Current state of cold hardiness research on fruit crops

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Palonen, P. and Buszard, D. 1997. Current state of cold hardiness research on fruit crops. Can. J. Plant Sci. 77: 399–420. This article gives an overview of the current state of cold hardiness research in fruit crops by reviewing the recently published studies on cold hardiness of both tree fruit and berry crops. Topics discussed include cold hardiness of fruit species, cultivars and different plant organs, biophysical and biochemical aspects of hardiness, evaluation of hardiness, as well as endogenous, cultural and environmental factors affecting cold hardiness in these species. Lack of cold hardiness is a major limiting factor for production of fruit crops in many regions of the world and improved cold hardiness one of the major objectives in numerous breeding programs and research projects. Screening cultivars or selections for cold hardiness is commonly done, and different methods applied to the evaluation of hardiness are discussed. The physical limit of deep supercooling may be a restricting factor for expanding the production of some fruit crops, such as Prunus species and pear. As for biochemical aspects, a relationship between carbohydrates and cold hardiness is most commonly found. Studies have also been made on different hardiness modifying cultural factors including rootstock, crop load, raised beds and application of growth regulators. The latter seems promising for some species. Cold hardiness is an extremely complex phenomenon and understanding different mechanisms involved is critical. Since hardiness is, however, primarily affected by genotype, developing cold-hardy fruit cultivars and effective screening methods for hardiness are essential. Finally, cultural practices may be improved to further enhance hardiness.

Key words: Berries, cold hardiness, fruits, small fruits, stress, winter hardiness


Mots clés: Petits fruits, résistance au froid, fruits de verger, stress, rusticité hivernale

Abbreviations: ABA, : DN, day neutral; DTA, differential thermal analysis; EC, electrical conductivity of diffused electrolytes; FDA, fluorescein diacetate; ENSO, El Niño/Southern Oscillation; INA, ice nucleation active; LTE, low temperature exotherm; NMR, nuclear magnetic resonance; PNA, Pacific/North America; r-PNA, reverse Pacific/North America; RFO, raffinose family oligosaccharides; TTC, tetrazolium chloride test
Lack of cold hardiness is a major limiting factor for production of fruit crops in many regions of the world. Low-temperature injuries cause serious economic losses. In Canada, major losses of tender fruit trees are estimated to occur once or twice every 5–10 yr depending on type of fruit and location (Brown and Blackburn 1987). Injuries to fruit trees are associated with either low fall or winter temperatures which kill trees or fruit buds, or with late spring frosts during bloom.

Cold hardiness of plants is a complex phenomenon, and is affected by temperature, daylength and conditions of a plant, such as maturity, water content, nutritional stage, physiological age and dormancy status (Stushnoff 1972). The maximum intensity of cold hardiness alone is not often crucial for survival. Time and rate of development of hardness, retention of hardness, time and rate of dehardening and ability to reharden, all contribute to winter hardiness and survival of a plant. This also makes evaluation of cold hardiness difficult. Research papers often deal with only one aspect of cold hardness, such as mid-winter hardness, but the early and late stages of cold acclimation are equally important. Also, breeders should pay attention to this when screening new plant material for cold hardiness.

Improved cold hardiness is one of the major objectives in numerous breeding projects throughout the world. Instead of relying on natural selection following test winters, breeders use artificial cold stress selection methods to accelerate breeding processes. Many breeding programs have been successful in developing new cold hardy cultivars; hardy blueberries and grapes bred in Minnesota are an example (Luby 1991). The availability of cold hardy genotypes is crucial for success in breeding. Germplasm maintenance and evaluation, as well as collection and evaluation of wild germplasm is of great importance.

Management and cultural techniques have been developed which allow the production of sensitive crops in some cold areas, although the risk of injury is ever present. In the long term, breeding new cultivars with improved hardness offers the best hope of success. Thus, it is important to evaluate the complex processes of hardening and frost resistance, and the mechanisms involved.

This paper aims to give an overview of the current state of cold hardiness research in fruit crops. Recently published studies on cold hardiness of tree fruit and berry crops including apple, pear, peach, nectarine, cherry, brambles, strawberry, blueberry, cranberry, currants and grape are reviewed. The emphasis is on more recent research but older studies have been included where they make a significant contribution.

GENERAL

The phenomenon of deep supercooling occurs in many deciduous fruit crops, such as apple, apricot, blackberry, blueberry, cherry, grape, peach, pear, plum and raspberry (Quamme 1991). Most of these crops exhibit deep supercooling in xylem tissues, and many in flower buds. When temperature is further lowered, the deep supercooled water finally freezes resulting in lethal injury to the tissue. Freezing of water releases heat causing a low temperature exotherm (LTE), which can be detected by differential thermal analysis (DTA). Xylem hardness can be estimated by DTA. Also, in dormant buds of many fruit species, the initiation point of the LTE indicates lethal injury to flower primordia. The flower buds of apple and pear are an exception; they do not exhibit a LTE. However, vegetative bud hardness and bark hardness, which are considered to be more crucial to survival, can not always be determined by thermal analysis. Limited capacity to deep supercool restricts the range of many fruit crops. Given the current desire to expand the production of these crops into colder regions, an improved knowledge of the mechanisms involved in deep supercooling is desirable (Quamme 1991).

Ashworth and Wisniewski (1991) described how various tissues of deciduous fruit trees respond to low temperatures. Low-temperature responses vary between different tissues within the same plant. Xylem tissues exhibit deep supercooling while bark tissue undergoes extracellular freezing. Despite these different survival strategies both cell types exhibit analogous seasonal changes in cell fine structure. In flower buds, extra-organ freezing occurs; ice crystals grow within the bud scales and the lower portion of axis tissue, but no ice is observed within developing floral organs. In apple and pear, water is withdrawn from floral organs to the ice crystals within the scales and axis, and buds are extensively dehydrated. In other species, such as grape, blueberry and several Prunus species, only a part of water is withdrawn, and the remaining undergoes supercooling.

RESEARCH TECHNIQUES

Studying cold hardiness of woody plants is complicated, because freeze injury occurring in the field usually only becomes visible in spring when growth commences. A range of different methods can be used to evaluate injury after artificial freezing in controlled conditions. According to Stushnoff (1972), the most frequently used methods are 1) regrowth test, 2) visual rating of injury, 3) electrical conductivity of diffused electrolytes (EC), 4) colour reaction tests, such as tetrazolium chloride test (TTC), 5) impedance measurement of intact tissues, and 6) exotherm analysis including DTA. Chlorophyll fluorescence measurement of leaves has also been used (Brennan and Jeffries 1990).

The reliability of these methods depends on plant species and experimental conditions. They often give slightly different lethal temperature (LT50) estimates. Visual rating of injury and EC of diffused electrolytes are the most frequently used methods for freeze injury assessment. The usefulness of the TTC test is limited. One of the newer methods, electrical impedance measurement, appears promising and has the advantage that it can be used without freezing the plant material (Coleman 1989; Privé and Zhang 1996). Chlorophyll fluorescence measurement may provide a screening method for spring frost hardness, as has been reported for black currant by Brennan and Jeffries (1990).

In some cases, lethal temperatures cannot be estimated using LTEs detected in DTA. For example, LTEs in blueberry stems are associated with xylem injury, but none of the LTEs is associated with injury to bark, which occurs at a much higher temperature (Quamme et al. 1972b).
Controlled freezing tests may also have a pitfall: blueberry flower primordia do not supercool under natural conditions, but water is withdrawn from the floret (Flinn and Ashworth 1994). LTEs are, however, observed at faster cooling rates in artificial freezing, and are probably caused by the experimental procedure rather than reflect natural conditions.

As an aid to studying freezing in a controlled environment, Wample et al. (1990) described a computerized system that controls freezing rate at variable rates of temperature decline and can collect, store and analyze data from multiple samples in LTE analysis. Wisniewski et al. (1990) described a microcomputer-based DTA system. Different freezing rates can be produced by programming aluminium block heat sinks independently. Each sink contains five samples. Exotherms from both floral buds and xylem tissue can be detected by the system. Khanizadeh (1991) described an application of a computer software program for temperature regulation in a controlled environment. It can control and record the temperature of the environment at selected time intervals. According to Brennan et al. (1993), nuclear magnetic resonance (NMR) imaging could be used to study the freezing events in black currant flowers.

According to Lindén et al. (1996), logit models offer a useful alternative for analysis of qualitative freeze survival data. Major benefits are that LT50 is easily estimated with confidence intervals, and that the effects of explanatory variables can be evaluated using model coefficients and odds ratios.

APPLE (*MALUS × DOMESTICA*)

Apple trees (*Malus × domestica* Borkh.) suffer frequently from cold winter temperatures. In both British Columbia and eastern Canada, severe freezes that kill apple trees or lower yields, occur every 5–7 yr (Coleman 1992; Hall and Quamme 1994). A severe winter in 1980–1981 caused significant apple tree losses in eastern Canada. For example, in Quebec about 25–30% of the apple trees either died or were severely injured (Brown and Blackburn 1987). The most common low-temperature injury to apple trees is blackheart injury, browning of the xylem tissue.

1. Cold Hardiness

1.1. Cold Hardiness of Species, Cultivars and Rootstocks

In South Sweden, frost damage to 129 apple cultivars was studied after a severe frost in April, which occurred when apple flower buds were in the early tight cluster stage (Nybom 1992). Variation in injury was almost negligible within cultivars, whereas between cultivars large variations were observed. Early-flowering cultivars were more seriously damaged than late-flowering ones. Interestingly, a relationship was found between frost hardiness of flower buds and country of origin. Winter hardiness estimation of cultivars from other studies reportedly did not correlate with the bud injury data from this study. Flower bud hardiness does not seem to correspond to hardiness of woody tissue. Thus, cultivars that are extremely hardy in a continental climate, may be subject to severe flower bud injury in late spring frosts of more variable maritime climates.

Imperial Red Mac/Antonovka was the hardiest and Imperial Red Mac/MM.111 the most tender cultivar rootstock combination among nine apple cultivars studied in New Brunswick by Coleman (1985). In March, LT50 for xylem tissue of apple cultivars Antonovka and Samo grown in Southern Finland were –46 and –43°C, respectively (Lindén et al. 1996). Hardening treatments failed to increase the resistance of either cultivar, but dehardening at –14°C decreased the resistance by 12–15°C. The authors concluded that both cultivars were close to their maximum possible hardiness.

In a study of Embree (1988), both root and trunk hardiness were greatest in rootstocks M.26, Ottawa 3 (O.3) and Alnarp 2 (A2). Also, root hardiness was good in rootstocks Budagovsky 118 (B.118), Budagovsky 490 (B.490) and a Polish rootstock (P.1), and trunk hardiness in Beautiful Arcade. According to Quamme (1990), M.26 rootstock is harder than M.9, whereas Callesen (1994) reported that they are equal in hardiness, but both agree that M.26 is harder than MM.106. According to Aaltonen (1995), the most commonly used apple rootstocks in Finland, A2 and Ylönien Piikkiö (YP), do not differ in winter hardness, both having an acceptable hardiness.

Root hardiness of several apple rootstocks and trunk hardness of a tender and a hardy cultivar were compared by Embree and McRae in 1991. Root survival did not differ between the scions, but root regrowth of the tender cultivar Gravenstein was greater than that of the hardy cultivar Wealthy. Trunk survival was dependent on the rootstock. The results provide some evidence for the occurrence of reciprocal effects; that the rootstock may have an effect on trunk hardness and the scion an effect on root hardness. Cultivars Sturdeespur Delicious and Empire with M.9 interstem had less scion and interstem wood injury on MM.111 rootstock than on Antonovka and Ottawa 11 rootstocks (Domoto 1986). Frost damage evaluated as tip die-back of branches was worse on trees grafted on MM.106 rootstock than on those grafted on M.9 or M.26 rootstocks (Callesen 1994). Blackheart injury of Starkspur Supreme Delicious apple trees growing on different rootstocks in different locations in North America was evaluated by Warmund et al. (1996). Generally, trees on B.9, P.2, P.16 and P.22 were more susceptible to blackheart injury than those on B.490, MAC.1, C.6 and MAC.39.

1.2. Cold Hardiness of Plant Parts and Organs

Bud and bark tissues of apple are harder than xylem in mid-winter, but in early autumn and late spring xylem and pith are hardier than buds and bark (Quamme et al. 1972a). Xylem becomes injured at –35 to –40°C, which is related to the initiation of LTE and to minimum temperatures at Northern limits of commercial production (Quamme 1976). Injury occurs in the shoot interior and spreads radially outwards, the secondary xylem being most cold resistant (Ketchie and Kammerreck 1987). Even if more than 50% of the xylem exhibits blackheart injury, it is possible for a tree to recover (Warmund et al. 1996). In the fall, hardening proceeds from terminal shoots downwards; thus the lower part of the trunk is the last part to harden and the least cold tolerant part of the tree.
2. Physiology of Cold Hardiness

2.1. Biophysical aspects of cold hardiness

Apple stem xylem parenchyma and pith cells avoid freezing by deep supercooling, whereas bark tissue and flower buds do not deep supercool (Quamme 1976, 1991; Ashworth et al. 1988; Malone and Ashworth 1990). Cell collapse next to voids formed by extracellular ice crystals was observed in apple bark tissue, but did not occur in xylem ray parenchyma or pith tissues (Ashworth et al. 1988; Malone and Ashworth 1990). Cell collapse throughout the bark was not uniform (Ashworth et al. 1988). The reduction in cell volume following cell collapse was greater in cortical cells than in the cells in periderm, phloem and cambium. Ristic and Ashworth (1995) used freeze substitution and TEM to study the response of apple xylem ray parenchyma to freezing stress. Two distinct responses were observed: formation of intracellular ice or fragmented protoplasm with indistinguishable cell ultrastructures, but there was no evidence of intracellular ice formation. Apparently, injury to xylem can be caused either by intracellular ice or by cavitation.

Vertucci and Stushnoff (1992) studied the interaction between sensitivity to desiccation and the presence of unfreezable water in acclimating vegetative apple buds. Buds were most hardy in January, and this characteristic was associated with maximum tolerance to desiccation. The amount of unfrozen water correlated with hardness. In buds that survived −45°C, the intracellular water content was reduced, but sufficient moisture content to avoid desiccation damage was maintained. Vertucci and Stushnoff (1992) concluded that hardening of vegetative apple buds includes an increase in tolerance to dehydration and an increase in the quantity of unfreezable water in the cell. According to Quamme et al. (1982a), the dehydration resistance in apple bark was not uniform (Ashworth et al. 1988; Stushnoff et al. 1993), cold hardness in Red Delicious apple cortical tissues and buds correlated with sorbitol, total sugars and raffinose family oligosaccharides (RFO), but contrary to Raese et al. (1978), not with sucrose. Khanizadeh et al. (1989, 1992) studied the effect of crop load on hardiness, carbohydrate, protein and amino acid content of apple flower buds. The absence of fruit in the previous year increased the cold hardiness of buds. Water-soluble reducing sugar content increased in apple flower buds from July to April-May, and starch increased during cold acclimation and decreased during late winter and early spring. Deblossomed trees had higher contents of glucose, sorbitol and starch in flower buds, than normally cropped trees (Khanizadeh et al. 1989). Also, the contents of hydrophilic and acidic amino acids from October to April were found to be higher in the flower buds of non-cropped trees (Khanizadeh et al. 1992).

Coleman et al. (1992) studied the effects of temperature on cold hardiness and carbohydrates separately by using plant growth regulators that alter the cold hardiness and carbohydrate status of apple shoots. A mixture of paclobutrazol, thidiazuron and flurprimidol was used. Treatment generally increased the hardness of shoots, but the contents of sucrose or sorbitol in bark or wood were not related to hardness levels. Air temperature had a clear effect on shoot hardiness, but to a much lesser degree on carbohydrate contents. Results indicate that the temperature effects on sorbitol or soluble sugar contents and on hardness may be independent, and can be experimentally separated by applying growth regulators.

When in vitro MM.106 apple shoot cultures were grown on media with elevated concentrations of sucrose (3–14%), they had reduced shoot moisture content and were up to 6°C harder than control shoots (Caswell et al. 1986). Whether hardening was caused by enhanced sucrose uptake of plants or by osmotic stress, was not determined.

3. Evaluating Cold Hardiness

Ketchie et al. (1972) found a close correlation between electrolyte leakage and survival of apple seedlings after natural frost. Apple bud survival was evaluated by visual observation, forcing and grafting by Spotts and Chen (1984). Test results from all these methods agreed to within 2.9°C. However, the forcing method for viability testing after
freezing is generally preferred. Root hardiness of apple rootstocks and trunk hardiness of cultivars were compared using two methods, tissue examination and regrowth measurement by Embree and McRae in 1991. The regrowth measurement used less labor and was found to be more objective than tissue examination. Calculating the ratio of discoloured xylem to total xylem area on a weight basis, was found to be a useful method in comparing the amount of blackheart injury among apple trees grafted on different rootstocks (Warmund et al. 1996).

Four exotherms were detected in apple twigs (Quamme et al. 1972a). The fourth exotherm arose from the xylem and pith tissues and was associated with lethal injury to xylem ray and pith parenchyma (Quamme et al. 1973, 1982a). Ketchie and Kammercek (1987), also observed four exotherms, but according to them, the third exotherm arose from the xylem and indicated injury to xylem. The third and fourth exotherms emerged during maximum hardiness in midwinter.

Electrical impedance measurement can be used to predict hardiness in apple wood throughout the winter period without freezing the material (Coleman 1989). By detecting changes in the cell wall fluids, electrical test reflects changes in membrane permeability of complex tissues. Privé and Zhang (1996) compared TTC, vital staining, EC and electrical impedance (Z) in assessing freezing injury of Beautiful Arcade field vs. cold stored roots. Electrical impedance was found to have the best ability to detect changes in cell viability with increasing cold stress, TTC having the worst. Z provided more detailed information than EC or TTC about the response of the roots to cold stress, and it may also provide some insight into freeze-thaw history before injury assessment.

### 4. Factors Affecting Cold Hardiness

#### 4.1. Endogenous

Cold hardiness of apple buds depends on the stage of dormancy (Spotts and Chen 1984; Coleman 1985). According to Ketchie (1985) cold resistance of xylem and bark of apple trees is related to both temperature and physiological stage, while cold resistance of leaves is more related to age than any other factor. Late-maturing cultivars suffered more damage in Eastern Canada during a severe winter in 1980–1981 than early-maturing ones (Brown and Blackburn 1987). The absence of fruit in the previous year was found to increase the cold hardiness of apple buds (Khanizadeh et al. 1992).

#### 4.2. Cultural and environmental

Growing Lobo and Transparent Blanche apple trees on raised beds covered with black plastic improved their winter survival, probably because of the improved moisture conditions and soil temperature compared with flat beds (Säkö and Laurinen 1986). Quamme and Brownlee (1989) reported that apple trees closest to sprinklers suffered less winter injury to roots than those more distant from sprinklers. Probably low temperatures developed in dry regions of the soil because of its lower heat capacity, resulting in root injury.

Hall and Quamme (1994) discussed the climate effects on apple trees in the Okanagan Valley, British Columbia. The relationships of temperature, precipitation and winter freezes to Pacific/North America (PNA) and El Niño/Southern Oscillation (ENSO) weather patterns were investigated. The PNA weather pattern is pronounced only in winter. Also a reverse PNA (r-PNA) pattern may occur. The ENSO is the best known teleconnection in the world. An r-PNA pattern combined with either a neutral or a cold event ENSO was related to the occurrence of fall and winter freezes. The authors concluded that the risk for winter freezes is low during ENSO warm events and high during an r-PNA pattern.

Kaukovirta and Syri (1985) analysed winter injury to apple trees in Finland during this century, and Coleman (1992) in New Brunswick, in an attempt to identify the environmental variables that lead to winter injury, and to provide a classification that would allow winter injury to be predicted. In both studies, the most important factor affecting the occurrence and severity of winter injury was mild weather during mid-winter, especially February, followed by low temperatures. According to Coleman (1992), low temperatures itself in December, January and February did not cause injury, whereas Kaukovirta and Syri (1985) concluded that severe frosts during November and December destroy flower and leaf buds and prolonged spells of severe frost injure xylem tissue. Also higher than average mean air temperatures in October, during the hardening-off process contributed to the incidence of injury in New Brunswick (Coleman 1992).

Mildew infected terminal apple buds were less hardy than healthy buds (Spotts and Chen 1984). This may be due to drying of bud scales in infected buds, which may influence their freezing pattern.

#### 4.3. Growth regulators

Cold hardiness of apple trees can be influenced by growth regulators. Autumn foliar application of 2000 ppm paclobutrazol increased the fruit set of Vista Bella apple, and may be used for spring frost protection (Kolodziejczak and Hamer 1985). Alar-85 and Dupont surfactant WK reduced freezing injury to Spur Mac/MM.111 apple, even though effects were not consistent from year to year (Coleman and Estabrooks 1985). Thidiazuron with or without VaporGard applied in September increased cold hardiness of Spur Mac/MM.106 apple trees (Coleman and Estabrooks 1988). A mixture of paclobutrazol, thidiazuron and flurprimidol greatly increased hardiness of shoots throughout the fall, winter and spring. The pattern of seasonal changes in hardness level was not affected, although treated shoots were less sensitive to temperature fluctuations during the winter period (Coleman and Estabrooks 1992).

**PEAR (PYRUS SPP.)**

Due to its limited cold hardness, pear cultivation is restricted to middle regions of North America (Quamme 1976). The nucleation temperature of supercooled water in pear xylem is related to minimum temperatures in the northern limit of pear production (Quamme 1976, 1977). Physical
limits of deep supercooling may impose a physiological barrier to improving cold hardiness of pear and the expansion of its production.

1. Cold Hardiness

1.1. Cold hardiness of species, cultivars and rootstocks

According to Granger (1982), it would be possible to establish a local pear industry in Quebec, Canada using cold resistant cultivars with acceptable commercial value, such as Flemish Beauty, Clapp’s Favorite, Miney, Parker and Guayot. The most cold hardy cultivars tested in Quebec were Gaspar and Moe (Granger and Rousseau 1984). Cultivars Flemish Beauty and Harrow (HW) 601 were very hardy at one test location but only fairly hardy at the other. The cultivars Beurre d’Anjou, O. 291, Kentville 14-24-15, Phileson, O. 301, HW 606, and Harvest Queen were also hardy. Flemish Beauty has, however, been found to be susceptible to spring frost injury (Lamb 1982). Early-blooming pear cultivars tend to be more severely injured by spring frost, although wide differences between cultivars within date-of-full-bloom classes occur (Lamb 1982).

1.2. Cold hardiness of plant parts and organs

In apple, xylem is the most susceptible tissue in pear trees, followed by bark and buds (Quamme 1976). Fruit buds of Abbé Fétel pear were investigated after a cold spell (below –20°C) in January in Italy by Filleti and Neri (1989). The number of tannin cells, and cells with calcium oxalate crystals in fruit buds was increased by frost. Cellular lysis was observed in the pith. Injuries extended to the spur at the bud base and parenchyma rays, as well as the bark and the border layers of phloem. Lamb (1982) found that pistils were the most commonly injured organs, when survival of pear blossoms in the “bud burst” to “green cluster” stage was studied after a –5°C frost.

2. Physiology of Cold Hardiness

2.1. Biophysical aspects of cold hardiness

Pear flower buds do not deep supercool (Quamme 1991). Xylem exhibits deep supercooling and the initiation of LTE in fully hardened twigs is –33 to –38°C, which corresponds to annual temperature minima at northern limits of commercial pear production (–28.9 to –34.4°C) (Quamme 1976, 1977). In stems of P. nivalis (Jacq.), P. cordata ((Desv.) Schneider) and P. elaeagnifolia (Pall.), supercooled water was found in both xylem and bark tissues (Rajashekar et al. 1982). LTEs detected in flower buds of some Pyrus species were likely to arise from the stem tissues associated with the buds.

2.2. Biochemical aspects of cold hardiness

When suspension-cultured pear (P. communis Bartlett) cells were cold acclimated at 2°C, changes in soluble extracellular polysaccharides occurred (Wallner et al. 1986). Extracellular accumulation of a relatively small, neutral polysaccharide was increased, and a larger molecular weight pectic polysaccharide decreased. The decrease in a pectic polysaccharide may have been due to inhibition of synthesis at low temperatures or to low-temperature-induced degradation. Also, the deposition of callose in the cell wall was observed, which may stabilize this region against stress induced disturbances.

3. Evaluating Cold Hardiness

Ketchie et al. (1972) found a close correlation between electrolyte leakage and survival of pear seedlings evaluated visually by tissue browning. According to Quamme (1976), the LTE detected in DTA indicates injury to the xylem. Montano et al. (1987) also reported that the initiation of the first LTE corresponded with accuracy of ±3°C to the temperature of incipient injury to the xylem in tests.

4. Factors Affecting Cold Hardiness

4.1. Cultural and environmental

In Bartlett pear trees, bloom delay through evaporative cooling reduced hardiness of flower buds and flowers, compared with controls at the same floral development stages (Strang et al. 1980). Montesinos and Vilardell (1989) pointed out the important role of Pseudomonas syringae in frost susceptibility of pear trees in Catalunya. Forty percent of the P. syringae strains isolated from buds were ice nucleation active (Montesinos and Vilardell 1991). Mean nucleation temperatures (NT90) of detached flower buds were dependent on population levels of P. syringae ranging from –2.7 to –6°C. According to Proebsting and Gross (1988), however, the population of ice nucleation active (INA) bacteria has no influence on frost injury to pear fruit buds, flowers or fruits, when buds are attached to the stem, because the intrinsic wood-associated ice nucleus is a source of ice nucleation at about –2°C.

4.2. Growth regulators

In a study by Ketchie and Murren (1976), polyvinylpyrrolidone K-30, glycerol, ethylene glycol and dimethyl sulphoxide in several concentrations and combinations, applied by terminal feeding, failed to increase the frost resistance of a hardy pear cultivar d’Anjou, but increased the resistance of the less hardy cultivar Bartlett.

PEACH, NECTARINE, APRICOT AND CHERRY (PRUNUS SPP.)

The most susceptible tissues of Prunus species avoid freezing by deep supercooling, and are injured by temperatures that are closely related to the average minimum temperatures at the northern limits of their distribution (Quamme et al. 1982b). The degree of deep supercooling may be a limiting factor for northern commercial production of these species. In Ontario, low temperatures cause some bud kill in peach (Prunus persica [L.] Batsch.) every winter (Brown and Blackburn 1987). Wood damage occurs less frequently and is usually significant only in temperatures below –25°C. An article on breeding cold hardy peaches and nectarines by Layne (1992) gives a good overview of cold hardiness in these species.

1. Cold Hardiness

1.1. Cold hardiness of species, cultivars and rootstocks

Arora et al. (1992) studied the seasonal pattern of cold hardiness in bark and xylem in related deciduous and evergreen
peach genotypes. Deciduous trees acclimated sooner, and in a fully dormant state, the bark and xylem of deciduous trees were almost 30 and 20°C harder, respectively, than the bark and xylem in evergreen trees.

Twenty-four peach and fourteen nectarine cultivars were screened for hardiness after a temperature drop from 5 to –11°C within 12 h on 2 November in Oklahoma (Smith et al. 1994). Fantasia and Sunfire nectarines were least injured. Three nectarine cultivars, Armking, Mayfire and Sungem were killed by the freeze. Of all the peach cultivars evaluated, Sentinel had the least damage, and Sam Houston was the only peach cultivar of which all the trees were killed. New peach cultivars Harson, Harrow Beauty and Harcrest, developed in a breeding program in Ontario, are equal or superior to Redhaven in flower bud and xylem hardiness (Layne 1989).

Flower bud hardiness in peach is influenced by rootstock through its influence on both timing and rate of flower bud development. Redhaven peach bud survival was greatest on Citation and Damas rootstocks, and lowest on GF655-2 and Amandier rootstocks among eight rootstocks studied by Brown and Cummins (1988). The influence of seven rootstocks on flower bud hardiness of Redhaven peach was evaluated during 2 yr by Durner (1990a). Flower bud survival was influenced by rootstock in both years but marketable yield was affected in only 1 yr. Siberian C rootstock was superior to all others in both years. The rootstock has an influence on both the time and the rate of flower bud development in peach (Durner and Goffreda 1992). In some cultivars, delayed bud development and late bloom avoided spring frost injury and resulted in an increased yield, while in other cultivars, no relationship between bloom delay and spring frost injury was found. Harber et al. (1992) found that rootstock affected the dormancy status of Redhaven peach slightly. However, effects on dormancy were not consistent with effects on cold hardiness.

For cherry, *P. mahaleb* (Mahaleb) rootstocks are usually harder than *P. avium* (Mazzard) (Westwood 1993). Howell and Perry (1990) found sweet cherry (*P. avium* L.) scion cultivars on the rootstock Colt (*P. pseudocerasus × P. avium*) less hardy than on either Mazzard or M × M 39 (Mazzard × Mahaleb). Scion cultivars on M × M 39 rootstock were harder than on Mazzard or Mahaleb rootstocks. However, the influence of scion cultivar may still be more important; cultivar Gold was ranked as hardest when grown on Mazzard and Colt. According to Strauch and Gruppe (1985), Colt was more frost sensitive and responded more readily to dehardening treatments in February than other genotypes.

1.2. Cold hardiness of plant parts and organs

Flower buds and xylem are the most frost sensitive tissues in *Prunus* species (Quamme et al. 1982b). Cold hardiness of peaches and nectarines was evaluated following a test winter by Layne (1982). There was no correlation between flower bud and xylem hardiness, indicating that cold hardiness of these tissues may be inherited independently. However, for 27 apricot (*P. armeniaca* L.) genotypes, flower bud hardiness seemed to correlate with shoot xylem hardiness (Layne and Gadsby 1995). On average, shoot xylem in midwinter was harder (LT50 = –35.3°C) than flower buds (LT50 = –28.7°C).

2. Physiology of Cold Hardiness

2.1. Biophysical aspects of cold hardiness

Freezing injury to xylem and flower buds of *Prunus* species is avoided by deep supercooling (Quamme 1974, 1978; Ashworth 1982; Quamme et al. 1982b; Ashworth et al. 1983). Bark tissues freeze extracellularly, and injury to bark is caused by extracellular ice formation and cellular hydration (Ashworth et al. 1983). The population of INA bacteria did not affect the temperature at which ice formed in peach shoots (Ashworth et al. 1985), flower buds, flowers or fruits of several *Prunus* species (Proebsting and Gross 1988). An intrinsic non-bacterial wood-associated ice nucleus, which is the primary source of ice nucleation and limits supercooling to about –2°C, has been found in stem tissue of several *Prunus* species (Gross et al. 1984; Ashworth et al. 1985; Proebsting and Gross 1988).

In peach flower buds, ice is formed in the bud scales and bud axis, but not in the flower primordium (Quamme 1978). It is proposed that the cuticle or epidermis prevents ice nucleation from the surface of the flower primordium, and a dry region at the base prevents ice from spreading into the primordium from the bud axis. In deacclimated peach flower buds exposed to –5°C, ice crystals were also observed within the developing floral organs (Ashworth et al. 1989). Deep supercooling in *Prunus* flower buds is related to vascular development (Ashworth 1982, 1984; Ashworth and Rowe 1982; Callan 1990). In supercooling buds, procambial cells have not yet differentiated into xylem vessel elements. Thus, the lack of xylem continuity between the primordium and the adjacent tissues is the morphological feature that allows deep supercooling. When xylem continuity is established during dehardening, buds lose their ability to deep supercool.

In Redhaven and Siberian C peaches, whole bud moisture content was not related to supercooling capacity (Quamme 1983). This is in accordance with findings of Warmund and George (1990) for brambles. Instead, the water content of the flower primordium and vascular traces was related to the level of supercooling. Water migrated from these tissues to bud scales and even to the exterior of the bud during freezing, and returned after thawing. However, at the same water content, the hardy cultivar Siberian C supercooled to lower temperatures than the less-hardy cultivar Redhaven. Also in sweet cherry, the ability to deep supercool was related to the water content of flower primordia and to a preceding minimum air temperature (Andrews and Proebsting 1987). The peach flower primordium in dormant buds has a lower osmotic potential than other parts, possibly as a consequence of high sucrose levels (Quamme and Gusta 1987). Lower water potential of the flower primordium may be involved in the recovery of water content of flower bud after freezing.

The extent of deep supercooling of living and dead flower buds of several *Prunus* species (almond (*P. dulcis* Mill.), peach, apricot (*P. armeniaca* L.) and cherry) was examined...
by Kadir and Proebsting (1993). When flower buds were killed by freezing, the DTA response of the dead flower primordia remained normal for more than 2 wk after the killing frost, indicating that dead flower primordia retained their ability to supercool. When living buds started to swell, the ability of dead buds to deep supercool was decreased and gradually lost. The authors concluded that when a dormant flower primordium is killed by freezing, the growth of ice crystals is prevented, because the tissue water remains compartmentalized.

The number of LTEs in sweet cherry selections and the cultivar Bing did not always correspond to the number of florets, the percentage of LTE per primordium being 75 to 90% (Kadir and Proebsting 1994). Due to earlier bud development in spring, flower primordia of tender selections ceased to supercool first. An increase in the LTE temperature, and an increased variation in LTE temperatures within a bud have been observed in deacclimating flower buds of sweet cherry (Andrews and Proebsting 1987), and sour cherry (P. cerasus L.) (Callan 1990). In sour cherry, all primordia within a bud ceased to deep supercool simultaneously (Callan 1990). Callan (1990) associated flower primordium deep supercooling after deacclimation with flower bud endodormancy, because deacclimated flower primordia ceased to deep supercool earlier when chill unit accumulation was accelerated.

According to Andrews et al. (1983), a frost-tolerant period occurs in peach and sweet cherry buds after the ability to supercool is lost during early bud swell in spring until petal tip emergence. During that period it is possible to reduce freezing injury by increasing the ice nucleating temperature. This was done either by wetting peach flower buds or by inoculating sweet cherry buds with INA P. syringae bacteria.

Despite their different mechanisms of cold hardiness, xylem parenchyma and cortical cells in peach twigs showed similar ultrastructural changes associated with changes in cold hardiness (Wisniewski and Ashworth 1986). During acclimation and deacclimation, plasmalemma infoldings and complex membrane aggregates were observed. During the period of maximum hardiness in midwinter, cells had a centrally located nucleus, many small vacuoles and no starch grains.

Schaffer and Wisniewski (1989) studied the development of an amorphous layer in the xylem of apple and peach (deep supercooling) and willow (extracellular freezing). They concluded that compositional differences may exist in this amorphous layer, which leads to the different freezing responses of these species. Wisniewski et al. (1991) studied the effect of externally applied cell-wall-degrading enzymes on the freezing profile and structure of the pit membrane and amorphous layer, and the relationship between these portions of the cell wall and the ability to deep supercool in current year twigs of peach. Macerase, an enzyme mixture rich in pectinase, caused a nearly complete digestion of the pit membrane and partial degrading of the underlying amorphous layer. Deep supercooling was almost completely prevented. Cellulysin caused a digestion of only the outermost layer of the pit membrane and reduced the ability to deep supercool. When a sodium phosphate buffer was applied, a very slight decrease in the ability to deep supercool was observed. The authors concluded that the pit membrane together with the underlying amorphous layer form a barrier to water movement and growth of ice crystals, which is a prerequisite for deep supercooling, and that pectins may regulate the permeability of that portion of the cell wall.

2.2. Biochemical aspects of cold hardiness

The total sugar and soluble protein contents in peach buds are correlated with frost resistance (Lasheen et al. 1970; Burak and Eris 1992), as also reported for apple (Raese et al. 1978; Khanizadeh et al. 1989, 1992; Stushnoff et al. 1993). Low total free amino acids were also associated with hardiness (Lasheen et al. 1970). Highest total lipid levels occurred in February and were concurrent with the lowest air temperatures and the highest bud survival of the peach cultivars (Burak and Eris 1992).

In related deciduous and evergreen peach genotypes seasonal changes in protein contents were quite similar in the two genotypes (Arora et al. 1992). Cold hardiness was highly correlated with seasonal fluctuations of three proteins (19, 70 and 80 kDa) in xylem of both genotypes. A 19-kDa polypeptide accumulated in bark of both genotypes during fall and decreased in spring, but the accumulation was less in evergreen trees. The accumulation of a 16-kDa protein was observed during fall in the deciduous genotype but not in the evergreen type.

3. Evaluating Cold Hardiness

The LTE detected in the stem tissue of 13 Prunus species and interspecific hybrids was closely related to xylem injury (Quamme et al. 1982b). According to Kadir and Proebsting (1994), the DTA was consistent with visual field observations of hardiness of sweet cherry genotypes and can thus be used for screening populations for floral bud hardiness. The possibility of LTE artifacts from dead flower primordia may affect the cold hardiness estimations of field collected samples and should be taken into account (Kadir and Proebsting 1993). Arora et al. (1992) found a close correlation between LTE and LT50 assessed by electrolyte leakage in related deciduous and evergreen peach genotypes.

4. Factors Affecting Cold Hardiness

4.1. Endogenous

Byrne (1986) studied the reasons for the superior fruit set of TEXITAR peach under spring freeze conditions. Large numbers of flowers, long bloom period and late bloom were the factors contributing to spring freeze injury avoidance. Differences in tissue moisture content explained about one-half of the constant hardiness difference between sweet cherry selections, the other half being associated with unknown factors (Kadir and Proebsting 1994).

4.2. Cultural and environmental

According to Seeley et al. (1992), defoliation of trees in late summer inhibited flower bud hardening and reduced endodormancy intensity, whereas prolonged leaf retention in the autumn increased the dormancy intensity and extend-
ed the dormant period. Harber et al. (1992) found that rootstock, time of pruning and soil fumigation affected the dormancy status of Redhaven peach slightly. However, effects on dormancy status were not consistent with effects on cold hardiness. Pruning peach trees after rest completion in January reduced flower bud hardiness, because pistils from pruned trees did not reharden after a thaw (Durner 1990b). Whitewashing entire peach trees in January reduced the size of pistils, delayed bud development, enhanced flower bud hardiness during bloom and enhanced yield, but did not affect bud hardiness in winter (Durner and Gianfagna 1992).

Lu and Rieger (1993) studied the effect of temperature preconditioning on freezing tolerance of fully opened peach flowers. The ovaries of cultivars Junegold and Loring trees grown in a cold regime were slightly harder than those of trees grown in a warm regime. No differences in hardiness of ovaries between cold-grown and warm-grown cultivar Redhaven trees were detected. While leaves and stems of cold-grown Junegold peach were more than 3°C harder than those of warm-grown trees, the difference in the LT50 of ovaries was only 0.38°C.

Bloom delay through evaporative cooling increased the hardiness of peach buds until early March, but decreased the hardiness and the number of viable buds by late March (Bauer et al. 1976). Byers and Marini (1994) found that blossom thinning and fruit thinning of peach trees to moderately light crop densities can enhance the cold tolerance of flower buds in late winter. This is in agreement with the findings of Khanizadeh et al. (1992) for apple. In peach, there was a negative relationship between the percent live buds after a spring freeze and crop load in the previous season (Byers and Marini 1994). Blossom thinning was more efficient in enhancing cold tolerance than hand-thinning trees 38 d after full bloom.

Cytophthora canker caused by Leucostoma personii had a negative effect on cold hardiness in peach (Chang et al. 1989).

4.3. Growth regulators
Numerous attempts have been made to increase cold hardiness of peach by application of growth regulators. Ethephon applied in the fall increases the hardiness of peach buds, delays bloom and enhances yield (Durner and Gianfagna 1988; Gianfagna et al. 1989). Ethephon enhances flower bud hardiness and prolongs dormancy of flower buds by increasing the chilling requirement, thus delaying late winter deacclimation and bloom in spring (Durner and Gianfagna 1991a). When dormant peach flower buds were treated with ethephon, buds on ethephon-treated trees grew more slowly than buds in control trees. Ethephon increased the ability of pistils to supercool, increased the number of buds that supercooled after deacclimation treatment and also retarded deacclimation. These effects were associated with increased pistil sorbitol and sucrose contents, reduced moisture content, pistil size and growth rates during deacclimation following ethephon application (Durner and Gianfagna 1991b).

Fall application of ethephon (100 ppm) to peach trees delayed bloom by 6 d, and whitewashing entire trees in January delayed bloom by an additional 1 to 2 d (Durner and Gianfagna 1990, 1992). Flower bud hardiness on ethephon-treated trees was slightly enhanced. In February, the mean LTE was –18.5°C for treated and –17.7°C for non-treated trees, but by March, no effects on hardiness were detected. The whitewashing of trees in January delayed pistil elongation in buds from non-ethephon-treated trees, but had no effect on ethephon-treated buds. Ethephon treatment did not prevent dehardening of pistils of trees pruned after rest completion in January, but it facilitated their rehardening after a thaw (Durner 1990b).

Gianfagna (1991) compared the effect of LAB 173711, a compound with ABA-like activity, and ethephon on time of flowering and cold hardiness of peach flower buds. In greenhouse experiments, LAB 173711 delayed bloom more effectively, when it was applied after a dormancy-breaking treatment. Ethephon was more effective when applied before the chilling requirement was satisfied, and caused flower bud abscission, if applied after the chilling requirement was complete. In field experiments, LAB 173711 delayed flowering by 2–3 d, but this was not long enough to avoid spring frost injury. Ethephon delayed flowering by 6–7 d, and no flower bud injury occurred on treated trees at –4.3°C, while 25% of flower buds in control trees were damaged at this temperature. According to Blanco (1990), paclobutrazol treatment advanced bloom the year after treatment, and at the same time enhanced the flower frost hardness resulting in almost a doubling of yield. Prebloom dormant oil application had no positive effects on peach flower bud hardiness, on the contrary; apparently because of decreased blossom hardiness, yield was reduced when compared to the control (Durner and Gianfagna 1992).

Seeley et al. (1992) reported that NAA delayed leaf senescence and increased Johnson Elberta peach flower bud hardness, while no hardness increase was observed for either Montmorency cherry or Redhaven peach. Decreased flower size may account for the increased cold hardness after NAA treatment. However, GA treatment also increased hardness to some extent although it did not affect flower size. NAA treatment applied to one side of the tree only prolonged leaf retention on the treated side, but affected the hardness of the entire tree. Results provide evidence for the existence of a translocatable cold-hardiness promoter produced in leaves under hardening conditions. Dormex (calcium cyanamid) did not enhance flower bud hardness of peach (Durner and Gianfagna 1988). The effectiveness of two commercial low-temperature protectants, Frost-Free (Plant Products, Vero Beach, FL) and Vapor Gard (Miller Chemical & Fertilizer, Hanover, PA) was examined (Aoun et al. 1993). Neither chemical was found to provide frost protection for peach flowers or developing fruits.

**BRAMBLES (RUBUS SPP.)**
Ideal conditions for red raspberry are cool summers and moderate winters. Despite this, raspberry is grown widely in different parts of the world. In colder climates, some loss of canes or flower buds usually occurs during winter (cane dieback), and injury to buds near the snow line is common (Brown and Blackburn 1987). Because of their late bloom,
raspberries do not usually suffer from spring frosts. Primocane fruiting cultivars avoid winter injury problems. Blackberry is more susceptible to cold than raspberry. Blackberry canes are injured at temperatures below −23°C (Moore and Skirvin 1990).

1. Cold Hardiness

1.1. Cold hardness of species and cultivars

Winter hardiness of 11 red raspberry cultivars was tested in Finland during a 4-yr trial (Dalman et al. 1991). Cultivar Ville, which is a cross between cultivar Ottawa and a Finnish wild raspberry strain (*Rubus idaeus* L.), proved to be the hardest, followed by the Canadian cultivars Ottawa, Muskoka and Boyne. The Scottish cultivars Glen Isla and Glen Clova were the most cold sensitive. Red raspberry cultivars and selections, including eight indigenous Norwegian *Rubus idaeus* L. populations, were screened for cold tolerance in Norway (Nestby 1992). In this study, the indigenous *R. idaeus* selections were no more hardy than the Norwegian cultivars and selections, so they can not be considered as a useful source of potential germplasm for freeze tolerance.

Hummer et al. (1995) screened more than 80 raspberry and 42 blackberry genotypes for cold hardiness with controlled laboratory freezing in January. Most red raspberries were found to be harder than black raspberries (*R. occidentalis* L.) or blackberries (*R. allegheniensis* Porter, *R. ursinus* Cham & Schldl.), while purple raspberries (*R. neglectus* Peck.) were intermediate between red and black raspberries. Burnetholm and Canby were the hardest red raspberry cultivars, their canes having the LT50 values of −34°C and −30°C, respectively. Brandywine and Royalty were the hardest purple raspberry cultivars. The hardiest blackberry cultivar in this study was Black Satin. Its canes had the LT50 value of −23°C and buds −19°C. Warmund and George (1990) found that among 11 blackberry (*Rubus subgen. Eubatus*) cultivars, floral buds of Darrow were found to be most hardy, with 45% of the primordia in primary buds surviving −33°C in January. In the study of Hummer et al. (1995) Darrow buds only survived −13°C. Among 10 red raspberry (*R. idaeus* L.) cultivars studied by Warmund and George (1990), Canby and Chilliwack had good primary bud hardiness in January, and they retained their hardiness longer than other cultivars, which may be associated with their longer rest requirement.

Zatylny et al. (1993) studied the cold hardness of red raspberry in vitro. Tissue cultured red raspberry plantlets were acclimated before controlled freezing. As also reported by Warmund et al. (1989b), the apical meristems were found to be the most cold hardy, and the older leaves the least cold hardy, when freezing injury was assessed by visual rating of tissue browning. Lowest survival temperatures varied from −12.5°C to −16.0°C for acclimated shoots, and from −11.0°C to −11.5°C for nonacclimated. Acclimated shoots exhibited varietal differences, which correspond to their known relative hardiness in the field. Cultivars in order of decreasing hardiness were Boyne, Gu72, Gu63 and Comox.

1.2. Cold hardness of plant parts and organs

In most *Rubus* genotypes studied by Hummer et al. (1995), canes were about 2 to 15°C hardier than buds. Usually the bud base was less hardy than the bud tissues inside the scales. According to Doughty et al. (1972), the vascular tissue at the base of the buds is the most susceptible tissue. Secondary buds of red raspberry and blackberry were found to be hardier than primary buds (Warmund and George 1990). In Shawnee blackberry, phloem tissue was injured at warmer temperatures than xylem (Warmund and George 1989). When the freezing tolerance of micropropagated *Rubus* plants grown in a greenhouse was tested, it was found that older leaves were more susceptible to injury than younger leaves (Warmund et al. 1989b). The apical meristem was the hardest plant part surviving at least −8°C.

1.3. Genetics of cold hardness

When offspring from 12 raspberry cultivars were examined in Norway, cultivars Zeva 1, Schönemann 3-6, and II-2LPG gave rise to the highest number of winterhardy progenies (Redalen 1982). In breeding *Rubus* species in Finland, the gene pools of wild raspberry (*R. idaeus* L.) have been exploited to obtain winter hardy selections (Hiiransalmi 1989). Cold hardiness of 12 seedling populations of complex hybrids of tetraploid blackberries was evaluated by Bourne and Moore (1992). Significant population effects were noted for xylem, phloem and bud cold hardiness. Seedling populations having cultivar Darrow as a parent were generally hardy. Populations having cultivar Brison as a parent were less hardy, except one population resulting from a cross between cultivars Brison and Darrow, which had consistently good hardiness. The authors conclude that cold hardiness is inherited quantitatively, but that dominance effects may also be involved in determining the cold hardiness of blackberries.

2. Physiology of Cold Hardiness

2.1. Biophysical aspects of cold hardiness

According to Kraut et al. (1986), in Smoothstem and Dirksen blackberries, stem tissue does not supercool. However, Warmund and George (1989) found that Shawnee blackberry stem xylem tissue can avoid freezing by supercooling. The floral primordia in primary buds of both raspberries and blackberries are known to survive freezing by supercooling (Kraut et al. 1986; Warmund et al. 1988, 1992; Warmund and George 1989, 1990). Supercooling has also been observed in some secondary buds of Dirksen black and Reveille red raspberry (Warmund and George 1990). Warmund et al. (1992) examined extracellular ice formation in differentiating buds of eastern thornless blackberry. Large extracellular ice crystals did not form in the inflorescence axis or in developing floral primordia. Instead, extracellular voids formed by freezing were observed in the scales surrounding the inflorescence and the bud axis. Among 10 red raspberry and 11 blackberry cultivars, whole-bud moisture content was not related to supercooling capacity, except in cultivar Nordic, which had very large LTEs when water content was high (Warmund and George 1990).

Only a single freezing event was observed in floral buds of Dirksen blackberry (Kraut et al. 1986). When Darrow blackberry buds were at an early stage of development, only
one LTE was detected, but when floral differentiation progressed, individual primordia froze independently and as many as 10 LTEs could be detected (Warmund et al. 1988). However, only one to four LTEs were detected in overwintering Black Satin and Hull thornless blackberry buds (Warmund et al. 1992) and one to three LTEs in Shawnee blackberry buds (Warmund and George 1989), indicating that several flower primordia froze simultaneously in buds that had multiple primordia. The possible explanation for different freezing characteristics may be that xylem development within the raceme of blackberry affects the freezing characteristics of floral buds (Warmund and George 1989) as is the case in *Primus* (Ashworth 1984).

3. Evaluating Cold Hardiness

Tissue browning was found to be a better screening method for freeze injury evaluation of red raspberry in midwinter than bud growth, which is more accurate and recommended in late winter when dormancy is extinguished (Nestby 1992). The field evaluation of freeze injury in May by observation of tissue browning is a convenient method and less time consuming than laboratory regrowth tests, but is subject to climatic fluxes.

The LTEs detected in primary buds of blackberry (*Rubus* subgen. *Eubatus*), and red raspberry (*Rubus idaeus* L.) cultivars are correlated with freezing injury, but the number of LTEs and the number of differentiated floral primordia seldom correspond (Warmund and George 1989, 1990). The hardiness of red raspberry buds determined from LTE values did not always correspond to earlier data about cultivars’ performance in the field (Warmund and George 1990). A LTE detected in stem tissue of Shawnee blackberry estimated the temperature of xylem injury with accuracy of ±3.2°C, but bark injury was not associated with a LTE (Warmund and George 1989).

4. Factors Affecting Cold Hardiness

4.1. Endogenous

In Cherokee blackberry, rest was found to play a major role in the deacclimation of both generative and vegetative tissues (Warmund et al. 1989a). During dormancy, floral primordia were harder and did not deacclimate as rapidly in 16°C as after cold treatment was fulfilled. Phloem and xylem were also harder before rest completion than after. In phloem the rate of deacclimation was slow regardless of the stage of rest, whereas in xylem, deacclimation rate increased after rest was completed.

In red raspberry, early growth cessation is positively correlated with winter hardiness (Jennings et al. 1972; Säkö and Hiirsalmi 1980). In three eastern thornless blackberry cultivars, the retention of leaves in the fall was negatively correlated with bud survival (Kraut et al. 1986). However, premature defoliation reduces photosynthetic activity and photoinduction responses, and decreases mid-winter hardness of stem tissue (Kraut et al. 1986), and bud tissue by reducing sugar and starch reserves (Doughty et al. 1972). Jennings and Cormack (1969) observed that premature defoliation prevented the water content of raspberry canes from dropping to the low level of untreated plants.

4.2. Cultural and environmental

Cultural practices that reduce vegetative growth may offer a possibility to improve winter survival of raspberry canes. Chemical primocane suppression early in the season improved winter survival of Festival, Latham and Newburgh raspberries, which may be due to the reduced length of primocanes (Buszard 1986). Cane thinning treatments were also found to affect winter survival advantageously; winter dieback seemed to correlate with cane density. The effect of different levels of broadcast and fertigated nitrogen and raised beds on yield and freeze injury of Veten red raspberry was studied by Nestby and Kongsrud (1993). Fertilization resulted in better exploitation of nitrogen leading to higher leaf nitrogen content and increased primocane height. Freeze tolerance was negatively correlated with cane growth and with leaf nitrogen content. As also reported for apple (Säkö and Laurinen 1986), raspberry plants on raised beds were found to suffer less winter injury than plants on flat beds, which could be an effect of the higher air volume in the soil and thus improved root conditions. Root rot (*Phytophtora fragariae var. rubi*) infection had no effect on cold hardiness of buds or canes in Willamette raspberry (Bristow and Hummel 1993).

4.3. Growth regulators

Only a few of the attempts to improve cold hardiness of raspberries or blackberries by using growth regulators have proved successful. Alar (N-dimethylamino succinic acid) (1000 ppm and 2000 ppm) reduced cane dieback of Latham and Trent raspberries, early application in June or July being more effective than later application in August (Granger and Hogue 1968). Succinic acid-2,2-dimethylhydrazide applied as 1000 and 2000 ppm sprays during growing season had no effect on bud survival or yield of Trent and Canby raspberries (Craig and Aalders 1973). ABA and benzyladenine increased the regeneration ability of Malling Promise canes after frost injury (Zraly 1978).

Veten red raspberries treated with 1500 ppm paclobutrazol had an earlier growth cessation, leaf fall and entered the dormant state early; however, paclobutrazol had little or no effect on winter injury (Maage 1986). Ethephon (1500 ppm) or ethephon with CaCl$_2$ (2%) also hastened leaf abscission of three eastern thornless blackberry cultivars, but had no effect on shoot dieback or yield (Kraut et al. 1986). Mefluidide spray (5 mg L$^{-1}$) did not enhance cold hardiness of 7-cm-tall micropropagated Shawnee blackberry plants grown in a greenhouse and exposed to controlled freezing (Warmund et al. 1989b).

**STRAWBERRY (Fragaria × ananassa)**

Strawberry (*Fragaria × ananassa* Duch.) is a semihardy evergreen and has relatively low tolerance to cold temperatures. It is widely grown despite its low cold tolerance, and is usually protected from cold by snow or other protective mulches, such as straw to enable it to survive the winter in colder climates (Brown and Blackburn 1987). Frost damage occurring during bloom is also a common problem.
1. Cold Hardiness

1.1. Cold hardiness of species and cultivars
Flower frost tolerance of 21 strawberry cultivars varied across a range of 3°C, and was not related to the origin of the cultivar (Ourecky and Reich 1976). Everbearing strawberry blossoms were found to be 2°C hardier in October than in September or during bloom in June (Boyce and Marini 1978). In June, the hardiness of blossoms was equal in both everbearing and June bearing cultivars. The hardiness of mother and daughter crowns of Redcoat strawberry were compared under field conditions and controlled freezing conditions (Turner et al. 1993). Crown placement in the soil may affect cold hardiness. Daughter plants were consistently harder than mother plants under field conditions, but in controlled freezing tests the mother plants were as hardy or harder than the daughter plants.

Exposure of Catskill strawberry plants to nonlethal low temperatures caused abnormal growth of leaves, early emergence of runners and browning of crown tissue (Marini and Boyce 1979). Blossom numbers were also decreased and flowering delayed. Bolduc and Paquin (1987) also reported that flowering may be affected by low nonlethal temperatures. Low temperature decreased total yield and fruit size in Elsanta runner plants lifted in late winter, although no visible frost injury symptoms were observed (Zurawicz and Dominkowski 1993).

1.2. Cold hardiness of plant parts and organs
Vascular tissue in a strawberry crown is the most resistant to freezing injury and most crucial to plant survival, cortex and medulla tissues being the most susceptible (Marini and Boyce 1977). In Redcoat and Bounty strawberries, vegetative buds were 3.8°C hardier than flower buds in January (Paquin et al. 1989). In September and May, vegetative and flower buds possessed equal levels of hardiness. Fruits are more susceptible to low temperature damage than flowers or buds (Ourecky and Reich 1976).

Ki and Warming (1992) studied the susceptibility to low temperature injury of styles, anthers and receptacles of Earliglow and Honeoye strawberry flowers. Styles and receptacles were found to be more susceptible to cold than anthers. Styles and receptacles of primary flowers were more susceptible than those of tertiary flowers. Anthers in primary flowers were more susceptible than in secondary or tertiary flowers.

2. Physiology of Cold Hardiness

2.1. Biophysical aspects of cold hardiness
Warmund (1993) characterized the distribution of extracellular ice in Earliglow strawberry crowns and tissue recovery after exposure to sublethal temperatures. Crown structure was disrupted temporarily at –5°C, but plants were not permanently injured. Voids formed by extracellular ice near the base of peduncle and vascular system remained in the tissue after thawing. After 15 wk incubation in a greenhouse, only very few and small voids were observed in crowns. Cell division and enlargement occurred near the voids. Due to the rosette morphology of a strawberry plant, a thermal ice propagation barrier sometimes occurs in the crown leading to independent freezing of individual leaves and flowers (Anderson and Whitworth 1993).

2.2. Biochemical aspects of cold hardiness
A relationship between strawberry crown sugar content and cold hardiness was reported by Paquin et al. (1989). Sucrose, reducing and total sugars increased in the crowns of Redcoat and Bounty strawberries during cold hardening and reached a maximum level in January, concurrently with maximum hardiness. Proline content also changed during hardening and dehardening, but the relationship with hardiness was not clear. The effect of fall fruiting on the cold tolerance of crowns of day neutral (DN) strawberry cultivars, and the importance of carbohydrate and nitrogen reserves on winter survival was studied by Gagnon et al. (1990). Cold hardiness was correlated with percent dry weight, accumulation of starch, total accumulation of carbohydrates and total nitrogen in roots. Root nitrogen content increased when fruits were removed on 15 or 30 September. Starch accumulation and total carbohydrate content were increased by removing fruits on 15 September.

3. Evaluating Cold Hardiness

According to Harris (1973), the electrical conductivity and the recovery method were both reliable in determining relative hardiness of four strawberry genotypes with stress temperature of –9°C in October, and a sequence of temperatures of –9°C, –10.5°C and –12°C in January and March. The electrical conductivity is preferred by the author, because it is quick and not affected by the differences in dormancy. Marini and Boyce (1977) reported that TTC reduction and tissue browning could both be used in evaluating strawberry crown tissue viability after freeze stress. Bolduc and Paquin (1987) suggested that evaluation of tissue browning should be combined with evaluation of regrowth and flowering to increase the reliability.

4. Factors Affecting Cold Hardiness

4.1. Cultural and environmental
The level of potassium (K) influenced significantly the hardiness of strawberry plants (Bédard and Therrien 1970). The ratio of nitrogen (N), phosphorus (P) and potassium (K) may be more important for hardiness than the absolute levels of single elements (Zurawicz and Stushnoff 1977). High P/K ratio correlated with hardiness of Redcoat strawberries in different fertilization treatments, whereas high P/N ratio did not. Plant survival had a positive correlation with P/K ratio in root and crown tissue, and a negative correlation with K/N ratio in same tissues. Nutrient deficiencies and high N with low P and K resulted in poor hardiness.

Removing fruits in September enhanced cold hardiness of the plants during the fall (Gagnon et al. 1990). When the hardiness of mother and daughter crowns of Redcoat strawberry were compared, mulching enhanced mother plant survival, but had no effect on daughter plant survival (Turner et al. 1993). Midway and Catskill strawberry plants on raised beds suffered more winter injury than plants grown on flat beds (Boyce and Reed 1983).

4.2. Growth regulators
The effect of Frost Gard (Custom Chemicides, Fresno, CA) on supercooling of strawberry plants in the presence and
absence of INA bacteria was evaluated by Anderson and Whitworth (1993). The supercooling of flowering Arking strawberry plants was not enhanced by Frost Gard, regardless of the presence or absence of INA bacteria. There was a negative linear relationship between the freezing temperatures of Frost Gard — infiltrated detached leaves and Frost Gard concentration. In leaves vacuum-infiltrated with 20% Frost Gard, the supercooling was enhanced by 1.7°C compared to control leaves.

Four cryoprotectants, Frost Free (Plant Products, Vero Beach, FL), Kocide (Griffin Corporation, Valdosta, GA), Cryoban (Great Lakes Chemical Corporation, West Lafayette, IN) and Frost Gard had no effect on the percentage of blossoms injured by low temperature, yield or berry size in Earliglow and Lateglow strawberries (Goulart and Demchak 1994).

Biological control of frost injury to strawberry blossoms has also been studied (Lindemann and Suslow 1987). Ice nucleation-deficient (INA–) strain of Pseudomonas syringae protected strawberry blossoms against freezing induced by other P. syringae strains, but was not able to protect against INA+ P. fluorescens. INA– P. fluorescens was a more effective inhibitor of P. syringae strains than of other P. fluorescens strains. Inhibition of one bacterial strain by its near-isogenic counterpart depended on dose rather than on strain. Strawberry blossoms sprayed with INA+ strain FG3 nucleated at −2°C, whereas the antagonistic strain FF1 lowered the nucleation point to −5°C (Leonardi et al. 1989). In the open field the FF1 strain could survive on sprayed plants for about 30 d.

**BLUEBERRY (VACCINIUM SPP.)**

In Northern climates, blueberry is subject to winter injury (dieback), spring frost injury to flower buds and blossoms, and also frost injury to fruit (Brown and Blackburn 1987). Highbush blueberry (Vaccinium corymbosum L.) is about as hardy as peach and may be killed by temperatures of −29°C (Westwood 1993). However, blueberry may escape frost by protective snowcover. A minimum of 30 cm of snow is necessary to insure fruit production of half-highbush blueberry cultivars in Northern Minnesota (Wildung and Sargent 1989). In 1968, in the Lac St. Jean area in Quebec, 50% of blueberry plants were destroyed because of dieback (Brown and Blackburn 1987).

1. **Cold Hardiness**

1.1. **Cold hardiness of species and cultivars**

All clones of lowbush blueberry (Vaccinium angustifolium Ait.) reached their maximum cold hardness in January or February (Cappiello and Dunham 1994). A selection of V. angustifolium and natural hybrids of V. angustifolium and V. corymbosum were found to be hardier than highbush cultivars (Quamme et al. 1972b). Southern highbush blueberry (Vaccinium spp.) and northern highbush blueberry (Vaccinium corymbosum) cultivars were found to have less winter freeze and spring frost damage in flower buds and flowers than rabbiteye (Vaccinium ashei Reade) blueberry cultivars (Patten et al. 1991).

When comparing five rabbiteye blueberry cultivars, flowers of cultivar Southland were found to be most tolerant to frost (Gupton 1983). When highbush blueberry cultivars were evaluated for their cold hardiness in Poland, all plants showed winter injury symptoms (Smolarz 1989). The hardiest cultivars were Concord, Rancocas and Weymouth. Older plants were found to be harder than younger ones.

1.2. **Cold hardiness of plant parts and organs**

In a Finnish blueberry cultivar Aron (cultivar Rancocas × (V. uliginosum L. × cv. Rancocas)), shoots were only slightly injured after a severe winter, whereas all flower buds were damaged (Hiirsimäki and Hietaranta 1989). In lowbush blueberry, stem tissue was equally or more hardy than bud tissue (Cappiello and Dunham 1994). Cold hardiness of vegetative buds was equal to that of floral ones in rabbiteye blueberry cultivars Tifblue and Woodard (Spiers 1978). Rabbiteye blueberry flower buds, formed on fall wood, were harder than buds formed on spring wood (Patten et al. 1991). Injury to flower buds increased as bud development advanced.

In highbush blueberries, V. corymbosum (Biermann et al. 1979; Hancock et al. 1987) and V. austral (Bittenbender and Howell 1976), lateral buds are harder than terminal ones. In lowbush blueberry, the two buds at the stem tip were more sensitive than buds 3 and 4 (Cappiello and Dunham 1994). Lowest survival temperatures varied from −25 to −35°C for the uppermost bud, and from −35 to −40°C for the fourth bud. Flower primordia at the stem tip also dehardened earlier and responded more readily to spring warming than flower primordia in lower buds. According to Cappiello and Dunham (1994), in major lowbush blueberry growing regions freeze damage is unlikely to occur in any other tissues than floral tissues in the uppermost bud.

In highbush blueberry, apical florets were found to be less hardy than median or basal florets within each bud (Biermann et al. 1979). Ovaries were the critical cold sensitive tissue (Bittenbender and Howell 1976). In rabbiteye blueberry cultivars, frost during bloom caused damage to pistils, thus reducing fruit set (Gupton 1983).

1.3. **Genetics of cold hardiness**

Fear et al. (1985) examined several Vaccinium species and interspecific hybrids and found that the heritability estimate for winter injury was low over years but high for individual years. In the inheritance of cold tolerance, additive variance is more important than nonadditive variance. In lowbush blueberry, general combining ability effects for winter injury were high in years with good snow cover but low in years without snow cover suggesting that lowbush blueberry clones are not inherently cold tolerant but depend on snow cover for their winter survival.

2. **Physiology of cold hardiness**

2.1. **Biophysical aspects of cold hardiness**

Blueberry xylem tissue exhibits deep supercooling (Quamme et al. 1972b). Contrasting reports exist about the ability of flower buds to deep supercool. LTEs have been observed in blueberry flower buds by Bittenbender and Howell (1976) and by Biermann et al. (1979). But according to Flinn and Ashworth (1994), blueberry flower primordia
do not supercool under natural conditions, but water is withdrawn from the floret towards ice sinks in the scales or bracts. LTEs observed at faster cooling rates in artificial freezing may be caused by the experimental procedure rather than reflect natural conditions.

2.2. Biochemical aspects of cold hardiness
In floral buds of Bluecrop and Tifblue blueberries, chilling-responsive 65-, 60-, and 14-kDa polypeptides which were identified as dehydrins or dehydrin like proteins, accumulated during cold acclimation (Muthalif and Rowland 1994). The largest increase in the level of polypeptides occurred at the same time as the largest increase in the level of cold hardiness. The maximum level of hardiness and the maximum level of chilling-responsive polypeptides was higher in Bluecrop than in Tifblue. However, chilling responsive polypeptides may also be related to the development of dormancy rather than the development of hardiness.

3. Evaluating Cold Hardiness
According to Bittenbender and Howell (1976), visual evaluation of browning of the ovary after controlled freezing at slow cooling rates successfully discriminates between hardy and susceptible blueberry plants.

LTEs in blueberry stems are associated with xylem injury, but none of the LTEs is associated with injury to bark, which occurs at a much higher temperature (Quamme et al. 1972b). Thus, DTA is not useful in determining stem hardiness of blueberry. According to Bittenbender and Howell (1976), estimating flower bud hardiness of seven highbush blueberry cultivars was less accurate by using exotherms than by using visual rating of tissue browning. According to Biermann et al. (1979) the DTA profile was rate dependent, each floret within an intact blueberry bud exhibiting one rate-dependent LTE that was correlated to lethal injury at slow cooling rates. Flinn and Ashworth (1994) found that blueberry (V. corymbosum) flower bud hardiness was not reliably estimated by the DTA in the cultivar Berkeley. The number of LTEs did not correspond to the number of injured florets. DTA profiles of excised flower buds were different from those of intact buds. The appearance of a DTA profile was also affected by the cooling rate. In attached buds cooled at 2° C per hour, no LTEs were detected.

Impedance measurement could be used to evaluate the hardiness of woody tissue in V. corymbosum blueberries but did not always detect differences in bud hardiness among cultivars (Doughty and Hemerick 1975).

4. Factors Affecting Cold Hardiness
4.1. Cultural and environmental
Blueberry shoots infected with blueberry canker caused by fungus Fusicoccum putrefaciens Shear. were more susceptible to frost than healthy shoots (Hiirsalmi and Hietaranta 1989). Because rabbiteye blueberry flower buds formed on fall wood were found to be harder than buds formed on spring wood, in southern regions it might be beneficial to enhance the production of fall wood by summer pruning after harvest (Patten et al. 1991).

4.2. Growth regulators
When cryoprotectants are applied, a protective effect of the cryoprotectant may be completely masked by a negative reaction to the surfactant (Robbins and Doughty 1980). Flowers and flower buds treated with surfactants X-77 (Chevron Chemical Company, Ortho Division, San Francisco, CA) and Surfactant WK (E. I. DuPont Wilmington, DE) were more susceptible to cold than controls. Multifilm (Colloidal Products, Petaluma, CA) had no effect.

CRANBERRY (VACCINIUM MACROCARPON)
Cranberry (Vaccinium macrocarpon Ait.) is grown in bogs, and due to its growth habit and growing sites is prone to frost injury. Plants survive in Northern climates because they are covered by water or snow in winter, and by water during frosts in the growing season. Only a few reports dealing with cold hardiness of cranberry were found. For example, there is no information about factors affecting its hardiness. Clearly, more research on cold hardiness of this quickly expanding crop is needed.

1. Cold Hardiness in General
Abdallah and Palta (1989) studied the frost resistance of Searles cranberry leaves, flower buds and fruits by controlled freezing. The lowest survival temperature of flower buds was –22°C in early November and changed sharply from –18°C in mid-April to –2°C in early to mid-May. Flowers and small fruits were only hardy to 0°C. Frost hardiness of fruits increased, as their anthocyanin content increased and chlorophyll content decreased. Leaves were the hardest plant part studied, being harder than –24°C in mid-December and losing their hardiness rapidly from late April (<–24°C) to early May (~–7°C). In native cranberry plants injured by frost in June, flower primordia development was delayed and the number of flower primordia and their final degree of development in the fall was lessened (Hall and Newbery 1972).

2. Physiology of Cold Hardiness
2.1. Biochemical aspects of cold hardiness
Larger increases occurred in non-reducing than in reducing sugars during cold hardening in cranberry leaves (Sieckmann and Boe 1978). Total sugar concentrations increased from 44 to 77 mg g–1 fresh weight in 6 d in low temperature and short day conditions. Reducing sugars increased from 22 to 33 mg g–1 fresh weight.

3. Evaluating Cold Hardiness
Only one report comparing methods to evaluate cold hardiness in cranberry was found. According to Eaton and Mahrt (1977), LTEs detected from DTA indicated greater hardiness than evaluating injury to cranberry buds visually through a dissecting microscope.

CURRANTS (RIBES SPP.)
Black currant (Ribes nigrum L.) and red currant (Ribes sativum [Rchb.] Syme) are generally very hardy and can be grown in areas with very cold winter temperatures.
However, spring frosts during bloom may sometimes be a problem.

1. Cold Hardiness in General

1.1. Cold hardness of species and cultivars

Since midwinter hardiness is not usually a problem for cultivars, it has not been a frequent subject in research. After a severe winter in Skierniwe, Poland, there was extensive flower bud damage to all currant cultivars (Gwozdecki 1989). The least damaged black currant cultivars were Karakol, Matchless, Pennylavkaya, Ri 1715, Smuglyanka and Typ 3 van Gotz. The least damaged red currants were Devinska, Velkoplodna, London Market, Neapolis, Cerveny and Pomona. Warnund et al. (1991) used the median LTE to estimate the LT$_{50}$ of red and black currant buds in Minnesota. LT$_{50}$ temperatures for buds of cultivar Red Lake in November, January and March were $-21.0$, $-18.1$ and $-22.1^\circ C$, respectively. For buds of cultivar Danka, the corresponding temperatures were $-26.7$, $-23.6$ and $-26.5^\circ C$, respectively. Black currant flower buds were able to reharden in winter (Dale and Heiberg 1984). However, this ability weakened as growth progressed. High spring temperatures caused flower buds to be less hardy at same stage of growth than low temperatures.

More research has been directed towards spring frost tolerance. Mather et al. (1980) studied differences in resistance to spring frost in an early-flowering black currant progeny and observed segregation of factors conferring frost resistance. Frost resistance was probably derived from the male parent, a selfed progeny of a Finnish wild black currant. According to Mather et al. (1980), good winter hardiness is possibly associated with spring frost resistance. Dale and Heiberg (1984) studied cultivars Baldwin, Ben Lomond and Ben More, and found a relationship between winterhardiness and spring frost tolerance, possibly because less hardy cultivars began to grow and deharden sooner. Cultivars related to Ben More and Ojebyn were tolerant to $-4.5^\circ C$ frost until after first flower. Baldwin and Magnus losing their tolerance at the grape stage, and Ben Lomond and its relatives being intermediate (Dale 1981). Consequently, cultivars should be tested for spring frost tolerance at several stages of growth. Night frosts in March and April damaged early- and midseason flowering black currant cultivars more severely than late-flowering ones (Keep et al. 1983).

The frost tolerance at flowering of 12 black currant genotypes of different origin, genetic background and times of flowering was studied in the United Kingdom (Brennan 1991). Scottish cultivars Ben Alder, Ben Tirran and Swedish cultivars Ojebyn and Stor Klas were found to be hardiest at flowering. Cultivars Baldwin and Narjadnaja were the least hardy at flowering. Narjadnaja is a derivative of Ribes nigrum sibiricum, the wild genotype regarded as a donor of winter hardiness of the vegetative parts. Contrary to the findings of Mather et al. (1980) and Dale and Heiberg (1984), Brennan (1991) concludes that the genes controlling spring frost tolerance and the genes controlling winter hardiness are not linked. In fact, using extremely hardy genotypes in breeding may increase spring frost susceptibility since, in milder climates, flowering and bud break may be advanced. This is in accordance with the findings of Jefferies and Brennan (1994). In their study, three black currant cultivars were exposed to low temperatures above freezing and low light intensity at the onset of growth. The more cold tolerant cultivar, Ben Lomond had lower base temperatures for leaf appearance and growth than the more cold sensitive cultivars Baldwin and Ben More. The primary effects of low temperature and low light intensity were reduced rates of leaf appearance and leaf expansion. The effects of low temperature on photosynthetic rate were minor. Dale (1987) found that frost tolerance at first flower was inherited additively and that Ojebyn and Goliath contributed highest parental values of tolerance.

1.2. Cold hardness of plant parts and organs

Brennan et al. (1993) used NMR imaging to study the freezing events in black currant flowers. The stigma, style and the ovarian tissues showed significantly increased signals in NMR images from freeze-damaged flowers. The water content of these parts was increased, probably as a consequence of tissue disruption after freezing. The authors conclude that stigma, style and ovarian regions are the most frost sensitive parts of the flower and the probable sites of spring frost damage leading to flower death and yield losses.

2. Physiology of Cold Hardiness

2.1. Biophysical aspects of cold hardiness

Freezing of supercooled water in the floral primordia was found to cause tissue injury in floral buds of Danka black and Red Lake red currants (Warmund et al. 1991). Floral buds had multiple, abrupt LTEs which were associated with injury to the inflorescence. As also observed in raspberry (Warmund and George 1990; Warmund et al. 1992), there was no relationship between the number of LTEs and the number of racemes or flowers per bud. Presumably several flowers froze simultaneously. Contrary to the findings of Warmund et al. (1991) and Takeda et al. (1993), Stone et al. (1993) found a significant relationship between the number of secondary exotherms resulting from lethal freezing of flower primordia and the number of racemes within black currant buds. From DTA profiles, it was concluded that the largest primordia in the base of the raceme froze first, followed by the smaller ones. Accumulation of needle ice was observed beneath the meristematic crown. The authors suggest that the crown is an ice nucleation barrier, but allows the migration of water from the primordia to facilitate their supercooling.

2.2. Biochemical aspects of cold hardiness

In Red Lake red currant, RFOs, raffinose and stachyose, were the only sugars that correlated with season-long cold hardness (Stushnoff et al. 1993).

3. Evaluating Cold Hardiness

According to Mather et al. (1980), frost room tests on single flowering nodes was economical on plant material and effective in testing genotypes with different resistance to spring frost. Injury was evaluated visually. Keep et al. (1983) found that response of buds and flowers to frost was dependent on cooling rate. A cooling rate of $-0.025^\circ C \text{ min}^{-1}$ with mini-
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Ribes assessing spring frost hardiness in black currant (Ribes nigrum L.) buds were injured (Quamme 1986). Sometimes a second LTE associated with injury to secondary buds was observed. Wolf and Cook (1994) compared DTA estimates of grape bud cold hardiness to field survival observations after a severe frost. It was found that DTA can confidently be used to estimate not only the relative hardness but also absolute cold hardness of buds. For seven of the nine cultivars, cold hardness measured by DTA corresponded well to actual bud hardness evaluated by visual examination of buds after a severe frost. In one cultivar, the differences were possibly due to primary buds having been previously destroyed by bud necrosis, which is a common phenomenon in grape. Thus, there is a possibility of misinterpreting exotherms from samples that have a significant number of previously destroyed buds.

4. Factors Affecting Cold Hardiness

4.1. Endogenous

Slater et al. (1991) determined the relationship between dormancy and winter hardness in Seyval Blanc grape buds and stem tissues. The buds did not seem to be fully dormant at any test date, but the number of days required for budbreak decreased as winter progressed. In January and February,
primary buds, phloem and xylem deacclimated when exposed to +16°C. Two warming episodes did not enhance the deacclimation of tissues more than one warming episode.

4.2. Cultural and environmental
Photoperiod influences on cold acclimation of *Vitis labruscana* Bailey and *Vitis riparia* Michx. were studied by Fennell and Hoover (1991). Short photoperiod caused reductions in growth, greater periderm development and onset of bud dormancy in both species, but only a little cold acclimation. *V. riparia* plants exposed to short days had 2 to 3°C, and *V. labruscana* 1°C greater freezing tolerance than control plants. It is generally expected that delayed harvest or leaving fruit on the vine would reduce cane and bud carbohydrate reserves and decrease bud cold hardiness, but in *V. vinifera* grapes Cabernet Sauvignon (Wample and Bary 1992), and Chardonnay and Riesling (Hamman et al. 1996), cold hardiness or carbohydrate reserves were not affected by early, late or no harvest treatments compared to normal harvest.

4.3. Growth regulators
The potential cryoprotectant activity of several chemicals on grapevines (*Vitis labruscana* Bailey) dormant buds and grape leaf tissue was determined by Himelrick et al. (1991). Chemicals included Dupont Surfactant WK (DEPEG) (DuPont de Nemours, Wilmington, DE), ethylene glycol (Fisher Chemical, Rochester, NY), BRIJ 35 (ICI Americas, Wilmington), Frost Gard (Custom Chemicals, Fresno, CA) and Frost Free (Plants Products Corp., Vero Beach, FL). Results indicated that cryoprotectant chemicals have potential to increase winter hardiness of dormant buds and developing shoots. For example, in April, BRIJ 35 and Surfactant WK were superior to others, LTEs in treated buds being 14.1°C and 12.1°C lower than in controls.

**SUMMARY**

A wide variety of different types of studies relating to cold hardiness of fruit crops have been published. *Prunus* species, especially peach, have been studied quite intensively. No studies on cold hardiness of plum were found in the literature.

Evaluation of cold hardiness of existing cultivars or new selections has been carried out in almost all the crops discussed in this paper. This information is useful for breeding programs. However, ability to withstand low winter temperatures often does not correlate with cold hardiness of flower buds in spring, as these characteristics do not seem to be controlled by the same mechanisms. Only in raspberry and apple has cold hardiness been studied in vitro, a technique which in future may offer an alternative to the time consuming field evaluations of cold hardiness.

Physical limits of deep supercooling may be a limiting factor for expanding the production of many fruit crops. Supercooling characteristics were studied in apple and pear stem tissues, stem and bud tissues of several *Prunus* species, stem and flower buds of blackberry, raspberry and blueberry, and in flower buds of currants and grapes. Usually the DTA was found to offer an estimation of cold hardiness in these tissues. Contradictory reports occur about the reliability of DTA in estimating cold hardiness of blueberry buds. Interestingly, in *Prunus* species, dead flower primordia were found to deep supercool, and their DTA response remained normal after a killing freeze.

Biochemical changes occur in plants during cold acclimation. The relationship between contents of some substances and cold hardiness has been studied in apple, pear, peach, strawberry, blueberry, cranberry, currant and grapes. Total sugars are related to season-long hardness clearly in apple, where sorbitol makes up the bulk of total sugar and is related to hardness. Total carbohydrates were associated with increase in hardness also in peach, strawberry, cranberry and grape. Raffinose and stachyose are also related to hardness. Contents of hydrophilic and acidic amino acids were associated with increase in hardness in apple buds, soluble protein contents and low total free amino acids in peach buds, and 65-, 60-, and 14-kDa polypeptides in blueberry buds. Total lipids in peach buds reached the maximum in midwinter. In strawberry, total nitrogen in roots correlated with cold hardiness, whereas, in raspberry there was a negative relationship between frost tolerance and leaf nitrogen.

Cold hardiness is determined primarily by genotype, but may be enhanced by different methods of management. The effects of cultural practices were studied in most of the crops discussed in this paper. Rootstock was found to have an effect on scion cultivar cold hardiness in apple, sweet cherry and peach. Heavy crop load had a negative effect on hardiness in both apple and peach. Whitewashing of trees provided protection to peach flower buds during spring. Raspberry plants and apple trees on raised beds suffered less winter injury than plants on flat beds, but for strawberry, the effect was reverse. Removing fruits in the fall enhanced winter survival of DN strawberry cultivars but had no influence on hardiness of grapevine.

Application of growth regulators is one possible way of enhancing cold hardiness. Understanding mechanisms by which growth regulators affect the plant hardness level would be important for further development of these techniques. The effects of growth regulators vary between plant species and even between cultivars. Paclobutrazol, thidiazuron and flurprimidol enhanced the cold hardiness of apple, while ethephon seems to have many beneficial effects on peach cold hardiness, including increased flower bud hardness during bloom. Also, paclobutrazol enhanced peach flower frost hardiness. NAA had a positive effect in some peach cultivars, but not in others. Several cryoprotectant chemicals had a positive effect on grape cold hardiness. Controlling INA bacteria populations in apple, pear and *Prunus* species does not seem to protect them from frost injury, since the intrinsic ice nucleus in the stem is still present.

Much research has been done on cold hardiness of fruit and berry crops. However, cold hardiness is an extremely complex phenomenon; species and cultivars vary in their response to low temperatures depending on location and other environmental factors. Since genotype is the most critical factor in determining hardness, developing cold hardy cultivars for different climatic conditions is essential. To
speed and facilitate long-term breeding the possibility of evaluating cold hardiness in laboratory conditions, such as screening for cold hardiness in vitro, should be studied carefully, and new techniques that could provide more rapid results must be developed. These may include chlorophyll fluorescence and electrical impedance. This would reduce the time-consuming and expensive field testing of selections. Cultural practices have a minor effect on cold hardiness but can be used to further enhance hardiness, once the appropriate cultivar is available.


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