EARLY RESULTS FROM A PILOT TEST OF PLANTING SMALL AMERICAN CHESTNUT SEEDLINGS UNDER A FOREST CANOPY

W. Henry McNab, Research Forester, USDA Forest Service,
Southern Research Station
Steven Patch, Professor, Mathematics Department,
University of North Carolina–Asheville
A. Amelia Nutter, Mathematics Department,
University of North Carolina–Asheville

INTRODUCTION

Successful development of American chestnut (*Castanea dentata*) hybrids that are resistant to chestnut blight (*Cryphonectria parasitica*) will require information about methods for effective and economical reintroduction

Figure 1. Natural understory vegetation on plots with the full canopy treatment consisted of scattered tree seedlings and sprouts that averaged 3 to 4 feet tall. The observer's left hand indicates height of a planted American chestnut seedling that has attained about half the height of a nearby red maple sprout (right hand) in four years. Heights of chestnut seedlings receiving the tree shelter treatment, in the adjacent row, were about the same as seedlings without shelters.

of this species in forests of the southern Appalachian Mountains (Boucher 2000). American chestnut regenerates naturally from seedlings that become established and gradually accumulate beneath a closed tree canopy (Paillet and Rutter 1989, Billo 1998). Chestnut seedlings on a partially shaded forest floor gradually develop well-established root systems through successive sprouting and dieback episodes, and eventually will initiate rapid growth upon receiving additional light resulting from disturbance in the overstory canopy (Billo 1998, Paillet 2002).

Planted seedlings can be an effective and inexpensive method of establishing blight resistant American chestnut seedlings on forested sites (Klinger 2002). Little information is available, however, on establishing seedlings on a forested site and particularly survival and growth of seedlings that receive no follow-up maintenance. To obtain such information we designed a study to determine survival and growth of planted American chestnut seedlings in relation to overstory canopy density. Our secondary objective was to determine if seedling survival and growth are influenced by cultural treatments applied at time of planting. Our's was a pilot study that will help us to plan and conduct a larger, more intensive study.

METHODS

This study was made in the Bent Creek Experimental Forest, located in the Pisgah National Forest, about 10 miles southwest of Asheville, NC. We followed methods outlined by Rutter (1992) to produce seedlings from nuts of American chestnut. Briefly, we obtained about 200 nuts in March 1998 and stratified them in damp peat moss for 2 months at 46°F. The nuts, which sprouted during stratification, were sown about 1 inch deep in raised nursery beds; germination was about 95 percent. Except for rainfall, the seedlings were seldom watered. Estimated nursery seedling mortality was < 5 percent. Total height of the 1-year-old nursery seedlings averaged 7 ± 2 in and ranged between 3 in and 12 in. For field planting we excavated the seedlings in December using a shovel. The root system of most seedlings was characterized by few lateral roots and a taproot that slightly exceeded length of the aboveground stem.

We planted the seedlings in a large intermountain basin with hilly terrain and deep (>40 in), well-drained soils characterized by clay accumulation in the B horizon. The site sloped slightly (5 percent) to the south. The site's overstory primarily is composed of xeric to subxeric species of oak (*Quercus*), including white (*alba*), scarlet (*coccinea*), and black (*velutina*). Chestnut oak (*Q. prinus*), a typical associate of American chestnut in the southern Appalachians, is rare on the study site, but is common on nearby, more steeply sloping mountainsides. The midstory canopy includes widely scattered red maple (*Acer rubrum*), sourwood (*Oxydendron arboreum*), and dogwood (*Cornus florida*). Basal area of the overstory and midstory averaged 110 ft^2/ac and 20 ft^2/ac, respectively. At the time of planting the sparse shrub layer was mostly tree seedlings and saplings (Fig. 1), although mountain laurel (*Kalmia latifolia*) occasionally was present. A portion of this forested site was clearcut in 1997 to salvage windthrown trees in a downburst area resulting from the remnants of Hurricane Opal on October 5, 1995; the stand on an adjacent part of the site was relatively undisturbed.

Two blocks, each consisting of three plots, were established in the study site. One plot of each block was situated in the clearcut portion of the stand, one in the undamaged stand, and one between the clearcut and undamaged areas. Each plot measured 12 ft x 18 ft and was planted with 20 seedlings (5 each in 4 rows) spaced 3 ft apart. In late December 1998, we manually planted the seedlings using a planting bar with a foot-long blade. One person planted all seedlings during a light rain, when air temperature was 40°F. Each seedling was planted in less than a minute, because the primary root was short (<10 in) and had few laterals. Seedlings with top lengths < 5 in were discarded. The small number of available seedlings allowed us to replicate the study in only two blocks, for a total of 120 seedlings.

We studied seedling survival and height growth in response to three canopy densities and four cultural treatments. The three canopy densities were: none (plots established in the clearcut), partial (plots placed at the edge between the clearcut and uncut areas), and full (plots located in the uncut stand). Each row of five seedlings received one of four randomly assigned cultural treatments: (1) fertilizer, (2) tree shelter, (3) fertilizer and shelter, or (4) no treatment (the control). The fertilization treatment consisted of a commercially produced soil amendment (Gromax™, forestry dry site formulation 17-3-5 with super-absorbent gel, minor elements, and biostimulants) contained in a premeasured 0.25-ounce packet. We applied the fertilizer treatment in early March 1999, using an 8-inch deep hole made with a planting bar about 4 in from each seedling. The opaque, corrugated white plastic tree shelters measured 3 in x 3 in x 24 in tall and were positioned to rest on the ground. We made no follow-up cultural treatment after planting, except to replace disturbed tree shelters.

Seedlings were examined for survival and measured for total height immediately after planting, 6 months after planting, and annually each early October. Because conventional wisdom suggests that survival should be lower among small, runty seedlings compared to large robust seedlings, we used a t-test to evaluate the hypothesis that first year survival was not associated with seedling size, as quantified by total height. At five points within each plot we measured mean photosynthetically active radiation—expressed as percent of full sunlight—once using a portable light meter positioned about two ft above ground level.

We used a split-plot design. The whole plots were a randomized complete-block design with two blocks containing each of the three canopy treatments. Four combinations of fertilizer and shelter were assigned to each of the six split plots. Twenty of the trees were planted in soil that later was found to be somewhat compacted by an old roadbed. Because the survival of those trees was much lower than that of other trees in the study ($P=0.002$ by chi-square test of independence) we dropped them from our analysis.

To analyze the effects of survival after five years, we applied a mixed-model methodology using a binomial error distribution. The model was fitted with Statistical Analysis System (SAS™) using the GLIMMIX macro to adapt to binomial response variable in a mixed model with the restricted maximum likelihood estimation method and the Satterthwaite approximation for degrees of freedom. Whole-plot error was used to test significance of canopy and within-plot error was used to test significance of shelter, fertilizer and any interactions. Because none of the interactions was significant, the reduced model with only main effects was then fit.

RESULTS

Overall seedling survival declined sharply during the first year, from 100 percent immediately after planting to 66 percent in October (Fig. 2). First-year survival was not associated ($P=0.53$) with initial seedling height; both live and dead seedlings averaged about 7.8 in. Survival declined little during the next 2 years and averaged about 58 percent at seedling age five.

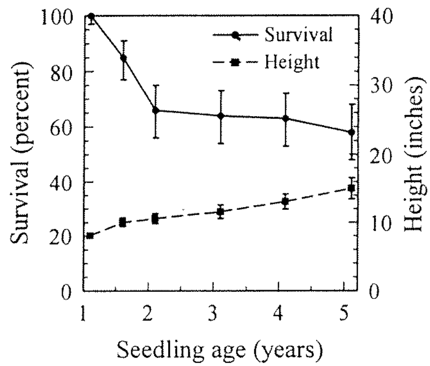


Figure 2. Mean (± 95 percent confidence intervals) survival and height of American chestnut seedlings at planting, when one-year of age, and periodically thereafter in Bent Creek Experimental Forest.

Total height of all surviving seedlings has almost doubled, from about 8 in at planting to 14.8 in after 4 years of field growth. We took light measurements on May 30, 2001; they averaged 97 percent, 45 percent, and 10 percent under canopy treatments of none, partial, and full, respectively.

At age 5, mean seedling survival ranged from 28 to 82 percent among canopy treatments (Table 1), but the means were not significantly different (P= 0.40). Confidence intervals for the three mean survival rates were large — ranging from almost 0 to 100 percent — and relatively consistent, indicating a high amount of variability. Among cultural treatments, survival was significantly lower (P=0.010) for seedlings receiving fertilizer (42 percent) than for those not fertilized (75 percent). However, survival was significantly higher (P=0.025) for seedlings receiving the shelter treatment (74 percent) than for those not receiving shelter (44 percent).

Overall seedling height averaged 15.7 in at age 5 and did not differ significantly (P=0.40) among any of the canopy or cultural treatments. In an unplanned investigation of the cause of slow height growth and high mortality of seedlings in some treatments, we excavated a small (10.6 inch) seedling in a full canopy, shelter and fertilizer treatment that had apparently died during the late summer of 2002 (Fig. 3). Examination of the seedling revealed that only the top was dead, the root system was alive, and it had top-died and root-sprouted at least twice since planting and likely would have sprouted again, in spring 2003. Using a diagnostic test, we found no evidence that this seedling was infected with *Phytophthora cinnamomi*. We observed little damage to seedlings from rodent girdling, rabbit clipping, or deer browsing.

DISCUSSION

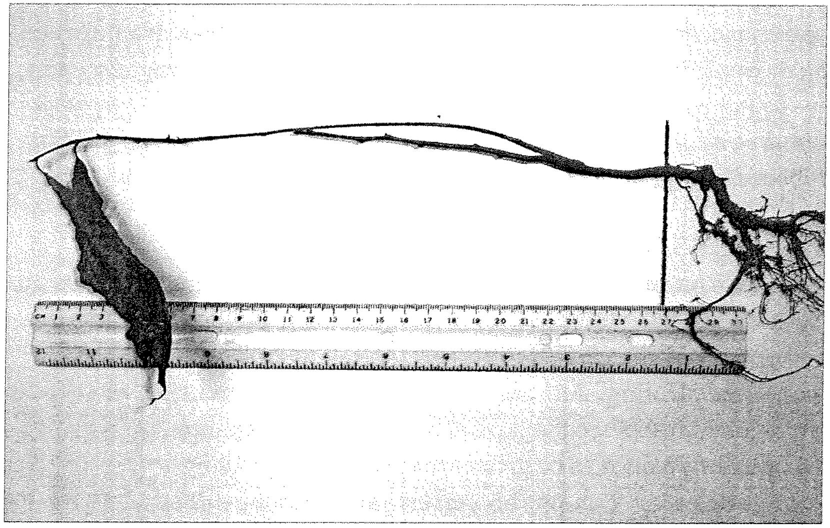
Results of our study suggest that small American chestnut seedlings can be successfully established in a forested environment with minimal investment of time, equipment, and no follow-up attention after planting. Although not statistically different, average survival of seedlings planted under the full canopy (82 percent) tended to be greater than survival of seedlings under the partial canopy (65 percent) or no canopy (28 percent) conditions. We speculate that increased replication of field plots would have allowed detection of a significant difference in survival among canopy treatments. Height growth of the seedlings was slow in all of the canopy treatments.

A likely contributing factor to the high level of mortality during the first growing season following planting was low soil moisture; precipitation was about half of normal from August through October. An introduced disease, Phytophthora root rot, can cause high mortality in American chestnut (Crandall et al 1945), however, we neither observed symptoms of this disease in our study plots nor detected sporangia on the roots of a small excavated seedling. We noted no mortality from chestnut blight, probably because the short seedling stems presented small target areas for infection (Paillet 2002). The only explanation we offer for the lower survival associated with the fertilization treatment is root desiccation caused by increased soil salt content during the dry summer after planting; we suggest additional study on this topic. Unlike other areas of the eastern U.S. where herbivory is a problem and must be dealt with (Griffin et al 1991, Klinger 1992), we observed little damage from deer and none that contributed to mortality. Although tree shelters increased overall survival and could

Table 1. Estimated percent survival (lower - upper 95 percent confidence limits) for the canopy, fertilizer, and shelter treatments 4 years after field planting of 1-yr-old American chestnut seedlings in Bent Creek Experimental Forest.

Treatment	Survival[a]
No canopy	27.7a (0.4 - 97.1)
Partial canopy	65.0a (2.5 - 99.3)
Full canopy	82.5a (5.5 - 99.7)
No fertilizer	74.8a (45.0 - 91.6)
Fertilized	42.8b (17.6 - 72.5)
No shelter	44.5a (17.9 - 74.6)
Sheltered	73.7b (44.0 - 90.8)

[a]Means in each of the three similar treatment groups followed by the same letter do not differ significantly at the 0.05 level of probability.



The above-ground portion of this 5-year old American chestnut seedling was dead in October 2002, but not the roots, which had resprouted at least twice since planting four years earlier. Now 10.6 inches tall, the seedling had doubled in size following planting in the full canopy treatment. (The vertical line at the seedling's root collar indicates ground level).

offer protection from deer, we observed little benefit in the full-canopy treatment and maintenance required considerable effort.

The co-occurrence of American chestnut and mountain laurel (Griffin et al 1991, Paillet 1996, personal communication Fred Hebard), or other ericaceous species (Griffin 1992) has been noted elsewhere. In our study, we observed that mountain laurel occurred on only one plot, which also had the highest survival of chestnut seedlings (100 percent). This coincidence is probably more interesting than important, but suggests that much remains to be learned about the ecology of American chestnut. For example, Vandermast et al (2002) found an allelopathic relationship with American chestnut for selected co-occurring species, particularly rosebay rhododendron (*Rhododendron maximum*).

Planting stock used in our study was small, 1-year old seedlings with equally small root systems. We did not design the study to investigate the effects of chestnut seedling size or vigor on survival and growth, although we found that seedling size apparently did not affect early survival. Competition from sprouting stumps of other vegetation has been intense in the no-canopy study plots; height of the largely hardwood-sprout stand averages about 6 ft compared to about 1.5 ft for the chestnut seedlings. Planting a larger chestnut seedling with a more vigorous root system would have likely allowed more successful competition, but would have required greater effort and possibly increased the likelihood of infection from root disease (Crandall et al 1945).

We know of no other study results with which to compare our findings. However, evidence suggests that American chestnut stands can "store" small resprouting seedlings for many years beneath an overstory until they are released by increased light resulting from disturbance in the canopy (Paillet and Rutter 1989, Paillet 1994, Billo 1998). The seedling we excavated had been among the smallest planted, only 5-in tall initially, but slowly was developing a root system in the limited light provided under the full forest canopy. Loftis (1990) proposed a shelterwood regeneration system for oak seedlings. By adjusting the mid- and over-story canopy density to stimulate continued development of understory seedlings, such a system could be adapted to chestnut. Although we did not design our study to determine if shaded American chestnut seedlings would respond to release, a rapid height growth response is likely (Griffin 1992, Paillet 1990, Paillet 1994, Paillet 1995).

CONCLUSION

Our study clearly demonstrates that American chestnut seedlings can be successfully and economically established by planting in a forested environment that simulates conditions favorable for natural regeneration. The 1-year-old seedlings we used averaged only about 7 in tall. Based on current standards, seedlings of this size would likely be discarded as too small to justify planting. It is likely that seeds of blight resistant American chestnut will be initially limited in quantity and when well-watered and fertilized nursery seedlings are produced, some will be naturally small. We suggest that small American chestnut seedlings could be used in a program of planting beneath an oak canopy, such as described in this study. We assume that hybrid American chestnut seedlings resistant to the blight will have survival and height growth characteristics similar to the seedlings we used. An essential part of this "pseudo-natural" regeneration system, however, would include monitoring development of seedlings and timely manipulation of the overstory. We suggest there is a need for larger, operation-scale studies to confirm our results, particularly on mountainous sites better suited to American chestnut.

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