FY13 New TAES Workplan Report

Disease resistance screening and breeding studies in *Juglans* L. species

UT Tree Improvement Program

East Tennessee Research and Education Center

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Background: Exotic forests pests have been a serious problem for North American forests for at least 160 years (cf. Campbell and Schlarbaum 1994, 2002). Bottomland populations of the American chestnut [*Castanea dentata* Marsh. (Borkh.)] first fell victim to an exotic root rot disease caused by *Phytophthora cinnamomi* Rands in the mid-1800s, followed by upland populations succumbing to the more well-known exotic chestnut blight [causal agent = *Cryphonectria parasitica* (Murrill) Barr]. The destruction of eastern and western white pine species (*Pinus* L. subgenus *Strobus* Lemmon) by white pine blister rust (causal agent = *Cronartium ribicola* A. Dietr.) prompted the federal government to pass the 1912 Plant Quarantine Act to stop the influx of exotic pathogens.

Exotic forest pests do not necessarily need to come from foreign shores. Pests native to different parts of North America, e.g., western or eastern forests, can be transported and introduced with devastating impacts to native vegetation. Pitch canker disease (causal agent = *Gibberella circinata* Nirenberg & O'Donnell) has been introduced to western forests from the East. More recently, Thousand Cankers Disease (TCD) caused by *Geosmithia morbida* sp. nov. *(*Kolarik et al. 2011) was discovered in eastern forests, first in Tennessee and subsequently in Virginia and Pennsylvania (Seybold et al. 2011).

In the century since the passing of that act, exotic forest pests have been accumulating in North American forests with alarming impact (Campbell and Schlarbaum, 2012). Virtually every major tree species or genera in eastern forests have exotic pest problems, with the exception of hickories (*Carya* L.) and yellow-poplar (*Liriodendron tulipifera* L.). Among the impacted species, are two of the most valuable timber species in the walnut genus, black walnut (*Juglans nigra* L.) and butternut (*J. cinerea* L.).]

North American *Juglans* species: Taxonomically, the species of the *Juglans* genus are divided into four sections (Manning 1978): *Rhysocaryon (*black walnuts, limited to the Americas), *Juglans* (Persian walnut), *Cardiocaryon* (Asian butternuts) and *Trachycaryon* (butternut). Other studies resolve only three sections, with butternut grouping with the Asian butternuts (Fjellstrom and Parfitt 1995). In North America, there are a number of *Rhysocaryon* section species with the majority occurring in mid-western and western states, e.g., *J. major* (Torr.) A. Heller (Arizona black walnut) and *J. microcarpa* Berland (Texas or little walnut). These species are generally small and aside from ecological importance, have no commercial value. Eastern forests host only two species: black walnut and butternut or white walnut. Both species are commercially valuable for timber and nut production. Black walnut is the most valuable tree species in eastern forests for furniture production.

Exotic diseases of eastern walnut species:

Thousand cankers disease This disease is native to southwestern North America and was only recently described (Kolarik et al. 2011). The disease was noticed when black walnuts planted in western states began to die. Prior to 2010, populations of black walnut within the natural range were not been affected by any serious exotic forest pest. Unfortunately, Knoxville, TN was the first location with confirmed TCD in the east. The symbiotic walnut twig beetle and pathogen have been confirmed in six eastern Tennessee counties. The twig beetle introduces the pathogen when feeding, usually on branches 2" or greater in diameter. Resulting

cankers eventually debilitate and girdle the infected tree causing mortality. Additional eastern TCD outbreaks were confirmed in Virginia and Pennsylvania during 2011, prompting establishment of quarantine zones around each location.

Dr. Ned Tisserat (personal communication) initiated TCD resistance tests using open-pollinated black walnut and butternut (*sensu lato*) families. He found different levels of resistance to the disease among the families, indicating that resistance has a genetic nature. This research indicates that seedlings and grafts of timber and nut selections, as well as promising wild trees, can be evaluated for resistance and for integrating into a breeding program.

Butternut canker disease (BCD) Butternut populations have had a serious decline over the last 90 years due to butternut canker disease (Nair et al. 1979) caused by the exotic fungus *Ophiognomonia clavigignenti-juglandacearum* Broders & Boland (formerly *Sirococcus clavigignenti-juglandacearum* Nair, Kostichka. & Kuntz). Infected trees develop black lesions, or cankers, that kill the tree by girdling the trunk. Trees of all ages and on all sites can be infected. Butternut canker has reportedly eliminated butternut from the Carolinas (Rink 1990), killed 77% of the butternut populations in other southern states (Schlarbaum et al. 1997), and has begun to dramatically affect populations in northern states and in Ontario, Quebec and New Brunswick (Nielsen et al. 2003). Severe losses, from 25% to 89% of the total population, have occurred in seven Midwestern states (Ostry and Pijut 2000) in the United States, where regional survival of the species is in danger (Fleguel 1996). In Mammoth Cave National Park, butternut populations have fallen from 1 in 10 trees in riparian areas to less than 100 specimens in the entire park (Thompson et al. 2006). Regeneration is rarely seen in heavily infected areas, as the disease kills butternut seedlings (Fleguel 1996).

The U.S. Fish and Wildlife Service currently lists butternut as a federal species of concern. The species is also listed as endangered on a number of state lists, as a 'sensitive species' in many National Forests, and as federally endangered species in Canada (Nielsen et al. 2003). Butternut is close to local extinction in parts of its native range, particularly in disjunct populations. Recent surveys of northern Mississippi and western Tennessee have discovered only two healthy trees (UT-TIP data on file). Some healthy butternut trees have persisted in the presence of highly diseased trees, suggesting a level of natural tolerance (Ostry and Pijut 2000; Schlarbaum et al. 2004). These suspicions have been confirmed in inoculation tests, showing that there are varying levels of resistance within butternut (Anagnostakis, personal communication).

***Melanconis* canker disease (MCD)** Butternuts also have been impacted by a native pathogen, *Melanconis juglandis* (Ellis & Everh.) A.H. Graves, which causes a canker disease. This pathogen was first thought to have been responsible for the dramatic decline in butternut populations in the 20th century. Identification of *O. clavigignenti-juglandacearum* as the causal agent for butternut canker disease shifted the blame away from *Melanconis*. Nevertheless, *Melanconis* is capable of killing juvenile and adult trees (Schlarbaum and Anagnostakis, personal observation), although regarded by some scientists as a secondary disease of butternut. As with butternut canker disease, inoculation tests have shown that there is genetic variation within butternut to *Melanconis* canker disease.

Thousand cankers disease (TCD) This disease was recently confirmed in the Knoxville, Tennessee area. Based on observations of TCD development in Colorado and other western states, it is very unlikely that any type of quarantine or mitigation strategy will now be effective. Therefore, the highest priority is to identify potential sources of resistance to the disease, preserve the most important sources of germplasm for timber and nut production, and evaluate the susceptibility of these sources, in the event TCD proves to be catastrophic to black walnut in its native range.

Objectives

1. Characterize genetic resistance in black walnut and butternut to Thousand Cankers Disease through inoculations of grafted clones and open-pollinated progenies.

2. Characterized genetic resistance in grafted clones and open-pollinated butternuts to butternut canker disease and *Melanconis* canker disease.

3. Develop seed orchards of disease resistant black walnuts and butternuts to generate seed for reforestation.

4. Develop breeding populations of disease resistant black walnut and butternut to study the genetic nature of resistance and to increase resistance.

Approach: Resolving exotic forest pest problems requires an integrated approach best achieved by assembling the resources required to conduct a scientific investigation(s) and a group of cooperators with different skills. The UT-TIP uses an integrated approach in virtually every project and has a strong cadre of internal and external scientists, who specialize in different research areas (see Research Team below). Technology transfer of results is ensured through existing MOUs with the Tennessee Division of Forestry, TWRA and the USDA Forest Service (pending).

Experimental Material: All federal and state quarantine regulations regarding movement of host species and pathogens will be adhered to in this project.

Host species: This project will draw upon existing black walnut and butternut genetic resources within Tennessee held by the UT-TIP (TAES Workplan #132), Tennessee Division of Forestry, and TVA and those living resources at the University of Missouri for scion wood and open-pollinated progenies. Grafting will take place, in part, at the University of Missouri, with the grafts transported to Tennessee. The primary and on understock planted at the East Tennessee REC. All grafts will be made on black walnut understock. In FY12, the raised nursery beds at the Plant Sciences Farm (TAES Workplan #136) were planted with black walnut understock from four genetic families for bench grafting. Additional seedlings for field grafting were planted by UT-TIP personnel proximal to the upper yellow-poplar orchard (TAES Workplan #129).

Grafts or seedlings from 20-30 different trees from across the native range of naturally occurring black walnut, Arizona black walnut , Hinds' walnut (*J. hindsii* (Jeps.) Jeps. ex R.E. Sm.), and little walnut will be screened primarily to verify the preliminary finding of a high level of resistance in Arizona and Hinds' walnuts, further investigate resistance in little walnut, and estimate the variability in response according to provenance. Another reason to screen a range-wide sample of each species, as well as the walnut cultivars, is to choose a set of controls representing the most susceptible and most resistant individuals. These controls can also be used for screening new isolates of the fungus, to detect differential responses and establish methods to identify races or biotypes

Pathogens: Cultures of *Geosmithia*, *Ophiognomonia*, and *Melanconis* will be (have been) developed by Drs. Gary Griffin (Virginia Tech) and Sandra Anagnostakis (Connecticut Experiment Station) and will be transferred to UT-TIP under appropriate state and federal regulations. Both Drs. Griffin and Anagnostakis have federal permits that allow interstate transfer of cultures.

Determination of butternut parentage: Butternut naturally introgresses with heartnut (*J. ailantifolia* var. *cordiformis*), a nut cultivar of Japanese walnut (*J. ailantifolia*). Heartnut were first introduced into the United States in the 1870s (Neilson 1930). Surveys in Connecticut and southern states have found many planted on private property and in naturalized areas. Investigators have repeatedly detected heartnut hybridization with butternut for over half a century (Reed 1936; McDaniel 1956; Jaynes 1969)**.** Hoban et al. (2007, 2009, 2010, 2011, 2012) and McCleary et al.(2008) developed molecular and chloroplast markers to detect F_1 and advanced generation hybrids in naturally occurring populations, working in Dr. Jeanne Romero-Severson's laboratory at the University of Notre Dame. Only grafts pure butternut will be used for screening. The UT-TIP has been

surveying Tennessee populations for surviving butternuts (*sensu lato*) and many trees have been tested for parentage. Dr. Romero-Severson (see below) has agreed to cooperate in various aspects of this project, including pedigree analysis of new or untested butternut trees intended for grafting.

Screening for disease resistance: Dr. Gary Griffin (see below) will provide on-site oversight of inoculation of experimental materials in consultation with Dr. Sandra Anagnostakis (see below). They will both advise on all aspects of evaluation.

Statistical Design: The statistical design will be determined by the number and type of successful grafts. At that time, Dr. Arnold Saxton (see below) will be requested to develop a design in consultation with project collaborators.

Technology Transfer within Tennessee: Resistant trees will be integrated into the UT-TIP and Tennessee Division of Forestry's Tree Improvement Program for further development and if warranted, construction of seed orchards to supply seed for the East Tennessee State Nursery. Transfer will be coordinated with Mr. Russell Cox and Ms. Diane Warwick, Tennessee Division of Forestry (see below). Seedlings produced by the State Nursery will be available for cooperators such as TWRA and the USDA Forest Service and Tennessee landowners in general.

Research Team: The cooperators listed below, in various combinations, have cooperated together on a number of projects on hardwood species for many years. Below is a brief summary of their expertise.

Internal

- Dr. Scott E. Schlarbaum has extensive experience in forest tree improvement, exotic forest pests, and also has extensive plant growth and artificial regeneration experience. He is responsible for the University of Tennessee's Tree Improvement Program (UT-TIP), a 53 year-old research program that has been linked with the Tennessee Division of Forestry since 1966 under a series of Memorandums of Understanding as well as a MOU with the Tennessee Wildlife Resources Agency since 2011 (http://treeimprovement.utk.edu/). He has worked closely with the USDA Forest Service, Southern Region since 1985 and with numerous state and federal agencies. He is the curator of all Norris, TN-based Tennessee Valley Authority forest tree improvement records. Under his leadership, the UT-TIP has coordinated a multi-institutional, multi-disciplinary effort to address butternut canker disease for the last two decades. Dr Schlarbaum is a co-PI on the NSF-funded Hardwood Genomics project (http://www.hardwoodgenomics.org/), as are Drs. Coggeshall and Romero-Severson, external cooperators listed below.

- Dr. Arnold M. Saxton has been an integral part of the UT-TIP since coming to the University of Tennessee. Dr. Saxton is one of the leading statisticians in the world and provides oversight for all UT-TIP experimental designs and statistical analysis.

External

- Dr. Sandra Anagnostakis is a senior scientist at the Connecticut Experiment Station. Dr. Anagnostakis is a plant pathologist and mycologist and has worked with forest tree diseases for many decades, most notably chestnut blight, Dutch elm disease, and butternut/*Melanconis* canker diseases. She has worked with Dr. Schlarbaum on chestnut blight and butternut canker research for many years.

- Dr. Mark V. Coggeshall is located at the University of Missouri and has worked on the genetic improvement of black walnut for both timber and nuts for 32 years. He is the foremost authority in the United States on black walnut improvement and silviculture, as well as nut cultivar germplasm. He is the curator of the largest collection of black walnut nut cultivars in the United States, and is currently serving as a member of the TCD Response Plan Task Force.

- Mr. Russell A. Cox is the senior forestry program specialist in tree improvement with the Tennessee Division of Forestry. He is primarily responsible for oversight, progeny testing, and contact with the North Carolina State Pine Improvement Cooperative for the TDF program. Mr. Cox has worked with UT-TIP geneticists, Drs. Thor and Schlarbaum, for over three decades.

- Dr. Thomas J. Hall is a Pennsylvania Bureau of Forestry's field pathologist and has worked with tree diseases for his entire career. He is responsible for monitoring walnut diseases in Pennsylvania. Dr. Hall has worked closely with Drs. Schlarbaum and Coggeshall on butternut and oak restoration and TCD research for many years.

- Dr. Gary Griffin is Professor *emeritus* in plant pathology at Virginia Polytechnic Institute and State University (Virginia Tech). Dr. Griffin has studied the impact of many forest tree pathogens, notably *Cryphonectria parasitica* (chestnut blight) and was asked by the State of Virginia to study the outbreak of TCD in Tennessee and Virginia. He has worked closely with Drs. Schlarbaum and Coggeshall on developing and implementing a strategy to screen TCD for disease resistance and worked with Dr. Schlarbaum on American chestnut restoration.

- Dr. Jeanne Romero-Severson is a molecular geneticist, who has worked closely with Drs. Schlarbaum and Coggeshall on developing molecular mapping populations of northern red oak and black walnut. In cooperation with Dr. Schlarbaum, she has conducted the definitive molecular biology research on butternut by developing molecular markers to determine advanced generation hybridization events with heartnut.

- Ms. Diane Warwick is a forestry program specialist in tree improvement with the Tennessee Division of Forestry who provides oversight for TDF seed orchard establishment, maintenance, and seed collection and processing. Ms. Warwick has worked closely with UT-TIP since her employment by TDF in 1997.

Research responsibilities: UT-TIP personnel and cooperators will be responsible for all research aspects of this project. UT-TIP personnel will be responsible for site preparation of areas to eventually house experimental materials for screening, breeding orchards, and clone banks (see next section). UT-TIP personnel will request assistance from the Tennessee Division of Forestry in removal of large and/or problem trees from experimental plots or immediately adjacent areas. UT-TIP will be responsible for all plant maintenance excluding mowing. UT-TIP will be responsible for procurement of all experimental material for screening and for eventual grafting of selections to form breeding orchards of butternut and black walnut.

Requested resources from East TN REC: This is a long-term project that will gradually expand to encompass land now assigned to the Yellow-poplar Breeding Orchards at the Plant Sciences Farm as declining yellow-poplar grafts are re-grafted and consolidated (TAES Workplan #129) and land on the peninsula adjacent to the lower Yellow-poplar Orchard. Eventually, this area will become breeding orchards for butternut and black walnut resistant selections. Additional land resources suitable for walnut growing and adjacent to the butternut clone bank (TAES Workplan #132) are also requested. Periodic mowing by REC personnel, as needed, is requested for in-between the rows of experimental materials and open areas prior to planting.

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