Cold Tolerance Assay Reveals Evidence of Climate Adaptation in American Elm

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BACKGROUND

American elm (*Ulmus americana* L.) historically occupied the rich, fertile soils of floodplain forests of northeastern and prairie regions of North America. American elm's distribution along waterways has been significantly reduced by Dutch Elm Disease (DED) -- a vascular wilt disease caused by *Ophiostoma ulmi* and *O. novo-ulmi* fungi [1] and vectored by several species of bark beetles. Despite the prevalence of DED, American elm persists throughout its historical range. Rare American elms with resistance to DED have been identified and are being used in breeding programs and horticultural plantings [2].

For elm restoration efforts to be successful, careful attention to climate suitability is critical, especially in cold regions at the northern limit of the species' range. Repeated episodes of winter shoot injury that ultimately impairs production of vegetative and reproductive tissues could limit the success of species restoration in northern New England.

The purpose of this study is to evaluate whether American elm trees are cold-adapted to the climate conditions where they originate, and if that manifests in differences in mid-winter shoot cold tolerance. Understanding this relationship will help inform recommendations for how far north it is possible to move trees without risking tree mortality due to maladaptation to cold temperatures.

VERMONT AND OHIO ELM SAMPLING

VERMONT
DED-resistant trees sampled from the planting in Vermont (University of Vermont Horticulture Research and Educational Center, South Burlington, VT, 44.4287, −73.2046, Figures 1 and 4) were established as part of the National Elm Trial [3] and included clonally propagated commercially available elms designed to test performance in different locations across the country. The geographic origin of these trees is ambiguous, imprecise or **unverified**.

OHIO
DED-resistant trees sampled from a resistance trial planting in Ohio (Westerville, OH, 40.1163, −82.8338, Figures 2 and 3) were established by the U.S. Forest Service, Northern Research Station, American Elm Breeding and Restoration Partnership and provided 11 families of clonally propagated survivor elms from **verified** locations in New England (Figure 3) as well as clonally propagated commercially available elms that serve as resistant controls, but whose source locations are **unverified**.

VERMONT AND OHIO ELM SAMPLING

Figure 1. John Butnor and Chris Hansen collecting elm shoot samples in S. Burlington, VT

Figure 2. Leila Wilson and Mikayla Bailey collecting shoot samples in Westerville, OH.

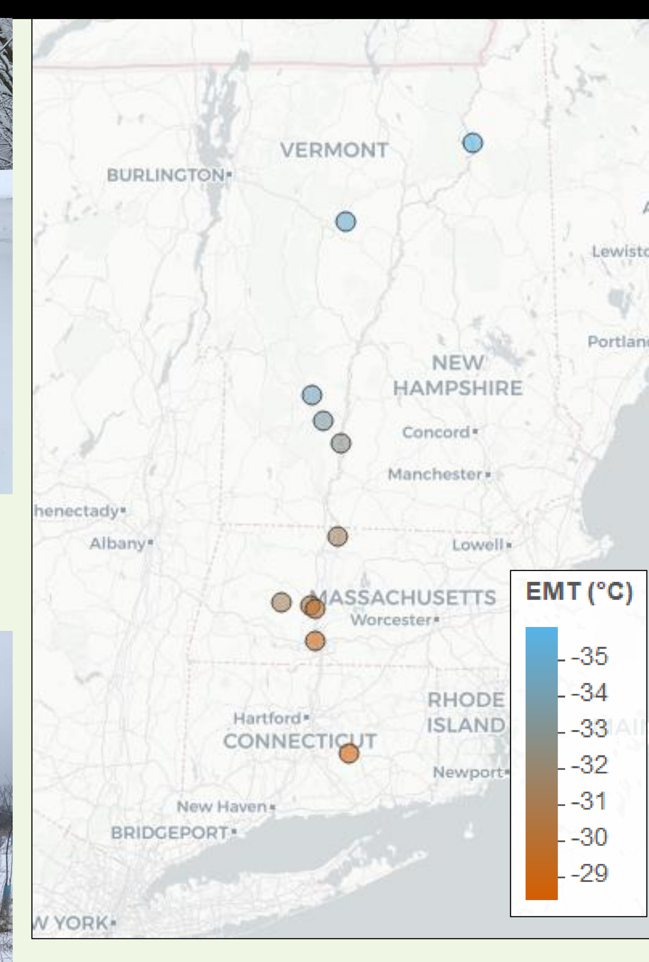

Figure 3. Map of sources for eleven elm genotypes with verifiable locations in New England. The color scale indicates 30-year (1991-2020) extreme minimum temperature (°C) for source location. Ohio source not shown.

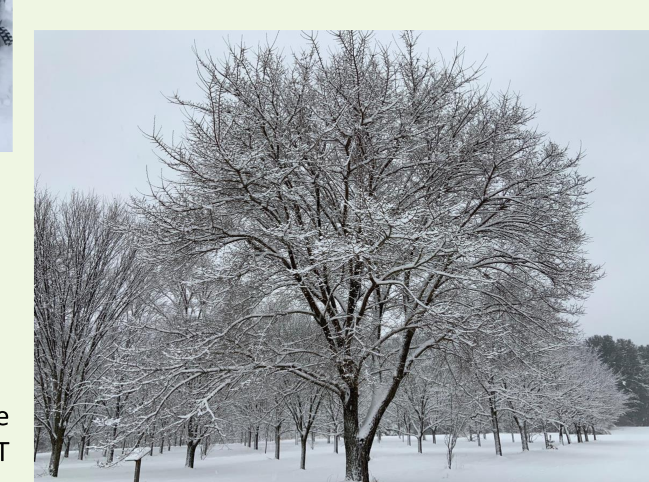

Figure 4. UVM Horticulture Center elm, Burlington, VT

COLD TOLERANCE MEASUREMENTS

In January 2023, a total of 11 genotypes from survivor elm trees with verified source locations across a north-south New England gradient as well as a DED-susceptible control (OH) were selected for measures of mid-winter cold tolerance via electrolyte leakage methods (Figure 5) [4]. These genotypes represent a calculated 30-year extreme minimum temperature (EMT) ranging from −35.9° to −27.7° C and a latitudinal range of 40.4° to 44.6° N.

Two additional DED-resistant and commercially available genotypes (of unverified origin), *Valley Forge* and *Princeton,* were collected in both OH and VT [5].

Figure 5. Processing shoot tissue in the laboratory for REL analysis.

COLD TOLERANCE OF NEW ENGLAND SOURCES WITH VERIFIED ORIGINS

The temperature at which 50% of cellular leakage occurred (LT_{50}) was calculated for all genotypes. The mean LT_{50} (+/- s.e.) for each genotype was plotted against source EMT and latitude (Figure 6). Genotypes from colder regions exhibited greater cold tolerance when grown in common garden, indicating genetic variation in susceptibility to mid-winter freezing injury that reflects the gradient in source climate.

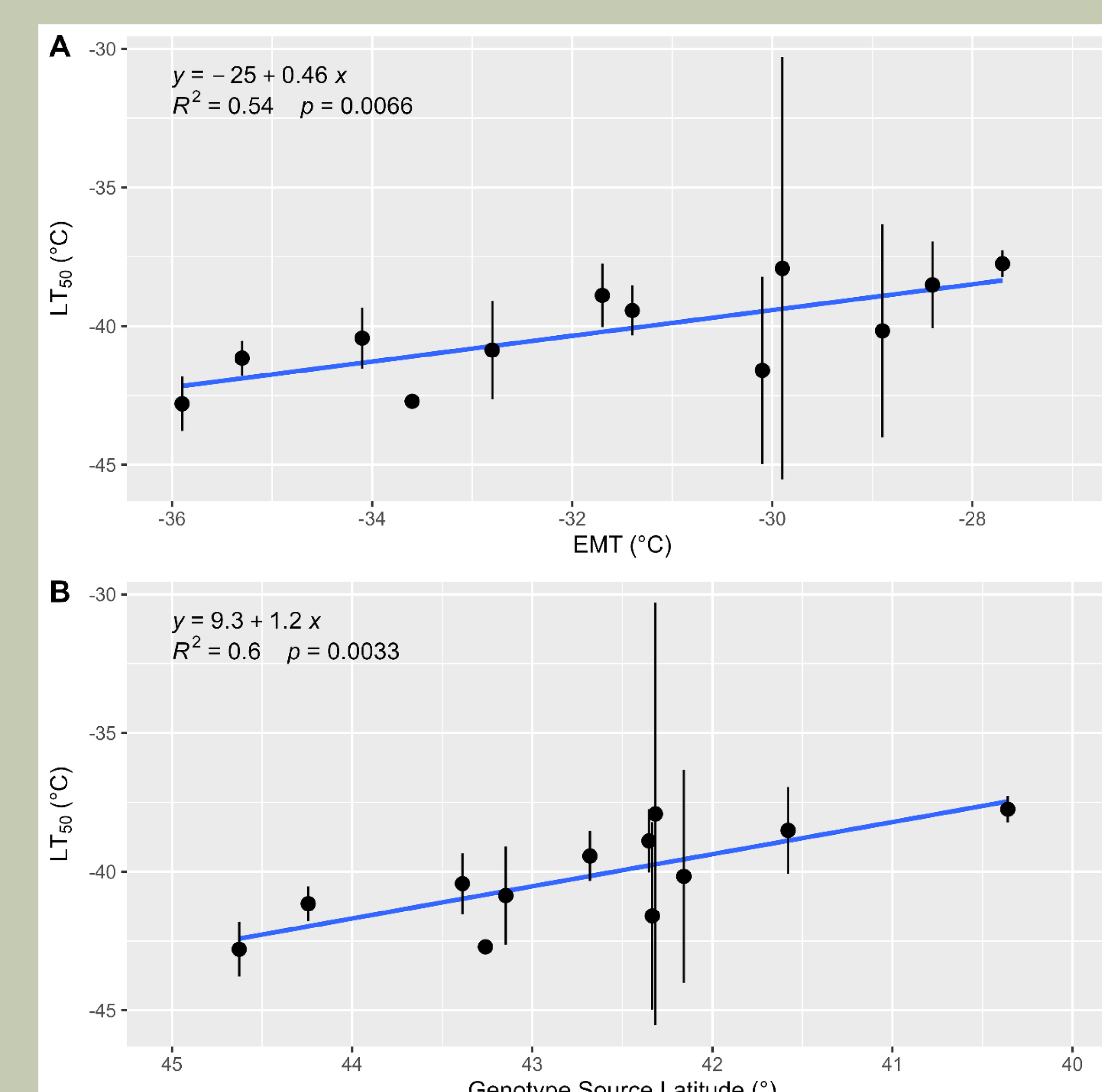

Figure 6. Linear regression of mean LT_{50} values (± s.e.) of 12 American elm genotypes with climate variable 30-year extreme minimum temperature (EMT) of genotype source location (A) and genotype source latitude (B).

COLD TOLERANCE OF COMMERCIALLY AVAILABLE SOURCES WITH UNVERIFIED ORIGINS

Princeton and *Valley Forge,* collected in both VT and OH, were directly compared by genotype and site effects on LT_{50} (Mann-Whitney rank-sum test). There was a significant difference between genotype means, but no difference between site means (Figure 7). During the two months leading up to sampling, winter air temperatures were consistently lower at the VT planting compared to the OH planting except on Dec 23 and 24, 2022 when minimum air temperature in OH was 32.3°C lower than temperatures recorded in VT. Despite air temperature differences, mid-winter cold tolerance measured by REL was not significantly different between sites.

COLD TOLERANCE OF COMMERCIALLY AVAILABLE SOURCES WITH UNVERIFIED ORIGINS

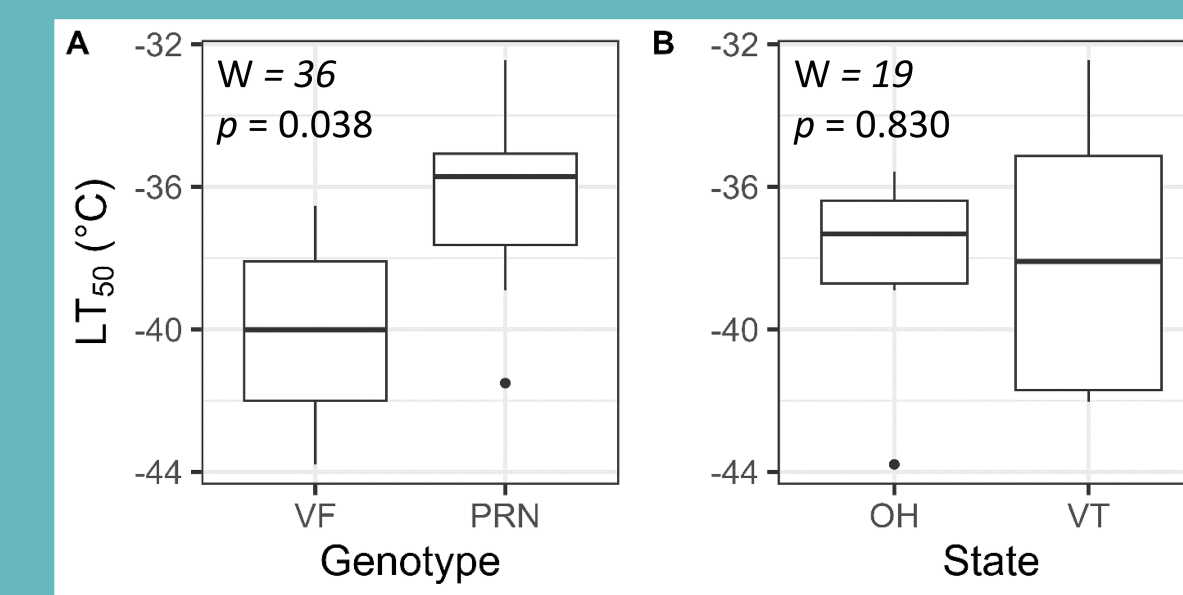

Figure 7. Boxplots of LT_{50} results for genotypes *Valley Forge* (VF) and *Princeton* (PRN)(A) planted in Ohio and Vermont (B). Boxplots display median and interquartile range, with whiskers showing the minimum and maximum of the range, excluding outliers which are indicated with a black dot.

SUMMARY

- American elm genotypes in this study exhibit clonal trait variation consistent with local adaptation to mid-winter conditions as assessed by laboratory cold tolerance methods.
- Genotypes that evolved in colder climates have greater cold tolerance in winter.
- Mid-winter cold tolerance of all New England genotypes was sufficient for survival at the coldest source location in northern Vermont. New research will examine the tolerance of flower and vegetative buds to freeze injury as they de-acclimate in warming spring temperatures.
- Findings suggest that planting American elms too far north from their origin location may result in lower fitness due to maladaptation to current local temperatures.

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