WINTER INJURY OF AMERICAN CHESTNUT SEEDLINGS GROWN IN A COMMON GARDEN AT THE SPECIES' NORTHERN RANGE LIMIT

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Abstract.—Hybridization of American chestnut (*Castanea dentata*) with Chinese chestnut (*C. mollissima*), followed by backcrossing to American chestnut, is conducted to increase the resistance of resulting stock to chestnut blight, caused by the fungal pathogen *Cryphonectria parasitica* (Murr.) Barr. Backcross breeding is being used to restore American chestnut throughout its range, including cold high elevation sites in southern and central regions, and along chestnut's northern range limits. Until now, a comparative analysis of the growth and cold hardiness of American chestnut seed sources grown in cold environments had not been conducted. We assessed first-year growth and winter shoot injury (terminal shoot mortality that reduces apical dominance and results in a shrubby form) of American chestnut seedlings from 13 genetic sources: four southern, four central, and five northern seed sources, each representing one or more half-sib families, grown in a common garden in Vermont. No differences in height or diameter growth or in winter shoot injury attributable to the region of seed source origin were detected. However, significant differences in growth and winter injury were detected among sources within each region. There appeared to be a tradeoff between growth and winter injury: sources that had the greatest growth were generally the most vulnerable to winter shoot injury.

INTRODUCTION

American chestnut (*Castanea dentata* (Marsh.) Borkh.) was once a dominant tree species in much of eastern North America where it represented up to 40 percent of the forest canopy (Keever 1953) and as much as 50 percent of the forest canopy in the central Appalachians (Braun 1950, Russell 1987, Smith 2000). American chestnut was fast growing (diameter growth as great as 2.5 cm/yr) and large (e.g., reaching heights of 37 m and diameters of 1.5 m) (Buttrick 1925, Kuhlman 1978). Furthermore, the straight-grained, rot-resistant wood; abundant production of nutritious nuts; and high tannin content made American chestnut a species with high commercial value (Anagnostakis 1987, Rice et al. 1980). However, about one century ago, chestnut blight (caused by the fungus *Cryphonectria parasitica* (Murr.) Barr) was introduced to the United States (Griffin 2000). The girdling cankers produced by the pathogen led to widespread tree mortality and the functional removal of American chestnut as an overstory tree throughout its range (Griffin 2000). Considering the economic and ecological value American chestnut once provided, various strategies of species restoration have been attempted. One approach that shows promise for providing blight-resistant trees in the near future involves hybridizing American chestnut with blight-resistant Chinese chestnut (*Castanea mollissima* Blume) or Japanese chestnut (*Castanea crenata* Sieb. and Zuc.) and then successively backcrossing blight-resistant progeny with American chestnut to produce blight-

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resistant trees with approximately 94 percent American chestnut germplasm (The American Chestnut Foundation [TACF] 2010). Although this breeding tactic addresses the primary challenge to American chestnut survival and productivity rangewide, it does not address the selection of adaptive traits needed to tolerate localized stresses, such as tolerance to freezing, that could benefit trees growing in cold montane or northern locations.

Field measurements indicate that some genetic sources of American chestnut, and potentially blight-resistant backcross stock, are vulnerable to winter freezing injury and dieback of terminal shoots, which often leads to a bushy form when apical dominance is disrupted (Gurney et al. 2011). This injury has been noted from Virginia northward in various American chestnut breeding orchards (personal communications with TACF orchard managers). One prominent factor that can influence cold tolerance is the genetics of plant tissues (Aitken and Hannerz 2001, Balduman et al. 1999). In particular, temperature gradients associated with the latitude of source material typically result in predictable variations in species adaptation to the cold consistent with genetic adaptations to native temperature regimes (Aitken and Hannerz 2001). To evaluate the influence of genetics on the field performance of American chestnuts in a cold environment, we established a planting of 13 genetic sources of American chestnut on the Green Mountain National Forest (GMNF) in Leicester, VT, and assessed seedling growth and winter shoot injury after 1 year of out planting.

STUDY AREA

American chestnut seeds (nuts) were collected by volunteers and TACF staff. Nuts were collected in fall 2008 and kept refrigerated at 3 °C for 3 months to satisfy stratification requirements. We used 54 nuts each from 13 open-pollinated American chestnut sources, each representing one or more half-sib families from three latitudinal regions in the eastern United States (Table 1). Sources include one each from Vermont and New Hampshire, two each from New York and Maine (northern sources); one from New Jersey and two each from Maryland and Pennsylvania (central sources); one each

Table 1.—Source codes, location information, latitude and longitude for open-pollinated American chestnut seed source (each representing one or more half-sib families) used in the Green Mountain National Forest silvicultural study

Code	County, State	Region	Latitude	Longitude	Elevation (m)
KY1	Metcalfe County, KY	South	37° 00' 16" N	85° 37' 34" W	269
MD1	Montgomery County, MD	Central	38° 57' 53" N	77° 5' 33" W	100
NC1	Jackson County, NC	South	35° 22' 21" N	82° 47' 29" W	1,387
NJ1	Monmouth County, NJ	Central	40° 36' 20" N	73° 07' 10" W	20
NY1	Westchester County, NY	North	41° 19' 41" N	73° 41' 10" W	94
PA1	Franklin County, PA	Central	39° 59' 38" N	77° 23' 55" W	600
PA2	Mercer County, PA	Central	41° 20' 58" N	80° 04' 58" W	384
VA1	Smyth County, VA	South	36° 49' 40" N	81° 25' 49" W	1,036
NY2	Wyoming County, NY	North	42° 37' 44" N	78° 03' 17" W	417
ME2	Knox County, ME	North	44° 10' 55" N	69° 08' 09" W	68
VT1	Chittenden County, VT	North	44° 31' 39" N	73° 12' 11" W	57
ME1	Piscataquis County, ME	North	145° 09' 35" N	69° 04' 58" W	101
VA2	Smyth County, VA	South	30° 51' 55" N	81° 26' 10" W	1,041

from North Carolina, Tennessee, and Virginia, and two from Kentucky (southern sources). Nuts were germinated and seedlings grown in the greenhouse located at the U.S. Forest Service in South Burlington, VT. Nuts were planted in small cone-shaped pots in a potting mix containing 1:1:1 peat/perlite/vermiculite (Gurney 2010). Seedlings were provided with supplemental lighting, water, and fertilizer (a one-time dose of Miracid© Plant Food 30-10-10; Scotts Miracle-Gro Products, Inc., Marysville, OH) to maximize greenhouse growth, and were outplanted into field plots on the GMNF in June 2009. The spacing of seedlings was approximately 2.5 by 2.5 m with variations based on topography and ground cover. Seedlings also received the following cultural treatments when planted: (1) 0.9 by 0.9 m black competition mats to reduce competition from other vegetation, (2) 7.5-cm-diameter, 25-cm-tall cylindrical aluminum shelters buried approximately 10.0 cm into the soil to protect seedlings from rodent damage, and (3) 1.2-m-high, 0.75-m-diameter welded-wire guards to protect seedlings from deer browse. No differences in snow accumulation or melt associated with seedling protection were noted.

METHODS

We measured the height (cm) from base to uppermost branch tip and diameter (mm) at the base of each seedling at the time of planting in June 2009 and again following a single growing season in October 2009. We calculated changes in height and diameter (October - June measurements) to evaluate the influence of region and source within region on growth.

Visual assessments of winter shoot injury were made in July 2010. Injury was quantified as the sum of the lengths (cm) of damaged (dark colored and sunken) stem on each terminal shoot (leader and branches) with no new growth.

Analyses of variance (ANOVA) were used to test for the significance of differences in seedling height and diameter growth, and winter shoot injury among the regions of nut origin and sources within region using JMP statistical software (SAS Institute, Inc., Cary, NC). Tukey HSD tests were used to test for differences among sources within each region. Homogeneity of variance was tested for each measurement parameter using the O'Brien's, Brown-Forsythe, Levene's and Bartlett's tests within JMP. 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 when $P \leq 0.05$. The linear associations of winter shoot injury with growth and nut cold tolerance (assessed separately; Saielli 2011) were quantified using regression analyses.

RESULTS

No differences in height or diameter growth attributable to the region of nut origin were evident (Fig.1). However, significant differences among the sources within the region were detected for height growth (in the northern region) and diameter growth (in the southern and central regions) (Fig. 1 and Table 2). Variability in height growth was quite large in the northern region, which included sources with some of the greatest (NY1) and least (ME1 and ME2) growth.

As was seen for growth, no differences in winter shoot injury attributable to region were found (Fig. 1). But like diameter growth, significant differences in winter injury among sources were found within two regions, the southern and northern regions (Fig. 1).

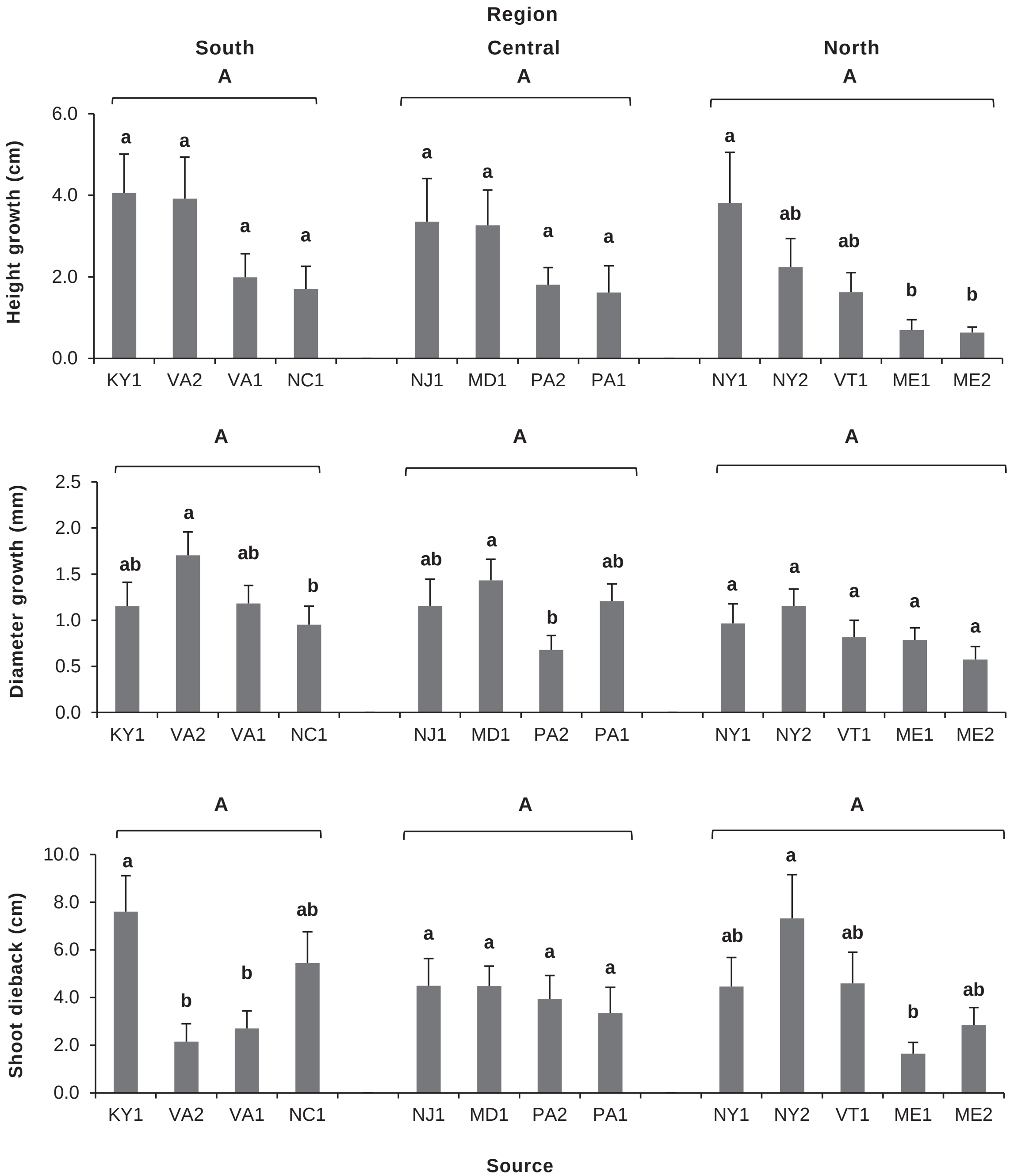

Figure 1.—Mean (± SE) height and diameter growth, and shoot dieback for American chestnut seedlings grown on the Green Mountain National Forest. Regional means with the same upper case letters are not significantly different. Source means within each region with different lower case letters are significantly different based on Tukey HSD tests ($P < 0.05$).

Table 2.—ANOVA tables showing the sources of variation (SOV), degrees of freedom (DF), sums of squares(SS), mean square (MS), and F and P values for the statistical tests used to evaluate differences in (A) height growth, (B) diameter growth, and (C) winter shoot dieback

(A) Height growth

SOV	DF	SS	MS	F	*P*
Region	2	144.19	72.10	1.053	0.384
Source [Region]	10	685.87	68.59	2.448	0.007
Error	640	17932.23	28.02		
Total	652	18767.29			

(B) Diameter growth

SOV	DF	SS	MS	F	*P*
Region	2	17.68	8.84	2.156	0.166
Source [Region]	10	41.07	4.11	1.960	0.035
Error	640	1341.05	2.10		
Total	652	1399.80			

(C) Winter shoot dieback

SOV	DF	SS	MS	F	*P*
Region	2	22.46	11.23	0.058	0.944
Source [Region]	10	1958.03	195.80	3.074	0.001
Error	640	40765.15	63.70		
Total	652	42745.64			

DISCUSSION

Considerable variation in growth and winter shoot injury was found among the 13 American chestnut seed sources evaluated. There was an approximate fourfold range in mean height growth, a threefold range in diameter growth, and a fourfold range in winter shoot injury among seed source means (Fig. 1). Although variation attributable to source was noteworthy, no broad influence of region of origin was evident. The apparent lack of regional influences on seedling growth and winter injury is similar to an earlier assessment of American chestnut nut cold tolerance that found significant source-to-source differences but no evidence of regional adaptation among the seven seed sources assayed (Schaberg et al. 2009). A lack of regional adaptation could be the result of massive tree and associated germplasm loss following blight introduction, so that the few reproductive American chestnut evaluated here better represented other genetic influences (e.g., founder effects, genetic drift, or inbreeding depression following steep population declines) rather than genetic adaptation to regional climate. However, various environmental factors (most notably elevation and proximity to large bodies of water) combine with latitudinal influences to exacerbate or moderate temperatures across the landscape. Considering this, it is possible that a division of sources into categorical groups more directly tied to temperature regimes at source locations (and that incorporate elevational and other influences) would do a better job of differentiating source adaptations to climate relative to the broad regional patterns evaluated here.

In general, there appeared to be a tradeoff between the average growth and winter injury among sources. Although not strong, a significant linear regression between the mean height growth (cm) and winter shoot injury (cm) ($P = 0.03$, $R^2 = 0.39$, Saielli 2011) indicated that sources with the greatest growth during the growing season also tended to experience the most shoot dieback the following winter. A tradeoff between growth and protection is a common theme in plant ecology, including the adaptation of species to the cold (Howe et al. 2003, Loehle 1998). However, a tradeoff between growth and freezing protection would impose an additional challenge to American chestnut breeding programs: identifying sources that counter overall trends and exhibit reasonable growth but that also have acceptable cold tolerance. The combination of good growth and adequate winter shoot protection is possible (e.g., see source VA2 – Fig. 1). However, identification of atypical sources that combine these traits would further complicate breeding efforts. Unfortunately, testing individual source performance in plantings is costly and time consuming, especially when assessing winter shoot injury, because results rely on stochastic exposures to ambient temperature lows that challenge physiological limits. As an alternative to growing and testing the winter shoot injury of seedlings, laboratory estimates of nut cold tolerance were evaluated for use as an indicator of the winter hardiness of seedling shoots. In a separate study (Saielli 2011), we measured the cold tolerance of nuts from 12 of the same seed sources that we assessed for winter shoot injury. Cold tolerance measurements estimate the temperature at which tissues exhibit freezing injury approximating 50 percent cell mortality, so a more negative temperature associated with injury indicates greater cold tolerance. The linear regression of mean nut cold tolerance and winter shoot injury for these sources was significant, positive, and strong ($P < 0.0025$, $R^2 = 0.67$, Saielli 2011), suggesting that nut cold tolerance measurements (that can be obtained in weeks rather than the years needed for plantation-based assessments of shoot injury) may be a reasonable screening tool for identifying sources with superior hardiness.

The substantial variation in growth and winter shoot injury we measured for the 13 seed sources evaluated suggests that there is meaningful genetic variation among remaining American chestnut populations. This variation highlights the potential for positive selection for these and likely other traits within American chestnut breeding programs. Breeding efforts have long focused on selection for blight resistance because this is by far the single greatest factor limiting the health and productivity of the species. However, as breeding trials progress and blight resistance is achieved, active selection for other traits that impart ecological or economic benefits (such as enhanced growth or shoot survival) should be incorporated into breeding efforts. Indeed, because much of the breeding for blight resistance has relied on germplasm from the heart of the species' range, it may be particularly important to identify sources of local adaptation that could foster species adaptation and survival at the limits of the species' environmental tolerances (such as cold high elevation and northern sites).

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