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To direct, fund, promote and communicate horticultural research, which increases the quality and value of ornamental plants, improves the productivity and profitability of the nursery and landscape industry, and protects and enhances the environment.

Midwinter Cold Hardiness of *Leitneria floridana* from Three Provenances[1]

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Abstract

Leitneria floridana Chapman (Florida corkwood) is native to the southeastern and south-central United States. This rare shrub has been cultivated north of its natural habitats. However, the cold hardiness of the species, and potential differences in hardiness among geographical provenances, have not been established. Our objective was to determine midwinter cold hardiness of stem segments from indigenous plants in Texas and Missouri, and from plants that had been cultivated for many years in northern Illinois, but were originally native to Missouri and Arkansas. After exposure to controlled low temperatures, the cortex and phloem were stained with 2,3,5-triphenyltetrazolium chloride (TTC) and compared with discoloration of the same tissues to determine midwinter cold hardiness. The two assays correlated positively, and marked provenance differences in cold hardiness were found. The estimated temperature at which 50% of stems were injured (T_{50}) was ≈ –20C (–4F) in December and –10C (14F) in January for samples from Texas. The estimated T_{50} for stems from Missouri and Illinois was at or below the lowest temperature tested [–35C (–31F) in December and –60C (–76F) in January]. Stems from cultivated plants in Illinois and from plants indigenous to Missouri were similarly hardy within the range of temperatures used. Autumnal acclimation and vernal deacclimation of *L. floridana* remain uncharacterized, but midwinter cold hardiness of *L. floridana* seems sufficient to permit its use north of its natural habitat. Substantial provenance differences in cold hardiness should be considered when selecting plants for landscaping outside the native range of *L. floridana*.

Index words: Leitneriaceae, Florida corkwood, environmental stress, TTC.

Significance to the Nursery Industry

Tolerance of midwinter temperatures is an important criterion for selecting plants for landscape use and for choosing germplasm for developing cultivars. *Leitneria floridana* (Florida corkwood), a rare shrub native to the southeastern and south-central United States, offers attractive, leathery foliage and an apparent tolerance of poorly drained soils, but its midwinter cold hardiness was not known until now. Results indicate that geographic origin (provenance) of *L. floridana* affects its tolerance of low temperatures. Stems from plants indigenous to Missouri and from plants cultivated in Illinois were the most tolerant and survived after exposure to –35C (–31F) in December and to –60C (–76F) in January. Stems from plants in Texas survived –10C (14F), which is much colder than the average minimum temperature encountered in this native provenance, but our data suggest that *L. floridana* in Missouri and Illinois have a greater capacity to tolerate harsh midwinter temperatures than do *L. floridana* from Texas. Additional research to characterize autumnal acclimation and vernal deacclimation is needed. However, results suggest that *L. floridana* can persist in many regions with harsh winters, and that provenance differences should be considered when selecting plants for landscaping.

Introduction

Temperature often limits the natural distribution and cultivation of woody plants. Shrubs capable of withstanding extreme temperatures are in great demand for use in northern landscapes. Several aesthetically pleasing but uncommon shrubs indigenous to North America are under-utilized in managed landscapes. Tolerance of low temperatures needs to be determined to define the regions where these shrubs can be used. By encouraging use of uncommon, native plants, horticulturists can contribute to preserving the overall genetic diversity of rare species. Selection and development of such plants for use in managed landscapes is needed to draw horticultural, ecological, and economic benefits.

Leitneria floridana is a deciduous shrub native to wooded or open swamps and wet thickets in the southeastern and south-central United States. Disjunct populations occur in Georgia, Florida, Texas, Arkansas, and Missouri. Plants grow to 5 m (16 feet) tall and have attractive, bright-green foliage and brown, slender stems that remain unbranched up to 1 to 2 m (3 to 7 ft). Maturing to about 5 m (16 ft) in its native habitat, the species tends to form thickets by producing suckers. *Leitneria floridana* also has potential in horticultural commerce because of its affinity for poorly drained soils. Other attributes such as ecotypic variability in growth habit and tolerance of cold and drought also could be useful for selecting forms suited for managed landscapes. In nature, this monotypic genus in the Leitneriaceae is regionally threatened, and populations may be declining (Kelly McPherson, Florida Department of Environmental Protection, personal communication). Evaluation of its potential for horticultural applications and subsequent use in landscapes can contribute to increasing awareness of the ecological status of wild populations of *L. floridana*.

For species with extensive and continuous natural ranges in North America, plants growing in northern latitudes and at higher elevations are typically more tolerant of low temperatures than are those native to more southern locations or those occurring at lower altitudes (8). This natural diversity among geographic ecotypes is useful when selecting plants for horticultural applications. Cultivation of a species outside its natural range can sometimes allow it to acclimate to

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low temperatures in response to seasonal cues (8). Evaluation of cold hardiness of plants cultivated in regions with harsher winters than where the species is native can help determine its capability to acclimate to low temperatures.

Documenting the extent of browning or discoloration of tissue is a common visual method for detecting injury in laboratory tests of cold hardiness. Injured tissues typically become yellowish-brown due to oxidation of polyphenols, and depending upon the extent of injury, the tissues may lose membrane integrity and become softened (5). Visual evaluation of tissues stained with chemical compounds such as 2, 3, 5-triphenyltetrazolium chloride (TTC) also can facilitate injury assessment. When the colorless TTC diffuses into actively respiring tissues, it accepts electrons from the mitochondrial electron transport chain, reducing it to formazan. The accumulation of formazan stains the tissue red, and the intensity of the staining is proportional to the rate of respiration (3). In woody plants, TTC staining of phloem and cortex is highly correlated to plant survival (7). In some studies, growth of callus along the cambial zone has also been used as an indicator of plant survival (7, 9). However, woody taxa vary considerably in their capacity to develop callus on wounded stems.

The following research was conducted to estimate midwinter low-temperature tolerance of *L. floridana* by conducting assays with stem segments from indigenous plants of the southern- and northern-most provenances of the species in Texas and Missouri. Estimates of injury via both tissue discoloration and TTC were compared; a third assay, callus formation, was attempted but proved unreliable with this species. Assays also were conducted with plants native to Missouri and Arkansas that have been cultivated for many years in northern Illinois to determine whether midwinter hardiness of *L. floridana* is enhanced by repeated, long-term exposure to temperatures lower than those of its native habitat.

Materials and Methods

Terminal portions [up to 30 cm (12 in) long] of stems formed in 2002 were collected on December 2, 2002, and January 21, 2003, from natural populations of *L. floridana* in Butler County, MO, and Brazoria County, TX. Plants originally from Missouri and Arkansas but cultivated for > 60 years at the Morton Arboretum in DuPage County, IL, also were sampled on the same dates (Fig. 1). The midwinter sampling dates were chosen to represent the time of the season when cold hardiness of stems likely would be maximal.

Collected stems were stored at 5C (41F) for up to 3 days until they were processed. Two pieces, each 5 cm (2 in) long, were cut from each stem after removing the terminal and basal nodes. Samples were wrapped individually in moist paper towelling and were assigned randomly to test tubes. One stem sample contained within a test tube represented an experimental unit. Ten experimental units were assigned to each of 42 treatment combinations, a factorial arrangement of three origins (two natural provenances [Missouri and

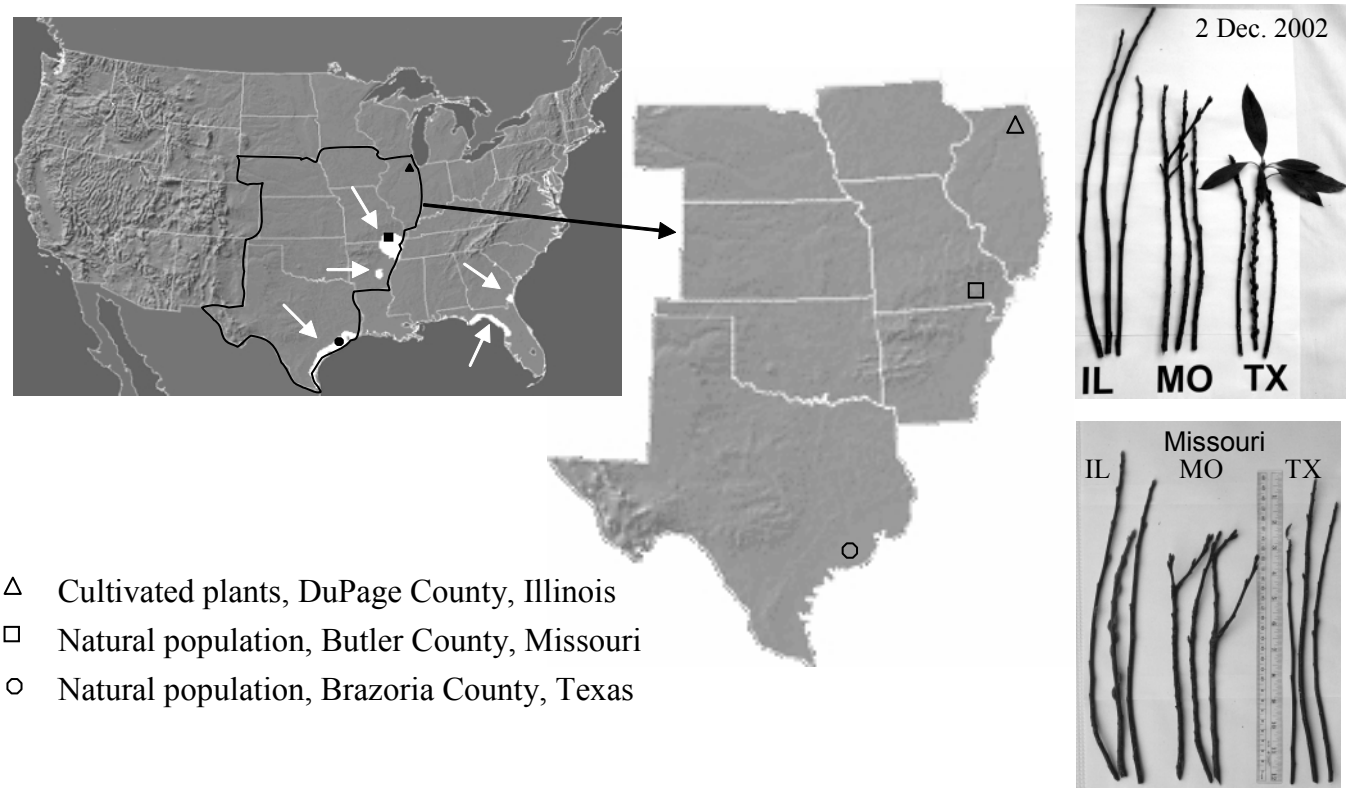

Fig. 1. Stems of *Leitneria floridana* were collected in December and January from plants growing in Illinois, Missouri, and Texas to assess cold hardiness. White arrows on the map of the United States indicate the complete natural distribution (highlighted in white) of *L. floridana*, which is restricted to the states of Georgia, Florida, Texas, Arkansas, and Missouri. The photographs show examples of terminal stems, which were divided into shorter sections for the assays. Leaves were present on unbranched samples collected from Texas in December. The stems from Illinois were highly pubescent and unbranched, whereas samples collected in Missouri often had short lateral branches. For scale, the ruler in the image on the bottom is 30.5 cm (12 in) long.

Table 1. Thirty-year average monthly minimum temperatures in regions where stems of *Leitneria floridana* were collected for assessing midwinter cold hardiness. Monthly averages were obtained on April 20, 2003, from the climatological database at http://www.weather.com/weather/climatology/monthly.

Location (County, state; longitude, latitude)	USDA Hardiness Zone	Average minimum temperature (C)			
		November	December	January	February
Morton Arboretum (DuPage County, Illinois; 41°48'43" N, 88°04'25" W)	5a	0	–7	–10	–7
Corkwood Conservation Area (Butler County, Missouri; 36°33'40" N, 90°32'09" W)	6b	3	–2	–5	–2
San Bernard National Wildlife Refuge (Brazoria County, Texas; 29°08'23" N, 95°47'50" W)	9a	11	7	7	8

Texas] and the site of cultivation in Illinois), two assessment methods (discoloration and TTC staining), and seven test temperatures. The test temperatures included a control (5C [41F]) and a range from –10C to –35C (14F to –31F) in December and –35C to –60C (–31F to –76F) in January. Ranges were selected based on the average monthly minima in late autumn and winter at locations where stems were collected (Table 1).

Before the freezing treatments commenced, all samples were equilibrated at 5C (41F) for up to 12 hr in a programmable chest freezer (ScienTemp, Adrian, MI). Temperature in the freezer was monitored by using six thermistors attached to a CR23X Micrologger (Campbell Scientific, Logan, UT). Thermistors were wrapped in moist paper towelling, inserted into empty test tubes, and placed in the freezer among stem samples. After the equilibration period, test tubes that contained the control treatments were removed, and temperature in the freezer was decreased at the rate of 2C/hr (3.6F/hr). Samples were ice-nucleated by adding ice crystals to the tubes when thermistors registered –2C (28F) and then holding this temperature for 1 hr. Remaining samples were removed sequentially as their respective test temperatures were attained.

Samples were thawed at 5C (41F) for 12 hr. The stem pieces to be assessed for discoloration remained intact, whereas those for the TTC test were cut longitudinally, wrapped in moist paper, and returned to the test tubes. All tubes were capped, and samples were incubated at 23C (73F) for 9 days. By dissecting the samples before incubation, we tested whether the stem pieces would produce callus, which could then be stained with TTC to determine survival of the cambial tissue. However, stems of *L. floridana* did not form callus readily, and callus grew inconsistently and only on stems from Illinois. Consequently, TTC staining of phloem and cortex was used as an indicator of survival after the 9-day incubation period. For this test, stems were incubated in the dark in 10 ml TTC solution (TTC at 2 g/liter in 0.05-M Na_2HPO_4-KH_2PO_4 buffer, pH 7.4) for 12 hr at 23C (73F).

After incubation, stem pieces for discoloration were dissected longitudinally and rated 1 to 3 for discoloration where 1 = obvious browning of phloem and cortex, 2 = slight or inconsistent browning (uncertain), and 3 = no browning. The TTC reaction also was rated 1 to 3 where 1 = no staining in the phloem and cortex, 2 = slight or inconsistent staining (uncertain), and 3 = obvious staining. Stems rated 3 were considered uninjured. The proportion of uninjured stems at each temperature was determined as the number of stems uninjured/total stems.

Analysis of variance models with two dependent variables (rating and proportion of uninjured stems) were used. Proportions were converted to percentages to report survival. Spearman's rank correlation coefficient was calculated to determine whether the two assessment methods were correlated. The Probit procedure was used to estimate the temperature at which 50% of the samples were uninjured (T_{50}) and to obtain 95% confidence intervals for T_{50} values (9). All statistical analyses were conducted by using SAS/STAT procedures (Version 8.02, SAS Institute, Inc., Cary, NC). Mean separation for the ratings was determined by using the Scott-Knott procedure (10), which was described further by Gates and Bilbro (2).

Results and Discussion

Visual methods for assessing injury from low temperature are considered reliable and are used commonly for studying cold hardiness in woody taxa (7, 9). In this study, ratings based on discoloration and staining of phloem and cortical tissues, which are essential to the survival of plants, were correlated ($P \leq 0.0001$) as estimated by Spearman's rank correlation coefficients (0.52 and 0.62 for December and January, respectively). Analysis of variance also indicated that results from both tests were similar.

At ≤ –15C (5F), mean ratings were lower (greater injury) for stems collected from Texas than for stems from Illinois and Missouri (Table 2). Mean ratings in December for stems from Illinois and Missouri did not decrease until test temperatures were ≤ –30C (–22F). In January, ratings were unaffected by temperature for samples from Missouri and Illinois, whereas stems from Texas rated lower at all test temperatures < 5C (41F, control). Survival percentages of stems of *L. floridana* from Illinois and Missouri were ≥ 50% at the lowest test temperature on both test dates (Fig. 2). However, survival was ≤ 20% below –20C (–4F) among samples from Texas in December, and ≤ 10% survival was observed at or below –35C (–31F) in January. Stem samples from all sources were consistently tolerant of temperatures lower than the average minima recorded in the regions where they were obtained (Tables 1 and 2, Fig. 2). Stems from the northern provenances were hardier than stems from Texas, but stems from Illinois and Missouri did not differ in hardiness to the lowest temperatures.

Estimated T_{50} values for stems from Texas were –12 and –9C (10 and 16F) in December and January, respectively, and the lower limit of the confidence intervals was near –20C (–4F) on both dates. The T_{50} values of stems from Illinois and Missouri were approximately 10C (18F) and 40C (72F)

Table 2. Mean ratings applied to longitudinally cut stems of *Leitneria floridana* after exposure to laboratory freezing tests and incubation at 23C (73F) for 9 days[z].

	December 2, 2002		January 21, 2003	
Provenance	**Temp (C)**	**Rating**	**Temp (C)**	**Rating**
Illinois	+5 (control)	2.90a[y]	+5	3.00a
	–10	2.90a	–35	2.80a
	–15	2.90a	–40	2.60a
	–20	2.85a	–45	2.75a
	–25	2.90a	–50	2.65a
	–30	2.65b	–55	2.35a
	–35	2.55b	–60	2.25a
Missouri	+5	2.85a	+5	2.85a
	–10	2.85a	–35	2.60a
	–15	2.85a	–40	2.60a
	–20	2.85a	–45	2.60a
	–25	2.75a	–50	2.60a
	–30	2.60b	–55	2.60a
	–35	2.55b	–60	2.40a
Texas	+5	2.95a	+5	2.80a
	–10	2.80a	–35	1.30b
	–15	2.65b	–40	1.25b
	–20	2.45b	–45	1.30b
	–25	1.95c	–50	1.35b
	–30	1.80c	–55	1.20b
	–35	1.25d	–60	1.35b

[z]Samples were evaluated by using two visual assessment methods [tissue discoloration of phloem and cortex and 2, 3, 5-triphenyltetrazolium chloride (TTC) staining]. Discoloration of stems was rated on a scale of 1 to 3 where 1 = obvious discoloration (browning) of phloem and cortex, 2 = slight or inconsistent browning (uncertain), and 3 = no discoloration. TTC staining also was rated on a scale of 1 to 3 where 1 = no staining of phloem and cortex, 2 = slight or inconsistent staining (uncertain), and 3 = obvious staining. Lack of discoloration and TTC staining indicated tissue viability. Because neither a main effect nor any interactions were detected at $P \leq 0.05$ for the assessment method, data were pooled over the two methods (n = 20).

[y]Means within a column were separated by using the Scott-Knott procedure at $\alpha = 0.05$.

lower, respectively, than the lowest tested temperature (Fig. 3). As other researchers have found (9), confidence intervals could not be calculated for all T_{50} values because insufficient injury of stems from Illinois and Missouri occurred at the lowest test temperature. The Probit procedure may be of more value when the lower temperatures to which samples are exposed results in substantial injury, as occurred among stems from Texas.

Assessing post-freezing growth of entire shoots and injury to individual tissues, such as buds or leaves, also can be useful for determining cold hardiness of woody plants. Floral buds were present on stems of *L. floridana* collected in Missouri and Texas. Bud-injury data were not analyzed statistically, but buds were damaged (complete disintegration of the tissues) at approximately 10C (18F) above the point where significant injury to stems was recorded. This implies that plants of *L. floridana* cultivated in regions colder than their native habitat may survive low-temperature events yet suffer compromised reproductive potential.

Cold hardiness develops in woody taxa in response to short days and decreasing temperatures (4), and local conditions strongly influence the extent of acclimation, deacclimation, or maximum midwinter tolerance (6). Large variations in midwinter cold hardiness exist in species with broad geographic distributions (1, 11). The geographic range of *L. floridana* is extensive, but populations are disjunct and small. Nonetheless, geographic variation in midwinter cold hardiness was evident in stems of *L. floridana*. Over 50% of stems from Illinois, Missouri, and Texas survived temperatures 20C (36F) lower than the average minima in December for the regions where samples were collected. By January, however, tolerance of stems from Texas was about 2C (4F) below the average minimum. In contrast, stems from Illinois and Missouri tolerated temperatures approximately 50C (90F) below the average low temperature recorded near the collection sites. Data herein suggest that *L. floridana* is adapted to

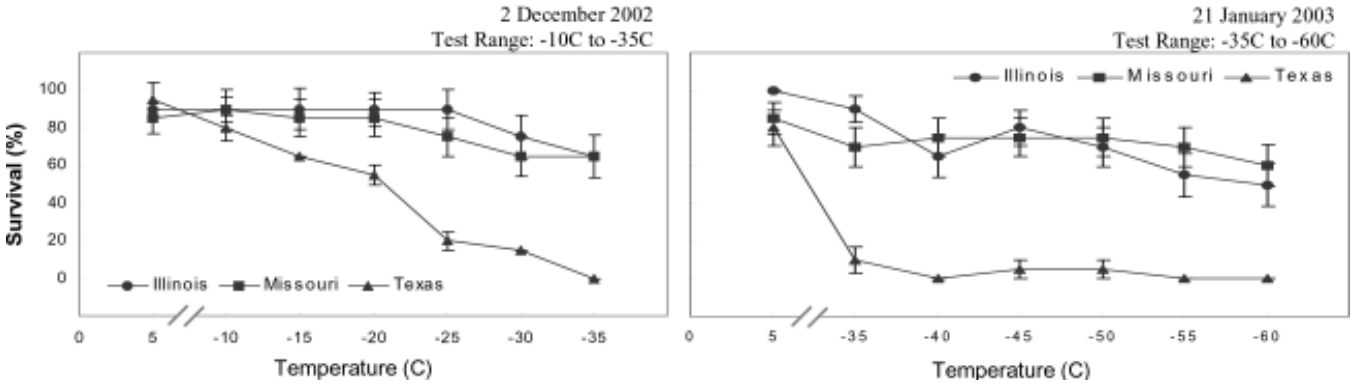

Fig. 2. Survival percentages for stems of *Leitneria floridana* collected on December 2, 2002, and January 21, 2003, and exposed to laboratory freezing tests and incubation at 23C (73F) for 9 days. Survival was determined by using two visual-assessment methods [tissue discoloration of phloem and cortex and 2, 3, 5-triphenyltetrazolium chloride (TTC) staining]. Discoloration was rated on a scale of 1 to 3 where 1 = obvious browning of phloem and cortex, 2 = slight or inconsistent browning (uncertain), and 3 = no browning. TTC staining also was rated 1 to 3 where 1 = no staining in the phloem and cortex, 2 = slight or inconsistent staining (uncertain), and 3 = obvious staining. Stems rated 3 were considered uninjured. Proportion of uninjured stems at each temperature was determined as the number of stems uninjured/total stems. Because neither a main effect nor any interactions were detected at $P \leq 0.05$ for the assessment method, data were pooled over the two methods (n = 20). The vertical bars about the data symbols represent ± 1 SE. Data symbols with no bars have an SE within the length of the symbol.

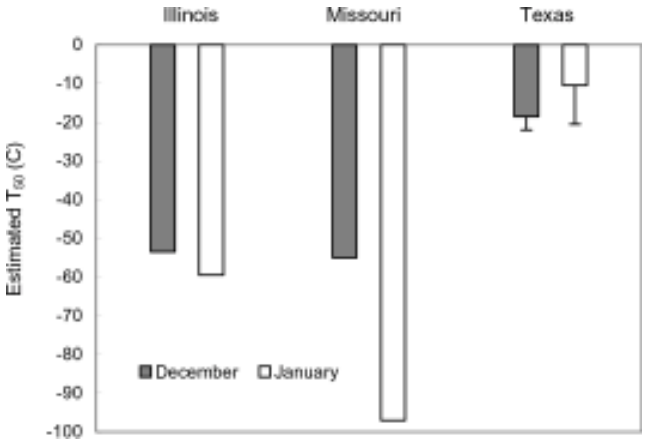

Fig. 3. **T_{50} for cold hardiness as estimated for stems of *Leitneria floridana* by using the Probit Procedure (SAS Inst., Inc.). Two visual methods (discoloration of stems and TTC staining) were used to determine the survival of stems during freezing tests. Because neither a main effect nor any interactions were detected at $P \leq 0.05$ for the assessment method, data were combined over the two methods. Stems rated 3 were considered uninjured. Error bars represent the upper limit of the 95% confidence interval (CI). CI values could not be calculated for T_{50} values showing no bars because insufficient injury occurred at the lowest test temperature.**

and can survive temperatures lower than those occurring in its native habitats, but that the extent of this capacity depends on the provenance. These results are consistent with the fact that plants of *L. floridana* native to Missouri and Arkansas have survived cultivation and have produced flowers and fruit at the Morton Arboretum in northern Illinois (USDA hardiness zone 5a).

Results indicate that, unless precluded by unsuitable seasonal accrual or loss of cold hardiness, *L. floridana* from Texas should be useful where winters are as harsh as those in USDA zone 6b (minima = –18 to –21C [0 to –5F]), and plants from Missouri should be cold-hardy in harsher USDA hardiness zones. These findings are a promising first step toward a complete characterization of cold hardiness of *L. floridana* that should reveal the timing and extent of autumnal acclimation and vernal deacclimation of plants from different provenances established in common settings that vary in winter severity.

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