Low-temperature Exotherms and Cold Hardiness in Three Taxa of Deciduous Trees

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Abstract. Although differential thermal analysis has been routinely used to evaluate cold hardiness, the relationship of deep supercooling ability and plant survival are not well understood. In this study, we compared the seasonal profiles of changes in low-temperature exotherm (LTE) occurrence with visually determined cold hardiness of *Acer rubrum* L. 'Armstrong', *Fraxinus americana* L. 'Autumn Purple' and *Zelkova serrata* (Thunb.) Mak. 'Village Green' growing in three locations representing plant cold hardiness zones 8b, 7b, and 5a. Between December and February, LTEs in *Acer rubrum* 'Armstrong' and *Fraxinus americana* 'Autumn Purple' occurred at temperatures around 10 to 25°C lower than the lowest survival temperatures. The mean difference between LTEs and lowest survival temperature was not significant for *Zelkova serrata* 'Village Green' from January to April and for *Acer rubrum* 'Armstrong' and *Fraxinus americana* 'Autumn Purple' in March. Data indicated that LTEs could be used as an estimate of lowest survival temperature in *Zelkova serrata* 'Green Village' but not in *Acer rubrum* 'Armstrong' and *Fraxinus americana* 'Autumn Purple'. This study demonstrated that LTEs may not reliably estimate cold hardiness in all species that deep supercool. Factors other than freeze avoidance ability of xylem may limit stem survival at temperatures above the LTE.

Deep supercooling of water in certain stem tissues such as axial and xylem ray parenchyma is a freezing-avoidance mechanism characteristic of many woody plants, especially deciduous hardwoods (Quamme, 1985, 1991). The deep supercooled water in these tissues freezes intracellularly as temperature decreases below a nucleation point, and subsequently causes lethal injury.

The freezing of deep supercooled water releases heat of fusion that can be detected by thermal analysis and is observed as a low-temperature exotherm (LTE) (Quamme, 1991). Occurrence of a LTE can be determined by differential thermal analysis (DTA). Although this technique has been routinely used to evaluate cold hardiness, the relationship between freeze tolerance and freeze avoidance of tissues and their effect on plant survival are not well understood.

DTA has usually been conducted at cooling rates exceeding those encountered in nature. The validity of such measurements in cold-hardiness evaluation relies on their correlation with freeze-tolerance tests conducted at slower rates (Quamme, 1991).

Comparisons of LTEs with visually determined freeze injury ratings demonstrate that DTA can often be used to measure cold hardiness of xylem (Arora et al., 1992; Ashworth et al., 1983; George et al., 1974; Quamme et al., 1972, 1973, 1982). There is, however, uncertainty whether the correlation of a LTE with xylem injury is simply coincidental or whether it indicates a causal relationship and applies to all species that deep supercool. Kaku and Iwaya (1978) found that xylem injury of several tree species indigenous to Japan did not agree with their LTE values. Similarly, George et al. (1974) noted that, although injury to xylem of many species of trees native to eastern North America frequently coincided with the LTE, in some species this injury occurred at a temperature below the LTE.

The ability of DTA to evaluate hardiness of a whole stem is even more questionable, because the LTE does not measure cold hardiness of tissues other than xylem, such as vegetative buds and bark tissues, both of which are often considered to be more critical to survival than xylem (Quamme, 1991). Hardiness of bark tissues in apple was not associated with the LTE (Quamme et al., 1972). In early fall and spring, the bark was injured at temperatures higher than the LTE, and in midwinter the relationship was reversed. In peach, however, injury to bark tissues occurred at about the same temperature as the LTE in midwinter (Quamme et al., 1982).

Using DTA to evaluate cold hardiness requires a more comprehensive understanding of the relationship between deep supercooling and cold hardiness of woody stems. We know of no published study that related seasonal changes in cold hardiness and supercooling ability of genetically identical plants growing under different climatic conditions. Therefore, the objective of this study was to compare seasonal profiles of changes in LTE occurrence and cold hardiness in stems of three taxa of deciduous trees growing at three locations representing different cold hardiness zones. We wanted to determine if the LTE provided a good estimate of cold hardiness in tree taxa that supercool.

Materials and Methods

Plant material. One-year-old stems of *Acer rubrum* 'Armstrong', *Fraxinus americana* 'Autumn Purple' and *Zelkova serrata* 'Village Green' were collected from trees growing in three locations: Athens, Ga.; Boring, Ore.; and Orono, Maine. Samples were sent via overnight express to the Georgia Experiment Station, Univ. of Georgia, Griffin. The freeze testing and DTA analysis were conducted monthly between December 1992 and April 1993.

DTA analysis. DTA analysis was performed on 2 cm internodal stem sections in which the bark and xylem were intact. The method used was adopted from Quamme et al. (1972). The differential temperature between a sample and a dried reference cooled in an aluminum block at a rate of 30°C/h was recorded by copper-constantan thermocouples placed in contact with the sample and wrapped in aluminum foil. The thermocouples were connected via a temperature compensation panel (T21; Strawberry Tree Com-

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puters, Sunnyvale, Calif.) to an analog-to-digital converter with 16-bit resolution (MINI-16; Strawberry Tree Computers) and data were retrieved using LABTECH NOTEBOOK software (Laboratory Technologies Corp., Wilmington, Mass.). Differential thermal analyses were determined on three samples for each taxon and location. Mean LTE values and their standard deviations were computed.

Ambiguity in reporting LTE occurrence exists in the literature. Authors reported the point of initiation of the LTE peak (George et al., 1974; Kaku and Iwaya, 1978; Montano et al., 1987; Quamme et al., 1972; Rajashekar and Reid, 1989), the middle point of the LTE peak (Arora et al., 1992), the initiation and middle points (Weiser and Wallner, 1988), or the points of initiation and termination of the LTE peak (Ashworth et al., 1983). Still, some investigators do not specify to what their LTE values refer (Hamman et al., 1990). The LTE temperature reported here is the temperature of the exotherm initiation determined according to Quamme (1976) as the point at which the differential temperature began to increase after the decline that follows the freezing of the bulk water.

Viability tests. Stem samples 4 to 6 cm long were prepared as described previously by Lindstrom and Dirr (1989) and subjected to freezing at the rate of 4C/h in a glycol bath (Forma Scientific, Marietta, Ohio). Four stem samples of each taxon and location were removed at 3C intervals and thawed in a refrigerator overnight. The samples were incubated for 7 days in water-saturated atmosphere at 25C and were evaluated visually under a dissecting microscope for injury. Oxidative browning was used as the criterion of injury to the whole stem and no distinction was made between various tissues. Lowest survival temperature (LST) was the lowest temperature at which no oxidative browning was observed in any of the stem tissues.

Results and Discussion

The LTE of *Acer rubrum* 'Armstrong' attained minimum values near -40C by December in Oregon and Maine (no data for

Fig. 1. Seasonal changes in the lowest survival temperature (LST) and low-temperature exotherm (LTE) of *Acer rubrum* 'Armstrong' from three locations (vertical bars represent standard deviation of the mean).

Georgia) and remained stable until February in Oregon and Maine and until March in Georgia (Fig. 1). The LST values indicated that stems did not fully acclimate until January and later maintained their hardiness at -25 to -30C until March. Lindstrom and Dirr (1989) reported that the LSTs of several *Acer rubrum* selections in Georgia reached a maximum hardiness of -23 to -29C by February. Some of these selections maintained this level of hardiness until March. Pellet et al. (1981) predicted a maximum cold hardiness of -29C for two *Acer rubrum* selections in Vermont.

In contrast, George et al. (1974) recorded a LTE for *Acer rubrum* in Minnesota in January at 45C and xylem-killing temperature below -54C. The authors, however, did not make any reference to bark-tissue survival. The difference between our LTE and LST estimates and the estimates by George et al. (1974) can be attributed to the fact that these authors collected their samples in plant cold-hardiness zone 3b (U.S. Dept. of Agriculture, 1990), while our locations in Oregon, Georgia, and Maine represent zones 8b, 7b, and 5a, respectively. In addition, George et al. (1974) stored their samples at -10C for up to 4 weeks before DTA and viability testing. In apple stems collected in mid-winter, prolonged exposure to subzero temperature did not affect LTE occurrence significantly (Quamme et al., 1973). On the other hand, Weiser and Wallner (1988) noted that a single freeze-thaw cycle lowered the LTE in *Fraxinus pennsylvanica* Marsh. from -27.4 to -38.5C. Similarly, Hong and Sucoff (1982) demonstrated that the exposure of *Malus pumila* Mill. stems to temperatures of -5C for 3 h lowered the LTE by 3 to 8C in winter and 4 to 11 C in spring. The exposure to freezing temperatures may also cause an increase in cold hardiness in plants (Sakai, 1970), and storage at subzero temperatures was used by Quamme (1976) and Montano et al. (1987) to induce the maximum level of cold hardiness. It is, therefore, likely that samples of *Acer rubrum* in the George et al. study were more cold hardy than these in our study as a result of prolonged exposure to low temperature, or because they represented the extreme northern provenance.

Fig. 2. Seasonal changes in the lowest survival temperature (LST) and low-temperature exotherm (LTE) of *Fraxinus americana* 'Autumn Purple' from three locations (vertical bars represent standard deviation of the mean).

The LTE of *Fraxinus americana* 'Autumn Purple' attained minimum values of -35 to -40°C by December and maintained them until January (Fig. 2). In all locations, the LTE shift to higher values began in February. It was greater in Oregon and Maine than in Georgia. The LST values reached their minimum of -25 to -30°C by December and remained so until March, with one dehardening episode in February in Oregon. Weiser and Wallner (1988) recorded LTE for *Fraxinus americana* in Colorado in January at around -30°C. Flint and Alexander (1982) tested *Fraxinus americana* from two provenances, with trees from Michigan developing -40°C midwinter hardiness and trees from Mississippi -30°C. George et al. (1974) recorded a LTE for *Fraxinus americana* in Minnesota at -42°C and xylem-killing temperature at -46°C. The difference between our estimate of LST and xylem-killing temperature determined by George et al. can be attributed to the analogous factors as for *Acer rubrum*.

The LTE and LST values of *Zelkova serrata* 'Village Green' attained minimum values of -20 to -27°C by January and fluctuated near this range until March (Fig. 3). A shift of about 5°C of the LTE and LST toward higher values began in April. The LTE for *Zelkova serrata* from native forests in Japan recorded by Kaku and Iwaya (1978) was -23°C in January and remained near this temperature until March. Temperature of xylem injury in this species determined by Sakai (1972, 1975) was -27°C, while the killing temperature of the cortex tissue was -30°C.

The seasonal changes of differences between the LTE and LST for *Acer rubrum* 'Armstrong' and *Fraxinus americana* 'Autumn Purple' resembled each other (Fig. 4). Between December and February, the LTE occurred at temperatures at least 10°C lower than the LST. In December, the LTE in *Acer rubrum* 'Armstrong' was recorded at over 25°C below the LST. On that date, despite deep supercooling down to near -40°C, stems tolerated freezing only to around -10 to -15°C. In a Lindstrom and Dirr (1989) study, several selections of *Acer rubrum* tolerated freezing to only -8 to -17°C in December.

Fig. 3. Seasonal changes in the lowest survival temperature (LST) and low-temperature exotherm (LTE) of *Zelkova serrata* 'Village Green' from three locations (vertical bars represent standard deviation of the mean).

In March the differences between LTE and LST were not significant for either *Acer rubrum* 'Armstrong' or *Fraxinus americana* 'Autumn Purple'. Greater differences in April were caused by deacclimation to about -10 to -15°C, while the LTE increased only to around -30°C. It has been reported previously that stems maintained their deep supercooling ability in spring and summer despite partial or complete deacclimation (Hamman et al., 1990; Hong and Sucoff, 1982; Rajashekar and Reid, 1989). In stems of *Juniperus virginiana* L. collected in late May, George (1982) observed a LTE near -40°C despite deacclimation of tissues to -20°C. George suggested that certain anatomical features necessary for deep supercooling are established during the first year of growth and later persist regardless of cold hardiness changes.

Mean difference between LTE and LST for *Zelkova serrata* 'Village Green' was not significant from January to March, as indicated by standard deviations overlapping the value of zero difference (Fig. 4). It is, however, possible that this difference would occur later in spring and summer when plants deacclimated. In a study by Kaku and Iwaya (1978) the LTE of *Zelkova serrata* remained at -19 to -22°C between May and September.

Considering LTE as a measure of cold hardiness was supported by studies that found a close relationship between the capacity to deep supercool in many woody plants and latitudinal and altitudinal limits of their distribution (Becwar et al., 1981; George et al., 1974; Kaku and Iwaya, 1978). The same studies, however, provide many observations that disagree with the universality of the LTE as an indicator of cold hardiness. George et al. (1974) concluded that the xylem-killing temperature of 25 tree species native to eastern North America frequently coincided with the LTE, despite the fact that, in 11 of these species, the LTE occurred at a temperature of 7 to >13°C higher than the xylem killing point. The

Fig. 4. Seasonal changes in low-temperature exotherm-lowest survival temperature difference for *Acer rubrum* 'Armstrong', *Fraxinus americana* 'Autumn Purple' and *Zelkova serrata* 'Village Green' (each point represents the mean of three locations, except for April data based only on Georgia location; vertical bars represent standard deviation of the mean).

authors speculated that xylem injury would be observed at LTE if detailed microscopic examination had been made. Becwar et al. (1981) reported that injury to the stem tissue of many woody timberline species of the Colorado Rocky Mountains was associated with the LTE, but they also observed LTE occurrence as low as 4°C below or as high as 6°C above the stem injury temperature in some species. Kaku and Iwaya (1978) showed a good correlation between LTE ranges and the northern limits of distribution in Japan for trees grouped according to their hardiness zones, but analysis of individual taxa indicated that LTE did not agree well with temperature of xylem injury as determined by Sakai (1972, 1975). Several of the species in their study had a LTE as much as 9°C below or above xylem injury temperature. Pukacki (1989), comparing 23 species of *Picea* A. Dietr., found that, in five species, the LTE differed from killing temperature by 8 to 11°C. Our study demonstrated that LTE could be used as an estimate of LST in *Zelkova serrata* 'Village Green', but not in *Acer rubrum* 'Armstrong' and *Fraxinus americana* 'Autumn Purple'.

Some reports that noted a close relationship between the LTE and stem cold hardiness were based on observations limited to a single month or even a single date. Rajashekar and Reid (1989) reported good correlation of the LTE and stem-killing temperature for several pecan cultivars based, however, on measurements conducted only on two dates in April. Incipient injury to xylem of several pear species tested by Montano et al. (1987) coincided with a LTE within 3°C, but testing was conducted only once in December.

Several investigators who studied seasonal changes in deep supercooling ability and in cold hardiness found a close relationship between LTE and stem-killing point for some dates but not for others. Arora et al. (1992) observed good correlation between LTE and xylem killing point in peach during midwinter but not in early fall or spring. On those later dates, LTE occurred at temperatures 4 to 11°C lower than the xylem-killing point. Freeze injury to peach bark tissue did not agree with the LTE even during the midwinter, when it appeared as much as 20°C below LTE. In contrast; Quamme et al. (1982) found that injury to bark tissues of peach and apple coincided with the LTE in midwinter. In our study, the LTE agreed with the LST in *Acer rubrum* 'Armstrong' and *Fraxinus americana* 'Autumn Purple' in March but not on any other dates.

There are no clear guidelines on how much discrepancy between survival or killing temperature and LTE is allowable for the latter to be regarded as a valid estimate of cold hardiness. Differences between LTE and killing temperature under 3°C usually indicate their close association (Arora et al., 1992; Montano et al., 1987; Quamme et al., 1972; Rajashekar and Reid, 1989). Differences in a range of 10 to 25°C, as occurred in the current study, make the LTE irrelevant for cold-hardiness evaluation in some species. The occurrence of the LTE at much lower temperatures than the LST suggests cold hardiness of bark tissues was limiting stem survival at temperatures above the LTE. This, however, does not exclude the possibility that xylem tissues were also lethally injured above LTE. Canes of *Vitis riparia* Michx. that had been killed by freezing still exhibited the LTE (Pierquet and Stushnoff, 1980). Similarly, water in heat-killed xylem, of *Prunus persica* (L.) Batsch. and *P. armeniaca* L. was able to deep supercool (Ashworth et al., 1983).

Additional difficulty in using DTA for cold hardiness evaluation arises with taxa that exhibit multiple LTEs. Rajashekar and Burke (1978) and Rajashekar et al. (1982) noted that, in several species of *Pyrus* with multiple LTEs, the injury appeared to be sustained at the temperature between exotherms but sometimes was associated with the first, second, or third LTE. Uncertainty as

to which of the multiple LTEs indicates injury and whether this association is constant over the winter season questions the applicability of using the LTE for cold hardiness evaluation.

This study demonstrated that the LTE may not reliably estimate cold hardiness in all species that deep supercool. Factors other than freeze-avoidance ability of xylem may limit stem survival at temperatures above the LTE. More insight is also needed into the extent to which plants can tolerate injury to xylem tissue and therefore survive below the LTE.

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