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Genetics and Silvicultural Treatments Influence the Growth and Shoot Winter Injury of American Chestnut in Vermont

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The backcross breeding of American chestnut (*Castanea dentata* [Marsh.] Borkh.) with Chinese chestnut (*Castanea mollissima* Blume) may provide an effective method to increase resistance against chestnut blight and help restore American chestnut throughout its historic range. However, the comparative adaptation (e.g., cold hardiness and growth) of American and Chinese chestnut source material in the north has been largely overlooked. We assessed first-year growth and shoot winter injury of 13 genetic sources of American chestnut and 2 sources each of Chinese chestnut and red oak (*Quercus rubra* L., a native competitor) under three silvicultural treatments (open, partial, and closed canopy overstories) in Vermont, USA. Seedlings grown under open canopies that provided greater access to light exhibited greater growth than seedlings grown under partial and closed canopies. However, open canopies also resulted in lower winter temperatures that increased winter injury. Chinese chestnut seedlings had significantly greater growth but experienced greater winter injury than American chestnuts, whereas red oaks generally grew the least and experienced intermediate levels of winter injury. There were also differences in growth and winter injury among American chestnut sources: seedlings from warm and moderate temperature zones (based on meteorological data recorded near source origins) grew more in height and diameter but experienced greater winter injury than seedlings from colder climates.

Keywords: *Castanea*, restoration, cold adaptation, physiological tradeoffs, crown closure, temperature zones

Before the early part of the 20th century, American chestnut (*Castanea dentata* [Marsh.] Borkh.) was a dominant component of eastern United States forests ranging from Maine to Georgia and west to the Ohio River valley (Little 1977), covering more than 800,000 km^2 (Braun 1950, Jacobs 2007). Within its native range, the species was thought to represent up to 40% of the forest canopy (Keever 1953) and as much as 50% in the central Appalachians (Russell 1987, Smith 2000). American chestnut is fast-growing compared with many of its competitors (diameter growth as great as 2.5 cm/year) (Buttrick 1925, Kuhlman 1978) and could achieve diameters of 1.5 m and heights of 37 m (Buttrick 1925). It also provided rot-resistant, straight-grained wood that was useful for construction, woodworking, furniture, railroad ties, telephone poles, mine timbers, and musical instruments (Anagnostakis 1987). Furthermore, the species annually provided abundant crops of nutritious nuts that were an important food source for wildlife, domestic animals, and humans alike (Rice et al. 1980).

Approximately one century ago, chestnut blight (caused by the fungal pathogen *Cryphonectria parasitica* [Murr.] Barr) was introduced to eastern forests. The disease results in girdling cankers that kill stems without harm to the root systems of American chestnut (Griffin 2000). Although scattered stump sprouts growing at the bases of long-dead trees may persist for some years, they typically succumb to the blight before reaching reproductive maturity. As a

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Table 1. Source codes, location, latitude, longitude, elevation, mean minimum winter temperatures (December through February), and temperature zones for open-pollinated American chestnut sources at the Green Mountain National Forest planting.

Code	Location (county, state)	Latitude	Longitude	Elevation (m)	Mean minimum temperature (° C)*	Temperature zone
KY1	Metcalfe County, KY	37°00′ 16″N	85°37′34″W	269	−2.50	Warm
MD1	Montgomery County, MD	38°57′ 53″N	77°05′33″W	100	−2.59	Warm
NC1	Jackson County, NC	35°22′ 21″N	82°47′29″W	1,387	−4.19	Warm
NJ1	Monmouth County, NJ	40°36′ 20″N	73°07′10″W	20	−5.05	Warm
NY1	Westchester County, NY	41°19′ 41″N	73°41′10″W	94	−6.02	Moderate
PA1	Franklin County, PA	39°59′ 38″N	77°23′55″W	600	−6.47	Moderate
PA2	Mercer County, PA	41°20′ 58″N	80°04′58″W	384	−7.15	Moderate
VA1	Smyth County, VA	36°49′ 40″N	81°25′49″W	1,036	−7.62	Moderate
VA2	Smyth County, VA	36°51′ 55″N	81°26′10″W	1,041	−7.62	Moderate
NY2	Wyoming County, NY	42°37′ 44″N	78°03′17″W	417	−8.65	Moderate
ME2	Knox County, ME	44°10′ 55″N	69°08′09″W	68	−10.81	Cold
VT1	Chittenden County, VT	44°31′ 39″N	73°12′11″W	57	−11.12	Cold
ME1	Piscataquis County, ME	45°09′ 35″N	69°04′58″W	101	−12.12	Cold

Each source represents one or two half-sib families, except for PA2, which was from three trees.
* Average minimum winter temperatures were calculated based on daily minimum air temperatures recorded over 10–30 years at local weather stations, averaged for each month, and adjusted for elevation between weather stations and the source location using the environmental adiabatic lapse rate of 6.5° C/1,000 m.

result, the blight functionally removed American chestnut as an overstory tree in North American forests within about 40 years of its first identification (Anagnostakis 1987, Griffin 2000). Considering the former ecological and economic value of American chestnut, many approaches to control chestnut blight and restore the species to its former prominence have been attempted (Beattie and Diller 1954, Elliston 1981, MacDonald and Fulbright 1991). One tactic that has shown significant promise of providing blight-resistant trees is the hybridization and backcrossing of American chestnut trees with blight-resistant Chinese chestnut (*Castanea mollissima* Blume) or Japanese chestnut (*Castanea crenata* Sieb. and Zuc.) (Griffin 2000, Hebard 2005, The American Chestnut Foundation [TACF] 2010). This method involves hybridizing Asian chestnut with American chestnut through controlled pollinations and then successively backcrossing blight-resistant progeny with American chestnut to produce blight-resistant trees that contain approximately 94% American chestnut germplasm (TACF 2010).

In the northern extremes of American chestnut's historic range, the restoration effort has an added complication: the limited cold hardiness of both native American chestnut and backcrossed stock (Gurney et al. 2011). This vulnerability to the cold leads to freezing injury and shoot dieback in the field, resulting in a shrubby form for affected seedlings (Gurney et al. 2011) that has been noted throughout New York and New England (Kendra Gurney, TACF, pers. comm., Feb. 20, 2008).

Two factors that can significantly influence plant cold tolerance are the genetics and environmental surroundings of plant tissues (Balduman et al. 1999, Aitken and Hannerz 2001). Environmental gradients in temperature over broad geographic areas, such as those influenced by latitude, elevation, and proximity to large bodies of water have been shown to result in similar, parallel variations in species adaptability to the cold, suggesting a genetic adaptation to in situ temperature regimes (Aitken and Hannerz 2001). In addition, nighttime temperatures during the winter months can be several degrees warmer under forest canopies than in open environments (Reifsnyder and Lull 1965). Forest canopies have been described as thermal blankets, which are capable of retaining warmer temperatures near the ground (Hough 1945). As a consequence, plants growing in the open (noncanopied) environments may have a significantly higher risk of experiencing shoot winter injury (Hadley and Smith 1986).

To better understand how genetics and canopy cover influence American chestnut in the northern portion of its range, we established plantings of 13 genetic sources of American chestnut along with two sources each of Chinese chestnut (used in breeding efforts to increase blight resistance) and red oak (*Quercus rubra* L., a native competitor) under three silvicultural treatments (open, partial, and closed canopies) in the foothills of the Green Mountains of Vermont. By assessing the influence of genetics (G), silvicultural treatment (environment [E]), and the interaction of the two (G × E) on seedling growth and shoot winter injury, we can help identify management options that best promote the development of large, single-stemmed seedlings that are well adapted for restoration in the northern forest.

Methods

Nut Sources

All American and Chinese chestnut nuts and red oak acorns were collected either by our research group or by volunteers and staff with TACF at various locations. Nuts were collected in fall 2008 and were stratified by refrigeration at 3° C for 3 months. We used almost 900 nuts, including 48 red oak acorns collected from each of two stands in Vermont and New Hampshire (acorns represented bulked collections without specifically identifiable parent sources). We also used approximately 54 nuts each from 13 open-pollinated American chestnut sources and 2 open-pollinated Chinese chestnut sources, each source represented by one or more half-sib families located throughout the eastern United States (Table 1). American chestnut were from native sources and included one from Vermont and two each from New York and Maine (northern sources), one each from New Jersey and Maryland and two from Pennsylvania (central sources), and one each from Kentucky and North Carolina and two from Virginia (southern sources) (Table 1).

Temperature Index to Estimate Source Cold Tolerance

Although nut sources were chosen to represent a wide variety of latitudes, past research with nut tissue established that latitude data alone did not help resolve broad differences in low temperature acclimation for American chestnut sources (Schaberg et al. 2009). Indeed, when nut cold tolerance for many of the same sources planted for this study was assessed, Saielli et al. (2012) found that "region" (south, central, or north based on latitude alone) did a poor job of isolating local adaptation to the cold. In contrast, "temperature zone," a new index of predicted low temperature acclimation based on local air temperature data (National Oceanic and Atmospheric Administration [NOAA] National Climatic Data Center 2005) that was adjusted for elevation using the environmental adiabatic lapse rate (Short 2011) better isolated sources with greater tolerance to the cold (Saielli et al. 2012). Accordingly, although we report latitudinal region in Table 1, we used temperature zone as a source of variation in statistical tests to assess patterns of possible local adaptation to temperature. To create temperature zone categories, mean minimum winter temperatures were calculated for the weather station nearest each source based on daily minimum temperatures recorded over 10–30 years and then averaged for each month. We focused on the mean temperature for December, January, and February at each location and adjusted for elevational differences between weather stations and the nut source location using the environmental lapse rate of 6.5° C/1,000 m (Table 1).

Planting Establishment

Seedlings were sown in a greenhouse located at the US Department of Agriculture (USDA) Forest Service in South Burlington, VT, in March 2009. Nuts were planted in 115-ml cone-shaped pots containing a potting mix of 1:1:1 peat/perlite/vermiculite (Gurney 2010). Seedlings were provided supplemental lighting to extend day length to 12 hours, were watered to keep soils moist, and were field planted on the Green Mountain National Forest in Leicester, Vermont (latitude 43.8°51′681″ and longitude −73.0°33′423″) in June 2009 after being acclimated for 1 month outside the greenhouse in South Burlington. Soils at the planting site included Sudbury fine sandy loams, Peru stony loams, and Peru gravelly fine sandy loams, and slopes ranged from 3 to 12% (USDA Natural Resources Conservation Service 2012).

Seedlings were planted in a replicated design under three levels of silvicultural overstory treatment: open canopy (no forest canopy cover), partial canopy (created via an oak regeneration harvest), and closed canopy. Open canopy sites have been mowed periodically to prevent tree regeneration and promote forage production. Partial canopy sites were generated by the first of three planned shelterwood harvests. This harvest was conducted in December 2006 through January 2007, and reduced forest stocking from 25.3 to 16.1 m^2 basal area/ha (a 37% reduction in canopy closure). Closed canopy sites have approximate stocking levels of 23.0 m^2/ha, and there are no records of harvests at closed canopy sites because the land was acquired by the USDA Forest Service in 1937. All plots exist within the oak-dominated forests of this region, an area where American chestnut was once a forest component (Paillet 2002). Indeed, scattered American chestnut stump sprouts still exist in the area, suggesting that species restoration here would be appropriate (Paul Schaberg, USDA Forest Service, pers. observ., July 14, 2013).

For American and Chinese chestnuts, six seedlings from each source were randomly planted in each of three replications per canopy treatment for a total of 54 seedlings per source. For red oak, six seedlings per source were planted in each canopy treatment for replication one, and five seedlings per source were planted in each canopy treatment for replications two and three, for a total of 48 seedlings per source. Spacing of seedlings was approximately 2.5 × 2.5 m with variations based on topography and ground cover in the partially open and closed canopy stands where existing overstory trees influenced spacing between seedlings. Seedlings also received the following three cultural treatments when planted: 0.9 × 0.9 m black competition mats to reduce vegetative competition, 7.5-cm diameter and 25-cm tall cylindrical aluminum shelters buried approximately 10.0 cm into the soil to protect seedlings from rodent damage, and 1.2-m high and 0.75-m diameter welded-wire guards to protect seedlings from deer browse.

Percent Canopy Openness

To quantify canopy cover for all of the treatments and replications, multiple hemispherical photographs were taken of the canopy throughout each treatment using an EOS Rebel digital camera (Canon USA, Inc., Lake Success, NY) with a 4.5-mm F2.8 EX DC circular fisheye lens (Sigma Corporation of America, Ronkonkoma, NY). Measurements were taken on overcast days or early/late in the day during diffuse light conditions in August and September 2009. The camera was attached to a tripod and leveled using a bubble level at 1.0 m above the ground and oriented so that the top of the photograph was pointing north. Percent canopy openness above each seedling was calculated using Gap Light Analyzer 2.0 (GLA 2.0) software (Simon Fraser University, Burnaby, BC, Canada, and the Cary Institute of Ecosystem Studies, Millbrook, NY). The threshold level for each image was adjusted by comparison with enlarged images of the digital negatives. Using hemispherical photography and GLA 2.0 software to produce a photo-negative of the canopy from horizon to horizon, percent canopy openness was determined for white (noncanopy) pixels relative to black (canopy) pixels. This system was used to compare the mean percentages of canopy openness among the three silvicultural treatments.

Temperature Data

Temperature data were recorded using 54 programmable temperature sensors (iButtons; Embedded Data Systems, Lawrenceburg, KY) that were placed at two heights (20 and 120 cm aboveground) adjacent to three randomly selected seedlings within each of the nine treatment replications. Each sensor was placed inside a small white container that protected the sensor from moisture but also reflected sunlight. Sensors were programmed to record temperature every 4 hours. Because shoots are vulnerable to low-temperature stresses (Gurney et al. 2011), temperature data were used to estimate the lowest temperatures seedlings were exposed to during December, January, and February, the period when American chestnut appears most vulnerable to shoot freezing injury (Gurney et al. 2011). Differences in temperature associated with the height of sensor placement helped us to infer the influence of snowpack within treatments and replications.

Height and Diameter Measurements

We measured height (cm) from the ground level to the uppermost tip and diameter (mm) at the ground level of each seedling at the time of field planting in June 2009 and again in October 2009, after a single growing season in the field. Measurements taken at the time of planting were used to evaluate the influence of species and source within species on the initial growth in the greenhouse, under which environmental conditions were considerably more uniform and moderate compared with field conditions. For field observations, we calculated height and diameter growth (October measurement − June measurement = growth) to evaluate the influence of species and source within species (G), silvicultural treatment (E), and G × E interactions on growth.

Table 2. Factor, source of variation, sample size, *df*, and the denominator term used for tests of significance for ANOVA model used to test for differences in field height and diameter growth and shoot winter injury among American chestnut sources.

Factor	Source of variation	*n*	*df*	Test used to determine F statistic and *P* value
Fixed	Temperature zone (TZ)	3	2	S[TZ] + (TZ × R)
Random	Source (S[TZ])	4	9	S[TZ] + R
Fixed	Treatment (T)	3	2	(S[TZ] × T) + (T × R)
Fixed	Replication (R)	3	2	S[TZ] × R
Fixed	TZ × T		4	(S[TZ] × T) + (TZ × T × R)
Fixed	TZ × R		4	S[TZ] + R
Random	S[TZ] × T		18	S[TZ] × T × R
Random	S[TZ] × R		18	T × R
Fixed	T × R		4	S[TZ] × T × R
Fixed	TZ × T × R		8	S[TZ] × T × R
Random	S[TZ] × T × R		36	Error
	Error		540	

Table 3. Canopy openness, minimum temperatures of coldest days in December, January, and February, field height and diameter growth (final − initial measurements), and percent shoot dieback for American chestnut seedlings grown at the Green Mountain National Forest planting, summarized by silvicultural treatment.

Silvicultural treatment	Canopy openness (%)	Mean minimum temperatures			Mean field growth and winter injury		
		December	January	February	Height (cm)	Diameter (mm)	Winter injury (% shoot dieback)
		. . . .	.(° C)				
Open canopy	58.0 (0.9) A	−26.4 (0.8) A	−23.8 (0.2) A	−21.4 (0.4) A	5.95 (0.57) A	2.28 (0.13) A	28.59 (2.05) A
Partial canopy	21.9 (0.3) B	−23.1 (0.9) B	−22.6 (0.2) B	−20.6 (0.1) B	0.68 (0.10) B	0.70 (0.05) B	11.39 (1.27) B
Closed canopy	15.5 (0.0) C	−22.8 (0.2) B	−22.3 (0.3) B	−20.0 (0.0) C	0.72 (0.12) B	0.30 (0.04) C	14.21 (1.72) B

Data are means (±SE). Means with different letters are significantly different based on Tukey HSD tests ($P \leq 0.05$).

Shoot Winter Injury

Seasonal cold tolerance assessments have shown that American chestnut shoots are fully capable of surviving fall and spring temperature lows without injury but are uniquely susceptible to freezing injury during winter (Gurney et al. 2011). Winter freezing injury leads to shoot dieback that is visually distinguishable as dark colored and sunken portions of stems in the spring (Gurney et al. 2011). Visual assessments of shoot winter injury were made in July 2010. Injury was identified after leaf-out as visible dieback (dark-colored and sunken portions of stems) on terminal shoots. Winter injury was quantified by measuring the length (cm) of damaged terminal shoots and expressed as a percentage of the total height for each seedling (percent shoot dieback).

Statistical Analyses

Analyses of variance (ANOVAs) were used to test for differences in canopy openness, winter air temperature lows, seedling height and diameter growth, and shoot winter injury using SAS statistical software (SAS Institute, Inc., Cary, NC). Differences in initial height and diameter among seedlings after greenhouse growth were tested with two fixed-effects statistical models: with species as the sole source of variation and for American chestnuts that had temperature zone, source within temperature zone, and the associated interaction as sources of variation. Percent canopy openness and minimum temperature data were used to evaluate differences in environmental parameters associated with silvicultural treatment and replication. Species differences in field-based height and diameter growth and shoot winter injury for all seedlings were tested with a fixed model that included the following sources of variation: species, silvicultural treatment, replication, and their interactions. Differences in field-based height and diameter growth and shoot winter injury among American chestnut sources were tested using a mixed-effects model that included temperature zone, source within temperature zone, silvicultural treatment, replication, and the interactions of these sources of variation (Table 2). For this analysis, all sources of variation were considered fixed except for source within temperature zone and interactions with this, which were considered random (Table 2). Tukey honestly significant difference (HSD) multiple comparisons were used to test for significant differences among means. Data were adjusted when needed using the Box-Cox transformation (Montgomery 2001) to satisfy the assumption of homogeneity of variances. For all tests, differences were considered statistically significant for $P \leq 0.05$.

Results and Discussion

Differences in Canopy Openness and Winter Temperature Lows

The open canopy treatment exhibited significantly greater canopy openness (58.0 ± 0.9%) than the partial canopy treatment (21.9 ± 0.3%), which showed somewhat greater openness than the closed canopy treatment (15.5 ± 0.3%) (Table 3). There were consistent differences in temperature minima between treatments as well, with the lowest temperatures recorded for all three winter months occurring in the open treatment (Table 3). For these periods, temperature sensors in all treatments that were closest to the ground consistently recorded lower temperatures than sensors deployed higher up. Snow can act as an insulator that limits exposure to ambient air temperature lows (Schaberg et al. 2008). Because lower sensors often recorded the lowest temperatures it is likely that there was little snow accumulation that would have helped buffer temperatures. Importantly, on the dates with the lowest temperatures of the winter (December 31 and January 1), temperature sensors closer to ground level (20 cm) recorded lower temperatures than the upper sensors (120 cm), indicating that there was little snowpack to protect seedlings during this freezing stress event. Depth of the snowpack averaged at the two closest NOAA weather stations was <8 cm on December 31 and January 1 (NOAA National Climatic

Table 4. Greenhouse height and diameter growth and field height and diameter growth (final − initial measurements), and percent shoot winter injury among species for seedlings grown at the Green Mountain National Forest planting.

	Mean greenhouse growth		Mean field growth and winter injury		
Species	Height (cm)	Diameter (mm)	Height (cm)	Diameter (mm)	Winter injury (% shoot dieback)
Chinese chestnut	27.63 (0.62) A	3.35 (0.13) B	4.22 (0.61) A	1.72 (0.19) A	32.25 (3.46) A
American chestnut	19.88 (0.22) B	3.89 (0.04) A	2.39 (0.21) B	1.06 (0.06) B	15.58 (1.03) B
Red oak	10.79 (0.90) C	3.22 (0.18) B	0.74 (0.26) B	0.50 (0.12) C	24.7 (6.17) AB

Data are means (±SE). Means with different letters are significantly different based on Tukey HSD tests ($P \leq 0.05$).

Data Center 2013), which supported temperature-based inferences of low snow levels that would leave seedlings vulnerable to freezing damage. Overall, the 2009–2010 winter snowfall total for these two closest weather stations averaged 177.8 cm, slightly lower than the 30-year average of 184.0 cm for these locations (NOAA National Weather Service Forecast Office 2014).

Growth in the Greenhouse

There were significant differences in height ($P < 0.0001$) and diameter ($P < 0.0001$) growth among the species after growth in the greenhouse (Table 4). Chinese chestnut seedlings were significantly taller than American chestnut and red oak seedlings and American chestnut were taller than red oak. Patterns of diameter growth differed from those for height; American chestnut diameters were greater than those for the other two species. Height differences corroborate past observations of fast early growth for Chinese chestnut relative to that for American chestnut (McKay and Crane 1953, Graves 1962). Indeed, McKay and Crane (1953) found that, in addition to being a fast early grower, Chinese chestnut is adapted to short winters and long growing seasons and breaks dormancy early in spring (which may also contribute to shoot damage from spring frosts). Graves (1948) found great variability in growth rates among different varieties of Asian and American cultivars, with Japanese chestnut being the slowest growing species observed and the American × Japanese hybrid (*C. dentata* × *C. crenata*) being the fastest.

Among American chestnut sources, significant differences in height and diameter growth were found among seedlings from the three temperature zones (Figure 1). In general, moderate-temperature sources showed elevated growth relative to that of sources from the warm zone, with sources from the cold zone being more intermediate in growth. It is possible that environmental conditions in the greenhouse provided a more moderate environment (e.g., intense sunlight muted by shade cloth during midday, warm temperatures moderated by venting and supplemental air exchange, and ample water) that may better reflect the environmental conditions of the moderate temperature zone than those of warm and cold zones. There were also significant differences in height and diameter growth among sources within each temperature zone (Figure 1). At least during early establishment in the greenhouse, temperature zone appears to account for only a small portion of the variation in growth for American chestnuts.

Growth in the Field

Almost 97% of all seedlings survived the first growing season, and they appeared healthy and robust when measured for growth in early autumn. There were no differences in early survival associated with any tested source of variation ($P > 0.20$ for all factors), in part because mortality levels were so low. There were significant differences ($P < 0.0001$) in height and diameter growth in the field for the three species (Table 4). Similar to greenhouse results, Chinese

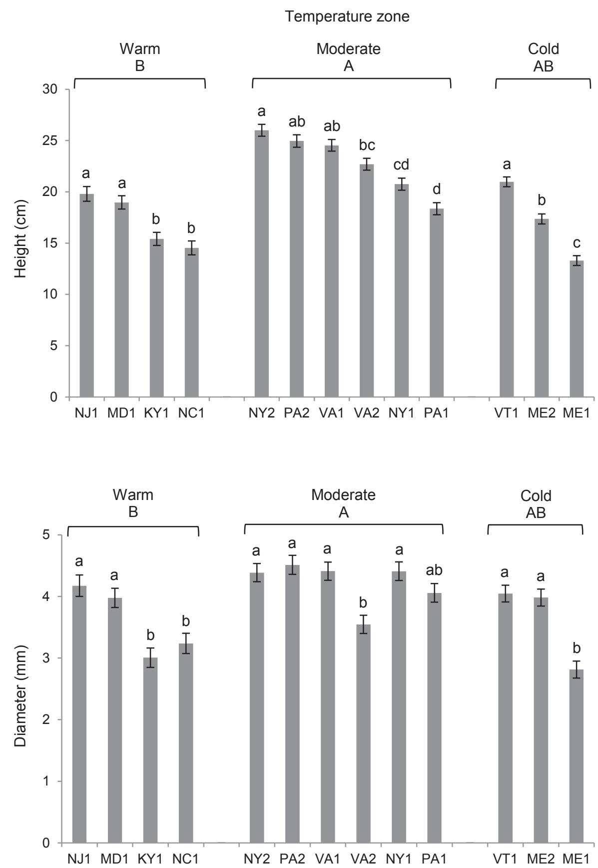

Figure 1. Mean (±SE) heights and diameters among American chestnut seedlings after greenhouse growth. Means with different uppercase letters are significantly different for temperature zone, and means with different lowercase letters are significantly different for source within the temperature zone, both based on Tukey HSD tests ($P < 0.0001$). Refer to Table 1 for source information.

chestnut experienced the greatest height growth, although in the field American chestnut and red oak height growth did not differ significantly. Diameter growth was greatest for Chinese chestnut, followed by American chestnut and red oak, respectively.

For all species (Saielli 2011) and particularly for American chestnut (Table 3), significant differences ($P < 0.0001$) in height and diameter growth were also identified among the three silvicultural treatments. Treatment accounted for almost 13% of the variation in height growth and 24% of the variation in diameter growth. In general, seedlings in the open canopy treatment experienced significantly greater height and diameter growth than seedlings in the partial and closed canopy treatments. Seedlings in the partial and closed canopy treatments differed only in diameter growth: seedlings under the partial canopy treatment were more than twice the diameter of seedlings grown under the closed canopy treatment.

A prominent and defining difference among the silvicultural treatments was the relative access to light afforded, with the open treatment providing substantially greater access to light that drives photosynthesis and growth (Table 3). American chestnut has been shown to respond well to high light conditions relative to the response of many sympatric species (Boring et al. 1981, Griffin 1989, Latham 1992, King 2003). Tindall et al. (2004) reported that American chestnut growth was positively correlated with canopy openness. However, the magnitude of the differences in aboveground height and diameter between the open canopy treatment and the partial and closed canopy treatments (Table 3) after one season was notable, considering that seedlings may also be expected to devote considerable resources to root growth immediately after field planting, especially in cooler northern environments (Smith et al. 1997). Our seedlings may have devoted more resources to aboveground growth because root competition from weeds and grasses around each seedling was reduced by the use of competition mats. Gersani et al. (2001) found that there is a corresponding increase in root growth relative to the amount of competition a plant is exposed to; consequently, when plants are faced with little competition, there is a tendency to put resources into aboveground biomass rather than roots.

Temperature zone accounted for only about 2% of the variation in American chestnut height and diameter growth. Nonetheless, growth was generally greater for seedlings associated with sources from warm and moderate temperature zones compared with growth of seedlings from the cold zone (Figure 2). The only significant difference in growth among sources within a temperature zone occurred for diameter growth and was attributable to differences among two of the six sources from the moderate zone: VA2 versus PA1 (Figure 2). Although this exception is notable, the broader consistency in growth in the field within temperature zones provides some validation that our temperature zone classifications captured variation in environmental adaptation that helped differentiate the growth potential among zones while minimizing within-zone variation. Consistent differences in field performance among the temperature zones suggests that this classification system may help predict growth rates of out-planted American chestnut; seedlings that originate in warm or moderate temperature zones are expected to experience greater height and diameter growth than seedlings originating from the cold temperature zone. However, temperature zone may be more valuable in identifying sources vulnerable to shoot winter injury.

Significant interactions between temperature zone and treatment (for height and diameter), and seed source within temperature zone and treatment (for height only) (Table 5) reflected the unusually high growth of seedlings from superior performing temperature zones and sources in the open relative to the other two silvicultural treatments.

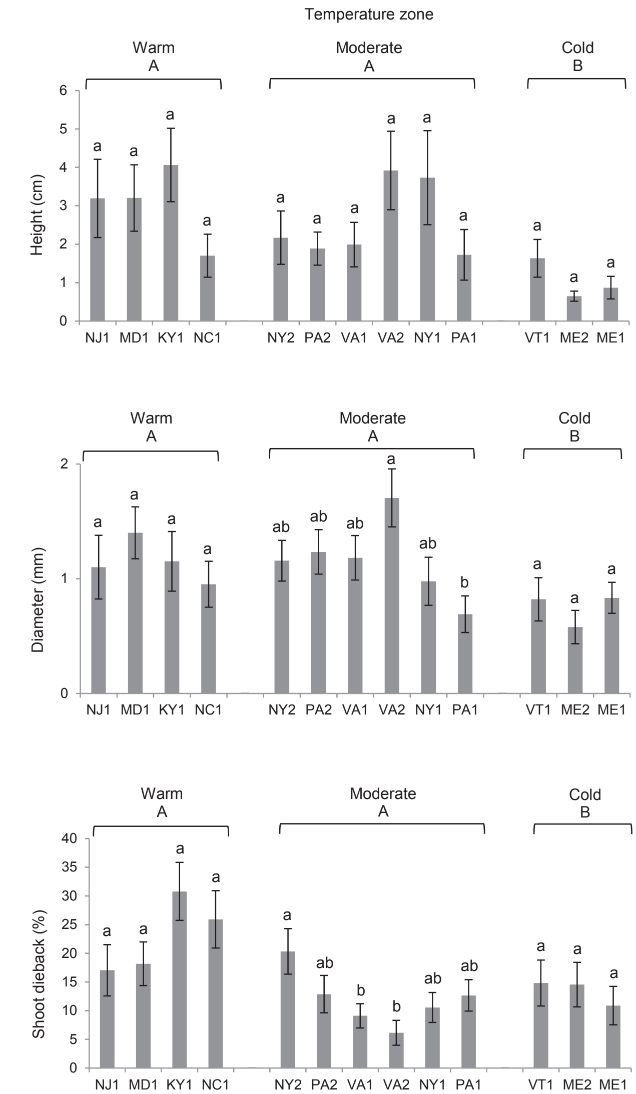

Figure 2. Mean (±SE) change in height and diameter growth and shoot dieback for American chestnut seedlings grown at the Green Mountain National Forest planting. Means with different uppercase letters are significantly different for temperature zone, and means with different lowercase letters are significantly different for source within the temperature zone, both based on Tukey HSD tests (*P* < 0.001 for height and diameter and *P* < 0.05 for shoot dieback). Refer to Table 1 for source information.

Shoot Winter Injury

Mortality of seedlings after the first winter was extremely low (~1.5% overall), and there were no differences in winter mortality associated with treatment, temperature zone, or source within temperature zone ($P = 0.87$, 0.37, and 0.21, respectively). Despite low winter mortality, shoot winter injury was common, especially among specific species, treatments, and genetic sources. Shoot winter injury was often greater than the amount of growth in the field, indicating that first-year growth was vulnerable to freezing injury regardless of where it was grown (greenhouse or field).

There were significant differences in shoot winter injury ($P < 0.0001$) among the three species, with Chinese chestnut exhibiting greater shoot dieback than American chestnut and red oak, which had indistinguishable levels of shoot injury (Table 4). Data showing the comparative sensitivity of Chinese chestnut to winter injury provide the first quantitative evidence to support observational data (McKay and Crane 1953, Jones et al. 1980) that Chinese chestnut has limited cold tolerance.

Significant differences in shoot winter injury ($P < 0.0001$) were also identified among the silvicultural treatments (Table 3). Similar to treatment differences in growth, open-grown seedlings had greater shoot winter injury than seedlings in the partial and closed canopy treatments. The greater injury for seedlings in the open canopy treatment is consistent with the lower winter temperatures experienced in this treatment (Table 3). Silvicultural treatment accounted for almost 11% of the variation in shoot winter injury.

Table 5. Sources of variation, degrees of freedom (df), mean squares, and *P* values for ANOVA assessments of height, diameter, and shoot winter injury (assessed as shoot dieback) measurements for American chestnut seedlings grown at the Green Mountain National Forest planting.

		Height		Diameter		Winter injury	
Source of variation	*df*	Mean square	*P* value	Mean square	*P* value	Mean square	*P* value
Temperature zone (TZ)	2	146.89	<0.001	9.23	<0.1	11,493.34	<0.025
Source (S[TZ])	10	41.48	<0.25	3.65	<0.001	1,473.36	<0.25
Treatment (T)	2	1,214.22	<0.001	165.90	<0.001	27,918.19	<0.001
Replication (R)	2	320.44	<0.001	24.76	<0.001	2,394.65	<0.25
TZ × T	4	135.66	<0.001	4.31	<0.025	6,411.64	<0.25
S[TZ] × T	20	35.51	<0.01	1.34	<0.25	1,288.89	<0.25
T × R	4	193.23	<0.001	19.57	<0.001	4,572.90	<0.005

Only sources that were statistically significant ($P \leq 0.05$) for at least one measurement parameter were included.

Temperature zone accounted for ~5% of the variation in shoot winter injury. Similar to patterns documented for height growth, there were significant differences in shoot winter injury among the temperature zones ($P < 0.025$). Overall, shoot dieback was greater for seedlings from warm and moderate temperature zones than for seedlings from the cold temperature zone (Figure 2). The minimal shoot dieback for seedlings from the cold zone is consistent with evidence that the cold adaptation of tree populations is not random and is strongly associated with climatic and geographic gradients (Howe et al. 2003). Our findings are also consistent with the many reports of an increased risk of freezing injury among genetic sources moved considerably northward (for a summary, see Wright 1976). Source within temperature zone accounted for <3% of the variation in shoot winter injury. Accordingly, we detected little variation in shoot dieback among sources within temperature zone classifications: only two groups of sources within the moderate temperature zone (NY2 versus VA1 and VA2) were statistically distinguishable from one another (Figure 2). Because American chestnut sources from the cold zone experienced significantly less winter injury, we propose that the temperature zone classification could be a useful tool for identifying locations where cold-adapted parent trees may preferentially be found. The functional value of temperature zone as a screening tool to better identify sources with greater cold hardiness awaits more robust evaluation that includes the assessment of more sources tested under a broad range of winter conditions.

The many differences in winter injury that we report were distinguishable despite the fact that winter temperature lows experienced in the first year of outplanting were comparatively moderate (~3.3° C warmer) compared with long-term seasonal averages (NOAA National Climatic Data Center 2010). In addition, the young seedlings that we worked with had comparatively few terminal shoot tips (which are more vulnerable to freezing injury; Gurney et al. 2011) compared with larger seedlings with more extensive branching. The relatively mild temperatures combined with the comparatively low supply of vulnerable tissues probably resulted in less injury and a more limited resolution of differences in injury expression than may be detectable after multiple years of assessment on larger seedlings. Because we found many meaningful differences in winter injury attributable to silvicultural treatment and temperature zone despite the limitations noted, the significant sources of variation we found are probably quite robust in their importance and influence, at least for seedlings planted at the northern limit of the American chestnut range.

Two points should be made regarding the possible influence of snowpack on shoot winter injury. First, because there was a low snowpack during the period with the lowest winter air temperatures at our study site, seedlings were probably damaged more than if they had been protected under an insulating snowpack. Unfortunately, winter snowpacks are generally declining as the climate of the northeastern US warms (Groffman et al. 2012); thus, freezing injury to vulnerable tree tissues that are typically protected by snow cover is likely to increase (Groffman et al. 2012, Comerford et al. 2013). Second, although low snowpack may influence the exposure of low-lying shoots to damaging freezing temperatures, shoot winter injury can occur even in years of high snowpack (Gurney et al. 2011), presumably because the shoots of larger plants exist above the snow.

Tradeoffs between Growth and Injury

In a general sense, it has been proposed that plants face an ecological tradeoff between diverting energy and resources toward growth versus the formation of protective compounds (Herms and Mattson 1992). This proposed conflict has been most intensively studied relative to the tradeoff between plant growth (usually height or mass) and defense from herbivory (e.g., Coley et al. 1985, Mole 1994, Fine et al. 2006). However, the tradeoff between growth and defense regarding the broad responses of tree species to temperatures that influence northern and southern range limits has also been examined (Loehle 1998), as well as differentiations in cold tolerance among populations within species (Howe et al. 2003).

Our data highlight this tradeoff between growth and cold tolerance at three levels: among species (Chinese versus American chestnut and red oak), among American chestnut sources (temperature zone and source within temperature zone), and with differences in the environment (open versus partial and closed canopy silvicultural treatments and significant treatment × replication interactions). We found that Chinese chestnut seedlings grew more but experienced greater shoot winter injury than American chestnut seedlings. In addition, American chestnuts from the warm and moderate temperature zone groups experienced greater height and diameter growth than seedlings from the cold zone, but also experienced more winter injury than seedlings from areas where low temperatures are more common. Finally, there was also a tradeoff between growth and shoot injury associated with silvicultural treatment: seedlings in the open canopy treatment experienced greater height and diameter growth but also more winter injury than seedlings in the partial and closed canopy treatments. Furthermore, this was most evident in replicates for which differences in canopy openness and winter temperature lows were most extreme. The consistency of this tradeoff between growth and winter tissue protection across many scales suggests that it is a fundamental and pervasive phenomenon that probably has strong implications for competition and adaptation in the field.

Sources from the warm and moderate temperature zones probably had an extended growing season because it took longer for them to reach the critical photoperiod required to set bud and begin winter dormancy (Wright 1976). Such sources with greater growth would also have more tissue that was vulnerable to winter injury (although this was at least partially corrected for by expressing shoot winter injury on a percentage basis). It is also reasonable to expect that American chestnuts from warm and moderate temperature zones would preferentially devote carbohydrates and other resources to growth at the expense of developing extensive cold hardiness because competition for light, water, and nutrients are pervasive and consistent, whereas elevated cold hardiness would be needed only occasionally to survive sporadic low temperature events. In contrast, chestnuts from more northerly and high elevation sites would need to balance resource allocation to foster both the growth needed to be competitive and protect vulnerable tissues from freezing injury that would be under more consistent selection pressure at these locations (Howe et al. 2003).

Implications for American Chestnut Restoration in the North

Chinese chestnut seedlings had a higher growth potential but were also quite vulnerable to shoot winter injury (Table 4). Consequently, inclusion of Chinese chestnut in the breeding program should enhance blight resistance but has the potential side effect of reducing the cold tolerance of resulting backcrossed stock. There was also measurable variation in shoot winter injury among American chestnut sources. However, our temperature zone index helped identify sources that have a high vulnerability to winter injury because of their adaptation to lower latitude and elevation (warm and moderate temperature) conditions. Although to a much smaller degree than differences in shoot winter injury, seedling growth varied somewhat among the temperature zones. Although seedlings from the cold temperature zone generally experienced the least winter injury, they also grew less than seedlings from the warm and moderate temperature zones. Perhaps the limited but significant differences in growth and winter injury among sources within the moderate temperature zone highlight the opportunity for source selection that can better balance growth and cold hardiness. For example, source VA2, a high elevation source from the moderate temperature zone, exhibited comparatively good growth but also had little shoot winter injury (Figure 2). Indeed, sources such as VA2 may not be that unusual: VA1 also exhibited comparably high growth and low shoot winter injury. In general, sources from more southern locations tend to grow better than native sources when planted together in the north, although they are more vulnerable to low temperature injury (Wright 1976). Sources such as VA1 and VA2 from the moderate zone may be particularly appropriate for possible assisted migration to the north as climates warm because they may best bridge the tradeoff between growth and protection from the cold.

Silvicultural treatment also had a significant influence on growth and winter injury. The open canopy treatment provided greater access to light and other resources that increased growth, but allowed exposure to lower temperatures in winter that provoked greater shoot dieback.

In this research, we only examined 13 sources of American chestnut and 2 sources of Chinese chestnut after one season in the field. The next phase of research should evaluate more American and Chinese chestnut populations to try to identify sources that better bridge the tradeoff between growth and cold tolerance (such as VA2 in this study) and examine long-term trends in growth and winter injury. Further research should also be conducted to evaluate alternative silvicultural treatments that provide for a buffered winter environment (as experienced in our partial and closed canopy treatments) when seedlings are small, followed by release cuts to provide for greater growth once plants are larger and have a more defined single stem (e.g., less likely to become shrubby in habit after winter injury kills terminal buds and releases dormant lateral buds) (Gurney et al. 2011).

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