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RESEARCH ARTICLE

Inadequate Cold Tolerance as a Possible Limitation to American Chestnut Restoration in the Northeastern United States

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Abstract

The American chestnut (*Castanea dentata* (Marshall) Borkh.), once a major component of eastern forests from Maine to Georgia, was functionally removed from the forest ecosystem by chestnut blight (an exotic fungal disease caused by *Cryphonectria parasitica* (Murr.) Barr), first identified at the beginning of the twentieth century. Hybrid-backcross breeding programs that incorporate the blight resistance of Chinese chestnut (*Castenea mollissima* Blume) and Japanese chestnut (*Castenea crenata* Sieb. & Zuc.) into American chestnut stock show promise for achieving the blight resistance needed for species restoration. However, it is uncertain if limitations in tissue cold tolerance within current breeding programs might restrict the restoration of the species at the northern limits of American chestnut's historic range. Shoots of American chestnut and hybrid-backcross chestnut (i.e., backcross chestnut) saplings growing in two plantings in Vermont were tested during November 2006, February 2007, and April 2007 to assess their cold tolerance relative to ambient low temperatures. Shoots of two potential native competitors, northern red oak (*Quercus rubra* L.) and sugar maple (*Acer saccharum* L.), were also sampled for comparison. During the winter, American and backcross chestnuts were approximately 5°C less cold tolerant than red oak and sugar maple, with a tendency for American chestnut to be more cold tolerant than the backcross chestnut. Terminal shoots of American and backcross chestnut also showed significantly more freezing damage in the field than nearby red oak and sugar maple shoots, which showed no visible injury.

Key words: **American chestnut, *Castanea dentata*, cold tolerance, restoration, winter injury.**

Introduction

The American chestnut (*Castanea dentata* (Marshall) Borkh.) once comprised up to 50% of basal area in portions of the Appalachian hardwood forest (Braun 1950). An extremely fast-growing species (diameter growth as great as 2.5 cm/year), it attained impressive proportions, reaching heights of 37 m and diameters of 1.5–3 m, with a maximum diameter of over 5 m reported in Pennsylvania (Buttrick 1925; Kuhlman 1978; Harlow et al. 1979). Chestnut was prized for its straight-grained, highly rot resistant wood, which made it useful for construction, woodworking, furniture, railroad ties, telephone poles, musical instruments, and mine timbers (Ronderos 2000). In addition, tannins from wood and bark were integral to a large leather tanning industry (Fowler 1944; Saucier 1973). Chestnut seeds—large, sweet, and highly nutritious—were an important source of food for wildlife, livestock, and humans and were even used for barter in rural communities (Rice et al. 1980). The magnificence, prevalence, and usefulness of this species secured it a place in American literature, folklore, and song.

The fungal pathogen *Cryphonectria parasitica* (Murr.) Barr, accidentally introduced from Asia and first identified in New York City in 1904, initiated a blight that functionally removed American chestnut as an overstory tree throughout its range within approximately 40 years (Griffin 2000). The blight produces girdling cankers, which eventually kill the trunk, but do not harm the root system. As a result of root collar sprouts from stems killed by blight, American chestnut populations continue to exist in many parts of the former range, mainly in the forest understory, and are considerably reduced in size, number, and reproductive success. Sprouts may reach diameters of 20 cm and heights of 15 m before they too are girdled and killed by the blight and in turn form new root collar sprouts (Paillet 2002).

Because of chestnut's former ecological, economic, and social importance, considerable effort has been applied to controlling chestnut blight and restoring the species to its

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former status. Aside from attempts to identify a suitable replacement species, three primary methods of restoration have been attempted: (1) breeding for resistance among pure American chestnut, (2) hypovirulence of the pathogen, and (3) hybridization of residual American chestnut with resistant Chinese chestnut (*Castenea mollissima* Blume) and Japanese chestnut (*Castenea crenata* Sieb. & Zuc.) followed by backcrossing with pure American chestnut (Griffin 2000).

The first method, controlled breeding among resistant American chestnut, has to date only produced trees with relatively low levels of blight resistance; however, work with this method is ongoing (Griffin 2000). The original parent trees associated with this method are grafted scions of large surviving American chestnuts showing field resistance, and resulting progeny are challenged with the fungus to assess blight resistance (Griffin 2000). The second method, hypovirulence, occurs when the fungus is infected with a virus, causing it to produce superficial cankers, which are typically non-fatal but may distort stems, reducing the tree's timber value. Though this method has met with some success in restoring European chestnut (*C. sativa* Mill.) following blight introduction to Europe, hypovirulence has currently proved to be unreliable for controlling chestnut blight in North America. This is partially due to high levels of vegetative incompatibility of the numerous strains of the hypovirulent fungus with the numerous virulent fungal strains found in the United States and poor mechanisms for the natural spread of hypovirulent strains between infected trees (Elliston 1981; MacDonald & Fulbright 1991).

In contrast to these first two methods, hybridization and backcrossing are believed to offer near-term promise as a mechanism for full ecological restoration. With this method, blight-resistant Chinese or Japanese chestnut is crossed with existing locally adapted American chestnut through controlled pollinations and then successively backcrossed with American chestnut until trees with blight-resistant genes and an average of about 94% American chestnut germplasm are produced. These offspring are then intercrossed to achieve the highest levels of blight resistance (Burnham et al. 1986). At each step, seedlings are challenged with the fungus to be certain that they contain the genes for blight resistance before being included in the next generation of breeding trials.

The native range of American chestnut stretched from Maine to Georgia and west to the Ohio Valley (Ronderos 2000), with few and scattered populations at the northern extreme, which suggests that this species may have limited cold hardiness. Restoration of the species to the northern reaches of its former range requires an examination of the cold tolerance of not only American chestnut, but also hybrid-backcross offspring to determine if cold tolerance will play a limiting role in the reintroduction of the species and assess how the hybridization process may influence the spread of American chestnut in colder climates. In addition to direct tissue damage, inadequate cold hardiness and associated physiological stress and winter injury may exacerbate the propagation of chestnut blight by weakening tree stress response systems or increasing stem wounding, thereby providing more avenues for infection (Jones et al. 1980; Griffin et al. 1993; Griffin 2000).

As a preliminary assessment of whether inadequate cold tolerance may limit the restoration of American chestnut to northern latitudes, we measured the cold tolerance of current-year shoots of pure American and hybrid-backcross chestnuts (hereafter referred to as backcross chestnuts) and compared these measurements to ambient air temperatures and cold tolerance levels for shoots of two potential native competitors—sugar maple (*Acer saccharum* L.) and northern red oak (*Quercus rubra* L.).

Methods

Site Selection and Description

Current restoration breeding efforts have focused on the hybridization and backcrossing of Asian chestnut with American chestnut predominantly from the central portion of the species' historic range. Indeed at the time of this study there were only two plantings of backcross American chestnut that include Vermont American chestnut germplasm, only one of which includes the third and final backcross (BC3F1) generation. These two plantings were used to (1) assess whether or not limited cold hardiness may restrict the restoration of American chestnut in the north, and (2) evaluate if differences in cold tolerance are consistent between sites and, thus, more broadly applicable to the region. Because limitations in cold hardiness during fall, winter, or spring can lead to tissue damage and tree decline (Levitt 1980), cold tolerance was measured once during each of these three seasons, with efforts made to assess hardiness at times of stable temperature that represented seasonal norms.

Shelburne Site

A breeding orchard established by The American Chestnut Foundation (TACF) in Shelburne, Vermont, located on private property at approximately 40-m elevation and in close proximity to the temperature-moderating influence of Lake Champlain, contains over 200 young American and backcross chestnut saplings. A majority of the saplings are third-backcross (BC3F1) offspring of Vermont mother trees (one from Shaftsbury, VT and one from Dummerston, VT) pollinated with backcross pollen from second-backcross (BC2F1) trees grown in Virginia for the TACF breeding program. This orchard is adjacent to a mixed hardwood forest containing sugar maple and red oak saplings that provided comparisons of the cold tolerance of potential native competitors. The NOAA National Climatic Data Center at the Burlington International Airport, located approximately 14 km from the Shelburne site, was used to estimate on-site temperature measurements for comparisons to shoot cold tolerance levels.

Sunderland Site

A small American chestnut test planting in Sunderland, Vermont, located on the Green Mountain National Forest (GMNF) at approximately 340 m in elevation, provided plant material for an initial assessment of differences in cold tolerance attributable to site. This planting contains pure American chestnut, as well as second-backcross (BC2F1) offspring of a Vermont mother tree and backcross pollen from the TACF breeding program. The BC2F1 chestnuts in the Sunderland planting are the offspring of the same Shaftsbury, Vermont, American chestnut common to many of the saplings in the Shelburne planting. The environment within the GMNF is much harsher than in Shelburne. The NOAA National Climatic Data Center at the Bennington William H. Morse State Airport, located approximately 23 km from the Sunderland site and approximately 90 m lower, was used to estimate temperature trends at this plantation.

Cold Tolerance Sampling

Measurements of cold tolerance of current-year shoots (an abundant tissue type that can be collected with low collateral damage to trees) were used as an indicator of cold hardiness. Measuring the cold tolerance of woody shoots is a standard method of assessing hardiness in hardwood species (e.g., Gregory et al. 1986; Zhu et al. 2002). Current-year shoots were harvested in November 2006, February 2007, and April 2007 to assess seasonal trends in cold tolerance. More frequent sampling was not possible because few saplings were large enough to withstand additional destructive sampling. Furthermore, limitations in available stock prevented including pure Chinese chestnut with these comparisons. In Shelburne, shoots from nine Shaftsbury BC3F1 saplings, three Dummerston BC3F1 saplings, and four pure American chestnut saplings from a Hopkinton, Massachusetts, source were collected per sample date. To provide a comparison of the hardiness level of chestnuts, current-year shoots of four similarly aged red oak and sugar maple saplings were also collected and assessed. Comparison saplings were selected from the forest edge at sites adjacent to the orchard and approximately the same distance from the moderating effect of Lake Champlain to minimize microclimate effects. In Sunderland, shoots were collected from four pure American chestnuts and two Shaftsbury BC2F1 chestnuts on the same November, February, and April sample dates. For all sources at both plantations, saplings were chosen for sampling at random and without replacement, to ensure the independence of measurements. Visibly damaged shoots were not collected.

Laboratory Cold Tolerance Assessment

Current-year shoots from each tree were rinsed in distilled water and chopped into 5-mm internodal segments to produce one bulked sample per tree. Subsamples of two 5-mm segments were placed into 64-cell styrene trays for freezing tests. Duplicate samples from each tree were included within each tray to produce mean electrical conductivity measurements used in later curve-fitting analyses. Freezing stress was imposed using well-established methods (Strimbeck et al. 1995; Schaberg et al. 2000, 2005). During fall and winter, 15 test temperatures were selected, with temperatures ranging from +5 to −64°C in fall and from +5 to −90°C in winter. During spring, 17 test temperatures were selected, ranging from +5 to −90°C. Freezer temperature was held for 30 min at each test temperature, after which one replicate tray was removed from the freezer, placed in a precooled styrene foam container, and transferred to either a refrigerator at 5°C (for test temperatures above −5°C), or a freezer (for test temperatures below −5°C). After trays in the freezer equilibrated to −5°C, they were transferred to a refrigerator at 5°C and held until thawed. A mild detergent solution (3.5 mL of 0.1% v/v Triton X-100 in deionized water) was added to each cell, and sample trays were stored in a high humidity cabinet and shaken at room temperature for 8 hours. The initial conductivity of effusate was measured using a multielectrode instrument (Wavefront Technology, Ann Arbor, MI). Samples were then dried for at least 48 hours at 40°C to kill the tissue, soaked in fresh detergent solution for 24 hours, and the final conductivity was measured. Relative electrolyte leakage (REL), a measure of cell injury calculated as the proportion of initial conductivity of samples following damage at each subfreezing test temperature relative to the final conductivity of fully killed, oven-dried tissue, was used to calculate T_m, the temperature at the midpoint of a sigmoid curve fit to REL data for all test temperatures (Strimbeck et al. 1995; Schaberg et al. 2000, 2005). T_m values were calculated via nonlinear curve fitting (JMP, SAS Institute, Cary, NC, U.S.A.) using the following equation (Anderson et al. 1988):

$$\mathrm{REL} = Y_{\min} + \frac{Y_{\max} - Y_{\min}}{1 + e^{k(T_{\mathrm{m}} - T)}} \quad (1)$$

where $Y_{\min}$ and $Y_{\max}$ are values of REL for uninjured and completely freeze-stressed tissue, respectively, k describes the steepness of the REL response to freezing stress, and T is the temperature in °C.

Winter Injury Assessment

In addition to laboratory testing, visual assessments of shoot winter injury were made in May 2007 at the Shelburne site. Injury was identified after leaf-out as visible die-back (dark colored and sunken portions of stems) on terminal shoots. Winter injury was classified relative to seedling size by comparing the number of terminal shoots overall on each seedling relative to the number of damaged terminals on a percentage basis (% of terminals injured) for all sources sampled.

Statistical Analysis

Analyses of variance (ANOVA) were employed to test for the significance of differences in shoot cold tolerance data (T_m) using JMP statistical software (JMP, SAS Institute, Cary, NC, U.S.A.). The ANOVA assumption of independence of observations was assured through the sampling of trees without replacement. Homogeneity of variance and normality assumptions for ANOVA were tested using JMP Statistical software. Assumptions for ANOVA were met for all data sets except the field-based winter shoot damage, which was analyzed using a nonparametric test (see below). Data from the Shelburne plantation were used to test for differences in cold tolerance attributable to species and seed source within each season. To assess specific differences among factor means, four mutually exclusive orthogonal contrasts were used: (1) American and backcross chestnut versus red oak and sugar maple, (2) American chestnut versus backcross chestnut, (3) Dummerston backcross chestnut versus Shaftsbury backcross chestnut and (4) red oak versus sugar maple. These contrasts maximized statistical power for evaluating potentially important differences in cold tolerance associated with: (1) chestnuts relative to native competitors, (2) the impacts of the hybridization process, (3) genetic differences in cold tolerance among offspring from regionally adapted mother trees, and (4) differences between native competitor tree species. Analyses of variance (ANOVA) were also used to compare data from the Shelburne and Sunderland sites on each sample date to test for differences in cold tolerance attributable to site. This comparison included American and Shaftsbury backcross sources only, because these sources were present at both sites.

Differences in field-based winter damage attributable to seed source or species were analyzed using the van der Waerden nonparametric test because data were not normally distributed (Conover 1980). This nonparametric test was selected because it was appropriate to the data and supported by JMP statistical software. For all tests, differences were considered statistically significant if $p \leq 0.05$.

Results

Source Differences in Cold Tolerance

Sampling was conducted in November 2006, February 2007, and April 2007 at times of stable and seasonally representative temperatures (Fig. 1). At the Shelburne plantation, five sources of seedlings were sampled: pure American chestnuts, Shaftsbury BC3F1 chestnuts, Dummerston BC3F1 chestnuts, northern red oak, and sugar maple. No differences in cold tolerance were detected among these sources in fall or spring (Fig. 2). However, significant differences in cold tolerance ($p < 0.0002$) were detected for winter (Fig. 2). Orthogonal contrasts defined two specific differences of note: (1) red oak and sugar maple were approximately 5°C more cold tolerant than American chestnut and backcross chestnut ($p < 0.0001$), and (2) a tendency for American chestnut to be approximately 3°C more cold tolerant than backcross chestnuts ($p = 0.0745$; Fig. 3).

Source Differences in Winter Injury

Field observations of shoot winter injury at the Shelburne site identified significant differences in winter damage between sugar maple and red oak compared to American and backcross chestnut ($p < 0.0001$; Fig. 4). Sugar maple and red oak showed no visible winter injury, whereas American chestnut experienced approximately 30% injury on average and backcross chestnut experienced approximately 60% mortality of terminal shoots (Fig. 4). Shoot winter injury resulted in the increased branching of injured chestnuts, presumably because of a suppression of terminal shoot dominance.

Site Differences

Both the Shelburne and Sunderland plantings include American and Shaftsbury backcross chestnuts, allowing for comparison of cold tolerance differences associated with geographical location (site). In the fall, Sunderland-grown American and backcross chestnuts were more cold tolerant than similar Shelburne-grown stock ($p \leq 0.05$; Fig. 5). No differences in cold tolerance were found between locations in spring or

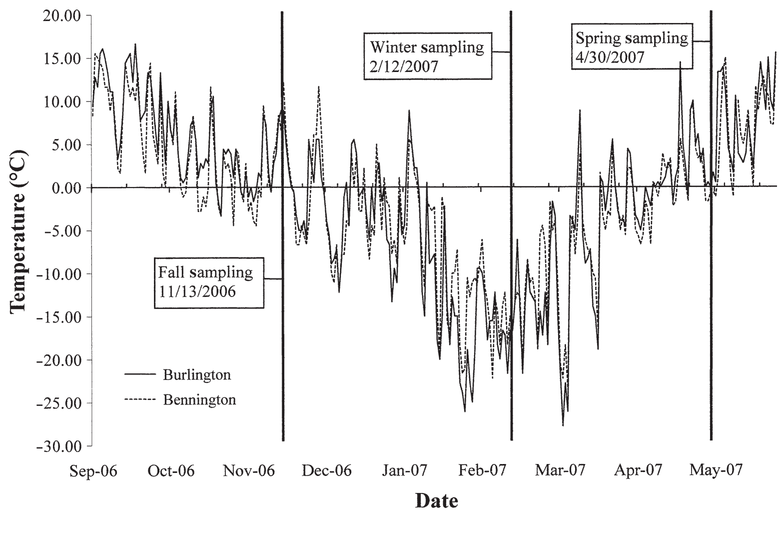

Figure 1. Minimum daily temperatures from September 2006 to May 2007 for Burlington and Bennington, Vermont. Temperature data are from NOAA National Climate Data Centers, collected at the Burlington International Airport and W.H. Morse State Airport in Bennington. The Burlington temperature data provided temperature estimates for the Shelburne site and the Bennington temperature data provided temperature estimates for the Sunderland site. Seasonal sampling dates are indicated.

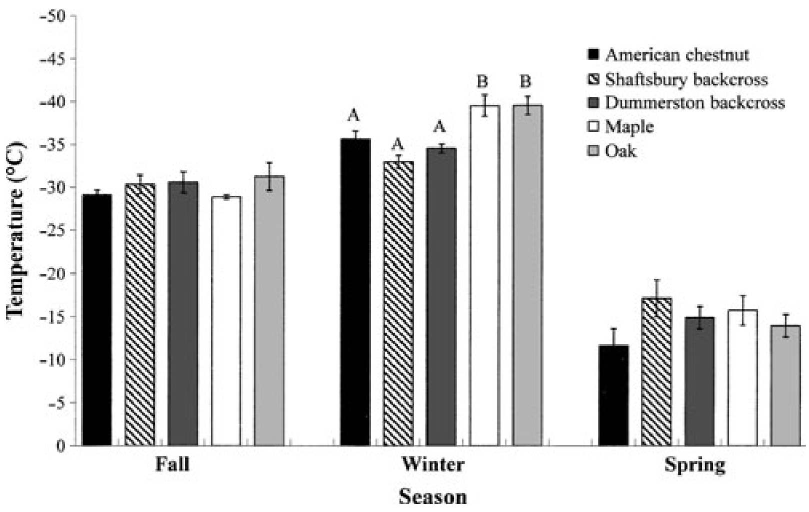

Figure 2. Differences in mean (±SE) shoot cold tolerance (T_m) measured in Shelburne, Vermont, from fall 2006 through spring 2007 for five species or sources. Source means with different letters are significantly different based on the orthogonal contrast of American chestnut, Shaftsbury and Dummerston backcross chestnut versus sugar maple and red oak ($p < 0.0002$).

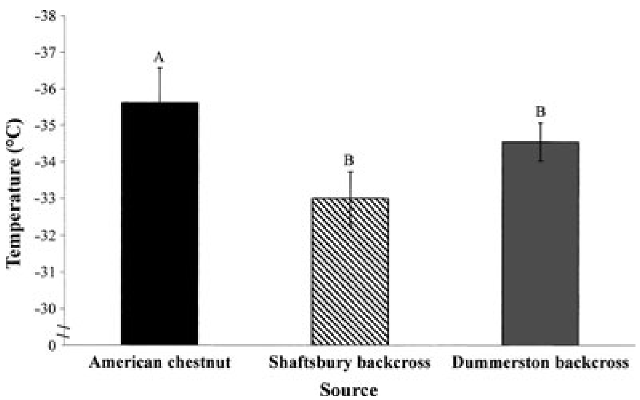

Figure 3. Differences in mean (±SE) shoot cold tolerance (T_m) of American and backcross chestnut in Shelburne, Vermont, during winter 2007. Source means with different letters are significantly different based on the orthogonal contrast of American chestnut versus Shaftsbury and Dummerston backcross chestnut ($p = 0.0745$).

winter—a time of particular vulnerability to winter injury for chestnut shoots relative to two native competitors (Fig. 2) and relative to ambient low temperatures (Fig. 1).

Discussion

Contemporary Limitations in Cold Tolerance

It is important to note that this study provided only a cursory look at the cold tolerance levels of American and backcross chestnuts at the northern edge of the historic American chestnut range. Sample sizes were small, only a few sources were assessed, and the number of individuals within sources sampled was not equal but was based on seedling size and availability. Additionally, sampling dates captured mid-season temperature trends well, but transition periods between seasons when shoots would be expected to be hardening or dehardening were not sampled. Nevertheless, even with these limitations, winter cold tolerance levels of American and backcross chestnuts in Shelburne were shown to be less than those of two common native competitors and close to ambient low temperatures experienced in the region.

It should also be noted that estimates of cold tolerance based on REL data often produce conservative estimates of cold tolerance (T_m values), because temperatures in laboratory tests are lowered slowly and at a constant rate, unlike the sudden drops and spikes found in nature (Schaberg & DeHayes 2000). This is particularly important because the T_m values of American and backcross chestnut were found to be approximately −32 to −35°C. However, winter temperatures in Shelburne did not appear to reach −30°C during the 2006–2007 sampling season, but shoot winter injury was still observed. This, coupled with the fact that visible assessments of winter injury mirrored patterns of winter cold tolerance estimates in the laboratory, raises the likelihood that the cold tolerance levels of American and backcross chestnut may be even less than those experimentally predicted. It is also noteworthy that no differences in winter cold tolerance between the Shelburne and Sunderland sampling sites were detected, suggesting that cold tolerance limitations of chestnut shoots during this critical period are not unique to one location. Range maps show the northern extreme of American chestnut in northern New England, whereas the botanical ranges of both red oak and sugar maple extend north into Canada (USDA NRCS 2008). This distribution is consistent with the possibility that limited cold tolerance influences the health and competitive success of chestnuts more than some common competitors within the Northern Forest. Although research into the cold hardiness of American chestnut is sparse, there is at least preliminary evidence indicating that insufficient cold hardiness may be an important factor limiting its competitive

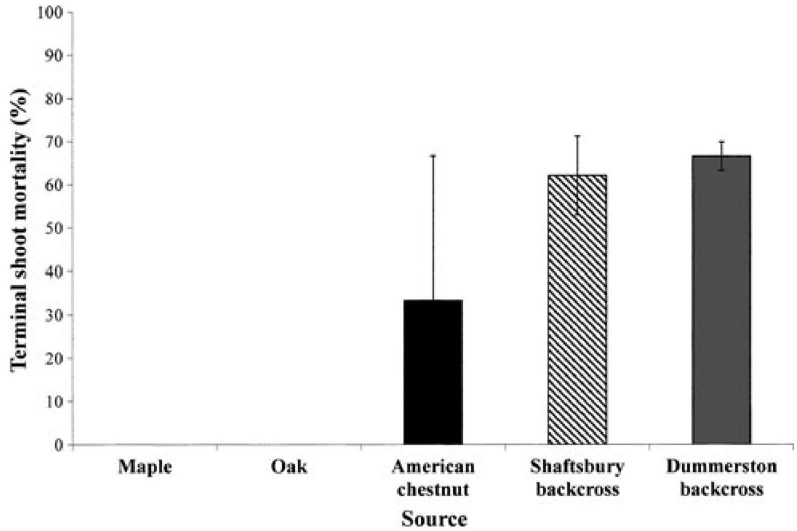

Figure 4. Differences in mean (±SE) terminal shoot winter injury measured in May 2007 on five species or sources in Shelburne, Vermont. Damage was quantified by comparing the number of terminal shoots overall on each seedling relative to the number of damaged terminals on a percentage basis (% of terminals injured). Differences in field-based freezing damage among all sources were significant ($p < 0.0001$) and were driven by the stark contrasts in injury between maple and oak (which exhibited no winter damage) and the chestnuts (which had mean damage levels between 30 and 60%). Data were analyzed using the van der Waerden nonparametric test due to a lack of homogeneity of variances among sources. The large error bar for American chestnut is a result of the low number of individuals available for sampling.

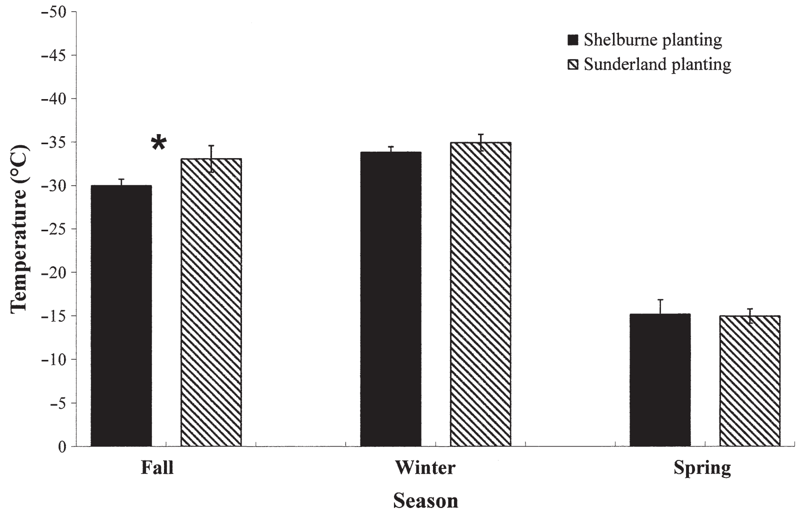

Figure 5. Differences in mean (±SE) shoot cold tolerance (T_m) of American and Shaftsbury backcross chestnut at Shelburne and Sunderland, Vermont, from fall 2006 through spring 2007. Significant differences in cold tolerance, as identified using analyses of variance (ANOVA), are denoted with an asterisk (*) ($p \leq 0.05$).

success in the north (Griffin & Elkins 1986). Furthermore, because the Chinese chestnut used in backcross breeding may be particularly vulnerable to freezing injury (Jones et al. 1980), genetic mixes containing Chinese chestnut genes could have hardiness levels even lower than pure American stock—a tendency suggested by our data.

The American and backcross chestnut at the Shelburne site were significantly less cold tolerant than those at the Sunderland site in the fall, the only season when a geographical difference was detected. NOAA temperature data indicate that the first frost occurred in Sunderland before it occurred in Shelburne, and that temperature minima were generally lower in Sunderland than Shelburne before fall sampling. Higher fall temperatures near the Shelburne plantation may have resulted from its low elevation (300 m below the Sunderland site) and/or its close proximity to the moderating influences of Lake Champlain. Whatever the cause(s), the most likely reason for observed differences in fall cold tolerance is that trees experienced colder temperatures in Sunderland and began acclimating to those temperatures sooner than the trees growing in Shelburne. Numerous studies with a variety of woody plant species have shown that decreasing photoperiod and low temperature exposure control cold acclimation (Bigras et al. 2001; Smallwood & Bowles 2002; Li et al. 2004).

The more limited cold tolerance of American and backcross chestnut relative to two potential native competitors may impair the near-term restoration of the species to northern forests. Although large chestnut trees exist in a few places in Vermont, cold damage has been observed on saplings and some pole-sized trees in Shelburne, Sunderland, and other locations throughout Vermont (Gurney 2007, author's personal observations). Terminal shoot die-back contributed to a shrubby growth habit at the Shelburne planting because extensive branching developed below damaged terminal tissues. Winter injury, although prevalent in young trees in Vermont and observed in juvenile plantings throughout the northeast (personal communication, TACF growers), does not seem to ultimately alter the form of at least some mature trees, because the few remaining mature American chestnuts observed are tall and straight with good timber form. Indeed, the prodigious capacity of American chestnuts to generate well-formed forest trees from amalgamations of stump sprouts attests to the ability of this species to overcome early setbacks in shoot dominance and branching habit. However, the potential impacts of winter injury as a drain on carbon stores (reduced photosynthetic area and lost stored resources within injured shoots), and the influence of this on seedling establishment and early competitive success have yet to be determined.

Implications for Future Restoration in Northern Latitudes

Limited winter shoot cold tolerance may complicate the early establishment of American chestnut in northern climates; however, further research is needed to determine the extent of this potential problem. It is unclear if the susceptibility to shoot winter injury is an issue largely confined to juvenile stock. The existence of a few large, well-formed American chestnut in the north could (1) result from natural selection for shoot cold hardiness (only the hardiest individuals compete well enough to reach dominant and co-dominant crown status), or (2) be an indication that American chestnuts increase in hardiness as they mature. It will be important to clarify these and other questions regarding the hardiness of American chestnuts before making firm recommendations regarding restoration in the north. Among other issues, further research could identify mechanisms for bolstering the cold tolerance of backcross chestnuts, thereby assisting restoration of the species to the northern forest. Various forms of seedling protection systems, including tree tube shelters, may increase temperatures around seedlings (Scowcroft & Jeffrey 1999), potentially protecting them from serious winter injury. Nutritional supplements, such as calcium (Ca) or nitrogen (N) fertilization, may also benefit young trees. Calcium has been shown to play an important role in stress response and cold tolerance in a number of species ranging from large trees such as red spruce (*Picea rubens* Sarg; Schaberg et al. 2001) to various herbaceous plants (Arora & Palta 1988; Dhindsa et al. 1993; Monroy et al. 1993; Pandey et al. 2000). Nitrogen has been shown to increase cold tolerance following short-term fertilization applied toward the end of the growing season, as well as in cases where plants were N-deficient prior to fertilization (Schaberg & DeHayes 2000). However, it should also be noted that excessive or prolonged N fertilization may contribute to the development of decline symptoms in forest trees (Aber et al. 1989, 1998). Finally, genetic selection for increased cold tolerance in backcross chestnut from northern-adapted mother trees could also boost the cold tolerance of future restoration plantings. Although various lines of experimentation may identify mechanisms to bolster shoot cold tolerance levels in the future, our results also suggest some Implications for Practice (below) that could reduce winter injury within restoration plantings under current climate conditions.

Climate change could also influence American chestnut restoration to the northern reaches of the native range. Current predictions of climate change in the northeastern United States cite the potential for proportionally more warming during winter than other seasons, resulting in shorter winters, reduced snow packs, and more freeze-thaw events, as well as smaller diurnal temperature fluctuations (Barron 2001, Christensen et al. 2007). These processes could combine to provide both positive and potentially negative influences on species restoration.

A potential positive impact on American chestnut restoration is the prediction of warming for the Northeast. It is predicted that the climate in the Northeast will increase as much as 2–3°C over the next 100 years (Barron 2001). As a result of this warming, forest species composition is expected to shift, with more cold-adapted species migrating north, to be replaced by more temperate species. In the northeastern United States, it is predicted that predominantly maple-beech-birch forests will be replaced by oak-hickory forests (Spencer 2001)—a forest type that historically included American chestnut. Furthermore, it is predicted that nighttime temperatures will warm more than those during the day, potentially raising temperature minima closer to those experienced throughout the heart of chestnut's historic range. However, shoot winter injury temperature thresholds have not yet been precisely identified, and a warming of only a few degrees may not be enough to dramatically alter terminal shoot survival.

Increases in air temperature in the Northeast may also cause a reduction in snow pack, which could negatively impact the restoration of American chestnut. It is predicted that the number of days per year with snow on the ground will decrease by an average of 7 days over the next 100 years (Barron 2001). Hydrologic modeling has estimated a reduction in snow cover by as much as 53%, with the greatest change predicted for areas that currently experience temperatures just below freezing (Federer 2001). Reductions in the duration and depth of snow pack could decrease survival of young chestnut seedlings because they would be less protected from animal herbivory and less insulated from winter temperature lows (Cox & Zhu 2003; Hennon et al. 2006).

Conclusion

The cold tolerance of American and backcross chestnut shoots was shown to be less than that of red oak and sugar maple, potential native competitors. In addition, although ambient temperatures during the 2006–2007 season did not reach estimated thresholds for winter injury identified in laboratory tests, ambient winter temperatures did damage terminal shoots of American and backcross chestnut, whereas native sugar maple and red oak remained uninjured. These findings support past evidence that laboratory methods produce a conservative estimate of cold tolerance and, more importantly confirm that chestnuts are vulnerable to winter injury within the northern forest. A tendency for the backcross chestnut tested to be less cold tolerant than American chestnut was also identified and could result from previous crosses with Asian chestnuts and American chestnuts from southern sources. However, further evaluation is needed to determine the practical importance of this tendency. Cold tolerance could potentially be improved through various cultural means including fertilization or through genetic selection for greater cold hardiness among sources within breeding programs. In addition, predicted climate change could interact with the limited cold tolerance of the species and thereby help (reduce winter injury) or hinder (reduce protective snow packs) restoration efforts.

Implications for Practice

Findings and regional observations suggest the likelihood of shoot winter injury. However, further research is needed to assess the extent to which this damage may constrain the restoration of American chestnut in northern locations. Although awaiting more definitive guidance provided by this research, restoration efforts in cold regions may consider the following options:

- Avoid planting in recognized "cold spots" (higher elevations, hollows prone to cold air drainage, etc.).
- Seek out planting locations that provide greater protection from excessive exposure to prevailing winds and temperature fluctuations during winter.
- Seek out planting stock from more northerly or high elevation seed sources.
- Maximize the nutrition and growth of seedlings to assure the optimal accumulation of cryoprotective sugars needed to foster maximum cold hardiness. However, late season fertilization should be avoided, because this may delay fall cold acclimation.
- Preferentially plant seedlings in the spring to allow for complete site acclimation and phenological synchronization.

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