FREEZING AVOIDANCE IN TWIGS OF BLACK OAK: A SUPERCOOLED AND DESICCATION RESISTANT FRACTION OF XYLEM WATER AT LOW TEMPERATURE AND LOW WATER POTENTIAL

Maria L. Carrasquilla and Milon F. George[1]

Abstract.--Differential thermal analysis (DTA) was used to investigate the freezing of tissue water in cold hardy twigs of black oak (*Quercus velutina* Lam.). Approximately 48% of tissue water was observed to freeze near -40°C. The freezing event at -40°C occurred in the xylem and was correlated with visual injury to the xylem. Frozen twig samples thawed near -2°C, suggesting that the water which freezes at low temperature is a supercooled fraction of tissue water that freezes near its homogeneous nucleation temperature. This supercooled water fraction resisted desiccation when exposed to 80% RH at 25°C for 20 days or when exposed to -12°C for 13 days. Freezing avoidance by supercooling of tissue water in cold hardy black oak twigs is therefore similar to that found in many temperate zone deciduous trees.

Additional keywords: Freezing avoidance, supercooling, desiccation resistance, xylem, water.

INTRODUCTION

Living xylem tissues of a number of woody species exhibit a pronounced low temperature freezing event during midwinter (Burke et al. 1975, Burke et al. 1976, Burke and Stushnoff 1979, George et al. 1974b, George et al. 1977, George and Burke 1980, Krasavtsev 1970, Pierquet et al. 1977, Quamme et al. 1972a, Quamme et al. 1972b, Rajashekar and Burke 1978, Sakai and Miwa 1979, Sakai and Hakoda 1979). For example, Krasavtsev (1970) found that up to 40% of water in cold hardy apple twigs remains unfrozen to -30°C at cooling rates as slow as 10°C/day. Similarly, Quamme et al. (1972a) found low temperature freezing events near -40°C in midwinter apple twigs in Minnesota. They also noted that the maximum cold hardiness of xylem parenchyma throughout the winter was about -41°C. Following these studies, George et al. (1974b) measured lethal low temperature freezing events near -40°C in the xylem of 25 woody species native to northern regions of the eastern deciduous forest. They also found that the northern distribution of these 25 woody species extended only to regions where -40°C temperature did not occur. Although Krasavtsev suggested that these freezing events were due to supercooling of tissue water, Weiser (1970) interpreted the freezing events as a "pulling away" of "vital water" from protoplasmic constituents to extracellular ice.

To characterize the biophysics of low temperature freezing, Burke et al. (1975) and George and Burke (1977) used differential thermal analysis (DTA),

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[1]Graduate Research Assistant and Assistant Professor of Forestry, Fisheries and Wildlife, University of Missouri, Columbia, MO 65211.

differential scanning calorimetry (DSC), pulsed nuclear magnetic resonance spectroscopy (NMR) and low temperature microscopy to study freezing in the xylem of shagbark hickory (Carya ovata (Mill.) K. Koch) twigs. The following is a summary of some of their DTA, DSC and NMR analyses: 1) hickory xylem exhibits essentially one major freezing peak which decreases to a limiting value near -45°C in midwinter; 2) rewarming of a frozen sample shows a gradual thawing of the tissues with a melting point near -2°C, similar to a 1 molar osmotic solution; 3) at -15°C the time constant for freezing in fully acclimated xylem is greater than 1 year; and 4) heat of fusion of tissue water freezing at low temperature is 51 $\pm$ 3 calories per gram, comparing favorably with the 61.1 calories per gram for the freezing of pure supercooled water at -38°C (Rasmussen and MacKenzie 1973). All of these results are analogous to the freezing behavior of supercooled, dilute aqueous solutions (Rasmussen and MacKenzie 1972) and support the concept of simple supercooling of water as the frost avoidance mechanism. DSC experiments also demonstrated that the low temperature freezing event in hickory is the sum of many small ice nucleations. This information, coupled with microscopic observation which showed that the predominant freezing occurs in the xylem ray parenchyma, led to the conclusion that the supercooled fraction of water is in the parenchyma. However, the microscopic observations were clouded by the fact that cross-sectioning caused freezing to occur at high temperature. This finding is not unexpected since xylem rays in hickory are large and multiseriate with heights often greater than 0.5 mm. Thin sectioning for microscopy would inevitably destroy the intactness of the ray and disrupt many cells. In a recent study conducted on a number of deciduous species, Hong and Sucoff (1980) conclude that the units which freeze at low temperature are single cells or small groups of cells within an individual ray.

George and Burke (1977) showed that the supercooling fraction of xylem water is resistant to desiccation at 20°C, even to water potentials as low as approximately -300 bars (80% RH). This resistance to desiccation at room temperature would seem to be a corollary of supercooling since under subfreezing conditions supercooled xylem water must resist the desiccation stress from extracellular ice. If this were not the case, tissue dehydration would occur via sublimation from the supercooled cellular water to extracellular ice. They also showed by D_2O exchange experiments that no impermeable barrier preventing water movement exists in hickory xylem. An "ink bottle" pore model was proposed to explain the resistance to desiccation at 20°C. Pores were suggested to be capillaries in the blanket of cell wall polymers which cover the ray cells and the cell lumina were suggested to be the "ink bottle" cavities.

The experiments reported below present results of similar studies, utilizing DTA, on the biophysics of freezing in winter hardy black oak (Quercus velutina Lam.) xylem.

MATERIALS AND METHODS

Plant Materials. - One year old twigs were collected from a black oak tree located at the University of Missouri, Columbia, on February 19, 1979. Twigs were stored at -10°C until tested. All experiments were completed by July 25, 1980.

Freezing and Thawing of Tissue Water. - Freezing and/or thawing of tissue water was monitored by DTA. Briefly, the DTA method involves cooling a sample twig section and a reference at the same rate in separate chambers. The sample is enclosed in a small aluminum foil cup and placed into one chamber to which a temperature sensor is attached. An empty aluminum foil cup is placed into a similar chamber as a reference. Freezing events occurring in the sample cause a warming of the sample chamber with respect to the reference chamber. The corresponding temperature difference is displayed on a strip chart recorder. Ambient temperature of the reference is also recorded. A DTA similar to that described by George et al. (1974a) was used, but thermistors were used as differential temperature sensors instead of thermocouples. Unless otherwise indicated, the cooling or thawing rate was 50 ± 5°C/hr. or 60 ± 5°C/hr.

Viability Testing. - Intact twig samples, approximately 3 cm. in length, were wrapped in wet cheesecloth and tinfoil with a copper-constantan thermocouple attached. The wet cheesecloth served to nucleate the samples at just under 0°C. The samples were then placed into insulated flasks and cooled in a low temperature freezer at a rate not exceeding 20°C/hr. Sample temperature was recorded on a multipoint recorder. At -20, -29, -40 and -48°C sample flasks were removed and then thawed slowly at +5°C. After thawing was complete, samples were wrapped in a wet paper towel and placed into polyethylene bags. The samples were incubated at 25°C for five days and then evaluated microscopically for browning injury. This is a routine viability test which has been described elsewhere (Palta et al. 1978, Pellett 1971).

Sample Desiccation. - The response of xylem water to desiccation above freezing was determined by placing twig sections without bark into humidity chambers at 25°C. Glycerol and water were mixed in the proper proportions to give 60, 70, 80, 90 and 100% relative humidities. Proportions were determined from the International Critical Tables of Numerical Data (1928). Xylem samples were evaluated after 20 days by DTA for freezing of tissue water. Two xylem sections were evaluated at each relative humidity.

Resistance of xylem water to dehydration at subfreezing temperatures was measured by DTA after holding a twig sample at -12 ± 3°C for 13 days. The twig was nucleated with a wet string during freezing to -12°C to ensure the presence of extracellular ice. DTA analysis was carried out without thawing at a cooling rate of 50 ± 5°C/hr.

RESULTS AND DISCUSSION

Freezing and Thawing of Tissue Water. - Freezing and thawing curves for an intact black oak twig are illustrated in Figure 1. The freezing event near -10°C is from extracellular ice formation and causes no injury to either the bark or xylem. This slight supercooling is typical of all DTA analyses in which artificial nucleation is not performed at 0°C. The freezing point between -40 and -50°C occurs in the xylem. Evaluation of Figure 1 plus two similar DTA curves indicates that 48 ± 11% of all observable freezing occurs at low temperature. DTA curves of twigs without bark displayed little or no high temperature freezing. The thawing curve shows that all tissue water melts near -2°C. This freezing and thawing behavior is similar to that described for shagbark hickory (George and Burke 1977). It is not typical of

melting point depression or eutectic phenomena which are equilibrium processes that have the same freezing and melting points, but characteristic of a supercooled liquid (Glasstone 1946). In this case the supercooled liquid is xylem tissue water.

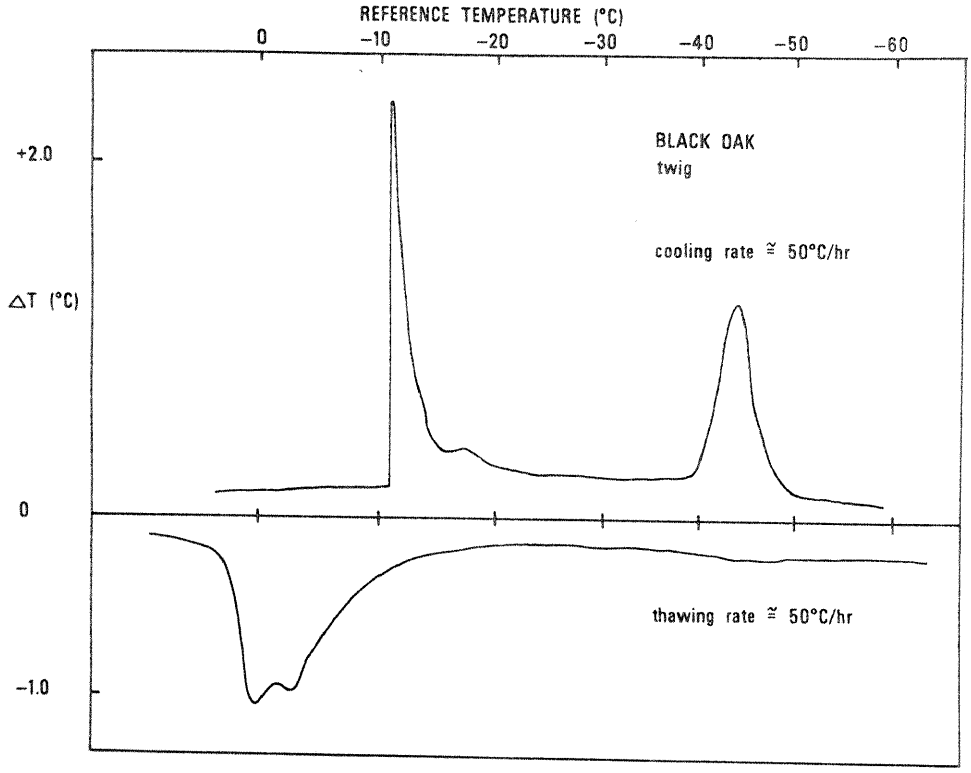

Figure 1.--DTA freezing and thawing curves for a winter hardy black oak twig. The high temperature freezing event is from extracellular ice formation in the bark and nonliving xylem. This freezing causes no damage to the twig. The low temperature freezing event is from crystallization of supercooled cellular water in the living xylem and produces freezing damage in the xylem.

Viability Testing. - Browning injury was observed in xylem tissues at -40°C or near the beginning of the low temperature freezing event. This result compares well with xylem damage reported in other woody species known to survive freezing by supercooling to low temperature (George et al. 1974b, Quamme et al. 1972a). Calorimetric and microscopic evidence reported by George and Burke (1977) and Hong and Sucoff (1980) suggests that freezing at low temperature occurs rapidly and intracellularly, instantly killing the cells in which it occurs. It should be noted, however, that no one has been able to directly observe intracellular freezing near -40°C under the microscope.

Partial injury was observed in bark tissue at -40 and -48°C. This result is unusual in that studies conducted on more northerly distributed species generally indicate that winter hardy bark tissues are resistant to freezing to temperatures as low as -196°C (Sakai and Weiser 1973, Weiser 1970). The reasons for this partial bark injury were not investigated further.

Sample Desiccation. - A DTA curve for a xylem section exposed to 80% RH for 20 days at 25°C is shown in Figure 2. By comparing DTA curves from desiccated samples with DTA curves from control xylem samples of fresh material, the loss or gain of freezable water was estimated. Freezable xylem water was estimated by integrating the area under the low temperature freezing curve and dividing this area by the sample dry weight. At 80% RH freezable water was approximately 0.95 that of the control. At 90 and 100% RH freezable water content increased by approximately 1.9 and 4.4 times, respectively. At 70 and 60% RH no freezable water was detected by DTA. In general, these results parallel those reported by George and Burke (1977) for shagbark hickory.

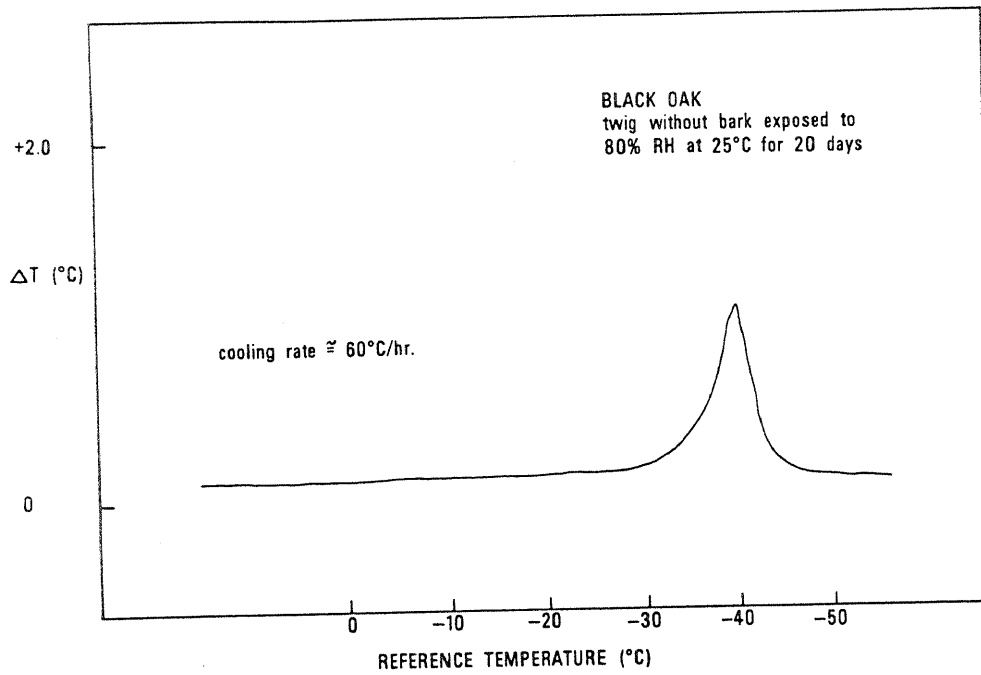

Figure 2. DTA freezing curve of black oak xylem exposed to 80% RH at 25°C for 20 days.

As for shagbark hickory the "ink bottle" pore model can be employed to hypothesize an explanation for the retention of a significant freezable water content at 80% RH in black oak xylem. In principle, the model would predict that as evaporation from a xylem section begins, water will be removed first from large vessel elements. After this water is removed, cell wall capillary

pores will begin to dehydrate and menisci will form in the pores. The radius of the largest capillary pore will determine the reduction in water potential of the system as specified by the Kelvin equation (Adamson 1966). Only in the capillary pores can the water potential be reduced significantly. If the water potential of the system becomes equal to the atmospheric water potential (at 80% RH, -310 bars in the above experiment), equilibrium will be achieved and no further desiccation will occur. To produce a water potential of -310 bars, the Kelvin equation requires a meniscus of approximately 50 Å radius. If the atmospheric water potential is lowered to the point at which all capillary pores are dehydrated (at 70% RH, -493 bars in the above experiment) the meniscus will move into the "ink bottle" cavity (the cell lumina) and become almost flat. This will lead directly to a much higher water potential in the cavity. Evaporation of water in the cavity to the atmosphere will then proceed rapidly. Despite the fact that the explanation is reasonable, many biophysical, anatomical and physiological details remain unresolved. George and Burke (1977) speculate on some of these details and also on "ink bottle" pore behavior at subfreezing temperature. That speculation will not be repeated here.

DTA analysis performed on an intact black oak twig section after 13 days at -12 ± 3°C is shown in Figure 3. Although the nature of the experimental procedure prevented an estimation of freezable water content as described above, the DTA curve displays a significant freezing event near -40°C. The result suggests that supercooled xylem tissue water can resist desiccation from extracellular ice in the bark or xylem vessels. The water potential difference between ice and supercooled pure water at -12°C would be approximately -141 bars. This result is again similar to data on shagbark hickory xylem (George and Burke 1977).

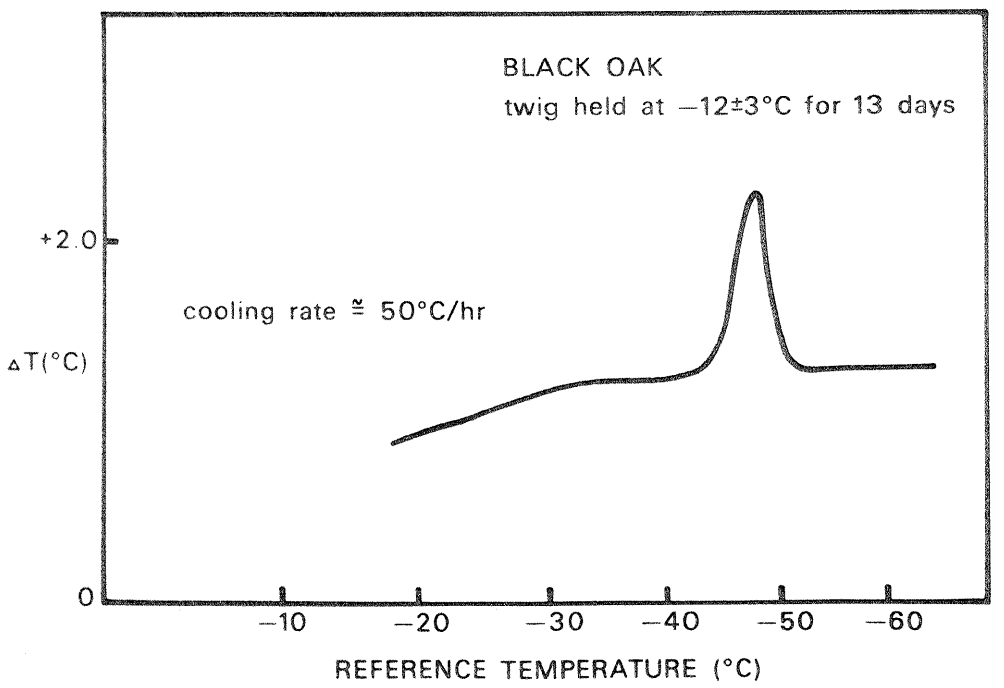

Figure 3. DTA freezing curve of a black oak twig held at -12 ± 3°C for 13 days. The sample was not thawed prior to DTA analysis.

CONCLUSION

The results reported here on black oak are similar to those reported previously for shagbark hickory and the following can be concluded: 1) as in many deciduous trees, freezing avoidance by supercooling of tissue water to temperatures just above its homogeneous necleation temperature is the mechanism of winter survival in one-year-old xylem and 2) as in shagbark hickory, supercooled xylem tissue water resists desiccation at low water potential, above or below 0°C, an apparent corollary of the freezing survival mechanism.

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