Silvicultural Considerations for the Reintroduction of American Chestnut,

Castanea dentata, to the Forests of the Eastern United States

A Dissertation Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

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August 2011

DEDICATION

I dedicate this dissertation to my father, Peter Cooper Pinchot, who instilled in me a deep respect for, and interest in, the natural world. For this I am immeasurably grateful.

ACKNOWLEDGEMENTS

I thank Dr. Scott Schlarbaum, my advisor, for encouraging me to pursue a doctoral degree, offering me an exciting research opportunity, and helping me develop myself professionally. I thank my committee for their support and guidance throughout my doctoral experience: Dr. Jennifer Franklin for attention to detail and for her scrutiny of my interpretation; Dr. David Buckley for teaching me the importance of scale; Dr. Stacy Clark for helping me connect my results to application; Dr. Saxton for providing statistical guidance, and Dr. Sandra Anagnostakis for supplying chestnut seedlings for the Yale-Myers study, and more importantly, for introducing me to American chestnut restoration.

I cannot fully express my gratitude toward the Tree Improvement Program crew for spending endless hours with me in the woods (in varying degrees of discomfort). Ami Sharp and Tracy Powers, in particular, helped me with every aspect of project implementation and data collection. David Griffin offered help and a great attitude throughout my project. Numerous student technicians helped record and enter data.

Dr. Callie Schweitzer was a source of guidance throughout my project. Robert Sitzlar and Ricardo Suarez, of the Daniel Boone National Forest, offered technical and field assistance. Dr. Mark Ashton and Richard Campbell, of the Yale-Myers Forest, supplied the planting site and field help for the Yale chestnut planting.

I thank Dr. Fred Hebard for supplying seed for my Daniel Boone National Forest study. I am grateful for funding provided by the Tennessee Tree Improvement Program, the University of Tennessee, Department of Forestry, Wildlife, and Fisheries, and by the United States Forest Service.

I am incredibly grateful for my husband, Matthew Wilson, for leaving his job, friends, and family, to join me in Knoxville.

ABSTRACT

In anticipation of widespread planting of putatively blight-resistant hybrid chestnuts (*Castanea* spp.), it is critical to understand the silvics and competitive ability of the species. This dissertation examines preliminary growth and survival of several species and genetic crosses of chestnut grown as 1-0 high-quality nursery seedlings and planted in two study sites: Southeastern Kentucky (Daniel Boone National Forest; chapter 2), and Northeastern Connecticut (Yale-Myers Forest; chapter 4). The effects of three silvicultural treatments on the Daniel Boone National Forest (DBNF), and four silvicultural treatments on the Yale-Myers Forest (YM) were evaluated. Furthermore, the effect of initial seedling size on seedling performance was tested. In both studies, seedlings grew largest in height and root collar diameter in silvicultural treatments with the most available light. High mortality at the DBNF planting was caused by *Phytophthora cinnamomi*, ink disease, and high mortality at the YM planting was caused by repeated deer, *Odocoileus virginianus*, browsing and competition from hay-scented fern, *Dennstaedtia punctilobula*. Additionally, seedlings at the DBNF study were repeatedly defoliated by *Craesus castaneae,* chestnut sawfly, during the first growing season (chapter 3). Seedlings with larger root collar diameters at planting demonstrated greater survival and growth in the DBNF study while seedlings larger in height and RCD added less growth and were more likely to die in the YM study, likely due to the high deer population and dense understory competition. These results indicate that silvicultural treatments that create high-light environments are ideal for chestnut, however the species can establish successfully under a variety of light conditions, from low light [10% photosynthetically active radiation (PAR)] to high light (65% PAR). Ink disease

will present a formidable obstacle to chestnut reintroduction efforts in the south, while northern efforts will be challenged by deer browsing due to overpopulated deer herds.

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Chapter 1: Introduction and Literature Review

Introduction

The American chestnut [*Castanea dentata* (Marsh.) Borkh] was a dominant forest tree in the eastern forests of the United States until the non-native chestnut blight fungus [*Cryphonectria parasitica* (Murr.) Barr] eliminated it as a dominant canopy tree species in the early 20th century. The tree was once ecologically important as a source of mast for wildlife (Diamond, et al. 2000; Minser, et al. 1995; Paillet 2005; Schlarbaum 1990), and valuable for its rot-resistant lumber, high-tannin content, and edible nuts (Ashe 1911; Brooks 1937; Emerson 1846; Frothingham 1924).

The chestnut blight fungus was discovered at the New York Botanical Garden in 1904 (Murrill 1906). By the 1930s, chestnut blight had spread throughout the tree's range (Brooks 1937. In response to the blight, G.F. Gravatt and R.B. Clapper of the USDA Office of Forest Pathology initiated a breeding program in 1922, primarily using American, Chinese (*Castanea mollissima* Blume.), and Japanese (*Castanea crenata* Siebold & Zucc.) chestnuts with a goal of producing a blight-resistant chestnut tree with good timber form (*cf.* Burnham, et al. 1986). Shortly thereafter, Dr. Arthur Graves, of the Brooklyn Botanical Garden, began a separate breeding program (Graves 1941; 1946; 1947), which became a cooperative effort with the Connecticut Agricultural Experiment Station (CAES) (Anagnostakis 2005; Burnham, et al. 1986). Neither program was ultimately successful in producing a blight-resistant chestnut with timber-type form, primarily because both backcrossed the initial filial generation with an Asian chestnut parent, never regaining the desired timber form characteristic of American chestnuts (Burnham 1988).

Currently, the American Chestnut Foundation (TACF) and CAES utilize a backcross breeding approach in attempt to develop a highly blight-resistant American chestnut selection (Anagnostakis 2005; Hebard 2001). Their shared approach incorporates an initial cross between a Chinese or Japanese chestnut and an American chestnut (F_1), followed by a series of backcrosses to American chestnuts (BC_1F_1, $BC_2F_{1,}$ and BC_3F_1) and an intercrossing generation (BC_3F_2). Each generation is screened for resistance and timber form. The final cross in the breeding scheme is among third generation backcross trees (BC_3F_2) to reach the BC_3F_3 generation. In theory, this final generation will have the growth habit of American chestnut and blight resistance of an Asian chestnut species (Anagnostakis 2005; Burnham 1988; Hebard 2001).

In anticipation of widespread planting of blight resistant hybrid chestnuts, it is critical to understand the silvics and competitive ability of the species for successful reintroduction to eastern North American forests. Forestry publications written while American chestnut was still a canopy dominant describe the species' rapid growth and prolific sprouting (Ashe 1911; Emerson 1846; Hough 1878; Mattoon 1909; Zon 1904); however they lack in-depth analysis of chestnut silvics. Although American chestnut has been planted since colonial days (Emerson 1846; Hough 1882; Neilson 1902), there were few experimental studies on chestnut silviculture and none on artificial regeneration of the species. Silvicultural studies that have been developed (Anagnostakis 2007; Clark, et al. 2011; Jacobs and Severeid 2004; McCament and McCarthy 2005; McNab 2003; Rhoades, et al. 2009) report varied results. In addition, no studies have evaluated artificial regeneration of blight-resistant backcross hybrid chestnut seedlings. Finally, there has been scant research to evaluate the effects of nursery practices, seedling size and genetics on chestnut establishment.

Research Objectives

This dissertation examines silvicultural strategies for, and the importance of initial seedling size to, the establishment of chestnut reintroduction plantings. Specific objectives include:

1. Evaluate early growth and survival of, and importance of initial seedling size to, American, BC_2F_3 hybrid, and Chinese chestnut seedlings among three silvicultural treatments; oak shelterwood, thinning, and shelterwood with reserves; on the Daniel Boone National Forest.
2. Evaluate the incidence of chestnut sawfly on chestnuts planted on the Daniel Boone National Forest.
3. Evaluate early growth and survival of, and importance of initial seedling size to, BC_3F_2 x BC_2F_3 hybrid chestnut seedlings among four silvicultural treatments; shelterwood, group selection, thinning, and control; on the Yale-Myers Forest in Northeastern Connecticut.

Literature Review

The American chestnut, a dominant species throughout much of its range, was hailed as one of the most important forest tree species in the eastern United States (Ashe 1911; Hawley and Hawes 1912; Mattoon 1909). Its rot resistant, straight-grained timber was used for railroad ties, telegraph and telephone poles, fence posts, musical instruments, furniture and shingles (Brooks 1937; Hawes 1906; Zon 1904). Tannins from its wood and bark were extracted for use by the leather tanning industry (Ashe 1911; Emerson 1846). Ashe (1911) estimated that annual revenues from chestnut timber in Tennessee exceeded $1,640,000 (present-day $37,888,323).

American chestnut produced copious amounts of nuts each year, which provided income, food, and livestock forage for many rural communities in the southern Appalachians (Brooks 1937; Emerson 1846; Lutts 2004). In addition to its numerous utilitarian values, chestnut was an ecologically important tree species. As a fast-growing tree, chestnut could achieve dominant canopy positions following disturbance in certain stands, and comprised upwards of 50% of eastern hardwood stands at the end of last century (Hawes 1906; Russell 1987). Many animals, including black bear (*Ursus americanus* L.), ruffed grouse (*Bonasa umbellus* L.), wild turkey (*Meleagris gallopavo* L.), white-tailed deer (*Odocoileus virginianus* Boddaert), among others, depended on its abundant and reliable nut crops (Diamond, et al. 2000; Paillet 2002).

Pre-blight Distribution

American chestnut historically grew from central Alabama north to Maine, westward to Tennessee, Ohio and southern Ontario (Hough 1878; Russell 1987; Figure 1-1). Disjunct populations were found as far south as northern Florida (Little 1977). Chestnut was considered a 'ubiquitous' species, for its ability to grow in most sites types, with the exception of very wet, clayey, or alkaline soils (Frothingham 1924; Russell 1987). Chestnut grew most abundantly on intermediate slopes, upland forests, and non-calcareous, deep, well-drained sandy soils (Ashe 1911; Frothingham 1924; Stephenson, et al. 1991). While chestnut was most abundant on slopes and upland forests, it attained its largest size in coves (Ashe 1911; Frothingham 1924; Figure 1-2). Fierce competition from other fast-growing species, such as yellow poplar (*Liriodendron tulipifera* L.) and black locust (*Robinia pseudoacacia* L.), however, prevented chestnut from becoming abundantly established in coves (Frothingham 1924). Chestnut tolerated a wide-range of elevations, from sea level in Connecticut and Massachusetts, to 1700 meters along the Smoky Mountains in North Carolina and Tennessee (Ashe 1911). Its frequency at the end of the 19th century had increased due to past land uses, specifically heavy cutting and forest fires of the 18th and 19th centuries, which favored chestnut establishment (Moss 1973; Paillet 2005; Russell 1987).

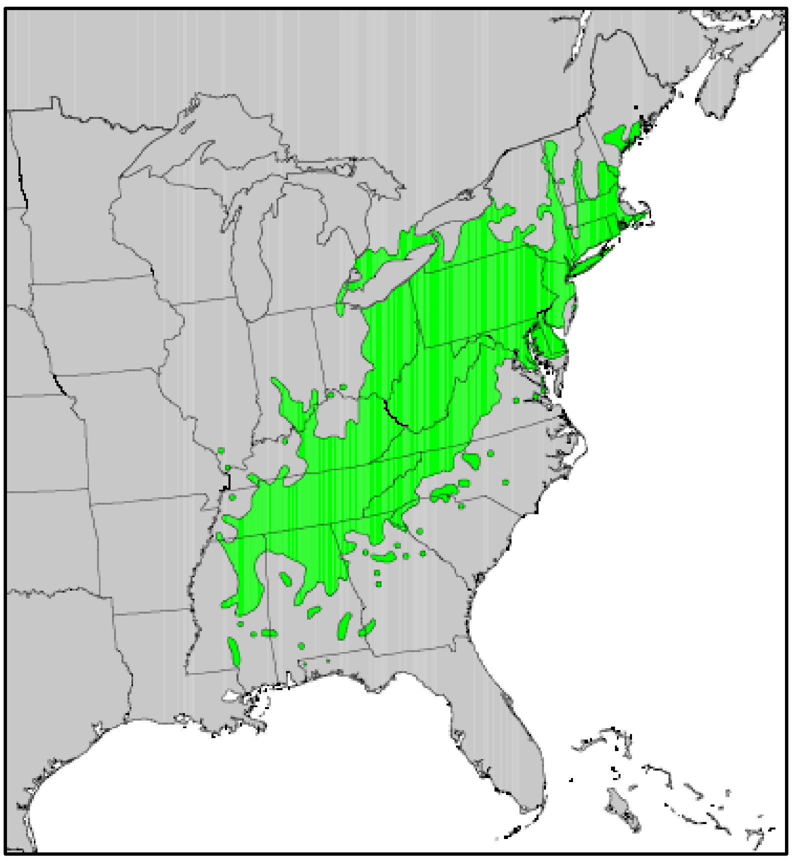

Figure 1-1: Historic range of American chestnut (USGS 1999).

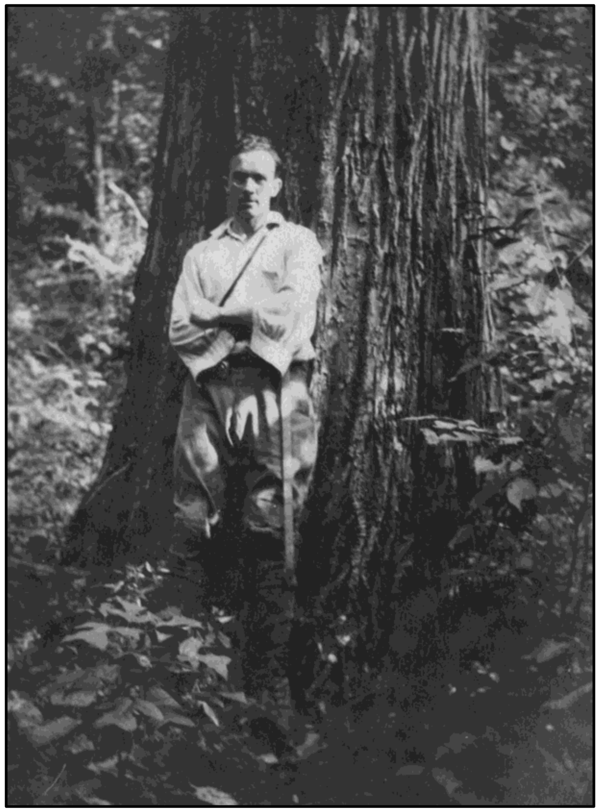

Figure 1-2: "Johnnie Keyes and a N.C. cove chestnut", 1912. Taken by Jonathan Keith Esser. Courtesy of the Forest History Society.

Sexual Reproduction

Chestnut produced annual seeds crops with heavy mast production every few years (Ashe 1911; Emerson 1846). Years of low production were rare because chestnut flowers in the summer, after threat of late spring frost has passed (Paillet 2005). Heavy predation by wildlife, and by humans after European colonization, limited reproduction by seed (Hawley and Hawes 1925; Toumey and Korstian 1931), although wildlife activities were primary pathways for broad distribution of the species (Thoreau 1996). Some authors reported that chestnut seedlings could survive for several years to several decades in shaded conditions (Ashe 1911; Hough 1878), while Hawley and Hawes (1925) wrote that chestnut could not survive long without direct sunlight. Russell (1987) hypothesized that chestnut seedlings did not grow well under heavy evergreen shade, but did establish under the lighter shade of deciduous shrubs. While seed predation drastically reduced the number of seedlings, the longevity of a few escaped seedlings, with the ability to respond quickly to release, allowed American chestnut to persist as an important canopy dominant throughout its range (Paillet 2005).

Competitive Ability and Tolerance

Chestnut's ability to sprout vigorously when injured, cut, or released, compensated for its low rate of seed regeneration (Hawley and Hawes 1925; Hough 1882; Paillet 1982; Mattoon 1909; Russell 1987; Zon 1904). Hawley and Hawes (1912) reported chestnut was one of the most rapidly growing trees in New England, and that sprouts often grew 1.5 - 2.5 m in the first year. Mattoon (1909) found that chestnut sprouts outgrew red maple (*Acer rubrum* L.), ash (*Fraxinus* spp. L), red oak (*Quercus rubra* L.), and chestnut oak (*Quercus prinus* L.) sprouts. Its prolific and rapid sprouting enables chestnut to form dense stands after clearcutting (Boring, et al. 1981). Several recent studies have shown that American chestnut seedling growth increases with increasing levels of irradiance (Joesting, et al. 2009; McCament and McCarthy 2005; Rhoades, et al. 2009; Wang, et al. 2006), although initial establishment may be favored by partial shade (Anagnostakis 2007). McCament and McCarthy (2005) planted American chestnuts in thinned and un-thinned plots and found that chestnut growth was greatest in the thinned sites.

Furthermore, they determined that light was more strongly related to increases in chestnut biomass than were soil nutrients, organic matter, soil moisture, or soil texture. Jacobs and Severeid (2004) evaluated the growth of American chestnut inter-planted with black walnut (*Juglans nigra* L.) and northern red oak in a plantation setting in Wisconsin. They found that American chestnut exhibited greater growth in height and diameter than either walnut or red oak, with chestnut adding an average of 0.83 cm/year in diameter growth and 0.84 m/year in height growth. In a study that examined the effects of several nutrient and light levels on six co-occurring hardwood species planted in containers, Latham (1992) found that American chestnut surpassed all other species, including yellow poplar and northern red oak, in height growth in nearly all light and nutrient treatments. The growth of American chestnut was less than that of other hardwoods only at very low light levels.

While chestnut displays optimal growth in high light conditions, the species also demonstrates an ability to live for prolonged periods in shade (Ashe 1911; Paillet 1982, 1984; Zon 1904). Emerson (1846) reported that in mature forests, chestnut grew slowly during the first 10 – 15 years, after which its growth increased significantly. Ashe (1911) reported that chestnut seedlings could establish and live for decades in low-light conditions of the understory and quickly grow into canopy gaps. In a 1982 study, Paillet examined core samples from American chestnut sprouts growing in Connecticut. He found that many sprouts exhibited 10 – 30 years of slow growth, followed by a rapid increase in growth, corresponding to natural release events. In another study, Paillet (1988) found that increased irradiance accompanied by release triggered new growth from the uppermost part of existing chestnut stems, causing a laterally growing suppressed sprout to grow into an apically oriented sapling. Paillet (1982) hypothesizes that this "sit-and-wait" strategy allowed chestnut to effectively compete with shrubs on poor sites, where chestnut was historically most abundant.

Several recent studies have examined the shade tolerance of American chestnut in an attempt to understand the physiological and morphological characteristics responsible for the species' "sit-and-wait" strategy (Joesting, et al. 2009; King 2003; McCament and McCarthy 2005; Wang, et al. 2006). These studies found that chestnut demonstrates high photosynthetic rates (Joesting, et al. 2009; Wang, et al. 2006), low respiration rates, and high specific leaf area (Joesting, et al. 2009; King 2003) when grown in the shade. These traits allow chestnut to survive in low light levels for long periods of time by maximizing carbon gain per unit leaf biomass. Based on these results, Joesting, et al. (2009) concluded that American chestnut is intermediate in shade-tolerance, while Wang, et al. (2006) characterized chestnut as a shade-tolerant species.

Pre-Blight Management

Many early silvicultural references, written during the development of forestry in the U.S., prescribed a coppice system for chestnut management, due to its ability to sprout rapidly and prolifically (Ashe 1911; Hawes 1906; Hawley and Hawes 1912; Hough 1882; Mattoon 1909; Zon 1904). Frequent light thinnings were encouraged to accelerate growth of the residual stand (Ashe 1911; Hough 1882). Even-aged stands were preferred to avoid irregular stems created by periods of suppression in mixed-age stands (Ashe 1911). Hawley and Hawes (1912) recommended a rotation of 80 years for mixed hardwoods stands that included American chestnut, and mentioned that the sprouting ability of chestnut and occasional seed regeneration was adequate for chestnut regeneration, therefore leaving seed trees was not necessary.

Relatively few pre-blight records of planting chestnuts exist, likely because chestnut regenerated so prolifically from sprouts. Emerson (1846) reported that chestnuts were successfully cultivated from seed, while Hough (1878) wrote that chestnut seedlings did not transplant well. Neilson (1902) described a chestnut planting established in 1822 on a Maryland farm that had previously been clearcut. Four-thousand chestnut seed were planted at a spacing of 20 by 6 feet (6.0 by 1.8 meters). Throughout the next eighty years, the chestnuts were cut occasionally, for rails and other uses, and by 1902, the largest trees were 35 inches (91 cm) DBH and were limb-free for 60 – 80 feet (18 – 24 meters).

Hough (1878) described a mixed-species planting that included American chestnut, established on prairie land in Elgin, Illinois in 1858. All but one of the chestnut seedlings had died by 1878. The lone survivor was 20 feet (6 m) tall and 6 inches (15 cm) in diameter at the time of the report. Hough concluded that prairie land was unsuitable for chestnut establishment.

Nonnative Pathogens and Pests of American Chestnut

While once a dominant and common tree throughout its range, American chestnut has suffered great reductions in numbers due primarily to chestnut blight fungus, as well as several other pathogens and pests. Below is a summary of these organisms and their effects on American chestnut.

Reports of unusual chestnut mortality in the southern United States began to appear in the early 1800s (*cf.* Anagnostakis 2006; Hough 1878; 1882). At the time, the causal agent was unknown, however, later studies have shown that chestnuts in bottomland or poorly drained sites were killed by ink disease, *Phytophthora cinnamomi* Rands (Crandall et al.1945), an exotic soil-borne oomycete that attacks and kills the root systems of American chestnut and the related Allegheny [*Castanea pumilla* (L.) Mill var *pumilla*) and Ozark chinquapins [*Castanea pumilla* var. *ozarkensis* (Ashe) Tucker]. Ink disease was most likely transported to the southern United States before 1824 in soil from Europe, and caused significant loss of chestnut in mesic sites in Gulf and Atlantic states (Anagnostakis 2006). Current attempts are underway to breed chestnuts that are resistant to both blight fungus and ink disease (Anagnostakis 2005).

The chestnut blight fungus, *C. parasitica*, formerly *Endothia parasitica* Murrill, was first discovered in 1904 at the New York Botanical Garden (Murrill 1906), although it was probably imported into the United States on Japanese chestnut nursery stock in the late 1800s (Anagnostakis 2008; 2009; Burnham 1988). Within 50 years of discovery, blight had killed about 3.6 million hectares of American chestnuts (Anagnostakis 2008). Most large chestnut trees throughout the species' range were dead or dying by 1950 (Burnham 1988). Occasional large survivors are located throughout chestnut's range; however most eventually succumb to blight.

Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu*)* is an exotic insect that infects all *Castanea species* (Anagnostakis 1997). The wasp lays eggs in the leaf and flower buds, where a gall subsequently develops (Itô 1967). Galls prevent normal development of shoots and flowers, thereby reducing growth and eliminating nut production. Severe cases of infection can kill small trees (Cooper and Rieske-Kinney 2007). The wasp is native to China and was introduced to Georgia in 1974 (Payne 1978). The gall wasp had spread to North Carolina by the early 1990s (Anagnostakis, et al. 2009). As of 2007, the gall wasp has been reported as far north as Maryland and Pennsylvania (Cooper and Rieske-Kinney 2007; Payne 1978).

Two nonnative ambrosia beetles (*Xylosandrus crassiusulus* Mot. and *X. saxeseni* Blandford) bore into chestnut trees, creating tunnels through which a symbiotic fungus grows, providing food for the insects (Oliver and Mannion 2001). Other fungi and bacteria can enter through these galleries and cause harm to the host tree (Oliver and Mannion 2001). These insects have been noted to cause severe mortality in open-field chestnut plantations, and damage to chestnuts planted in forest settings (Schlarbaum, personal communication, 2011).

Breeding Chestnut for Blight Resistance

Van Fleet was the first scientist to cross American chestnut with the moderately resistant European (*Castanea sativa*), and highly resistant Chinese (*C. mollissima*) and Japanese (*c.* crenata) chestnut trees. However the progeny from his F_1 generation crosses, made during the turn of the 19th century, were killed by blight (Van Fleet 1914). The Office of Forest Pathology, USDA Bureau of Plant Industry initiated a program to breed a resistant chestnut with timber form in 1922. Asiatic and European chestnut species were bred with American chestnuts to improve timber form of the tree. In 1929 Dr. Arthur Graves established a similar breeding program in Connecticut, which became a cooperative effort with the Connecticut Agricultural Experiment Station in 1936 (CAES) (Anagnostakis 1987; Burnham, et al. 1986). These two programs, however, ultimately failed to produce sufficient numbers of backcrosses (BC_1F_1) with an American chestnut parent, thereby failing to regain American characteristics (Burnham 1988). The majority of the F_1 crosses between American and either Chinese or Japanese chestnuts were crossed back to the Asian parent to increase resistance, failing to produce a tree with the desired timber form (Burnham, et al. 1986).

In the early 1980s Dr. Charles Burnham, a retired maize cytogeneticist at the University of Minnesota, and colleagues suggested using a backcross breeding scheme, a common agronomic breeding approach, for chestnut restoration (Burnham, et al. 1986). In this method, based on the assumption that resistance was expressed by two or three genes, the susceptible American chestnut is crossed with a resistant Chinese or Japanese chestnut. Instead of crossing the progeny back with an Asian parent to regain resistance, the F_1 generation trees are crossed with

American chestnuts, producing a BC_1F_1 generation, to increase the desirable American characteristics of growth and form, retain or partially retain genes for resistance, and decrease undesirable Chinese chestnut characteristics. Following two additional backcross generations, two intercrossed generations are made (BC_3F_2 and BC_3F_3) to consolidate resistance. Screening for resistance is conducted in each breeding generation. Theoretically, the final product of the breeding program is progeny that are approximately $15/16^{th}$ American chestnut and are highly resistant to the chestnut blight fungus (Burnham 1988).

Currently The American Chestnut Foundation (TACF) and CAES each utilize the backcross breeding program in efforts to develop blight-resistant American chestnut trees. CAES's program utilizes resistance from Chinese and Japanese chestnuts and will produce the final generation of chestnut trees, putatively resistant to both chestnut blight and ink disease within the decade (Anagnostakis, personal communication, 2011). Another research goal of CAES's chestnut restoration program is to examine the effectiveness of hypovirulence, a virus of the blight pathogen, in reducing the impact of chestnut blight (Anagnostakis 2001). Hypovirulence has not yet exhibited large-scale effectiveness; largely due to slow spread of the infected virus from tree to tree (Double and MacDonald 2002).

The American Chestnut Foundation uses resistance only from Chinese chestnuts in their breeding program. Putatively resistant chestnut seed from the BC_3F_3 generation was collected in 2006 (Diskin, et al. 2007), and the first experimental forest plantings were established in 2009 (Clark, et al. 2011).

Dr. Eyvind Thor, professor of forestry at the University of Tennessee, initiated a breeding program to increase American resistance by intercrossing American chestnuts possessing low levels of resistance (Samman and Thor 1975). This program eventually was abandoned due to lack of survival of progeny (Schlarbaum, personal communication, 2009). The American Chestnut Cooperator's Foundation (ACCF), a non-profit organization, headed by Dr. Gary Griffin of Virginia Tech University and Lucille Griffin, currently employs a similar approach in breeding pure American chestnut with low levels of blight resistance. Survival of ACCF trees is enhanced with the aid of hypovirulent strains of the blight fungus that debilitate the more virulent fungal strains (Griffin, et al. 1983; Griffin 2000). Griffin has found this strategy to be effective at controlling blight on trees planted on low-altitude mesic sites, but suggests increased resistance is necessary for larger scale restoration (Griffin 2000; Robbins and Griffin 1999).

Molecular Approaches to Blight-Resistance: transgenes and genomics

A gene encoding oxalate oxidase (OXO), an antifungal enzyme, was recently introduced into American chestnut via *Agrobacterium*-mediated transformation of embryogenic cultures. OXO degrades oxalic acid produced by fungal pathogens, and produces hydrogen peroxide, which acts as a fungicide (c*f.* Wheeler and Sederoff 2009). OXO has been found to confer resistance to the pathogen *Septoria musiva* when engineered into poplar hybrids (*Populus* x *euroamericana*) (Liang, et al. 2001). Because chestnut blight fungus produces oxalic acid, which kills chestnut tissue, the overexpression of the OXO gene in transgenic American chestnut may confer blight-resistance (*cf*. Merkle, et al. 2007).

Research is underway to sequence American and Chinese chestnut genes (Margulies, et al. 2005) and develop molecular markers indicating blight-resistance, among other traits, using the sequence data (Wheeler and Sederoff 2009). Researchers utilizing the genomics approach are working to obtain a high-resolution genetic and physical map of Chinese chestnut in effort to detect blight-resistance genes. The detection of resistance genes would augment both traditional breeding and transgenic approaches to blight resistance in American chestnut.

Current Distribution

Because chestnut blight does not generally infect the root system of American chestnut trees (Hepting 1974), due to deleterious effects of an unidentified soil organism(s) (Weidlich 1978), the American chestnut is still a common component of the understory throughout much of its native range, existing as stump or seedling sprouts from root systems of trees and seedlings killed by chestnut blight (Griffin 2000; Paillet 1982; Russell 1987). Boring (1981) found that chestnut was among the most dominant woody species in recently clear-cut forests in the southern Appalachians. Sprouts occasionally escape blight long enough to develop into sexually mature trees.

Reintroduction

In anticipation of the release of blight-resistant American chestnut trees, several studies have examined methods to successfully reintroduce American chestnut to the forest (Clark, et al. 2011; McCament and McCarthy 2005; McNab 2003; Rhoades, et al. 2009). In a test planting of American chestnut established on the Daniel Boone National Forest (Rhoades, et al. 2009), seedling growth was greatest in shelterwood treatments, as compared to seedlings planted in midstory removal treatments. McCament and McCarthy (2005) similarly found that American chestnut added the most growth in silvicultural treatments with the greatest available light. McNab (2003), however, found that silvicultural treatment did not affect chestnut height growth after five years.

Only two American chestnut forest studies, Rhoades, et al. (2009) and Clark, et al. (2009), have examined the effect of initial seedling size on growth. Rhoades, et al. (2009) found that over a two-year interval, chestnut seedling growth in shelterwood treatment sites was positively related to initial seedling size, while growth in midstory removal treatment sites (less available light) was not related to initial size. Furthermore they found that the number of first order lateral roots on seedlings at the time of planting was unrelated to seedling growth. Clark, et al. (2009) found that first year height and diameter growth of large seedlings were negatively related to planting size due to the planting shock that larger trees have to overcome in the first year after planting. Clark, et al. (2009) speculated that the seedlings sacrificed height growth at the expense of rebuilding their root systems after damage sustained during lifting and planting. While McNab (2003) did not directly examine the effect of seedling size on growth and survival, competing hardwoods were able to outgrow his planting of relatively small American chestnut seedlings (18 cm in height on average).

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Chapter 2: Early establishment of chestnut (*Castanea* Mill.) seedlings on the Cumberland Plateau of Eastern Kentucky

First year results presented in this chapter are based partly on a paper written by Cornelia C. Pinchot, Scott E. Schlarbaum, Jennifer A. Franklin, David S. Buckley, Stacy L. Clark, Callie J. Schweitzer, Arnold M. Saxton, and Frederick V. Hebard:

Pinchot, C.C.; Schlarbaum, S.E.; Franklin, J.A.; Buckley, D.S.; Clark, S.L.; Schweitzer, C.J.; Saxton, A.M.; Hebard, F.V. Early results of a chestnut planting in Eastern Kentucky illustrate reintroduction challenges. In: Proceedings of the 16th Biennial Southern Silvicultural Conference. February 15 – 17, 2011. In press.

My primary contribution to this paper includes (i) identification of the study objectives, (ii) collection, analysis, and interpretation of data, and (iii) manuscript preparation.

Abstract

Putatively blight-resistant hybrid chestnut seedlings will soon be available for outplanting on a regional scale. Few studies, however, have examined the importance of seedling size in artificial regeneration of American [*Castanea dentata (*Marsh.) Borkh] or hybrid chestnuts in different silvicultural treatments. This paper examines two-year results from a study of American, hybrid (BC_2F_3 generation) and Chinese chestnut (*Castanea mollissima* Blume.) 1-0 nursery seedlings planted under three silvicultural treatments; shelterwood with reserves (SW; high-light), thinning (TH; moderate light) and oak shelterwood (OS; low-light); in the Cumberland Plateau of eastern Kentucky. Additionally, the effect of initial seedling size; height, RCD and root volume; on establishment success was evaluated. Chestnut seedlings in the SW treatment sites added 2- and

3-times more height growth as those in the TH and OS treatment sites, respectively. Root collar diameter growth was also largest in the SW sites for all chestnuts. Seedlings that were larger at planting suffered less mortality and demonstrated better height and RCD growth compared to smaller seedlings. Mortality among American and hybrid seedlings was primarily caused by the non-native root-rot disease, *Phytophthora cinnamomi* (Rands), which was confirmed at the site. This study illustrates chestnut's ability to successfully establish under varying abiotic conditions with pressure from various biological stressors.

Introduction

Limited artificial regeneration methods for American chestnut, [*Castanea dentata (*Marsh.) Borkh], stands were developed before the chestnut blight fungus, *Cryphonectria parasitica*, was introduced into the United States (Ashe 1911; Hawes 1906; Hawley and Hawes 1912; Hough 1882; Mattoon 1909; Zon 1904). Although artificial regeneration of the species was carried out in the 19th century (Emerson 1846; Hough 1878; Neilson 1902), there were no prescriptions developed for planted stock. In anticipation of the availability of blight-resistant chestnut seedlings, there has been increased interest in the growth requirements of American chestnut (Anagnostakis 2007; Bauerle, et al. 2006; Clark, et al. 2009; 2011; in press; Jacobs and Severeid 2004; Jacobs 2007; Joesting, et al. 2007; Joesting, et al. 2009; Latham 1992; McCament and McCarthy 2005; McNab 2003; Rhoades, et al. 2009; Wang, et al. 2006). American chestnut has been found to more grow more quickly with increased light availability (Latham 1992; McCament and McCarthy 2005; Rhoades, et al. 2009), although Anagnostakis (2007) found that chestnut seedlings growing under 65% shade-cloth outperformed those in full-sunlight. Several studies have found that American chestnut growth is more sensitive to light availability than to either soil water or nutrient availability (Latham 1992; McCament and McCarthy 2005). All of these studies, however, have evaluated pure American chestnut, leaving the backcross hybrid chestnut largely unstudied. While the backcross hybrid chestnuts have been bred to maximize American chestnut characteristics, they have been found to differ somewhat from pure American chestnuts in several attributes, such as timing of budbreak and bud morphology (Hebard 1994; Diskin, et al. 2007). Such differences may affect the ecological behavior of the hybrid. Understanding which silvicultural techniques appropriately manipulate light levels for backcross hybrid chestnut establishment and growth will augment reintroduction efforts.

While studies examining competitive ability of chestnut are sparse, even fewer studies have examined the influence of initial seedling size and pedigree on chestnut establishment and growth (Clark, et al. 2009; 2011; Rhoades, et al. 2009). Initial seedling size has been found to significantly affect seedling establishment in studies of other hardwood tree species (Dey and Parker 1997a; Jacobs, et al. 2005; Johnson 1984). Seedlings with larger diameters and root systems are better able to re-establish root-to-soil contact, reducing the probability that the seedling will experience transplant shock (Burdett 1990; Grossnickle 2005). The overall goal of this study is to evaluate the effects of three silvicultural prescriptions; oak shelterwood, thinning, and shelterwood with reserves; and initial seedling size on early chestnut seedling establishment on the Cumberland Plateau of eastern Kentucky.

More specifically, we aim to:

1. Evaluate the effects of silvicultural treatment, species, and family treatments on chestnut survival and growth.

2. Assess above-ground competition among silvicultural treatments and its effect on seedling growth.

3. Evaluate the relationship between canopy cover and available photosynthetically active radiation (PAR).

4. Assess below-ground competition among silvicultural treatments and its effect on seedling growth.

5. Evaluate the effects of silvicultural treatment, species and family on timing of budbreak, and the effect of budbreak on seedling growth.

6. Evaluate the relationship between initial seedling height, RCD and root volume on overall seedling establishment.

Methodology

Study Site

This study is located on the London Ranger District of the Daniel Boone National Forest (DBNF) on the Cumberland Plateau in Southeastern Kentucky (37°03' N, 84°11' W, elevation 370 m; Figure 2-1). The forest type is classified as upland hardwood and is dominated by mixed oak species (Schweitzer, et al. 2008). Braun (1950) described this area of Kentucky as part of the mixed-mesophytic forest region, abundant with beech (*Fagus grandifolia* Ehrh.), white oak (*Quercus alba* L.), black oak (*Quercus velutina* Lam.), and hickory (*Carya* spp Nutt.). Other common hardwoods include chestnut oak (*Quercus prinus* L.), particularly on ridges, maple (*Acer* spp. L.), and black gum (*Nyssa sylvetica* Marsh). Before the chestnut blight, American chestnut was a dominant timber tree on the Cumberland Plateau, particularly at higher elevations (Braun 1950). The historic widespread distribution of chestnut in the area is further evidenced by its continued presence in the understory (personal observation).

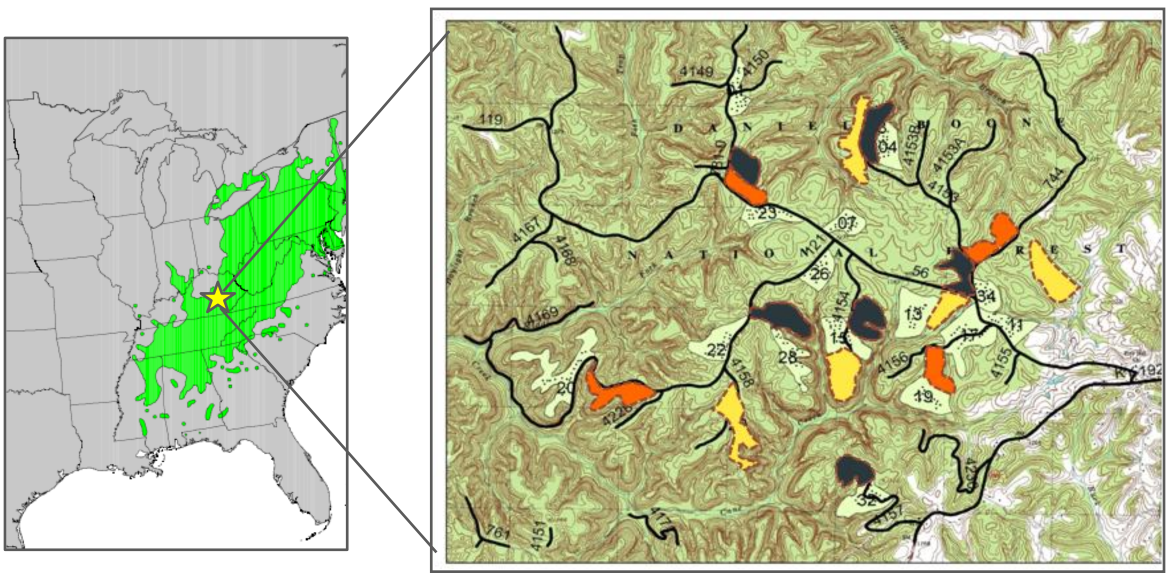

Figure 2-1: Map on left depicts the range of American chestnut. Map on right depicts the study site. Yellow sites are shelterwood with reserves treatments, orange sites are thinning treatments, and black sites are oak shelterwood treatments.

Silvicultural Treatments

This study was nested within a larger USDA Forest Service study, referred to as the Cold Hill Study, which was established with the goal of improving oak regeneration and forest health prior to the anticipated arrival of gypsy moth (*Lymantria dispar* L.) to the area (Schweitzer, et al. 2008). Our study utilized three of the five silvicultural treatments implemented in the Cold Hill study: oak shelterwood (OS), thinning (TH), and shelterwood with reserves (SW). For the OS treatment sites, all stems greater than 3 cm diameter at breast height in suppressed and intermediate (i.e. midstory) canopy positions were killed using triclopyr herbicide injection (c*f.* Loftis 1990), leaving a basal area of 22 m^2/ha of intact overstory (Schweitzer, et al. 2008). This treatment increases light on the forest floor to favor oak regeneration, while retaining enough overstory to inhibit shade intolerant species, such as yellow poplar (*Liriodendron tulipifera* L.). The overstory will be removed four to five years following midstory removal. The TH treatment left stands thinned to the B-level of Gingrich stocking (Gingrich 1967), with a basal area of 18 m^2/ha of overstory (Schweitzer, et al. 2008). While thinning is not a standard regeneration treatment, this may provide adequate light for seedling establishment or recruitment of species that are moderate in shade tolerance (e.g. *Quercus* spp.), while discouraging the least shade-tolerant species (e.g. yellow poplar). The SW treatment was a commercial tree harvest that left a residual basal area of 5 m^2/ha of overstory. Overstory trees that promoted "good forest health conditions" and wildlife habitat were left uncut (Schweitzer, et al. 2008). All harvest treatments were completed between August, 2007 and February, 2009.

Experimental Materials

American, BC_2F_3 generation hybrid (Hebard 2001), and Chinese chestnut (*Castanea mollissima* Blume.) seedlings were used in this study. The open-pollinated American and hybrid chestnut nuts were harvested at The American Chestnut Foundation's Meadowview Research Farms, Meadowview, VA, in the fall of 2007, and manually sown at the Georgia Forestry Commission's Flint River Nursery in Byromville, GA in January, 2008 at a density of 65 nuts per square meter. Fertilization and irrigation of the seedlings followed guidelines developed by Kormanik and others (1994). The 1-0 seedlings were lifted in February, 2009, and stored in a cold room (~1º C) until they were planted. The bare-root 1-0 Chinese chestnut seedlings were purchased from Forrest Keeling Nursery (PO Box 135, Elsberry, MO) in February, 2009. Seedlings were processed for planting in February, with roots trimmed to 15 centimeters from the main tap root to facilitate planting. Seedlings were individually tagged to maintain the genetic identification and unique records for each.

Experimental Design

The study was designed to examine the effect of species, family, and silvicultural treatments and initial seedling size on chestnut establishment. The use of 'species' in this chapter refers to the two separate species of chestnut; American and Chinese; and the hybrid (BC_2F_3), used in this study. Silvicultural treatments in the USFS's Cold Hill Study (Schweitzer, et al. 2008) were arranged in a completely randomized design. Within these silvicultural treatments, the chestnut plots were arranged in a randomized block design. Thus the experimental design incorporates a split plot, with silvicultural treatment in the main plot and species and family in the subplot.

Three hundred American, 300 hybrid and 150 Chinese chestnut seedlings were planted on the experimental sites between March 2nd and 9th, 2009. Table 2-1 contains information on the families planted. The 750 seedlings in the study were planted in 15 different sites on the DBNF. Each of the three silvicultural treatments was replicated three to five times, with the seedlings evenly distributed among the three treatment types. Treatments were not replicated an equal number of times because not all proposed sites in the Cold Hill Study had been treated at the time of planting. Seedlings were planted in linear plots at a spacing of 2.5 m in one transect per site. The chestnuts were planted in blocks of five seedlings, each representing two American seedlings, one Chinese seedling, and one of each of the hybrid families. Table 2-16 (in appendix 1) shows the distribution of seedlings among experimental units.

Table 2-1: Mean (± standard error) initial height, root collar diameter, and root volume of species and families used in this study.

Species	**Family**	**No. planted**	**HT (cm)**	**RCD (mm)**	**Root vol. (cm^3)**
American	Bulk	300	96 ± 1	11.3 ± 0.2	51 ± 2
BC_2F_3	SA330 x OP	150	85 ± 2	10.6 ± 0.2	53 ± 3
BC_2F_3	SA417 x OP	150	103 ± 1	12.6 ± 0.2	81 ± 4
Chinese	Dunstan	150	111 ± 2	12.2 ± 0.2	97 ± 4

Survival, Growth, and Dieback

Bare-root seedling height, root collar diameter (RCD) and root volume (by water displacement; Novoselov 1960) were measured in February, 2009 prior to planting. Height and RCD were measured again, with mortality, at the end of the 2009 and 2010 growing seasons. Height growth, using only positive growth values, for year one (2009), year two (2010), and over both years (two-year) was used in analysis. Negative growth in height (2009, 2010 and two-year total) was analyzed as dieback. RCD growth for year two (2010) was also used, however 2009 and two-year RCD growth values were not used due to inconsistencies of RCD measurement between the greenhouse and field. Seedlings with stems that died back to the ground and re-sprouted were removed from the analysis.

Above-ground competition measurements

A 2.60 meter diameter plot was centered on each surviving chestnut seedling and competition data on understory woody plants collected at the end of the 2009 and 2010 growing seasons. Data collected included species and height of the largest understory competitor [> 0.3 m in height and < 3.8 cm diameter at breast height (DBH)], and the number of understory competitors in each plot. In 2009, woody plants and vines in the understory were recorded as an understory competitor, including green briar (*Smilax* spp. L.) and blackberry (*Rubus* spp. L.). In 2010, only arborescent species were recorded as understory competitors.

The silvicultural treatments in this study were expected to vary in the amount of irradiation available to the chestnut seedlings. Light levels were also expected to differ within treatments, as light can vary greatly under a forest canopy due to spatiotemporal heterogeneity (Parent and Messier 1996; Washitani and Tang 1991) and sunflecks (Washitani and Tang 1991). In order to capture this variation and analyze its effect on chestnut growth and survival, photosynthetically active radiation (PAR) and canopy cover were measured during the 2009 and 2010 growing seasons. Instantaneous PAR measurements were taken for each chestnut seedling in June, 2009 and July, 2010 on all fifteen treatment sites, on either completely cloudless or overcast days when possible (Klinka, et al. 1992; Parent and Messier 1996; Smith 1991). Light intensity was quantified with an AccuPar linear PAR/LAI ceptometer (Decagon Devices, Inc., Pullman, WA). The ceptometer contains 80 independent photodiodes that measure photosynthetically active radiation in the 400-700 nm waveband, which are expressed in units of micromoles per meter squared per second ($\mu mol\ m^{-2} s^{-1}$) (Decagon Devices, Inc., Pullman, WA). A total of three readings were taken at each seedling: one measurement at the midcanopy of the seedling on the south side of each tree taken in the morning (9:30 am - 11:30 am), midday (12:30 pm - 2:30 pm), and in the evening (3:30 pm - 5:30 pm). An additional ceptometer was placed in a nearby open field for a continuous PAR reading in full sunlight. On days with occasional cloud cover, readings were avoided during periods of cloudiness. PAR measurement at each seedling was divided by the corresponding full light measurement to obtain percent available PAR. The three measurements of available PAR for each seedling were averaged to create one average available PAR per seedling.

The fifteen study sites were located up to four kilometers from the field in which overstory PAR was measured. In order to reduce the effect of differences in cloud cover between the understory and overstory PAR measurement sites, a regression slope was calculated using time (minutes into the day) to predict overstory (continuous) PAR (Equations 2-1 and 2-2 in appendix). Before fitting regression slopes to the continuous PAR data, abnormally low readings (300 µmol $m^{-2}s^{-1}$ below the half-hour average) were removed so that the regression equation would not be impacted by periods of cloudiness, during which PAR was generally not measured. One OS and one SW replicate were removed from 2009 measurements, and one TH replicate was removed from the 2010 measurements due to inconsistencies in the data.

Canopy cover is defined as "the proportion of the sky hemisphere obscured by vegetation when viewed from a single point" (Jennings, et al. 1999). Canopy cover can be measured with a spherical densitometer (Lemon 1956), and has been found to be strongly correlated with light transmittance (Buckley, et al. 1999; Jenkins and Chamber 1989; Lhotka and Lowenstein 2006). Due to its ease and low cost, this method is more commonly used by researchers as a means of estimating available light than are PAR measurements. This measurement may be used to compare sites in the study with other potential chestnut reintroduction sites. A convex spherical densitometer was used to estimate canopy cover on ten randomly selected treatment sites, representing three to four replications of each treatment type (Table 2-17). Readings were taken on the south side of each chestnut seedling in each of the four directions (Buckley, et al. 1999; Lhotka and Lowenstein 2006). Readings were taken during the 2009 and 2010 growing seasons and an average closure for each seedling was used in the analysis.

Below-ground Competition

The SPAD-502 chlorophyll meter is a well-established instrument for estimating foliar nitrogen in both crop plants and forest trees (Follett, et al. 1992; Moreau, et al. 2004). In order to estimate nutrient levels in the planted chestnut seedlings, a sample of 94 seedlings from six sites (two of each treatment type) was taken in August 2009 and a sample of 74 seedlings from the same sites was taken in 2010 (Table 2-18 in appendix). The third healthy (no visible damage) leaf toward the ground from the terminal bud was taken from each sampled seedling and temporarily placed in a cooler for transport to the lab. Leaves were stored in a -70° C freezer until processing, at which point they were removed and approximately 3 g of the midsection was cut from each leaf. Leaf tissue was crushed with a mortar and pestle in 85% acetone. The extract was then centrifuged to remove suspended solids and then enough 85% acetone was added to make 15 ml of extract. The optical density of the extract was measured with a Thermospectronic Biomate 3 spectrometer (Thermo Electron Scientific Instruments Corp., Madison, WI), and chlorophyll and carotenoid concentrations estimated using McKinney equations (Sestak, et al. 1971). Chlorophyll a, b and total (a + b) estimations were used to evaluate relative foliar nitrogen content for each leaf sample. These measurements were used as indirect evaluations of soil nutrients available to the chestnut seedlings.

To indirectly measure the availability of soil water, the midday (11:30 am-1:30 pm) stomatal conductance rate was recorded for each chestnut seeding in ten randomly chosen sites, representing 2-3 replicates of each treatment type (Table 2-19). Stomatal conductance was measured using a LiCor 1600 steady-state porometer in July 2009 and 2010. The LiCor porometer calculates stomatal conductance by measuring the flow rate of dry air required to maintain the humidity within the cuvette (Li-Cor, Inc., Lincoln, NE). One sun-exposed leaf on each seedling in the selected transects was sampled in a two hour period surrounding solar noon.

Budbreak

Two replicates from each of the three silvicultural treatments were randomly selected for budbreak evaluation (Table 2-20). These sites were monitored on five separate dates, approximately a week apart between March 31st and May 5th, 2009, and four dates during the spring of 2010, between March 29th and April 14th. Each seedling was examined and assigned a rating, according to the budbreak ratings in Tables 2-2 and 2-3. In 2009 buds were rated on a scale of 1 – 6, based on the most developed bud (Tables 2-2). The rating system was refined in 2010 to reduce observer bias and subjectivity. In 2010, the rating system in Table 2-3 was used to evaluate both the most developed bud and the terminal bud. In addition to the numbers assigned according to this table, a decimal, between 0 and 0.9, to describe the percentage of the seedling that was as developed as the most developed bud, was recorded (West and Wein 1971). The final rating, between 0 and 10.9, was calculated by adding these three numbers together.

Table 2-2: 2009 Budbreak rating system.

Rating	Description
1	Just starting to break; scales loosened
2	Buds swelled, starting to break
3	Bud scales nearly shed, leaves still tight
4	Leaves just visible
5	Leaves separated; less than 5 cm long
6	Leaves 5 cm or longer

Table 2-3: 2010 Budbreak rating system.

Rating	Description
1	Buds dormant, no sign of breaking
2	Buds display silver/green tip
3	Buds expanding, leaves unfolding from bud
4	Internodes visible, leaves hanging but not enlarged
5	Internodes visible, leaves enlarged

Initial Seedling Size

To analyze the effect of seedling size on seedling growth and survival, seedlings were divided, post-planting, into three root collar diameter classes according to their initial RCD (Table 2-4). RCD ranges were chosen to create classes with similar numbers of seedlings. These RCD classes, as well as initial RCD, height, and root volume, were used to evaluate the importance of seedling size to establishment.

Table 2-4: Number of observations, initial RCD range, and mean initial RCD for each RCD class.

RCD Class	No.	Range (mm)	Mean RCD (mm)
1	183	5.3 – 10.0	8.6
2	204	10.1 – 12.5	11.2
3	194	12.6 – 20.9	14.4

Biotic Stressors

Seedlings were visually inspected for disease, browsing, and insect damage annually in August. Severe predation by chestnut sawfly (*Craesus castaneae*, Rohwer) larvae was noticed on both planted chestnut seedlings and naturally occurring chestnut sprouts during the 2009 growing season, specifically June through August. To evaluate the effect of predation on chestnut growth, and to evaluate the frequency of insect predation among silvicultural and species treatments (described in Chapter 3), the presence/absence of leaf predation was recorded towards the end of the 2009 growing season (August 12 - 15, 2009). Predation presence was recorded for seedlings having at least one leaf with evidence of insect predation.

Statistical Analysis

All analyses for this study were processed using SAS 9.2 software (SAS Institute, 2007). Seedling response was analyzed using a mixed model analysis of variance (ANOVA; Table 2-5) to determine significant effects of silvicultural, species, and family treatments and their interactions on the following dependent variables: yearly and two-year total height growth, yearly and two-year total RCD, and 2010 RCD growth. The 2009 and two-year total RCD growth were not analyzed due to inconsistencies between location on the stem where RCD was measured after lifting and where it was measured after planting. The effects of RCD size classes (1 – 3) on the dependent variables listed above were tested using a separate model that included RCD class, silvicultural treatment, species and their interactions as fixed terms. Because the Chinese chestnut seedlings were larger on average than the American and hybrid seedlings, and because Chinese chestnut seedlings suffered less mortality, the RCD class analysis excluded Chinese chestnuts. Mixed model ANOVA was used to test the effects of silvicultural treatment and species on foliar chlorophyll, stomatal conductance, and 2010 budbreak rating. The effects of silvicultural treatment on yearly canopy cover, available PAR, and the height of the tallest competitor and density of competing seedlings in competition plots were also tested with mixed model ANOVA.

PROC GLIMMIX was used to test the effect of silvicultural, species, and family treatments and their interactions on seedling survival, dieback (binomial distribution), and 2009 budbreak rating (normal distribution).

Table 2-5: ANOVA table for experimental design

Source	DF	Type
Silvicultural treatment [silv]	2	fixed
Species	2	fixed
Silv*Species	4	interaction
Family(species)	1	fixed
Rep(silv)	12	random (error)
Block(rep*silv)	149	random (error)
Rep(family*species*silv)	76	random (error)
Block(rep*family*species*silv)	503	random (residual)
Total	749	

Chestnut sawfly predation (yes/no) was added ad hoc as a treatment variable to the analysis. Mixed model ANOVA was used to determine if predation affected yearly, and two-year total survival, height growth, yearly RCD, and 2010 RCD growth. To avoid biasing the effect of predation on height and RCD growth, only American and hybrid seedlings were included in this analysis, as Chinese chestnuts suffered fewer incidences of insect predation (chapter 3). Significant main effects for all ANOVA analyses ($\alpha = 0.05$) were further analyzed using least significant difference for mean separation. Standard errors are reported in tables.

The relationships between measurements of resource availability (available PAR, canopy cover, density of seedlings in competition plots, stomatal conductance, chlorophyll a, b and a + b), measurements of seedling size (initial height, initial RCD, initial root volume) and budbreak rating; and 1) two-year total height growth and 2) 2010 RCD growth were evaluated, individually, through Pearson correlation analysis.

Multiple regression, using treatments as dummy variables, was used to predict both two-year total height growth (with all dieback removed) and 2010 RCD growth (separately), using a randomly selected sample of the data. Predictor variables were related to seedling size and resource availability (Table 2-6). Interactions between treatments and variables, and polynomial terms were also tested. Variables with p-values > 0.05 were removed and the analysis repeatedly conducted until all variables included in each model were significant. The model was then tested on a validation dataset, which consisted of observations not used in the sample dataset.

Normality and equal variance assumptions for dependent variables were tested. The following variables required transformations to satisfy assumptions of equal variance. Transformation type is listed in parentheses after the variable name: 2009 height growth (square root), 2009 canopy cover (rank), 2009 total chlorophyll (square root), 2010 height growth (square root), two-year height growth (rank), 2010 RCD (rank), 2010 canopy cover (cubic power), 2010 stomatal conductance (square root), 2010 chlorophyll a (square root), 2010 chlorophyll b (square root), and 2010 carotenoids (square root).

Table 2-6: Variables tested in multiple regression to predict 1). Two-year total height growth and 2). 2010 RCD growth (separately). In addition to these variables, interactions between treatments and seedling qualtity and resource availability variables, as well as polynomial terms, were tested.

Treatments	**Seedling size**	**Resource availability**
Silvicultural treat.	Initial height	Canopy cover
Species	Initial RCD	Available PAR
Family	Initial root volume	Stomatal conductance
Height class		
Foliar predation		

Results

Survival

First year survival averaged 70% across all treatments. Survival rates differed among species; 95% of the Chinese chestnut seedlings survived the first growing season, compared to 61% of American and 67% of hybrid seedlings ($p < 0.0001$; Table 2-7). Survival between hybrid families also differed ($p < 0.0001$). Eighty-two percent of seedlings in family SA 417 survived the first growing season, compared to 52% of seedlings in family SA 330 (Table 2-21). Survival among silvicultural treatments did not differ ($p = 0.9699$; Table 2-22).

Seventy-six percent of the seedlings alive at the end of the 2009 growing season survived the 2010 growing season. As in 2009, Chinese chestnuts suffered less mortality than did American and hybrid seedlings ($p < 0.0001$; Table 2-7). No differences were found between hybrid families ($p = 0.4021$; Table 2-21). Silvicultural treatment did not affect survival ($p = 0.4012$; Table 2-22).

Species again differed in two-year survival ($p < 0.0001$; Table 2-7), as did hybrid families ($p < 0.0001$), while no significant differences were found among silvicultural treatments ($p = 0.2425$; Table 2-22). Forty-one percent of the 300 planted American seedlings, 48% of 300 hybrid seedlings, and 90% of 150 Chinese were alive at the end of the 2010 growing season. Family SA 417 had better two-year survival than SA 330 (Table 2-21).

Table 2-7: 2009, 2010, and two-year mean (± standard error) percent survival among species. Species means within each column followed by the same letter are not significantly different ($\alpha = 0.05$).

Species	**2009**	**2010**	**Two-year**
American	61 b ± 5	67 b ± 5	41 b ± 4
Hybrid	67 b ± 5	73 b ± 5	48 b ± 5
Chinese	96 a ± 2	92 a ± 2	90 a ± 3

Growth

At the time of planting, American chestnut seedlings averaged 96 cm in height and 11 mm in RCD; hybrid seedlings averaged 94 cm in height and 11 mm in RCD; and Chinese seedlings averaged 110 cm in height, and 12 mm in diameter. After one growing season, no differences were found in height growth among silvicultural treatments, species, or family treatments (p = 0.0979, p = 0.4634, p = 0.8457, respectively; Table 2-24 and Table 2-25 in appendix 1).

In 2010, height growth differed among silvicultural treatments (p = 0.0036; Figure 2-2 and Table 2-23). Seedlings growing in SW sites added more growth on average (34 cm) than those in TH sites (20 cm) or OS sites (8 cm). Height growth did not differ among species (p = 0.5908; Table 2-24) or between hybrid families (p = 0.7769; Table 2-25).

Two-year height growth also differed among silvicultural treatments (p < 0.0001; Figure 2-2). Seedlings growing on SW sites again demonstrated the greatest height growth (40.12 cm) compared to those in TH and OS sites (24 cm, 12 cm, respectively; Figure 2-2, Table 2-23). Species and family treatment did not affect two-year height growth (p = 0.5908, p = 0.7769, respectively; Tables 2-24 and 2-25).

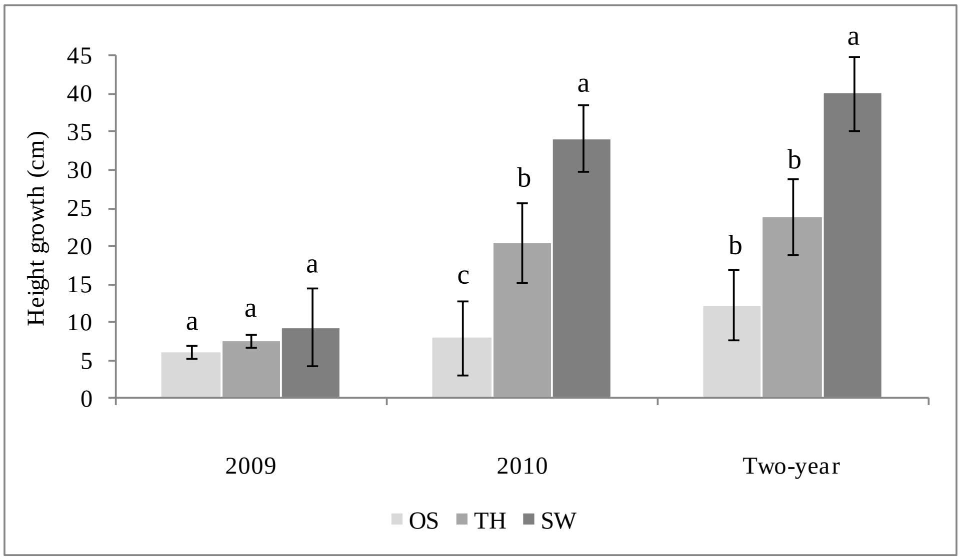

Figure 2-2: Mean 2009, 2010, and two-year height growth among silvicultural treatments. Error bars indicate standard error. Treatment bars within year with the same letter are not significantly different ($\alpha = 0.05$).

Mean 2009 Chinese RCD was greater than that of American and backcross chestnut seedlings ($p < 0.0001$; Figure 2-3). RCD did not differ among silvicultural treatments ($p = 0.2680$; Table 2-27) or between hybrid families ($p = 0.1397$; Table 2-26). Initial RCD was strongly related to final RCD ($p < 0.0001$).

Again in 2010, mean Chinese chestnut RCD was greater than those of American and hybrid chestnuts ($p < 0.0001$; Figure 2-3). Seedlings in SW sites exhibited the greatest RCD growth (5.0 mm), followed by seedlings in TH sites (2.1 mm), and then by seedlings in OS sites (0.7 mm) ($p < 0.0001$; Figure 2-4 and Table 2-27). Initial RCD was strongly related to final RCD ($p < 0.0001$). Chinese chestnut seedlings added the most RCD growth in 2010 (1.4 mm; p =

0.0236). Hybrid chestnuts grew 0.9 mm on average, and American chestnuts grew 0.9 mm on average. RCD growth did not differ among hybrid families (p = 0.3339; Table 2-26).

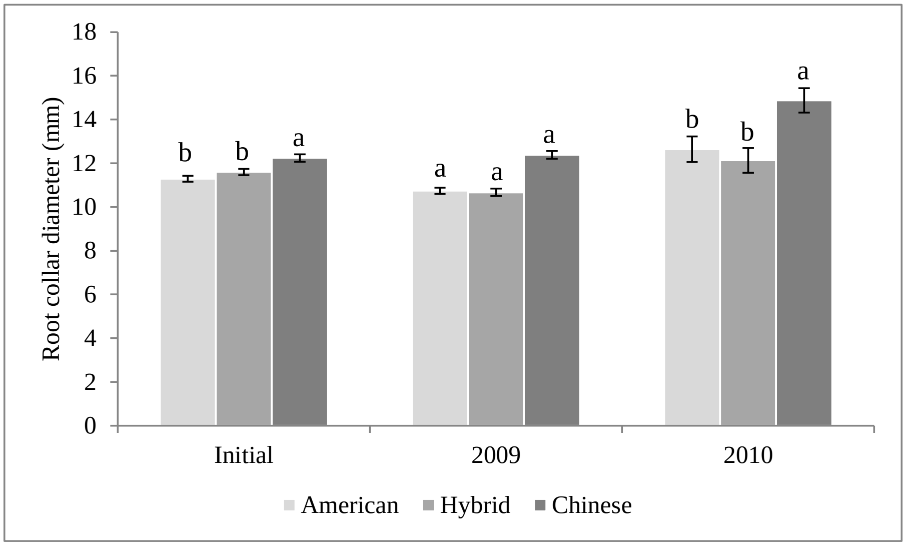

Figure 2-3: Mean initial, 2009 and 2010 RCD among species. Error bars indicate standard error. Treatment bars within measurement period with the same letter are not significantly different (α = 0.05).

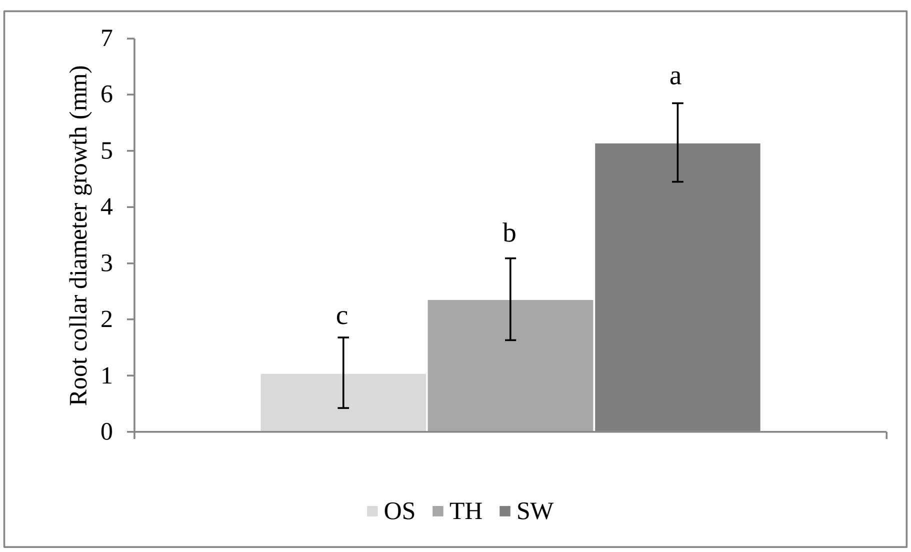

Figure 2-4: 2010 RCD growth among silvicultural treatments. Error bars indicate standard error. Treatment bars with the same letter are not significantly different (α = 0.05).

Dieback

American and Chinese chestnut seedlings experienced greater occurrence of dieback in 2009 than did hybrid chestnut seedlings (p = 0.0004; Table 2-8). No differences in dieback were found between hybrid families (p = 0.7720; Table 2-28), nor among silvicultural treatments (p = 0.2060; Table 2-9).

More seedlings in the OS and TH sites experienced dieback in 2010 than those growing in the SW sites (p = 0.0097; Table 2-9). Species did not differ in frequency of dieback (p = 0.8145; Table 2-8), nor did families (p = 0.2544; Table 2-28).

Overall instances of dieback, from the time of planting to the end of the 2010 growing season, differed among silvicultural treatments (p = 0.0298; Table 2-9) but not among species (p = 0.0832; Table 2-8) or between family treatments (p = 0.0871; Table 2-28). Again, seedlings in SW sites exhibited the lowest frequency of dieback.

Table 2-8: Mean (± standard error) 2009 and 2010 frequency of dieback (percent) among species. Species means within each column followed by the same letter are not significantly different (α = 0.05).

Species	2009	2010	Two-year
American	43 a ± 4	28 a ± 5	30 a ± 5
Hybrid	26 b± 5	30 a ± 4	26 a ± 4
Chinese	55 a ± 4	32 a ± 4	39 a ± 5

Table 2-9: Mean (± standard error) 2009 and 2010 frequency of dieback (percent) among silvicultural treatments. Treatment means within each column followed by the same letter are not significantly different (α = 0.05).

Treatment	**2009**	**2010**	**Two-year**
OS	49 a ± 5	44 a ± 5	45 a ± 5
TH	38 a ± 6	30 ab ± 5	34 ab ± 5
SW	37 a ± 5	19 b ± 4	19 b ± 5

Above-ground Competition

Silvicultural treatments did not affect the height of the tallest understory competitor (p = 0.6383) or the density of competing seedlings (p = 0.2275) within competition plots in 2009 (Table 2-29). Red maple (*Acer rubrum* L.) was the most common tallest competitor in 2009 (38% of all plots) and averaged 176 cm in height. Green briar was the next most common tallest competitor, representing 17% of all plots and averaging 84 cm in height. Red maple was also the most abundant species in the understory, representing 30% of plots, followed by green briar (26% of plots; Table 2-30).

Again in 2010, the height of the tallest competitor within competition plots did not differ among silvicultural treatments (p = 0.3165; Table 2-29). The density of competing seedlings, however, did differ among silvicultural treatments (p = 0.0022). Greater densities of competing seedlings were found in SW and TH sites (17 and 12 seedlings per plot, respectively) as compared to OS sites (9 seedlings per plot).

Red maple was the most common tallest understory competitor in 2010 (42% of plots). Sassafras (*Sassafras albidum* L.) was the next most common tallest competitor, representing 13% of all plots. Red maple was also the most abundant seedling in competition plots (42% of plots) across silvicultural treatments (Table 2-31).

In 2009 SW sites demonstrated the lowest canopy cover, followed by TH sites, and OS sites ($p < 0.0001$; Figure 2-5). Available PAR also differed significantly among silvicultural treatment ($p < 0.0001$), with the least percent full sun light available to seedlings in OS sites, followed by TH sites, and SW sites (Figure 2-6).

In 2010 canopy cover again differed among silvicultural treatments ($p > 0.0001$). OS sites averaged 97% canopy cover; TH sites 91% closure, and SW sites 65% closure (Figure 2-6). Silvicultural treatments also differed in the amount of PAR available to seedlings ($p < 0.0001$) (Figure 2-6).

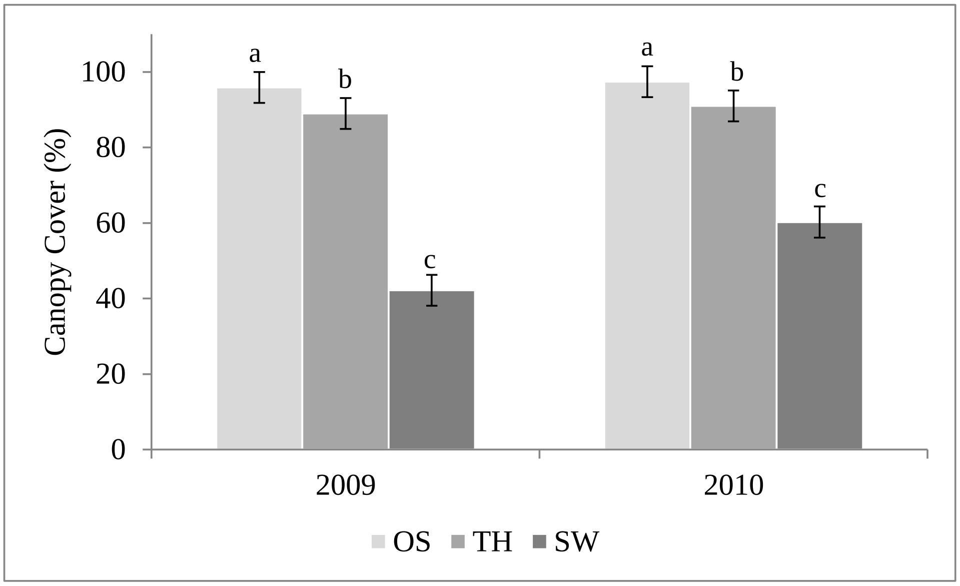

Figure 2-5: 2009 and 2010 mean percent canopy cover among silvicultural treatments. Error bars indicate standard error. Treatment bars within year with the same letter are not significantly different ($\alpha = 0.05$).

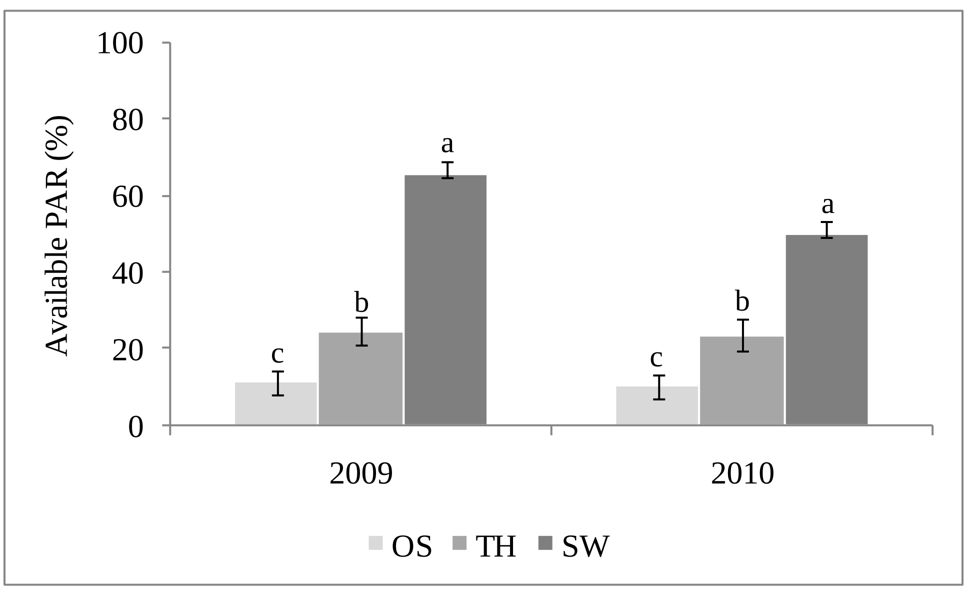

Figure 2-6: Mean 2009 and 2010 available photosynthetically active radiation among silvicultural treatments. Error bars indicate standard error. Treatment bars within year with the same letter are not significantly different ($\alpha = 0.05$).

Relationship between canopy cover and available PAR:

Canopy cover was a moderate predictor of the square root of available PAR in 2009 and 2010 (Figure 2-7 and Figure 2-8).

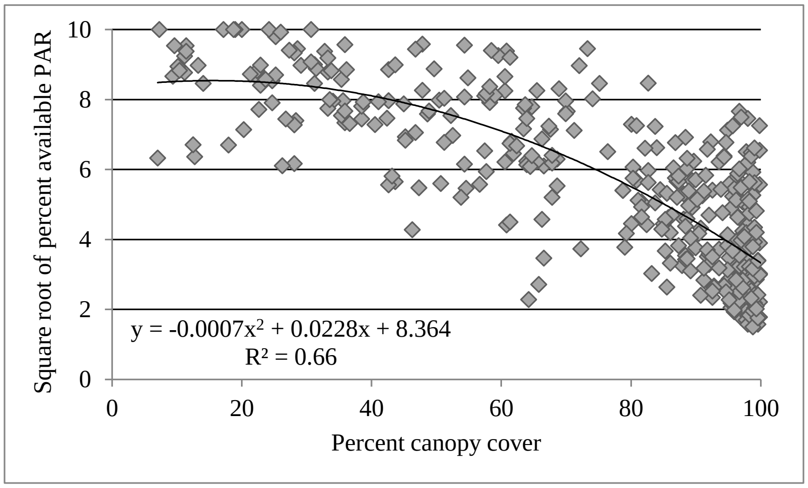

Figure 2-7: Relationship between percent canopy cover and the square root of percent available PAR for 2009 data.

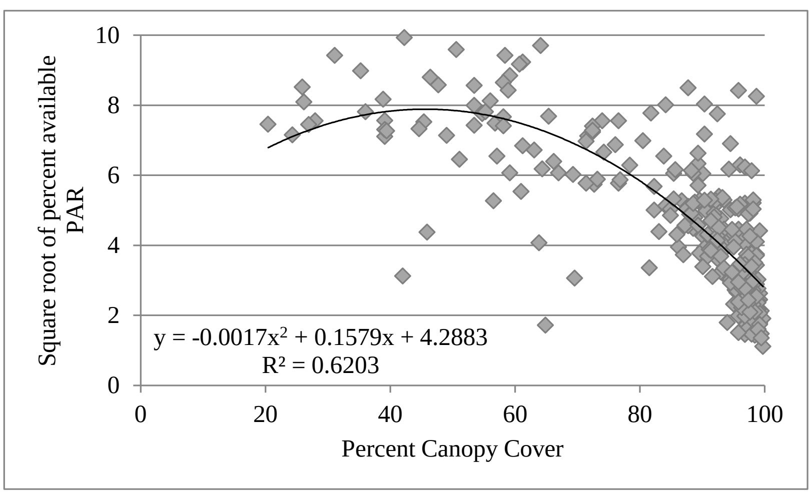

Figure 2-8: Relationship between percent canopy cover and the square root of percent available PAR for 2010 data.

Below-ground Competition

Stomatal conductance did not differ statistically among silvicultural treatments in 2009 (p = 0.0659; Figure 2-9) or species (p = 0.5284; Table 2-32). In 2010, however, mean stomatal conductance was greater in seedlings growing in SW and TH sites compared to those growing in OS sites (p = 0.0023; Figure 2-9).

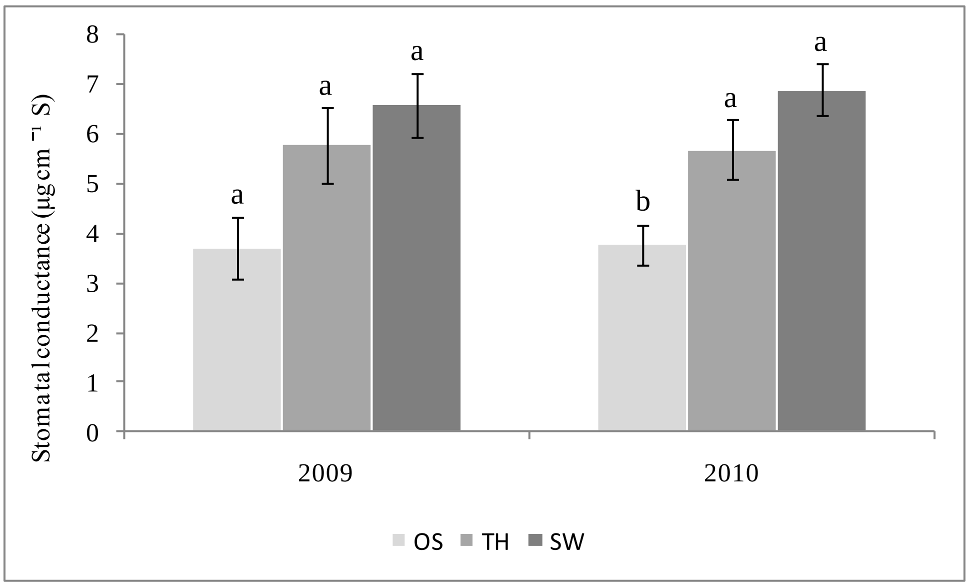

Figure 2-9: 2009 and 2010 mean stomatal conductance among silvicultural treatments. Error bars indicate standard error. Treatment bars within year with the same letter are not significantly different ($\alpha = 0.05$).

Chlorophyll a, b, and total chlorophyll (a + b) all differed significantly among silvicultural treatments in 2009 (p = 0.0103, p = 0.0289, p = 0.0042, respectively), with all three parameters greatest in OS sites (Figure 2-10). Chlorophyll parameters did not, however, differ among species (p = 0.9033, p = 0.4423, and p = 0.5877, respectively; Table 2-33). Species by silvicultural interaction was significant for all three variables (p = 0.0345, p = 0.0106, and p = 0.0338, respectively). Chinese chestnuts tended to have lower chlorophyll parameters in the SW sites than the other two chestnut species. Carotenoids did not differ among species (p = 0.6401) or silvicultural treatments (p = 0.2495; Table 2-33).

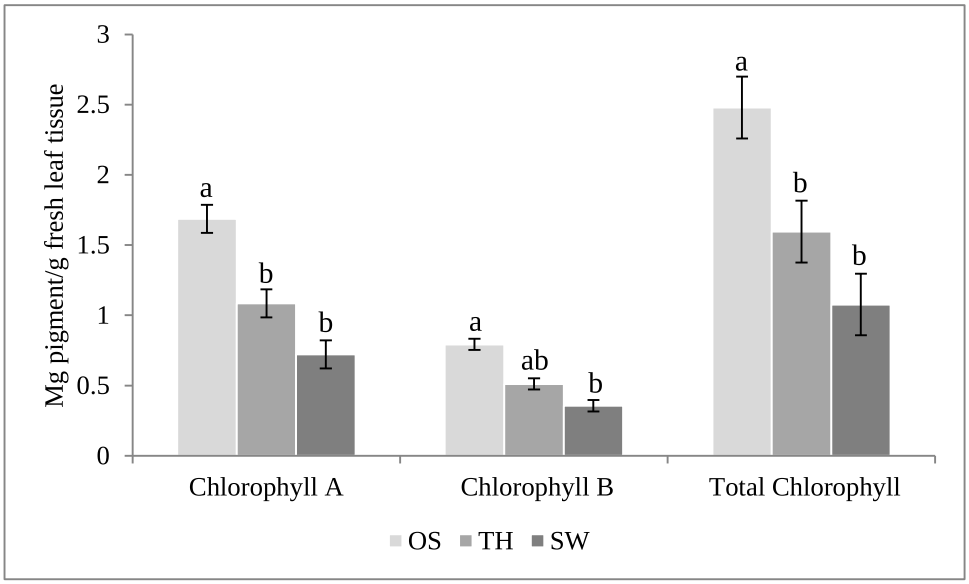

Figure 2-10: 2009 mean chlorophyll a, b, and total chlorophyll (a + b) among silvicultural treatments. Error bars indicate standard error. Treatment bars within each variable with the same letter are not significantly different (α = 0.05).

The four pigment variables did not differ among silvicultural treatments (chlorophyll a: p = 0.5077; chlorophyll b: p = 0.7053; total chlorophyll: p = 0.2885; carotenoids: p = 0.4656) or species (chlorophyll a: p = 0.1435; chlorophyll b: p = 0.0702; total chlorophyll: p = 0.1209; carotenoids: p = 0.7152) treatments in 2010 (Tables 2-34 and 2-35).

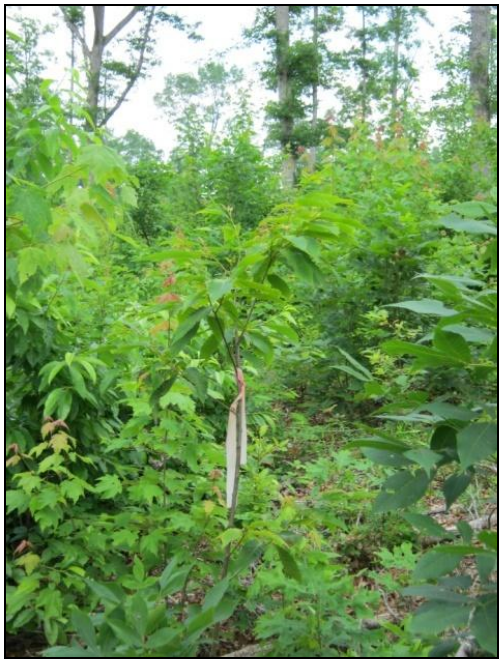

Figure 2-11: Planted chestnut seedling growing in a shelterwood with reserves treatment site.

Figure 2-12: Shelterwood with reserves site. Note the density of competing sprouts.

F**igure 2-13:** Planted chestnut seedling growing in a thinned treatment site.

Figure 2-14: Thinned treatment site.

Figure 2-15: Planted chestnut seedling growing in an oak shelterwood treatment site.

Figure 2-16: Oak shelterwood treatment site. Note the dense forest canopy and relatively sparse understory.

Correlations

Available PAR and canopy cover were the only variables which demonstrated relatively strong relationships with two year height growth (r = 0.35, -0.49, respectively; Table 2-10). Seedling density (of competition plots), height of tallest seedling, stomatal conductance, and budbreak rating all produced significant, but weak correlations with height growth (Table 2-10). Additionally, all four foliar pigment variables yielded low r values.

Of the variables correlated with 2010 RCD growth, again only available PAR and canopy cover demonstrated significant relationships (r = 0.38, -0.46, respectively; Table 2-10). Stomatal conductance was weakly related to RCD growth (r = 0.17). Again, none of the foliar pigment variables were related to growth (Table 2-10).

No moderate or strong relationships were found among the variables related to above-ground and belowground competition (Table 2-11). Stomatal conductance was weakly related to canopy cover (r = -0.33; $p < 0.0001$), and available PAR (r = 0.28; $p < 0.0001$).

Table 2-10: Correlation estimates between two-year height growth and 2010 RCD growth, and the variables listed below.

Variable	**N**	**Two-Year ht. growth**	**N**	**2010 RCD growth**
Seedling density	270	0.18 ($p = 0.0022$)	312	0.26 ($p < 0.0001$)
Ht of tallest competitor	270	0.20 ($p = 0.0008$)	313	0.11 ($p = 0.0437$)
Available PAR	272	0.35 ($p < 0.0001$)	313	0.38 ($p < 0.0001$)
Canopy cover	181	-0.49 ($p < 0.0001$)	209	-0.46 ($p < 0.0001$)
Stomatal conductance	180	0.21 ($p = 0.0042$)	205	0.17 ($p = 0.0115$)
Budbreak rating	114	0.21 ($p = 0.0282$)	135	0.05 ($p = 0.5458$)
Chlorophyll a	59	-0.05 ($p = 0.6984$)	62	0.08 ($p = 0.5257$)
Chlorophyll b	59	0.08 ($p = 0.5529$)	62	0.07 ($p = 0.5481$)
Total chlorophyll	59	0.01 ($p = 0.9274$)	62	0.09 ($p = 0.4594$)
Carotenoids	59	0.05 ($p = 0.6840$)	62	0.18 ($p = 0.1675$)

Table 2-11: Correlation estimates between variables related to above-ground and below-ground competition.

Variable One	Variable Two	N	Estimate
Canopy cover	Chlorophyll a	68	0.08 (p = 0.5130)
Canopy cover	Chlorophyll b	68	0.01 (p = 0.9216)
Canopy cover	Total chlorophyll	68	0.04 (p = 0.6942)
Canopy cover	Carotenoids	68	-0.10 (p = 0.3964)
Canopy cover	Stomatal conductance	191	**-0.33 (p < 0.0001)**
Available PAR	Chlorophyll a	79	-0.15 (p = 0.1833)
Available PAR	Chlorophyll b	79	-0.20 (p = 0.0738)
Available PAR	Total chlorophyll	79	-0.19 (p = 0.0817)
Available PAR	Carotenoids	79	0.10 (p = 0.3599)
Available PAR	Stomatal conductance	263	**0.28 (p < 0.0001)**
Stomatal conductance	Chlorophyll a	79	0.14 (p = 0.2570)
Stomatal conductance	Chlorophyll b	79	0.03 (p = 0.7916)
Stomatal conductance	Carotenoids	79	0.08 (p = 0.5010)
Stomatal conductance	Total chlorophyll	70	0.10 (p = 0.3966)

Predicting Growth

Of the five treatment and eight continuous variables tested using dummy regression to predict two-year positive height, only canopy cover, initial root volume, family, and family by root volume interaction significantly contributed to the model ($p < 0.0001$). This model, based on 80 observations, accounted for 54% of the variation in positive height growth. Using the model on the validation dataset, with 64 observations, produced an R-square value of 35% ($p = 0.0002$). Family and family by volume interaction were not significant ($p = 0.2285$, $p = 0.1122$, respectively), therefore, the final model to predict growth contained only canopy cover and root volume. This model accounted for 45% of the variation in positive height growth in the sample dataset, and 30% of the variation in the validation dataset. Canopy cover and root volume both significantly contributed to the model, $p < 0.0001$, $p = 0.0027$, respectively, for the sample dataset, and ($p < 0.0001$, $p = 0.0135$, respectively) for the validation dataset.

Canopy cover, canopy cover squared, initial RCD, and the interaction between initial RCD and canopy cover explained 50% of the variation in 2010 RCD growth using the sample data set ($p < 0.0001$). This model was significant when used on the validation data ($p = 0.0055$), however only canopy cover was significant and thus the other variables were dropped. The revised model explained 39% of the variation in the sample dataset (model $p = < 0.0001$), and 16% of the variation in the validation dataset ($p = 0.0013$).

Budbreak

First year budbreak ratings did not differ among silvicultural treatments ($p = 0.3517$), or families ($p = 0.9891$), but did differ among species ($P < 0.0001$; Table 2-12). Chinese seedlings were found to break bud earlier than hybrid and American seedlings, and hybrid seedling broke bud earlier than American seedlings. Budbreak ratings differed among the five dates ($p < 0.0001$; Table 2-36), with each successive date increasing in budbreak rating. The interaction of date by species ($P < 0.0001$) was significant (Figure 2-18).

Again in 2010, budbreak ratings differed among species ($p < 0.0001$; Table 2-12), and not silvicultural treatments ($p = 0.6449$). Ratings increased significantly over each of the four measurement dates ($p < 0.0001$; Table 2-36). Species by date interaction was significant ($p < 0.0001$; Figure 2-19).

Table 2-12: Budbreak rating averaged over all seedlings and dates among each species for 2009 and 2010. Species means within year followed by the same letter are not significantly different ($\alpha = 0.05$).

Species	**2009**	**2010**
American	1.57 c ± 0.09	2.90 c ± 0.26
Hybrid	2.17 b ± 0.10	3.76 b ± 0.27
Chinese	3.34 a ± 0.15	5.71 a ± 0.32

Effect of Seedling Size

Survival at the end of the 2009 growing season was greatest for seedlings in RCD class 2 (medium) and 3 (large) ($p = 0.0401$; Table 2-13). RCD class did not affect 2010 survival or two-year survival ($p = 0.3515$, 0.4099, respectively; Table 2-13).

Table 2-13: 2009, 2010, and two-year survival (percent) among RCD classes. Class means within year followed by the same letter are not significantly different ($\alpha = 0.05$).

RCD Class	**2009**	**2010**	**Two-year**
1 (5.3 – 10.0 mm)	57 b ± 6	71 a ± 6	41 a ± 5
2 (10.1 – 12.5 mm)	64 ab ± 5	78 a ± 5	46 a ± 5
3 (12.6 – 20.9 mm)	72 a ± 5	68 a ± 5	48 a ± 5

Dieback in 2009 did not differ among RCD classes ($p = 0.1548$), but did differ among RCD class in 2010 ($p = 0.0149$). Fewer large (class 3) seedlings died back (14%) than either class 2 (40%), or class 1 (31%). RCD class did not affect frequency of two-year dieback ($p = 0.0670$).

Initial height, 2009 and 2010 height, and 2009 and 2010 RCD differed significantly among RCD classes ($p = <0.0001$ for each; Table 2-14 and 2-15). Large seedlings added the most height growth in 2010 and over the two-year period since planting ($p = 0.0015$, $p = 0.0012$, respectively; Figure 2-17). Large and medium seedlings added the most RCD growth in 2010 ($p < 0.0001$; Table 2-15).

Table 2-14: Mean 2009 and 2010 height in centimeters among RCD classes Class means within each column followed by the same letter are not significantly different (α = 0.05).

RCD class	**Initial**	**2009**	**2010**
1 (5.3 – 10.0 mm)	79 c ± 1	82 c ± 3	86 c ± 4
2 (10.1 – 12.5 mm)	95 b ± 1	100 b ± 2	101 b ± 4
3 (12.6 – 20.9 mm)	111 a ± 1	112 a ± 2	132 a ± 4

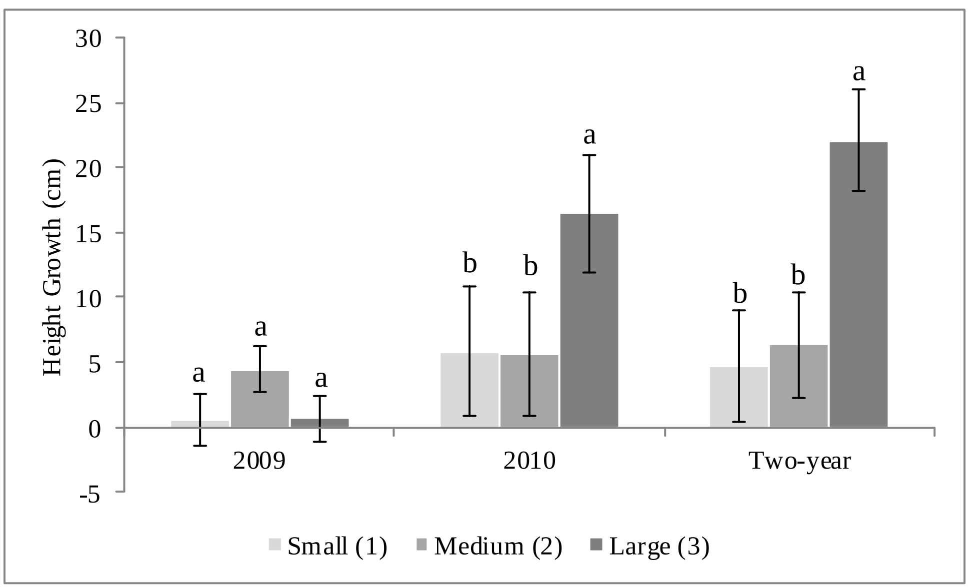

Figure 2-17: Mean 2009, 2010, and two-year height growth among RCD classes. Error bars indicate standard error. Treatment bars within each growth period with the same letter are not significantly different (α = 0.05).

Table 2-15: Mean 2009 and 2010 RCD, and 2010 RCD growth in milimeters among height class treatments. Class means within each column followed by the same letter are not significantly different (α =0.05).

RCD Class	2009 RCD	2010 RCD	2010 RCD Growth
1 (5.3 – 10.0 mm)	8.1 c ± 0.2	9.1 c ± 0.7	1.5 b ± 0.6
2 (10.1 – 12.5 mm)	10.3 b ± 0.2	11.9 b ± 0.6	2.4 ab ± 0.6
3 (12.6 – 20.9 mm)	12.8 a ± 0.2	15.1 a ± 0.6	2.9 a ± 0.6

Biotic Stressors

Four American chestnuts in 2009 and five in 2010 demonstrated evidence of blight fungus. Due to the high mortality in 2009, and symptoms associated with *Phytophthora cinnamomi*, withered leaves and shriveled black roots, we sent five dead or dying seedlings samples during the 2009 growing season to be tested for *P. cinnamomi* (Dr. Steven Jeffers, Clemson University). All five seedlings tested positive for the pathogen.

Chestnut sawfly predation did not significantly affect 2010 survival or any height or RCD variables in 2009 or 2010 (Table 2-38).

Discussion

Survival

Seedling survival was lower than expected for the American and hybrid chestnut seedlings in this study (41% and 49%, respectively). While low survival has been a problem for northern red oak forest plantings (Kormanik, et al. 1997), survival rates of 80% or greater for plantings using high quality NRO seedlings is not uncommon (Dey and Parker 1997a; Jacobs, et al. 2005; Johnson 1984; Teclaw and Isebrands 1993). Survival of chestnut forest plantings has varied. Rhoades, et al. (2009) recorded 57% survival of American chestnut seedlings planted under two silvicultural treatments in Kentucky; McNab (2003) recorded 66% second-year survival of American chestnut seedlings planted in clearcut and control sites; and Clark, et al. 2011, recorded 80 - 93% survival among a mix of chestnut species and hybrid generations planted in Virginia on well-drained sites, and 44% for a planting on a site with a high-water table. McCament and McCarthy (2005) reported low two-year survival rates (44% - 51%) for a planting of American chestnut in Ohio. The low rates of survival of American chestnuts presented in these studies were due, in part, to chestnut blight fungus. Very few trees in our study, however, were infected with chestnut blight. We believe the high mortality of American and hybrid chestnuts was due to presence of *Phytophthora cinnamomi* Rands at our study site. Reports of unusual chestnut mortality in the southern United States began to appear in the early 1800s (Anagnostakis 2006). At the time, the causal agent was unknown, yet the disease symptoms were well described (Hough 1882). More recent studies have shown that chestnuts in bottomland or poorly drained sites were being killed by ink disease, *P. cinnamomi* (Crandall, et al. 1945), a soil-borne Asian oomycete that attacks and kills the root systems of American chestnut and the related Allegheny

[*Castanea pumilla* (L.) Mill var *pumilla*) and Ozark [*Castanea pumilla* var. *ozarkensis* (Ashe) Tucker] chinquapins. Chinese chestnuts, in general, are more resistant to *P. cinnamomi* than American chestnuts (Anagnostakis 2006). Ink disease was most likely transported to the southern United States before 1824, and caused significant loss of chestnut in the Gulf and Atlantic states (Anagnostakis 2006). Evidence of *P. cinnamomi* and the greater incidence of mortality among American and hybrid compared to Chinese chestnut seedlings (60%, 52%, and 10%, respectively), leads us to believe that mortality among the American and hybrid chestnuts was caused in part by ink disease. While nursery seedlings may carry ink disease to new locations, soil tests located at least five meters from our planting transects also tested positively for *P. cinnamomi*, indicating the pathogen was already present on the sites. This is troubling, as *P. cinnamomi* has not commonly been found on well-drained upland sites.

Growth

Height growth did not differ among chestnut species, however more Chinese and American chestnut seedlings experienced height dieback during the first year than hybrid seedlings. This may indicate heterosis of the two hybrid families tested compared to the American and Chinese chestnuts. The viability of acorns, and likely chestnuts, is sensitive to excessive drying, freezing, and heating (*cf.* Bonner 1984). Differential seed handling prior to planting may have contributed to the superior survival and growth rates demonstrated by family SA 417.

Chinese chestnut seedlings added more RCD growth than did the American and hybrid seedlings. This may have been caused by the larger initial size, in height and RCD, of the

Chinese chestnut seedlings. Furthermore, the Chinese chestnut seedlings used in our study were grown in a different nursery than the American and hybrid chestnut seedlings, which may have caused the initial larger size of the Chinese chestnut seedlings and subsequent superior RCD growth. Clark, et al. (in press) found that Chinese chestnut seedlings grew less in the first year after planting than did American and hybrid (BC_3F_3) seedlings. It is possible that the Chinese chestnut seedlings in our study will slow in growth, compared to the American and hybrid chestnuts, as the nursery effect diminishes. Schlarbaum, et al. (1992) evaluated 13 Chinese and Asian hybrid chestnut plantings established before 1960 and found that most demonstrated poor survival and poor growth compared to the competing hardwood overstory, indicating that Chinese chestnut does not compete well in North American forests.

Hybrid family SA 417 demonstrated superior survival compared to family SA 330 over the course of this study. This may indicate a genetic difference between the two families, or again may reflect differential seed handling prior to planting. Greater survival of SA 417 likewise may indicate superior resistance to *Phytophthora cinnamomi*. Additional studies are necessary to test if true genetic differences exist between the two back-cross hybrids. If SA 417 is indeed more resistant to *Phytophthora cinnamomi* than SA 330, SA 417 will be a better family to plant in areas where the pathogen is already established or may become established.

Silvicultural Treatments

Overall, chestnut seedlings growing in the SW treatments added the most height and diameter growth. The competition parameters that differed most significantly among silvicultural treatments were canopy cover and available PAR, which were least and greatest (respectively) in SW sites. Chestnut growth has been found to correlate strongly with light (Boring 1981; Griffin 1989; Latham 1992; Billo 1998; McCament and McCarthy 2005; Paillet 1984; Rhoades, et al. 2009). Our results agree with these studies. While the SW treatment had the greatest amount of available light at the time of measurement, this treatment also had the greatest number of understory hardwood stems, a large proportion of which were stump sprouts. Stump sprouts generally have large root systems that enable them to grow more quickly than seedlings of the same species (Bond and Midgley 2001). Available light in the SW sites will presumably decline as the competing sprouts continue to grow. Although American chestnut has been found to grow as fast as or faster than many hardwood competitors (Ashe 1911; Frothingham 1924; Hawes and Hawley 1912; Jacobs and Severeid 2004; Latham 1992; Mattoon 1909), it is not known if planted chestnut seedlings can successfully compete with hardwood stump sprouts. McNab (2003) found that planted chestnut seedlings were outgrown by competing hardwood sprouts in clearcuts. Initial seedling height in that study, however, averaged 18 cm, while seedlings in the present study averaged 96 cm at planting. The large size of these seedlings will likely enable them to compete more effectively with fast-growing sprouts, as has been found with high quality NRO oak seedlings (Teclaw and Isebrands 1993).

Chestnut seedlings growing in the OS treatment sites added the least growth, on average, during the first two years after planting. American chestnut can survive in the forest understory for long periods of time and then quickly respond when light becomes available (Paillet 2002). In several years the overstory will be removed from the OS treatment sites, greatly increasing the amount of light available to the chestnut seedlings. OS treatments had fewer competing seedlings in the understory at the time of our study. While release in the OS treatment will likely result in the regeneration of many hardwood sprouts, if they are not treated, the chestnuts in this treatment will have had several years to establish, compared to those in the SW sites that were planted among stump sprouts. Based on chestnut's ability to rapidly respond to increases in light, the planted chestnuts should successfully compete with other seedlings and stump sprouts and become dominant stems in the successive stand.

The TH sites presented intermediate light conditions; less light was available than in the SW sites, and more light was available than in OS sites. While SW and TH sites had similar numbers of competing stems in the understory, growth was two-times greater in the SW treatment sites than in the thinned sites. The moderate TH harvest may have made enough light available to favor stump sprouts, with large established root systems, but not enough light to optimize transplanted chestnut seedling growth. Without a future release, the chestnut seedlings in the TH treatment may not successfully compete with fast-growing sprouts, particularly those of red maple and yellow poplar. In the event of a future release, the established chestnut seedlings will likely respond vigorously to increased light availability and become dominant stems in the successive canopy.

Above-ground Competition

Chestnut growth was most strongly related to the two light variables tested in our study: canopy cover and available PAR. While PAR is a direct, and canopy cover an indirect, measurement of available light, our results suggest that canopy cover in fact was more closely related than PAR to growth. The relatively long distance (up to 4 km) between seedling sites, where understory PAR was measured, and the field where overstory (continuous) PAR was measured may have reduced the accuracy of available PAR. While we tried to avoid taking measurements while clouds were overhead, it is possible that clouds that were blocking light at the overstory PAR measurement site may not have been simultaneously blocking PAR at the understory sites.

We found canopy cover to be a relatively strong predictor of available PAR ($R^2 = 0.66$ in 2009 and 0.62 in 2010). Lhotka and Loewnstein (2005) also found that canopy cover predicted light transmittance relatively accurately ($R^2 = 0.68$). Buckley, et al. (1999) found canopy cover to have a stronger predictive ability than we did ($R^2 = 0.93$). That study, however, averaged PAR measurements among multiple plots, decreasing the effect of each PAR measurement on the site average. Our results indicate that canopy cover, measured with a spherical densitometer, is a relatively accurate measurement of available light.

Below-ground Competition

Our stomatal conductance and foliar chlorophyll results suggest that silvicultural treatments differed in below-ground resource availability, however, correlation and regression results indicate that chestnuts responded most favorably to decreases in canopy cover, and increases in available PAR. Growth was only weakly affected by variation in soil moisture (measured by stomatal conductance); and not at all affected by variation in soil nutrients (measured by foliar chlorophyll and carotenoid levels). This concurs with other studies that have found that chestnut growth is more strongly related to light than available nutrients or moisture (Latham 1992, McCament and McCarthy 2005). These studies have found that chestnut is positively related to nutrient availability, specifically soil nitrogen (McCament and McCarthy2005; Rhoades, et al. 2009), magnesium and potassium (McCament and McCarthy 2005), however growth was more strongly related to light than nutrient or water availability. Rhoades et al.'s (2009) study evaluated growth of planted chestnuts under two silvicultural treatments (shelterwood and midstory removal), each containing two soil types: mesic soils (more available nutrients and soil water) and xeric soils (fewer available nutrients and less soil water). They found that chestnut growth was better on mesic shelterwood than xeric shelterwood sites. No differences in growth were found between mesic and xeric midstory removal sites. Trees added the most growth overall in the shelterwood sites, similarly to our trees. Our study did not, however, evaluate growth on different soils within treatments. These results suggest that while chestnut can compete successfully in high-light nutrient-poor environments, growth is best in high-light, high-nutrient environments.

Budbreak

McGee (1986) evaluated fifteen hardwood tree species for timing of budbreak under a canopy and in the open. Twelve species initiated budbreak earlier when growing under a canopy. Difference in budbreak timing may affect long-term survival and success of seedlings. Trees that break bud earlier may experience longer growing seasons, but may also be more susceptible to frost damage. Budbreak did not differ among silvicultural treatments in our study, indicating that shoot growth was initiated at nearly the same time across the treatments. Budbreak did, however, differ among species. Chinese chestnut broke bud first, followed by hybrids, and then by American chestnuts. This consistent pattern, occurring in both 2009 and 2010, may indicate that the hybrid chestnut still retains a portion of the Chinese genome responsible for timing of budbreak. Hebard (1994) also found that Chinese chestnut broke bud earlier than American, and that second backcross hybrids were intermediate in timing of budbreak. He hypothesized that either that different origins of American chestnut parents, or low heritability of leaf emergence caused the earlier than expected development of hybrid buds. Since Hebard's study, Clark, et al. (in press) has also found earlier budbreak in BC_3F_3 hybrid chestnuts compared to American chestnut. Based on these results, it seems likely that the early leaf emergence is a Chinese chestnut trait and is not related to the origin of American parents. While the timing of budbreak did not affect growth in our study, American chestnut is particularly sensitive to frost (Parker et al.1993; Zon 1904) and early leaf emergence could be disadvantageous to hybrid chestnuts in years with late spring frosts.

Impacts of Initial Seedling Size on Survival and Growth

Initial height and initial RCD were more strongly correlated with each other (r = 0.62) than either were with initial root volume. Initial height and RCD were also more strongly related to height and height growth than was root volume. Initial RCD exhibited the strongest relationship to first and second-year RCD, however initial height and root volume were also moderately related to these variables. Based on these findings, initial RCD was the best predictor for future growth. These results agree with Dey and Parker (1997b), who found that of initial northern red oak seedling height, RCD, and first order lateral roots, RCD was most strongly related to two-year stem height (r = 0.29, 0.18, 0.16, respectively). Jacobs, et al. (2005) also found that root volume is a better predictor of second-year stem height than first order lateral roots, but did not compare root volume with initial RCD. Dey and Parker (1997b) hypothesized that RCD is a good predictor for future growth because it is highly correlated with root characteristics that influence seedling performance.

Seedlings in the larger RCD size classes demonstrated better survival and second year height growth than did smaller seedlings. Growth of transplanted seedlings is often slow during the first year after planting due to transplant shock (Struve and Joly 1992). The process of lifting seedlings severs lateral roots, can sever the tap root depending on the depth set on the lifting machine, and kills many of the fine roots (Nambiar 1980). Root loss reduces the ability of seedlings to absorb water and mineral nutrients (Burdett 1990), and causes the loss of carbohydrate and mineral nutrients stored in the roots (Larimer and Struve 2002). It is critical to survival and establishment that seedlings regain root-soil contact by producing new fine roots immediately after planting (Burdett 1990; Grossnickle 2005). The risk of transplant shock increases between budbreak and root regeneration (Stuve and Joly 1992) due to the initiation of transpiration, which demands large quantities of water, creating a stressed condition of physiological drought (Rietveld 1989). The superior survival demonstrated by larger seedlings in this study supports results found by other studies that examine the importance of seedling size to survival and growth to bare-root northern red oak forest plantings (Dey and Parker 1997a; Jacobs, et al. 2005; Kormanik, et al. 1997; Rhoades, et al. 2009). Larger seedlings were presumably able to restore root to soil contact and gain access to soil water and nutrients more rapidly than smaller seedlings.

The results of this study indicate that chestnut can establish successfully under varying light levels, however that they produce the most growth in high light (65% PAR) environments. High light environments also encourage fast-growing seedlings and stump sprouts, however after two-years chestnuts in the SW sites were able to compete successfully, based on growth and survival, with advanced regeneration. Seedlings with large RCD at planting demonstrated better survival and growth than smaller seedlings across all silvicultural treatments. These findings suggest that large 1-0 nursery-grown chestnut seedlings, at least 14 mm in RCD and 110 cm in height, should be used in backcross hybrid chestnut forest plantings.

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Appendix 1

Table 2-16: Distribution of seedlings among experimental units. Treatment indicates silvicultural treatment.

Treatment	No. sites	No. blocks/treatment	No. blocks/site	No. trees/site	No. trees/treatment
OS	6	50	8-9	50-55	250
TH	4	50	12-13	60-65	250
SW	5	50	10	50	250

Equation 2-1: 2009 equations to smooth full sunlight PAR: y = full sunlight, x= minutes into the day, 0 = midnight.

Date	Equation
June 22	$y = 1024105.25 + 3034.22(x) + 2.00(x)^2 = 0.001(x)^3$
June 23 (when cloudy)	$y = 2392.27 – 1.46(x)$
June 23 (when cloud-free)	$y = 117.86 + 1.05(x)$
June 24	$y = -9132.95 + 27.18(x) = 0.01(x)^2$
June 25	$y = -5874.50 + 17.55(x)– 0.01(x)^2$

Equation 2-2: 2010 equations to smooth full sunlight PAR: y = full sunlight, x = minutes into the day, 0 = midnight.

Date	Equation
July 12	$Y = y = -34769 + 88.20(x) – 0.06(x^2)$
July 14	$Y = 695.92 + 7.96 X -0.01 X^2$
July 15	$Y = -2240.20 + 4.94 X$

Table 2-17: Silvicultural treatments, sites and number of seedlings per site sampled for canopy cover measurements in 2009 and 2010.

Silv.	Site	No. 2009	No. 2010
OS	3	40	24
	10	45	34
	14	40	27
	24	40	25
TH	21	65	31
	25	60	34
	33	60	34
SW	2	50	30
	12	50	14
	16	50	34
Total		500	292

Table 2-18: Silvicultural treatment, sites and number of seedlings sampled for chlorophyll and carotenoid measurements in 2009 and 2010

Silv.	**Site**	**No. Seedlings 2009**	**No. Seedling 2010**
OS	3	16	11
	24	16	12
TH	21	15	9
	25	14	20
SW	2	17	16
	29	16	11
Total		94	79

Table 2-19: Silvicultural treatment, sites and number of seedlings sampled for stomatal conductance (transpiration) in 2009 and 2010.

2009			**2010**		
Silv.	**Site**	**No. Seedlings**	**Silv.**	**Site**	**No. Seedlings**
OS	10	34	OS	3	23
	24	25		10	33
	27	24		14	20
TH	25	37		24	25
	21	29	TH	25	37
SW	12	15		18	49
	29	22	SW	2	29
	35	16		16	31
				29	20
Total		202			267

Table 2-20: Silvicultural treatment, sites, and number of seedlings sample for budbreak ratings in 2009 and 2010.

		2009	2010
Silv.	**Site**	**No. Samples**	**No. Samples**
OS	10	45	33
	24	40	24
TH	18	65	55
	29	65	23
SW	2	50	31
	29	50	23
Total		215	189

Table 2-21: 2009, 2010, and two-year mean (± standard error) percent survival for each hybrid family. Family means within column followed by the same letter are not significantly different ($\alpha = 0.05$).

Family	**2009**	**2010**	**Two-year**
SA 330	52 b ± 6	68 a ± 7	35 b ± 5
SA 417	82 a ± 4	77 a ± 5	62 a ± 5

Table 2-22. 2009, 2010, and two-year mean (± standard error) percent survival among silvicultural treatments. Treatment means within each column followed by the same letter are not significantly different (α =0.05).

Treatment	**2009**	**2010**	**Two-year**
OS	82 a ± 5	83 a ± 5	68 a ± 6
TH	81 a ± 6	84 a ± 5	71 a ± 7
SW	80 a ± 7	73 a ± 7	55 a ± 7

Table 2-23: Mean (± standard error) 2009, 2010, and two-year height growth in centimeters among silvicultural treatments. Treatment means within each column followed by the same letter are not significantly different (α = 0.05).

Treatment	**2009**	**2010**	**Two-year**
OS	6 a ± 1	8 c ± 5	12 b ± 5
TH	8 a ± 1	20 b ± 5	24 b ± 5
SW	9 a ± 1	34 a ± 5	40 a ± 5

Table 2-24: Mean (± standard error) 2009, 2010, and two-year height growth in centimeters among species. Species means within column followed by the same letter are not significantly different (α = 0.05).

Species	**2009**	**2010**	**Two-year**
American	7 a ± 1	21 a ± 4	24 a ± 4
Hybrid	8 a ± 1	15 a ± 3	22 a ± 3
Chinese	7 a ± 1	27 a ± 3	30 a ± 4

Table 2-25: Mean (± standard error) 2009, 2010, and two-year height growth in centimeters for each backcross hybrid chestnut family. Family means within each column followed by the same letter are not significantly different (α = 0.05).

Family	2009	2010	Two-year
SA 330	9 a ± 1	13 a ± 5	21 a ± 4
SA 417	8 a ± 1	18 a ± 4	22 a ± 3

Table 2-26: Mean (± standard error) 2009 and 2010 RCD and 2010 RCD growth in millimeters for each backcross hybrid chestnut family. Family means within each column followed by the same letter are not significantly different (α = 0.05).

Family	2009 RCD	2010 RCD	2010 RCD Growth
SA 330	10.9 a ± 0.2	12.2 a ± 0.7	2.1 a ± 0.6
SA 417	10.5 a ± 0.2	12.8 a ± 0.6	2.6 a ± 0.5

Table 2-27: Mean (± standard error) 2009 and 2010 RCD and 2010 RCD growth in millimeters among silvicultural treatments. Treatment means within each column followed by the same letter are not significantly different (α = 0.05).

Treatment	2009 RCD	2010 RCD	2010 RCD Growth
OS	10.9 a ± 0.2	11.4 b ± 0.8	0.7 c ± 0.7
TH	11.2 a ± 0.2	12.6 b ± 0.9	2.1 b ± 0.8
SW	11.6 a ± 0.2	16.2 a ± 0.8	5.0 a ± 0.7

Table 2-28: Mean (± standard error) 2009, 2010 and two-year frequency of dieback (percent) for each backcross hybrid chestnut family. Family means within each column followed by the same letter are not significantly different (α = 0.05).

Family	**2009**	**2010**	**Two-year**
SA 330	25 a± 6	36 a± 7	31 a ± 7
SA 417	27 a ± 5	26 a ± 5	23 a ± 5

Table 2-29: 2009 and 2010 mean (± standard error) height of tallest competitor and density of competing seedlings among silvicultural treatments. Treatment means within each column followed by the same letter are not significantly different (α = 0.05).

	2009		**2010**	
Treatment	**Ht. (cm)**	**Density**	**Ht. (cm)**	**Density**
OS	157 a ± 26	10 a ± 1	170 a ± 25	9 b ± 1
TH	125 a ± 28	12 a ± 2	155 a ± 30	12 ab ± 1
SW	136 a ± 25	15 a ± 2	221 a ± 27	16 a ± 1

Table 2-30: 2009 percentage of most abundant species in competition plots.

Species	2009
Red maple (*Acer rubrum* L.)	29
Green Briar (*Smilax* spp. L.)	26
Sassafras [*Sassafras albidum* (Nutt.) Nees]	12
Red oak spp. (*Quercus* spp. L.)	8
Blackberry (*Rubus* spp. L.)	5
Hickory spp. (*Carya* spp. Nutt.)	4

Table 2-31: 2010 percentage of most abundant species in competition plots.

Species	2010
Red maple (*Acer rubrum* L.)	42
Sassafras [*Sassafras albidum* (Nutt.) Nees]	13
Yellow poplar (*Liriodendron tulipifera* L.)	12
Red oak spp. (*Quercus* spp. L.)	10
Hickory spp. (*Carya* spp. Nutt.)	6
Sourwood [Oxydendrum arboreum (L.) DC.]	2

Table 2-32: 2009 and 2010 mean (± standard error) stomatal conductance (µg cm^{-1} S) among species. Species means within each column followed by the same letter are not significantly different (α = 0.05).

Species	**2009**	**2010**
American	5.56 a ± 0.45	5.52 a ± 0.32
Hybrid	5.23 a ± 0.42	5.40 a ± 0.31
Chinese	5.27 a ± 0.43	5.20 a ± 0.30

Table 2-33: 2009 mean (± standard error) chlorophyll a, b, and total chlorophyll (a + b) among species. Units are mg pigment per gram fresh leaf tissue. Species means within each column followed by the same letter are not significantly different (α = 0.05).

Species	**Chlorophyll a**	**Chlorophyll b**	**Total chlorophyll**	**Carotenoids**
American	0.60 a ± 0.06	0.27 a ± 0.03	1.01 a ± 0.13	0.73 a ± 0.07
Hybrid	0.51 a ± 0.04	0.21 a ± 0.02	0.76 a ± 0.05	0.64 a ± 0.05
Chinese	0.45 a ± 0.04	0.19 a ± 0.02	0.70 a ± 0.06	0.68 a ± 0.05

Table 2-34: Mean (± standard error) 2010 chlorophyll a, b, total (a + b), and carotenoids, among silvicultural treatments. Units are mg pigment per gram fresh leaf tissue. Treatment means within each column followed by the same letter are not significantly different ($\alpha = 0.05$).

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
OS	0.56 a ± 0.05	0.23 a ± 0.03	0.94 a ± 0.09	0.68 a ± 0.07
TH	0.47 a ± 0.04	0.21 a ± 0.02	0.72 a ± 0.08	0.62 a ± 0.6
SW	0.52 a ± 0.05	0.23 a ± 0.02	0.81 a ± 0.09	0.76 a ± 0.07

Table 2-35: Mean (± standard error) 2010 chlorophyll a, b, total (a + b), and carotenoids among species. Units are mg pigment per gram fresh leaf tissue. Species means within each column followed by the same letter are not significantly different ($\alpha = 0.05$).

Treatment	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
American	0.60 a ± 0.06	0.27 a ± 0.03	1.01 a ± 0.13	0.73 a ± 0.07
Hybrid	0.51 a ± 0.04	0.21 a ± 0.02	0.76 a ± 0.05	0.64 a ± 0.05
Chinese	0.45 a ± 0.04	0.19 a ± 0.02	0.70 a ± 0.06	0.68 a ± 0.05

Table 2-36: 2009 and 2010 mean (± standard error) budbreak ratings among dates. Means within each column followed by the same letter are not significantly different (α = 0.05)

Date	2009 Rating	Date	2010 Rating
3/31/09	0.55 e ± 0.10	3/29/10	0.61 d ±0.24
4/8/09	1.45 d ± 0.10	4/5/10	2.49 c ± 0.24
4/17/09	2.17 c ± 0.10	4/11/10	5.27 b ±0.24
4/24/09	2.99 b ± 0.10	4/19/10	8.14 a ± 0.24
5/5/09	4.62 a ± 0.10		

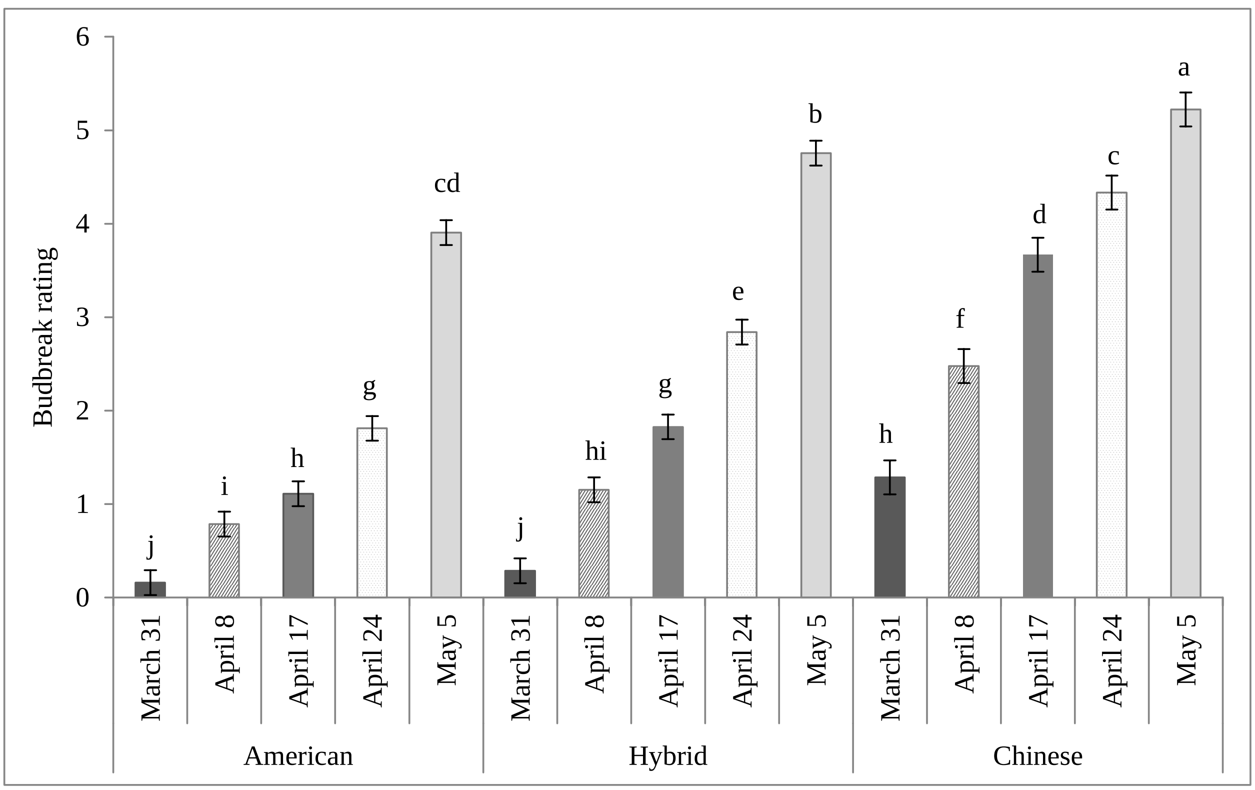

Figure 2-18: 2009 budbreak rating species by date interaction. Error bars indicate standard error. Treatment bars with the same letter are not significantly different (α = 0.05).

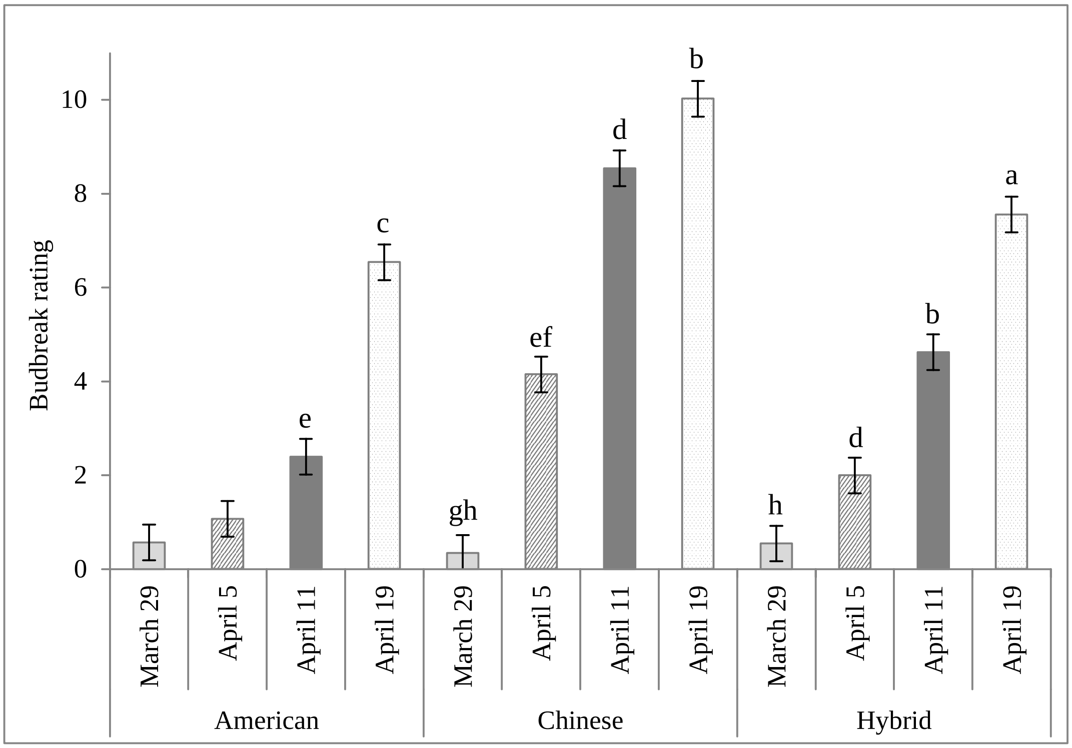

Figure 2-19: 2010 budbreak rating species by date interaction. Error bars indicate standard error. Treatment bars with the same letter are not significantly different (α = 0.05).

Table 2-37: Mean (± standard error) 2009, 2010, and two-year height growth in centimeters among height classes. Class means within year followed by the same letter are not significantly different (α = 0.05).

RCD class	**2009**	**2010**	**Two-year**
1 (5.3 – 10.0 mm)	1 a ± 2	6 b ± 5	5 b ± 4
2 (10.1 – 12.5 mm)	4 a ± 2	6 b ± 5	6 b ± 4
3 (12.6 – 20.9 mm)	1 a ± 2	16 a ± 5	22 a ± 4

Table 2-38: Mean (± standard error) 2009, 2010 height; 2009, 2010, and two-year height growth; 2009 and 2010 RCD; 2010 RCD growth, and 2010 survival for seedlings with and without evidence of insect predation. Means are followed by p-values. Means within each row followed by the same letter are not significantly different (α = 0.05).

Variable	**Predation evident**	**Predation non evident**
2009 height (cm)	100 a ± 2 (0.4354)	102 a ± 2
2009 height growth (cm)	2 a ± 2 (0.9079)	1.87 a ± 1.68
2009 RCD (mm)	10.4 a ± 0.2 (0.9702)	10.4 a ± 0.1
2010 survival (%)	75 a ± 5 (0.7060)	83 a ± 4
2010 height (cm)	100 a ± 4 (0.1282)	108 a ± 4
2010 height growth (cm)	5 a ± 4 (0.9550)	8 a ± 4
2010 RCD (mm)	11.3 a ± 0.5 (0.5668)	11.4 a ± 0.4
2010 RCD growth (mm)	1.7 a ± 0.6 (0.7030)	2.0 a ± 0.5
Two-year height growth (cm)	7 a ± 4 (0.0969)	17 a ± 3

Chapter 3: Incidence of *Craesus castaneae* (Hymenoptera: Tenthredinidae) on Chestnut Seedlings Planted on the Daniel Boone National Forest, Kentucky

This chapter is based on a paper written by Cornelia C. Pinchot, Scott E. Schlarbaum, Arnold M. Saxton, Stacy L. Clark, Callie J. Schweitzer, David R. Smith, Alex Mangini and Frederick V. Hebard:

Pinchot, C.C., Schlarbaum, S.E., Saxton, A.M., Clark, S.L, Schweitzer, C.J., Smith, D.R., Mangini, A., and Hebard, F.V. 2011. Incidence of *Craesus castaneae* (Hymenoptera: Tenthredinidae) on Chestnut Seedlings Planted in the Daniel Boone National Forest, Kentucky. Journal of Entomological Science. 46(3): 265-268.

My primary contributions to this paper included: (i) development of experiment and objectives, (ii) gathering and analyzing and interpreting data and (iii) manuscript preparation.

Abstract

American chestnut [*Castanea dentata* (Marsh.) Borkh], hybrid chestnut (BC_2F_3 generation), and Chinese chestnut (*Castanea mollissima* Blume) 1-0 bare-root seedlings were planted in the Daniel Boone National Forest in southeastern Kentucky in March, 2009. Seedlings were planted under three silvicultural treatments ranging from high light to low light conditions. Severe leaf predation by C*raesus castaneae* larvae was noticed on chestnut seedlings and presence/absence of leaf predation was recorded towards the end of the growing season. PROC GLIMMIX in SAS 9.2 (SAS Institute, Cary, NC) was used to conduct an analysis of variance on the presence/absence data using a binomial distribution. Seedlings in the lower light conditions experienced greater predation incidence than seedlings growing in the high light treatment.

Additionally, American and hybrid seedlings exhibited greater predation frequency than did Chinese chestnut seedlings. Site selection for restoration plantings should consider the potential impact of chestnut sawfly.

Introduction

American chestnut was a dominant forest tree in the eastern forests of the U.S. until it was eliminated as a canopy tree species by two exotic pathogens. Ink disease, a root rot caused by *Phytophthora cinnamomi* Rands (Pythiales: Pythiaceae), began to destroy chestnut populations on bottomland and poorly-drained sites in the mid-1800s, and the chestnut blight fungus [*Cryphonectria parasitica* (Murrill) Barr, Diaporthales: Cryphonectriaceae] reduced the species to short-lived sprouts on upland sites in the first half of the 20^{th} Century (*cf.* Campbell and Schlarbaum 2002). Various organizations have employed a backcross breeding approach to integrate blight resistance from Asiatic chestnut species into American chestnut in an effort to restore the species to eastern forests (Anagnostakis 2005; Hebard 2001). Putatively blight-resistant hybrid chestnuts became available for planting in 2008 (Clark, et al. 2011).

American chestnut was eliminated from eastern forests before the species' silvics were clearly defined, leaving the silvicultural parameters important to the initial stages of restoration largely unknown. Consequently, an increasing number of studies have examined the silvicultural requirements for chestnut restoration (Anagnostakis 2007; Clark, et al. 2011; Jacobs and Severeid 2004; McCament and McCarthy 2005; McNab 2003; Schlarbaum, et al. 2006; Rhoades, et al. 2009). These experiments will further our understanding of the silvicultural requirements of chestnut, but may also yield information on other factors critical to its successful establishment**.** Aside from ink and chestnut blight diseases, other exotic organisms such as the chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu, Hymenoptera: Cynipidae) and the Asian ambrosia beetle (*Xylosandrus crassiusculus* Motschulsky, Coleoptera: Curculionidae) have become resident pests in eastern forests and may affect restoration (Anagnostakis 1997; 2006; Oliver and Mannion 2001). Little is known, however, about native pests affecting American chestnut, particularly in the seedling phase. Native pests, such as the twolined chestnut borer (*Agrilus bilineatus* Weber, Coleoptera: Buprestidae) may pose significant challenges to species restoration. Here we report the significant impact of a rarely observed native defoliating insect, *Craesus castaneae* Rohwer, in a silvicultural study of chestnut species and hybrids.

Methods

Three hundred American chestnut, 300 hybrid chestnut (BC_2F_3 generation) (Burnham, et al. 1986; Hebard 2001), and 150 Chinese chestnut (*Castanea mollissima* Blume) 1-0 bare-root seedlings were planted on the Daniel Boone National Forest in southeastern Kentucky in March 2009. Seedlings were planted in a completely randomized design with a split-plot treatment arrangement. Silvicultural treatments were whole plots, and species and family were planted in a randomized block design in the sub-plots. Seedlings were planted under three silvicultural treatments: (1) oak shelterwood (Loftis 1990) that left an average residual basal area of 22 m^2/ha and average 96% canopy cover; (2) thinning to the B-level of Gingrich stocking with an average residual basal area of 18m²/ha and an average 89% canopy cover (Gingrich 1967), and; (3) shelterwood with reserves, with an average residual basal area of 5 m²/hectare and an average 39% canopy cover. The silvicultural treatments were a mixture of regeneration treatments and intermediate stand treatments and created a gradient of light conditions on the forest floor, from high light (shelterwood with reserves) to low light (oak shelterwood) (Schweitzer, et al. 2008). The oak shelterwood treatment was replicated six times, the shelterwood with reserves five times, and the thinning was replicated four times. Seedlings were planted in single line transects in 50 blocks per silvicultural treatment, with two American, two hybrid and one Chinese chestnut seedling per block at a spacing of 2.43 m. Severe herbivory by *C. castaneae* larvae was noticed on both planted chestnut seedlings and naturally-occurring sprouts during the 2009 growing season, specifically June through August (Figure 3-1). Predation ranged from minimal, evident only on the leaf edges, to complete, with only leaf midribs and petioles remaining. The presence/absence of leaf predation was recorded towards the end of the growing season (August 12-15, 2009). Predation presence was recorded for seedlings having at least one leaf with evidence of insect predation. Few instances of leaf predation and no *C. castaneae* larvae were observed during the 2010 field season (April – September), therefore no 2010 defoliation data were recorded. PROC GLIMMIX SAS® 9.2 (SAS Institute, Cary, NC) was used to conduct an analysis of variance on the 2009 presence/absence data using a binomial distribution. Fisher's least significant difference was used to separate treatment means.

Figure 3-1: *Craesus castaneae* consuming an American chestnut sprout.

Results

Craesus castaneae larvae were confirmed to be consuming the leaves of seedlings at the time that defoliation presence/absence data were collected. No other insect species were observed consuming the leaves at this time or during other site visits. Seedlings in the oak shelterwood and thinning treatments experienced greater predation incidence ($p = 0.003$) than seedlings growing in the high light treatment (Figure 3-2). Additionally, American and hybrid seedlings exhibited greater predation frequency ($p = 0.007$) than did Chinese chestnut seedlings (Figure 3-3). No significant interaction between species and silvicultural treatments was found ($p = 0.822$).

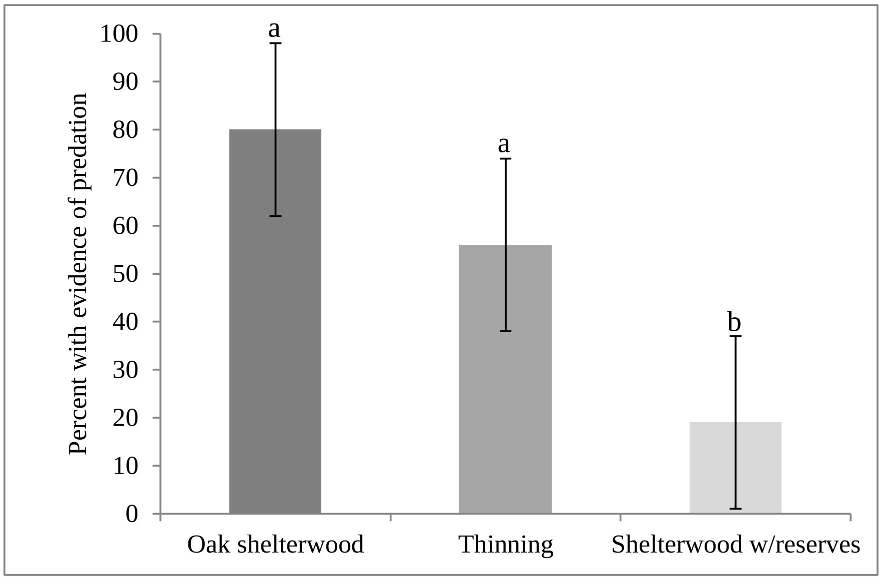

Figure 3-2: Percent of seedlings with evidence of *C. castaneae* predation among silvicultural treatments. Error bars represent standard error. Treatment bars with the same letter are not significantly different (α = 0.05).

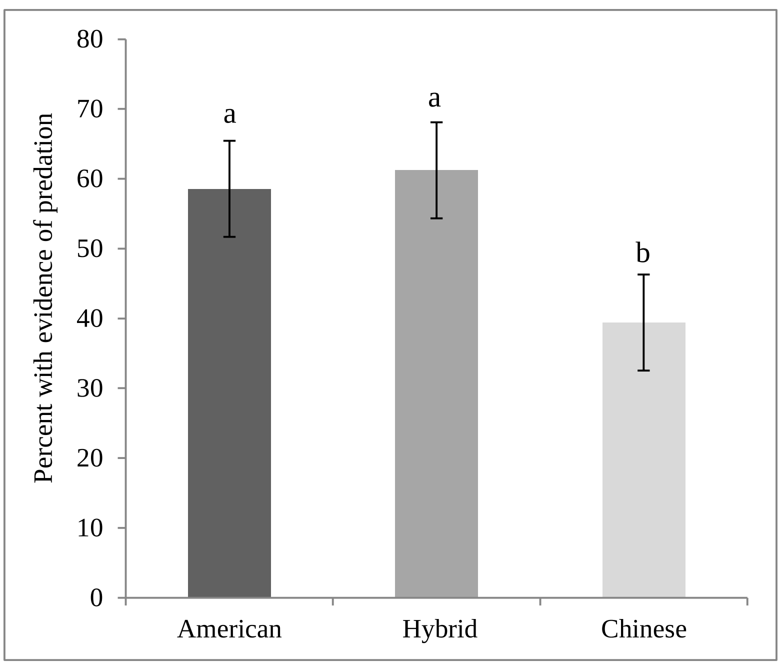

Figure 3-3: Percent of seedlings with evidence of *C. castaneae* predation among species. Error bars represent standard error. Treatment bars with the same letter are not significantly different (α = 0.05).

Discussion

Little information about *C. castaneae* biology and predation habits has been published, probably because the pest became rare with the decimation of American chestnut. *Craesus castaneae* has been previously recorded in Virginia, Maryland, New York, Kentucky, and Pennsylvania (Rohwer 1915; Middleton 1922; Smith 1972). It is described as feeding gregariously on the edges of chestnut leaves during the summer and entering the ground for pupation in early fall. Defoliation from *C. castaneae* may present a challenge to the restoration of American chestnut as seedlings could be weakened or killed due to leaf loss.

Our results show that chestnuts planted in areas with more available light experienced significantly fewer incidences of predation from *C. castaneae* than in areas with less light. Several studies have found that hardwood species, including yellow polar (*Liriodendron tulipifera* L., Magnoliidae: Magnoliaceae), flowering dogwood (*Cornus florida* L., Cornales: Cornaeae), and sessile oak [*Quercus petraea* (Mattuschka) Lieblein, Fagales: Fagaceae], increase production of defense compounds with increased light availability, presumably due to greater carbohydrate production (Dudt and Shure 1994; Kelly 2001; Roberts and Paul 2006). These studies suggest that the lower predation rates found in chestnut seedlings with increased light availability in the present study may result from an increase in defense compound production. Further testing is necessary to assess this theory.

Our results indicate that site selection for restoration plantings should consider the potential impact of *C. castaneae* and that a pest management strategy may be beneficial to ensure seedling establishment. Further research is needed to gain a better understanding of *C. castaneae* biology and how predation on chestnut seedlings affects establishment, growth and survival.

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Chapter 4: Hybrid Chestnut Seedling Establishment on Yale-Myers Research Forest in Northeastern Connecticut

Abstract

Putatively blight-resistant backcross American chestnut (*Castanea dentata*) seedlings, locally adapted to Connecticut, will soon be available for outplanting. This study examines second-year results from a planting of 432 1-0 nursery-grown hybrid chestnut seedlings established in four silvicultural treatments; shelterwood, group selection, thinning, and an uncut control; on the Yale-Myers forest in Northeastern Connecticut. Repeated browsing by deer (*Odocoileus virginianus* Boddaert) and intense competition with hay-scented fern [*Dennstaedtia punctilobula* (Michx.) T. Moore] caused high mortality (63%) among the seedlings. Seedlings in shelterwood sites, however, averaged 30% mortality, compared to 70% and greater morality found in each of the other treatments. Likewise, seedlings growing in the shelterwood sites added more height and RCD growth than those in the remaining treatments. In the group selection, thinning, and control treatments, greater numbers of larger seedlings died, compared to smaller seedlings, while large seedlings exhibited superior survival compared to small seedlings in the shelterwood treatment sites. Shelterwood treatments provide a suitable environment for chestnut plantings. Group selection treatments, with control of fern and other competition, may also be suitable for chestnut. Protection from deer is a critical component of chestnut reintroduction in areas with overabundant deer herds.

Introduction

American chestnut [*Castanea dentata* (Marsh.) Borkh] made up nearly half of Connecticut's standing timber in the late 19th century and was considered the most important hardwood timber tree in the state (Hawes 1906). By 1920, most mature chestnut trees in Connecticut were dead or dying from the chestnut blight fungus (Moss 1973). The Connecticut Agricultural Experiment Station (CAES) responded by initiating a blight-resistance breeding program in 1930, which is currently the oldest chestnut research program in the United States. Early attempts to breed a blight-resistant chestnut with good timber form ultimately failed because the first generation hybrids were backcrossed with an Asian chestnut parent, to increase blight-resistance, but resulted in producing a tree with few American characteristics (Burnham 1988). A new approach to chestnut backcross breeding was initiated in the 1980s (Burnham, et al. 1986). This breeding program incorporates an initial cross between a blight-resistant Asian chestnut and an American chestnut, followed by a series of backcrosses of the resulting F_1 progeny to American chestnut and two additional interbreeding generations, with screening for resistance at each generation. The final breeding generation, BC_3F_3, is projected to have the growth habit of American chestnut and blight resistance of an Asian chestnut species (Burnham 1988). After 25 years of breeding following this method, CAES has produced the BC_3F_2 generation for reintroduction to New England forests (Anagnostakis 2005; Anagnostakis, personal communication, 2011).

Blight resistance, however, is only one aspect of American chestnut reintroduction. Methods for artificial regeneration of the species are not well-established and are an essential component to successful reintroduction (*cf.* Jacobs 2007). Establishing chestnut test plantings now presents an opportunity to develop artificial regeneration and silvicultural recommendations for, and assess potential challenges to, reintroduction prior to the release of blight-resistant material. In addition to considering optimal outplanting and silvical strategies for chestnut, a successful reintroduction plan must consider current landowner objectives. Especially in Connecticut, where 80% of forest lands are privately owned, developing recommendations that integrate forest management practices currently utilized by private landowners will be critical to the successful restoration of American chestnut. The present study evaluates reintroduction success of high-quality chestnut hybrid seedlings established under four silvicultural treatments: shelterwood, group selection, thinning, and an uncut control.

Materials and Methods

Experimental Material

Full-sibling chestnut seedlings (432) from one family representing the BC_3F_2 x BC_2F_3 generation from the Connecticut Agricultural Experiment Station's chestnut breeding program (Anagnostakis 2005) were used in this study. The control pollinated seeds were harvested in the fall of 2007. After stratification, they were manually planted at the Flint River Nursery in Byromville, GA in January, 2008 at a density of 65 nuts per square meter. Fertilization and irrigation of the seedlings followed guidelines developed by Kormanik, et al. (1994). The 1-0 seedlings were lifted in February, 2009 and stored in a cold room (~1° C) until they were outplanted.

Study Site

This study was implemented on the Yale Myers Forest in Windham and Tolland counties in northeastern Connecticut. The forest type is classified as the central hardwoods – hemlock and white pine cover type (Westveld 1956) and is dominated by mixed oak species (*Quercus spp* L), hickory (*Carya spp* Nutt), eastern hemlock [*Tsuga canadensis* (L) Carrière], and sugar maple (*Acer saccharum* Marsh.). Prior to chestnut blight, American chestnut was a dominant tree in this forest type (Westveld 1956).

Silvicultural Treatments

This study was embedded into recently implemented silvicultural treatments: shelterwood harvest, group selection, thinning, and an un-harvested control. The three harvested treatments were established between 2005 and 2009. The group selection treatments were established within thinned stands, and were located in a separate section of the Yale-Myers Forest from the shelterwood and control sites (Figure 4-1).

The shelterwood and group selection treatments were designed to favor growth of oak advanced regeneration. The group selection treatments were established within stands abundant with eastern hemlock in anticipation of hemlock mortality caused by hemlock woolly adelgid (*Adelges tsugae* Annand). Group selection treatments consisted of elliptical gaps, approximately 0.16 hectares in size, that were cut within the thinnings to provide growing space for oak regeneration within the hemlock stands. Gaps that are 0.12 hectares or larger have been found to provide ample light for intolerant species (Dale, et al. 1995). Oak seed trees were left to provide an acorn crop. The thinning treatment left stands thinned to the B-level of Gingrich stocking (Gingrich 1967) and was implemented to increase resource availability to residual stems.

The shelterwood treatments retained individual and group oak trees (17 m^2/ha basal area). The harvest increased available light to advanced regeneration and emergent seedlings, while the shelterwood left sufficient canopy cover to inhibit fast-growing shade-intolerant species. Each paired control was located within 200 meters of a shelterwood harvest replicate (Figure 4-1). Control treatments were left un-harvested.

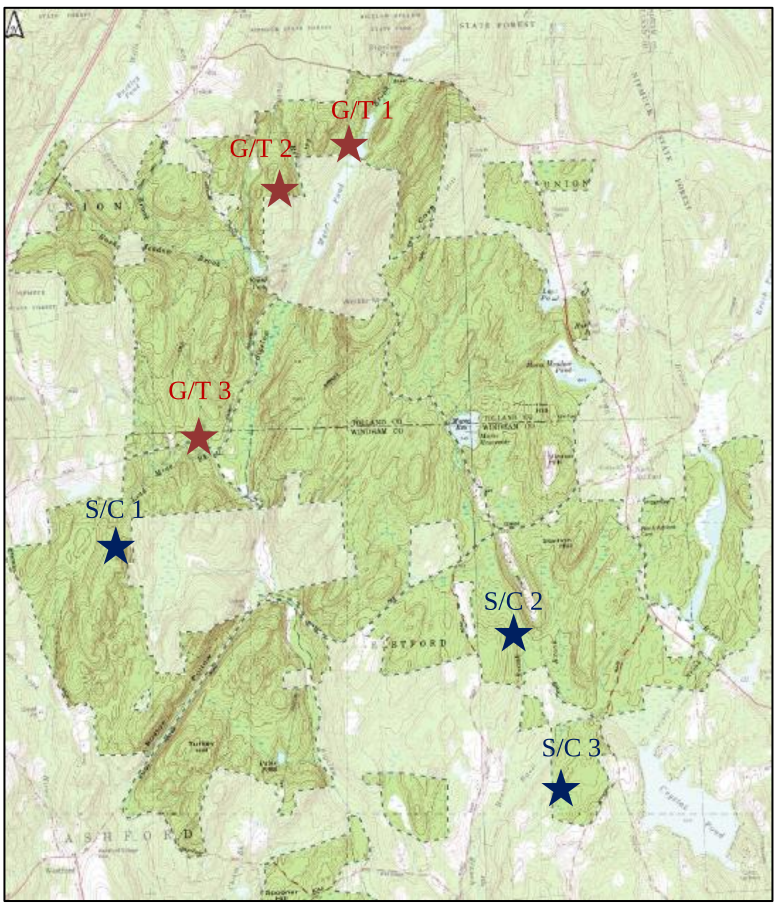
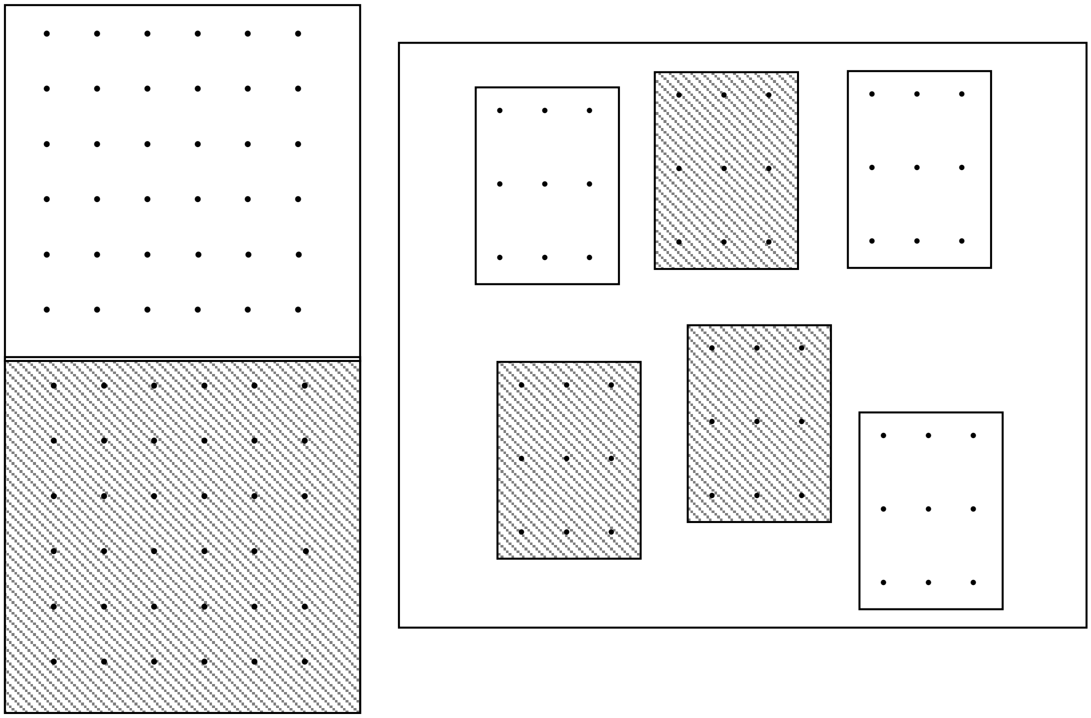


Figure 4-1: Map of study site showing layout of treatments. G/T refers to the group selection and thinning treatments, and S/C refers to the shelterwood harvest and control treatments.

Experimental Design

Seedlings were planted in a completely randomized design with a split plot treatment arrangement. Silvicultural pairings (shelterwood with paired control, group selection with paired thinning) were whole plots and the four individual silvicultural treatments (shelterwood, group selection, thinning, and control) constituted the sub-plots. Each silvicultural pairing was replicated in three locations. Each of the replicates consisted of either a shelterwood plot (1) with a paired control plot (1), or group selection plots (3) with paired thinning plots (3) (Figure 4-2). Paired treatments at each replicate were located within 200 meters of each other. Each shelterwood treatment and paired control plot consisted of 36 trees arranged in a 6 tree by 6 tree square. Each of group selection treatment replicates consisted of three group selection and three thinned plots, with 12 trees arranged in a 4 tree by 3 tree rectangle in each plot. Group selection planting plots had fewer trees than shelterwood plots due to the relatively small gap size in the group selection treatments (0.16 hectares). Seedlings were planted in 2.43 m by 2.43 m spacing. Seedlings were planted April 3-4, 2009 using a Jem Gem KBC© bar, modified by adding five cm to each size of the blade, creating a blade 15 cm at the top, tapering to the tip.



A B

Figure 4-2: Each shelterwood pairing replicate (A), consisted of two plots: a shelterwood plot and a control plot, with 36 chestnuts planted in each. Each group selection pairing replicate (B), consists of six plots: three group selection plots and three thinned plots, with 12 trees planted in each plot. Each dot (•) in the figures below represents one seedling. Shaded plots represent control plots (for shelterwood replicates) and thinned plots (for group selection replicates).

Measurements

Seedling height and root collar diameter (RCD) were measured immediately after planting and at the end of the 2009 and 2010 growing seasons. In 2009, a convex spherical densitometer was used to estimate canopy cover above each seedling (Lemon 1956) within four randomly selected treatment units, representing two shelterwood and two group selection pairings. Readings were taken on the south side of each seedling in each of the four ordinal directions and averaged (Buckley, et al. 1999).

Statistical Analyses

To evaluate the effect of initial seedling size on survival and growth, seedlings were categorized after planting by initial height. Seedlings were divided into three height classes, each with a similar number of observations: 1) 24 – 70 cm, 2) 71-100 cm, and 3) 101-200 cm (Table 4-1). Initial height size class was used as a fixed treatment variable.

A mixed model analysis of variance (PROC MIXED) was used to detect the effects of silvicultural pairing (shelterwood and paired control, group selection and paired thinning), silvicultural treatment (shelterwood, group selection, thinning, control), and height class on yearly 2010 mortality, height change (calculated by subtracting planting height from end of season heights), RCD, and canopy cover. The effect of height class on 2009 and 2010 height was also tested. RCD growth was not tested due to inconsistencies in location of RCD measurements on the stem taken at planting and those taken at the end of the 2009 and 2010 growing seasons.

All analyses for this study were processed using SAS 9.2 software (SAS Institute, 2007). PROC MIXED was used to conduct an analysis of variance (ANOVA) on continuous variables. Any significant main effects ($\alpha = 0.05$) were further analyzed using least significant difference tests for means comparisons. PROC GLIMMIX was used to conduct an analysis of variance on mortality (alive or dead) using a binomial distribution. All residuals satisfied tests of normality and equal variance.

Table 4-1: Height range, mean initial height and RCD, and number of seedlings planted of each height class.

Ht class	Ht Range (cm)	Ht (cm)	RCD (mm)	N
1 (24 -70 cm)	24 -70	55	8	70
2 (71 – 100 cm)	71 - 100	86	10	178
3 (101 – 200 cm)	101 - 200	118	13	184

Results

Mortality

Few seedlings died in 2009 (Figure 4-3, Table 4-5 and 4-6 in appendix 2). Silvicultural treatment pairings (shelterwood and paired control, group selection and paired thinning) did not differ in rates of mortality ($p = 0 .6939$). Within pairings, shelterwood control sites experienced greater mortality (5.62%) than did shelterwood sites (0%; $p = 0.0060$), and thinning sites suffered greater mortality (7.62%) than did group selection sites (1.26%; $p = 0.0060$).

Sixty-three percent of the seedlings had died by the end of the 2010 growing season (Figure 4-3; Table 4-7 and Table 4-8 in appendix). Again, silvicultural treatment pairings types did not affect mortality ($p = 0.2743$), however, treatments within pairings did differ ($p < 0.0001$). Shelterwood treatments averaged 30% mortality, compared to 77% mortality in control sites. Group selection and thinning treatments each suffered approximately equivalent mortality (76%) in 2010.

Deer Browsing

In 2009, 85% of the seedlings exhibited evidence of browsing by deer. In 2010, all but one living seedling showed signs of browsing.

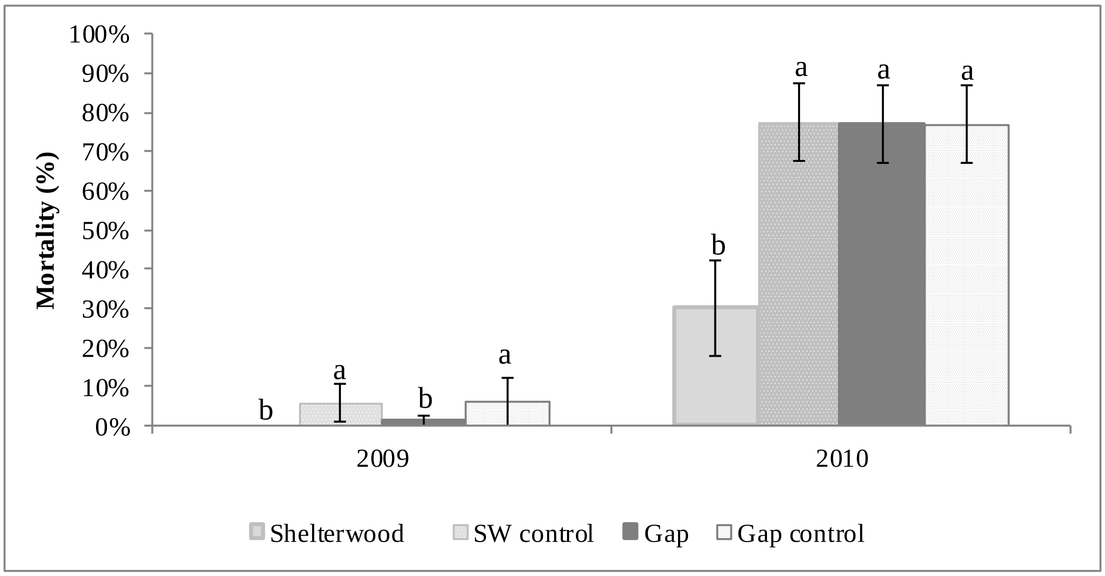


Figure 4-3: 2009 and 2010 mortality among silvicultural treatments. Error bars indicate standard error. Treatment bars within each year with the same letters are not significantly different ($\alpha = 0.05$).

Height and RCD

Silvicultural treatment pairings differed significantly in 2009 height change ($p = 0.0210$), while treatments within pairings did not differ ($p = 0.7437$; Figure 4-4; Tables 4-5 and 4-6 in appendix 2). Seedlings growing in the shelterwood/control pairings added more height growth on average (7 cm) than trees growing in the group selection/thinning pairings (-1 cm average). There were no significant differences in RCD between treatment effects (silvicultural pairing $p = 0.7340$, treatment $p = 0.5484$; Figure 4-4; Tables 4-5 and 4-6).

Height growth in 2010 was also greater in the shelterwood pairing (-1 cm) than the group selection pairing (-32 cm; p = 0.0344; Figure 4-4; Table 4-7 in appendix 2). As in 2009, height growth did not differ between silvicultural treatments within pairings (p = 0.2075; Table 4-7). RCD did not differ between silvicultural pairings (p = 0.1752). Within the silvicultural pairing, RCD was larger in the shelterwood sites than the control sites (p > 0.0001; Figure 4-5; Table 4-8).

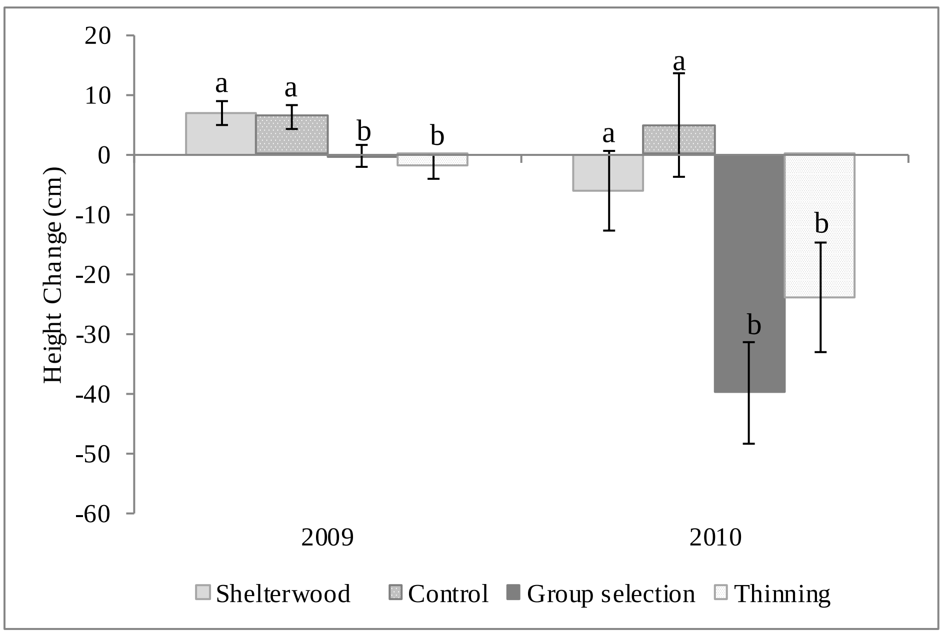


Figure 4-4: 2009 and 2010 height change among silvicultural treatments. Error bars indicate standard error. Treatment bars within each year with the same letters are not significantly different (α = 0.05).

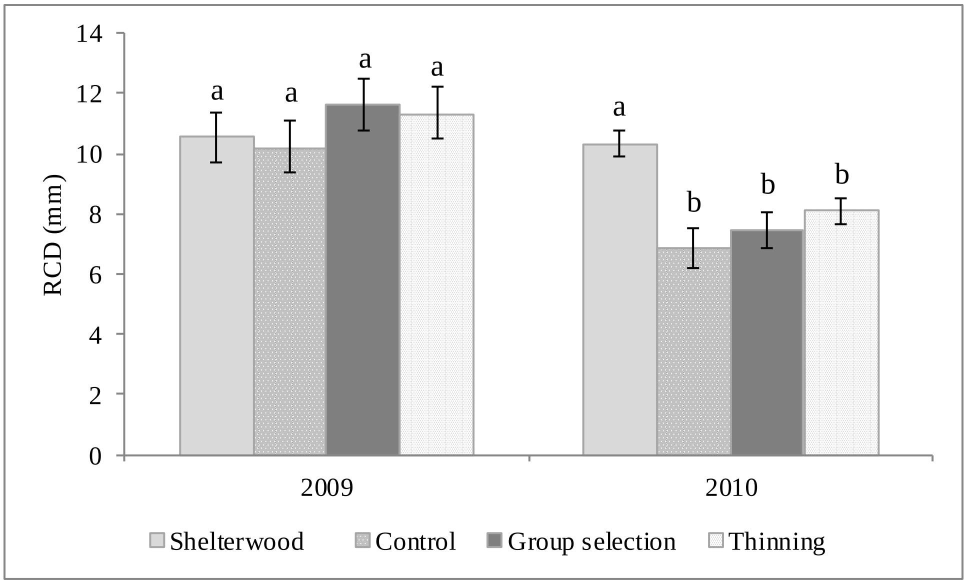


Figure 4-5: 2009 and 2010 Mean RCD among silvicultural treatments. Error bars indicate standard error. Treatment bars within year with the same letters are not significantly different ($\alpha = 0.05$).

Height classes

Mortality did not differ among height classes ($p = 0.5470$; Table 4-2) in 2009. The height and RCD of size classes segregated according to their original class designation ($p < 0.0001$, $p < 0.0001$, respectively; Table 4-2). There were no significant differences in height change among the seedling height classes ($p = 0.1655$; Table 4-2).

A greater number of large (class 3) seedlings died in 2010 than did medium (class 2) and small (class 1) size classes ($p = 0.0496$; Table 4-3). Silvicultural treatment by initial height class interaction was significant ($p = 0.0290$), indicating a difference in rates of mortality among the height classes over the silvicultural treatments (Figure 4-6). Within the group selection, thinning and control treatments, more large (class 3) seedlings died than did smaller seedlings. However, within shelterwood treatments, large and medium seedlings suffered less mortality than did small seedlings.

Table 4-2: Mean (± standard error) 2009 mortality, height, RCD, and change in height among height class treatments. Class means within each column followed by the same letter are not significantly different (α = 0.05).

Ht class	Ht (cm)	RCD (mm)	Ht change (cm)	Mortality (%)
1 (24 – 70 cm)	63 c ± 2	7.6 c ± 0.3	5 a ± 2	1 a ± 1.
2 (71 – 100 cm)	89 b ± 2	9.5 b ± 0.3	3 a ± 1	5 a ± 2
3 (101 – 200 cm)	120 a ± 2	12.6 a ± 0.3	1 a ± 1	5 a ± 3

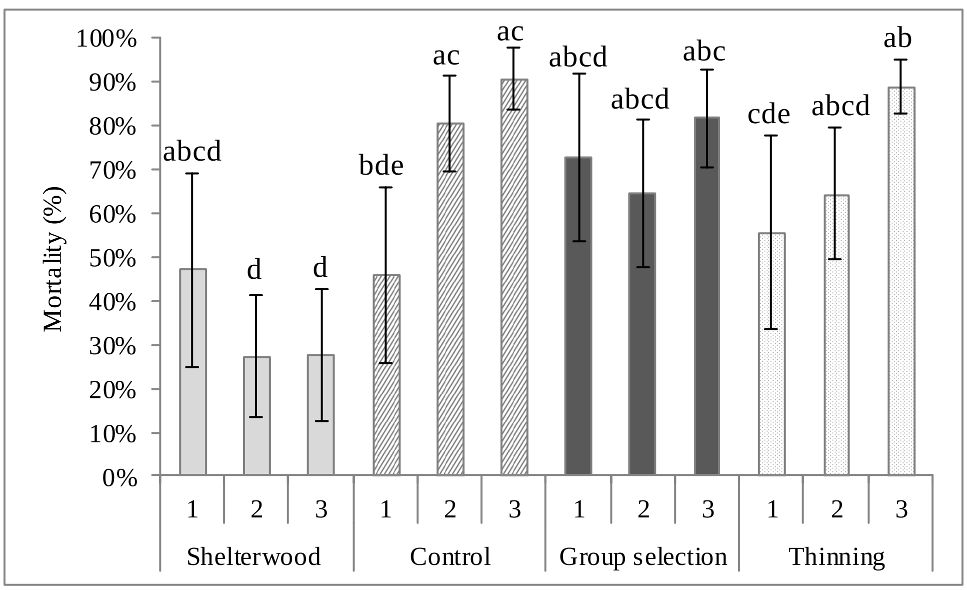


Figure 4-6: 2010 mortality for silvicultural treatment by height class (1-3) interaction. Error bars indicate standard error. Treatment bars with the same letters are not significantly different (α = 0.05).

Height and RCD in 2010 were greatest for large seedlings, followed by medium seedlings, and small seedlings ($P < .0001$; Table 4-3). Large seedlings grew less on average than did the medium and small seedlings ($p = 0.0162$).

Table 4-3: Mean (± standard error) 2010 mortality, height, RCD, and height growth among height classes. Class means within each column followed by the same letter are not significantly different ($\alpha = .05$)

Ht class	Ht (cm)	Ht change (cm)	RCD (mm)	Mortality (%)
1 (24 – 70 cm)	49 c ± 8	-4 a ± 8	6.1 c ± 0.6	57 b ± 12
2 (71 – 100 cm)	71 b ± 6	-14 a ± 7	8.4 b ± 0.4	63 b ± 10
3 (101 – 200 cm)	90 a ± 8	-31 b ± 8	10.4 a ± 0.6	79 a ± 8

Canopy cover

Canopy cover did not differ between silvicultural treatment pairings ($p = 0.3627$), but did differ between silvicultural treatments within pairings ($p = 0.0437$; Table 4-4). Control sites had greater canopy cover than shelterwood sites and thinning sites has greater canopy cover than group selection sites.

Table 4-4: Mean (± standard error) 2009 percent canopy cover between silvicultural pairings and treatments within pairings. Treatment means within each column followed by the same letter are not significantly different (α = 0.05).

Treatment pairing	Canopy cover	Treatment	Canopy cover
Shelterwood/ Control	80 a	Shelterwood	55 b
		Control	97 a
Group selection/ thinning	73 a	Group selection	56 c
		Thinning	81 ab

Discussion

Environmental conditions varied quite markedly among the four treatments. Shelterwood and group selection sites had much less canopy cover than the control and thinning treatments, allowing greater amounts of light to reach the seedlings. While canopy cover, and presumably available light, was similar in group selection and shelterwood treatments, many of the group selection treatment sites harbored thick populations of hay-scented fern [*Dennstaedtia punctilobula* (Michx.) T. Moore], which colonized the sites after harvest and prevented hardwood seedlings from establishing. Hay-scented fern is a fierce competitor for above- and below-ground resources and has been found to reduce ectomycorrhizal infection frequency and root-branching in greenhouse planted northern red oak, *Quercus rubra* L. (Lyon and Sharpe 1996). Horsley (1993) found that hay-scented fern reduced black cherry seedling survival by shading the seedlings. In a study on the Yale-Myers forest, Kittridge and Ashton (1990), however, found that hay-scented fern had little effect on hardwood regeneration. Hay-scented fern was waist-high at many of the group selection sites in this study and likely reduced the amount of light available to chestnut seedlings in these sites, resulting in reduced growth and survival in group selection versus shelterwood treatment sites.

The higher rates of mortality in 2009 in the control and thinning sites, as compared to the shelterwood and group selection treatments, can presumably be explained by the lower light availability in the control and thinning sites. High rates of mortality across all sites in 2010 were likely caused by repeated browsing by deer during both years and a late spring frost in 2010. What is of note is the relatively low rate of morality (30%) found in the shelterwood treatment, as compared to the other three treatments types. This discrepancy in mortality is likely due to greater available light and absence of hay-scented fern in the shelterwood treatment. Presumably these advantages enabled seedlings in the shelterwood treatment to better survive repeated deer browsing and to produce new flushes of leaves after browsing. The combined effect of low light in the control and thinning sites, and heavy fern competition in the group selection sites, with repeated deer browsing likely prevented seedlings from flushing following browsing. Without leaves to produce sufficient photosynthate for maintenance respiration, many of these seedlings died.

The difference in mortality among the seedling size classes among silvicultural treatments is also of interest. While more of the large seedlings died than medium and small seedlings over all treatments, more large seedlings than small and medium survived in the shelterwood treatment.

This can likely be explained by greater availability of light to large seedlings, enabling them to produce sufficient carbohydrates to sustain maintenance respiration. Large seedlings in the other treatments, with higher competition, in combination with deer browsing presumably did not have sufficient photosynthate to allocate to maintenance respiration, eventually leading to death.

The early patterns of establishment, mortality and growth in this study provide preliminary guidance for chestnut reintroduction in Connecticut upland forests. High rates of mortality across all treatments demonstrate the need to protect the seedlings from deer browsing. The shelterwood harvest treatment appears to offer a more suitable growing environment for early chestnut establishment and will likely yield better results than planting chestnuts in thinned or uncut stands. Large seedlings suffered less mortality in the shelterwood sites than small seedlings and therefore these results suggest that large high-quality seedlings be planted within shelterwood cuts. Group selection cuts, with control of hay-scented may be a reasonable, albeit less productive, choice for chestnut establishment.

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Appendix 2

Table 4-5: Mean (± standard error) 2009 height, height change, and root collar diameter between silvicultural pairings. Family means within each column followed by the same letter are not significantly different (α = .05).

Treatment pairing	Ht (cm)	Ht Change (cm)	RCD (mm)	Mortality (%)
Shelterwood/ Control	100	7 a	10.4 a ± 0.7	3 a
Group Selection/ thinning	95	-0.96b	11.5 a ± 0.7	2 a

Table 4-6: Mean (± standard error) 2009 height change, root collar diameter, and mortality between silvicultural treatments within pairings. Family means within pairings for each column followed by the same letter are not significantly different (α = 0.05).

Treatment	Ht Change (cm)	RCD (mm)	Mortality (%)
Shelterwood	7 a ± 2	10.5 a ± 0.8	0 b ± 0
Control	6 a ± 2	10.2 a ± 0.9	6 a ± 5
Group selection	-1a ± 2	11.6 a ± 0.8	1 b ± 1
Thinning	-2 a ± 2	11.3 a ± 0.9	8 a ± 6

Table 4-7: 2010 mean height, height change, and root collar diameter between silvicultural pairings. Family means within each column followed by the same letter are not significantly different (α = 0.05).

Treatment pairing	Ht (cm)	Ht Change (cm)	RCD (mm)	Mortality (%)
Shelterwood/ Control	89	-1 a ± 7	8.6 a ± 0.4	77 a ± 10
Group Selection/ Thinning	58	-32 b ± 8	7.8 a ± 0.5	55 a ± 14

Table 4-8: 2010 mean height, height change, and root collar diameter between silvicultural treatments within pairings. Family means within each column followed by the same letter are not significantly different (α = 0.05)

Treatment	Ht (cm)	Ht change (cm)	RCD (mm)	Mortality (%)
Shelterwood	93	-6 a ± 7	10.3 a ± 0.4	30 b ± 12
Control	75	5 a ± 9	6.9 b ± 0.7	77 a ± 10
Group selection	51	-40 a ± 8	7.5 b ± 0.6	77 a ± 11
Thinning	64	-24 a ± 9	8.1 ab ± 0.4	77 a ± 11

Chapter 5: Conclusion: Management Implications

Silvicultural Treatments for Chestnut Reintroduction

The success of chestnut reintroduction plantings will differ from site to site, based on site quality, amount of competition, species composition, nursery quality of the seedlings, abundance of deer, and follow-up site management, among other factors. Successful establishment of chestnut will require both an understanding of the management tools available, such as silvicultural manipulation and the use of large seedlings, and an understanding of potential biotic stressors the planted seedlings will encounter.

Preliminary results from the Daniel Boone National Forest (DBNF) study (chapter 2) and Yale-Myers Forest (YM) study (chapter 4), demonstrate that chestnut seedlings show greatest initial growth when planted in silvicultural treatments that create high-light environments. This finding concurs with other silvicultural studies of American chestnut (McCament and McCarthy 2005; Rhoades, et al. 2009). The DBNF study shows that chestnut seedlings can also survive in low-light environments. Chestnut's ability to establish in both low-light and high-light environments may give forest managers flexibility in which silvicultural treatment they choose to implement in preparation for chestnut reintroduction. While a clear-cut or shelterwood with a low residual basal area may yield the best initial growth of chestnut seedlings, forest managers may choose to use other silvicultural treatments to satisfy additional management objectives. For example, an oak shelterwood treatment may be desirable in stands that are not yet ready for an overstory harvest.

A shelterwood with low residual basal area, a seed-cut, or clearcut treatments are the appropriate silvicultural treatments when fast growth of chestnut seedlings is desired. These treatment types will likely be most effective on xeric sites, where fast-growing site-sensitive species, such as yellow poplar (*Liriodendron tulipifera* L.) are not abundant, however mesic sites, with appropriate control of competition, may yield superior growth. It is important to plant seedlings immediately after harvest, so as not to give stump sprouts, if not controlled, time to grow and monopolize resources before seedlings are planted. On sites with substantial sprout and seedling competition, control of competition, through mechanical or chemical means, may be necessary during the first several years of establishment, as has been found for some NRO plantings (*cf.* Dey, et al. 2008).

Control of competition will be especially important on mesic sites, where fertile, moist soils sustain fast-growing, site-sensitive species. While American chestnut has been found to grow faster than yellow poplar in high-resource soils (Latham 1992), the growth of planted high-quality chestnut seedlings versus hardwood sprouts has not been directly tested. Chestnut seedlings on the DBNF planting were generally not as tall as competing sprouts, even in the SW treatment sites. Most treatment sites were cut at least one year before the chestnut seedlings were planted, giving the stump sprouts a tremendous growth advantage over the seedlings. Furthermore planted hardwood seedlings spend the first growing season after planting recovering from transplant shock and grow roots at the expense of height growth (Clark, et al. 2009).

Shelterwood harvest sites with higher residual basal areas may also be appropriate for chestnut, and will inhibit the fast growth of shade-intolerant hardwood sprouts (Johnson, et al. 2002). This may be an effective method of securing chestnut recruitment on mesic sites with shade-intolerant competitors. This treatment will also be suitable for landowners who wish to retain part of the overstory, to satisfy other management objectives, or to allow the residual overstory to accrue value until the final harvest is implemented. Once this harvest is implemented, chestnut's ability to grow rapidly once released will allow it to outcompete other seedlings and sprouts that had been slowly growing under the residual overstory.

Chestnut seedlings growing in group selection harvest sites in the YM study demonstrated high rates of mortality and low growth. These results, however, do not necessarily indicate that group selection treatments are unsuitable for chestnut reintroduction. The high mortality and poor growth on these sites at Yale-Myers were caused by repeated deer browsing and severe competition from hay-scented fern. No other study has examined the suitability of group selection harvests for chestnut reintroduction. I speculate that selection cuts that are large enough to preclude side-shading (>0.12 hectares) will likely offer suitable growing environment for chestnut restoration if deer browsing and fern competition can be ameliorated. This may be a useful treatment for forest managers who wish to regenerate chestnut using a targeting approach, rather than transforming the entire stand through clear-cutting or shelterwood pairings, and for. small landowners who want to introduce chestnut to their land, but do not want to harvest timber.

The long-term suitability of midstory removal sites for chestnut reintroduction has yet to be evaluated. Our preliminary results suggest that chestnut can survive under shade on the subxeric sites were studied on the DBNF and will grow, albeit slowly. Based on chestnut's ability to live for long periods of time under hardwood overstory, this will likely prove a useful method for establishing a mixed chestnut-oak stand, where oak regeneration is abundant.

Seedling size

Seedling size is an important aspect of chestnut reintroduction. The results from both the DBNF and YM studies demonstrate that larger seedlings yield survival than smaller seedlings when planted in high-light silvicultural treatments. Larger seedlings in the DBNF study added more height growth regardless of silvicultural treatment. Larger seedlings in the YM forest, even in the shelterwood sites, lost more height than smaller seedlings, which was likely an artifact of intense deer browsing. Presumably, repeated defoliation impeded large seedlings from acquiring sufficient photosynthate to maintain the respiration requirements of a large stem and root system. Smaller seedlings demonstrated better survival in treatments with less light in the YM study, perhaps due to escape from deer. These studies suggest that chestnuts seedlings at least 14 mm in DBH and 110 cm in height, should be used for chestnut reintroduction plantings in forested settings.

Challenges to Reintroduction

The studies described in this dissertation elucidate some of the challenges facing the reintroduction of blight-resistant hybrid chestnut. In Southern states, the greatest biological challenge is *Phytophthora cinnamomi*, ink disease. As demonstrated in the DBNF study, ink disease may cause substantial mortality among blight-resistant hybrid chestnut seedlings. Furthermore, this study demonstrated that *P. cinnamomi* can live in xeric soils, where it historically was not prevalent. Potential chestnut reintroduction sites should be thoroughly tested for the presence of ink disease, and if confirmed, should not be planted.

In Northern states, the greatest biological challenge to chestnut restoration is repeated deer browsing by overabundant deer. Protection from deer, in the form of tree shelters, repellents or fencing on forests with large populations will be critical to the successful establishment of blight resistant American chestnut. Deer protection, in all forms, is expensive, therefore chestnut reintroduction to areas with large deer populations will require a significant investment.

Craesus castaneae, chestnut sawfly, may prove to be a hindrance to chestnut reintroduction, although survival and growth were not found to be affected by defoliation by chestnut sawfly on our seedlings. The superior quality of our seedlings likely aided their recovery. Lower quality seedlings, access to fewer above-ground and below-ground resources, may not recover from defoliation as successfully. Very little is known about this insect. As chestnut reintroduction efforts progress, and chestnut becomes increasing abundant in the forests, chestnut sawfly populations, believed to feed only on American chestnut, may increase in numbers.

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VITA

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