Two-year response of American chestnut (*Castanea dentata*) seedlings to shelterwood harvesting and fire in a mixed-oak forest ecosystem

Corinne L. McCament and Brian C. McCarthy

Abstract: The American chestnut (*Castanea dentata* (Marsh.) Borkh.) was once an important tree species in the eastern United States prior to its devastation by the chestnut blight. The American Chestnut Foundation will soon release seeds that are blight resistant. However, the necessary site requirements for restoration efforts have not yet been explored. The goal of this study was to evaluate the survival and growth of chestnut seedlings within a diverse forest management regime. Seedlings were experimentally grown for 2 years in three mixed-oak forests subjected to thinning, burning, thinning followed by burning, and an untreated control. Seedling biomass parameters were most influenced by treatments that increased light availability. Soil chemistry and texture parameters were also correlated ($p < 0.05$) with chestnut biomass. Thus, site fertility should also be considered in reintroduction efforts. While site quality may influence growth, light conditions appear to be overwhelmingly important. Therefore, we recommend that American chestnut seeds be planted in areas with moderate to high light conditions (recently disturbed), with low surrounding competing vegetation (possibly after a burn) for optimal growth benefits.

Résumé : Avant d'être décimé par la brûlure, le châtaignier d'Amérique (*Castanea dentata* (Marsh.) Borkh.) était une espèce importante dans l'est des États-Unis. La « American Chesnut Foundation » va bientôt distribuer des graines de châtaignier résistant à la brûlure. Cependant, les conditions de station requises pour orienter les efforts de restauration n'ont pas encore été examinées. Le but de cette étude consistait à évaluer le taux de survie et la croissance de semis de châtaignier en fonction de divers régimes d'aménagement forestier. Les semis ont été cultivés expérimentalement pendant deux ans dans trois forêts mélangées de chênes soumises à une éclaircie, à un brûlage et à une éclaircie suivie d'un brûlage avec un témoin non traité. Les paramètres de biomasse des semis étaient surtout influencés par les traitements qui augmentaient la disponibilité de la lumière. Les paramètres de chimie et de texture du sol étaient également corrélés ($p < 0,05$) à la biomasse du châtaignier. Par conséquent, la fertilité de la station devrait aussi être considérée dans les efforts de réintroduction. La qualité de station peut influencer la croissance mais les conditions de lumière ont une importance prédominante. Par conséquent, les auteurs recommandent que les graines de châtaignier d'Amérique soient plantées dans des endroits où les conditions de lumière vont de modérées à élevées (endroits récemment perturbés) avec peu de végétation compétitrice environnante (possiblement après un brûlage) pour bénéficier d'une croissance optimale.

[Traduit par la Rédaction]

Introduction

The American chestnut, *Castanea dentata* Marsh. (Borkh.), was an important forest species throughout the eastern United States for the last several millennia (Delcourt and Delcourt 1998). Chestnut was the leading dominant hardwood in some forests, often comprising more than 50% of the basal area of these stands (Braun 1950). When chestnut blight (*Cryphonectria parasitica*) was introduced in 1904 on a cargo shipment in New York, it spread rapidly throughout the oak–chestnut and mixed mesophytic forests of the eastern United States (Merkle 1906; Harlow 1996). By 1940, the blight had reached virtually every forested area throughout the Appalachian region, devastating mature chestnuts in these stands. By 1953, the oak–chestnut association was dropped as a viable vegetation type in the eastern United States (Keever 1953).

Historically, American chestnuts thrived on upland habitats composed of acidic and well-drained sandy soils in mixed forests (Stephenson et al. 1991). Russell (1987) conducted a postblight analysis of chestnut in Virginia forests and believes that chestnut was abundant on submesic or subxeric sites. Throughout most of its range, chestnut routinely reached 24–30 m in height and 0.6–1.2 m in diameter (Woods and Shanks 1959). Trees of old-growth forests may have grown considerably larger. Historically, chestnut trees reproduced by root sprout and rarely through seed, because of intensive predation and frost damage to nuts (Pinchot 1905; Paillet 2002). Sprouts have been shown to be a leading competitor after clear-cutting and in sparse canopies, possibly because

Received 23 March 2004. Accepted 16 December 2004. Published on the NRC Research Press Web site at http://cjfr.nrc.ca on 8 April 2005.

C.L. McCament and B.C. McCarthy.[1] Department of Environmental and Plant Biology, Ohio University, Athens, Ohio 45701, USA.

[1]Corresponding author (e-mail: mccarthy@ohio.edu).

 doi: 10.1139/X05-002

of chestnut having a strong positive response to high light conditions as compared with other hardwoods (Boring et al. 1981; Griffin 1989; Latham 1992). Billo (1998) also found that canopy disturbance initiated rapid growth in chestnut seedlings. However, McNab (2003) reported that chestnut sprout seedlings were out-competed in clearcuts by intense sprouting of other species. This suggests that thinning and burning would have a positive effect on chestnut sprouting and survival, because the combined effect would create canopy gaps, kill off competitors, and let newly formed chestnut sprouts grow with less competition (Russell 1987).

Since *Cryphonectria parasitica* is a phloem pathogen that does not affect roots, chestnut sprouts still occur throughout most of its native range. However, these sprouts rarely grow into the mid-story and rarely reach sexual maturity, because blight will eventually girdle the stem and kill larger chestnut saplings (Paillet 1988). Although *C. dentata* is still important in the understory of many forest systems, it has been rendered functionally extinct as a canopy tree (Parker 1992). If chestnut saplings did not die of blight infection, they probably would out-compete other species and fill newly opened canopy gaps (Paillet 1982).

Three major areas of research have focused on the restoration of American chestnut: the propagation of hypovirulence strains of *Cryphonectria parasitica*, blight-resistant pure American chestnut intercrosses, and the hybridization of Chinese and American chestnuts. A strain of *Cryphonectria parasitica* was first identified on European chestnuts and had been parasitized by a virus (Anagnostakis 2000). This hypovirulent strain was thought to be successful in treating the virulent *Cryphonectria parasitica* on trees by slowing the blight enough for infected trees to recover on their own and survive (Anagnostakis 2000). The primary difficulty has been the slow dissemination of the hypovirus to trees infected with the fungus (Double and Macdonald 2002). The method of breeding resistance from pure American chestnuts has not been effective, because there are so few naturally resistant trees and those that are available lose some resistance when crossed with susceptible parents to maintain diversity. Hybridization of the blight-resistant Chinese chestnut (*Castanea mollissima* Blume) and the blight-susceptible American chestnut (*Castanea dentata*) has been another approach to breeding blight-resistant American chestnuts. The American Chestnut Foundation (TACF) has now produced a tree through a series of intercrosses that has the genetic material responsible for blight resistance and preserves the genetic heritage of the American species (Hebard 2001). Hybrids were chosen for their resistance and form characteristics, and then backcrossed with other American chestnuts, making them 15/16ths pure American chestnut (Burnham 1981). TACF has been performing these intercrosses since 1989 and will be ready for planting the final progeny from them by 2005 (Hebard 2001). The hybrids are showing considerable hope for successful reintroduction.

As preparation for the release of blight-resistant seeds begin, more information is needed about the microsite conditions most conducive to chestnut establishment and growth (Phares 1978) to maximize the success of chestnut restoration efforts. In fact, because the blight occurred before the advent of modern ecological methods (Paillet 2002), very little is known about even the most basic silvicultural characteristics of *C. dentata*. It is also important to focus on microenvironmental features, because these are the most important factors contributing to a plant's existence and growth (Geiger 1950). Therefore, the overall objective of this work was to characterize and quantify silvicultural characteristics most conducive to chestnut success. We examined two specific questions: (1) What silvicultural treatments typically used in the mixed-oak forest landscape have the greatest effect on chestnut growth? (2) What environmental factors (e.g., light and soil characteristics) best account for these patterns of chestnut growth? Both questions are being addressed in a diverse management regime and will ultimately provide proper management tools for reintroduction of hybridized seeds?

Materials and methods

Study sites

The USDA Forest Service Fire and Fire Surrogate (FFS) research project (http://www.fs.fed.us/ffs) is one of the largest restoration experiments in the continental United States. The Ohio Hills sites were chosen to represent the entire central hardwoods region and are located in Zaleski State Forest, Tar Hollow State Forest, and Raccoon Ecological Management Area.

Experimental design

The FFS study sites provided the reintroduction trials with a preexisting field experiment infrastructure that offers a variety of environmental and silvicultural treatments to study American chestnut restoration. Each forest contains a control (undisturbed) and three treatments (approx. 20 ha each): burned (prescribed burn in spring 2001), thinned (30% basal area reduction from merchantable overstory), and a thin + burn treatment (combined 30% cut in winter 2000–2001 followed by a prescribed burn in spring 2001). Details about the treatments (e.g., burn dates, weather conditions, flame heights, maximum temperatures, etc.) and environmental conditions created can be found in Iverson et al. (2004*a*, 2004*b*). Early spring burns in mixed-oak forests of southeastern Ohio do not characteristically result in significantly increased understory light conditions (Robison and McCarthy 1999*a*; Iverson et al. 2004*a*), but thinning did significantly increase light availability (Iverson et al. 2004*a*). Pretreatment regeneration in the immediate region has been well categorized (Hutchinson et al. 2003). In this experiment, treatments dramatically influenced the regeneration layer in a complex fashion (M. Albrecht and B.C. McCarthy, unpublished data). Generally, burning treatments resulted in at least a 100% increase in understory cover. Burning treatments resulted in a decrease in sapling density, but an increase in seedling density. Density and cover of thin only treatments also increased, but the response was delayed by a year.

At each of the 12 forest × treatment combinations, there exist ten 0.1-ha vegetation test plots (120 plots total). Plots were 2 m × 5 m and delineated 15 m from the 10 vegetation plots located in each forest × treatment combination. Within those plots, 10 American chestnut seeds were planted 1 m apart, for a total of 1200 seeds.

Field methods

Fifteen-hundred pure American chestnut seeds were collected from a natural remnant stand of American chestnut in southwestern Wisconsin. Seeds were then stratified in cold storage for 16 weeks at 5 °C to mimic forest conditions (Young and Young 1992). They were stored in drainable flats containing a base of peat moss potting mix, saturated with water, and covered to retain soil moisture. Seeds were planted in the spring of 2002 under predator-proof wire cages to monitor germination, establishment, and subsequent growth.

Predator-proof cages were constructed out of aluminum gutter screening (C.H. Keiffer, personal communication, 2002). Cages were 15 cm in diameter and 30 cm in height and were capable of protecting seeds from fossorial and surface predators (Barnett 1977; Bendfeldt et al. 2001). Each cage was placed in a 5 cm deep hole and held in place with 2.5 cm of soil. The seed was placed in the cage and covered with 2.5 cm of soil (Anagnostakis 1997). A wire flag was weaved into the seam of the cage, holding the cage together and to the ground. The top of the cage was crimped when the seeds were planted and uncrimped when the seedling approached the top of the cage (C.H. Keiffer, personal communication, 2002). A numbered aluminum tag was attached to the cage for identification.

Fifty percent of each plot's germinated seedlings were harvested from each test plot in September 2002, leaving 50% of the remaining germinated seedlings in each plot for the 2003 harvest. Protocols for seedling biomass allocation follow Robison and McCarthy (1999*b*). Seedlings were dug up and washed with water to remove soil from root tissue. Harvested stems and roots were separated from each other and sealed in plastic bags, placed in a cooler, and brought to the laboratory where stem, primary root lengths, and basal diameter were immediately measured. The stems, roots, and leaves were placed in a drying oven at 80 °C and weighed after 72 h. Leaves were harvested from all surviving seedlings in September of 2002 (N = 579 plants) and 2003 (N = 288 plants), kept in coolers in the field, and brought back to the lab for leaf area measurement (LI-COR, Lincoln, Nebraska). Specific leaf area was determined by coring a 0.01-cm^2 disc from each healthy leaf, drying the disc in the oven at 80 °C, and weighing it after 48 h. American chestnut seedlings were given a health rating of one through four at the end of each growing season. A rating of one was given for a healthy seedling, a two if it was showing early signs of disease and (or) defoliation, a three if it was diseased, and a four if it was defoliated. Germination was recorded in July of 2002 and calculated as a percentage. Survival was recorded in September 2002 and again in September 2003 and recorded as a percentage.

The available light at each site was measured by use of hemispherical photography. A 35-mm Nikon digital camera with a Sigma 8-mm fish-eye lens was used to take photographs 1 m from the ground. Photographs were taken in mid-July 2003 at the center of all American chestnut plots. Percentage of open sky relative to canopy cover and global radiation was determined through use of digitally analyzed images using Gap Light Analyzer software (Robison and McCarthy 1999*a*; Frazer et al. 2001).

Soil parameters were measured to determine their effects on plant growth. Soil collection, processing, and extractive methods all follow McCarthy (1997). One soil sample was taken from the A horizon at each of the American chestnut plots (N = 120), placed in a cooler, dried at room temperature, ground through a 2-mm brass sieve, and stored at room temperature (McCarthy 1997). Phosphorus, magnesium, and calcium in the soil were determined for each sample via Mechlich III extraction and atomic absorption spectrometer methods (Varian 1989; McCarthy 1997). Potassium was determined using a Mehlich III extraction then analyzed using colorimetric spectrophotometry (McCarthy 1997). Nitrate was measured using ion exchange resins that measure long-term nitrate availability in soil (Binkley and Matson 1983; Binkley and Hart 1989; Hart and Firestone 1989). Resin bags containing 50 g wet mass of ion-exchange resin were placed in the A horizon from June 2003 to August 2003 (Rexyn 300 (H-OH), Fisher Scientific, Fair Lawn, New Jersey). Nitrate was extracted with 2 mol·L^{-1} KCl solution and analyzed colorimetrically on a spectrophotometer (Spectronic 20D, Unicam, Rochester, New York) using Nitrate Reagent Powder Pillows (Nitra Ver, Hach, Loveland, Colorado). Soil texture was calculated using the hydrometer method to determine the percentage of sand, silt, and clay (Sheldrick and Wang 1993). Soil moisture was calculated from soil texture results (Saxton et al. 1986). Soil pH was calculated with the glass electrode method using 2:1 water:soil mixture (pH/Ion Analyzer 350, Corning Inc., Corning, New York). Organic matter and ash content were determined using the dry-ash method (Shepard et al. 1993).

Statistical analysis

Our experimental design was such that forest was treated as a random effect (block), and treatment was a fixed effect in all analyses. Germination data were analyzed using a general linear model analysis of variance (GLM ANOVA) using percentage of germinated seedlings per plot (Zar 1999). Survivorship data (measured annually) were analyzed for year 2002 and 2003 separately using a GLM ANOVA with percentage of survivors per plot. Health ratings were analyzed using the same GLM ANOVA model. All data were transformed to meet the D'Agostino Omnibus test for normality (D'Agostino et al. 1990) and pass the modified levene equal variance test.

Silvicultural treatment influences on American chestnut biomass data were analyzed using a multivariate analysis of variance (MANOVA) followed by GLM ANOVAs to determine differences in years and treatments (Scheiner 1993) for total biomass, basal diameter, stem height, root length, leaf area, specific leaf area, and leaf, stem, root, and fine root mass. MANOVA significance was evaluated using Wilks' λ. Highly correlated variables were not used in the analysis. Forest was treated as a random effect, and treatment and year were fixed effects in both models. Bonferroni post hoc analyses were used to assess differences among the two years and four treatments. All 2002 and 2003 biomass parameters were $\log_{10}$ transformed to smooth data and pass normality, but in using this transformation, all data did not pass the homoscedasticity assumption. However, because of the robustness of the MANOVA (Zar 1999) and as it is useful to $\log_{10}$ transform biomass data (McCune and Grace 2002), the MANOVA was performed using only this transformation.

The impacts of microenvironmental parameters such as light and soil nutrients on American chestnut biomass parameters were analyzed using multiple regression (Philippi 1993). Each plot's biomass parameters were calculated separately by using means to compare with each plot's environmental factors (light and soil measurements). Environmental factors were the independent variables, and biomass parameters were the dependent variables. Data were screened for multicollinearity and transformed to pass assumptions of normality. All analyses were performed using NCSS (Hintze 2000).

Results

As a consequence of the thin and thin + burn silvicultural treatments applied to forests, results showed that there was a statistically significant difference in light availability among treatments ($F = 27.15$, $p < 0.001$). Plots subjected to thinning had more available light than plots that were not thinned (Fig. 1). However, burning had no effect on light (Fig. 1, $p < 0.001$).

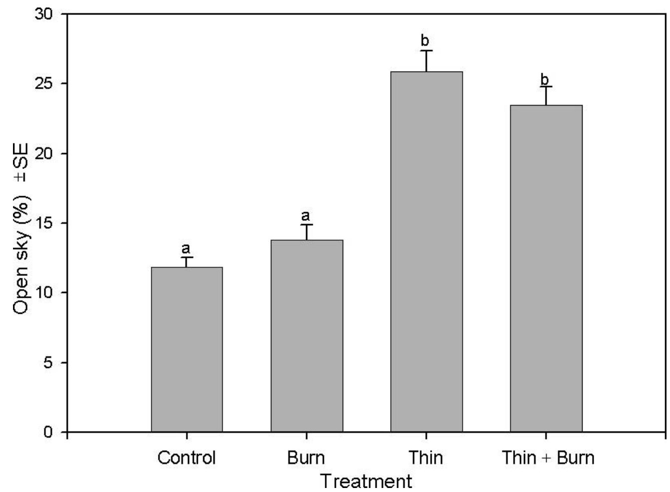
The 2002 harvest (1-year-old seedlings) revealed that thinning and burning had a large effect on plant biomass variables. Plants grown in burn treatments were significantly larger than the control treatments in most leaf parameters ($p < 0.05$). Plants that were grown in the thin and thin + burn treatments were larger in almost all aspects than those grown in the control and burn treatments alone, except for specific leaf area where it was significantly smaller in the thin + burn treatments (Figs. 2 and 3*d*).

The 2003 harvest (2-year-old seedlings) continued to reveal that the thinning and burning treatments affected plant biomass significantly. Plants grown in the thin + burn treatments were significantly larger in all aspects of growth than those grown in the control and burn treatments, except for specific leaf area (Fig. 3*d*) which was significantly smaller ($p < 0.05$). In stem (Figs. 2*a*, 2*b*) and root (Figs. 2c, 2*d*) variables, the thin treatments were significantly larger than the control and burn treatments. In leaf biomass variables (leaf area, leaf mass, and number of leaves) there was no significant difference between the burn and thin treatments, but there was an increase in leaf biomass in the thin and thin + burn treatments (Figs. 3*a*–3*d*).

Total plant biomass response after 2 years (2002 and 2003) increased significantly ($p < 0.05$) among treatments with all aspects of plant growth in the thin and thin + burn treatments being larger than in the control and burn treatments (Table 1). Exceptions included: specific leaf area (Fig. 3*d*), which decreased significantly from the control, burn, thin, and thin + burn treatments, and root length (Fig. 2*b*) where there was no significant difference between the control and burn treatments. These results demonstrate that as the plants grew older, the silvicultural treatment had an increased effect on plant growth in the thin and thin + burn treatments (Figs. 2*a*–2*f* and 3*a*–3*d*).

Although silvicultural treatment was shown to have a significant impact on biomass variables, germination ($F = 0.44$, $p = 0.09$), survival ($F = 0.56$, $p = 0.11$), and health ratings ($F = 2.13$, $p = 0.19$) did not differ significantly among silvicultural treatments (Table 2).

Fig. 1. Mean percentage of open sky (±SE) by silvicultural treatment (ANOVA). Means with a different letter indicate a significant difference ($p < 0.05$). Light measurements were taken in 2002, 2 years after burning and thinning.



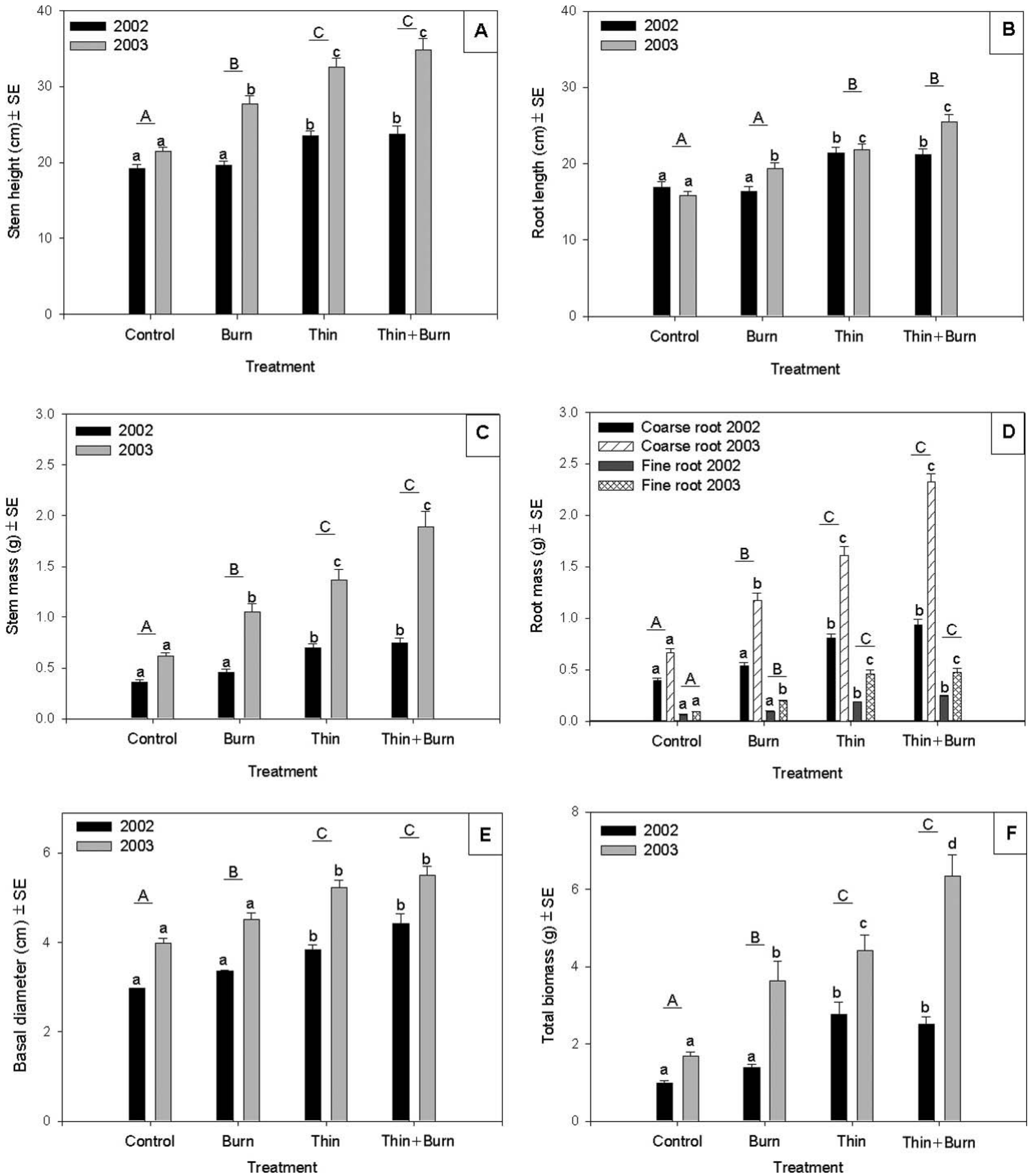
Out of the environmental variables measured, (light, calcium, magnesium, nitrate, potassium, phosphorus, organic matter, soil moisture, and soil texture) light was most strongly correlated with biomass parameters in years 2002 and 2003 in the multiple regression model. The regression model revealed a significant positive correlation ($p < 0.05$) in 2002 between light and almost all biomass parameters (Table 3). In 2003, this trend continued with light showing a significant positive correlation ($p < 0.05$) between all biomass parameters (Table 3). These results concur with the increase in biomass in the thin and thin + burn treatments, where there was also greater light availability.

The multiple regression model also revealed significant positive correlations ($p < 0.05$) between several soil parameters and biomass parameters (Table 3). In 2002, nitrate was positively correlated with stem and root variables as well as specific leaf area, and in 2003, it continued to be positively correlated with root variables. In 2003, magnesium was positively correlated with leaf variables and root mass. Potassium was positively correlated with basal diameter in 2002 and then with specific leaf area in 2003. In 2002, sand had a positive correlation with specific leaf area, but had a negative correlation with stem mass.

Discussion

American chestnut has been cited to be both a broad generalist and a strong competitor (Latham 1992). In our study, chestnut seedlings responded very strongly to only certain environmental variables. Seedlings had a significant positive response to light, as was the case in other studies (King 2003; Boring et al. 1981). However, because germination, health, and mortality were similar in areas of low light and high light, seedlings showed their best ability to germinate and survive in low light levels (control and burn treatments) and grow rapidly when light then became available (thin and thin + burn treatments). Chestnut seedlings also responded to decreased light by increasing their specific leaf area. This characteristic allows *C. dentata* to survive in a shaded envi-

Fig. 2. Growth measurements of *Castanea dentata* seedlings. Different lowercase letters indicate a significant difference among silvicultural treatments within a year ($p < 0.05$). Different underlined uppercase letters indicate significant differences among silvicultural treatments across years 2002 and 2003 ($p < 0.05$).



ronment. King (2003) also found that specific leaf area increased in *C. dentata* saplings with decreased light. American chestnuts have survived the blight with similar responses to light, by using their ability to survive in the understory of forests and then responding when light becomes available through canopy gaps (Paillet 2002). Chestnut seedlings in our study were not subjected to canopy gaps after being planted in low light levels, but did show an increase in biomass in areas with greater light conditions. The evidence suggests that *C. dentata*'s general response to light is that of an intermediate shade-tolerant species — it has the ability to survive in a shaded environment and the ability to respond when exposed to increased light levels.

American chestnut seedling growth was maximized under a high-light environment, which was created by thinning followed by prescribed burning. Boring et al. (1981) showed that chestnuts grew well in clearcuts. In contrast, McNab (2003) reported that chestnut sprouts were surpassed in

Fig. 3. Leaf measurements of *Castanea dentata* seedlings. Different lowercase letters indicate a significant difference among silvicultural treatments within a year ($p < 0.05$). Different underlined uppercase letters indicate significant differences among silvicultural treatments across years 2002 and 2003 ($p < 0.05$).

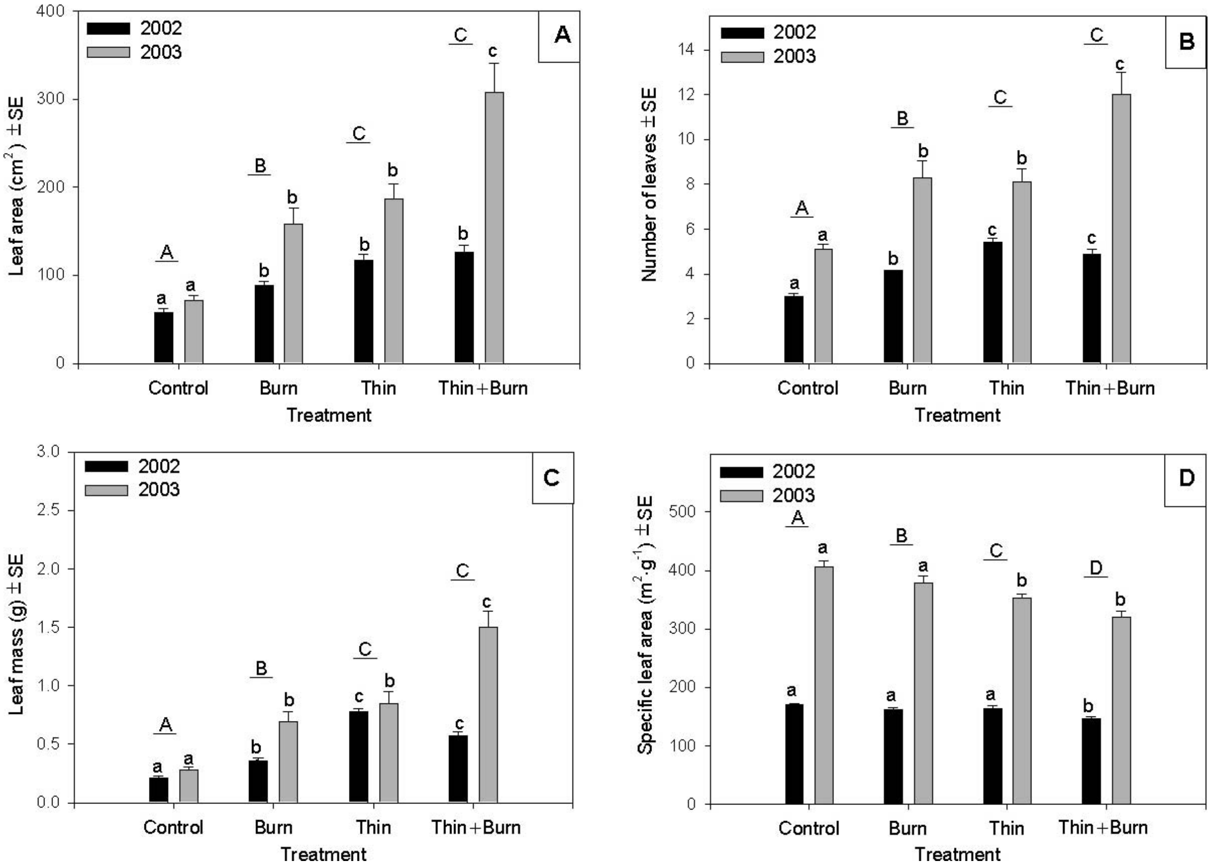


Table 1. Biomass parameters for *Castanea dentata* seedlings in 2002 and 2003.

Variable	Effect	df	*N*	*p*
No. of leaves	Forest	2	3357	<0.001
	Treatment	3	3357	<0.001
Leaf mass	Forest	2	494	0.06
	Treatment	3	494	<0.001
Leaf area	Forest	2	500	0.03
	Treatment	3	500	<0.001
Specific leaf area	Forest	2	415	<0.001
	Treatment	3	415	<0.001
Basal diameter	Forest	2	540	<0.001
	Treatment	3	540	<0.001
Stem height	Forest	2	540	0.49
	Treatment	3	540	<0.001
Stem mass	Forest	2	540	0.02
	Treatment	3	540	<0.001
Root length	Forest	2	543	0.41
	Treatment	3	543	<0.001
Root mass	Forest	2	543	0.006
	Treatment	3	543	<0.001
Fine root mass	Forest	2	543	<0.001
	Treatment	3	543	<0.001
Total biomass	Forest	2	543	0.006
	Treatment	3	543	<0.001

Note: Forest was treated as a random effect and silvicultural treatment was treated as a fixed effect in the GLM ANOVA.

clearcuts by intense sprouting of other species. Although there are conflicting published results on the effects of clear-cutting and chestnut, it is known that forest burning is an effective means by which to reduce vegetative competition that occurs after clear-cutting (Rieske 2001). This may be why there was an increase in many biomass parameters in year 2003 in the burn treatment versus control. Fire likely decreases competing vegetation for a brief window of time. This period may be critical for seedling establishment in restoration efforts. Even though in our study the burn was not as effective as it could have been, it still suggests that long-term fire management prescriptions may be necessary to maintain optimal conditions for good chestnut growth. In the treatments where there was thinning alone, seedling performance increased as compared with fire alone treatments, suggesting that competing vegetation may limit light near the forest floor, but that light from above will be the primary determinate of seedling growth response. The combined effects of fire and thinning yielded the greatest net growth for the largest number of seedling growth metrics. The removal of competing vegetation (and possible light limitation near the ground) and the addition of light from above (via shelterwood cutting) initiated a strong response for most seedlings (cf. Perry 2003).

In addition to light, American chestnut seedlings were also responsive to a number of soil parameters. Soils were sampled ca. 2 years following the treatments, thus any transitory differences in labile nutrients (e.g., nitrogen) would

Table 2. Mean percentages of germination, survival, and health rates of *Castanea dentata* seedlings (±SE) of each silvicultural treatment by year.

	2002			2003	
Treatment	Germination	Survival	Health	Survival	Health
Control (%)	71.33±5.06	50.33±5.08	2.46±0.12	51.33±5.42	2.37±0.25
Burn (%)	70.33±4.75	59.00±4.96	2.38±0.11	47.31±6.38	2.14±0.25
Thin (%)	62.00±5.64	53.00±5.73	1.79±0.09	45.37±6.05	2.23±0.20
Thin + burn (%)	64.33±5.48	53.66±5.53	2.27±0.15	44.60±6.48	1.89±0.15

Note: Health rates: 1, healthy; 4, defoliated. No significant differences were observed among treatments ($p > 0.05$, ANOVA).

Table 3. Multiple regressions relating *Castanea dentata* biomass metrics to environmental variables.

Dependent variable	Independent variable	Year significant	β	R^2	p
Basal diameter (cm)	Potassium (mg·g^{-1})	2002	0.723	0.415	0.045
	Light (%)	2003	2.834	0.306	<0.001
Stem height (cm)	Light (%)	2002	5.905	0.34	0.021
	Nitrate (mg·g^{-1})	2002	2.447	0.34	0.022
	Light (%)	2003	0.422	0.418	<0.001
Root length (cm)	Light (%)	2002	10.627	0.478	<0.001
	Nitrate (mg·g^{-1})	2002	3.348	0.478	<0.001
	Light (%)	2003	0.389	0.454	<0.001
	Nitrate (mg·g^{-1})	2003	0.001	0.454	0.01
Stem mass (g)	Light (%)	2002	0.425	0.459	<0.001
	Nitrate (mg·g^{-1})	2002	0.123	0.459	0.024
	Sand (%)	2002	–0.733	0.459	0.042
	Light (%)	2003	0.773	0.429	<0.001
Root mass (g)	Light (%)	2002	0.622	0.452	<0.001
	Nitrate (mg·g^{-1})	2002	0.144	0.452	0.016
	Light (%)	2003	0.898	0.524	<0.001
	Magnesium (mg·g^{-1})	2003	0.215	0.524	0.016
	Nitrate(mg·g^{-1})	2003	0.17	0.524	0.026
Fine root mass (g)	Light (%)	2002	0.747	0.36	<0.001
	Light (%)	2003	1.227	0.341	<0.001
Specific leaf area (m^2·g^{-1})	Magnesium (mg·g^{-1})	2002	0.219	0.33	0.042
	Nitrate (mg·g^{-1})	2002	0.22	0.33	0.016
	Sand (%)	2002	1.509	0.33	0.016
	Light (%)	2003	0.001	0.446	0.007
	Potassium (mg·g^{-1})	2003	0.004	0.446	0.006
Leaf mass (g)	Light (%)	2002	0.305	0.357	0.034
	Magnesium (mg·g^{-1})	2002	0.299	0.357	<0.001
	Light (%)	2003	1.235	0.41	<0.001
	Magnesium (mg·g^{-1})	2003	0.297	0.41	0.032
Leaf area (cm^2)	Light (%)	2002	0.562	0.378	<0.001
	Magnesium (mg·g^{-1})	2003	0.941	0.319	0.04
	Light (%)	2003	3.173	0.34	0.005
Leaves (no.)	Light (%)	2003	2.152	0.34	0.003

Note: Only terms that have a statistically significant slope ($p < 0.05$) are included.

not be manifested at this time. Chestnut seedlings showed a significant response to magnesium, potassium, nitrogen, and soil texture. Magnesium was positively correlated with leaf mass, leaf area, and root mass. This might be due to the central role of magnesium in chlorophyll construction (Shaul 2002). American chestnut seedlings in our study also showed that as potassium increased, so did basal diameter and specific leaf area. Such a response might be because potassium is used by plants in activating enzymes used in photosynthesis and respiration (Taiz and Zeiger 1998; Walker et al. 1996). Stems of potassium-deficient plants are often slender as compared with plants with a healthy supply of potassium (Taiz and Zeiger 1998), suggesting that American chestnut seedlings had an adequate supply of potassium, because their stems were healthy and robust. Nitrate was also positively correlated with stem, root, and leaf parameters in 2002 and 2003. Many studies have demonstrated that when there is an increase in nitrogen, there is an increase in stem mass, root mass, and specific leaf area (Freijsen and Veen 1990; Konings 1990; McDonald 1990; Chapin et al. 1998; Masarovicova et al. 2000), because nitrogen frequently limits plant growth and is needed for the production of new foliage. In a study testing the effects of added nitrogen on pure American chestnuts and Chinese × American hybrids, Rieske et al. (2003) found that although both species showed a positive response to added nitrogen, the hybrid demonstrated a greater response than the pure American chestnut (Rieske et al. 2003). These combined results may indicate that nitrogen could significantly impact the health of hybrid seedlings and should be carefully considered during reintroduction efforts.

Oddly, the percentage of sand in the soil had a negative correlation with stem mass in chestnut seedlings in 2002, even though past research has shown that American chestnuts grow best in well-drained sandy soils (Russell 1987; Stephenson et al. 1991). This may be due to a decrease in ability for sandy soil to retain moisture (Taiz and Zeiger 1998). Ashe (1911, 1922) found that chestnuts grew most robustly in lower coves with rich, deep, moist soils in Tennessee. This implies that chestnuts are adapted to a wide range of soil conditions. Since there was no correlation with sand in 2003, this indicates that sand and a potential water deficit may only impact seedlings during the initial recruitment stages.

In conclusion, stand level management had significant impacts on chestnut growth, suggesting that silvicultural practices could play a key role in chestnut's return to the eastern forests. In our study, American chestnut seedlings grew best in stands that had been subjected to a shelterwood thinning, followed by prescribed fire. This suggests that with the aid of fire in forests that have already been thinned, hybrid chestnuts could have an increased rate of success at reclaiming their dominance in the overstory. Results also suggest that American chestnut seedlings prefer sunlight rather than shade (as in the thin and thin + burn treatments), even though they are capable of germinating and surviving under lower light conditions (as in the control and burn treatments). Therefore, it is recommended that American chestnut seeds be planted in areas with moderately high light conditions, with low surrounding competing vegetation, for optimal growth benefits. However, adequate nutrients such as magnesium, nitrogen, and potassium have also been shown to affect chestnut growth and can significantly contribute to an American chestnut seedling's growth and vigor. Thus, site fertility should also be considered in reintroduction efforts.

Acknowledgements

We thank the Ohio Biological Survey, Ohio University's John Houk Memorial Grant Fund, Program in Environmental Studies, and the Department of Environmental and Plant Biology for funding this research. While direct funding was not provided, we also thank the National Fire and Fire Surrogate Research program for providing the experimental design and infrastructure necessary to make this project possible. This is contribution number 44 of the Fire and Fire Surrogate research initiative. The American Chestnut Foundation graciously provided the seeds for this study. Dr. Kim Brown and Dr. Geoffrey Buckley provided many helpful remarks and thoughts on this project. We also thank Matthew Albrecht, Jill Brown, Ben McCament, Ryan McEwan, Audrey Larrimer, Aswini Pai, and Zack Rinkes for field and laboratory assistance — without them this research would not have been possible. We also appreciated Dr. Carolyn Keiffer's many insights and contagious interest in chestnut.

References

Anagnostakis, S. 1997. Starting chestnut from seed. Fact sheet files. The Connecticut Agricultural Experiment Station, New Haven, Conn.

Anagnostakis, S. 2000. American chestnut sprout survival with biological control of the chestnut-blight fungus population. For. Ecol. Manage. **152**: 225–233.

Ashe, W.W. 1911. Chestnut in Tennessee. Bulletin of the Tennessee State Geological Survey, Nashville, Tenn.

Ashe, W.W. 1922. Forest types of the Appalachians and White Mountains. J. Elisha Mitchell Sci. Soc. **37**: 3–4.

Barnett, R.J. 1977. The effect of burial by squirrels on germination and survival of oak and hickory nuts. Am. Midl. Nat. **98**: 319–330.

Bendfeldt, E.D., Feldhake, C.M., and Burger, J.A. 2001. Establishing trees in an Appalachian silvopasture: response to shelters, grass control, mulch, and fertilization. Agrofor. Syst. **53**: 291–295.

Billo, T.J. 1998. Excerpts from a study of the past and present ecology of the American chestnut (*Castanea dentata* (Marsh.) Borkh.) in a northern hardwood forest. J. Am. Chestnut Found. **12**: 27–45.

Binkley, D., and Hart, S.C. 1989. The components of nitrogen availability assessments in Forest soils. Adv. Soil Sci. **10**: 57–112.

Binkley, D., and Matson, P.A. 1983. An evaluation of an ion exchange resin bag method for assessing forest soils nitrogen availability. Soil Sci. Soc. Am. J. **47**: 1050–1052.

Boring, L.R., Monk, C.D., and Swank, W.T. 1981. Early regeneration of a clear-cut southern Appalachian forest. Ecology, **62**: 1244–1253.

Braun, E.L. 1950. Deciduous forests of eastern North America. The Blakiston Co., Philadelphia, Penn.

Burnham, C.R. 1981. Blight-resistant American chestnut: there's hope. Plant Dis. **65**: 459–460.

Chapin, F.S., Lambers, H., and Pons, T.L. 1998. Plant physiological ecology. Springer-Verlag Inc., New York.

D'Agostino, R.B., Belanger, A., and D'Agostino, R.B., Jr. 1990. A suggestion for using powerful and informative tests of normality. Am. Stat. **44**: 316–321.

Delcourt, P.A., and Delcourt, H.R. 1998. The influence of prehistoric human set fires on oak-chestnut forests of the southern Appalachians. Castanea, **63**: 337–345.

Double, M.L., and MacDonald, W.L. 2002. Hypovirus deployment, establishment and spread: results after six years of canker treatment. *In* Proceedings of the 2002 Annual Meeting of the American Phytopathological Society, 27–31 July 2002, Milwaukee, Wisconsin. American Phytopathological Society, St. Paul, Minn. pp. 92–94.

Frazer, G.W., Fournier, R.A., Trofymow, J.A., and Gall, R.J. 2001. A comparison of digital and film fisheye photography for analysis of forest canopy structure and gap light transmission. Agric. For. Meteorol. **109**: 249–263.

Freijsen, A.H.J., and Veen, B.W. 1990. Phenotypic variation in growth as affected by N-supply: nitrogen productivity. *In* Causes and consequences of variation in growth rate and productivity of higher plants. *Edited by* H. Lambers, M.L. Cambridge, H.

Konings, and T.L. Pons. SPB Academic Publishing, The Hague, Netherlands. pp. 19–34.

Geiger, R. 1950. The climate near the ground. 2nd ed. Harvard University Press, Cambridge, Mass.

Griffin, G.J. 1989. Incidence of chestnut blight and survival of American chestnut in forest clear-cut and neighboring understory sites. Plant Dis. **73**: 123–127.

Harlow, W.M. 1996. Textbook of dendrology. 4th ed. McGraw Hill Inc., New York.

Hart, S.C., and Firestone, M.K. 1989. Evaluation of three in situ soil nitrogen availability assays. Can. J. For. Res. **19**: 185–191.

Hebard, F.V. 2001. Backcross breeding program produces blight-resistant American chestnuts (Virginia). Ecol. Restor. **19**: 252–254.

Hintze, J. 2000. NCSS 2000 user's manual. Syntec, Kaysville, Utah.

Hutchinson, T.F., Sutherland, E.K., and Scott, C.T. 2003. Composition and abundance of tree regeneration. *In* Characteristics of mixed-oak forest ecosystems in southern Ohio prior to the reintroduction of fire. *Edited by* E.K. Sutherland and T. Hutchinson. USDA For. Serv. Gen. Tech. Rep. NE-299. pp. 85–98.

Iverson, L.R., Prasad, A.M., Hutchinson, T.F., Rebbeck, J., and Yaussy, D.A. 2004*a*. Fire and thinning in an Ohio oak forest: grid-based analyses of fire behavior, environmental conditions, and tree regeneration across a topgraphic moisture gradient. *In* Upland oak ecology symposium: history, current conditions, and sustainability. *Edited by* M.A. Spetich. USDA For. Serv. Gen Tech. Rep. SRS-73. pp. 190–197.

Iverson, L.R., Yaussy, D.A., Rebbeck, J., Hutchinson, T.F., Long, R.P., and Prasad, A.M. 2004*b*. A comparison of thermocouples and temperature paints to monitor spatial and temporal characteristics of landscape-scale prescribed fire. Int. J. Wildland Fire, **13**: 311–322.

Keever, C. 1953. Present composition of some stands of the former oak-chestnut forest in the southern Blue Ridge Mountains. Ecology, **34**: 44–54.

King, D.A. 2003. Allocation of above-ground growth is related to light in temperate deciduous saplings. Funct. Ecol. **17**: 482–488.

Konings, H. 1990. Physiological and morphological differences between plants with a high NAR or a high LAR as related to environmental conditions. *In* Causes and consequences of variation in growth rate and productivity of higher plants. *Edited by* H. Lambers, M.L. Cambridge, H. Konings, and T.L. Pons. SPB Academic Publishing, The Hague, Netherlands. pp. 101–123.

Latham, R.E. 1992. Co-occurring tree species change rank in seedling performance with resources varied experimentally. Ecology, **73**: 2129–2144.

Masarovicova, E., Welschen, R., Alexander, L., Lambers, H., Argalasova, K., Brandsteterova, E., and Caniova, A. 2000. Photosynthesis, biomass partitioning, and perozisomicine A_1 production of *Karwinskia* species in response to nitrogen supply. Physiol. Plant. **108**: 300–306.

McCarthy, B.C. 1997. Lab protocols for the testing of eastern deciduous soils [online]. Ohio University, Department of Environmental and Plant Biology, Research Resources Section. Available from http://www.plantbio.ohiou.edu/epb/soils/oils.htm [updated 1997; cited 21 January 2004].

McCune, B., and Grace, B. 2002. Analysis of ecological communities. MjM Software Design, Gleneden Beach, Ore.

McDonald, J.A. 1990. Phenotypic variation in growth rate as affected by N-supply: its effects on NAR, LWR, and SLA. *In* Causes and consequences of variation in growth rate and productivity of higher plants. *Edited by* H. Lambers, M.L. Cambridge, H. Konings, and T.L. Pons. SPB Academic Publishing, The Hague, Netherlands. pp. 35–44.

McNab, H.W. 2003. Early results from a pilot test of American chestnut seedlings under a forest canopy. J. Am. Chestnut Found. **16**: 32–41.

Merkle, H.W. 1906. A deadly fungus on the American chestnut. Annual report. Vol. 10. New York Zoological Society, Bronx, N.Y. pp. 97–103.

Paillet, F.L. 1982. The ecological significance of American chestnut *Castanea dentata* in the Holocene forests of Connecticut. Bull. Torrey Bot. Club, **109**: 457–473.

Paillet, F.L. 1988. Character and distribution of American chestnut sprouts in southern New England woodlands. Bull. Torrey Bot. Club, **115**: 32–44.

Paillet, F.L. 2002. Chestnut: history and ecology of a transformed species. J. Biogeogr. **29**: 1517–1530.

Parker, A.C.G. 1992. Decline of understory American chestnut *Castanea dentata* in a southern Appalachian forest. Can. J. For. Res. **23**: 259–265.

Perry, J. 2003. The impact of fire on chestnut in the central hardwood region. J. Am. Chestnut Found. **17**: 34–41.

Phares, R.R. 1978. New challenges in chestnut research. *In* Proceedings of the American Chestnut Symposium, Morgantown, West Virginia, 4–5 January 1978. West Virginia University and the Forest Service Department of Agriculture Northeastern Forest Experiment Station, Parsons, W. Va. pp. 26–29.

Philippi, T.E. 1993. Multiple regression: herbivory. *In* Design and analysis of ecological experiments. *Edited by* Samuel M. Scheiner and Jessica Gurevitch. Chapman and Hall, New York. pp. 183–210.

Pinchot, G. 1905. A primer of forestry. USDA Dep. For. Bull. 24.

Rieske, L.K. 2001. Wildfire alters oak growth, foliar chemistry, and herbivory. For. Ecol. Manage. **168**: 91–99.

Rieske, L.K., Rhoades, C.C., and Miller, S.P. 2003. Foliar chemistry and gypsy moth *Lymantria dispar* (L.), herbivory on pure American chestnut, *Castanea dentata* (Fam: Fagaceae), and a disease resistant hybrid. Environ. Entomol. **32**: 359–365.

Robison, S.A., and McCarthy, B.C. 1999*a*. Potential factors affecting the estimation of light availability using hemispherical photography in oak forest understories. Bull. Torrey Bot. Club, **126**: 344–349.

Robison, S.A., and McCarthy, B.C. 1999*b*. Growth responses of *Carya ovata* (Juglandaceae) seedlings to experimental sun patches. Am. Midl. Nat. **141**: 69–84.

Russell, E.W. 1987. Pre-blight distribution of *Castanea dentata* (Marsh.) Borkh. Bull. Torrey Bot. Club, **114**: 183–190.

Saxton, K.E., Rawls, W.J., Romberger, J.S., and Papendick, R.I. 1986. Estimating generalized soil-water characteristics from texture. Soil Sci. Soc. Am. J. **50**: 1031–1036.

Scheiner, S.M. 1993. MANOVA: multiple response variables and multispecies interactions. *In* Design and analysis of ecological experiments. *Edited by* S.M. Scheiner and J. Gurevitch. Chapman and Hall, New York. pp. 93–137.

Shaul, O. 2002. Magnesium transport and function in plants: the tip of the iceberg. Biometals, **15**: 309–323.

Sheldrick, B.H., and Wang, C. 1993. Particle size distribution. *In* Soil sampling and methods of analysis. *Edited by* M.R. Carter. Canadian Society of Soil Science, Lewis Publishers, Ann Arbor, Mich. pp. 499–557.

Shepard, M.I., Tarnocai, C., and Thibault, D.H. 1993. Chemical properties in organic soils. *In* Soil sampling and methods of analysis. *Edited by* M.R. Carter. Canadian Society of Soil Science, Lewis Publishers, Ann Arbor, Mich. pp. 461–463.

Stephenson, S.L., Adams, H.S., and Lipford, M.L. 1991. The present distribution of American chestnut in the upland forest communities of Virginia. Bull. Torrey Bot. Club, **118**: 24–32.

Taiz, L., and Zeiger, E. 1998. Plant physiology. 2nd ed. Sinauer Associates, Inc., Sunderland, Mass.

Walker, D.J., Leigh, R.A., and Miller, A.J. 1996. Potassium homeostasis in vacuolated plant cells. Proc. Natl. Acad. Sci. U.S.A. **93**: 10 510–10 514.

Woods, F.W., and Shanks, R.E. 1959. Natural replacement of chestnut by other species in the Great Smokey Mountains National Park. Ecology, **40**: 349–361.

Varian. 1989. Flame atomic absorption spectrometry analytical methods. Varian Australia Pty. Ltd., Mulgrave, Victoria, Australia. Publ. 85-100009-00.

Young, J.A., and Young, C.G. 1992. Seeds of woody plants in North America. Timber Press Inc. Portland, Ore.

Zar, J.H. 1999. Biostatistical analysis. 4th ed. Prentice Hall, Upper Saddle River, N.J.