Response of Fruit Tree Tissues to Freezing Temperatures

E.N. Ashworth

Department of Horticulture, Purdue University, West Lafayette, IN 47907

M.E. Wisniewski

*U.S. Department of Agriculture, Agricultural Research Service, Appalachian Fruit Research Station,
Kearneysville, WV 25430*

Low winter temperatures are a major limiting factor in deciduous fruit production in North America. Economic losses can occur due to very low temperatures during dormancy or due to untimely freezing temperatures before acclimation in the fall or after deacclimation in the spring. Due to the importance of cold hardiness in fruit production, this characteristic is often a selection criterion in breeding programs. In addition, numerous research programs have focused on the mechanisms of freezing injury and temperature acclimation. The rationale for these studies has been that knowledge of the factors limiting freezing resistance and controlling acclimation and deacclimation may result in potential new strategies for preventing freezing injury or for developing more cold-hardy germplasm.

One intriguing concept developed from the aforementioned research was that different tissues within the same plant respond in distinctly different ways to subzero temperatures. In this paper, we review the literature on how the various tissues of deciduous fruit trees respond to low temperatures and, we hope, provide insights on how this response may influence strategies to enhance plant freezing resistance.

RESPONSE OF XYLEM TISSUES

Experiments using differential thermal analysis (DTA) and nuclear magnetic resonance (NMR) have provided evidence that the bark and wood tissues of apple, peach, and other deciduous hardwoods exhibit contrasting freezing behavior (Ashworth et al., 1983; George and Burke, 1977; Hong et al., 1980; Quamme et al., 1972, 1973, 1982). When cold-hardened xylem tissues were exposed to subzero temperatures, two distinct freezing events were detected (Ashworth et al., 1983; George and Burke, 1977; Quamme et al., 1972). The first corresponded to the freezing of water within the xylem vessels and was not correlated with tissue injury. The second freezing event occurred near -40°C and was correlated with the

death of the xylem ray parenchyma cells (Ashworth et al., 1983; Hong et al., 1980; Quamme et al., 1972, 1973). Although this second fraction of water froze near -40°C , melting occurred just below 0°C . This difference suggested that a portion of the tissue water had supercooled before freezing (Burke et al., 1976; Quamme et al., 1972, 1973). Injury was absent when water within these tissues remained supercooled and, presumably, cells were killed by intracellular ice formation when freezing was initiated (Burke et al., 1976). Xylem ray parenchyma cells appeared to freeze as individual units or as small groups of cells (Ashworth et al., 1983; Hong and Sucoff, 1980; Wisniewski and Ashworth, 1985). Since the supercooling of water was limited by the homogeneous nucleation point, cell survival was limited to about -38°C . The distribution of tree species as related to the deep supercooling characteristic has been examined in several studies (Becwar et al., 1981; George et al., 1974; Quamme, 1976).

It is not known how water in xylem parenchyma cells can supercool to temperatures approaching the homogeneous ice nucleation temperature (-38°C) when ice is present in adjacent tissues. These cells must be free of heterogeneous ice nuclei, and properties of the tissue must prevent both the spread of ice from adjacent tissues and the loss of cellular water to extracellular ice. Properties of the cell wall have been implicated in facilitating supercooling and preventing the spread of ice from adjacent tissues (Ashworth and Abeles, 1984; George, 1983; George and Burke, 1977). Since water in small-diameter pores has a lower melting point than bulk water, it has been hypothesized that restricted pore size (6–10 nm in diameter) in the cell wall may facilitate supercooling and prevent the spread of ice from adjacent tissues (Ashworth and Abeles, 1984; George, 1983; George and Burke, 1977). Recently, Wisniewski et al. (1987a, 1987b) used lanthanum nitrate to characterize cell wall permeability in relation to tissue freezing response. Their data indicated that the pore structure and/or permeability of the pit membrane and underlying "protective layer", rather than the overall

cell wall, appeared to play a limiting role in determining the spread of ice and the rate of water movement in response to a water potential gradient. Therefore, the structure and permeability of this portion of the cell wall may largely determine whether or not a species would exhibit deep supercooling.

Seasonal changes in the cold hardiness of xylem tissues appeared to result from changes in the extent of supercooling (Ashworth et al., 1983; George and Burke, 1977). The level of the low-temperature exotherm decreased in the fall during acclimation, reached a minimum in midwinter, and increased in the spring during deacclimation. Properties of the tissue that changed seasonally and influenced the ice nucleation temperature have not been identified.

The results of studies using low-temperature scanning electron microscopy (LTSEM) and freeze substitution techniques were consistent with the concept that water within xylem parenchyma cells supercooled (Ashworth et al., 1988). Xylem ray parenchyma cells from apple twig specimens freeze-stressed to -5 , -20 , or -45°C appeared the same as cells from unfrozen control tissues. No evidence of cell collapse, large extracellular ice crystals, or tissue disruption was noted. Examination of specimens in summer, fall, and midwinter provided similar results, indicating that the pattern of freezing was the same despite large differences in cold hardiness. These observations were consistent with the idea that water within xylem parenchyma cells of apples supercooled and that these tissues did not undergo extracellular freezing. When the critical temperature was reached, intracellular ice formation would be expected and cellular collapse would not be anticipated.

RESPONSE OF BARK TISSUES

The pattern of freezing in cold-acclimated bark tissues was different from that observed in xylem tissues. DTA and NMR studies on apple and peach bark tissues revealed only a single freezing event initiated a few degrees below 0°C (Ashworth et al., 1983; Quamme et al., 1972, 1982). The temperature at which water froze in the bark was not correlated with the cold hardiness of the tissue. It appeared that water within the bark tissues froze extracellularly. Presumably, ice formation was initiated in the intercellular spaces. As the ice crystals grew, a water potential gradient was established between the extracellular ice and the intracellular solution (Mazur, 1969). The formation of this gradient resulted in the movement of intracellular water through the plasma membrane to the growing extracellular ice crystal. As the temperature decreased, water would be withdrawn from the cells, cell volume would be reduced, and the internal solute concentration would increase (Burke et al., 1976; Mazur, 1969). Based on the above pattern, several possible mechanisms of injury have been proposed. These include: the concentration of intracellular components to toxic levels (Levitt, 1980; Lovelock, 1953; Mazur, 1969); decreased water activity that leads to a precipitation of proteins (Levitt, 1980); alterations in hydrophilic-hydrophobic interactions within membranes (Steponkus, 1984); and establishment of a minimum critical volume (Meryman, 1971) or surface area (Steponkus, 1984). Each of these proposed mechanisms of injury was related to the dehydration of the tissue during freezing. The survival of bark tissues would depend on the capability of the cell to tolerate or ameliorate the numerous stresses that accompany extracellular ice formation.

LTSEM and freeze substitution studies corroborated the proposal that bark tissue underwent extracellular freezing. Large extracellular ice crystals were observed in bark tissues exposed to a freezing stress (Ashworth et al., 1988). Cortical cells had collapsed and large reductions in cell volume were noted. The large reductions in cellular volume were accompanied by evidence of buckling and wrinkling of cell walls.

Neither the distribution of ice crystals nor the extent of cell collapse was uniform in apple bark exposed to a freezing stress. Large ice crystals were noted in the cortex, but were not observed within the phloem and cambial regions (Ashworth et al., 1988). The region where the large extracellular ice crystals formed corresponded to the area of the bark where the cells were less densely packed and had larger intercellular spaces. Likewise, the extent of cell collapse was not uniform throughout the bark tissues. Cortical cells exhibited

extensive reductions in cell volume, whereas periderm and phloem parenchyma cells changed little compared to unfrozen controls. The diverse response of cell types may have resulted from several factors, including differences in a) the path length that water must migrate from cells to the growing ice crystals, b) the water potential of different cell types; and c) the structural features that may influence the extent and direction of cell collapse.

Despite large seasonal changes in freezing resistance, ice formation in bark tissues occurred at the same temperature throughout the year (Ashworth et al., 1983). Likewise, the distribution of ice crystals and tissue response was similar at different stages of cold acclimation (Ashworth et al., 1988). The combined results suggested that seasonal changes in cold hardiness reflected changes in tissue tolerance to extracellular ice formation and the accompanying stresses, rather than changes in the temperature or nature of ice formation. Neither the means by which plant cells tolerate a freezing stress nor the mechanisms of temperature acclimation and deacclimation have been established.

Parenchyma cells within bark and xylem tissues exhibited distinct responses to subzero temperatures. Likewise, the strategies used to increase cold hardiness in each tissue was different. Acclimation in bark cells involved increasing tolerance to extracellular ice formation and the accompanying stresses, whereas acclimation in xylem parenchyma cells involved lowering the nucleation temperature of the intracellular solution. Despite these apparently dissimilar strategies, a comparative ultrastructural study demonstrated that both cell types exhibited analogous seasonal changes in cell fine structure (Wisniewski and Ashworth, 1986). In both tissues, a gradual breaking up of large vacuoles into smaller ones, an increase in the proportion of cytoplasm, and the movement of the nucleus to the central portion of the cell were observed. Changes in membrane structure were also noted. Plasmalemma infoldings, multivesicular bodies, and complex membrane aggregates were associated with the cell membrane during both acclimation and deacclimation. These observations suggested that alterations in cell membrane and wall properties occurred during these periods. It was not clear whether the observed changes were directly associated with establishing levels of cold hardiness or whether they simply reflected changes associated with dormancy that enable cells to function at low temperatures (Wisniewski and Ashworth, 1986). However, the observations in the aforementioned study do illustrate that despite the obvious differences in cellular response to subzero temperatures, bark and xylem tissues presumably share many common features as well.

RESPONSE OF FLOWER BUDS

The pattern of freezing in dormant flower buds was different than that observed in woody tissues. In buds, freezing was initiated within the bud scales and the tissues subtending the developing floral organs (Ashworth, 1982; George et al., 1974; Sakai, 1979; Quamme, 1978). Ice crystals did not propagate throughout the bud tissue, but instead were segregated into localized sites. Examination of frozen buds revealed large ice crystals within the scales and the lower portions of the subtending axis tissue (Ashworth et al., 1989; Dorsey, 1934; Quamme, 1978; Wiegand, 1906). No evidence of ice formation was observed within the developing floral organs at temperatures above the lethal level.

Formation of ice crystals within the bud axis and scales resulted in the formation of a water potential gradient within the bud tissues (Sakai, 1979; Quamme, 1978). Water withdrew from the developing floral organs and pedicel region and migrated to the growing ice crystals within the bud axis and scales (Sakai, 1979; Quamme, 1978). The segregation of ice crystals into discrete regions appeared to be critical for bud survival. Sakai (1979) has termed this pattern "extraorgan freezing".

The formation of large ice crystals within the bud scales and axis tissue created large intercellular voids within the tissue and disrupted adjacent cells (Ashworth et al., 1989). A large split in the transverse plane was observed midway up the subtending bud axis. In addition, voids were created within the bud scales. In both cases, cells adjacent to the wounds were disrupted; however, this injury

did not appear to reduce the viability of the bud. Evidence of mechanical disruption persisted, and upon refreezing, ice apparently formed in the same locations, such that the original wounds were reopened. Increased mechanical injury was noted as winter progressed and buds were exposed to repetitive freeze-thaw cycles (Ashworth et al., 1989).

In the dormant buds of many woody tree species, including apple and pear (Sakai, 1979; Wiegand, 1906), water appeared to be withdrawn from the developing floral organs and to migrate to the growing ice crystals within the scales and axis. These buds underwent extensive dehydration, such that ice formation within the developing floral organs was not observed even at the lethal temperature. When killed, these buds were apparently killed by excessive dehydration rather than freezing per se. This pattern of freezing was characteristic of buds from the most cold-hardy species. Presumably, these species have developed mechanisms to tolerate the extreme levels of dehydration observed during extraorgan freezing.

In other species, including grape (Pierquet et al., 1977), blueberry (Biermann et al., 1979), and several *Prunus* spp. (Quamme, 1974), only a portion of the water within the developing floral organs was withdrawn during extraorgan freezing and the remainder persisted as a supercooled liquid. DTA studies of dormant peach flower buds detected two distinct freezing events (Ashworth, 1982; Quamme, 1978). The first one occurred a few degrees below 0°C and corresponded to the freezing of water within the bud axis and scales. The second freezing event, often referred to as the low-temperature exotherm, corresponded to the freezing of a supercooled fraction of water and was closely correlated to the temperature at which the developing floral organs were killed (Quamme, 1978). The developing floral organs apparently had no tolerance for ice formation, and once freezing was initiated within the floral organs, ice spread rapidly and the tissue was killed as a unit (Quamme, 1978). In species having multiple flowers within a single bud, such as sour cherry, sweet cherry, and azalea, individual florets froze as independent units within the same bud. DTA of these buds revealed multiple low-temperature exotherms, each corresponding to the lethal freezing of an individual floret (Graham and Mullin, 1976; Quamme, 1974).

The mechanism by which water in dormant flower buds supercooled when ice was present in adjacent tissues has not been established. Several features of dormant buds that facilitate supercooling have been identified. These included structural (Ashworth, 1982; George et al., 1974), physiological (Ashworth, 1982; Biermann et al., 1979; Quamme, 1978), and morphological features (Ashworth, 1982, 1984). Presumably the capacity to deep supercool was a composite of these and other features. It also was not clear what characteristics of the bud limited the extent of supercooling and therefore limited hardiness. The supercooled water within peach buds always froze well before the homogeneous ice nucleation temperature (Quamme, 1974, 1978), yet the factor responsible for initiating ice formation within the floral organs has not been identified.

As flower buds broke dormancy and resumed development in the spring, the capacity to deep supercool was lost (Ashworth, 1982) and cold hardiness progressively diminished (Proebsting, 1963). Eventually, floral tissues completely lost the capacity to survive subfreezing temperatures and were killed as soon as ice formation was initiated. The distribution of ice crystals in frozen buds also changed during deacclimation. Large ice crystals had still formed within the bud scales and subtending axis tissues, but evidence of ice crystals within the developing floral organs was also noted. Large ice crystals formed predominantly in the lower portions of the developing flower and peduncle and caused a separation of the epidermal layer from adjacent cells (Ashworth et al., 1989). It has been proposed that morphological changes during spring bud development lead to the loss of the capacity to supercool and the change in the freezing pattern (Ashworth, 1982, 1984). Further research will be required to test this hypothesis adequately.

CONCLUSIONS

The response of deciduous fruit tree tissues to subzero temperatures is both varied and complex. Plants have evolved various strat-

egies to cope with low temperatures and, in the case of deciduous fruit trees, different tissues within the same plant respond differently. The mechanisms by which plant cells respond to a freezing stress are influenced by the unique physiological and anatomical features of the tissue. Overlaying this complex process are cyclic transitions in cold hardiness as plants acclimate and reacclimate seasonally. One can become impressed with the elegant means by which plants adapt and respond to low temperatures. Unfortunately, the resistance expressed is not sufficient for our needs, and means for increasing plant freezing resistance are desirable.

Literature Cited

- Ashworth, E.N. 1982. Properties of peach flower buds which facilitate supercooling. *Plant Physiol.* 70:1475-1479.
- Ashworth, E.N. 1984. Xylem development in *Prunus* flower buds and the relationship to deep supercooling. *Plant Physiol.* 74:862-865.
- Ashworth, E.N. and F.B. Abeles. 1984. Freezing behavior of water in small pores and the possible role in the freezing of plant tissues. *Plant Physiol.* 76:201-204.
- Ashworth, E. N., G.A. Davis, and M.E. Wisniewski. 1989. The formation and distribution of ice within dormant and reaclimated peach flower buds. *Plant, Cell & Env.* 12:521-528.
- Ashworth, E. N., P. Echlin, R.S. Pearce, and T.L. Hayes. 1988. Ice formation and tissue response in apple twigs. *Plant, Cell & Env.* 11:703-710.
- Ashworth, E. N., D.J. Rowse, and L.A. Billmyer. 1983. The freezing of water in woody tissues of apricot and peach and the relationship to freezing injury. *J. Amer. Soc. Hort. Sci.* 108:299-303.
- Becwar, M. R., C. Rajashekar, K.J. Hansen Bristow, and M.J. Burke. 1981. Deep undercooling of tissue water and winter hardiness limitations in timberline flora. *Plant Physiol.* 68:111-114.
- Biermann, J., C. Stushnoff, and M.J. Burke. 1979. Differential thermal analysis and freezing injury in cold hardy blueberry flower buds. *J. Amer. Soc. Hort. Sci.* 104:444-449.
- Burke, M. J., L.V. Gusta, H.A. Quamme, C.J. Weiser, and P.H. Li. 1976. Freezing and injury in plants. *Annu. Rev. Plant Physiol.* 27:507-528.
- Dorsey, M.J. 1934. Ice formation in the fruit bud of the peach. *Proc. Amer. Soc. Hort. Sci.* 31:22-27.
- George, M.F. 1983. Freezing avoidance by deep supercooling in woody plant xylem. Preliminary data on the importance of cell wall porosity, p. 84-95. In: D.D. Randall, D.G. Blevins, R.L. Larson, and B.J. Rapp (eds.). *Current topics in plant biochemistry and physiology*. Univ. of Missouri Press, Columbia.
- George, M.F. and M.J. Burke. 1977. Cold hardiness and deep supercooling in xylem of shagbark hickory. *Plant Physiol.* 59:319-325.
- George, M. F., M.J. Burke, H.M. Pellett, and A.G. Johnson. 1974. Low temperature exotherms and woody plant distribution. *HortScience* 9:519-522.
- George, M. F., M.J. Burke, and C.J. Weiser. 1974. Supercooling in overwintering azalea flower buds. *Plant Physiol.* 54:29-35.
- Graham, P.R. and R. Mullin. 1976. The determination of lethal freezing temperatures in buds and stems of deciduous azalea by a freezing curve method. *J. Amer. Soc. Hort. Sci.* 101:3-7.
- Hong, S. and E. Sucoff. 1980. Units of freezing of deep supercooled water in woody xylem. *Plant Physiol.* 66:40-45.
- Hong, S., E. Sucoff, and O.Y. Lee-Stadelmann. 1980. Effect of freezing deep supercooled water on the viability of ray cells. *Bet. Gaz.* 141:464-468.
- Levitt, J. 1980. *Responses of plants to environmental stresses*. Academic, New York.
- Lovelock, J. 1953. Haemolysis of human red blood cells by freezing and thawing. *Biochem. Biophys. Acta.* 10:414-426.
- Mazur, P. 1969. Freezing injury in plants. *Annu. Rev. Plant Physiol.* 20:419-448.
- Meryman, H.T. 1971. Osmotic stress as a mechanism of freezing injury. *Cryobiology* 8:489-500.
- Pierquet, P., C. Stushnoff, and M.J. Burke. 1977. Low temperature exotherms in stem and bud tissues of *Vitis riparia* Michx. *J. Amer. Soc. Hort. Sci.* 102:54-55.
- Proebsting, E.L. 1963. The role of air temperature and bud development in determining hardiness of dormant Elberta peach fruit buds. *Proc. Amer. Soc. Hort. Sci.* 83:259-269.
- Quamme, H.A. 1974. An exothermic process involved in freezing injury to flower buds of several *Prunus* species. *J. Amer. Soc. Hort. Sci.* 99:315-318.
- Quamme, H.A. 1976. Relationship of the low temperature exotherm to apple and pear production in North America. *Can. J. Plant Sci.* 56:493-500.

- Quamme, H.A. 1978. Mechanism of supercooling in overwintering peach flower buds. J. Amer. Soc. Hort. Sci. 103:57-61.
- Quamme, H. A., P.M. Chen, and L.V. Gusta. 1982. Relationship of deep supercooling and dehydration resistance to freezing injury in dormant stem tissues of 'Starkrimson Delicious' apple and 'Siberian C' peach. J. Amer. Soc. Hort. Sci. 107:299-304.
- Quamme, H. A., C. Stushnoff, and C.J. Weiser. 1972. The relationship of exotherms to cold injury in apple stem tissue. J. Amer. Soc. Hort. Sci. 97:608-613.
- Quamme, H. A., C.J. Weiser, and C. Stushnoff. 1973. The mechanism of freezing injury in xylem of winter apple twigs. Plant Physiol. 51:273-277.
- Sakai, A. 1979. Freezing avoidance mechanism of primordial shoots of conifer buds. Plant Cell Physiol. 20:1381-1390.
- Steponkus, P. 1984. Role of the plasma membrane in freezing injury and cold acclimation. Annu. Rev. Plant Physiol. 35:543-584.
- Wiegand, K.M. 1906. Some studies regarding the biology of buds and twigs in winter. Bot. Gaz. 41:373-424.
- Wisniewski, M. and E.N. Ashworth. 1985. Changes in the ultrastructure of xylem parenchyma cells of peach (*Prunus persica*) and red oak (*Quercus rubra*) in response to a freezing stress. Amer. J. Bot. 72:1364-1376.
- Wisniewski, M. and E.N. Ashworth. 1986. A comparison of seasonal ultrastructural changes in stem tissues of peach (*Prunus persica*) that exhibit contrasting mechanisms of cold hardiness. Bot. Gaz. 147:407-417.
- Wisniewski, M., E.N. Ashworth, and K. Schaffer. 1987a. The use of lanthanum to characterize cell wall permeability in relation to deep supercooling and extracellular freezing in woody plants: I. Intergeneric comparisons between *Prunus*, *Cornus* and *Salix*. Protoplasm 139:105-116.
- Wisniewski, M., E.N. Ashworth, and K. Schaffer. 1987b. The use of lanthanum to characterize cell wall permeability in relation to deep supercooling and extracellular freezing in woody plants: II. Intrageneric comparisons between *Betula lenta* and *Betula papyrifera*. Protoplasm 141:160-168.
-