RESTORATION OF HARDWOODS: EFFECTS OF FERTILIZER SUPPLEMENT ON OAK AND OF OVERSTORY DENSITY AND FERTILIZER SUPPLEMENT ON AMERICAN CHESTNUT SEEDLINGS

by

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A THESIS

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This thesis is dedicated to mom and dad.
Restoration of hardwood forest ecosystems usually involves planting oak species and more recently American chestnut (*Castanea dentata*). American chestnut is currently a rare understory component of forests where it once dominated the overstory. An introduced fungal pathogen, chestnut blight (*Cryphonectria parasitica*), decimated the species last century. Hybrids that are resistant to this pathogen have been developed and are expected to be widely released within 10 years. This research project aims to improve establishment of several hardwood species. American chestnut seedlings were planted in the open, light shade, and heavy shade. They were also treated with foliar fertilizer supplement zero, one, or seven times. Cherrybark, Nuttall, and swamp chestnut oak seedlings planted in the open were treated zero, one, or two times. Survival and growth of the chestnuts was followed for two years and of oaks for one year.

Growth of American chestnut and oaks was not consistently improved by the fertilizer supplement. The 88% mortality of American chestnut seedlings in the open after two years was greater than in the shaded areas and unacceptably high. Planting American chestnut under light shade appears to be more effective than planting in full sunlight, but the factors affecting this probably depend on the site and are not well understood yet.

Keywords: American chestnut, *Castanea dentata*, Accele-Grow-M, restoration
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CHAPTER 1
INTRODUCTION

Forests in northern Alabama and much of the eastern United States have historically been dominated by mixed hardwood species with a smaller pine component. Dominant canopy species included oaks (Quercus spp.), hickories (Carya spp.), American chestnut (Castanea dentata (Marsh.) Borkh.), and pines (Pinus spp.) (Braun, 1947). Less common species included maples (Acer spp.), ashes (Fraxinus spp.), walnuts (Juglans spp.), eastern hemlock (Tsuga canadensis (L.) Carr.), American beech (Fagus grandifolia Ehrh.), sweetgum (Liquidambar styraciflua L.), American basswood (Tilia americana L.), magnolias (Magnolia spp.), black cherry (Prunus serotina Ehrh.), elms (Ulmus spp.), and birches (Betula spp.) (Braun, 1942; Hinkle, 1989). In the past few hundred years, the extent and composition of these forests has changed drastically due to the practices of European settlers. Much of the forested area was first cleared for timber and agriculture. Later, American chestnut, a species that used to dominate many eastern forests, was virtually eliminated (Russell, 1987) by the introduced Chestnut blight (Cryphonectria parasitica (Murr.) Barr.). More recently, many mixed species hardwood forests were replaced with loblolly pine (Pinus taeda L.) plantations for timber production (Evans et al., 2002; Zak et al., 2010). Additionally, fire suppression in the hardwood forests was substantially intensified since about the 1940s.
Native Americans practiced controlled burning for the past few thousand years that helped maintain a strong oak component in forests (Foster et al., 1998; McEwan et al., 2007). European settlers continued frequent burning, but in the decades after around 1940, fire was suppressed under the assumption that all fire was destructive and harmful for forests (Nowacki and Abrams, 2008). Under this strict regimen, regeneration of longleaf pines farther south and of many hardwood species such as oaks in Cumberland Plateau forests has become either suppressed by competitors or less common (Frost, 1993; Abrams, 1996). Because of fire suppression, maples have become more common canopy species, and their seedlings often dominate the forest understory (Hart and Grissino-Mayer, 2008; Nowacki and Abrams, 2008).

More recently, focus on reforestation and afforestation has shifted to more ecologically sound mixed hardwood forests (Keddy and Drummond, 1996). In the Cumberland Plateau and southern Appalachian region of northern Alabama, hardwood restoration efforts will include both American chestnut in the uplands and oaks in alluvial bottoms and terraces. American chestnut has been studied in these areas in Alabama and will likely be a popular tree for restoration when blight resistant hybrids are ready (Clark et al., 2007; Jacobs, 2007). Planting oaks as part of restoration efforts is increasingly popular (Allen and Kennedy, 1989). Oaks are a desirable primary component of these forests due to their quality timber, benefit to wildlife, and greater resilience to disease than pure pine plantations. Both oaks and American chestnut have historically provided habitat and a quality food source for a variety of wildlife species including white-tailed deer, turkey, black bear, and squirrels (Paillet, 2004). American chestnut, likewise, has also historically provided quality timber and forage for wildlife in addition to being used
as food and income source for rural communities (Baxter, 2009). The quality and quantity of its nut are greater than that of oaks, and its reintroduction will benefit wildlife (Diamond et al., 2000), including favored game animals such as white-tailed deer and American turkey (*Meleagris gallopavo*). Consideration of wildlife is not limited to restoration efforts, and it often influences decisions for private landowners who are primarily interested in timber (Jones, 2008). Some government programs offer payments or tax deductions for planting oaks but not much for pine and for non-keystone tree species (NRCS, 2000, 2011).

Restoration of hardwood forest ecosystems often depends on the survival rates and fast initial growth of the outplanted tree seedlings. Obtaining high survival rates and fast growth is strongly influenced by our knowledge of the silvics of the planted species, availability of high quality nursery material, and seedling response to enhancement treatments (Jacobs et al., 2004). Hardwood restoration efforts are continually hampered by poor early survival and growth. Light, water, and nutrient limitations must be addressed by establishment treatments. The silvical characteristics of American chestnut are largely unknown due to its disappearance in the early part of the 19th century (Jacobs, 2007). The knowledge gap about the optimal conditions for restoration of American chestnut is bound to reduce the success of any afforestation efforts using this species.

Blight resistant chestnut hybrids will be reintroduced into forests of eastern North America within 10 years, and studies on *C. dentata* establishment will aid these efforts (Jacobs, 2007). Since little research has been conducted on the best propagation practices for *C. dentata* in the Southeast, the species should be studied to help determine the most efficient ways to manage the blight resistant hybrids or in case genetically modified *C.*
dentata with blight and Phytophthora cinnamomi resistance become available in the future.

Oaks do not grow as well in the understory or sprout as well as maple, so in the absence of fires combined with a major stand disturbances, maintenance of an oak component on the productive sites is hindered (McEwan et al., 2007). Artificial regeneration is often desirable to ensure a strong oak component, and in some situations it is necessary. Planting only large size high quality seedlings is one of the best methods for bringing oaks back into the composition (Kormanik et al., 2002). However, planting such large seedlings is often not possible when using machine planting. Machine planting is the common method used for large scale restoration efforts, especially in the bottomlands, when natural regeneration is insufficient (Allen, 1997; Battaglia et al., 2008). If machine planting of smaller seedlings is used, then survival and growth can be improved by competition control and fertilization.

Competition, seedling health, and herbivory must be monitored and addressed to insure the success of forest restoration efforts (Allen and Kennedy, 1989; Meiners and Handel, 2000; Stanturf et al., 2001). Competition control enhances the growth and survival of oak seedlings (Ezell et al., 2007). Increasing early growth through the use of site preparation, genetically superior seedlings, and optimal fertilization will improve establishment success and decrease the need for repeated treatment of competitors.

Some evidence suggests that young hardwood plantations are nutrient deficient, and optimal fertilization may improve early survival and growth (Jacobs and Seifert, 2004). Fertilizers promote the growth of seedlings from oak species and other hardwoods, but there are mixed results about the growth of field planted seedlings following
application of soil fertilizer (Vaitkus et al., 1993) and little is known about fertilizer effect on chestnut (Auchmoody, 1972; Phillips and Fahey, 2007; Salifu et al., 2008). Studies on soil-applied fertilizer found no increased chestnut growth or survival, which is likely due to increased root disease (Rieske et al., 2003; Herendeen, 2007). Controlled release fertilizer applied before planting increased root necrosis, and it negated the beneficial effect on roots conferred by ectomycorrhizal inoculation (Herendeen, 2007). Alternative forms of fertilization such as a targeted application leaf spray have not been tested. Foliar sprays may be desirable due to the possible decreased severity of root to shoot ratio decline that is observed with soil fertilizers (Sileshi et al., 2007). A decline in root to shoot ratio following soil fertilization is observed in oaks (Vaitkus et al., 1993), while in American chestnut, the root to shoot ratio similarly declines but also contributes to increased mortality (McNab et al., 2003; Herendeen, 2007; Miller, 2010). The targeted foliar application method is desirable over broadcast soil fertilizer because it enables repeated application on individual trees while reducing off-target fertilization that would enhance competition growth.

Additionally, because chestnut is intermediate in shade tolerance and at the southern, and warmest, extent of its natural range in the Cumberland Plateau, its best early survival and growth may be under sheltered conditions, such as in thinned stands or stands where the establishment cut of a shelterwood regeneration method was recently carried out (McCament and McCarthy, 2005; Jacobs, 2007; Joesting, 2009; Rhoades et al., 2009). The oaks commonly used in restoration efforts in bottomlands of the Cumberland Plateau region, such as swamp white oak, Nuttall oak, cherrybark oak, and water oak are shade-intolerant and thrive best under full sun.
This study aimed to determine the effect of 1) shade level, a foliar fertilizer supplement, and their interaction on *C. dentata* seedling survival and growth and 2) a foliar fertilizer supplement on oak survival and growth. The results will help guide chestnut and oak restoration efforts in the Cumberland Plateau.

**Objectives**

The objectives of this study are to improve our knowledge and restoration practice by:

1. Investigating the potential effect of single or multiple applications of a foliar fertilizer supplement, Accele-Grow-M®, on American chestnut and oak seedling survival and growth.
2. Determining the effect of three shade levels on American chestnut seedling survival and growth.
3. Examining if there is an interaction between the two treatments for American chestnut.

**Justification**

*Castanea dentata* was once a major component of eastern North American forests (Russell, 1987). It provided more food and habitat for wildlife than oaks (Diamond *et al.*, 2000). The timber is valuable, and the growth rate exceeds other hardwood species (McEwan *et al.*, 2006). Blight resistant hybrid chestnuts share most morphological, and possibly ecophysiological (Bauerle *et al.*, 2006), characteristics with *C. dentata*, so studying *C. dentata* establishment and early growth will help develop more effective methods for establishing blight resistant hybrids in the wild. This research will also be valuable if blight resistant pure *C. dentata* are found, and if future use of gene therapy
allows the reintroduction of genetically modified *C. dentata* or a hypovirus that is useful at a population scale (Andrade *et al.*, 2009; Wheeler and Sederoff, 2009).

The three shade levels were chosen to represent a range of light levels from full sunlight to a level that should facilitate growth and maximize water efficiency (Wang *et al.*, 2006). Both American chestnut and many oaks have intermediate shade tolerance. The lowest light level of 50% overstory is likely sufficient for noticing an effect of fertilizer, since northern red oak fertilization increases growth only in 30% or greater of full sunlight (Phares, 1971). The conversion of old agricultural fields to forests, especially by planting oaks and other hardwood tree species, is an increasingly common restoration activity. It is desirable to study these sites to find better restoration efforts in addition to studying regeneration methods in forests. More land will be available for reforestation on old agricultural fields than in national forests, and private landowner demand for blight resistant hybrids will be high (Jacobs, 2007). For cherrybark oak (*Quercus pagoda* Raf.), planting mixtures with sweetgum and underplanting beneath sweetgum are successful methods of establishment that result in high survival and quality timber (Gardiner and Yeiser, 2006; Lockhart *et al.*, 2006). Similarly, Nuttall oak (*Quercus texana* Buckl.) performs well when underplanted beneath eastern cottonwood (*Populus deltoids* Bartr. ex Marsh.) (Gardiner *et al.*, 2004). Since underplanting in stands of short rotation woody crops has been a successful method of establishing oaks, such underplanting may be desirable for improving initial tree establishment and growth for American chestnut.

Nuttall oak, cherrybark oak, and swamp chestnut oak (*Quercus michauxii* Nutt.) are dominant overstory species in bottomland forests. They are valuable timber species
and provide important food for wildlife. Fertilization is a promising method for improving establishment success, but more research needs to be done on effective limited fertilization and nutrient hardening (Trubat et al., 2008). Limited fertilization may also be useful for helping with defense against pathogens such as sudden oak death. The establishment success of large nursery seedlings is high, and effective fertilization in the nursery is well understood (Kormanik et al., 2003). However, the success of fertilizer applied in the field on bareroot seedlings has been limited (Taylor and Golden, 2002; Ponder et al., 2008). These types of seedlings are increasingly popular in restoration efforts since they are cheaper than more mature seedlings and can be machine-planting.

For afforestation of large plots such as agricultural fields, machine planters are used almost exclusively (Schoenholtz et al., 2001). When blight resistant chestnut hybrids are widely available, landowners would likely use young bare root seedlings that are machine planted on such fields.

Accele-Grow-M® fertilizer supplement (Accelegrow Technologies, West Point, GA) can be applied as a leaf spray. It contains a 3-0-3 fertilizer that provides 0.117, 0, and 0.097 g/L of N, P, K, respectively, in a solution with a preservative, stabilizer, activator, carrier system, and a large concentration of the amino acid sarcosine. The leaf spray fertilizer supplement provides an equivalent of approximately 0.5 kg N and 0.4 kg K per acre (1.2 kg N and 1.0 kg K per hectare) per application. Surfactants containing sarcosine may increase adherence to leaves since the surface tension will be reduced (Nnanna et al., 2001); this is the same purpose as part of spreader-sticker products. Spreader-sticker products have been used to increase foliar fertilizer absorption in slash
pine (Eberhardt and Pritchett, 1971). The products help to improve adherence to waxy and pubescent leaf surfaces (Whitemore, 1983).

**Literature Review**

**American Chestnut**

*Castanea dentata* (Marsh.) Borkh. (American chestnut) was formerly a primary component of eastern North American forests. The chestnut blight, *Cryphonectria parasitica* (Murr.) Barr., an accidentally introduced fungal pathogen from Asia, decimated the species in the early 1900s (Russell, 1987). The American Chestnut Foundation (TACF) has been breeding hybrids of *C. dentata* and *C. mollissima* (Chinese chestnut) that retain most *C. dentata* characteristics but are resistant to blight (Diskin et al., 2006).

A major root pathogen of *Castanea* species is *Phytophthora cinnamomi*, a water mold normally found in the soil. *C. dentata* was historically widespread, but the introduction of *P. cinnamomi* will likely decrease the conditions where *C. dentata* can be established and is therefore an important consideration in restoration. *P. cinnamomi* resistance has not been considered in the American Chestnut Foundation breeding program, and resistance is not genetically related (Sisco, 2009). Young leaves of chestnut are particularly susceptible to multiple *Phytophthora* spp. (Balci et al., 2008b). However, Hewitt et al. (2004) found that one year after planting, there was no significant difference in *P. cinnamomi* infection rate of live and dead *C. dentata* seedlings, indicating that at least in their conditions *P. cinnamomi* might not be a major cause of mortality in young seedlings one growing season after outplanting. The authors also found that site
preparation involving application of fungicide increased survival significantly. *C. dentata* is most resistant to *P. cinnamomi* on well drained sandy loam soils (Rhoades 2003). Therefore, when blight resistant hybrid seeds are available, dry ridge tops may be among the best places for chestnut restoration. Since the introduction of *P. cinnamomi* and *P. cambivora* from Asia in the 1900s, some populations of *Castanea sativa* (European chestnut) have evolved resistance to the fungus (Robin *et al.*, 2006). Significant change in *C. dentata* populations has also occurred during this time (Stilwell *et al.*, 2003).

Two fungicides have been successfully used to control *P. cinnamomi* in other host species. Metalaxyl (Ridomil® , Syngenta, Wilmington, DE, USA) successfully controlled *P. cinnamomi* in avocado and *C. dentata* (Coffey, 1987; Hewitt *et al.*, 2004). It is possible that this fungicide may be useful in the short term for treating roots of blight resistant chestnut hybrids before planting, but it cannot provide long term resistance to *P. cinnamomi*. When *P. cinnamomi* was introduced from Asia, some of the strains were resistant to metalaxyl, and these strains reach high enough concentrations to destroy avocado trees after years of metalaxyl treatment (Goodwin *et al.*, 1996).

If longer term control of *P. cinnamomi* is desired for planted blight resistant chestnut hybrid trees, another fungicide, phosphonate, is the most promising fungicide. *Phytophthora* resistance to phosphonate has not yet been observed in avocado. Semiannual application through the bole should be sufficient for control in *C. dentata*, but one yearly application after root and leaf shoot growth and before flower bud development may be sufficient (Giblin *et al.*, 2007). The trees should not be treated during early spring growth since the phosphonate will likely be concentrated in the leaves instead of the roots (Whiley *et al.*, 1992).
A major problem is the delivery of the fungicide to the root system. Pentra-Bark is a bark penetrating surfactant used to deliver fungicides, fertilizers, and other chemicals into mature trees (Quest Products, Louisburg, Kansas). Injections are currently used to deliver some chemicals, especially pesticides and fungicides for trees attacked by invasive pests. This practice damages the tree, especially when it is conducted annually (Shigo and Campana, 1977). Furthermore, repeated drillings and injections may become less effective due to wound healing and reverse sap flow near wounds (Campana, 1977). The damage from injections to small trees is so great that injected fertilizers do not have a positive impact on growth (Mayhead and Bole, 1994).

Unlike bark injections, Pentra-Bark does not damage tree boles, but long term effects are unknown. In Australian avocado trees, Pentra-bark was not as successful as bark injections in delivering phosphonate for Phytophthora resistance. However, sufficient phosphonate was delivered for root survival for both treatments (Giblin et al., 2005). Pentra-bark also delivered sufficient insecticide for control of calico scale on an ornamental tree (Potter et al., 2007).

Keeping C. dentata trees vigorous and less susceptible to pathogens can be achieved through providing them with the necessary nutrients through fertilization. This applies especially for poor quality sites with lower incidence of P. cinnamomi on which C. dentata may be planted. Standard fertilization, however, leads to fertilization of competing vegetation.

Most seedling mortality occurs during the first years following planting in the field, before the root system is well established. Ensuring the success of planting and other reforestation efforts at an acceptable cost will require avoiding the need for
replanting an area many times (Keeton, 2008). Initial treatment of soils before planting seedlings has a great effect on survival and growth of a variety of species (Archibold et al., 2000; Karlsson, 2002; Hewitt et al., 2004; Knapp et al., 2006). Mechanically preparing the site and controlling Phytophthora could result in very high seedling survival (Hewitt et al., 2004). This is especially desirable when no replanting is intended and when there is enough funding and time for such soil treatments. Some examples of cases when time invested for the initial planting is not a major consideration are tree planting volunteer activities with conservation organizations, urban forestry, and homeowner planting.

Although few studies have been conducted, standard fertilization has not proved promising for C. dentata. Weekly N fertilization did not significantly enhance the growth of C. dentata over control in a greenhouse study (Rieske et al., 2003). Another greenhouse study found that treatment with controlled release fertilizer resulted in higher mortality and lower growth rates compared to control, probably due to the increase in root disease (Herendeen, 2007). Seedlings planted on mine reclamation sites that had previously been fertilized did not grow more than those on unfertilized soils (Herendeen, 2007). However, other chestnut species have been found to respond positively to fertilization. Organic compound fertilizer increased sapling C. mollissima height growth about 1.5x over control (Zeng et al., 2007). Fertilization also increased shoot growth by approximately 3x over control in C. sativa seedlings (Kohen and Mousseau, 1994).

The amount of fertilizer applied off-target can be reduced by using a fertilizer that is delivered directly into the plant instead of through the soil or through aerial spraying. Accele-Grow-M® (Accelegrow Technologies, West Point, GA) is a new fertilizer
supplement that is delivered directly into the plant leaves or roots. It can be delivered to hardwoods by dipping the plant roots or spraying the leaves. I was able to find study results only from drought years, and the fertilizer supplement may be more useful with overcoming water stress than with increasing growth when resources are abundant (Dawson, 1993). Application only to the target plant also prevents direct fertilization of neighboring competing plants, possibly making the application process more efficient and cost effective. Fertilization of large trees under attack by exotic insects or fungi could have a similar effect, but foliar application in such cases would be labor intensive.

Availability of more resources that can be devoted to defensive compound production would help trees survive attack by pathogens (Sayler and Kirkpatrick, 2003). Targeted fertilization through the leaves of _C. dentata_ may help to prevent the decline in root system vigor observed with standard fertilization. The interaction of shade and fertilizer treatments has not been studied in _C. dentata_, and the results may differ from other species due to its susceptibility to pathogens.

_C. dentata_ has intermediate shade tolerance (Joesting, 2009). It responds well to release from overstory competition and grows rapidly in full sun. However, it survives well in the understory. When the main stem dies, the trees resprout well and do so repeatedly, so seedling size trees in the understory may have a 100 year old root system (Paillet, 2002). Historical literature suggests that survival is higher under partial shade than in the open for the first two years and that this method was a good way of establishing _C. dentata_ in the forest (Russell, 1987). Seedlings in a 30% shelterwood treatment grew better but had similar germination, vigor, and survival to seedlings planted in intact forest (McCament and McCarthy, 2005). Rhoades et al. (2009) found
that seedling mortality did not differ but visible root disease was twice as common in a 30% shelterwood than in a midstory removal treatment. *Phytophthora* incidence was higher in an oak shelterwood than in clearcuts in Alabama, but no infection was observed in Tennessee (Clark *et al.*, 2009). Survival of seedlings may be greater under moderate shade (Griffin, 1989; Anagnostakis, 2007). Examination of growth patterns in a Wisconsin stand suggest that planting blight resistant *Castanea* hybrids in clumps in canopy gaps or with silvicultural treatments will be a good method of re-establishing the species (McEwan *et al.*, 2006). If this method is used, fertilizing the seedlings would be easier.

**Oaks**

Nuttall, cherrybark, and swamp chestnut oaks are common bottomland overstory species in the southeastern United States. Due the decline of oaks in natural forests as well as landowner incentives, there has been increasing interest in planting bottomland agricultural fields to oak-dominated hardwood forests (Allen and Kennedy, 1989). Oaks are often planted for their valuable timber and wildlife benefit. The US Natural Resources Conservation Service’s Conservation Reserve Program aims to facilitate the conversion of fields used for row cropping into hardwood forests. The program goals include providing habitat for wildlife, improving air and water quality, reducing soil erosion, and reducing flood damage. Other government programs with similar goals include the Conservation Reserve Enhancement Program and the Wetlands Reserve Program.

Natural oak regeneration in bottomland hardwood forests has been declining (Young *et al.*, 1995; Nowacki and Abrams, 2008). Oak decline is a disease complex
increasingly affecting trees in parts of the Southeast by causing crown dieback and mortality. It includes sudden oak death which is caused by *Phytophthora ramorum*, a fungus of the same genus as the water mold that contributed to the decline of American chestnut and will hinder re-establishment of blight resistant hybrids (Oak et al., 2004; Linderman et al., 2007). The causes of oak decline are not well understood (Oak et al., 1996). In the Ozarks, the decline is more associated with oak abundance than site factors (Kabrick et al., 2008). As with *P. cinnamomi* on American chestnut, oaks growing in more acidic, sandy soils are more resistant to *Phytophthora* species associated with oak decline across continents (Jung et al., 2000; McDonald et al., 2002; Thomas et al., 2002; Balci and Halmschlager, 2003; Balci et al., 2010). Unlike most *Phytophthora* species, *P. ramorum* spreads aerially in addition to through soil and water. Factors influencing *Phytophthora*-induced sudden oak death, such as soil pH and latitude, vary within the United States and between the United States and Europe (Brasier, 2003; Garbelotto, 2004; Balci et al., 2007). Oak decline may become a more important consideration for management decisions with reforesting and afforesting oaks.

Planting former agricultural fields to oaks is an increasingly common practice, but survival is sometimes low (Ponder et al., 2008). Discovering better establishment methods is desirable for converting these areas to productive forests (Lockhart et al., 2003). Soil applied fertilizer has been a somewhat effective method for improving establishment, and response varies among oak species (Dunn et al., 1999). Response also varies due to site factors such as nutrients present and soil compaction. In a greenhouse study, nitrogen uptake from soil applied fertilizer is decreased by compacted soils for northern red oak (*Quercus rubra* L.) and scarlet oak (*Quercus coccinea* Muencch).
(Jordan et al., 2003), so foliar fertilizer application could help seedlings uptake the nutrients more effectively on compacted soils like former mine lands and agricultural fields.

Ectomycorrhizal colonization with certain fungal species helps improve the root function of several hardwoods and improves plant defense against various pathogens (Harley and Smith, 1983). Bauman and others found that increasing ectomycorrhizal colonization helped American chestnut and blight resistant hybrids grow and survive better (Bauman et al., 2010), and the effect of ectomycorrhizae on defense against *P. cinnamomi* has been well demonstrated in European chestnut (Branzanti et al., 1999; Blom et al., 2009). In addition, sudden oak death from a different *Phytophthora* species is an increasing problem and will probably become more prevalent in Alabama or at least the Southeast (Oak et al., 2004; Linderman et al., 2007). A variety of other *Phytophthora* species are associated with oak decline, and new species are being discovered in the Southeast (Balci et al., 2008a). Little research is available about the interaction among oaks, ectomycorrhizal colonization, and *Phytophthora* species or other pathogens, but given the similarities between chestnut and oaks, enhancing ectomycorrhizal colonization of both oaks and chestnuts may enhance restoration success.

Foliar fertilizer may increase ectomycorrhizal colonization by increasing leaf area and the carbon allocated to roots (Wallander, 2006). The ectomycorrhizal colonization of fine roots by a Tuberaceae fungus (truffle species) is increased by foliar but not soil applied fertilizer for holm oak (*Quercus ilex* L.) seedlings in a European plantation established on former agricultural land (Suz et al., 2010). Therefore, in addition to improving the nutrient status of the young seedlings, foliar fertilization may improve
hardwood restoration efforts through increasing plant defense mechanisms against root-borne pathogens.

Soil fertilizer increases height and root collar diameter growth for water (Q. nigra L.) and swamp chestnut oaks, and the optimal rate for root and shoot growth differs for the different species (Vaitkus et al., 1993). Fifth-year growth of outplanted cherrybark oak was increased when the seedlings were first fertilized while they were in the nursery (Howell and Harrington, 2004). Nuttall oak first year growth increased after a single soil fertilization at planting (Taylor and Golden, 2002), and work on European oak species suggests that limited fertilization may be an effective method (Trubat et al., 2008).

Northern red oak responds well to soil fertilizer, and unlike in sugar maple (Acer saccharum L.), the root biomass is not significantly reduced compared to control (Phillips and Fahey, 2007). Repeated soil fertilization of northern red oak and white oak (Quercus alba L.) increases biomass and decreases leaf chlorosis, and exponential nutrient loading performs best (Birge et al., 2006). Exponential nutrient loading in the nursery improves above and below ground growth of those species planted on a former mine site, and a medium fertilizer rate resulted in the best performance (Salifu et al., 2008). Off-target fertilization of competing plants is an undesirable effect of soil fertilization.

Competition control has had less positive results for establishing oaks than for some other species such as most pines (Groninger et al., 2000). Controlling competition by mowing or herbicide within 1 m of seedlings or for the entire plot before planting did not increase the survival of Nuttall or cherrybark oak seedlings (McLeod et al., 2000). Pre-planting competition control with herbicide did not increase the survival or three year growth of cherrybark oak in a thinned forest (Gardiner and Yeiser, 2006); however,
midstory removal in an intact forest increased cherrybark oak advanced regeneration growth and survival at nine but not three years. Multiple herbicide applications on the competition improved survival of Nuttall and cherrybark oak seedlings (Ezell et al., 2007). Off target application of herbicide on oak seedlings complicates results. Mulching for competition control improves establishment more than no competition control or herbicide application (Adams, 1997). Repeated mowing or diskng for competition control increases the growth of oak seedlings at fifteen years, and control of overtopping vegetation of young seedlings is most important for improving establishment and growth (Kennedy, 1981; Krinard and Kennedy, 1987). Greenhouse studies have demonstrated that competition control may increase survival and growth during the first year, especially when browsing occurs, for the studied oak species (Facelli and Pickett, 1991; Frost and Rydin, 1997; Meiners and Handel, 2000).
CHAPTER 2
EFFECT OF FERTILIZER SUPPLEMENT ON OAK AND OF OVERSTORY DENSITY AND FERTILIZER SUPPLEMENT ON FIRST YEAR AMERICAN CHESTNUT GROWTH

Introduction

American chestnut (Castanea dentata (Marsh.) Borkh.) was formerly a primary component of eastern North American forests. The chestnut blight (Cryphonectria parasitica (Murrill) Barr), an accidentally introduced fungal pathogen from Asia, decimated the species (Russell, 1987). The American Chestnut Foundation (TACF) has been breeding hybrids of C. dentata and C. mollissima Blume (Chinese chestnut) that retain most C. dentata characteristics but are resistant to the blight (Diskin et al., 2006).

A major root pathogen of Castanea spp. is Phytophthora cinnamomi Rands, a water mold normally found in the soil. C. dentata is historically widespread, but the introduction of P. cinnamomi will likely decrease the conditions where C. dentata can be grown and is therefore an important consideration in restoration. P. cinnamomi resistance has not been considered in TACF breeding program, and resistance is not genetically related (Sisco, 2009). C. dentata is most resistant to P. cinnamomi on well drained sandy loam soils (Rhoades 2003). Ridge tops usually have such drained drier soils. Young leaves of chestnut are particularly susceptible to multiple Phytophthora spp. (Balci et al., 2008b). However, Hewitt et al. (2004) found that P. cinnamomi was not the main source
of mortality in young \textit{C. dentata} seedlings, whose growth was impaired by it, one year after planting. When blight resistant hybrid seeds are available, dry ridge tops may provide a good opportunity for reforestation of chestnut. Since the introduction of \textit{P. cinnamomi} and \textit{P. cambivora} from Asia in the 1900s, some populations of \textit{Castanea sativa} Mill. (European chestnut) have evolved resistance to the fungus (Robin et al., 2006). Significant change in \textit{C. dentata} populations has also occurred during this time (Stilwell et al., 2003).

Vigor of \textit{C. dentata} trees and therefore a decrease in susceptibility to pathogens can be enhanced through providing the trees with the additional nutrients through fertilization. This applies especially for poor quality sites where a lower incidence of \textit{P. cinnamomi} is expected and on which \textit{C. dentata} may be planted. I was unable to find any studies that found increased growth or survival of \textit{C. dentata} with fertilization on forested sites or former agricultural fields.

Most seedling mortality occurs during the first years following planting, before the root system is well established. Ensuring the success of planting the first time requires less investment than replanting an area many times (Keeton, 2008). Initial treatment of soils before planting seedlings has a great effect on survival and growth of a variety of species (Archibold \textit{et al.}, 2000; Karlsson, 2002; Hewitt \textit{et al.}, 2004; Knapp \textit{et al.}, 2006; Rhoades \textit{et al.}, 2009). Fertilizing seedlings along with mechanically preparing the site could result in very high seedling survival (Hewitt \textit{et al.}, 2004). This is especially desirable when no replanting is intended and time invested in planting is not a major consideration. Some examples of cases when time invested for the initial planting is not a
major consideration are tree planting volunteer activities with conservation organizations, urban forestry, and homeowner planting.

Results from several studies on soil fertilization of *C. dentata* show that the effect is marginal - weekly nitrogen fertilization does not significantly enhance the growth of *C. dentata* over control in greenhouse conditions (Rieske et al., 2003). Additionally, treatment with controlled release fertilizer in greenhouse conditions results in higher mortality and lower growth rates compared to control, probably due to the increase in root disease (Herendeen, 2007). The results from such greenhouse experiments are consistent with results from experiments with open grown seedlings planted on mine reclamation sites that had previously been fertilized - *C. dentata* seedling growth on the fertilized soils is not significantly different from growth on unfertilized soils (Herendeen, 2007). I was unable to find any studies that report a significant increase in growth or survival of *C. dentata* seedlings with soil fertilization on forested sites or former agricultural fields. It is likely that limited nutrient supplementation will increase *C. dentata* performance since it has in other species (Trubat et al., 2008), but that the adequate combination or amounts have not been applied. Results with other chestnut species consistently show an increase in growth with fertilization. Organic compound fertilizer increases sapling *C. mollissima* height growth about 1.5 times over control (Zeng et al., 2007). Similarly, fertilization increases shoot growth by approximately 3 times over control in *C. sativa* seedlings (Kohen and Mousseau, 1994).

Increasing nutrient leaching in the runoff after fertilizing is an undesirable side effect of such forest restoration efforts. The amount of fertilizer in the runoff can be reduced, however, if the fertilizer is delivered directly into the plant instead of through
the soil or through aerial spraying from a helicopter or airplane. Accele-Grow-M® (Accelegrow Technologies, West Point, Georgia) is a new fertilizer supplement that is delivered directly onto the plant leaves or roots. This application method also prevents direct fertilization of neighboring competing plants, possibly making the application process more efficient and cost effective. Avoiding fertilization of the competitors can be achieved if the seedlings are sprayed or dipped before outplanting, or if each of the outplanted target plants is sprayed individually, or if the entire planted area is sprayed at a time when the only vegetation present is the targeted seedlings. Such delivery of the fertilizer is also possible for large trees. Such fertilization could increase tree vigor and its defenses against attacks by insects or fungi, including those that are new or exotic. Greater availability of resources to the tree allows it to increase its defensive compound production and improve its chance of surviving such attacks (Sayler and Kirkpatrick, 2003). Targeted fertilization through the leaves of *C. dentata* may have another benefit – it may help to prevent the decline in root system vigor observed with standard fertilization (Sileshi *et al.*, 2007). Information on the response of such fertilizer delivery is needed, and so is information on the possible interaction of shade levels and fertilizer treatments.

*C. dentata* has intermediate shade tolerance (Joesting, 2009). It responds well to release from overstory and grows rapidly in full sun. However, it survives well in the understory. When the main stem dies, the tree resprouts well and does so repeatedly, so seedling size trees in the understory may have 100 year old root systems (Paillet, 2002). Historical literature suggests that survival is higher under partial shade than in the open for the first two years and that this method was a good way of establishing *C. dentata* in
the forest (Russell, 1987). Partial shade appears to be better for growth not only when compared to the open, but also when compared to full shade – \textit{C. dentata} seedlings in 30\% shelterwood treatment can have approximately three times greater annual height growth and twice as large a diameter growth than seedlings in an intact forest (McCament and McCarthy, 2005). However, germination, vigor, and survival do not improve significantly in shelterwood (McCament and McCarthy, 2005). Rhoades et al. (2009) found that seedling mortality does not differ significantly between 30\% shelterwood and midstory removal treatments. Seedling annual height growth was over 3 times and diameter growth about 4 times as great in shelterwood versus in midstory removal treatments. Visible root disease was twice as common in shelterwood treatments as in midstory removal. Survival of seedlings may be greater under moderate shade (Griffin, 1989; Anagnostakis, 2007). Examination of growth patterns in a Wisconsin stand suggest that planting blight resistant \textit{Castanea} hybrids in clumps in canopy gaps or after certain silvicultural treatments will be a good method of re-establishing the species (McEwan et al., 2006). If this method is used, fertilizing the clumps of seedlings would be easier than treating more scattered seedlings.

Nuttall, cherrybark, and swamp chestnut oaks are common bottomland overstory species in the southeastern United States. Due the decline of oaks in natural forests as well as landowner incentives, there has been increasing interest in planting bottomland agricultural fields to oak-dominated hardwood forests (Allen and Kennedy, 1989). Oaks are often planted for their valuable timber and wildlife benefit. The US Natural Resources Conservation Service (NRCS) Conservation Reserve Program aims to facilitate the conversion of fields used for row cropping into hardwood forests. The program goals
include providing habitat for wildlife, improving air and water quality, reducing soil erosion, and reducing flood damage. Other government programs with similar goals include the Conservation Reserve Enhancement Program and the Wetlands Reserve Program.

Soil fertilizer increases height and root collar diameter growth for water (*Q. nigra* L.) and swamp chestnut oaks, and the optimal rate for root and shoot growth differs for the different species (Vaitkus *et al.*, 1993). Fifth-year growth of outplanted cherrybark oak was significantly increased when the seedlings were first fertilized while they were in the nursery (Howell and Harrington, 2004). Nuttall oak first year growth significantly increased after a single soil fertilization at planting (Taylor and Golden, 2002), and work on European oak species suggests that limited fertilization may be an effective method (Trubat *et al.*, 2008). Repeated soil fertilization of northern red oak and white oak (*Quercus alba* L.) increases biomass and decreases leaf chlorosis, and exponential nutrient loading performs best (Birge *et al.*, 2006). Exponential nutrient loading in the nursery improves above and below ground growth of those species planted on a former mine site, and a medium fertilizer rate resulted in the best performance (Salifu *et al.*, 2008). Off-target fertilization of competing plants is an undesirable effect of soil fertilization.

This study aims to determine shade level and fertilizer supplement effects and their interaction on pure *C. dentata* seedling survival and growth. The effect of the fertilizer supplement will also be examined on three bottomland oak species. The results will help guide chestnut hybrid restoration efforts and oak restoration.
Materials and Methods

Study Site

The study was conducted at the Alabama A&M University’s Winfred Thomas Agricultural Research Station in Hazel Green, Alabama, on the southern Cumberland Plateau (34.53.50N, 86.34.34W). Stands of sweetgum (*Liquidambar styraciflua* L.) were planted in February - March 1995 at 5 x 10 ft (1.5 x 3.05 m) spacing. In February 2009, the stands were thinned. One-third of each stand was left unthinned (no seedlings were planted in the unthinned areas), one-third had approximately 50% overstory removal, and the other one-third had approximately 66% overstory removal. The operation was thinning from below with primary removal of suppressed and intermediate trees, as well as diseased trees and trees with lower stem quality. The *C. dentata* seedlings were planted in the thinned areas and in the open. The cut sweetgum trees resprouted, but the sprouts were not removed or treated in any way. Root and stump sprouts were fairly common by the end of the study. Soils are eroded, undulating, Decatur and Cumberland silty clay loams and silty clays. Soils are classified as fine, thermic Rhodic Paleudults and Paleudalfs (Soil Survey Staff, 2010).

The oak restoration study was conducted at the RW Jones property on the southern Cumberland Plateau (34.41.05N, 86.19.58W). The land was recently converted from row cropping to three species of bottomland oaks planted in 2010 at a 12 x 12 ft (3.65 x 3.65 m) spacing in accordance with the Conservation Reserve Program. The field is near the Paint Rock River. Soils are Melvin silty clay and Sequatchie fine sandy loam, undulating phase, and classified as fine-silty, mixed, active, nonacid, mesic Fluvaquentic
Endoaquepts and fine-loamy, siliceous, semiactive, thermic Humic Hapludults (Soil Survey Staff, 2010).

Experimental Design and Sampling

Four-hundred American chestnut bare root 1-0 seedlings were purchased from Michigan. The seedlings were planted in April 2009 at 5 x 5 ft (1.5 x 1.5 m) spacing in a modified randomized complete block split plot design inside the thinned portions of the L. styraciflua plantations and in the open. The seedlings were planted under three shade levels: open conditions (no overstory trees), 50% residual overstory, and 34% residual overstory. Each one of these three shade levels was replicated three times, and there were 44 seedlings planted in each of the 9 plots. The seedlings planted in three plots in the open were located to the south, east, and west, respectively, of the sweetgum stands at a distance of about 75 ft (23 m) from the stand edge. Seedlings were planted with dibble bars.

The seedlings surviving after 8 weeks within each shade level were randomly assigned one of three fertilizer levels: no fertilizer, single application, and two applications during the growing season. The first fertilizer supplement application was done on August 13, and the subsequent application on the two application treatment was on September 30, 2009. Accele-Grow-M® fertilizer supplement was provided free of charge by Accelegrow Technologies, West Point, Georgia and was applied as a leaf spray. The fertilizer supplement is a mixture of a 3-0-3 fertilizer (NPK), preservative, stabilizer, activator, and carrier system that contains a large concentration of the amino acid sarcosine. Seedlings were sprayed until all the leaves were covered with fertilizer.
solution. Competitors from shrub, vine, and herbaceous species located up to 0.5 ft (15 cm) from the planting location of each seedling were removed. All trees were sprayed regularly with Liquid Fence deer and rabbit repellent due to initial herbivory. Invasive species and other competitors were removed near seedlings approximately ten times during the growing season.

In the oak study, the experiment plot contains 499 oak seedlings that were planted in February 2010 in a random species arrangement in accordance with Conservation Reserve Program regulations. The seedlings were stratified by species and randomly assigned among the three fertilizer levels. The study was conducted on 149 *Quercus pagoda*, 91 *Q. texana*, and 159 *Q. michauxii*. Accele-Grow-M® fertilizer supplement was applied as a leaf spray on July 1 and again on September 20, 2010 for the two treatment group of seedlings. The controls received no fertilizer.

The root collar diameter (RCD) and height of all chestnut trees was measured before and immediately after planting. The RCD was measured with digital calipers, and height was measured as vertical distance from the ground to the highest point on the stem with a tape measure. For each chestnut seedling I also recorded the number of first order lateral roots with diameter over 1 mm (0.04 in) at the base. All living trees were measured again at the end of the 2009 growing season for chestnuts and the 2010 growing season for oaks.

**Hypotheses and Statistical Analysis**

I tested whether fertilizer supplement application, shade, and their interaction have an impact on the survival and on the absolute and relative seedling growth in height
and RCD after the first growing season. Seedlings that died or were browsed were excluded from the analyses of seedling growth. Analysis of variance (ANOVA), as a split plot design, was used to test if there were differences in the post-treatment (with fertilizer supplement) seedling mortality in each subplot. The different causes of seedling death were not considered in the analyses. ANOVA was used to test if seedling browse by 1) any herbivore, or 2) rabbit only, or 3) deer only, was different among the treatments. Pre-treatment (with fertilizer supplement) and overall mortality was analyzed with ANOVA as a randomized complete block design using shade as the predictor variable.

For the number of first order lateral roots, a log transformation was used to improve normality. Due to values of zero, 1 was added to the number of laterals. In the rest of the chapter, the number of first order lateral roots refers to this log transformed variable. Linear regression was used with each measure of growth as the response variable and the initial seedling attributes (height, RCD, and number of first order lateral roots) as the predictor variables.

I used mixed models to test for differences among the groups of fertilizer supplement treatment and shade levels. When I use the treatment name “fertilizer” in the rest of the chapter, I refer to treatment with fertilizer supplement. The dependent variables are absolute and relative growth of root collar diameter and height. The relative growth is the growth expressed as a proportion of the initial root collar diameter and height, respectively. Each seedling is treated as an observation in the mixed model. The seedlings are biologically independent due to the planting spacing, application of fertilizer supplement on individual trees, and interspersion of fertilizer treatment (subplot factor) within each shade block (whole plot factor).
In the oak study, analysis of variance (ANOVA) was used to test for differences among the groups of treatment levels and species. The effects are fertilizer, species, and the fertilizer by species interaction. The dependent variables are absolute and relative root collar diameter and height growth. The relative growth variables are more appropriate for measuring the effects of fertilizer and shade than absolute growth. Furthermore, these variables are more normal and have fewer outliers. For chestnuts, the fixed effects are fertilizer, shade, and the fertilizer by shade interaction. The random effects are replication and the replication by shade interaction. Restricted estimate of maximum likelihood (REML) methods were used to decrease bias in the mixed model. Type III sum of squares was used due to missing data from mortality. Tukey-Kramer method was used for comparing marginal means. I considered results to be significant if $p < 0.1$. Statistical analyses were performed in SAS software version 9.1.3 (PROC MIXED procedure; SAS Institute, Cary, NC, 2006).

**Results**

Seventy of the 396 chestnut seedlings had died by the time of first fertilizer treatment in August, which was four months after planting. Chestnut mortality by August among the three shade levels was not significantly different ($p=0.29$; Figure 2.1). By the end of the first growing season the average mortality in the open was 57%, while in the light and heavy shade it was 9% and 17%, respectively. However, the mortality was not significantly different among the three shade levels tested as a randomized complete block design (RCBD; $p=0.13$; Figure 2.1).
Figure 2.1. Percent mortality in each shade level split by replication. The hatched portion is mortality that occurred after the first fertilizer supplement treatment and the open portion is mortality before that treatment.

Seedlings were not significantly different in size at planting or at the time of treatment assignment. However, after removing from the analysis the 41 seedlings that died or were browsed after treatment, seedlings that received two treatments were initially significantly shorter than those that received a single spray. Despite that difference in height, their root collar diameters were not different.

Fertilizer, shade, and their interaction all had a significant effect on the relative root collar diameter growth (Table 2.1). However, none of the factors affected relative height growth (Figure 2.2; Table 2.1). Additionally, increased shade consistently decreased absolute root collar diameter growth (Figure 2.3). Linear regression with each
dependent variable used in the mixed model showed that number of first order lateral roots was not a significant predictor for any of the growth measurements (all p>0.16).

Seedlings in the open grew 26% in relative RCD, which was significantly more than the 17% growth of the seedlings in the heavy shade (Table 2.2). The seedlings in the light shade had intermediate growth, which was statistically the same as the growth in the open and in heavy shade. The pairwise comparisons of relative RCD and height among seedlings treated with fertilizer twice, once, or not sprayed were not different from each other (Table 2.2).

Although there were no significant differences, there was a consistent trend that the single fertilizer application resulted in less growth in the open and heavy shade but

Table 2.1. Effect of the treatments on the four measures of chestnut seedling growth. Relative growth is the growth as a proportion of the original size. An “*” indicates the interaction term. Shade levels are no overstory, 34% residual overstory, and 50% residual overstory. Fertilizer levels are no application, single application, and two applications.

<table>
<thead>
<tr>
<th>Dependent and predictor variables</th>
<th>F (df)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute root collar diameter growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>6.15 (2,4)</td>
<td>0.060</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>2.17 (2,240)</td>
<td>0.116</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>1.66 (4,240)</td>
<td>0.159</td>
</tr>
<tr>
<td>Relative root collar diameter growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>6.40 (2,4)</td>
<td>0.057</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>2.72 (2,240)</td>
<td>0.068</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>2.45 (4,240)</td>
<td>0.047</td>
</tr>
<tr>
<td>Absolute height growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>0.09 (2,4)</td>
<td>0.918</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1.71 (2,240)</td>
<td>0.183</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>1.09 (4,240)</td>
<td>0.364</td>
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<tr>
<td>Relative height growth</td>
<td></td>
<td></td>
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<tr>
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<tr>
<td>Shade*fertilizer</td>
<td>1.15 (4,240)</td>
<td>0.336</td>
</tr>
</tbody>
</table>
more growth in the light shade for absolute and relative RCD (Table 2.3; Figure 2.4). In the discussion I elaborate on the possible implications of this trend, particularly if there were more replications.

Figure 2.2. Mean ± SE for each fertilizer supplement treatment level for each dependent variable. Relative growth is the growth as a proportion of the original size. Letters indicate significant difference with Tukey-Kramer adjustment for multiple comparisons (α = 0.1).
0=no fertilizer supplement application, 1=single application, 2=two applications
Figure 2.3. Mean ± SE for each shade level for each dependent variable. Relative growth is the growth as a proportion of the original size. Letters indicate significant difference with Tukey-Kramer adjustment for multiple comparisons (α = 0.1).

0=open, 1=light shade, 2=heavy shade

Browsing and mortality between the time of fertilizer application and the end of the first growing season were unaffected by treatment. However, this is based only on 41 seedlings that died after fertilizer application (out of the 326 seedlings alive at the time of
the initial fertilizer application). Mortality after fertilizer application was not different among the three shade levels tested as a RCBD (p=0.32).

Rabbit browse was 9.1% in the heavy shade, 3.8% the light shade, and it was absent in the open. There was no significant main or interaction effect of the nine treatments on percent browsing in the split plot analysis. Hence, I used RCBD to test if the percentage of browsed seedlings was different in the three shade levels before and after treatment. Deer, rabbit, and cumulative browsing at the end of the growing season were unaffected by shade. Only 4 seedlings were browsed before treatment assignment, all by rabbit, so pre-treatment browsing was not analyzed separately.

For the oaks, initial RCD and height were significantly different across species (p<0.01). *Quercus pagoda* had significantly lower initial RCD (p<0.01), 5.24 mm, than both *Q. michauxii* and *Q. texana*, which were not significantly different from each other.

### Table 2.2. Relative seedling growth of chestnuts. Tukey-Kramer method was used to identify significant differences (α = 0.1) between marginal means of the relative growth of each main effect group found significant with ANOVA. Means on the first three lines for the same response variable and with the same letter are not significantly different. It is analogous for the last three lines in the table.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Root collar diameter</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Shade Fertilizer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0† All</td>
<td>0.26 0.03</td>
<td>0.069 0.020</td>
</tr>
<tr>
<td>1 All</td>
<td>0.23 0.02</td>
<td>0.061 0.017</td>
</tr>
<tr>
<td>2 All</td>
<td>0.17 0.02</td>
<td>0.057 0.018</td>
</tr>
<tr>
<td>All 0‡</td>
<td>0.20 0.02</td>
<td>0.059 0.017</td>
</tr>
<tr>
<td>All 1</td>
<td>0.20 0.02</td>
<td>0.053 0.017</td>
</tr>
<tr>
<td>All 2</td>
<td>0.25 0.02</td>
<td>0.075 0.016</td>
</tr>
</tbody>
</table>

†Shade 0=open, 1=light shade, 2=heavy shade, All=compared across all fertilizer levels
‡Fertilizer 0=no fertilizer supplement application, 1=single application, 2=two applications, All=compared across all shade levels
Table 2.3. Marginal mean estimates for chestnuts of the change (growth) in dependent variables at the end of the first growing season following treatments. Relative growth is the growth as a proportion of the original size. Tukey-Kramer adjustment was used for multiple comparisons ($\alpha = 0.1$). Means in the same row with same letter are not different.

<table>
<thead>
<tr>
<th>Abs RCD (mm)</th>
<th>Open, no spray</th>
<th>Open, 1 spray</th>
<th>Open, 2 sprays</th>
<th>Light shade, no spray</th>
<th>Light shade, 1 spray</th>
<th>Light shade, 2 sprays</th>
<th>Heavy shade, no spray</th>
<th>Heavy shade, 1 spray</th>
<th>Heavy shade, 2 sprays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs RCD (mm)</td>
<td>1.185&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.910&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.469&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.958&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.117&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.986&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.769&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.656&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.911&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rel RCD</td>
<td>0.240&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.193&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.336&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.205&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.262&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.218&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>0.163&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.139&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.198&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abs HT (cm)</td>
<td>3.826</td>
<td>2.322</td>
<td>3.880</td>
<td>3.293</td>
<td>2.669</td>
<td>3.562</td>
<td>1.898</td>
<td>3.185</td>
<td>3.853</td>
</tr>
<tr>
<td>Rel HT</td>
<td>0.080</td>
<td>0.048</td>
<td>0.079</td>
<td>0.063</td>
<td>0.051</td>
<td>0.070</td>
<td>0.035</td>
<td>0.060</td>
<td>0.076</td>
</tr>
</tbody>
</table>

Abs=absolute, Rel=relative
Relative growth is calculated as a proportion of the initial size.
RCD=root collar diameter, HT=height
(p>0.89) and had a mean of 8.24 mm. *Q. texana* was initially about 9 cm taller than both *Q. pagoda* and *Q. michauxii*, which had an overall mean of 47 cm.

The absolute height growth at the end of the growing season was significantly different only among species (p=0.01) and not for fertilizer or the interaction (Table 2.4). *Q. texana*, the species with the greatest initial height, had about 2.9 cm less height growth than both *Q. pagoda* and *Q. michauxii*, both of which did not have different height growth from each other (Figure 2.5; Table 2.5). For relative height growth, both species (p=0.01) and the species*fertilizer interaction effects were significant. *Q. texana* had the lowest relative height growth, about 5% compared to 18% for the other species, and provided the only significant difference (Table 2.6). The interaction effect was significant.

![Treatment combination means with SE bars](image)

Figure 2.4. Marginal means of each fertilizer level within each shade level. Letters indicate significant difference with Tukey-Kramer adjustment for multiple comparisons (α = 0.1).
in ANOVA, and there was no consistent response to fertilizer supplement (Table 2.4).

Absolute RCD growth was not different across fertilizer levels within each species.

However, there were significant differences in relative RCD growth (Table 2.5).

Fertilizer did not have a significant overall effect for any of the measures of growth (Table 2.4).

Table 2.4. Effect of the treatments and species on the four measures of oak seedling growth. Relative growth is the growth as a proportion of the original size. An “*” indicates the interaction term. Species are Nuttall, cherrybark, and swamp chestnut oak. Fertilizer levels are no application, single application, and two applications.

<table>
<thead>
<tr>
<th>Dependent and predictor variables</th>
<th>F (df)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute root collar diameter growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>18.75 (2,300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>0.55 (2,300)</td>
<td>0.578</td>
</tr>
<tr>
<td>Species*fertilizer</td>
<td>1.37 (4,300)</td>
<td>0.246</td>
</tr>
<tr>
<td>Relative root collar diameter growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>11.44 (2,300)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>0.42 (2,300)</td>
<td>0.659</td>
</tr>
<tr>
<td>Species*fertilizer</td>
<td>2.56 (4,300)</td>
<td>0.039</td>
</tr>
<tr>
<td>Absolute height growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>4.65 (2,300)</td>
<td>0.010</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>2.20 (2,300)</td>
<td>0.112</td>
</tr>
<tr>
<td>Species*fertilizer</td>
<td>1.44 (4,300)</td>
<td>0.221</td>
</tr>
<tr>
<td>Relative height growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Species</td>
<td>5.20 (2,300)</td>
<td>0.006</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1.87 (2,300)</td>
<td>0.157</td>
</tr>
<tr>
<td>Species*fertilizer</td>
<td>2.05 (4,300)</td>
<td>0.088</td>
</tr>
</tbody>
</table>
Figure 2.5. Marginal means of each fertilizer level within each oak species. Relative growth is the growth as a proportion of the original size. Letters indicate significant difference with Tukey-Kramer adjustment for multiple comparisons ($\alpha = 0.1$).
Table 2.5. Marginal mean estimates for oaks of the change (growth) in dependent variables at the end of the first growing season following treatments. Tukey-Kramer adjustment was used for multiple comparisons ($\alpha = 0.1$). Means in the same row with same letter are not different.

<table>
<thead>
<tr>
<th></th>
<th>Q. michauxii, no spray</th>
<th>Q. michauxii, 1 spray</th>
<th>Q. michauxii, 2 sprays</th>
<th>Q. texana, 1 spray</th>
<th>Q. texana, 2 sprays</th>
<th>Q. pagoda, no spray</th>
<th>Q. pagoda, 1 spray</th>
<th>Q. pagoda, 2 sprays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs RCD (mm)</td>
<td>0.915$^c$</td>
<td>0.639$^c$</td>
<td>0.821$^c$</td>
<td>1.99$^a$</td>
<td>1.49$^{ab}$</td>
<td>1.84$^{ab}$</td>
<td>0.585$^c$</td>
<td>0.997$^{bc}$</td>
</tr>
<tr>
<td>Rel RCD</td>
<td>0.123$^{bc}$</td>
<td>0.090$^{c}$</td>
<td>0.119$^{bc}$</td>
<td>0.264$^a$</td>
<td>0.186$^{abc}$</td>
<td>0.234$^{ab}$</td>
<td>0.120$^{bc}$</td>
<td>0.234$^{ab}$</td>
</tr>
<tr>
<td>Abs HT (cm)</td>
<td>5.65$^a$</td>
<td>5.32$^a$</td>
<td>4.74$^{ab}$</td>
<td>-0.185$^b$</td>
<td>2.50$^{ab}$</td>
<td>4.08$^{ab}$</td>
<td>3.06$^{ab}$</td>
<td>6.46$^a$</td>
</tr>
<tr>
<td>Rel HT</td>
<td>0.144$^a$</td>
<td>0.120$^a$</td>
<td>0.109$^{ab}$</td>
<td>0.003$^b$</td>
<td>0.053$^{ab}$</td>
<td>0.090$^{ab}$</td>
<td>0.061$^{ab}$</td>
<td>0.156$^a$</td>
</tr>
</tbody>
</table>

Abs=absolute, Rel=relative
Relative growth is calculated as a proportion of the initial size.
RCD=root collar diameter, HT=height
Table 2.6. Relative seedling growth of oaks. Tukey-Kramer method was used to identify significant differences ($\alpha = 0.1$) between marginal means of the relative growth of each main effect group that was found significant with ANOVA. Means on the first three lines for the same response variable and with the same letter are not significantly different. It is analogous for the last three lines in the table.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Root collar diameter</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Species</td>
<td>Fertilizer</td>
<td>Mean</td>
</tr>
<tr>
<td>$Q. \text{ michauxii}$</td>
<td>All</td>
<td>0.111$^b$</td>
</tr>
<tr>
<td>$Q. \text{ texana}$</td>
<td>All</td>
<td>0.228$^a$</td>
</tr>
<tr>
<td>$Q. \text{ pagoda}$</td>
<td>All</td>
<td>0.190$^a$</td>
</tr>
<tr>
<td>All</td>
<td>0‡</td>
<td>0.169</td>
</tr>
<tr>
<td>All</td>
<td>1</td>
<td>0.170</td>
</tr>
<tr>
<td>All</td>
<td>2</td>
<td>0.190</td>
</tr>
</tbody>
</table>

‡Fertilizer 0=no fertilizer supplement application, 1=single application, 2=two applications, All= compared across all fertilizer or species levels, respectively.

**Discussion**

Light intensity is known to have a significant effect on tree growth. This was indeed observed for some of the response variables, especially when comparing growth in the open with growth in the heavy shade (for all fertilizer treatment levels combined; Table 2.2; Figure 2.3). Unfertilized seedlings had decreased growth with increased shade, but the difference was not statistically significant. However, fertilized seedlings did not exhibit the same growth trend with increased sunlight. This is most likely due to the observed interaction between light and fertilizer supplement. Additionally, disease, especially *Phytophthora cinnamomi*, could be another reason. Some of the increase in root collar diameter may be due to basal swelling that occurs with infection by *P. cinnamomi* and *Cryphonectria parasitica*. Observations of the seedlings appeared to
indicate that such infections, and corresponding swelling, were more common in the open.

The greatest growth in absolute RCD was achieved in the open for seedlings treated twice, 1.47 mm. However, it was significantly greater than the absolute RCD growth of only two out of the eight other treatments (Table 2.3). The control seedlings in the heavy shade and the seedlings in the heavy shade treated once both had lower growth, an average of 0.71 mm, which is 51% lower growth than the seedlings in the open treated twice. The non-significant differences ranged from 38% less, for the seedlings in the heavy shade treated twice, to 19% less, for control seedlings in the open (Figure 2.4).

Although the differences were not significant, there was a consistent trend that in the light shade, the single fertilizer treatment resulted in greater growth than both the no fertilizer and the two fertilizer treatments. However, in the heavy shade and in the open, the single fertilizer treatment resulted in lower growth than both the no spray and the two sprays treatment. This could indicate that increasing fertilizer supplement in the heavy shade may help to offset the growth disadvantage from being shaded. Although I did not observe a significant difference in growth, a significant effect might have been observed with a larger sample size. This is because the power of the statistical test would increase and a smaller percentage difference in growth would be required for statistical significance. For instance, for relative RCD, a 64% increase in growth was significant but a 54% increase was not (Table 2.3). Since the seedlings that did not follow the same growth patterns are the ones in light shade, and the difference might be attributable to an effect of the fertilizer supplement, further study of this fertilizer supplement may be
warranted when underplanting blight resistant hybrid chestnuts in light levels near 66% of full sunlight.

Due to the great variation in survival, it cannot be claimed that any of the shade levels resulted in a better survival than any of the other two, even though the value for average mortality of seedlings in the open was approximately three to six times greater than that of the seedlings in the heavy and light shade, respectively (the test for the difference among them had a p=0.13). Nevertheless, considering this result and the results from other studies, it can be recommended that at least some care be taken when considering planting in fully open conditions. American chestnut has consistently survived well when planted under some shade (Jacobs, 2007). Afforestation of chestnut on agricultural fields has been studied little, but unpublished work in Ohio has found very high mortality (McCarthy, personal communication, August, 2010). However, a study in Indiana found negligible American chestnut mortality two years after planting on a former agricultural field (Selig et al., 2005). It is possible that the prevalence of P. cinnamomi on the site has a large impact on mortality in agricultural field afforestation. On sites in the South with P. cinnamomi in the soils, converting former agricultural fields directly into chestnut plantations is not recommended. Therefore, prior soil testing for P. cinnamomi may be useful.

Quercus michauxii and Q. texana had relative and absolute RCD growth patterns with changing fertilizer supplement that were similar to chestnut seedlings in the open and heavy shade - single fertilizer treatment resulted in the lowest growth but was not statistically different from the other treatments. Q. pagoda growth patterns more closely resembled those of chestnut in the light shade than other oak species.
CHAPTER 3
TWO YEAR RESPONSE OF AMERICAN CHESTNUT TO OVERSTORY DENSITY
AND FERTILIZER SUPPLEMENT

Introduction

American chestnut (Castanea dentata (Marsh.) Borkh.) was formerly a primary component of eastern North American forests. The chestnut blight (Cryphonectria parasitica (Murrill) Barr), an accidentally introduced fungal pathogen from Asia, decimated the species (Russell, 1987). The American Chestnut Foundation (TACF) has been breeding hybrids of C. dentata and C. mollissima Blume (Chinese chestnut) that retain most C. dentata characteristics but are resistant to the blight (Diskin et al., 2006). In addition to the chestnut blight, a major root pathogen of Castanea spp. is Phytophthora cinnamomi Rands, a water mold normally found in the soil. C. dentata is historically widespread, but the introduction of P. cinnamomi will likely decrease the conditions where C. dentata can be grown and is therefore an important consideration in restoration. P. cinnamomi resistance has not been considered in TACF breeding program, and resistance is not genetically related (Sisco, 2009).

Vigor of C. dentata trees and therefore a decrease in susceptibility to pathogens can be enhanced through providing the trees with the additional nutrients through fertilization. This applies especially for poor quality sites where a lower incidence of P. cinnamomi is expected and on which C. dentata may be planted. I was unable to find any
studies that found increased growth or survival of C. dentata with fertilization on forested sites or former agricultural fields.

Most seedling mortality occurs during the first years following planting, before the root system is well established. Ensuring the success of planting the first time requires less investment than replanting an area many times (Keeton, 2008). Initial treatment of soils before planting seedlings has a great effect on survival and growth of a variety of species (Archibold et al., 2000; Karlsson, 2002; Hewitt et al., 2004; Knapp et al., 2006; Rhoades et al., 2009). Fertilizing seedlings along with mechanically preparing the site could result in very high seedling survival (Hewitt et al., 2004). This is especially desirable when no replanting is intended and time invested in planting is not a major consideration. Some examples of cases when time invested for the initial planting is not a major consideration are tree planting volunteer activities with conservation organizations, urban forestry, and homeowner planting.

Results from several studies on soil fertilization of C. dentata show that the effect is marginal - weekly nitrogen fertilization does not significantly enhance the growth of C. dentata over control in greenhouse conditions (Rieske et al., 2003). Additionally, treatment with controlled release fertilizer in greenhouse conditions results in higher mortality and lower growth rates compared to control, probably due to the increase in root disease (Herendeen, 2007). The results from such greenhouse experiments are consistent with results from experiments with open grown seedlings planted on mine reclamation sites that had previously been fertilized - C. dentata seedling growth on the fertilized soils is not significantly different from growth on unfertilized soils (Herendeen, 2007). I was unable to find any studies that report a significant increase in growth or
survival of *C. dentata* seedlings with soil fertilization on forested sites or former agricultural fields. It is likely that supplementation with limited nutrients will increase *C. dentata* performance, but that the adequate combination or amounts have not been applied. Results with other chestnut species consistently show an increase in growth with fertilization. Other *Castanea* species, however, have been found to respond well to fertilization. Organic compound fertilizer increases sapling *C. mollissima* height growth about 1.5 times over control (Zeng et al., 2007). Similarly, fertilization increases shoot growth by approximately 3 times over control in *C. sativa* seedlings (Kohen and Mousseau, 1994).

Increasing nutrient leaching in the runoff after fertilizing agricultural crops, and possibly trees during restoration, is an undesirable side effect. The amount of fertilizer in the runoff can be reduced, however, if the fertilizer is delivered directly into the plant instead of through the soil or through aerial spraying from a helicopter or airplane. Accele-Grow-M® (Accelegrow Technologies, West Point, Georgia) is a new fertilizer supplement that is delivered directly into the plant leaves or roots. This application method also prevents direct fertilization of neighboring competing plants, possibly making the application process more efficient and cost effective. Avoiding fertilization of the competitors can be achieved if the seedlings are sprayed or dipped before outplanting, or if each of the outplanted target plants is sprayed individually, or if the entire planted area is sprayed at a time when the only vegetation present is the targeted seedlings. Such delivery of the fertilizer is also possible for large trees. Such fertilization could increase tree vigor and its defenses against attacks by insects or fungi, including those that are new or exotic. Greater availability of resources to the tree allows it to increase its defensive
compound production and improve its chance of surviving such attacks (Sayler and Kirkpatrick, 2003). Targeted fertilization through the leaves of *C. dentata* may have another benefit – it may help to prevent the decline in root system vigor observed with standard fertilization (Sileshi *et al.*, 2007). Information on the response of such fertilizer delivery is needed, and so is information on the possible interaction of shade levels and fertilizer treatments.

*C. dentata* has intermediate shade tolerance (Joesting, 2009). It responds well to release from overstory and grows rapidly in full sun. However, it survives well in the understory. When the main stem dies, the tree resprouts well and does so repeatedly, so seedling size trees in the understory may have 100 year old root systems (Paillet, 2002). Historical literature suggests that survival is higher under partial shade than in the open for the first two years and that this method was a good way of establishing *C. dentata* in the forest (Russell, 1987). Partial shade appears to be better for growth not only when compared to the open, but also when compared to full shade - *C. dentata* seedlings in 30% shelterwood treatment can have approximately three times greater annual height growth and twice as large a diameter growth than seedlings in an intact forest (McCament and McCarthy, 2005). However, germination, vigor, and survival do not improve significantly in shelterwood (McCament and McCarthy, 2005). Rhoades et al. (2009) found that seedling mortality does not differ significantly between 30% shelterwood and midstory removal treatments. Seedling annual height growth was over 3 times and diameter growth about 4 times as great in shelterwood versus in midstory removal treatments. Visible root disease was twice as common in shelterwood treatments as in midstory removal. Survival of seedlings may be greater under moderate shade (Griffin,
1989; Anagnostakis, 2007). Examination of growth patterns in a Wisconsin stand suggest that planting blight resistant *Castanea* hybrids in clumps in canopy gaps or after certain silvicultural treatments will be a good method of re-establishing the species (McEwan et al., 2006). If this method is used, fertilizing the clumps of seedlings would be easier than treating more scattered seedlings.

This study aims to determine the effect of shade level, treatment with a fertilizer supplement, and their interaction on pure *C. dentata* seedling survival and growth. The results will help guide chestnut hybrid restoration efforts and restoration of pure chestnuts that have some degree of natural resistance to the blight. There have been isolated cases of blight resistant individual *C. dentata* trees, and geneticists may engineer blight and *Phytophthora* resistant pure American chestnut trees. Unpublished work from the manufacturer suggests that this fertilizer supplement has demonstrated promising agricultural results in drought years, but it has not been tested on hardwoods (http://www.accelegrow.com/testing.html, accessed February 28, 2011). No work has been done on its effectiveness under different light conditions. I hope to find if the fertilizer supplement may be useful in restoration efforts involving tree underplanting after different silvicultural treatments or in afforestation efforts.

**Methods**

**Study Site**

The study was conducted at the Alabama A&M University’s Winfred Thomas Agricultural Research Station in Hazel Green, Alabama, on the southern Cumberland Plateau (34.53.50N, 86.34.34W). Stands of sweetgum (*Liquidambar styraciflua* L.) were
planted in February - March 1995 at 5 x 10 ft (1.5 x 3.05 m) spacing. In February 2009, the stands were thinned. One-third of each stand was left unthinned (no seedlings were planted in the unthinned areas), one-third had approximately 50% overstory removal, and the other one-third had approximately 66% overstory removal. The operation was thinning from below with primary removal of suppressed and intermediate trees, as well as diseased trees and trees with lower stem quality. The *C. dentata* seedlings were planted in the thinned areas and in the open.

**Experimental Design and Sampling**

Four-hundred American chestnut bare root 1-0 seedlings were purchased from Michigan. The seedlings were planted in April 2009 at 5 x 5 ft (1.5 x 1.5 m) spacing in a modified randomized complete block split plot design inside the thinned portions of the *L. styraciflua* plantations and in the open. The seedlings were planted under three shade levels: open conditions (no overstory trees), 50% residual overstory, and 34% residual overstory. Each one of these three shade levels was replicated three times, and there were 44 seedlings planted in each of the 9 plots. Seedlings were planted with dibble bars.

The seedlings surviving after 8 weeks within each shade level were randomly assigned one of three fertilizer levels: no fertilizer, single application, and multiple applications during the growing season. The first fertilizer supplement application was done on August 13, 2009, and the subsequent application on the multiple application treatment was on September 30, 2009. In 2010, the multiple application treatment seedlings were sprayed on May 30, June 30, August 10, September 13, and October 9. Accele-Grow-M<sup>®</sup> fertilizer supplement was provided free of charge by Accelegrow.
Technologies, West Point, Georgia and was applied as a leaf spray. The fertilizer supplement is a mixture of a 3-0-3 fertilizer (NPK), preservative, stabilizer, activator, and carrier system that contains a large concentration of the amino acid sarcosine.

The leaf spray fertilizer supplement provides an equivalent of approximately 0.5 kg N and 0.4 kg K per acre (1.2 kg N and 1.0 kg K per hectare) per application. This figure was calculated by using the amount of fertilizer supplement solution used to treat all the multiple spray trees for the last two treatments of the 2010 growing season. The square feet occupied by those seedlings was converted to acres. Therefore, the figure is the nutrient equivalent per acre for seedlings planted at a 5 x 5 ft spacing; an estimate of nutrient equivalent per acre of seedling crown would be comparable to broadcast fertilizer rates in agriculture. Since this figure was calculated based on the amount applied at the end of the 2010 growing season, it is an overestimate of the amount applied to the seedlings when they were younger. Due to the foliar application method, seedlings receive more fertilizer supplement as their crowns grow larger and more leaves are available to spray. If the fertilizer supplement works, this characteristic is desirable since seedlings of other species have been shown to benefit from increasing nutrient loading as they grow larger (Kormanik et al., 2003).

Competitors from shrub, herbaceous, and vine species located up to 0.5 ft (15 cm) from the planting location of each seedling were removed about 15 times across both growing season. All trees were sprayed regularly with Liquid Fence® Deer and Rabbit Repellent (Liquid Fence, Brodheadsville, PA, USA) due to initial herbivory. Invasive species competitors near seedlings were removed about every month.
The root collar diameter (RCD) and height of all trees was measured before and immediately after planting. The RCD was measured with digital calipers, and height was measured as vertical distance from the ground to the highest point on the stem with a tape measure. For each seedling I also recorded the number of first order lateral roots with diameter over 1 mm (0.04 in) at the base. The survival of seedlings was recorded six times throughout the two growing seasons. All living trees were measured again at the end of the 2009 growing season and again at the end of the 2010 growing season.

Hypotheses and Statistical Analysis

I tested whether fertilizer supplement application, shade, and their interaction had an impact on the survival and on the absolute and relative seedling growth in height and RCD after the second growing season. Seedlings that died or were browsed were excluded from the analyses of seedling growth. I used analysis of variance (ANOVA), as a split plot design, to test if there were differences in the percent post-treatment (with fertilizer supplement) seedling mortality in each subplot. The different causes of seedling death were not considered separately in the analyses. Since mortality in the open was so high and therefore too few seedlings were available, all seedlings in the open were excluded from the growth analyses. Thus, only the growth of the seedlings in the heavy and light shade levels was compared. All seedlings were included in the survival analyses. Mortality was tested as both percent mortality and days to death. Percent mortality was used for the blocks. The days to death variable was used to analyze death with each seedling that died as an observation. It was created using six observations of seedling survival throughout the two growing seasons. The number of days from planting
until the seedling was first recorded as dead is the days to death. It is treated as a continuous variable. Cox proportional-hazards regression was used to analyze days to death.

The amount of fertilizer supplement applied was estimated by measuring the amount of solution sprayed into a graduated cylinder for a sample of seedlings at the end of the second growing season. Linear regression was used with RCD as the predictor variable and milliliters of fertilizer supplement spray as the response variable. Crown area was also used with this information to estimate the nutrient equivalents per acre.

For the number of first order lateral roots, a log transformation was used to improve normality. Due to values of zero, one was added to the number of laterals. In the rest of the chapter, the number of first order lateral roots refers to this log transformed variable. Linear regression was used with each measure of growth as the response variable and the initial seedling attributes (height, RCD, and number of first order lateral roots) as the predictor variables.

I used mixed models to test for differences among the groups of fertilizer supplement treatment and shade levels. When I use the treatment name “fertilizer” in the rest of the chapter, I refer to treatment with fertilizer supplement. The dependent variables are absolute and relative growth of root collar diameter and height. The relative growth is the growth expressed as a proportion of the initial root collar diameter and height, respectively. Each seedling is treated as an observation in the mixed model. The seedlings are biologically independent due to the planting spacing, application of fertilizer supplement on individual trees, and interspersion of fertilizer treatment (subplot factor) within each shade block (whole plot factor).
The relative growth variables are more appropriate for measuring the effects of fertilizer and shade than absolute growth. Furthermore, these variables are more normal and have fewer outliers. The fixed effects are fertilizer, shade, and the fertilizer by shade interaction. The random effects are replication and the replication by shade interaction. Restricted estimate of maximum likelihood (REML) methods were used to decrease bias in the mixed model. Type III sum of squares was used due to missing data from mortality. I considered results to be significant if $p < 0.1$, and differences discussed in the text are significant unless otherwise noted. Tukey-Kramer post-hoc tests were used for multiple comparisons between groups that had a significant difference in ANOVA. Statistical analyses were performed in SAS software version 9.1.3 (PROC MIXED procedure; SAS Institute, Cary, NC, 2006).

**Results**

At the end of the second growing season the average mortality in the open was significantly higher, 88% ($p \leq 0.01$), than in the light and heavy shade where it was 22% and 29%, respectively (Figure 3.1). When compared to seedlings in the light shade, those in the heavy shade had similar hazard ratios and those in the open were 10.5 times greater (Figure 3.2).

The original height and RCD were significantly different among the seedlings that survived in each treatment combination by the end of the first growing season. However, neither of those measures of initial size were different for the survivors at the end of the second year (all $p > 0.49$). Seedlings grew more in the second growing season than the
Figure 3.1. Percent mortality in each shade level split by replication. The shaded portion is mortality that occurred during the second year and the open portion is mortality that occurred during the first year.

first, but the difference was not compared statistically. The percent growth of the seedlings in RCD was greater than in height (Figures 3.3 and 3.4).

The two shade levels did not have a different effect on any of the growth measures (all $p>0.11$; Table 3.1). Fertilizer had a significant effect on the relative RCD ($p=0.086$). Seedlings that were unfertilized increased only 41% in relative RCD, versus 55% for seedlings that were sprayed once (Table 3.2). Seedlings that were sprayed multiple times (twice in 2009 and five times in 2010) had intermediate growth values and were not significantly different from either of the other two groups (Table 3.2).

There was a significant shade by fertilizer interaction effect on the absolute and relative RCD (Table 3.1). The differences in relative growth were more significant than
Figure 3.2. Kaplan-Meier curves showing the proportion of seedlings surviving over time in each shade level with 90% confidence intervals.
those in absolute growth (Figure 3.5). For the seedlings in the light shade, those treated once grew 72% in relative RCD, which was more than the growth of 45% for non-fertilized seedlings, but was not different from the 52% growth of seedlings receiving multiple fertilizations.

Unfertilized seedlings in the heavy shade and seedlings in the heavy shade sprayed once grew at about the same rate in absolute and relative RCD. Additionally, these seedlings both grew less in absolute and relative RCD than the seedlings in the light shade treated once (Table 3.3).

Linear regression was used to express the milliliters (mL) of fertilizer solution sprayed per application for seedlings of a given RCD size. The equation (3.1) is given by:

![Figure 3.3](image)

**Figure 3.3.** Height marginal means of each fertilizer level within each shade level. The bars show the height at planting, the first year growth increment, and the second year growth increment.
Figure 3.4. RCD marginal means of each fertilizer level within each shade level. The bars show the RCD at planting, the first year growth increment, and the second year growth increment.

\[
\text{mL solution} = 9.1 \times \text{RCD (mm)} - 14.8
\]  

That is, a 1 cm difference in root collar diameter resulted in approximately 9 mL change in fertilizer solution being applied each time. This function is for the RCD size at the end of the second growing season. Therefore, seedlings received more fertilizer as they grew, and larger seedlings received more fertilizer. With this in mind, seedlings that were treated with fertilizer multiple times received up to 7 times the amount given by the equation since they were treated 7 times.

Linear regression with each measure of growth after two years as the dependent variable and the initial seedling measurements as predictors (height, RCD, number of first
Table 3.1. Effect of the treatments on the four measures of 2nd year chestnut seedling growth. Relative growth is the growth as a proportion of the original size. An “*” indicates the interaction term. Shade levels are 34% residual overstory and 50% residual overstory. Fertilizer levels are no application, single application, and seven applications over both growing seasons.

<table>
<thead>
<tr>
<th>Dependent and predictor variables</th>
<th>F (df)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute root collar diameter growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>7.54 (1,2)</td>
<td>0.111</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>1.41 (2,161)</td>
<td>0.247</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>2.44 (2,161)</td>
<td>0.091</td>
</tr>
<tr>
<td>Relative root collar diameter growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>6.90 (1,2)</td>
<td>0.120</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>2.49 (2,161)</td>
<td>0.086</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>3.47 (2,161)</td>
<td>0.033</td>
</tr>
<tr>
<td>Absolute height growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>0.25 (1,2)</td>
<td>0.665</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>0.45 (2,161)</td>
<td>0.639</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>0.33 (2,161)</td>
<td>0.720</td>
</tr>
<tr>
<td>Relative height growth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shade</td>
<td>0.39 (1,2)</td>
<td>0.595</td>
</tr>
<tr>
<td>Fertilizer</td>
<td>0.44 (2,161)</td>
<td>0.646</td>
</tr>
<tr>
<td>Shade*fertilizer</td>
<td>0.33 (2,161)</td>
<td>0.717</td>
</tr>
</tbody>
</table>

Table 3.2. Relative seedling growth. Tukey-Kramer method was used to identify significant differences (α = 0.1) between marginal means of the relative growth of each main effect group that was found significant with ANOVA. Means for the same response variable and with the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Root collar diameter</th>
<th>Height</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SE</td>
</tr>
<tr>
<td>Shade</td>
<td>Fertilizer</td>
<td></td>
</tr>
<tr>
<td>1†</td>
<td>All</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>All</td>
<td>0.41</td>
</tr>
<tr>
<td>All</td>
<td>0‡</td>
<td>0.41</td>
</tr>
<tr>
<td>All</td>
<td>1</td>
<td>0.55</td>
</tr>
<tr>
<td>All</td>
<td>7</td>
<td>0.50</td>
</tr>
</tbody>
</table>

†Shade 1=light shade, 2=heavy shade, All=compared across all fertilizer levels
‡Fertilizer 0=no fertilizer supplement application, 1=single application, 7=two applications during 2009 plus 5 applications in 2010, All=compared across both shade levels
order lateral roots) showed that number of first order lateral roots was a significant predictor only of height growth (p=0.09) and relative height growth (p=0.07). For the first year growth, the number of first order lateral roots was not a significant predictor for any of the variables (all p>0.16).

Figure 3.5. Marginal means of each fertilizer level within each shade level for the four measures of growth. Relative growth is the growth as a proportion of the original size. Letters indicate significant difference with Tukey-Kramer adjustment for multiple comparisons (α = 0.1).
Table 3.3. Marginal mean estimates of the change (growth) in dependent variables at the end of the second growing season following treatments. Relative growth is the growth as a proportion of the original size. Tukey-Kramer adjustment was used for multiple comparisons of significant treatment effects (α = 0.1). Means in the same row with same letter are not different.

<table>
<thead>
<tr>
<th>Light shade, no spray</th>
<th>Light shade, 1 spray</th>
<th>Light shade, 7 sprays</th>
<th>Heavy shade, no spray</th>
<th>Heavy shade, 1 spray</th>
<th>Heavy shade, 7 sprays</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abs RCD (mm)</td>
<td>2.24&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>3.20&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>1.849&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.82&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rel RCD</td>
<td>0.451&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.721&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.520&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.373&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.375&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abs HT (cm)</td>
<td>12.30</td>
<td>17.09</td>
<td>14.25</td>
<td>11.71</td>
<td>12.58</td>
</tr>
<tr>
<td>Rel HT</td>
<td>0.230</td>
<td>0.317</td>
<td>0.271</td>
<td>0.214</td>
<td>0.225</td>
</tr>
</tbody>
</table>

Abs=absolute, Rel=relative
Relative growth is calculated as a proportion of the initial size.
RCD=root collar diameter, HT=height

Discussion

The very high mortality of seedlings in the open was unexpected. It is important to consider how the statistics changed with the exclusion of all seedlings in the open, especially for the main effect shade. Since the seedlings in the open had to be excluded from the growth analyses, the degrees of freedom used for the F test changed, lowering the power of the shade test. Shade would have been a significant predictor for absolute and relative RCD growth with the original (2,4) degrees of freedom. With (1,2) degrees of freedom, the relative RCD growth of seedlings in the light shade would have needed to be about 2% higher (58% vs 56%) on average to be significantly different. With (2,4) degrees of freedom, the relative RCD growth in the light shade would have been significantly different from the heavy shade at only 53% (versus 41% in the heavy
More replications for shade would have been desirable in this study, and it is likely that it would have found more differences to be significant due to the increased power of the test. However, only three plots of thinned sweetgum were available for this study. Had three levels of thinning been tested instead of two levels and no shade, the mortality probably would have been lower, and the degrees of freedom would have remained the same for the shade test across years. Since seedlings were treated as observations for the test of fertilizer, there were sufficient replications for mortality to not influence the statistics as strongly. The change from 240 denominator degrees of freedom for first year growth to 161 for the second year analyses does not change the calculated p-value much. Having more replications for shade with a similar number of seedlings in each would have been more desirable than increasing the number of seedlings in each replication.

Seedlings in the light shade fertilized once grew best over two years. I was unable to find any studies on *C. dentata* or blight resistant hybrids that compared different fertilizer rates. The only study that reported increased growth with the addition of fertilizer also found decreased survival. Controlled release fertilizer applied at planting increased second year growth of *C. dentata* planted on a former quarry site (Miller, 2010). The study was conducted on a nutrient deficient site that probably had a lower prevalence of *P. cinnamomi*. A study on a forested site in Tennessee found greater survival and growth after one year than seedlings planted in Alabama, partially due to the much lower prevalence of *P. cinnamomi* on the Tennessee site. Additionally, the seedlings in Alabama were fertilized, and the ones in Tennessee were not (Clark *et al.*, 2009). The interaction of fertilizer and *P. cinnamomi* on the survival and growth of *C.
dentata deserves further study. The prevalence of *P. cinnamomi* is an important consideration for reforestation and afforestation of blight resistant chestnut hybrids, and its importance varies in different areas (Rhoades *et al.*, 2003; Bowles, 2006; Herendeen, 2007; Clark *et al.*, 2009).

Seedlings in the light shade were expected to grow better than those in the heavy shade. Although this was not found with the statistical test used for second year growth, the seedlings in the light shade treated once did grow more than seedlings in the heavy shade treated once or not treated with fertilizer for most growth measures (Table 3.3). A previous study in Alabama did not find increased growth with increased light in forested conditions, but this was mostly due to low overall seedling growth and high mortality (Clark *et al.*, 2009). In other areas, increased light in forested conditions increased growth consistently. In Kentucky, seedlings planted in a midstory removal treatment with about 27% of full sunlight grew less than seedlings planted in a shelterwood treatment with about 47% of full sunlight. Survival did not differ significantly between the shade levels, and site had a strong effect (Rhoades *et al.*, 2009). In Ohio, seedlings planted in a thinned stand with about 30% of full sunlight grew more than seedlings planted in intact forest (McCament and McCarthy, 2005). A greenhouse experiment found increased growth of *C. dentata* with increased light (Wang *et al.*, 2006).

The number of first order lateral roots was a stronger predictor of second year growth than of first year growth. For linear regression, those roots were not a significant predictor of any measure of first year growth. However, they were for some measures of second year growth even though fewer seedlings were tested due to mortality. This delayed response was expected (Ward *et al.*, 2000). Previous studies have not found the
number of first order lateral roots to be a significant predictor of first or second year growth (Clark et al., 2009; Rhoades et al., 2009). However, a delayed effect has been observed in other species (Ward et al., 2000).
CHAPTER 4
CONCLUSIONS

While shade level did not affect chestnut seedling mortality in the first year, results from other plantings suggest that higher mortality may occur in the open, possibly as a result of presence of *P. cinnamomi* in the soil. The mortality at the end of the second growing season was highest in the open at 88%. Therefore, some care should be taken when considering planting in fully open conditions. While growth in the open was generally greater than growth in the shade, high mortality in the open may make it more appropriate and less risky to plant in light shade where survival is greater than in the open and growth is not significantly less than in the open.

The difference in percent mortality in the light shade and the heavy shade did not change much from end of the first growing season to the end of the second. This is in contrast to the mortality in the open. This further suggests that the non-significant difference in mortality in the light shade and heavy shade observed during the first year was due to the higher rabbit browse in the heavy shade before treatment assignment. The mortality in the open increased enough during the second year after planting to make the difference between it and the shaded areas significant. Few studies have been conducted on chestnuts planted on former agricultural fields. A study in Indiana found negligible mortality of *C. dentata* seedlings planted on a former agricultural field after two years (Selig *et al.*, 2005). However, unpublished work in Ohio has found that mortality was very high, similar to levels in this study, of seedlings planted in the open, which was in contrast to those planted in forest habitats (McCarthy, personal communication, August,
The factors that influence the varying mortality in these plantings on former agricultural fields are unclear, and disease is likely an important factor. More research is needed in this regard to help managers predict if planting chestnut seedlings in this manner is appropriate on the site.

Underplanting American chestnut seedlings beneath a thinned forest or tree plantation was a successful method of establishment. For the first two growing seasons in this study, it worked much better than planting seedlings in full sunlight. The current body of scientific evidence on chestnut plantings suggested that planting on forested sites is a good idea, but planting them in the open may not be depending on the site. Short rotation woody crop plantations have been successfully used to help establish oaks (Gardiner et al., 2004; Gardiner and Yeiser, 2006). The mycorrhizae found in short rotation woody crops probably differ depending on species and also from natural forests. Since this is an important consideration for American chestnut and blight resistant hybrids (Bauman et al., 2010), mycorrhizae should be studied to help ascertain the best species under which to plant chestnuts.

The fertilizer supplement Accele-Grow-M® applied at the rates in this study did not have a significant positive or negative impact on seedling growth over one or two growing seasons. If its effect is delayed, changes in growth may be observed in the future after additional growing seasons. Unless this occurs, one can conclude that the use of the supplement is not helpful for increasing survival or growth of American chestnut seedlings. A delayed effect of the number of first order lateral roots was observed at the end of the second growing season. Similarly, the supplement did not increase seedling
growth of any of the three bottomland oak species and therefore cannot be recommended for use on them, unless the results change in the subsequent growing seasons.

Due to the observed significant interaction with shade, the fertilizer supplement appears to have a different effect on American chestnut seedling growth under the different shade levels. Further investigation in this aspect of the study is recommended. If the fertilizer supplement consistently increases growth of chestnut seedlings underplanted in light shade as much as in this study, it will be useful for managers. Results from more replications, more growing seasons, or both would help in shedding more light in this regard. An interaction was also observed between fertilizer supplement and oak species, so similar testing in this regard will be of interest.
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