EARLY RESULTS OF A CHESTNUT PLANTING IN EASTERN KENTUCKY ILLUSTRATE REINTRODUCTION CHALLENGES

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ABSTRACT

This paper examines the first year results from a silvicultural study of American, hybrid (BC_2F_3) and Chinese chestnut seedlings (*Castanea* spp. Mill.) on the Daniel Boone National Forest in southeastern Kentucky. After one year, no significant differences in growth were found among the silvicultural treatments. Hybrids and Chinese seedlings added significantly more height growth than the American seedlings. American chestnut suffered nearly 40 percent mortality, hybrids 34 percent, while only 5 percent of Chinese seedlings died during the first growing season. High mortality among American and hybrid seedlings is thought to have been caused by the native chestnut sawfly, *Craesus castaneae* (Rowher) and the non-native *Phytophthora cinnamomi* (Rands.), both of which were present at the site. These results illustrate potential challenges facing the reintroduction of American chestnut.

INTRODUCTION

The American chestnut [*Castanea dentata* (Marsh.) Borkh] was a dominant forest tree in the eastern forests of United States until the nonnative chestnut blight fungus [*Cryphonectria parasitica* (Murr.) Barr] virtually eliminated it as a canopy tree species in the early 20th century. The tree was once ecologically important as a source of mast for wildlife (Minser and others 1995, Paillet 2005, Schlarbaum 1989), and economically valuable for its rot-resistant lumber, high-tannin content, and edible nuts (Burnham 1988, Moss 1973).

Limited silvicultural prescriptions for chestnut management were developed before the blight epidemic (Paillet 2002), and the effects of competition on growing space, light, water, and nutrients have not been well defined.
In anticipation of widespread planting of blight resistant American chestnuts, it is important to understand the silvics and competitive ability of the species in order to successfully reintroduce it within the eastern North American forests. Forestry manuals written while American chestnut was still a canopy dominant describe the species' rapid growth and prolific sprouting (Ashe 1911, Mattoon, 1909, Zon 1904), however they lack in-depth analysis of the chestnut silvics. Relatively few experimental studies on chestnut silvics and optimal planting methods have been developed (Anagnostakis 2007, MCCamment and McCarthey 2005, Jacobs and Severeid,2004, McNab 2003 Rhoades and others 2009), and those that exist do not all support the same results.

The overall goal of this study is to assess early establishment success of chestnut seedlings on the Cumberland Plateau of eastern Kentucky by evaluating growth and survival of American, BC_2F_3 hybrids, and Chinese chestnut seedlings grown under three silvicultural treatments: oak shelterwood, thinning, and shelterwood with reserves.

METHODS

STUDY SITE

This study is located on the London Ranger District of the Daniel Boone National Forest (DBNF) on the Cumberland Plateau in southeastern Kentucky (37°03' N, 84°11' W, elevation 370 m). The forest type is classified as upland hardwood and is dominated by mixed oak species (Schweitzer and others 2008). Braun (1950) described this part of Kentucky as part of the mixed-mesophytic forest region, abundant with beech (*Fagus grandifolia* Ehrh.), white oak (*Quercus alba* L.), black oak (*Quercus velutina*

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Lam.), and hickory (*Carya* spp Nutt.). Other common hardwoods include chestnut oak (*Quercus prinus* L.), particularly on ridges, maple (*Acer* spp. L.), and black gum (*Nyssa sylvetica* Marsh). Before the chestnut blight, American chestnut was a dominant timber tree in these forests, particularly in higher elevations (Braun 1950). The former importance of chestnut in the area is further evidenced by its continued presence in the understory (personal observation).

SILVICULTURAL TREATMENTS

This study is nested within a larger USDA Forest Service study, referred to as the Cold Hill Study, which established the goal of improving oak regeneration and forest health prior to the anticipated arrival of gypsy moth to the area (Schweitzer and others 2008). This study utilizes three of the five silvicultural treatments implemented in the Cold Hill study: oak shelterwood (OS), thinning (TH), and shelterwood with reserves (SW). For the OS treatment sites, all stems in the midstory and understory were removed using triclopyr herbicide injection, leaving a basal area of 22 m^2/ha of intact overstory (cf. Loftis 1990). This treatment increases light on the forest floor to favor oak regeneration, while retaining enough overstory to inhibit shade intolerant species, such as yellow-poplar (*Liriodendron tulipifera* L.). The overstory will be removed four to five years following midstory removal. The TH treatment left stands thinned to the B-level of Gingrich stocking (Gingrich 1967), with a basal area of 18.6 m^2/ha of overstory. While thinning is not a standard regeneration treatment, this may provide adequate light for seedling establishment while discouraging shade-intolerant species. The SW treatment left a residual basal area of 5 m^2/ha or overstory. All harvest treatments were completed between 2007 and February 2009.

EXPERIMENTAL MATERIALS

American, BC_2F_3 hybrid, and Chinese chestnut seedlings were used in this study. The open-pollinated American and hybrid nuts were harvested at The American Chestnut Foundation's Meadowview Research Farms, Meadowview, VA, in the fall of 2007, and manually planted at the Flint River Nursery in Byromville, GA in January 2008 at a density of 65 seeds per square meter. Fertilization and irrigation of the seedlings followed guidelines developed by Kormanik and others (1994). The 1-0 seedlings were lifted in February 2009, and stored in a cold room (~1º C) until they were planted. The Chinese chestnut seedlings were purchased from Forrest Keeling Nursery (PO Box 135, Elsberry, MO) in February 2009. Seedlings were processed in February, and the lateral roots were trimmed to 15 centimeters to facilitate planting.

EXPERIMENTAL DESIGN

This study examines the effect of species and silvicultural treatments on chestnut establishment. Silvicultural treatments, arranged in a completely randomized design, were implemented for the USFS's Cold Hill Study (Schweitzer and others 2008). Within the silvicultural treatments, chestnut species were arranged in a randomized block design. Thus the experimental design incorporates a split plot, with silvicultural treatment in the main plot and species in the subplot. Three hundred American, 300 BC_2F_3 hybrid and 150 Chinese chestnut seedlings were planted on the experimental sites between March 2nd and 9th, 2009, using a Jim-Gem© KBC Bar, modified by adding 5 cm to each side of the blade, creating a blade 15 cm at the top, tapering to the tip. The 750 seedlings in the study were planted in transects on 15 different sites. Each of the three silvicultural treatments was replicated three to five times, with the seedlings evenly distributed among the three treatment types. Seedlings were planted at a spacing of 2.44 meters in one transect per site. Transects were located at least 30 m from the treatment edge. The chestnuts were planted randomly in complete blocks of five seedlings.

MEASUREMENTS

Seedling height and root collar diameter (RCD) were measured prior to planting. The height and RCD of each seedling was measured again at the end of the 2009 growing season. The density of naturally regenerated seedlings and height and species of the tallest seedling within a 0.0005 hectare plot surrounding each planted chestnut seedling was recorded. A convex spherical densitometer was used to estimate canopy closure on ten randomly selected treatment sites, representing three to four replicates of each treatment type. Readings were taken on the south side of each chestnut seedling in each of the four cardinal directions and an average canopy closure was recorded. To evaluate the availability of soil water, midday (10:30 am – 12:30 pm) stomotal conductance was recorded on one mature sun-exposed leaf on each chestnut seeding in the same sites sampled for canopy closure. Stomatal conductance was measured with a LiCor 1600 steady-state porometer (LiCor, Inc., Lincoln, NE). To evaluate the availability of soil nutrients, the third healthy leaf down from the terminal bud from 92 seedlings from nine treatment units (three of each treatment type) was taken in August. Leaves were stored in a -70° C refrigerator until processing, at which point 0.2 - 0.4 grams of tissue was cut from each leaf. The leaf tissue was crushed with a mortar and pestle in 85 percent acetone. The extract was centrifuged to remove suspended solids and then enough 85 percent acetone was added to make 15 ml of extract. The optical density of the extract was measured with a Thermospectronic Biomate 3 spectrometer (Thermo Electron Scientific Instruments Corp., Madison, WI), and chlorophyll and total carotinoid concentrations estimated using McKinney equations (Sestak and others 1971). Chlorophyll a and b estimations were used to predict relative foliar nitrogen content for each leaf sample.

Data Analyses SAS software (SAS Institute Inc., Cary, NC, USA) was used for all data analysis. Mixed model analysis of variance (ANOVA) was used to evaluate the effect of silvicultural and species treatments on end-of-season height and RCD, height change (end-of-season height minus initial height), frequency of dieback (negative height change), as well as the effect of silvicultural treatment on canopy cover, stomatal conductance, and chlorophyll and carotinoid levels. Any significant main effects ($\alpha = .05$) were further analyzed using least significant distance tests for means comparisons. Proc Glimmix with binomial distribution was used to evaluate the effect of silvicultural and species treatments on seedling survival and dieback. To ensure the validity of the assumptions of ANOVA, tests for normality and equal variance of the residuals were performed. The square-root of the end-of-season RCD was used in analysis to correct unequal variance.

RESULTS

SURVIVAL

The seedlings experienced substantial mortality during the first growing season. Survival rates differed among species; 40 percent of American and 34 percent of hybrid and seedlings had died by the end of the growing season, while only 5 percent of Chinese chestnuts died ($p < 0.0001$). Survival among silvicultural treatments did not differ ($p=0.9699$).

GROWTH

At the time of planting, American chestnut seedlings averaged 95.68 cm in height and 11.29 in RCD, hybrid seedlings averaged 94.06 cm in height and 11.61 cm in RCD, and Chinese seedlings averaged 110.74 cm in height, and 12.24 cm in diameter. After one growing season, hybrid chestnut seedlings averaged 103.35 cm in height, which was significantly greater than the 98.44 cm average height exhibited by American chestnuts ($p = 0.0355$, but was not different from the 99.6 cm average height of Chinese chestnuts. No differences in height were found among silvicultural treatments ($p=0.4419$; Table 1). Initial height covariate was included in this analysis ($p < 0.0001$).

Hybrid and Chinese chestnut seedling height change was significantly greater than that of the American chestnut seedlings ($p = 0.0010$; Figure 1). Height change did not differ significantly among silvicultural treatments ($p = 0.4419$), however trees in the oak shelterwood decreased in height, on average, by 0.73 cm, while seedlings in the thinning sites added 1.51 cm on average, and seedlings in the shelterwood with reserve sites added 2.86 cm.

Chinese chestnuts exhibited greater RCD than did American and backcross chestnut seedlings ($p < 0.0001$; Figure 2). RCD did not differ among silvicultural treatments ($p = 0.2680$; Table 1). Initial RCD covariate was significant ($p < 0.0001$). Change in RCD from planting to September 2009 was not analyzed due to inconsistencies in RCD measurement between bare-root measurement (before planting) and planted measurement (September 2009).

DIEBACK

American and Chinese chestnut seedlings experienced greater occurrence of dieback than did hybrid chestnut seedlings (42 percent, 54 percent, 26 percent, respectively; $p = 0.0004$). No differences in dieback among silvicultural treatments were found ($p = 0.2060$).

UNDERSTORY COMPETITION

The height of the tallest competitor and the density of competing seedlings within competition plots were not significantly different among silvicultural treatments. Red maple (*Acer rubum* L.) was the tallest competitor in 38 percent of all plots, averaging 176 cm in height. Green briar (*Smilax* spp. L.) was the next most common tallest competitor (17 percent of all plots), averaging 84 cm in height. Red maple was also the most abundant species in the understory (30 percent of plots), followed by green briar (26 percent of plots).

MEASUREMENTS OF AVAILABLE RESOURCES

SW sites exhibited the least canopy closure (47 percent), followed by TH sites (87 percent), followed by OS sites (94 percent; $p < 0.0001$). Transpiration rates did not differ statistically among silvicultural treatments ($p = 0.0679$) or species ($p = 0.5282$). Seedlings in OS sites averaged 3.71 $\mu g\ cm^{-1}\ S^{-1}$, seedlings in TH sites averaged 4.55 $\mu g\ cm^{-1}\ S^{-1}$, and seedlings in SW sites averaged 6.58 $\mu g\ cm^{-1}$ S. Chlorophyll a, b, and total chlorophyll (a + b) all differed significantly among silvicultural treatments ($p = 0.0103$, $p = 0.0289$, $p = 0.0042$, respectively), with all three parameters decreasing from OS to SW treatments (Table 2). Chlorophyll parameters did not, however, differ among species ($p = 0.9033$, $p = 0.4423$, and $p = 0.5877$, respectively). Species by silvicultural interaction was significant for all three variables ($p = 0.0345$, $p = 0.0106$, and $p = 0.0338$, respectively). Carotinoids did not differ among species ($p = 0.6401$) or silvicultural treatment ($p = 0.2495$).

DISCUSSION

While stomatal conductance rates did not differ statistically among silvicultural treatment, the results exhibit a pattern of increasing transpiration with decreasing canopy. Treatment differences in residual basal area may well have affected water balance on these sites through altered evapotranspiration, however seedling response appears to be highly variable. Greater quantities of chlorophyll in leaf samples, from the oak shelterwood sites than those in the thinning and shelterwood with reserve sites may indicate that the oak shelterwood sites have more available soil nutrients than the other silvicultural treatments.

Harvesting usually causes increased rates of organic matter decomposition and nitrogen mineralization, while decreasing evapotranspiration, which together create an initial increase in the amount of available nitrogen (Vitousek and Matson 1985). However in the short term, a high input of woody debris alters soil C:N ratios, resulting in a temporary immobilization of nitrogen (Turner 1977). Based on the amount of canopy cover observed, OS sites clearly provide the least available light, TH sites provide slightly more light, and the SW provided the most light. Seedlings demonstrated a (non-significant) pattern of increased height growth from OS to SW treatments. Future years of data will determine if this pattern reflects a true effect. McCament and McCarthy (2005) found that light was more closely related to increase in chestnut biomass in a forest planting than were soil nutrients, organic matter, soil moisture, and soil texture. In a greenhouse study examining the effect of several light and nutrient treatments on chestnut seedlings, Latham (1992) also found that chestnut growth exhibited a greater sensitivity to light than nutrient availability. More accurate measurements of soil water and nutrients at our sites and future years of growth data are necessary to test this hypothesis for our study.

Reports of unusual chestnut mortality in the southern United States began to appear in the early 1800s (Anagnostakis 2006). At the time, the causal agent was unknown, however, later studies have shown that chestnuts in bottomland or poorly drained sites were being killed by ink disease, *Phytophthora cinnamomi* Rands (Crandall and others 1945), a soil-borne Asian oomycete that attacks and kills the root systems of American chestnut and the related Allegheny and Ozark chinquapins. Chinese chestnuts, in general, are more resistant to *P. cinnamomi* than American chestnuts. Ink disease was most likely transported to the southern United States before 1824, and caused significant loss of chestnut in the Gulf and Atlantic states (Anagnostakis 2006). Ink disease was confirmed in five dead seedlings from this study. This confirmation, and the greater incidence of mortality among American and hybrid, compared to Chinese chestnut seedlings (40 percent, 34 percent, and 5 percent, respectively), leads us to hypothesize that mortality among the American and hybrid chestnuts was caused in part by ink disease. While nursery seedlings may carry ink disease to new locations, soil tests located at least 5 meters from our planting transects also tested positively for *P. cinnamomi*, indicating the pathogen was already present on the sites. This is troubling, as *P. cinnamomi* has not commonly been found on well-drained upland sites. Mortality among American and hybrid chestnuts may have also been aggravated by repeated defoliation by chestnut sawfly (*Craesus castaneae* Rohwer), which favored these species over the Chinese chestnut (Pinchot and others, In Press).

IMPLICATIONS FOR AMERICAN CHESTNUT REINTRODUCTION

The presence of *P. cinnamomi* on these sites indicates that this pathogen may be able to survive in well-drained upland areas, areas where chestnut was once a dominant species and where reintroduction efforts will likely be targeted. Based on the high mortality observed in this study, it is recommended that all reintroduction sites be tested for presence of *P. cinnamomi*, and chestnut planting excluded for sites that test positive. Furthermore, plantings should be observed for presence of chestnut sawfly and if found, seedlings may benefit from the application of a foliar pesticide treatment. The presence of *P. cinnamomi* and *C. castaneae* and the high mortality among chestnut seedlings illustrates additional biotic challenges facing the reintroduction of American chestnut.

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Table 1—2009 mean height, height change, and RCD among the silvicultural treatments. Treatment means followed by the same letter are not significantly different (α = 0.05).

Treatment	Ht (cm)	Ht change (cm)	RCD (mm)
OS	98.47a ± 1.81	-2.14a ± 1.69	10.43b ± 0.17
TH	100.70a ± 1.40	0.09a ± 1.84	10.43b ± 0.17
SW	102.31a ± 1.55	1.70a ± 1.96	12.18a ± 0.17

Table 2—2009 Differences in mean chlorophyll a, chlorophyll b, total chlorophylls (a + b) and carotenoids in mg leaf pigment per g fresh leaf tissue among silvicultural treatments. Treatment means followed by the same letter are not significantly different (α = 0.05)

Treatment	Chlorophyll a	Chlorophyll b	Total Chlorophyll	Carotinoids
OS	1.64a ± 0.10	0.88a ± 0.04	2.40a ± 0.22	1.25a ± 0.07
TH	1.04b ± 0.10	0.70ab ± 0.04	1.48b ± 0.22	1.00a ± 0.08
SW	0.67b ± 0.10	0.55b ± 0.04	0.92c ± 0.22	1.01a ± 0.08

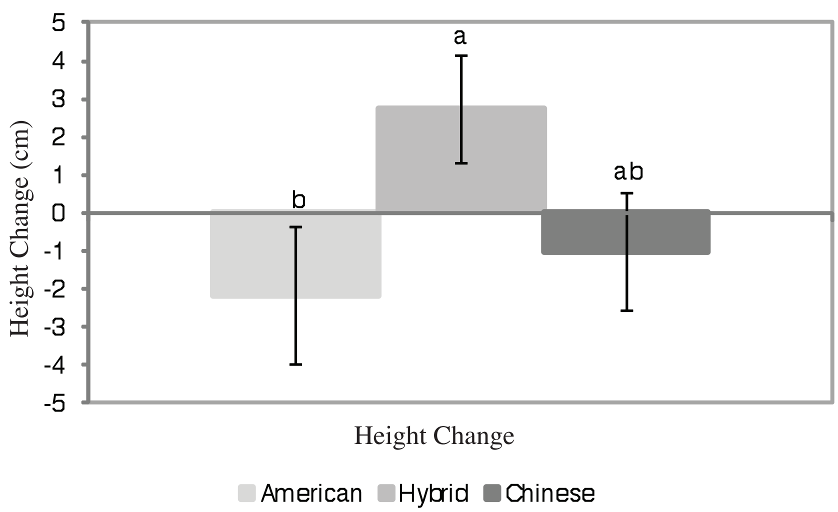

Figure 1—Height at the time of planting, at the end of one growing season, and height change among species after one growing season. Bars with the same letter are not significantly different (α = 0.05)

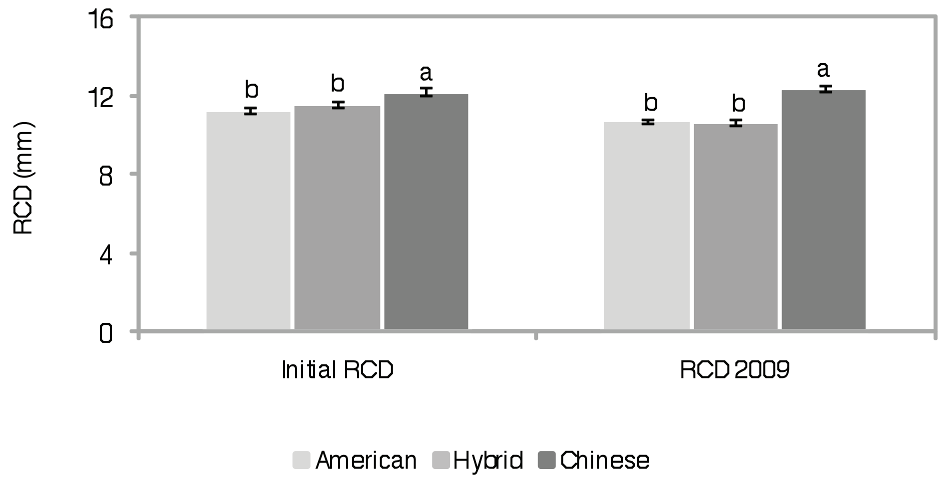

Figure 2—Root collar diameter among species at the time of planting and after one growing season. Bars with the same letter are not significantly different (α = 0.05).