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NOTE

Preliminary assessments of shoot cold tolerance for American elm bred for enhanced tolerance to Dutch elm disease

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Abstract: Although Dutch elm disease (DED) is the primary threat to American elm (*Ulmus americana* L.), we hypothesized that shoot freezing injury may also limit tree productivity and survival in the north. We assessed shoot cold tolerance and field winter injury of American elm bred for DED tolerance planted in Lemington, Vermont. We tested for differences in cold tolerance associated with date, maternal DED tolerance sources, paternal sources from plant hardiness zones 5a, 6a, and 6b (determined using data from 1996 to 2005), and the interactions of these. Cold tolerance was greatest in the winter, followed by fall and then spring. For all dates, cold tolerance never differed between maternal DED tolerance sources. However, in mid-winter, paternal sources from zone 5a (coldest zone) were significantly more cold tolerant than sources from zone 6b (warmest zone), and sources from zone 6a were intermediate. Field freezing injury confirmed that shoots were only marginally cold tolerant relative to ambient temperature lows.

Key words: cold hardiness, genetic sources, local adaptation, *Ulmus americana*, winter injury.

Résumé : Bien que la maladie hollandaise de l'orme (MHO) soit la principale menace pour l'orme d'Amérique (*Ulmus americana* L.), Nous avons émis l'hypothèse que les dommages causés par le gel des pousses pourraient également limiter la survie et la productivité des arbres dans le nord. Nous avons évalué la tolérance au froid des pousses et les dommages hivernaux chez des ormes d'Amérique sélectionnés pour la tolérance à la MHO et plantés à Lemington, au Vermont. Nous avons testé les différences de tolérance au froid associées à la date, aux sources maternelles tolérantes à la MHO, aux sources paternelles provenant des zones de rusticité des plantes 5a, 6a et 6b (déterminées à l'aide de données de 1996 à 2005) ainsi que les interactions entre ces facteurs. La tolérance au froid était la plus élevée en hiver, suivie de l'automne puis du printemps. Peu importe la date, la tolérance au froid ne différait pas entre les sources maternelles tolérantes à la MHO. Cependant, au milieu de l'hiver, les sources paternelles de la zone 5a (zone la plus froide) étaient significativement plus tolérantes au froid que les sources de la zone 6b (zone la plus chaude) et les sources de la zone 6a étaient intermédiaires. Les dommages dus au gel sur le terrain ont confirmé que les pousses n'étaient que légèrement tolérantes au froid par rapport aux minima de température ambiante. [Traduit par la Rédaction]

Mots-clés : résistance au froid, sources génétiques, adaptation locale, *Ulmus americana*, dommages hivernaux.

Introduction

American elm (*Ulmus americana* L.) was once an important component of wetland and floodplain forests from the eastern coast of North America (Florida through Nova Scotia) west to southeastern Saskatchewan down through central Texas (Burns and Honkala 1990). American elm helped to supply critical ecosystem services (e.g., wildlife habitat, soil improvement, carbon sequestration, etc.) within wet and mesic forests, and elm wood (hard and with strong interlocking grain) was used to make furniture, flooring, construction and mining timbers (Burns and Honkala 1990). Because it is fast growing, tolerant to many stresses, and has a pleasing vase-shaped crown, American elm was once the most common urban tree in North America (Gerhold et al. 1993; Plotnik 2000). However, the many benefits provided by American elm faded from the landscape with the tree's population crash following the introduction of two non-native fungal pathogens (*Ophiostoma ulmi* and *O. novo-ulmi*) that cause Dutch elm disease (DED) (Brasier 1991) and are spread in North America primarily by two elm bark beetles: the native *Hylurgopinus rufipes* and the introduced *Scolytus multistriatus* (Schreiber and Peacock 1979).

Considering the multifaceted benefits provided by American elms, and their vast decline in numbers and areal extent, many methods for controlling DED outbreaks have been pursued, including the reduction of vector populations, the use of fungicides for high-value trees, and breeding via the creation of elm hybrids with resistant non-native elm species and the identification of disease-tolerant American elm (Hubbes 1999). This last approach — the generation of a diverse group of American elm selections with durable tolerance to DED — has been the focus of long-term research by the USDA Forest Service and its partners (e.g., Slavicek 2012; Knight et al. 2017). Considering the large native range of the species, it could also be important for breeding efforts to also consider issues of local adaptation to a suite of environmental factors (e.g., temperature extremes, differences in day length, duration of the growing season, and variations in water availability) that can

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constrain the health and productivity of trees, especially near range limits. This includes adaptations to low winter temperatures that can directly injure sensitive tissue such as the youngest cohorts of woody shoots (e.g., Slavicek 2012; Knight et al. 2017). American elm grows from Florida (USDA Plant Hardiness Zone 9b) to Canada (Zone 2b) — with 30-year average annual extreme minimum temperatures ranging from –1 to –42.9 °C (USDA Agricultural Research Service 2018). Thus, while breeding efforts must focus on fostering maximum DED tolerance, they also need to be mindful of the cold hardiness of breeding stock to match with the temperature conditions of sites being restored. Maximizing local adaptation in cold tolerance is necessary for current restoration in the north, and may be particularly important for assuring the adaptive capacities of northern populations for continued range expansion as the climate warms. Indeed, an easing of cold restrictions is an important predictor of the projected expansion of American elm's northern range limit with climate change (e.g., Shafer et al. 2001; Prasad et al. 2007-ongoing).

As a preliminary assessment of whether inadequate cold tolerance may limit the restoration of American elm to northern latitudes, we measured the cold tolerance of current-year shoots of American elm selections created by crossing two maternal sources of DED tolerance (Valley Forge and R18-2) with paternal sources from three plant cold hardiness zones (5a, 6a, and 6b: USDA Agricultural Research Service 2018) grown together in Lemington, Vermont (plant hardiness zone 3b). Laboratory-derived cold hardiness measurements were compared with ambient air temperatures near planted stock and shoot winter injury measurements made in the field. Our hypotheses were that (*i*) American elm shoots would exhibit cold hardiness levels that were marginal compared with winter low temperatures experienced in the field, (*ii*) few differences in cold tolerance would be detected between the two maternal DED-tolerant sources that underwent extensive selection for DED tolerance only, (*iii*) paternal sources from colder hardiness zones would exhibit greater cold tolerance, and (*iv*) current-year shoots in the field would exhibit signs of freezing injury in the spring.

Methods

Site

An American elm restoration trial was established in an old cornfield located in the rich soils of the Connecticut River floodplain in Lemington, Vermont (Lat: 44.905320, Long: –71.495429, elevation: 307 m). American elm, silver maple (*Acer saccharinum* L.), and boxelder (*Acer negundo* L.) were the dominant canopy tree species of floodplain forests on this river prior to the spread of DED (Marks and Canham 2015). American elm saplings were planted following a randomized design with elm surrounded by dense natural regeneration of boxelder saplings throughout the planting to possibly provide some shelter from cold winds in winter. Lemington is in cold hardiness zone 3b, which had an average annual extreme minimum temperature range of –37.2 to –34.4 °C from 1976 to 2005. All material sampled for cold tolerance and other assessments here were planted as saplings in 2014 from seed collected in 2011 or 2012. Sample trees were chosen to represent the following breeding crosses: two maternal sources of DED tolerance (Valley Forge and R18-2), four paternal sources from plant hardiness zone 5a, three paternal sources from plant hardiness zone 6b, and four sources from zone 6a (Table 1). The R18-2 line represents one of 17 survivors of a 21 000 seedling screen of DED tolerance conducted by Cornell University and the Boyce Thompson Institute, whereas the Valley Forge line represents a survivor from a chemical test that showed high DED tolerance (Knight et al. 2012). Although the scientific origins of the R18-2 and Valley Forge lines are known, the specific geographic origins of these sources cannot be confirmed (Haugen and Bentz 2017).

Table 1. Maternal and paternal genetic sources, paternal hardiness zones, and the number of trees assessed for shoot cold tolerance.

Maternal DED tolerance source	Paternal source	Paternal hardiness zone	No. of trees
Valley Forge	Whale Tails	5a	3
	Cunningham	5a	3
	Rainbow Beach 1	6a	4
	Hadley	6a	2
	Podunk River	6b	4
R18-2	Whale Tails	5a	3
	Cunningham	5a	2
	Rainbow Beach 1	6a	3
	Hadley	6a	3
	Podunk River	6b	3
	Goff Brook	6b	3

Starting 16 November 2016, temperatures at the site were measured using programmable sensors (i-Buttons; Embedded Data Systems, Lawrenceburg, Kentucky, USA) that were placed at ~1.5 m above ground level in six randomly selected elms within the planting. Each sensor was placed inside a small open-ended white container that protected the sensor from moisture but also reflected sunlight. Sensors were programmed to record temperature every 3 h.

Cold tolerance

Measurements of the cold tolerance of current-year shoots (an abundant tissue type that can be collected with low collateral damage to trees and are the tissue most vulnerable to freezing injury for many hardwood species (e.g., Gregory et al. 1986; Zhu et al. 2002; Gurney et al. 2011)) were used as an indicator of cold hardiness. Shoots (≤8 mm in diameter) from the upper third of crowns (to avoid deer browse — described later) were harvested on 6 December 2016, 14 February 2017, and 24 April 2017 to assess seasonal trends in cold tolerance. No visibly injured shoots were collected. Harvested shoots were transported to the laboratory in sealed plastic bags within insulated coolers.

Cold tolerance assessments duplicated well-established methods (for details see Strimbeck et al. 1995; Schaberg et al. 2000, 2005; Gurney et al. 2011). In brief, current-year shoots were chopped into 5 mm internodal segments to produce one bulked sample per tree. Tree-specific subsamples were exposed to gradually decreasing low-temperature treatments and then cellular integrity was assessed using electrical conductivity measurements. For samples collected in December and February, 15 test temperatures were selected, with temperatures ranging from +5 °C to –64 °C in fall and +5 °C to –90 °C in winter. In April, 17 test temperatures were selected ranging from +5 °C to –90 °C. Electrical conductivities were measured using a multielectrode instrument (Wavefront Technology, Ann Arbor, Michigan). Relative electrolyte leakage (REL), a measure of cell injury calculated as the proportion of the electrical conductivity of samples following exposure to each subfreezing test temperature relative to the final conductivity of oven-dried, killed 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 non-linear curve fitting software (JMP, SAS Institute, Cary, North Carolina, USA).

Field winter injury

In addition to controlled freezing tests, visual assessments of shoot winter injury in the field were made in July 2017. Injury was identified after leaf-out as visible dieback (dark colored and sunken portions of stems) on current-year shoots (Saielli et al. 2014). Winter injury was classified relative to sapling size by comparing the number of current-year shoots overall on each sapling

Fig. 1. Differences in mean (±SE) shoot cold tolerance (T_m) of American elm from all sources across months in 2016 and 2017. Means with different letters are significantly different at $p \leq 0.05$ based on results from a Tukey HSD test.

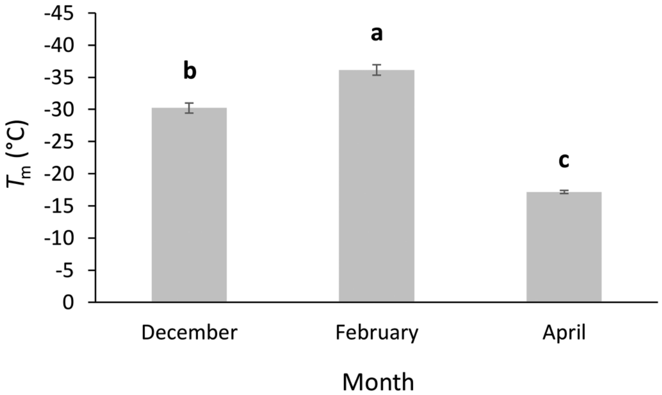


Fig. 2. Differences in mean (±SE) shoot cold tolerance (T_m) between the two Dutch elm disease tolerant maternal sources regardless of date. Means with different letters are significantly different at $p \leq 0.05$ based on results from a Tukey HSD test.

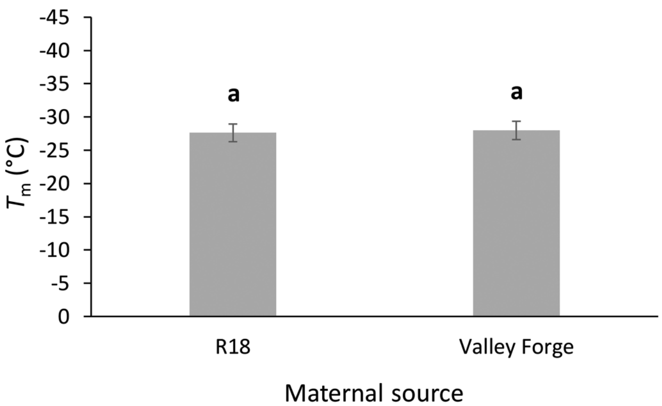


Fig. 3. Differences in mean (±SE) shoot cold tolerance (T_m) between the three paternal source hardiness zones regardless of date. Means with different letters are significantly different at $p \leq 0.05$ based on results from a Tukey HSD test.

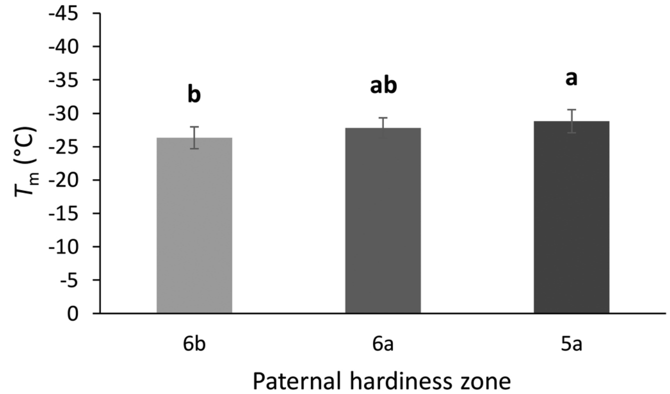
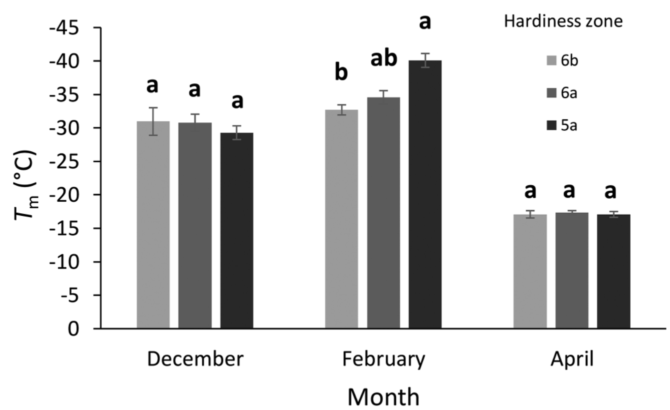


Fig. 4. Differences in mean (±SE) shoot cold tolerance (T_m) between the three paternal source hardiness zones across 3 months in 2016 and 2017. Means with different letters are significantly different at $p \leq 0.05$ based on results from a Tukey HSD test.



with the number of damaged laterals on a percentage basis (% of shoots injured). Relative amounts of damage from deer browse (rated as low (<1/3), medium (1/3 to 2/3), or high (>2/3) of lateral shoots) was also noted because browsing removed current-year shoots and prevented their inclusion in field winter injury assessments.

Statistical analyses

Repeated measures analysis of variance (ANOVA) was used to test for differences in shoot cold tolerance (T_m) using JMP statistical software. The statistical model included "date", "maternal source", "paternal hardiness zone", and the interactions of these as sources of variation. Differences among means were considered significant if p was ≤0.05. Differences among cold tolerance means were tested using the Tukey honestly significant difference (HSD) test.

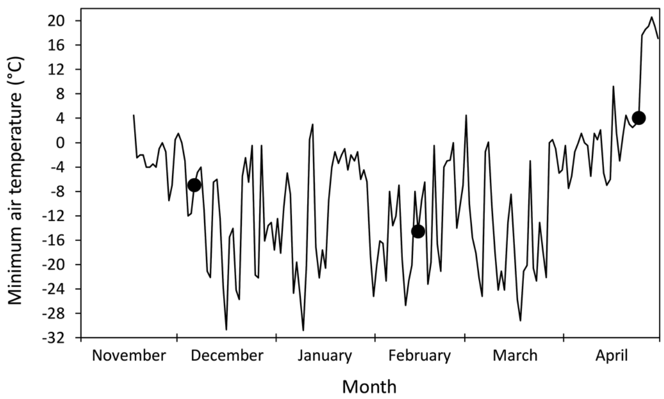
Results

Regardless of genetic source, shoots were most cold tolerant in mid-winter, followed by late fall and then early spring (Fig. 1). Regardless of date, there were no differences in shoot cold tolerance associated with the maternal sources of DED tolerance (Fig. 2). Also, regardless of date, trees from paternal sources in hardiness zone 5a (the coldest zone) were significantly more cold tolerant than trees from zone 6b (the warmest zone), whereas trees with paternal heritage from zone 6a were intermediate in cold tolerance (Fig. 3). The only significant interaction among main sources of variation was for date × paternal hardiness zone, which showed that overall differences in shoot cold hardiness associated with paternal hardiness zone were driven by differences in mid-winter (Fig. 4). Temperature measurements detected multiple periods that approached levels that could have injured sensitive shoots (Fig. 5). Field assessments of freezing injury indicated that over 83% of the trees sampled for cold tolerance experienced shoot winter injury. However, detailed quantification of the percentage of shoots per tree with winter injury could not be calculated because many remaining current-year shoots had been browsed by deer prior to winter injury assessment. Indeed, deer browse was extremely common; almost 84% of the 368 trees in the overall planting were browsed, and approximately 88% of these showed signs of moderate to heavy browse damage.

Discussion

Seasonal variation in shoot cold tolerance was expected and matched regional trends in the likelihood of low-temperature exposure. As expected, winter was the time of greatest shoot cold tolerance. However, this does not mean that plants were less vulnerable to freeze-induced damage at this time because this

Fig. 5. Daily minimum air temperatures recorded at the Lemington, Vermont, field planting in November 2016 through April 2017. Temperatures are the means of data from six sensors. Field collection dates are identified with black circles.



period is also when ambient temperatures reach their yearly minimums (U.S. Climate Data 2018), which can approach the limits in hardiness that we estimated here. In addition, laboratory-based measures slightly overestimate tissue cold tolerance levels because they take place in the absence of other environmental stresses (e.g., rapid freezing or repeated freeze–thaw cycles) found in the field that enhance damage (Schaberg and DeHayes 2000).

The unknown geographic origins of the two maternal sources of DED tolerance meant that there was uncertainty whether these may differ in cold tolerance, which provided the justification for specific testing here. Nonetheless, it could be argued that it is not surprising that no differences in cold tolerance were detected between the two maternal sources tested because these were the product of extensive selection for DED tolerance (e.g., R18-2 resulted from 21 000 individuals tested) that was not based on local adaptation to the cold. At least 22 American elm selections have shown elevated levels of DED tolerance in recent tests (Haugen and Bentz 2017). We evaluated only two of these (R18-2 and Valley Forge). It is possible that other American elm selections have shoot hardiness levels different (either higher or lower) from the sources assessed here. This possibility should be experimentally tested because increasing both DED and cold tolerance at the same time through selective breeding could speed the development of American elm best suited for restoration in the north. Cold hardiness testing will also help in deciding where it is appropriate to plant these selections.

Regardless of sampling date, shoots of trees with paternal sources from hardiness zone 5a were significantly more cold tolerant than the shoots of trees with paternal sources from hardiness zone 6b, with trees with paternal sources from zone 6a being intermediate. This pattern was driven by differentiations among paternal hardiness zones in mid-winter. At this time, the difference between zone 5a and zone 6b cold tolerance means was about 7.4 °C, which was within 1 °C of the 8.3 °C average divergence in mean winter minimum temperature experienced between these zones over a 30-year period (1976–2005; USDA Agricultural Research Service 2018).

Field assessments made after budbreak showed that all genetic crosses experienced at least some winter freezing injury that was visually distinguishable as areas of darkened necrotic lesions on shoots with associated bud mortality. Indeed, over 83% of the 33 trees sampled for cold tolerance experienced shoot winter injury. However, primarily because deer browse damage was so high (nearly 84% of trees in the planting overall, with near 100% of shoots browsed on some trees), no accurate estimate of the percent of current-year shoots that were winter injured could be calculated. Despite this inability to fully quantify levels of winter shoot injury, broad documentation of the presence of freezing injury in the field verified that many of the current-year shoots of the American elm that we studied were fatally damaged by temperatures that went as low as −30.8 °C with several other events approaching this level. This further highlights that laboratory-based cold tolerance measurements (that estimated shoot cold tolerance levels to be slightly less than −30 °C in December and between −30 and −40 °C in February) generally overestimate the hardiness levels of tissues relative to damaging conditions in the field. Field measurements also highlight that freezing injury can be compounded by the highly damaging impact of deer browse — a phenomenon also noted in other American elm restoration plantings (e.g., Adams et al. 2015; Knight et al. 2017). The combined loss of shoots to freezing injury and browse damage can significantly limit the productive capacity of restoration stock — at least when young.

Lemington is in cold hardiness zone 3b where average low temperatures ranged from −37.2 to −34.4 °C from 1976 to 2005. Considering this, levels of cold tolerance that we estimated indicate that planted stock appeared marginally adequate for restoration at the Lemington site or similar locations in zone 3b or lower. Indeed, American elm's native range extends into locations in North America rated as low as zone 2b, with 30-year average annual low temperatures of −42.8 to −40.0 °C. To accommodate restoration under such cold extremes, it may be helpful to locate and incorporate new sources of American elm from zones as low as 2b (e.g., locations like central Manitoba, Canada) into the restoration breeding program to maximize the hardiness of stock planted in cold locales near the species' northern range limits.

Conclusions

Laboratory and field injury assessments verified that genetic sources bred for DED tolerance can differ in their shoot cold tolerance in winter. Severe or repeated losses of current-year shoots to freezing injury can reduce crown fullness and the carbon capture potential of trees that are already carbon limited in cold locales with short growing seasons. The current study did not detect any differences in cold tolerance associated with maternal sources of DED tolerance. However, a broader survey of this is warranted because simultaneously improving upon DED and cold tolerance could meaningfully assist restoration near the species' northern range limits. Differences in cold tolerance consistent with the temperature zone of paternal sources suggest that improvements in local adaptation to the cold can be transferable via selective breeding. This favorable trend evident in our initial analyses should be tested with a broader range of sources, including ones from the lowest temperature zone in which American elm grows (zone 2b). Breeding efforts involving genetic stock from cold hardiness zones similar to or colder than target planting sites may help ensure adaptation to potentially damaging winter temperatures. In addition to breeding, the development and deployment of cultural practices that bolster cold tolerance (e.g., fertilization of potentially limited nutrients; Halman et al. 2008) or better protect tissues from low-temperature exposures (e.g., silvicultural treatments that buffer exposures to winter temperature lows; Saielli et al. 2014) may also assist restoration in the north. In the absence of new information, restoration efforts may similarly benefit from the avoidance of planting in recognized "cold spots" (higher elevations, hollows prone to cold air drainage, etc.), instead targeting plantings toward locations that provide greater protection from prevailing winds and temperature fluctuations during winter — conditions that could exacerbate cold damage.

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