Nursery Quality and First-Year Response of American Chestnut *(Castanea dentata)* Seedlings Planted in the Southeastern United States

REFEREED PAPER

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Abstract

We examined nursery seedling quality and 1-yr field performance of American chestnut [*Castanea dentata* (Marshall) Borkh.] seedlings planted in Alabama (AL study) and Tennessee (TN study). Root-collar diameter (RCD) had the highest correlations to nursery seedling quality and first-year field performance for both studies. Survival was low in the Alabama study (18 percent) due to ink disease, caused by *Phytophthora cinnamomi*, and rabbit damage. Seedling growth was not affected by genetic family in either study, and seedling size class and silvicultural treatment did not affect growth at the AL site. On the TN site, survival was 86 percent; relative height and diameter growth were negatively related to nursery height, indicating planting shock was a factor. Restoration of blight-resistant American chestnut will depend on early establishment success, which can be affected by factors not easily controlled.

Introduction

The American chestnut [*Castanea dentata* (Marshall) Borkh.] was an important component of the eastern hardwood forest for thousands of years until a blight-causing fungus, *Cryphonectria parasitica* (Murrill) Barr, was introduced in the early 1900s (Delcourt and others 1998, Anagnostakis 2001). Loss of the American chestnut has resulted in large-scale shifts in species composition in forests of the Southeastern United States, particularly in well-drained upland stands where the species was most competitive (Braun 1950, McCormick and Platt 1980, Stephenson and others 1991). Remnant chestnut trees manage to stay alive through stump and root sprouting and occasionally live long enough to flower and bear fruit, providing genetic material for reintroduction efforts. Blight-resistant trees are produced through backcross breeding with the resistant Asian chestnut (*Castanea mollissima* Blume or *C. crenata* Siebold & Zucc.), in hopes of breeding for the Asian resistance while maintaining the desired form and other silvical characteristics of the American chestnut (Hebard 2001).

The American Chestnut Foundation (ACF) will have limited numbers of resistant hybrid chestnut trees available in 2009 for research testing (Hebard 2001), but little information is available on how seedlings will respond in "real world" forest conditions. The first years of hardwood plantation establishment are the most crucial, because this is when mortality is generally highest and seedlings are most vulnerable to biotic and abiotic factors. Successful establishment of American chestnut will require information on effects of nursery seedling quality, genetics, and silvicultural requirements of the species, yet no or very limited testing of these factors has been conducted. Chestnut may have silvicultural requirements similar to oak (*Quercus* spp.) with the ability to persist in the absence of disturbance, but stimulated growth and photosynthesis with increased light (McNab and others 2003, McCament and McCarthy 2005, Wang and others 2006, Joesting and others 2007). To our knowledge, no study has specifically examined how nursery seedling quality, together with early genetic and silvicultural treatments, affects growth and survival of American chestnut.

Our objectives were to determine:

- The best indicator of nursery seedling quality for nursery managers.
- The effects of nursery seedling quality and genetic family on first-year growth and survival in the field.

- First-year growth and survival differences in seedlings planted into contrasting light environments created through silvicultural prescriptions.

Methods

Our research was conducted at two study areas: Jackson County, Alabama (AL study), and the Cherokee National Forest in Polk County, Tennessee (TN study).

Site-specific descriptions, seedling production, and study design. *Alabama study*. The AL study is located on properties owned by Stevenson Land Company and by the Alabama Department of Conservation and Natural Resources, State Lands Division, on the escarpment of the Mid-Cumberland Plateau (Smalley 1982). The site is characterized by generally short, moderately steep side slopes, with elevations averaging 1,600 ft (488 m). Soils were field tested by the Natural Resources Conservation Service, U.S. Department of Agriculture (USDA), and identified as loamy, derived from sandstone with a small component of residuum from limestone. Dominant tree species before silvicultural manipulations consisted of oaks, sugar maple (*Acer saccharum* Marsh.), and hickories (*Carya* spp.); remnant American chestnut sprouts were present in the understory.

Five silvicultural treatments were implemented in the fall of 2001 (Schweitzer 2004). For this study, we examined the oak shelterwood and the clearcut treatments. The oak shelterwood treatment consisted of an herbicide injection (imazapyr) designed to remove 25 percent of the total basal area by treating trees >1 in (2.5 cm) in diameter at breast height (dbh) and in a suppressed or intermediate social position. The oak shelterwood method creates transient conditions (typically lasting a few years) where light-seeded species [e.g., yellow poplar (*Liriodendron tulipifera* L.)] are restricted in their ability to establish and grow, but where enough light penetrates the forest floor to stimulate growth of desirable regeneration, e.g., oak (Loftis 1990). The clearcut treatment, used to regenerate shade-intolerant species, involved removing all trees >4 in (10.2 cm) dbh by chainsaw felling and grapple skidding along predesignated trails.

Photosynthetically active radiation (PAR) was measured in each treatment unit three times during the 2006 growing season with an AccuPar Linear Par ceptometer, model PAR-80 (Decagon Devices, Inc., Pullman, WA). The PAR measurements taken inside the treatment units were compared to PAR measurements taken at the same time in adjacent areas receiving full sun.

Seedlings for planting were grown from nuts gathered from seven open-pollinated, pure American chestnut families. Seedlings were grown 1 yr in nursery beds managed by the Alabama Chapter of TACF in Muscle Shoals, AL. Seedlings were watered as needed throughout the growing season and were fertilized once with 13-13-13 fertilizer at approximately 25 lb ac^{-1} (28 kg ha^{-1}).

Nursery managers sometimes use visual grading of hardwood seedlings by quick assessments of overall seedling size to improve seedling quality (Oswalt and others 2006, Tennessee Department of Agriculture 2006). To mimic these methods, we visually graded seedlings into "Large" and "Small" size classes by assessing their overall size (Clark and others 2000).

A total of 288 seedlings was planted in February 2006 on a 5 × 5 foot (1.5 × 1.5 m) spacing into the oak shelterwood and clearcut treatments (three replications of each) in a randomized-block, split-plot design. Silvicultural treatment was the whole plot factor; a 7 × 2 factorial (family × seedling grade) was the subplot factor.

Planting spots in the clearcut units were cleared with machetes before planting, and herbicide (glyphosate) was applied in April 2006 to sprouting vegetation within a 3-ft (0.9 m) radius around each planted seedling. Care was taken to protect planted seedlings from herbicide drift. Deer Away® animal repellent was applied twice during the growing season to a rope fence surrounding the plantings.

Tennessee study. The TN study area is located on the Ocoee Ranger District of the Cherokee National Forest, within the Blue Ridge Mountains Section of the Central Appalachian Broadleaf Forest Province (Bailey 1995). The planting site was 1,240 ft (372 m) in elevation on a site previously dominated by planted Virginia pine (*Pinus virginiana* Mill.) and shortleaf pine (*Pinus echinata* Mill.) decimated by the southern pine beetle (*Dendroctonus frontalis*) in summer 2005. All dead trees were harvested or downed by chainsaw felling, and the site was prescribed-burned in November 2005.

Seedlings were obtained from nuts grown from seven open-pollinated pure American families located at the American Chestnut Foundation Meadowview Seed Orchard in Meadowview, VA. Seedlings were grown for 1 yr and received regular fertilization and irrigation throughout the growing season according to protocols developed by Kormanik and others (1994).

We planted 167 seedlings on an 8 × 8 ft (2.4 × 2.4 m) spacing in February 2006, using an incomplete block design. Three to four trees were planted within each incomplete block, with a single tree plot for each family. Single tree plots were used to reduce variation in family performance between incomplete blocks, as compared with using multiple tree plots. We used an incomplete block design because the space required for a complete block composed of all seven families would have been too large to assume constant variability within a block. Deer repellent was not used in the TN study.

Seedling measurements and damage assessment. Seedlings were lifted in February for the AL study and in January 2006 for the TN study. After lifting, we measured seedling root-collar diameter (RCD) to the nearest 0.001 in (0.1 mm) approximately 1 in (2.5 cm) above the root collar, using a digital caliper. We measured total height from the root collar to the top of the terminal bud to the nearest 0.4 in (1 cm), using a standard height pole. We counted the number of first-order lateral roots (FOLR), defined as a root connected to the main tap root and at least 0.04 in (1 mm) diameter at the proximal end. Because FOLR count can be subjective, the same person counted roots on seedlings for each study separately.

Because planting height differs from nursery height due to differences in planting depth of each tree, total height and ground-line diameter (GLD) were measured immediately after planting. Height and GLD were measured again when trees were dormant in October 2006. Relative height and GLD growth were computed as the 1-yr growth increment divided by initial planting height or GLD, respectively.

We recorded damage from deer (*Odocoileus virginianus*), rabbit (*Sylvilagus floridanus*), chestnut blight, and ink disease, caused by *Phytophthora cinnamomi*. Deer browse was identified as a jagged cut to the main stem; rabbit damage was identified as a clean-cut, angled clipping of the main stem or as gnawing on the outer cambium layer (University of Nebraska Cooperative Extension 1994). Chestnut blight was identified as a sunken and dried spot on the stem, sometimes accompanied by orange fruiting bodies. Ink disease was identified by wilted leaves followed by tree death and accompanied by an inky blue exudate on the roots (Crandall and others 1945). Ink disease was confirmed through testing by the Connecticut Agriculture Experiment Station in New Haven.

Data analysis. All data were analyzed in SAS version 9.1 (SAS 2004). An error level of 5 percent was used to indicate significance in all tests. For the AL study, *t*-tests were conducted to determine if large and small seedlings differed significantly in nursery seedling characteristics. We excluded seedlings damaged by deer browse, rabbit browse, and ink disease from statistical analysis involving first-year field measurements, excluding computations of raw means, because these factors influence seedling growth responses to independent variables being tested.

Pearson correlation coefficients were used to test the linear relationships among nursery seedling characteristics and between these characteristics and first-year height and GLD. We were interested in determining if initial nursery seedling characteristics could predict total first-year absolute height and GLD, which, along with survival, are the primary criteria for successful seedling establishment (Ward and others 2000). Growth may be a better measure than absolute size in determining seedling response to biotic and abiotic factors during early plantation establishment, however, because absolute size largely depends on initial seedling size in the early years (Dey and Parker 1997). Therefore, linear regression analysis (PROC REG) was used to determine the usefulness of initial seedling characteristics in predicting first-year absolute height, relative height growth, absolute GLD, and relative GLD growth. The best regression models were identified by stepwise selection procedures. Graphical analysis of residuals conducted to assess normality and homogeneity of variance assumptions found no problems with these assumptions; therefore, variables were not transformed.

We conducted logistic regression (PROC LOGISTIC) to determine if initial nursery seedling characteristics (both studies) and seedling size class (AL study only) could be used to predict the probability of survival. We used analysis of variance (ANOVA; PROC MIXED) to test for fixed effects of silvicultural treatment (AL study only), family (both studies), and size class (AL study only) and their interactions on relative height, relative GLD growth, and survival. Relative, rather than absolute, height and GLD growth were used in the ANOVAs in order to understand how independent variables affect growth of seedlings while controlling for effects of initial seedling size. For both studies, replication (AL study) and incomplete block (TN study) were random. If fixed effects were significant, least-square means were computed and mean separations performed with the PDIFF option.

Results and Discussion

Seedling quality and growth. Nursery seedling quality depended on study site. Seedlings used in the AL study were approximately 22 in (57 cm) shorter and 0.2 in (4.6 mm) smaller in RCD than seedlings in the TN study (table 1). RCD was a good indicator of nursery seedling quality and was highly correlated with both height (R=0.76, AL; R=0.75, TN) and number of FOLR (R=0.69, AL; R=0.62, TN). These results are similar to the findings of Ruehle and Kormanik (1986) and Clark and others (2000), who found high correlations between RCD and other nursery characteristics for northern red oak (*Quercus rubra* L.). Number of FOLR had the lowest correlations with both height (R=0.56, AL; R=0.47, TN) and RCD. The similarity in correlation coefficients among studies suggests that RCD may be the best indicator of nursery seedling quality, regardless of nursery production methods. This finding is important to nursery and forest managers because RCD has been shown to be a good predictor of growth 2 yr after planting for other hardwood species (Dey and Parker 1997, Jacobs and others 2005).

Seedlings planted at the AL site, regardless of size class, were below minimum standards for other species of hardwoods (Johnson 1981). Nursery height, RCD, and number of FOLR were all significantly larger in the Large size class than in the Small in the AL study (table 1). Despite their relatively small size, variation in quality among nursery seedlings in the AL study was large enough to allow us to visually distinguish two grades of seedlings (Clark and others 2000) that may perform differently after several years in the field (Kormanik and others 2002, Jacobs and others 2005, Oswalt and others 2006).

Height and RCD measurements taken in the nursery were not significantly different from those taken at planting, but individual measurements could be as much as 9 in (23 cm) different in height and 0.2 in (5.1 mm) different in diameter because of differences in planting depth and occasional stem damage during transportation to field. Researchers should not use nursery measurements as a surrogate for measurements taken at planting because use of the former may erroneously reduce correlations to subsequent field growth. Additionally, distinguishing between nursery and planting measurements was important for computing accurate measurements of first-year growth.

For all seedlings in the AL study, relative height growth was 32 percent and relative GLD growth was 19 percent

Table 1. Nursery seedling characteristics, planting measurements, and first-year measurements and survival, expressed as arithmetic means (standard error), for American chestnut seedlings planted in Jackson County, Alabama (AL), and the Cherokee National Forest, Tennessee (TN). Results for the AL study are for two seedling size classes and for all seedlings combined. Nursery measurement means for the AL Large and Small seedlings followed by the same letter do not differ significantly (t-test, p<0.05).

	AL			TN
	Large	Small	All	
Nursery measurements				
Height (in)	19.5 (0.4)a	12.5 (0.3)b	16.0 (0.3)	38.0 (0.9)
(cm)	50 (1)	32 (1)	41 (1)	98 (2)
Root-collar diameter (in)	0.35 (0.01)a	0.25 (0.01)b	0.30 (0.01)	0.48 (0.01)
(mm)	8.9 (0.2)	6.3 (0.1)	7.6 (0.1)	12.2 (0.3)
Number of first-order lateral roots	9 (<1)a	6 (<1)b	8 (<1)	7 (<1)
Measurements at planting				
Height (in)	19.0 (0.4)	12.5 (0.3)	16.0 (0.3)	38.0 (0.9)
(cm)	48 (1)	32 (1)	40 (1)	98 (2)
Ground-line diameter (in)	0.34 (0.01)	0.25 (0.01)	0.30 (0.01)	0.44 (0.01)
(mm)	8.8 (0.2)	6.4 (0.2)	7.6 (0.1)	11.3 (0.2)
First-year field measurements				
Height (in)	22.0 (1.2)	14.0 (0.8)	17.5 (0.8)	44.0 (0.9)
(cm)	56 (3)	37 (2)	45 (2)	113 (2)
Ground-line diameter (in)	0.34 (0.01)	0.29 (0.01)	0.31 (0.01)	0.57 (0.01)
(mm)	8.8 (0.3)	7.3 (0.4)	7.9 (0.3)	14.7 (0.3)
Relative height growth (percent)	36 (5)	30 (5)	32 (4)	21 (2)
Relative ground-line diameter growth (percent)	14 (5)	23 (5)	19 (4)	33 (3)
Survival (percent)	15 (3)	21 (3)	18 (2)	86 (3)

(table 1). Seedlings in both size classes grew more in height than they did below ground, indicating that the smaller nursery stock from AL may have grown stems at the expense of the root system. For the larger seedlings in the TN study, in contrast, seedlings grew more in relative GLD than in relative height. Growth of seedlings at the TN site was similar to that reported by Struve and Joly (1992), who found that hardwoods devote growth to the root system just after the first flush.

Percent full sunlight in the clearcut and the oak shelterwood was 82 and 11 percent, respectively, corresponding to 504 and 57 μmol m^{-2} s^{-1} PAR, respectively. Despite the large differences in amount of available sunlight between silvicultural treatments, we detected no differences in relative growth among silvicultural treatments, families, and seedling size class in the AL study or among families in the TN study. American chestnut seedlings have exhibited increased growth in higher light areas than in lower light (McCament and McCarthy 2005, Wang and others 2006), but this difference was not significant in the AL study, likely due to the low numbers of surviving seedlings. We conducted a *post-hoc* ANOVA and removed silvicultural treatment from the model. In these analyses, neither family nor seedling size class affected relative height and GLD growth.

Survival, disease, and animal damage. Survival in the AL study was low (table 1); mortality was primarily related to ink disease, documented on 51 percent of trees (table 2). Chestnut blight and ink disease were more common in the oak shelterwoods than in the clearcuts, which contradicts a previous study that found chestnuts were more susceptible to chestnut blight in clearcuts than in undisturbed areas (Griffin and others 1991). American chestnuts show little to no resistance to ink disease, which, unlike chestnut blight, destroys the root system and kills the entire tree (Anagnostakis 2001, Rhoades and others 2003). The disease is not easily treated in the field, so planting chestnuts for reforestation should be avoided where the pathogen exists until resistant seedlings can be produced; however, TACF is only in the early stages of breeding for ink disease resistance.

Animal damage was documented on 34 percent of trees in the AL study and was an additional factor controlling survival, particularly in clearcut units (table 2). Rabbit browse was greater in clearcuts than in oak shelterwoods, while deer browse occurrence was similar for both treatments. Our results indicate that open canopy areas may leave seedlings more vulnerable to predators, especially small mammals. Deer repellent as applied in the AL study was not effective; however, the repellent was not applied directly to the stem because it damages newly developing leaves in oak (Scott Schlarbaum, unpublished data).

First-year survival was higher in the TN study than in the AL study (table 1), likely due to the lack of animal damage and disease. No ink disease was documented at this site, and chestnut blight and deer browse were documented on only 9 percent and 3 percent of trees, respectively (table 2).

Survival probability could not be explained by seedling size class or initial nursery seedling measurements by logistic regression (χ^2=1.8, p=0.77, AL; χ^2=5.5, p=0.14, TN), contradicting studies that have found seedling size to be positively related to survival (Zaczek and others 1996, Kormanik and others 2002, Spetich and others 2002).

Relationships of nursery characteristics to first-year growth. Nursery seedling height, RCD, and FOLR were all positively correlated to first-year absolute height and GLD (table 3). For the AL study, only RCD was a significant predictor of relative height growth, but the overall model was not significant (P>0.05); relative RCD growth could not be explained by the variation in nursery seedling size. For the TN study, initial nursery seedling characteristics were significant, but weak predictors of relative height or GLD growth (R^2<0.27; table 4). This result was similar to the work of Jacobs and others (2005), who found low predictive power of initial seedling characteristics to growth increment.

Table 2. Occurrence of animal browse and disease of American chestnut seedlings planted at two field studies in Jackson County, Alabama (AL), and at the Cherokee National Forest, Tennessee (TN). The AL results are shown for seedlings planted in clearcut and oak shelterwood silvicultural treatments and for all seedlings combined.

	AL			TN
	Clearcut	Shelterwood	All	
	Percent			
Rabbit browse	55	1	28	0
Deer browse	6	5	6	3
Ink disease	45	56	51	0
Chestnut blight disease	9	20	15	9

Variation in absolute height was best explained by nursery height for the AL study (R^2=0.74) and by both nursery height and RCD for the TN study (R^2=0.89). Variation in absolute GLD was best explained by nursery RCD (R^2=0.40, AL; R^2=0.41, TN) (table 4). A higher number of FOLR has been shown to improve field performance after outplanting (Schultz and Thompson 1990, Ward and others 2000, Kormanik and others 2002), but in this study FOLR was the variable least correlated with first-year height and GLD and could not significantly explain variation associated with absolute size, relative growth, or survival in either study. Our results agree with others who have found that early seedling size after planting is strongly related to nursery seedling height or diameter (Dey and Parker 1997) and that number of FOLR may not correlate well with field performance until after a few years of establishment (Ward and others 2000).

The negative relationship between relative height growth and GLD to nursery seedling height in the TN study (table 4) indicates that taller nursery seedlings may be more likely to experience planting shock. Johnson and others (1984) found that shoot dieback was more noticeable in large 1-0 oak seedlings than in smaller seedlings, which they attributed to shoot dehydration during cold storage. Although we tried to minimize stress from shoot dehydration by using a root wetting agent after lifting, the seedlings for the TN site were in cold storage for a month before planting, increasing the opportunity for shoot dehydration. Bareroot hardwood seedlings typically grow slowly the first 1–2 yr after planting while they overcome planting shock and roots become established (Struve 1990), and we expect seedlings to increase growth rate in subsequent years. Future measurements and analysis will help us determine if the benefit of growing and planting larger seedlings will outweigh the increased stress of planting shock in larger seedlings, as it does in oak species (Johnson 1981).

Conclusions

Preliminary results from these two studies indicate that factors not specifically tested *a priori*, such as animal damage, disease, and planting shock, can control early establishment success of artificially regenerated American chestnut seedlings. Results on the response of American chestnut to genetics, seedling size class, and silvicultural treatment were not clear due to low survival and plant-

Table 3. Pearson correlation coefficients and associated p-values for the linear relationship between nursery seedling characteristics and first-year height and ground-line diameter for American chestnut seedlings planted in Jackson County, Alabama (AL), and at the Cherokee National Forest, Tennessee (TN).

First-year measurements	Nursery seedling characteristic					
	Height		Root-collar diameter (RCD)		Number of first-order lateral roots (FOLR)	
	AL	TN	AL	TN	AL	TN
Total height	0.86 (<0.001)	0.96 (<0.001)	0.71 (<0.001)	0.75 (<0.001)	0.37 (0.026)	0.46 (<0.001)
Ground-line diameter (GLD)	0.42 (0.009)	0.49 (<0.001)	0.63 (<0.001)	0.64 (<0.001)	0.33 (0.048)	0.45 (<0.001)

Table 4. Regression models used to explain variation in first-year seedling performance of American chestnut using initial nursery characteristics for two field studies in Jackson County, Alabama (AL), and the Cherokee National Forest, Tennessee (TN).

Linear regression model	R^2	F	P
AL study			
Height (in)=1.59 + 1.13(HtNurs)	0.74	100.74	<0.0001
Height (cm)=4.06 + 1.13(HtNurs)			
GLD (in)=0.11 + 0.68(RCDNurs)	0.40	23.62	<0.0001
GLD (cm)=2.92 + 0.68(RCDNurs)			
TN study			
Height (in)=11.25 + 0.76(HtNurs) + 8.29(RCDNurs)	0.89	558.08	<0.0001
Height (cm)=28.81 + 0.76(HtNurs) + 0.83(RCDNurs)			
GLD (in)=0.21 + 0.77(RCDNurs)	0.41	96.04	<0.0001
GLD (mm)=5.42 + 0.779(RCDNurs)			
RHtG=30.34 – 0.26(HtNurs) + 0.59(RCDNurs)	0.26	23.54	<0.0001
RGLDG=65.52 – 0.31(HtNurs)	0.08	12.04	0.0007

Height=absolute first-year height; GLD=absolute first-year ground-line diameter growth; HtNurs=nursery seedling height; RCDNurs=nursery seedling root-collar diameter; RHtG=relative first-year height growth; RGLDG=relative first-year ground-line diameter growth.

ing shock. The results from this study, not surprisingly, indicate that first-year seedling size is largely a function of nursery seedling size, and that first-year height growth will be minimal (21–32 percent). Managers may need to provide provisional protection to seedlings from animals, disease, and competing vegetation until seedlings can overcome planting shock. Once established, American chestnuts are expected to have higher growth rates than other hardwoods (Ashe 1911, Jacobs and Severeid 2004, McEwan and others 2006), so additional efforts to protect seedlings may only be required for the first couple of years.

Higher-quality nursery seedlings, like those used in the TN study, may initiate root growth more rapidly than lower quality seedlings, like those used in the AL study. Our results indicate that RCD may be the best criterion for selecting high-quality seedlings; however, planting shock may be related to larger size at planting, resulting in decreased stem growth. American chestnut seedlings should be planted immediately after lifting from the nursery to minimize shoot dehydration and planting shock stress.

Although family was not a significant effect, we speculate that family differences in growth and survival will become important after seedlings establish a strong root system (Zobel and Talbert 1984). Because resistance to chestnut blight can be inherited, tracking family identification in chestnut restoration efforts will provide the ability to link field performance to resistance. Testing early field performance of pure American chestnut will provide researchers and managers with important information to improve success of blight-resistant field plantings that will be established in 2009.

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Acknowledgments

We acknowledge the following for assistance with field work and logistical concerns and for providing locations for the plantings: Greg Jansen, Stevenson Land Company; Daryl Lawson, Alabama A&M University; Dave Griffin and Tracy Binder, University of Tennessee; Ryan Sisk and Jennifer Rice, Forest Service, Southern Research Station; and Tim Dalton, Cherokee National Forest. We thank Sandra Anagnostakis, Connecticut Agriculture Experiment Station, for providing advice throughout the study, for ink disease testing, and for reviewing the manuscript. We thank Geoff Wang, Clemson University, for reviewing the manuscript and Doug McClendon (now deceased), USDA, Natural Resources Conservation Service, for conducting soil field tests. Arnold Saxton, University of Tennessee, provided assistance with statistical design for the Cherokee National Forest study. The American Chestnut Foundation provided partial funding for this project.

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