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EFFECT OF SHADE AND FERTILIZER SUPPLEMENT ON SURVIVAL AND GROWTH OF AMERICAN CHESTNUT SEEDLINGS

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Abstract

American chestnut (*Castanea dentata*) was once a dominant overstory species of eastern North American forests before it was decimated by chestnut blight. Blight resistant hybrid chestnuts share most morphological characteristics with *C. dentata*, so studying its establishment and early growth will help develop effective methods for establishing blight resistant hybrids. Supplementation with nutrients that are not sufficient in the soil likely will increase early *C. dentata* performance, but optimal amounts and combination of nutrients have not been determined. We examined the effect of three shade levels and a novel leaf spray fertilizer supplement on pure *C. dentata* seedlings. Shade levels were 0 %, 50 %, and 34 % overstory of sweetgum (*Liquidambar styraciflua*). After one growing season, fertilizer had a significant effect only on relative root collar diameter growth. However, due to interaction with shade, the growth was not different among the three fertilizer levels. Root collar diameter growth was about 53 % greater in the open than in stands with 50 % residual overstory. The effects were non-significant for relative height growth. By the end of the first growing season, seedling mortality was 57 %, 9 %, and 17 % in the open, light shade, and heavy shade, respectively. Planting of chestnuts in light shade had the most acceptable combination of survival and growth.

Key words: *Castanea dentata***,** shade, leaf spray 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 introduced fungal pathogen from Asia, decimated the species in the early part of the 20th century (Russell 1987). Over the last decades, breeding has been carried out of hybrids of *C. dentata* and Chinese chestnut (*Castanea mollissima* Blume) that retain most *C. dentata* characteristics but are resistant to the blight (Diskin et al. 2006).

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 positive 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 increase seedling survival (Hewitt et al. 2004).

Results from several studies on soil fertilization of *C. dentata* show that the growth effect is marginal. (Rieske et al. 2003) showed that weekly nitrogen fertilization does not significantly enhance the growth of *C. dentata* over control in greenhouse conditions. Additionally, treatment with controlled release fertilizer in greenhouse conditions resulted in higher mortality and lower growth rates compared to control seedlings, probably due to an 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. On these reclamation fields, *C. dentata* seedling growth on fertilized soils was not significantly different from growth on unfertilized soils (Herendeen 2007). The following four studies are the only ones that found any increase in growth or survival of *C. dentata* seedlings with soil fertilization: Latham (1992) observed greater growth with increasing soil nutrient availability, and increased growth was also found with greater availability of magnesium, potassium (McCament and McCarthy 2005), and nitrogen (Rieske et al. 2003, McCament and McCarthy 2005). It is likely that proper supplementation with nutrients that are limited on the site can increase *C. dentata* performance because it does so in other hardwoods (Trubat et al. 2008). However, in most experiments this has not been observed with *C. dentata*, possibly because the adequate combination or amounts have not been applied. Results with other chestnut species, however, consistently show an increase in growth with fertilization. Organic compound fertilizer increased *C. mollissima* sapling height growth about 1.5 times over control (Zeng et al. 2007). Similarly, fertilization increased shoot growth approximately 3 times 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 onto the plant instead of through the soil. Such application method also prevents fertilization of neighboring competing plants, making the application process more efficient and possibly more 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. This type of delivery of the fertilizer is also possible for large trees. Successful fertilization generally increases 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 insect or fungal attacks (Sayler and Kirkpatrick 2003). Targeted fertilization through the leaves of *C. dentata* may also 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 et al. 2009). It responds well to release from the overstory and grows rapidly in full sun. However, it also survives well in the understory. When the main stem dies, the tree resprouts well and does so repeatedly, so seedling-sized trees in the understory may have 100-year-old root systems (Paillet 2002). Historical literature suggests that survival is greater 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). Examination of growth patterns in a Wisconsin stand suggest that planting blight-resistant chestnut hybrids in clumps in canopy gaps or after certain silvicultural treatments is a good method of re-establishing the species (McEwan et al. 2006). If such planting configuration is used, fertilizing the clumps of seedlings through foliar application would be easier than treating more scattered seedlings. The objectives of this study were to determine the effect of shade level and fertilizer supplement and their interaction on pure *C. dentata* seedling survival and growth. Knowledge about the first year survival and performance of American chestnut is crucial due to the frequently observed high levels of mortality during the first growing season after outplanting.

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 (34º53'50"N, 86º34'34"W). Stands of sweetgum (*Liquidambar styraciflua* L.), ca. 46 x 91 m, were planted February – March 1995 at 1.5 x 3.05 m spacing. In February 2009, the stands were thinned. One-third of each stand was left unthinned, one-third had approximately 50 % overstory removal, and the other one-third had approximately 66 % overstory removal. The thinning was from below with primary removal of trees from the overtopped and intermediate crown classes. The cut *L. styraciflua* 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 2010).

Experimental design

Four-hundred American chestnut bare root 1-0 seedlings purchased from the nearest available source, a nursery in Freesoil, Michigan, were planted in April 2009 at 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 23 m from the stand edge. All 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, 2009 and the second application on the two-application treatment was on September 30, 2009. Each fertilizer application consisted of Accele-Grow-M® fertilizer supplement (Accelegrow Technologies, West Point, Georgia) 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 (Accelegrow Technologies 2008). Seedlings were sprayed until all the leaves were covered with fertilizer solution. We removed competitors from shrub, vine, and herbaceous species located up to 15 cm from the planting location of each seedling. All trees were sprayed regularly with Liquid Fence® deer and rabbit repellent (The Liquid Fence Company, Brodheadsville, PA) due to initial herbivory. Invasive species and other competitors within 15 cm of the seedlings were removed approximately ten times during the growing season.

We measured the root collar diameter (RCD) and height of all trees 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. Before planting we also recorded the number of first order lateral roots (FOLR) with diameter over 1 mm at the proximal end. The roots were pruned to approximately 30 cm. Basal diameter and height of all living trees were measured again after the end of the 2009 growing season.

Statistical analysis

We 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) was used to test if there were differences in the post-fertilization 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. Deer browse was identified by missing parts of the main stem or branches with frayed stem ends, and rabbit browse was identified by clipping at an angle near the base up to about 20 cm from the ground. Pre-fertilization and overall mortality were analyzed with ANOVA as a randomized complete block design (RCBD) using shade as the predictor variable.

For the number of FOLR, 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 article, the number of FOLR refers to this log transformed variable. Linear regression was used with each growth measure as the response variable and the initial seedling attributes (height, RCD, and number of FOLR) as the predictor variables.

We used mixed models to test for differences among the groups of fertilizer supplement treatment and shade levels.

When we use the treatment name “fertilizer” in the rest of the article, we refer to treatment with fertilizer supplement. The dependent variables were absolute and relative growth in RCD and height. The relative growth is the growth expressed as a proportion of the initial RCD and height, respectively. 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. 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 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 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 means. We considered results to be significant if $p<0.1$, but we also provide the actual p-values. 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 died by the time of first fertilizer treatment in August (four months after planting). This mortality did not differ among the three shade levels tested as a RCBD ($p=0.29$). By the end of the first growing season the average mortality of seedlings in the open was 57 %, while in the light and heavy shade it was 9 % and 17 %, respectively. However, the differences were still not significant ($p=0.13$; Figure 1).

Seedlings were not significantly different in size at planting or at the time of treatment assignment. Fertilizer, shade, and their interaction all had a significant effect on the relative RCD growth (Table 1).

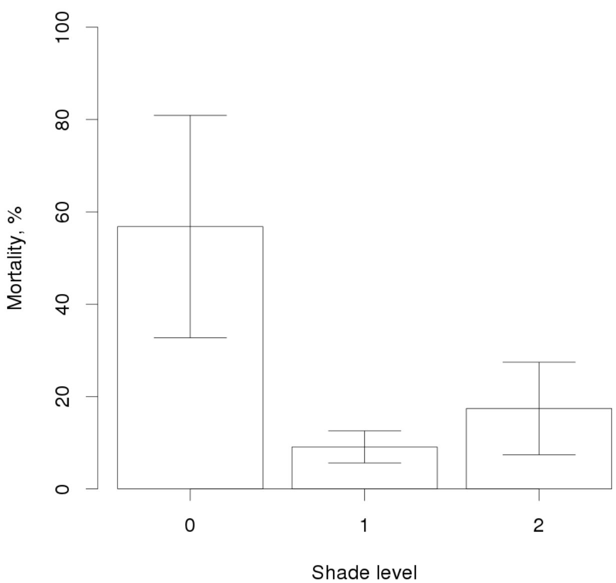

Fig. 1. Percent mortality with SE bars at the end of the growing season in each shade level.

Note: 0=open, 1=light shade, 2=heavy shade.

Table 1. Effect of the treatments on the four measures of chestnut seedling growth.

Dependent and predictor variables	*F* (*df*)	*P*
Absolute root collar diameter growth		
Shade	6.15 (2.40)	0.060
Fertilizer	2.17 (2.24)	0.116
Shade*fertilizer	1.66 (4.24)	0.159
Relative root collar diameter growth		
Shade	6.40 (2.40)	0.057
Fertilizer	2.72 (2.24)	0.068
Shade*fertilizer	2.45 (4.24)	0.047
Absolute height growth		
Shade	0.09 (2.40)	0.918
Fertilizer	1.71 (2.24)	0.183
Shade*fertilizer	1.09 (4.24)	0.364
Relative height growth		
Shade	0.19 (2.40)	0.831
Fertilizer	1.94 (2.24)	0.147
Shade*fertilizer	1.15 (4.24)	0.336

Note: 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 2. Relative seedling growth as a proportion of the initial size.

Predictor		Root collar diameter		Height	
Hade	**Fertilizer**	**Mean**	***SE***	**Mean**	***SE***
0†	All	0.26[a]	0.03	0.069	0.020
1	All	0.23[ab]	0.02	0.061	0.017
2	All	0.17[b]	0.02	0.057	0.018
All	0‡	0.20[a]	0.02	0.059	0.017
All	1	0.20[a]	0.02	0.053	0.017
All	2	0.25[a]	0.02	0.075	0.016

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

Note: Tukey-Kramer method was used to identify significant differences (α=0.1) between means of the relative growth of each main effect group found significant in the ANOVA tests. 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.

However, none of the factors affected relative height growth (Figure 2; Table 1). Additionally, increased shade consistently decreased absolute RCD growth (Figure 2). Linear regression with each dependent variable used in the mixed model showed that number of FOLR 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). 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).

Fertilizer significantly affected only relative RCD growth (Table 1). However, due to the existence of interaction between fertilizer and shade, none of the pairwise comparisons were different (Table 2). Fertilization did not have an effect on any of the other measures of seedling growth during the period.

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 than in the light shade for absolute and relative RCD (Figure 2).

Browsing and mortality between the time of fertilizer application and the end of the first growing season were unaffected by treatment. Mortality after fertilizer application did not differ among the

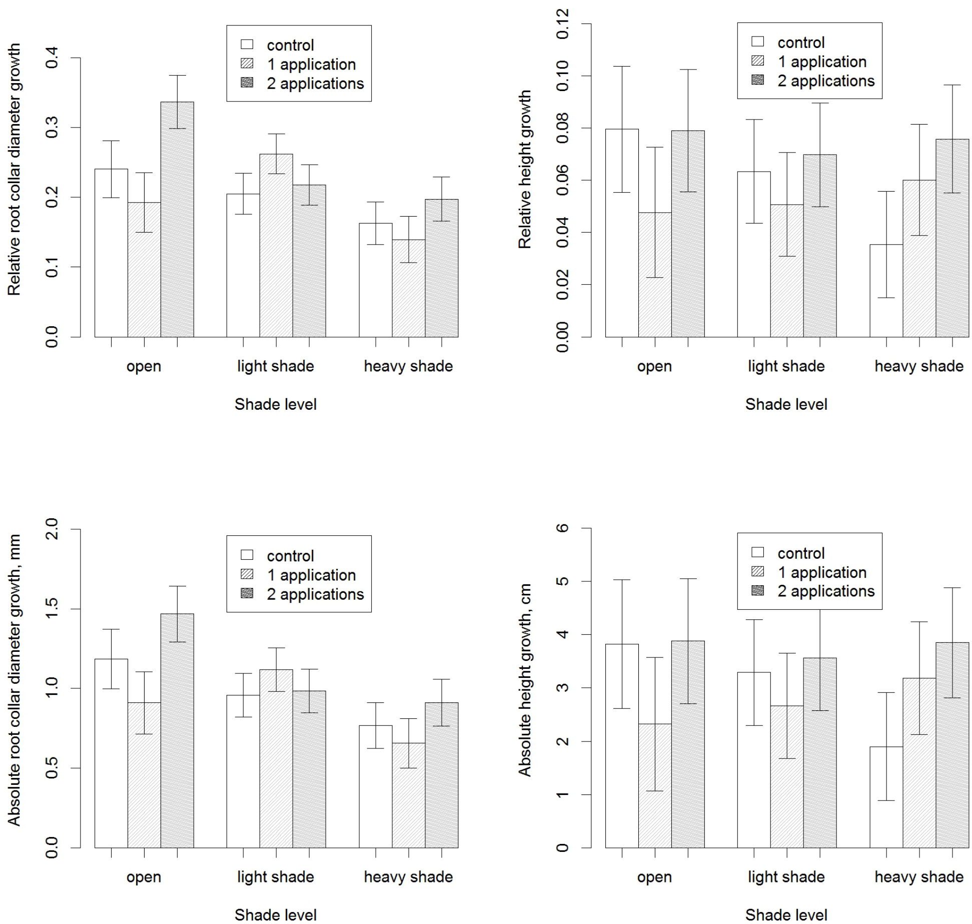

Fig. 2. Mean ± *SE* for each fertilizer supplement treatment level within each shade level for each dependent variable.

Note: 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).

three shade levels tested as a RCBD (*p*=0.32).

Rabbit browse was 9.1 % in the heavy shade, 3.8 % in the light shade, and 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, we 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 all unaffected by shade. Only 4 seedlings were browsed before treatment assignment, all by rabbit, so pre-treatment browsing was not analyzed separately.

Discussion

The greatest growth in absolute RCD was achieved in the open for seedlings treated twice. However, it was significantly greater than the absolute RCD growth of only two out of the eight other treatments (Figure 2). Greater growth in partial shade when compared to full shade has been found consistently, e.g., *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 noted but not identified in some seedlings. This was twice as common in shelterwood treatments as in midstory removal. Survival of seedlings may be greater under moderate shade (Anagnostakis 2007, Griffin 1989).

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 (the test for the difference among them had a *p*=0.13), 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. 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 (Brian McCarthy, pers. comm., Ohio University, August 4, 2010). However, a study in Indiana found negligible American chestnut mortality two years after planting on a former agricultural field (Selig et al. 2005). Unless there is a delayed growth response that may be seen in subsequent growing seasons, fertilization of the kind used in this study is not recommended. In addition to a longer term response, future studies in the region should also consider the potential impact of *Phytophthora cinamomi* on the American Chestnut Foundation's blight-resistant hybrids. There were indications that this water mold caused some of the seedling mortality in our study.

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