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The influence of silvicultural treatments and site conditions on American chestnut (*Castanea dentata*) seedling establishment in eastern Kentucky, USA

Chuck Rhoades [a,*], David Loftis [b], Jeffrey Lewis [c], Stacy Clark [d]

U.S.D.A. Forest Service

[a] Rocky Mountain Research Station, 240 W. Prospect, Fort Collins, CO 80526, USA
[b] Southern Research Station, Upland Hardwood Ecology and Management Research Work Unit, Asheville, NC, USA
[c] Daniel Boone National Forest, Morehead Ranger District, Morehead, KY, USA
[d] Southern Research Station, Upland Hardwood Ecology and Management Research Work Unit, Huntsville, AL, USA

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ABSTRACT

After more than 50 years of research and selective breeding, blight-resistant American chestnut (*Castanea dentata*) trees will soon be available for planting into the species' pre-blight range. Increased understanding of the regeneration requirements of pure American chestnut (*C. dentata* [Marsh.] Borkh.) will increase the success of future efforts to establish blight-resistant chestnut. We quantified survival and initial growth of bare-root American chestnut seedlings at five locations in eastern Kentucky, USA. We used a split-plot design to compare seedlings planted within adjacent mesic and xeric sites treated with either a two-age shelterwood overstory treatment or a midstory removal treatment. The silvicultural treatments and topographic settings allowed us to evaluate chestnut seedling performance under four light and site productivity combinations. Seedling survival was 57% and seedling height averaged 94 cm following two growing seasons. Seedling survival was negatively related to sand and coarse fragment content, but was unrelated to silviculture treatment or topographic position. Chestnut seedlings grew best in shelterwood overstory treatments areas on mesic sites. Seedlings growing in shelterwood overstory treatment areas added 3- and 3.5-times more height and stem increment compared to seedlings planted after midstory removal. Seedling leaf mass and foliar nitrogen (N) content were also greatest in shelterwood plantings on mesic sites. The high-light environment created by shelterwood overstory removal resulted in better initial seedling growth, but the moderate-light of the midstory removal treatment may ultimately provide chestnut seedlings a greater advantage over competing vegetation.

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1. Introduction

The former abundance of American chestnut (*Castanea dentata* [Marsh.] Borkh.) in forests of eastern North America is well documented by historic stand descriptions and land records (Braun, 1950; Keever, 1953; Stephenson et al., 1991; Rhoades and Park, 2001; McEwan et al., 2005). The loss of overstory chestnut due to the chestnut blight (*Cryphonectria parasitica* Murr. Barr) during the first half of the 20th century has had significant and enduring effects on the species composition and successional development of forests across its native range (Keever, 1953; Paillet, 1984). After decades of tree breeding and pathology research, the first blight-resistant chestnut hybrids will be available from The American Chestnut Foundation for planting on U.S. Forest Service land in 2009. These seedlings are produced through a backcross breeding technique that seeks to combine the blight-resistance traits of Chinese chestnut (*C. mollissima* Blume) with desired form, stature and nut characteristics of the American chestnut (Burnham, 1981; Hebard, 2001; Jacobs, 2007). Increased understanding of the regeneration requirements of native American chestnut will increase the success of future efforts to establish the blight-resistant chestnuts.

The pre-blight range of American chestnut extended south from New England through the Appalachian Mountains into northern Alabama (Russell, 1987). In Kentucky, chestnut was common in both mixed-mesophytic and western mesophytic forest types (Braun, 1950; McEwan et al., 2005). The tree was abundant throughout the Cumberland Plateau in the eastern portion of the state, and reached its highest densities in the Cumberland Mountains along Kentucky's eastern border with the Ridge and

* Corresponding author. Tel.: +1 970 498 1250; fax: +1 970 498 1212.
E-mail address: crhoades@fs.fed.us (C. Rhoades).

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Valley Region of Tennessee and Virginia (DeFriese, 1884; Braun, 1950; Rhoades and Park, 2001). Historically, chestnut was most abundant on well-drained, acidic soils and rare on limestone-derived soils (Saucier, 1973; Russell, 1987). In both eastern and west-central Kentucky, chestnut was concentrated on ridge-top and south-facing upper topographic positions with sandstone parent material (Braun, 1935, 1950; Hussey, 1884; McEwan et al., 2005). However, large-diameter chestnuts also occurred on mesic lower slopes and coves on a variety of soil types (DeFriese, 1884; Braun, 1935; Muller, 2003; Vandermast and Van Lear, 2002). Experimental trials will help determine which portions of the southern Appalachian landscape are best-suited for chestnut reintroduction (Fei et al., 2007).

The rapid growth of American chestnut seedlings and sprouts was well-recognized across the Appalachian Region prior to arrival of the blight (Zon, 1904; Hawley and Hawes, 1912; Ronderos, 2000) and has been documented in controlled experiments (Latham, 1992; Joesting et al., 2007). After clearing in pre-blight forests, chestnut was reported to sprout vigorously, outgrow many species and increase in relative abundance with successive harvests (Hawley and Hawes, 1912; Wacker, 1964; Smith, 1977; Griffin, 1989). Under controlled greenhouse conditions, chestnut seedlings have been shown to outgrow a variety of eastern North American forest species, including yellow-poplar (*Liriodendron tulipifera* L.; Latham, 1992). In a recent study on fine-silty and coarse-loamy soils in Wisconsin's unglaciated Driftless Region, direct-seeded American chestnut outgrew both black walnut (*Juglans nigra* L.) and northern red oak (*Quercus rubra* L.) during an eight-year study (Jacobs and Severeid, 2004).

The performance of American chestnut seedlings planted after various overstory and midstory silvicultural manipulations has not been widely tested (Jacobs, 2007). Based on physiological and morphological parameters, American chestnut is characterized as tolerant or intermediately-tolerant to shade (Wang et al., 2006; Joesting et al., 2007, 2009). Field studies have noted greater survival of chestnut sprouts and seedlings under moderate shade compared to full-sun (Griffin, 1989; Anagnostakis, 2007), though greater initial seedling growth is typically measured in high-light environments (McNab, 2003; McCament and McCarthy, 2005). In full-sun openings, competition with yellow-poplar reduces establishment success of another intermediate shade tolerant species, northern red oak (*Quercus rubra*) (Loftis, 1990; Oswalt et al., 2006) and yellow-poplar is likely to have a similar competitive influence on chestnut seedlings (McNab, 2003; Jacobs, 2007). As has been learned for northern red oak, successful artificial regeneration of American chestnut may require silvicultural techniques that combine the species' tolerance of moderate-light with its rapid growth in high-light environments.

A silvicultural method has been developed that is intended to enhance growth of intermediate-shade-tolerant species, while hindering establishment of shade-intolerant species (Loftis, 1993; Smith, 1993; Buckley et al., 1998). The method is a two-stage shelterwood in which the first step is a non-commercial removal of the midstory canopy, resulting in a moderate (e.g., 10–15%) increase in light reaching the forest floor. Removal of the overstory is delayed until the desired naturally-regenerating or planted seedlings gain a height advantage over shade-intolerant species that establish and grow once the overstory is removed (i.e., 8–10 years for northern red oak). American chestnut seedlings have not been planted in stands following midstory removal, and it is unclear how the species will respond to the midstory light environment or if this silvicultural technique can promote chestnut establishment by restricting competition.

In anticipation of future efforts to restore chestnut to its native range, this study evaluates initial establishment of seedlings planted on two distinct topographic positions and a range of conditions typical of forest ecosystems of the southern Appalachian ecoregion. Since back-crossed, blight-resistant hybrid chestnut share more than 93% of their genetic traits with pure American chestnut (Burnham, 1981; Hebard, 2001), trials conducted using native American chestnut provide a useful strategy to develop guidelines for introduction of hybrid chestnut. Our specific objectives were (1) to compare survival and physiological and morphological growth of pure American chestnut seedlings planted in two silvicultural treatments on productive, mesic sites and on xeric sites of intermediate productivity; and (2) to determine relationships between abiotic and biotic factors and American chestnut seedling response.

2. Methods

2.1. Study areas

Chestnut seedlings were planted at five locations dispersed throughout the Northern Cumberland Plateau Physiographic Region (Braun, 1950; Smalley, 1986) of eastern Kentucky (Table 1). The study areas support mixed-mesophytic overstory species ranging from 80 to 100 years in age. Naturally-occurring chestnut sprouts were present at each of the sites. Annual precipitation and temperature for three weather stations distributed between the planting locations average 118 cm and 12.9 °C (Kentucky Climate Center, 2008; Grayson, Berea, Jackson stations, 30 year record). Average monthly rainfall is the greatest in May and July and the lowest during September and October. Surface soils are silt loams, silty clay loams and sandy loams formed from shale, siltstone and sandstone parent material common throughout the Cumberland Plateau. Soils at the five locations are classified as Typic Dystochrepts, Ultic Hapludalfs or Typic Hapludults (Newton et al., 1973; Kelley et al., 1983; Hayes, 1998) with pH ranging from 4.3 to 5.6, 7 to 18% clay and 5 to 59% sand in the upper 15 cm.

At each study area, we located a pair of neighboring stands (i.e., $\leq$0.5 km of one another), each 2 ha in size, that differed in site productivity based on topographic position, but had similar stand age and disturbance history. Xeric stands were south- and west-facing, along upper slope positions, and supported a mixture of oak species (*Quercus alba* L., *Q. coccinea* Münchh., *Q. prinus* L., *Q. velutina* Lam.). Mesic stands were generally north- and east-facing, along mid to lower slope positions, and were dominated by yellow-poplar, sugar maple (*Acer saccharum* Marsh.), white ash (*Fraxinus americana* L.), and a mixture of oaks (*Q. alba*, *Q. prinus*, *Q. rubra*, *Q. velutina*). Each stand was divided in half and each half was randomly selected to be treated with one of two silvicultural procedures. Both treatments were even-aged, shelterwood methods used to regenerate hardwood stands. The two-age shelterwood treatment was a commercial harvest that removed 65–70% of total stand basal area (Loftis, 1990), and retained 2–4 dominant overstory trees per hectare (Smith et al., 1997). Fresh cut tree stumps were treated with herbicide to control sprouts. The midstory removal treatment was the first step of a two-stage shelterwood harvest designed to reduce stand basal area by approximately 30% by mechanically removing suppressed and intermediate canopy trees $\geq$3 cm in diameter at 1.7 m height (Loftis, 1990, 1993). Removal of the overstory will occur if and when planted chestnut seedlings add sufficient height to outgrow the shade-intolerant species that establish after overstory removal.

Within each silvicultural treatment area, 49 chestnuts were planted during March 2001 (2 sites) and 2002 (3 sites) at a 3 m $\times$ 3 m spacing; planting occurred within 2 months of the stand manipulations. Planting stock consisted of 1-0 bare-root seedlings grown from nuts collected from pure American mother trees in the Mississippi Palisades region of Illinois (F. Hebard, American

Table 1
Soil properties of eastern Kentucky chestnut planting locations (0–15 cm depth; $n = 5$ per site type; SE in parentheses)[a].

Location Latitude, longitude, elevation	Site type	pH	P (μg/g)	Ca^{2+} (μg/g)	Mg^{2+} (μg/g)	K^{+} (μg/g)	N (%)	C (%)	C:N	Sand (%)	Silt (%)	Clay (%)
Bear Mountain	Mesic	5.6	4.1	1252.4	230.5	93.2	0.2	2.2	11.7	10.8	71.2	18.0
Berea College Forest		(0.2)	(0.5)	(180.1)	(19.7)	(10.5)	(0.02)	(0.3)	(0.4)	(2.3)	(2.2)	(2.0)
37°32′19″N, 84°14′51″W	Xeric	4.6	2.3	56.5	25.8	66.4	0.1	2.0	24.5	5.1	78.2	16.8
335 m.a.s.l.*		(0.1)	(0.3)	(7.0)	(4.1)	(7.5)	(0.01)	(0.2)	(1.1)	(0.5)	(1.7)	(2.0)
Carpenter Branch	Mesic	5.0	16.0	408.2	97.1	107.2	0.3	3.5	13.5	27.1	66.2	6.7
Daniel Boone National Forest		(0.1)	(1.2)	(57.4)	(14.6)	(10.8)	(0.03)	(0.4)	(0.1)	(1.5)	(1.4)	(0.2)
38° 20′ 7″N, 83° 28′45″ W	Xeric	4.3	4.6	57.9	22.6	54.0	0.1	2.1	20.3	16.9	69.0	14.1
305 m.a.s.l.		(0.1)	(0.5)	(5.3)	(2.6)	(2.8)	(0.01)	(0.3)	(1.2)	(0.9)	(1.2)	(1.3)
Cox Branch	Mesic	4.7	5.9	177.2	65.2	77.3	0.2	1.8	11.8	12.7	75.0	12.2
Daniel Boone National Forest		(0.1)	(0.5)	(19.1)	(6.8)	(4.0)	(0.01)	(0.1)	(0.3)	(0.5)	(1.2)	(0.8)
38° 20′ 46″N, 83° 28′29″ W	Xeric	4.5	2.9	44.6	14.3	55.5	0.1	2.1	22.2	11.9	77.4	10.7
305 m.a.s.l.		(0.0)	(0.2)	(6.5)	(1.5)	(4.1)	(0.01)	(0.2)	(1.3)	(1.0)	(1.1)	(0.4)
Robinson Forest	Mesic	5.5	5.6	909.8	145.1	119.7	0.3	3.4	12.8	23.1	58.7	18.3
University of Kentucky		(0.1)	(0.2)	(110.3)	(17.5)	(12.0)	(0.03)	(0.3)	(0.4)	(1.5)	(1.2)	(1.1)
37° 28′ 9″N, 83° 8′3″W	Xeric	4.8	3.8	155.8	37.7	56.0	0.1	2.6	21.2	58.7	30.7	10.7
427 m.a.s.l.		(0.1)	(0.3)	(28.4)	(5.0)	(3.8)	(0.01)	(0.4)	(1.9)	(1.9)	(1.5)	(0.5)
Tygart State Forest	Mesic	5.2	11.1	731.4	52.3	87.3	0.2	2.8	13.7	39.2	51.0	9.7
Kentucky Division of Forestry		(0.1)	(1.1)	(178.1)	(9.7)	(10.6)	(0.04)	(0.5)	(0.4)	(1.4)	(1.3)	(0.8)
38° 23′ 42″N, 83° 9′ 3″ W	Xeric	4.3	7.4	44.2	12.6	52.9	0.1	2.4	27.0	42.2	45.9	12.0
305 m.a.s.l.		(0.1)	(1.6)	(8.1)	(1.8)	(4.2)	(0.01)	(0.3)	(0.6)	(0.9)	(0.7)	(0.3)

[a] m.a.s.l. = meters above sea level.

Chestnut Foundation, pers. comm.). Seedlings were propagated at the Kentucky Division of Forestry's Morgan County Nursery. Nursery stock was planted within 2 weeks of lifting from the planting beds. Diseased and poorly-formed individuals were discarded, and the height, root collar diameter, and number of first-order suberized lateral roots (≥2 mm proximal diameter) were recorded for each seedling selected for planting.

2.2. Seedling measurements

Total seedling height, basal stem diameter and annual height increment were measured in mid-August after one and two seasons of seedling growth using measuring tapes and digital calipers. Visual indicators of seedling health, insect damage and disease were documented annually. Ten undamaged leaves and petioles were excised from the upper one-half of the canopy of 5–10 randomly selected trees per planting block at the end of two growing seasons. Excised leaves were flattened, transported to the laboratory in coolers and the area of individual leaves was measured within 24 h with a LICOR 3100 area meter (Lincoln, NE). Leaf plane length was measured along the midrib and mass was determined after drying samples at 60 °C for 48 h. Specific leaf area was derived from projected leaf area per unit dry mass (Reich et al., 1998). Leaf N was measured on dried, ground subsamples using dry combustion (LECO 1000 CHN analyzer, LECO Corporation, St. Joseph, MI) and used to report foliar N per unit leaf mass.

2.3. Site characterization

Photosynthetically active radiation (PAR: 400–700 nm wavelengths) was measured within each shelterwood and midstory planting block and in an adjacent full-sun clearing. PAR was measured 1 m above the ground using a quantum sensor (LICOR 191SA line quantum sensor, Lincoln NE) between 10:00 and 14:00 h on cloud-free days on three growing season dates (June–August) per site. At each planting site, PAR was measured at 3-m intervals along seven parallel transects in each planting block. Percent of full sunlight was calculated from PAR measured in planting sites and adjacent full-sun openings; full-sun PAR was averaged from measurements collected <10 min before and after PAR was measured in adjacent planting areas. We also compared PAR in treated sites and full-sun openings to PAR measured in adjacent mature hardwood forests with no recent sign of harvesting or other canopy disturbances.

Soil analyses characterized differences in seedling nutrient availability, soil chemistry and physical conditions across the topographic site types and five study locations. For each planting block, mineral soil from the 0–15 cm layer was composited from five subsamples collected with a 5-cm diameter, slide-hammer corer (Giddings Co., Ft. Collins, CO). Composited samples were passed through a 2-mm sieve to separate fine and coarse soil fractions. The coarse fraction and a 15-g subsample of the fine fraction were dried at 105 °C for 24 h and weighed and used to calculate percent coarse fraction. Soil texture was measured with the Bouyoucos hydrometer method (Gee and Bauder, 1986).

Mineral soil total C and N were analyzed by Dumas dry combustion (LECO CHN 2000; St. Joseph, MI). Soil pH was analyzed in a 1:1 soil to de-ionized water slurry after 1 h of agitation (Thomas, 1996). Exchangeable phosphorus and cations were extracted with Mehlich-III reagents (0.2N CH_3COOH, 0.25N NH_4NO_3, 0.015N NH_4F, 0.13N HNO_3, and 0.001 M EDTA; Mehlich, 1984) and analyzed by colorimetery for P and atomic absorption for cations. To assess patterns of N availability we measured an index of the production of plant available soil N (Waring and Bremner, 1964; Bundy and Meisinger, 1994). For each soil sample, one 10-g air-dried sample was incubated in 50 ml of de-ionized water for seven days at 40 °C. After incubation, 50 ml of 2 M KCl was added and samples extracted, filtered and analyzed for NH_4^+. Production of NH_4^+ was calculated as the difference between initial and incubated subsamples and reported per unit of oven-dry soil.

2.4. Statistical analysis

The study was analyzed as a randomized block design with a split-plot treatment arrangement. Each study location represented a block, site type (xeric vs. mesic) represented the whole-plot treatment factor, and silvicultural treatment (shelterwood vs. midstory removal), represented the sub-plot treatment factor. Each tree represented a sample.

Seedling response was analyzed using a mixed model analysis of variance to determine significant effects of study locations site type, silviculture treatment and their interactions on two-year survival, seedling growth and foliar characteristics at $\alpha = 0.05$ (PROC GLIMMIX, SAS version 9.1, SAS Institute, Cary, NC). Silvicultural treatment, site type and their interaction were analyzed as fixed effects and study location was treated as a random effect. Silvicultural treatment and site type were nested within study locations. Data were checked for homogeneity of variance with Levene's test and normality using the Shapiro–Wilks statistic prior to analysis. Where significant differences ($p \leq 0.05$) occurred, the Tukey's LSD procedure was used to compare specific means (PROC GLIMMIX). Spearman's Rho was used to assess non-parametric correlations between initial nursery seedling growth parameters and two-year height and diameter growth. Relations between soil resources and light with seedling growth were evaluated by multiple regression. Mean second year seedling measures for each plot ($n = 20$; planting location by site/aspect by silvicultural treatment combinations) were treated as dependent variables and compared with plot-level means for soil and light as independent variables.

3. Results and discussion

Differences in soil conditions between mesic and xeric site types were consistent across the five Kentucky planting locations (Table 1). Overall, soils on mesic sites have higher pH (up to 1 pH unit) and contain on average twice the extractable P, 70% more K^+, and four and nine times more extractable Mg^{2+} and Ca^{2+}, respectively than xeric sites. The three assays of soil N availability all document higher N fertility on mesic sites; total N was 150% higher, the anaerobic N index was 1.8-fold higher and C:N was 45% lower on mesic compared to xeric sites. At most locations soils were classified as silt loams or silty clay loams and with the exception of one location, soil texture differed little between site types. At that study location (Robinson Forest), the xeric site was twice as sandy as its mesic pair and was the only site classified as a sandy loam.

3.1. Seedling survival

Chestnut seedling survival averaged 57% after two growing seasons across the five locations. Canopy treatment had no effect on seedling mortality (oneway ANOVA; $p = 0.905$), although average survival differed widely (26–90%) among the five study locations. Mortality was highest in the midstory (92%) and shelterwood (88%) plantings on the Robinson Forest xeric site. Seedling mortality was also high at a second location (Carpenter Branch xeric site) that underwent extensive canopy breakage during a severe windstorm (Fig. 1).

Basal phloem cankers associated with chestnut blight were present on 23% of the dead seedlings; canker incidence was nearly two-fold higher on xeric compared to mesic sites (31% vs. 16%, respectively). Visible symptoms of root disease (i.e., root collar necrosis or root detachment in the absence of blight cankers) occurred on 11% of dead seedlings and were twice as common on deceased seedlings found in shelterwood (15%) compared to midstory (7%) sites. Eighty percent of all seedlings suffered mild insect herbivory; severe defoliation that removed the majority of the photosynthetic surface affected 4% of the seedlings.

Across all sites and aspects, chestnut survival was negatively related to both sand and coarse fragment content (Spearman's rho: −0.54, and −0.68 for percent sand and coarse fragment respectively; $p \leq 0.01$). Excluding the midstory and shelterwood sites affected by severe canopy damage, coarse fragment and sand content explained 46 and 31% of the variability in seedling survival,

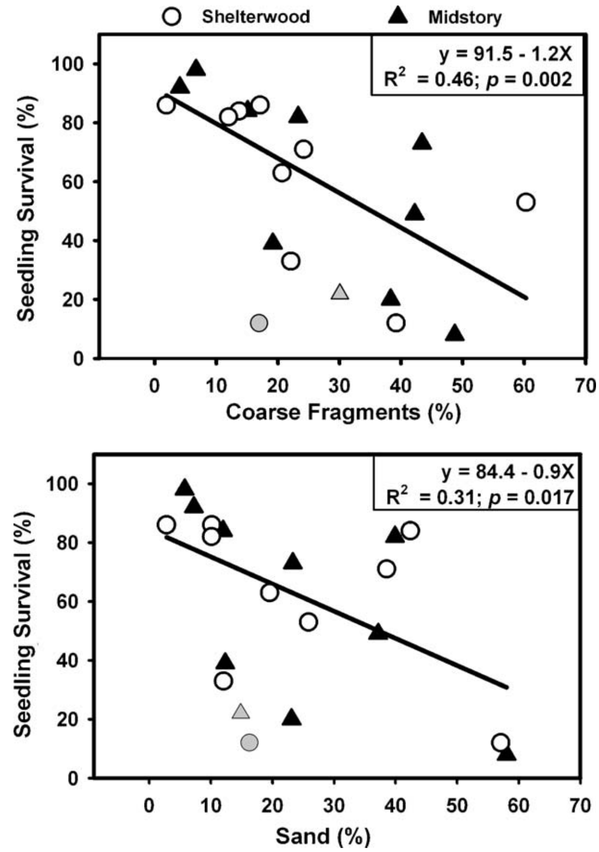

Fig. 1. Linear relationship between chestnut seedling survival after two growing seasons and (a) coarse fragments (>2 mm) and (b) sand content in the upper 15 cm at five planting sites in eastern Kentucky. Regression results exclude a xeric shelterwood and a midstory site (Carpenter Branch site; gray shaded symbols) that suffered heavy mortality due to mechanical damage from a severe wind storm.

respectively (Fig. 1). Survival exceeded 80% in sites with <13% sand and 17% coarse fragments. The Robinson Forest xeric plantings, where only 10% of the seedlings survived, had 57% sand and 45% coarse fragments.

3.2. Seedling growth

After two growing seasons, seedlings averaged 94 cm in height and 10 mm in stem caliper diameter; the largest seedling was 261 cm tall (Table 2). Chestnut seedling growth responded consistently to silvicultural treatment and occasionally to site type (Table 2). Growth was significantly highest on mesic sites treated with a shelterwood treatment. Overall, seedlings growing in shelterwood openings added 3- and 3.5-times more height and stem increment compared to midstory seedlings. On mesic sites, seedling height and caliper growth were 3.4-fold and 5.3-fold greater in shelterwood as compared to midstory plots. On xeric sites, seedlings growing in shelterwood openings added 2-fold more height and stem increment compared to midstory seedlings. Significant interactions between silvicultural treatment and site type indicated that seedling height growth was similar in midstory removal areas on mesic and xeric sites, but that it differed between mesic and xeric shelterwood sites.

Similar to seedling growth, leaf N mass was significantly higher on mesic shelterwood opening compared to the other three treatment combinations, leaf mass was greater on mesic shelterwoods compared to xeric or mesic midstory sites, and foliar N concentration was higher on mesic shelterwoods compared to either treatment on xeric sites (Table 3). Silvicultural treatment

Table 2
Silvicultural treatment and site type (aspect) effects on chestnut seedlings after two growing seasons at five eastern Kentucky. Within columns, similar letters indicate that silviculture treatment and site type combination means are not significantly different based on Tukey's means separation test ($\alpha = 0.5$).

Site type	Canopy treatment		Height cm	Caliper mm	Height-year 2 Annual Increment cm yr^{-1}	Height-year 2 Relative Growth %	Caliper-year 2 Annual Increment mm yr^{-1}	Caliper-year 2 Relative Growth %
Mesic	Shelterwood	Mean	118.1 a	12.1 a	37.0 a	29.7 a	3.6 a	43.2 a
		SE	3.7	0.4	2.1	1.5	0.3	4.0
		Max	261	28	116	82	16	282
	Midstory	Mean	86.1 bc	9.0 c	10.9 c	11.2 c	0.7 c	10.1 c
		SE	3.0	0.3	1.2	1.1	0.3	3.2
		Max	200	25	70	80	10	126
Xeric	Shelterwood	Mean	90.9 b	10.1 b	19.5 b	20.8 b	1.9 b	26.6 b
		SE	2.6	0.3	1.7	1.6	0.3	3.7
		Max	201	22	112	92	16	272
	Midstory	Mean	81.0 c	8.9 c	9.7 c	10.9 c	0.5 c	9.4 c
		SE	2.8	0.3	1.0	1.0	0.3	3.5
		Max	175	19	75	92	13	198
Fixed effects		*F* test						
Silviculture treatment			9.0**	7.3*	12.2**	10.3**	9.7**	19.0**
Site type			3.3	2.1	7.2**	5.4*	2.0	1.1
Silviculture × Site Type			9.7**	5.3*	14.2***	7.3**	3.8*	3.6*

* $p < 0.1$.
** $p < 0.05$.
*** $p < 0.01$.

significantly affected leaf morphology, although the effect of site type was only marginally significant (i.e., $p \leq 0.1$) and limited to foliar N attributes. In contrast to seedling height and diameter growth (Table 2), the lack of interactions between the fixed effects for foliar characteristics showed a consistent influence of silvicultural treatments on both site types.

3.3. Controls on seedling performance

Chestnut seedling growth was related to the initial size of transplanted seedlings for some silvicultural treatments. Height growth of two-year-old seedlings grown in shelterwood areas, for example, related positively ($p \leq 0.01$ for two-tailed correlation) to initial seedling height and stem caliper (Spearman Correlations: 0.24 and 0.26, respectively). In contrast, initial seedling size and growth were unrelated for the midstory plantings. The number of first-order lateral roots was closely related to initial seedling height and stem caliper (Spearman Pearson Correlations: 0.14 and 0.47, respectively; $p \leq 0.001$), but were unrelated to seedling growth. For a number of other hardwood species, seedling growth has been shown to be highly correlated to both root collar diameter and the number of first-order lateral roots (Clark et al., 2000; Ruehle and Kormanik, 1986).

The large difference in growth between the shelterwood and midstory treatments resulted primarily from the influence of the canopy manipulations on the forest floor light environment. Seedlings planted in shelterwood openings received 47% of the PAR measured in full-sun openings on average, compared to 27% in the midstory plantings (Fig. 2). For comparison, only 7% of the PAR measured in full-sun openings reached the forest floor in adjacent undisturbed forest stands. Site type had no consistent influence on PAR. However, for the midstory plantings, light transmittance explained 52% of the variability in seedling height increment (Height Increment = 55.6 + 0.5X; $p = 0.018$; Fig. 2a) and 33% of the seedling stem increment (Stem Increment = 87.8 + 0.9X; $p = 0.082$; not shown). Site differences in light penetration within the midstory plantings relate to residual canopy, topographic position and at one site the formation of an overstory canopy gap following plantation establishment. In contrast, seedling height growth was unrelated to light transmittance within the high-light, shelterwood openings.

The leaf area produced per unit leaf mass (e.g., specific leaf area—SLA) was significantly lower in the high-light shelterwood planting areas (Table 3; Fig. 2b). Specific leaf area (SLA) declined linearly with increasing light transmittance for seedlings planted in both silvicultural treatments (SLA = 196 − 1.38X; $p < 0.001$; $R^2 = 0.75$; for both treatments; Fig. 2b). Our findings match the relationship between foliar morphology and incident light documented for deciduous and conifer species that span a range of shade tolerance categories (Walters and Reich, 2000). Similarly,

Table 3
American chestnut foliage characteristics. Means and SE calculated from leaf samples from 10 seedlings per planting site. Within columns, similar letters indicate that silviculture treatment and site type combination means are not significantly different based on Tukey's means separation test ($\alpha = 0.05$).

Site type	Canopy treatment	Mass g leaf^{-1}	Leaf length cm	Specific leaf area cm^2 g^{-1}	N %	Leaf N mass mg N leaf^{-1}
Mesic	Shelterwood	0.59 (0.03) a	18.99 (0.4) a	125.21 (2.5) b	1.88 (0.03) a	11.27 (0.75) a
	Midstory	0.43 (0.02) bc	17.48 (0.4) a	167.69 (6.7) a	1.72 (0.06) ab	7.84 (0.63) b
Xeric	Shelterwood	0.49 (0.03) ab	17.61 (0.5) ab	132.92 (3.7) b	1.72 (0.04) b	8.69 (0.61) b
	Midstory	0.35 (0.02) c	15.85 (0.5) b	166.48 (3.8) a	1.50 (0.01) c	5.26 (0.33) c

Fixed Effects	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Silviculture treatment	5.3	0.039	14.7	0.000	10.2	0.006	54.9	0.000	6.2	0.029
Site type	0.2	0.196	3.0	0.158	0.1	0.788	4.5	0.100	3.5	0.086
Silviculture × site type	0.0	0.921	0.1	0.768	0.1	0.714	1.3	0.262	0.0	0.998

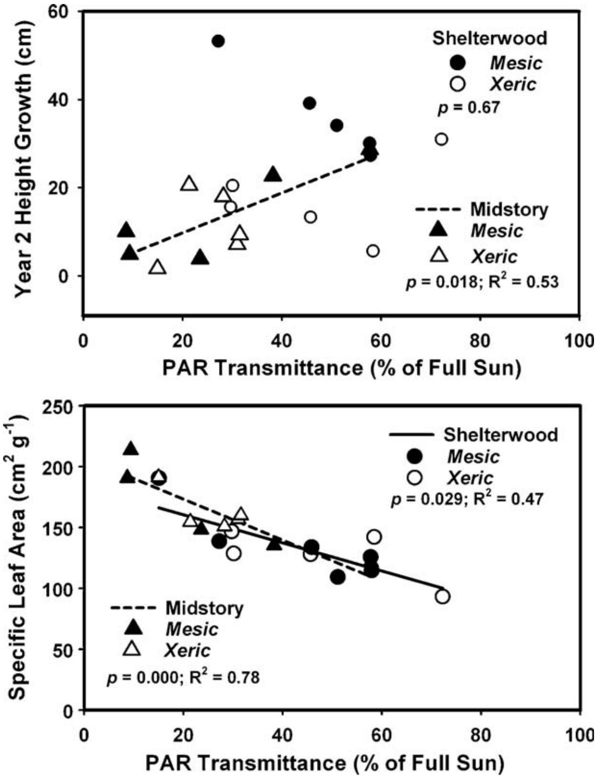

Fig. 2. Relationship between PAR transmittance and (a) second year height growth and (b) specific leaf area of chestnut seedlings.

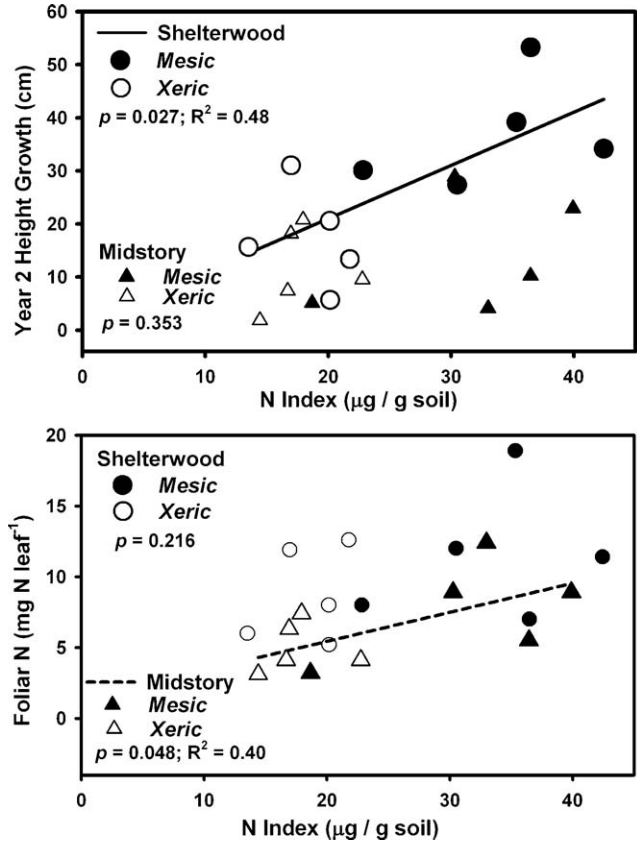

Fig. 3. Linear relationship between an index of plant available nitrogen and the (a) second-year height growth and (b) leaf N content of chestnut seedlings in eastern Kentucky.

a study using direct-seeded chestnut seedlings also measured lower SLA in thinned and burned areas that received greater light transmittance compared to untreated areas (McCament and McCarthy, 2005).

Higher soil fertility and moisture probably also contribute to better seedling performance in the mesic shelterwood openings (Table 2). Similar relations between soil productivity and topographic aspect and slope position are common in temperate deciduous forests (Muller, 1982; Newman et al., 2006; Fabio et al., 2009). Species composition, microenvironment and geologic substrate often combine to influence soil resources, nutrient dynamics and ecosystem productivity (Newman et al., 2006; Fabio et al., 2009). The relation we measured between soil N availability and chestnut seedling growth and foliar nutrition (Fig. 3) indicates that seedling height growth responded positively to increased soil fertility for the shelterwood light environment and foliar N responded to soil N fertility in the midstory treatment areas. Specific leaf area did not differ between xeric and mesic site types (Table 3), but it declined linearly with increasing foliar N ($SLA = 185 - 4.8X$; $p = 0.001$; $R^2 = 0.44$) confirming the relationship between leaf structure and N that is known to influence net photosynthetic capacity (Reich et al., 1998). Similar to our Kentucky study, the growth of direct-seeded chestnut seedlings responded positively to higher soil nitrogen availability in Ohio forest sites and SLA was related to soil nitrate (McCament and McCarthy, 2005).

Our initial results indicate that the performance of bare-root chestnut seedlings compares favorably to other economically important hardwood species and that chestnut seedlings respond well to a range of site conditions typical of the Cumberland Plateau. For example, northern red oak added 34 cm of height over two growing seasons in Tennessee shelterwood openings (Oswalt et al., 2006), compared to similar height growth for chestnut

Table 4
Density of hardwood seedlings within chestnut planting blocks at five locations in eastern Kentucky.

Site Type	Canopy Treatment	Species	Mean trees m^{-2}	SE trees m^{-2}	Maximum trees m^{-2}
Mesic	Shelterwood	*Liriodendron tulipifera*	2.4	2.3	11.7
		Acer rubrum	1.6	0.5	3.4
		Total	5.5	2.7	15.8
	Midstory	*Acer rubrum*	1.3	0.5	2.9
		Acer saccharum	0.7	0.7	3.3
		Total	3.0	0.5	4.1
Xeric	Shelterwood	*Sassafras albidum*	1.2	0.6	3.7
		Acer rubrum	0.6	0.2	1.3
		Total	3.5	0.5	5.1
	Midstory	*Acer rubrum*	1.8	1.1	6.2
		Quercus prinus	1.7	1.6	8.0
		Total	5.4	1.5	10.2

seedlings (e.g., 20–37 cm in year 2; Table 2) during a single season in Kentucky shelterwood openings. Poor seedling survival on rocky, sandy sites, and better height growth and foliar nutrition on more fertile, mesic sites provide guidance for plantation site selection.

The blight-resistant chestnut seedlings intended for outplanting during the coming years will be less susceptible to blight disease than the native chestnuts planted in our study, but risks from other pathogens and pests are less certain. For example, gypsy moth (*Lymantria dispar* L.) herbivory was higher on blight-resistant American × Chinese backcrossed hybrids compared to pure American chestnut seedlings in one greenhouse study (Rieske et al., 2003). Another concern, the common root rot fungus, *Phytophthora cinnamomi*, responsible for significant pre-blight American chestnut mortality (Crandall et al., 1945; Anagnostakis, 1995) was a likely contributor to mortality in the current study. A greenhouse trial using soils from our planting locations documented high susceptibility of pure American chestnut to *P. cinnamomi*, even under moderate levels of soil compaction and moisture (Rhoades et al., 2003); soil baiting techniques combined with molecular approaches (i.e., PCR amplification with DNA sequencing) confirmed the presence of *P. cinnamomi* at these sites (Adank et al., 2008). Asian chestnuts (*C. mollissima* and *C. crenata* Siebold & Zucc.) are known to resist Phytophthora root rot (Crandall, et al., 1945), but Phytophthora resistance traits are not selected by chestnut-blight breeding efforts and are not likely to be inherited by blight-resistant chestnut hybrids (F. Hebard, American Chestnut Foundation, *pers. comm.*).

It is uncertain how performance during the initial two years following planting will predict long-term establishment success under the different silvicultural treatments and site types. For northern red oak, competition with fast-growing tree species is known to reduce long-term success of plantings on mesic sites (Loftis, 1993; Povak et al., 2008). For our Cumberland Plateau study locations, chestnut transplants grew best in the high-light mesic shelterwood openings (Table 2), where the density of potential hardwood competitors was highest (Table 4). Yellow-poplar seedlings were abundant in mesic shelterwood sites and red maple (*Acer rubrum* L.), another fast-growing species that is becoming increasingly abundant in eastern North American forests (Abrams, 1998; McDonald et al., 2002), was common in all our study locations (Table 4). Where left untreated, competition from stump sprouts could present an even greater obstacle for planted chestnut (Loftis, 1985). In high-productivity sites where competitors are well-stocked, chestnut plantation success may require a period of initial growth in the moderate-light environment of the midstory removal treatment. Future plantation resurvey will allow us to evaluate both the potential for chestnut to achieve dominance in high-light shelterwood openings and to maintain itself in the moderate-light environment of the midstory removal treatment.

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