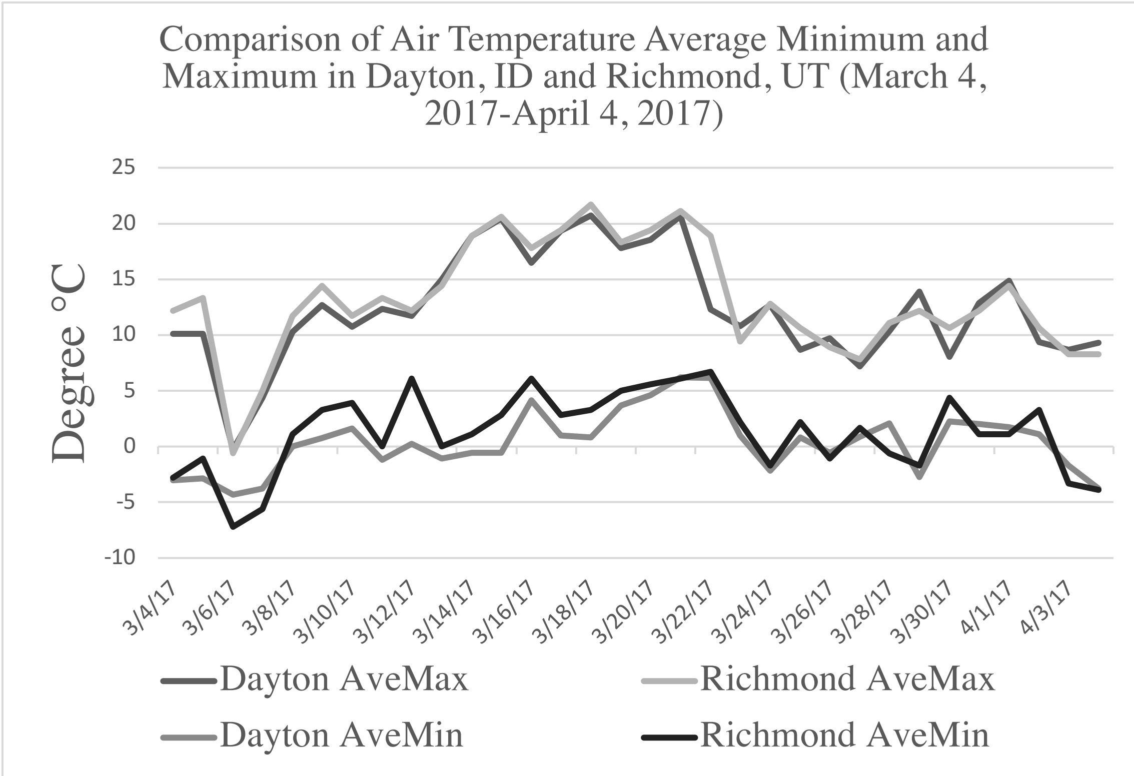

Figure 13. Comparison of the range of temperatures experienced at each weather collection site.

Discussion

Horticulture Traits

Because of the genetic diversity of the collection, trees exhibited a wide range of characteristics. Canopy shape was likely the most variable with most of the trees having a round canopy, but the collection contained trees with oval, pyramidal and, potentially, a columnar form (proximity of neighboring trees may have influenced the development of this form). The majority of the trees planted were directly sown black walnut seedlings on 3-foot centers (0.9meters). Management practices prior to the undertaking of this project in 2015 were unknown, but many trees in both locations were overgrown and had encroached on neighboring trees causing irregular development of the canopy and long, spindly trunks that were susceptible to wind damage. The Dayton, ID location was co-planted with alfalfa in a cropping system focused on the legume rather than the tree and subsequent mechanical damage to the trees was not an uncommon issue and several inoculated branches were lost each year. Proximity of the Richmond, UT location to native lands had the additional challenge of wildlife that would damage and even kill trees. Bird strike injury was noted on several branches at the wounds in spite of protocol techniques (parafilm and tape wrapped). Finally, less than ideal growing conditions contributed to plant stress and likely resulted in reduced vigor and increased susceptibility to pests and diseases. The Cache Valley, located in northern Utah and extending into southeastern Idaho, experiences a range of extreme winter and summer temperatures. Other conditions that may have contributed to plant stress include; low rates of precipitation and high winds as well as soils that are arid and alkaline. Frost damage was the most common environmental injury followed by southwest winter injury. These factors together played a role in the development of the trees and as such these characteristics may not be expressed under more favorable growing conditions.

Differential Thermal Analysis

DTA of bud tissue yielded no significant results. The protocol utilized for this project, has been used with good success for *Prunus* and other fruit crops but did not meet the same expectations with black walnut. A study in 2010 successfully utilized DTA of Persian walnut (Aslamarz et al., 2010) and with some adjustment may be a viable protocol in future analysis. Aslamarz et al. (2010) used Persian walnut buds with twigs attached (small section of phloem tissue), that were then directly embedded with a thermocouple. Due to the smaller bud size in black walnut, this method would not have been suitable for determining cold tolerance. Exothermic readings that were recorded were observed to peak most often between -9 °C and -15 °C suggesting that black walnut may be sensitive at these temperatures, but sample size and results were limited. However, these observations did coincide with observed cold injury of the trees.

Weather Data Collection

Weather data was significantly different in Dayton, ID from the nearest (11.25km) weather station in Preston, ID. This information was valuable in determining the cause of decline in some trees. Most information regarding climate extremes and disease susceptibility focus on a single climatic element (e.g., precipitation, wind, humidity, or temperature) and little is known about what influences these elements combined may have on disease susceptibility and development (Coakley, Scherm, & Chakraborty, 1999). Recent research indicates that the WTB experiences high rates of mortality when winter temperatures drop below -15 °C and experience 100% mortality at -23 °C (Luna, Sitz, Cranshaw, & Tisserat, 2013). The black walnut is generally considered hardy to USDA Cold Hardiness Zone 4 (-35 °C) (Dirr, 1977). This discrepancy in temperature range may be a potential management technique that can be employed by growers, especially if more frost tolerant trees can be incorporated into existing programs. Additionally, the monitoring of weather data from these locations could potentially explain the variability of the spread of TCD in areas that exceed the black walnuts preferred range.

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CHAPTER 4

CONCLUSION

The vector-pathogen complex, TCD, has the potential to affect *Juglans* species worldwide and has already negatively affected several regions within the USA causing widespread mortality of the trees. Concern for sensitive ecosystems, timber and nut industries, native, naturalized and urban forest health, and landscape aesthetics warrants the utilization of tools that will mitigate disease pressure for *Juglans*. Breeding programs that incorporate naturally resistant trees will be an intricate part of the long-lasting solution for control.

The focus of this project was to determine if natural genetic resistance existed in a relatively small subset (<10% of the reported ~4,000 *Juglans* trees located across the collection) of genetically variable trees within a set geographic location (Richmond, UT and Dayton, ID). A common limitation to screening for resistance is the range of genetic material. The collection at the Cyril Reed Funk Research Farms contains specimens from all over the world and includes hybrids and genotypes that will likely not be encountered in a single location. This collection is invaluable to the continuation of the efforts to discover genetic resistance to TCD. In addition, the genetic diversity of the collection exhibits a wide range of physical characteristic that, when coupled with the potential for resistance to TCD, provide valuable solutions for improvement of the species. Many of these traits, including canopy form, branch angles, and bark texture, were recorded in conjunction with resistant phenotypes.

Rates of resistance to TCD were variable each year with the overall rate of resistance at 0.003% (based on an average of 336 trees inoculated each year and one consistently resistant tree) and 0.04% when trees that exhibited partial resistance for two years were included (15 trees total from the average of 336). These trees exhibited an overall average increase in DBH (diameter of tree at breast height) of 5.42% in a growing season and have round canopies with wide branch angles all of which are favorable traits in a potential breeding stock candidate.

Resistance was surprisingly equally distributed throughout the two most common phenotypes; black walnut and Persian walnut. Susceptibility of black walnut to TCD is well known and documented, while Persian type trees are generally thought to be less susceptible. These results indicate that Persian types may not be any less susceptible but may be more tolerant of the disease. The reduction of canker size in Persian type walnut trees over the course of the project indicate that the potential for tolerance within this *Juglans* species may be worth investigating and be a trait to consider for breeding programs.

Tolerance of weather conditions within the group indicated that the tree is adaptable to a range of weather extremes and, especially where winter temperatures approach the range in which WTB are detrimentally affected, this information can be used to aid growers, breeder and nurserymen. Differential thermal analysis has been an effective tool in determining cold resistance in many valuable crops but did not produce the expected results in black walnut. With some adjustment, it may prove equally as effective for black walnut, but additional testing is needed with a more effective protocol.

Continued evaluation of these trees will aid in determining the resistance type of the trees, specifically if resistance is complete (qualitative) or incomplete (quantitative). The direction of future related projects should include the monitoring of trees, both within the collection and in native populations, for resistance to the causal agent of TCD as well as resistance to its vector, the WTB. Additionally, only a small percentage of the total collection at the Cyril Reed Funk Research Farm was inoculated, leaving thousands of black walnut, hybrid, and other *Juglans* species untested that may potential contain resistance to TCD.

Continued testing of the identified resistant trees would aid in the determination of durability of resistance, type of resistance and could be easily and economically achieved through vegetative propagation (budding, grafting). While the testing of resistant trees under similar environmental conditions is an important aspect of continuing the evaluation of resistance for these trees, a variety of environmental conditions could be beneficial and increase our understanding of this response.

Because of the long perennial nature of the tree, genetic analysis of resistant type trees can aid in identifying the gene(s) responsible for resistance and ensure the trait is passed to progeny in a shorter period of time. However, the breeding of trees for resistance will need to undergo extensive testing in long-term trials to ensure the efficacy and “durable resistance” (prolonged effective resistance across a wide susceptible environment) of the developed tree(s) while maintaining genetic diversity of the species (Sniezko & Koch, 2017). As a long-term endeavor, other management practice will need to be investigated for short-term control, including the use of pest control measures.

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