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I am submitting herewith a dissertation written by Sunshine Liberty Brosi entitled "Steps Toward Butternut (*Juglans cinerea* L.) Restoration." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Natural Resources.

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Steps Toward
Butternut (*Juglans cinerea* L.) Restoration

A Dissertation Presented for
The Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Sunshine Liberty Brosi

August 2010

DEDICATION

I dedicate my dissertation to Summer Ela Shaper, my daughter, my heart, and my inspiration to preserve species for the future. Summer, this dissertation was written for you and includes so much of you. You stood in the rain with your umbrella, avoided the copperheads, drew pictures while I collected data, and read to me as I typed. This journey has been your entire life and was only possible with you on my back, in the truck, walking along side in the woods, and always on my mind. May you live life knowing that you are as strong as the mightiest trees in the forest.

ACKNOWLEDGMENTS

E. Lucy Braun was on my mind throughout this journey, imagining her in the same woods with her model T many decades before, inspired me tremendously. My sincerest gratitude goes to Dr. Scott E. Schlarbaum, for serving as my major professor and mentor for many years. This dissertation would not have been possible without his support, guidance, friendship, patience and kindness. I thank him for seeing my potential and pushing me to meet goals. I also thank the other members of my committee, Dr. Frank van Manen, Dr. David Buckley, and Dr. Nathan Sanders. I am greatly indebted to Dr. Arnold Saxton for his support with statistical analysis. The University of Tennessee Tree Improvement Program (UT-TIP) personnel have been invaluable in their assistance, especially support from David Griffin. I would like to thank the late Gene Johnston for giving perspective, purpose, and being an invaluable mentor in my life; his advice will stay with me forever. Thanks to Allison Mains, Tracy Binder, Allison Houser, Shinara Taylor, Laura Beth Jones, Laura Thompson, and Wes Tilley. I express my appreciation to my fellow past and present graduate students in Annex B for friendship and support, especially Dr. Stacy Clark, Belinda Esham, and Ami Sharp.

I would like to thank Dr. Sandra Anagnostakis and Dr. Jeanne Romero-Severson for advice and support. My appreciation is given to several current and retired employees of the USDA Forest Service. Edwin Manchester has been a teacher, an inspiration and a friend. Dr. Robert L. Anderson taught me a great deal about butternut and answered many questions along the way. I would also like to thank the following for their valuable assistance with technical advice and field work: Paula Spaine, Carol Young, John Knighten, Dr. Paul Kormanik, Tim Dalton, Ted Oprean, Dave Casey, Jessica Beir, Jeff Lewis, John Brooks, Bill Jones, Robyn Taylor, and Clay Logan. Thanks to Tom Tibbs for inadvertently introducing me to the Tree Improvement Program. I would also like to thank the land managers at planting sites including John Perry, Scott Friedhoff, George Bullard, Bobby McCormick, Stephen Beason, Jonathan Wild, Ron Myers, David Cozzo, John Brittan, Brice Leech, and Mark DePoy.

Funds for this project have been secured from grants through the USDA Forest Service State and Private Forestry, The National Park Service, and The Revitalization of

Traditional Cherokee Artisan Resources subsidiary of the Cherokee Preservation Foundation. Cooperators for this project include: U.S. Geological Survey (Southern Appalachian Field Laboratory), USDA Forest Service (Southern Region, Forest Health Protection and Genetic Resources Program, Daniel Boone, Jefferson, Pisgah, Nantahala and Cherokee National Forests, Bent Creek Experiment Forest), National Park Service (Mammoth Cave National Park), CT Agricultural Experiment Station, The Nature Conservancy (Kentucky Chapter), Healing Stones Foundation, TN Division of Forestry, US Army Corps of Engineers (Nashville District), NC Division of Forest Resources, NC Department of Environmental and Natural Resources, CT Department of Environmental Protection, KY Department of Fish and Wildlife Resources, TN Department of Environment and Conservation and Notre Dame University.

Thanks to Dr. William C. Davis, my first forestry professor, and the 4-H Forestry Camp in Jabez, KY for inspiring my love of being in the woods. I would like to thank the support of my entire extended family, for field assistance, childcare, and advice. Thanks for being such a large part of my life's journey and helping me reach many of my goals. I would especially like to thank Brook Brosi, Dr. Berry Brosi, Blossom Brosi, Sky Marietta, Glade Brosi, Eagle Brosi and my parents George and Connie Brosi. My sincerest thanks go to Steven Shaper, for financial and emotional support along my college career and for help with fieldwork. Thanks to my friends, for babysitting, being tough, teaching, rousing, and studying with me for comprehensive exams, for proofreading drafts, and for my absence at so many social events. I thank Annie's Natural Foods for healthy options on those long research days.

Special thanks to people who have shared many aspects of this professional and personal journey including old and new chapters from my transition from forester to naturalist. This dissertation would not have been written without the persistent encouragement and support of my husband and fellow scholar, Daniel J. Feller. I look forward to many more years of research collaboration with him by my side. My greatest thanks are also given to my stepson, Caeman Feller, for letting me hog the computer and for cooking so many great lunches on snow days. I wish him success in school and beyond.

ABSTRACT

Butternut (*Juglans cinerea* L.), a lesser-known relative of black walnut (*Juglans nigra* L.), is a native tree species beneficial for wildlife, valuable for timber, and part of the great diversity of species in the eastern forests of North America. Populations of butternut are being devastated by butternut canker disease, caused by the fungus *Sirococcus clavigignenti-juglandacearum* (V.M.G. Nair, Kostichka, & Kuntz), which is thought to be introduced to North America. The disease causes multiple branch and stem cankers that eventually girdle trees. Lack of sprouting and shade intolerance exacerbates the disease and results in permanent losses of butternut across the native range. Fortunately, healthy, canker-free butternut trees have been found proximal to diseased trees, indicating that a breeding approach could be a feasible strategy for producing and reintroducing resistant butternuts. A successful restoration program will require an understanding of genetic variation in open-pollinated seedlings, disease resistance, seedling establishment procedures, site requirements, a greater understanding of disease development over time and levels at various populations.

This dissertation is divided into six parts, with the overall goal of insight into butternut ecology and management techniques which could be used to guide restoration decisions for this important species. The first two parts are an introduction and a literature review. In the third section, butternut seedlings were propagated in nursery progeny plantings to determine the genetic and phenotypic variability among one-year-old seedlings in a controlled environment. Part four outlines the disease development of butternut seedlings across progeny in resistance screening plantings at various locations. Part five aims at aiding restoration techniques by determining the impact of phenotypic and genetic variables on establishment success across various planting sites. Part six describes the dynamics of large populations of healthy and diseased butternut trees including comparisons of tree conditions and health. The information gained from this research will be directly used in gene conservation strategies, the construction of disease resistant breeding orchards, determining appropriate restoration techniques, and prioritizing populations at greatest threats to losses.

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PART I. INTRODUCTION, GOALS, AND OBJECTIVES

Introduction

The introduction and establishment of invasive exotic (nonnative, non-indigenous, or alien) organisms, including plants, pathogens, insects, and nematodes, has resulted in dramatic changes in North American forests (Ayres and Lombardero 2000, Campbell and Schlarbaum 2002). Exotic species displace and disturb native species and exotic pests decimate native hosts with the overall effect of degrading diversity, structure and function of entire forest ecosystems (Campbell and Schlarbaum 2002, Simberloff *et al.* 2005, Clavero and Garcia-Berthou 2005). Specifically in United States forests, more than twenty exotic pathogens attack woody plants resulting in dramatic impacts on diversity and ecosystem function (Liebold *et al.* 1995). The economic loss of forest products attributed to introduced forest pests in the United States is estimated to exceed $2.1 billion per year (Pimentel *et al.* 2000). However, the environmental and social impacts of exotic pathogens over time are incalculable and ultimate ramifications are unknown.

One of the greatest ecological disasters to eastern North American forests was caused by an introduced pathogen, *Cryphonectria parasitica* (Murrill) M.E. Barr, causing chestnut blight disease on American chestnut, *Castanea dentata* (Marsh.) Borkh. The disease reduced a once dominant forest tree to an occasional sprouting remnant (Paillet 1982). Chestnut blight is one of several exotic diseases that are modifying eastern North American forest ecosystems. In addition, sudden oak death is a new disease impacting a number of woody species in California resulting from an introduced species of *Phytophthora* (*P. ramorum* S. Werres, A.W.A.M. de Cock) (Rizzo *et al.* 2002). This disease has the potential to cause major changes to multiple trees and shrubs in eastern forests where it has been recently introduced (Cohen *et al.* 2003, Stokstad 2004).

For many species affected by exotic organisms, research is needed on species abundance, population biology, habitat requirements, habitat availability, soil-plant interactions, disease impacts and restoration biology. There is also a need to develop strategies for maintaining genetic diversity of extant tree population (Ayres and Lombardedo 2000). Exotic pests can spread rapidly and decades of research are often

needed to understand and successfully manage the introduced pest and to reintroduce a host species into an altered environment.

Single species restoration, however, may be possible for some tree species impacted by exotic diseases (Schlarbaum *et al.* 1997). For example, the restoration of American chestnut appears possible as multiple approaches have been developed to combat the disease. The American Chestnut Foundation's (TACF) breeding program has used a backcross breeding method has reached their final stage of producing resistant nursery stock (Burnham 1981, Hebard 2002, 2005). Other methods are also being investigated, including intercrossing of pure American trees (Griffin *et al.* 2005), inoculation with non-transgenic and transgenic hypervirulent strains (Anagnostakis 1982, 2001, MacDonald and Double 2005 Root *et al.* 2005), and transgenic resistance (Powell *et al.* 2005). In addition, research to understand the silvics of the species and reintroduction methods are being explored (Brosi 2001, Rhoades *et al.* 2003, Schlarbaum *et al.* 2006, McCament and McCarthy 2005, Clark *et al.* 2009), resulting in the probable successful reintroduction in the species within the coming decades.

Another probable introduced fungus, (*Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz)), causes butternut canker disease and is devastating populations of butternut, (*Juglans cinerea* L.), across eastern North America (Ostry 1996, Schlarbaum *et al.* 1997). Butternut, also called white walnut, is native to the eastern forests of North America and is a close relative of eastern black walnut (*Juglans nigra* L.). Butternut is a fast-growing, shade-intolerant, and relatively short-lived tree (Rink 1990, Ostry *et al.* 1994). Though a minor component of many ecosystems, butternut adds to landscape level diversity and is one of a few large nut bearing trees important to various wildlife species. The fungus causes multiple cankers that girdle and eventually kill trees (Ostry *et al.* 1994). Butternut canker disease infects all sizes and ages classes of trees on a variety of sites (Anderson and LaMadeleine 1978, Ostry *et al.* 1994). Loss of butternut populations range-wide has been attributed to butternut canker disease (Tisserat and Kuntz 1984, Cummings Carlson and Guthmiller 1993, Ostry *et al.* 1998, Nair 1999).

The first report of widespread dying of butternut was in 1967 in southwestern Wisconsin (Renlund 1971) and was attributed to *Melanconis juglandis* (Ell. et Ev.), a weak parasite that primarily causes branch dieback and rarely cankers on the main stem. In 1979, the responsible pathogen was identified as a new species of *Sirococcus*, *S. clavigignenti-juglandacearum* (Nair *et al.* 1979). The fungus is believed to be an introduced species due to the lack of genetic variation, highly aggressive qualities, and rapid spread (Furnier *et al.* 1999, Ostry *et al.* 2003). Variation in disease resistance among *Juglans* L. species, with Japanese walnut (*J. ailantifolia* Carr. synonyms: *J. cordiformis* Maxim., *J. sieboldiana* Maxim) appearing the most resistant. Heartnut, (*J. ailantifolia* var. *cordiformis* (Maxim.) Rehd.) is a nut cultivar of Japanese walnut that has been imported for over 130 years, indicating that the disease may have been introduced from Japan through the nursery trade (Orchard 1984).

The initial report on butternut canker disease in Wisconsin by Nair *et al.* (1979) has misled some to believe that the fungus was first introduced in northern populations. However, the disease was probably introduced in the southeastern portion of butternut's natural range in the 1920s (Anderson and LaMadeleine 1978), more specifically in southeastern Virginia (*pers. comm.*, R.L. Anderson, 2002), and spread west and north. In 1976, cankers on butternuts in Wisconsin were aged, and the disease was determined to be present since 1965 (Kuntz *et al.* 1979). In 1978, butternut canker was not reported in Vermont or New Hampshire (Anderson and La Madeleine 1978), but in 1994 was observed throughout the northeastern states (Ostry *et al.* 1994). Butternut canker disease is still spreading northward in Canada (Carter 2004, Harrison *et al.* 2004). The first report of butternut canker disease in Quebec was in 1990 and 1992 in Ontario. The disease was noted in New Brunswick in 1997, and was believed to have been present at least seven years (Harrison *et al.* 1998, Ostry *et al.* 2003). In November 2003, butternut was added to the federal endangered species list in Canada (COSEWIC 2005). In 2004, Harrison *et al.* (2004) reported that butternut canker had spread sufficiently to approach the northern limit of the native range of butternut.

Nonlethal infections are produced through inoculation of *S. clavigignenti-juglandacearum* on many *Juglandaceae* species that occur in sympatry with butternut including black walnut (*J. nigra* L.), bitternut hickory (*Carya cordiformis* (Wangenh.) K.Koch), pecan (*Carya illinoinensis* (Wangenh.) K. Koch) and shagbark hickory (*Carya ovata* (P.Mill.) K. Koch). *Sirococcus clavigignenti-juglandacearum* has also been recovered three- to five-months post inoculation in oaks and chestnuts, although the trees showed no signs of infection (Ostry and Moore 2007).

The disease attacks butternut trees at all ages and, unlike chestnut blight, the fungus attacks below-ground portions of the tree. Therefore, sprouts may not survive resulting in the eventual extirpation of populations (Ostry 1998). Poor vigor of individual trees is probably augmenting the disease, as butternut is being eliminated from ecosystems as a result of natural forest succession and limited harvesting in riparian areas (Ostry and Pijut 2000, Schultz 2003). There are many unknowns about the current status of butternut populations due to limited study prior to, and after, infection and because specific butternut information has not been collected at most locations by USDA Forest Service Forest Inventory and Analysis.

Butternut populations were thought to be particularly threatened in the southern portions of the range where there are historically fewer trees and higher infection rates. In 1996, Ostry concluded from reports of infection that "viable populations of butternut are probably no longer present in the southern portions of its range." However, in some areas of the south, butternut has been commonly found (personal observation). Additionally, genetic analysis of populations throughout the species range (Hoban *et al.* 2010) found reduced genetic diversity in the northern section of the range with lower disease pressure as a product of range shifts.

Over three decades ago, Anderson and LaMadeleine (1978) stressed the need for a response to butternut canker disease. Hope for restoration of the species lies in speculation that genetic resistance to the disease may exist, as healthy trees have been found in close proximity to dead or dying trees (Ostry *et al.* 1994). If resistance is genetically-based, a breeding approach could be a feasible strategy to produce trees that

are resistant to butternut canker disease (Ostry *et al.* 1994, Schlarbaum *et al.* 1997, 2006). However, the relationship between punitively resistant trees in the field and actual genetic-based resistance has yet to be confirmed (Millikan *et al.* 1990, Ostry *et al.* 1994, Davis and Meyer 1997, McIlwrick *et al.* 2000, Ostry and Moore 2007). Anagnostakis (*pers. comm.*) has detected differences in specific individual butternuts to inoculations with *S. clavigignenti-juglandacearum*, similar to differential response among chestnut seedlings inoculated with chestnut blight fungus (Anagnostakis 1992). However, additional tests are needed to determine if these results indicate resistance in the field as differences among chestnuts were not consistent in field conditions.

Surviving butternut trees could have escaped from the disease as a result of natural resistance within butternut, tree vigor and/or site conditions. Survival may also be the result of hybridization with the closely related heartnut cultivars. Introgression of exotic genes due to the lack of reproductive barriers may have occurred as early as 1870, when heartnut was readily available in the nursery trade (Neilson 1930). Indication of Japanese heartnut origin can be determined using nuclear microsatellites and chloroplast markers (Hoban *et al.* 2009). Hybridization alone, however, may not result in disease resistance as Japanese walnuts may have various levels of disease resistance (Orchard 1984) similar to the variation in resistance to chestnut blight that has been found in American and Chinese (*C. mollissima* Blume) chestnuts (Anagnostakis 1992).

Restoration of butternut will require more than disease resistance alone. A restoration program for the species should incorporate an understanding of the ecology and silvics of butternut and factors involved in seedling establishment and subsequent growth. Butternut is found in deep, moist, loamy areas and is most commonly associated with cove hardwood and riparian sites (Clark 1958, Rink 1990). In southeastern North America, butternut has mainly been found on floodplains in valleys with streams or rivers (van Manen *et al.* 2002, Thompson *et al.* 2006). Butternut relies on periodic disturbances to promote regeneration and maintain dominance in a stand, and young trees can tolerate shade from the side, but do not survive when shaded from above (Rink 1990). Therefore, butternut is often found in disturbed areas along streams, roads, and the borders of

agricultural fields (Schultz 2003). Artificial regeneration of butternut through seeds and seedlings can be adversely affected by shading from competing hardwood spouts and heavy shrubs (Ostry *et al.* 2003). Additional challenges, in more recent years, will significantly increase the difficulty of planting butternut seedlings. Fast-growing invasive exotic species, e.g., Japanese honeysuckle (*Lonicera japonica* Thunb.), oriental bittersweet (*Celastrus orbiculatus* Thunb.), Japanese stilt grass (*Microstegium vimineum* (Trin.) A. Camus), and tree-of-heaven (*Ailanthus altissima* (Mill.) Swingle) are aggressive competitors in light-saturated environments. Increasing white-tailed deer (*Odocoileus virginianus* (Boddaert)) populations are impacting seedling establishment and plant species composition in many eastern forests (Rossell *et al.* 2005, Augustine and McNaughton 1998, Stromayer and Warren 1997). Hardwood seedling establishment has been negatively impacted by these factors, resulting in planting failures on multiple locations (Gottschalk and Marquis 1983, Gordon *et al*. 1995, Buckley *et al*. 1998, Oswalt *et al.* 2004). Success of planted seedlings could be aided by using high quality and larger seedlings that will compete vigorously with surrounding competition and rapidly grow above deer browse level.

Seedling establishment success has proven to be increase with the quality of the nursery stock for northern red oak (*Quercus rubra* L.) (Zaczek *et al.* 1997, Dey and Parker 1997, Ward *et al.* 2000, Jacobs *et al.* 2004). Over the past decade, nursery practices have adjusted density of plantings and fertilization and irrigation applications in order to produce high quality hardwood species (Kormanik *et al.* 1994a, 1994b). Out-planting large material shifts the competitive advantage toward the seedlings and reduces losses due to herbivory (Ward *et al.* 2000, Oswalt *et al.* 2006). Large nursery stock can quickly become established in the field and can be produced by adjusting nursery practices (Kormanik *et al.*1994a). Selecting high quality seedlings has increased establishment success for a variety of hardwood species including oak species *Quercus alba, Q. falcata, Q. michauxii, Q. pagoda,* and *Q. rubra* (Kormanik *et al.* 1994a, 1994b, Schlarbaum 1993). Ostry *et al.* (1994) observed that vigorously growing butternut saplings may have outgrown the girdling effects of the canker. Therefore, a strong

relationship may exist between seedling height and survival and the detrimental impact of the butternut canker disease. However, aspects of production and establishment of butternut seedlings have yet to be scientifically explored.

Objectives

The focus of this dissertation is multidimensional in nature, reflecting the need to understand butternut ecology, seedling biology and the silviculture of early plantation establishment and disease development. This research has been directed toward yielding information that will provide the foundations for a resistance breeding program and to define establishment protocols for butternut restoration efforts. Specific objectives are:

1. Evaluation of seedling characteristics and genetic and phenotypic variability among one-year-old seedlings in a commercial nursery environment;
2. Determine impacts of seedling characteristics and genetic variation in disease development on growth and survival of pedigreed butternut seedlings at various locations;
3. Determine the impact of seedling size and genetic variables on initial establishment success across various planting sites; and
4. Examine the dynamics of large populations of healthy and diseased butternut trees including disease development across phenotypic, temporal, and spatial scales.

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PART II. LITERATURE REVIEW

Butternut

Species description

Butternut, *Juglans cinerea* L., is a native tree species across much of eastern North America (Rink 1990, Little 1971). The tree is also called white walnut due to the light-colored wood and bark (Clark 1958) or oilnut, referring to the kernel of the nut from which oil can be extracted (Ashworth 1965). The botanical name '*cinerea*' refers to the ashy-gray bark color that discriminates the butternut from the darker-bark of the related species black walnut (*Juglans nigra* L.) (Clark 1958). Butternut trees average around 30m tall and 90cm in diameter (Rink 1990). Butternut differs from black walnut in reaching reproductive maturity at a younger age and having a shorter life span, ca. 70-90 years (Samuelson and Hogan 2003).

Butternut is easily distinguished by foresters and taxonomists from black walnut by four main phenotypic characteristics including 1) bark that is usually light-gray, closely furrowed with flat ridges, 2) oblong-sticky fruits, 3) twigs with thick pith chambers nearly black in color; and 4) leaf scars with hairy upper margins (Rink 1990, Harlow and Harrar 1996). Field observations, however, indicate that there are different bark color/furrow combinations within the butternut species (Ostry *et al.* 2003). Four different types are distinguished: 1) light gray and lightly furrowed; 2) light gray with deeper, more compressed furrows; 3) gray with light furrows; and 4) gray with deeper, more compressed furrows (Ostry *et al.* 2003).

Taxonomy

Butternut belongs to the Juglandaceae L. family, which has many species of economic and ecological significance. Other members of the Juglandaceae in forests of the eastern United States include hickories and pecans (*Carya* Nutt.). Butternut is a member of the walnut genus *Juglans* L. Twenty-one distinct known species of *Juglans* exists throughout the world growing in North, Central, and South America, the West Indies, southern Europe, and Asia (Manning 1978). The four generic sections of the walnuts are based on

morphological characteristics and include Cardiocaryon Dode, Dioscaryon Dode, Rhysocaryon Dode, and Trachycaryon Dode (Mann) (Manning 1978, Stanford *et al.* 2000). Section Trachycaryon only includes butternut (Manning 1978), however, nuclear RFLP (restriction fragment length polymorphism) placed the section Trachycaryon in a monophyletic group of the *Cardiocaryon* (Fjellstrom and Parfitt 1995, Stanford *et al.* 2000). *Cardiocaryon*, the Asian butternut clade, also includes species native to northeastern Asia: Japanese walnut (*J. ailantifolia* Carr; synonyms: *J. cordiformis* Maxim, *J. sieboldiana* Maxim) and Chinese or Manchurian walnut (*J. mandschurica* Maxim. synonyms: *J. cathayensis* Dode, *J. hopeiensis* Dode, *J. stenocarpa* Maxim.) (Manning 1978, USDA, ARS, National Genetic Resources Program 2001). Recent genetic research using microsatellite technology found butternut clustering nearer to Rhysocaryon than Cardiocaryon (Ross-Davis and Woeste 2008). However, this analysis is inconsistent with incompatibilities among species in each group.

Section Dioscaryon includes English (Carpathian) or Persian walnut (*J. regia* L.), a species native to southern Europe and western Asia and iron walnut, *J. sigillata* Dode, which is indigenous to China (Orel *et al.* 2003). Rhysocaryon section (black walnut clade) includes sixteen species and two subclades including the temperate and tropical walnuts of North, Central, and South America. Eleven species are native to South and Central America: *J. australis* Griseb, *J. brasiliensis* Dode, *J. mollis* Engelm, *J. neotropica* Diels, *J. olanchana* Standl. & L.O.Williams, *J. hirsuta* Manning, *J. peruviana* Dode, *J. steyermarki* Manning*i, J. jamaicensis* C.DC, *J. soratensis* Manning, *J. venezuelenis* Manning, *and J. major* (Torrey) Heller. Six species of *Juglans* are native to the Great Plains and western North America: *J. californica* S. Wats., *J. hindsii* (Jepson) R.E. Smith, *J. microcarpa* Belandier, and *J. major* (Torrey) Heller. The section is represented in eastern North America by only black walnut, which also extends into the Great Plains.

Section Rhysocaryon is thought to have diverged from the Dioscaryon (Eurasian butternut clade), approximately 50 million years ago based on DNA analysis techniques (Stanford *et al.* 2000). The lineage of *J .cinerea* is more closely related to the Eurasian butternuts, including, *J. regia* and *J. ailantifolia*, as the subgroup Dioscaryon is thought to

have originally evolved in North America, and then migrated along the Beringian land bridge 30 million years ago to Europe and Asia (Stanford *et al.* 2000).

Due to their economic importance, black walnut and Persian walnut are cultivated across a wide geographic range (Jaynes 1969, Woodruff 1979). Hinds' walnut, *J. hindsii* [(Jepson) R.E. Smith], and *Juglans x paradox* Burbank (hybrid of *J. hindsii x J. regia*) are of economic importance as rootstocks for nut cultivars of *J. regia* and producer of high-quality burl wood (USDA, ARS, National Genetic Resources Program 2001). Butternut and most Latin American walnut species are sparsely collected and rarely propagated (USDA, ARS, National Genetic Resources Program 2001).

Natural range and abundance

Butternut is native to eastern North America and is one of the most winter hardy *Juglans* species. The natural range extends north from southern Ontario and Québec to New Brunswick; west to southeast Minnesota; and south to western South Carolina and Georgia, including northern Mississippi and Arkansas (Graves 1913, Clark 1965, Brown 1975, Rink 1990, Majcen 1995 *viz*. Ostry *et al.* 2003, Little 1971, Figure II-1). The species is found farther north and not as far south or west as black walnut (Rink 1990). The current range and abundance of butternut may not reflect the historical range due to changes in land use and the impact of butternut canker disease, as discussed below.

Historical distribution and abundance of butternut

Research on relative pollen abundance after the Wisconsinan glaciations for North American forests has resulted in conflicting evidence of abundance and has often failed to distinguish black walnut and butternut at the species level (Finkelstein *et al.* 2006). Pollen records for abundance and distribution from black walnut cannot be applied to butternut because individual species of *Juglans* have been shown to follow different trends of increasing and decreasing abundance over time. For example, canopy openings 500 years ago in southern Ontario forests showed increases in black walnut pollen and decreases in butternut pollen percentages (Finkelstein *et al.* 2006). However, some studies have differentiated to the species level. Pollen records from southeastern Kentucky 7300-4800

years before present, account that butternut constituted 1-2 percent of the forest (Ison 2000). Butternut populations were highest during this time period, than from any other period from 9500 years ago to present, and butternut was more frequent than black walnut (Ison 2000). Pollen records for western North Carolina include butternut as abundant prior to the early 20th century (Delcourt and Delcourt 1997). Birks (2003) found butternut pollen in percentages equivalent to black walnut and most other non-dominant forest species in Minnesota. Butternut was found in the southern margins of boreal forest with low expansion rates with climatic warming (Birks 2003).

Limited and contradictory information also exists within the more recent historical documentations of forest composition. From the time of European settlement and expansion westward limited documentation is available on the abundance of butternut. Individual sources of historical data may be biased and therefore not accurate indications of historical occurrences (Whitney and DeCant 2001). However, a collection of multiple sources of data can indicate the presence of a species at a particular location, and give some insight into potential relative abundance compared with other similar species (Rhoades and Park 2001). Historical records indicate species abundance at a particular time and butternut abundance could be higher in certain time periods after heavy cutting, similar to other early-succession species, due to butternut's ability to colonize.

Clark (1958) indicates butternut was a sparse component of the forest and seldom found in pure stands. However, in specific locations, butternut can be very abundant (Harlow *et al.* 1978, Braun 1950, Cambell 1989, Ison 2000, Ostry and Pijut 2000, COSEWIC 2006). In the United States, Braun (1950) noted bottomland locations in western Kentucky where ten percent of the forest was butternut. In a review of early historical accounts and data of the forests of the Bluegrass Region of Kentucky from 1750 to 1919, Campbell (1989) found that butternut was mentioned with the same frequency as black walnut, *Juglans nigra* L. in fertile areas, and more frequency on less fertile areas, when the species were differentiated in the records. Short (1828) noted that around Lexington, KY butternut was even more abundant than black walnut (*viz*. Campbell 1989). An evaluation of witness trees from land deeds in Edmonson County, KY from 1824-1877 found butternuts listed less often

than black walnuts and a minor component of the forest; mentioned with equal frequency as sassafras and ironwood (McEwan *et al.* 2005). Bearing-tree data from land survey records from 1847-1850 in south-central Minnesota show butternut to be three times as common as black walnut and more common than black cherry (Grimm 1984). However, butternut made up less than one percent of the large variety of tree species mentioned (Grimm 1984). It has been suggested that southern states had historically less butternut than northern states; however, in the 1940s Tennessee was in top four states in the production of butternut wood (Betts 1945).

Ecological significance

Butternut is an important source of hard mast for wildlife, particularly in the northern sections of its range where black walnut is not present (Rink 1990). Protein content in butternut is 29.2 percent, 7.09 calories per gram and 64.1 percent lipids (Talalay *et al.* 1984). When comparing butternut to fourteen other abundant nut crops, butternut and black walnut have the highest amounts of calories per gram and percent protein (Abrams and Nowacki 2008). Values for protein are twice as high as hazelnuts (*Corylus americana* Walter) and hickories (*Carya glabra* (Mill.) Sweet*, Carya laciniosa* (Michx. f.) G. Don*, and C. ovata* (Mill.) K.Koch*, Carya alba* (L.) Nutt.) and five times the content found in American chestnut (Abrams and Nowacki 2008). Butternuts were only slightly lower in lipid content than hickories, pecan (*C. illinoinensis* (Wangenh.) K. Koch), and hazelnuts (Abrams and Nowacki 2008).

The high-quality of butternuts as a food source may be extremely important for overwintering for a number of animals, as whitebark pine (*Pinus albicaulis* Engelm.) seeds are for grizzly bears (*Ursus arctos* Ord). Eastern gray squirrels, (*Sciurus carolinensis* Gmelin) have been documented to eat butternut (Davison 1964). Northern flying squirrels, (*Glaucomys sabrinus* Shaw), nests were situated near particular mast producing tree species as a food source including butternut (Muul 1974). Butternuts have commonly been found in caches of the Allegheny woodrat (*Neotoma magister* Baird), a species which is extirpated, endangered, and threatened in the northern parts of its range (*pers. comm.* Daniel J. Feller, MD Department of Natural Resources).

The production of these nuts during particular mast year periods is thought to be a strategy evolved in response to scatterhoarding animals (Stapanian and Smith 1978). Production of nuts in varying annual abundance results in squirrels burying more than they can eat in high mast years resulting in seed stock. Successive low mast years help reduce populations of scatterhording animals, thereby lowering losses to herbivory.

Butternut is one of several host species to the luna moth (*Actias luna* L.), which has adapted specific detoxification enzymes that are tolerant of juglone (Lindroth 1989). Another species of insect that uses butternut as a host include the two-marked tree hopper, *Enchenopa binotata* Say (Guttman *et al.* 1981). *Eubulus parochus* (Herbst), a weevil whose host tree is the butternut, may be solely dependent on butternut (Halik 2006).

Anthropogenic uses

Butternut was historically used by Native Americans in the United States and Canada as a food source, for dye, for medicinal purposes, and as a building material (Krochmal and Krochmal 1982). In the southern Appalachians, the Cherokee tribe used the sweet oil from butternuts extensively according to William Bartram in 1789 (*teste* Williams 1928). Butternut was one of 46 traditional foods served at Cherokee traditional feasts (Ulmer and Beck 1951). The Cherokee also used butternut for dye accents for white oak baskets, as a cathartic, and for toothaches (Hamel and Chiltoskey 1975, Hill 1997). Northern tribes in the United States around the Great Lakes, including the Menominee, Meskwaki, Ojibwa, and Potawatomi Indians used butternut bark to make a brown dye, syrup, and as a cathartic and laxative (Gilmore 1933, Smith 1923, 1928, 1932, 1933). The Iroquois used butternut for multiple medical purposes and as a dye (Herrick 1977). Maritime Indians in eastern Canada used butternut as an herbal remedy for stomach ailments (Chandler *et al.* 1979). Historical accounts as a medicine are supported by laboratory studies where extracts of butternut bark produced antimicrobial activity against multiple bacteria and fungi (Omar *et al.* 2000).

The palatable nuts were considered by Burbank (1915) to be the best tasting of the walnuts, and cultivars have been selected for orchard production (Jaynes 1969). The sap of butternut has high sugar content and has been tapped to create syrup used in confections (Hough 1884, Clark 1958, Lauriault 1989, Harlow and Harrar 1996, Cirelli *et al.* 2008).

Butternut sap, however, could not be made into granulated sugar like sap from sugar maple (*Acer saccharum* Marsh.) (Hough 1884). Maple sugar candy, embedded with butternut kernels, was popular in New England (Rink 1990). Pioneers extracted a dye from the husks for clothing and during the Civil War, Confederate troops were called "butternuts" due to butternut dye used on their uniforms (Clark 1958). Butternut dye was used in colonial America to create various colors and one bushel of butternut bark was used to create an olive brown to black color on wool (Bronson and Bronson 1977:1817).

Butternut can be a valuable source of lumber (Watts 1941, Clark 1958). High-quality butternut wood commands a high market price and is used for veneer, furniture, cabinets, specialty products and carving (Peattie 1950). In the 1970s, veneer butternut lumber was second only to black walnut in economic value (Peterson 1977). Butternut is becoming increasingly valuable due to the increasing scarcity of the wood and increased demand for specialty products such as carvings (Ostry *et al.* 2003).

Regeneration of butternut

It is reported that butternut reaches reproductive maturity by twenty years of age, (Brinkman 1974, McIlwrick *et al.* 2000, Ostry and Pijut 2000), although seed production has been observed in trees less than 10 years old (personal observation). Butternut is monoecious and produces inconspicuous flowers between April and June (Clark 1958, Rink 1990). Most nut crop trees, including butternut, are dichogamous; having wind-pollinated pistils and stamens that mature at different times, thus promoting cross-pollination rather than self-pollination (McDaniel 1956). As a result, different varieties are needed for cross-pollination (McDaniel 1956). Unlike many forest trees, production of self-fertilized nuts is possible for most walnut species.

Butternut flowers from April to June, depending upon location. Male pollen is produced on slender catkins that develop from auxiliary buds and female flowers are short terminal spikes borne on current year's shoots. Flowers of both sexes do not usually mature simultaneously on any individual tree. Yellow-green catkins are single-stemmed, six to 14 inches long, and often produced in midsummer. Female green-yellow flowers appear on a short spike near the end of the twig in mid to late summer.

The nut develops in one year and is oblong with an indehiscent husk or hull covering, usually 5cm in length, occurring in clusters from 2 to 5 and maturing in September and October (Clark 1958). The husk usually remains on the tree until after leaf fall and is glandular pubescenct on the surface, lemon-shaped, and exhibits a citrus-like odor. The kernel or seed of the nut is sweet, oily, and edible. The species has mast years, with good crops occurring every two to three years (Ostry and Pijut 2000). The large nuts are dispersed by gravity, rodents and water (Rink 1990).

Though less common than reproduction by seed, butternut can reproduce asexually by sprouting from dormant buds on the root collar, an important reproductive and competitive strategy that allows seedlings the ability to withstand periodic disturbances. Butternut can be propagated asexually through grafting (Kaeiser and Funk 1971, Ostry and Pijut 2000, Pijut 2004), rooted cuttings (Pijut and Barker 1999, Pijut and Moore 2002) and in-vitro culture (Pijut 1997, Beardmore and Vong 1998).

Riparian site butternuts

Many reports describe butternut in deep, moist, loamy areas and most commonly associated with cove hardwood and riparian sites (Clark 1958, Smith 1952, Larsen 1942, Peattie 1950, Clark 1965, Schroeder 1972, and Strode 1977). Butternut is generally considered a floodplain species, found with cottonwood (*Populus deltoides* Bartr. ex Marsh.) and sycamore (*Platanus occidentalis* L) (Conard 1952). The species also can be associated with other mesic cove hardwood species including: black walnut, white ash (*Fraxinus americana* L.), American beech (*Fagus grandifolia* Ehrh.), American elm (*Ulmus americana* L.), basswood (*Tilia americana* L.), black cherry (*Prunus serotina* Ehrh.), common hackberry (*Celtis occidentalis* L.), red and white oaks (*Quercus* spp.), and sugar maple (*Acer saccharum* Marsh.) (Rink 1990, Schultz 2003).

Verry *et al.* (2000) report butternut as being associated with the second bottom of the outwash plain, which is normally a well-drained ridge with sugar maple, northern red oak (*Quercus rubra* L.), and swamp white oak (*Quercus bicolor* Willd.). Butternut is generally found on streambanks and well-drained gravelly soils (Krochmal and Krochmal 1982). Butternut grows on loam and sandy loam soils, but does not tolerate soils with over 30

percent moisture (Cogliastro *et al.* 1997). Butternut has been associated with wetter sites than black walnut (Krochmal and Krochmal 1982). Butternut plantings in southwest Quebec had the greatest mortality in plantations in which water availability was lowest (Cogliastro *et al.* 1993).

Butternut can be found on a pH range from 5.9 to 7.6 (Cogliastro *et al.* 1997). When compared to other hardwood species, butternut was the most responsive to nitrogen and had the highest level of nitrate reductase activity (Lambert *et al.* 1994). Butternut has also been shown to respond poorly to mulching and have the highest foliar concentration of nitrogen when compared with other hardwoods (Truax and Gagnon 1993). Associated species with high nutrient requirements include elm and sugar maple (Rink 1990). Samuelson and Hogan (2003) report black walnut grows on fertile soils with sugar maple, ash, hickory and persimmon and butternut on moist sites with red maple, a less nutrient demanding species. In depth evaluation of nutrient and site requirements are unknown.

Dry site butternuts

Reports of butternut on dry, rocky soils (Rink 1990, Harlow *et al.* 1996) indicate that butternut may be capable of growing on non-riparian sites. This claim has been repeated in the modern literature (Harlow *et al.* 1996), but is generally lacking in older, i.e., pre 1900, literature. One exception is in a review of early historical accounts and data from 1750 to 1919, Campbell (1989) found that butternut was mentioned more frequency on less fertile areas, then black walnut. Dry site butternuts may be an artifact of forest disturbance, or differences across the range of the species. A more recent description of butternut in southern areas of its range indicates that it is a riparian species found on moist slopes and coves (Samuelson and Hogan 2003). Butternuts can often be found at the base of talus or outcroppings in areas that superficially appear dry, but have high soil moisture.

Successional role of butternut

Butternut is fast growing, shade intolerant, and relatively short-lived with an average timber maturity of 75 years (Rink 1990). Butternut is considered early successional due to low plasticity of leaf absorbance and establishment dynamics in forest stands (Doyon *et al.*

1998). The species relies on periodic disturbances to promote regeneration and maintain dominance in a stand, and young trees can tolerate shade from the sides but do not survive when shaded from above (Rink 1990). Butternut is often found along streams, roads, and agricultural fields with higher levels of periodic disturbance (Schultz 2003). In the absence of disturbance, butternut trees are replaced over time with shade-tolerant species through autogenic successional processes (Ostry *et al.* 2003). In mesic forests, the order of canopy stratification by species is closely linked to the number and intensity of disturbance events (Oliver and Stephens 1977). Shade-intolerant species, such as butternut, are able to move further into the canopy with each new disturbance, while shade-tolerant species are restricted to lower canopy strata (Oliver and Stephens 1977, Foster 1988). Increasing forest age and lack of cutting, particularly in riparian areas, has been reported to reduce the ability of butternut to naturally regenerate (Ostry *et al.* 2003). Pollen records from Upper Michigan showed butternut reappearing after other species declines created canopy openings (Solomon and Bartlein 1992). Lack of regeneration of butternut is problematic for the species and has been noted throughout the southern United States (*pers. comm.* E. Manchester, R.L. Anderson; UT Tree Improvement Program, date on file).

Even age forests stands, resulting from management, may result in areas where there are no small light gaps as in old growth forests to allow for natural regeneration. Pollen records for western North Carolina include butternut along with hemlock, beech, and sugar maple prior to the early 20th century (Delcourt and Delcourt 1997). This association with climax species may indicate the importance of colonization of butternut in light gaps of mature forests. Butternut may be adapted to regenerating in late-successional forests in riparian areas prone to single tree disturbances. Planted butternut seedlings are very responsive to herbicide application of competing vegetation (Cogliastro *et al.* 1993, Lambert *et al.* 1994).

In riparian areas, flooding causes an increase in fertility by depositing organic and inorganic materials that contribute to productivity (Verry *et al.* 2000). Large-seeded species are often dispersed relatively close (10-30m) to the parent tree where the soil-site characteristics and conditions are likely to be similar to where the parent became established

(Barnes *et al.* 1998). The adaptation of butternut with large seeds may indicate the importance of site conditions for growth and survival and this may be a function of a requirement for high moisture or high nutrient content or both. The importance of dispersal to appropriate sites overrides the importance of other adaptations to allow them to compete with the plants and animals of these sites (Barnes *et al.* 1998).

Challenges to Butternut

Loss of species identity through hybridization

The species integrity of butternut may be at risk from hybridization with nonnative walnut species. Butternut readily hybridizes with Japanese walnut and a nut cultivar of Japanese walnut, heartnut (*J. ailanthifolia* var. *cordiformis* (Maxim.) Rehd.) to produce buartnut, *J.* x *bixbyi* Rehd. (Bixby 1919, Woodworth 1930, Clark 1958, McGranaham and Catlin 1987). Nut growers in the eastern United States have planted heartnut since the 1870s (Neilson 1930), and buartnut since at least 1919 (Bixby 1919). Heartnut hybridization with butternut has been document for over half a century (Reed 1936, McDaniel 1956, Jaynes 1969).

Butternut is difficult to distinguish and may often be confused with hybrids (Ross-Davis and Woeste 2008, Woeste *et al.* 2009). Some subtle differences are evident with direct comparison of butternut, Japanese walnut, and hybrids including pith color on young trees and leaf scar shape (Woeste *et al.* 2009). Phenotypic characteristics, including tree form, bark color, pith structure and color, and nut shape, are sometimes used as diagnostic features to differentiate *J. cinerea*, *J. ailantifolia*, and first generation hybrids (Ross-Davis *et al.* 2008). Some studies, however, have demonstrated considerable plasticity in these traits among *Juglans* species (Busov *et al.* 1997). In addition, some traits have overlapping characteristics; ie. nut clusters: 1-5 in butternut, 3-7 in hybrids (Woeste *et al.* 2009). Other traits are evident when the various species are present for comparison, which is not always possible in field taxonomy, i.e. pith color dark brown in butternut, dark brown, medium brown or light brown in hybrids (Woeste *et al.* 2009). In addition, nuts are present only

during certain years, and the positive identification of a parent tree does not indicate if the seedlings would be hybrids.

It has been speculated that much of the host resistance to butternut canker disease is from integration with heartnut, as discussed below (Michler *et al.* 2006, Woeste *et al.* 2009). Allozyme variation has been used to distinguish between butternut, heartnut and hybrids (Busov *et al.* 1997), but was unable to distinguish beyond the F_1 generation. Isoenzyme polymorphisms found distinct banding in butternut and bands similar to *Cardiocaryon* walnuts (Germain *et al.* 1993). Preliminary results using polymorphisms in the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA were reported by Michler *et al.* (2006) to detect hybrids.

In addition to determining the presence of private alleles, maternal parentage can be determined through evaluation of markers from the chloroplast genomes (cpDNA). In *Juglans*, cpDNA is maternally inherited providing the opportunity to detect hybrids, even in the event that complete homogenization of sequencing has occurred, of the maternal parent (Potter *et al.* 2002). With other Juglans species (*J. california, J. hindsii, J.nigra* and *J. microcarpa*), unique chloroplast genome sequencing markers have been found for each species. *J. cinerea* and *J. ailantifolia* individuals as well the hybrids formed between them may lie within the normal range of phenotypic variance for one or another of the parent species (Ashworth 1965, Ross-Davis *et al.* 2008). To detect genetic ancestry reliably, a test kit consisting of 12 butternut nuclear microsatellites was developed (Hoban *et al.* 2008) that amplify and have high information content in both species and a microsatellite marker originally developed for black walnut (Robichaud *et al.* 2006). Cleaved amplifiable polymorphic sequence (CAPS) markers have also been developed, each of which definitively distinguishes the *J. ailantifolia* from the *J. cinerea* chloroplast (McCleary *et al.* 2008).

Extensive hybridization between the *J. cinerea* and *J. ailantifolia* has recently been documented using chloroplast and nuclear DNA markers (Hoben *et al.* 2009). F_1 and F_2 and backcrosses with *J. cinerea* were extensive in all seven distinct populations evaluated (Hoben *et al.* 2009). Hybridization occurred at proportions from 0.05 to 0.92 and most maternal parents were *J. ailantifolia* (Hoben *et al.* 2009). Results from Hoben *et al.* (2009) concluded

that hybrids persist and interbreed with native species in natural settings. The general term used to describe butternut trees may actually describe trees that include hybrids with heartnut as discussed below.

Other walnut species can hybridize with butternut, but none are an introgression threat. Butternut hybridizes with Persian walnut producing *J. x quadrangulata* (Carr.) Rehd. (Woodworth 1930, McGranaham and Catlin 1987), and hybridization with Manchurian walnut (*J. mandschurica* Maxim.) has also been reported (Funk 1979). Butternut can hybridize with the native little walnut or Texas walnut (*J. microcarpa* Berland var. *microcarpa*; synonym *J. rupestris*), although the natural range of the two species does not overlap. A hybrid of butternut and black walnut was reported from Germany by Gervais (1963), but natural or controlled pollinated hybrids have never been substantiated or found in the United States in forest or orchard settings (Rink 1990).

Historical progression of butternut canker disease

Widespread mortality of butternut trees has been attributed to butternut canker disease, caused by the fungus *Sirococcus clavigignenti-juglandacearum* (Orchard *et al.* 1982, Tisserat and Kuntz 1984, Cummings Carlson and Guthmiller 1993, Ostry *et al.* 1997, Ostry 1998, Nair 1999). The fungus causes multiple branch and stem cankers that eventually girdle infected trees (Ostry *et al.* 1994).

Symptoms of butternut canker disease, actually caused by *S. clavigignenti-juglandacearum* may have been mistakenly attributed to a native fungus, *Melanconis juglandis* (Ellis & Everh.) A.H. Graves, delaying the detection of the primary disease agent for many years. *Melanconis* is usually a secondary pathogen that often rapidly invades dying portions of the tree (Nicholls *et al.* 1978), although Anderson and Schlarbaum (unpublished) observed severe *Melanconis* damage in a young butternut plantation. Infection of butternut by *Melanconis* was first reported in 1923 and was considered not a serious threat to the tree (Graves 1923). Significant decline and mortality of butternut were mentioned in literature as early as 1958 (Clark), which may have actually been caused by *S. clavigignenti-juglandacearum*. Clark (1958, 1965) noted cankers caused by *Nectria* spp., and *M. juglandis*

resulted in dead branches which could be used to distinguish the butternut species. In addition, Peattie (1950) noted symptoms of a disease on butternut "leaves are often sparse . . . and . . . many dead branches detract from its appearance." Ashworth, 1965, mentions a "dead limb disease" on butternut. These types of descriptions, however, could have been describing *Melanconis* damage prior to identification of *S. clavigignenti-juglandacearum* as the causal agent.

Widespread dying of butternut was in southwestern Wisconsin during 1967 (Renlund 1971), was also attributed to *M. juglandis*. In 1979 the pathogen was identified as a new species of *Sirococcus*, based on examination of the asexual stage (Nair *et al.* 1979). The fungus is believed to be an introduced species, though there is no known date of introduction (Ostry *et al.* 2003). The lack of genetic variation, highly-aggressive qualities and rapid spread of the disease, offer support that *S. clavigignenti-juglandacearum* was recently introduced to North America (Ostry *et al.* 1994; Ostry 1997, Furnier *et al.*1999). However, the species has not been found on any other continent (Nair *et al.* 1979, Nair 1999). Inoculation tests with butternut and heartnut have found that heartnut is more resistant than butternut to the fungus (Orchard *et al.* 1982). Therefore it has been speculated that the fungus was introduced on heartnut trees sometime in the 1900s, even though the fungus was not isolated on heartnut until 1997 (Ostry 1996).

There is debate about the point of origin of butternut canker disease. The first identification of *S. clavigignenti-juglandacearum* was in Wisconsin, and therefore confusion exists between the diagnosis location with the point(s) of entry into the United States. Though the first reports of butternut canker disease were from Wisconsin, the disease is believed to have been introduced on the east coast in the southern section of butternut's natural range (Anderson and LaMadeleine 1978). Butternut canker disease has infected populations throughout most of the species range, with the greatest impacts on southern populations (Ostry 1996). Double-stranded RNA analysis of *S. clavigignenti-juglandacearum* isolates found that dsRNA occurs more often in isolates from southern states than northern states (Spaine *et al.* 2001).

Butternut canker disease is believed to have impacted trees in the south since at least the 1920s. Investigations using stem analysis of dead butternut trees also support this assumption (non-published data UT-Tree Improvement Program and personal investigations of R.L. Anderson). This evidence suggest that *S. clavigignenti-juglandacearum* was introduced through an eastern port in the southern sections butternut's range 80-90 years ago, perhaps in Virginia, and spread north and south. The first report of butternut canker disease in Quebec was in 1990, Ontario in 1992 and New Brunswick in 1997, where it was believed to have been present at least seven years (Harrison *et al.* 1998, Harrison *et al.* 2004).

Sirococcus biology

Sirococcus clavigignenti-juglandacearum belongs to the division Deuteromycotina and the class Coelomycetesis (Nair 1999). It is only known in the asexual stage and the perfect stage of the fungus has not yet been reported (Nair *et al.* 1979, Nair 1999). In 1979, the pathogen was isolated in pure culture and proven to be the causal agent of butternut canker disease through Koch's postulates by repeated inoculations of seedlings, saplings, and mature butternut trees (Nair *et al.* 1979, Nair 1999). Spores produced throughout the growing season can be spread by rainsplash (Tisserat and Kuntz 1984), insects (Stewart *et al.* 2004), and on seed husks (Orchard 1984). The fungus infects trees through open wounds and bud and leaf scars (Davis *et al.* 1997, Davis and Meyer 1997). The obvious symptom of the disease is elongated, sunken cankers, which exude inky-black fluid in the spring (Orchard *et al.* 1981). Peeling of the bark reveals the dead, black cambium underneath (Ostry *et al.* 1994). *S. clavigignenti-juglandacearum* is active during the tree's dormant season and is dependent upon abundant moisture for growth. Butternut canker disease can cause current year annual cankers to form during the winter months, which actively grow in the early spring and late fall (Orchard *et al.* 1982). On many small branches the canker then is infected by secondary pathogens and therefore *S. clavigignenti-juglandacearum* is hard to isolate and may not be present or a main source of future injury to the tree (Nicholls *et al.* 1978) However, butternut canker can be a perennial canker as well (Nair 1999, Cummings Carlson 2004). Through annual growth ring counts, older stem cankers have been shown to persist for up to 13 years (Nair 1999). Often cankers form in the upper sections of the tree

and are spread by rainsplash to lower sections of the stem. Cankers commonly occur on the branches, bole, and on exposed roots (Ostry *et al.* 1994). Butternut canker infects all sizes and ages of trees, including sprouts on all sites (Ostry *et al.* 1994, Ostry 1996).

Sirococcus clavigignenti-juglandacearum is very difficult to raise in culture (Harrison and Hurley 2004). This difficulty has often resulted in evaluation of sunken cankers without the verification of isolation of *S. clavigignenti-juglandacearum.* However, new cankers on stems, in which cultures could be collected, can be concealed by bark fissures and difficult to distinguish from other wounds. Older cankers may be more obvious but are often colonized by secondary fungi, making isolation difficult. *Melanconis juglandis* cankers are also common on small branches of the tree and difficult to distinguish from *S. clavigignenti-juglandacearum* in the field (Cummings Carlson 2004). *Armillaria* root rot also causes dieback on trees and, like *Melanconis*, is associated with trees experiencing stress conditions (Cummings Carlson 2004).

Pathogen transmission and spread

Initially, the pathogen usually attacks in the crown of the tree, spreads throughout the branches, and finally down the main stem, causing numerous cankers which eventually girdle the tree (Ostry *et al.* 1994). Black lesions rupturing the outer bark on branches or stems are from hyphal pegs, or stromatal columns, which expose the pycnidia of the fungus and cause necrosis, resulting in canker formation (Tisserat and Kuntz 1983). Conidia are released from pycnidia during periods of rain or high humidity (Cree 1995) and are spread from the infected crown down the stem by rain wash (Anderson 1996, Ostry *et al.* 1994). Spore survival is favored by cool temperatures (13°C) (Cummings Carlson 2004).

The spores infect trees through lenticels, open wounds, bud and leaf scars. At least seventeen beetle species have been found to be vectors of the pathogen (Halik and Bergdahl 2002). Conidia have been proven to travel far, ca. 40 m, from the nearest inoculum source and infections have been found over 100 m from the nearest infected tree (Tisserat and Kuntz 1983). A relationship between disease incidence and spatial measures exists within a 40 m radius of an infected tree, but not beyond (unpublished data, D. Bergdahl, University of Vermont, *viz*. Cummings Carlson 2004). The fungus continues to sporulate for 20 months

following the death of a tree (Tisserat and Kuntz 1984). Over 35 insects families associated with butternut trees could be potential vectors for the disease (Katovich and Ostry 1998). Stewart *et al.* (2004) found three insect species carried viable conidia up to 16 days with numbers substantially decreasing with time. Tisserat and Kuntz found that conidia can survive in the airborne environment on a spider web in field conditions for at least eight hours (1983). Potentially becoming dormant, at 35 percent relative humidity and 13° C, conidia were found to remain viable for 32 hours (Tisserat and Kuntz 1983). In a laboratory setting, conidia remained viable on an insect exoskeleton for up to 384 hours (Stewart *et al.* 2004).

Butternut canker disease has spread rapidly and covered a significant part of the butternut range. In 1978, butternut canker was found in 14 of 16 states in a region-wide survey in eastern states (Anderson and LaMedeleine, 1978). Range-wide population impacts are not known due to the lack of surveys and because butternut canker disease is still spreading into the northernmost sections of the range (Harrison and Hurley 2004). Once the disease is present in a butternut population, there is a danger of extirpation and permanent loss of a particular gene pool.

Plant hosts

Inoculation trials have found pecans (*Carya illinoinensis* (Wangenh.) K. Koch), hickories (*Carya spp.* Nutt), and Persian walnut to be susceptible (Orchard *et al.* 1982). In inoculation trials of 10- to 20-year old trees, English walnut was the most susceptible and heartnut was the least (Orchard *et al.* 1982). Additional inoculation trials of black walnut, Japanese walnut, heartnut, Persian walnut, and their hybrids resulted in infection (Nair 1999). Seedlings of these species were susceptible to the disease (Federspiel and Nair 1982). It is unknown if the pathogen is present on other species which have been inoculated and show limited disease development. The disease could have devastating impacts on other species as well and could threaten walnut plantations in the western United States if introduced (USDA, ARS, National Genetic Resources Program 2001).

Two alternate hosts have been found infected with limited disease development, including the native black walnut (*Juglans nigra* L.) and the introduced heartnut variety of

Japanese walnut (Ostry 1997; Ostry 1996). Although infection by butternut canker in natural stands has been limited, artificial inoculation of black walnut with *S. clavigignenti-juglandacearum* has caused infections (Ostry *et al.* 1997). Butternut and black walnut nurseries in Quebec have been infected by butternut canker, but the disease has less of an impact on black walnut (Innes and Rainville 1996, Innes and Rainville 1998). Black walnut and heartnut have been found infected in plantings in the United States and the fungus causes a twig blight but not stem cankers (Ostry 1997; Ostry *et al.* 1997). *Sirococcus clavigignenti-juglandacearum* was isolated from the fruits of both butternut and black walnut (Innes 1997). Butternut canker disease also is of international concern due to a potential impact on other Juglandaceae species occurring worldwide (Nair 1999).

Butternut Population Impacts

The overall impact of butternut canker on original butternut populations appears to be significant, but specific details on the progression of the disease remain fragmentary. Since the determination of *S. clavigignenti-juglandacearum* as the causal agent of the fungus in 1979, the United States Department of Agriculture (USDA) Forest Service and other federal, state, and private entities have indicated dramatic decreases in the number of healthy live butternut trees (Nair 1999). Reports from these agencies on the loss of butternut are not systematic due to many factors, including the lack of ability to identify the less common tree. In addition Forest Service FIA (Forest Inventory and Analysis) data only took specific butternut data in certain areas. Considering the length of time since *S. clavigignenti-juglandacearum* was identified and the range of the species; very few investigations of its impacts on butternut have been conducted.

USDA inventory data indicate that in a 15 year period from 1984 to 1999, there was a decrease of living butternut trees in all size classes of 58 percent in Michigan and 84 percent in Wisconsin (Nair 1999). In 1976, butternut canker was found on 89 percent of trees examined in southwestern Wisconsin (Prey and Kuntz 1982). In 1993, 91 percent of all living butternut trees of all age classes were diseased or cankered throughout Wisconsin (Cummings Carlson 1993). U.S. Forest Service Forest Inventory and Analysis (FIA) plot

data for the eastern states, albeit fragmented across the range, showed significant differences in number of butternuts by ecoregion province and section in some northern states (Gottschalk *et al.* 2002).

Surveys in Vermont and Wisconsin have shown greater than 90 percent infection rates with less than 30 percent mortality (Bergdahl *et al.* 1996, Cummings Carlson and Guthmiller 1993). Extensive surveys have taken place in Wisconsin, of butternuts occurring within and outside USDA Forest Service Forest Inventory and Analysis plots (Cummings Carlson and Guthmiller 1993, Cummings Carlson 2004). In 1976, 2882 butternut trees were surveyed and butternut canker was found in 18 counties, 30 percent of the trees were cankered, and 8.5 percent of the trees were dead. In 1992, 1394 butternut trees were surveyed and butternut canker was in 45 counties, 91 percent of the trees were cankered and 30 percent of the trees were dead (Cummings Carlson 2004). In Vermont, Bergdahl *et al.* (1996) examined 1317 trees and found 94 percent cankered and 4 percent were non-cankered. From 1993 until 1996 mortality was 12 percent and infection rates were 96 percent (Schmalz and Bergdahl 2006). In 2001-2002, mortality increase to 41 percent and infections increased to 96 percent (Schmalz and Bergdahl 2006). In 1999, USDA Forest Service Forest Inventory and Analysis data indicated that 92 percent of the butternuts in CT were infected and up to 84 percent of the butternuts in Michigan were killed in a fifteen year period (USDA Forest Service 1999, Schultz 2003).

Butternut populations are particularly threatened in the southern portions of the range where there are fewer trees. In 1995, Ostry concluded from reports of infection that "viable populations of butternut are probably no longer present in the southern portions of its range". Forest Inventory and Analysis (1995) data for North Carolina and Virginia show a 77 percent reduction in butternut population, over a 30-year period (USDA Forest Service 1995). Tainter and Baker (1996) found that in Virginia and North Carolina the butternut population was reduced from 7.5 million in 1966 to 2.5 million in 1986. Over a 74-year period in West Virginia, Schuler (1997) found significant reductions in various hardwood species including butternut. These reductions in butternut cannot be attributed solely to disease and may be a result of land use changes over time. Butternut has been extirpated from some of the disjunct

populations in southern portion of the range and present in small populations, ca. < 10 trees, in other locations at the southern border of the continuous range (unpublished surveys, UT-Tree Improvement Program, data on file). In 1999, butternut canker disease was believed to have been in the southeastern states at least 40 years, and is believed to have killed 75 percent of the butternuts in North Carolina and Virginia (USDA Forest Service 2003).

In the Bluegrass Region, butternut was said to be more abundant than black walnut in 1828, however, butternut today comprises less than 0.1 percent of the overstory (Campbell 1989). Survey work by Braun in western Kentucky at Mammoth Cave National Park revealed that butternut comprised ten percent of the forest bottomland canopy (1950). Butternut surveys conducted at Mammoth Cave National Park from 2001 through 2003 revealed less than 100 surviving butternuts indicating that population declines due to disease and succession have reduced butternut populations by as much as 98 percent (Thompson *et al*. 2004). Surveys of an uneven aged stand in central Tennessee showed population with only 8 percent new mortality six years after extensive cankers were reported on over half of the butternut trees (see Chapter VI, data on file, UT TIP). Overall, the reports collective show progression from the southern Atlantic coast westward and northward.

Butternut canker disease resistance

Inoculation experiments of Juglans species including butternut, heartnut, Japanese walnut, black walnut, and Persian walnut, show that no species are immune to *S. clavigignenti-juglandacearum* (Nair 1999). Heartnut and Japanese walnut are more resistant to attack by the pathogen exhibiting smaller cankers (Nair 1999) and the restriction of canker development by sealing with callus tissue (Orchard *et al.*1982). The thick periderm and heavy deposits of cellulose on the cell walls of Japanese walnut and heartnut are thought to be a physical barrier to pathogen penetration (Nair 1999). Other mechanisms of resistance include the production of phenolics upon wounding which degrade the fungus and the restriction of the pathogen through gums and tyloses produced in xylem vessels (Nair 1999). Periderm thickness is 35-45 cells thick in Japanese walnut, 10-12 cells thick in black walnut, 8-9 cells thick in butternut, and 6-7 cells thick in Persian walnut (Nair 1999). Black walnut appears to produce phenolics and tyloses upon wounding; resulting in a hypersensitive

response to inoculation and infrequently disease in natural stands (Nair 1999). Butternut and Persian walnut are highly susceptible to reactions with infected tissues exhibiting cell maceration and mycelial penetration of phloem and xylem tissues (Nair 1999). However, butternut does produce tyloses and gums in infected areas (Nair 1999). Artificial inoculation has shown variation in date of inoculation but with limited effect of accession on susceptibility during times of ideal inoculation (Ostry and Moore 2007). In addition, accessions showing fewer numbers of cankers also had longer canker lengths (Ostry and Moore 2007).

Presently, there is no known control for butternut canker. Fungicides are not practical for forestry use due to the economic costs and feasibility of application. Antagonistic agents to the fungus, such as hypovirulent fungal strains debilitated by a virus are not known (*pers. comm.*, S.L. Anagnostakis).

Resistance to butternut canker

Ostry *et al.* (1994) first reported the presence of healthy, putative resistant butternut trees growing near diseased trees in the northern sections of the range. Subsequently, putatively resistant trees have been located in southern sections of the range as well and in 19 states (Anderson 1996, Thompson *et al.* 2006, Schmalz and Bergdahl 2006, Schlarbaum *et al.* 2004). Surveys to locate surviving butternut trees in the south began at The University Tennessee's (UT), Tree Improvement Program in 1993. Preliminary work began with surveying for surviving trees, since little information was known about the number of surviving butternuts. Since then putatively resistant trees have been located in Tennessee, North Carolina, Virginia, and Kentucky (Schlabaum *et al.* 2004).

Putative resistance in the field may be somewhat misleading, as certain environmental conditions, either abiotic or biotic, may predispose a tree to infection by butternut canker disease. Butternut canker is one of many factors, including shading and maturity, which may result in decline and death. The presence of living, heavily infected trees in the forest indicate that butternut canker may not be the primary agent in tree mortality. Butternut may be able to survive for long periods after initial and intensive infection by butternut canker

disease, and open-grown trees in general often appear healthier, even when infected. Infection and mortality estimates vary by location.

A proven methodology for increasing the ability to locate surviving butternuts is through the use of a geographic information system (GIS)-based predictive model, e.g, van Manen *et al.* 2002, Thompson *et al.* 2006. A GIS-based approach to locate extant butternut trees has been implemented on various land bases in the southeastern United States. Under this project, predictive habitat models have been developed for the Great Smoky Mountains National Park and expanded to the Southern Blue Ridge Mountains, Mammoth Cave National Park and the St. Francis National Forest (van Manen *et al.* 2002, Thompson *et al.* 2004, UT-Tree Improvement Program, unpublished data). Habitat models for butternut have focused on specific public lands and have been successful at finding new trees and restoration locations based on habitat characteristics such as elevation, aspect, and indices that help determine site moisture availability. Using genetically resistant trees, a backcross breeding approach could be a feasible strategy to produce resistant butternut trees (Schlarbaum *et al.* 1997, 2004).

Additional challenges to butternut persistence

Butternut is threatened by changing land use, seed predation, and lack of suitable conditions for reproduction (Ostry *et al.* 2003). Consequences of previous land management can result in changes to natural autogentic succession resulting in the loss of butternut from the forest. Lesser abiotic and biotic threats can have a cumulative impact on butternut trees already debilitated by butternut canker disease. Damming and channeling of riparian areas where butternut typically grows has resulted in a massive loss of habitat during the 20^{th} century, particularly in the Tennessee River Valley.

One potential significant impact to streams and rivers within the eastern United States has been the creation of small dams to make lakes and grist mills, and later larger dams by the Tennessee Valley Authority (TVA) to provide power and flood control. The TVA was established in 1933 and manages 54 owned dams that impact 105,930km^2 of the Tennessee River Basin in seven southern states (Miller *et al*. 1998). Richter *et al.* (1997) found that dams and impoundments are one of the three leading threats to aquatic systems, along with

agricultural non-point source pollution and exotic species. Water control was one of the primary causes of impacts, fourth behind collecting, grazing, and development for 98 plant species listed as endangered or threatened in the US (Schemske *et al.* 1994). Butternut populations may have already been fragmented with much of their historical habitat destroyed. The impacts of butternut canker disease, therefore, are acting on already impacted populations.

Butternut, like many hardwood trees, is attacked by many insects, fungi, and bacterial pests which could have significant impacts in certain locations. However, the three main pests that significantly impact butternut are: *Phytophthora* root rot, bunch disease, and *Melanconis*. Root and crown rot disease caused by *Phytophthora spp.* are of concern for many species, including most walnuts (Olson and Buchner 2002, Matheron and Mircetich 1985). Butternut is susceptible to bunch diseases, including walnut witches broom (phytoplasma rrnB AF190227, phytoplasma rrnA AF190226) (Hutchins and Wester 1947, Seliskar 1976, Hiruki 1988). *Xanthomonas campestris* also causes black rot in butternut (Belisario *et al.* 1999) and butternut is susceptible to anthracnose (Black and Neely 1978, McLaughlin 2000). The butternut curculio, *Conotrachelus juglandis* LeConte, is also a pest of the tree (Corneil and Wilson 1979). Disease caused by *Melanconis* is common on butternut. *Melanconis* is a common secondary pathogen that usually does not have a significant impact on the trees health (Hepting 1971), although plantings of butternut have seen serious impacts due to *Melanconis* (UT TIP, data on file).

There are additional fungi that can adversely affect butternut, but are poorly understood. Cultures of samples taken from small, dark lesions were found on the twigs and stems of living seedlings revealed the presence of *Phomopsis* sp. Lokoyae and *Diaporthe eres* Nitschke, Pyren. Germ. (Anagnostakis 2007). *Diaporthe eres* is an exotic pathogen of *Juglans ailantifolia* and *J. regia* var. *orientes* in Japan (Kobayashi 1970); this was the first report on *J. cinerea*. Schmalz and Bergdahl (2006) found infections by *Armillaria gallica* Marxmuller &Romagn. root rot and a variety of other root rots and heart/trunk rots. Occurrence of butternut canker increases infections with secondary pathogens (Schmalz and Bergdahl 2006).

Butternut Conservation

In 2001, the *Juglans* Crop Germplasm Committee of the National Plant Germplasm System recommended several actions for conservation research for butternut (USDA, ARS, National Genetic Resources Program 2001). The committee recommended: 1) identifying the most threatened populations and determining conservation strategies for these populations; 2) germplasm collection and evaluation of potential resistance or tolerance to butternut canker through disease screens; 3) genetic and phenotypic characterization of the germplasm with the long-term strategy of introduction of canker resistant genotypes into state and private nurseries and seed orchards (USDA, ARS, National Genetic Resources Program 2001). Trees exhibiting putative resistance have been found in Arkansas, North Carolina, Tennessee, Kentucky and Virginia (USDA 2003).

Preservation of butternut germplasm has been suggested by Millikan and Stefan (1989) and McIlwrick *et al.* (2000). Clonal archives of potential resistant trees have been made of a limited number of trees in the northern United States (Ostry 1998). Entire remaining populations of butternut have been grafted from populations at the Mammoth Cave National Park in Kentucky and the St. Francis National Forest in Arkansas (Hoban *et al.* 2008). Clonal archives, however, are impeded by complications with vegetative propagation of this species. Graft failures with butternut are common and have resulted in significant losses (Kaeiser and Funk 1971). One method of genetic preservation is with cryogenic preservation of embryos, which has been successful for butternut (Beardmore and Vong 1998). Accessions of butternut are housed at the USDA National Plant Germplasm System repositories (Postman *et al.* 2006).

Conservation status

Loo (1998) developed the following criteria for need to conserve a species: 1) is naturally rare, 2) has an uncertain viable seed source, 3) is impacted by loss of habitat, 4) has poor regeneration after forestry practices, 5) is threatened by hybridization, 6) is in demand

for special purposes, and 7) is threatened by a serious disease. Butternut meets many of the above criteria. Butternut has a global ranking of apparently secure, G4, according to NatureServe (2009, Table II-1). In the United States, butternut has been listed by the U.S. Fish and Wildlife Service under the Endangered Species Act of 1973 due to significant ongoing population decline. Butternut was previously been as a Category 2 species under the Federal Endangered Species Act and is currently listed as a "species of special concern" under the revised nomenclature. In over half of the states where butternut is a common tree, it is listed through State Natural Heritage Programs (Table II-2). Butternut is listed as an endangered species by Indiana, Missouri, North Carolina, and Tennessee, and as an extremely rare in Alabama, rare in Arkansas, Georgia, Maryland, Mississippi, and West Virginia. Butternut is classified as potentially threatened in Ohio and as a species of special concern by the states of South Carolina and Minnesota. Eight of the 27 states within the range of butternut, however, have no state listing for the species. The status of butternut is unknown in New York and there is no listing in Connecticut, Delaware, Illinois, Iowa, Kentucky, Massachusetts, and Michigan. In 1992, Minnesota banned harvesting healthy butternuts on state lands. The USDA Forest Service restricted harvesting healthy butternut on national forests in 1993 (Ostry *et al.* 2003) and has classified butternut as a "Regional Forester Sensitive Species" for 13 of the 16 National Forests in the eastern region. In November of 2003, butternut was listed in Canada as a federal endangered species (Carter 2004).

Genetic diversity

Signs that indicate vulnerability in rare plants include population size, degree of isolation, and fitness (Ellstrand and Elam 1993). Butternut is often found in small populations and increased mortality due to butternut canker disease may have dire genetic consequences. Factors that impact genetic diversity and fitness in rare plants include genetic drift, inbreeding, and gene flow (Ellstrand and Elam 1993). Loss of individuals or populations due to disease can increase the risk of genetic isolation and thereby, lower diversity. Butternut canker disease is reducing neighborhood size, which could result in increased risk of reproductive failure and genetic isolation.

Distance to seed source appears to be a key determinant of patch colonization of *Juglans* species, including butternut (Hewitt and Kellman 2002). Hewitt and Kellman (2002) found that butternut shows a high positive correlation (0.73) between seedling densities in openings and tree densities in connected areas. Due to the large seed of butternut, openings unconnected to seed sources are rarely colonized and distances as little as 50 m appear to be isolating (Hewitt and Kellman 2002). Human disturbances have caused fragmentation that can increase genetic isolation and decrease local population size (Ledig 1988, 1992, Ellstrad and Elam 1993, Sork *et al.*, 2002). The remaining population can be in put in jeopardy based on the pollen distances and the number of effective pollinators in a general area (Ledig 1988, 1992, Ellstrad and Elam 1993). Other species of *Juglans* are declining due to fragmentation and small local populations. In the United States, local and regional extensions of eastern black walnut have been reported (Curtis 1956, DeAngelis *et al.* 1979) and could be a result of increased fragmentation of systems (Hewitt and Kellman 2002). West Indian walnut, *Juglans jamaicensis* (C. DC.), from the islands of Hispaniola, Cuba, and Puerto Rico is federal listed as an endangered species. West Indian walnut populations are at risk due to habitat loss and the status of most of the Juglans species in Central and South America is unknown (USDA, ARS, National Genetic Resources Program 2001). Due to value for timber and limited geographic distributions, at least three species are probably endangered: *J. pyriformis, J. olanchana, and J. mollis* (USDA, ARS, National Genetic Resources Program 2001).

Pollen travel distance is inversely proportional to pollen grain size. In the genus *Juglans*, pollen has distinct scabrate sculpture with pollen grain size ranging from 30.3-42.6 μm depending on species (Crepet *et al.* 1975). *Juglans* species are anemophilous, wind-pollinated, and pollen grain size is relatively large compared with oak species. Eastern black walnut pollen size is 32.6μm. The surface of butternut pollen is also sculpted, and therefore pollen may not travel as far.

It is unknown if the effective population size is close to the census for butternut populations. There is a need to determine if a handful of super parents are producing the majority of the population. For example, land use changes in California have jeopardized the endemic species California Valley oak (*Quercus lobata* Née). In a population with a good

census of 60 trees, the effective number of pollen donors was less than 4 and average effective pollen movement was 65 m (Sork *et al.* 2002). For this species there are fewer effective fathers than elsewhere observed for a wind-pollinated species (Sork *et al.* 2002, Dutech *et al.* 2005). Estimates of pollen dispersal suggest a small reproductive neighborhood size and the historical gene flow for the species was a larger than the current distance (Dutech et al, 2005).

A number of studies on the genetic diversity of butternut have produced inconsistent or limited results. Evaluations of genetic diversity present in wild populations of butternut through the use of isozymes found estimates much lower than other species of *Juglans* in Quebec, New Brunswick and Vermont (Morin *et al.* 2000). They hypothesized that the loss of genetic diversity in northern populations could be an indication of low diversity throughout the range or the impact of postglacial colonization. Variation is accumulated more rapidly in DNA than in proteins.

An evaluation of butternut genetic diversity in the center of the range using *J. nigra* microsatellites found high diversity and low structure (Ross-Davis *et al.* 2008). However, this evaluation did not test for hybridization with *J. ailantifolia* which could result in impacts on diversity and structure composition. The use of microsatellites developed from a species within a different section of the genus, may lead to departures from expected allele distributions. Hoban *et al.* (2008) developed the first set of microsatellites specific to butternut applied them to population samples from different portions of the range (Hoban *et al.* 2009). Hoban *et al.* (2008) investigated a wild population of 63 individual trees in central Kentucky and found four loci with fewer heterozygotes than expected under Hardy-Weinberg equilibrium . McCleary *et al.* (2008) identified unique chloroplast for heartnut and butternut that allows for identification of first and advanced generation hybrids. A range-wide evaluation of genetic diversity in North America found reduced genetic diversity in the northern sections of butternuts range (Hoban *et al.* 2010). Hoban *et al.* (2010) discarded hybrids in order to accurately evaluate genetic diversity. The overall impact of butternut canker disease on diversity was less than the bottleneck that occurred during postglacial colonization (Hoban *et al.* 2010). Diversity was greatest in locations where

population declines due to disease were highest, showing resilience due to overlapping generations and surviving individual trees (Hoban *et al*. 2010). Diversity was reduced in northern populations, and high diversity was present at the southern and eastern perimeters of the range indicating the historical range shift hypothesis (Hoban *et al.* 2010). Northern populations may have increased vulnerability to mortality due to the additive impact of snow and ice on cankered limbs and may be less able to adapt to changing climate.

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Appendix: Tables and Figures

Table II-1. Global and state ranking system for rare, threatened, and endangered species (NatureServe and State Heritage Programs)

Ranking Abbreviation	*Description*
S1	Critically imperiled in state because of extreme rarity (5 or fewer occurrences).
S2	Imperiled in state because of rarity (6 to 20 occurrences).
S3	Rare or uncommon in state (on the order of 21 to 100 occurrences).
S4	Apparently secure in state (of no immediate conservation concern).
S5	Demonstrably secure in state.
G1	Critically imperiled globally because of extreme rarity (5 or fewer occurrences).
G2	Imperiled globally because of rarity (6 to 20 occurrences).
G3	Rare and local throughout range or in a special habitat or narrowly endemic (on the order of 21 to 100 occurrences).
G4	Apparently secure globally (of no immediate conservation concern).
G5	Demonstrably secure globally.
?	uncertainty with rank

Table II-2. Butternut conservation status in states within the native range of the species (State Natural Heritage Programs, 2009)

State	*Status Description*	*Status Abbreviation*
Alabama	Extremely Rare	S1
Arkansas	Rare	S3
Connecticut	None	SNR
Delaware	None	S3
Georgia	Rare	S2
Illinois	None	S2
Indiana	Endangered	S3
Iowa	None	SU
Kentucky	None	S3
Maryland	Rare	S3
Massachusetts	None	S4?
Michigan	None	S3
Minnesota	Special Concern	S3
Mississippi	Rare	S2
Missouri	Endangered	S2
New Hampshire	None	S3
New Jersey	None	S3S4
New York	Unknown	S4
North Carolina	Endangered	S2S3
Ohio	Potentially Threatened	S4
Pennsylvania	None	S4
South Carolina	Special Concern	S3
Tennessee	Endangered	S3
Vermont	None	S3
Virginia	None	S2
West Virginia	Rare	S3
Wisconsin	None	S3?

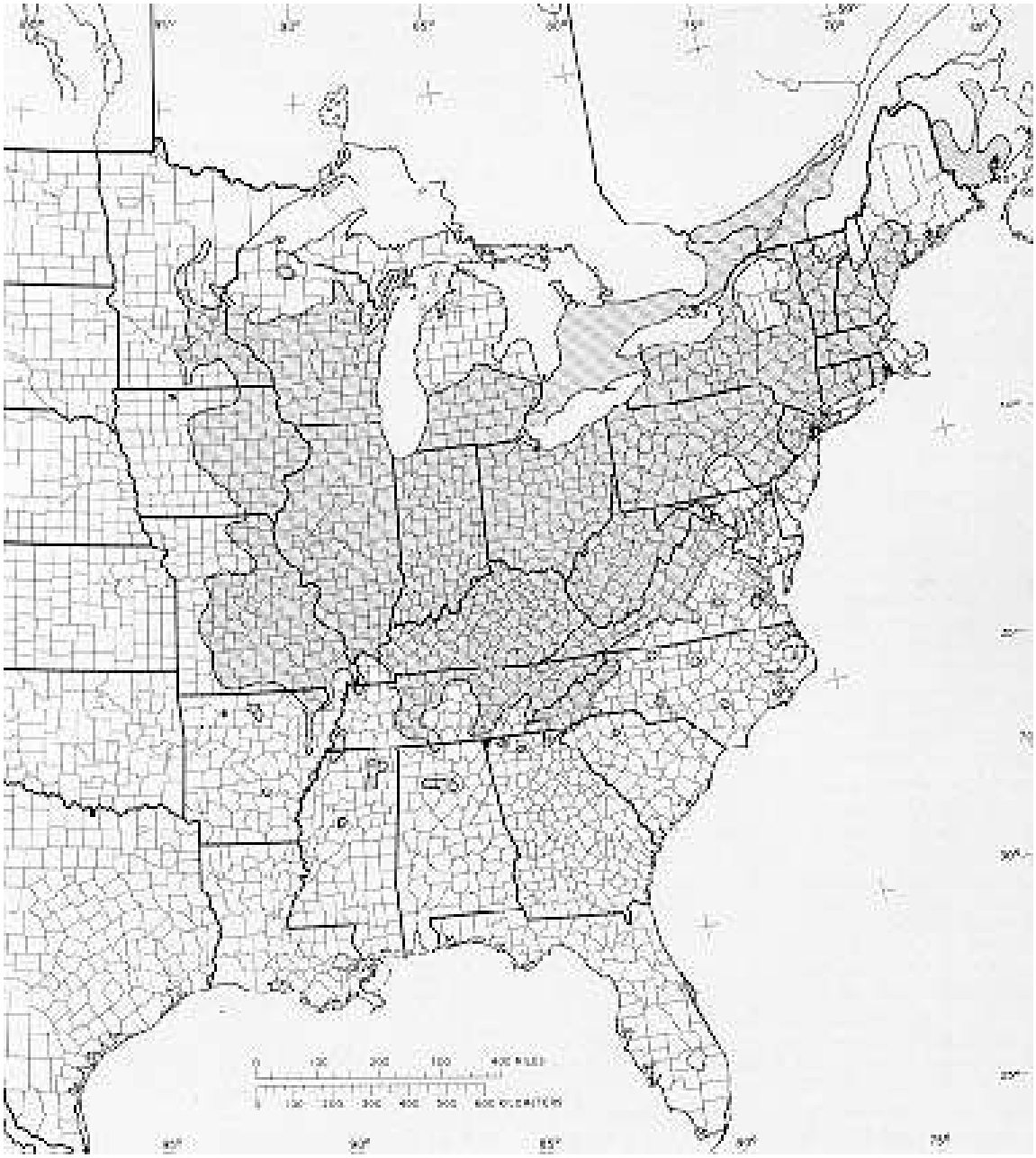

Figure II-1. Range of butternut in the United States (Little, E.L. 1971).

PART III. DIFFERENCES IN SEEDLING CHARACTERISTICS BETWEEN BUTTERNUT FAMILIES FROM SOUTHERN APPALACHIAN PROVENANCES

Abstract

Butternut, *Juglans cinerea* L, grows mainly in riparian zones in eastern North America and is important in providing hard mast for a number of wildlife species. Butternut canker disease, caused by an introduced pathogen, *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka & Kuntz), is devastating populations of butternut. Ecological restoration of the species hinges on several key factors associated with butternut nursery production and seedling establishment. In this study, nuts were collected from butternut trees originating in southern Appalachian provenances in Tennessee and surrounding states and grew them into large seedlings at the Georgia Forestry Commission's Flint River Nursery. Over 7,700 seedlings were grown for one year in a nursery across four different growing seasons or nursery trials. Annual differences in sizes of butternut seedlings varied seasonally and depended on placement within the nursery in relation to the location of irrigation risers. Overall seedling height (HT) was quite large and averaged 78.95 cm. Planting locations that were farthest from irrigation risers tended to produce the most plantable seedlings, which had the least damage during lifting. Seedlings from known parents of either pure *J. cinerea,* pure *J. ailantifolia,* F_1 hybrids (*J.* X *bixbyi*), and unknown families were significantly different each year and across multiple years for all seedling variables including HT, root collar diameter (RCD), and first-order lateral root number (FOLR). For family analysis and correlations, seedling from known *J. ailantifolia* and *J.* X *bixbyi* were excluded. Annually, open-pollinated seedling families had significant differences across provenances and families in initial seedling size after one year of nursery. This genetic influence was apparent for all individual years. Strong phenotypic and genetic correlations persisted across individual years for seedling variables. Germination rate was approximately 60% in the largest nursery trial (#2), which produced 4,834 seedlings. Family heritabilities were calculated by removing known *J. ailantifolia* and F_1 hybrids (*J.* X *bixbyi*). Heritabilities were strong for RCD (0.56), FOLR (0.52), VOL (0.47) and HT (0.36). Characteristics that have the potential to influence establishment success are heritable for butternut with potential gains from selection. RCD should be considered as a visual indicator of seedling quality and a two-way

selection approach is most appropriate for improvement of seedling quality. This work represents the first application of large seedling production techniques and seedling characterization for this species.

Keywords: butternut, *Juglans cinerea*, seedlings, nursery production, genetics

Introduction

Butternut or white walnut, *Juglans cinerea* L, is hardwood species usually inhabiting riparian zones in eastern North America, although occasionally occurring on drier limestone shelves. The species is related to black walnut, *J. nigra* L., but is in a taxonomically distinct section of *Juglans* L. (Stanford *et al.* 2000). Butternut is used for veneer, cabinets, and carving and is an important source of hard mast for wildlife. Populations of butternut have been declining due to an introduced pathogen, *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka & Kuntz), which is the causal agent for butternut canker disease. The disease causes multiple stem and branch cankers that girdle and kill trees of all ages and size classes (Ostry *et al.* 1994). Once a butternut population is infected lack of sprouting can result in permanent loss of a particular gene pool. Reduced genetic diversity has been found in portions of the overall range of butternut (Busov *et al.* 1997, Fjellstrom and Parfitt 1995, Morin *et al.* 2000; Hoban *et al.* 2010), whereas other locations have shown high diversity (Hoban *et al.* 2010).

Before complete extirpation of the species occurs, efforts to conserve genetic diversity of sensitive populations are needed to aid restoration of this species to former levels of abundance throughout its range. Restoration of the species may depend on genetic resistance to the disease, as healthy trees have been found in close proximity to dead or dying trees (Ostry *et al.* 1994). If resistance is genetically based, a breeding approach could be a feasible strategy to produce resistant butternut trees for restoration of the species in habitats where it has been extirpated (Schlarbaum *et al.* 1997).

Butternut canker disease is just one challenge faced in restoring butternut. Hardwood seedlings face many challenges. One major obstacle to seedling establishment includes

browsing and rubbing by white-tailed deer (*Odocoileus virginianus* Boddaert) and losses to other herbivores (Marquis 1974; Martin and Baltzinger 2002). In addition, herbaceous and hardwood competition can overtop outplanted seedlings in open areas (Cogliastro *et al.* 1990, Kolb *et al.* 1990, Willoughby and McDonald 1999). Variability in planting stock quality of hardwood seedlings often results in planting failures and low vigor of seedlings during the initial stages of establishment (Johnson 1981, Johnson *et al.* 1986, Wendel 1980). Traditionally, nurseries have grown hardwood seedlings in dense spacing and infrequent fertilization regimes (Dey and Buchanan 1995), which generally produced small, uniform size seedlings to facilitate shipping and planting (Johnson 1981, Kormanik and Ruehle 1987). Establishment success of northern red oak (*Quercus rubra* L.) seedlings increases with increased height and caliper of nursery stock (Zaczek *et al.* 1997, Dey and Parker 1997, Ward *et al.* 2000, Jacobs *et al.* 2004, Kormanik *et al.* 1994a, 1994b, Schlarbaum 1993).

Artificial regeneration using large seedlings with highly developed root systems shifts the competitive advantage toward planted seedlings and reduces losses to herbivory due to the location of terminal buds above the height of deer browse (Ward *et al.* 2000). Large nursery stock can be produced by adjusting nursery practices and can quickly become established in the field (Kormanik *et al.*1994a). Selecting large seedlings has increased establishment success for a variety of hardwood species including oak species such as *Quercus alba* L.*, Q. falcata* Michx.*, Q. michauxii* Nutt.*, Q. pagoda* Raf.*,* and *Q. rubra* L. (Kormanik *et al.* 1995, Kormanik *et al.* 1997, Schlarbaum 1993). Seedling genetic and phenotypic characteristics prior to outplanting have been used to determine certain seedling attributes of black walnut and many other species that are valuable for predicting survival (Thompson and Schutz 1995). The production of high-quality stock for black walnut seedling production has been investigated through seed selection and nursery techniques since at least 1947, and has continued for decades (Chase 1947, Beineke 1989, Schlutz and Thompson 1990). Over the past decade, nursery practices have adjusted seed density and fertilization and irrigation applications in order to produce large hardwood seedlings (Kormanik *et al.* 1994a, Kormanik *et al.* 1994b). This approach may be especially useful for butternut, where nuts are limited due to extirpation of natural populations from butternut

canker disease and a lack of seed orchards. Family and seedling quality are important qualities to consider along with resistance in a breeding program (Schlarbaum *et al.* 2004).

Seedling height (HT), root collar diameter (RCD), and/or number of first-order lateral roots (FOLR) have been used as an indicator of seedling quality (Russell and Kormanik 1986, Kormanik 1994a, Thompson and Schultz 1995). First-order lateral roots have been shown to endure after planting (Thompson and Schultz 1995) and are a heritable trait of many hardwood species associated with seedling competitiveness (Schutz and Thompson 1997, Kormanik *et al.* 1997). Nursery-grown hardwood seedlings with larger RCDs prior to outplanting have exhibited better survival and growth after outplanting than smaller stock (Olson and Hopper 1972, Zaczek *et al.* 1997).

Production of high-quality seedlings in the nursery and selecting seedlings with specific characteristics may increase establishment success of butternut in the field. The feasibility of hardwood genetic improvement has been well-documented by research in the 1970s (Bey 1973, Funk 1973), however, application of tree improvement programs has lagged behind (Rink and Stelzer 1982). Many of the above characteristics, as well as establishment success, are strongly controlled by genetics for several species, including black walnut (Bresnan *et al.* 1994).

Butternut has been cultivated since 1633; a half-century before black walnut and other North American *Juglans* species (cf. Brinkman 1974). Artificial regeneration of butternut, however, has never been widely practiced in the United States, due to abundant natural regeneration in some areas and biased selection of black walnuts for timber and nut production (Ostry and Pijut 2000). Butternut has a quickly developing root system that requires transplanting early in seedling development (Rink 1990), although information on specific seedling characteristics has not been published. Visual comparisons of nursery-grown, high-quality butternut and black walnut bare-root seedlings (*personal observation*) indicate that both species have very different root systems than oak (*Quercus* L.) species. The root system of black walnut is dominated by a relatively large, thick, deep-growing tap root with comparatively short lateral roots. Butternut has a relatively smaller and shorter tap root, but has very large (ca. > 1 cm diameter) lateral roots that often approach 1 m in length.

If resistance to butternut canker disease has a genetic basis, pedigree will be vital to restoration efforts. In addition, understanding genetic variability and potential gains in growth from selecting specific genetic families, and the impact of seedling quality and seedling characteristics on establishment will greatly enhance restoration efforts. Studies on heritability of these characteristics and the impacts on establishment success have yet to be completed for butternut.

The Georgia Forestry Commission's Flint River Nursery consistently produces high-quality seedlings of various hardwood species including butternut (Clark *et al.* 2000, Brosi *et al.* 2007). In the autumns of 1999, 2000, 2002, and 2004, there were crops of butternuts produced throughout the southern Appalachian region, and open-pollinated progenies were collected from many trees. This seed formed the basis of four nursery trials; Trial 1 (1999 crop), Trial 2 (2000 crop), Trial 3 (2002 crop), and Trial 4 (2004 crop). Each year the seed was planted by genetic family at the Flint River Nursery in December, 2000 and grown for one season to achieve the following objectives:

- *Objective a)* compare annual variation in seedling characteristics grown over four years (all trials);
- *Objective b)* compare species variation in seedling characteristics for all years combined and on individual years with known species differences (Trials 2, 3, and 4);
- *Objective c)* determine provenance and family variation in various seedling characteristics (all trials);
- *Objective d)* calculate phenotypic and genetic correlations for all seedling variables (all trials);
- *Objective e*) determine seed germination for provenances and families and seed lot sizes (Trial 2); and
- *Objective f)* calculate individual tree and family heritabilities for seedling characteristics (Trial 2).

This study represents the first application of high-quality seedling production techniques for this species.

Methods

Seed collection and handling

Throughout this manuscript butternut, *J. cinerea,* is defined by whole-tree characteristics for identification. For each tree overall phenotype was evaluated based on dendrological characteristics and nut characteristics that tend to distinguish the species. It is unknown if whole-tree characteristics can distinguish between pure butternut or hybrids of butternut and heartnut of various generations. Trees classified as butternut (*sensu lato*) could actually be the result of hybridization between *J. cinerea* and *J. ailantifolia* Carr or *J. ailantifolia* var. *cordiformis* (Maxim.) Rehd.) or hybridization with advanced generation hybrids, *J.* x *bixbyi* Rehd. Progeny of all parent trees could also be hybrids, depending on the presence of a heartnut or hybrid as the pollen source(s) (see Chapter II). A limited number of specific maternal parent trees used in this study were DNA genotyped using 12 microsatellite loci and specific chloroplast markers to determine recent maternal ancestry (Hoban *et al.* 2009). Parent trees consisted of *J. cinerea*, *J. ailantifolia* and F_1 hybrids *J.* x *bixbyi*. No offspring were genetically tested. Not all parent trees were tested, so they were labeled as unknown families.

Seedlings were produced in a set of four nursery trials. The first nursery trial was conducted with nuts collected in the fall of 1999, grown in the summer of 2000, and lifted in early spring 2001 when seedling characteristics were measured. In addition, butternuts were also collected in the fall of 2000, 2002, and 2005 with resulting seedlings measured in 2002, 2004, and 2007 (Table III-1, note tables and figures appear in appendixes). Evaluations over all five years included a total of 7,732 1-0 seedlings.

Open-pollinated nuts were collected by individual trees in the southern Appalachian region during the autumn season. Provenances were distinguished using Bailey's Ecoregions (1995, Table III-2). The nuts were gathered from the ground under fruiting trees, regardless of disease condition. The parent trees were found in a variety of sites and had large variation in nut production. Parent trees from the same provenance were often collected within 25 km of each other with some families from concentrations of neighboring trees. Trees in all provenances were located in areas where butternuts were occasionally found in forest stands.

However, trees in forest stands have small crowns and produce fewer nuts, so often nuts were collected from trees on the edge of forests and in yards adjacent to forests. All nuts were collected by hand and placed in mesh bags, labeled by family name and collection date. Nuts were stored under refrigeration (5.6^{o} C) and temporarily removed in early November to be dehusked by a commercial machine (Brinkman 1974). The machine was run empty and cleaned between families to ensure family separation. Following dehusking, the nuts were returned to cold storage for approximately one month until planting.

For Nursery Trial 1, nuts were collected in 1999 from one region in central Tennessee from 18 different genetic families; all parent trees were verified to be pure *J. cinerea*. For Nursery Trial 2, nuts were collected in 2000 from six general provenances: east Tennessee, central Tennessee, southwest Virginia, western North Carolina, northwestern North Carolina, and northern Georgia. Nuts from up to 11 trees per location (41 parents in total) were kept separate by family of origin. In Nursery Trial 2, from one location in western North Carolina maternal parent trees were determined to be either J. *cinerea* or F_1 *J.* X *bixbyi*. Three parent trees from east Tennessee were identified as *J. ailantifolia* through whole-tree characteristics.

For Nursery Trial 3, nuts were collected in 2002 from one location in western North Carolina from 6 families, one maternal parent trees was determined to be a *J. ailantifolia* and one was and F_1 *J.* X *bixbyi* . For Nursery Trail 4, nuts were collected in 2005 from four locations, with a total of 15 families, one maternal parent tree was determined to be a *J. ailathifolia* and one was *J. cinerea*. Provenances were from eastern Kentucky, central Tennessee, western North Carolina, and northwestern North Carolina.

Nursery protocols

The nuts were sown by hand in early December at the Georgia Forestry Commission's Flint River Nursery, located near Byromville, GA at a spacing of approximately six per linear foot or 24 per running foot of nursery bed. The seedlings were grown according to fertilization and irrigation protocols developed by the USDA Forest Service's Institute for Tree Root Biology (Kormanik *et al.* 1994a). Nursery beds were treated with methyl bromide and fertilized prior to planting. The Georgia Nursery is designed to have six nursery beds between irrigation risers, with beds 1 and 6 next to a riser. In Nursery

Trials 1 and 4, butternuts were planted in bed 1, immediately next to the irrigation risers. For Nursery Trial 3, seedlings were sown in row 4. For Nursery Trial 2, the families were sown in two replications in an incomplete block design, with three feet of empty bed space between each family to facilitate retention of pedigree during lifting. The replications for trial 2 were sown in the same middle row (row 3) in adjacent nursery compartments, each containing a total of six rows between irrigation risers.

Seedlings were grown for one growing season in the nursery. In late January, the seedlings were undercut to 30 cm in length and machine lifted. The seedlings were bundled by family and transported to Knoxville, TN and placed in cold storage. Lateral roots were pruned to 15 cm in length to assist in planting and the stems were inspected for disease. The seedlings were observed to be disease-free with no evidence of butternut canker or root rot disease.

Seedling characteristics

Measurements were taken on the following two parameters annually: stem height (HT) and root-collar diameter (RCD) (all trials). Height was measured from the root collar to the terminal bud to the nearest 0.5 cm using a meter stick and RCD was measure at 1.3 mm above the root collar using digital calipers. In addition, first-order lateral root number (FOLR) (Trials 1, 3, and 5) and root system volume (VOL) were also measured (Trail 2). A FOLR was defined as a lateral root at least 1 mm in diameter at the point of junction from the main taproot (*sensu* Kormanik 1994b). Due to the large seedling size and extensive root systems, some FOLR were broken off at the tap root in the lifting process in some seedlings, leaving a scar. For these seedlings, an 'absent' FOLR category was also recorded (Trail 2). Root system volume was measured by water displacement in specially constructed graduated cylinders. Root system volume was measured to provide a better approximation of root system size than FOLR due to the large size differences in lateral roots, i.e., 1 mm to 1+ cm. There were 5,387 seedlings with FOLR measurements during Nursery Trials 1 and 2 and 4,834 seedlings with VOL in Nursery Trial 2.

Statistical analyses

Statistical analyses were conducted using SAS version 9.1 (for Windows, © 2005 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Additional macro program codes to maximize programming efficiency developed by Saxton (1998, 2004) were used for means separation, heritability analysis and genetic correlations. Macros included pdmix800 for means separation using multiple range tests (Saxton 1998), Mixed Model Analysis of Variance Macro (mmaov) uses Fisher's Least Significant Difference (LSD) test conducted at the 5% significance level, determines if standard deviations are within five fold of each other, calculates Levene P values to determine equality of variances, and a Shapiro-Wilk W test for normality (Saxton ©2002). The additional macro (herit) was used for genetic correlations (Saxton 1998). Percentage data underwent arc sign transformations. In all analyses seedling characteristics of HT, RCD, and FOLR were treated as independent variables. Years, species, species*year, provenance, provenance*family, and family were considered fixed effects. Mixed model analysis was conducted using (PROC MIXED).

Objective a). A mixed model analysis using a randomized complete block design was conducted for annual effects on seedling characteristics. *Objective b).* A mixed model analysis using a randomized complete block design was conducted for species effects on seedling characteristics. Analysis was conducted on all years combined and then on individual years with known species differences (Trial 2, 3, and 4). *Objective c).* A mixed model analysis using a randomized complete block design was conducted for provenance and family effects on seedling characteristics for individual years. All known heartnut and hybrid parent families were removed from the sample prior to analysis. Analysis of provenance effects was conducted when families were collected from multiple provenances (Trail 2 and 4). Family effects only were included in years with just one provenance collected (Trial 1 and 3).

Objective d). For seedling variables, phenotypic and genetic correlations were determined for all years individually and combined. All known heartnut and hybrid parent families were removed from the sample prior to analysis. Phenotypic correlations among all

growth variables were generated using Pearson's correlation coefficients (PROC CORR). Genetic correlations were calculated to indicate the degree in which one trait will change as a result of another trait and help determine how selecting for a specific trait will impact other traits (Zobel and Talbert 1984).

Objective e). For Nursery Trial 2, percent seed germination was calculated for each family and summed across provenances. Percent germination underwent inverse trigonometric sine transformations for homogeneity of error variance, which reduces deviations from additivity on the transformed scale (Bogyo and Becker 1965, Snedecor and Cochran 1998). A Chi-squared test was used for provenance and family effects. Regression values between the number of seeds and germination rate were calculated.

Objective f). For Nursery Trial 2, analyses were conducted to determine individual tree and family heritabilities on specific traits and are used to determine potential gains from genetic selection. All known heartnut and hybrid parent families were removed from the sample prior to analysis. Individual-tree heritability and family heritability calculations were based on the assumption that the butternut seedlings from one genetic family are half-siblings (Zobel and Talbert 1984). The least square difference (LSD) values of the family means were averaged to produce a single value for the mean separation test. Tukey-Kramer multiple comparisons of the family means was used due to determine specific differences due to unequal sample sizes. Values for heritabilites were calculated for the additive genetic variance using $y_{ij} = \mu + F_i + T(F)_{ij}$, means squared values were calculated between families for: $G^2_E + nG^2_F$ and within families for: G^2_E, Genetic values were calculated using: ($G^2_{F = ¼}$ G^2_A) + ($G^2_E = ¾$ G^2_A + G^2_E) / G^2_p, h2=G^2_A / G^2_A + G^2_E with $r_p = r_A + r_E$ (Saxton 2004).

Results

Annual effects

Objective a). For all years combined HT, RCD, and FOLR were 66, 13.5, and 14.9 respectively (Table III-3). Nursery Trial 1 resulted in 553 seedlings averaging 16 mm in diameter and 112 cm in height with the largest seedling over 2 meters tall (Table III-4). Nursery Trial 4 resulted in 1,995 seedlings also averaging over 1 meter tall in height and over

15 mm in diameter. In Nursery trial 2, 4,834 seedlings were produced with an average height of 66.8 cm and diameters of 14 mm. Average seedling heights in trial 3, with 350 seedlings, was 58 cm and 13mm in diameter. Root collar diameters followed the same trend as height with largest averages produced in Trials 1 and 4 followed by Trials 2 and 3 (Table III-4). Trial 1 had only one seedling over 2 meters (0.18%) and trial 4 had over eight seedlings over 2 m (0.35%). All trials, except for trial 3, had seedlings over 175 cm in height. 5.28% of seedlings in trial 1 were over 175 cm and 4.05% in trial 4, but only 0.12% of seedlings were over 175 cm tall in trial 2. Analysis of variance on the combined data revealed significant annual variation in HT, RCD, but not FOLR (Table III-5). HT was significantly different in all years (Figure III-1). RCD was significantly larger in trials 1 and 4 then trials 2 and 3 (Figure III-2). Though height in Trial 2, on average, was 40% shorter than in Trial 1, FOLR number did not vary significantly across these two nursery trials (Figure III-3).

Species effects

Objective b) For all years, the combined seedlings showed significant variation in seedling attributes across species of origin of maternal parent (Table III-5). For HT, *J. cinerea* offspring were significantly taller than all others (n=27), *J. ailantifolia* (n=4), and F_1 *J.* X *bixbyi* (n=2) were not significantly different from each other, and unknown families were smaller than all other groupings except for F_1 s (Figure III-4, $p<0.0001$). For RCD, F_1 *J.* X *bixbyi* was significantly larger than all other groups, followed by *J. cinerea* families. *J. ailantifolia* and unknown families were not significantly different (Figure III-5, $p<0.0001$). FOLR number was largest for F_1 *J.* X *bixbyi* and smallest for *J. cinerea* families. There was no significant difference between *J. ailantifolia* and unknown families (Figure III-6, $p<0.0001$).

For Nursery Trial 1, all seedlings were from maternal parents of *J. cinerea* and therefore species effects were not evaluated. For Nursery Trail 2, HT was significantly shorter for unknown families (n=24) than known *J. ailantifolia* (n=3), F_1 *J.* X *bixbyi* (n=2), and J. *cinerea* (n=11) families. Each group was significantly different in RCD with F_1 *J.* X *bixbyi* the largest followed by *J. ailantifolia*, *J. cinerea*, and unknown families. Values for FOLR followed the same order from smallest to largest with no significant difference

between unknown families and *J. ailantifolia*. For Nursery Trial 3, seedlings from the unknown families (n=4) were significantly taller (62.01) than both the F_1 (n=1, 51.84) and *J. ailantifolia* (n=1, 54.46) families (p=0.0007). The same was true for RCD (p<0.0001), and FOLR (p<0.0001). For Nursery Trial 4, the seedlings with maternal parent *J. cinerea* (n=1) were significantly shorter than both the unknown families but not the *J. ailantifolia* offspring. RCD followed the same statistical outcome with the largest values for unknown families followed by *J. ailantifolia* then *J. cinerea.*

Provenance and family effects

Objective c) **All known heartnut and hybrid parent families were removed from the samples prior to analysis. Across all years, seedling characteristics were significantly different across both provenance and family of origin (Table III-6). Provenances varied in HT (**Figure III-7**, RCD (**Figure III-8**), and FOLR (**Figure III-9**). Analysis for years combined showed significance family effects on HT (**Figure III-10**), RCD (**Figure III-11**), and FOLR (**Figure III-12

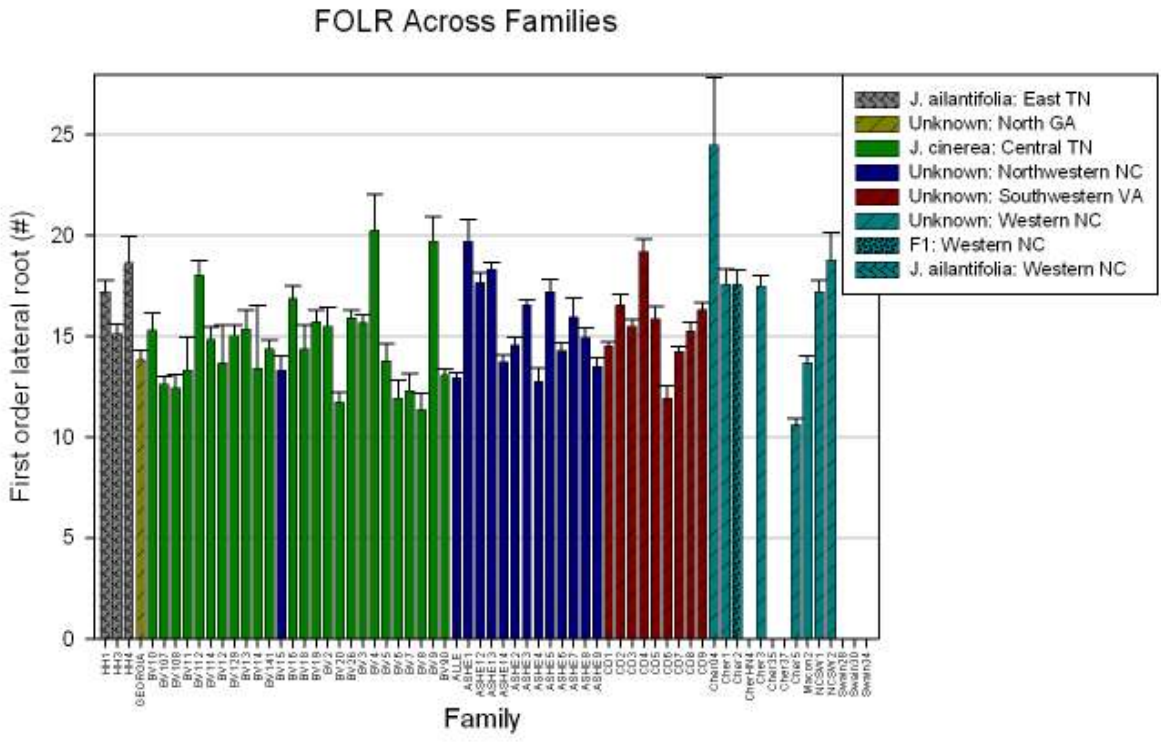

Figure III-12). Large variation existed in these variables with a number of very large seedlings (Figure III-26). Analysis for each year showed significance provenance and

family effects on all seedling variables for each individual year ($p<0.05$). Families BV9, BV10 and Cher30 were consistently larger in both HT and RCD. However, more often families were either tall with lower RCD values (BV19, CherLP) or shorter with large RCD values (Ashe13, Ashe7, Cher7).

For Trial 1, family effects were significant for all seedlings characteristics ($p<0.0001$, Table III-12). Thre were no provenance effects as all families were collected from the same location. Average HT ranged from over 140 cm for the tallest family (BV9) to under 50 cm for the shortest family (BV14) (Figure III-13). RCD was greatest in the tallest family (BV9) but the shortest family (BV14) had larger RCD averages than many other families (Figure III-14). Some families (BV9, BV10, and BV13) were both tall and had large RCD values. Other families were tall with smaller RCD values (BV5, BV19) or short with larger RCD values (BV3, BV8). Other families were both short and small diameter (BV2, BV7). FOLR was greatest in a family with lower than average values for HT and RCD (BV4). The tallest and largest diameter family had the second largest FOLR value (Figure III-15).

Nursery Trial 2 was the largest trial with 4,834 seedlings. Overall height ranged from 14-185 cm with a mean of 66.7 cm. Mean root collar diameter was 13.5 mm with a range of 1.2-38.7 mm. The average number of first-order lateral roots was 15 with a maximum of 37 (Table III-10). Less than half of the seedlings were greater than the mean for the characteristics of HT and RCD, but just over half were larger for FOLR (Table III-10). The percentage of seedlings above the mean was 43 for HT, 41 for RCD, and 52 for FOLR number (Table III-10). Provenances varied significantly for all variables ($p<0.0001$, Table III-11). Provenance of origin significantly impacted HT (range 59.6-84.6 cm), RCD (range 12.8-15.2 mm), and FOLR number (range 13.8-16.3), though the differences in provenance were less pronounced than family differences. Family differences were significant for all four seedling parameters ($p<0.0001$, Table III-12); HT (Figure III-16), RCD (Figure III-17), FOLR number (Figure III-18), and VOL. Significant differences existed among families in HT (range 42-110 cm), RCD (range 11-20 mm), and FOLR number (range 11-19). The largest genetic family (CD4) was over twice the size of the smallest in terms of height (CD2). The same trend was present for RCD and FOLR.

For Nursery Trial 3, all families were collected from one provenance in western North Carolina. Family differences were significant for both HT and RCD ($p<0.0001$). One family was (Swain28) significantly larger than all other families in height (Figure III-19). Multiple families varied significantly in RCD (Figure III-20). Two families (Swain28, Swain30) were over 15 mm in RCD with the smallest family less than 12 mm (Cher24).

For Nursery Trial 4, families varied significantly in both HT and RCD ($p<0.0001$). Two families from western NC were significantly larger than all other families (Cher30, CherLP, Figure III-21). RCD averaged over 20 mm for the largest family (Cher30) and under 12 mm for the smallest (CHLake) (Figure III-22).

Phenotypic and genetic correlations

Objective d). For all years combined, phenotypic correlations were strongest for RCD's relationship with HT (0. 65, $p<0.0001$, Figure III-23) and relationship with FOLR number (0.52, $p<0.0001$, Figure III-25). Over half of the variation expressed in height and FOLR number could be accounted for in variation of root collar diameter variation. A significant relationship also existed between height and FOLR number (0.28, $p<0.0001$,

Figure **III-24**, Table III-7). Genetic correlations showed the strongest relationship between root collar diameter and FOLR ($r_{rcd.folr}=0.39$). RCD and HT also had significant genetic relationship ($r_{rcd.ht}=0.36$). The genetic correlation between HT and FOLR number was weakly negative ($r_{ht.folr}=-0.16$) (Table III-7).

Analysis of phenotypic and genetic correlations within each individual year showed very similar annual trends (Table III-8). Trial 1 with 553 seedlings had very similar phenotypic and genetic correlations as Trial 2 with 4,834 seedlings. The largest value for genetic correlations was found in trial 3 with the smallest number of observations and families evaluated. Nursery Trial 1 had phenotypic relationships between 0.42 for HT-FOLR and 0.61 for HT-RCD with genetic correlations all 0.35 or under.

Within Nursery Trial 2: all seedling characteristics were significantly correlated with each other ($p<0.0001$) (Table III-14). Phenotypic correlations were strong for all relationships with RCD: VOL (0.77), HT (0.66), and FOLR (0.45). Phenotypic correlations were pronounced for HT with VOL (0.50) and less so with FOLR (0.28). The phenotypic

correlation for FOLR and VOL were also relatively weak (0.45). Genetic correlations were strong only for VOL and RCD (0.68), RCD and FOLR (0.45); and less so for HT and RCD (0.26) (Table III-14). Within Nursery Trial 3, RCD had strong correlations with HT phenotypically (0.49, $p<0.0001$) and genetically (0.82). Within Nursery Trial 4, RCD had stronger significant correlations with HT phenotypically (0.62, $p<0.0001$) and genetically (0.55).

Germination

Objective e) For Nursery Trial 2, the average germination percentage was 59% (Table III-9). Provenances ranged in germination percentage from 46.32 to 79.55 and were significantly different (Figure III-27, $p<0.05$). Families ranged from 13.06 to 94.30% and were significantly different (Table III-9, $p<0.05$). Only three provenances had all families having greater than 40% germination (Figure III-29). Nuts collected from central Tennessee had an average germination percentage of 77.11%. 90% of the families from central Tennessee had germination rates above the mean for all families combined and greater than 65%. Nuts collected from southwest Virginia, western North Carolina and northwest North Carolina had germination percentages closer to the average (50%, 46%, and 53% respectively). Only 33% of the trees in northwestern Virginia had germination rates above the mean. In western North Carolina only 33% of the families had germination above that of the mean. In northwestern North Carolina 67% of the families had germination averages above the mean. The family range in germination varied from 13 to 94%.

The relationships between number of seeds sown in the nursery per family and germination was statistically significant ($r^2=0.1592$, $p=0.019$) (Figure III-28). Germination average was 30% in families with greater than 400 seeds, with the exception of CD7, which had 90% germination and 423 seeds. Average germination was 60% for families with 29-366 seeds. Germination was 75% in families with less than 100 seeds, 69% in less than 150 seeds, 65% in less than 200 seeds, 64% in less than 300 seeds, 63% in less than 400 seeds, and 60% in less than 500 seeds, and 59% with all families. Families with less than 100 seeds had the greatest percent germination (Figure III-29).

Germination rate had a phenotypic effect on seedlings. Family Ashe7 experienced low germination, which resulted in low density and seedlings being shorter with larger RCDs then would be expected. High germination families resulted in high density with taller and thinning seedlings as with Ashe12. CD4 had fewer than 40% germination and resulted in the tallest and largest diameter family.

Heritabilities

Objective f) For Nursery Trial 2: Family heritability estimates were calculated for all variables and ranged from 0.36 to 0.56 (Table III-13). Family heritabilities were strong for RCD (0.56), FOLR (0.52), and VOL (0.47), but weaker for HT (0.36). Individual tree heritabilities were similar and lower for all variables: HT (0.12), RCD (0.16), FOLR (0.16) and VOL (0.14).

Discussion

Annual seedling production

The Georgia Forestry Commission's Flint River Nursery consistently produce large, high-quality butternut seedlings using techniques primarily developed for other hardwood species (Kormanik *et al.*1994a). Butternut was among the top tree species in the nursery for both height and diameter (unpublished data, UT-Tree Improvement Program) with seedlings reaching over 2 m in height. Correspondingly, butternut as a species can be challenging to nursery management. The incredible annual growth rate of the species results in spreading lateral roots up to 1 m in length; which cause seedlings to be extremely difficult to lift from the nursery beds and pose severe problems for packing. Additionally, the seedlings are virtually unplantable without severe root pruning. Root pruning also removes fine root structure, which is located at the ends of the lateral roots.

Results for this study suggest that placement within a nursery compartment can impact seedling size. In nursery trials 1 and 4, 2001 and 2007 seedlings, butternuts were planted in bed 1 closer to the irrigation risers at the nursery where they received ample moisture. Most of these seedlings had root systems so massive that they would be impossible

to plant without a large auger or planting shovels, even with lateral root pruning. Seedlings which were greater than 1 m in height would be extremely cost inhibitive to plant due to the few seedlings in each nursery bag, increased space in transport, and additional handling difficulties. The reduced height of butternuts in trials 2 and 3 was due to placement in the middle beds (beds 3 and 4) in the nursery compartment. These beds are farthest from the risers and receive relatively less moisture. Seedlings produced in these beds were fairly large with increased competitive ability, without the excessive size of the seedlings by the risers. Selecting seedlings between 80 and 100 cm in height would be manageable for transplanting without the loss of competitive ability. Nursery plantings of butternut should be strategically placed in beds within compartments that receive the least water in order to minimize height and root system growth.

Species variation

This is the first investigation of variation of offspring with the known identity of the material parent for *J. cinerea, J. ailathifolia,* and F_1 *J.* X *bixbyi.* Seedlings from F_1 parents were shorter than seedlings from *J. cinerea* parents, but were larger than all other groups for RCD and FOLRs. The larger RCD size and FOLR numbers of F_1 offspring (F_2 seedlings) indicates hybrid vigor may be expressed in root systems, as opposed to affecting the overall seedling. *Juglans cinerea* seedlings were taller than both *J. ailantifolia,* and F_1 seedlings, indicating that traits responsible for greater capacity for height growth may be more pronounced in *J. cinerea* than in *J. ailantifolia* and *J.* X *bixbyi*. In addition, smaller average lateral roots and diameters were apparent in F_1 and *J. ailantifolia* families, indicating more resources allocated to below-ground biomass. This might reflect indirect selection in heartnut, where the primary selection is nut-oriented and for nut/fruit orchard tree shape. The root systems may be inadvertently selected resulting in reduced height growth.

Evaluation of hybrids has shown less hybridization in forested and forest edge settings and increased hybridization in areas with greater human influences and disturbances (Hoban *et al.* 2010). The increased height of *J. cinerea* could be a critical factor for establishment in forest settings. Therefore, though hybrids may have increased resistance to

butternut canker disease (Orchard *et al.* 1982), they may be selected against in forest stands due to lack of height growth.

Provenance and family impacts

Specific genetic provenances and families produce superior seedlings in terms of all seedling variables. No trends exist in latitude or longitudinal differences in HT, and there does not appear to be a negative impact of growing more northern provenances in southern Georgia. In terms of HT, families from provenances in southwestern VA and Northwestern NC represented the farthest eastern provenances, and all had lesser heights. KY and GA provenances were the next shortest in terms of height and represented the farthest north and south provenances. Western North Carolina, east Tennessee, and central Tennessee provenances generally produced the tallest seedlings. RCD followed the same trend, as the three provenances were also the largest in diameter. However RCD was smallest in the KY provenance, next smallest in the VA provenance, and the northwestern NC and GA provenances were intermediate. Provenances originating near the center of the range of butternut exhibited the best performance. These locations have high concentrations of butternut, which may result in increased pollen flow and increased seedling size.

Within the same provenance, differences among families in all characteristics were very large, indicating that family identities should be kept separate for potential gains from selection. Differences in family rankings in all variables indicate that some specific families may allocate a larger percentage of resources to below-ground biomass and some allocate more resources to apical growth. Determining the relationships that these specific variables have on field performance in butternut (Chapters IV and V) will aid in determining which families are ideal candidates for incorporation into breeding and/or restoration programs. Families with larger seedlings will help overcome the many challenges with hardwood seedling restoration, including deer browse and competition with invasive herbaceous species. However, extremely large seedlings are not well suited for the nursery and transplanting.

Correlations

Phenotypic and genetic correlations showed strong relationships between initial seedling variables, indicating a two-way selection process should be used for assessing seedling quality. The strong relationships of RCD with HT and FOLR indicate this variable is extremely important for determining quality of seedlings. VOL of seedlings was highly correlated to RCD, but was too labor intensive to measure for operational use. Therefore, RCD should be first considered as a visual indicator of seedling quality. As height was more weakly correlated with RCD than the other root systems characteristics, it should be a secondary consideration for inclusion in a breeding program and when culling for operational plantings.

The high genetic correlation between root volume and root collar diameter (0.68) and phenotypic correlations (0.77) also indicate that the extra time spent in measuring root volume was unnecessary. Strong correlations indicate that selection based on root collar diameter and not first-order lateral root number will result in selecting seedlings with larger root systems and increased height. For rapid selection of the best seedlings, root collar diameter should be the initial focus of quality selection, followed by height.

Germination concerns

The overall germination rate was similar (59%) than the previously reported value of 65% (Brinkman 1974). Bare-root nurseries using butternut seed collected from naturally occurring or planted trees, without insect control, should consider a potential 40% loss. Adjustments to amounts of nuts to collect and nursery spacing should be made to generate an approximate number of seedlings and conserve nursery space.

Variation in germination was a function of provenance and family differences. Provenances from areas of high concentrations of butternut had higher pollen flow and higher germination rates. Reduced germination could be a result of a number of seedlings being pollinated by closely related individuals or impacts on the seed by insects. Though not included in the analysis, some seedlings were found with altered leaf phenotype, variegated leaves, and leaves lacking chlorophyll as a potential result of self-pollination or inbreeding depression (UT-Tree Improvement Program unpublished data). A number of these seedlings

experienced mortality prior to lifting from the nursery beds.

Germination percentage generally decreased as seed number increased; a large number of seeds could imply a significant pollen flow from other trees, whereas low numbers would suggest the opposite. Low germination in families with high numbers of seeds could be a result of increased impacts by nut weevils and other insects including the butternut curculio, *Conotrachelus juglandis* (LeConte) (Hay and Donley 1966, Wilson and Corneil 1978). There were, however, some families with a large amount of nuts that had good germination. This suggests that population numbers of damaging insects are cyclical, which may result in significant damage to nuts at some locations and a virtual lack of damage to nut crops in other locations.

Other factors, however, may be involved. Trees from the central Tennessee location may give additional insight into variability in germination. This population is the largest known concentration of butternut in southern states, with over 200 butternuts along a 4-mile stretch of creek in a relatively narrow valley with steep slopes. Due to the high concentration of trees, pollen flow and populations of nut weevils should be relatively uniform in this butternut population. Within this particular location, seed number did not directly correlate to percent germination. For instance, in the two families with the lowest percent germination, one family had a very small number of nuts and the other family had a large number of nuts collected. Both trees were located very within one kilometer from each other. This suggests that factors other than pollen flow or cyclical insect populations are present that affects seed germination. More intensive studies are needed on the relationships among nuts, insect damage, and pollen flow before conclusions can be reached.

Future studies should extensively study insect diversity and damage impacts to nuts. For black walnut, the black walnut curculio, *Conotrachelus retentus* (Say), is a major insect pest, as well as the codling moth, *Cydia pomonella* (L.), the walnut husk maggot, *Rhagoletis suavis* (Loew) and walnut husk fly, *R. completa* (Cresson). Though no studies have been conducted on butternut, over fifty percent of the annual nut production in black walnut was impacted by *C. retentus* in a two year study in Missouri (Blair and Kearby 1979). Many of these species can be controlled by rapid removal and destruction of nuts that have fallen in

early summer, before insects migrate to the soil (Katovich 2004). Therefore butternut orchards should aggressively address practices to reduce damaging impacts to nuts.

Heritability estimates

High heritabilities in butternut seedlings indicate that these characteristics are influenced substantially by additive genetic variances when environmental influences are minimized. Heritability analyses estimate the relative contributions of differences in genetic and non-genetic factors to the total phenotypic variance in a population. Traits with high heritabilities indicate that gains in growth could be made through selection and breeding to improve production. Root collar diameter was the seedling variable with the strongest heritability estimate for each year, indicating the greatest gains in seedling quality can be made by RCD selection, as that characteristic is highly correlated with other root system characteristics and also correlated with height. Findings from butternut are similar to studies on heritabilities for related species *J. regia* and *J. nigra.* In *J. regia* found stronger values for height (0.77) than diameter (0.65) for the first growing season (Aleta *et al.* 2004). Heritiablities for root volume for *J. nigra* were (0.54) but values for height and diameter were greater than one due to high standard errors (Jacobs 2005). *J. regia* and *J. nigra* have been more intensively studied in terms of nursery production, nut production, and seedling characteristics. Understanding areas in which butternut is similar to these species may allows for additional gains of using related species as references for future research.

Conclusions

Practical applications

Production of high quality seedlings of butternut with robust root systems is possible, although the sheer size produces many challenges in the nursery and field. A partial solution may be to reduce size by planting the seedlings in nursery beds within compartments that receive the least water, i.e., middle rows of a compartment. This may also reduce the impacts of *Phytophthora* root rot, which severely impacts butternut seedlings. Phenotypic and genetic correlations indicated that a two-way selection approach based first on RCD and

then on HT and FOLR would result in higher quality seedlings. High heritabilities indicate that gains from selection could be highest for RCD followed by FOLR number, and seedling HT. Seedlings selected from specific families will increase germination and result in larger seedlings. Selecting these families may reduce losses to deer browse and competition. In practice, increased survival and growth decreases the number of seedlings needed to plant to achieve future stocking densities. Additional studies, described in Chapters IV and V, discuss the impact of initial seedling condition on outplanting success.

Biological findings

The impacts of introgression with *J. ailantifolia* may result in seedlings with reduced height growth, though hybrid vigor may result in larger root systems and diameters. Though introgression has been reported to be dominant throughout butternut's range; reduced height growth of hybrids may result in less hybridization in forested stands. Screening of parent trees will aid in determining maternal parents for open-pollinated seed though will not ensure pure butternut selections. Butternut, like walnut, is capable of large height gains when grown from seeds in nurseries. Artificial regeneration using large seedlings offers great potential to increase success of restoration efforts. However, extremely large seedlings are not ideal for nursery production, transport and transplanting logistics. A seedling with extensive lateral roots can be damaged in lifting. Ideally seedling height between 80 and 100 cm should be competitive without excessive practical drawbacks.

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Appendix: Tables and Figures

Table III-1. Nursery Trials Total: Trial Number and number of seedlings produced from each nut crop.

Nursery Trial	Seed Year	Seedling Year	Seedling (#)	Provenances (#)	Families (#)
1	1999	2001	553	1	18
2	2000	2002	4834	6	41
3	2002	2004	350	1	6
4	2005	2007	1995	4	15
Totals/Mean			**7,732**	**6**	**80**

Table III-2. Butternut seedlings across provenances, families of origin, and species.

Seedling Year	Provenance Name	Family Abbreviation	Species
2001	central Tennesee	BV	*J. cinerea*
2002	northern Georgia	Raba1	Unknown
	western North Carolina	Cher2	F_1: *J.* X *bixbyi*
		Cher22	F_1: *J.* X *bixbyi*
		Macon2	*J. cinerea*
		Cher	Unknown
		Swain	Unknown
	northwestern North Carolina	Ashe	Unknown
	central Tennessee	BV	*J. cinerea*
	east Tennessee	HH	*J. ailantifolia*
	southwest Virginia	CD	Unknown
2004	western North Carolina	Cher24	*J. ailantifolia*
		Cher37	F_1: *J.* X *bixbyi*
		Cher	Unknown
		Swain	Unknown
2007	western North Carolina	Cher24	*J. ailantifolia*
		Nant1	*J. cinerea*
		Cher	Unknown
	northwestern North Carolina	Ashe	Unknown
	central Tennessee	JD/CHL	Unknown
	Eastern Kentucky	Law	Unknown

Table III-3. Nursery Trials Total: Average nursery stock (1-0) butternut seedling characteristics, including height, root collar diameter and first order lateral root number.

Characteristic	Mean	Range	Standard Deviation
HT[1] (cm)	66.75	4-209	38.30
RCD[2] (mm)	13.53	1.6-38.7	4.46
FOLR[3] (#)	14.87	1-37	5.15

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table III-4. Nursery Trials Total: Annual nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Nursery Trial	HT[1] (cm)	RCD[2] (mm)	FOLR[3] (#)
1	111.72 (20-209)	15.73 (5-29)	14.83 (2-39)
2	66.75 (4-185)	13.53 (1-39)	14.50 (1-37)
3	57.59 (25-135)	13.42 (7-38)	--
4	103.73 (20-211)	15.04 (3-22)	--
Average	**84.94**	**14.43**	**14.67**

Range in parentheses

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table III-5. Nursery Trials Total: Analysis of variance of species differences between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Variables	Maternal Parent Species	Mean	F value	P value
HT[1]	*J. cinerea*	92.03	269.24	<0.0001
	J. ailantifolia	68.06		
	*J.*X *bixbyi*	61.79		
	Unknown	61.50		
RCD[2]	*J. cinerea*	14.23b	19.02	<0.0001
	J. ailantifolia	13.36c		
	*J.*X *bixbyi*	15.28a		
	Unknown	13.40c		
FOLR[3]	*J. cinerea*	14.11c	19.41	<0.0001
	J. ailantifolia	15.68b		
	*J.*X *bixbyi*	17.68a		
	Unknown	15.06b		

Table III-6. Nursery Trials Total: Analysis of variance of provenance, family, and annual differences between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Variables	Effects	Degrees of Freedom	F value	P value
HT[1]	Provenances	6	6.57	<0.0001
	Families	83	19.01	<0.0001
	Years	3	375.90	<0.0001
RCD[2]	Provenances	6	25.41	<0.0001
	Families	83	14.42	<0.0001
	Years	3	32.36	<0.0001
FOLR[3]	Provenances	5	12.86	<0.0001
	Families	55	13.87	<0.0001
	Years	1	19.09	<0.0001

Table III-7. Nursery Trials Total: Phenotypic and genetic correlations between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Variables	Observations (n)	Phenotypic Correlations		Genetic Correlations
		r^2	P value	
HT[1]-RCD[2]	7,707	0.64885	<0.0001	0.35850
HT-FOLR[3]	5,387	0.27818	<0.0001	-0.15855
RCD-FOLR	5,387	0.52258	<0.0001	0.38558

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table III-8. Nursery Trials Individually: Phenotypic and genetic correlations between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Year	Variables	Observations (n)	Phenotypic Correlations		Genetic Correlations
			r^2	P value	
2001	HT[1]-RCD[2]	553	0.60658	<0.0001	0.345782
	HT-FOLR[3]		0.41794	<0.0001	0.27911
	RCD-FOLR		0.57323	<0.0001	0.35104
2002	HT-RCD	4834	0.65617	<0.0001	0.25936
	HT-FOLR		0.27715	<0.0001	-0.17481
	RCD-FOLR		0.53408	<0.0001	0.45216
2004	HT-RCD	345	0.48609	<0.0001	0.81470
2007	HT-RCD	1975	0.61822	<0.0001	0.55007

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table III-9. Nursery Trial 2: Percent germination of butternut seeds across provenances and families of origin, bold indicates values above the mean of all families.

Provenance Name	Provenance Germination (%)	Family Name	Family Germination (%)	Seeds per family (#)	Seedlings per family (#)
Northern Georgia	**79.55**	Raba1	**79.55**	88	70
Western North Carolina	46.32	Cher1	13.66	**644**	88
		Cher2	16.67	**420**	70
		Cher3	28.43	**496**	**141**
		Cher5	**71.38**	**283**	**202**
		Nant1	55.26	**342**	**189**
		Swai1	**92.54**	**295**	**273**
Northwestern North Carolina	53.00	Ashe1	**72.73**	55	40
		Ashe2	**70.79**	89	63
		Ashe3	**65.14**	**479**	**312**
		Ashe4	**67.57**	74	50
		Ashe5	38.01	200	76
		Ashe6	**65.73**	143	94
		Ashe7	23.00	100	23
		Ashe8	**60.31**	131	79
		Ashe9	**61.00**	**241**	**147**
		Ashe12	19.95	**366**	73
		Ashe14	**78.70**	**230**	**181**
		Alle1	13.06	**444**	58
Central Tennessee	**77.11**	BV3	**69.33**	150	104
		BV16	**65.52**	29	19
		BV26	42.13	**254**	107
		BV90	**84.29**	**337**	**284**
		BV107	**85.29**	68	58
		BV108	**84.21**	38	32
		BV112	**89.74**	39	35
		BV114	**83.33**	30	25
		BV129	**72.94**	96	70
		BV141	**94.30**	158	**149**
East Tennessee	**69.22**	HH1	**66.10**	118	78
		HH3	**88.24**	102	90
		HH4	53.33	30	16
Southwest Virginia	49.79	CD1	44.97	**994**	**447**
		CD2	37.43	187	70
		CD3	**60.63**	**348**	**211**
		CD4	32.84	134	44
		CD5	35.06	154	54
		CD6	22.34	197	44
		CD7	**90.14**	**426**	**384**
		CD8	48.57	210	**102**
		CD9	**76.13**	222	**169**
Mean			**59.03**	**224**	**118**

Table III-10. Nursery Trial 2: Average nursery stock (1-0) butternut seedling characteristics, including height, root collar diameter and first order lateral root number.

Characteristic	Mean	Range	% seedlings ≥ mean
HT (cm) [1]	66.7	4-185	43.8
RCD (mm) [2]	13.5	1.2-38.7	41.2
FOLR (#)[3]	14.9	1-37	51.5

Table III-11. Nursery Trial 2: Average nursery stock (1-0) butternut seedling characteristics, including height, root collar diameter and first order lateral root number, across provenance of origin.

Provenance Name	Family (#)	Seedling (#)	HT (cm)[1]	RCD (mm) [2]	FOLR (#)[3]
1. Northern Georgia	1	70	70.3 b	13.8 c	13.8 cd
2. Western North Carolina	8	758	63.4 c	14.5 b	14.7 cd
3. Northwestern North Carolina	10	1414	63.0 c	13.6 c	14.9 bcd
4. Central Tennessee	10	883	84.6 a	13.4 c	14.9 bcd
5. East Tennessee	3	184	82.0 a	15.2 a	16.3 a
6. Southwest Virginia	9	1525	59.6 c	12.8 d	15.0 bc
Total or Average	**41**	**4834**	**70.5**	**11.6**	**14.9**

Means followed by different letters indicate significant differences at p=0.05 level based on Tukey-Kramer means separation analysis.

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table III-12. Nursery Trial 2: Average nursery stock (1-0) butternut seedling characteristics, including height, root collar diameter and first order lateral root number, across family of origin.

Provenance Name	Family Name	Seedling (#)	HT (cm) [1]	RCD (mm)[2]	FOLR (#)[3]
Northern Georgia	Raba1	70	63	13	13
Western North Carolina	Cher1	88	43	14	**18**
	Cher2	70	**74**	**18**	**18**
	Cher3	141	**66**	**15**	**18**
	Cher5	202	**69**	14	11
	Nant1	189	43	14	**18**
	Swai1	273	51	12	13
Northwestern North Carolina	Ashe1	40	**68**	13	**18**
	Ashe2	63	53	13	14
	Ashe3	312	**73**	**15**	**16**
	Ashe4	50	**74**	14	12
	Ashe5	76	61	14	**17**
	Ashe6	94	62	13	14
	Ashe7	23	**87**	**20**	**16**
	Ashe8	79	**78**	**15**	15
	Ashe9	147	**64**	13	14
	Ashe12	73	46	**15**	**18**
	Ashe14	181	54	12	14
	Alle1	58	**71**	11	**16**
Central Tennessee	BV3	104	**82**	**15**	**16**
	BV16	19	**65**	**15**	**17**
	BV26	107	**105**	**15**	**16**
	BV90	284	**85**	**12**	13
	BV107	58	**72**	14	14
	BV108	32	**72**	13	14
	BV112	35	**65**	14	12
	BV114	25	**73**	13	**17**
	BV129	70	52	11	13
	BV141	149	**99**	14	14
East Tennessee	HH1	78	**89**	**17**	**17**
	HH3	90	**69**	13	15
	HH4	16	**110**	**18**	**17**
Southwest Virginia	CD1	447	60	13	15
	CD2	70	42	12	**17**
	CD3	211	**67**	13	**16**
	CD4	44	**88**	**17**	**19**
	CD5	54	**68**	**17**	**16**
	CD6	44	49	13	12
	CD7	384	55	12	14
	CD8	102	**77**	14	15
	CD9	169	57	12	**16**
Mean		**118**	**63**	**14**	**15**

Bold indicates values above the mean of all seedlings**.**

[1] Height of the seedling from the root collar to the first live terminal bud [2] Diameter taken 1.3 mm above the root collar[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

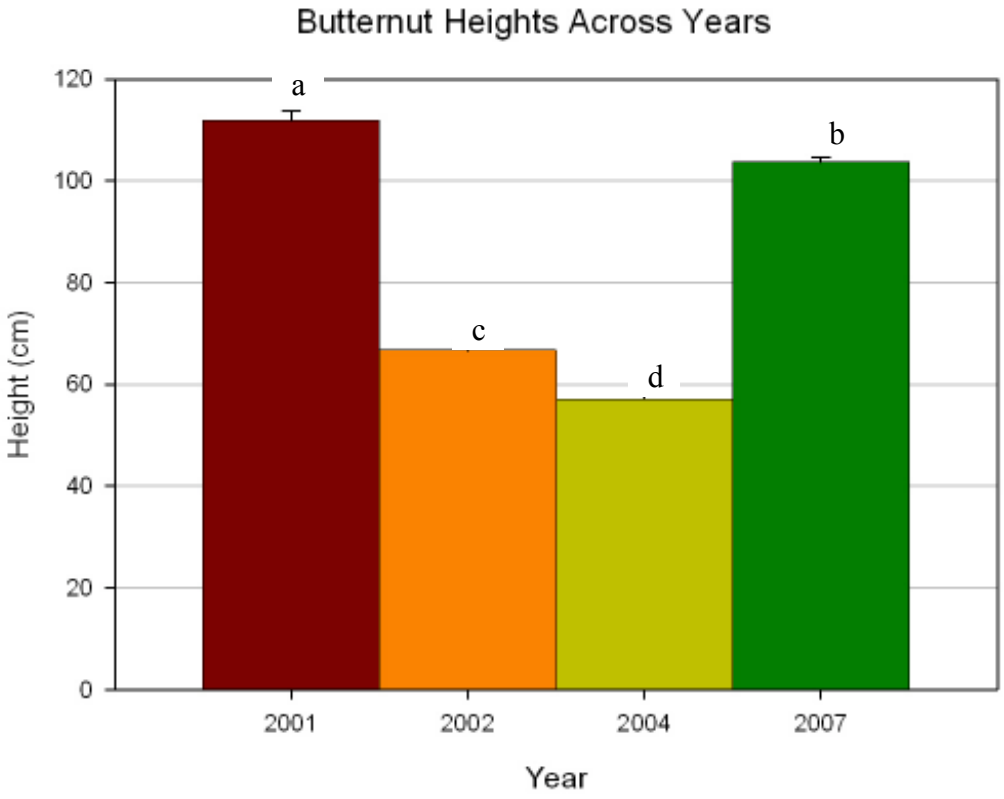
Table III-13. Nursery Trial 2: Genetic heritabilities of nursery stock (1-0) butternut seedling characteristics, including height, root collar diameter and first order lateral root number, across family of origin and individual seedlings.

Variable	Provenance Range	Family Range	Heritabilities of family means (h^2)	Individual seedling heritabilities (se_h^2)
HT[1]	56.1—89.4	42.5—110.3	0.35590	0.11727
RCD[2]	11.4—16.1	11.3—20.2	0.55523	0.16341
FOLR[3]	12.9—16.7	10.6—19.3	0.52455	0.15671
VOL[4]	19.3—24.6	19.7—72.6	0.45606	0.14122

Table III-14. Nursery Trial 2: Phenotypic and genetic correlations between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, first-order lateral root numbers, and root volume.

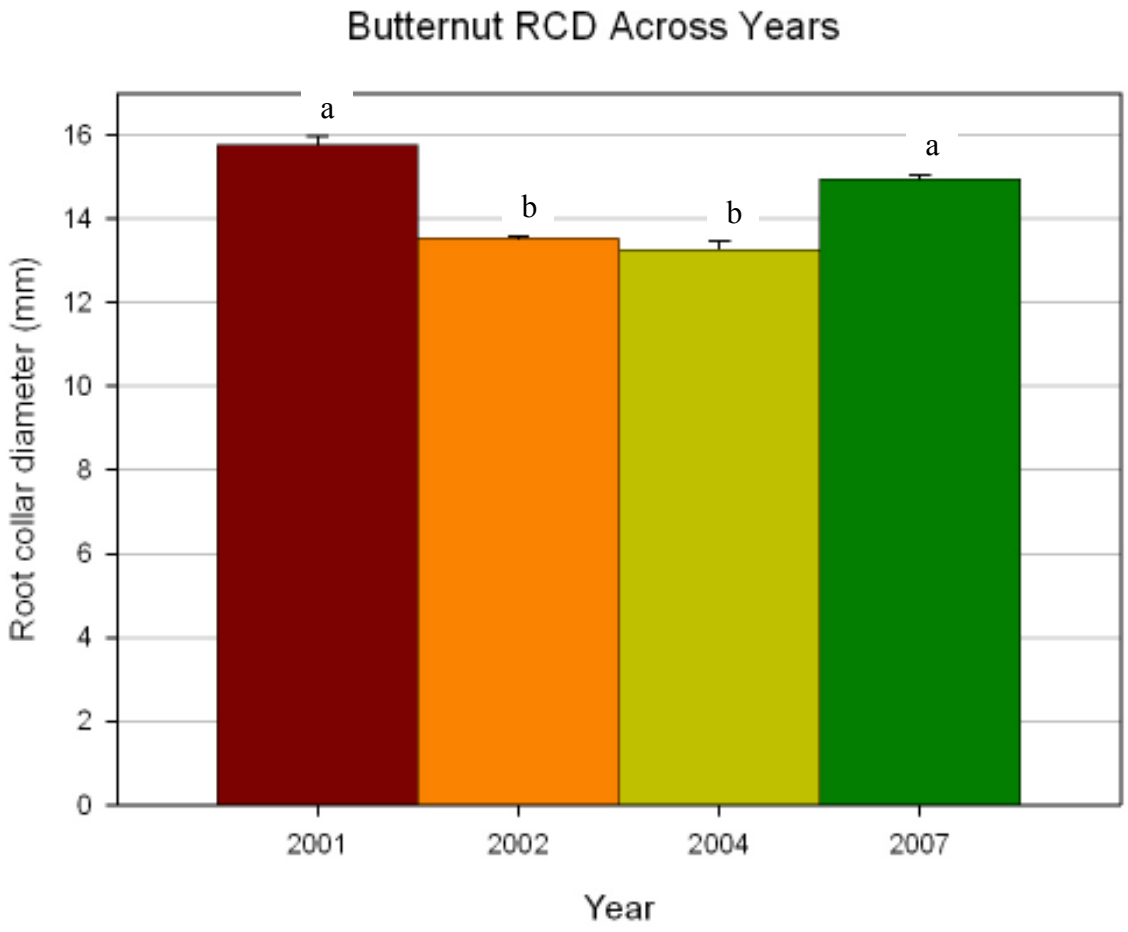
Variables	Phenotypic Correlations		Genetic Correlations
	r^2	P value	
HT-RCD	0.66	p<0.0001	0.53
HT-FOLR	0.29	p<0.0001	0.05
HT-VOL	0.50	p<0.0001	0.06
RCD-FOLR	0.53	p<0.0001	0.54
RCD-VOL	0.77	p<0.0001	0.68
FOLR-VOL	0.45	p<0.0001	0.04

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root
[4] Root volume measured by water displacement



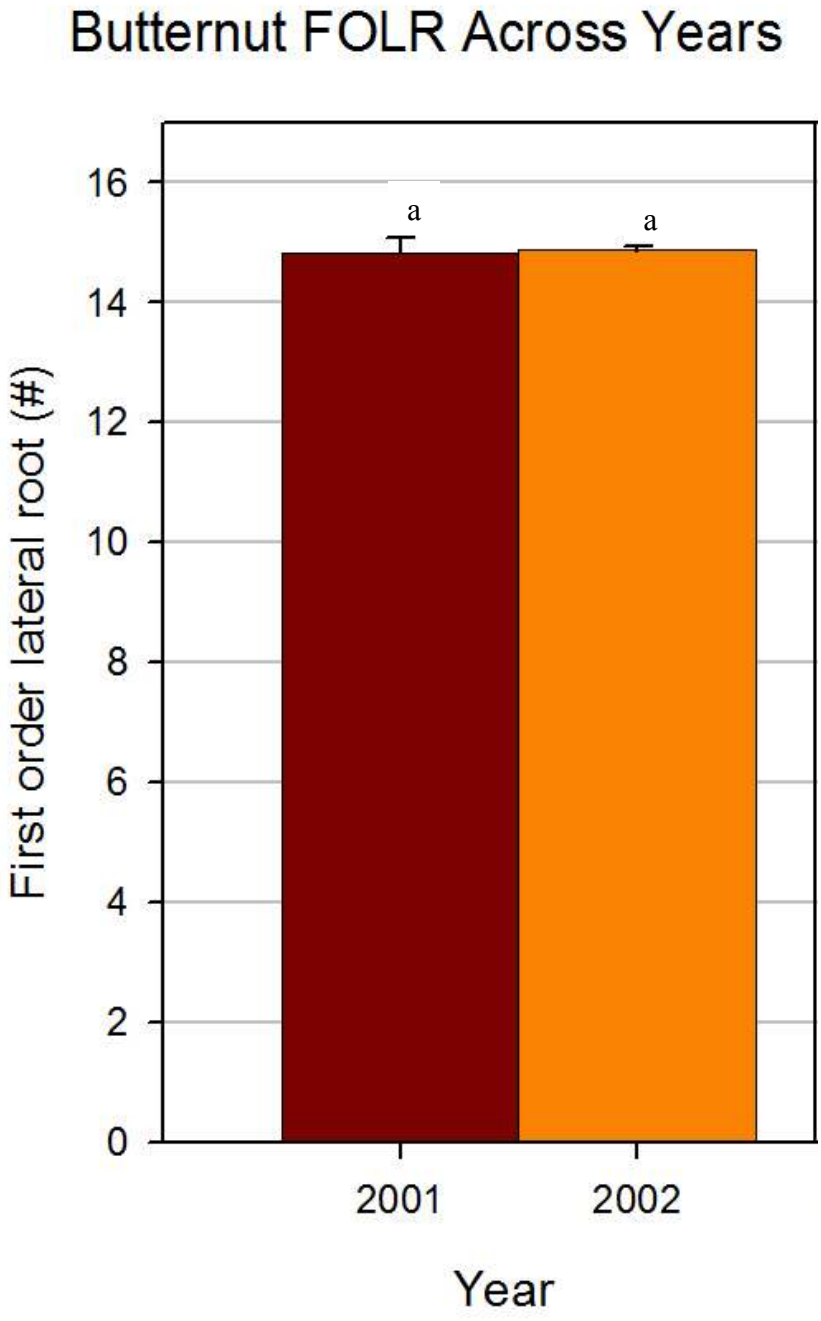
Means followed by different letters indicate significant differences at p=0.05 level based on Tukey-Kramer means separation analysis.

Figure III-1. Nursery Trial Totals: Annual variation in initial height.



Means followed by different letters indicate significant differences at p=0.05 level based on Tukey-Kramer means separation analysis.

Figure III-2. Nursery Trial Totals: Annual variation in initial root collar diameters.



Means followed by different letters indicate significant differences at p=0.05 level based on Tukey-Kramer means separation analysis.

Figure III-3. Nursery Trial Totals: Annual variation in initial first order lateral root number.

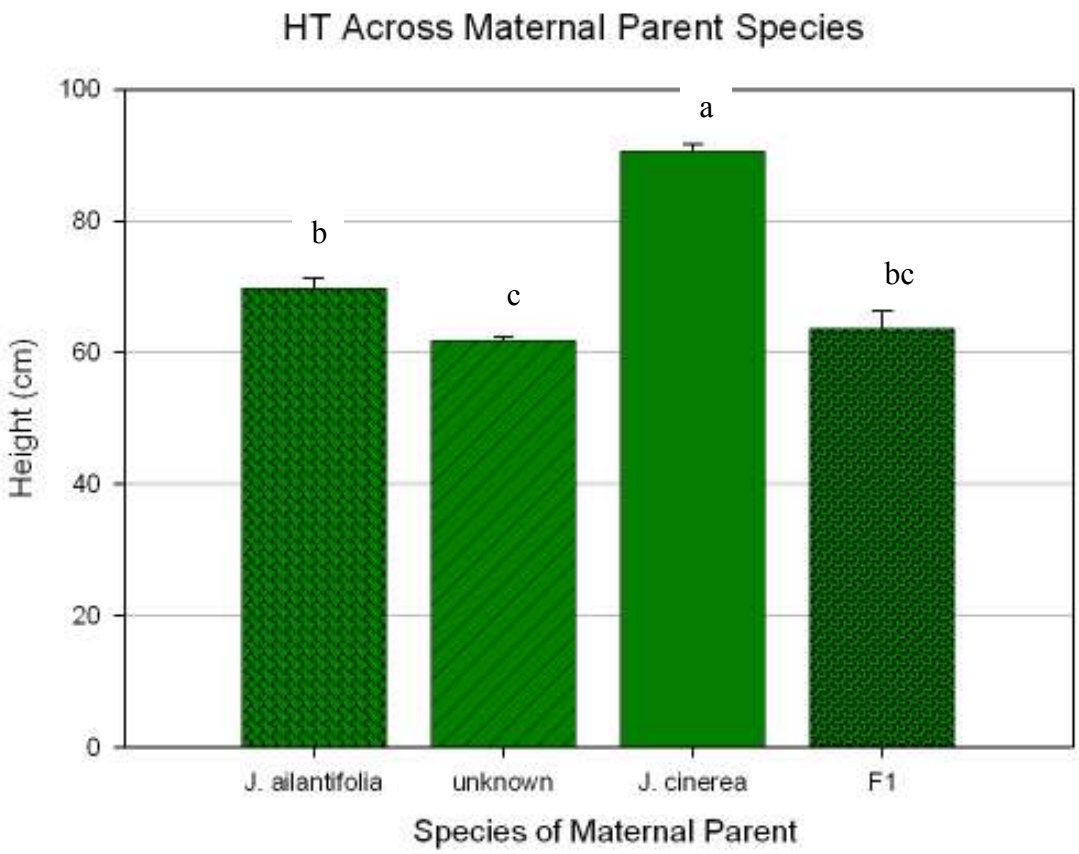


Figure III-4. Nursery Trial Totals: Species variation in heights.

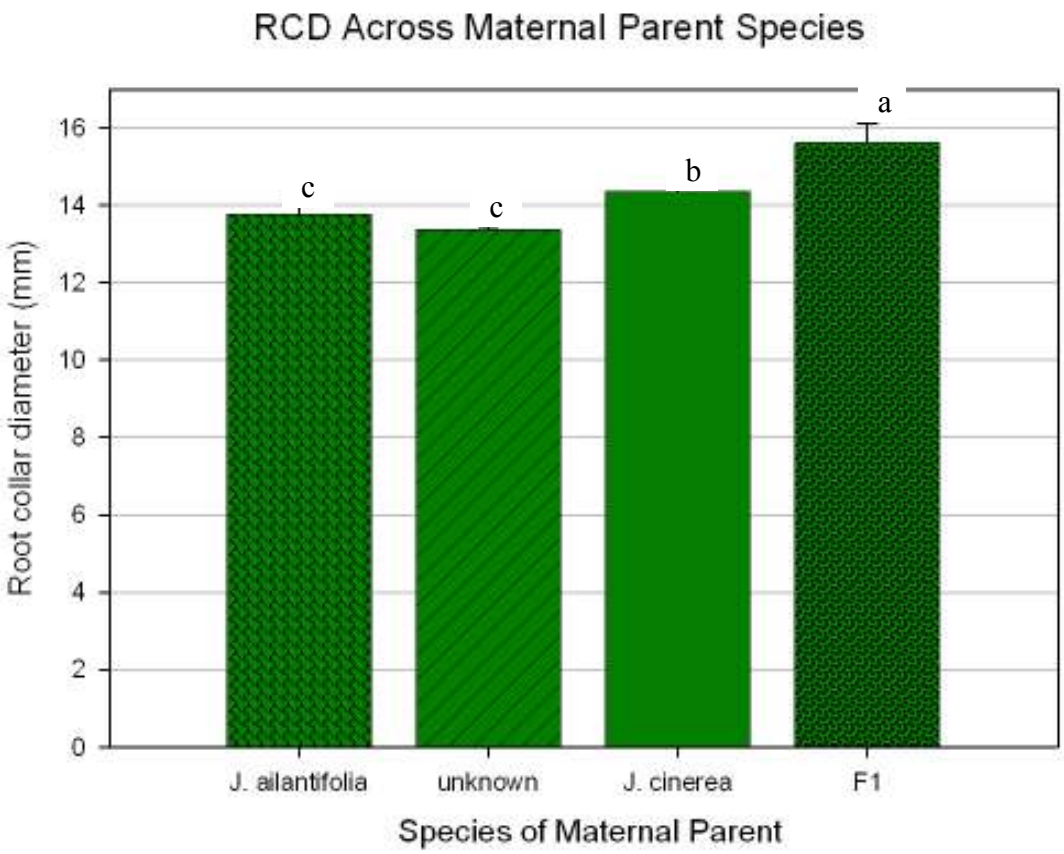


Figure III-5. Nursery Trial Totals: Species variation in root collar diameter.

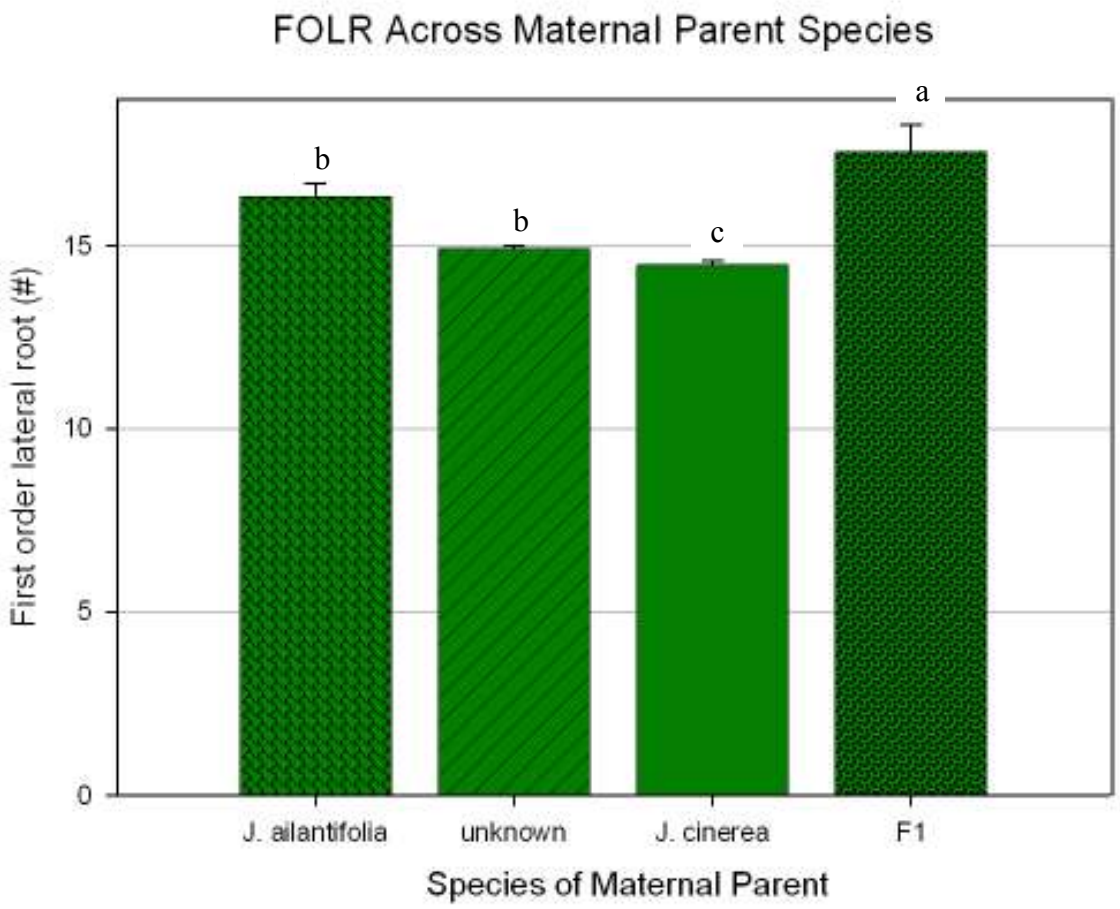


Figure III-6. Nursery Trial Totals: Species variation in initial first order lateral root number.

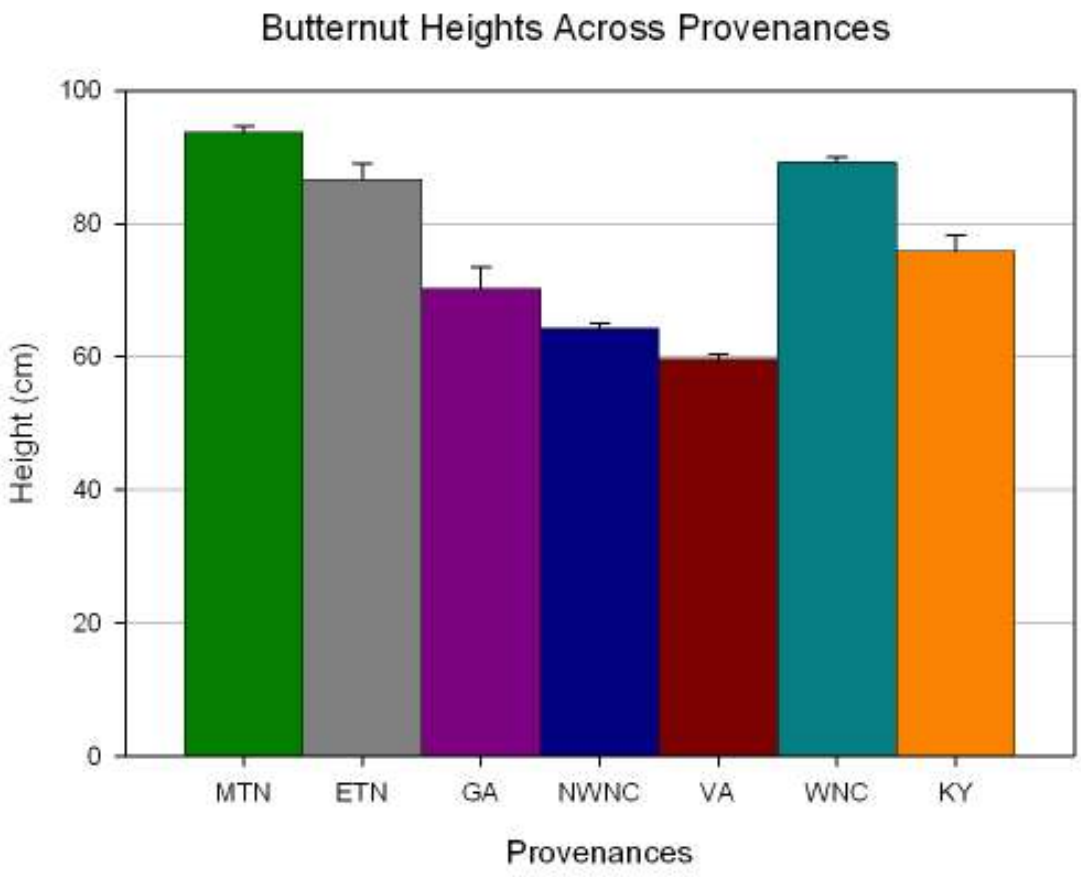


Figure III-7. Nursery Trial Totals: Provenance variation in initial height.

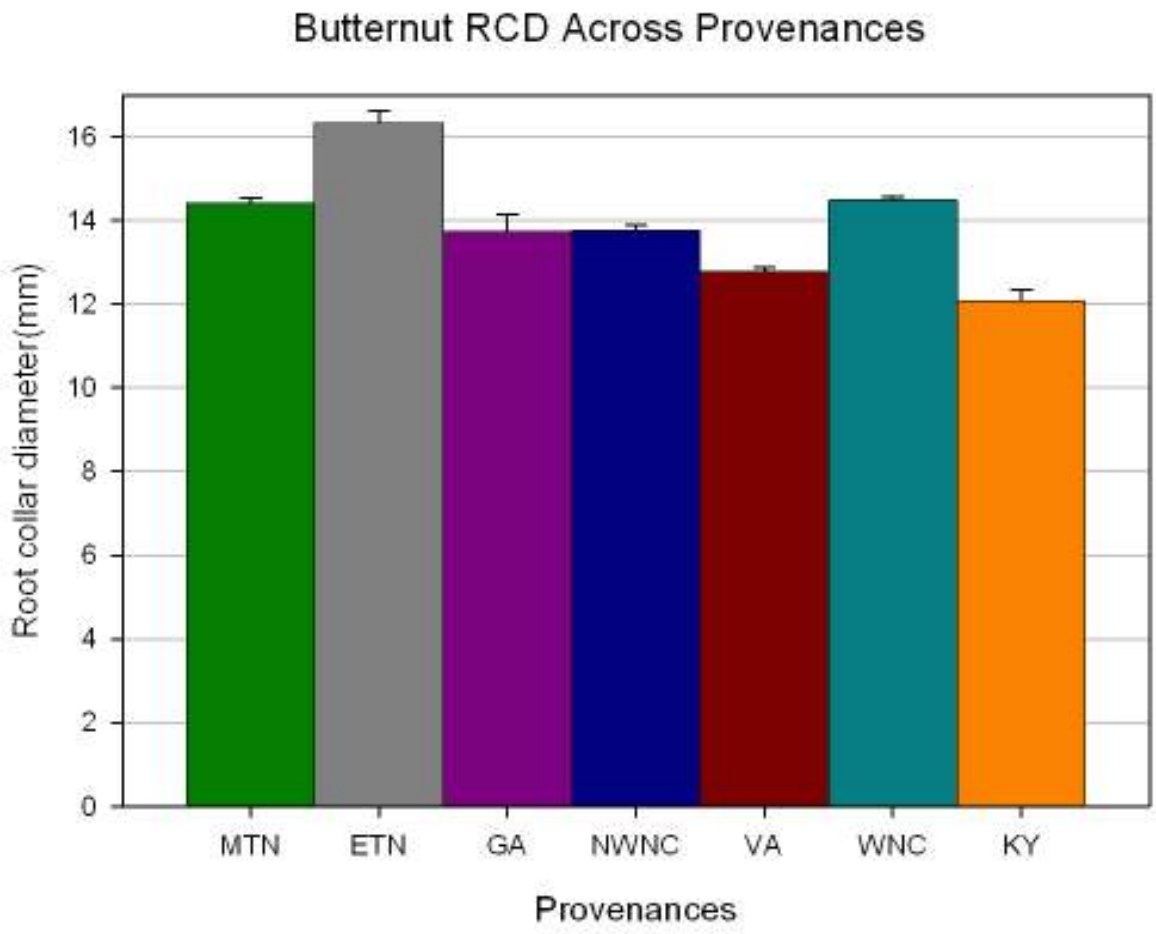


Figure III-8. Nursery Trial Totals: Provenance variation in initial root collar diameters.

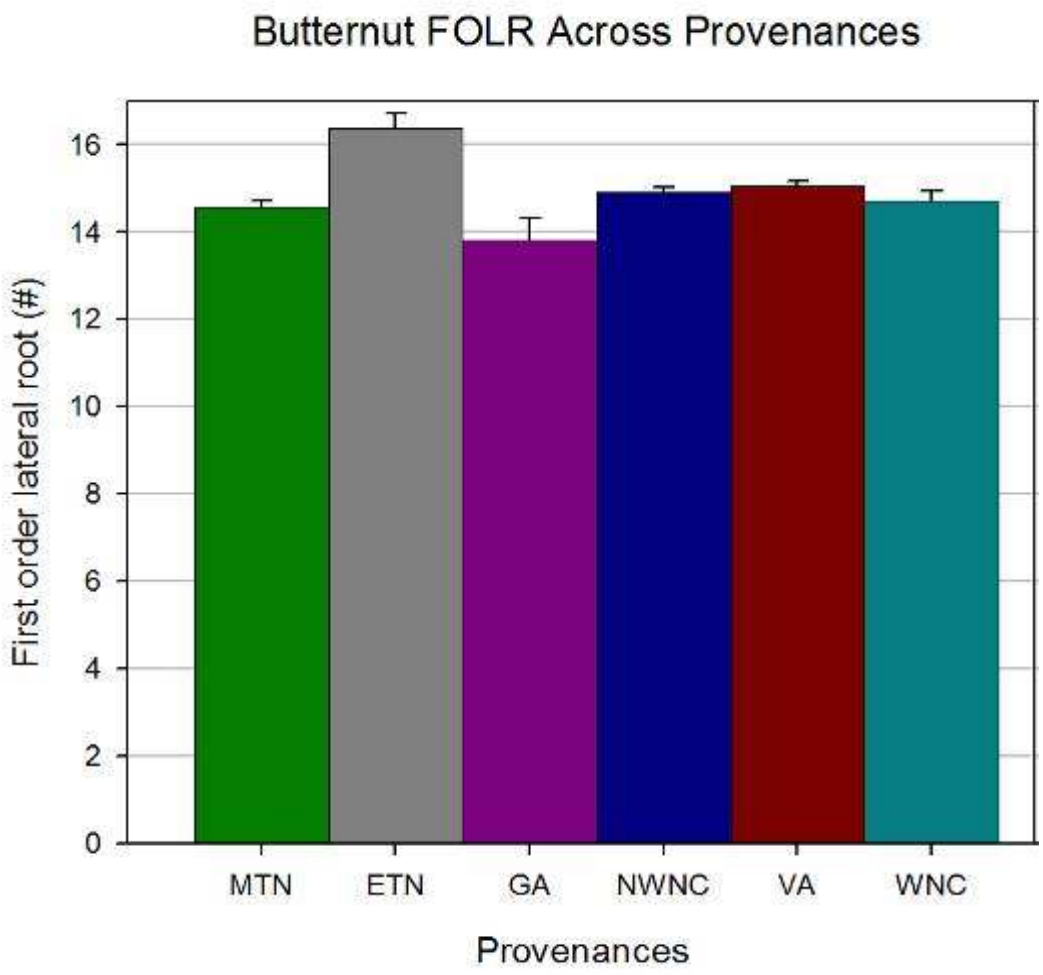


Figure III-9. Nursery Trial Totals: Annual variation in initial first order lateral root number.

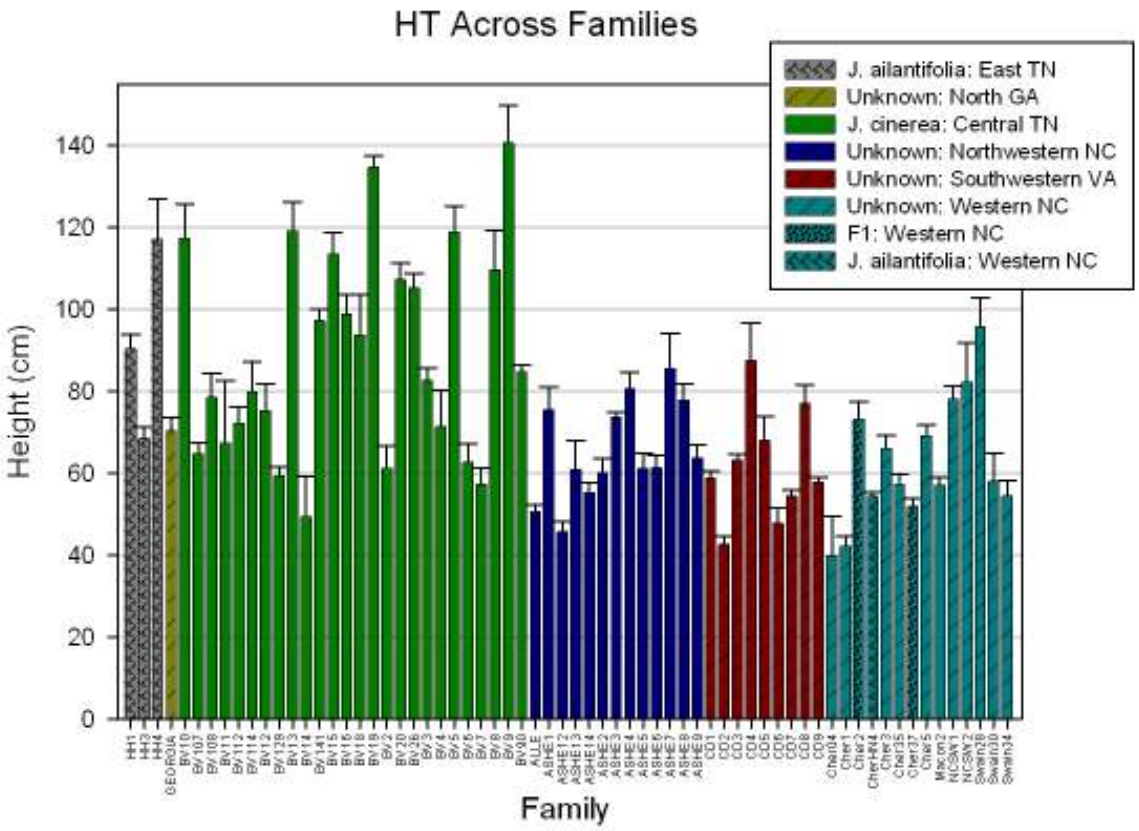


Figure III-10. Nursery Trial Totals: Family variation in initial height.

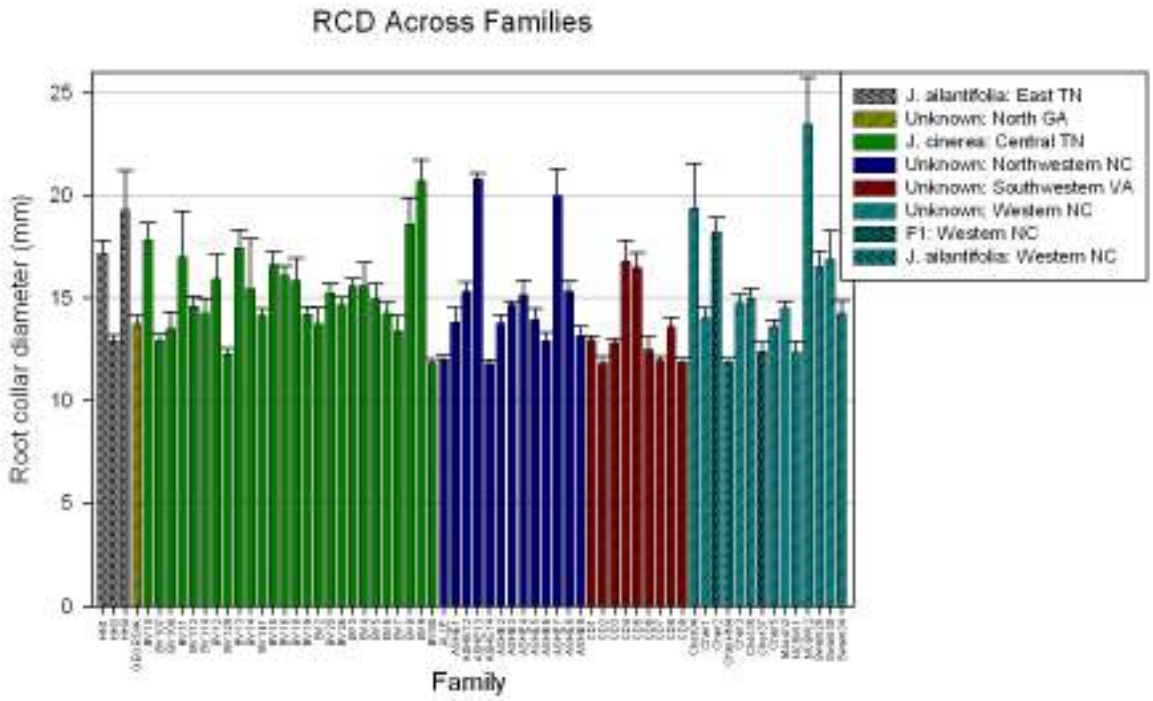


Figure III-11. Nursery Trial Totals: Family variation in initial root collar diameters.

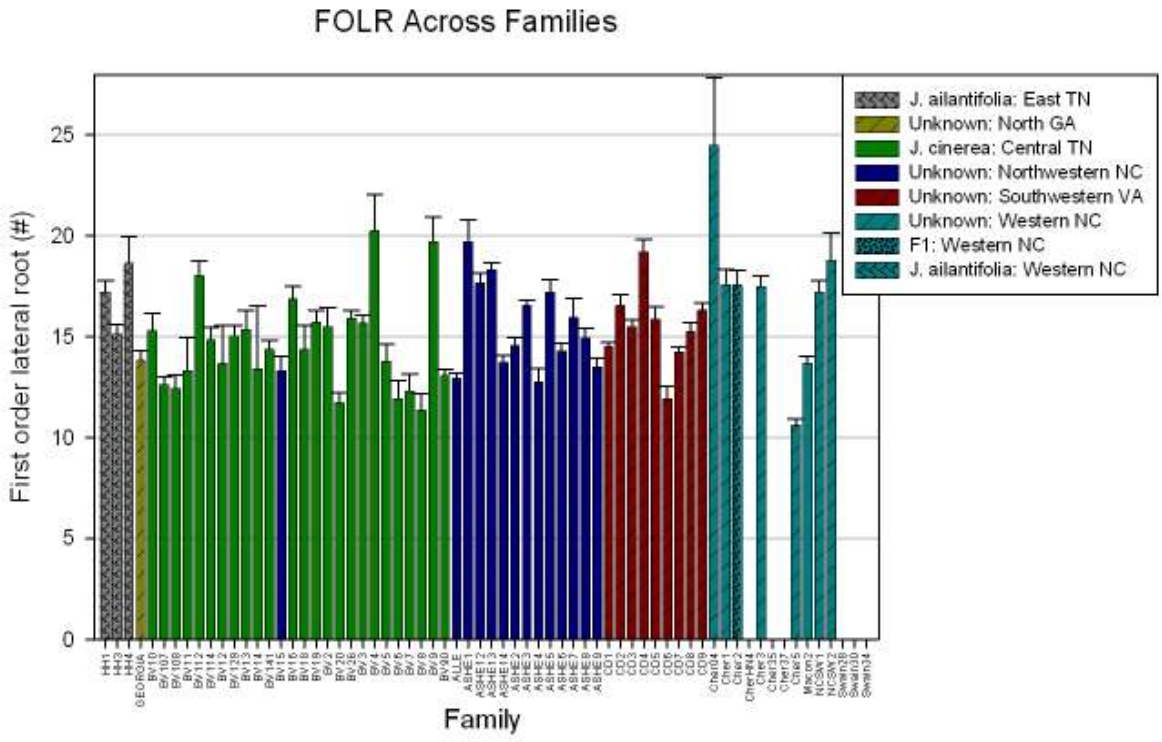


Figure III-12. Nursery Trial Totals: Family variation in initial first order lateral roots.

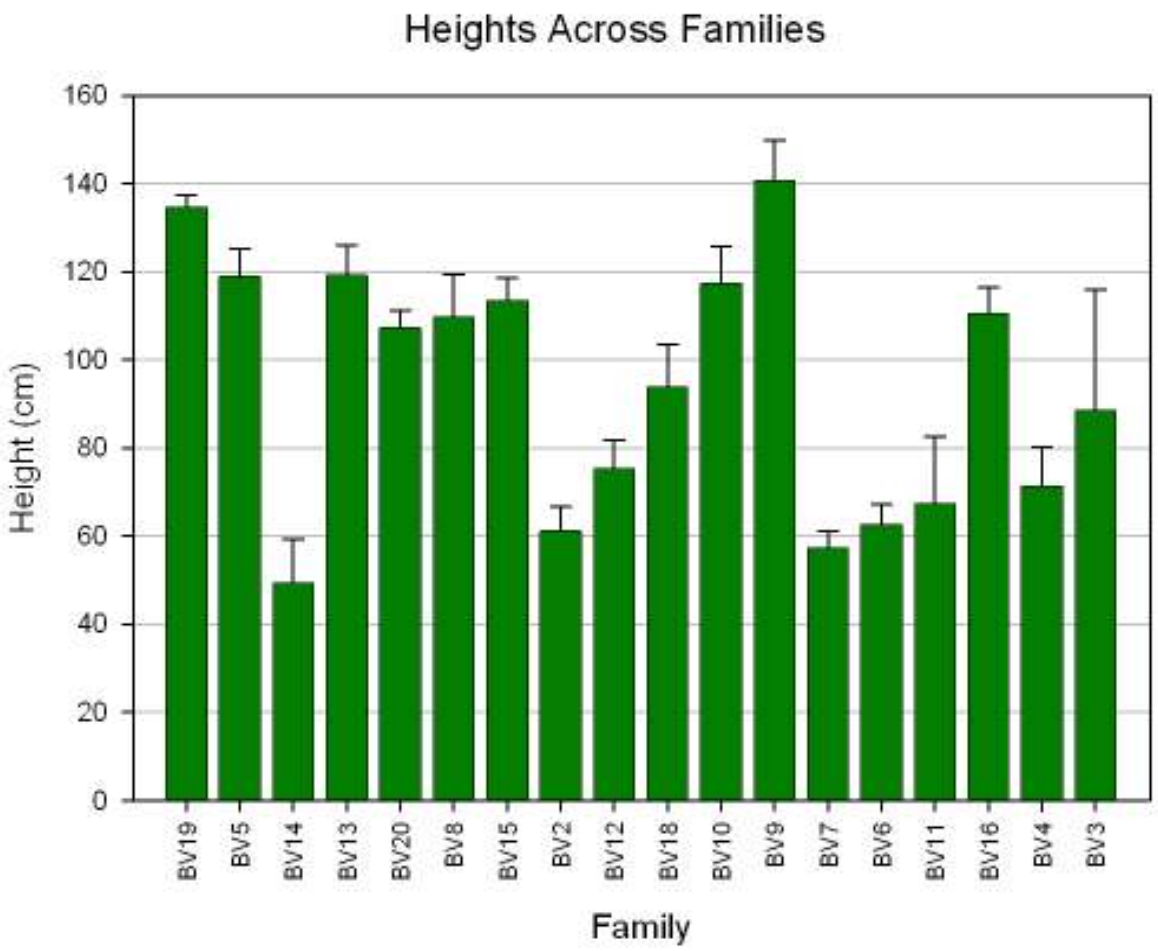


Figure III-13. Height of 1-0 butternut seedlings from across family of origin for Nursery Trial 1.

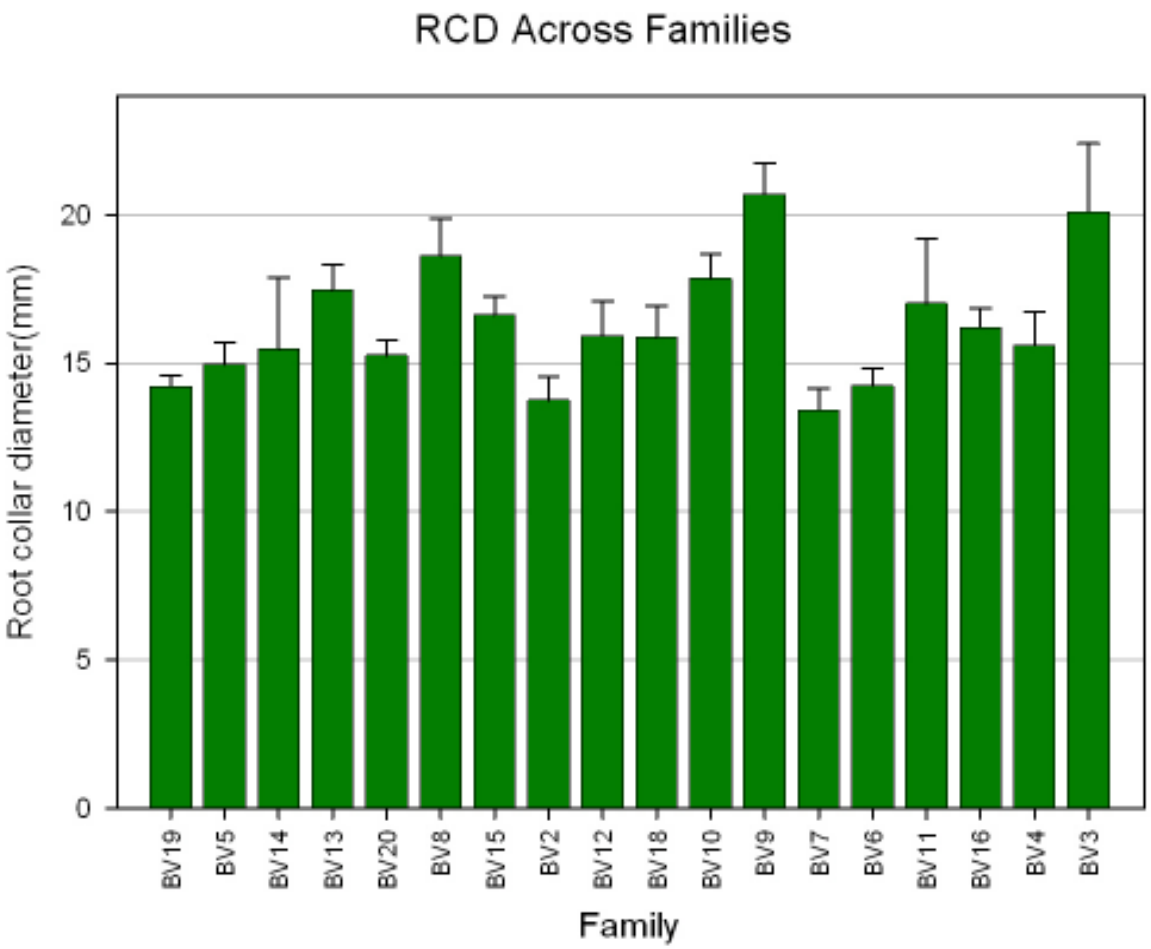


Figure III-14. Root collar diameters of 1-0 butternut seedlings from across family of origin for Nursery Trial 1.

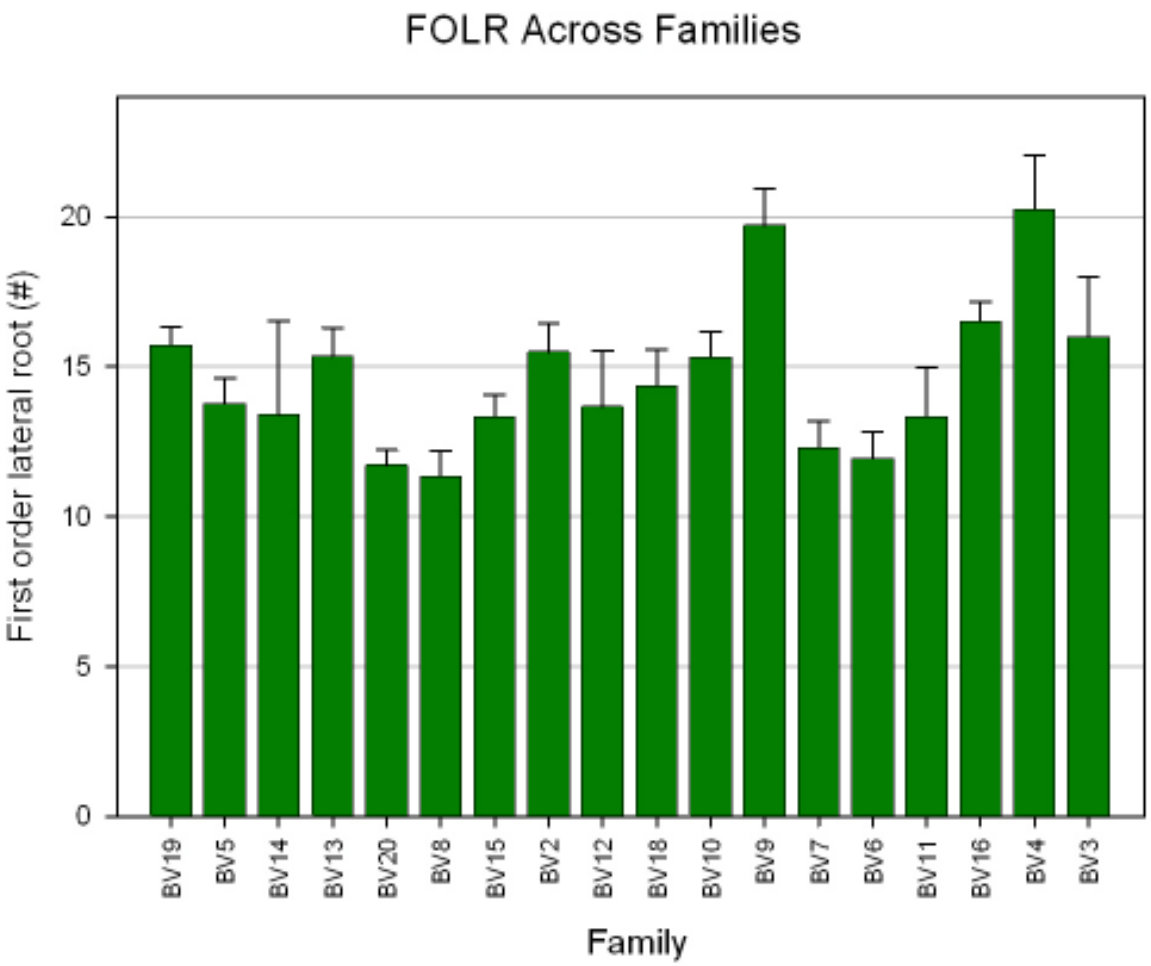


Figure III-15. First-order lateral root number of 1-0 butternut seedlings from across family of origin for Nursery Trial 1.

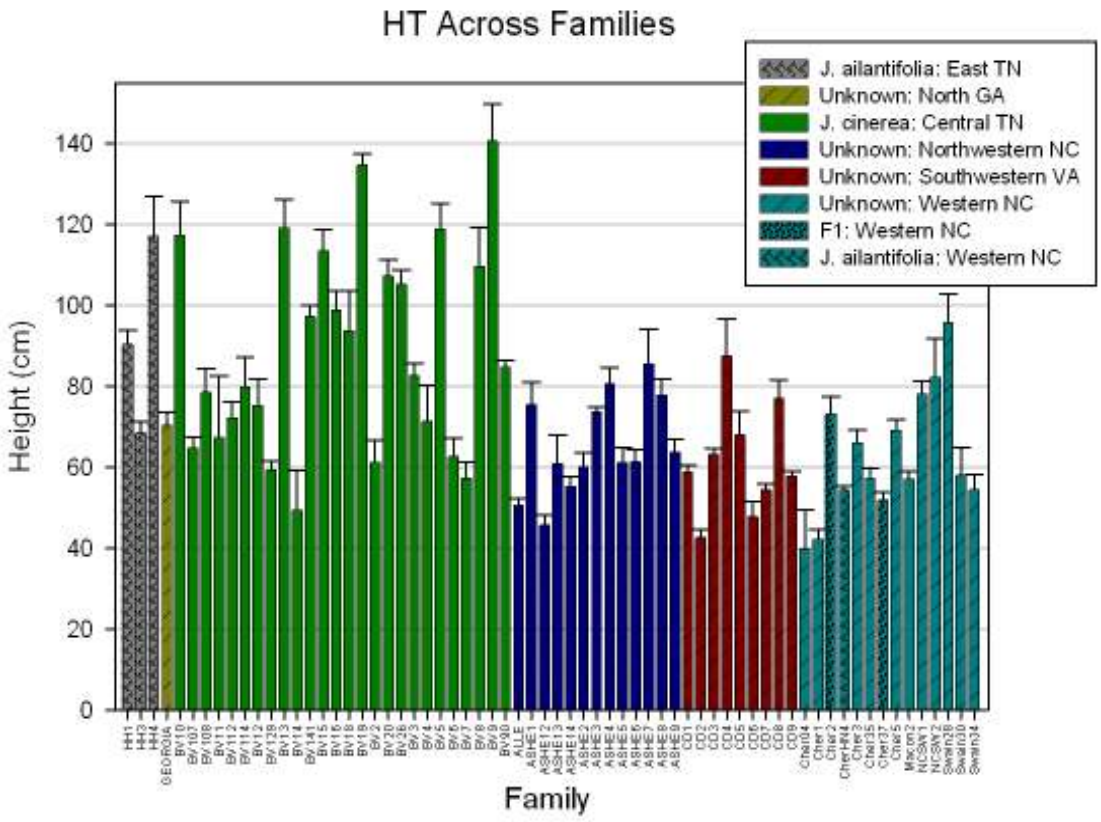


Figure III-16. Height of 1-0 butternut seedlings from across family of origin for Nursery Trial 2.

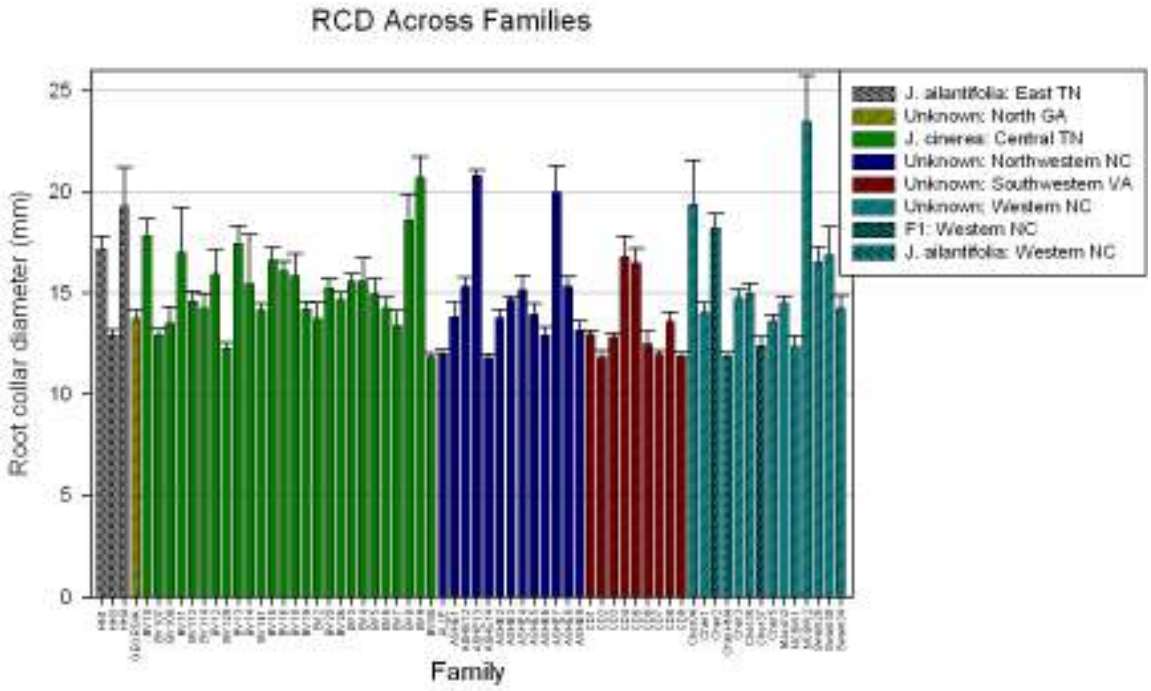


Figure III-17. Root collar diameter of 1-0 butternut seedlings from across family of origin for Nursery Trial 2.

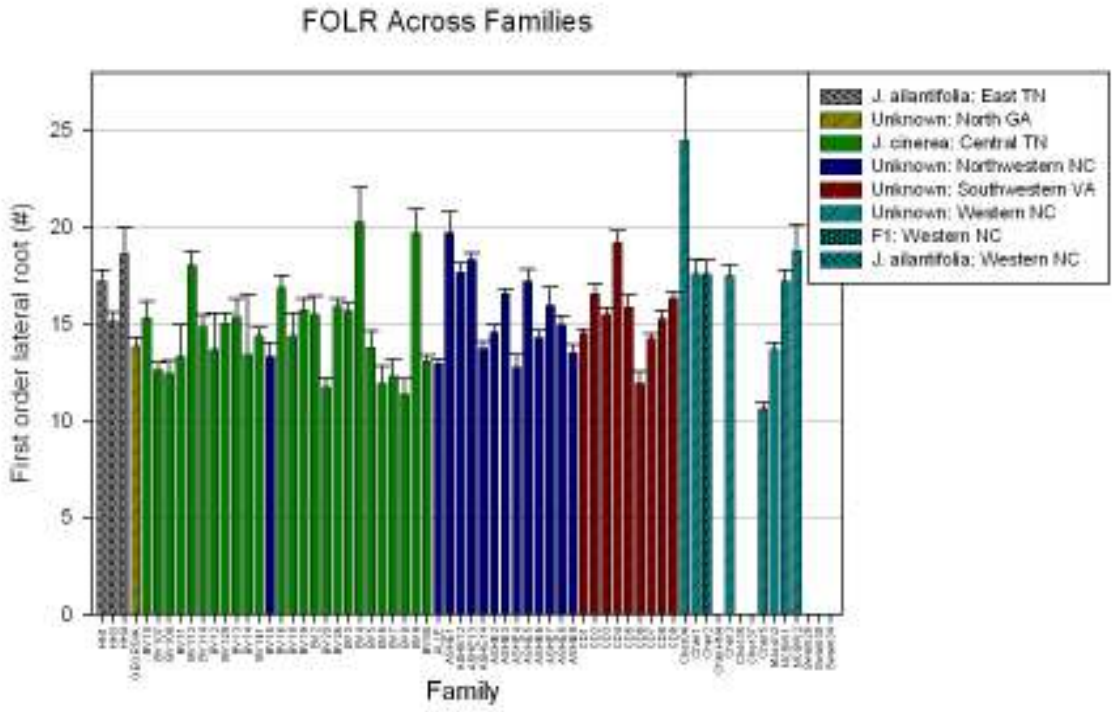


Figure III-18. First-order lateral root number of 1-0 butternut seedlings from across family of origin for Nursery Trial 2.

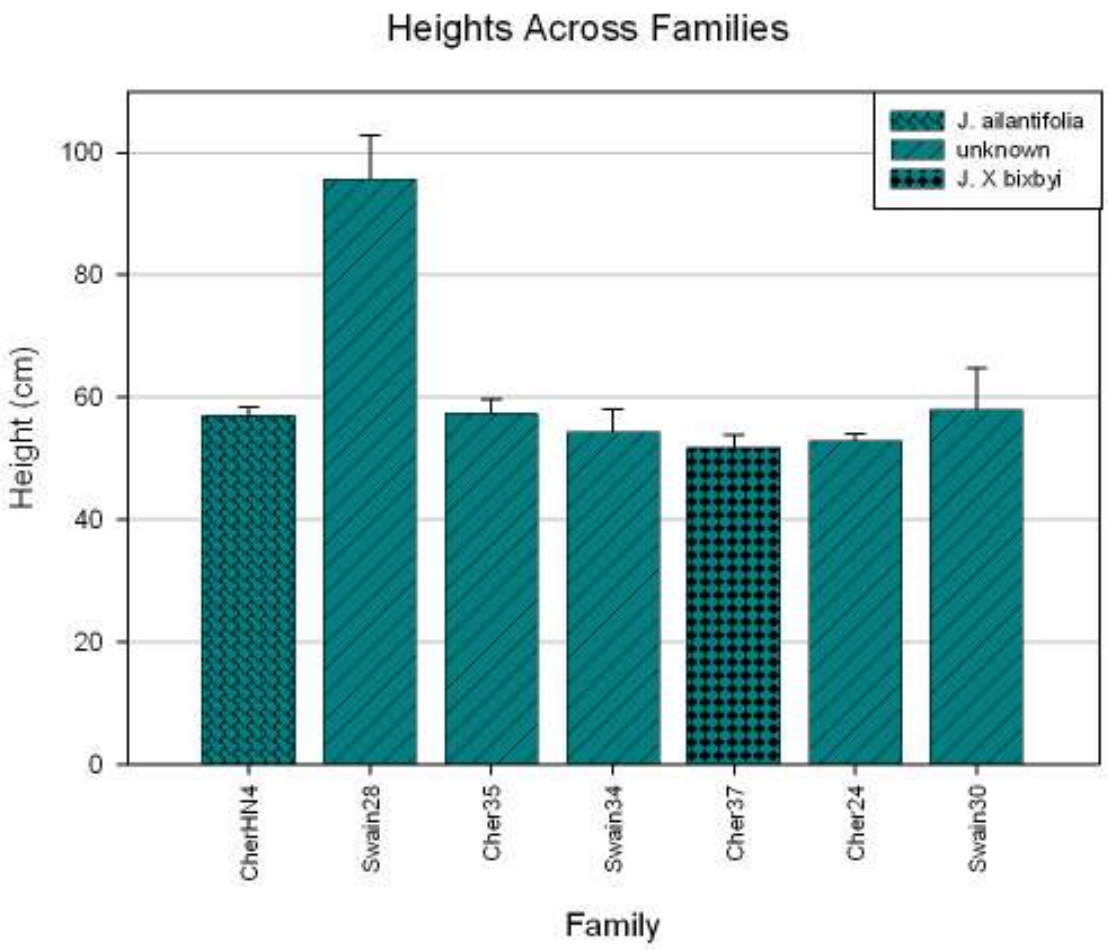


Figure III-19. Height of 1-0 butternut seedlings from across family of origin for Nursery Trial 3.

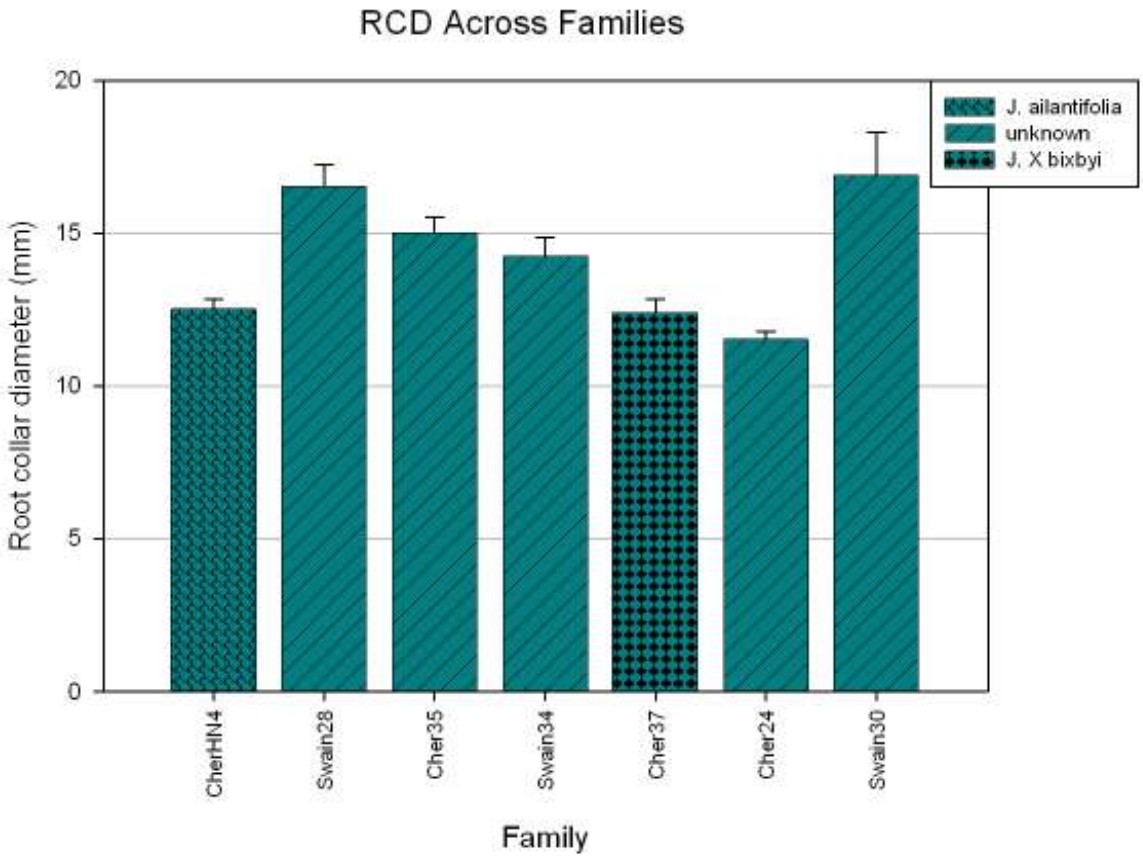


Figure III-20. Root collar diameter of 1-0 butternut seedlings from across family of origin for Nursery Trial 3.

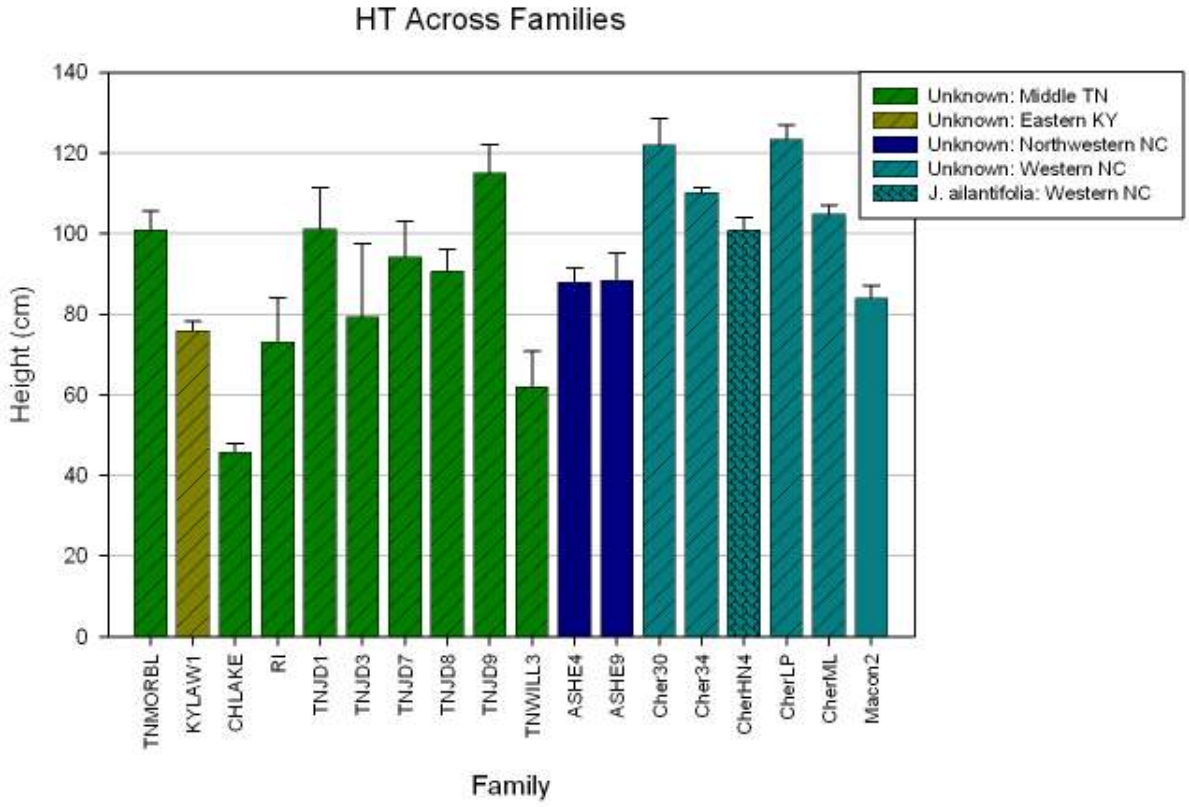


Figure III-21. Height of 1-0 butternut seedlings from across family of origin for Nursery Trial 4.

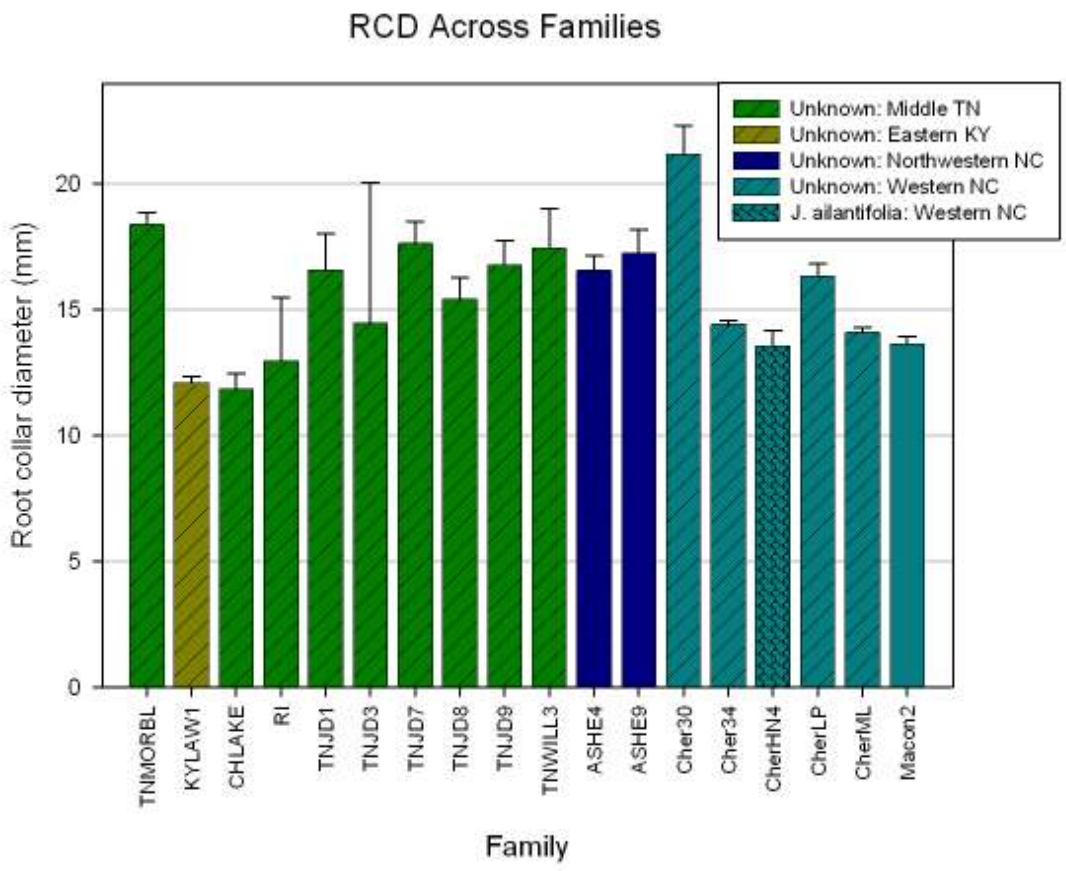


Figure III-22. Root collar diameter of 1-0 butternut seedlings from across family of origin for Nursery Trial 4.

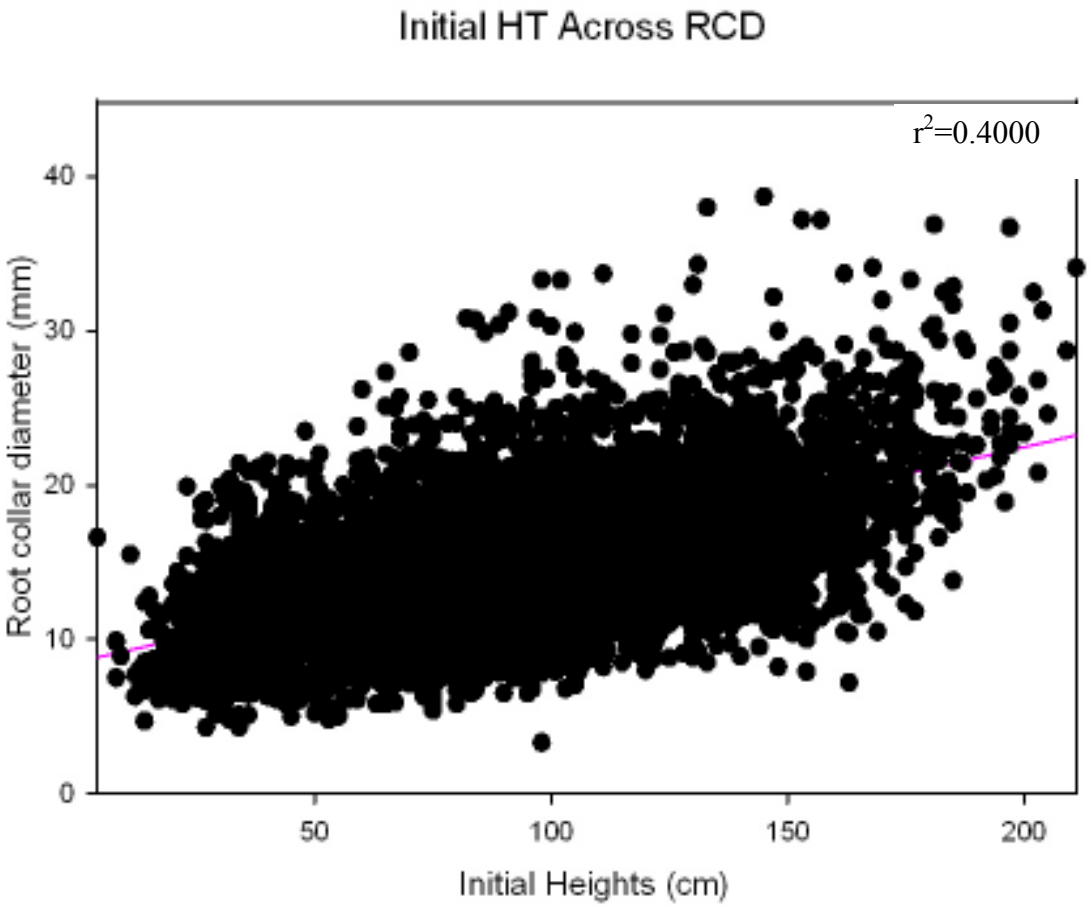


Figure III-23. Nursery Trial Totals: Variation in initial heights across initial root collar diameters.

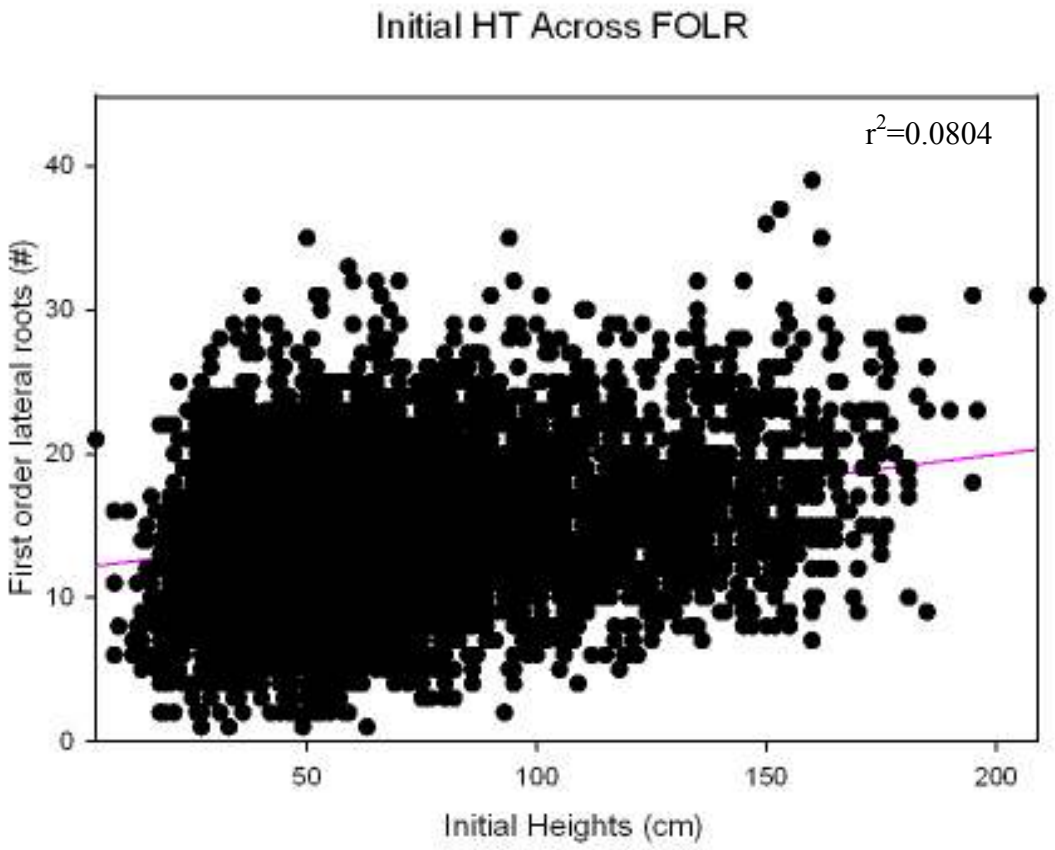


Figure III-24. Nursery Trial Totals: Variation in initial heights across initial first order lateral root numbers.

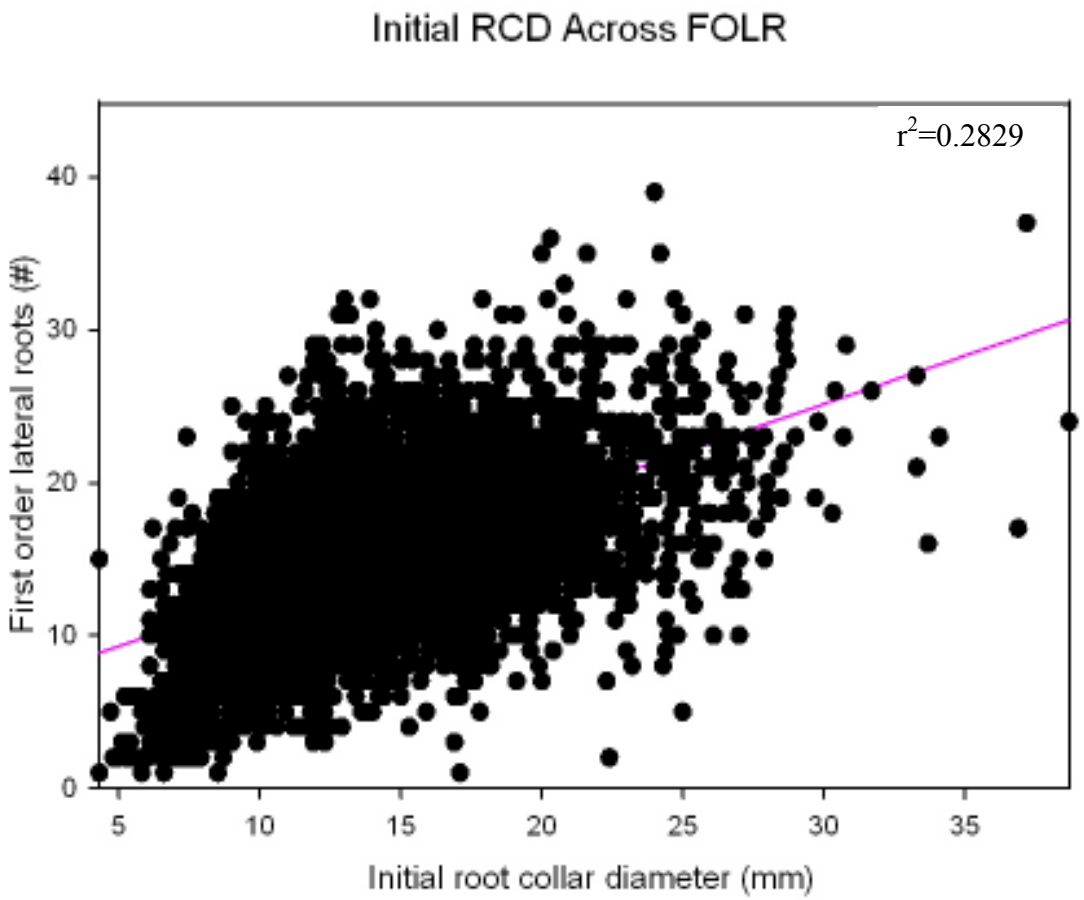


Figure III-25. Nursery Trial Totals: Variation in initial root collar diameters across initial first order lateral root numbers.

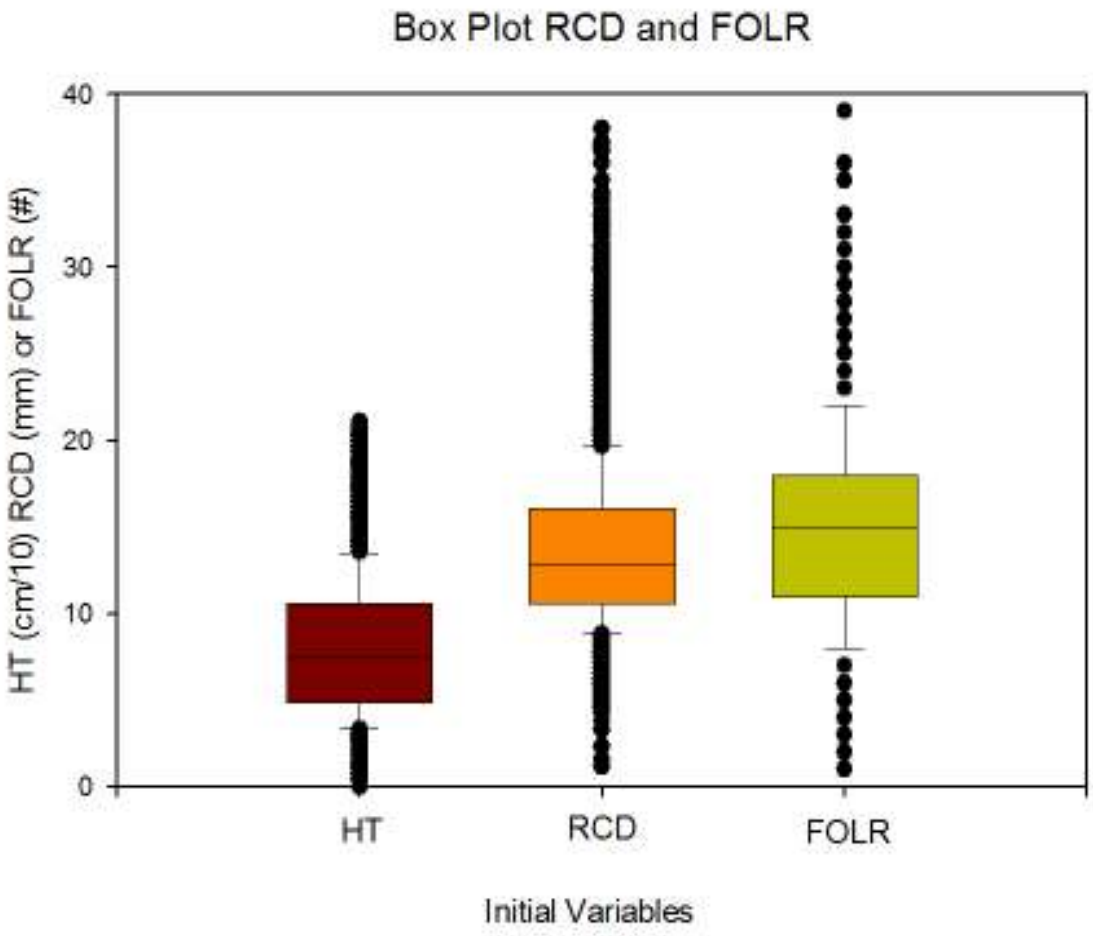


Figure III-26. Nursery Trial Totals: Distribution of initial characteristics of height, root collar diameter and first order lateral root number.

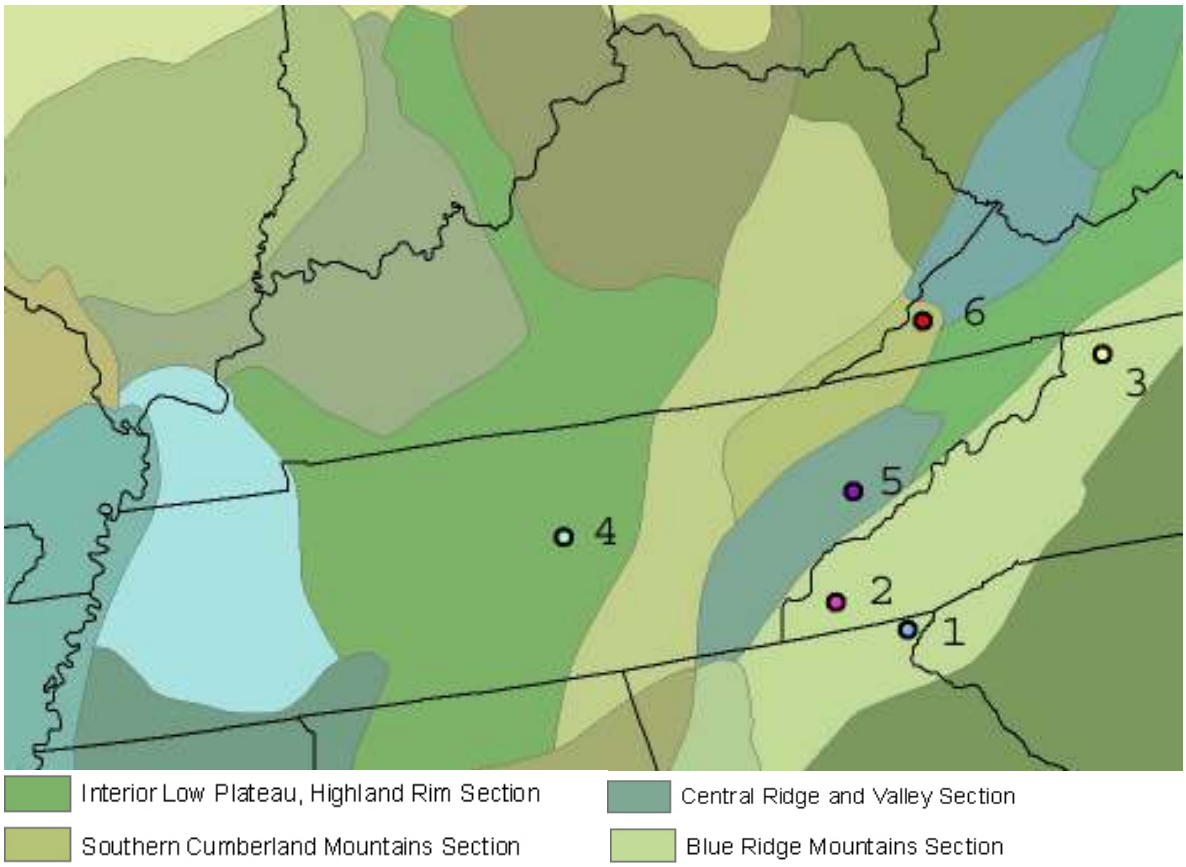


Figure III-27. Nursery Trial 2, origins of provenances for the 42 parent trees where butternut seeds were collected delineated by Bailey's Ecoregions (Bailey 1995) in the Southern Appalachians.

1. Northern Georgia, N-GA, Blue Ridge Mountains
2. Western North Carolina, W-NC, Blue Ridge Mountains
3. Northwestern North Carolina, N-NC, Blue Ridge Mountains
4. Central Tennessee, M-TN, Interior Low Plateau, Highland Rim
5. East Tennessee, E-TN, Central Ridge and Valley
6. Northwestern Virginia, N-VA, Southern Cumberland Mountains

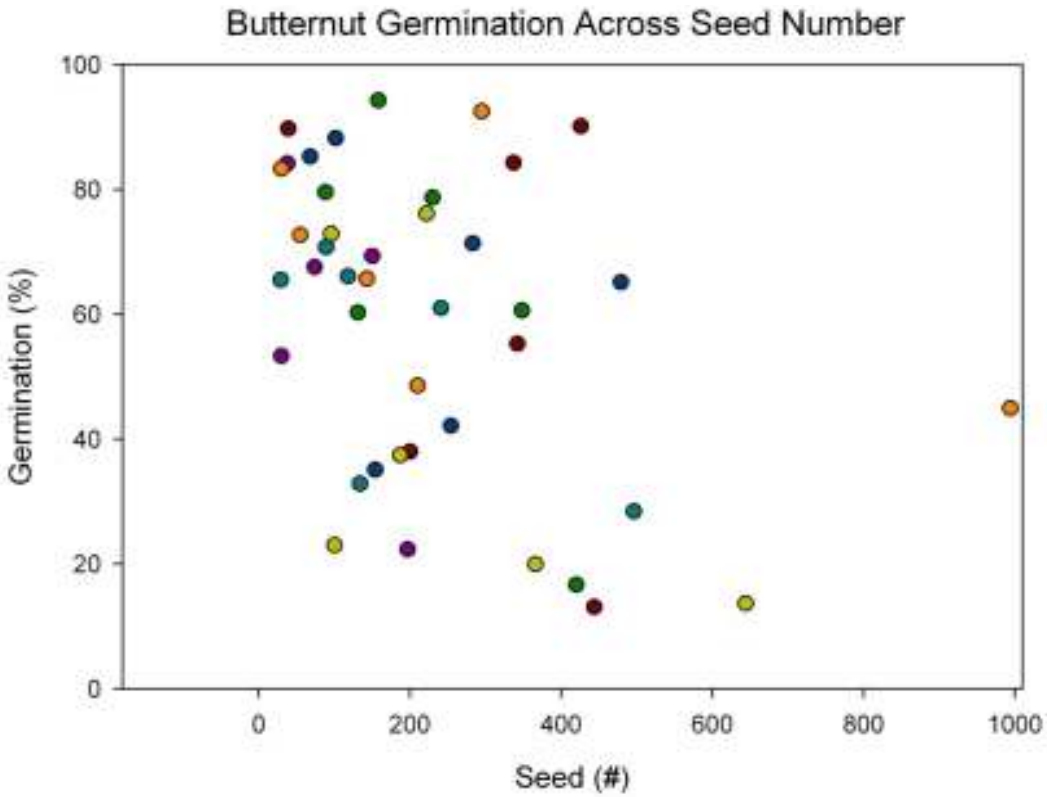


Figure III-28. Nursery Trial 2, percent germination of butternut seed across numbers of seed collected. $R^2 = 0.1592$

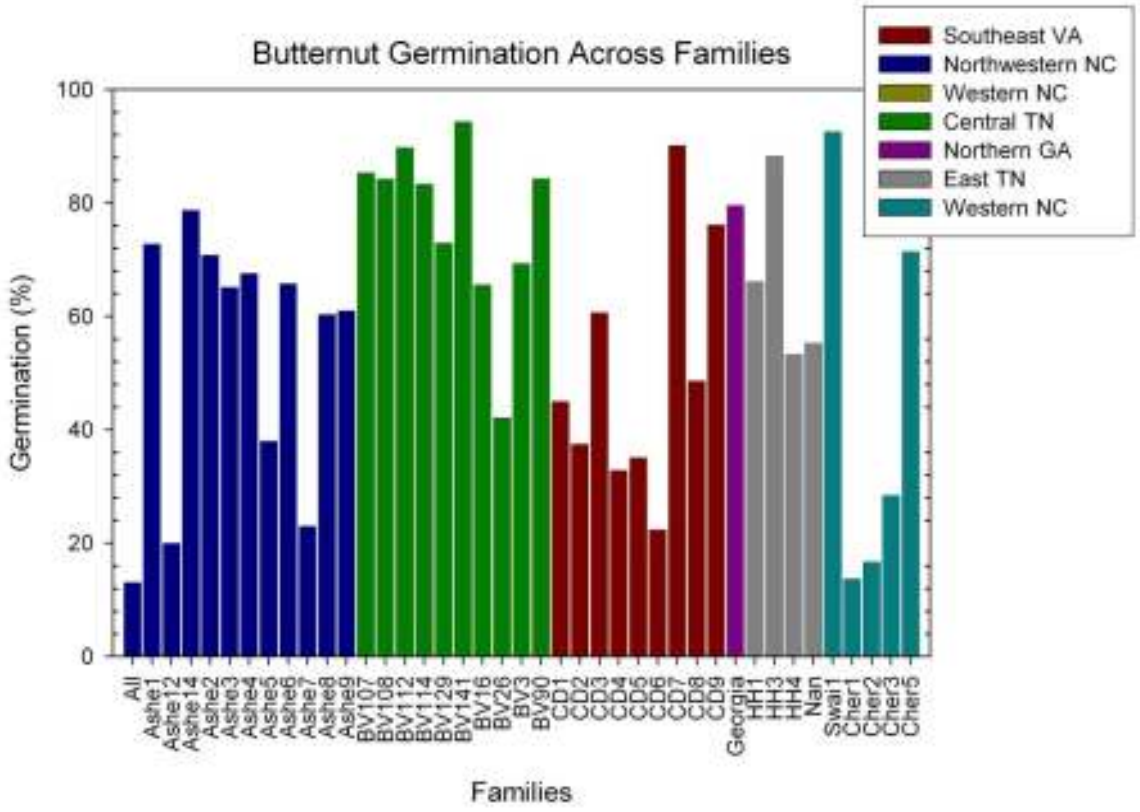


Figure III-29. Nursery Trial 2, percent germination of butternut seed across provenance and family of origin.

PART IV. SURVIVAL, GROWTH, AND DISEASE OF BUTTERNUT PROGENY IN RESISTANCE SCREENING PLANTINGS

Abstract

Butternut, *Juglans cinerea* L, a tree native to eastern North America, is another addition to the list of species being eliminated from forests due to an introduced fungal pathogen. Butternut canker disease, caused by *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka & Kuntz) a species identified in the late 1970s, causes stem and branch cankers that can girdle trees of all sizes and ages (Nicholls 1979, Ostry *et al.* 1994). However, hope for restoration lies in observations of non-infected or healthy trees proximal to infected, diseased, and dying trees (Ostry *et al.* 1994). The healthy trees could hold genetic-based resistance mechanisms that would allow for restoration using a breeding approach (Schlarbaum *et al.* 1997). Research has yet to link putative field condition and genetic-based resistance through screenings (Millikan *et al.* 1990, Davis and Meyer 1997, McIlwrick *et al.* 2000). Thirty-six open-pollinated families from six provenances were placed in an area of high inoculation potential to screen for genetic resistance. Three families from heartnut (*J. ailantifolia* var. *cordiformis* (Masim.) Rehd.) parents and one verified F_1 hybrid (*J.* X *bixbyi*) were used as controls. Offspring of the four *J. ailantifolia* and *J.* X *bixbyi* experience no evidence of cankering, but high rates of mortality and limited height growth. For families from *J. cinerea* parents and unknowns; family and site differences existed for cankering. Specific genetic families with no cankering and greater than average survival were concentrated in origin from one specific provenance in central Tennessee. Many individuals from this population were screened for hybridization resulting in no evidence of *J.* X *bixbyi* (Hoban *et al.* 2009). This indicates that resistance may not be primarily a result of introgression.

After four years since outplanting, survival and growth under conditions of disease varied significantly across families and provenances of origin. Though the average survival was 45%, specifically, four families had over 80% survival and two families had less than 20%. Two provenances had over twice the average height of the remaining provenances. Initial seedling sizes, including height, root collar diameter, and first-order lateral root (FOLR) number showed statistically significant correlations with future seedling sizes and

survival, during the four years since outplanting. Taller seedlings were consistently larger after four years, but showed no difference in survival. Seedlings with RCD less than nine mm had 70% mortality, and seedlings under 11mm when planted were 34% shorter than seedlings greater than 20mm in diameter. Seedlings with less than nine FOLRs had over 70% mortality and were 20% shorter and 27% smaller in diameter. Continual gains from additional FOLR numbers were not apparent. Planting site were also statistically significant with higher growth and increased survival in sites with increased light, including 95% survival of seedlings planted in full sun. Resistance screening plantings are hindered by the inability of butternut to establish and grow in the understory of a forest canopy. Overall, seedling establishment was made difficult by transplant shock and infection by *Phytophthora* root rot, rooting by non-native wild hogs, and flooding. Future restoration efforts should focus on determining adequate sites for restoration.

Introduction

Butternut, *Juglans cinerea* L, is a lesser known relative of black walnut, *J. nigra* L., but is in a taxonomically distinct section of *Juglans* L. (Stanford *et al.* 2000). Butternut is utilized for veneer, cabinets, and carving and is an important source of hard mast for wildlife. Populations of butternut have been declining due to an introduced pathogen, *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka & Kuntz), which is the causal agent for butternut canker disease. The disease causes multiple stem and branch cankers that girdle and kill trees of all ages and size classes (Ostry *et al.* 1994). Once a butternut population is infected, the lack of sprouting can result in permanent loss of a particular gene pool. Low genetic diversity has been found in parts of the range of butternut (Busov *et al.* 1997, Fjellstrom and Parfitt 1994, Morin *et al.* 2000; Hoban *et al.* 2010), so any additional loss of trees could contribute to inbreeding depression.

Before extirpation of the species occurs, efforts can be made to conserve genetic diversity of sensitive populations in order to aid the process of restoring butternut to its former levels of abundance. Restoration of the species may rely on genetic resistance to butternut canker disease, as healthy trees have been found in close proximity to dead or dying trees (Ostry *et al.* 1994). Butternut canker disease has been impacting populations since at

least the 1920s, and therefore the remaining populations are often disjunct (Ostry *et al.* 2004, Hoban et al. 2009; non-published data UT- Tree Improvement Program). The presence of surviving trees could be related to site factors, although habitat models did not suggest specific habitat requirements for putatively resistant trees compared to diseased trees (UT-Tree Improvement Program records data on file). The surviving trees may also be the result of hybridization with a nut cultivar of Japanese walnut, heartnut (*J. ailantifolia* var. *cordiformis* (Masim.) Rehd.). Nut growers in the United States have planted hybrid buartnuts, *J.* x *bixbyi* Rehd. since the early 1900s (Bixby 1919) and F_1, F_2, and hybridization has been found to be extensive in seven distinct populations (Hoban *et al.* 2009).

Regardless of the influence of hybridization, if resistance is genetically-based, a breeding approach could be a feasible strategy to produce resistant butternut trees for restoration of the species into habitats where it has been extirpated (Schlarbaum *et al.* 1997). Development of butternut canker resistant trees depends on the identification of resistant genotypes and incorporation of the genotypes into a breeding program.

The crippling effects of butternut canker disease represent just one challenge to restoring the species. Hardwood seedlings face many challenges including browsing and rubbing by white-tailed deer (*Odocoileus virginianus* Boddaert), effects of other herbivores (Marquis 1974; Martin and Baltzinger 2002), and herbaceous and hardwood competition, which overtop seedlings planted in open areas (Cogliastro *et al.* 1990, Kolb *et al.* 1990, Willoughby and McDonald 1999). Variability in planting stock quality of hardwood seedlings often results in planting failures and low vigor of seedlings during the initial stages of establishment (Johnson 1981, Johnson *et al.* 1986, Wendel 1980). Artificial regeneration using large seedlings with developed root systems enhances the competitive advantage of seedlings and minimizes the effects of deer herbivory as the terminal buds are above the height of deer browsing(Ward *et al.* 2000). Large nursery stock can quickly become established in the field and can be produced by adjusting nursery practices (Kormanik *et al.*1994).

Production of high quality butternut seedlings has only occurred during the last decade. However, genetic tests have been established with black walnut seedlings that were

grown under optimal nursery conditions (Schultz and Thompson 1990, 1997). These studies demonstrated that seedlings with the highest FOLR numbers had increased height and survival five years after planting, and seedlings with less than seven FOLR should not be planted (Schultz and Thompson 1990, 1997). FOLR number is a heritable trait in butternut (see Chapter III); however, the potential impact on establishment success has yet to be documented over time. Seedling vigor can delay the effects of pathogens (Ostry *et al.* 1994), so large butternut seedlings may have decreased mortality from butternut canker disease.

The Georgia Forestry Commission's Flint River Nursery consistently produces high-quality seedlings of various hardwood species, including butternut (Clark *et al.* 2000, Brosi *et al.* 2007). Seedling height (HT), root collar diameter (RCD), and/or number of first-order lateral roots (FOLR) have been used as an indicator of seedling condition for butternut (see Chapter III). Understanding the variability and potential gains from selecting specific genetic families, and the impact of seedling characteristics on growth, disease development and survival are unknown.

In 1995 and 1996, plantings of butternut seedlings were established in an orchard setting and in a forest setting in Tennessee, North Carolina and Kentucky (Spaine et al. 1997, Brosi et al. 2007). Butternut plantings were established using pure butternuts and putative hybrids between *J. ailantifolia* and butternut. Plantings of butternut seedlings in orchard settings had little disease development and therefore, no family differentiation (Schlarbaum *et al.* 2004). From this study, butternut canker disease did not readily develop on seedlings grown in optimal conditions with minimal competition. At two locations, plantings were established in a forest setting using 36 open-pollinated families at a narrow spacing under the canopy of an infected butternut tree to expose the seedlings to heavy disease pressure. The intent was for spores from infected branches to spread through rain splash onto seedlings planted under the drip line of the infected trees, as occurs naturally (Kuntz *et al.* 1979, Tissert and Kuntz 1982). This technique allowed monitoring of disease development on seedlings in a stressed, disease-enhanced environment. The plantings were periodically evaluated for survival, growth and disease resistance as indicated by canker development. Varying

amounts of cankering were seen on individual seedlings, with family relationships (Spaine *et al.* 1997, Schlarbaum *et al.* 2004, Brosi *et al.* 2007).

The above results indicated that a resistance screening technique could be applied to other seedlings from various seed sources throughout the southeastern Appalachian region, in order to identify resistant genotypes. To further evaluate this approach, progeny tests were established in 2002 under infected butternut trees at different locations within the southeastern range of butternuts. The plantings were established for these specific objectives:

- *Objective a)* determine if variation occurs between progeny from maternal parents of unknown families, *J. cinerea, J. ailantifolia*, F_1 *J.* X *bixbyi* in cankering, early growth (height (HT) and diameter (RCD)), and seedling survival; and
- *Objective b)* determine site differences in cankering, early growth (HT and RCD), and seedling survival;
- *Objective c)* determine if genetic variation of provenances and families exists in cankering, early growth (HT and RCD), and seedling survival within specific sites and across multiple planting locations;
- *Objective d)* determine if certain seedling attributes relate to survival and vigor after field planting and in turn relate to disease resistance. Seedlings will be categorized by initial variable to determine impacts of initial size on seedling growth (HT and RCD) and survival.

Methods

Throughout this manuscript butternut, *J. cinerea,* is defined by whole-tree characteristics for identification. For each tree overall phenotype was evaluated based on dendrological and nut characteristics that tend to distinguish the species. It is unknown if the whole-tree characteristics can distinguish between pure butternut or hybrids of butternut and *J. ailantifolia* of various generations. Trees classified as butternut (*sensu lato*) could

actually be the result of hybridization between *J. cinerea* and *J. ailantifolia* Carr or *J. ailantifolia* var. *cordiformis* (Maxim.) Rehd.) or hybridization with advanced generation hybrids, *J.* x *bixbyi* Rehd. Progeny of all parent trees could also be hybrids, depending on the presence of a *J. ailantifolia* or *J.* x *bixbyi* as the pollen source(s) (see Chapter II). A limited number of maternal parent trees used in this study were DNA genotyped using 12 microsatellite loci and specific chloroplast markers to determine recent maternal ancestry (Hoban *et al.* 2009). Parent trees consisted of *J. cinerea*, *J. ailantifolia* and F_1 hybrids *J.* x *bixbyi*. No offspring were genetically tested. Not all parent trees were tested, so they were labeled as unknown families. From one location in western North Carolina maternal parent trees were determined to be a combination of J. *cinerea* and F_1 *J.* X *bixbyi*. Three parent trees from east Tennessee were determined to be *J. ailantifolia* through whole-tree silvics. Known *J. cinerea* parent trees included ten families from central Tennessee (BV) and one family from western North Carolina (Macon 2). Known *J. ailantifolia* were from two families in east Tennessee (HH). One family in western North Carolina was a F_1 hybrid (*J. bixbyi,* Cher2). All other species the maternal parent was unknown. Offspring of these maternal parent trees could by the results of selfing or could be outcrossing with either species producing F_1 hybrids or backcrosses to either species.

Open-pollinated nuts for plantings were collected in the 2000 autumn from 41 butternut trees, which occurred in eight ecoregions (*sensu* Bailey 1995) or provenances, in Tennessee, Virginia, and North Carolina (Table IV-1). The butternuts were dehusked and planted at the Georgia Forestry Commission's Flint River Nursery near Byromville, Georgia in December, 2000. The resulting seedlings were grown for one growing season (2001) using fertilization and irrigation regimes developed by the USDA Forest Service's Institute of Tree Root Biology (Kormanik *et al*. 1994). The seedlings were lifted in February, 2002 using a Fobro® lifter-shaker (Bartschi-Fobro, Huswil, Switzerland) that undercut the root systems at 30 cm.

Seedling measurements were taken on 1-0 nursery stock prior to planting (Table IV-5). Three parameters were measured: stem height (HT) in cm, root-collar diameter (RCD) in mm, and first-order lateral root number (FOLR) in cm. Height was measured from

the root collar to the terminal bud to the nearest 0.5 cm using a meter stick and RCD was measure at 1.3 mm above the root collar using digital calipers. A FOLR was defined as a lateral root at least 1 mm in diameter at the point of junction from the main taproot (*sensu* Kormanik 1994). The lateral roots extended up to 1.2 meters in each direction from the seedling base, which caused some FOLR were broken in the lifting process. Absence data was taken on FOLR based on broken root locations. All remaining lateral roots were clipped to 4"-6" in length.

Over 2,000 seedlings from the half-sibling families were sorted into experimental plantings using an incomplete block within complete block design and single tree plots. Plantings were established at twelve locations, ten in Tennessee (TN) and adjacent states Virginia (VA), North Carolina (NC), and Kentucky (KY), one at the western edge of the range in south central Missouri (MO) and one in the northeastern part of the range in Connecticut (CT). (Figure IV-1, Table IV-1).

At each location, the experiments were established at a 61 cm spacing (2 foot by 2 foot) directly under surviving butternut trees. The seedlings were manually planted in 30 cm deep holes made by gas powered augers with six inch auger bits. All of the plantings were established under butternut trees with butternut canker disease, as evidenced by extensive stem and branch cankers. One site on the Jefferson National Forest in Virginia was established in an open field near diseased trees. Sites in the National Forest in NC and TN were established under trees in forested settings. Sites on private land and Army Corps of Engineer land in TN were established in open areas under the shade of butternut trees.

Evaluation of plantings

Sirococcus infects and grows during the dormant season (Tissert and Kuntz 1982). Therefore, the plantings were evaluated at the beginning and end of the dormant season from 2002 through 2005. At each evaluation, seedlings were monitored as to the presence and number of cankers on the main stem of the seedling. A canker was defined as a sunken dark area on the bark with or without peeling bark. Canker number from the main stem of the seedling was recorded. Butternut canker disease is the main cause of cankers on the bark of young seedlings and all cankers were classified as potential butternut canker disease. Other

injuries from lifting or insect damage that were not clearly cankers were not recorded. Survival and total height was measured in the fall after the onset of dormancy for four growing seasons, 2002, 2003, 2004, and 2005.

Statistical analyses

Statistical analyses were conducted using SAS version 9.1 (for Windows, © 2005 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Analysis of variance was completed using PROC MIXED. Additional macro program codes to maximize programming efficiency developed by Saxton (1998, 2002) were used for means separation. Macros included pdmix800 for means separation using multiple range tests (Saxton 1998), Mixed Model Analysis of Variance Macro (mmaov) uses Fisher's Least Significant Difference (LSD) test was conducted at the 5% significance level, determines if standard deviations are within five fold of each other, calculates Levene P values to determine equality of variances, and a Shapero-Wilk W test for normality (Saxton ©2002). . The following analyses were conducted on individual planting locations and on combined data from all locations where survival was greater than 50%. Locations where survival was less than 50% for each year were removed from the analysis. Least-square survival and height means of each provenance and family were converted to relative survival and heights, expressed as a percentage of the plantation mean. All percentage data, derived from count data, including survivorship and presence of cankering, underwent inverse trigonometric sine transformations due to lack of homogeneity of variances (Snedecor and Cochran 1998).

Objective a). Yearly seedling variables were analyzed across groups based on maternal parent source of unknown, *J. cinerea, J. ailantifolia,* and *J.* X *bixbyi* using analysis of variance. Plantings were planted in an incomplete block within complete block design. Fixed effects included species, sites, provenances and families: location, rep (location), block (location*rep), location*family (provenance), provenance, family (provenance). Seedling response, in terms of growth, infection and mortality, were treated as independent variables. Tukey's pairwise means comparisons were used to separate means when a statistically significant overall effect was established (SAS 2005). Seedling measurements (HT, RCD, survival and cankering) were analyzed to species differences within and across sites (SAS

2005).

Objective b). Yearly seedling variables were analyzed across planting sites using analysis of variance. Sites with greater than 50% mortality due to other causes were removed from analysis. Remaining intact sites were evaluated for consecutive years until mortality reached greater than 50%.

Objective c). Yearly seedling variables were analyzed across provenances and families using analysis of variance. Analyses were conducted to determine family impacts on height and survival. All known *J. ailantifolia* and *J.* x *bixbyi* parent families were removed from the sample prior to analysis. A mixed model analysis using a randomized complete block design was used to determine provenance and family (provenance) effects. The least square difference (LSD) values of the family means were averaged to produce a single value for the mean separation test. Tukey-Kramer multiple comparisons of the family means was used due to determine specific differences due to unequal sample sizes.

Objective d). Phenotypic correlations were calculated using Pearson's correlation analysis for year to year family means on survival, cankering, and height (PROC CORR). Correlations were also calculated between year to year seedling variables and between year-before height, RCD, and FOLR to specific-year survival, cankering and height. Seedlings were grouped based on initial seedling characterizations. Categories for initial HT were small (under 50 cm), medium (50-100 cm), and large (over 100 cm). Categories for RCD were small (under 11 mm), medium (11-20 mm), and large (over 20 mm). Categories for FOLR number were small (less than nine) and large (greater than nine). Analyses of variance was conducted on groupings of initial seedling variables.

Results

Plantation survival

The overall mortality at the end of the first growing season, in 2002, was four percent and attributed to transplanting shock. In the early spring 2003, three planting locations were lost; one site in Connecticut (CT) burned due to a fire in a nearby structure and two plantings

at Mammoth Cave National Park (KY-MC 1 & 2) were almost completely killed by root rot disease, likely *Phytophthora cinnamomi* Rands, which was promoted by an unusually high amount of precipitation during the summer. Two additional plantings had greater than 95% mortality by the fall of 2003. The site in Kentucky on Nature Conservancy Property (KY-TNC) was destroyed by flooding, and the Pisgah National Forest site (NC-PI) suffered heavy mortality probably due to *P. cinnamomi*. In addition, one site in the Ocoee District of the Cherokee National Forest in Tennessee (TN-OC1) had 77% mortality in 2003. At this location there was evidence of repeated damage by European wild boars (*Sus scrofa* L.) including rooting of young seedlings to uncover attached nuts, which occasionally remained after nursery lifting. Nine plantings at seven locations remained viable at the end of 2003 (Table IV-2). By the fall of 2004, the sites in North Carolina along the New River (NC-NR) and in the Daniel Boone National Forest of Kentucky (KY-DB) also suffered heavy mortality due to flooding. Cumulative survival varied by planting location and decreased annually for all planting sites from 85.3%, 65.5%, 55.9% to 31.2% in 2005 (

Table IV-4). Heights were measured annually on seedlings in sites with greater than 90% survival (Figure IV-2).

Overall disease development

The plantings under butternut trees developed cankers in their first growing season and continued to become diseased and die throughout the study. The number of cankers varied on individual trees from one to twelve. Very few individual trees and families experienced cankering. Overall, there was no significant relationship in the presence of cankering in a previous year and the presence of cankering in following years ($r^2<0.05$ for all years).

Species effects

Objective a). Survival Significant differences existed for survival across all years across families of various species of origin. Seedlings from *J. ailantifolia* and *J. bixbyi* families all had low survival. In 2005, all *J. ailantifolia* and *J. bixbyi* families averaged under 40% survival. In 2005 there were five *J. cinerea* families with greater than 50% survival and six *J. cinerea* families under 50% survival. Variation in known *J. cinerea* survival ranged in 2005 from 27.8 to 67.08%. Great variation also occurred in unknown families ranging from 4.6% to 100%.

Height Height was also significantly different across parent species of origin for each individual year. Initially the height of seedlings was tallest for *J. cinerea* families, followed by F_1 *J.* X *bixbyi* and *J. ailantifolia*. In 2005, the known *J. cinerea* and *J.* X *bixbyi* from western North Carolina were both shorter than average in height; reversing their initial status. In 2005, *J. ailantifolia* parent trees resulted in the 6th and 7th tallest families, both averaging over 180 cm tall. Of the ten families from central Tennessee with known parents of *J. cinerea*, half were over 150 cm in height in 2005 and half were under this value. The percent height change averaged largest for the *J. ailantifolia* families (60%), followed by the known *J.* X *bixbyi* family (34%) with the least percent increase in the *J. cinerea* families (32%, Table IV-5). Initial RCD were largest for *J.* X *bixbyi* followed by *J. ailantifolia*, and *J.*

cinerea families. In 2005, RCD was largest for *J. ailantifolia* families (24), followed *J. cinerea* families (20), and then *J.* X *bixbyi* families (18).

Cankering Known *J. cinerea* and unknown families experienced cankering each year from 2002 until 2005. There were no cankers observed on known *J. ailantifolia* and known F_1 *J.* x *bixbyi* throughout the four years.

Site effects

Objective b). Survival First year mortality (2002 autumn) averaged 4% and ranged from planting location from 0 to 10.3%. In the spring of 2003 four sites were excluded for having 90% or great mortality (Table IV-2). The site at Mattatuck State Forest (CT) was lost due to fire, the site at Horse Lick Creek (KY-TNC) was flooded and the sites at Mammoth Cave National Park (KY-MC1 and MC2) were lost due to infection with *P. cinnamomi*. The mean mortality of the remaining sites in the fall of 2003 of the plantings was 42%;sites ranging from 0- 95 ($p<0.0001$,

Table IV-6). By the end of 2003 an additional one site was removed due to over 90% mortality; Pisgah National Forest (NC-PI). This site was located in the deep shade of woods on a higher elevation position in a swale.

Mean mortality on remaining planting sites in the fall of 2004 was 68%. Mortality at the end of 2004 varied according to planting site (0-92%, $p<0.0001$). In 2004, the planting on the New River State Forest (NC-NR) and the planting on the Daniel Boone National Forest (KY-DB) had significant loss due to flooding. In September 2004, heavy flooding throughout the southeast had large impacts on riparian areas from remnant hurricanes Frances and Ivan. At the end of 2004, there were nine out of 15 sites remaining sites with less than 90% mortality.

At the end of 2005, the average mortality on sites was 60%, slightly lower than the previous year (Figure IV-4). Mortality varied significantly across planting site (5-94%, $p<0.0001$). Two additional sites were lost and there were only seven remaining sites at six locations with greater than 90% survival (Table IV-3). KY-DB had over 90% mortality due to flooding and one site on the Cherokee National Forest (TN-OC1) was planted in heavy shade. Over half of the initial plantings had been eliminated from the study for greater than 50% mortality.

The Jefferson National Forest site (VA), planted in an open field, and had the least amount of mortality each year (0-5%). Of the plantings established under butternut trees, mortality in 2005 was lowest on the Wayah District of the Nantahala National Forest in North Carolina (NC-WA2: 31%), at the Healing Stones Foundation Property in central Tennessee (TN-HS: 36%), and at the Centerhill Lake site in central Tennessee (TN-CHL: 37%) (Table IV-3).

Height In the fall of 2002, the average height of all plantings was 88.07 cm and ranged from 60 to 112 cm (Table IV-2). In 2003, mean height at all sites with greater than 90% survival in the previous year was 97.52 cm, a significant increase from the year before. Average height ranged from 72-126 across planting sites. Average height in 2004 was 100.71 cm and ranged from 73-171 across sites. Percent change in height varied significantly according to planting site for each year ($p<0.001$). At some locations including

VA-CL, TN-OC2, NC-WA1, and NC-WA2, the average height of seedlings was almost identical or even shorter than in the previous year. Dieback was noted on a large number of seedlings, and was responsible for this difference.

Significant differences existed in site for height growth at the end of the 2005 growing season (Figure IV-3). Height varied significantly across planting location with the average height of 153 cm, a large gain in height over the previous year. At individual planting locations height increases were noticeable and sometimes extremely large. For example, the average height at the Butternut Valley site (TN-HS) was 145 cm in 2004 and 185 cm one year later. One site on the Ocoee District (TN-OC2) had an average change in height from 78 to 128 cm. The Clinch District site (VA) in full sun had an average height of over 2.5 meters tall. VA had the tallest average seedlings each year and the individually largest seedling each year.

Cankering There were significant site differences each year in the number and percentage of trees experiencing cankering ($p<0.001$). The site in the full sun experienced the least amount of cankering.

Provenance and family effects

Objective c). Survival Significant differences existed for provenances in degree of mortality each year (2002: $p<0.0001$, 2003: $p< 0.0001$, 2004: $p=0.0104$, and 2005: $p=0.0192$

Table IV-6). Percent survival at the end of 2004 varied across provenances from 36 to 83%. Virginia and middle Tennessee as well as northwestern North Carolina resulted in seedlings with higher survival than other provenances (Figure IV-6).

Family effects did not impact mortality during the first two years, 2002 and 2003 ($p=0.4414$ and 0.2843 respectively). Family effect on mortality in 2004 and 2005 were significant ($p=0.0464$ and 0.0266 respectively), but less so than provenance and site (

Table IV-6). Families CD4, CD6, Ashe5 and Ashe6 had over 80% survival at the end of four years (Figure IV-9). Percent survival ranged across families from 21 to 100%. Four families, two from southeastern Virginia and two from central Tennessee, had both high rates of survival and larger than average heights (BV108, BV16, CD4, and CD6). Ashe6 had high survival but averaged half the height of other families. BV14 and BV26 averaged taller seedlings with low percentages of survival.

Height Provenances were significantly different for all variables ($p<0.05$) except for initial RCD, HT 2004, and RCD 2004 ($p=0.3684$, 0.4556 and 0.2268 respectively,

Table IV-6). At the end of 2005, seedlings from provenances in Virginia and east Tennessee had greater heights; as well as middle Tennessee and Georgia (Figure IV-5).

Each year families were significantly different in average height ($p<0.01$,

Table IV-6). The tallest four families in 2003 were also tallest in 2005 and included BV16, CD4, CD6, and CD8 (Figure IV-7, Figure IV-8). 2004 height varied across families from 27 to 149 cm ($p<0.001$). 2005 height averaged 154 cm and ranged significantly across families from 62 to 273 ($p=0.0116$, Table IV-5). Percent height change also varied from -34 to 68 with an average increase of 34%. RCD in 2005 ranged significantly across families from 13 mm to 46 mm ($p=0.0003$).

Cankering In 2002 and 2003, the tests exhibited a lack of significant differences in the number of cankers per tree or the percentage of trees with cankers in each family experiencing cankering ($p<0.05$). For 2004 there were significant differences in presence of cankering among families ($p=0.0432$, Figure IV-10). In 2005, there were also significant differences in cankering among families ($p=0.0215$, Figure IV-11). Canker incidence was variable among families ($p=0.0374$), and the half of the families impacted by cankers in 2004 were impacted again in 2005.

Initial planting size effects

Objective d). Survival At the end of the 2005 planting seedlings, mean survival at all remaining planting locations was 44.5%. Average seedling HT was 153 cm and RCD was 22.67 mm in planting sites with less than 50% mortality. Consistently significant correlations were seen for both initial HT and initial RCD with future seedling sizes (Table IV-9, Table IV-10). FOLR number was consistently significant for seedling sizes. However, unlike seedling HT and RCD, FOLR was significantly correlated with survival from the second growing season to the fourth (Table IV-10).

Height A comparison of seedlings in various initial height categories showed significant impact of initial height on 2005 HT and RCD (Table IV-8, $r^2=0.0433$, $p=0.0143$, Figure IV-12, Figure IV-15). Seedlings which were under 50cm when planted contained the smallest 24% of seedlings and average 138.53 cm over 14cm shorter than the average seedling height. Seedlings with initial heights between 50 and 100 cm in height, average 162.87 cm at the end of the fourth growing season. Seedlings over 100 cm in height prior to planting consisted of 22% of the planting stock and averaged 184.76 cm in height at the end of 2005. These seedlings were over 25% taller (46 cm) than the seedlings under 50 cm.

However, these three height categories were not statistically different in percent survival (Table IV-8). Root collar diameters were 25% larger (26.49 mm) in the larger height category than in the smallest category (19.96 mm).

Impacts of initial RCD follow the same trend as height, but were even more pronounced. Overall initial RCD significantly impacted 2005 height (r^2=0.0590, p=0.0091, Figure IV-13**,** Figure IV-16). Seedlings under 11mm in diameter when planted had an average height 34% shorter (67 cm) than seedlings equal to or over 20mm in diameter when planted. Seedlings in the middle category between 11 and 20 mm were 35 cm taller than the smaller category and 30 cm shorter than the larger category. In terms of RCD in 2005, seedlings in the largest initial RCD category average 40% larger in diameter (29.79 mm) than seedlings in the smallest category (17.98 mm) and 24% larger for the middle category (22.67) (Table IV-8). Initial root collar diameter significantly impacted survival (p=0.0279); with the greatest mortality in seedlings under nine mm in initial RCD 70% compared to 55% with seedlings over nine mm in diameter.

Initial first-order lateral root number was significantly lower in seedlings that died in both 2004 and 2005 (p=0.032, p=0.041). FOLR number averaged 16.22 for living seedlings and 15.36 for dead seedlings in 2005 and 16.13 and 15.14 in 2004. Initial FOLR number was significantly related to 2005 height (r^2=0.0128, p=0.0368, Figure IV-14). Average FOLR number was 15.74 and seedlings greater than the mean had heights in 2005 24% larger (173.36cm) than seedlings less than the mean (148.61). This relationship was even more pronounced with seedlings with less than nine FOLRs compared to seedlings with over 9 FOLRs. Seedlings with less than nine FOLRs were 40 cm shorter in HT and 6mm smaller in RCD (Figure IV-17). Seedlings with less than nine FOLR numbers had 70% mortality as compared to 55% in seedlings with greater than nine FOLRs (Figure IV-18).

Discussion

Resistance testing

The original purpose of this research was to investigate whether these tests would reveal genetic differences in resistance and then identify disease-free seedlings for use in a resistance breeding program. The loss of multiple sites due to environmental factors greatly impacted this study. Developing reliable procedures that can identify resistance at a seedling stage is extremely crucial to butternut restoration. These tests were able to show family and site differences in cankering. *J. ailantifolia* and *J.* X *bixbyi* families were reliable controls which did not develop any cankers. However, the number of families impacted by cankering was quite small, seven to eight. This may also indicate some form of juvenile resistance. It is possible, however, that conditions were not favorable for disease formation as there is evidence that the disease may have specific years in which cankering is extensive and other years in which it is less extensive (Clark *et al.* 2008, R.L. Anderson, *pers. comm.*).

The number of cankers on each individual seedling was very difficult to determine. At many of the planting locations, deer rubs were frequent and often contributed to the inability to distinguish scaring from cankering. Many sites were selected in riparian areas and frequent flooding of the areas caused scars to develop where debris had injured seedlings. Therefore, even though several attempts were made to count the number of cankers each year, overall data on cankering could not be acquired with confidence.

The plantings in 1995-6 were able to show family differences in cankering. Those plantings were located in fairly open areas under the overstory of just one butternut tree. The seedlings were experiencing few other negative influences besides cankering. These seedlings developed clear boles in which cankers were easily distinguished. In the studies established in 2002, low light conditions were impacting seedlings and resulting in dieback and less uniform height growth where reword this - awkward cankers could be monitored. As anticipated, *J. ailantifolia* and *J.* x *bixbyi* genetic families had no evidence of cankering, though mortality for these families for other reasons was very high

Resistance screening tests can be an accurate method for determining resistance to butternut canker disease. *J. ailantifolia* and *J.* x *bixbyi* offspring are extremely valuable for

controls. However, critical site selection is essential. As known surviving open- grown butternut seedlings on public lands are limited, sites for this study were already greatly reduced. In addition, planting butternuts in areas prone to flooding reduces their value in resistance screening. Planting in areas within the forest, with reduced light, results in heavy mortality and decreased light growth. Determination of cankering on seedlings with dieback, less vigorous growth, and deer rub can be extremely difficult. Therefore, future resistance screening plantings need to be established on specific sites based on enough light to provide relatively good health and limited growth of test seedlings, avoidance of sites prone to flooding or *Phytophthora cinnamomi,* protection from deer, and the heavy disease pressure of a surviving infected tree.

Species

J. ailantifolia and *J.* x *bixbyi* families were effective controls by not developing cankers. Therefore, as previously reported by Orchard (1982), one of the origins of potential resistance to butternut canker disease could be through a backcross breeding approach using resistant hybrids (Schlarbaum 2004). However, offspring from these families had large amounts of mortality. Though initially shorter than the *J. cinerea* families; they had the largest percent change in height and were taller in later years. This could be due to cankering in the infected *J. cinerea* families resulting in dieback and reduced growth. Deer rub could be another contributing factor, as deer would have initially targeted the larger *J. cinerea* seedlings. The high mortality in *J. ailantifolia* and *J.* x *bixbyi* families may be due to reduced initial height and the inability to compete in shaded environments.

Plantation mortality

The cause of whole plantation mortality was varied, but was not influenced by butternut canker disease. Plantings were destroyed by fires, floods, root rot disease, and invasive exotic animals. Within plantations that survived, butternut canker disease did not cause mortality, although it did cause damage to a limited number of families.

The study's primary objective (*Objective c*) shifted to determining survival and growth under shaded conditions (*Objective a, b,* and *d*). These conditions are becoming

increasingly more prevalent in riparian areas. Riparian systems have been harvested in the past, and now many stands are currently single-aged with limited natural light gaps due to the earlier successional stage. Clear site differences were evident for both survival and growth, and comparisons among sites indicate that increased light will increase establishment success.

Many seedlings probably died due to lack of light growing under the canopy of a mature tree, but not butternut canker disease. All plantings under butternut trees experienced a large degree of mortality three years after outplanting. This may have been the point when seedlings were unable to tolerate the heavy shade from above and sides at some of the planting locations. Seedlings grown in an open field on the Clinch District of the Jefferson National Forest in Virginia (VA-CL) had 95% survival at the end of three years, probably due to the increase in light. Butternut seedlings grown in full sun were able to have high survival and large increases in height. These seedlings were planted at two foot spacing, and had little mortality, which resulted in strong apical growth. This type of growth in butternut is congruent with classification of the species as intolerant (Doyon *et al.* 1998, Rink 1990). The difficulty in establishing butternut in heavily shaded areas may largely impact restoration efforts. Current restrictions for harvesting in riparian area make shaded conditions more prevalent in areas where restoration of butternut may occur, particularly on National Forests and National Park lands. For instance, Thompson *et al.* (2006) found very few areas within Mammoth Cave National Park that were both suitable habitat for butternut and were in areas with enough light for establishment.

Families and provenances

Large differences in families and provenances existed for all variables. There are clear reasons to keep family identify separate in order to benefit from the inclusion of particular families in a breeding or restoration program with superior establishment and growth traits. Certain individual families and provenances had increased survival rates and larger seedlings over all sites. At the end of four years, with 51% average mortality, three families had over 100% survival. These specific families were from three different provenances: central TN, northwestern NC, and southwestern VA. Only one (BV129) had

greater percent height change than the average. There were also 13 families with above the average survival; i.e. greater than 60%. Of these six families were from central TN, four from southwestern VA, and three from northwestern NC. The largest percentage of resistant offspring is from a population that was screened for introgression with *J. ailantifolia*. Genetic analysis of the population in central TN revealed no *J. ailantifolia* or *J.* X *bixbyi* individuals (Hoban *et al.* 2009). Therefore, genetic resistance in this population is probably not due to hybridization. In addition, many individuals from the western North Carolina population were determined to be hybrids and no families from this area had greater than average survival. Height change was up to 68% in some families and as low as -34% in others. Overall there were ten families with both greater height gains than average and greater than average mortality.

Seedlings from specific provenances and families have increased changes of survival and increased growth rates. With butternut, it is important to monitor specific progeny that are sown in the nursery and established in the field. Field testing will allow the selection of specific maternal parents who consistently produce nuts with high germination rates, seedlings with larger RCD and greater heights, and with higher probabilities of survival after outplanting. This selection process will aid restoration by providing material with greater changes of establishment success. Selection of ideal maternal sources of progeny for restoration should also consider the impacts of hybridization with *J. ailantifolia*. The specific parent trees for this study were of unknown genetic identity. However, recent advancements have allowed for the screening of maternal parents prior to incorporation into a breeding program (Hoban *et al.* 2009). Genetic analysis of the identity of the parent tree and of surrounding trees would increase the chances of hybrid offspring.

Initial seedling characteristics

Strong relationships indicate that initial seedling variables are useful predictors for seedling success after planting, even in harsh planting conditions. Due to the strong correlations between RCD and FOLR, the FOLR variable could be considered an extraneous measure of initial seedling quality. However, due to the more significant impact of root number on mortality continued evaluation of seedlings based on this parameter could be

beneficial. First-order lateral root number was slightly, but significantly higher in seedlings that had survived in 2004 and 2005. Schultz and Thompson (1990, 1997) found that seven FOLR number or greater was ideal for black walnut seedling establishment. This study shows the same relationship with FOLR and survival. Butternut seedlings in this study with over nine FOLRs and greater than nine mm in diameter resulted in seedlings more likely to survive. Future outplantings should select only seedlings with these attributes for increased establishment success.

The seedlings in this study showed very little increase in growth over the first growing season. However, in the second growing season, height growth increased significantly. Then with heavy mortality in the third growing season, height growth was minimal. During the fourth year height growth again increased significantly. Percent height change was drastically larger during the second growing season than the first. This lag in establishment time could be due to several factors including the large size of the seedlings resulting in extensive root pruning prior to planting. However, the larger seedlings continued to be larger even after outplanting, indicating that transplant shock was not more severe in larger seedlings or they had more reserves to recover faster.

Larger initial RCDs and HTs resulted in larger seedlings heights and diameters after four years. Seedlings over 100cm tall at planting average almost 46cm taller than seedlings less than half of that height. This may be extremely important to reduce time vulnerable to deer browse and lessen the impact of rubbing. Deer rubbing was significant at many of the planting locations. In a recent study, butternut seedlings were found to be preferentially used as a species for rubbing by white-tailed deer (Hygnstrom *et al.* 2009). In a non-timber forest product planting in east-central Nebraska, rubbing in areas with 48 deer per square mile occurred primarily in fall and winter and resulted in reduced vigor of seedlings (Hygnstrom *et al.* 2009). Among eight tree species, butternut was the 4th most frequently rubbed, 8th of 26 species total including shrubs (Hygnstrom *et al.* 2009). White-tailed deer were found to preferentially rub aromatic trees, trees on higher fertility sites, and greater than 78% of rubbing occurred on seedlings less than 27 mm in diameter at the midpoint of the rub (Hygnstrom *et al.* 2009). The increased preference for butternut as a species for rubbing,

may not only reduce vigor in seedlings, preferentially for seedlings under 30mm in diameter, but may also increase susceptibility to butternut canker disease as the fungus can infect trees through wounds (Davis *et al.* 1997, Davis and Meyer 1997). The use of tree shelters is often limited to species preferentially browsed by deer, but with butternut may help prevent rubbing until seedlings are larger in size and can better withstand the impact of rubbing.

Conclusions

Resistance screening plantings, using *J. ailantifolia* and hybrids as controls, can be effective in determining resistance to butternut canker. Specific genetic families with increased survival in heavily diseased environments were from *J. cinerea* parent trees and from locations without extensive introgression. Seedlings with greater than 9 FOLRs and larger than 11 mm in diameter had increased growth and greater chances for survival. However, butternut seedlings experiences heavy mortality in partial shade environments. Low survival in relatively low light conditions indicates potential problems for restoration in riparian areas of state and national parks. Dendrochronology evidence indicates that butternut recruitment occurs up to seven years post clearcutting and in old agricultural stand (Clark *et al.* 2008). Butternut may be an ideal species for Conservation Resource Enhancement Program (CREP) plantings or other conversion plantings, including agroforestry applications on mesic sites along riparian areas. However, growth form in these types of openings may not be ideal for timber production but may be good for nut production for mast for wildlife. Additional research into locations suitable for restoration needs to be explored.

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Appendix: Tables and Figures

Table IV-1. Experimental designs of butternut resistance screening plantings.

Site (# & abbr.[1])	**Families** (#)	**Seedling per family (#)**	**Incomplete blocks** (#)	**Families per block** (#)	**Seedling planted** (#)
1) CT	9	5-6	20	5-6	120
2) MO	12	6	20	6	117
3) VA	10	4-5	20	4-5	88
4) KY-DB	21	7	27	7	186
5) KY-TNC	15	7	30	7	210
6) KY- MC1	12	4	30	4	119
MC2	8	4	30	4	120
7) TN-OC1	16	5	31	5	160
OC2	10	5	20	5	200
8) TN-HS	15	5	20	5	95
9) TN-CHL	16	6	27	6	144
10) NC-NR	16	7	30	7	208
11) NC-PI	16	5	29	5	151
12) NC-WA1	20	6-7	20	6-7	126
WA2	10	6	30	6	179
TOTAL (Range)	**41 (8-21)**	**(4-7)**	**(20-31)**	**(4-7)**	**2,043 (95-210)**

[1] See Figure IV-1 for location information.

Table IV-2. Average annual mortality and height for individual and combined butternut plantings from 2002 until 2004.

Plantation (no.)	2002 Fall		2003 Spring	2003 Fall		2004 Fall	
	Mortality (%)	HT (cm)	Mortality (%)	Mortality (%)	HT (cm)	Mortality (%)	HT (cm)
1) CT	1.9	85.93	100	-	-	-	-
2) MO	0.0	59.74	24	30	91.00	36	73.27
3) VA	0.0	112.33	0	0	126.01	0	170.90
4) KY-DB	19.0	95.14	25	85	107.01	84	108.07
5) KY-TNC	1.1	82.67	93	-	-	-	-
6) KY- MC1	7.4	84.35	100	-		-	-
TN-MC2	4.2	85.63	100	-	-	-	-
7) TN-OC1	1.3	79.51	24	77	98.16	66	108.00
TN-OC2	1.2	71.58	25	43	71.48	73	78.39
8) TN-HS	0.0	98.63	6	8	123.02	19	145.27
9) TN-CHL	1.4	78.47	17	31	106.00	25	118.23
10) NC-NR	7.3	86.91	26	43	83.19	92	103.44
11) NC-PI	10.3	89.23	37	96	-	-	-
12) NC-WA1	12.0	92.65	23	36	92.89	79	75.85
NC-WA2	0.0	90.86	5	12	90.10	20	87.70
MEANS (Ranges)	**4.00 (0-10.3)**	**88.07 (60-112)**	**40.00 (0-100)**	**41.91 (0-96)**	**97.52 (72-126)**	**68 (0-92)**	**100.71 (73-171)**

Note: sites with greater than 90% mortality were considered destroyed and not measured in sub sequential years.

Table IV-3. Status and average annual mortality and height for individual and combined butternut plantings until 2005.

Plantation (#)	2005	2005 Fall		2006
	Status	Mortality (%)	HT (cm)	Status
1) CT	Destroyed	-	-	Destroyed
2) MO	Viable	88	94.38	Viable
3) VA	Viable	5	256.05	Viable
4) KY-DB	Viable	92	160.21	Destroyed
5) KY-TNC	Destroyed	-	-	Destroyed
6) KY-MC1	Destroyed	-	-	Destroyed
KY-MC2	Destroyed	-	-	Destroyed
7) TN-OC1	Viable	94	101.46	Destroyed
TN-OC2	Viable	89	128.32	Destroyed
8) TN-HS	Viable	36	185.32	Viable
9) TN-CHL	Viable	37	175.70	Viable
10) NC-NR	Destroyed	-	-	Destroyed
11) NC-PI	Destroyed	-	-	Destroyed
12) NC-WA1	Viable	65	104.52	Viable
NC-WA2	Viable	31	96.78	Viable
MEANS (Ranges)		**59.67 (5-94)**	**144.75 (94-256)**	

Note: sites with greater than 90% mortality were considered destroyed and not measured in sub sequential years.

Table IV-4. Survival for individual sites and combined butternut plantings until 2005.

Interval (start and end) YR	# at risk at start of interval	# censored during interval	# at risk at end of interval	# who died at end of interval	proportion surviving this interval	cumulative survival at end of interval	SITE
2001-2002	1195	0	1195	176	0.8527	0.8527	TOTAL
2002-2003	1019	0	1019	236	0.7684	0.6552	TOTAL
2003-2004	783	0	783	187	0.6900	0.5587	TOTAL
2004-2005	783	0	783	98	0.6300	**0.3122**	TOTAL
2001-2002	144	0	144	17	0.8819	0.8819	TN-CHL
2002-2003	127	0	127	9	0.9291	0.8194	TN-CHL
2003-2004	118	0	118	8	0.9322	0.6737	TN-CHL
2004-2005	110	0	110	19	0.8273	**0.4028**	TN-CHL
2001-2002	186	0	186	24	0.8710	0.8710	KY-DB
2002-2003	162	0	162	88	0.4568	0.3978	KY-DB
2003-2004	74	0	74	35	0.5270	0.1826	KY-DB
2004-2005	39	0	39	24	0.3846	**0.0243**	KY-DB
2001-2002	95	0	95	6	0.9368	0.9368	TN-HS
2002-2003	89	0	89	2	0.9775	0.9158	TN-HS
2003-2004	87	0	87	11	0.8736	0.7495	TN-HS
2004-2005	76	0	76	15	0.8026	**0.5161**	TN-HS
2001-2002	117	0	117	30	0.7436	0.7436	MO
2002-2003	87	0	87	15	0.8276	0.6154	MO
2003-2004	72	0	72	25	0.6528	0.2987	MO
2004-2005	47	0	47	29	0.3830	**0.0523**	MO
2001-2002	304	0	304	49	0.8388	0.8388	NC-WA
2002-2003	255	0	255	15	0.9412	0.7895	NC-WA
2003-2004	240	0	240	34	0.8583	0.5684	NC-WA
2004-2005	206	0	206	56	0.7282	**0.2741**	NC-WA
2001-2002	179	0	179	37	0.7933	0.7933	NC-WA1
2002-2003	142	0	142	11	0.9225	0.7318	NC-WA1
2003-2004	131	0	131	31	0.7634	0.4432	NC-WA1
2004-2005	100	0	100	75	0.2500	**0.0643**	NC-WA1
2001-2002	126	0	126	12	0.9048	0.9048	NC-WA1
2002-2003	114	0	114	4	0.9649	0.8730	NC-WA1
2003-2004	110	0	110	3	0.9727	0.7683	NC-WA1
2004-2005	107	0	107	20	0.8131	**0.4934**	NC-WA1
2001-2002	88	0	88	1	0.9886	0.9886	VA
2002-2003	87	0	87	0	1.0000	0.9886	VA
2003-2004	87	0	87	0	1.0000	0.9774	VA
2004-2005	87	0	87	3	0.9655	**0.9224**	VA

Table IV-5. Average initial seedling variables and annual mortality and height for 2005 across provenance and genetic family.

Provenance Name	Family Name	Parent Species	Initial				2005			
			(#)	HT (cm) [1]	RCD (mm)[2]	FOLR (#)[3]	HT (cm)	RCD (mm)	HT Change (%)	Survival (%)
North Georgia	Raba1	Unknown	70	63	13	13	149	23	27	25
Western North Carolina	Cher1	Unknown	88	43	14	**18**	104	13	**68**	10
	Cher2	*J.* X *bixbyi*	70	**74**	**18**	**18**	107	18	34	27
	Cher3	Unknown	141	**66**	**15**	**18**	67	13	8	23
	Cher5	Unknown	202	**69**	14	11	62	**25**	30	37
	Maco2	*J. cinerea*	189	43	14	**18**	103	15	**41**	41
	Swai1	Unknown	273	51	12	13	94	17	-34	50
Northwestern North Carolina	Ashe1	Unknown	40	**68**	13	**18**	--	--	--	--
	Ashe2	Unknown	63	53	13	14	**180**	**26**	**56**	11
	Ashe3	Unknown	312	**73**	**15**	**16**	103	19	0	28
	Ashe4	Unknown	50	**74**	14	12	--	--	--	--
	Ashe5	Unknown	76	61	14	**17**	--	--	--	--
	Ashe6	Unknown	94	62	13	14	105	17	**47**	**87**
	Ashe7	Unknown	23	**87**	**20**	**16**	100	14	5	53
	Ashe8	Unknown	79	**78**	**15**	15	**181**	22	26	**100**
	Ashe9	Unknown	147	**64**	13	14	109	17	2	35
	Ashe12	Unknown	73	46	**15**	**18**	137	21	**40**	45
	Ashe14	Unknown	181	54	12	14	117	17	**42**	**60**
	Alle1	Unknown	58	**71**	11	**16**	84	15	**42**	26
Central Tennessee	BV3	*J. cinerea*	104	**82**	**15**	**16**	**191**	**25**	**37**	**53**
	BV16	*J. cinerea*	19	**65**	**15**	**17**	**226**	**27**	**66**	**60**
	BV26	*J. cinerea*	107	**105**	**15**	**16**	**174**	22	10	29
	BV90	*J. cinerea*	284	**85**	**12**	13	126	16	14	30
	BV107	*J. cinerea*	58	**72**	14	14	138	18	**57**	**82**
	BV108	*J. cinerea*	32	**72**	13	14	**196**	**26**	**49**	**60**
	BV112	*J. cinerea*	35	**65**	14	12	142	18	4	**63**
	BV114	*J. cinerea*	25	**73**	13	**17**	**198**	**24**	**39**	32
	BV129	*J. cinerea*	70	52	11	13	145	16	**43**	**100**
	BV141	*J. cinerea*	149	**99**	14	14	136	16	-15	44
East Tennessee	HH1	*ailantifolia*	78	**89**	**17**	**17**	**208**	**26**	**54**	44
	HH3	*ailantifolia*	90	**69**	13	15	**199**	22	**63**	37
	HH4	*ailantifolia*	16	**110**	**18**	**17**	--	--	--	--
Southwest Virginia	CD1	Unknown	447	60	13	15	**203**	**29**	**48**	55
	CD2	Unknown	70	42	12	**17**	**201**	**26**	**67**	53
	CD3	Unknown	211	**67**	13	**16**	**173**	**24**	1	52
	CD4	Unknown	44	**88**	**17**	**19**	**269**	**46**	33	**100**
	CD5	Unknown	54	**68**	**17**	**16**	107	19	26	25
	CD6	Unknown	44	49	13	12	**273**	**40**	**68**	**60**
	CD7	Unknown	384	55	12	14	**171**	**23**	**40**	**58**
	CD8	Unknown	102	**77**	14	15	**270**	**37**	**62**	56
	CD9	Unknown	169	57	12	**16**	**159**	**23**	**44**	**70**
Mean			**118**	**63**	**14**	**15**	**154**	**22**	**34**	**57.31**

Bold indicates values above the mean of all seedlings. [1] Height of the seedling from the root collar to the first live terminal bud [2] diameter taken 1.3 mm above the root collar[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root. Families from sites with greater than 90% mortality were excluded.

Table IV-6. Analysis of variance of provenance, family, and planting sites between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Variables	Effects	F value	P value	Variables	Effects	F value	P value
Initial HT[1]	Provenances	10.26	<0.0001	HT04	Provenances	0.94	**0.4556**
	Families	9.95	<0.0001		Families	2.30	0.0002
	Site	11.75	<0.0001		Site	49.68	<0.0001
Initial RCD[2]	Provenances	3.56	0.0034	RCD04	Provenances	1.39	**0.2268**
	Families	8.83	<0.0001		Families	3.42	<0.0001
	Site	12.82	<0.0001		Site	61.98	<0.0001
Initial FOLR[3]	Provenances	1.08	**0.3684**	MORT04	Provenances	3.38	0.0104
	Families	7.70	<0.0001		Families	1.47	0.0116
	Site	6.58	<0.0001		Site	128.34	<0.0001
HT02[4]	Provenances	10.47	<0.0001	HT05	Provenances	3.07	0.0104
	Families	4.25	<0.0001		Families	1.74	0.0116
	Site	29.62	<0.0001		Site	56.88	<0.0001
MORT02[5]	Provenances	5.84	<0.0001	RCD05	Provenances	2.91	0.0143
	Families	1.02	**0.4414**		Families	2.27	0.0003
	Site	5.53	<0.0001		Site	57.46	<0.0001
HT03	Provenances	3.25	0.0067	MORT05	Provenances	2.70	0.0192
	Families	2.64	<0.0001		Families	1.55	0.0266
	Site	22.79	<0.0001		Site	112.41	<0.0001
MORT03	Provenances	11.66	<0.0001				
	Families	1.13	**0.2843**				
	Site	44.71	<0.0001				

Bold indicates non-significant results at the p=0.05 level

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root
[4] Height of the seedling at the end of the 2002 growing season
[6] Mortality of the seedling at the end of the 2002 growing season
Continued for years 2003, 2004, and 2005

Table IV-7. Survival in 2004 and 2005 of butternut seedlings across initial characteristics, including height, root collar diameter and first order lateral root number.

Initial Variable	2004		2005	
	Alive	**Dead**	**Alive**	**Dead**
HT[1]	76.09a	78.61a	77.08a	77.10a
RCD[2]	14.94a	14.39a	14.90a	14.56a
FOLR[3]	16.14a	15.14b	16.22a	15.36b

Unique letters indicate significantly different values using Tukey's Mean Separation Analysis across individual rows.

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table IV-8. Survival and size in 2005 of butternut seedlings across initial characteristics categories, including height, root collar diameter and first order lateral root number.

Initial Variable	Category Variable	% of seedlings	2005 mortality (%)	2005 HT (cm)	2005 RCD (mm)
HT[1]	< 50 cm	24.20	54.9a	138.53c	19.98c
	50-100 cm	54.02	55.4a	162.87b	22.23b
	> 100 cm	21.77	56.2a	184.76a	26.49a
RCD[2]	< 11 mm	18.43	53.81b	129.47c	17.98c
	11-19 mm	59.12	56.77a	167.87b	22.67b
	> 20 mm	22.45	50.00b	196.46a	29.79a
FOLR[3]	< 9	7	70.01a	124.48b	16.77b
	> 9	93	54.49b	163.97c	22.94a

Unique letters indicate significantly different values using Tukey's Mean Separation Analysis across individual columns for each variable.

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table IV-9. Phenotypic correlations between nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers n=2,043.

Variables	Phenotypic Correlations	
	r^2	P value
HT[1]-RCD[2]	0.60688	<0.0001
HT-FOLR[3]	0.23383	<0.0001
RCD-FOLR	0.49191	<0.0001

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken 1.3 mm above the root collar
[3] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

Table IV-10 .Phenotypic correlations between seedling growth and survival and nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Variables	Phenotypic Correlations	
	r^2	P value
Initial HT[1]- HT02[2]	0.45313	<0.0001
- HT03[3]	0.37746	<0.0001
- HT04[4]	0.16039	<0.0001
- HT05[5]	0.21573	<0.0001
- RCD04[6]	0.21953	<0.0001
- RCD05[7]	0.21573	<0.0001
- MORT02[8]	0.01009	**0.7278**
- MORT03[9]	-0.02547	**0.3793**
- MORT04[10]	-0.1377	**0.6347**
- MORT05[11]	-0.03269	**0.2591**
Initial RCD[12]- HT02	0.36382	<0.0001
- HT03	0.36498	<0.0001
- HT04	0.18954	<0.0001
- HT05	0.24291	<0.0001
- RCD04	0.26222	<0.0001
- RCD05	0.30434	<0.0001
- MORT02	-0.00435	**0.8808**
- MORT03	-0.03404	**0.2399**
- MORT04	-0.06366	0.0278
- MORT05	-0.04386	**0.1299**
Initial FOLR[13]- HT02	0.23656	<0.0001
- HT03	0.2703	<0.0001
- HT04	0.11593	0.0061
- HT05	0.11341	0.0213
- RCD04	0.014906	0.0004
- RCD05	0.16692	0.0007
- MORT02	-0.03061	**0.2908**

- MORT03	-0.06731	0.0200
- MORT04	-0.07585	0.0087
- MORT05	-0.06916	0.0168
HT02- HT03	0.69556	<0.0001
- HT04	0.42571	<0.0001
- HT05	0.38321	<0.0001
- RCD04	0.42000	<0.0001
- RCD05	0.43939	<0.0001
- MORT02	-0.04412	**0.1595**
- MORT03	-0.24021	<0.0001
- MORT04	-0.25813	<0.0001
- MORT05	-0.29015	<0.0001
HT03- HT04	0.60448	<0.0001
- HT05	0.53992	<0.0001
- RCD04	0.54545	<0.0001
- RCD05	0.51227	<0.0001
- MORT03	0.04671	0.1917
- MORT04	-0.029004	<0.0001
- MORT05	-0.36601	<0.0001
HT04- HT05	0.83266	<0.0001
- RCD04	0.80915	<0.0001
- RCD05	0.69213	<0.0001
- MORT04	0.06535	**0.01231**
- MORT05	0.42976	<0.0001
HT05- RCD05	0.78452	<0.0001
- MORT04	-0.10748	0.0292
- MORT05	.	.
RCD04- RCD05	0.83266	<0.0001
- MORT04	0.06535	**0.1231**
- MORT05	-0.42970	<0.0001
RCD05- MORT05	.	.

Bold indicates not significant at the p=0.05 level

[1] Height of the seedling from the root collar to the first live terminal bud at time of planting
[2] Height of the seedling at the end of the 2002 growing season
[3] Height of the seedling at the end of the 2003 growing season
[4] Height of the seedling at the end of the 2004 growing season
[5] Height of the seedling at the end of the 2005 growing season
[6] Root collar diameter at the end of the 2004 growing season
[7] Root collar diameter at the end of the 2005 growing season
[8] Mortality of the seedling at the end of the 2002 growing season
[9] Mortality of the seedling at the end of the 2003 growing season
[10] Mortality of the seedling at the end of the 2004 growing season
[11] Mortality of the seedling at the end of the 2005 growing season
[12] Diameter taken 1.3 mm above the root collar at time of planting
[13] Number of first order lateral roots, greater than 1 mm in diameter attached to the tap root

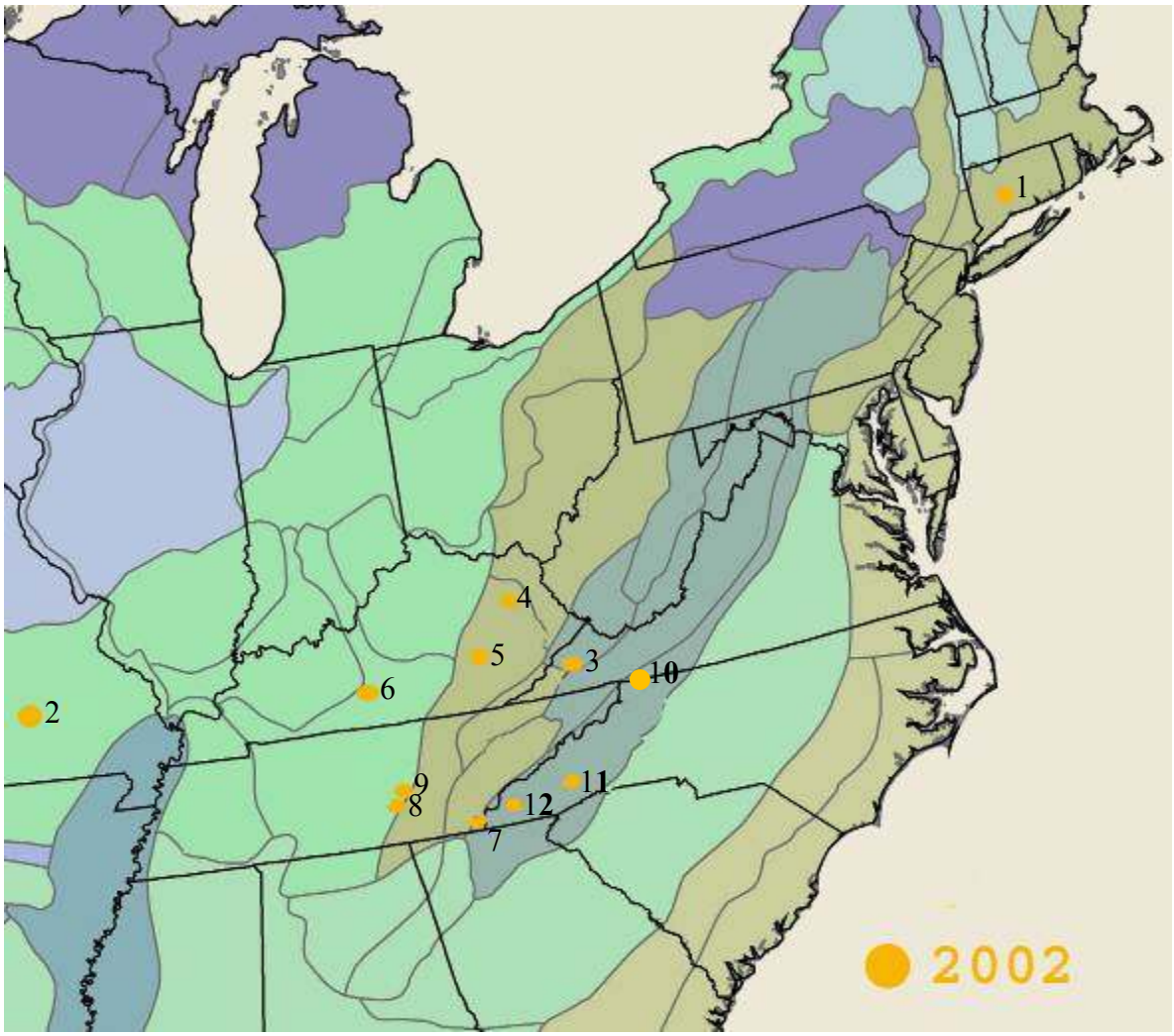

Figure IV-1. Locations of butternut resistance screening plantings established in 2002.

Number, location, nearest town, state (abbreviation)

1. Mattatuck State Forest, Terryville, CT (CT)
2. South Central Missouri, Thomasville, MO (MO)
3. Jefferson National Forest: Clinch River District, Pound, VA (VA)
4. Daniel Boone National Forest, Morehead District, Farmers, KY (KY-DB)
5. The Nature Conservancy: Horse Lick Creek, Sand Gap, KY (KY-TNC)
6. Mammoth Cave National Park, Cave City, KY (KY-MC1 & 2)
7. Cherokee National Forest: Ocoee District, Reliance, TN (TN-OC1 & 2)
8. Healing Stones Foundation, Smithville, TN (TN-HS)
9. US Army Corp Center Hill Lake, Smithville, TN (TN-CHL)
10. New River State Park, West Jefferson, NC (TN-NR)
11. Pisgah National Forest: Brevard District, Brevard, NC (NC-PI)
12. Nantahala National Forest: Wayah District, Bryson City, NC (NC-WA1 & 2)

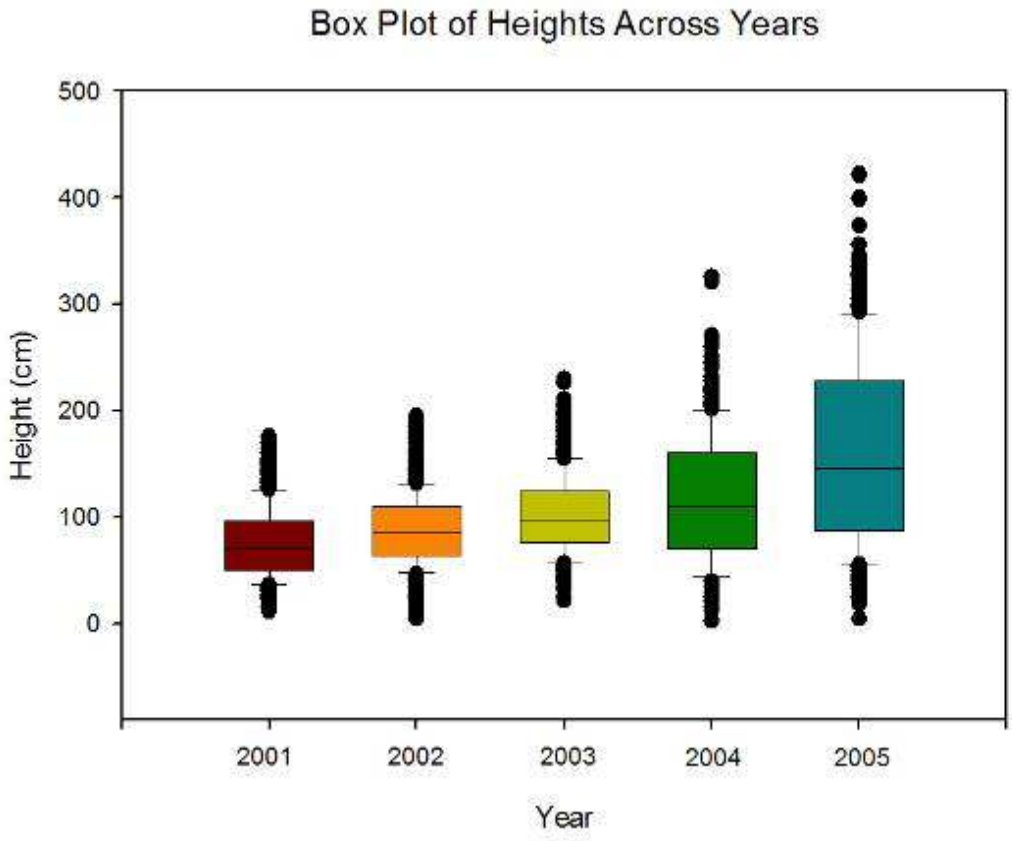

Figure IV-2. Annual heights of butternut seedlings established in 2002.

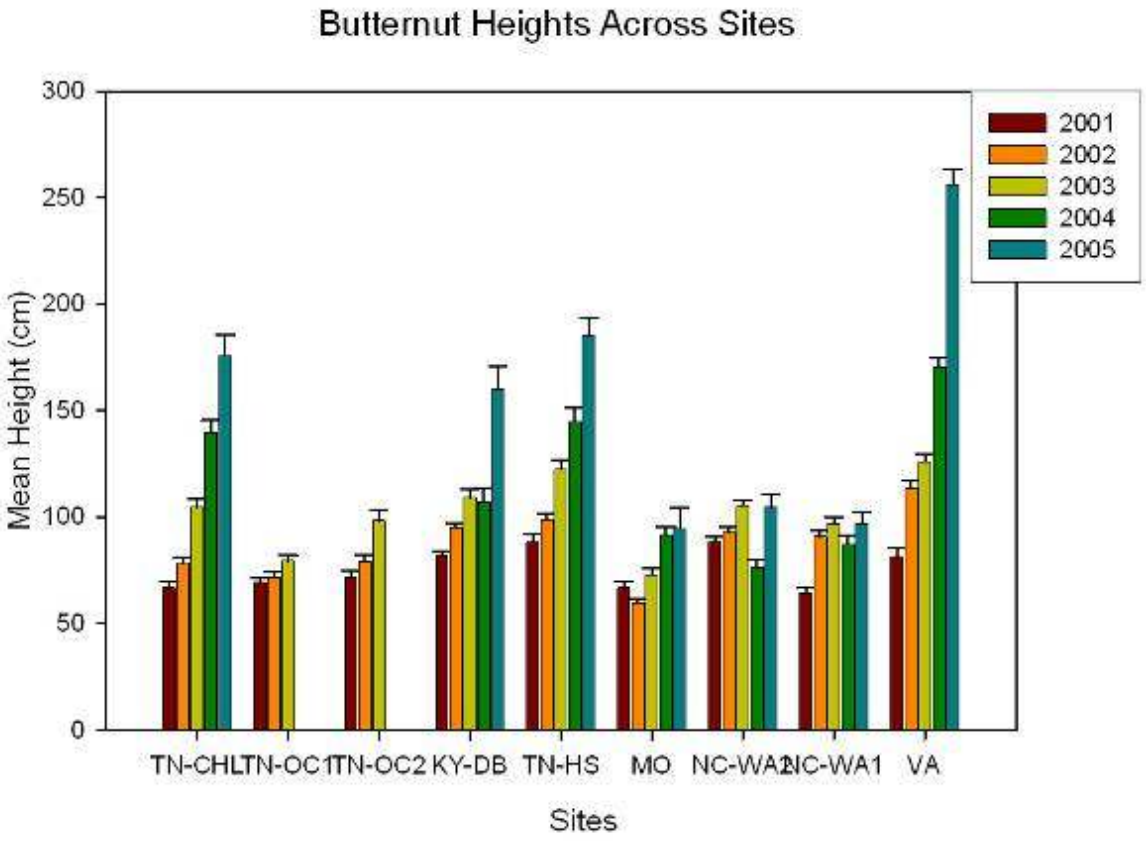

Figure IV-3. Average height of butternut seedlings across surviving planting sites from 2001 until 2005.

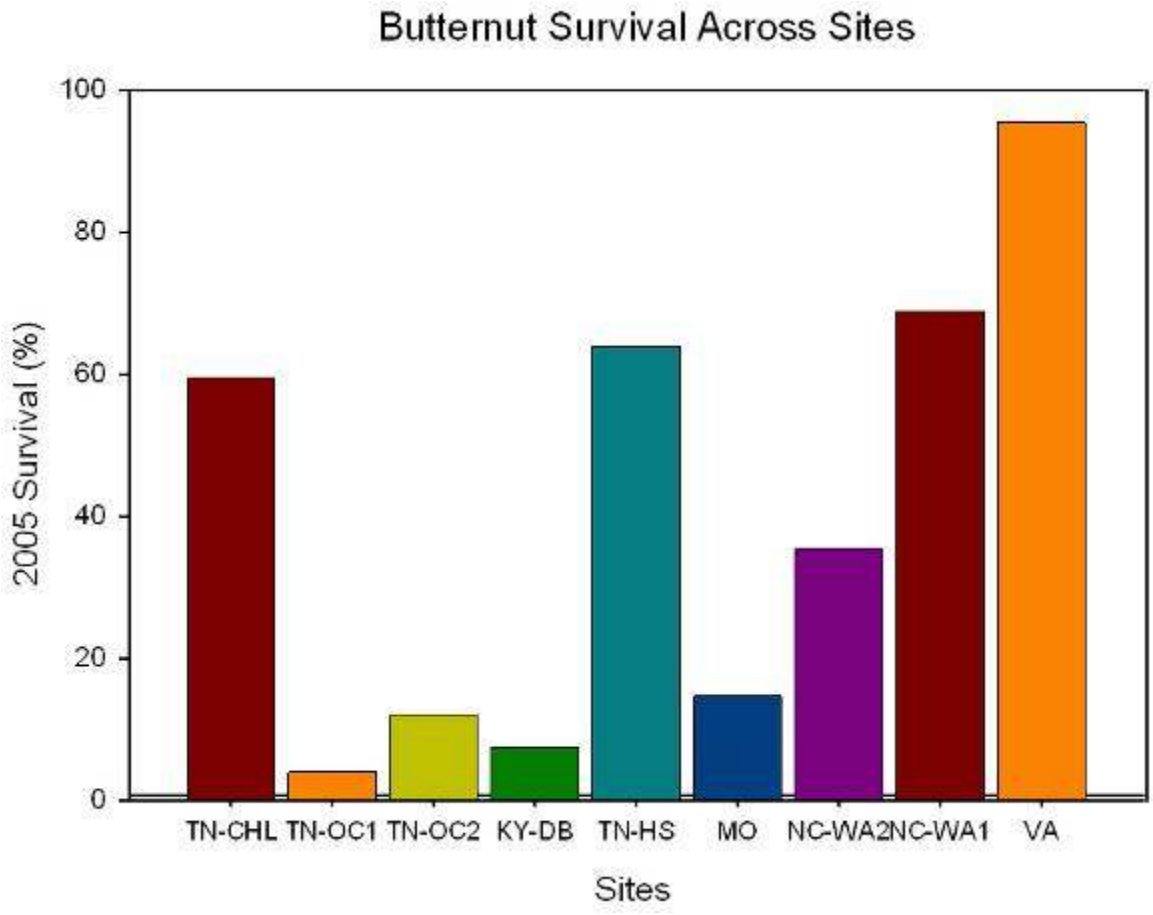

Figure IV-4. Percent of butternut seedlings surviving in 2005 across remaining planting sites.

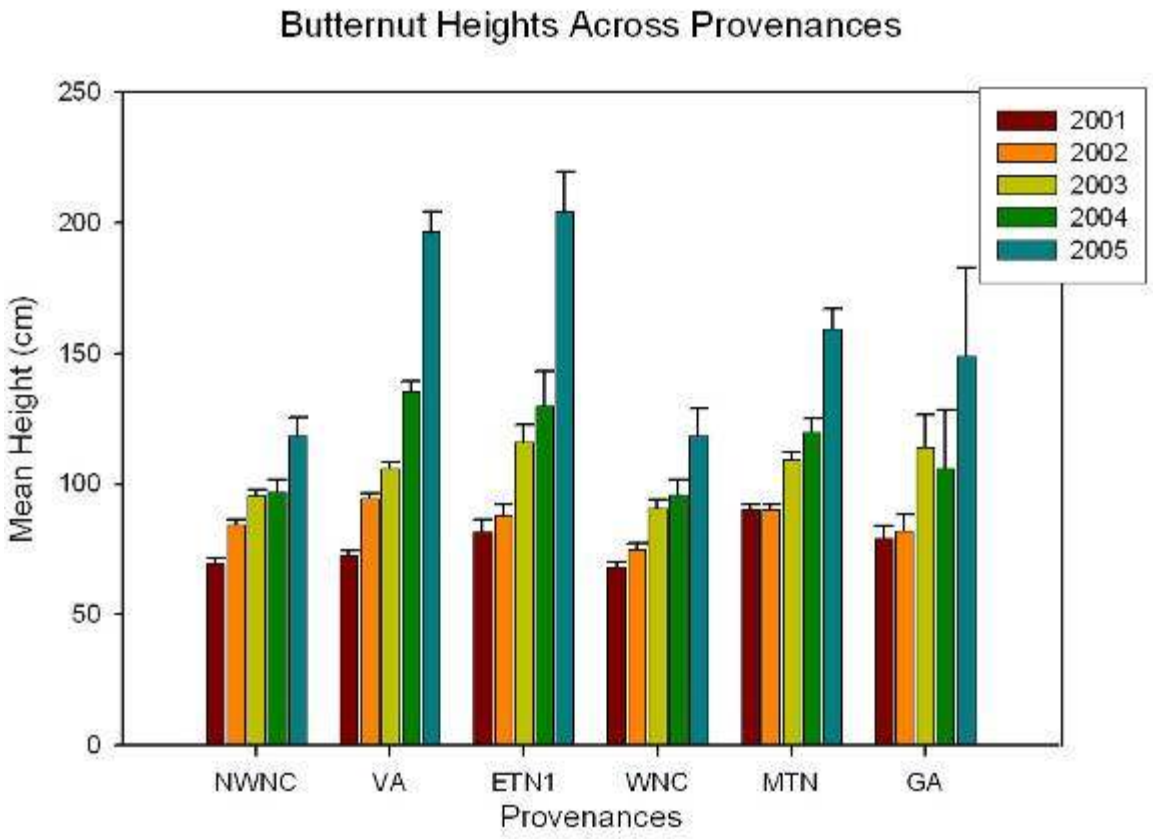

Figure IV-5. Height of butternut seedlings across provenances of origin from 2001 until 2005.

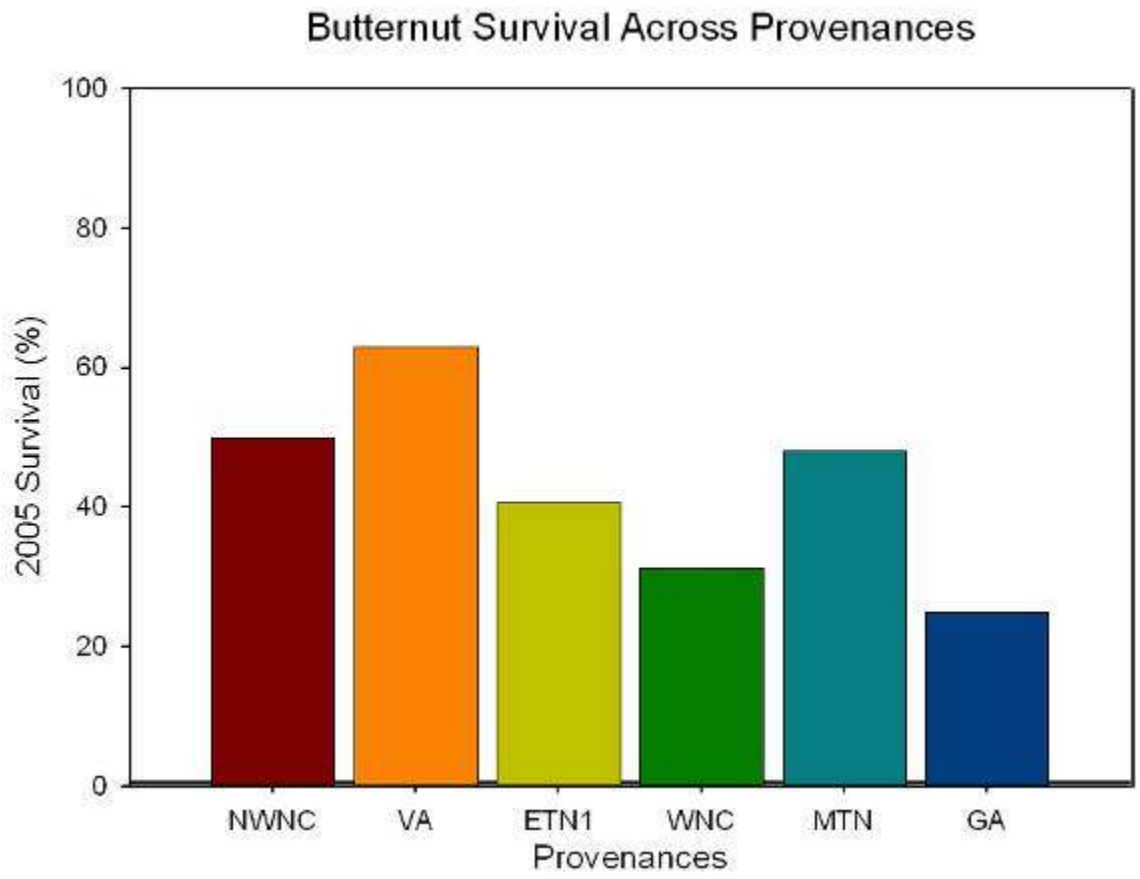

Figure IV-6. Percent of seedlings surviving in 2005 across provenance of origin.

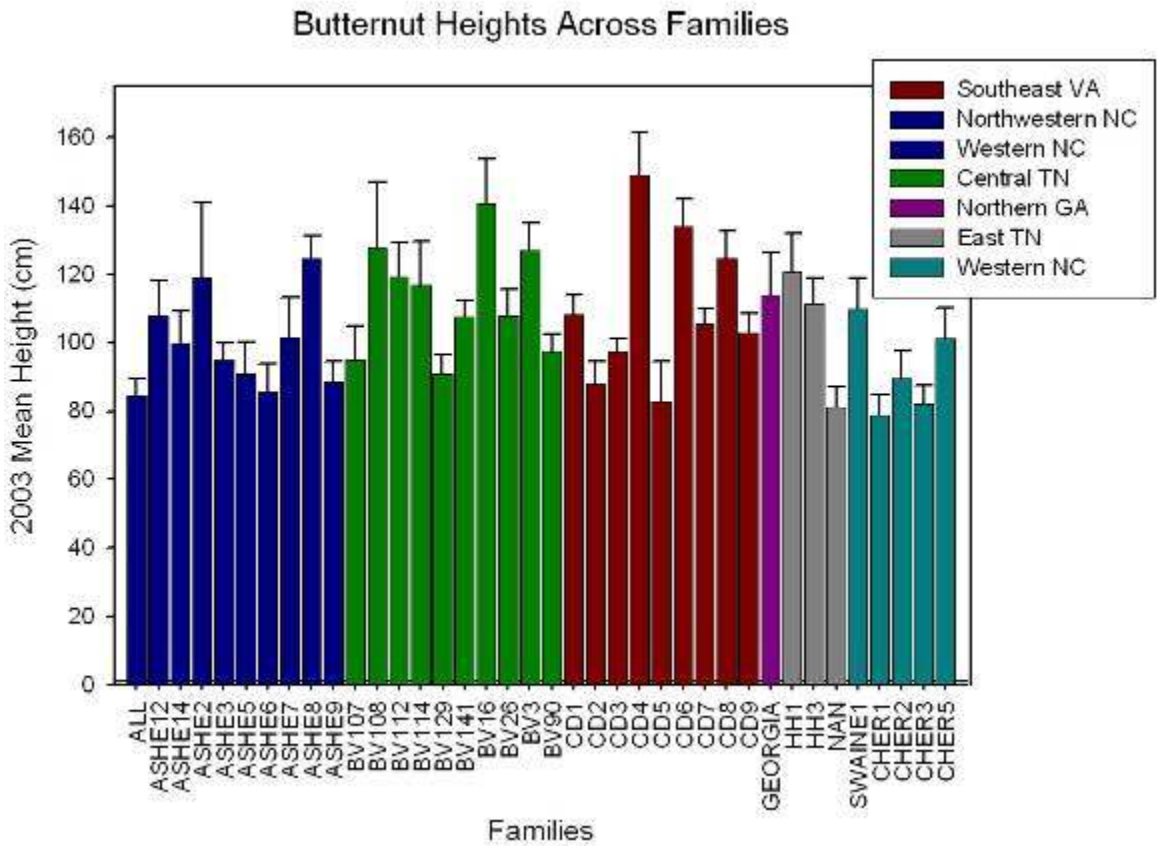

Figure IV-7. Height of butternut seedlings in 2003 across provenance and family of origin.

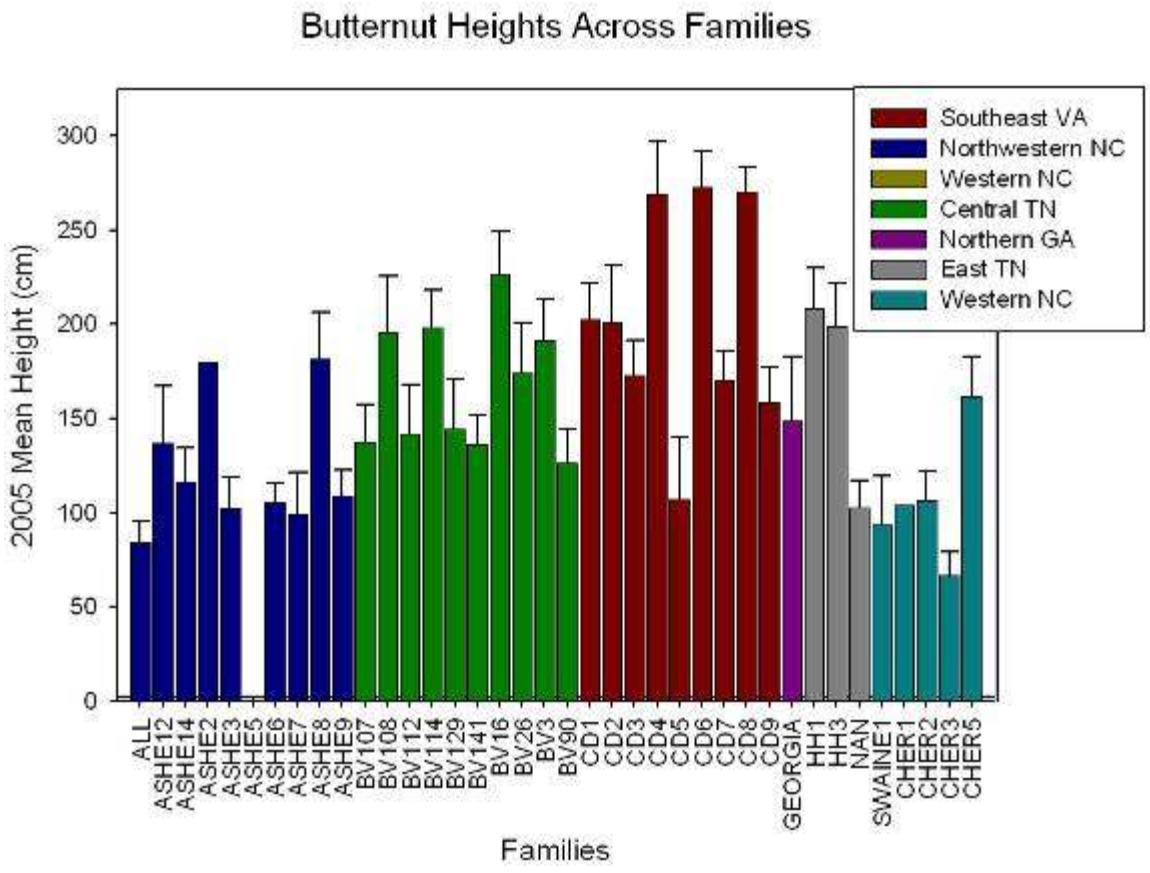

Figure IV-8. Height of butternut seedlings in 2005 across provenance and family of origin.

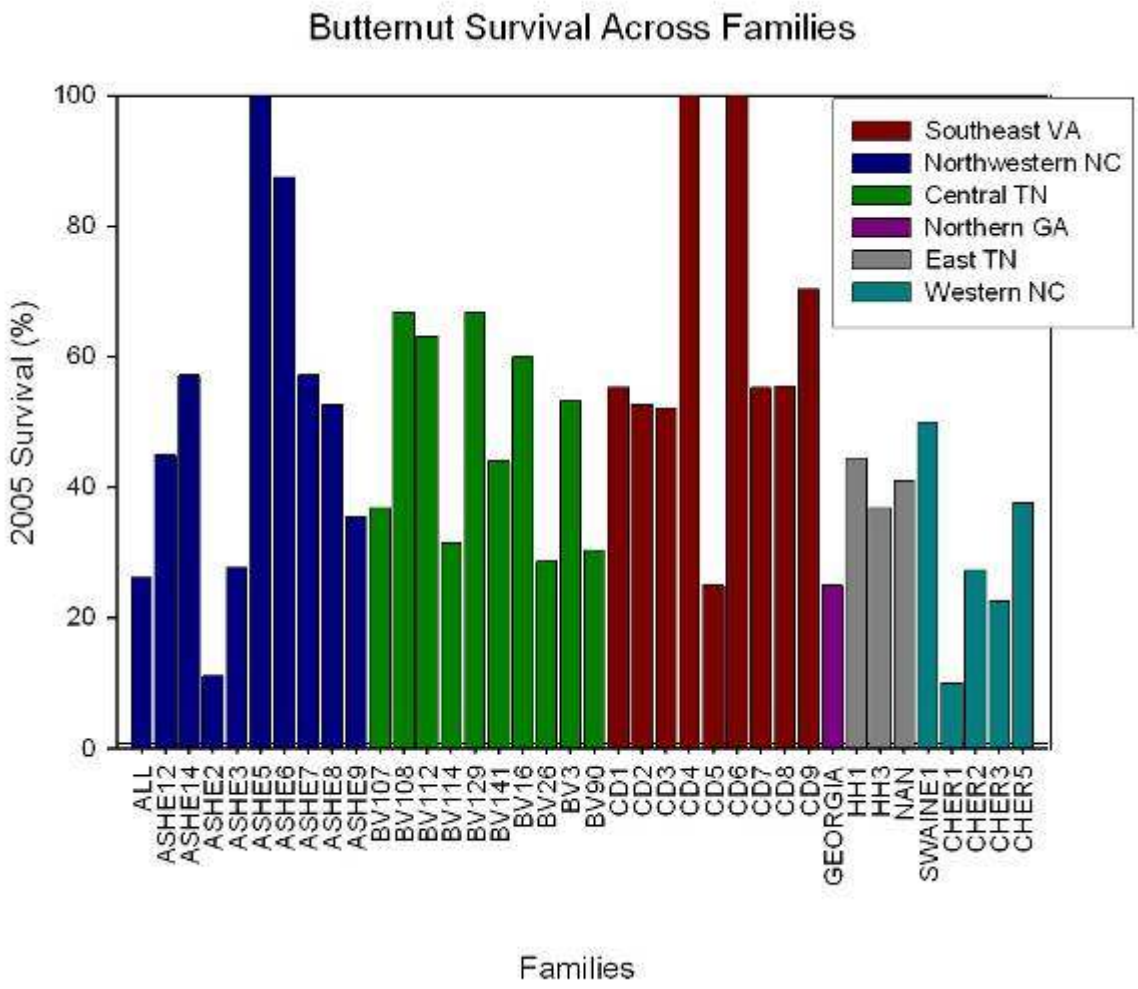

Figure IV-9. Survival of butternut seedlings in 2005 across provenance and families of origin.

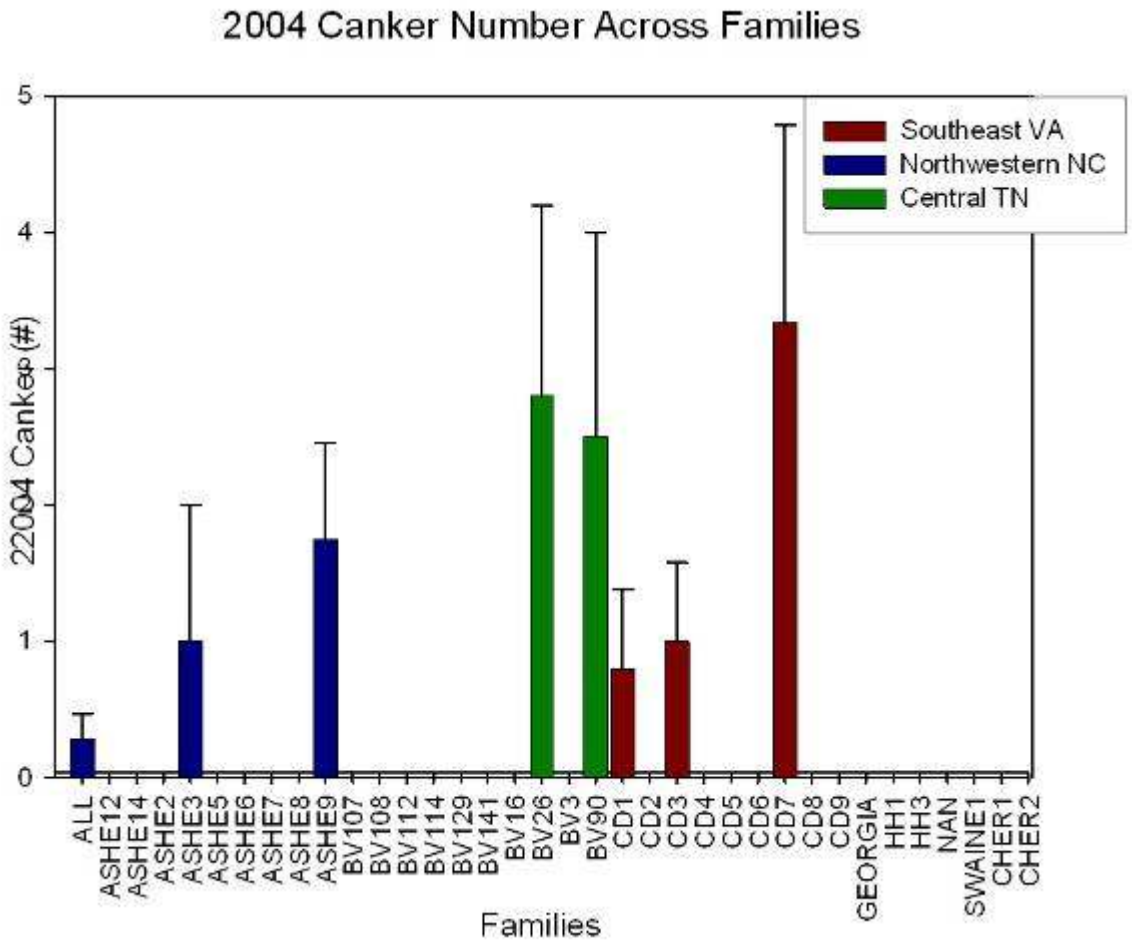

Figure IV-10. Cankering of butternut seedlings in 2004 across provenance and families of origin.

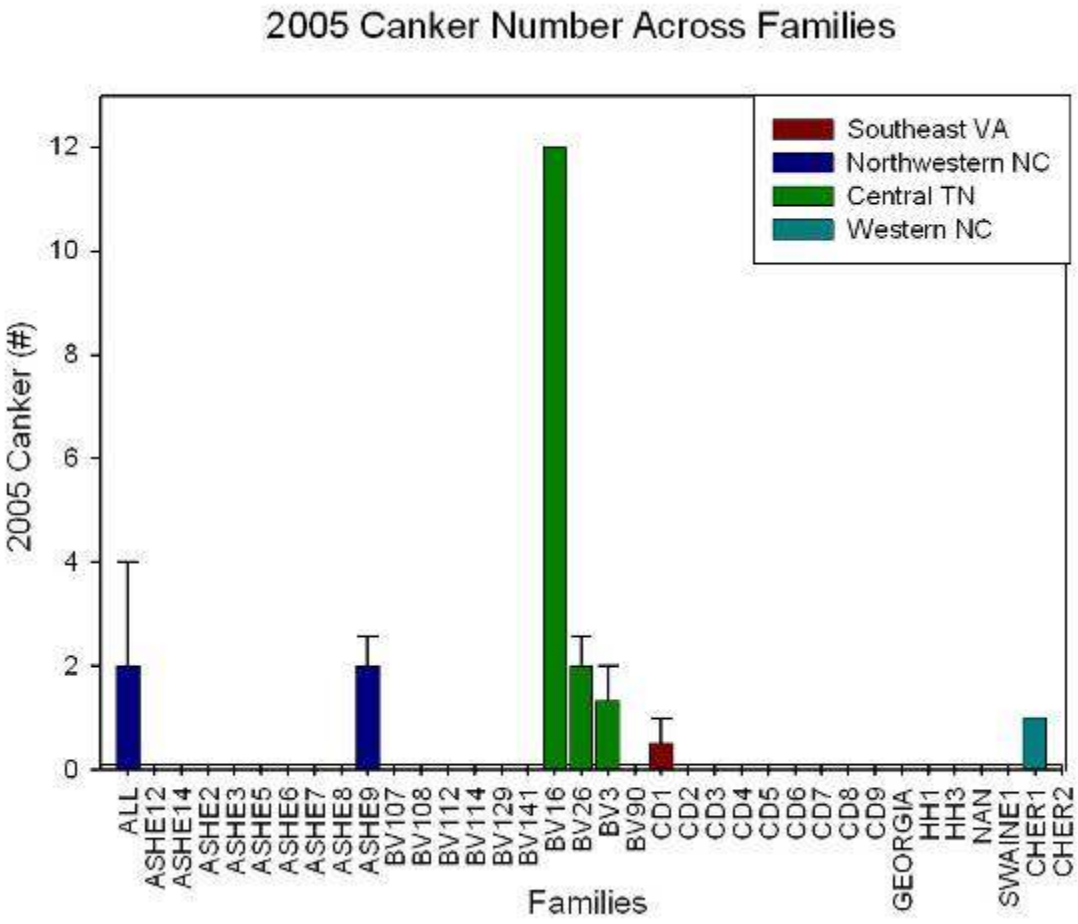

Figure IV-11. Cankering of butternut seedlings in 2005 across provenance and families of origin.

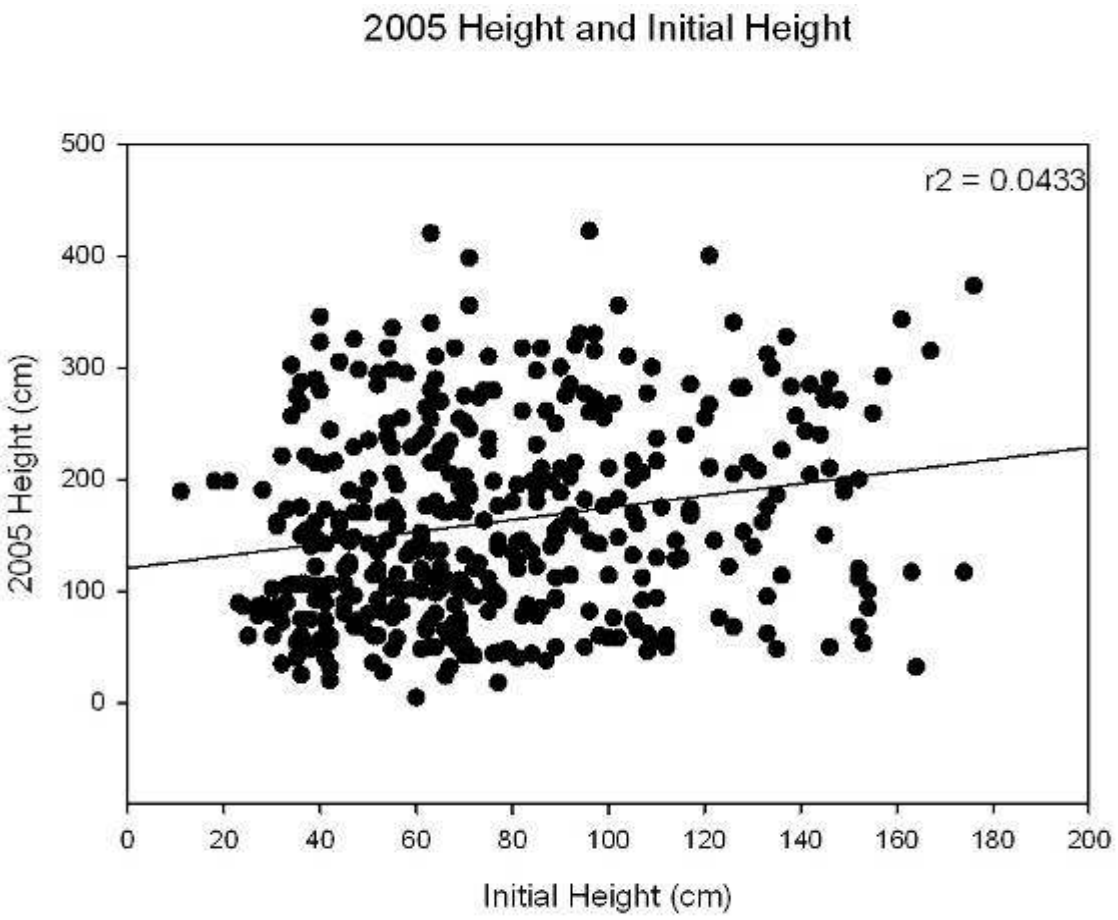

Figure IV-12. Height of butternut seedlings in 2005 across initial height for seedlings prior to planting.

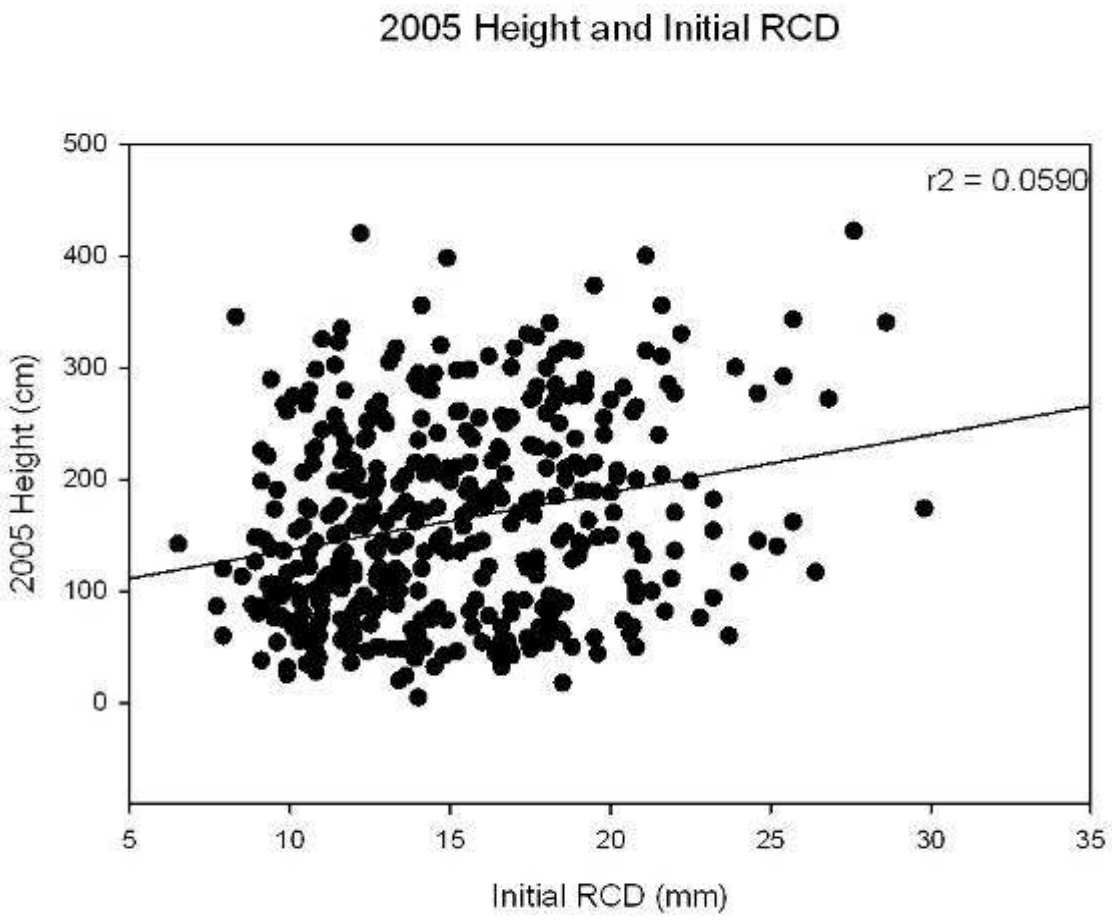

Figure IV-13. Height of butternut seedlings in 2005 across initial root collar diameter for seedlings prior to planting.

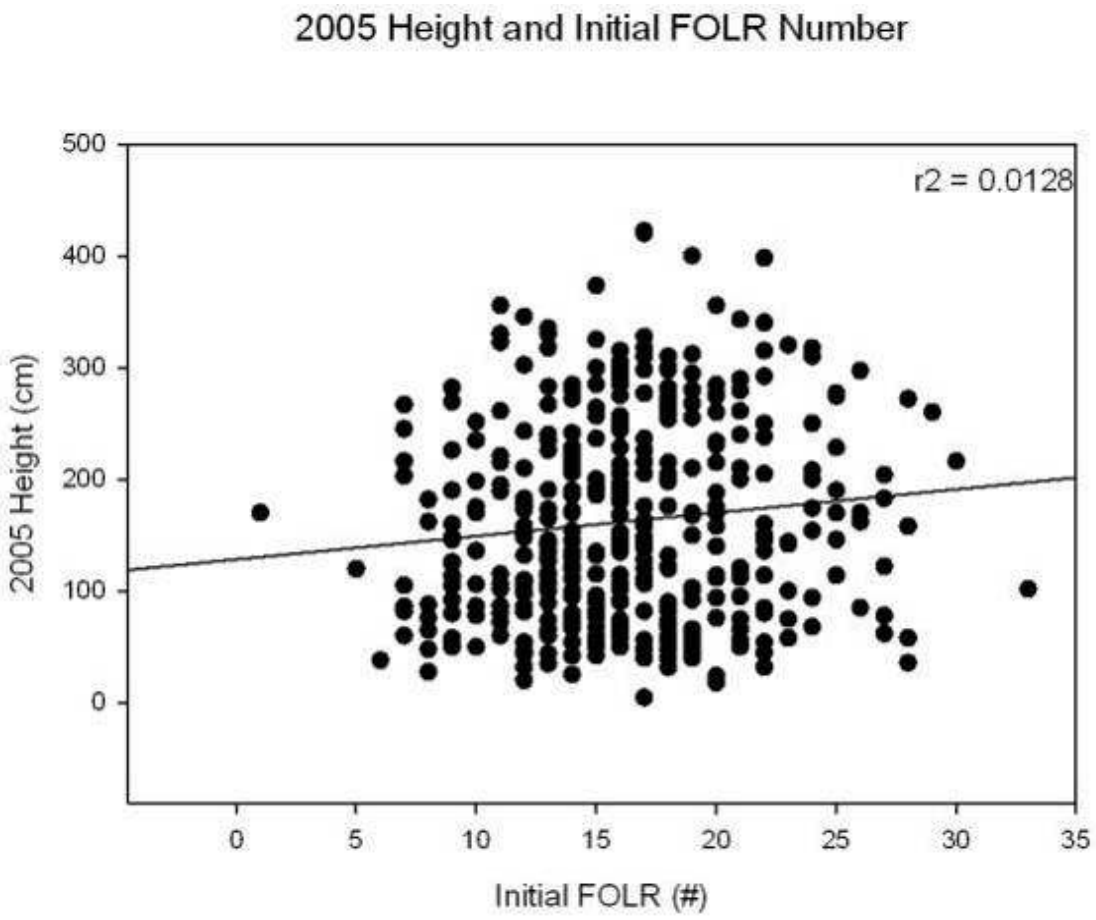

Figure IV-14. Height of butternut seedlings in 2005 across initial number of first-order-lateral root number for seedlings prior to planting.

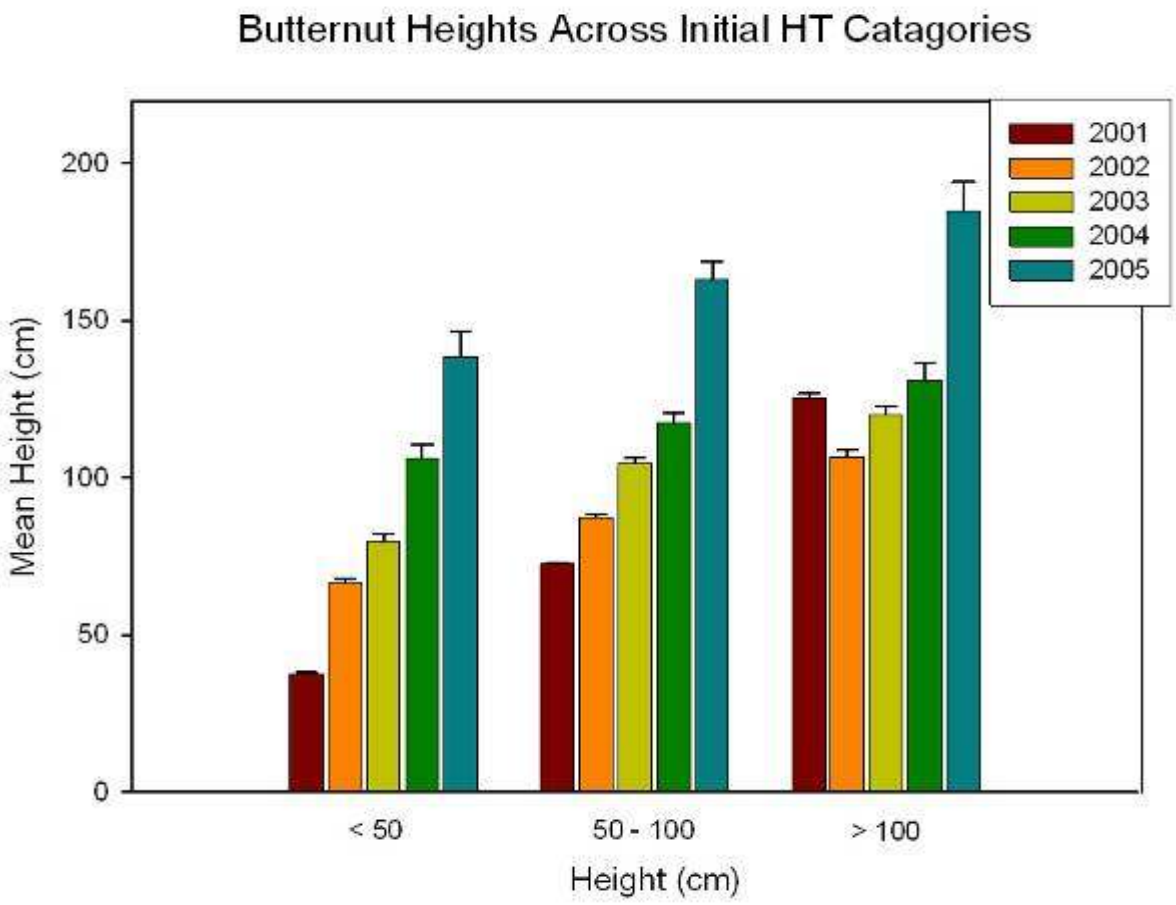

Figure IV-15. Height of butternut seedlings from 2001 until 2005 across initial height categories for seedlings prior to planting.

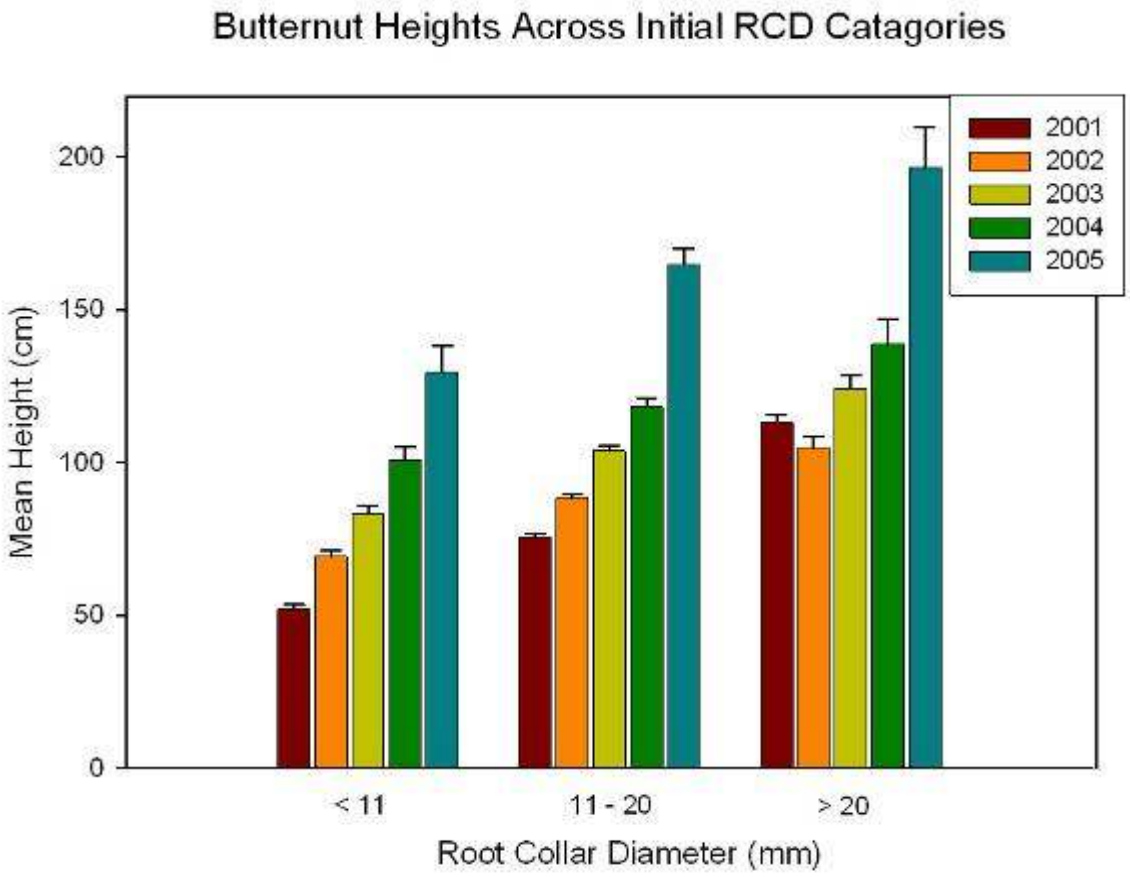

Figure IV-16. Height of butternut seedlings from 2001 until 2005 across initial root collar diameter for seedlings prior to planting.

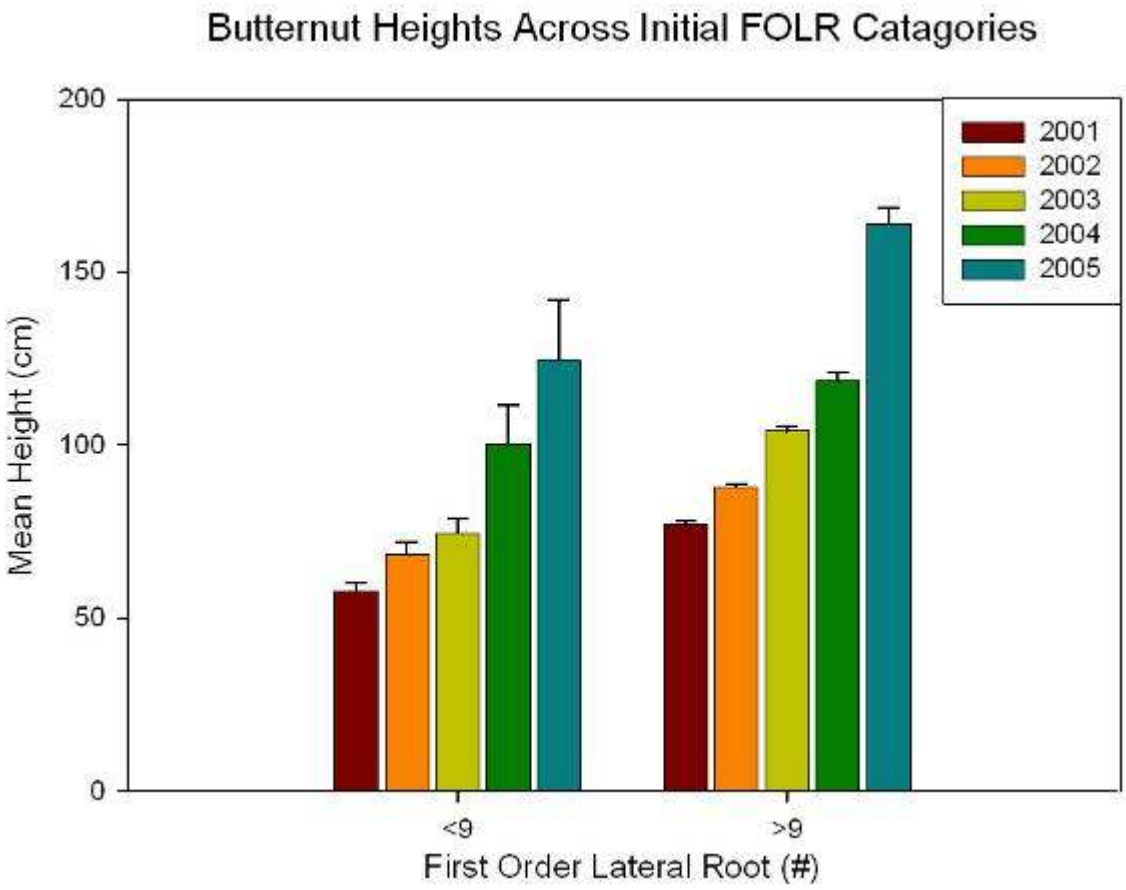

Figure IV-17. Height of butternut seedlings from 2001 until 2005 across initial first-order lateral root number for seedlings prior to planting.

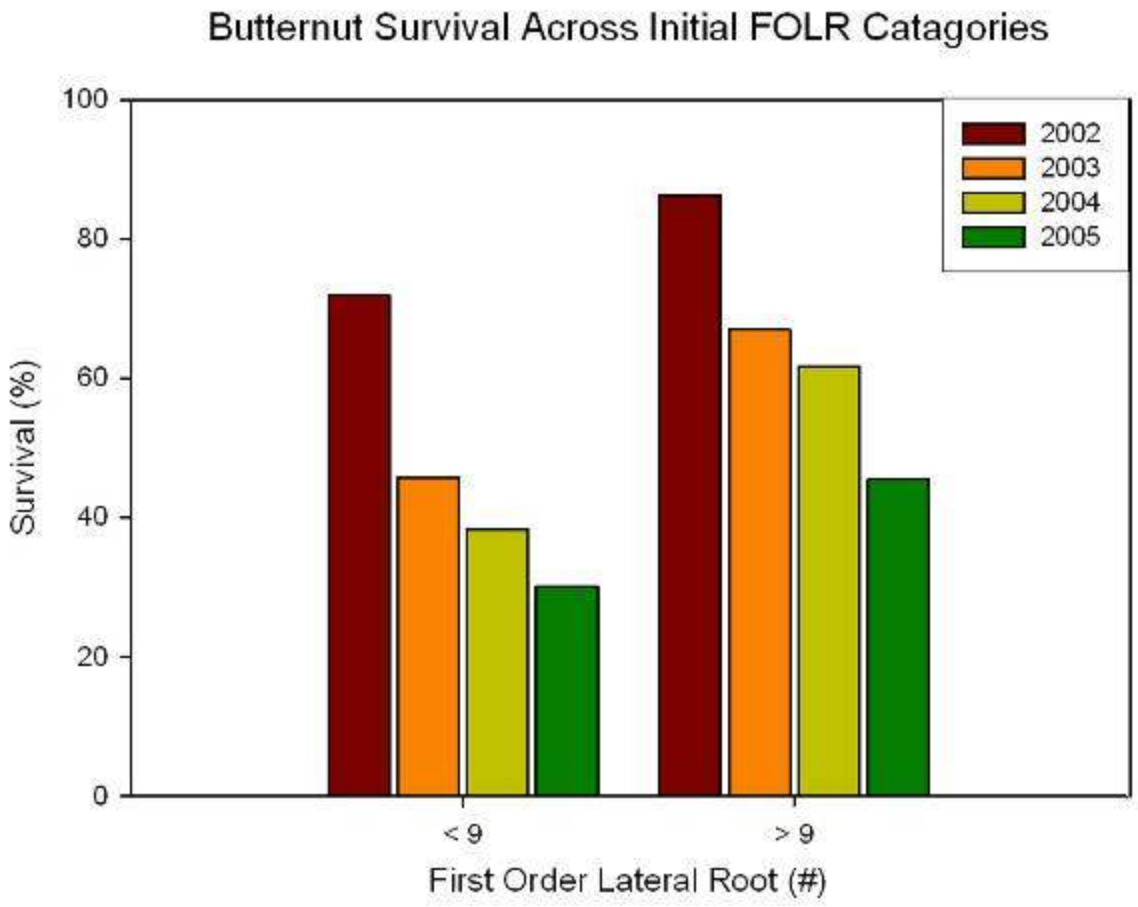

Figure IV-18. Survival of butternut seedlings from 2002 until 2005 across first-order lateral root number for seedlings prior to planting.

PART V. SURVIVAL AND GROWTH OF BUTTERNUT PROGENY FROM DIFFERENT FAMILIES ACROSS VARIOUS PLANTING SITES

Abstract

Butternut, *Juglans cinerea* L., is currently being eliminating from various locations particularly in the southeastern United States. *Sirococcus clavigignenti-juglandacearum (*V.M.G. Nair, Kostichka, & Kuntz), the causal fungus of butternut canker disease, is suspected to have been impacting butternut since at least the 1920s. Butternut is a short-lived, early successional tree species. As a result, it is also being eliminated due to crown closure of even-aged stands in riparian areas. Locations adequate for enrichment plantings will guide restoration protocols for this important mast producing species. Over two thousand seedlings were planted in stands with various histories and manipulations in Tennessee and surrounding states (Kentucky, Virginia, and North Carolina). Nursery seedlings were grown from open-pollinated nuts collected from eleven distinct families. Seedlings were planted under four scenarios: in open grasslands, in open grasslands with seedlings in tree shelters, in the understory of shelterwoods, and on reclaimed surface mines. Survival and height growth varied across sites, families, and initial seedling conditions. Mortality was 19% in the first year and 26% in the second year with the greatest mortality on surface mines and the least on open grasslands. Seedlings planted in shelterwoods averaged taller at the end of the second year (130 cm) than seedlings in all other planting types (92 cm). Seedlings in shelterwoods were competing with seedlings and sprouts for light, which encourages strong apical dominance and height growth. Two specific genetic families were taller than all others, and averaged over 120 cm tall. In addition, four families had less than 20% mortality, whereas one family had 60% survival. Initial seedling size greatly impacted establishment success. Seedlings < 9 mm in root collar diameter (RCD) when planted were twice as likely to experience mortality and were 26% shorter than larger diameter seedlings. Seedlings <50 mm in height also experienced additional mortality and were 36% shorter after two years than larger seedlings. Results of this study suggest that selecting larger diameter and taller seedlings from specific genetic families would be very worthwhile for increasing butternut establishment success.

Introduction

Butternut canker disease is caused by the exotic fungal pathogen *Sirococcus clavigignenti-juglandacearum (*V.M.G. Nair, Kostichka, & Kuntz). This fungus is killing butternut (*Juglans cinerea* L.) trees throughout their native range. A crucial first step in the process of restoring butternut is to determine if genetic resistance to butternut canker disease exists (see Chapter IV). However, successful establishment and growth of resistant seedlings will require an understanding of artificial regeneration procedures and the influences of site, seed source, and seedling quality on establishment success. Success of reintroduction of resistant butternut may be increased by incorporating knowledge of the impact of initial conditions on growth and survival (see Chapter IV). Potential planting areas on public land include old fields, riparian areas with an absence of harvesting, and forested areas with various silvicultural manipulations. Given the costs of producing and planting butternut seedlings for restoration, a greater understanding of the best site types where seedling establishment and growth are maximized would substantially aid restoration efforts.

Artificial regeneration of butternut

Butternut has been cultivated since 1633 a half-century before black walnut and other North American *Juglans* species (cf. Brinkman 1974). Artificial regeneration of butternut, however, has never been widely practiced in the United States or Canada, due to abundant natural regeneration in some areas and the selection of other walnuts for timber and nut production (Ostry and Pijut 2000). Butternut has been noted to have a rapidly developing root system that requires transplanting early in seedling development (Rink 1990). A few recent plantings of butternuts have occurred, but artificial regeneration of butternut has had mixed results. Establishment of 2-0 butternut stock in tree shelters in 1998 was unsuccessful due to shading from competing hardwood spouts and heavy shrubs (Ostry *et al.* 2003). Success of planted seedlings could be aided by using large seedlings that will compete vigorously with surrounding competition and rapidly grow above the reach of deer.

Site selection

Historically, artificial regeneration of butternut was conducted on old field sites for reforestation and for species conversion in order to support timber and nut production goals (Ostry *et al.* 2003). When butternut seedlings were planted in five different canopy openings, greater height and diameter occurred on with more light (Cummings Carlson 1997). A planting of 2-0 butternut nursery stock in Wisconsin was out-competed by hardwood sprouts and heavy shrubs and mortality, unrelated to disease, was high (Ostry *et al.* 2003). However, seedlings growing on clearcut or heavier thinning treatments were more vigorous (Ostry *et al.* 2003). The few attempts at direct seeding of butternut has failed (Ostry *et al.* 2003).

Progeny plantings from 1995 to 2002, in which butternut seedlings were planted under the canopy of trees, have shown the ability of butternuts to survive and grow in various light environments (Brosi *et al.* 2007, Chapter IV). Butternut seedlings were able to persist and grow under the overstory of mature trees, however, mortality was high. In light-saturated environments, butternut seedlings grow, but encounter competition. Overall, the greatest survival has consistently occurred in areas with more sunlight with the lowest survival in the areas with the most shading. Butternuts on sites without competing vegetation lose apical dominance and become more bush-like in habit. While that form may be ideal for nut production, it is not be suitable for timber and may not be competitive over time if vegetation control would lapse and other woody species invade the site.

Establishment success of butternut for timber production or as a component of the surrounding forest would be optimized by selecting sites with characteristics that allow seedlings to become initially established and subsequently have enough competition for the seedlings to retain apical dominance, yet vigorously compete with surrounding vegetation. For butternut in particular, initial seedling height coupled with a correspondingly large root system may be paramount to survival. Ostry *et al.* (1994) observed that vigorously growing saplings may have outgrown the girdling effects of the canker. Therefore, a strong relationship may exist among seedling quality, survival and competitive ability, and delaying the impact of the butternut canker disease.

Seedling origin

Seed source and genetic family can impact survival and growth of butternut seedlings (see Chapter IV). However, multiple genetic families have not been evaluated over a large number of sites. Extensive research has shown that variation in establishment success of black walnut (*Juglans nigra* L.) and English walnut (*Juglans regia* L.) can be partially attributed to genetic family and individual genotypes within families (Bey 1973, Rink *et al.* 1994, Bresnan *et al.* 1994, Geyer and Rink 1998). Black walnut progeny plantings, after 17, 21, 22, and 35 years have shown significant differences in height growth and heartwood production between open-pollinated families, indicating the opportunity for gains from selection (Bresnan *et al.* 1994, Geyer and Rink 1998). In black walnut progeny plantings, selection of superior progenies to improve rotation height and diameter may occur at age 4 to 6 years (Kung 1975, McKeand *et al.*1979, Rink *et al.* 1994). Heritability of height growth increased steadily with age until age 10 (Rink 1984), and leveled off from age 10 on (Rink and Kung 1995). Evaluation of a 20-year old planting determined that any time after age 8 was acceptable for selection (Rink and Kung 1995). However, in a spatially non-replicated study Michler *et al.* (2003) found rank shifting and increases in heritability occurring between ages 10 and 15. Butternut genetic families need long-term evaluation to determine if specific families exhibit superior height growth over time.

Seedling characteristics

Low hardwood plantings success often results from damage by animal browsing (Marquis 1974; Martin and Baltzinger) and herbaceous and hardwood competition which overtop artificial regeneration planted in open areas (Cogliastro *et al.* 1990, Kolb *et al.* 1990, Willoughby and McDonald 1999). Variability in the quality of hardwood seedling planting stock often results in planting failures and low vigor (Johnson 1981, Johnson *et al.* 1986, Wendel 1980). Seedling establishment success often increases with the quality of the nursery stock for northern red oak (*Quercus rubra* L.) (Zaczek *et al.* 1997, Dey and Parker 1997, Ward *et al.* 2000, Jacobs *et al.* 2004). Traditionally, nurseries have grown hardwood

seedlings according to their own procedures on dense spacing to produce small, uniform seedlings that facilitate shipping and planting (Johnson 1981, Kormanik and Ruehle 1987). Production of large stock for black walnut seedling production has been investigated through seed selection and nursery techniques since at least 1947 (Chase 1947). Over the past decade, nursery practices have adjusted density of plantings and fertilization and irrigation applications in order to produce large hardwood seedlings (Kormanik *et al.* 1994a, Kormanik *et al.* 1994b).

Field establishment of large seedlings shifts the competitive advantage toward the seedlings and reduces losses due to herbivory (Ward *et al.* 2000). Large nursery stock can quickly become established in the field and can be produced by adjusting nursery practices (Kormanik *et al.*1994a). Selecting large seedlings has increased establishment success for a variety of hardwood species including oak species (*Quercus* L.) (Kormanik *et al.* 1997, Schlarbaum 1993, Clark *et al.* 2000). In addition to height (HT), root collar diameter (RCD) measured at 1.3 cm above the root collar, has been used as an indicator of seedling condition (Ruehle and Kormanik 1986). Nursery-grown hardwood seedlings with larger RCD, prior to outplanting, have been shown to result in better survival and growth after outplanting than smaller stock (Olson and Hopper 1972, Zaczek *et al.* 1997). RCD and stem height can also predict establishment success for black walnut seedlings (Schultz and Thompson 1990, 1997).

Objectives

Seedling characteristics, including initial seedling height and RCD, were found to impact survival and growth of butternut seedlings on particular sites (see Chapter IV). Those plantings, however, were designed as butternut canker resistance tests rather than restoration experiments. Butternut restoration plantings were established in this experiment with the overall objective of evaluating the effects of site, genetic family, and nursery characteristics on initial butternut seedling survival and growth. The overarching goal of this project is to aid butternut restoration by generating information on site requirements, seed source selection, and the impacts of seedling quality.

- *Objective 1).* Determine establishment success across various individual sites and site categories;
- *Objective 2).* Determine if some genetic families are superior in terms of growth and establishment success
- *Objective 3).* Determine if relationships between initial seedling variables (HT and RCD) and establishment success are present. Seedlings will be categorized by initial variable to determine impacts of initial size on establishment success.

This is the first evaluation of butternut progeny tests across various sites using high quality seedlings.

Methods

Progeny collection

In the fall of 2003, 10,984 butternuts were collected from 17 open-pollinated genetic families from Virginia, Tennessee and North Carolina (*sensu* Bailey 1995, Figure V-1, note tables and figures appear in appendixes). Seven open-pollinated families had over 600 nuts per family. The butternuts were dehusked and planted at the Georgia Forestry Commission's Flint River Nursery in Byromville, Georgia in December, 2003. The resulting seedlings were grown for one growing season (2004) using fertilization and irrigation regimes developed by the USDA Forest Service's Institute of Tree Root Biology (Kormanik *et al*. 1994a). The seedlings were lifted in February, 2005 using Fobro® lifter-shaker (Bartschi-Fobro, Huswil, Switzerland) that undercut the root systems at 30 cm.

Planting sites

Over 2,000 seedlings from the half-sibling families were sorted into experimental plantings using an incomplete block within complete block design and single tree plots. Plantings were established on sites with four varying site histories across four southern states. Plantings occurred on 15 sites in seven locations on public and private land in Tennessee,

Virginia, North Carolina, and Kentucky (Table V-1, Figure V-2). Seedlings were planted on various sites after various stand manipulations and site histories. The plantings took place under four scenarios: 1. open grassland, 2. open grassland using tree shelters, 3. beneath shelterwoods, and 4. on reclaimed surface mines. Plantings in open grasslands (1) included a former Forest Service Work Center (TN-OC1), former recreational area (VA-CL), and former hay fields (NC-KA1, KA2). The open grassland with tree shelters (2) included a new orchard site (NC-HO). Shelterwoods (3) included previous year timber harvesting with 30% basal area retention (NC-GF1, GF2, AP1, and AP2) and a site located near a beaver pond with heavy thinning due to beaver activity (TN-OC2). Shelterwood sites had commercial harvest that removed 70% of the total stand basal area (Loftis 1990, 1993). Sites in surface mines (4) were on ripped mine spoil benches near artificial ponds with limited grading to reduce compaction. Tree shelters were manufactured by TreePro© and were 4.5 inches in diameter (11.4 cm) and five feet tall (1.5 m). Seedlings planted in tree shelters are in old fields free from any competition. Seedlings were placed in tree shelters at this site due to heavy deer browsing pressure in the planting area. This site was in an area where hunting is prohibited and herd sizes are artificially large due to lack of natural predators. The two plantings in old agricultural fields close to each other had one site where competition was controlled (KA-2) and one where competition was not controlled (KA-1).

The plantings were established in a completely random design using single-tree family plots on 1.5m (5 ft) spacing. The seedlings were manually planted in 30 cm deep holes made by gas powered hand augers with six inch auger bits. The seedlings were measured for initial HT and RCD just after planting. HT was measured from the ground to the terminal bud to the nearest 0.5 cm using a meter stick and RCD was measured at ground level using digital calipers. After the first growing season (2005) and the second growing season (2006), the seedlings were measured for their responses, in terms of survival, HT, RCD, condition, and growth form. Seedling condition, i.e. evidence of dieback, disease, or deer browse, was evaluated. Seedling condition classes were developed based on initial seedling size into three HT classes and two RCD classes. Seedling condition classes

included: HT classes were > 50 cm, 50-100 cm, and >100 cm. RCD classes were < 9 mm or ≥ 9 mm.

Statistical analyses

Statistical analyses were conducted using SAS version 9.1 (for Windows, © 2005 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Additional macros developed by Saxton (1998, 2004) were used for means separation. Macros included pdmix800 for means separation using multiple range tests (Saxton 1998), Mixed Model Analysis of Variance Macro (mmaov) uses Fisher's Least Significant Difference (LSD) test was conducted at the 5% significance level, determines if standard deviations are within five fold of each other, calculates Levene P values to determine equality of variances, and a Shapero-Wilk W test for normality (Saxton ©2002). Least-square survival and HT means of each family were converted to relative survival and heights, expressed as a percentage of the plantation mean. All percentage data, derived from count data, including survivorship and presence of cankering, underwent inverse trigonometric sine transformations due to lack of homogeneity of variances (Snedecor and Cochran 1998).

Objective 1). Yearly seedling variables (percent HT growth, percent RCD growth, and percent survival) were analyzed across planting sites using analysis of variance (PROC MIXED). Fixed effects included sites and families: site, rep (site), block (site*rep), site*family. Seedling response, in terms of growth and survival, were treated as independent variables. Tukey's pairwise means comparisons were used to separate means when a statistically significant overall effect was established (SAS 2005).

Objective 2). Yearly seedling variables (percent HT growth, percent RCD growth, and percent survival) were analyzed across families using analysis of variance (PROC MIXED). Seedling response, in terms of growth and survival, were treated as independent variables. Tukey's pairwise means comparisons were used to separate means when a statistically significant overall effect was established (SAS 2005).

Objective 3). Phenotypic correlations were calculated using Pearson's correlation analysis for year to year family means on survival and growth (PROC CORR). Correlations were also calculated between year to year seedling variables and between year-before HT

and RCD to specific-year survival and HT. Analyses of variance were conducted on categories of initial seedling variables described previously.

Results

Seedling production

The 2004 growing season had high levels of precipitation, which promoted *Phytophthora* root rot disease [causal agent: *P. cinnamomi* Rands] at the Flint River Nursery. The disease impacted the seedlings and caused rapid mortality, reducing the number of families and seedlings available for planting. After culling seedlings affected by root rot, only ten families remained with a total of 2,201 seedlings suitable for planting. The studies were measured immediately following planting for HT and RCD. These data correspond to HT and RCD at the end of the 2004 growing season. Just after planting, the seedlings had a mean HT of 87.61 cm (variance 10.7) and a RCD of 12.61 mm (variance 1.69). Unlike other years (2001, 2002, 2004, and 2007) where seedlings were measured prior to planting (see Chapter III), these seedlings were measured after outplanting.

Overall growth and survival

The average HT at the end of the 2005 growing season was 89.7 cm (variance 11.61) and the average RCD was 14.96 mm (variance 3.54. The seedlings average an increase of HT of 2 cm since planting, not significant, and a significant increase of 2.35 mm in RCD in the first year after planting ($p>0.001$). Overall survival was 81.82% for the first year.

At the end of the seedlings second year in the planting locations (2006), the average HT of the seedlings was 110.02 cm; greater than initial HT and first year HT growth ($p>0.001$, Figure V-3, Figure V-4). The average RCD, 18.68 mm, was larger than the previous years ($p>0.001$). Over twenty percent of the seedlings were over 2 meters in HT, and 45% of the seedlings were larger than the mean. The seedlings have had a 19% increase in HT (20 cm) and a 21% increase in RCD (3.9 mm) since the previous year. Since planting the seedlings had increased 20 and 33% for HT and RCD respectively. Overall survival was 71.85%.

Objective 1: Planting sites

Height growth and survival varied in percent among sites (Table V-3). During the first year survival varied across planting sites with 33% of the sites with greater than 90% survival, 53% with greater than 80% survival, and 73% with greater than 70% survival during the first year (Table V-2). First year survival was over 70% on all sites except for two sites on a surface mines and two forested sites **(**Figure V-6). HT also varied among planting locations in 2005. At both the TN-OC and the NC-AP sites, increased HT was found on sites with greater mortality where competition for with competing vegetation was high.

Second year survival varied across planting sites with the highest survival at 90% and the lowest at 20%. Just under half of the sites had over 80% survival, with 20% of the sites with under 60% survival (Figure V-9). The tallest sites had seedlings averaging 50% taller (159 cm) compared to the shortest sites (80 cm). RCDs were also 36% larger on one site (25.17 mm - 16.20 mm, Table V-2).

Grouping sites by site types showed that for both years, the greatest survival occurred on open grasslands (9, 17%) and the least on surface mines (29, 47%) (Figure V-6). The sites in open grasslands had the lowest percent mortality for both 2005 and 2006 (9.48 and 16.63%, Table V-3). Percent mortality in 2005 and 2006 was similar in open grasslands with tree shelters (23.80, 25.54) and in shelterwoods (20.85, 28.13). All sites on surface mines had higher mortality for both years compared to all other sites (29.47, 47.38%, Figure V-7).

Total HT in 2006 was largest in sites in shelterwoods (Table V-3). Seedlings planted in shelterwoods were taller at the end of the second year (130 cm) than seedlings in all other planting types (92cm). Sites located on grasslands had the second tallest average HT, followed by sites on surface mines and in tree shelters. Only sites on surface mines and in tree shelters had a decrease in HT over the two growing seasons (Figure V-8). On surface mines dieback during the first year (13.06% decrease in HT), continued though was less dramatic in the second year (1.79% decrease in HT). Reduction in HT also occurred at the one site with tree shelters. In 2006, over six sites had seedlings averaging over 120cm, five of these sites were in forested stands and the other site was in an open grassland with competition controlled: KA-2 (Figure V-5).

The one open grassland with tree shelters had only 74% survival and an average seedling HT of 80.6cm (15-178) and average RCD of 17.13 mm (8-35). This is an increase in RCD of almost 5 mm since outplanting. None of the seedlings are over 2 meters in HT, only 8.52% are over 1.5 meters, 33.52% are over 1 meter, and 49.35% of the seedlings are larger than the mean. The seedlings have RCD growth rates similar to the other plantings, but not similar rates of HT growth.

The KA-1 old field site without competition controlled had 83.33% survival and an average HT of 106.52 (16-192) and a RCD of 19.11mm (3-41). Only 8% of the seedlings were over 1.5 meters and 56% were larger than the mean. The KA-2 site with competition controlled had over 81% survival, lost only 10% in one year, and an average seedling HT of 123.93 cm (15-240) and average RCD of 25.17 mm (3.9-62.5). Five percent of the seedlings at this site were over 2 meters tall, 33% over 1.5 meters, 53% greater than the mean, and over 67% of the seedlings were larger than 1 meter in HT.

Objective 2: Family effect

The impact of genetic family was statistically significant for all variables (Table V-4). Initial seedling HT and RCD varied among families of origin. Prior to planting some genetic families were nearly twice the average HT of others. These family differences continued for the 2005 and 2006 growing season (Figure V-10) . Two specific genetic families were taller than all others and averaged over 120 cm tall in 2006 (Figure V-11). In addition, four families had under 20% mortality compared to one family with greater than 60% (Figure V-12).

Objective 3: Seedling variables

Overall relationships Overall, the impact of initial characteristics on 2006 height was significant for HT (Figure V-13) and RCD (Figure V-14). All phenotypic variables were significantly correlated except for 2006 HT and mortality (Table V-5). Similar to results found in other years, initial HT and RCD were significantly correlated (0.42), but with a lower value than found when measuring seedlings prior to planting in other nursery trials (see

Chapter III). The strongest relationships were between HT of consecutive years: (initial HT and HT05: 0.60, HT05 and HT06 0.56).

Relationships by seedling size category Initial seedling size class greatly impacted establishment success (Table V-6). Seedlings < 9 mm in RCD when planted were twice as likely to experience mortality and were 26% shorter. Seedlings < 50 mm in HT also experience additional mortality and were 36% shorter after two years.

Discussion

Site conditions

Butternuts were successfully established on a variety of site conditions. Large differences in HT over the various planting sites indicate variation in sites for height growth. The greatest survival occurred on old agricultural fields, and the greatest height gains occurred on shelterwoods. Sites with tree shelters and mine spoils experienced heavy mortality and reduced height growth.

Old agricultural fields appear to be particularly good locations for restoration, although long-term survival and growth is not known. Surveys for butternut, however, found many locations in fence rows and by houses on non-typical butternut sites (UT-Tree Improvement Program, data on file). Dendrochronology evidence indicates butternut recruitment in old agricultural fields (Clark *et al.* 2008). Therefore butternut may be an ideal species for Conservation Resource Enhancement Program (CREP) plantings. Timber production on these sites may not be an option, as form becomes more favorable for nut production for hard mast. Butternut may also be an ideal species for agroforestry applications. Temperate agroforestry involves multicropping of agricultural crops under high value plantations and intercropping of high values tree species with other commercially valuable crops and animal grazing (Gold and Hanover 1987). Butternut may also provide the increased profit of nuts prior to harvesting for timber. The impacts of juglone in walnut species, including butternut, have limited companion planting for *Juglans*. However, agroforestry applications of *J. nigra* have shown great potential for reforestation, timber and polyculture applications (Scott and Sullivan 2007). Many non-timber forest products that

require shade are tolerant to juglone and the juglone could act as a resource reducing unwanted competition (Scott and Sullivan 2007). Production of black walnut in combination with shade-tolerant agroforestry crops could be possible with *Ribes* L., *Sambucus nigra* L. ssp. *canadensis* (L.) R. Bolli, *Morus rubra* L., *Astisima triloba* (L) Dunal, and *Diospyros virginiana* L. Species such as American ginseng (*Panax quinquefolius* L.) have also been found in association with black walnut (Apsley 2004, Carroll 2004). Black walnut may also provide shade for mushroom production (Scott and Sullivan 2007). In addition mixed species plantings of black walnut have show the ability to plant juglone-tolerant species for increased height growth in walnut and production of *Robinia pseudoacacia* L., *Pinus strobus* L., *Fraxinus americana* L., *Quercus rubra* L. and *Acer rubrum* (Scott and Sullivan 2007). Planting butternut in association with juglone tolerant species on open sites may increase profitability for landowners and result in ideal conditions for growth form and establishment. This is especially important as plantings in open areas may require extensive competition control and the application of fertilizers. Agroforestry can offer landowners options for quicker returns on investments in high quality timber species.

Shelterwoods with 30 basal area retention were also suitable sites for butternut plantings. There was lower survival than open field plantings, but increased HT growth was present in surviving seedlings. The seedlings in forested stands are competing with seedlings and sprouts for light, which may result in increased mortality, but also encourages strong apical dominance and HT growth. Mortality on these sites was primarily in smaller seedlings; under 50 cm in height and under 9 mm in diameter. The larger seedlings were able to establish with limited mortality after the first year. Shelterwoods have been important for establishment of *Quercus rubra* L. (Loftis 1990, 1993). Increased height growth of butternuts in shelterwoods is similar to studies conducted with American chestnut (Rhoades *et al.* 2009). Two years after planting chestnut seedlings were 3.5 times taller when planted on shelterwoods verses midstory removal treatments (Rhoades *et al.* 2009).

At the end of the second growing season, the seedlings on shelterwood sites averaged 85 cm at planting, 95 cm after the first year and 130 cm after two years. The height of the

seedlings after two years was above the reach of deer , in southern states with limited snowpack. Deer can be a major obstacle to successful hardwood seedling establishment (Kolb *et al.* 1990, Gillespie *et al.* 1996). Gillespie *et al.* (1996) found decreased growth on *Juglans nigra* three years after planting due to heavy deer browsing on unprotected seedlings. Planting high quality seedlings on sites capable of supporting height growth beyond the reach of deer in the second year after outplanting will increase establishment success. Survival in the forest shelterwoods indicates potential success for restoration in gap dynamics in riparian areas of state and national parks. Clark (1958) mentions butternut as a component of old-growth stands in forest gaps.

Considering that there was only one site that used tree shelters, it is difficult to make general statements about restoration using this approach for planting butternuts. The use of shelters resulted in large RCD increases in seedlings but also high mortality and decreased HT compared to other sites. Tree shelters cause a general shading of the seedlings with the exception of the open top. Studies using intolerant oak species (*Quercus* L.) and American chestnuts (*Castanea dentata* (Marsh.) Borkh.) have shown that tree shelter shading plus general forest shade can reduce seedling growth and ultimately can cause mortality (UT-TIP data on file). Studies on *Quercus rubra* L. found that shelters increased height growth until seedlings emerged from the shelters (Gillespie *et al.*1996). Tree shelters have also increased height growth in black walnut (Ponder 1991, Ward and Stevens 1995). However, Gillespie *et al.* (1996) had the greatest amount of mortality two years after planting on seedlings in tree shelters (12%) compared to control (0%). The use of tree shelters for butternut needs to be further explored with additional studies.

The surface mines plantings were on areas that were not intensively graded and were ripped to decrease compaction. Philo *et al.* (1982) found increased survival of *J. nigra* seedlings in areas with limited intensive grading and ripping. Second year survival on the seedlings were 85% and 65% (Philo *et al.* 1982). Ashby (1996) found survival of *J. nigra* on ripped mine spoils to be 74% twelve years after outplanting, with seedlings averaging 550 cm tall. Butternut experienced lower survival rates than black walnut indicating that the seedlings are more negatively impacted by the highly compacted spoil. Though surface

mine sites may superficially appear to provide habitat for many riparian tree species; planting in mine spoil soils is very different than planting in undisturbed riparian habitats. Butternut showed lower survival than previous black walnut studies on similar sites. Long-term survival on these sites is unknown. In two years almost half of the seedlings planted had died and HT had decreased since outplanting. The root system structure of butternut, with larger lateral roots may have resulted in the lack of suitability on mine spoils.

Though tree shelters and mine spoils appear to negatively impact height growth in butternut, the results suggest that butternut can readily establish on old agricultural sites and in shelterwoods. Long-term survival and growth on each site, however, is yet to be determined as site conditions will change over time.

Families

Family identity impacts survival and growth of butternut seedlings. Seedlings from certain genetic families were significantly taller and therefore, more likely to survive. Seedlings from one particular provenance, east Tennessee, had families with very different survival and height than other families. The RF family had the highest average height of seedlings and very little mortality. Another family, Sevier2, came from the same east Tennessee area and was the shortest of all of the seedlings and had by far the greatest amount of mortality. The four families with less than 20% mortality were from two neighboring counties in western North Carolina. One of these families was the second tallest family in 2006. With butternut, it is important to monitor specific progeny that are sown in the nursery and established in the field. Field testing will allow the selection of maternal parents that consistently produce nuts with high germination rates, seedlings with larger RCD and greater heights, and with higher probabilities of survival after outplanting. The maternal trees can be grafted and planted on seed orchard spacing to aid restoration by providing seeds that will produce seedlings with a higher probability of establishment success and vigorous growth.

Seedling characteristics

Selecting larger RCD seedlings and taller seedlings from specific genetic families will increase establishment success for butternut. Survival was increased by 50% for

seedlings having a RCD of at least nine mm at the time of planting. This is consistent with my studies from previous years (see Chapter IV). Planting of high-graded seedlings will increase survival and reduce planting costs, as you would plant at a different density. Nursery costs per seedling would increase with respect to the potential survival of each seedling. Refinement in high-graded nursery seedlings offers the ability to purchase a seedling with a high probability of success. Public land managers and private landowners alike could base the grade of the seedling they select on scientific estimates of the establishment potential of individual seedling groups as done in this study. However, smaller seedlings could be given to landowners who can offer the additional attention to establish the smaller seedlings.

Transplant shock is a common obstacle in bare-root hardwood seedlings (Struve and Joly 1992). In establishment of *J. nigra*, reduced height and RCD growth has been documented even with or without the addition of fertilizer (Jacobs *et al.* 2005). If the transplant shock continues for several years increased mortality results as the competing vegetation continues to overtop seedlings. Butternut seedlings in this study only experienced this problem in the first growing season. The seedlings showed very little increase in growth over the first growing season. In the second growing season, however, both HT and RCD growth increased significantly. Percent HT change was drastically larger during the second growing season than the first. This lag in establishment time could be due to several factors including the large size of the seedlings resulting in extensive root pruning prior to planting. However, the larger seedlings continued to be larger even after outplanting, indicating that transplant shock or recovery rate was not a significant problem in larger seedlings. Based on this study's results, outplantings of butternut seedlings will require competition control only during the first-growing season because of this lag time in establishment. Completion control after the first year may not be as necessary.

A potential solution to the impact of transplant shock is planting of seed instead of seedlings. Direct seedling of butternut was tried by Ostry *et al.* (2003) and failed. Generally, butternut seedlings have been used in reforestation due to the potential for seeds being eaten by various animals. Barrier systems to protect nuts have been investigated for American

chestnut. However, their effectiveness has yet to be proven. One unique solution that might be applied to direct seedling of butternut is the use of predator odors (Rosell 2001). The use of red fox (*Vulpes vulpes*) and raccoon (*Procyon lotor*) scent deterred foraging by gray squirrels (*Sciurus carolinensis*) (Rosell 2001). Fox and raccoon scent were more effective than human or white-tailed deer (*Odocoileus virginianus*) scent (Rosell 2001). If nuts could be protected, by readily available fox scent, there may be an increased benefit in planting individual nuts to reduce transplant shock and transportation costs. Future studies should compare the benefits of direct seeding of butternut, protected by fox scent, compared with transplanting 1-0 nursery stock.

Conclusions

Butternuts were successfully established on a variety of sites, with the greatest survival on old agricultural fields and the greatest height growth in shelterwoods. Specific genetic families had increased survival and growth. Maintaining genetic family identity may assist by selecting maternal parents with increased establishment success of offspring. Transplant shock in butternut during the first year resulted in limited height growth. Competition control during this time period will increase chances for survival.

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Appendix: Tables and Figures

Table V-1. 2005 Butternut planting distribution and experimental design.

Map #	Opening Type[1]	State	Location	Site	Abbr.	Seedlings
1	1	TN	Cherokee National Forest	Ocoee	TN-OC1	142
	3				TN-OC2	148
2	3	NC	Pisgah National Forest	Grandfather	NC-GF1	147
					NC-GF2	144
3				Appalachian	NC-AP1	146
					NC-AP2	149
4	1	VA	Jefferson National Forest	Clinch1	VA-CL	288
5	3	KY	Yatesville WMA	Yatesville1	KY-YA1	150
				Yatesville2	KY-YA2	168
6	4	TN	Private Surface Mine	Mine Spoil	TN-SM1	32
					TN-SM2	30
					TN-SM3	33
7	1	NC	Qualla Boundary	Kituwah	NC-KA1	120
					NC-KA2	259
8	2		North Carolina Forest Service, State Forest	Holmes Educational SF	NC-HO	237
	TOTAL	**3**	**7**	**15**		**2093**

[1] 1.Grassland sites on edge of forest in open areas: includes former recreation, work center, agricultural areas. 2. Glassland sites with trees in tree shelters. 3. Forested sites in shelterwoods: includes 30% basal area retention harvest and partial tree removal caused by beaver activity. 4. Reclamation project after surface mining.

Table V-2. 2005 Butternut planting survival and growth across all sites.

Map #	Opening Type[1]	Site Abbreviation	% Survival		Height (cm)			RCD (mm)		
			2005	2006	2004	2005	2006	2004	2005	2006
1	1	TN-OC1	92.95	88.89	87.65	90.07	105.64	11.19	12.66	16.20
	3	TN-OC2	71.62	59.46	87.87	100.37	124.92	11.09	13.17	17.87
2	3	NC-GF1	80.95	20.41	85.35	81.48	104.27	10.89	12.56	16.31
		NC-GF2	90.97	88.89	87.63	85.77	110.02	11.35	14.36	16.20
3	3	NC-AP1	94.44	83.56	82.71	92.37	158.57	11.08	14.63	20.37
		NC-AP2	87.08	89.93	80.34	104.40	132.79	10.98	14.39	16.47
4	1	VA-CL	92.26	90.13	81.80	95.04	112.94	11.30	14.40	18.41
5	3	KY-YA1	60.61	79.12	105.72	93.28	129.98	16.03	18.24	24.96
		KY-YA2	83.33	79.53	84.87	83.63	130.14	13.33	15.32	18.03
6	4	TN-SM1	63.36	53.13	105.72	93.33	81.05	16.03	18.24	20.81
		TN-SM2	80.00	61.29	84.87	83.62	87.44	13.33	14.67	17.68
		TN-SM3	66.66	45.45	89.55	70.82	74.93	14.52	16.49	18.27
7	1	NC-KA1	95.00	83.33	73.38	78.25	106.52	12.24	13.10	19.11
		NC-KA2	90.73	81.08	104.04	92.91	123.93	13.25	18.16	25.17
8	2	NC-HO	77.31	73.53	72.60	100.21	80.6	12.60	14.04	17.13
Mean			**81.82**	**71.85**	**87.61**	**89.70**	**110.02**	**12.61**	**14.96**	**18.86**
Range					**9-196**	**4-200**	**15-370**	**1-29**	**2-34**	**2-42**
Standard Deviation			**0.39**	**0.44**	**29.03**	**32.84**	**48.91**	**3.68**	**4.19**	**6.13**

1.Grassland sites on edge of forest in open areas: includes former recreation, work center, agricultural areas. 2. Grassland sites with trees in tree shelters. 3. Forested sites in shelterwoods: includes 30% basal area retention harvest and partial tree removal caused by beaver activity. 4. Reclamation project after surface mining.

Table V-3. 2005 Butternut planting survival and growth across planting types.

Site Description	Opening Type[1]	Mortality (%)		HT (cm)		
		2005	2006	2004	2005	2006
Open Grasslands	1	9.48	16.63	82.10	88.32	114.31
Grasslands, Tree Shelters	2	23.80	25.54	72.86	99.65	79.80
Shelterwood	3	20.85	28.13	84.97	94.57	129.78
Surface Mines	4	29.47	47.38	93.55	82.45	82.30
Mean		**18.52**	**25.61**	**83.43**	**92.72**	**117.16**

1. Grassland sites on edge of forest in open areas: includes former recreation, work center, agricultural areas. 2. Grassland sites with trees in tree shelters. 3. Forested sites in shelterwoods: includes 30% basal area retention harvest and partial tree removal caused by beaver activity. 4. Reclamation project after surface mining.

Table V-4. Analysis of variance of provenance, family, and planting sites between nursery stock (1-0) butternut seedling characteristics including heights and root collar diameters.

Variables	Effects	F value	P value
Initial HT[1]	Families	22.48	<0.0001
	Site	1.38	**0.2267**
Initial RCD[2]	Families	17.50	<0.0001
	Site	2.83	**0.0971**
HT05[3]	Families	12.84	<0.0001
	Site	5.59	0.0038
MORT05[4]	Families	4.13	<0.0001
	Site	5.52	0.0041
HT06[5]	Families	5.90	<0.0001
	Site	6.76	<0.0001
MORT06[6]	Families	2.18	0.0105
	Site	4.41	0.0004

Bold indicates not significant at the p=0.05 level

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken at ground level at time of planting
[3] Height of the seedling at the end of the 2005 growing season
[4] Height of the seedling at the end of the 2006 growing season
[5] Mortality of the seedling at the end of the 2005 growing season
[6] Mortality of the seedling at the end of the 2006 growing season

Table V-5. Phenotypic correlations between seedling growth and survival and nursery stock (1-0) butternut seedling characteristics including heights, root collar diameters, and first-order lateral root numbers.

Variables	Observations (n)	Phenotypic Correlations	
		r^2	P value
Initial HT[1]- RCD[2]	1,849	0.42263	<0.0001
- HT05[3]		0.60203	<0.0001
- HT06[4]		0.35197	<0.0001
- MORT05[5]		-0.11021	<0.0001
- MORT06[6]		-0.10964	<0.0001
Initial RCD- HT05	1,849	0.46573	<0.0001
- HT06		0.24312	<0.0001
- MORT05		-0.19285	<0.0001
- MORT06		-0.17498	<0.0001
HT05- HT06	1,561	0.56184	<0.0001
- MORT05		-0.08191	0.0012
- MORT06		-0.26171	<0.0001
HT06- MORT06		-0.04901	**0.0649**

Bold indicates not significant at the p=0.05 level

[1] Height of the seedling from the root collar to the first live terminal bud
[2] Diameter taken at ground level at time of planting
[3] Height of the seedling at the end of the 2005 growing season
[4] Height of the seedling at the end of the 2006 growing season
[5] Mortality of the seedling at the end of the 2005 growing season
[6] Mortality of the seedling at the end of the 2006 growing season

Table V-6. Survival and size of butternut seedlings across initial characteristics categories, including height and root collar diameter.

Initial Variable	Category Variable	% of seedlings	2005 mortality (%)	2006 mortality (%)	2004 HT (cm)	2005 HT (cm)	2006 HT (cm)
HT[1] (cm)	< 50	13.84	22.7a	30.67a	36.89	63.03a	87.14a
	50-100	54.27	17.1b	25.4b	76.16	85.39b	113.88b
	> 100	31.89	12.1c	19.6c	116.21	116.10 c	135.49c
RCD[2] (mm)	< 9 mm	18	26.94a	41.51a	60.35a	64.54a	90.68a
	> 9 mm	82	13.89b	21.01b	88.64b	98.13b	122.28b

Unique letters indicate significantly different values using Tukey's Mean Separation Analysis across individual columns for each variable.

[1] Height of the seedling from the root collar to the first live terminal bud at time of planting
[2] Diameter taken at ground level at time of planting

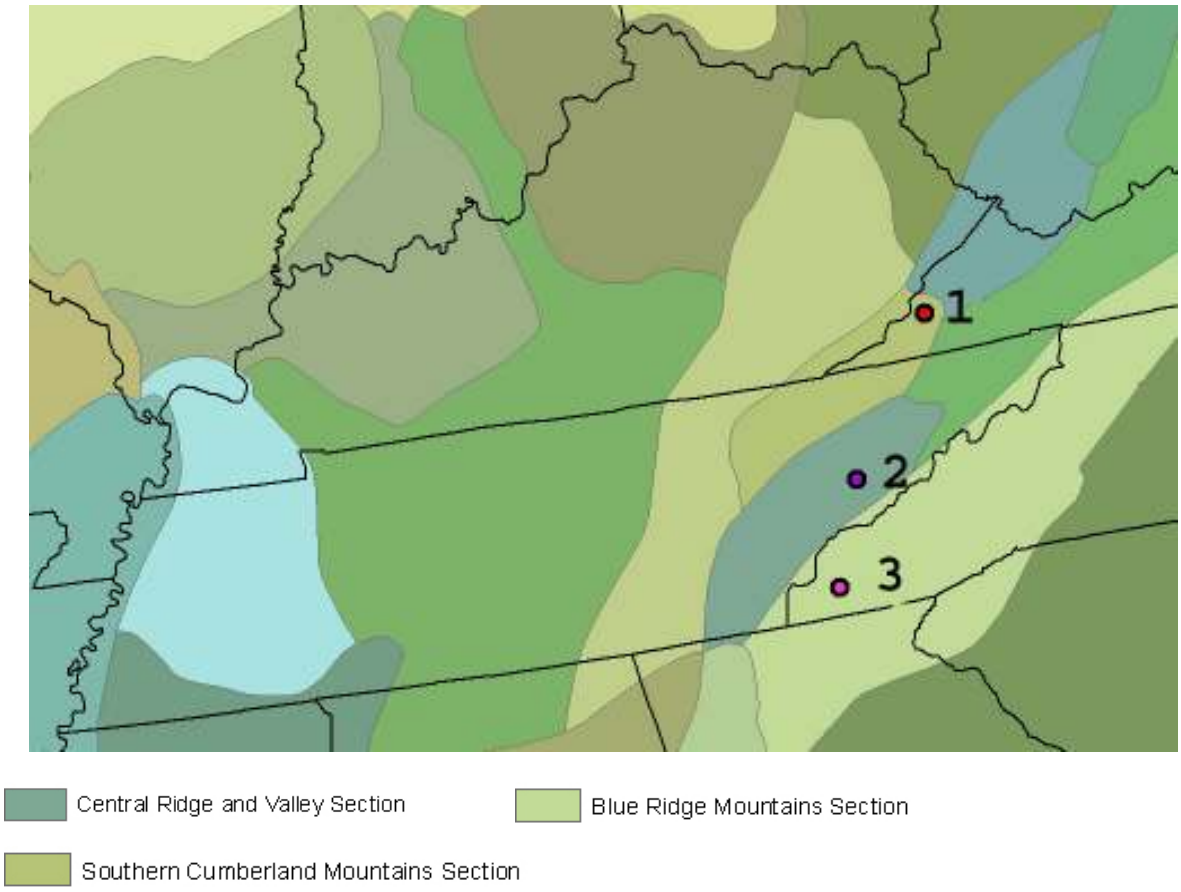

Figure V-1. Origins of provenances for the 10 parent trees, delineated by Bailey's Ecoregions (Bailey 1995) in the Southern Appalachians.

1. Northwestern Virginia, N-VA, Southern Cumberland Mountains
2. East Tennessee, E-TN, Central Ridge and Valley
3. Western North Carolina, W-NC, Blue Ridge Mountains

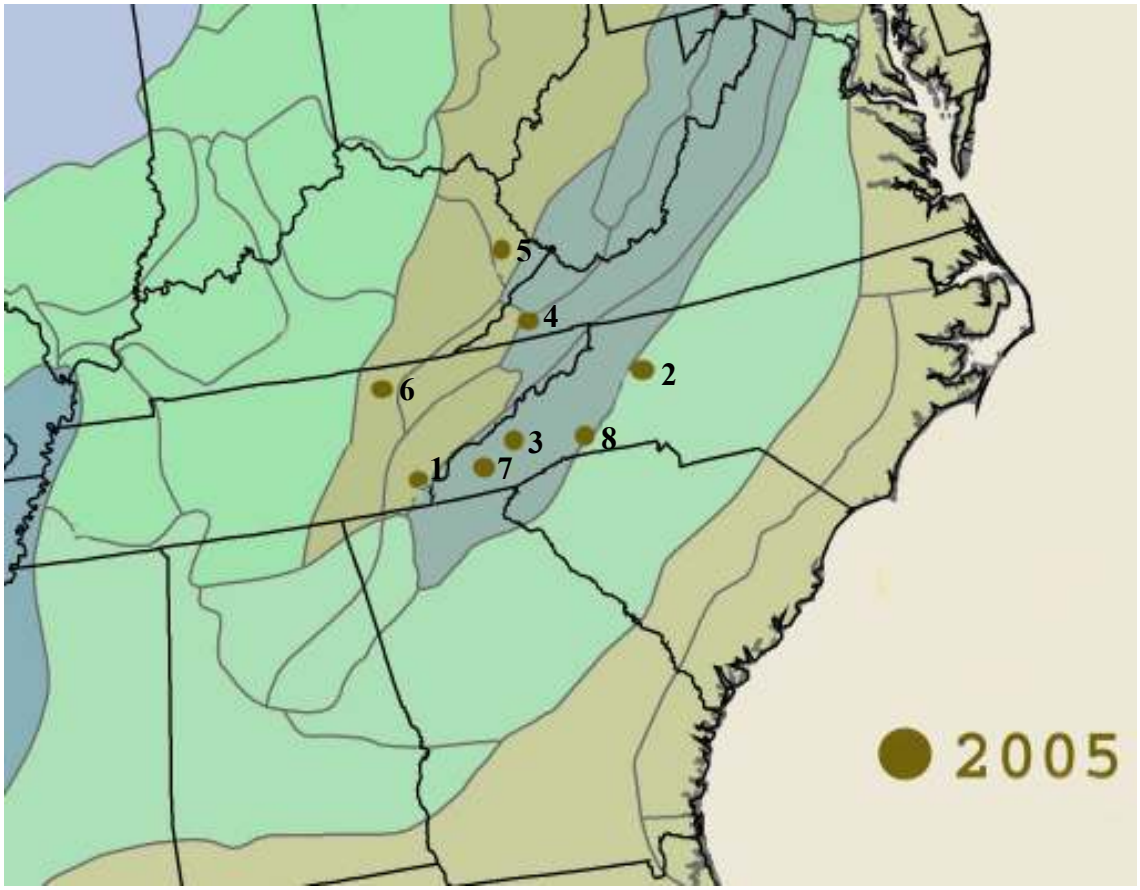

Figure V-2. Locations of 2005 butternut plantings delineated by Bailey's major physiographic provenances (Bailey 1995) in the Southern Appalachians.

Number, location, nearest town, state (abbreviation)

1. Cherokee National Forest, Ocoee District, Reliance, TN (TN-OC1 & OC2)
2. Pisgah National Forest: Grandfather District, Old Fort, NC (NC-GF1 & GF2)
3. Pisgah National Forest: Appalachian District, Waterville, NC (NC-AP1 & AP2)
4. Jefferson National Forest: Clinch River District, Pound, VA (VA-CL)
5. Yatesville Lake Wildlife Management Area, Louisa, KY (KY-YA1 & YA2)
6. Surface Mine: Private Mining Company Land, Oak Ridge, TN (TN-SM1, SM2, SM3)
7. Eastern Band of the Cherokee Indians: Qualla Boundary, Ela, NC (NC-KA1 & KA2)
8. North Carolina Forest Service: Holmes Educational State Forest, Hendersonville, NC (NC-HE)

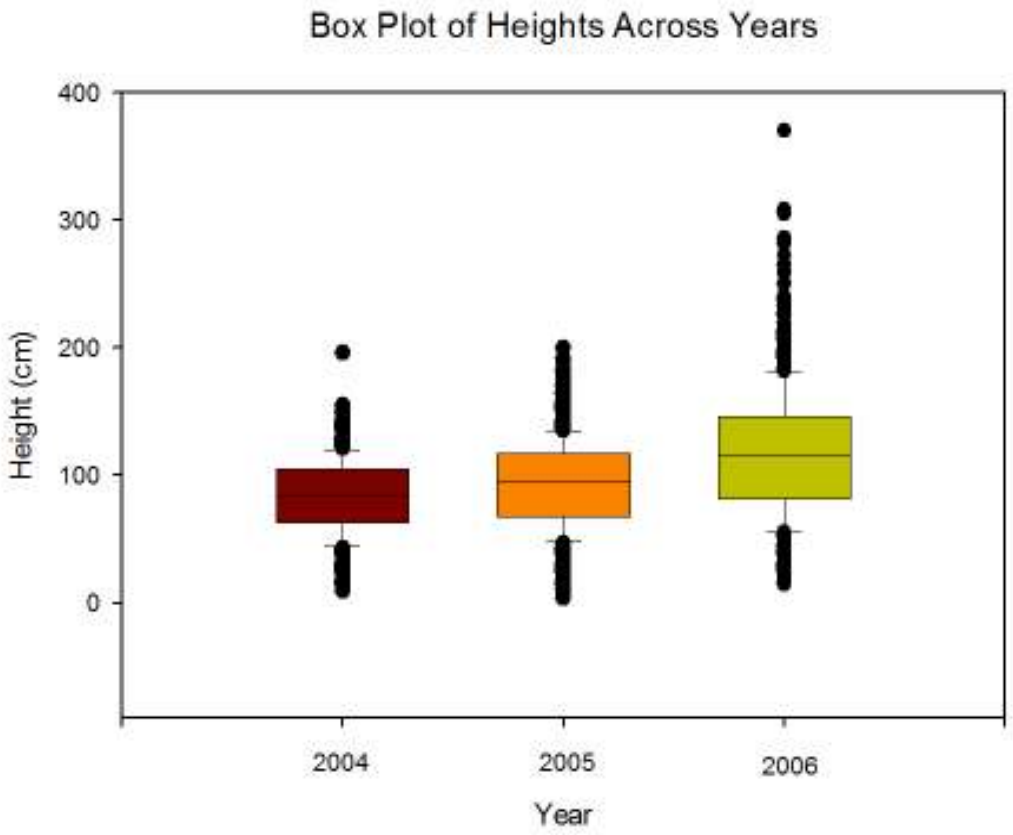

Figure V-3. Average annual heights of butternut seedling established in 2005.

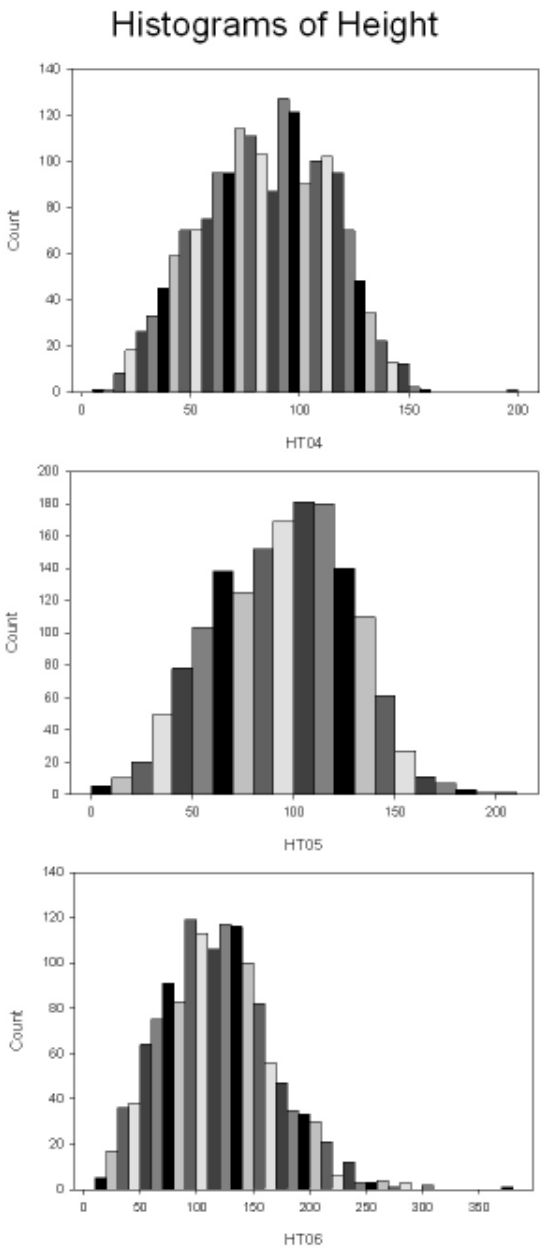

Figure V-4. Distribution of annual heights of butternut seedling established in 2005.

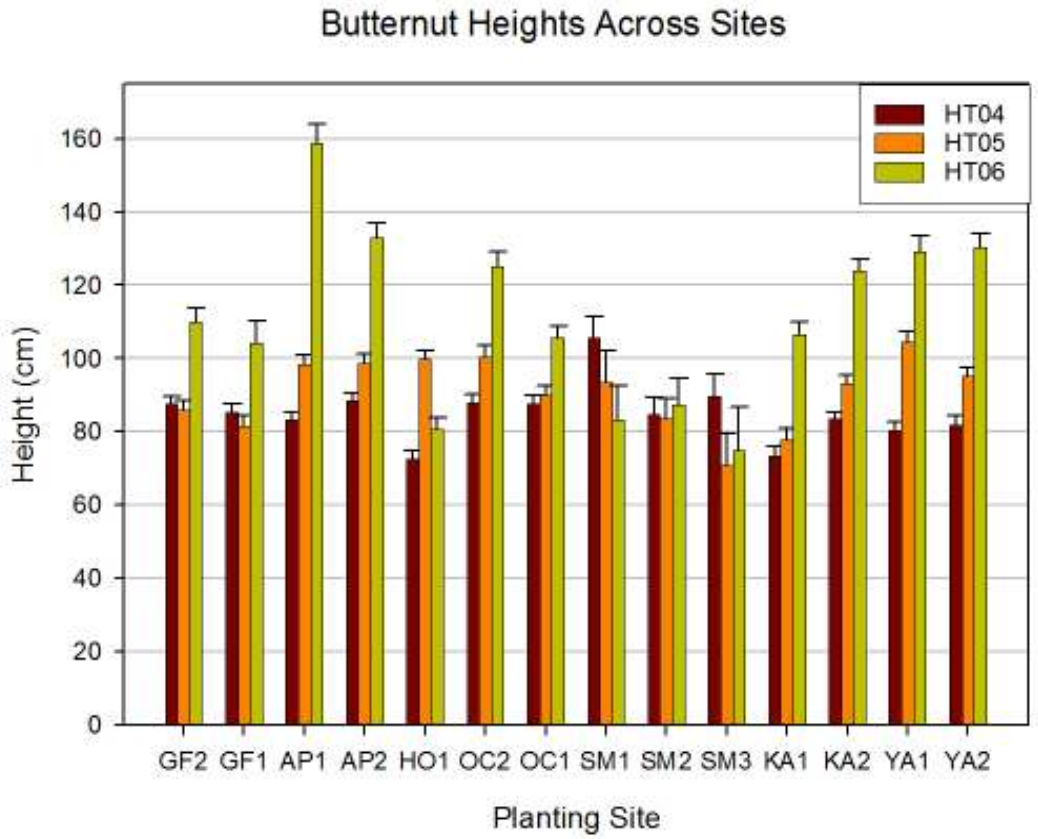

Figure V-5 . Average height of butternut seedlings across planting sites from 2004 until 2006.

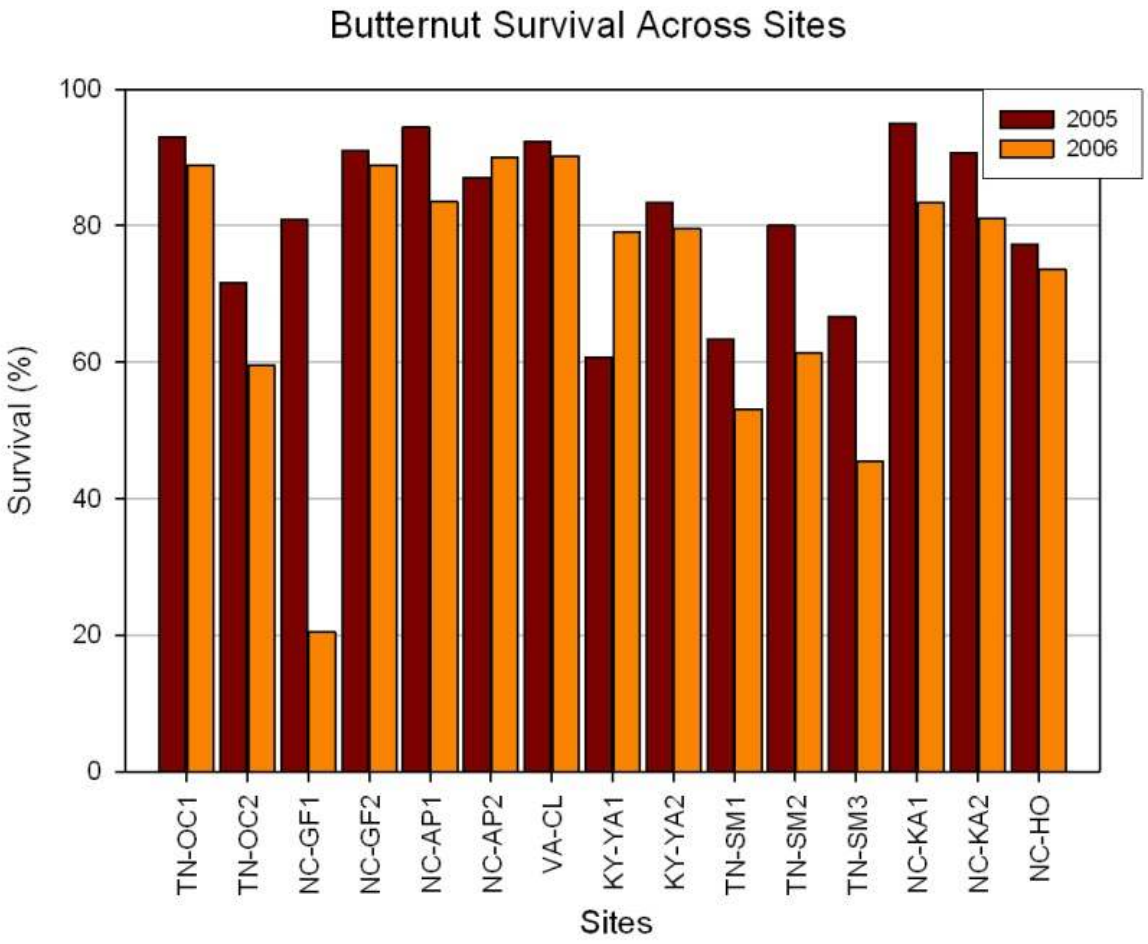

Figure V-6 . Average survival of butternut seedlings across planting sites from 2005 until 2006.

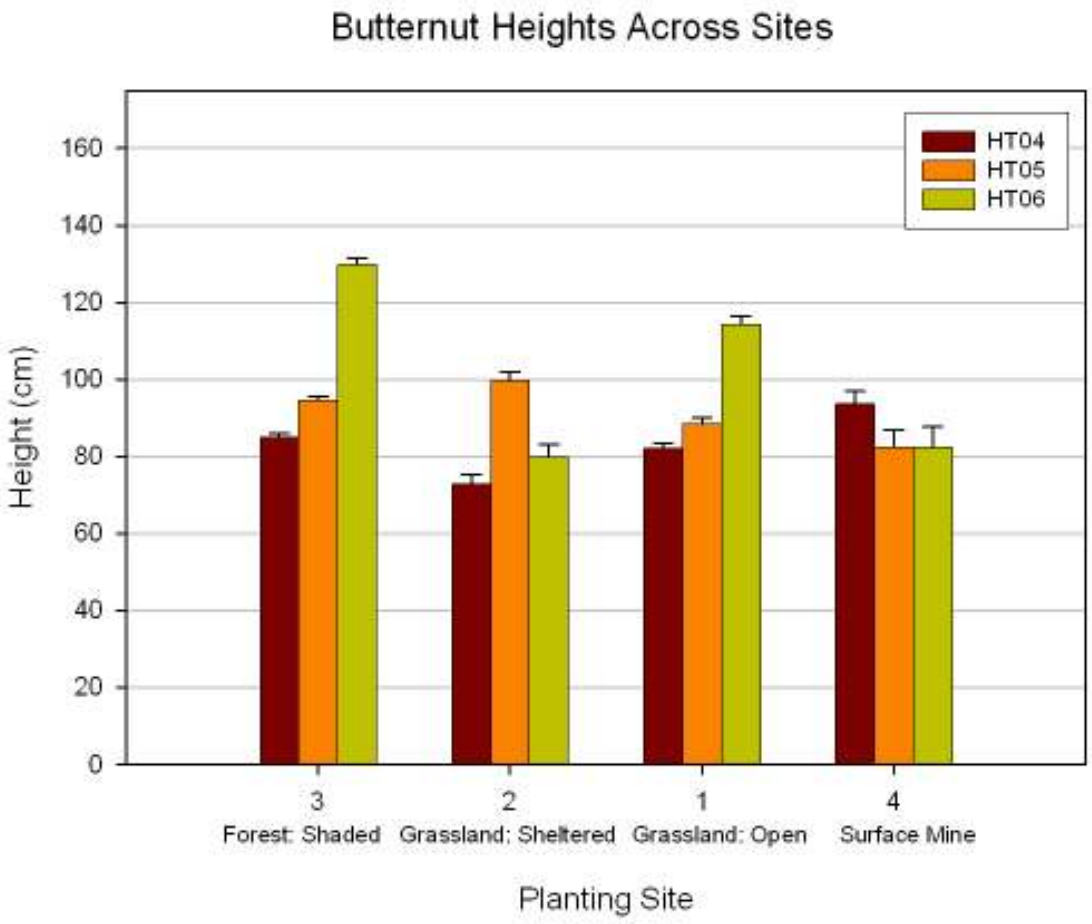

Figure V-7 . Average height of butternut seedlings across planting sites from 2004 until 2006.

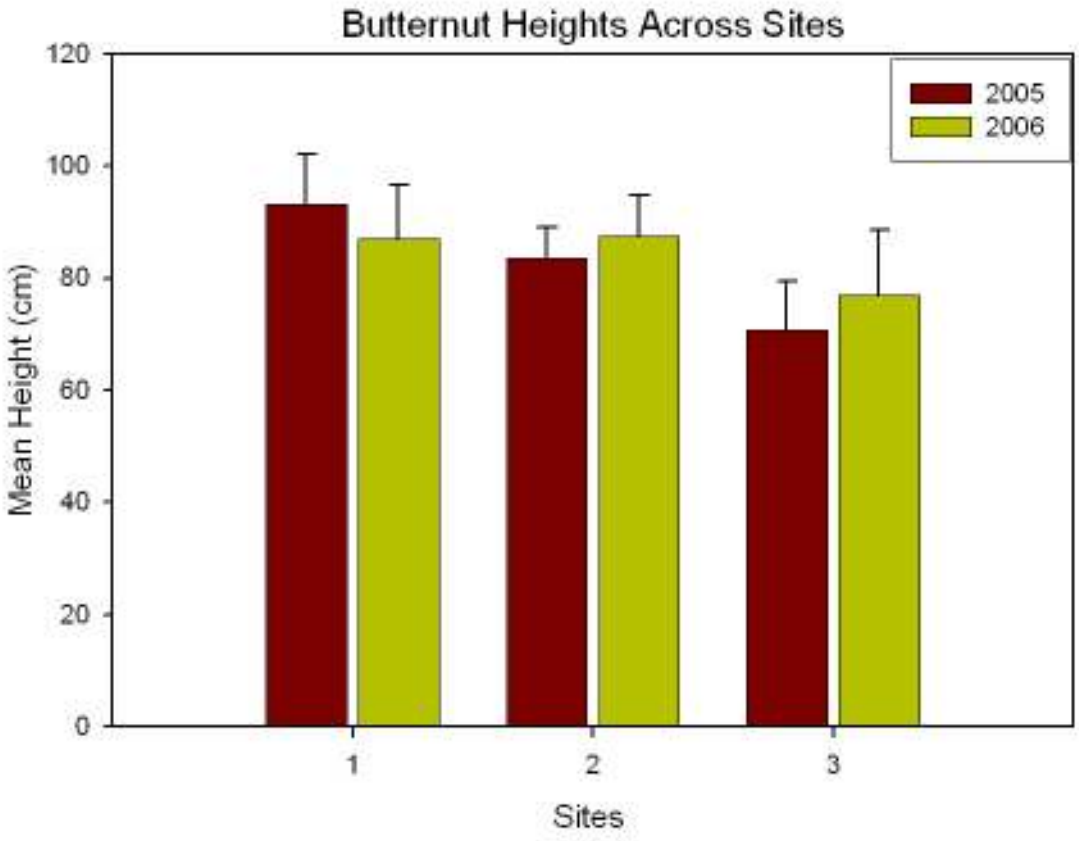

Figure V-8. Height of butternut seedlings at the three sites on surface mines in 2005 and 2006.

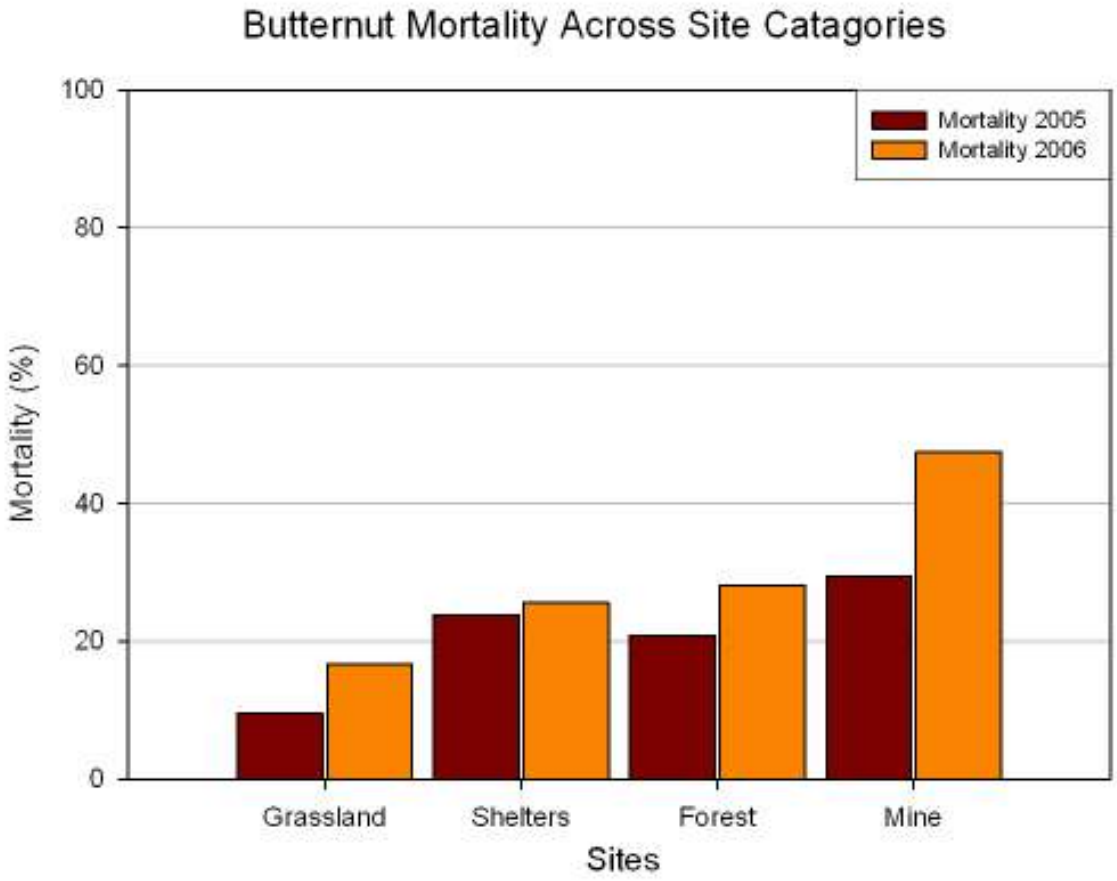

Figure V-9 . Average mortality of butternut seedlings across planting sites from 2005 until 2006.

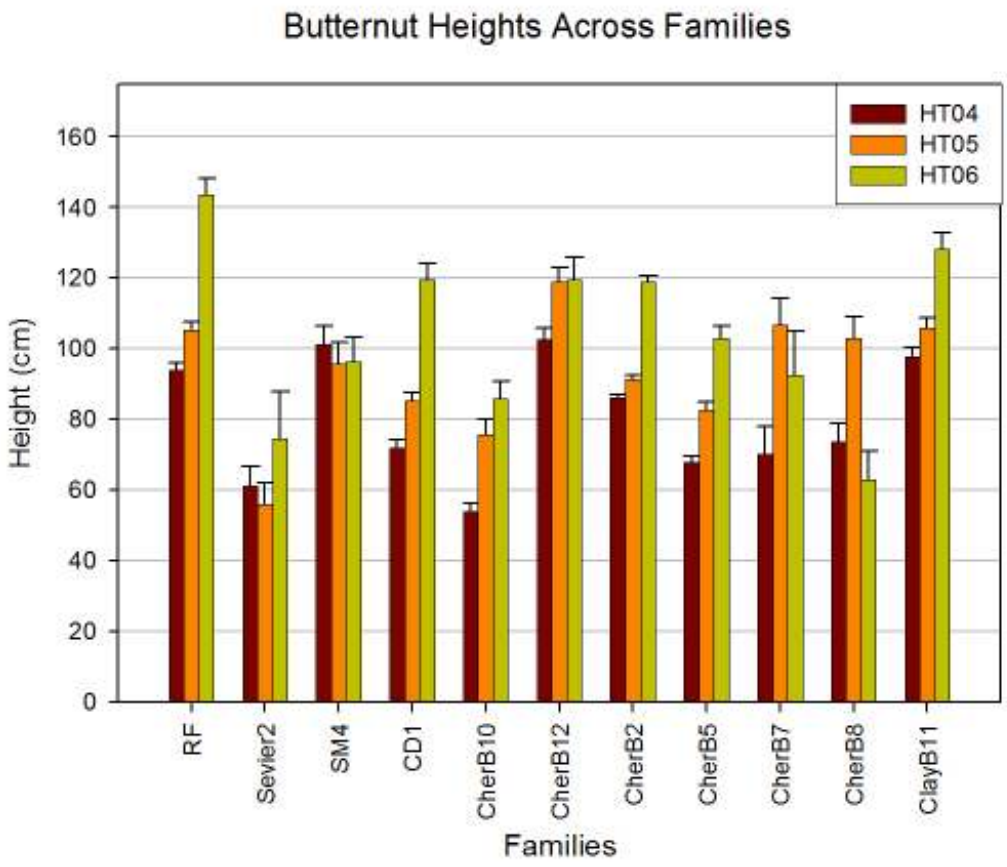

Figure V-10. Height of butternut seedlings across families of origin from 2004 until 2006.

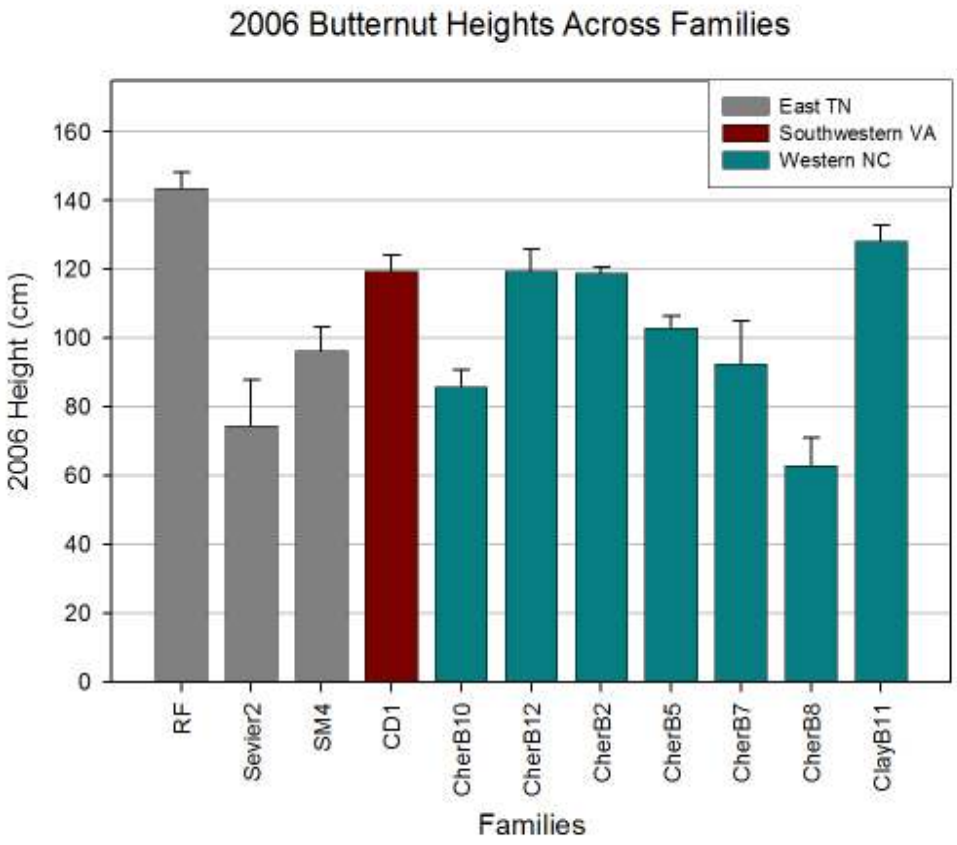

Figure V-11. Height of butternut seedlings across families of origin in 2006.

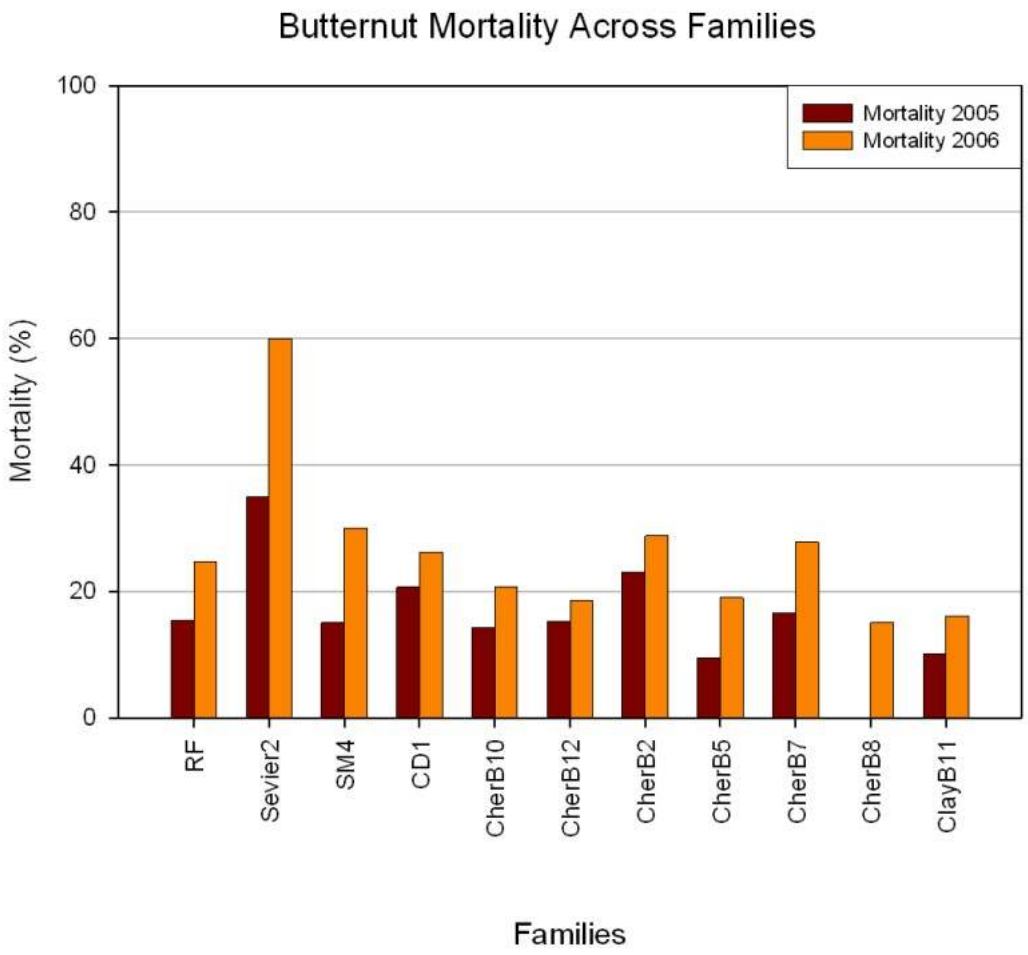

Figure V-12. Average mortality of butternut seedlings across families of origin in 2006.

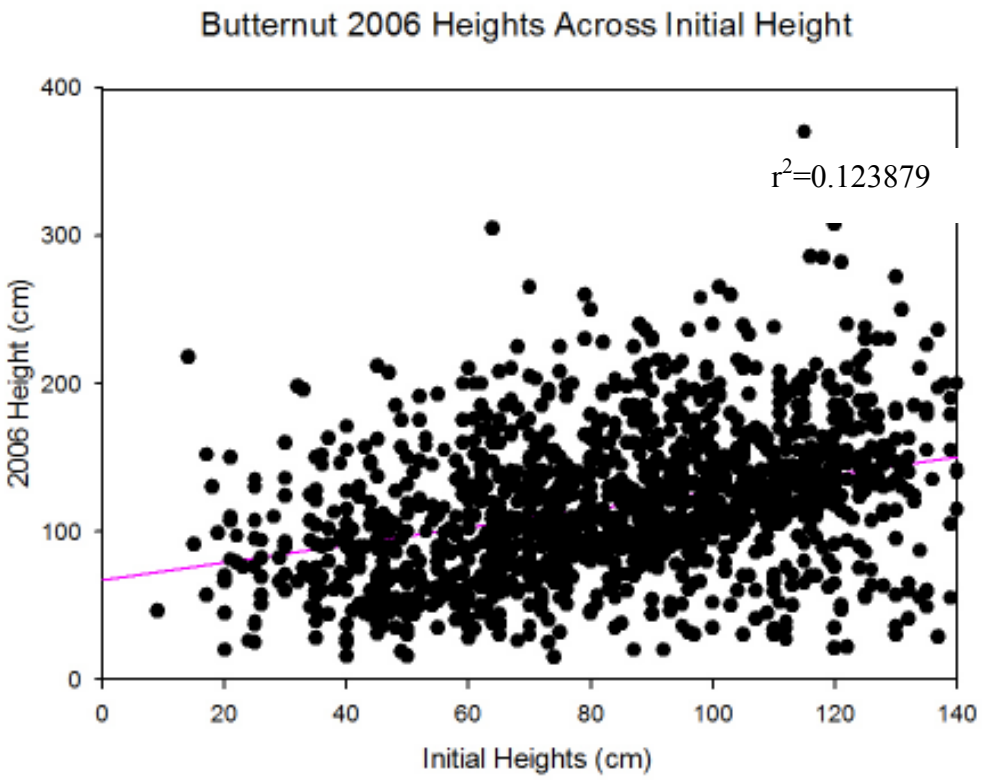

Figure V-13. Height of butternut seedlings in 2006 across initial seedling heights.

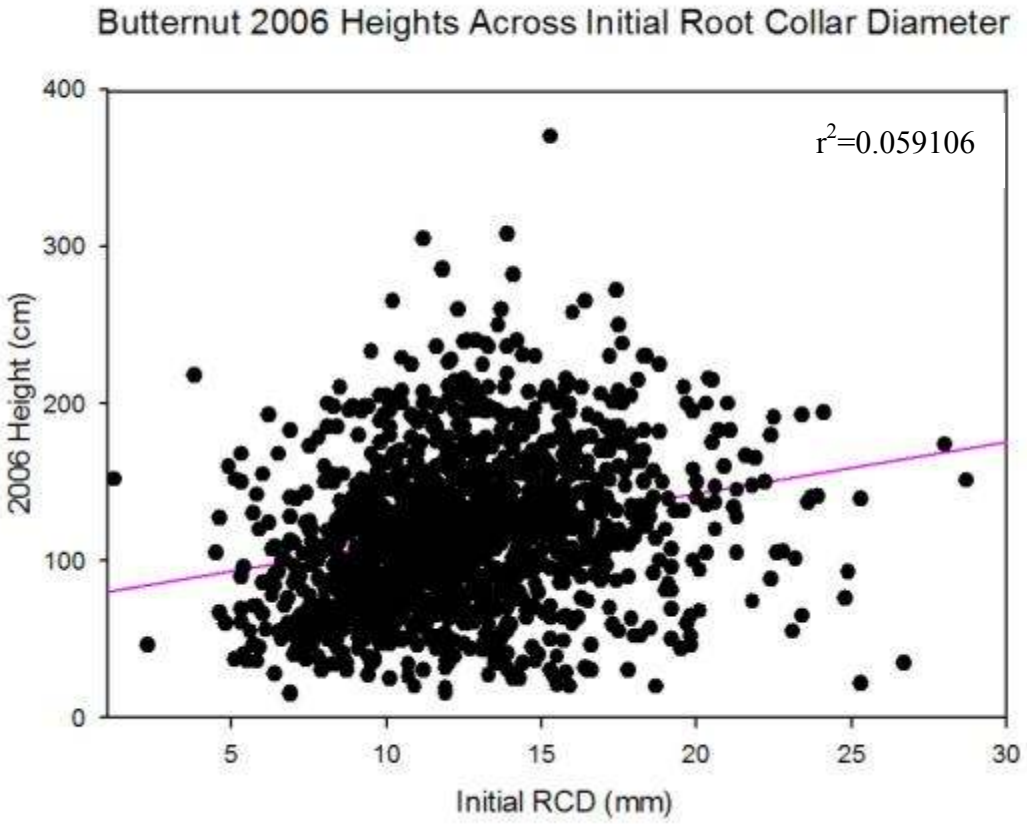

Figure V-14. Height of butternut seedlings in 2006 across initial seedling root collar diameter.

PART VI. CLASSIFICATIONS OF SIZEABLE BUTTERNUT POPULATIONS IN TENNESSEE, MISSOURI, AND ARKANSAS

Abstract

Butternut, *Juglans cinerea* L., has shown drastic declines due to butternut canker disease caused by *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz), throughout its native range. Many populations are thought to have been lost. However, there are a very few locations in the southern United States where butternut is still a significant species of the forest community. The current rate of butternut mortality in communities, however, is unknown. Butternut Valley in central Tennessee is so named due to a high concentration of butternut trees. The four-mile stretch of Dry Creek contains over 200 individual trees which were examined for disease development in 1998 and then again six years later, in 2005, to determine percent survival and its relationship to tree size and disease condition. Mortality in the butternut population was less than 9% over a six-year period, even though more than 30% of the trees were initially considered heavily diseased. A numerical canker classification system was developed for comparability between these populations. Trees were ranked on a scale of 0-10 with 10 indicating the worst disease condition. Trees that died had a significantly higher average disease rating of 8.22 in the initial survey as opposed to 5.92 for living trees. Trees that died in general existed in lower canopy positions and were significantly smaller in size. Dead trees averaged over 3 m shorter and had a mean diameter at breast height of 17 cm compared to 25 cm for living trees. Disease development may be exacerbated by lack of vigor and in lower canopy positions. The large number of surviving small trees indicates that shade-intolerance alone is not causing mortality; however, butternut canker may act within the stem-exclusion stage of stand development to remove diseased butternuts during canopy closure. Determining those individuals within populations that are at greatest risk for mortality could guide managers in removing surrounding trees to reduce competition and increase chances of survival.

Determining individual populations at greatest risk is also essential to butternut restoration. The relative health and tree characteristics of the trees in Tennessee were compared to two other large southern populations in Missouri and Arkansas. A numerical canker classification system was developed for comparability between these populations.

Phenotypic variables related to tree crown condition were measured to determine if the canker classification was related to tree health. In addition, phenotypic bark characteristics were quantified to determine their relationship with other variables including potential resistance. The three populations were not different in diameter but were different in height. The average canker score was lowest at the Arkansas population, which consequently also had the largest amount of heavily diseased trees. The Missouri population, with a larger overall average disease rating, had a significantly greater percentage of trees in the healthiest disease ranking, but still fewer than ten percent of the population. In the Arkansas population there was no difference in diameter between resistant trees and trees greater than the average disease ranking. In Missouri, resistant trees were smaller in diameter than resistant trees in Tennessee. Therefore, height and crown position are not universal indicators of disease condition.

Phenotypic variables were measured to determine if the canker classification was related to health. In addition, phenotypic bark characteristics were quantified to determine their relationship with other variables including potential resistance. Resistant trees were larger in diameter and had deeper bark fissures. Bark fissure depth was closely related to canker classification. However it was also positively related to crown diameter, crown position, height to first live branch, crown light exposure, crown density, and crown dieback. Increased fissuring occurs on trees with healthier canker classifications and healthier crown conditions. Quantifying canker classification, crown position and health and bark fissure depth allows managers indications of relative disease resistance. These measures are important in selecting potentially resistant trees for breeding programs and monitoring disease development over time.

Introduction

Butternut, *Juglans cinerea* L., is currently a minor component of eastern North American hardwood forests. Populations of butternut are have been significantly reduced or extirpated by butternut canker disease caused by an exotic fungus, *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz). Some individual locations, however, contain

butternut trees in relatively large numbers, e.g., 100+ individuals. These populations afford the opportunity to study butternut condition and dynamics across spatial scales. In addition, little is known about the impact of this canker disease across temporal scales or on phenotypic tree condition.

Temporal classification

Many questions still remain about the reaction of butternut trees to butternut canker disease including: what are the physical and climatic conditions that increase susceptibility of the host; and what is the impact of butternut canker on tree growth and vigor? Answering these questions will aid conservation programs for butternut by leading to a greater understanding of disease-host relationships. The impact and epidemiology of any disease is dependent many factors including the environment, the condition of the host, and the attributes of the fungal pathogen causing the disease (Agrios 1997). The complexities in decline and mortality etiology are very difficult to assess especially across temporal scales. Butternut canker is one of many factors, including shading and old-age that may result in tree mortality. The presence of living, heavily diseased trees in the forest that persist over time indicate that butternut canker may not be the primary agent in tree mortality.

Butternuts may be able to survive for long periods after initial and intensive infection by butternut canker disease. Surveys in Vermont and Wisconsin have shown over 90% infection rates and less than 30% mortality (Bergdahl *et al.* 1996, Cummings Carlson and Guthmiller 1993 respectively). Ostry *et al.* (2003) evaluated a stand of butternut on the Nicolet National Forest in Wisconsin since 1993 and found that previously identified healthy trees remain in good vigor. In these stands, butternuts were identified using whole tree characteristics, however, they could be the product of hybridization with heartnut (*Juglans ailantifolia* var. *cordiformis* (Maxim.) Rehd.)), a nut cultivar of Japanese walnut.

In Tennessee, there is a location, Butternut Valley, with the largest concentration of butternuts in southeastern North America. At this location, trees have been monitored for a seven-year time period to gain insight into the progression of butternut canker disease including the degree of cankering and mortality over time across trees of various ages, crown positions, and initial health conditions. A primary goal of this study is to create methods for

uniform measurements of tree health and canker condition that can be applied throughout the native range of butternut.

Population comparisons

The impacts and loss of butternut in general have been reduced to mostly incidental personal accounts. In order to assess the overall impact of butternut canker it is essential to compare dynamics of multiple locations. Comparing multiple populations through the southern part of the range of butternut will aid the development of management guidelines for butternut in existing stands. The amount of seedlings that are potentially resistant or heavily infected may vary across populations and indicate populations at greatest threat.

Phenotypic classification

The relationships between butternut canker disease and phenotypic characteristics of trees are poorly understood. It is believed that butternut canker disease may be behaving like a stress canker disease and have a larger impact on trees that are stressed by other factors (*pers. comm.,* R.L. Anderson). Insight into the dynamics of butternut canker disease can be determined by evaluation of disease impact or vigor as well as other characteristics, such as tree size and crown position.

In addition, phenotypic variables may indicate potential resistance or health condition. Specific crown condition measurements may indicate the level of impact the disease is having on trees or conditions that result in increased susceptibility. There has been speculation that certain phenotypic traits may be used as criteria for increased susceptibility to butternut canker disease (Ostry *et al.*2003). Ostry *et al.* (2003) noticed that in geographically diverse populations of butternuts, in general, smoother bark type was often associated with an increase in the number of cankers. Ostry *et al.* (2003) investigated a 40 – acre woodlot containing 544 butternut trees, which were classified as butternut by whole tree characteristics. Of these trees, 17% percent were noted to be disease-free. They classified these trees as having two different bark types: dark gray and deeply fissured or light gray and shallow fissured. The dark-deep bark phenotype was present on 73% of the diseased free butternuts compared with 21% of the diseased or dead butternuts (Ostry *et al.* 2003).

Therefore, trees with the phenotypic trait of deeper bark furrows may have resistance to butternut canker disease. Many of these characteristics, including disease condition and bark characteristics are not uniform. Therefore, subjective measures need to be quantified in order to be repeated in the same stand at various points of time and for comparison of multiple stands.

Objectives

The butternut population in Butternut Valley was evaluated to determine population reduction over time and relate canker condition to phenotypic characteristics of the trees including disease condition and bark type with the following objectives:

Objective a) Temporal classification. Examine temporal aspects of disease development within the Butternut Valley population to determine percent mortality over a seven-year time period across different tree characteristics and canker classifications.

Objective b) Population comparisons. Quantify canker classification across three large existing populations to determine the percentage of trees which are potentially resistant or highly diseased as indicators of populations at greatest risk. In addition, tree characteristics will be compared across disease conditions between and among populations to determine universal characteristics related to disease development.

Objective c) Phenotypic classification. Determine if relationships exist between canker classification measures and tree phenotype which would indicate overall tree health and putative resistance to butternut canker disease.

Methods

Butternut Valley

"Butternut Valley" is located in central Tennessee at approximately 36.1716° N, -86.7848° W around 450 meters elevation on private property owned by the Healing Stones Foundation (Figure VI-1, , note tables and figures appear in the Appendix at the end of the text). The area contains over 300 individual butternut trees occurring along a four-mile stretch of creek in rural DeKalb County, TN. The valley is located in the Interior Low

Plateau of the Eastern Highland Rim Section of the Eastern Broadleaf Forest Provenance (Bailey 1995). Soils are deep, well-drained, cherty silt loam of the Staser series and the site is characterized by steep hillsides. The area contains many abandoned homesteads and a few non-residential homes. There are several agricultural subsistence farms that were established by several families from the 1870s to the middle 1900s. By 1960, all families had moved away from the area allowing the old fields to be colonized by early successional species, predominantly butternut. The Butternut Valley population begins along the headwaters of a small creek that periodically goes underground (Figure VI-2). Some old fields are currently kept open through mowing while others have been allowed to return to forest. Trees used in this study were DNA genotyped using 12 microsatellite loci and specific chloroplast markers to determine recent maternal ancestry (Hoban *et al.* 2009). The butternut trees at Butternut Valley were all pure *J. cinerea*.

Butternut Valley contains butternut concentrated in a narrow creek bed bounded by steep sided ridges with limited terracing due to the narrowness of the valley. Here butternut exists as natural regeneration in various aged, small, old fields in the riparian zone along the creek. The age, crown class, and health conditions of the trees are highly variable. Some trees have no stem cankers and appear to be naturally resistant to butternut canker disease, but on closer examination have numerous branch cankers in the upper canopy, which are symptomatic of butternut canker disease. This location offers an exceptional opportunity for research on the species. Cataloging and monitoring of the butternut trees at Butternut Valley began in 1996 and continues to the present time. Classification of this unique population across multiple scales will provide new information about population dynamics and disease development over time

Population comparisons

In 2004, a Butternut Canker Classification System (Appendix B) was developed in order to provide a more consistent and uniform measurement of overall tree health and the amount of cankering on a tree. Based on the number of bole and branch cankers per linear foot, a tree is given a canker classification rating of 0 to 10. A rating of three indicates three cankers per linear foot. With this rating system, a score of 0 indicates a tree with no evidence

of disease, and a score of 10 is the worse disease rating, with at least one canker per foot. This system was field-tested and incorporated into the University of Tennessee's Tree Improvement Butternut Program in 2005. One goal of the classification system was to determine if canker number was indicative of overall tree vigor and compare multiple populations.

Sites for comparison with the Butternut Valley site were chosen based on personal accounts and preliminary vegetation surveys that identified unusually high abundance of butternut in these areas (Figure VI-3). The St. Francis National Forest in Arkansas and the Ozark National Scenic Riverway in Missouri are two additional locations where a large number of butternut trees are surviving in various health conditions. In all three areas, butternut was found close to drainages and streams, indicating typical habitat for the species. All three stands, however, had atypically high numbers of surviving butternut trees.

The St. Francis National Forest is located at approximately 34.545702° N, -90.644346° W in eastern Arkansas (AR) on the Mississippi River. The Forest rises from the delta plains of Arkansas and Mississippi with the highest ridge at around 91 meters elevation. The St. Francis Lowland Ecoregion is part of the Mississippi Alluvial Plain. River terraces provide the main elements of relief with poorly drained soils. Much of the hydrologic system has been impacted by levees and river channel dredging projects. Much of the area was clearcut in the mid-1970s, resulting in recruitment of a single-aged stand. Based on microsatellite and chloroplast analysis, all trees in the St. Francis National Forest were pure *J. cinerea* (Hoban *et al.* 2009).

The Ozark National Scenic Riverways in Missouri (MO) is located at approximately 37.323390° N, -91.959775° W along the Current River and Jacks Fork streams, which drain northeast into the Mississippi River. The area is at around 350 m elevation and has irregular physiography. It is mostly forested with soils developing mainly from chert and shale. The Current River Hills ecoregion of the Ozark Highlands has chert ridges that are deeply dissected with steep-sided hills. Trees at the Ozark Riverways in Missouri consisted of primarily of *J. cinerea* with one F_1 *J.* x *bixbyi* individual and one with a 75% or greater

probability of not being a pure butternut (Hoban *et al.* 2009). These two individuals were removed from the study and not included in the analysis.

Phenotypic classification

Butternut trees were measured for their relative condition and characteristics in terms of canker numbers as well as factors that express a tree's health condition and phenotypic variables that may be related to disease resistance. At the Butternut Valley location, 224 butternut trees occurring along a four-mile stretch of creek were permanently tagged, measured for height, diameter, and form. Initial measurements were taken in 1998. The trees were evaluated for disease in the limbs and boles (0-5) and overall (0-10 with 0 = no cankers present to 10 = heavily diseased) and some trees were cored for age. In February of 2004 (resurvey), the trees were reevaluated to determine mortality since the previous survey. Additionally, the health of each tree was assessed using the uniform canker classification system (Appendix B). This rating technique is based on the number of cankers on the main stem and branches. Based on this system, trees that exhibited potential resistance to disease (little or no cankers) were recorded. Dead trees were noted to provide an estimate of mortality over time. New individual seedlings that were either not present or very small in size in 1998 were added to the survey, but not included in the reported analyses. The canker score on a scale from 0-10, with 0 being healthy and 10 being heavily diseased and the bole canker score were evaluated.

A suite of measurements were collected from each tree including survival, height, and diameter at breast height. *Bark fissure depth* was determined using a tire wear gauge to create a consistent measurement between deeply-furrowed and smoother bark types (Appendix A). Trees with deeper furrows had higher numbers of bark depth. *Height to the first live branch* was measured in meters. Crown measurements were completed using protocols developed and used by the USDA Forest Service, Forest Inventory and Analysis as indicators of tree health (Schomaker *et al.* 2007). *Crown diameter* is the average of two diameter measurements: (1) widest distance anywhere in the crown between the driplines of two live branches, and (2) the distance perpendicular to the widest measurement; taken in meters. *Crown light exposure*: estimates the amount of a tree's foliage receiving direct

sunlight; crowns are rated from zero to five depending on the number of crown sections exposed to direct sunlight (0=no sections exposed, 5=all sections exposed. *Crown position*: estimates the position of a tree's crown relative to the stand overstory canopy zone; Codes 1 to 4 represent the superstory, overstory,understory, and open-grown crown positions, respectively. Many of the percentage data are recorded in 5-percent classes and coded as 0, 05, 10, 15, . . . , 100, where the code represents the upper limit of the class, e.g., 1 to 5 percent is code 05. *Crown density*: estimates the percentage of light blocked by branches, reproductive structures, and foliage within a tree's crown; recorded in percent classes. *Crown dieback*: estimates recent branch mortality in the upper and outer portions of the live crown; recorded in percent classes. *Foliage transparency*: estimates the amount of skylight visible through the live, normally foliated portion of the crown; recorded in percent classes; recorded in percent classes (Schomaker *et al.* 2007).

Statistical analysis

Statistical analyses were conducted using SAS version 9.1 (for Windows, © 2005 SAS Institute Inc., SAS Campus Drive, Cary, North Carolina 27513, USA). Additional macros developed by A. Saxton (1998, 2004) were used for means separation and genetic correlations. Macros included pdmix800 for means separation using multiple range tests (Saxton 1998), Mixed Model Analysis of Variance Macro (mmaov) uses Fisher's Least Significant Difference (LSD) test was conducted at the 5% significance level, determines if standard deviations are within five fold of each other, calculates Levene P values to determine equality of variances, and a Shapiro-Wilk W test for normality (Saxton ©2002). All percentage data, derived from count data, underwent inverse trigonometric sine transformations due to lack of homogeneity of variances (Snedecor and Cochran 1998).

Objective a). Surviving and dead trees were compared using t-tests for differences in height, diameter, tree crown position, and previous disease measurement.

Objective b). For all three populations individually trees were grouped by ratings of either greater than or lesser than the mean, *healthy* (0-rating), *resistant* (0 and 1 ratings), and *heavily diseased* (10 rating). Groupings of trees were compared to determine differences in

percentages across the three populations using an analysis of variance (PROC ANOVA). Butternut trees from each of the three populations, TN, MO, and AR were compared using a multivariate analysis of variance (PROC MANOVA) for variation in bark depth and crown conditions. Canker classification groups were analyzed for the impacts of canker classification on various other tree measurements including DBH (diameter at breast height), bark fissure depth (mm), and indicators of crown condition.

Objective c). Ordinal logistic regressions (PROC LOGISTIC) were conducted on the relationship between canker classification (0-10) and phenotypic tree variables including height, DBH, crown class position, bark fissure depth, and variables indicative of crown condition. All canopy measurements were analyzed for correlations (PROC CORR).

Results

Temporal classification

In 1998, 224 butternut trees were measured for disease condition on limbs, on the main bole, and overall disease condition. Trees averaged 26.83 cm (variance 2.16) diameter at breast height (DBH) and 15.96 m (1.67 variance) in height. Disease condition on limbs was significantly higher than the condition on the main boles (Figure VI-5). A level 3 disease condition was present on 30% of trees on limbs and 20% on boles. A level 4 disease condition was present on 33% of the trees on limbs and 23% on boles (Figure VI-5). Numerous limb cankers may have less of an overall impact of a tree than a few large bole cankers. Taller and larger trees had significantly lower disease ratings than smaller size classes of trees (Figure VI-6). Trees in the lowest limb disease rating of 0 average 30.91 cm DBH and 17.32 m tall compared and consistently larger at the 2-4 disease limb rating (Figure VI-6). However at the worse limb disease rating, this correlation was consistent for DBH but not for height: 13.29 cm DBH and 20.36 cm tall (Figure VI-6). There was less of a downward trend on disease ratings of boles for height; however, the downward trend was evident for DBH except for the worse disease rating for the one site in TN(Figure VI-7).

In the initial survey, overall level of disease was fairly consistent across height and diameter of trees (Figure VI-8). There were no relationships among tree size, age, form, and

disease rating for the stem, bole, and overall. This indicates that trees with varying levels of disease could be found in any size, form or age class. For the entire population disease pressure was equal across a wide range of tree conditions. Overall disease level was essentially bell-shaped with a slight skew towards the score of 7 out of 0-10 (Figure VI-9).

Objective a). The resurvey found overall mortality in the butternut population was 8.7 percent. This percentage was considered to be low, as over 30 percent of the trees received disease ratings of 8-10 in initial survey. Dead trees in 2004 had a significantly higher average disease rating of 8.22 in 1998, as opposed to 5.92 for living trees ($p=0.00106$, Figure VI-11). Average canker values on the branch were higher in dead trees (4.44) compared to live trees (3.03, $p<0.0001$, Figure VI-11). Average canker values on the main stem were also higher on dead trees (3.78, variance 0.76) compared to live trees (3.03, variance 0.64, $p<0.0001$, Figure VI-11). The highest amount of mortality occurred among small trees with high disease rankings. 20 percent of surviving trees received a very high disease rating six years prior.

The dead trees in 2004 were smaller trees in the 1998 survey than trees that remained alive (Figure VI-12). Trees averaged over 3 m shorter; height of 11 m for dead trees and 15 m for living trees ($p<0.0001$). The mean diameter at breast height of dead trees was 17 cm compared to 26 cm for living trees ($p<0.0001$). Within the Butternut Valley population, bark fissure depth is higher in trees with lower canker ratings and lower in trees with the most amount of cankering.

There are 53 butternut trees in butternut grove, with an average DBH of 24.7 cm and an average height of 19.5 m. The average butternut canker score on a scale from 0 (most resistant) to 10 (highly susceptible) for this area is 3.88 and ranges from 1 to 10. The second concentration is known as the "Old Field" and is another abandoned agricultural field that has not been disturbed in approximately five years. There are 41 seedlings in the old field, with an average DBH of 3.9 cm and an average height of 3.4 m. The butternut canker score for this population is less than 1 (0.70) with a maximum canker score of 4. These two areas provide evidence of regeneration in two distinct time periods and could provide important

information in terms of genetic relatedness and parental lineage across two regeneration episodes within the population.

Population comparisons

Objective b). Canker classification The average health rating of the Tennessee population (TN) was compared with populations in the St. Francis National Forest in Arkansas (AR) and the Ozark National Scenic Riverways in Missouri (MO) (Table VI-1). The mean canker rating, on a scale from 0 to 10, of these three populations was: 3.038 for the TN and 3.945 for AR and 2.96 for MO (Table VI-1, Table VI-1, Figure VI-13). The classification system was consistant between the Tennessee and Missouri populations in mean health rating of trees and the percentage of trees with healthy and resistant ratings. The AR population was different that both the TN and MO populations for canker classifications. Cankers on the main bole of the tree were consistant with overall canker score (Table VI-2, Figure VI-14). MO had the largest percent of trees with the healthy rating (9.77), however it was still under ten percent of the population. AR had the healthiest overall rating of trees, but also had the highest percentage of heavily diseased trees (9.52). The AR population all canker classification catagories had the same DBH. In the TN population resistant trees were larger in DBH than trees less than or greater than the mean health rating. The opposite was true in the MS population with the largest trees with the greatest amount of diseased.

Tree characteristics All three populations did not differ in diameter at breast height ($p= 0.4142$, Table VI-2, Figure VI-15). The AR population had the tallest trees followed by TN and then MS (19, 16, 12 m, respectively, Figure VI-16). AR also had a greater percentage of trees in more dominant crown position and decreased height to first branch. Crown light exposure, height to first live branch, and crown diameter were all greatest in the MS population, followed by AR and then TN. The bark depth overall is less furrowed in the TN and AR populations with the deepest furrows in the MS population (Table VI-3). At all populations trees with greater than average canker scores had lower fissure depth (Figure VI-21), increased dieback, increased foliage transparency, and decreased crown diameter.

Phenotypic classification

*Objective c).*The combined average height was consistent across canker scores (Figure VI-17), as was diameter (Figure VI-18), crown positions (Figure VI-19), and crown light exposure (Figure VI-20). Relationships exist between canker classification ratings and indicators of tree health and vigor (Table VI-3). Increasing canker condition resulted in increased dieback, foliage transparency, and decreased foliage density and diameter, as expected. Canker condition was not related to crown position, crown light exposure, or height to first branch; indicating that disease was present in all crown classes. Canker classification was not significantly related to DBH or height (Table VI-4). Correlations for crown condition indicated their reliability in assessing crown health (Table VI-5, Figure VI-22, Figure VI-23, Figure VI-24).

Bark fissure depth was closely related to canker classification (Table VI-4). However it was also positively related to crown diameter, crown position, height to first live branch, crown light exposure, crown density, and crown dieback (Table VI-5). At all populations increased fissuring occurred on trees with healthier canker classifications and healthier crown conditions.

Discussion

Temporal classification

Results of the temporal study at Butternut Valley show very little mortality over the six-year time period investigated. This indicates that butternut canker alone may not be the primary agent in tree mortality. A large number of surviving trees are small trees in the suppressed age class indicating that shade-intolerance is also not the sole agent of mortality. The relationship between survival and canopy position as well as disease ranking reflects the greatest mortality among trees experiencing a loss of vigor due to suppression from canopy closure and larger negative effects of butternut canker disease. At this individual population of *J. cinerea* species, natural resistance to the disease appears to be fairly high as mortality appears to be primarily caused by stand dynamics.

Population comparisons

The mean rating values and the percentages of seedlings in each rating class are fairly consistent across multiple populations. Therefore, the canker classification system can be used to determine relationships in the average score and number of potentially resistant trees across populations. In addition, it provides evidence that the Butternut Valley population has a similar amount of disease and health trees as other known concentrations of butternut. However, the increased disease among smaller trees may be an artifact of location in TN as the opposite is true in the MO population and no difference occurs in the AR population. This variation could be due to dynamics of both genetic resistance and environmental controls. In a fairly resistant population, the most vulnerable individuals may be suppressed trees that are dying due primarily to stand dynamics. In a less resistant population, trees in upper canopy positions may be highly susceptible and surviving due to other conditions optimal for growth.

Phenotypic classification

Phenotypic characterization of the individual trees, if related to genetic resistance, can then be used in breeding efforts and to understand patterns of diversity within the species. This study developed and applied a standardized method of determining disease condition and phenotypic traits of butternut. These evaluations will create a base-line measurement for continued evaluation of tree health over time.

Trees with a large number of cankers also had increased dieback, increased foliage transparency and reduced crown density and diameter. The canker classification rating, therefore, can be used as an indicator of overall tree health. Phenotypic variables give indications of individual tree vigor. Quantifying canker classification, crown condition, and bark fissure depth allows managers indications of relative disease resistance. These measures are important in selecting potentially resistant trees for breeding programs and monitoring disease development over time.

Given the relationship between smooth bark type and cankering, smooth barked trees may be more susceptible to cankering, which is similar to observations by Ostry *et al.* (2003).

However, this may also be an artifact of reduced light exposure and lower canopy conditions which may increase susceptibility to the disease. Ostry *et al.* (2003) did not discuss the relationship between canopy position and bark phenotype. Increased mortality found in more northern sections of the range (Bergdahl *et al.* 1996, Cummings Carlson and Guthmiller 1993) could be a result of increased damage of snow and ice on cankered stems.

Conclusions

Assessment of a single population six years after initial assessment showed only eight percent mortality; concentrated in lower crown positions. Canker condition is consistent across a variety of tree sizes and canopy positions. The canker classification system developed for assessment can determine the proportion of resistant seedlings in a population. Canker condition also gives insight into the health of the trees crown. Heavily cankered individuals occurred across all canopy positions with variation between sites on the degree of cankering and canopy dynamics. Trees with smooth bark types have lower canker scores, but also appear in higher crown positions with greater exposure to light. This phenotypic variable is related to tree health as well as degree of cankering, with increased health in the deeply furrowed bark type. This is the first report of health over time on a population genetically verified as *J. cinerea.*

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Appendix: Tables and Figures

Table VI-1. Disease conditions of three butternut populations using the numerical canker classification rating system.

Location	Number of Trees	Mean Health Rating	% trees < mean	% trees with 0 rating: HEALTHY	% trees with 0 and 1 rating: RESISTANT	% trees with 10 rating: HEAVILY DISEASED
St. Francis, AR	162	3.945a	40.14c	4.08b	11.57b	9.52a
Butternut Valley, TN	153	3.038b	53.59b	3.27c	13.07a	2.61c
Ozark, MO	512	2.957b	64.44a	9.77a	13.77a	3.33d
TOTAL/MEAN	**827**	**3.314**	**52.72**	**5.71**	**12.80**	**5.15**

Unique letters indicate significantly different values using Tukey's Mean Separation Analysis across individual columns for each variable.

Table VI-2. Variation between sites with butternut tree characteristics.

Variables	Arkansas	Missouri	Tennessee	F value	P value
Height (m)	18.59a	12.21c	15.91b	40.53	<0.0001
Diameter at breast height (cm)	24.04a	24.01a	25.46a	0.88	**0.4142**
Bark Depth[1]	1.33b	2.36a	1.24c	71.85	<0.0001
Canker Score[2]	3.95a	2.96b	3.04b	29.91	<0.0001
Main Bole Cankers[3]	1.90b	6.92a	7.76a	34.67	<0.0001
Crown Position[4]	2.91a	2.64b	2.76ab	3.93	<0.0001
Crown Light Exposure[5]	1.86b	2.00a	1.62b	4.75	0.0090
Height to 1st live branch (m)	1.07c	8.99a	5.27b	73.24	<0.0001
Crown Diameter (m)	2.05c	8.17a	5.12b	84.22	<0.0001
Crown Dieback (%)	--	25.24a	20.51a	2.67	**0.1452**
Crown Density (%)	--	58.87a	54.71a	1.04	**0.3084**
Foliage Transparency (%)	--	35.51a	39.26a	1.54	**0.2160**

Bold indicates not significant at the p=0.05 level

Unique letters indicate significantly different values using Tukey's Mean Separation Analysis across individual rows for each variable.

[1] bark fissure depth measured with a tire gauge
[2] on a scale from 0 healthy to 10 heavily diseased on the main bole and smaller limbs
[3] on a scale from 0 healthy to 10 heavily diseased on the main bole
[4] Codes 1 to 3 represent the superstory, overstory, and understory crown positions, respectively
[5] crowns are rated from zero to five depending on the number of crown sections exposed to direct sunlight

Table VI-3. Butternut tree characteristics in three southern populations using the numerical canker classification rating system.

Location	Trees % (#)	DBH[1] (cm)	Crown Density[2] (%)	Crown Diameter (m)	Foliage Transparency (%)	Dieback (%)	Bark Fissure Depth[3] (mm)
St. Francis, Arkansas							
Resistant	11 (17)	24.5a	33.3c	7.4a	34.1b	15.3b	3.67a
Health Rating < Mean	36 (58)	24.4a	41.67b	7.5a	33.3b	15.4b	3.61a
Health Rating >Mean	54 (87)	24.6a	43.57a	6.5b	39.3a	22.0a	2.90b
Total/ Mean	**(162)**	**24.5**	**39.5**	**7.1**	**35.6**	**17.6**	**3.40**
Butternut Valley, Tennessee							
Resistant	12 (20)	38.0a	63.0a	11.9a	36.3b	15.6c	7.58a
Health Rating < Mean	47 (82)	31.5b	65.0a	10.0b	33.0c	19.3b	6.10b
Health Rating >Mean	41 (71)	27.5c	56.0b	8.2c	44.0a	27.4a	4.92c
Total/ Mean	**(173)**	**32.3**	**61.3**	**10.0**	**37.8**	**20.8**	**6.20**
Ozarks, Missouri							
Resistant	12 (62)	17.0c		5.7a		8.0c	
Health Rating < Mean	57 (290)	22.5b		5.9a		19.4b	
Health Rating >Mean	31 (160)	23.2a		6.0a		21.2a	
Total/ Mean	**(512)**	**20.9**		**5.9**		**16.2**	
OVERALL	**(847)**	**25.9**	**50.4**	**7.7**	**36.7**	**18.2**	**4.80**

Unique letters indicate significantly different values using Tukey's Mean Separation Analysis across individual columns for each variable at each specific location.

[1] diameter at breast height, [2] crowns are rated from zero to five depending on the number of crown sections exposed to direct sunlight, [3] bark fissure depth measured with a tire gauge

Table VI-4. Canker score, DBH, and Crown positions across measures of crown condition and bark depth.

Variables	Regression	
	r^2	P value
Canker Score[2]—Dieback (%)	0.0094	0.0335
Crown Density (%)	0.0339	0.0205
Foliage Transparency (%)	0.0475	0.0063
Bark Depth[1]	0.0356	<0.0001
DBH—HT	0.5471	<0.0001
HT to 1st live branch	0.0532	<0.0001
Crown Diameter (m)	0.1110	<0.0001
Crown Position[3]	0.0137	0.0054
Light Exposure[4]	0.0095	0.0279
Bark Depth	0.0691	<0.0001
Crown Position—Ht to 1st live branch (m)	0.1241	<0.0001
Crown Density (%)	0.1016	<0.0001
Crown Diameter (m)	0.1021	<0.0001
Light Exposure	0.0322	<0.0001
Bark Depth	0.1244	<0.0001

[1] bark fissure depth measured with a tire gauge
[2] on a scale from 0 healthy to 10 heavily diseased on the main bole and smaller limbs
[3] Codes 1 to 4 represent the superstory, overstory, understory, and open-grown crown positions, respectively
[4] crowns are rated from zero to five depending on the number of crown sections exposed to direct sunlight

Table VI-5. Correlations between measurements of crown conditions and measurements of bark fissure depth.

Variables	Correlations	
	r^2	P value
Bark Depth[1] —Ht to first branch (m)	0.38935	<0.0001
Crown Diameter (m)	0.46290	<0.0001
Crown Positon[2]	0.35274	<0.0001
Crown Light Exposure[3]	0.25706	<0.0001
Crown Density (%)	0.23815	0.0031
Crown Dieback	0.09559	0.0373
Crown Diameter—Crown Density (%)	0.40130	<0.0001
Foliage Transparency (%)	-0.22193	0.0058
Crown Dieback	-0.29513	<0.0001
Crown Position—Crown Density (%)	-0.31871	<0.0001
Crown Light Exposure	-0.17955	<0.0001
Crown Diameter (m)	0.1021	<0.0001
Light Exposure	-0.17955	<0.0001
Crown Light Exposure—Crown Density (%)	0.21888	0.0059
Crown Dieback (%)	-0.9224	0.0438
Crown Dieback— Ht to first branch (m)	0.12267	0.0073
Crown Dieback (%)	0.42167	<0.0001
Crown Density— Foliage Transparency (%)	-0.70377	<0.0001

[1] bark fissure depth measured with a tire gauge
[2] Codes 1 to 4 represent the superstory, overstory, understory, and open-grown crown positions, respectively
[3] crowns are rated from zero to five depending on the number of crown sections exposed to direct sunlight

Figure VI-1. Location of Butternut Valley in DeKalb County, TN.

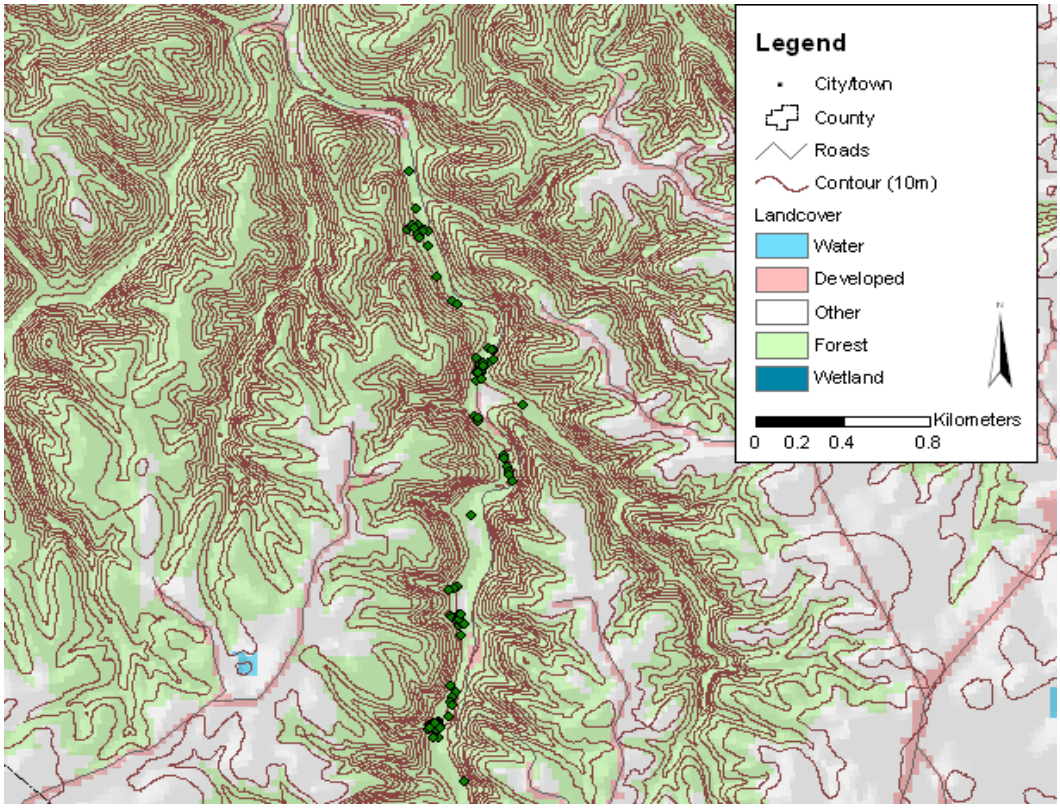

Figure VI-2. Locations of butternut trees along Dry Creek in DeKalb County, TN. Map courtesy of Laura Thompson

Figure VI-3. Study areas on range of butternut in southern United States, as adapted from Rink (1990).

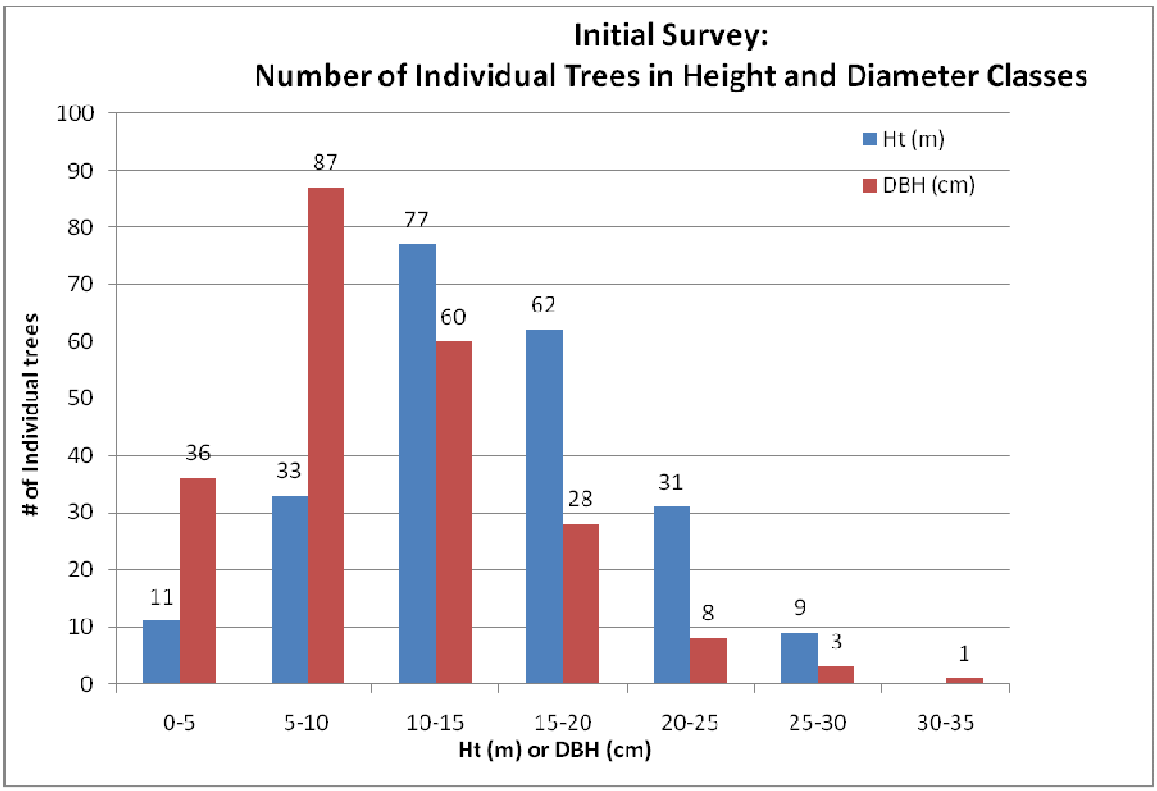

Figure VI-4. Number of individual butternut trees in each tree height and diameter class in initial survey at Butternut Valley.

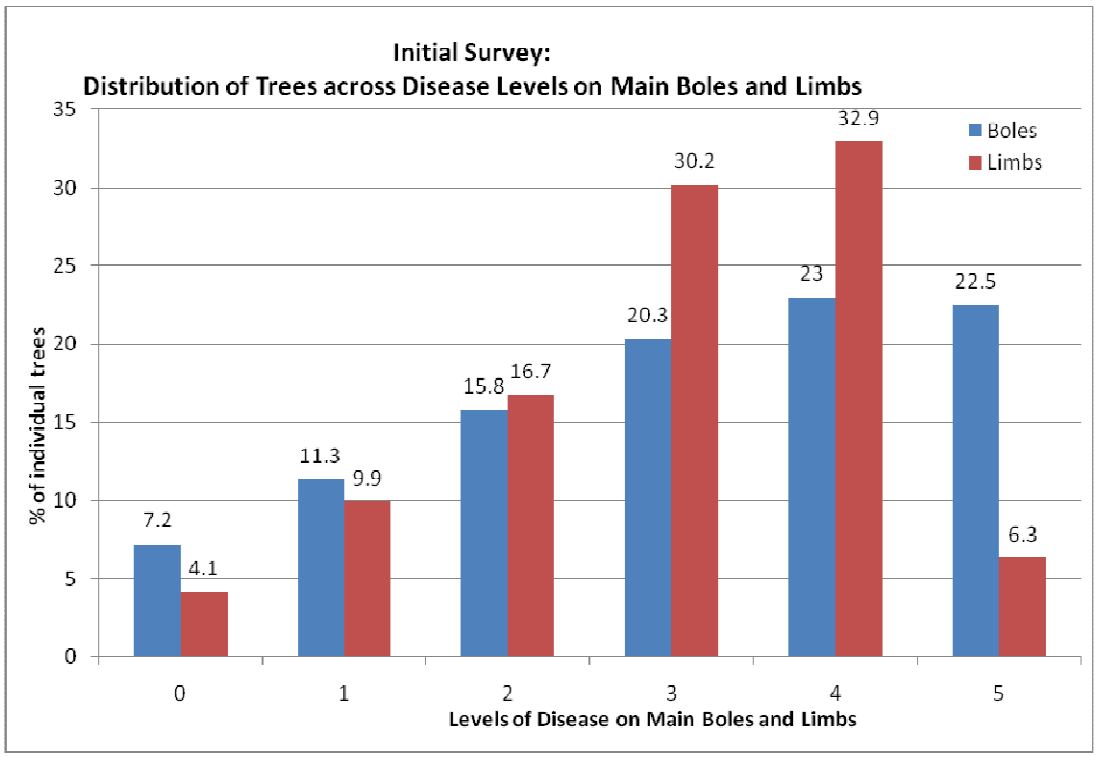

Figure VI-5. Percentages of individual butternut trees with levels of disease on main boles and tree limbs in initial survey at Butternut Valley.

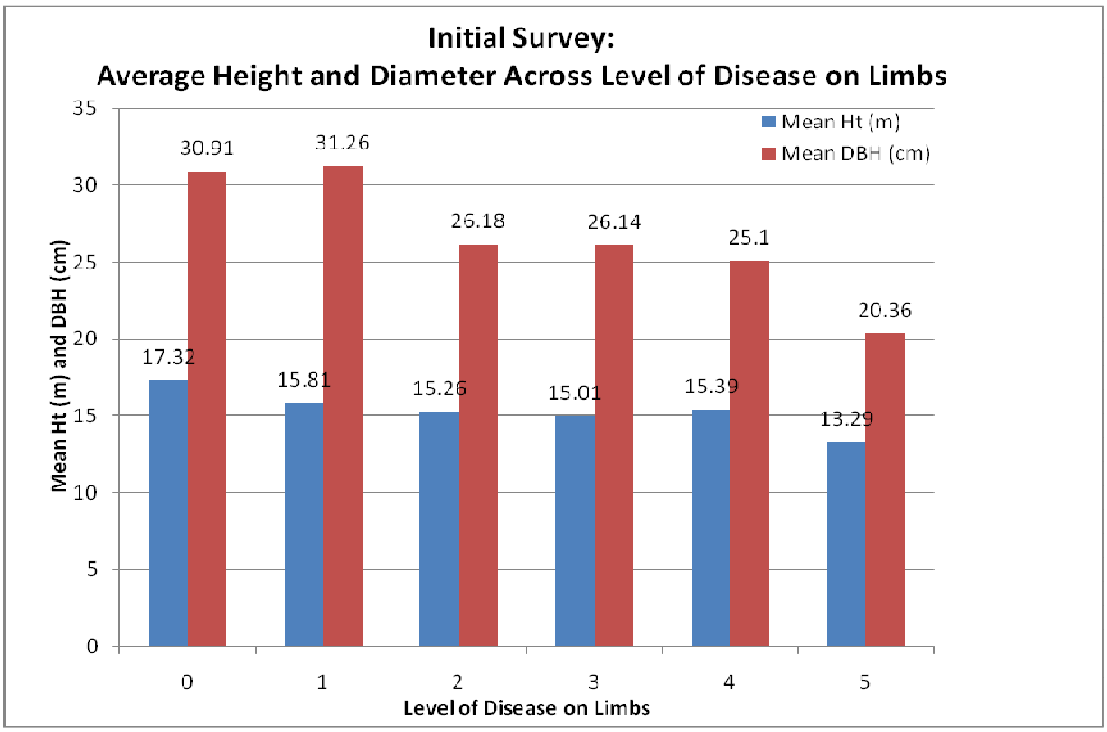

Figure VI-6. Average tree height and diameter across level of disease on limbs in initial survey at Butternut Valley.

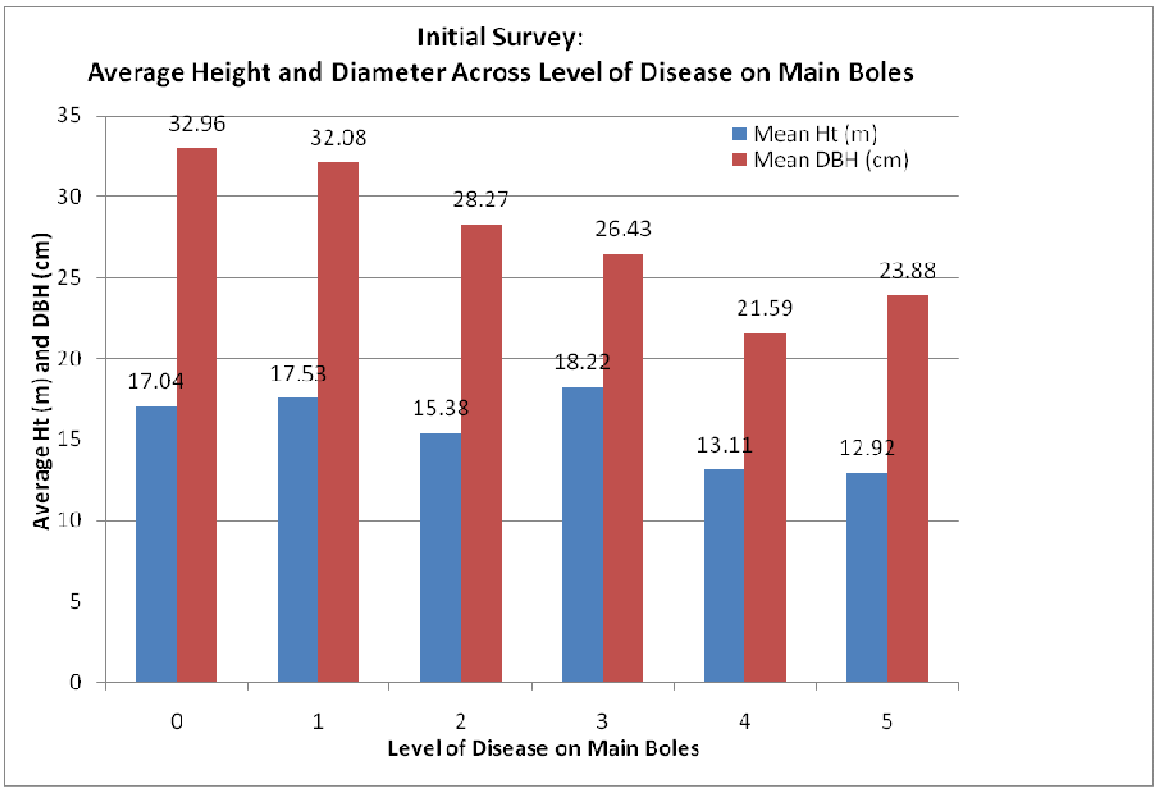

Figure VI-7. Average butternut tree height and diameter across level of disease on main boles in initial survey at Butternut Valley.

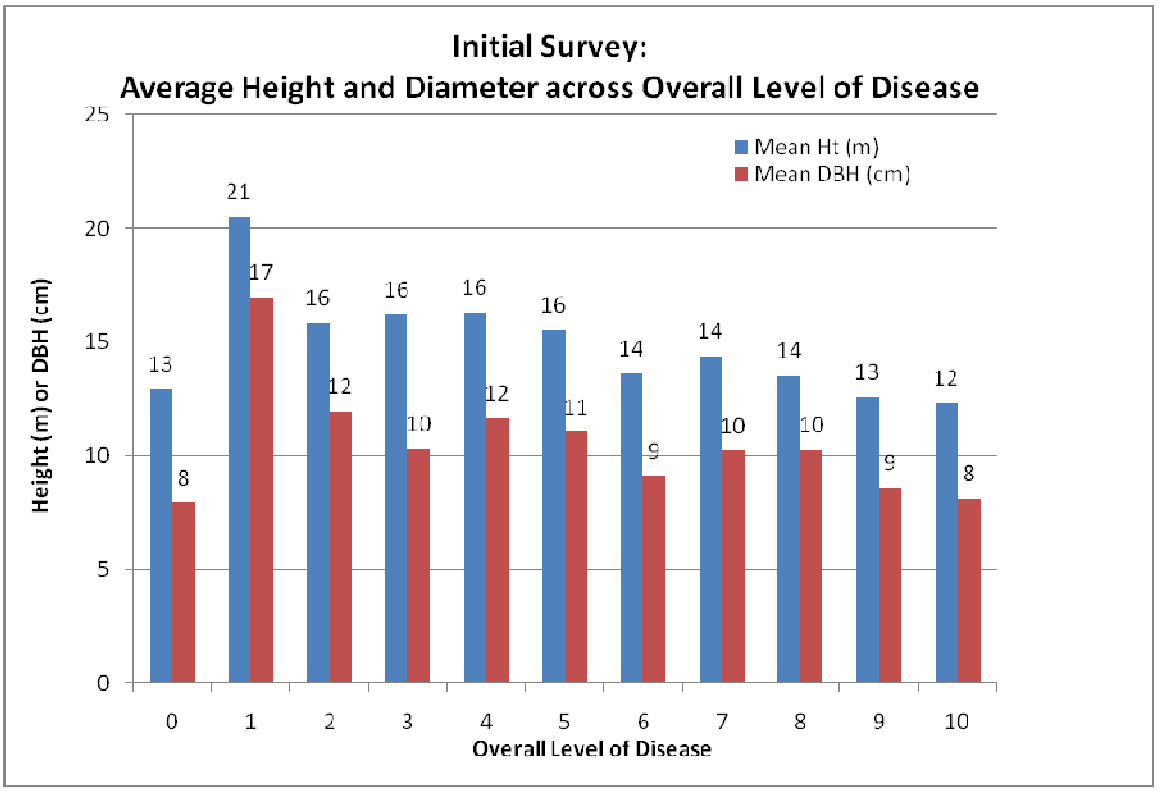

Figure VI-8. Average butternut tree height and diameter at breast height across overall level of disease in initial survey at Butternut Valley.

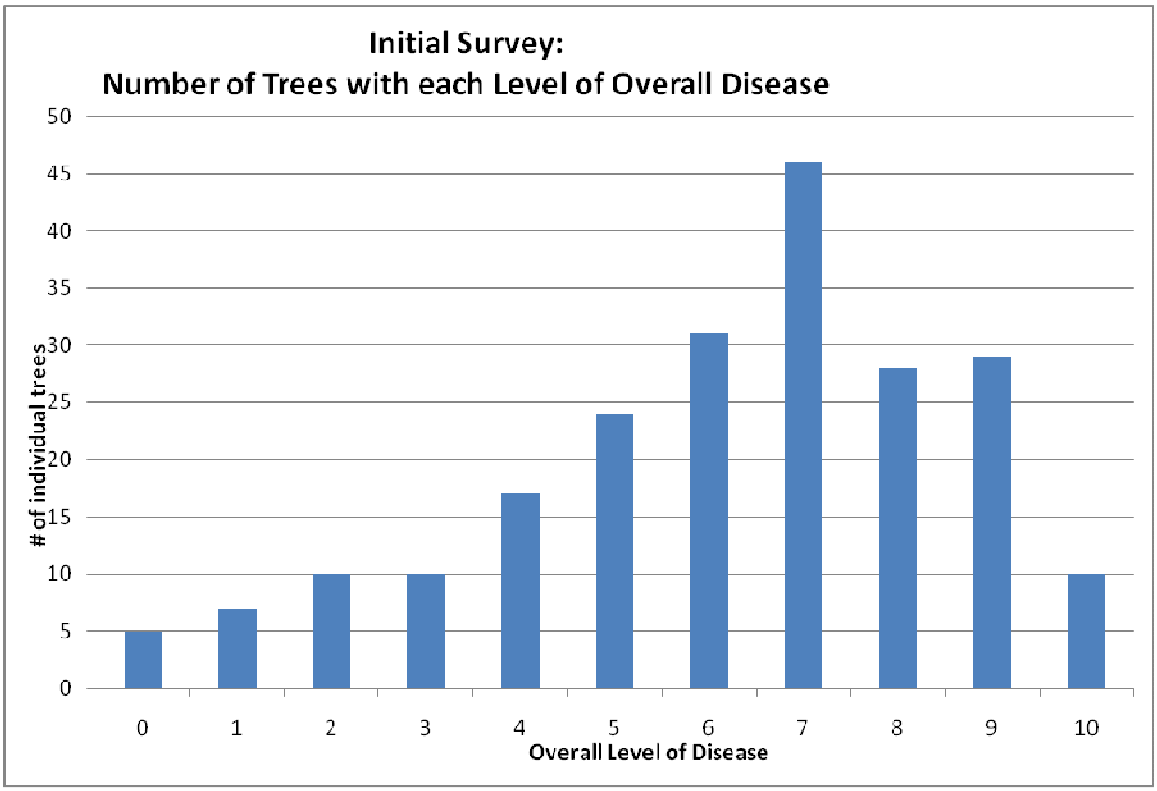

Figure VI-9. Number of individual butternut trees in overall levels of disease in initial survey at Butternut Valley.

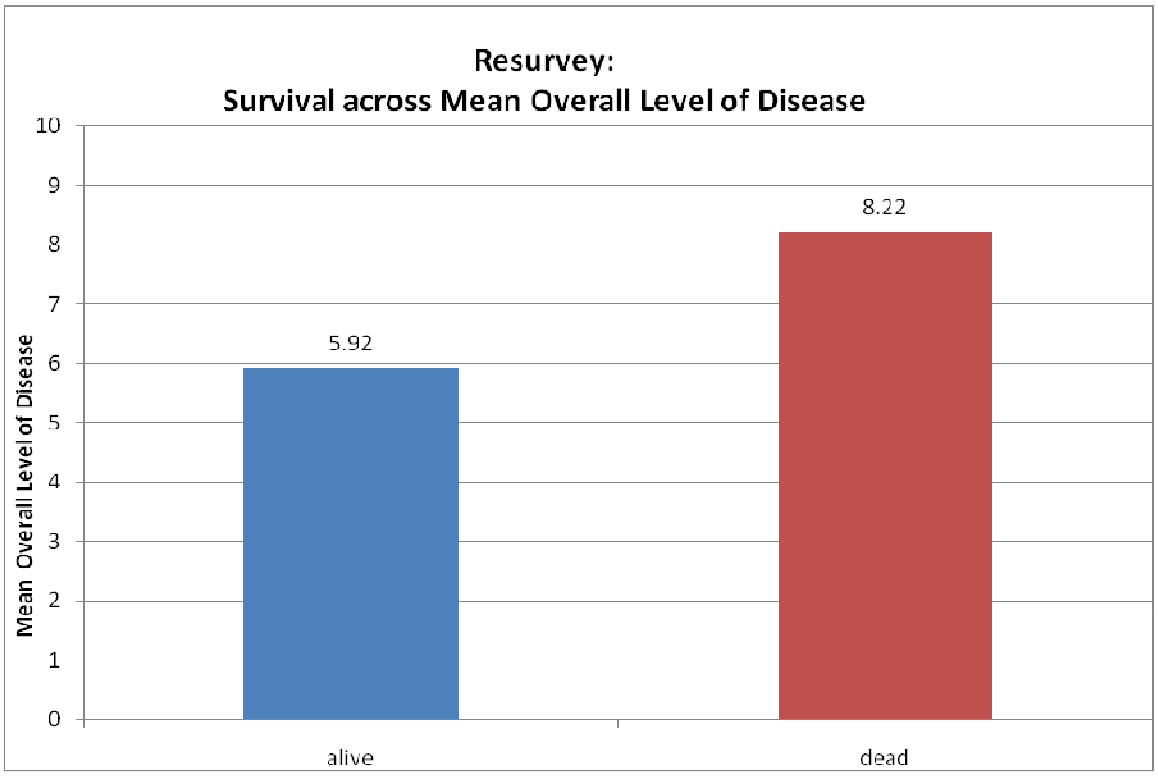

Figure VI-10. Average overall level of disease in initial survey of butternut trees across survival condition in resurvey at Butternut Valley.

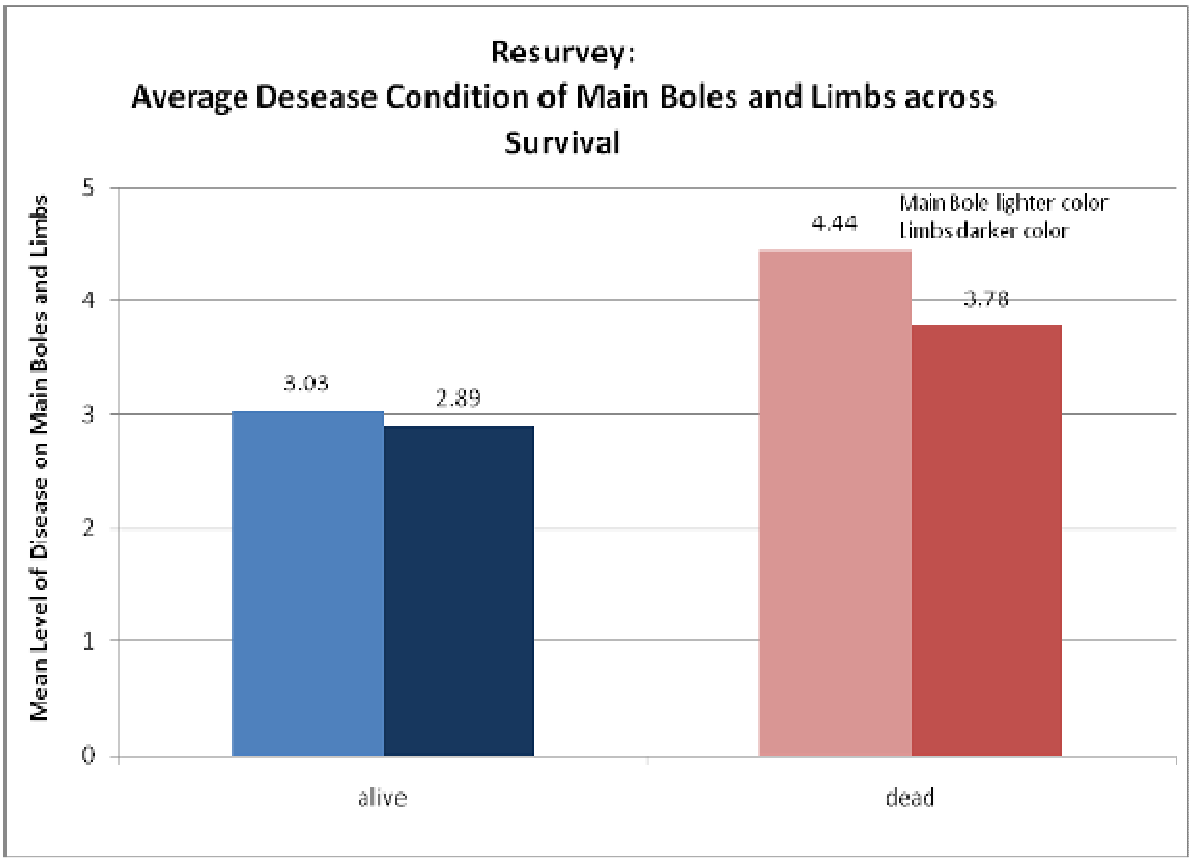

Figure VI-11. Average levels of disease on main boles and limbs of butternut trees across survival condition in resurvey at Butternut Valley.

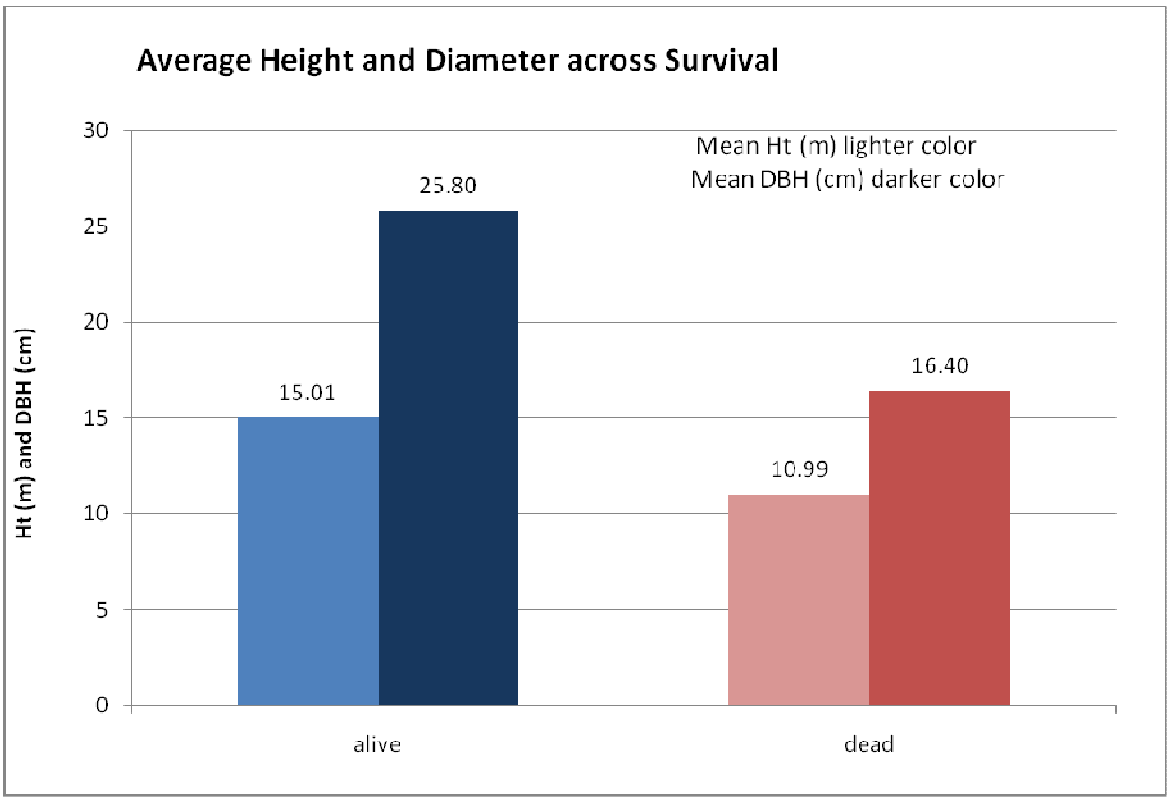

Figure VI-12. Average mean heights and diameter at breast heights of butternut trees across survival condition in resurvey at Butternut Valley.

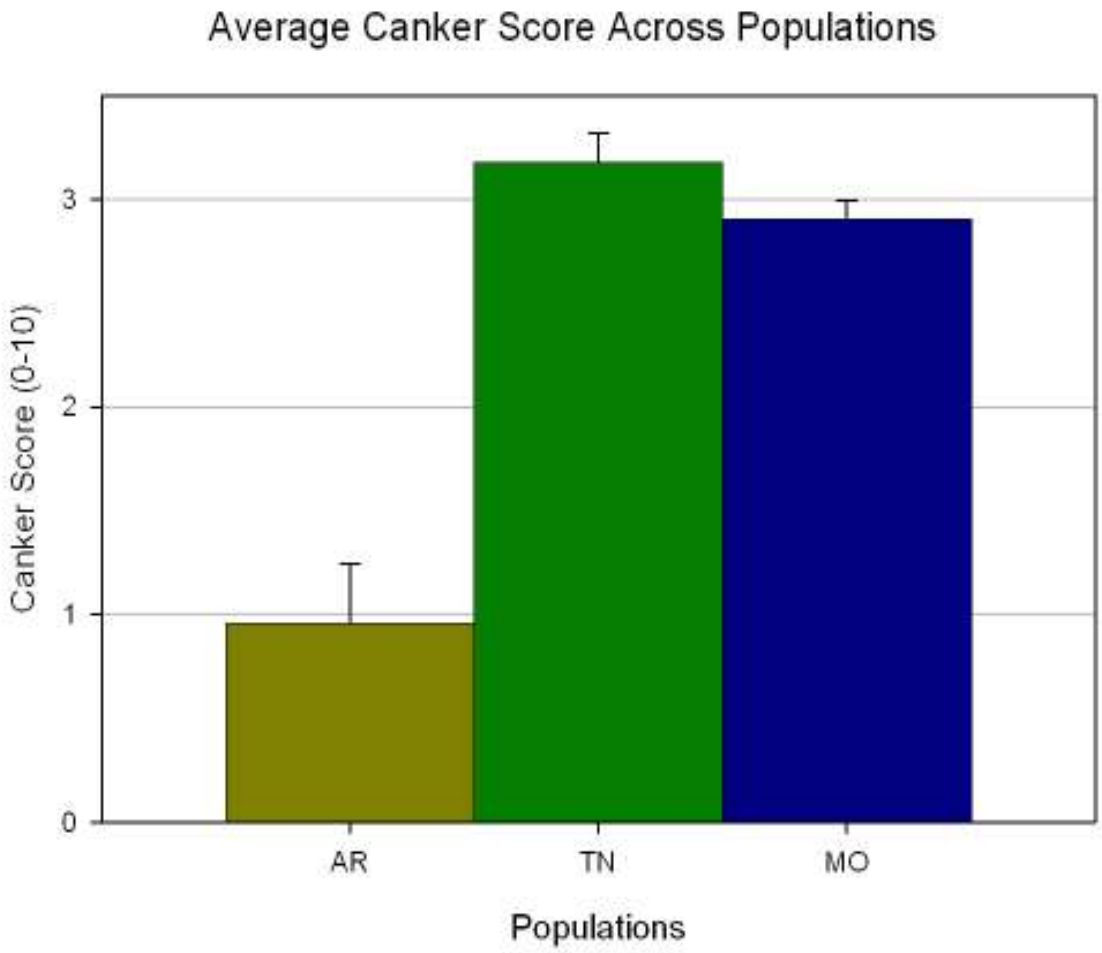

Figure VI-13. Canker score across populations.

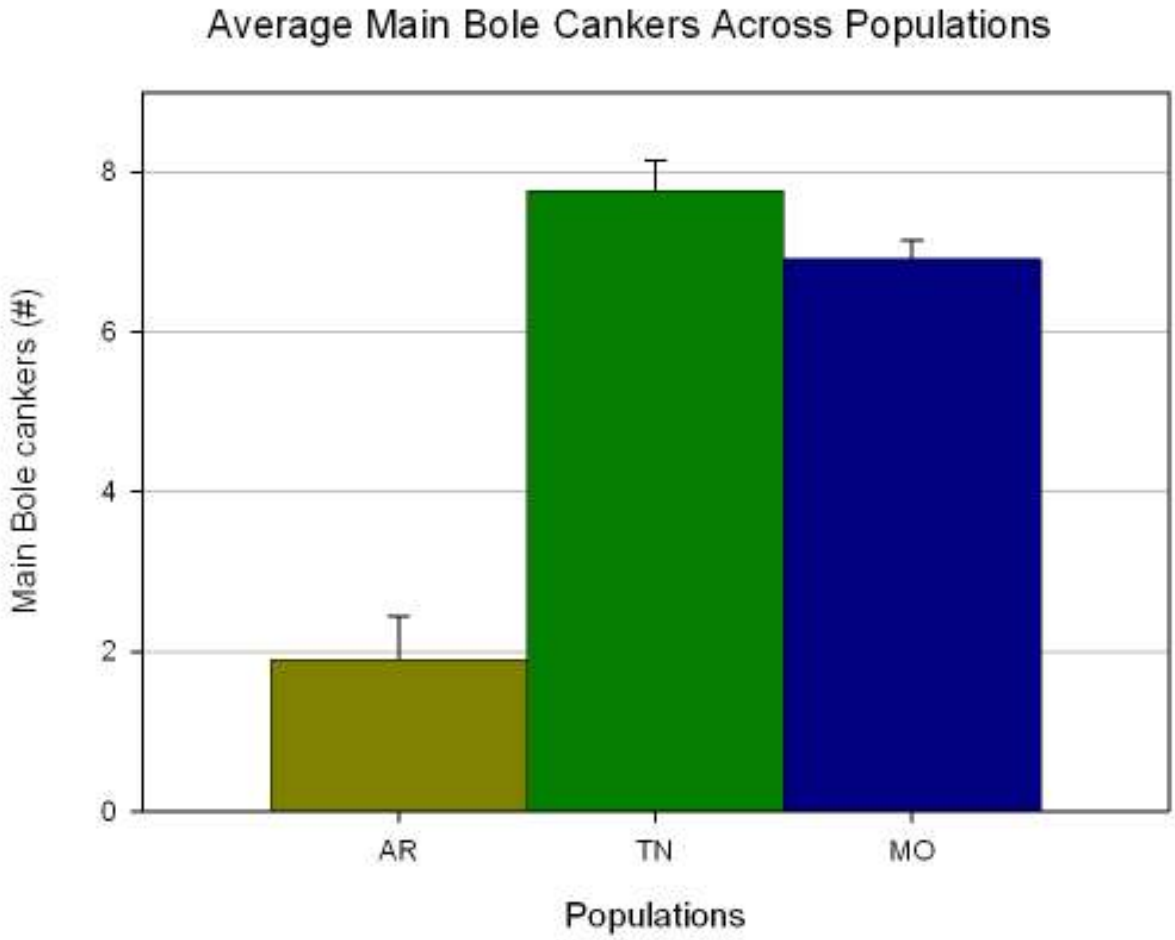

Figure VI-14. Number of main bole cankers across populations.

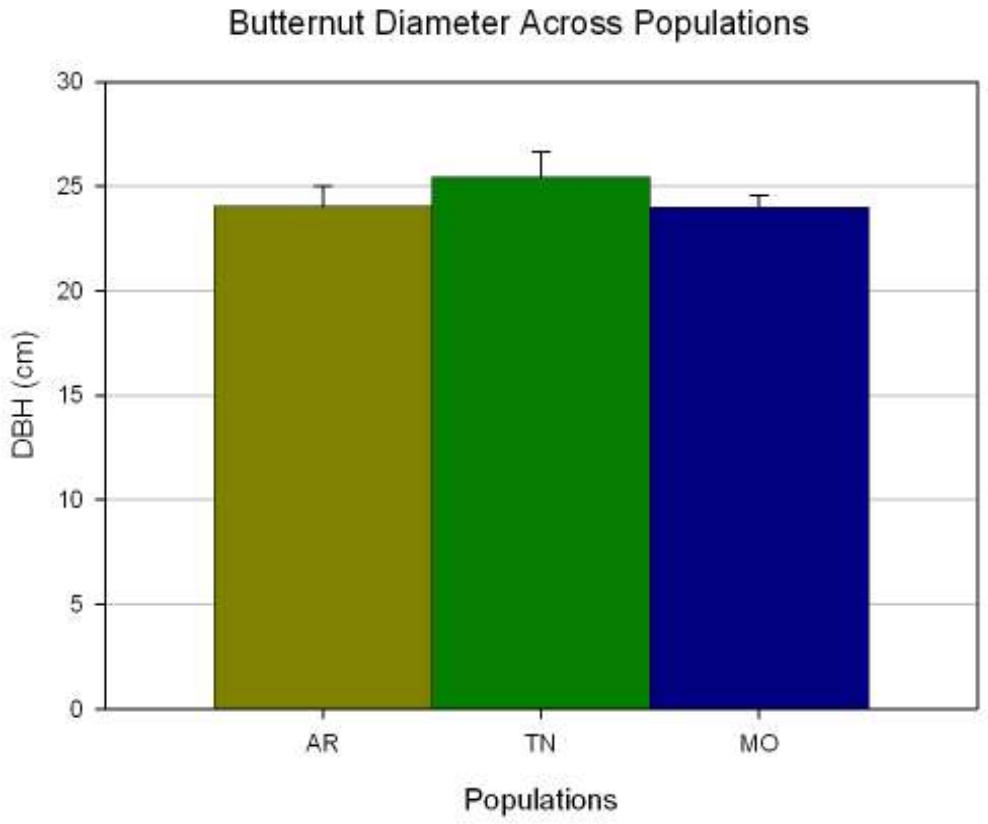

Figure VI-15. Diameter of butternut trees across populations.

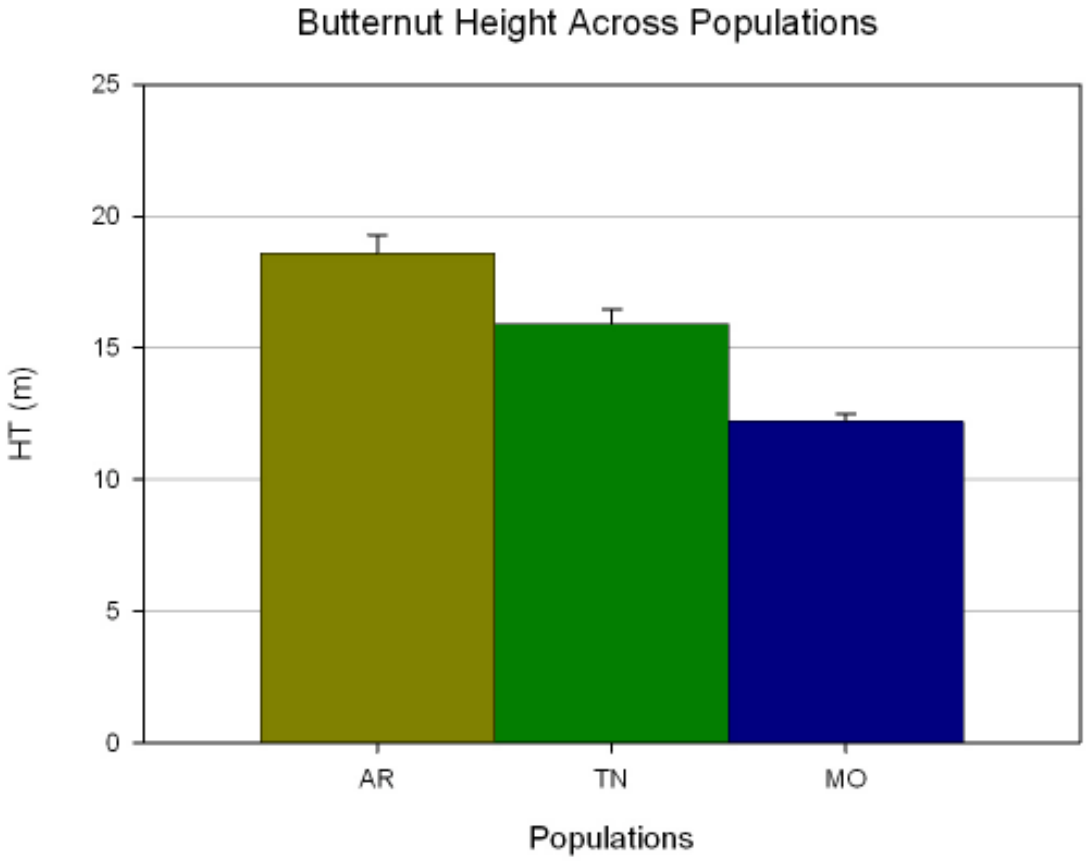

Figure VI-16. Height of butternut trees across populations.

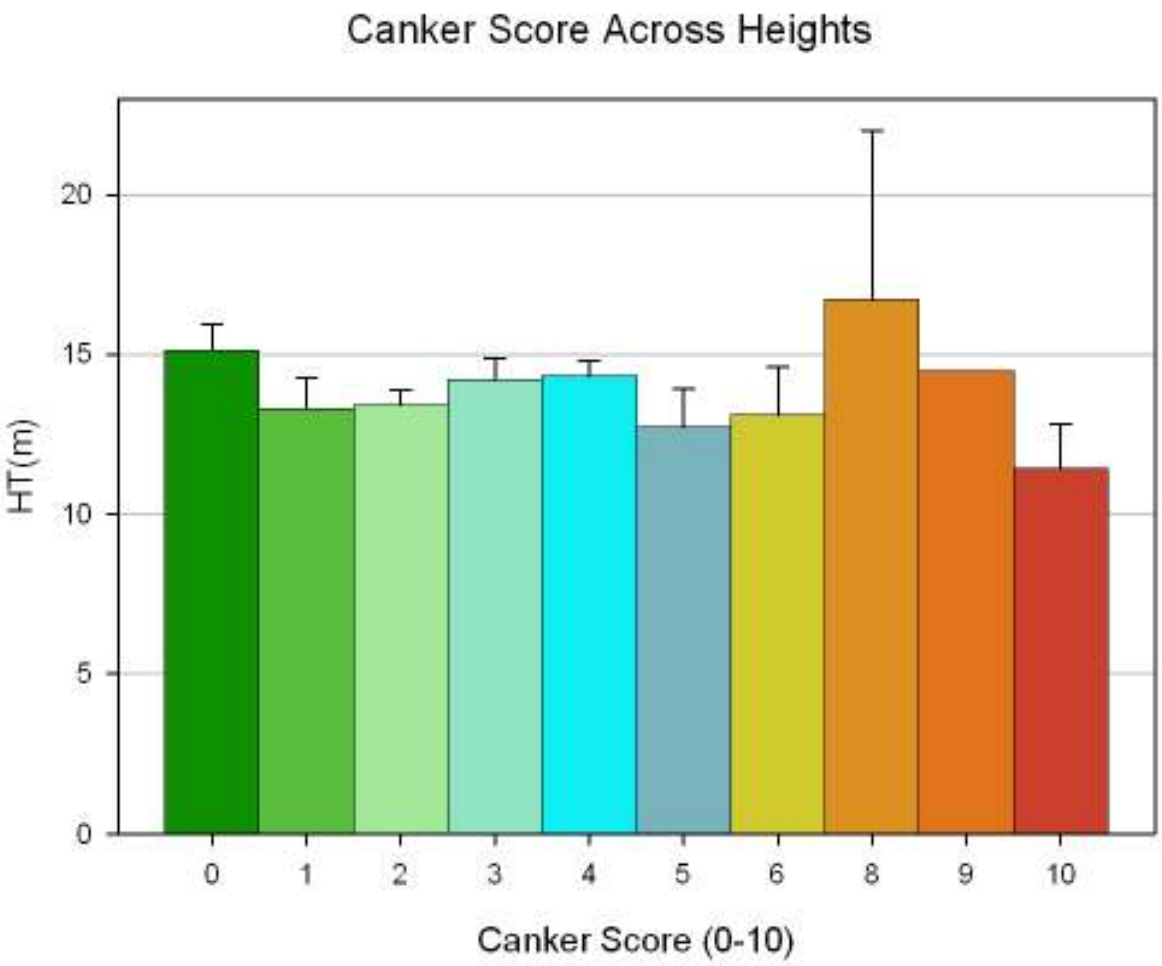

Figure VI-17. Average height of butternut trees at each canker score.

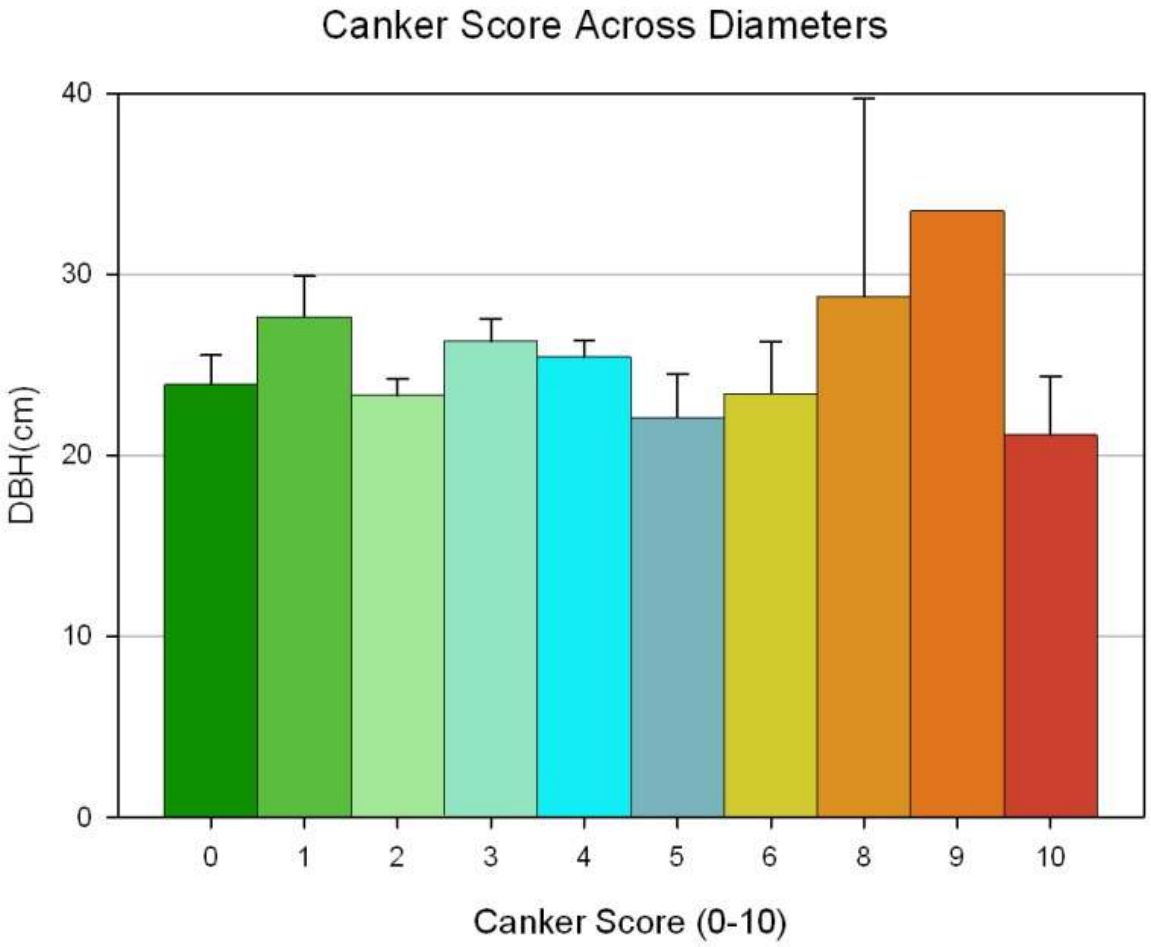

Figure VI-18. Average diameter at breast height of butternut trees at each canker score.

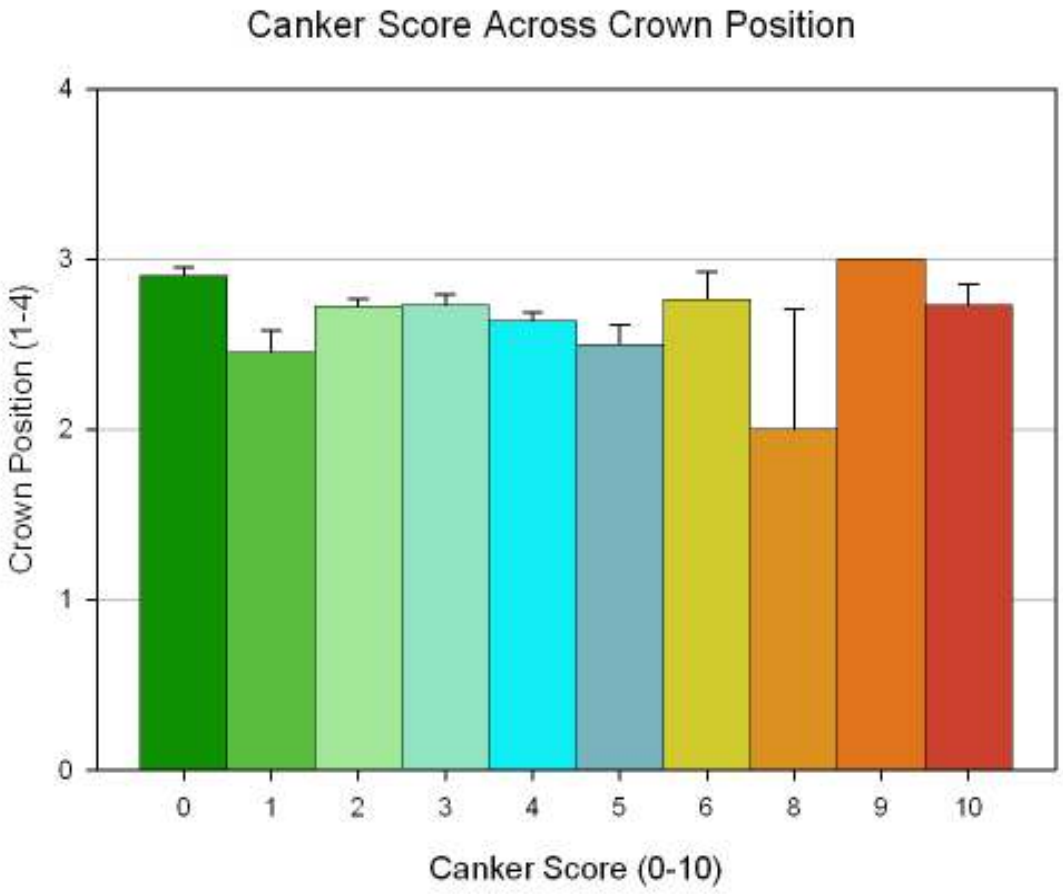

Figure VI-19. Average crown position of butternut trees at each canker score.

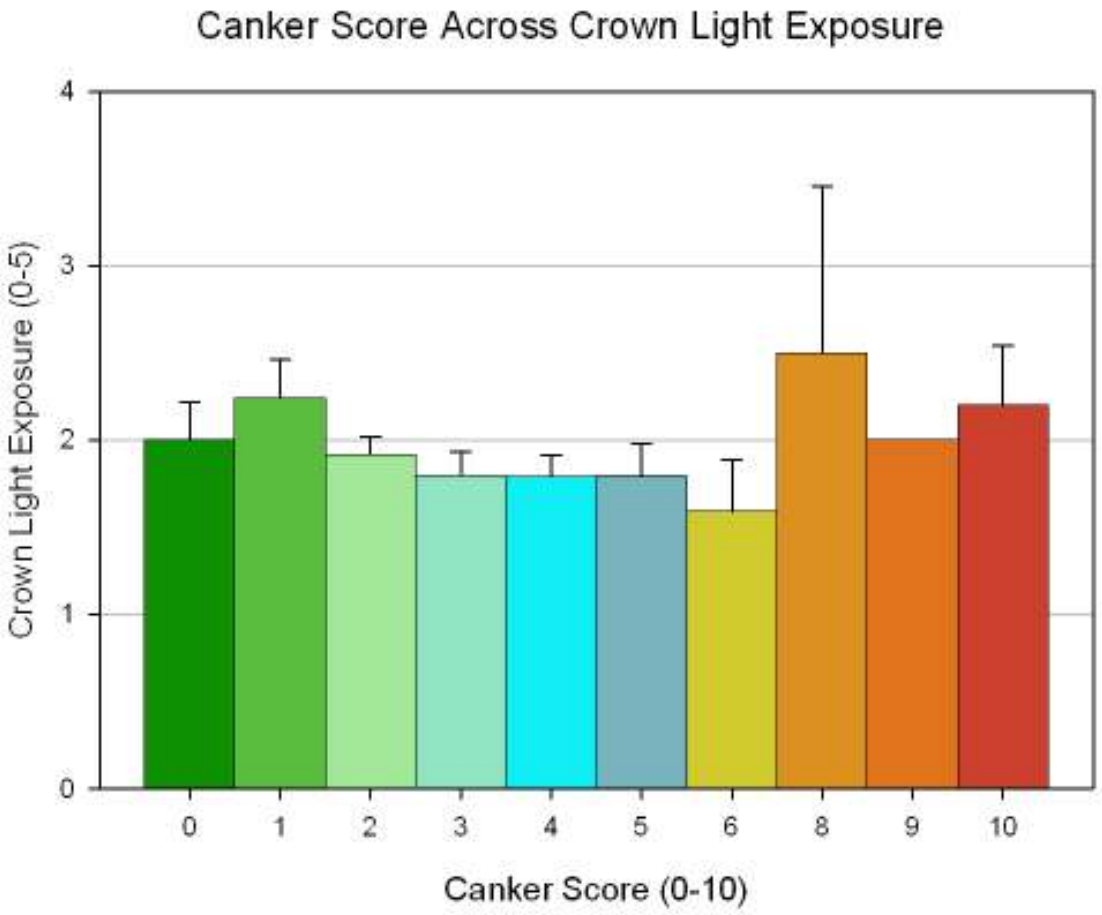

Figure VI-20. Average crown light exposure of butternut trees at each canker score.

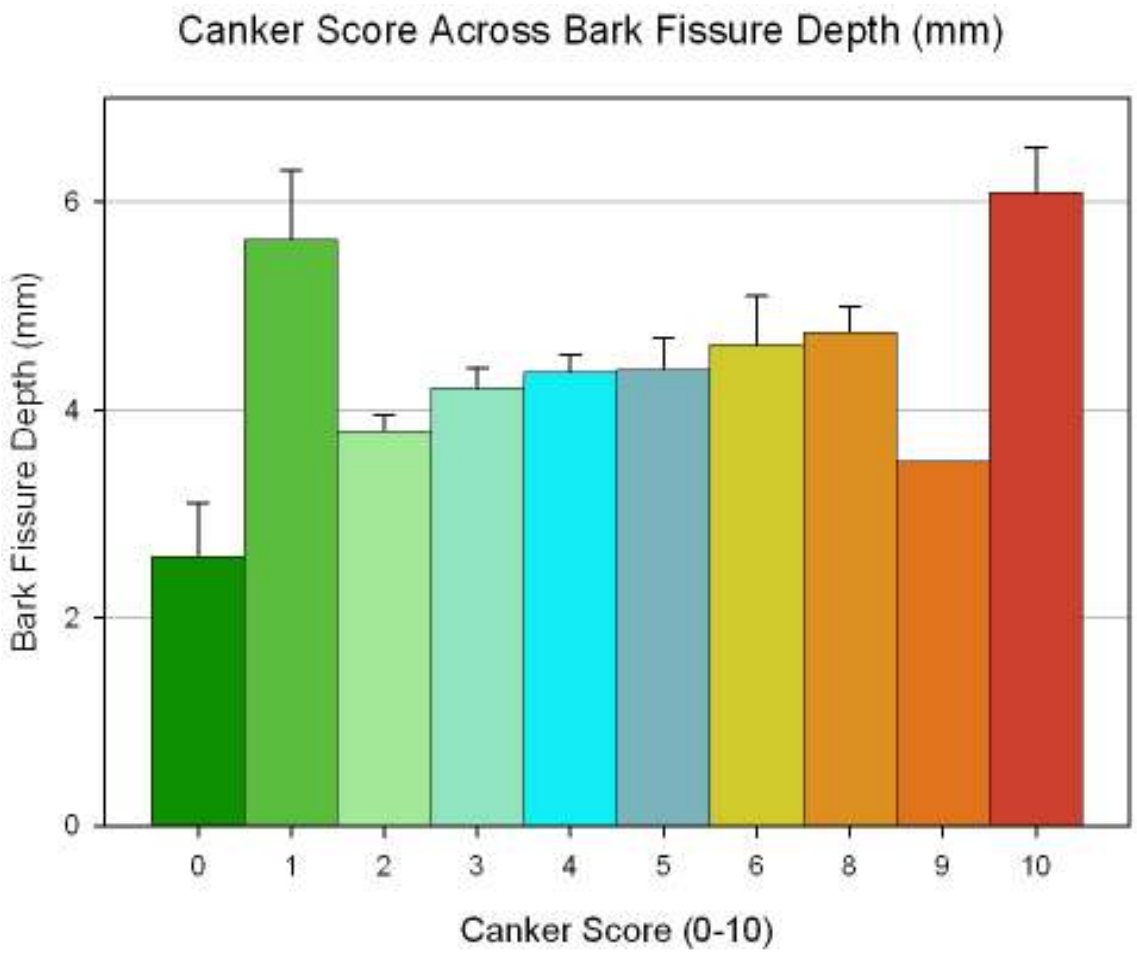

Figure VI-21. Average bark fissure depth of butternut trees at each canker score.

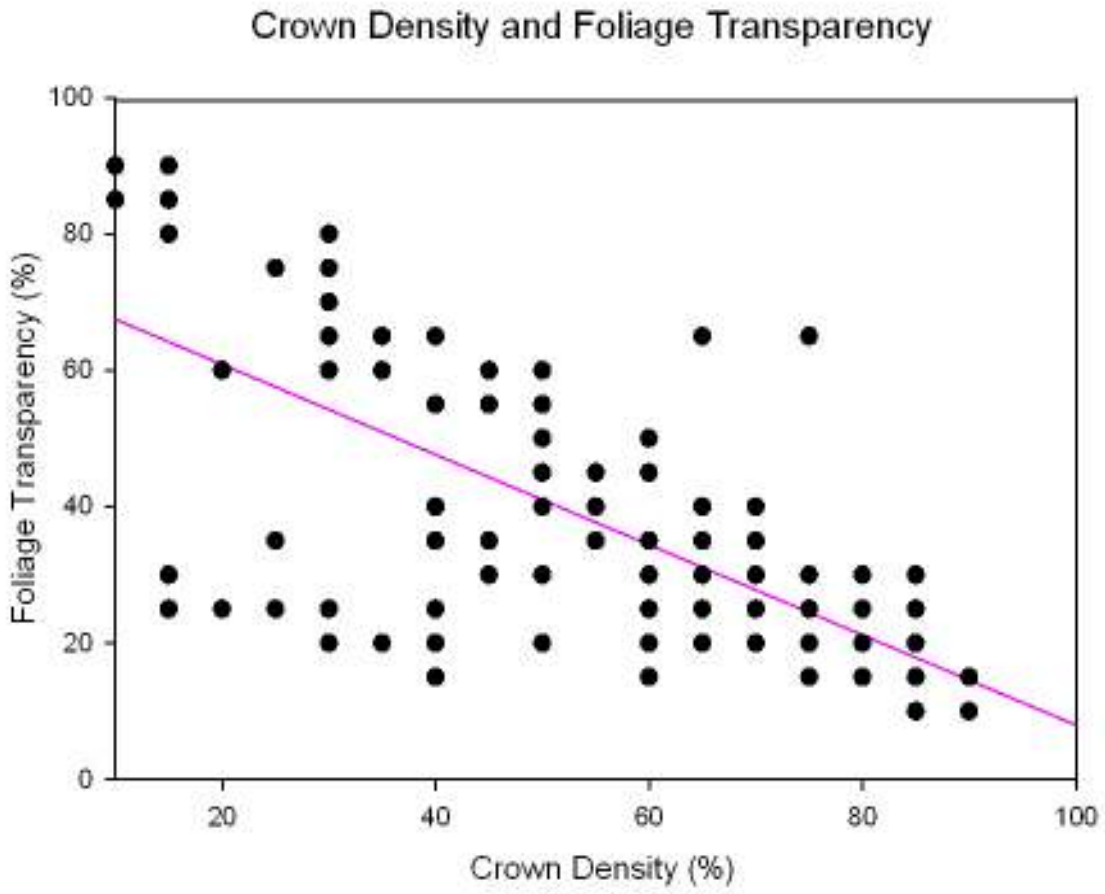

Figure VI-22. Relationship between crown density and foliage transparency.

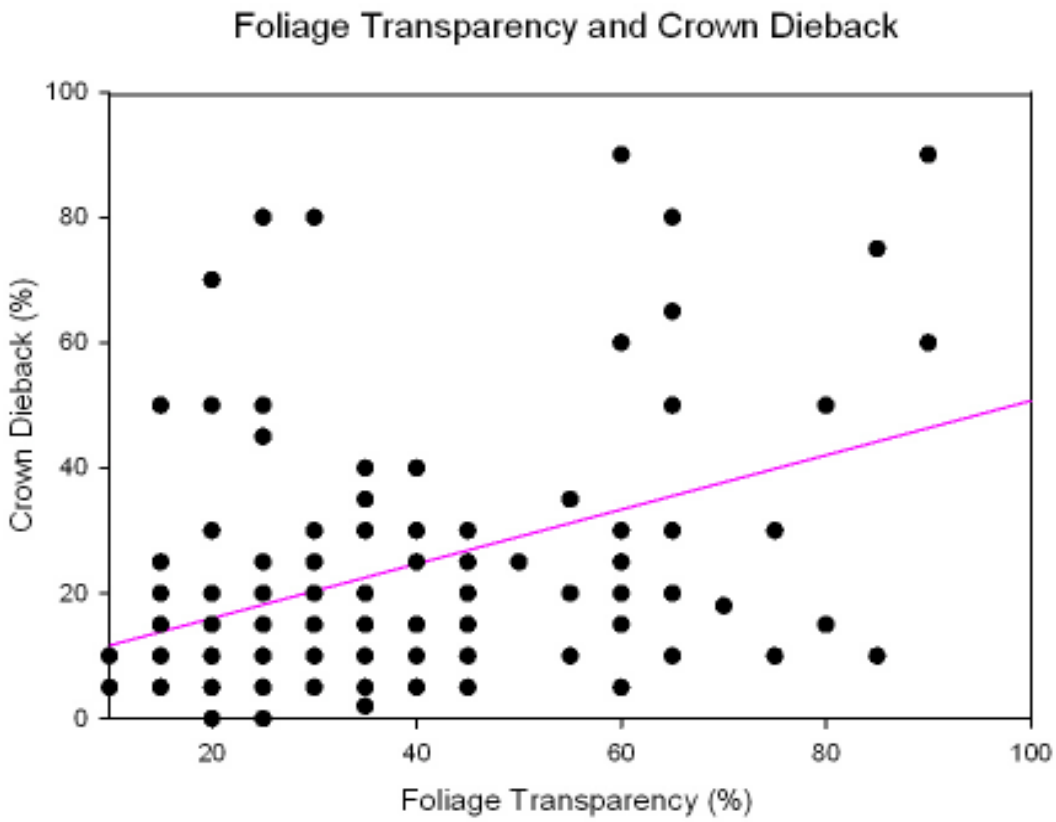

Figure VI-23. Relationship between crown dieback and foliage transparency.

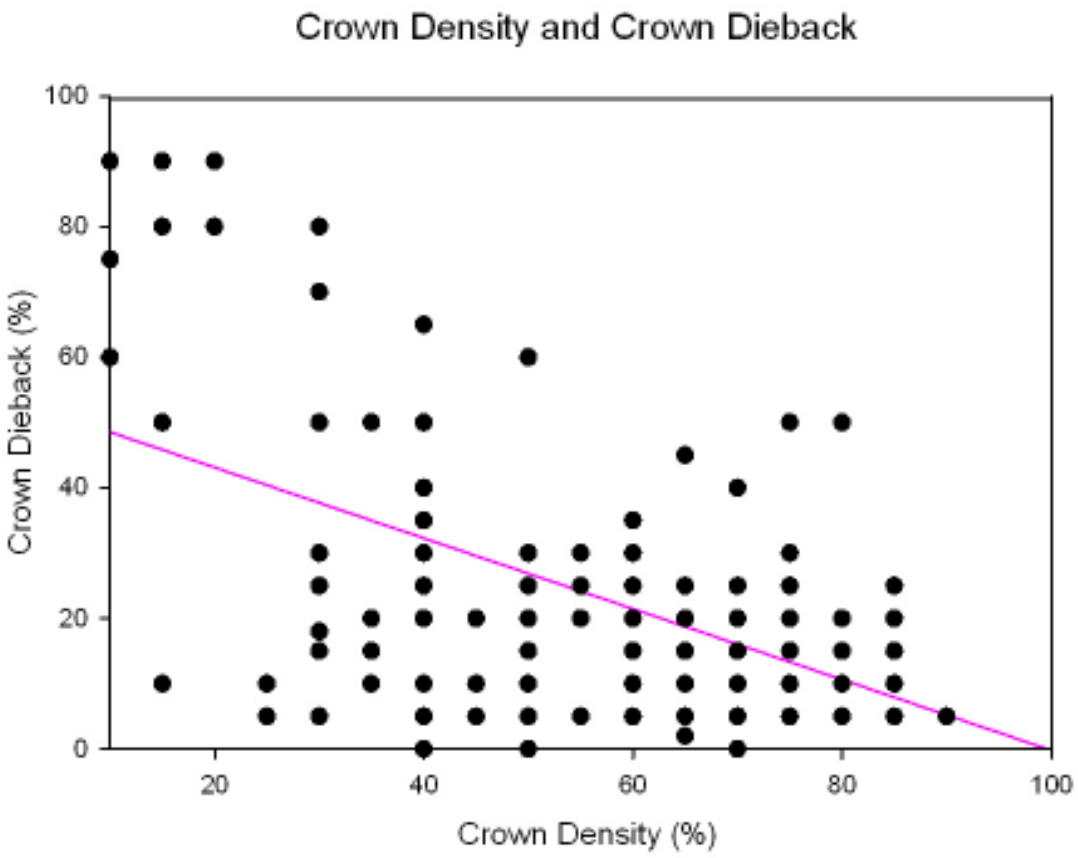

Figure VI-24. Relationship between crown dieback and crown density.

PART VII. SUMMARY OF CONCLUSIONS

Butternut has shown drastic declines due to butternut canker disease, causal fungus *Sirococcus clavigignenti-juglandacearum* (Nair, Kostichka, & Kuntz), throughout the native range. Previous investigations into disease resistance, artificial regeneration, and silvics of the species have been limited, although they are critical to eventual restoration efforts. Even if resistance to butternut canker disease can be developed in planting stock, there were still many unknowns around nursery growth, seedling characteristics, and establishment protocols. This research addressed these areas in butternut, though four different investigations.

These comprehensive studies result in an overview of the status of the current scientific understanding on butternut. Major results of these studies include an understanding of the genetic basis of disease resistance, heritability of seedling characteristics that relate to seedling establishment, and a greater understanding of factors involved in butternut mortality.

Seedlings from open-pollinated genetic families had drastically varying germination rates with low overall germination. Mutated leaves and albino seedlings indicated evidence of inbreeding depression and/or self-pollination from some families. Seedling size, including height (HT), root collar diameter (RCD) and number of first-order lateral roots (FOLR) were found to be heritable and indicated that significant gains could be made through keeping pedigree identity. Genetic correlations indicated that RCD should be considered as the primarly visual indicator of seedling quality, followed by height, and a two-way selection approach is most appropriate for improvement of seedling quality. These seedling variables impacted establishment success of seedlings in two types of plantings; resistance tests and restoration plantings. Gains in percent survival, height, and diameter growth were found four years after planting in resistance tests for seedlings with > 9 mm in RCD and with > 9 FOLRs. In restoration plantings, seedlings < 9 mm in diameter when planted were twice as likely to experience mortality and were 26% shorter than larger diameter seedlings. Seedlings <50 mm in height also experience additional mortality and were 36% shorter after

two years than larger seedlings. Selecting larger diameter and taller seedlings from specific genetic families will increase establishment success for butternut.

Multiple year nursery testing has shown the importance of placement of butternut within the nursery for production of plantable seedlings. The rapid growth of butternut seedlings requires placement further from irrigation risers and extensive pruning of lateral roots. Ideal butternut seedlings are large enough to have an increased competitive ability without the extraneous handling of extremely large seedlings. Ideal sizes of butternut seedlings are from 80-100 cm in height.

Investigations into survival and growth show successful establishment of butternut on a variety of different land management histories. Butternut is able to establish on 30 basal area retention harvests, converted grasslands, and surface mine sites. Increased survival was found in areas with increased light. However, training through companion planting with river cane or other species is necessary for the development of straight boles.

Resistance to butternut canker disease has a genetic basis and varied among open-pollinated progenies when seedlings were planted under infected trees. Seedlings from known heartnuts, (*J. ailantifolia* var. *cordiformis* (Maxim.) Rehd.), and hybrids, *J.* x *bixbyi* Rehd., did not develop cankers and are suitable controls for testing. As offspring from these parent trees were resistant they could be a source of resistance in butternut (*sensu lato*) populations. In addition, some butternut families did not develop cankers and had very high survival under heavy disease conditions. This technique is adequate in determining genetic resistance to butternut canker. However, high mortality of seedlings under these conditions was attributed to many factors aside from cankering. Ideal testing conditions would be in locations that are protected from flooding and with adequate sunlight. Since these conditions are limited in public lands, resistance test could be done in State Forest or State Park lands.

Additional factors other than butternut canker disease may have large impacts of butternut survival. An evaluation of a population of mature butternuts over a six-year time period in middle Tennessee resulted in only 8% mortality, even though a large percentage of the seedlings were diseased. This trend may contradict mortality reported in northern sections of the range where the impacts of ice and snow may result in additional damage to

cankered limbs. The mortality occurred primarily in suppressed trees during the stem exclusion stage of stand dynamics, indicating that butternut canker alone may not be the primary agent in mortality. A consistent canker classification system was o determine if this population was unique with a higher than average percentage of resistant trees. Comparing various southern populations found little variation in the number of potentially resistant trees in Tennessee, compared to populations in Arkansas and Missouri. However canker classification was related to phenotypic characteristics used to assess tree health. A disjunct population in Arkansas had a three-fold increase in trees with the highest disease rating than populations in Missouri and Tennessee. A wave of mortality that has already caused mortality on heavily disease trees at other locations may soon impact this population. Thoug the average disease ratings were very similar in Tennessee and Missouri, the Missouri population had over twice as many trees with the healthiest disease rating. Comparison of population conditions can help to assess which populations are in greatest threat of becoming extinct. Trees with smooth bark types have lower canker scores but also appear in higher crown positions with greater exposure to light. This phenotypic variable is related to tree health as well as degree of cankering, with increased health in the deeply furrowed bark type.

Classification systems for disease condition need to be expanded to include populations of butternuts throughout the range. This system will help to determine mortality rates and health condition and allow for determinations of which populations are in greatest need of conservation. Continued evaluation of populations over longer periods of time will allow for a greater understanding of disease dynamics across several gradients.

Research needs

Through research presented in this dissertation, there is now a great understanding of how to begin the restoration process with butternut. Research has resulting in knowing where butternut trees are and the development of effective habitat models to predict locations of finding new butternuts. Research has also developed genetic analysis to determine if individual trees are butternuts or hybrids. Studies show that pockets of hybrid trees and pure

stands of butternut exist. My research has shown that decreasing vigor, primarily due to shading, may increase chances of mortality and infection by butternut canker disease in both seedlings and large trees. Management for increased survival should take into account actions of increase vigor of the seedlings. In addition, nursery and seedling establishment protocols have been developed for butternut. My research has shown the types of sites which will result in increase establishment success. My research has also resulting in knowledge of a genetic basis of resistance to butternut canker disease and verified the resistance of hearnut and hybrid seedlings.

The next steps for restoration include incorporating resistant pure butternuts into orchards for restoration. Prioritizing restoration sites based on knowledge of butternut performance, and reestablishing butternut throughout the range with adequate material on ideal butternut sites.

PART VIII. APPENDICES

Appendix A: Butternut Field Data Sheet

TREE NUMBER_____ **DATE**_____

STUDY AREA_____

GENERAL LOCATION_____
X UTM_____ **Y UTM**_____ **GPS ERROR**_____

TREE MEASUREMENTS:

DBH (cm) _____ Crown diameter (m) [1]_____×_____Ave. crown diameter_____
Height (m) _____Height/1st live branch (m) _____
No. of nuts collected_____ Tree living/dead_____

FOREST HEALTH MONITORING CROWN CLASSIFICATION SYSTEM: [2]

% Crown dieback_____% Crown density_____% Foliage transparency_____
Crown position_____ Crown light exposure_____

CANKER CLASSIFICATION SYSTEM:

#/main stem cankers (>5 inches) in first 20 feet of bole_____#/20_____
#/in-hand branch cankers (>1 inch) _____length/branch material_____
Butternut rating[3]_____

BARK CHARACTERISTICS[4]_____

[4]Bark Characteristics: 1 = white flat bark, 2 = white bark deep furrow, 3 = gray flat bark, 4 = gray bark deep furrow or enter bark fissure depth measured with a tire gauge

COMMENTS_____

---

1) Measure the width of the crown in 2 (perpendicular) directions and average the 2 widths
2) Refer to FHM crown classification guidelines at the following website: www.na.fs.fed.us/spfo/fhm/
3) Butternut rating:

Branch Cankers per Foot									
	0.0	**0.1**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**	**0.9**
Stem Cankers per Foot									
0.0	0	1	2	3	3	4	5	6	8
0.1	1	2	3	4	4	4	5	6	8
0.2	2	2	3	4	4	5	6	7	9
0.3	2	3	3	4	5	5	6	7	9
0.4	3	4	4	4	5	6	6	8	9
0.5	4	4	5	5	5	6	7	8	9
0.6	4	5	5	6	6	6	7	8	9
0.7	5	5	6	6	7	7	7	8	9
0.8	6	6	7	7	8	8	8	8	9
0.9	8	8	9	9	9	9	9	9	9
1.0	10	10	10	10	10	10	10	10	10

Appendix B: Butternut Canker Classification System

Developed and used by: S. L. Brosi, L. M. Thompson, R. L. Anderson, S. L. Clark and S. E. Schlarbaum

Background

Butternut trees are under attack by an exotic fungal disease called butternut canker. The disease is caused by *Sirococcus clavigignenti-juglandacearum* and is killing butternut trees (*Juglans cinerea* L.) throughout its range in North America. The disease has killed up to 80 percent of the butternut in some states. The primary hope for control of this disease lies with host plant resistance through utilizing selections with genetic resistance. Healthy trees and infected trees that apparently have resistance have been found in severely affected forest stands in a number of States. Clonal and seedling propagation of trees exhibiting resistance is being used to evaluate breeding and future restoration efforts. Hybrids with heartnut ((*J. ailantifolia* var. *cordiformis* (Maxim.) Rehd.), a nut cultivar of Japanese walnut, are also being evaluated as alternatives for development of resistant nursery stock.

Surviving butternut trees need to be comparatively evaluated for disease based on the number of cankers. Initially, a system that classified butternuts in the southern United States was based on the following categories: no cankers, less than five cankers, and more than five cankers on the tree. This system was effective in selecting for trees in the field that appear to be resistant, but limited the comparison of trees in the field with results of progeny plantings and laboratory screening methods. The following methodology provides a quick, reliable method for assessing the canker status of an individual butternut tree according to its relative number of butternut cankers on the main trunk and branches.

Methods

This system evaluates the number of cankers on the bole or main stem of the tree and adjusts this number based on tree height. If possible a branch of the tree is also evaluated and then used in addition to the stem ranking to create a linear ranking for the tree (Figure 1).

Evaluation of the main stem:

1. Choose a tree that is alive and only evaluate the portion of the tree that is alive. The system is not reproducible using dead trees.

2. If possible, clear around the base of the tree to ensure good visibility of the main stem.

3. It is recommended that two people evaluate the tree and average their results. If there is a large variation, they should discuss the difference and agree on a rating.

4. Count the number of obvious cankers on the lower twenty feet of the main stem (Figures 2 and 3). These cankers should be at least 5 inches long. Be sure to look at the tree from three different angles to ensure all cankers are found. Different bole lengths pose no problem, since the number of cankers is divided by the bole length. Count only cankers five inches or greater in length, as counting every small canker on a stem resulted in large variations between observers.

5. Divide the number of cankers by the length of bole observed and place the number in the table at the end of this paper to get a ranking. The trees will rank from between 0 (no disease) to 10. This number is the ranking for the tree if a branch is not available for evaluation.

Using this system, the tree can be classified using the bole information only if branch counts are not available. If an in-hand branch can be obtained, evaluating branches for the presence of butternut canker will improve the overall ranking of the tree. This process, though not always possible, is important for trees that have no cankers on the main stem. Trees have been found that have heavily cankered branches, but do not have stem cankers. It is not recommended that the number of cankers on branches in the crown be evaluated using binoculars. Estimation of the number of cankers using binoculars and using a branch sample in hand showed little correlation. If possible, obtain an in-hand branch (es) from the tree for examination. Ideally, approximately ten linear feet of branch material should be used. The

branch (es) should have a proximal end between 1 and 3 inches in diameter and evaluated as follows.

Evaluation of a branch:

1. On the in-hand branch (es), count the number of obvious cankers at least one inch in length on all living shoots (Figures 4, 5, and 6). Add up the total length of the branch material, being sure to count all small shoots.

2. Divide the number of cankers by the length of branch material and place the number in the table to refine the estimate that has been made from the bole material.

3. The estimate for the tree can be refined by merging the two codes to give a clear picture of the tree status (Table 1). For example, if the trees had 0.7 cankers per linear foot of main stem and 0.5 cankers per linear foot of branch, it would be classified as a 7. This coding system allows the users, access to the actual data and gives them the flexibility to set up a different linear ranking system for their particular data set if needed.

Butternut rating using data from both the main stem and the branch can be used to create a linear ranking of the cankered trees with more weight given to the cankers on the main stem. This numeric code is uniform and can be used to compare a single population over a period of time, or compare multiple populations.

Appendix B: Tables and Figures

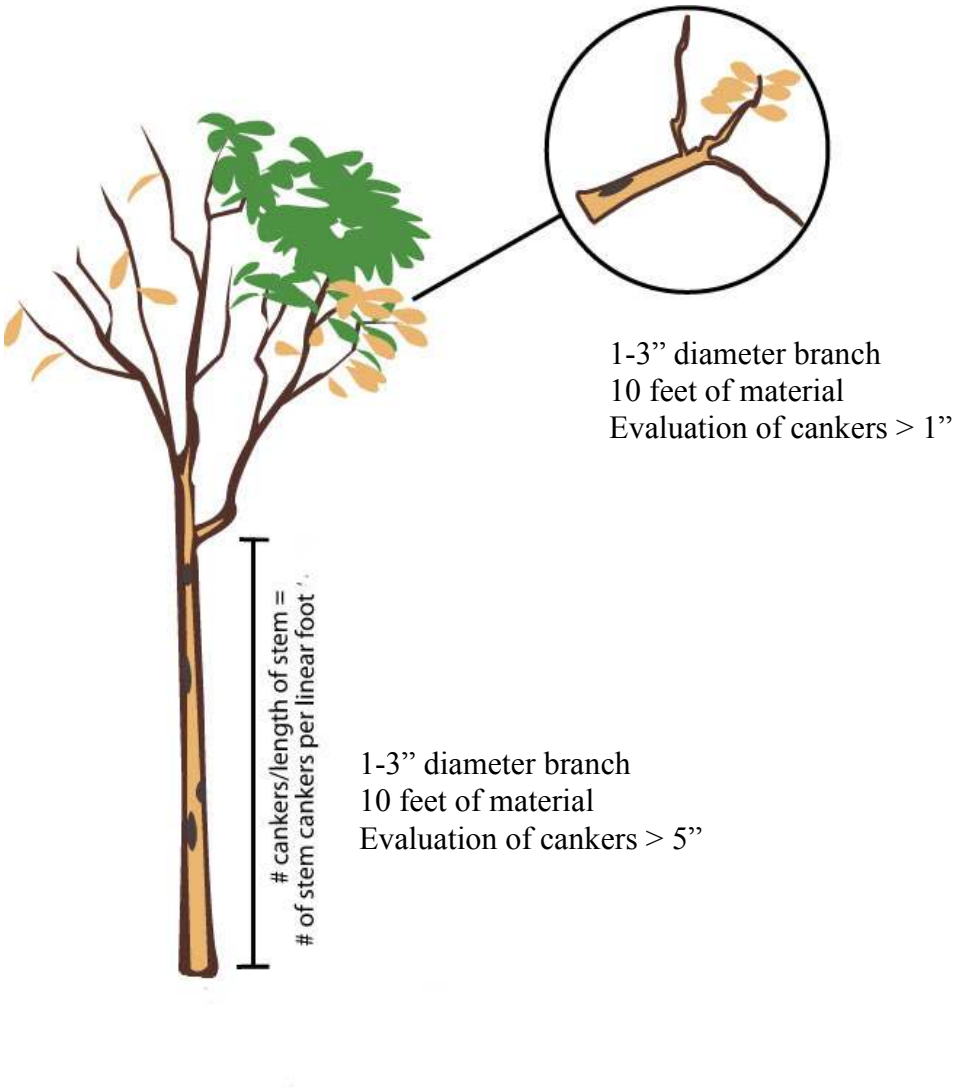

Figure 1: Schematic of material for evaluation under the numerical rating system for disease conditions on mature butternut trees.

Table 1: Numerical rating system for disease conditions on mature butternut trees with heavier weight given to main stem cankers.

 # branch cankers per linear foot

# main stem cankers per linear foot	**0**	**.1**	**.2**	**.3**	**.4**	**.5**	**.6**	**.7**	**.8**	**.9**	**1+**
0	0	1	1	2	3	3	4	5	6	8	10
.1	1	2	2	3	4	4	4	5	6	8	10
.2	2	2	3	3	4	4	5	6	7	9	10
.3	2	3	4	3	4	5	5	6	7	9	10
.4	3	4	4	4	4	5	6	6	8	9	10
.5	4	4	5	5	5	5	6	7	8	9	10
.6	4	5	5	5	6	6	6	7	8	8	10
.7	5	5	6	6	6	7	7	7	8	9	10
.8	6	6	7	7	7	8	8	8	8	9	10
.9	8	8	9	9	9	9	9	9	9	9	10
1+	10	10	10	10	10	10	10	10	10	10	10

Figure 2: Cankers on main stem

Figure 3: Multiple old main stem cankers

VITA

Sunshine Liberty Brosi was born on December 8, 1977 in Franklin County, Tennessee to George Ralph Brosi and Connie Carol Fearington Brosi. Sunshine is one of seven children and was raised in Berea, Kentucky. She graduated valedictorian a year early from Berea Community High School in May of 1995. In the fall of that same year, she began an undergraduate program at Warren Wilson College in Swannanoa, N.C. She received her Bachelor's of Arts degree in Environmental Studies with a concentration in Forest Resource Conservation in May 1999. The following fall she began her graduate work at the University of Kentucky in Lexington. Sunshine completed her Master's of Science degree in Forestry in May 2001 with a project on American chestnut restoration. In May 2002, Sunshine entered The University of Tennessee, Knoxville graduate program and began to work towards a Ph.D. in Natural Resources in the Department of Forestry, Wildlife and Fisheries. Sunshine received her Ph.D. in August 2010 under the direction of Scott E. Schlarbaum in the Tree Improvement Program on butternut and chestnut restoration.

In August 2007, Sunshine was hired as a tenure-track assistant professor in the Department of Biology at Frostburg State University in Frostburg, Maryland. Sunshine teaches and coordinates the undergraduate Bachelor's of Science program in Ethnobotany, one of only two such programs in the United States. Sunshine also teaches and advises graduate students in the Master's of Science program in Applied Ecology and Conservation Biology. Sunshine lives in rural Garrett County, MD with her daughter Summer Ela Shaper, stepson Caeman Thomas Feller, and husband Daniel Joseph Feller. Sunshine plans to continue her work on the conservation of tree species at risk due to exotic diseases as a means to help conserve threatened species for future generations.