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PHYSIOLOGIC AND GENETIC STUDIES ON WOOD HARDINESS
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PHYSIOLOGIC AND GENETIC STUDIES ON WOOD HARDINESS OF PEACHES (PRUNUS PERSICA (L.) BATSCH) GROWN NEAR THE NORTHERN LIMITS OF COMMERCIAL ADAPTATION

By

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ABSTRACT

PHYSIOLOGIC AND GENETIC STUDIES ON WOOD HARDINESS OF PEACHES (PRUNUS PERSICA (L.) BATSCH) GROWN NEAR THE NORTHERN LIMITS OF COMMERCIAL ADAPTATION

By

David Wayne Cain

Based on their orchard survival, 'Velvet', 'Redhaven' and 'Siberian C' were considered representative of tender, intermediate and hardy peach cultivars, respectively. Controlled freezing tests on 13 dates in 2 winters showed that 'Siberian C' always had less inner bark and xylem injury than 'Redhaven', while, for both tissues, 'Velvet' had slightly more injury than 'Redhaven'. In the fall, both inner bark and xylem injury increased rapidly as temperature decreased, but during midwinter, inner bark injury increased slowly with temperature decline, while xylem injury still increased rapidly with temperature decline. This was indicative of changes in freezing processes. Fully acclimated xylem of all 3 cultivars was always killed near -37°C, indicating that it may have deep supercooled. Within dates, cultivar injury ratings paralleled moisture content. Increasing tissue moisture content prior to freezing did not affect xylem injury, but increased inner bark injury more in 'Redhaven' than in

'Siberian C'. High temperature pretreatment increased xylem injury in both cultivars, and increased inner bark injury more in 'Siberian C' than in 'Redhaven'.

An electrophoretic mobility technique was used to evaluate tissue freezing patterns. Even though bark moisture was twice that of the xylem, bark exhibited equilibrium freezing patterns while xylem produced nonequilibrium patterns. This indicated a greater interaction between water and cellular components in the bark than in the xylem. Freezing patterns of corresponding tissues in 'Redhaven' and 'Siberian C' were similar.

Inner bark, xylem and vegetative bud injury were assessed for 7 parents and 5 progenies differing in hardiness. Mean progeny inner bark and xylem hardiness could be predicted from average parental performance. Relative progeny vegetative bud hardiness did not correspond to that of bark or xylem, and it could not be predicted from parental performance. Injury, as measured within dates and tissues, was highly heritable, while environmental variation estimates were very low. Based on individual tree observations, correlations among injury ratings for different tissues, temperatures and dates were low, indicating poor repeatability of individual genotypes. Correlations based on family means were higher. The low individual tree correlations indicate that selection based on individual phenotypic performance in a single test might be ineffective.

This thesis is dedicated to my parents for their
years of devotion and encouragement

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INTRODUCTION

Freezing damage to peach flower buds and woody tissues is a major factor limiting peach production in most of North America. Injury to woody tissues is sometimes dramatic, for a single freeze may kill thousands of trees (Bradford and Cardinell, 1952). Such freezes may occur only a few times in a century but low temperature stress causes some injury nearly every winter. While such chronic damage may appear innocuous, it often causes increased susceptibility to diseases and may reduce productivity. Freezing damage has been implicated in increasing tree susceptibility to perennial canker (Weaver, 1963; Tekauz and Patrick, 1974; Layne et al., 1976). In southern production areas freeze damage has been associated with peach tree short life (Daniell and Crosby, 1971; Nesmith and Dowler, 1976; Brittain and Miller, 1976). Loss of whole trees and major scaffold limbs can reduce orchard bearing surface to such a degree that, as an economic unit, the productive life of an orchard may be only 7 or 8 years (Brittain and Miller, 1976). Winter injury also causes occlusions in xylem vessels (Daniell and Crosby, 1968) and causes wood to become brittle, resulting in limb breakage under heavy crop loads (Campbell, 1948; Campbell and Hadle, 1960).

Improved wood hardiness is essential for the successful long-term commercial production of peaches in Michigan and other regions having serious winter injury and should be of prime concern in breeding new cultivars. Genealogical information derived from cultivar descriptions reveals that most commercial freestone peach cultivars have been derived from a limited number of progenitors (Hedrick, 1917; Savage and Prince, 1972). Hesse (1975) notes the severely restricted base used for cultivar improvement in the United States, and the tendency for breeding programs to become highly inbred.

Recently, very cold hardy germplasm has been obtained from nothern China (Pieniazek, 1968). This material offers a new source of genes to peach breeders and is being used in breeding programs in Michigan, New York and other states, and in Ontario, Canada. Of this material, 'Siberian C' has been the most extensively studied (Chaplin and Schneider, 1974; Layne, 1974, 1976; Ormrod and Layne, 1974, 1977; Quamme et al., 1975; Layne et al., 1976; Layne et al., 1977). Under Canadian conditions, 'Siberian C' rootstock causes earlier than usual defoliation of scion cultivars, increases scion cold hardiness of phloem, cambium and xylem in fall and enhances midwinter flower bud hardiness (Layne,et al., 1977). The roots of 'Siberian C' are also very cold hardy (Layne, 1974), and they increase scion survival through their own survival (Layne et al., 1976; Ormrod and

Layne, 1977). In Kentucky, hardiness as assessed by electrolytic conductance tests indicated 'Siberian C' transmitted less hardiness to 'Redhaven' scions than did 'Harrow Blood' but was generally better than 'Rutgers Red Leaf'.

'Siberian C' is presently used as a rootstock but breeders are interested in utilizing it as a source of cold hardiness for both scion and rootstock breeding programs. However, its fruits are very small, soft, bitter, and white-fleshed and have no commercial value. Therefore, its valuable hardiness characteristics must be recombined with the high fruit quality of commercial cultivars via hybridization. Very little is known about the physiological or genetic features which cause 'Siberian C' to possess superior hardiness. My thesis research focused on 3 related questions:

1. When does 'Siberian C' exhibit its superior cold hardiness?
2. What are some physiological causes of this superiority?
3. What breeding value does this germplasm have for creation of commercially adapted scion cultivars with improved cold resistance?

LITERATURE REVIEW

Siminovitch et al. (1968) define winter hardiness as "the capacity of living cells to respond without injury to the sum total of freezing stresses experienced over the winter". For perennial crops the definition must be expanded to include many winters. In its general definition winter hardiness becomes an exceedingly complex trait. There is voluminous literature dealing with many facets of cold hardiness. Many good books and reviews cover various aspects of the subject (Chandler, 1954; Olien, 1967; Mazur, 1969; Weiser, 1970; Stushnoff, 1972; Levitt, 1972; Burke et al., 1976).

Since plant breeders have finite resources in terms of money, land, time and plant material, efficient use of these resources is necessary for maximum breeding progress. The relative importance of plant characters contributing to cold hardiness needs to be known in order to develop suitable screening techniques and appropriate breeding approaches. Stushnoff (1972) has classified the seasonal hardiness cycle in terms of (a) time of tolerance development, (b) rate of development, (c) level of intensity developed, (d) retention of tolerance, (e) onset of loss of tolerance, (f) rate of loss, and (g) ability to regain tolerance. He points out that in some situations it may be more useful to breed for a timing mechanism rather than for maximum level of expression. This may be true for peaches in northern regions where much injury often occurs in late fall (Bradford and Cardinell, 1926; Rollins et al., 1962; Andersen, 1974).

Differences in cold injury among geographic clones of Cornus stolonifera during late fall are associated with differences in phenological responses (Smithberg and Weiser, 1968). Evidence for a two stage acclimation mechanism exists in apple (Howell and Weiser, 1970a). The first stage is induced by short photoperiod and the second by killing frost. Photoperiod has an effect on flower bud hardiness at warm temperatures, but temperature effects overcome photoperiod effects, especially at lower but above freezing temperatures. Therefore, peaches appear to follow the general acclimation pattern for woody plants (Ormrod and Layne, 1974).

Ambient air temperature prior to stressing markedly affects injury. Ketchie and Beeman (1973) find high negative correlation between cold resistance of apple bark and air temperature during the preceding 7 days. Short term changes in cold resistance of apple bark during spring dehardening are related to air temperature of the preceding day (Howell and Weiser, 1970b). Peach and blueberry flower buds also deharden upon exposure to warm temperature (Proebsting, 1970; Bittenbender and Howell, 1975). The 'Latham' raspberry, a classic example of a plant considered very hardy during prolonged cold, but loses its hardiness very quickly under fluctuating temperature conditions (Brierley and Landon, 1946, 1954). Blake (1935) describes differential varietal response to several environmental conditions. Some cultivars are resistant to consistently low winter temperatures while others are better at resisting variable temperatures. Prunus davidiana and P. kansuensis are extremely hardy during midwinter but are subsequently injured because they begin growth very early in the spring.

Hardiness differences among tissues

Different tissues and regions of the tree do not acclimate at the same rate or attain the same maximum level of hardiness. During the summer all tissues are quite sensitive. Flower buds begin to develop hardiness early in the fall. Xylem also develops some degree of hardiness earlier than bark tissues but it acclimates more slowly than flower buds. By early winter the situation is reversed. Now, flower buds are most sensitive, xylem is intermediate, phloem tissues are slightly hardier while cortex and cambium are most resistant in most field conditions (Chandler, 1913; Bradford and Cardinell, 1926; Fogle and Overley, 1954; Edgerton, 1960). The early acquisition of flower bud hardiness might be explained by the fact that they undergo deep supercooling (Quamme et al., 1975).

During deep supercooling, isolated water may remain liquid at normally subfreezing temperatures down to the homogeneous nucleation point of pure water which is about -38° to -40°C (Fletcher, 1962). Xylem tissues of many species can also deep supercool (Quamme et al., 1972a, 1973; George et al., 1974). Vegetative buds are usually hardier than flower buds and are usually not killed except when wood and bark tissues are also severely injured (Dorsey and Strausbaugh, 1923). However, late fall freezes can kill vegetative buds while not injuring flower buds (Overley and Overholser, 1936).

Even the same tissue can exhibit very different levels of resistance in different parts of the tree. In fall, bark tissues are often injured in the trunk and major scaffolds, yet identical tissues in twigs may not be injured (Chandler, 1913; Bradford and Cardinell, 1926; Blake, 1938; Fogle and Overley, 1954; Edgerton, 1960). Trunks may also suffer severe sunscald or southwest injury while twigs are not damaged (Savage et al., 1976). Narrow crotch angles also increase injury (Blake, 1935; Edgerton, 1960). Apical sections are less hardy than basal sections of the same twig, and twigs from lower shaded portions of the tree are less hardy than those from upper well-exposed areas (Cain and Andersen, 1976).

Effects of cultural practices

Cultural practices have a dramatic effect on hardiness of fruit trees. Practices such as late nitrogen application and clean cultivation, which stimulate late growth, increase the chance of winter injury in northern regions (Bradford and Cardinell, 1926; Edgerton, 1960; Rollins et al., 1962). However, late fall nitrogen applications in southern regions may decrease spring cold injury by delaying bud development (Chandler, 1913; Savage et al., 1976). Fall pruning (Bradford and Cardinell, 1926; Potter, 1938; Edgerton, 1960; Nesmith and Dowler, 1976), heavy fruit load, and late fruit maturity may reduce hardiness (Chandler, 1913; Potter, 1938; Edgerton, 1960).

Factors affecting recovery

Environmental and physiological factors affect recovery. A cool moist spring aids recovery, whereas hot dry weather creates additional stress which can kill the tree (Bradford and Cardinell, 1926; Potter, 1938). Nitrogen and potassium fertilizer applications may aid recovery (Potter, 1938). Some varieties such as 'Redhaven' may recover better even though suffering more apparent injury than other cultivars (Fogle and Overley, 1954). Injured barley tissues can produce toxic substances which cause secondary degeneration of adjoining uninjured tissues (Olien and Marchetti, 1976).

Mechanisms of freezing injury

Histological effects. Directly or indirectly, freezing injury involves water transitions and associations within the plant. The amount and distribution of water greatly affect freezing processes. Freezing may occur intracellularly or extracellularly. Intracellular freezing is a nonequilibrium process and is nearly always fatal (Mazur, 1969; Levitt, 1972). If the cell membrane is not sufficiently permeable, if supercooling displaces the system far from equilibrium, or if freezing rate is sufficiently rapid, sudden intracellular freezing may result (Mazur, 1969).

Nonacclimated plants may completely lack tolerance to ice formation in their tissues, while acclimated plants can tolerate ice formation and increase in resistance to intracellular freezing (Single and Olien, 1967). The protoplasmic membrane provides a barrier against ice inoculation into the cell (Asahina, 1963). Plots of tritiated water flux from dead and living cortex cells of *Cornus stolonifera* verified that membranes limited water flux (McKenzie et al., 1974). During acclimation, protoplasmic permeability increases (Krasavtsev, 1967; McKenzie et al., 1974). Reduction or elimination of ice nucleating centers in the cell may also be involved in the acclimation process (Burke et al., 1976). Ice crystal growth can cause histological damage by physical disruption of tissues (Olien, 1968). Adhesion energies can cause ice to adhere to regions of the protoplasmic membrane resulting in mechanical stress (Olien, 1974).

Cytological effects. In acclimated tissues, when freezing occurs as an equilibrium process ice forms first in the extracellular spaces because of the protoplasmic membrane barrier (Asahina, 1967; Siminovitch, 1967; Levitt, 1972). Water is withdrawn from the cell to the growing ice nucleus as a result of an extracellular vapor pressure deficit (Levitt, 1972). Injury is associated with this dehydration or desiccation but the exact reasons for injury are not known. Removal of water causes stiffening and coagulation of the protoplast (Siminovitch et al., 1968). Reduction of cell volume to a critical level and/or exceeding a maximum tolerable osmotic pressure may be important (Meryman, 1970; Williams and Williams, 1976). Cold resistant tissues often tolerate having a greater proportion of their water frozen than nonresistant tissues (Burke et al., 1974, 1975, 1976). Electrolytes may precipitate as solute concentration increases if their solubilities differ, and such differential precipitation may result in large pH changes (Mazur, 1969). Formation of intermolecular disulfide bonds due to close association of protein sulfhydryl groups in dehydrated cells may cause protein precipitation (Levitt, 1962). Changes in starch, sugars, water soluble protein, RNA, lipids and other cellular components have been associated with development of freezing resistance (Siminovitch et al., 1967, 1968). Samygin (1963) found that hardened kale cells die without deformation of the protoplasts, suggesting death results from dehydration rather than mechanical damage. Certainly no one mechanism can explain all types of freezing injury.

Measurement of freezing processes. Several methods have been used to measure water distribution in plants. Calorimetric methods can be used to measure the amount of heat given off during freezing and thus the amount of ice forming (Krasavtsev, 1966; Olien, 1974; George and Burke, 1977a). Both exotherm analysis and differential thermal analysis also detect gross rises in temperature at points where relatively large quantities of water freeze (Quamme, 1972a, 1972b, 1975; George and Burke, 1977a). Pulsed nuclear magnetic resonance spectroscopy can be used to directly measure liquid water content as a function of temperature (Burke et al., 1974; George and Burke, 1977a). Electrophoretic methods have been used to measure the extracellular water content (Olien, 1961).

Using electrophoretic techniques, Olien (1961) found 3 basically different freezing patterns. Reversible equilibrium freezing develops in hardened tissues where no sudden readjustment of water content occurs. Equilibrium freezing is controlled by regulating temperature. When the system is displaced farther from equilibrium, qualitative changes occur which result in nonequilibrium freezing. Nonequilibrium freezing is associated with a sudden readjustment of liquid water between the protoplasts as freezing progresses, and is not controllable by temperature regulation. A nonequilibrium pattern characterized by an abrupt rise in water content with freezing is typical of nonhardened plants. The rise in water content is due to rupture of the protoplasts (Olien, 1964). Water content and structural features affect the freezing pattern. Ice tends to form explosively in water not closely associated with living protoplasts. This often occurs in senile or dead cells such as xylem vessels and in boundaries between different tissue types. At high moisture levels nonequilibrium freezing may occur in all tissues (Olien, 1964). Different freezing processes can occur simultaneously in different regions of a single plant (Olien, 1967).

The type of freezing process affects the size and distribution of ice crystals. Fine ice structure is often found in undifferentiated tissues, while large ice masses often occur in highly differentiated tissues and at transitional zones. Injured areas often contain large perfect crystals while uninjured areas correspond to regions containing imperfect crystalline structures. There appear to be varietal differences in crystal size and dispersion (Olien, 1964, 1967, 1971; Olien et al., 1968). Cooling rate, minimum temperature reached, and nature and concentration of the solute determine the type of ice formation which develops (Luyet, 1970). Because small imperfect ice crystals have a high surface free energy they can recrystallize into larger, more perfectly structured crystals having a lower surface free energy. Crystals do not have to be in direct contact with liquid water but will grow as long as water vapor is provided.

Polysaccharide polymers extracted from cereals can affect crystal structure and distribution by competing with water for positions in the ice lattice. The specific molecular configuration of a polymer determines its effectiveness and is a varietal characteristic (Olien, 1965, 1967).

Structural features of individual cell types and the degree of association between water and cellular components are important in determining the type of freezing pattern produced by a tissue. Water is dynamically oriented in polylayers around cellular components. The physical properties of the water vary with the distance from the interface. Fluidity and freezing point decrease as the interface is approached. During freezing, solutes, hydrophylic substances and the intervening liquid water compete for positions in the ice lattice (Olien, 1973). In xylem vessels where cells are dead or senile most water is not closely associated with cellular components and non-equilibrium patterns are common (Olien, 1964; Dennis et al., 1972). In supercooled wheat stems structural features cause freezing boundaries to be immobilized at the nodes (Single and Olien, 1967).

Deep supercooling. Flower buds and xylem ray cells of some woody plants undergo deep supercooling. In such cells the liquid water content is a weak function of temperature (Burke et al., 1974). The system is displaced far from equilibrium. Freezing occurs as an explosive nonquilibrium process and is probably intracellular (Burke et al., 1976). Azalea florets supercooled to as low as -43^{o} during midwinter (Graham, 1971), while apple, pear, blueberry and grape xylem have exotherms between -30^{o} and -48^{o} (Quamme et al., 1972a, 1972b, 1973; Quamme, 1976; Pierquet et al., 1977). Twenty-five of 49 temperate woody species had exotherms between -41^{o} and -47^{o} (George et al., 1974). Freezing in these tissues occurs near the homogeneous nucleation point of pure water which is around -38^{o} to -40^{o} (Fletcher, 1962; Rasmussen and MacKenzie, 1972). Deep supercooling depends upon structural features of the tissues. Very hardy tissues capable of surviving freezing in liquid nitrogen do not exhibit deep supercooling (Burke et al., 1976). Plants without low temperature exotherms have diffuse porous or nonporous xylem, while plants that deep supercool tend to be ring porous. State, living or dead, has little effect on the low temperature exotherm, but structural integrity is important. Finely ground samples exhibit no exotherm, nor do freeze dried twigs unless they are rehydrated (Quamme et al., 1973). Thin (0.5 mm) xylem sections do not supercool indicating that morphological features are important (Burke et al., 1976). In azalea, the floret deep supercools but adjacent stem and bud scales do not (Graham, 1971; George and Burke, 1977b). Deep supercooled water may remain stable for long periods of time (George and Burke, 1977b). Therefore, some physical or thermodynamic barrier must separate this water from other cellular water, or else it would slowly migrate to exterior ice crystals due to vapor pressure differences.

Moisture content has a dramatic effect on freezing patterns as well as on hardiness. In xylem, a decrease in tissue hydration allows supercooling to lower levels (George and Burke, 1977a). In barley crowns nonequilibrium freezing processes occur when water content is greater than 2.6 g of water per g dry weight (Olien, 1964). A close relationship exists between crown moisture and regrowth of frozen wheat and barley cultivars; small changes in crown moisture result in a large difference in plant survival. Different freezing processes are observed at low moisture levels (Metcalf et al., 1970). Lumis et al. (1972) attribute differential survival of two azalea species to differences in moisture content. Splits in the vascular and cortex tissues caused by ice crystals are eliminated when stem moisture is reduced from 54 percent to 46 percent. However, in this case freezing patterns of the two species differ little despite differences in moisture content. Reducing moisture content of blueberry flower buds increases hardiness, while increasing moisture content reduces hardiness (Bittenbender and Howell, 1975).

Moisture content appears to be a varietal characteristic which is relatively stable throughout the winter. Chandler (1913) found no consistent differences in moisture content of 'Elberta' peach buds, bark and entire twigs, from November to May. Johnston (1923) found moisture content of 'Elberta', 'Late Crawford' and 'Greensboro' peach flower buds to be negatively correlated with hardiness. Moisture content was stable from September to mid-November, rose slightly and remained stable until mid-February, then it slowly began to rise as buds developed.

Moisture content of 2- to 3-year-old apple twig tissues is not constant for individual complex tissues (bark, outer xylem, inner xylem, etc.) from December to January but the total twig moisture content is practically constant (Traub, 1927). Winter moisture content of the hardy 'Duchess' apple remains practically constant while that of the less hardy 'Jonathan' shows a tendency to fluctuate more (Hildreth, 1926). Wildung et al. (1973) find apple root hardiness is negatively correlated with soil temperature and moisture. Stem moisture content of azaleas remains relatively constant during midwinter (Lumis et al., 1972). In contrast, moisture content of plum twigs fluctuates markedly during the winter and is positively correlated with air temperature at collection. Artificial dehydration results in greater water loss from semihardy twigs than from hardy twigs (Strausbaugh, 1921).

Genetics of cold hardiness

Most knowledge of the genetics of cold hardiness is empirical and has been gained by observation of cultivars after test winters. Because of the dynamic nature of cold hardiness, genotype by environment interactions are important. As cited earlier, different genotypes may acclimate and deacclimate at different rates and some may perform well in some climates but not in others. To be useful, genetic potential for cold hardiness must be expressed phenotypically in a wide variety of environmental conditions occurring over many seasons.

Tissue by genotype interactions may also be important Some cultivars, e.g. 'Elberta', are wood hardy but lack bud hardiness, while others, e.g. 'Chinese Cling', are bud hardy but very tender in wood (Chandler, 1913). Both Blake (1938) and Layne et al. (1977) find that hardiness of the lower trunk and bark are not always correlated with hardiness of peach flower buds. However, phloem, cambium and xylem injury are highly correlated with each other (Layne et al., 1977). Flower bud injury in interspecific plum hybrids is correlated with wood injury and twig dieback. No individuals have hardy flower buds and tender bark or wood (Dorsey and Bushnell, 1925).

Inheritance of cold hardiness in wheat is quantitative in nature. Both high and low intensity freezing are controlled by partially dominant genes which are mostly additive in their effect. Changes in relative ranking of some varieties under different freeze intensities indicate different genes may operate under different freezing conditions. Broad and narrow-sense heritabilities are estimated as 77.09 and 63.90 percent respectively. No maternal differences have been found (Gullord, 1974).

Some progenies of interspecific plum hybrids show bimodal groupings (Dorsey and Bushnell, 1925). When both parents are hardy nearly all the progeny are as hardy as the parents. When one parent is hardy a large proportion of the F_1 offspring are hardy. Winter hardiness is dominant in crosses between cultivated and hardy wild strawberries (Hildreth and Powers, 1941). Lapins (1962) finds injury to apple seedlings is approximately normally distributed. One progeny gives some evidence of maternal influence or specific combining ability. Morrison et al. (1963) rate hardiness and vigor of over 130,000 apple seedlings from crosses involving 24 parents. Progenies were grown at 8 locations in Alberta, Saskatchewan and Manitoba. No progeny was outstanding at all locations. Some trees of all progenies had satisfactory ratings. They suggest that selection of parents should be based on average performance of their progenies. Mowry (1964) finds peach flower bud hardiness to be quantitatively inherited. Progenies generally have normal distributions but 'Redskin' progenies are skewed, indicating that 'Redskin' may have prepotency for transmitting hardiness. Hardier parents transmit more hardiness than tender parents, and Mowry suggests that parents be chosen on the basis of their individual performance. Watkins and Spangelo (1970) have studied inheritance of 5 traits and 2 composite traits contributing to plant survival in 2 apple dialleles. Additive genetic variance accounts for 59 percent to 100 percent of the total genetic variance. Fejer (1976), using other apple dialleles, finds specific combining ability to be slightly higher than reported by Watkins and Spangelo, but additive variance is still high. Watkins and Spangelo (1970) conclude that breeding progress can be achieved by exploiting additive genetic variance, total genetic variance or by progeny selection. Potential parents can be screened phenotypically and so eliminate the need for genetic evaluation.

SECTION I

COMPARISON OF RELATIVE FREEZING INJURY IN 'VELVET', 'REDHAVEN' AND 'SIBERIAN C' PEACHES FOLLOWING CONTROLLED FREEZER TESTS AT SELECTED DATES DURING TWO WINTERS

INTRODUCTION

Phenological response of a plant in a specific environment is an important aspect of hardiness. As stated earlier, many North American peach breeders are interested in utilizing 'Siberian C' as a parent in rootstock and scion cultivar breeding programs. Much is known about its fall and midwinter influence on increasing wood and flower bud hardiness of budded scion cultivars. However, little is known concerning its behavior as a scion cultivar, especially during late winter and spring. Nurserymen and growers have expressed concern about its spring hardiness status, because as a scion cultivar under northern conditions it blooms earlier than most commercial cultivars.

This study was undertaken to determine the hardiness of 'Siberian C' relative to a medium hardy and a tender commercial cultivar over the course of the dormant season, and also to determine the reasons for its superior hardiness.

MATERIALS AND METHODS

Plant Material

'Velvet', 'Redhaven' and 'Siberian C' peaches were selected to represent tender, medium hardy and very hardy genotypes, respectively, with regard to field observations of tree hardiness. During the 1975-76 winter (year 1) the 3 cultivars were tested on 7 dates. During the 1976-77 winter (year 2) only 'Redhaven' and 'Siberian C' were tested because the 'Velvet' orchard had been removed. Six tests were conducted in year 2. All trees were in commercial orchards near Hartford, Michigan. 'Velvet' and 'Redhaven' were located on comparable sites within one-half mile of each other. The 'Siberian C' seed orchard was within a mile of the other orchards. Preceding year 1, 'Velvet' and 'Redhaven' bore a nearly full crop. 'Siberian C' bore a heavy crop and was not thinned, which reduced the amount of vegetative growth. In year 2, 'Redhaven' bore a very light crop while 'Siberian C' had no crop due to spring frosts. In year 2 'Siberian C' grew more than 'Redhaven'.

Sample Preparation

Current season twigs 30 to 70 cm long were removed from the upper exposed portion of randomly chosen trees (Cain and Andersen, 1976). Excised twigs were placed into plastic bags contained in a large plastic can. During midwinter twigs were packed with snow or ice to prevent excessive warming during transport to the laboratory. Twigs were usually frozen within 5 to 15 hours after excision. Before freezing, 20-twig groups of each cultivar were placed in plastic bags with the basal ends left exposed. Four to 9 such samples were frozen at each date (Table 1). All samples were placed in a programmable walk-in freezer. Temperature change was controlled by either an evaporator pressure regulator (EPR) valve activated by 2 time clocks or by a Partlow cam-controlled solenoid valve. In year 1 only the Partlow system was used. During year 2 the EPR system was utilized, as it gave a smoother temperature decrease and reduced temperature fluctuations associated with cycling of the solenoid system. Fans inside the freezer prevented air stratification.

Freezing Procedure

The general freezing procedure was to lay the prepared samples on a wooden rack in the chamber. The exposed

Table 1. Collection dates, injury rating dates, number of temperatures used and temperature ranges used for comparing relative hardiness of 'Velvet','Redhaven' and 'Siberian C' peach twigs during 2 winters.

Date of collection	Date of injury evaluation	Number of temperatures		Temperature range	
				Maximum	Minimum
		1975-76			
Nov 9	Nov 17	4		-23.3	-37.2
Nov 29	Dec 7	6		-23.3	-40.6
Dec 12	Dec 17	7		-23.3	-41.1
Jan 1	-[z]	6		-26.1	-40.6
Feb 7	-	6		-26.1	-40.6
Mar 8	Mar 19	9		-17.8	-40.0
Apr 10	Apr 18	8		-3.9	-23.3
		1976-77			
Oct 3	Oct 12	6		-6.7	-21.1
Nov 5	Nov 11	6		-17.8	-32.8
Jan 6	Jan 16	8	Field[y]	-26.1	-43.9
Mar 7	Mar 15	8	Field	-23.3	-38.5
Mar 26	-	9	Field	-21.7	-40.0
Apr 13	Apr 18	8	Field	-3.9	-20.0

[z] Exact date not known

[y] Field control

twig bases were covered with snow or crushed ice to induce ice nucleation and prevent excessive supercooling. All samples were allowed to equilibrate at -2.2°C for 2 to 4 hours before the temperature was decreased at 1.7° to 2.8° per hour. Samples were held for at least 2 hours and occasionally up to 10 hours at each test temperature. Generally samples were removed at 2.8° intervals. Twig temperature was monitored with 24-gauge copper-constantan thermocouples inserted into the twigs and attached to a Honeywell Electronik Potentiometer. Chamber air temperature was recorded on a Partlow recorder and checked against a low temperature alcohol thermometer. Generally there was little difference between air temperature and twig temperature. Twenty-four to seventy-two hours were required to reach the minimum temperature. Frozen samples were held at 0° chamber until all samples appeared to be thawed.

Thawed samples were left in warm humid conditions to oxidize for 2 to 10 days. Preliminary results indicated tissue browning was complete after 48 hours and no visible changes could be detected for several weeks if twigs remained under cool humid conditions to prevent desiccation and growth of microorganisms.

Tissue browning ratings

A thin cross section of each twig was rated separately for browning of cambium and xylem to ascertain the relative level of injury in each cultivar. Cambium was rated on a 1 to 5 scale (Cain and Andersen, 1976). Xylem ratings used were: 1- no injury; 2- slight browning of the xylem near the pith; 3- no more than half the area brown; 4- completely brown except at the outer edge adjoining the cambium region; 5- completely brown and considered dead. Cultivars were compared by averaging injury across temperatures at which at least one cultivar had a rating greater than 1.0 or less than 5.0.

Callusing and TTC Tests

In addition to browning ratings, other viability tests were performed on selected dates to check the reliability of the browning ratings. After assigning browning ratings to twigs on sampling dates December 10, 1975, March 7, 1977 and April 13, 1977 the twigs were dipped in a 100 ppm benomyl solution and the bases were placed into sand in a greenhouse with intermittent misting to prevent desiccation. When sufficient callus had formed, usually 23 to 35 days after initial harvest, each twig was rated for extent of callusing based on a 1 to 5 scale, 1 indicating prolific callus, 5 indicating death. Data were analyzed in a manner similar to browning data.

A tetrazolium chloride (TTC) test was performed on 2.0 to 5.0 mm twig sections removed from basal portions of twigs used on March 7, 1977. The twig sections were placed

in test tubes and covered with approximately 2 ml of a 0.6% W/V 2,3,5-triphenyl-2H-tetrazolium chloride solution containing 0.05 M Na_2PO_4-KH_2PO_4 buffer (pH 7.4) and 0.05% V/V ortho X-77 wetting agent. Tubes were placed in a bell jar and vacuum infiltrated at 24^oC for 15 hours. Five to 6 twig sections were used per cultivar and temperature. Infiltrated twig sections were dissected and examined microscopically. Extent, intensity and location of red coloration were recorded, but the amount of TTC was not quantified spectrophotometrically.

Moisture Content of Excised Twig Internodes

At each test date moisture content of each cultivar was measured. The basal 3 to 4 internodes were discarded and the next 4 internodes were used as the sample because this same region was used for browning determinations. The nodal sections with vegetative and flower buds were discarded. Five twigs per replicate were used. Three to 9 replicates were used per date (Table 4). In year 1 the sections were placed in cork stoppered test tubes during weighing; during drying they were transferred to weighing tins. Because of excessive moisture changes during transfer and weighing, in year 2 twigs were placed into glass weighing jars whose covers were sealed with silicone grease. Twigs were sectioned at 4.4^o to reduce moisture loss during sectioning. After recording fresh weight, samples were

dried at 70° to constant weight. Moisture content was expressed as percent dry weight.

Data were analyzed within dates via analysis of variance (AOV). In year 1 means were compared using Duncan's Multiple Range or its approximation when there were uneven replicates (Steel and Torrie, 1960). Two treatment contrasts (year 2) were compared using the F values from the AOV. Browning injury was correlated with moisture content.

Moisture Content of Bark and Xylem

To determine if differences in tissue moisture content could account for differences in injury, moisture content of bark and xylem tissues of 'Redhaven' and 'Siberian C' was determined on October 3, 1976, January 6, 1977 and March 7, 1977. Basal internode sections were used as in whole twig moisture determinations. The bark and xylem were separated and placed into weighing jars. On October 3, 1976, 3 replicates per cultivar were used while on January 6, 1977 and March 7, 1977, 4 replicates were used.

Effect of Varying Moisture Content on Tissue Hardiness

On November 5, 1976 samples of 'Redhaven' and 'Siberian C' were placed under 2 moisture and 2 temperature regimes in a factorial design to determine the effect that these environmental variables had on hardiness. For the low moisture treatment, twigs were kept sealed in dry plastic bags. High moisture twigs were allowed to stand in about 5 cm of water for 24 hours. High and low test temperatures were 27.3°C and 1.5° respectively. Fifteen twigs of each cultivar per treatment combination were frozen to -26.1°. Inner bark and xylem were rated via browning. Another 15 twigs of each treatment combination were used for moisture determination. Four basal internodes from each of 5 twigs constituted a replicate, giving 3 replicates per cultivar per treatment combination. Differences among means were tested using Duncan's multiple range at $p = 0.05$.

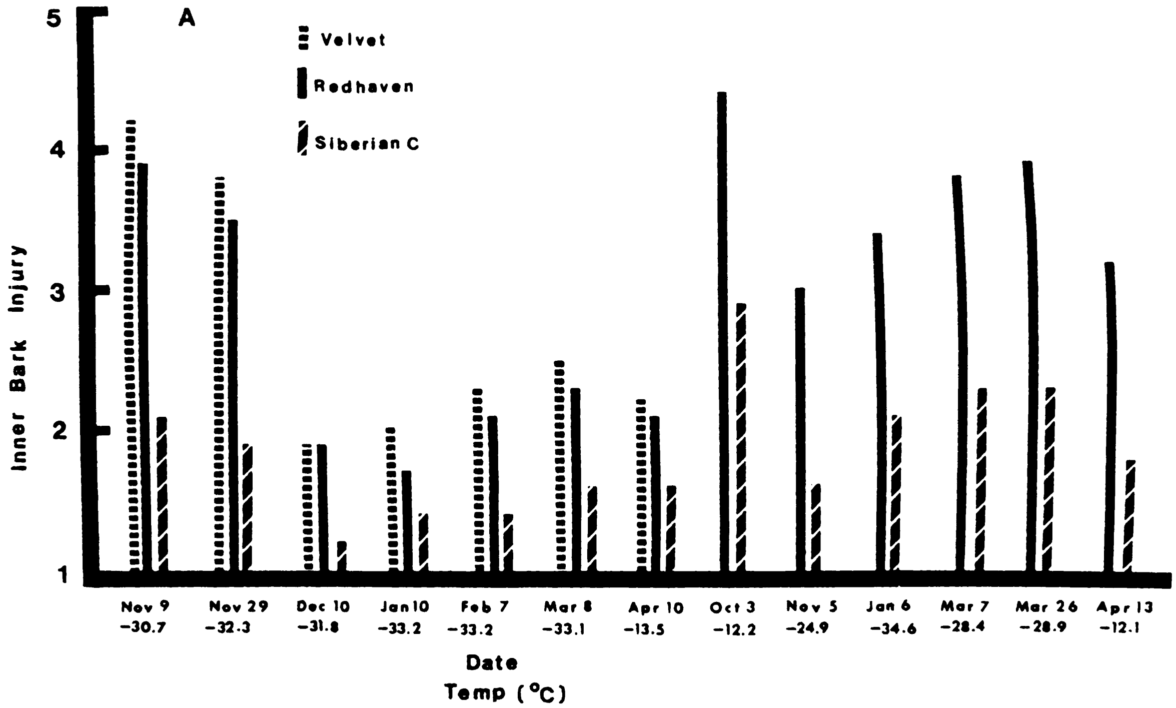
RESULTS

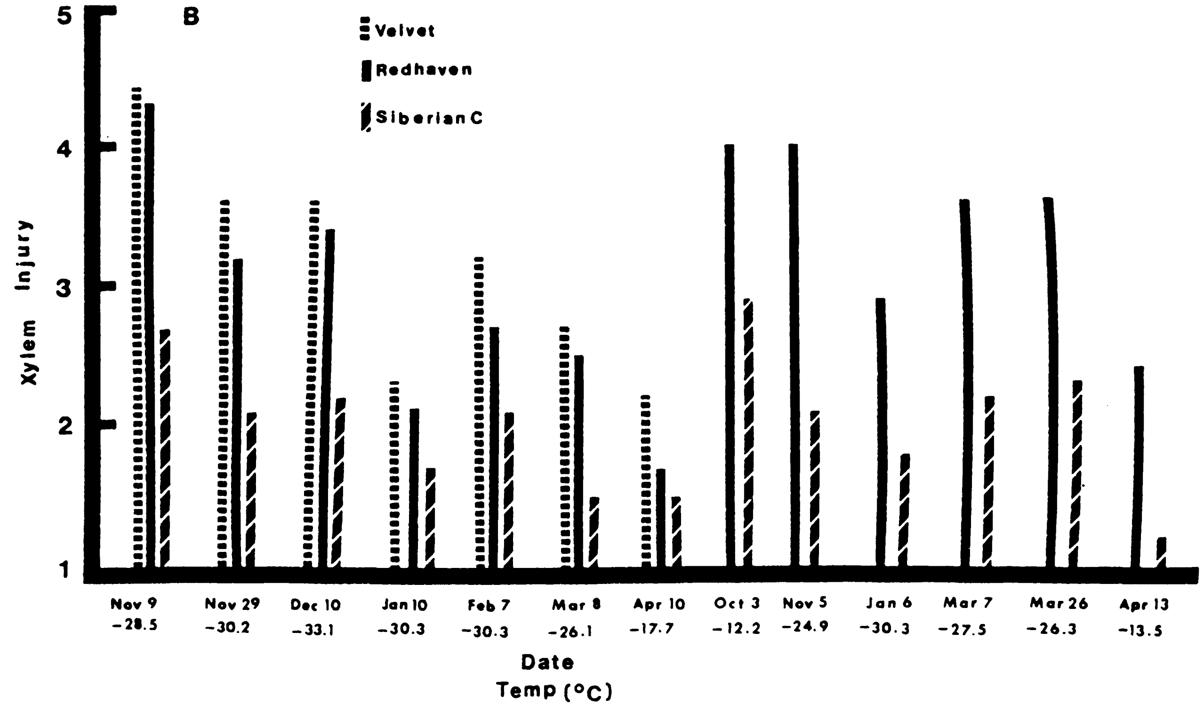
Cultivar browning ratings compared at 13 dates

Inner bark and xylem ratings of 'Siberian C' were much lower than comparable ratings of either 'Redhaven' or 'Velvet' at all 13 test dates during both winters (Figure 1). Average injury to 'Velvet' was slightly higher than 'Redhaven' for both tissues at all dates during year 1, except for inner bark on December 10, 1975, when both means were 1.5 (Figure 1). Inner bark ratings for 'Velvet' were 0.0 to 0.3 units higher than for 'Redhaven' and 0.6 to 2.1 units more than 'Siberian C'. Xylem ratings for 'Velvet' ranged from 0.1 to 0.5 and 0.6 to 1.7 units above 'Redhaven' and 'Siberian C' respectively. 'Redhaven' inner bark was 0.3 to 1.8 units greater than 'Siberian C' while its xylem was 0.2 to 1.9 units higher. In year 1, for both inner bark and xylem, the greatest difference between 'Siberian C' and the other cultivars occurred during the fall, especially in November. Relative differences were much more variable in xylem than in bark tissues. In year 2, differences between 'Redhaven' and 'Siberian C' were more uniform for both tissues.

Injury ratings to both tissues in year 1 were generally

Figure 1. Mean inner bark (A) and xylem (B) injury ratings of 'Velvet', 'Redhaven' and 'Siberian C' averaged over test temperatures where at least 1 cultivar had a mean injury rating greater than 1.0 and less than 5.0.


A
Velvet
Redhaven
Siberian C
Inner Bark Injury
5
4
3
2
1
Nov 9 −30.7
Nov 29 −32.3
Dec 10 −31.8
Jan 10 −33.2
Feb 7 −33.2
Mar 8 −33.1
Apr 10 −13.5
Oct 3 −12.2
Nov 5 −24.9
Jan 6 −34.6
Mar 7 −28.4
Mar 26 −28.9
Apr 13 −12.1
Date
Temp (°C)


B
Velvet
Redhaven
Siberian C
Xylem Injury
5
4
3
2
1
Nov 9 −28.5
Nov 29 −30.2
Dec 10 −33.1
Jan 10 −30.3
Feb 7 −30.3
Mar 8 −26.1
Apr 10 −17.7
Oct 3 −12.2
Nov 5 −24.9
Jan 6 −30.3
Mar 7 −27.5
Mar 26 −26.3
Apr 13 −13.5
Date
Temp (°C)

lower than in year 2 (Figure 1, Appendix A). In year 2, field injury occurred in both tissues prior to January 6, 1977. Overall, inner bark tissues sustained less damage than xylem tissues. Xylem of all cultivars was always killed at temperatures below -37.2°C (Appendix A-Table 1). When fully acclimated, inner bark often suffered only slight damage at -41.1° (Appendix A).

During both winters, tissues of all genotypes did not deacclimate as rapidly as expected. On both April 10, 1976 and April 13, 1977, flower buds were in pink tip, and developing leaves were about 1.3 cm long. Bark and xylem were assumed to be fully active. However, the average level of injury to both tissues was low (Figures 1A and B). In fact, tissues of all genotypes suffered only minor to moderate damage at -23.3° (Appendix A-Table 1).

Shoots of all cultivars had partially defoliated on November 9, 1975 with 'Siberian C' being somewhat more advanced. At this time, inner bark of 'Velvet' and 'Redhaven' were completely killed at -33.3°C (Figure 2), while 'Siberian C' already exhibited considerable resistance to temperatures as low as -37.2°. During midwinter, all cultivars exhibited greater resistance. 'Velvet' and 'Redhaven' suffered moderate injury, while 'Siberian C' was only slightly injured at -41.1°, which was the lower limit of the freezing chamber. Although the magnitude of the differences between 'Velvet' and 'Redhaven' compared with 'Siberian C' decreased during midwinter, 'Siberian C' always exhibited less injury.

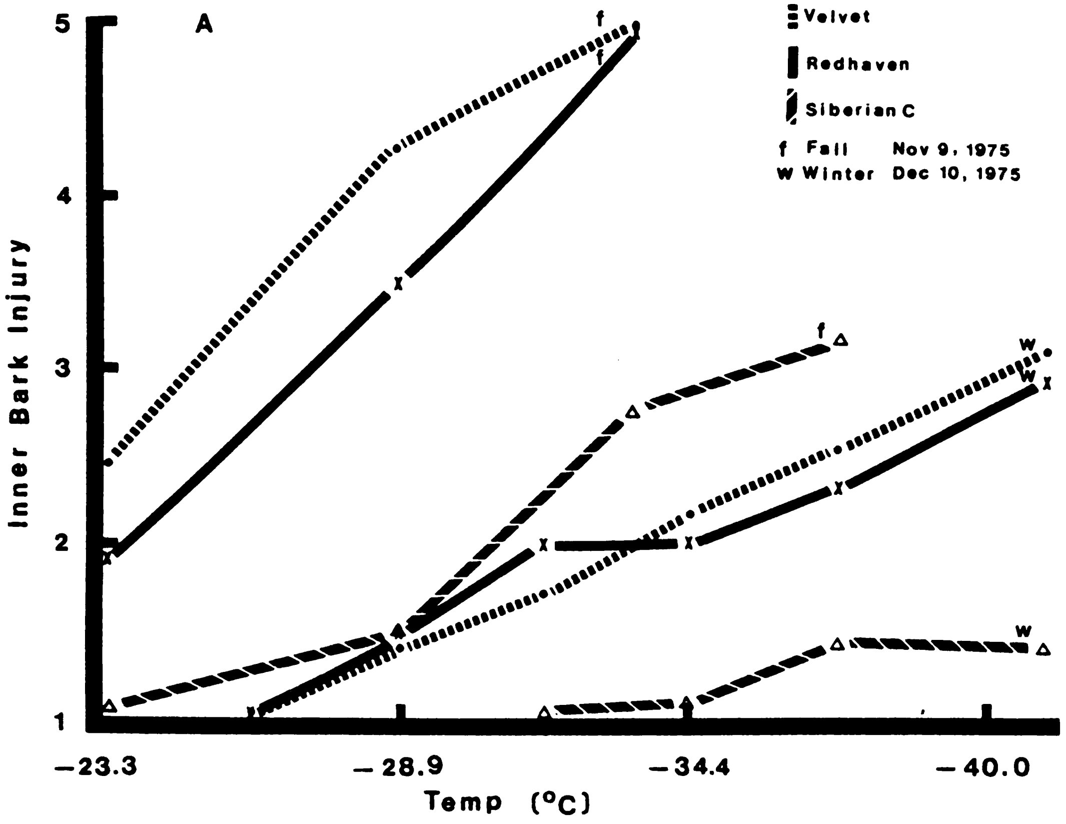
During fall, hardiness of the xylem was quite similar to that of the inner bark (Figures 2A and B). 'Velvet' and 'Redhaven' had considerable damage at -23.3°C and were killed by -28.9°. 'Siberian C' was not injured at -23.3°, but was killed between -33.3° and -37.2° (Figure 2B). By midwinter, initial injury of all cultivars occurred at lower temperatures. Although a considerable difference was still apparent in temperature of initial injury, 'Siberian C' had a killing temperature only slightly lower than that of 'Velvet' or 'Redhaven'.

In midwinter, bark differed considerably from xylem in response to temperature. While injury changed little with temperature in the inner bark, xylem still exhibited an abrupt increase in injury within a narrow temperature range (Figures 2A and B).

Callusing and TTC tests

Proliferation of callus about the cut base of the twig was used as another criterion of viability. Figures used to obtain the correlations were mean injury ratings at each test temperature for each date. Correlation coefficients between browning ratings and callus ratings for each cultivar and the overall correlation were significant at the 0.05 level (Table 2). The correlations indicate good agreement between the 2 methods of injury assessment. Callus tests provided additional proof that inner bark tissues were

Figure 2. Changes in temperature response patterns of inner bark and xylem of 3 peach cultivars from late fall to winter.


A
Velvet
Redhaven
Siberian C
f Fall Nov 9, 1975
w Winter Dec 10, 1975
f
f
f
w
w
w
Inner Bark Injury
5
4
3
2
1
−23.3
−28.9
−34.4
−40.0
Temp (°C)

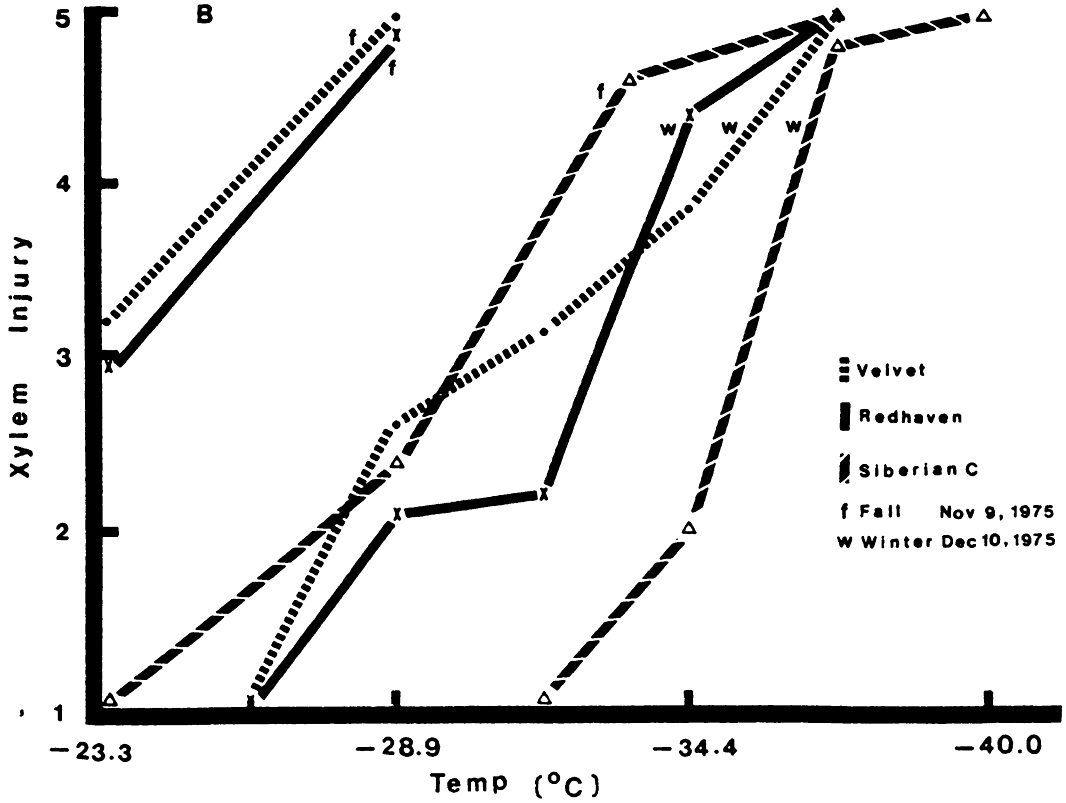

B
Velvet
Redhaven
Siberian C
f Fall Nov 9, 1975
w Winter Dec 10, 1975
f
f
f
w
w
w
Xylem Injury
5
4
3
2
1
−23.3
−28.9
−34.4
−40.0
Temp (°C)

Table 2. Correlations between callus proliferation and inner bark browning ratings following freezing of twigs of 3 peach cultivars.

Date	Velvet		Redhaven		Siberian C		Overall	
	r	n[z]	r	n	r	n	r	n
12-10-75	.94**	7	.86*	7	.89**	7	.69**	21
3-7-77	-	-	.98**	7	.84*	7	.93**	14
4-13-77	-	-	.85*	6	.89**	7	.83**	13

[z] n is the number of observations involved, r is the correlation coefficient.

*Significant at 5%

**Signifcant at 1%

alive at -41.1°C. 'Redhaven' forms more callus than 'Siberian C' when both are uninjured (Figure 3), but progressively less than 'Siberian C' as temperature decreases. At the lowest temperatures, 'Redhaven' produces no callus, while 'Siberian C' continues to produce some even at -41°. Thus, inherent callusing capacity is important in comparing genotypes.

A TTC reduction test performed on twigs from the March 7, 1977 sampling date provided another method of injury assessment (Table 3). The extent of TTC reduction (red coloration) agreed closely with browning ratings. Because TTC tests were not conducted until March 17, 1977, tissues had already undergone oxidative browning. The brown pigments reduced the ability to discern faint differences in pink coloration, especially in more severely injured tissues. Lack of red color in the xylem at -31.7°C to -34.4° indicated that death occurred in this range. This is the same range in which xylem death occurs as assessed via browning (Figure 2B). Inner bark tissues showed moderate red color at -38.5°, indicating that they were still alive but had been injured. These observations agree with browning and callusing results.

Relationship between moisture content and injury

Moisture content of basal internodes was measured at each test date. Relative cultivar order within each date

Figure 3. Callusing of 'Redhaven' (left in each set of 6) and 'Siberian C' (right) collected on Nov. 5, 1977 following exposure to stress temperatures (°C) of (upper left to lower right): -17.8°, -20.6°, -23.3°, -26.1°, -28.9° and -32.8°.

Table 3. Color of 'Redhaven' and 'Siberian C' twig tissues treated with triphenyl tetrazolium chloride following controlled freezing on March 7, 1977.

Temperature (°C)	Cultivar	Tissue			
		Cortex	Outer phloem	Cambium region	Xylem
Field control	Redhaven	Dark red[z]	Dark red	Dark red	Light pink
	Siberian C	Dark red	Dark red	Dark red	Light pink
-23.3	Redhaven	Dark red	Dark red	Dark red	Light pink
	Siberian C	Dark red	Dark red	Dark red	Red
-26.1	Redhaven	Dark red	Pink, some browning	Dark red	Light pink some brown
	Siberian C	Dark red	Dark red	Dark red	Red
-28.9	Redhaven	Dark red	Pink to brown	Dark red	Pink near cambium
	Siberian C	Dark red	Red	Dark red	Light pink
-31.7	Redhaven	Red	Brown	Red to light red	Brown
	Siberian C	Red	Red	Dark red	Brown, pink at cambium
-34.4	Redhaven	Dull brown	Brown	Light pink to dark red	Brown
	Siberian C	Brown to light pink	Pink to brown	Dark red	Brown
-36.7	Redhaven	Brown	Brown	Dark red near xylem only	Brown
	Siberian C	Light red to brown	Brown	Dark red	Brown
-38.5	Redhaven	Brown	Brown	Red near xylem only	Brown
	Siberian C	Brown with red areas	Brown	Dark red to light pink	Brown

[z] Lighter red and more browning indicate greater injury.

is quite consistent, 'Velvet' having the highest moisture content, 'Redhaven' being intermediate and 'Siberian C' having the lowest moisture content (Table 4). During year 1, moisture change occurred as twig sections were transferred between stoppered test tubes and weighing tins. In year 2, glass weighing jars were used; as a result, between date differences were reduced. On certain dates, e.g. November 29, 1975, twigs were collected while wet from rain,snow or frost. Much of the external water was wiped off but external residues may have masked internal moisture content differences. However, precipitation and temperatures preceding and during collection did not explain all such differences.

Within test dates, a strong relationship exists between moisture content and cultivar injury ratings (Table 5). Each correlation coefficient is based on only 3 observations, thus, only 1 degree of freedom. Correlations were not calculated for year 2 because only 2 cultivars were available. However, comparison of moisture content data in year 2 (Table 4) with injury ratings (Figure 1) shows the same consistent relationship; 'Redhaven' was less hardy than 'Siberian C' and generally had a higher tissue moisture content. Correlations between xylem injury and moisture content were usually slightly higher than those between inner bark injury and moisture (Table 5).

Table 4. Moisture content[z] of peach twigs at each test date.

Date	Velvet		Redhaven		Siberian C	
	n[y]	$\bar{x}$	n	$\bar{x}$	n	$\bar{x}$
1975-76[x]						
Nov 9	4	78.01a	4	74.84b	4	63.81c
Nov 29	4	96.49a	4	93.27a	4	93.42a
Dec 12	4	88.11a	5	86.30b	5	84.61c
Jan 1	3	93.53a	5	91.23b	3	87.52c
Feb 7	5	87.45a	5	82.48ab	5	79.72b
Mar 8	5	91.68a	5	88.08b	5	85.54c
1976-77[w]						
Oct 3			10	80.65a	10	79.04b
Nov 5			6	81.19a	6	81.24a
Jan 6			9	83.97a	9	80.55b
Mar 7			9	85.53a	9	79.84b
Mar 26			9	80.37a	9	80.02a
Apr 13			9	95.24a	9	93.10a

[z] Percent dry wt

[y] Number of replicates

[x] Within rows, means followed by the same letter are not significantly different at $P_{.05}$ by Duncan's multiple range test.

[w] Within rows, means followed by the same letter are not significantly different by $F_{.05}$.

Table 5. Correlations between internodal moisture content and mean tissue injury ratings of all trees for each sampling date during 1975-76.

Date	Cambium	Xylem
Nov 9	.997*	.990
Nov 29	.602	.685
Dec 12	.860	.904
Jan 10	.996	.989
Feb 7	.909	.969
Mar 8	.872	.918

*Significant at $P_{.05}$

Table 6. Moisture content^z of bark and xylem of 'Redhaven' and 'Siberian C' at selected dates in 1976-77.

		Tissue	
Date	Cultivar	Bark	Xylem
Oct 3^y	Redhaven	124.70a	64.62c
	Siberian C	115.10b	67.98c
Jan 6	Redhaven	122.23a	56.99d
	Siberian C	116.14b	60.65c
Mar 7	Redhaven	131.77a	53.93c
	Siberian C	114.00b	54.46c

^{z}Percent dry wt

^{y}Within dates, means followed by the same letter are not significantly different at $P_{.05}$ by Duncan's multiple range test.

Determination of bark and xylem moisture

On 3 selected dates during year 2 , moisture content was determined separately for bark and xylem tissues (Table 6). Bark tissues of 'Redhaven' contained significantly more water than those of 'Siberian C' at all 3 dates. 'Redhaven' xylem tissues had slightly, but not significantly, lower moisture content than 'Siberian C'. Moisture content of bark tissues was approximately twice that of the xylem.

Effect of temperature and moisture adjustment on hardiness

Both temperature and moisture pretreatment significantly affected hardiness (Table 7). Twigs took up more water at high temperature than at low temperature, and 'Redhaven' took up more than 'Siberian C' at both temperatures. Twigs stored at high temperature suffered more injury than those stored at low temperature. 'Siberian C' xylem suffered significantly less injury than 'Redhaven', but moisture content did not affect xylem injury. Cambium showed significant temperature by cultivar, and moisture by cultivar interactions. High storage temperature increased injury more in 'Siberian C' than in 'Redhaven', while soaking increased injury more in 'Redhaven' than in 'Siberian C'.

'Siberian C' xylem pretreated at high temperature and moisture had less injury than 'Redhaven' pretreated at low

Table 7. Effect of moisture and temperature pretreatments on moisture content and injury to 'Redhaven' and 'Siberian C' inner bark and xylem following freezing to -26.1°C on November 5, 1976.

	Pre-treatment		Injury rating		Moisture content
Cultivar	Temp	Moisture	Inner bark	Xylem	(% d.w.)
Redhaven	0.5	high	4.3f[z]	4.5c	90.78c
		low	3.0d	4.3c	82.11a
	27.6	high	5.0g	5.0d	98.99e
		low	3.6e	5.0d	81.81a
Siberian C	0.5	high	1.9b	1.6a	89.04b
		low	1.3a	1.8a	81.78a
	27.6	high	3.1d	2.2b	95.87d
		low	2.3c	1.9ab	81.21a
			Main effects		
Effect:					
Redhaven			4.0a[y]	4.7a	88.42a
Siberian C			2.2b	1.9b	86.98b
	0.5		2.6a	3.0a	85.93a
	27.6		3.5b	3.5b	89.47b
		high	3.6a	3.3a	93.67a
		low	2.6b	3.3a	81.73b

---

[z] Within columns, means followed by the same letter are not significantly different at $P_{.05}$ by Duncan's multiple range test.

[y] Within each column of each main effect, means followed by the same letter are not significantly different at $F_{.05}$.

temperature and moisture. Similarly treated inner bark of 'Siberian C' had no more injury than 'Redhaven' bark held at low temperature and moisture, even though twig moisture content of 'Siberian C' was 13.8 percent higher.

DISCUSSION

Seasonal hardiness comparisons

At all test dates inner bark and xylem of 'Siberian C' suffered less injury than similar tissues of 'Redhaven' or 'Velvet'. This supports conclusions by Ormrod and Layne (1974) and Layne (1974) that 'Siberian C' is relatively cold hardy compared to other rootstocks and scion cultivars grown under Canadian conditions. The average injury to 'Velvet' was slightly greater than for 'Redhaven'. This agrees with the general description by peach growers that 'Velvet' is a "tender" tree while 'Redhaven' is "hardy", although not the hardiest of the commercial types.

The relationship between temperature decline and injury changes as plants acclimate from late fall to midwinter. Fall temperature response patterns of inner bark and xylem are quite similar (Figures 2A and B). They are characterized by a rapid rise in injury over a narrow temperature decline. In contrast, winter temperature response patterns of the inner bark and xylem are quite different (Figures 2A and B). The major change in the xylem pattern is a shift in the overall pattern to a lower series of temperatures. Injury still increases rapidly over a narrow temperature range. The inner bark also shows a downward shift in the temperature at which initial injury occurs but injury levels rise very slowly with declining temperatures.

The characteristic changes in temperature response patterns may indicate that different stresses are developing in the inner bark and xylem at different dates. The rather abrupt killing of the partially acclimated inner bark and xylem indicate direct injury by ice. In nonacclimated or partially acclimated wheat, freezing often occurs in a nonequilibrium pattern. In such cases protoplasts are disrupted by crystallization, causing irreversible damage (Olien, 1964). Intracellular freezing may occur in such a situation and is nearly always fatal (Mazur, 1969).

Xylem ray cells of many woody plants including black cherry (Prunus serotina Ehrh.) exhibit deep supercooling (Quamme, 1972a, 1972b, 1976; George et al., 1974; George and Burke, 1977a). Freezing occurs near the homogenous nucleation point of water, which is approximately -38 to -40°C (Fletcher, 1962; Rasmussen and MacKenzie, 1972). The abrupt rise of xylem injury over a narrow temperature decline and the fact that xylem of all cultivars never survived temperatures below -37.2° (Appendix A-Table 1) suggest that peach xylem undergoes deep supercooling.

The slow increase in cambium injury with temperature decline suggests that the cambium is undergoing equilibrium freezing. During an equilibrium freeze ice formation occurs

extracellularly because the cell membrane provides an effective barrier against intracellular ice inoculation (Asahina, 1963). Water is withdrawn from the cell to extracellular ice nuclei near the cell wall, due to an extracellular vapor pressure deficit. This results in protoplasmic dehydration (Asahina, 1963; Krasavtsev, 1966; Mazur, 1969; Levitt, 1972). Injury is associated with protoplasmic dehydration (Burke, 1974). Cold resistant tissues seem to tolerate having a greater proportion of their water frozen than non-resistant tissues (Burke et al., 1975). Inner bark injury during midwinter may be due to desiccation effects rather than direct effects of ice formation. 'Siberian C' may be able to tolerate removal of greater amounts of its cellular water than 'Velvet' or 'Redhaven', or the temperature for removal of a critical amount of water may be lower for 'Siberian C' than for 'Velvet' or 'Redhaven'.

Hardiness of inner bark was unexpectedly high, especially during year 1 (Figure 1A). Viability, however, was verified by callus regrowth (Table 2) and TTC tests (Table 3). Wood injury and even complete tree death often occur at warmer temperatures in the field (Chandler, 1913; Bradford and Cardinell, 1926; Fogle and Overley, 1954). However, under some conditions commercial cultivars have survived field temperatures as low as -35.6°C (Campbell, 1948) and hardy Chinese germplasm has survived -38° (Pieniazek et al., 1968). However, temperature alone

gives no indication of the types of stresses developing in the plant (Olien, 1974a). Thus, absolute comparisons between injury levels to specific tissues produced in different situations should be interpreted with caution. Still, relative ranking of the three cultivars according to freezer tests agree with field performance rankings. The greater injury in year 2 as compared with year 1 may have been a result of the field injury occurring in year 2. This injury may have made the tissues more susceptible to damage during subsequent freezer tests. Olien (1974b) has shown that cyclic freezing and thawing greatly increases injury from ice crystal growth, which raises the killing point of barley crowns.

Relationship between moisture content and injury

Although tissues of the hardy cultivar consistently contained less water, seasonal hardiness patterns within cultivars did not parallel moisture content (Table 4). Changes in moisture content did not follow any discernible pattern from late October to mid April. Moisture content of each cultivar remains nearly constant throughout the dormant season (Table 4). Chandler (1913) likewise found moisture content of 'Elberta' twigs to be fairly constant throughout the winter. His moisture estimates were similar but slightly higher than moisture estimates in Table 4.

However, he included buds, used a different cultivar and used a higher drying temperature. His bark moisture estimates are very similar to bark moisture estimates in Table 6. Stem moisture content of two azalea clones remained relatively constant during midwinter (Lumis et al., 1972). Winter moisture content of the hardy 'Duchess of Oldenburg' apple was constant but the less hardy 'Jonathan' showed much greater fluctuation (Hildreth, 1926).

Maximum difference in water content between cultivars was 14 percent ('Velvet' and 'Siberian C' on November 9, 1975) but averaged 6.8 percent. 'Redhaven' averaged 3 percent higher than 'Siberian C' and 3 percent less than 'Velvet'. Such differences may be small compared to the total moisture content but can be very important biologically. Gullord (1974), using 9 wheat cultivars with a maximum difference in content of 3.6 percent, showed that moisture content explained 68.9 and 72.3 percent of the variation in freezing hardiness during high and low intensity freezes, respectively. Moisture content was a heritable trait. Metcalf et al. (1970) demonstrated that a small increase in the percent crown moisture could result in a large decrease in survival of wheat and barley. Differential stem injury of 2 azalea clones was correlated with water content (Lumis et al., 1972).

Differences in freezing characteristics of bark and xylem

The lower moisture content of 'Siberian C' bark (Table 6) may be an important factor contributing to its superior hardiness, since, as pointed out above, small differences in moisture among cultivars may affect injury.

Xylem behavior is more difficult to explain. Even though water content of the xylem was slightly, but not significantly, lower in 'Redhaven' than in 'Siberian C' (Table 6), 'Siberian C' xylem had less injury than 'Redhaven'. Location of this water within the xylem is not known. Differences in amount of water in mature xylem vessels may not be critical. This water freezes a few degrees below $0^{o}C$ (Kitaura, 1967), but this freezing appears to cause no damage. In acclimated peach xylem, injury occurs only at low temperatures, indicating that xylem ray cells are deep supercooling. Moisture content may have minimal influence on the deep supercooling process. Effectiveness of the protoplasmic membrane as a barrier against ice growth into the cytoplasm (Asahina, 1963) might be more important, or xylem ray cell volume may be important, since the temperature at which homogenous nucleation occurs is a function of droplet size (Fletcher, 1962). Concentration and type of solutes present may also affect nucleation temperature.

Artificially increasing twig moisture content significantly increased injury to inner bark but not to xylem. Both temperature and cultivar affected moisture uptake and injury.

Freezing stress is affected by the rate of freezing, the amount of water involved and the resultant amount of free energy released (Olien, 1971,1973). High freezing rates and moisture contents increase the probability that nonequilibrium freezing will occur (Olien, 1974).

Moisture distribution and redistribution during freezing were not studied in this experiment. Much of the absorbed water probably occurred in the large xylem vessels as bulk water, only weakly associated with cellular constituents.

'Siberian C' scions defoliate and acclimate earlier in the fall and transmit these characteristics to budded scions. It also promotes fall and midwinter flower bud hardiness of budded scion cultivars (Layne et al., 1977). In Ontario, field survival of scions budded to 'Siberian C' seedlings was superior to those budded onto more tender stocks (Layne et al., 1976; Ormrod and Layne, 1977). The present study confirms the superior wood hardiness of 'Siberian C' compared to 'Velvet' and 'Redhaven' during fall and midwinter.

'Siberian C' and other peaches from northern China evolved in a climate with long, consistently cold winters. Zagaja (1974) expressed concern that the trees might be susceptible to fluctuating winter and spring temperatures.

Continued observation of this Chinese germplasm indicates it has a chilling requirement comparable to most commercial types (S.W. Zagaja, personal communication). Since 'Siberian C', under northern conditions, blooms earlier than most cultivars, growers and nurserymen have been concerned that this stock and scions budded to it may be susceptible to flower bud and wood injury in early spring. Therefore, it was not known if the superior fall and midwinter wood hardiness of 'Siberian C' would be exhibited during late winter and spring.

In this study, 'Siberian C' continued to have less injury than 'Velvet' or 'Redhaven' up to pink tip (Figure 1). Both inner bark and xylem of 'Siberian C' had less injury than 'Velvet' or 'Redhaven' on April 10, 1976 and April 13, 1977. Callus regrowth verified browning results on April 13, 1977 (Appendix A-Table 2). Injury to inner bark and xylem was unexpectedly low at both dates, for all cultivars were expected to have deacclimated to a greater extent. Similarly, Howell (1970) found that apple bark remained somewhat hardy even during bud and leaf expansion. Blake and Steelman (1945) reported that peach flower buds in medium pink stage survived $-12.8^{\circ}C$. In Missouri, Chandler (1913) found no wood injury to 'Elberta' twigs frozen to -24.7° on April 5, 1913. The reasons for survival at such low temperatures in early spring are not known.

This study indicates that 'Siberian C' is hardier than commercial cultivars in the spring. Also, 'Siberian C' has not caused early blooming of scion cultivars in rootstock trials at South Haven, Michigan (unpublished data) or at Harrow, Canada (R.E.C. Layne, personal communication); in fact, reports from Georgia and California indicate that 'Siberian C' delays bloom of budded scion cultivars (S. Dowd and W. Krause, personal communication). Thus, 'Siberian C' does not appear to be unduly susceptible to fluctuating late winter and spring temperatures, and indeed may be more tolerant than other rootstocks and scion cultivars.

Summary

'Siberian C' was hardier than 'Velvet' or 'Redhaven' at 13 dates during 2 winters. Within test dates, cultivar injury was positively correlated with moisture content. Moisture content of bark was twice that of the xylem; however, xylem suffered more injury than bark. Differential response of fully acclimated bark and xylem to temperature decrease suggested that bark underwent equilibrium freezing while xylem deep supercooled

Because of its distinctly different geographic origin and its consistently superior freezing resistance compared to commercial genotypes, 'Siberian C' represents a new germplasm source for cold hardiness breeding.

SECTION 2

COMPARATIVE FREEZING PATTERNS OF BARK AND XYLEM OF 'SIBERIAN C' AND 'REDHAVEN' PEACH TWIGS

INTRODUCTION

Electrophoretic mobility is a reliable means of studying water redistribution and resultant stresses arising in plant tissues as ice forms (Olien, 1961). It has been used to study freezing patterns in winter cereals and woody perennials (Olien, 1961; Single and Olien, 1967; Dennis et al., 1972; Lumis and Mecklenburg, 1974). It provides a simple means of continuously monitoring changes in extracellular liquid water during freezing and thawing. Three basic patterns have been found, as described in the literature review. This study was undertaken to determine the types of freezing patterns occurring in acclimated twig tissues of 'Redhaven' and 'Siberian C' peaches.

MATERIALS AND METHODS

Plant material

The twigs of 'Redhaven' and 'Siberian C' used were subsamples of those used to study seasonal hardiness (Section 1). Twigs were sealed in plastic and stored at 4°C for several days until used. The basal region of the twig used for hardiness ratings in Section 1 was used in these tests. Intact twig sections, longitudinally split twig wedges, and excised bark and xylem were tested. Bark sections were prepared by removing a 3 to 5 mm strip of bark, xylem sections by removing all bark tissues and excising a longitudinal wedge about 3 mm wide. All sections used were 40 to 50 mm long.

Freezing patterns

All sections were coated with silicone grease and wrapped with plastic film (Saran wrap) to prevent desiccation and to confine current flow to within the section. About 5 mm on each end was left exposed to provide a contact surface. The prepared section was placed on the freezing block (see Dennis et al., 1972, Figure 1). A fine thermocouple was placed under the section and connected to a manual potentiometer to monitor temperature. An aluminum block cooled by an ethanol-ice bath and a thermoelectric block were placed over the section. Temperature was controlled by regulating the ice bath temperature and by applying current to the thermoelectric block. Electrical contacts with the twig section were made by coating the exposed section ends with a paste of finely divided moistened carbon. A flat platinum plate was inserted into the paste and connected to a 22.5 V dry cell. Cotton wicks inserted in water-filled test tubes and in contact with the paste prevented drying of the paste. An ammeter and voltage regulator were included in the circuit. To prevent polarization of cellular electrolytes, a low frequency (less than 5 cycles per second) direct current was used, as use of low frequency current gives the same conductivity data as direct current (Olien and Chao, 1973). The test section was cooled to -2°C to -3°. Here temperature was stabilized and ice nucleation was induced by touching a cold probe to the tissue. Temperature change did not exceed 2.8° per hour except during the initial exotherm after inoculation.

Voltage (V) and amperage (C) were recorded at each temperature and used to calculate resistance (I) by the equation $I = \frac{V}{C}$. Resistance data were expressed relative to an arbitrarily selected prefreeze temperature. Observed values were corrected for the viscosity of bulk water at that temperature (NS). Viscosity was also expressed on a relative basis (NS_r). The product of relative resistance and relative viscosity was used to estimate the relative extracellular liquid water content at a given temperature

(M_r). This was multiplied by the grams of water per gram of dry weight for the particular tissue in question to estimate the grams of liquid water per gram of dry weight in the tissue at a given temperature. Sample data are shown in Table 1.

Table 1. Sample data showing calculations used to estimate the amount of liquid water at a given temperature

Parameter	Temperature (° C)		
	+1.7	-1.4	-14.2
(a) Observed resistance (I)	1.7600	1.2800	0.0975
(b) Relative resistance (I_r)	1.0000	0.7273	0.0554
(c) Water viscosity (centipoise) (NS)	1.7000	1.8750	3.1380
(d) Relative viscosity (NS_r)	1.0000	1.1029	1.8459
(e) (b)x(d) Relative extracellular water (M_r)	1.00	0.80	0.10
(f) (e)x g H_2O/ g dry wt[z]	0.65	0.52	0.06

[z] Moisture content = 0.6462 g H_2O/g dry wt.

Contact resistance

Contact resistance was measured by preparing twigs as described above and measuring current flow (C) at a known voltage while varying the distance between contacts from 119 mm to 1.0 mm. The resistance (I) was then plotted as a function of distance and extrapolated to 0 distance to estimate contact resistance. The contact resistance was expressed as a percent of the total predicted resistance at 40 mm because all sections tested were at least 40 mm long. Six separate tests were conducted.

Dye flow experiments

Amaranth, a negatively charged dye, was used to determine the path of current flow in the sections. The sections were prepared as for the freezing pattern determinations but a finely ground cellulose paste replaced the carbon paste and carbon electrodes replaced the platinum ones. A 1 percent dye solution was used to moisten the negative terminal. A 22.5 V battery connected with a variable resistance provided a stable current from 0 to 30 μA applied for up to 12 hours. Dye location and movement were determined by microscopic examination of the tissues.

Moisture content

Since preliminary experiments indicated that moisture content remained stable for several weeks when twigs were sealed in plastic bags and stored at low temperatures, moisture values determined for hardiness tests in Section 1 were used as estimates of moisture content in these experiments (see Table 6, Section 1).

RESULTS AND DISCUSSION

Use of whole twigs resulted in unstable contacts. Contact resistance accounted for up to 60 percent of the total resistance. In longitudinally split twig sections contact resistance accounted for no more than 11 percent of the total resistance (Table 2) and had little effect on the freezing pattern. Therefore, data were not corrected for contact resistance. Twig wedges produced patterns similar to those of whole twigs. Contact resistance of 'Redhaven' and 'Siberian C' were similar and both cultivars produced identical freezing patterns.

Table 2. Calculation of contact resistance of longitudinally split twig sections in 6 separate experiments

Contact resistance	Slope	R^2	Estimated Resistance (at 40 mm)	Contact Resistance (% of total)
0.0824	0.0208	0.999	0.9144	9.0
0.0473	0.0096	0.998	0.4313	11.0
0.0455	0.0118	0.997	0.5175	8.8
0.0033	0.0159	0.996	0.6393	0.5
- 0.0241	0.0175	0.998	0.6759	-
0.0269	0.0172	0.997	0.7129	3.8

The current was proportional to the voltage applied within the range used in the tests. Therefore, the currents used did not appear to cause cellular injury.

Diffusion of indicator dye revealed that the main current path was through the outer xylem area near the cambium region. Very little movement occurred in the phloem and cortex. The current path in peach is much different than that in azalea stems where the cortex appears to be the main path (Dennis et al., 1972; Lumis and Mecklenburg, 1974). Rate of dye movement was proportional to current flow. When the cellulose paste was very wet, mass water flow and transpirational effects influenced dye movement. When the paste was quite dry, dye moved only 1 to 2 mm into sections with no current flow. In bark sections dye moved throughout the entire phloem and cortex region.

Freezing patterns of intact sections or twig wedges with bark attached were very similar to patterns produced when only xylem tissue was used. This evidence and that from the dye flow experiments indicate that twig wedges were essentially measuring freezing of xylem water.

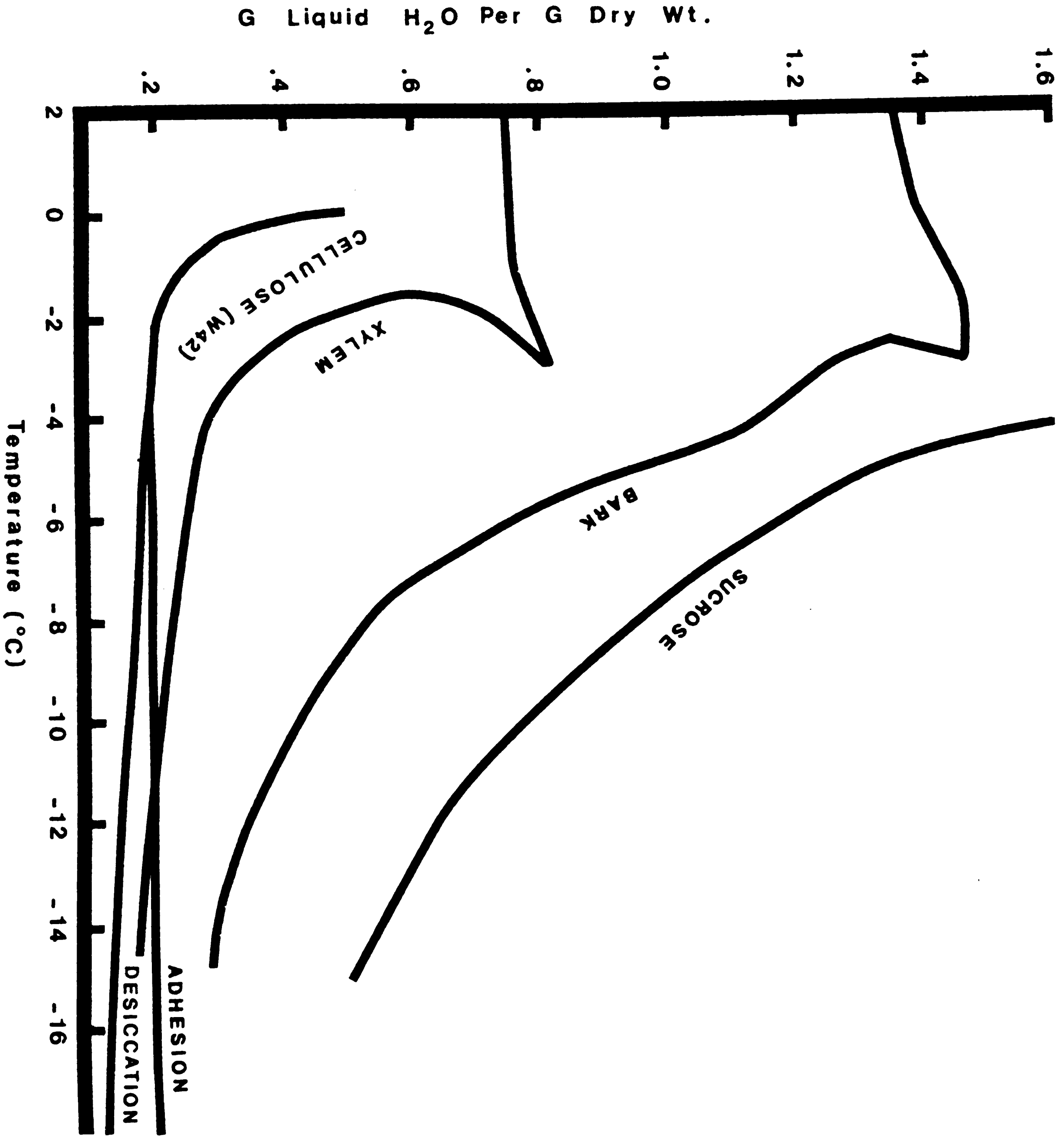
Water content can have a marked effect on the type of freezing pattern in a specific tissue (Olien, 1963, 1964). Some tissues of 'Siberian C' contained as much as 18 percent less water than similar tissues of 'Redhaven'; however, this difference did not result in qualitatively different freezing patterns. Freezing patterns of bark, xylem or twig wedges of 'Redhaven' were very similar to those of similar tissues of 'Siberian C.'

Major differences in freezing processes were evident

in bark and xylem tissues (Figure 1). Freezing curves for sucrose and cellulose (from Olien, 1977) are given for comparison. Data were corrected for mobility. Both bark and xylem were supercooled to -2.8°C before inoculation. Upon inoculation, temperature rose rapidly in both tissues as ice formed throughout the system releasing heat of fusion. During this initial freeze only about 14 percent of the bark tissue water froze, as compared with about 58 percent of the xylem water, before tissue temperature returned to that of the test environment (Figure 1). The slight rise in water content prior to freezing is probably due to water movement from the carbon paste into the tissue.

The initial moisture content of the bark was nearly twice that of the xylem (see Table 6, Section 1). However, bark water must be intimately associated with the living protoplast, while much of the xylem water is less closely associated with cellular components. Cellular substances can interact competitively with ice for water at interfaces. Cellular water orients itself around hydrophilic substances within the cell. The degree of orientation is a function of the distance from the interface. When the system has a large interfacial area, as in the dispersed sucrose system, there is more opportunity for intimate association between water and other molecules. Where interfacial contact area is small, as in the cellulose system, there is less interaction between water and other molecules. Bark water behaves much like the dispersed sucrose solution, while xylem water

Figure 1. Typical freezing patterns of bark and xylem from acclimated peach twigs. Patterns for sucrose and cellulose model systems (from Olien, 1977) are given for comparison.


G Liquid H_2O Per G Dry Wt.
.2
.4
.6
.8
1.0
1.2
1.4
1.6
Temperature (°C)
2
0
-2
-4
-6
-8
-10
-12
-14
-16
CELLULOSE (W42)
XYLEM
BARK
SUCROSE
ADHESION
DESICCATION

resembles that associated with the cellulose model.

During equilibrium freezing of pure water associated in colloidal interfaces, net energy is dissipated by shifts in activation energies as ice crystals grow and the ice-liquid interface approaches the interface between liquid and the cellular components. Competitive structuring of water causes a lowering in the activation energy required for a water moelcule to escape from the ice lattice, resulting in a lowering of the activation limit for melting. This shifts the equilibrium towards melting and reduces the freezing point. Energy for adhesion arises primarily from the lowering of the activation limit for melting. The competitively structured interstitial water acts as an adhesive, binding hydrophilic substances to the ice. Such adhesions between ice and the protoplasmic membrane can cause distortion and disruption of the membrane, while adhesions between ice and soluble proteins or polysaccharides can protect vital regions by modifying structure and location of ice crystal growth (Olien, 1965).

Structure formers and structure breakers can affect transition patterns not only by increasing or decreasing ordering of water, but also by affecting the surface characteristics of the polymer or cellular component. They can also create complex interactions between themselves, ice, water, and cellular components.

Addition of sucrose or other substances also produce colligative effects by reducing the density of water

molecules in the polylayer at the ice-liquid interface. The density of water molecules in the liquid phase is reduced while the density in ice is not decreased. More water molecules are available to escape from the ice lattice than to enter it, resulting in net melting.

When ice and water are separated by a vapor phase, the amount of water associated with each is determined by the vapor pressure, a density function. As temperature slowly decreases, the vapor pressure over ice decreases, producing a difference between the vapor pressure over water associated with plant components and that over ice. This difference is an index of the energy available for vapor desiccation. Water associated with the tissues moves to the ice lattice until the vapor pressures are again in equilibrium. As the water is removed from the cell to extracellular ice crystals, cells become dehydrated. Injury apparently results from removal of some critical amount of water rather than by direct effects of ice, but the exact causes of injury are not clear.

During nonequilibrium freezing, large amounts of available free energy allow explosive ice formation to occur in liquid water not closely associated with cellular components. This results in large perfect crystals which can physically disrupt tissues (Olien, 1964). In acclimated peach xylem tissues this initial nonequilibrium freezing appears to cause little damage. Xylem injury as assessed by browning did not begin until -28.9°C to -31.7°.

and complete death did not occur until -37.2° (Figure 4, Section 1). The living xylem ray cells appear to undergo deep supercooling similar to that in other woody plants (Quamme et al., 1973; Geroge et al., 1974; Burke et al., 1976). The water in the ray cells is probably isolated from water in the xylem vessels by a freezing barrier which is related to the structural integrity of the tissues (Quamme et al., 1973; George and Burke, 1977a). The cumulative effect of repeated freezing and thawing might allow such stresses to become injurious at some point. Also, while initial freezing may not result in apparent damage, it could set up conditions in the tissue for increased damage at lower temperatures by limiting time for diffusion of water to sites where less injury would occur.

The patterns of water redistribution during freezing indicate that cambium and xylem differ in their control of water. The resultant freezing stress is a function of freezing rate, amount of water involved and the free energy produced by freezing (Olien, 1971). Moisture content is a heritable trait in wheat (Gullord, 1974). Moisture content of peach twigs is a relatively stable cultivar trait (Section 1). Differences in control of tissue water can affect hardiness by modifying stress energies. Kinetics inhibitors can modify freezing stress by controlling structure and location of ice. The effectiveness of a specific polysaccharide inhibitor is related to its molecular structure and is a cultivar characteristic (Olien, 1967). If these and other component traits of overall plant hardiness are heritable, and genetic variation exists for them in peach germplasm, they should have simpler inheritance patterns than overall plant hardiness. Their identification would enable plant breeders to develop plants resistant to specific types of stresses, enhancing rate of breeding progress.

No differences were found in initial freezing patterns of similar tissues in 'Redhaven' and 'Siberian C.' Therefore, the superior freezing resistance of 'Siberian C' tissues is not explained by initial redistribution of water. The different freezing patterns of bark and xylem may contribute to observed hardiness differences between these tissues. Different genes may control resistance to these very different types of stresses in peach. Evidence for such separate genetic control exists in wheat (Gullord, 1974).

Future research should explore other reasons for observed cultivar differences. Such differences might arise from differences in resisting or modifying adhesion or desiccation stress. Kinetics inhibitors might also modify structure and location of ice crystals.

SECTION 3

INHERITANCE OF WOOD HARDINESS AMONG HYBRIDS OF COMMERCIAL AND EXOTIC PEACH GENOTYPES

INTRODUCTION

The need for improved wood hardiness in peaches is well documented (Chandler, 1913; Bradford and Cardinell, 1926; Campbell, 1948; Fogle and Overley, 1954). Although artificial freezing has long been used to assess hardiness (Chandler, 1913), most early cultivar improvement was the result of identifying hardy genotypes after test winters. A number of detailed studies of hardiness in other crops indicate that hardiness is a genetically complex trait (Dorsey and Bushnell, 1925; Watkins and Spangelo, 1970; Gullord, 1974; Fejer, 1976). Field observations and intuitive reasoning suggest that the same is true for peach. There are few detailed genetic analyses of quantitative traits in peach. French (1951) investigated several characters, and Hansche et al. (1972) calculated heritability estimates for a number of traits for peaches used in the California Agriculture Experiment Station breeding program. Mowry (1964) concluded that inheritance of flower bud hardiness in peach is quantitative in nature. Hardy parents tended to transmit a greater level of hardiness to their offspring than did tender parents. Some evidence of specific combining ability was also indicated. Recently, very cold hardy peach germplasm has become available from northern China (Pienazek et al., 1968; Zagaja, 1974). As described in the literature review, this germplasm has very poor quality fruits with no commercial value. Therefore, hybridization with genotypes possessing commercially desirable fruit is necessary to obtain recombinant genotypes with improved hardiness and commercially acceptable fruit. Because of the long breeding cycle involved, this process may require 20 or more years. The extent and rate of expected hardiness improvement must be balanced against the time and expense involved when determining merit and feasibility of such a project.

This study was undertaken to examine inheritance of wood hardiness in crosses between commercial cultivars and recently introduced cold hardy peaches. Plant materials were provided by Dr. R.E.C. Layne of the Agriculture Canada Research Station, Harrow, Ontario, Canada.

MATERIALS AND METHODS

Plant material

Seven parents were used. Two, 'Harken' and 'Canadian Harmony' are scion cultivars developed at the Harrow station, while 'Garnet Beauty' is an early ripening mutation of 'Redhaven' found in Ontario, Canada. All 3 are well adapted to northern growing conditions. 'Siberian C' and 'Harrow Blood' are rootstocks that are very hardy under northern conditions (Layne, 1974, 1976; Layne et al., 1976, 1977). 'Siberian C' was selected at Harrow from seeds originating in China. 'Harrow Blood' is a chance seedling found near Harrow in 1938. 'Harrow 6116-256' and 'Harrow 6116-292' (henceforth designated 256 and 292 respectively) are cold hardy, yellow fleshed, freestone siblings selected from an open pollinated F_2 progeny derived from the cross 'Valiant' x _P_. _persica_ var _Mandschurica_. _Mandschurica_ is a very hardy wild Chinese peach. Pollen for the initial cross was obtained from Poland by Harrow research personnel.

The crosses were 'Siberian C' x 'Harrow Blood' (progeny 1), representing very hardy x hardy parents; 'Siberian C' x 'Harken' (progeny 2), representing very hardy x medium hardy parents; 'Garnet Beauty' x 'Harken' (progeny 3),

representing medium hardy x medium hardy parents; 'Canadian Harmony' x 'Harrow 6116-256' (progeny 4) and 'Harrow 6116-292' x 'Harken' (progeny 5), both representing backcrosses to a medium hardy genotype from a medium hardy x very hardy cross.

All progeny trees were planted in 1971. 'Siberian C' and 'Harrow Blood' were in a seed orchard while the commercial cultivars were in a test orchard. Tree age varied but all were 4 to 8 years old except 256 and 292 which were 2-year-old trees. All trees were grown under clean cultivation with a winter cover crop of oats being sown in July.

Sampling procedure

Each progeny contained 50 seedlings, except for progeny 5 with 29. Only healthy trees, free from major cankers, were selected initially. The same seedlings were sampled at each test date. For each sampling date and test temperature, each seedling was represented by a sample of 5 twigs, collected, when possible, from the upper exposed part of the tree (Cain and Andersen, 1975). Each sample was labeled, placed in a plastic bag and packed with snow in a large plastic can to prevent excessive warming during transport to East Lansing.

Ten twigs were collected from each tree of the parent cultivars. On Jan. 16, 1976, 5 trees of 'Harken', 'Siberian C' and 'Harrow Blood' and 4 trees of 'Canadian Harmony' and 'Garnet Beauty' were sampled. Parents 256 and 292 were not available on January 16, 1976. On November 18, 1976 and

February 7, 1977, 3 trees each of 'Harken', 292 and 256, 4 trees of 'Canadian Harmony' and 5 trees of 'Harrow Blood' and 'Siberian C' were sampled, the same trees being sampled on both dates.

Freezing of twigs

Twigs were kept in a cold room at 0°C while being separated into temperature replicates, then were placed into large plastic bags. Twig bases remained exposed and were covered with snow or crushed ice to induce ice inoculation and prevent excessive supercooling. Freezing was done in a 2.4 x 3.0 m freezer. Temperature was controlled by a Partlow cam system on January 16, 1976, and by an evaporator pressure regulator valve on November 18, 1976 and February 7, 1977 (Section 1). All samples were allowed to equilibrate at -2.2°C for at least seven hours, then the temperature was lowered at 1.7° to 2.8° per hour. Samples were held at test temperatures for at least 2 hours, then were transferred to 0°. Test temperatures were -32.2° on January 16, 1976; -26.1° and -31.7° on November 18, 1976 and -33.8° on February 7, 1977. Twigs were placed under warm humid conditions for 36 to 48 hours to allow browning development before being evaluated.

Evaluation of injury

A thin cross section from the basal region of each of the 5 twigs per progeny tree, or 10 twigs per parent

tree, was examined under a binocular microscope. Cambium was rated on an arbitrary 1 to 5 scale (Cain and Andersen, 1976). Xylem was rated on an arbitrary 1 to 5 scale (Section 1).

Vegetative buds were examined on November 18, 1976 at -31.7° and on February 7, 1977 after exposure to -21.7° Observations on 5 buds per twig were taken on each seedling and 10 observations per twig were taken on each parent tree. A 1 to 4 scale was used as follows: 1- no injury; 2- less than one-half of the leaf primordia dead (brown); 3- more than one-half the leaf primordia killed but a few remaining green; 4- all leaf primordia brown (considered dead).

Crop load and ripening date

On August 6, 1976 the amount of fruit borne by each seedling was estimated on a 1 to 5 scale as follows: 1- no fruit; 2- a few scattered fruit; 3- a near commercial crop with no thinning needed; 4- needed a light thinning; 5- needed heavy thinning. None of the seedlings were thinned.

The week of ripening was recorded relative to August 6, 1976 which was designated week 4 to avoid negative numbers. Dates of fruit ripening before August 6, 1976 were estimated by the extent of deterioration of fruit which had fallen from the tree. The earliest ripening trees were estimated to be four weeks earlier than those ripe on August 6, 1976. Ripening dates after August 6, 1976 were

estimated by approximate maturity. The latest ripening trees were estimated to ripen at least four weeks after August 6, 1976. The effect of these variables on previous and subsequent wood hardiness was examined.

Canker ratings

Extent of canker was rated for each seedling on April 26, 1977. Two ratings, one from each side, were taken on each tree and averaged. All cankers were assumed to be symptomatic of infection by perennial canker fungus (Leucostoma spps.). A 1 to 9 rating scale developed by Dr. R.E.C. Layne of Agriculture Canada was used as follows:

1 - absence of visible cankers

2 - evidence of gum and/or a few small insignificant cankers

3 - evidence of distinct cankers, few in number and having little adverse effect on the tree

4 - distinct cankers with one capable of having a debilitating effect on the tree

5 - distinct cankers with more than one causing visible damage

6 - presence of cankers in the trunk and scaffold region

7 - large scaffold and/or trunk cankers likely to kill the tree

8 - large scaffold and/or trunk cankers killing many branches

9 - death of the tree from trunk and scaffold cankers.

The data presented may not be truly representative of the populations, for trees were initially chosen because they had few serious cankers.

Bloom type

On April 26, 1977 each seedling was classified as having showy or nonshowy bloom. Bloom type is a monogenic trait with nonshowy being recessive (Bailey and French, 1942). Where possible, this trait was used as one indication that seedlings were true hybrids.

Data analysis

Data on injury, crop load, ripening date and cankering were analyzed via analysis of variance (AOV) and means were separated using Duncan's Multiple Range test or its uneven replicate approximation (Steel and Torrie, 1960). Population distributions of each progeny for each trait measured are presented in Appendix B. As a population approaches the scale limits, it becomes distinctly skewed and its variance may be reduced. Nonnormality and heterogeneous variance can affect the mean separation tests. Test temperatures were chosen to induce a 3.0 level of injury which allows a normal dispersion of ratings about the mean. Gullord et al. (1975), rating freezing injury of wheat crowns, found that when the populations were kept well within scale limits all varieties had a normal distribution and homogeneous variance; however, populations became skewed when the population mean approached either scale limit.

Heritability Estimates

Broad-sense heritability estimates of injury ratings were calculated for progenies and parents. Progeny estimates were calculated using the equation:

$$h^2_{BS} = (\hat{\sigma}_{BP}^{2} + \hat{\sigma}_{WP}^{2} - \hat{\sigma}_{WC}^{2})/(\hat{\sigma}_{BP}^{2} + \hat{\sigma}_{WP}^{2})$$

where h^2_{BS} is the broad-sense (BS) heritability estimate; $\hat{\sigma}_{BP}^{2}$ is the between progeny variance; $\hat{\sigma}_{WP}^{2}$ is within progeny variance; and $\hat{\sigma}_{WC}^{2}$ is within parental clone variance used as an estimation of environmental variation.

Broad-sense heritability estimates obtained from parent data were calculated as follows:

$$h^2_{BS} = (\hat{\sigma}_{BC}^{2} - \hat{\sigma}_{WC}^{2})/\hat{\sigma}_{BC}^{2}$$

where σ_{BC}^{2} is between clonal parent variation and $\hat{\sigma}_{WC}^{2}$ is within clonal parent variation.

RESULTS

Parent injury ratings

Significant differences among parent cultivars for injury of the cambium region, xylem and vegetative buds were found at every test date (Table 1).

Cambium. 'Siberian C' consistently exhibited the least injury (Table 1). 'Harrow Blood' sustained significantly more injury than 'Siberian C' except on January 16, 1976. The backcross selections 256 and 292 were comparable to 'Harrow Blood.' Significant differences existed among the 3 commercial cultivars, all of which were injured more than the rootstocks or backcross parents, except on February 7, 1977 when injury to 'Harken' was not significantly different from 256 and 292. 'Harken' and 'Canadian Harmony' were never significantly different from each other, and 'Garnet Beauty' always exhibited the most injury.

Xylem. At all dates 'Siberian C' suffered less injury than the commercial and backcross cultivars (Table 1). 'Harrow Blood' was similar to 'Siberian C' on January 16, 1976 and February 7, 1977 but had more injury at both temperatures on November 18, 1976 when it suffered as much injury as the commercial cultivars. The two backcrosses

Table 1. Injury ratings of parents' cambium, xylem and vegetative buds subjected to controlled freezer tests on 3 dates

Tissue	Parents						
	Siberian C	Harrow Blood	Harken	Canadian Harmony	Garnet Beauty	256	292
	January 16, 1976 (-32.2°)						
Cambium	1.1a[z]	1.2a	1.8bc	1.7b	2.1c	-	-
Xylem	1.1a	1.5a	2.1b	2.9c	2.8c	-	-
	November 18, 1976 (-26.1°)						
Cambium	1.4a	1.8b	2.4c	2.4c	3.0d	1.5ab	1.8b
Xylem	1.1a	3.1de	3.1de	3.4e	2.9d	1.4b	2.2c
	(-31.7°)						
Cambium	2.3a	3.4c	3.3c	3.5c	4.0d	2.5ab	2.7b
Xylem	3.6a	5.0b	5.0b	5.0b	5.0b	4.8b	5.0b
Vegetative buds	2.2b	1.7a	3.5c	3.6cd	3.9d	3.8cd	2.2b
	February 7, 1977 (-33.8°)						
Cambium	1.7a	2.2b	2.8cd	3.1d	3.8e	2.4bc	2.3bc
Xylem	3.2a	3.3a	4.8b	5.0b	5.0b	4.7b	4.4b
Vegetative buds	2.5b	2.0a	3.4c	3.6c	3.7c	3.7c	2.8b

[z] Within rows, means followed by the same letter are not significantly different at P.05 by Duncan's multiple range test.

were similar to each other and intermediate between the hardy rootstocks and the commercial cultivars at -26° C on November 18, 1976. On November 18, 1976, at -31.7° and February 7, 1977, all cultivars except 'Siberian C' were nearly or completely killed, thus no differences could be detected among them. At -26.1° on November 18, 1976, 'Harmony' had more injury than 'Garnet Beauty' while 'Harken' was intermediate.

Vegetative buds. In contrast to woody tissues, vegetative buds of 'Siberian C' showed significantly more injury than those of 'Harrow Blood'. 'Siberian C' and 292 were similar while 256 suffered as much injury as the commercial cultivars. At -31.7° on November 18, 1976, 'Harken' had less injury than 'Garnet Beauty'. This was the only significant difference between the commercial types.

Mean family hardiness

Cambium. In general, progeny 1 was the most hardy in all tests, while progenies 3 and 4 were least hardy (Table 2). Progenies 2 and 5 were intermediate and differed significantly in only one case.

Xylem. Hardiness of the xylem generally paralleled that of the bark tissues, but more injury occurred at the same temperature (Table 2). Progeny 1 was hardier than all others except on January 16, 1976, while progenies 3 and 4 were least hardy except on February 7, 1977 when

Table 2. Mean freezing injury of cambium, xylem and vegetative buds tested on 3 dates, and crop load, ripening date and canker ratings for 5 progenies.

Tissue	Progeny				
	1	2	3	4	5
	Siberian C X Harrow Blood	Siberian C X Harken	Garnet Beauty X Harken	Canadian Harmony X 256	292 X Harken
			January 16, 1976 (-32.2°)		
Cambium	1.1a^{z}	1.3b	1.5c	1.5c	1.2ab
	(.11)	(.34)	(.33)	(.32)	(.19)
Xylem	1.2a	1.5b	1.9c	2.3d	1.4ab
	(.23)	(.41)	(.46)	(.52)	(.38)
			November 18, 1976 (-26.1°)		
Cambium	1.5a	2.0c	2.1d	2.2d	1.9b
	(.27)	(.26)	(.24)	(.27)	(.42)
Xylem	1.7a	1.9b	2.6d	2.9e	2.3c
	(.34)	(.45)	(.41)	(.46)	(.69)
			(-31.7)		
Cambium	2.8a	3.0b	3.0b	3.0b	3.0b
	(.27)	(.21)	(.25)	(.20)	(.19)
Vegetative buds	3.0a	3.1ab	3.5d	3.3cd	3.2bc
	(.40)	(.34)	(.43)	(.37)	(.47)
			February 7, 1977 (-33.8°)		
Cambium	1.5a	1.9b	2.3d	2.1c	1.9b
	(.26)	(.42)	(.37)	(.43)	(.30)
Xylem	3.2a	3.5b	4.4d	4.2c	4.6d
	(.49)	(.58)	(.48)	(.52)	(.41)
Vegetative buds	2.5a	2.4a	3.6b	3.4b	3.4b
	(.47)	(.38)	(.40)	(.58)	(.42)
			1976 crop load^{y}		
	3.6bc	3.8c	2.7a	3.3b	3.4bc
	(1.05)	(1.19)	(1.21)	(1.08)	(1.08)
			1976 ripe date^{x}		
	8.0c	6.3c	3.5a	6.4b	6.0b
	(0.00)	(2.05)	(2.59)	(1.93)	(1.83)
			Canker rating^{w}		
	6.3c	5.9c	7.0d	5.3b	4.5a
	(0.96)	(1.88)	(0.89)	(1.78)	(1.98)

[z] Within rows, means followed by the same letter are not significantly different at P.05 by Duncan's multiple range test. Figures in parentheses are standard deviations used as a measure of population dispersion.

[y] Crop load, amount of fruit borne by each seedling, 1 = no crop to 5 = heavy crop.

[x] Ripe date, estimated weeks to fruit maturity after July 6.

[w] Canker rating, 1 = no cankers to 9 = death of tree from cankers.

progency 3 did not differ from progeny 5. Progeny 2 was intermediate between progeny 1 progenies 4 and 5 except on January 16, 1976 when progeny 2 did not differ significantly from progeny 5.

Vegetative buds. Progenies 1 and 2 were similar and generally hardier than the other progenies. No differences were detected between progenies 3, 4 and 5 on February 7, 1977. On November 18, 1976, progeny 3 was slightly but not significantly worse than progeny 4, while progeny 5 was intermediate, not differing from either progeny 1 or 4.

Crop load 1976. Progeny 3 had significantly fewer fruit than other progenies in 1976 (Table 2) and was the least hardy. Progenies 1, 4 and 5 bore similar amounts, while yield of progeny 2 was significantly greater than that of progeny 1, but not significantly different from that of 4 and 5. Very low negative correlations existed between crop load and hardiness (Tables 6 and 7).

Ripening date 1976. Large differences in ripening date were apparent among the progenies (Table 2). Fruits of progeny 1 were estimated to be 4½ weeks later than those of progeny 3, and much later than those of most commercial varieties. Progenies 2, 4 and 5 included a majority of midseason seedlings with similar ripening dates. Most seedlings in progeny 3 were early to midseason. Very low negative correlations existed between ripening date and hardiness (Tables 6 and 7).

Canker ratings. Progeny 5 had fewest cankers while progeny 3 had the most (Table 2). Progenies 1, 2 and 4 were intermediate, 4 having significantly less cankers than the other two. The 7.0 rating of progeny 3 indicates very serious cankering. No correlation existed between canker ratings and hardiness

Bloom type. All seedling trees were evaluated for bloom type. 'Harken', 'Canadian Harmony', 'Garnet Beauty' and progeny 3 seedlings all had nonshowy flowers, while 'Siberian C', 'Harrow Blood', 256, 292 and progeny 1 seedlings had showy blooms (Table 3). Progenies with showy x nonshowy parentage (progenies 2, 4 and 5) all segregated in 1:1 ratios, indicating that 'Siberian C', 256 and 292 have the genotype Sh/sh. 'Harrow Blood' is Sh/Sh and the commercial cultivars are all homozygous recessive.

Table 3. Inheritance of bloom type in parents and progenies. Showy bloom (Sh) is dominant to nonshowy bloom (sh)

Parent	Genotype	Phenotype
Harken	sh/sh	nonshowy
Canadian Harmony	sh/sh	nonshowy
Garney Beauty	sh/sh	nonshowy
Siberian C	Sh/sh	showy
Harrow Blood	Sh/Sh	showy
256	Sh/sh	showy
292	Sh/sh	showy

Progeny	expected	observed
Siberian C X Harrow Blood	All showy	All showy
Siberian C X Harken	1:1	27 showy : 23 nonshowy
Garnet Beauty X Harken	All nonshowy	All showy
Canadian Harmony X 256	1:1	24 showy : 26 nonshowy
292 X Harken	1:1	17 showy : 12 nonshowy

Population dispersion. Standard deviations (SD) of each trait for each progeny indicated that no progeny had a consistently large or small variance (Table 2). Progenies whose mean approached the scale limits (progeny 1, January 16, 1976 cambium) tended to have lower variances. Population distributions are illustrated graphically in Appendix B.

Genetic analysis of progenies. Separate parent and progeny broad-sense heritability estimates were calculated for each tissue at each test date (Table 4). All estimates except for the cambium on November 18, 1976, -37.7°C were in the 0.65 to 0.96 range. Estimates based on parent data

Table 4. Progeny and parent broad-sense heritability estimates for cold injury assessed for individual tissues, temperatures, and dates

Date and tissue test temperature (°C)	Progeny $\hat{h}^2_{BS}$	Parent $\hat{h}^2_{BS}$	σ^2_{WC}[z]
Jan 16 cambium[y] -32.2	.65	.77	.04
Nov 18 cambium -26.1	.69	.82	.05
Nov 18 cambium -31.7	.05	.84	.06
Feb 7 cambium -33.8	.73	.86	.06
Jan 16 xylem -32.2	.65	.80	.12
Nov 18 xylem -26.1	.92	.96	.04
Nov 18 xylem -31.7	-	.86	.05
Feb 7 xylem -33.8	.83	.84	.10
Nov 18 vegetative buds -31.7	.74	.94	.05
Feb 7 vegetative buds -33.8	.85	.84	.08
Jan 16 average injury -32.2	.69	.84	.05
Nov 18 average injury -26.1	.90	.95	.03
Nov 18 average injury -31.7	-	.94	.02
Feb 7 average injury -23.8	.86	.91	.05
Nov 18 total injury -31.7	-	.94	.02
Feb 7 total injury -23.8	.94	.96	.02
Overall cambium	.82	.96	.01
Overall xylem	.93	.99	.02
All tissues	.94	1.0	.01

[z] σ^2_{WC} is within clonal parent variation taken as an estimate of environmental variation.

[y] Average injury is the average of the cambium and xylem; total injury is the mean of cambium, xylem and vegetative buds; overall cambium and xylem is the mean rating across all dates and temperatures; all tissues is the mean rating across all tissues, temperatures and dates.

tended to be slightly higher in most cases. The extremely low heritability estimate for cambium on November 18, 1976, -31.7° is due to small differences between progenies (Table 2 and Appendix B). Environmental variation, as estimated from within parent variance, was consistently very small.

Deviations of progeny means from their midparent means (Table 5) are generally small negative values, indicating progenies had slightly less injury than would be expected from parent performance. Values for progeny 4 were more positive than other progenies indicating that at several dates it was injured more than expected based on parent ratings. Comparing progeny means in Table 2 with parent means in Table 1 shows a number of cases where the progeny mean is lower than the least injured parent. Vegetative bud injury at -31.7° on November 18, 1976,is the only case in which the progeny had more injury than the most severely injured parent.

Correlations between injury ratings for separate tissues and samples were based on individual tree ratings of all progenies (Table 6). Nearly all correlation coefficients were significantly different from zero, but they tended to be very low. On November 16, 1976 a correlation of only 0.41 was obtained when cambium ratings at -26° and -31.7°C were compared. If progeny means were used (Table 7), values of correlation coefficients were increased, but significance levels decreased because they were based on 5 observations rather than 229 (Table 6).

Table 5. Deviations of progeny mean injury ratings from midparent ratings calculated for each tissue, temperature and date

Tissue	Siberian C X Harken	Siberian C X Harrow Blood	Garnet Beauty X Harken	Canadian Harmony X 256	292 X Harken
		January 16, 1976 (-32.2)			
cambium	-0.1[z]	-0.1	-0.5	-	-
xylem	-0.1	-0.1	-0.5	-	-
		November 18, 1976 (-26.1)			
cambium	+0.1	-0.1	-0.6	+0.1	-0.1
xylem	-0.2	-0.4	-0.4	+0.1	+0.1
			(-31.7)		
cambium	+0.2	0.0	-0.6	-0.1	+0.1
vegetative buds	+0.3	+1.0	-0.2	+0.4	-0.4
		February 7, 1977 (-33.8)			
cambium	-0.3	-0.5	-1.0	-0.6	-0.7
xylem	-0.5	0.0	-0.5	-0.5	-0.2
vegetative buds	-0.6	+0.3	0.0	+0.2	-0.1

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[z] Positive values indicate progenies sustained more injury than the parents, negative values indicate the opposite.

Table 6. Correlation coefficients[z] between individual tree ratings across all progenies for injury, crop load, ripening date and canker ratings

	Date and temperature (°C)								
	Jan 16, 1976 (-32.2)		Nov 18, 1976 (-26.1)		Nov 18, 1976 (-31.7)		Feb 7, 1977 (-33.8)		
	cambium	xylem	cambium	xylem	cambium	vegetative buds	cambium	xylem	vegetative buds
Jan 16 (-32.2)									
xylem	.61[y]								
Nov 18 (-26.1)									
cambium	.34	.39							
xylem	.29	.50	.64						
Nov 18 (-31.7)									
cambium	.07	.16	.41	.20					
vegetative buds	.29	.23	.26	.30	.08				
Feb 7 (-33.8)									
cambium	.27	.35	.46	.40	.32	.22			
xylem	.21	.36	.38	.52	.25	.27	.40		
vegetative buds	.34	.38	.35	.53	.14	.48	.35	.60	
1976 crop load	-.15	-.18	-.15	-.28	.04	-.10	-.22	-.30	-.29
1976 ripe date	-.22	-.15	-.34	-.29	-.16	-.26	-.48	-.34	-.30
canker rating	.03	.03	-.03	-.02	.01	.07	.04	-.02	.07

[z] 229 observations per correlation.

[y] All correlations $\geq |.16|$ and $\geq |.21|$ are significant at $P = .01$ and $P = .001$, respectively

Table 7. Correlation coefficients^z among progeny means for injury, crop load, ripe date and canker ratings

	Date and temperature (°C)									1976 crop load	1976 ripe date
	Jan 16, 1976 (-32.2)		Nov 18, 1976 (-26.1)		Nov 18, 1976 (-31.7)		Feb 7, 1977 (-33.8)				
	cambium	xylem	cambium	xylem	cambium	vegetative buds	cambium	xylem	vegetative buds		
Jan 16 (-32.2) xylem	.95^y (.01)										
Nov 18 (-26.1) cambium	.93 (.06)	.82 (.09)									
xylem	.86 (.06)	.89 (.04)	.81 (.10)								
Nov 18 (-31.7) cambium	.84 (.08)	.70 (.19)	.98 (.001)	.76 (.14)							
vegetative buds	.82 (.09)	.73 (.16)	.82 (.09)	.90 (.04)	.80 (.11)						
Feb 7 (-33.8 cambium	.91 (.03)	.77 (.13)	.91 (.03)	.85 (.07)	.87 (.05)	.96 (.01)					
xylem	.52 (.37)	.47 (.43)	.63 (.25)	.81 (.10)	.70 (.19)	.86 (.06)	.73 (.16)				
vegetative buds	.58 (.30)	.60 (.29)	.57 (.31)	.89 (.04)	.58 (.31)	.88 (.04)	.74 (.15)	.94 (.02)			
1976 crop load	-.58 (.30)	-.51 (.38)	-.46 (.44)	-.71 (.18)	-.40 (.50)	-.86 (.06)	-.78 (.12)	-.67 (.22)	-.81 (.10)		
1976 ripe date	-.66 (.23)	-.43 (.47)	-.72 (.17)	-.61 (.28)	-.74 (.15)	-.89 (.05)	-.91 (.03)	-.70 (.19)	-.65 (.24)	.80 (.11)	
canker rating	.20 (.74)	.05 (.93)	0.0 (1.0)	-.13 (.83)	-.12 (.85)	-.12 (.85)	.23 (.71)	-.34 (.57)	-.20 (.74)	-.40 (.50)	-.35 (.56)

^{z}5 observations per correlation.

^{y}Values in parentheses indicate significance level.

DISCUSSION

The relative hardiness of 'Velvet', 'Redhaven' and 'Siberian C', (Section 1) and parents used in this study, as assessed by controlled freezer tests, matched their field hardiness rankings. This indicates that controlled freezing is a reliable method of assessing relative hardiness of different genotypes.

For all tissues at all dates tested, 'Siberian C' was significantly hardier than any of the commercial cultivars used. 'Siberian C' generally suffered less injury to cambium and xylem than did 'Harrow Blood', which is in agreement with Layne's (1974) and Ormrod and Layne's (1974) observations on wood hardiness of these rootstocks. These results agree with the effects these rootstocks have in increasing flower bud, phloem, cambium and xylem hardiness of scion cultivars budded onto them, as measured by browning tests (Layne,et al., 1977). However,they disagree with findings of Chaplin and Schneider (1974) who, using electrolytic conductance tests on 1-year-old trees found that 'Harrow Blood' promoted greater wood hardiness of budded scion cultivars than did 'Siberian C.'

The greater hardiness of 'Harrow Blood' vegetative buds compared to 'Siberian C' may indicate a different

physiological stress is occurring in this organ in comparison with stresses occurring in cambial and xylem tissues. Data presented in Section 2 showed that stresses arising in the xylem are different from those in bark tissues. Layne et al. (1977) found that injury ratings of phloem, cambium and xylem were closely correlated with each other in the fall, but were not correlated with flower bud injury on the same shoots. Blake (1938) also noted that hardiness of the lower trunk and bark tissues was not always correlated with fruit bud hardiness. Flower buds undergo different freezing processes than adjacent stem tissues (Graham, 1971; Quamme et al., 1975). The inconsistency in relative hardiness of various tissues in different genotypes may indicate that separate genes control reaction to stresses occurring in different tissues. Gullord (1975) has shown that different groups of genes may be responsible for reaction to different types of stresses in wheat.

The hypothesis that separate groups of genes are controlling stress reactions in different tissues is not supported by progeny data. If 'Harrow Blood' has more favorable alleles for vegetative bud hardiness than 'Siberian C' it should contribute more favorable alleles to its progeny than 'Siberian C.' 'Harken', having more injury than either 'Siberian C' or 'Harrow Blood', should have fewer favorable alleles to contribute. Therefore, the 'Siberian C' x 'Harrow Blood' progeny should have more favorable alleles than the 'Siberian C' x 'Harken' progeny and thus should have a lower injury rating, however, both have similar injury. A similar inconsistency occurs with the backcross parents. Specific combining ability could account for such discrepancies. All interactions between tissues, dates and genotypes must be interpreted cautiously, since even within one date and tissue, data obtained at different temperatures yielded different results. Different stresses developing within tissues at different temperatures may explain such differences on a physiological basis, but differences could also be due to poor repeatability of the rating system. Repeatability of the rating system, using different plant material, was previously found to be about 0.8 (Cain and Andersen, 1976).

Based upon parent performance, vegetative buds of progeny 4 were expected to have more injury than those of progeny 5; however, ratings were nearly identical. Again vegetative bud response differed from that of cambium and xylem. This again suggests that genetic control of hardiness in vegetative buds differs from that in cambium and xylem.

Except for vegetative buds, the 'Siberian C' x 'Harken' (very hardy x medium hardy) cross was intermediate between 'Garnet Beauty' x 'Harken' (medium hardy x medium hardy) and 'Siberian C' x 'Harrow Blood' (very hardy x hardy). The standard deviations (Table 2) of each progeny are reasonably similar except when scale limits are approached. Cambium ratings show somewhat less dispersion

than those of xylem or vegetative buds. Distributions approximated the normal distribution except where scale limits are approached (Appendix B), which is in agreement with the data of Gullord et al. (1975). Parent and progeny mean performance and generally normal distributions of progenies indicate that inheritance of resistance to freezing stress is quantitative. This is to be expected in a physiologically complex trait and agrees with other reports (Watkins and Spangelo, 1970; Federer, 1976; Dorsey and Bushnell, 1925).

Deviations of progeny means from midparent values were usually small negative values (Table 5). This may indicate presence of some dominant genes for hardiness, in agreement with the data of Hildreth and Powers (1941), who found winter hardiness in strawberries to be dominant. Gullord (1974) found low moisture content and high and low intensity freezing hardiness in wheat to be controlled by partially dominant genes mostly additive in their effect. However, hardiness appears to be recessive in crosses between other hardy Chinese peaches and commercial types, with most seedlings tending to be closer to the commercial parent in hardiness (Zagaja, personal communication). In a number of cases, progeny means are lower than the least injured parent (Tables 1 and 2); this may indicate presence of overdominance or transgressive segregation. However, comparisons between parent and progeny data should be interpreted very cautiously because differences may be due to physiological differences resulting from divergent cultural

practices and tree age. These preliminary findings should be investigated further when more suitable populations become available for genetic analysis.

Cultural practices are known to affect subsequent winter hardiness (Hildreth, 1926; Cooper, 1953; Edgerton, 1960; Chandler, 1913). Significant differences exist among progenies regarding crop load and ripening date (Table 2.) These differences were not highly correlated with differences in injury on either an individual tree basis (Table 6) or a progeny basis (Table 7). Since none of the fruits were removed, excessive crop set on some seedlings was expected to reduce hardiness. It was suspected that late maturity might also reduce hardiness. The small negative correlations for both crop size and ripening date vs. injury ratings may be explained by the fact that both 'Siberian C' and 'Harrow Blood' ripen later and their flower buds are hardier than the commercial parents, and these traits are transmitted to their offspring. Hansche (1972) estimated narrow-sense heritability for ripe date and crop to be 0.84 and 0.08 respectively. Because no estimate of environmental variation was available in my study, no heritability estimates were calculated for these traits. However, large differences were noted among progenies for both traits (Table 2).

Winter injury has been implicated in increasing susceptibility of peaches to perennial canker (Weaver, 1963). Trees with more injury were therefore expected to have more

serious cankering. Tables 6 and 7 show no correlation between canker ratings and injury ratings either on a progeny or single tree basis. Backcross progenies having commercial and exotic parentage have fewer cankers than progenies having entirely commercial or exotic parents (Table 2). Several explanations are possible. The *Mandchurica* ancestor of progenies 4 and 5 may contribute some resistance apart from improved hardiness. 'Siberian C' and 'Harrow Blodd' have narrow crotch angles of scaffold limbs, leading to more bark inclusions in these crotches which provide sites for canker infection. Narrow crotches are also more susceptible to winter injury than are wide crotches (Weaver, 1968; Blake, 1935). Hybrids between commercial and exotic types may have fewer cankers because recombination has incorporated improved hardiness of the exotics with wider crotch angles of the commercial types. Weaver (1968) found large differences in crotch angle between cultivars.

Crop load, ripening date and canker ratings were used in a multiple regression to see if they affected previous or subsequent tissue injury ratings. All R^2 values were 0.15 or less, and therefore accounted for virtually no variation among injury ratings.

Broad-sense heritability estimates the relative importance of genetic and environmental influence on phenotypic expression of a trait. Narrow-sense heritability is the ratio of the additive portion of the genetic variance

to the total variance; therefore, broad-sense heritability is always equal to or greater than narrow-sense heritability. A trait's heritability may be increased by either increasing genetic variation or by reducing non-genetic variation. Non-genetic variation can be decreased by increasing accuracy and precision of measurements through increased replication or improved sampling procedures and experimental technique. Cain and Andersen (1976) suggest sampling procedures for minimizing non-genetic wood hardiness variation in peach. Hansche et al. (1972), estimating narrow-sense heritabilities for several traits in peach, found ripe date (.84) was very high, bloom date (.39), amount of bloom (.38) and fruit cheek (.26) were high, fruit firmness (.13) and acidity (.19) were moderate, and percent soluble solids (.01) was very low.

In this study, broad-sense heritability estimates (Table 2) indicate that within dates and tissues, injury (browning) is a highly heritable trait. Heritability estimates of composite traits were also high. The environmental variation was consistently very low and may have been under estimated, since seedlings were subjected to different cultural practices and growth response may have varied. Environmental variation was much lower than that found in previous experiments using different material (Cain and Andersen, 1976). The amount of additive genetic variance is not known. Genetic variance for winter survival of apples was found to be mainly additive (Watkins and

Spangelo, 1970), but significant specific combining ability effects were also found in this species (Fejer, 1976). In wheat, genetic variation for high and low intensity freezing was found to be largely additive in nature (Gullord, 1974). The progenies had full and half-sib relationships and narrow-sense heritabilities were calculated. However, because estimates were based on only a single degree of freedom, they were not accurate enough to be useful and are not presented.

Phenotypic correlations of injury to individual tissues between each date and temperature were used to estimate repeatability of individual trees (Table 6) or progenies (Table 7). Heritability is a measure of the repeatability of the genotypic expression. The correlations presented, while they are not heritability *per se*, contain both the genetic and environmental variation and estimate phenotypic repeatability over time, temperature or tissue. Correlation coefficients were higher when based on progeny performance. This was expected, as progeny means were based on many more observations than individual tree means. Correlations within similar tissues were generally higher than correlations between different tissues. The low correlation coefficients obtained for individual trees indicate repeatability of individual seedlings is quite low when measured across tests. Watkins and Spangelo (1970) obtained correlation coefficients ranging from 0.84 to 0.50 for several measures of tree survival in two apple

dialleles. Fejer (1976), using other apple dialleles, also found low correlations between electrical impedance values and other measures of plant injury. Correlation coefficients for impedance values measured at three different dates were also low. French (1951) has found low year to year correlations in other quantitative traits in peaches.

Considering the limited environmental variation for an individual tissue sample, the lack of correlation may indicate presence of large genotype by environment interactions. These interactions may reflect the inability of the rating system to discriminate between genotypes in which injury actually differs or they may indicate true differential response of individual tissues to stresses produced at different temperatures and dates. In these experiments,separation of interactions due to experimental technique from those due to physiological and genetic causes was not possible. Large numbers of values near the scale limits during some tests, and the narrow range of some progenies undoubtedly also contributed to the low correlations.

While important cultivar differences for tree survival occur among commercial peaches (Chandler, 1913; Fogle and Overley, 1954; Campbell and Hadle, 1960), wild peaches from northern China are far hardier then commercial types. This germplasm represents a source of new alleles and/or increased gene frequencies for wood hardiness improvement.

Since these wild peaches have small, low quality fruit, their favorable hardiness genes need to be recombined with the excellent fruit quality characters of commercial cultivars. The question, then, is: what is the most efficient breeding method for recombining these two groups of characters into new superior cultivars? After the initial crosses are made to produce an F_1 progeny, several alternative methods are available.

Logical alternatives would be backcrossing the hybrids to commercial cultivars or intermating of the hybrids using some form of recurrent selection. Allard (1960) states that in backcrossing the character being transferred should be highly heritable since the certainty with which the character can be identified in segregating populations determines the speed and efficiency with which it can be transferred. A useful intensity of the character must be maintained through several generations of backcrosses.

The high broad-sense heritabilities found in this study suggest that a high level of cold resistance could be maintained in a backcrossing program. Yet, conflicting low correlations indicate that many misidentifications might be made. The mean performance of the two backcross populations (Table 2) indicates that the overall performance of progeny 5 was superior to the commercial cross (progeny 3) while that of the other backcross progeny (progeny 4) was not. Parents 256 and 292 were selected primarily for a high level of cold hardiness combined with commercial characteristics, leading to a rapid approach toward restored commercial fruit type. If selection were to be based primarily on hardiness, more improvement might be expected in cold resistance while slower return to commercial fruit quality might be expected.

Linkage groups are a major factor in determining how readily cold hardiness and high fruit quality characters can be recombined. Hybrid progeny with the greatest cold resistance often have the poorest fruit quality, and *vice versa* (S. Zagaja and R.E.C. Layne, personal communication). Since maximum chance for recombination between chromosomes from opposite parents occurs at F_1 synapsis, growing large F_2 populations before beginning a backcross program would aid in producing desirable recombinant types.

The alternative to the backcrossing procedure is some form of recurrent selection. Here the F_1 constitutes a base source population from which selections can be intermated to produce a second cycle. Unless very large populations were grown, this method would probably result in slower approach to the reconstituted commercial type, but it would provide a greater chance for recombination between the genomes.

Andersen (1970) has discussed the relative merits of several recurrent selection schemes as they relate to clonally propagated crops. Recurrent mass selection based on clonal family performance (RMSCF) was inferior to simple recurrent mass selection (RMS) except when heritability was

very low, and even then gains resulting from RMSCF could be nullified if clonal propagation lengthened the breeding cycle substantially. Another disadvantage of RMSCF is that fewer genotypes can be grown on the same unit of land. The high heritabilities for tissue injury found here indicate that RMS would be superior to RMSCF. Even though correlation coefficients of injury ratings between tissues, temperatures or dates were quite low, the mean performance of an individual seedling tested over an entire winter should increase the accuracy of the estimate of net genetic worth without lengthening the breeding cycle. The low environmental variation (Table 3) indicates that, if plots are reasonably uniform, replicating over locations (within a limited climatic region) would not significantly improve accuracy of estimates of genotype performance. This is also supported by earlier work (Cain and Andersen, 1976) which showed that between-tree variation was only a minor contributor to overall environmental variation.

Other alternatives to RMS are recurrent full-sib family selection (RFSFS), recurrent selection among half-sib families (RSHSF) and selfed progeny family selection (SPS). RFSFS is superior to RMS only when heritability is very low, while RSHSF has utility only where large full-sib families are difficult to obtain, which is not true in this case. SPS is more competitive with RMS at higher levels of dominance and at higher gene frequencies.

The small number of families involved in this study

and the conflicts between the heritability estimates and the correlation data make it impossible to state with reasonable assurance which breeding approach would produce most rapid improvements in peach tree cold resistance.

More suitably designed populations are needed to obtain more accurate estimates of the heritability and magnitude of genotype by environment interactions. Also, more physiological research is needed to identify components of the complex trait of cold hardiness. Such components should have simpler modes of inheritance and be easier to identify and manipulate genetically.

SUMMARY AND CONCLUSIONS

Freezing tests were conducted on 'Velvet', 'Redhaven' and 'Siberian C' peach cultivars on 13 dates during the winters of 1975-76 and 1976-77. 'Siberian C' suffered least injury at all test dates, 'Velvet' suffered most and 'Redhaven' was intermediate. This agrees with field assessment of the relative hardiness of the cultivars.

Xylem tissues were injured at higher temperatures and over a narrower range than the inner bark. During acclimation, the major change in xylem temperature response pattern was a downward shift in temperatures necessary to cause injury. In fall, inner bark injury increased rapidly over a narrow temperature range in a manner similar to the xylem. In midwinter, injury increased slowly as temperature declined. This shift may reflect a change in the type of freezing stress occurring at different seasons. Xylem appeared to deep supercool.

Within test dates, twig injury was correlated with overall twig moisture content. 'Velvet' generally had the highest moisture content, 'Redhaven' was intermediate and 'Siberian C' had the lowest moisture content. Injury and moisture content were not correlated over dates because moisture content remained quite stable throughout the

winter while injury changed dramatically. Increasing twig moisture content and preconditioning twigs at warmer temperatures increased subsequent freeze injury to bark. Increased moisture content did not affect xylem injury but high temperature increased xylem injury in both cultivars. Increasing the moisture content increased inner bark injury more in 'Redhaven' than in 'Siberian C', while high temperature pretreatment increased injury more in 'Siberian C' than in 'Redhaven'. The superior hardiness of 'Siberian C' was not explained by moisture content alone.

Moisture content of bark tissues was nearly twice that of the xylem. Equilibrium freezing occurred in bark tissues, nonequilibrium freezing in xylem tissues, indicating that water in bark tissues was more closely associated with cellular components than was that in the xylem. Freezing patterns of corresponding tissues in 'Redhaven' and 'Siberian C' were similar. Thus, cultivar hardiness differences were not explained by differences in water redistribution during initial freezing.

Injury to inner bark, xylem and vegetative buds was determined for parents and progenies of crosses among medium hardy commercial cultivars, very hardy rootstock cultivars, and two backcross progenies. As a parent, 'Siberian C' inner bark and xylem suffered significantly less injury than the same tissues in all commercial cultivars tested, and generally had less injury than 'Harrow Blood'. Vegetative buds of 'Harrow Blood', however,

suffered less injury than those of 'Siberian C'. Vegetative bud performance in the progenies also did not correspond with that of inner bark and xylem, nor did relative ranking correspond with predicted ranking based on parent performance. Relative ranking of progeny means as to inner bark and xylem injury could be predicted based on average performance of the parents, with hardier parents producing hardier progenies. All populations had similar distributions except where scale limits were approached.

Progenies differed significantly in canker rating, ripening date and crop load, but these factors had no significant effect on injury. Backcross progenies had lower canker ratings than progenies of very hardy cultivars.

Broad-sense heritability estimates indicated that within dates and tissues, freeze injury is a highly heritable trait. Environmental variation estimates were very low. Correlation coefficients among tissues, temperatures and dates based on individual tree observations indicated repeatability of individual genotypes was very low. Correlation coefficients based on progeny family means were higher. Relative ranking of parents was quite consistent over time for a given tissue. This suggested limited replication of individual seedlings may have been a problem. The low correlations indicate many misidentifications of individual genotypes would be made if selection were based on individual phenotypic performance. This is in conflict with the high heritability estimates. The true

heritability of the trait would markedly affect the breeding procedure to be used. With high heritability, most rapid progress would be made by backcrossing or recurrent mass selection based on individual performance. Low heritability would dictate that some form of family selection should be employed.

APPENDICES

APPENDIX A

Table 1. Means and standard errors for cambium and xylem injury ratings to 'Velvet', 'Redhaven' and 'Siberian C' at each sampling date and test temperature, 1975-77.

Date	Temp (°C)	Velvet				Redhaven				Siberian C			
		cambium		xylem		cambium		xylem		cambium		xylem	
		$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Nov 9	-23.3	2.4	.11	3.3	.15	2.0	.11	3.0	.17	1.0	.00	1.0	.00
	-28.9	4.2	.14	5.0	.00	3.6	.17	4.9	.07	1.5	.15	2.4	.15
	-33.3	5.0	.00	5.0	.00	5.0	.00	5.0	.00	2.8	.17	4.7	.11
	-37.2	5.0	.00	5.0	.00	5.0	.00	5.0	.00	3.2	.17	5.0	.00
Nov 29	-23.3	1.0	.00	1.0	.00	1.0	.00	1.0	.00	1.0	.00	1.0	.00
	-26.1	1.4	.11	1.7	.11	1.5	.11	1.1	.05	1.1	.05	1.0	.00
	-28.9	3.1	.27	2.8	.25	2.5	.20	1.8	.16	1.0	.00	1.1	.05
	-31.7	4.6	.18	5.0	.00	3.6	.14	5.0	.00	1.6	.21	2.2	.20
	-34.4	4.9	.07	5.0	.00	4.8	.10	5.0	.00	2.4	.15	4.2	.16
	-40.6	5.0	.00	5.0	.00	5.0	.00	5.0	.00	3.6	.22	5.0	.00
Dec 10	-23.3	1.0	.00	1.0	.00	1.1	.05	1.0	.00	1.0	.00	1.0	.00
	-26.1	1.0	.00	1.0	.00	1.1	.05	1.0	.00	1.0	.00	1.0	.00
	-28.9	1.4	.11	2.5	.11	1.5	.11	2.1	.17	1.0	.00	1.1	.00
	-31.7	1.8	.14	3.0	.23	2.1	.11	2.2	.12	1.0	.00	1.0	.00
	-34.4	2.2	.09	3.8	.17	2.1	.15	4.4	.09	1.2	.09	2.1	.18
	-37.2	2.6	.13	5.0	.00	2.4	.11	5.0	.00	1.5	.11	4.8	.09
	-41.1	3.2	.11	5.0	.00	3.1	.10	5.0	.00	1.4	.11	5.0	.00
Jan 10	-26.1	1.1	.11	1.1	.05	1.1	.05	1.0	.00	1.0	.00	1.0	.00
	-28.9	1.3	.11	1.8	.12	1.2	.11	1.8	.09	1.0	.00	1.0	.00
	-31.7	1.4	.11	2.4	.11	1.2	.09	2.2	.09	1.0	.00	1.3	.11
	-34.4	2.1	.05	3.8	.18	1.8	.10	3.4	.17	1.6	.11	3.4	.11
	-37.2	2.4	.11	5.0	.00	1.9	.07	5.0	.00	1.6	.13	5.0	.00
	-40.6	4.0	.16	5.0	.00	3.0	.14	5.0	.00	2.0	.11	5.0	.00
Feb 7	-26.1	1.8	.16	1.6	.18	1.6	.11	1.1	.07	1.2	.08	1.1	.05
	-28.9	2.4	.21	2.8	.19	2.1	.09	2.2	.16	1.6	.14	1.1	.05
	-31.7	2.6	.11	3.2	.14	2.6	.11	2.4	.17	1.8	.14	1.4	.14
	-34.4	2.4	.15	5.0	.00	2.5	.11	5.0	.00	2.2	.08	4.6	.13
	-37.2	2.8	.12	5.0	.00	2.2	.12	5.0	.00	2.2	.08	5.0	.00
	-40.6	2.4	.11	5.0	.00	2.6	.11	5.0	.00	2.1	.10	5.0	.00
Mar 8	Field^{z}	1.8	.14	1.1	.07	1.6	.13	1.1	.05	1.1	.07	1.0	.00
	-20.6	1.9	.11	1.1	.05	1.6	.11	1.0	.00	1.2	.11	1.1	.05
	-23.3	1.6	.13	1.1	.07	1.6	.13	1.0	.00	1.1	.07	1.1	.07
	-26.1	2.1	.05	2.7	.15	2.0	.00	2.2	.12	1.1	.05	1.0	.00
	-28.9	2.1	.07	3.6	.15	2.2	.08	3.1	.11	1.4	.11	1.4	.14
	-31.7	2.6	.11	4.9	.07	2.5	.16	4.0	.00	1.7	.12	3.0	.15
	-34.4	2.5	.11	5.0	.00	2.2	.10	5.0	.00	1.6	.11	5.0	.00

^{z}Field control

Table 1 (cont'd.)

	-37.2	2.7 .13	2.0 .00	2.6 .11	5.0 .00	2.0 .05	5.0 .00
	-40.0	3.1 .07	5.0 .00	2.9 .10	5.0 .00	2.1 .19	5.0 .00
Apr 10	-3.9	1.4 .11	1.0 .00	1.2 .10	1.0 .00	1.2 .12	1.1 .05
	-6.7	1.7 .13	1.1 .05	2.0 .12	1.0 .00	1.4 .11	1.1 .05
	-8.9	2.0 .15	1.0 .00	1.7 .13	1.0 .00	1.2 .10	1.0 .00
	-12.2	2.3 .10	1.0 .00	2.2 .13	1.0 .00	1.3 .11	1.1 .10
	-15.0	2.2 .16	1.6 .17	2.3 .11	1.8 .20	1.8 .14	1.4 .17
	-17.2	2.3 .11	2.2 .19	2.2 .13	1.6 .21	1.7 .11	1.4 .15
	-20.6	3.2 .13	3.0 .14	2.4 .15	1.9 .19	2.0 .19	1.6 .18
	-23.3	2.8 .18	3.2 .16	2.4 .15	2.2 .19	1.9 .16	1.9 .23
Oct 3	-6.7			3.1 .28	2.4 .21	1.3 .13	1.3 .12
	-9.4			3.8 .25	2.7 .31	1.7 .23	2.0 .21
	-12.2			5.0 .00	5.0 .00	2.8 .20	2.8 .19
	-15.0			5.0 .00	5.0 .00	3.9 .19	3.4 .21
	-17.8			5.0 .00	5.0 .00	4.9 .07	4.8 .09
	-21.1			5.0 .00	5.0 .00	5.0 .00	5.0 .00
Nov 5	-17.8			1.8 .10	1.9 .14	1.0 .00	1.0 .00
	-20.6			2.2 .11	3.4 .14	1.0 .00	1.0 .00
	-23.3			2.9 .07	4.4 .17	1.0 .00	1.1 .07
	-26.1			3.2 .10	4.7 .13	1.4 .11	1.8 .09
	-28.9			3.4 .11	4.9 .10	2.3 .10	3.0 .10
	-32.8			4.2 .09	5.0 .00	3.0 .14	4.7 .13
Jan 6	Field			1.9 .12	1.0 .00	1.2 .08	1.0 .00
	-26.1			2.4 .13	1.3 .10	1.2 .09	1.0 .00
	-28.9			2.8 .10	1.8 .13	1.4 .11	1.1 .07
	-31.7			3.0 .15	3.6 .20	1.4 .13	1.6 .11
	-34.4			3.2 .08	5.0 .00	2.1 .07	3.5 .14
	-37.2			3.4 .14	5.0 .00	2.4 .11	5.0 .00
	-40.0			4.0 .15	5.0 .00	2.6 .11	5.0 .00
	-43.9			5.0 .00	5.0 .00	4.0 .15	5.0 .00
Mar 7	Field			2.2 .15	1.1 .07	2.1 .18	1.1 .10
	-23.3			2.2 .12	1.6 .15	1.8 .18	1.1 .05
	-26.1			3.0 .11	3.2 .17	1.8 .14	1.2 .08
	-28.9			3.3 .13	5.0 .00	2.0 .14	1.8 .18
	-31.7			3.8 .17	4.8 .20	2.7 .16	4.7 .10
	-34.4			4.0 .17	5.0 .00	2.4 .11	5.0 .00
	-36.7			4.4 .11	5.0 .00	3.0 .18	5.0 .00
	-38.5			4.6 .11	5.0 .00	3.3 .18	5.0 .00
Mar 26	Field			2.1 .20	1.4 .13	2.0 .21	1.0 .00
	-21.7			2.5 .11	1.5 .14	1.6 .15	1.1 .07
	-23.3			3.1 .19	2.3 .25	1.5 .17	1.2 .08
	-26.1			3.2 .16	4.0 .14	2.0 .17	1.7 .10
	-28.9			3.8 .13	5.0 .00	2.2 .20	2.8 .17
	-31.7			4.8 .08	5.0 .00	2.8 .14	4.8 .20
	-34.4			4.8 .12	5.0 .00	3.0 .14	5.0 .00
	-36.1			5.0 .00	5.0 .00	3.2 .16	5.0 .00

Table 1 (cont'd.)

Apr 13	Field	2.2	.14	1.4	.11	1.5	.14	1.1	.05
	-3.9	2.4	.11	1.4	.11	1.6	.17	1.1	.05
	-6.7	2.3	.13	1.8	.14	1.4	.14	1.2	.08
	-9.4	2.9	.07	2.2	.10	1.6	.11	1.1	.05
	-12.2	2.8	.16	2.2	.17	1.7	.10	1.0	.00
	-15.0	3.9	.10	2.9	.14	1.8	.17	1.2	.11
	-17.8	3.4	.20	2.1	.18	2.2	.13	1.2	.10
	-20.0	3.8	.17	3.4	.17	2.3	.10	1.4	.11

Table 2. Means and standard errors for callus regrowth ratings for 'Velvet', 'Redhaven' and 'Siberian C' at each test temperature on 3 dates, 1975-77.

Date	Temp	Velvet		Redhaven		Siberian C	
	(°C)	$\bar{x}$	SE	$\bar{x}$	SE	$\bar{x}$	SE
Dec 10, 1975	-23.3	1.1	.07	1.2	.08	1.6	.20
	-26.1	1.0	.00	1.0	.00	1.0	.00
	-28.9	1.2	.11	1.2	.12	1.2	.12
	-31.7	1.1	.05	1.2	.14	1.2	.12
	-34.4	2.1	.17	1.4	.11	1.4	.19
	-37.2	2.2	.16	1.4	.11	2.4	.21
	-41.1	2.6	.24	2.2	.16	1.9	.22
Mar 30, 1977	Field[z]			1.1	.05	1.4	.15
	-23.3			2.0	.19	1.6	.22
	-26.1			3.0	.20	2.5	.22
	-28.9			3.4	.24	2.9	.36
	-31.7			4.4	.13	2.8	.21
	-34.4			4.8	.09	3.2	.18
	-36.7			4.9	.07	3.7	.26
	-38.5			5.0	.00	3.8	.20
Apr 13, 1977	Field			2.2	.19	2.3	.15
	-3.9			2.6	.23	2.4	.21
	-6.7			2.2	.19	2.0	.12
	-9.4			2.8	.30	2.0	.09
	-12.2			2.6	.22	2.1	.22
	-15.0			4.2	.24	2.1	.22
	-17.7			5.0	.00	3.3	.16
	-20.0			5.0	.00	3.0	.20

[z] Field control

APPENDIX B

Figure 1. Progeny distributions according to cambium injury class. A to D are: January 16, 1976 (-32.2°C); November 18, 1976 (-26.1°), (-31.7°); February 7, 1977 (-33.8°), respectively.

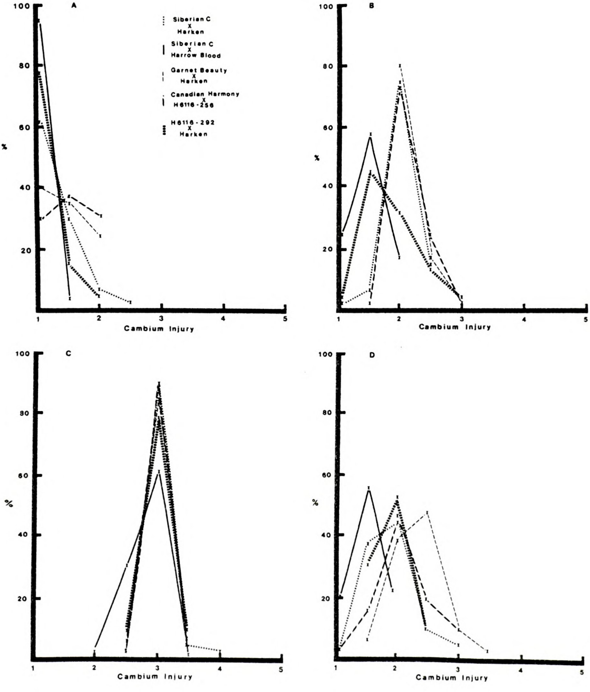

A
Siberian C
X
Harken
Siberian C
X
Harrow Blood
Garnet Beauty
X
Harken
Canadian Harmony
X
H6116-256
H6116-292
X
Harken
B
C
D
100
80
60
40
20
%
1
2
3
4
5
Cambium Injury

Figure 2. Progeny distributions according to xylem injury class. A to C are: January 16, 1976 (-32.2°C); November 18, 1976 (-26.1°); February 7, 1977 (-33.8°), respectively.

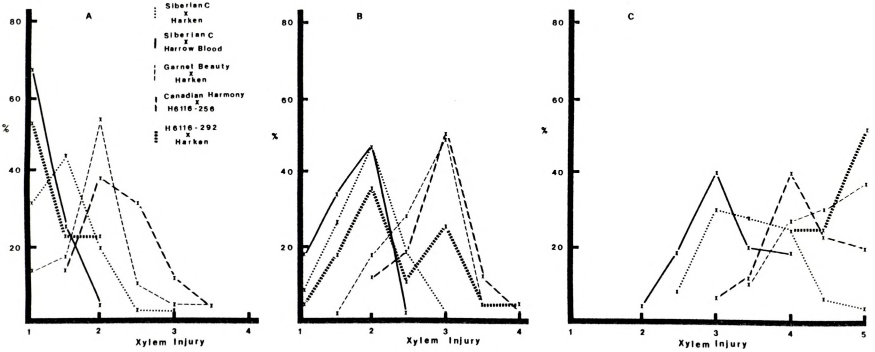

A
B
C
Siberian C x Harken
Siberian C x Harrow Blood
Garnet Beauty x Harken
Canadian Harmony x H6116-256
H6116-292 x Harken
%
80
60
40
20
1
2
3
4
5
Xylem Injury

Figure 3. Progeny distributions according to vegetative bud injury class. A and B are: November 18, 1976 (-31.7°C) and February 7, 1977 (-33.8°), respectively.

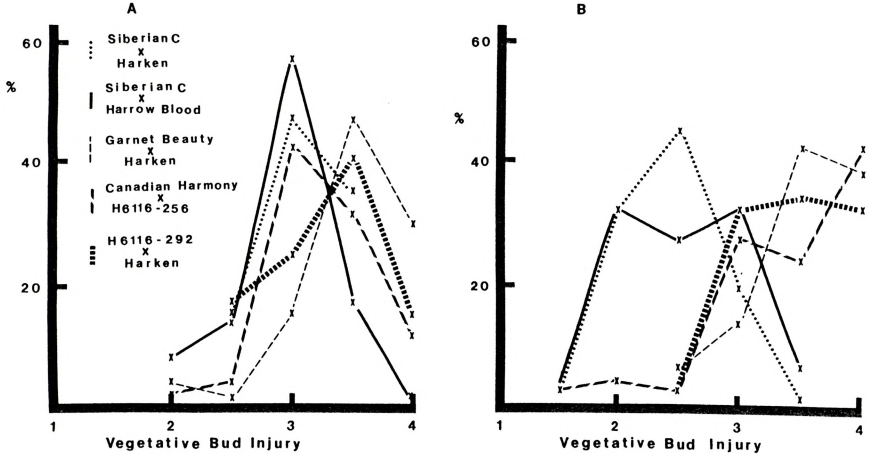
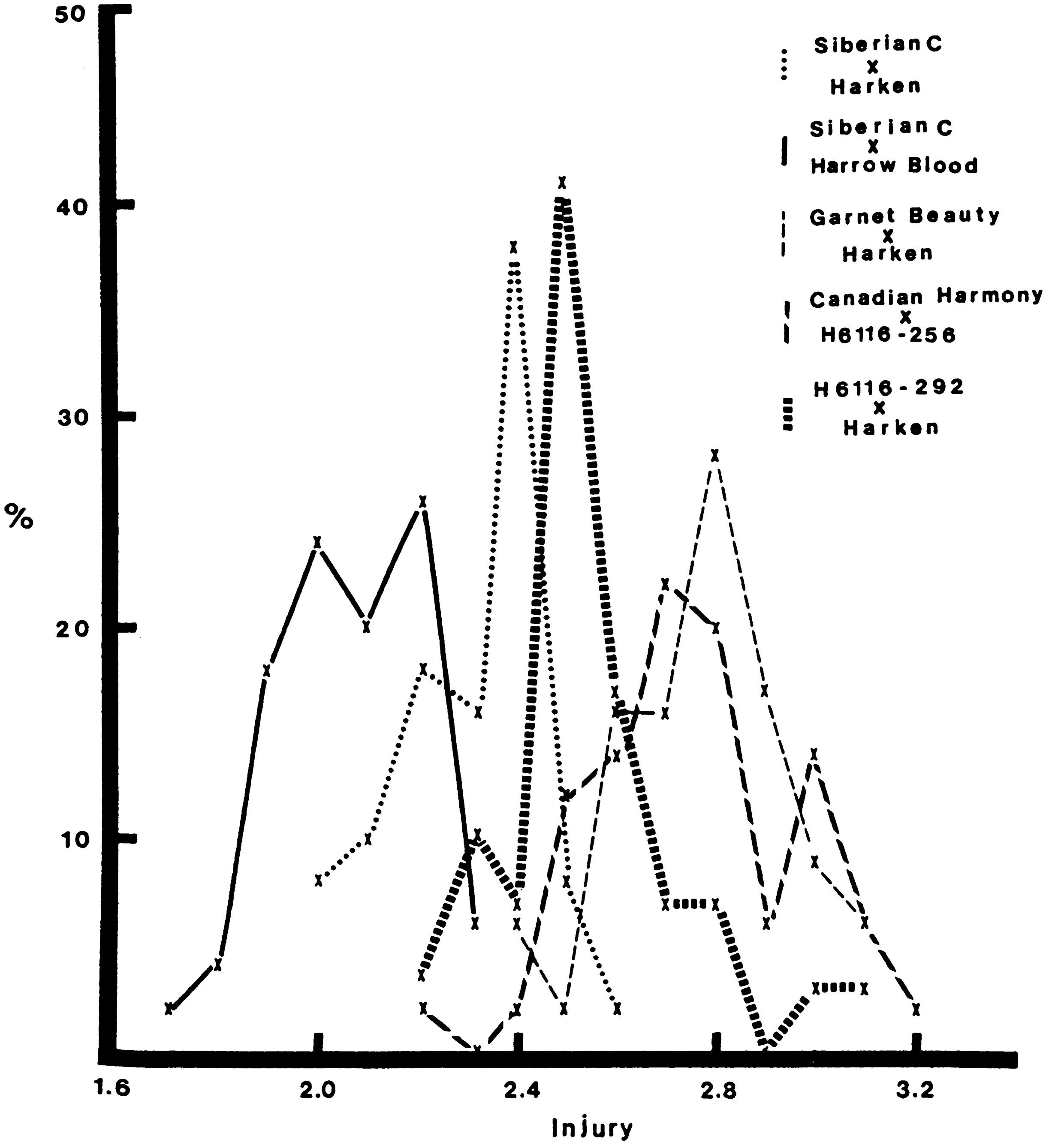

A
B
60
40
20
%
1
2
3
4
Vegetative Bud Injury
Siberian C
x
Harken
Siberian C
x
Harrow Blood
Garnet Beauty
x
Harken
Canadian Harmony
x
H6116-256
H6116-292
x
Harken

Figure 4. Progeny distributions according to overall mean injury ratings of each seedling as assessed by averaging individual seedling performance across all tissues, temperatures and dates.


50
40
30
20
10
%
1.6
2.0
2.4
2.8
3.2
Injury
Siberian C x Harken
Siberian C x Harrow Blood
Garnet Beauty x Harken
Canadian Harmony x H6116-256
H6116-292 x Harken

BIBLIOGRAPHY

BIBLIOGRAPHY

Allard, R.W. 1960. Principles of plant breeding. Wiley & Sons Inc., New York.

Andersen, R.L. 1970. Some quantitative genetic considerations pertinent to breeding asexually propagated crops. Ph.D. thesis, University of Minnesota.

_____________. 1974. Peach hardiness and varieties. Proc. 33rd Ann. Conv. Nat'l. Peach Council, St. Louis, Mo. Feb 10-13. pp. 43-49.

Asahina, E. 1967. A principle of the frost-resistance mechanism in plant and animal cells. In Prosser, C.L. The cell and environmental temperature (English trans.) Pergamon Press, New York. pp. 15-23.

Bailey, J.S. and A.P. French. 1942. The inheritance of blossom type and blossom size in peach. Proc. Amer. Soc. Hort. Sci. 40: 248-250.

Bittenbender, H.C. and G.S. Howell. 1975. Interactions of temperature and moisture content on spring de-acclimation of flower buds of highbush blueberry. Can. J. Plant Sci. 55: 447-452.

Blake, M.A. 1935. Types of varietal hardiness in the peach. Proc. Amer. Soc. Hort. Sci. 33: 240-244.

__________. 1938. Hardy rootstocks for the peach should extend well above the surface of the soil. Proc. Amer. Soc. Hort. Sci. 36: 138-140.

__________, and C.H. Steelman. 1945. Preliminary investigations of the cold resistance of peach fruit buds at the pink bud stages of development. Proc. Amer. Soc. Hort. Sci. 44: 37-41.

Bradford, F.C. and H.A. Cardinell. 1926. Eighty winters in Michigan orchards. Mich. Agr. Exp. Sta. Bul. 149: 1-103.

Brierley, W.G. and R.H. Landon. 1946. Some relationships between rest period, rate of hardening, loss of cold resistance and winter injury in the Latham raspberry. Proc. Amer. Soc. Hort. Sci. 47: 224-234.

__________ and __________. 1954. Effects of dehardening and rehardening treatments upon cold resistance and injury of Latham raspberry canes. Proc. Amer. Soc. Hort. Sci. 63: 173-178.

Brittain, J.A. and R.W. Miller. 1976. Managing peach tree short life in South Carolina. Clemson Univ. cir. no. 568.

Buchanan, D.W., R.H. Biggs and J.F. Bartholic. 1976. Cold acclimation of Florida peach and nectarine cultivars. HortScience 11: 398-400.

__________, __________, and __________. 1974. Cold hardiness of peach and nectarine trees growing at 29-30° N latitude. J. Amer. Soc. Hort. Sci. 99: 256-259.

Burke, M.J., R.G. Bryant and C.J. Weiser. 1974. Nuclear magnetic resonance of water in cold acclimating ed sier dogwood stem. Plant Physiol. 54: 392-398.

__________, M.F. George and R.G. Bryant. 1975. Water in plant tissues and frost hardiness. In R.B. Duckworth (ed.) Water relations in foods. Academic Press, New York. pp. 111-135.

__________, L.V. Gusta, H.A. Quamme, C.J. Weiser and P.H. Li. 1976. Freezing and injury in plants. Ann. Rev. Plant Physiol. 27: 507-528.

Cain, D.W. and R.L. Andersen. 1976. Sampling procedures for minimizing non-genetic wood hardiness variation in peach. J. Amer. Soc. Hort. Sci. 101: 668-671.

Campbell, R.W. 1948. More than thirty peach varieties survived minus thirty-two degrees fahrenheit. Proc. Amer. Soc. Hort. Sci. 52: 117-120.

__________, and F.B. Hadle. 1960. Winter injury to peaches and grapes. Proc. Amer. Soc. Hort. Sci. 76: 332-337.

Chandler, W.H. 1913. Killing of plant tissues by low temperature. Univ. of Mo. Res. Bul. 8: 141-309.

__________. 1954. Cold resistance in horticultural plants: A review. Proc. Amer. Soc. Hort. Sci. 50: 552-572.

Chaplin, C.E. 1948. Some artificial freezing tests of fruit buds. Proc. Amer. Soc. Hort. Sci. 52: 121-129.

__________, and G.W. Schneider. 1974. Peach rootstock/scion hardiness effect. J. Amer. Soc. Hort. Sci. 99: 231-234.

Daniell, J.W. and F.L. Crosby. 1968. Occlusion of xylem elements in peach trees resulting from cold injury. Proc. Amer. Soc. Hort. Sci. 90: 128 - 134.

__________, and __________. 1971. The relation of physiological stage, preconditioning and rate of fall of temperature to cold injury and decline of peach trees. J. Amer. Soc. Hort. Sci. 96: 50-53.

Dennis, F.G. Jr., G.P. Lumis and C.R. Olien. 1972. Comparative freezing patterns in stems of cherry and azalea. Plant Physiol. 50: 527-530.

Dorsey, M.J. and P.D. Strausbaugh. 1923. Plum investigations. I. winter injury to plum during dormancy. Bot. Gaz. 76: 113-143.

__________, and J. Bushnell. 1925. Plum investigations. II. The inheritance of hardiness. Minn. Agr. Expt. Sta. Tech. Bul. 32.

Edgerton, L.J. and M.B. Hoffman. 1952. The effect of thinning peaches with bloom and postbloom sprays on the cold hardiness of the fruit buds. Proc. Amer. Soc. Hort. Sci. 60: 155-159.

__________. 1960. Studies on cold hardiness of peach trees. Cornell Agr. Expt. Sta. Bul. 958.

Fejer, S.O. 1976. Combining ability and correlations of winter survival, electrical impedance and morphology in juvenile apple trees. Can. J. Plant Sci. 56: 303-309.

Fletcher, N.H. 1962. The physics of rain clouds. Cambridge Univ. Press, London.

Fogle, H.W. and F.L. Overley. 1954. Winter hardiness of stone fruit varieties in irrigated central Washington. Wash. Agr. Expt. Sta. Bul. 553.

George, M.F., M.J. Burke, H.M. Pellett, and A.G. Johnson. 1974. Low temperature exotherms and woody plant distribution. HortScience 9: 519-522.

__________, __________, and C.J. Weiser. 1974. Supercooling in overwintering azalea flower buds. Plant Physiol. 54: 29-35.

__________, and __________. 1977a. Cold hardiness and deep supercooling in xylem of shagbark hickory. Plant Physiol. 59: 319-325.

__________, and __________. 1977b. Supercooling in overwintering azalea flower buds. Plant Physiol. 59: 326-328.

Graham P. 1971. Cold injury and its determination in selected Rhododendron species. M.S. thesis, University of Minnesota.

Gullord M. 1974. Genetics of freezing hardiness in winter wheat (Triticum aestivum L.). Ph.D. thesis, Michigan State University.

__________, C.R. Olien and E.H. Everson. 1975. Evaluation of freezing hardiness in winter wheat. Crop Sci. 15: 153-157.

Hansche, P.E., C.O. Hesse and V. Beres. 1972. Estimates of genetic and environmental effects on several traits in peach. J. Amer. Soc. Hort. Sci. 97: 76-79.

Hedrick, U.P. 1917. The peaches of New York. J.B. Lyon Co., Albany, New York.

Hesse, C.O. 1975. Peaches. In Janick, J. and J.N. Moore (eds.) Advances in fruit breeding. Purdue Univ. Press, Lafayette, Indiana. pp. 285-335.

Hildreth, A.C. 1926. Determination of hardiness in apple varieties and the relation of some factors to cold resistance. Minn. Agr. Expt. Sta. Tech. Bul. 42.

Hildreth, A.C. and L. Powers. 1941. The rocky mountains strawberry as a source of hardiness. Proc. Amer. Soc. Hort. Sci. 38: 410-412.

Howell, G.S. and C.J. Weiser. 1970a. The environmental control of cold acclimation in apple. Plant Physiol. 45: 390-394.

__________, and __________. 1970b. Fluctuations in the cold resistance of apple twigs during spring dehardening. Proc. Amer. Soc. Hort. Sci. 95: 190-192.

Johnston, E.S. 1923. Moisture relations of peach buds during winter and spring. Univ. Maryland Agr. Expt. Sta. Bul. 255.

Ketchie, D.O. and C.H. Beeman. 1973. Cold acclimation in 'Red Delicious' apple trees under natural conditions during four winters. J. Amer. Soc. Hort. Sci. 98: 257-261.

Kitaura, K. 1967. Supercooling and ice formation in mulburry trees. In Asahina, E. (ed.) Cellular injury and resistance in freezing organism. Bunyeido Printing Co., Sapporo. pp. 143-156.

Krasavtsev, O.A. 1967. Frost hardening of woody plants at temperatures below zero. In Asahina, E. (ed.) Cellular injury and resistance in freezing organisms. Bunyeido Printing Co., Sapporo. pp. 131-141.

Lapins, K. 1962. Artificial freezing as a routine test of cold hardiness of young apple seedlings. Proc. Amer. Soc. Hort. Sci. 81: 26-34.

Layne, R.E.C. 1974. Breeding peach rootstocks for Canada and the northern United States. HortScience 9: 10-12.

__________. 1976. Influence of peach seedling rootstocks on perennial canker of peach. HortScience 11: 509-511.

__________, G.M. Weaver, H.O. Jackson and F.D. Stroud. 1976. Influence of peach seedling rootstocks on growth, yield and survival of peach scion cultivars. J. Amer. Soc. Hort. Sci. 101: 568-572.

__________, H.O. Jackson and F.O. Stroud. 1977. Influence of peach seedling rootstocks on defoliation and cold hardiness of peach cultivars. J. Amer. Soc. Hort. Sci. 102: 89-92.

Levitt J. 1962. A sulfhydryl-disulfide hypothesis of frost injury and resistance in plants. J. Theor. Biol. 3: 355-391.

__________. 1972. Responses of plants to environmental stresses. Academic Press, New York.

Lumis, G.P., R.A. Mecklenburg and K.C. Sink. 1972. Factors influencing winter hardiness of flower buds and stems of evergreen azaleas. J. Amer. Soc. Hort. Sci. 97: 124-127.

__________, and __________. 1974. Freezing patterns in twigs of evergreen azalea. J. Amer. Soc. Hort. Sci. 99: 564-567.

Luyet, B.J. 1970. Physical changes occurring in frozen solutions during rewarming and melting. In Walstenholme, G.E.W. and M. O'Connor (eds.) The frozen cell. J. & A. Churchill Publications, London. pp. 27-50.

Marshall, H.G. 1976. Genetic changes in oat bulk populations under winter survival stress. Crop Sci. 16: 9-15.

Mazur P. 1966. Physical and chemical basis of injury in single-celled micro-organisms subjected to freezing and thawing. In Meryman H.T. (ed.) Cryobiology. Academic Press, New York. pp. 214-315.

__________. 1969. Freezing injury in plants. Ann. Rev. Plant Physiol. 20: 419-448.

Mckenzie, J.S., C.J. Weiser, E.J. Stadelmann and M.J. Burke. 1974. Water permeability and cold hardiness of cortex cells in *Cornus stolonifera* Michx. - A preliminary report. Plant Physiol. 54: 173-176.

Meryman, H.T. 1970. The exceeding of a minimum tolerable cell volume in hypertonic suspension as a cause of freezing injury. In Wolstenholme, G.E.W. and M. O'Connor (eds.) J. & A. Churchill Publishers, London.

Metcalf, E.L., C.E. Cress, C.R. Olien and E.H.Everson. 1970. Relationship between crown moisture content and killing temperature for three wheat and three barley cultivars. Crop Sci. 10: 362-365.

Morrison, J.W., C.R. Ure, R.H. Anderson, R.E. Harris and P.D. Hargrave. 1963. Hardiness and vigor of apple seedlings grown on the Canadian prairies. Proc. Amer. Soc. Hort. Sci. 83: 113-119.

Mowry, J.B. 1964. Inheritance of cold hardiness of dormant peach flower buds. Proc. Amer. Soc. Hort. Sci. 85: 128-133.

Nesmith, W.C. and W.M. Dowler. 1976. Cultural practices affect cold hardiness and peach tree short life. J. Amer. Soc. Hort. Sci. 101: 116-119.

Olien, C.R. 1961. A method of studying freezing stresses occurring in plant tissues during freezing. Crop Sci. 1: 26-28.

__________. 1964. Freezing processes in the crown of 'Hudson' barley, *Hordeum vulgare* (L., emend. Lam.) Hudson. Crop Sci. 4: 91-95.

__________. 1965. Interference of cereal polymers and related compounds with freezing. Cryobiology 2: 47-54.

__________. 1967a. Preliminary classification of polysaccharide freezing inhibitors. Crop Sci. 7: 156-157.

__________. 1967b. Freezing stresses and survival. Ann. Rev. Plant Physiol. 18: 387-408.

__________, B.L. Marchetti and E.V. Chomyn. 1968. Ice structure in hardened winter barley. Quart. Bul. Mich. Agr. Expt. Sta. 50: 440-448.

__________. 1970. Freezing of plant tissues and the protective effect of naturally occurring substances. Symposium, Michigan State Univ. March 23-25, 1970.

__________. 1971. A comparison of desiccation and freezing as stress vectors. Cryobiology 8: 244-248.

__________. 1973. Thermodynamic components of freezing stress. J. Theor. Biol. 39: 201-210.

__________, and Su-En Chao. 1973. Liquid water content of cell walls in frozen tissues evaluated by electrophoresis of indicators. Crop Sci. 13: 674-676.

__________. 1974a. Energies of freezing and frost desiccation. Plant Physiol. 53: 764-767.

__________. 1974b. Winter hardiness in barley. Mich. State Univ. Res. Rep't. 247.

__________, and B.L. Marchetti. 1976. Recovery of hardened barley from winter injuries. Crop Sci. 16: 201-204.

__________. 1977. Barley: patterns of response to freezing stress. U.S.D.A. Tech. Bul. 1558.

Ormrod, D.P. and R.E.C. Layne. 1974. Temperature and photoperiod effects on cold hardiness of peach scion-rootstock combinations. HortScience. 9: 451-453.

Ormrod, D.P. and R.E.C. Layne. 1977. Scion and rootstock influence on winter survival of peach trees. Fruit Varieties J. 31: 30-33.

Overley, F.L. and E.L. Overholser. 1936. Low temperature injury of fruit trees in central Washington during 1935-36. Proc. Wash. State Hort. Assn. 32: 147-151.

Pienazek, S.A., S.W. Zagaja and A. Wojniakiewicz. 1968. Winter-hardy peaches (P. vulgaris L.) from China. Bul. Acad. Pol. Sci. 16: 715-717.

Pierquet, P., C. Stushnoff and M.J. Burke. 1977. Low temperature exotherms in stem and bud tissues of *Vitis riparia* Michx. J. Amer. Soc. Hort. Sci. 102: 54-55.

Potter, G.F. 1938. Low temperature effects on woody plants. Proc. Amer. Soc. Hort. Sci. 36: 185-195.

Proebsting, E.L. Jr. 1970. Relation of fall and winter temperatures to flower bud behavior and wood hardiness of deciduous fruit trees. HortScience 5: 22-24.

Quamme, H., C. Stushnoff and C.J. Weiser. 1972a. The relationship of exotherms to cold injury in apple stem tissues. J. Amer. Soc. Hort. Sci. 97: 608-613.

__________, __________, and __________. 1972b. Winter hardiness of several blueberry species and cultivars in Minnesota. HortScience 7: 500-502.

__________, C.J. Weiser and C. Stushnoff. 1973. The mechanism of freezing injury in xylem of winter apple twigs. Plant Physiol. 51: 273-277.

__________, R.E.C. Layne, H.O. Jackson and G.A. Spearman. 1975. An improved exotherm method for measuring cold hardiness of peach flower buds. HortScience 10: 521-523.

__________. 1976. Relationship of the low temperature exotherm to apple and pear production in North America. Can. J. Plant Sci. 56: 493-500.

Rasmussen, D.H. and A.P. Mackenzie. 1972. Effect of solute on ice-solution interfacial free energy; calculation from measured homogeneous nucleation temperatures. In Jellinek H.H.G. (ed.) Water structure at the water-polymer interface.Plenum Press, New York.

Rollins, H.A. Jr., F.S. Howlett and F.H. Emmert. 1962. Factors affecting apple hardiness and methods of measuring resistance of tissue to low temperature injury. Ohio Agr. Expt. Sta. Res. Bul. 901.

Samygin, G.A. 1967. Protoplasmic dehydration as one of the causes of cell death from the formation of extracellular ice. In Prosser, C.L. (ed.) The cell and environmental temperature (English trans.) Pergamon Press, New York. pp. 44-52.

Savage, E.F. and V.E. Prince. 1972. Performance of peach cultivars in Georgia. Univ. of Georgia Res. Bul. 114.

__________, R.E. Jensen and R.A. Hayden. 1976. Peach tree micro-climate and methods of modification. Univ. of Georgia Agr. Expt. Sta. Res. Bul. 192.

Siminovitch, D., B. Rheaume and R. Sachar. 1967. Seasonal increase in protoplasm and metabolic capacity in tree cells during adaptation to freezing. In Prosser, C.L. (ed.) Molecular Mechanisms of Temperature Adaption Amer. Assoc. Adv. Sci. Pub. 84.

__________, __________, K. Pomeroy and M. Lepage. 1968. Phospholipid, protein and nucleic acid increases in protoplasm and membrane structures associated with development of extreme freezing resistance in black locust tree cells. Cryobiology 5: 202-225.

Single, W.V. and C.R. Olien. 1967. Freezing processes in wheat stems. Aust. J. Biol. Sci. 20: 1025-1028.

Smithberg, M.H. and C.J. Weiser. 1968. Patterns of variation among climatic races of red-osier dogwood. Ecology 49: 495-505.

Steel, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. Inc., New York.

Strausbaugh, P.D. 1921. Dormancy and hardiness in the plum. Bot. Gaz. 71: 337-357.

Stushnoff, C. 1972. Breeding and selection methods for cold hardiness in deciduous fruit crops. HortScience 7: 10-13.

Tekauz, A. and Z.A. Patrick. 1974. The role of twig infections on the incidence of perennial canker in peach. Phytopathology 64: 683-688.

Traub, H.P. 1927. Regional and seasonal distribution of moisture, carbohydrates, nitrogen and ash in 2-3 year portions of apple twigs. Univ. of Minn. Agr. Expt. Sta. Tech. Bul. 53.

Watkins, R. and L.S.P. Spangelo. 1970. Components of genetic variance for plant survival and vigor of apple trees. Theor. and App. Gen. 40: 195-203.

Weaver, G.M. 1963. Influence of rootstock susceptibility of peach to peach canker. Fruit Var. and Hort. Digest 17: 43-44.

__________. 1968. Crotch angle development in peach trees as influenced by scion and rootstock cultivars. Can. J. Plant Sci. 48: 419-421.

Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science 169: 1269-1278.

Wildung, D.K., C.J. Weiser and H.M. Pellett. 1973. Temperature and moisture effects on hardening of apple roots. HortScience 8: 53-55.

Williams, J.M. and R.J. Williams. 1976. Osmotic factors of dehardening in *Cornus florida* L. Plant Physiol. 58: 243-247.

Zagaja, S.W. 1974. Breeding cold hardy fruit trees. Proc. Int'l. Hort. Cong. 19: 9-15.