The First Research Plantings of Third-Generation, Third-Backcross American Chestnut (*Castanea dentata*) in the Southeastern United States

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Abstract

Production of American chestnut (*Castanea dentata*) resistant to the chestnut blight fungus (*Cryphonectria parasitica*) is being conducted currently through traditional breeding and genetic transformation. Sufficient material for field testing is currently available from The American Chestnut Foundation's backcross breeding program. We planted approximately 4500 chestnut seedlings into forest test plantings on three National Forests over three years, beginning in 2009. Early survival and growth was dependent on disease pressure from exotic pathogens, primarily, root rot caused by *Phytophthora cinnamomi*. Plantings that contained seedlings not exhibiting symptoms of this disease had high survival (>75%) and fast rates of height growth (0.5 m/yr). We documented other non-native pests negatively affecting chestnuts including Asiatic oak weevil (*Cyrtepistomus castaneus*) and the Asian chestnut gall wasp (*Dryocosmus kuriphilus*). Native pest problems included browsing of the terminal leader by deer (*Odocoileus virginianus*) and defoliation caused by the chestnut sawfly (*Craesus castaneae*). Restoration of American chestnut will require not only blight-resistance, but adaptation to forest environments with intense vegetation competition and strategies to address other native and exotic insects and pathogens.

INTRODUCTION

American chestnut, *Castanea dentata* (Marsh.) Borkh., was a keystone tree species in eastern North America for thousands of years until it was ecologically extirpated by two exotic diseases, ink disease caused by *Phytophthora cinnamomi* Rands. and chestnut blight caused by the fungus, *Cryphonectria parasitica* (Murr.) Barr (cf. Anagnostakis, 2002; Paillet, 2002). Efforts to restore American chestnut have focused predominately on development of blight-resistant trees through breeding techniques with Chinese (*Castanea mollissima* Blume) and/or Japanese chestnut (*Castanea crenata* Siebold and Zucc.) (Clapper, 1952; Jaynes and Graves, 1963; Hebard, 2005), breeding low levels of resistance using pure American parents (Griffin, 2000), biological control of the blight through hypovirulence (Anagnostakis, 2001; Milgroom and Cortesi, 2004), and genetic transformation (Merkle et al., 2007; Wheeler and Sederoff, 2009). Development of blight-resistant chestnut progeny is only the first step in successful restoration. Blight-resistant trees have to compete in natural forest settings which contain abiotic and biotic challenges to survival and growth. The goal of our study was to examine early growth, survival, and adaptability of American chestnut seedlings traditionally bred for blight resistance and planted in commercially harvested forest sites in the southeastern United States. This report will discuss overall survival and height of each planting for all parental species and generations combined to provide an estimate of overall plantation success.

MATERIALS AND METHODS

The American Chestnut Foundation (TACF) is a non-profit organization attempting to restore this species using a backcross breeding program that was first initiated in the 1980s (Burnham et al., 1986; Hebard, 2005). In theory, the first putatively blight-resistant generation, the BC_3F_3 generation, is 94% American chestnut, 6% Chinese chestnut, and is predicted to have the desired phenotypic characteristics of the American chestnut parent while maintaining blight resistance of the Chinese chestnut parent (Hebard, 2005). Recent tests have indicated the BC_3F_3 is less blight-resistant than predicted, but does have more blight-resistance than the American parent (Hebard et al., 2014). TACF provided nuts to the United States Department of Agriculture Forest Service for establishment of eleven field plantings in 2009, 2010, and 2011 (Table 1). We planted a total of 3957 trees, of which 45% were the from the BC_3F_3 generation. The remaining seedlings at each planting represent a mixture of progeny from the American and Chinese parental species, and the BC_1F_3, BC_2F_3, and BC_3F_2 generations.

All plantings were established on sites which have not been recently disturbed and were selected for future harvest due to forest maturity. Sites had northerly or easterly aspects and relatively high site quality (site index for *Quercus rubra* ≥24 m, base age 50). The planting sites were located within the Blue Ridge Province of the Appalachian Highland Region (Fenneman, 1938). A total of eleven plantings were established. Three in 2009 (A-C sites), two in 2010 (D and E sites), and three in 2011 (F, H, and J sites), were established on sites where the majority of the over-story was removed through a commercial harvest. The harvests reduced the original basal area by 70-80%, leaving a residual basal area of approximately 5-7 m^2/ha in trees greater than 14 cm in diameter at breast height (DBH). Three plantings (G, I, and K plantings) were established in non-harvested forests that had the mid-story removed (Loftis, 1990). The mid-story-removal method is the first stage of a shelterwood regeneration harvest, designed to improve light quantity and quality to the understory to promote seedling recruitment until the commercial harvest is conducted approximately four years later. The original stand basal area (for trees >3 cm DBH) of approximately 23 m^2/ha was reduced 25-35% by treating stems using an herbicide applied using a hack and squirt method. Approximately 1 mm of Triclopyr was applied to hatchet marks of trees hacked once for every 6 cm dbh. Only stems in the understory and mid-story canopy strata were treated to avoid creating openings in the over-story canopy that could increase light on the forest floor to favor shade-intolerant species.

Seedlings were grown as 1-0 bareroot nursery stock by genetic family and grown according to prescriptions to produce a high-quality seedling that will more efficiently escape deer browse pressure and compete with natural vegetation (Kormanik et al., 1994; Clark et al., 2012). Seedlings were planted using gas powered augers with 15-20 cm bits or using a KBC planting bar modified to be 30 cm wide and 45 cm deep to accommodate the large root system of high-quality seedlings. Within each planting year, seedlings were planted using similar experimental material across sites.

We measured total height and survival at the end of each growing season and noted impacts from abiotic and biotic factors for each seedling planted. Height growth was calculated as the difference between total height at the end of the growing season and height at planting. Approximately 200 seedlings that exhibited signs of root rot caused by *P. cinnamomi* were excavated and roots were assayed at Clemson University, Department of Ornamental Plant Pathology and at the Connecticut Agricultural Experiment Station. Defoliating insects and galls were collected or photographed and identified at the United Stated Department of Agriculture Forest Service, Southern Research Station.

RESULTS

Chestnuts in the 2009 plantings had high survival (81%) and grew approximately 80 cm in height by the end of the third growing season. At sites A and B, deer browse to the terminal leader on 80 and 46% of the seedlings, respectively, caused a reduction or minimal gain in total height in the first year. Deer browse also caused mortality at site A where it was most intense. Seedlings at sites A and B were sheltered after the first growing season to protect from deer browse, and subsequently, survival stabilized and height growth increased by the second year. After three growing seasons, seedling total height ranged between 15 to 407 cm and height growth ranged between 144 and 300 cm across all three 2009 plantings. Negative height growth was due to dieback of the main stem related to planting shock, injury, or other unknown sources. Some seedlings grew more than 150 cm in a single growing season. The incidence of chestnut blight was low (<5%) in the 2009 plantings. Defoliation caused by Asiatic oak weevil (*Cyrtepistomus castaneus* Roelofs) was noted on less than 10% of trees in planting C in Sept. 2011. Galls from the Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu) were noted on two trees in sites A and B.

Survival was more modest in the 2010 plantings, where second-year survival varied from 70 to 49%. The relatively low survival at site E was speculated to be related to root rot from *P. cinnamomi*. Speculation was later confirmed through positive identification of the pathogen from assays from roots taken from seedlings exhibiting signs of the disease. Site E had a high water table and poor draining soil compared to site D, which likely contributed to site E's lower survival and growth rate. Seedlings at site D grew 65 cm on average by end of the second growing season, and some seedlings grew more than 150 cm in the second year. Tree shelters were erected at the time of planting for site D due to high deer populations near the planting site. Deer browse pressure was low at both plantings (<3%). The incidence of chestnut blight was low at both 2010 plantings (<2%). Defoliation caused by Asiatic oak weevil was noted on 36% of trees in site D in Sept. 2011. Galls from the Asian chestnut gall wasp were noted on eleven trees in site E.

The 2011 plantings were compromised to varying degrees by root rot caused by *P. cinnamomi*. The variation in disease pressure was probably related to differences in soil texture and drainage among sites. The shelterwood harvest plantings had similar survival to the mid-story-removal plantings. Shelterwood harvest plantings all had positive height growth and mid-story-removal plantings had negative height growth. Deer browse pressure was mitigated by use of deer repellant at the 2011 plantings, and by using high-quality seedlings at planting that were mostly above browse. Deer browsed less than 1% of seedlings at any planting. We noticed defoliation caused by the chestnut sawfly (*Craesus castaneae* Rowher) on one tree in planting K. The incidence of chestnut blight was low (<5%) in the 2011 plantings.

Differences in survival and growth among species and generation were minimal, and will be presented in future publications.

DISCUSSION

The future of chestnut restoration in the southern United States will require much more than blight resistance. For our plantings, early mortality was primarily linked to disease pressure from root rot caused by *P. cinnamomi*. The pathogen was brought into the United States in the early 1800s, and is transferred most easily on low-lying valley sites historically used for agricultural purposes (Russell, 1987). This disease is most virulent in poorly drained soils, such as site E, or compacted or clayey soils (Anagnostakis, 2002; Rhoades et al., 2003). We postulate that the pathogen was transferred to the site from the roots of the nursery seedlings. It is highly unlikely that the soils of the planting sites previously contained *P. cinnamomi*, as the sites were never tilled for agriculture and are predominately high elevation sites where the disease would be unlikely to exist (Russell, 1987). We tested soils from several sites prior to planting for presence of this pathogen, and tests were all negative. Nursery soils are known to contain *P. cinnamomi* (Crandall et al., 1945), and the use of fungicides, as was used by nurseries that grew seedlings for this study, will mask disease symptoms. Currently no adequate control measures exist for large-scale plantings. The future of chestnut restoration will require growing seedlings for planting using soil and water free of the disease (e.g., containers) because the disease is coming from the nursery and no adequate control measures exist to ensure complete protection from this disease in southern seedling nurseries. The use of containerized seedlings will be most important in the southern United States where annual minimum temperatures are less than -20°C (Balci et al., 2007; Benson, 2002). Production of high-quality containerized stock will add considerable costs to planting.

Planting seedlings tall enough to escape deer browse pressure also will be important to successful chestnut restoration. Heavy browse pressure, as was present at site A, can cause mortality and retard height growth. The use of high quality seedlings at planting can improve the probability of a tree escaping deer browse (Oswalt et al., 2006). If smaller seedlings are planted, the time and resources needed to protect seedling from deer browse increase and could be cost prohibitive. Production of high-quality chestnut seedlings in containers that can quickly escape deer browse is currently being tested (C.C. Pinchot, pers. commun.).

Other exotic pests affecting seedlings include the Asiatic oak weevil at two plantings and the Asian chestnut gall wasp seen at three plantings. These species will have negative impacts on chestnut restoration (Anagnostakis et al., 2011), although the impacts are not well understood at this time. The Asiatic oak weevil is a mid- to late-season leaf defoliator and the larvae feed on roots (Roling, 1979; Triplehorn, 1955). The gall wasp will inhibit flower and nut production and could weaken already stressed trees, causing death in the most severe cases. Currently, there are no adequate control methods for these insects for large-scale restoration efforts as being proposed by The American Chestnut Foundation. We also have observed native pests including the rarely observed chestnut sawfly (*C. castaneae*). This insect has only been recorded in one other chestnut planting, and the insect favored American chestnut over Chinese chestnut (Pinchot et al., 2011). In planning for the future of chestnut restoration, an integrated approach will be essential. Success will require a balance among high seedling quality at planting, competition control, disease resistance, and forest management practices to control native and non-native pests and pathogens (including blight).

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Tables

Table 1. Survival and height growth for American chestnut research plantings. SHW=shelterwood harvest, MID=mid-story-removal treatment.

Planting	Silvicultural prescription	Year planted	N	Total planting Ht* (cm)	Growing season 2009			Growing season 2010			Growing season 2011		
					Survival	Total Ht* (cm)	Height growth (cm)	Survival	Total Ht* (cm)	Height growth (cm)	Survival	Total Ht* (cm)	Height growth (cm)
A	SHW	2009	442	97	82	83	-14	75	107	10	73	163	66
B	SHW	2009	369	97	93	100	3	88	144	47	85	188	91
C	SHW	2009	344	96	89	110	14	87	145	49	85	188	92
D	SHW	2010	579	136				80	148	12	70	201	65
E	SHW	2010	514	134				56	147	13	49	166	32
F	SHW	2011	300	135							83	144	9
G	MID	2011	282	133							72	118	-15
H	SHW	2011	293	135							67	142	7
I	MID	2011	288	133							51	118	-15
J	SHW	2011	248	135							64	144	9
K	MID	2011	298	132							76	130	-2

*Ht=Height.