FEASIBILITY OF A TIP GRAFTING SYSTEM FOR FRUIT BREEDING AND ITS EFFECTS ON COLD HARDINESS AND JUVENILITY

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ABSTRACT

The cost of new cultivar development is high due to long juvenile periods and large tree size in tree fruit breeding programs. For apples, sour cherries, and saskatoon berries, grafting seedling scions onto the tips of branches of mature plants was hypothesized to shorten the juvenile period and reduce land use under the Canadian prairie conditions.

For apples, a tip grafting system (tip grafting onto mature crabapple rootstocks) was compared with the traditional grafting system (grafting onto young 'Ottawa 3' rootstocks). Apple scions of 'Golden Delicious', 'McIntosh', and 'SK Prairie Sun' which exhibit a range of inherent cold hardiness, were grafted in the spring of 2001. Over a two year period, winter survival of the scions was improved by 37% by the tip grafting system as compared to the traditional grafting system making it not feasible for evaluation of cold hardiness of scions. Vegetative growth of scions approximated the rootstocks on which the scions were grafted. Winter survival was highly correlated with shoot growth cessation ($r = +0.83$) and terminal bud stage ($r = +0.85$) observed around the time of first frost.

Juvenile seedlings of saskatoon berry and sour cherry hybrids were tip grafted onto mature plants of their own species in the spring of 2000. After two growing seasons, the tip grafting system in sour cherries had reduced flowering by 69.7%, shoot length by 84%, and shoot diameter by 76% compared with the juvenile seedlings on their own roots (scion donors). Tip grafting saskatoon berry seedlings increased flowering by 68%, shoot length by 257%, and shoot diameter by 42% compared with

scion donors. For sour cherries, the tip grafting system reduced winter dieback by 99.6%, hastened terminal bud development and leaf drop compared with the scion donors. Tip grafting of saskatoon berry seedlings had little effect on terminal bud development and cold hardiness of scions perhaps due to the cold hardy character of this species.

For apples and sour cherries, the tip grafting system tested in this study enhanced cold hardiness of scions when combined with the appropriated rootstocks and may be useful for maintaining germplasm that otherwise would not be hardy in northern locations.

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1. INTRODUCTION

The long sunny days and the cool nights of the Canadian prairies are ideal for developing good colour and high sugar content in fruit. Fruits that can be mechanically harvested and those that can be produced organically have a high economic potential. The three fruit crops in this study, apple, sour cherry, and saskatoon berry fit one or both criteria (Bors et al., 2003). They have great potential as commercial fruit crops for the fresh fruit market and/or the processing industries. Approximately 550 cultivated hectares were reported for commercial fruit production in Saskatchewan in 2002 with saskatoon berry accounting for a cultivated area of 66% of this acreage (Anonymous, 2003). However, apples and sour cherry are being widely tested throughout the prairies.

The low acreage of tree fruit cultivation in Saskatchewan can be partly attributed to extremely low winter temperatures. Consequently, breeding and selection for improved winter survival have received a great deal of attention. Winter survival has been gradually improved and development of advanced varieties with good fruit quality has occurred in the tree fruit breeding programs at the University of Saskatchewan (Bors et al., 2003). The cost of new variety development in tree fruit crops, however, is very high due to long juvenile periods and large tree size.

Shortening the juvenile period can reduce the selection cycle, thereby decreasing the associated costs of breeding programs. Tree fruit breeders have developed many

methods to accelerate flowering to obtain an earlier assessment of new seedlings. These methods include: girdling the tree trunks, pruning lateral branches, pruning the roots, reorienting branches horizontally, knotting the stems, and grafting inverted bark rings on the trunk or branches. None of these methods are capable of reducing the juvenile period if used on very young seedlings (Brown, 1975; Sherman and Lyrene, 1983).

Shortening of the juvenile period was claimed when scions of sweet cherry seedlings were grafted onto the tips of the branches of mature cherry trees (Burbank, 1921). It is possible that this grafting method allowed the scions to transform from a juvenile phase to an adult phase. Unfortunately, the tip grafted shoots were not compared to the control seedlings, nor was the age of the scions reported, so this claim cannot be substantiated.

Tip grafting would reduce land requirements, thereby decreasing the cost to tree fruit breeders. This method has been used in pecan breeding programs for saving land requirement (Sherman and Lyrene, 1983). A reduction in tree size would also directly correlate to land requirements. Grafting of seedling scions onto dwarf rootstocks has been used successfully in apples to reduce the size of the trees (Zimmerman, 1972).

The technique of tip grafting was developed for fruit trees in milder climates than the Canadian prairies. In Saskatchewan, the winters are extremely cold with lows of -35 to -40^{o}C. Only very cold resistant rootstocks can grow into mature trees under these conditions. Therefore, the tip grafting systems used in the breeding program at the University of Saskatchewan are based on very cold hardy rootstocks. Graft transmission of a variety of traits or agents from rootstock to scion has been reported in many plants. Traits transferred across grafts include: branching in poinsettia (Dole and Wilkins,

1992); virus resistance genes in tobacco (Smironv et al., 1997) and in sweetpotato (Okada et al., 2001); phytochrome-sensitive flowering in peas (Weller et al., 1997); and phloem protein transportation in heterografts of Cucurbitaceae (Golecki et al., 1998). Cold hardiness could also be graft transmitted from rootstocks to apple scions. Reports attributing enhanced cold hardiness of scions to the use of cold hardy rootstocks are found for cherry (Palonen and Buszard, 1997), peach (Layne, 1994; Layne and Jui, 1994) and apple trees (Westwood, 1970). Unfortunately the extent of cold hardiness potentially induced by a tip grafting system is not known.

The way that a tip grafting system enhances cold hardiness of the scions is probably through accelerated growth cessation as this growth cessation is a prerequisite for cold acclimation (Weiser, 1970; Hurme et al., 1997). In most woody deciduous perennials, the first stage of cold acclimation is induced by shortening the photoperiod, which causes growth cessation (Weiser, 1970). Low night temperatures trigger the second stage of cold acclimation. The transition from the first to the second stage of acclimation generally occurs during leaf fall (Weiser, 1970; Westwood, 1993). The ability of rootstocks to cease vegetative growth of scions and induce timely leaf drop is a potential index of cold hardiness. If there is a correlation between either the timing of terminal bud formation or leaf drop with cold hardiness, these cold acclimation factors could be used as physiological markers for the evaluation of cold hardiness.

Vegetative growth responses of scions might be also important for cold hardiness, as too vigorous or too weak vegetative growth can affect the tree quality, thereby influencing the quality of cold acclimation. Consequently vegetative growth can

affect the cold hardiness. It is unknown how a tip grafting system affects the vegetative growth of scion and how the vegetative growth relates to cold hardiness of the scions.

The specific objectives of this study were:

1. To compare the winter survival of apple scions either tip grafted onto mature crabapple rootstocks or traditionally grafted onto young ‘Ottawa 3’ rootstocks;
2. To determine the relationship between winter survival and vegetative growth, terminal growth cessation and leaf drop in apple trees;
3. To determine the effects of a tip grafting system on cold hardiness and juvenility of scions of sour cherries and saskatoon berries.

2. LITERATURE REVIEW

2.1 Apples, Sour Cherries and Saskatoon Berries on the Canadian Prairies

The apple (*Malus domestica* Borkh.) is adapted to different climates and is grown commercially from the tropics to the high latitudes where winter temperatures may fall to -40^{o}C (Lakso, 1994). The center of origin for apple is thought to be in either southern China or in Georgia and Armenia (Janick et al., 1996). There are more than 10,000 documented apple cultivars, but only a few dozen of the cultivars are grown on a worldwide, commercial scale (Janick et al., 1996). The best known of these cultivars are from seedlings that appeared by chance, most of which were derived in North America (Janick et al., 1996). 'Golden Delicious' is a chance seedling which originated in West Virginia (Janick et al., 1996; Hampson and Kemp, 2003). 'Golden Delicious' is a tender cultivar with a low temperature limit for –26^{o}C in November and –33.0^{o}C in December tested in British Columbia, Canada (Chilton et al., 1994). 'McIntosh' is a chance seedling discovered in Ontario, Canada (Hampson and Kemp, 2003). This cultivar is cold hardy with a low temperature limit for –33^{o}C in November and –37.2 ^{o}C in December tested in BC, Canada (Chilton et al., 1994). Traditionally, apple cultivars are grafted onto standard rootstocks, such as crabapple seedling rootstocks to improve their winter hardiness, as crabapple rootstocks are very cold hardy (Palmer et al., 2003). There is no evidence suggesting 'Golden Delicious' or 'McIntosh' can grow on the

Canadian prairies even if grafted on cold hardy crabapple rootstocks. Most of the apple cultivars at the University of Saskatchewan are currently grafted onto 'Ottawa 3', a dwarfing clonal apple rootstock. 'Ottawa 3' can dwarf trees to approximately one fourth the size of trees on seedling rootstocks. 'Ottawa 3' was developed at the Agriculture and Agri-Food Canada Research Station, Ottawa, Ontario by crossing 'Robin' and 'Malling 9 (M.9)' (Webster and Wertheim, 2003) and it is moderately cold hardy (Palmer et al., 2003). In the 1960's Dr. Nelson added 'Ottawa 3' to the Saskatchewan apple breeding program and it is now the major dwarf rootstock recommended for the Canadian prairies (Bors et al., 2003). In 1999, the domestic fruit development program at the University of Saskatchewan released the apple cultivar, 'SK Prairie Sun', which is cold hardy with high commercial quality and is propagated by grafting onto 'Ottawa 3' rootstocks.

Sour cherry (*Prunus cerasus*) is native to Southeast Europe (cold hardiness zones 6-10) and usually cannot survive in the Canadian prairies (cold hardiness zones 1-3). Mongolian Cherry (*Prunus fruticosa*) is native to Siberia (cold hardiness zone 2) and grows only 30-60cm tall (Olden and Nybom, 1973). In the late 1940's, Dr. Lester Kerr at Agriculture and Agri-Food Canada's Morden Research Centre, Manitoba (cold hardiness zone 3) began to intercross *P. cerasus* and *P. fruticosa*, which resulted in cold hardy sour cherry that grow 0.6 to 1.0m tall (Bors et al., 2003). In the 1970's, Dr. Stewart Nelson and Rick Sawatzky at the University of Saskatchewan began evaluating hybrids of *P. cerasus* x *P. fruticosa* imported from Siberia. In the 1980's Dr. Kerr donated his germplasm to the University. The goals of the ongoing breeding program were to combine cold hardiness, dwarf stature and fruit quality through crossing

between *P. cerasus* and *P. fruticosa*. Progress in the area of introgressing cold hardiness from *P. fruticosa* into *P. cerasus* has occurred. Dwarf sour cherry lines bred in Saskatchewan now survive winter temperatures of -40°C (Bors et al., 2003). As the goals for cold hardiness and dwarf stature have been met, in the ongoing breeding program, fruit quality has become the new emphasis.

The saskatoon berry (*Amelanchier alnifolia* Nutt.) is a native shrub that is widely distributed across North America. It produces a flavorful fruit, known as the saskatoon, saskatoon-berry, Juneberry, or serviceberry. The saskatoon berry was a dietary staple for aboriginal people (St. Pierre, 1999). Several cultivars of saskatoon berry have been selected from the superior native genotypes (Kaurin et al., 1984; Steeves and Steeves, 1990). The saskatoon berry is well adapted to the cold northern climates. The flower buds are able to escape low temperature injury (from -50 to -60^{o}C) when at maximum cold hardiness (Kaurin et al, 1984). Several papers have been published on cold hardiness of this crop during cold acclimation (Karurin et al., 1984; Friesen and Stushnoff, 1989) and deacclimation (Junttila et al., 1983). The timing of cessation of growth, bud development and cold acclimation significantly affected cold hardiness in this crop (Junttila et al., 1983; Kaurin et al., 1984; Friesen and Stushnoff, 1985; Friesen and Stushnoff, 1989). The cold hardy nature and cold acclimation patterns of this species make it a good model to study the relationship between phenological development and cold hardiness.

The development of new cultivars of tree fruits, such as apples, sour cherries, and saskatoon berries, are desirable to broaden grower's choices and/or better adapt to the low temperatures in cold hardiness zone 2. Breeding programs must screen many

plants to select improved cultivars. Due to the long juvenile period and large size of fruit trees, a more rapid and efficient method of evaluating seedling populations is needed.

2.2 Cold Hardiness

Low temperature has profound effects on all aspects of tree fruit production. It determines the northern boundaries of production areas, controls the length of the growing season, and alters the rate of phenological development (Palmer et al., 2003). Freezing resistance is the ability of plants to maintain their functions following freezing temperatures and it involves freezing avoidance and freezing tolerance (Levitt, 1980). To survive low temperatures, most well-adapted plants respond to environmental stimuli in synchrony with the season for proper growth and development (Quamme and Stushnoff, 1983). During autumn and early winter, temperate trees undergo a transition from low to high level of freezing resistance (Levitt, 1980; Sakai and Larcher, 1987). Cold acclimation which involves both freezing avoidance and freezing tolerance (Sakai and Larcher, 1987) is used to describe the transition from tender to hardy status (Burke et al., 1976; Chen, 1994; Chen et al., 1995). Levitt (1980) defined cold acclimation as the exposure of plants to a low but non-freezing temperature that usually results in a greater ability to survive a lower temperature stress that otherwise would be lethal. During cold acclimation, phenological changes, such as cessation of growth and leaf drop occurs. If these phenological changes are closely related to cold resistance, they may be used as a physiological marker for early selection in a breeding program.

2.2.1 Freezing Injury

Frosts in the late spring and early fall, low mid-winter temperatures, long periods of low temperature, as well as rapid temperature changes can cause various types of injury in woody plants (Faust, 1989a). Among the most commonly recognized types of freezing injury are: sunscald on the trunks of thin-barked trees; blackheart and frost cracking in the xylem of trees; death of vegetative shoots in late maturing trees; and the death of buds and bark in plants which deharden rapidly during transient warm spells in winter. Flower buds may also be killed due to frost in spring occurring after the buds begin to grow (Faust, 1989a).

The response of organs and tissues of woody plants to subzero temperature are both varied and complex. Leaves of deciduous fruit trees abscise and consequently do not need to cold acclimate. The lower part of the trunk is the least cold hardy among the organs above the ground because in the fall hardening progresses basipetally from the terminal shoots (Palonen and Buszard, 1997). Roots are less hardy than stems but snow cover can insulate the soil and thereby decrease damage to the roots (Faust, 1989a). Freezing resistance may change significantly with season and the stage of development (Burke et al., 1976). Quamme (1976) reported that different tissues within the same stem of apple and pear responded differently to low temperatures in different seasons. In the early autumn and the late spring, the xylem and pith were the hardiest tissue, but in the winter they were the most susceptible. Hardy trees and shrubs which can survive -196^{o}C in liquid nitrogen during winter dormancy may be killed at -3^{o}C during active spring growth (Burke et al., 1976).

Freezing injuries are both directly and indirectly associated with the freezing of the water in plant tissues (Burke et al., 1976). When a specific plant tissue freezes, ice forms either inside (intracellular) or outside (extracellular) the cell walls (Burke et al., 1976; Levitt, 1980). Intracelluar freezing is defined as ice formation in the living protoplasm (Burke et al., 1976; Sakai and Larcher, 1987). When the temperature drops below a tissue's survival point, intracellular ice formation can occur suddenly causing the tissue to be injured. This injury probably results from cataclysmic mechanical stresses. Intracellular freezing destroys the plasmalemma, disrupts the integrity of the cells and is invariably lethal (Burke et al., 1976). This phenomenon is common in tender plants, such as tropical plants, which lack the capacity to acclimate. It also occurs in hardy plants if rapid freezing occurs before the plants have acclimated (Burke et al., 1976). In temperate woody plants the causes of injury in woody tissues are generally attributed to intracellular freezing (Burke et al., 1976).

Extracellular freezing is defined as ice formation between the protoplasm and the cell wall or in the intercellular space (Sakai and Larcher, 1987). Injury from extracellular freezing is due to dehydration of plant cells. As the ice crystals grow in the extracellular space, a water potential gradient is established between the extracellular ice and the intracellular solution. This gradient results in the outward flow of intracellular water through the plasmalemma to the growing extracellular ice crystal. The cell continues to dehydrate as the temperature decreases (Burke et al., 1976). Dehydration results in the denaturation of membrane-bound protein and the disruption of the plasma membrane (Burke et al., 1976; Wisniewski et al., 2003). Plants which are tolerant to freezing generally undergo extracellular freezing (Burke et al., 1976). The

freezing injury in bark tissues of temperate woody plants is usually caused by extracellular ice formation (Burke et al., 1976; Quamme and Stushnoff, 1983). Most freezing injury results from the severe cellular dehydration that occurs with freezing (Levitt, 1980; Thomashow, 1998). In nature, the air temperature rarely decreases more than a few degrees an hour. At such slow rates of freezing, ice forms first outside the cytoplasm of cells (Weiser, 1970).

2.2.2 Mechanisms of Freezing Resistance

The ability of a plant to survive freezing stress is a complex phenomenon. The mechanisms that plants use to survive freezing temperatures have been organized into two categories: freezing avoidance and freezing tolerance (Levitt, 1980).

Avoidance mechanisms include the evasion of intracellular ice formation through desiccation, freezing point depression, supercooling, and deep supercooling (Burke et al., 1976). Annual plants with little or no frost resistance survive by means of dehydrated seeds which are very hardy (Burke et al., 1976). Freezing point depression due to high solute concentrations within the cell can increase freezing avoidance by a few degrees (Burke et al., 1976). Some plant tissues maintain their cellular water in a deep supercooled state or metastable equilibrium, thus avoiding intracellular ice formation (Ashworth, 1986; Ashworth and Wisniewski, 1991; Ashworth et al., 1998). Deep supercooling can be defined as the ability of a population of cells or entire organs to retain cellular water in a liquid phase at subfreezing temperatures (Wisniewski et al., 2003). Flower buds and xylem tissues in temperate woody fruit crops have the ability to maintain supercooled cellular water (Ashworth and Wisniewski, 1991). Deep supercooling of xylem tissues is a common characteristic of many temperate woody

plants (Wisniewski et al., 2003). The degree to which deep supercooling occurs in the xylem tissues appears to determine the northern limits of native woody plants (George et al., 1974), and the northern extent of temperate fruit trees (Burke et al., 1976; Quamme, 1991).

Freezing tolerance is the ability to tolerate ice formation in tissues without injury (Levitt, 1980). Those tissues with freezing tolerance have the ability to lose cellular water to extracellular ice during freezing and are able to tolerate the resulting dehydration (Chen et al., 1995). The mechanisms responsible for freezing tolerance are not well understood but may include preventing freeze-induced denaturation of proteins, preventing molecules from precipitating, and lessening direct physical damage caused by the accumulation of intercellular ice (Thomashow, 1998). Bark tissues (cambium, phloem, cortex, and epidermis) exhibit freezing tolerance and can survive temperatures below -38^{o}C, and in some cases as low as -196^{o}C (Burke et al., 1976; Faust, 1989a; Ristic and Ashworth, 1997).

2.2.3 Cold Acclimation

In a natural environment, cold acclimation, a non-genetic adjustment of an individual organism in response to changing environmental conditions, is used to describe the transition from tender to hardy status (Chen et al., 1995). During active summer growth, woody plants are very susceptible to freezing, and injury can occur at -2 to -3^{o}C. In the autumn and winter after acclimation, the plant tissues become more freezing resistant (Burke et al., 1976).

Weiser (1970) classified cold acclimation into two stages. The first stage of cold acclimation is triggered by short days and the second stage of cold acclimation is induced by low temperatures.

The first stage involves two distinct events: growth cessation and initiation of metabolic changes (Weiser, 1970). Growth cessation is a necessary prerequisite to cold acclimation in woody plants as an actively growing woody plant does not acclimate (Levitt, 1980), and it is used as an indicator of the initiation of cold acclimation (Chen et al., 1995). Early winter freezes tend to injure the tissues that are last to start cold acclimation (Faust, 1989a). In apple trees, the cessation of growth in different parts of the tree is not synchronized. In these plants cessation of growth occurs early in some shoots, but vigorous shoots continue to grow until the late summer or early fall (Proebsting, 1978). These actively growing shoots are less hardy than those that have stopped growing (Arora et al., 1992). For visual observation, the cessation of vegetative growth is indicated by the terminal bud set since the cessation of shoot growth is generally concomitant with the formation of terminal buds (Guak and Fuchigami, 2001). In this thesis, the term of "terminal growth cessation" refers to cessation of active production of new leaves by the meristems located on the terminal ends of shoots.

During the first stage of cold acclimation, carbohydrates accumulate (Pomeroy and Siminovitch, 1971; Coleman et al., 1992; Fujikawa et al., 1999; Gilmour et al., 2000), osmotic pressure increases (Chen et al., 1995), the levels of fatty acid desaturation in membrane phospholipids increase (Wang and Faust, 1990; Thomashow, 1998; Fujikawa et al., 1999), cell walls thicken (Ashworth et al., 1998), shoots become more rigid as the percentage of water declines (Coleman et al., 1992), and hydrophilic

proteins accumulate (Arora et al., 1992; Arora and Wisniewski, 1994; Arora et al., 1996; Lim et al., 1999). These metabolic changes facilitate the plant's responses to low temperatures during the second stage of cold acclimation (Weiser, 1970).

In the second stage, the hardiness promoting factors move from the leaves, through the bark to the overwintering stems (Fuchigami et al., 1970). Hardy plants become freeze resistant, tissue hydration decreases, but little metabolic activity occurs (Weiser, 1970). These events usually happen during leaf fall (Westwood, 1993). Early cold acclimation is often associated with low vigor and early natural defoliation (Guak and Fuchigami, 2001). Trees are less cold resistant if their leaves are killed by frost before they naturally abscise (Chandler, 1954; Faust, 1989a). Arora et al. (1992) reported that deciduous peach trees acclimated sooner and to a greater extent than evergreen ones, and consequently the deciduous trees attained a two-fold greater level of cold hardiness than the evergreen types.

The level of hardiness achieved by cold acclimation varies considerably among plant species and cultivars (Chen, 1994). Many temperate trees possess the ability to resist severe freezing stress, but lacking proper timing of acclimation limits their ability to survive winter (Weiser, 1970). The initiation of cold acclimation of plant species at high latitude is related to the timing of growth cessation (Hurme et al., 1997). At high latitudes where summers are short, the timing of growth cessation when freezing weather begins is more likely to be the determining factor for successful winter survival than at lower latitudes (Hurme et al., 1997). Trees native to warm regions usually cannot be moved to cold regions because they do not acclimate fast enough to survive and do not develop enough hardiness to cold (Kozlowski et al., 1991). Apple trees from

milder climates moved to northern areas typically grow vigorously until the late fall and then encounter cold injury. To increase the cold hardiness of apple trees modifications are required to accelerate growth cessation, trigger timely leaf fall, causing the apple trees to enter dormancy in synchrony with the season.

2.2.4 Evaluation of Cold Hardiness

The identification of cold hardy genotypes is an essential component in the success of fruit breeding programs with the objective of improving cold hardiness. Fruit breeders in cold climates usually have to screen many plants and conduct repeated cycles of selection to develop hardy cultivars, so an easy, rapid and efficient method of evaluating cold hardiness is required. Evaluation of cold hardiness can be done in the laboratory or field or both.

A widely used laboratory-based method of determining cold hardiness is to freeze plants or plant parts in controlled environment chambers and evaluate damage by recovery, tissue browning, and conductivity tests (Quamme and Stushnoff, 1983). Recovery from freezing injury can be directly observed after forcing the cold-stressed plants or plant parts under optimal growing conditions (Stushnoff, 1972). However, as temperate fruit trees have a chilling requirement for bud break, recovery testing cannot be used to evaluate seasonal cold hardiness and relative cold hardiness if the plants are in deep rest, because the plants or the detached parts of the plants cannot be forced (Quamme and Stushnoff, 1983).

Browning of woody tissue due to the oxidation of polyphenols in the cell is an alternative method to measure cold injury. The degree of browning can be assessed visually and given a numerical rating (Quamme and Stushnoff, 1983). The evaluation of

browning requires the tissue to be cut, so this method is both time consuming and destructive. Calculating the ratio of discolored xylem to total xylem area on a weight basis has been used to compare the amount of blackheart injury among apple trees grafted on different rootstocks (Warmund et al., 1996; Palonen and Buszard, 1997), but scoring systems are subject to individual bias. Thus, objective methods such as conductivity tests have been developed to avoid scoring bias.

Freezing stress results in the leakage of cellular electrolytes due to membrane damage (Quamme and Stushnoff, 1983). The conductivity test measures the electrical conductivity of the plant extract providing an estimate of cellular leakage and thus membrane damage from cold injury (Levitt, 1980). Although this method has been used widely (Stuart, 1941; Wilner, 1960; Ketchie et al., 1972; Brown, 1975; Raese, 1983; Coleman and Estabrooks, 1992), the correlation between electrolyte leakage and the survival of plant tissue after natural freeze is not consistent (Proebsting, 1978).

Freezing resistance in plant tissues with the ability to undergo deep supercooling may be tested with exotherm analysis (Quamme, 1991). In these plant tissues, cold injury is associated with a sudden freezing of a supercooled fraction of water (Quamme et al., 1972, 1973). The sudden freezing releases heat of fusion which is measured as an exotherm (Quamme et al., 1972). Differential Thermal Analysis (DTA) has been used for detecting exotherms. In DTA, the temperature of the sample is compared to a dried reference during freezing (Quamme et al., 1972). When cold-hardened xylem tissues were exposed to subzero temperatures, two distinct freezing events were detected (Quamme et al., 1972). The first one, high-temperature exotherm (HTE), appears to correspond with the freezing of the water in the xylem vessels and extracellular spaces.

This exotherm occors at temperatures between -1°C to -4°C (in the field) or -5°C to -15°C (in the laboratory). The second freezing event, the low-temperature exotherm (LTE), corresponds to the freezing of a fraction of supercooled water at temperatures between -37°C to -40°C, and is correlated to the injury in the living cells of the woody tissue (Quamme, 1976). Measurements of the LTE can provide a convenient way of studying freezing injury and resistance in xylem of woody plants (Quamme et al. 1972) and can be used to estimate the lowest survival temperature for many deciduous trees (Quamme, 1976; Quamme et al., 1982; Quamme 1991; Lindstrom et al. 1995). Coleman et al. (1992) analyzed shoot hardiness with DTA and found that the percent injury at a stress temperature of -25 or -35°C was closely related to the previous 3-day mean air temperatures. This indicates that DTA is sensitive to the time of tissue collection. Furthermore, Proebsting (1978) found the length and the diameter of stems influenced the DTA. This limits comparisons to the materials at the same physical parameters. The utility of DTA is limited by the need for sample specificity and the destructive and time consuming nature for large sample numbers (Quamme and Stushnoff, 1983).

Because of the limitations of laboratory testing methods, cold hardiness testing for deciduous fruit trees is based mostly on evaluation of winter injury in the field, bud phenology and cold acclimation developed on woody species (Proebsting, 1978; Westwood, 1993; O'Neill et al., 2000).

In the field, natural winter injury is visually assessed during the subsequent growing season (Proebsting, 1978). Despite the disadvantages of variability among locations, years and the lack of objectivity (Proebsting, 1978; Quamme and Stushnoff,

1983), assessment of the damage occurring in the field facilitates cultivar evaluation, determination of the nature of the injury and its frequency (Quamme and Stushnoff, 1983). The major advantages of field testing are the potential for simultaneous testing of a large number of plants, the evaluation of whole plant rather than plant tissue performance, and the elimination of the risk of non-standardized laboratory systems. Winter survival is used to evaluate relative cold hardiness of a variety or cultivar by direct comparison with the known resistant and susceptible check cultivars (Van Adrichem, 1970). Cold hardiness is evaluated by visual observations after a natural freeze by scoring the degree of injury with a numerical rating system (Morrison et al., 1963). Cold hardiness of a single shoot or cane can be assessed as the percent of bud death after spring flush (Morrison et al., 1963; Van Adrichem, 1970). For young seedlings, dieback as a percentage of shoot length is more practical and provides a better estimate of winter survival than percent bud death (Zatylny et al., 1996).

Cold hardiness is achieved by cold acclimation and the two stages of cold acclimation are indicated by growth cessation and leaf drop (Weiser, 1970; Sakai and Larcher, 1987; Westwood, 1993). Timing of growth cessation may be an important indicator for comparing the relative cold hardiness of cultivars. In raspberries, the early growth cessation of raspberry canes was associated with increased cane survival in the field (Van Adrichem, 1970). Guak and Fuchigami (2001) reported that ABA applied to Fuji/M.26 apple nursery plants accelerated growth cessation and improved bud dormancy by the advancement of the early stage of bud cold acclimation. The time of leaf drop has also been proposed an indicator of cold hardiness. The percentage leaf drop in raspberries, calculated as the portion of the cane with leaf abscission out of the

total cane length, was associated with winter survival (Van Adrichem, 1970). Wood and Reilly (2001) reported that leaf retention based on the percentage of leaflets retained was usable as a symptom of cold damage to pecan. However, Zatylny et al. (1996) found the length of time required for half of the leaves in the upper third of the cane to abscise was not correlated with field survival. If there are high correlations between the cold acclimation indicators and cold hardiness, indicators of early acclimation are useful in ranking cultivars for cold hardiness.

2.3 Phase Change

Plants developed from seeds display four phases: the embryonic phase, in which the shoot and root meristems are formed; the juvenile phase, which is incapable of sexual reproduction; the adult vegetative phase, in which reproductive competency is established; and the adult reproductive phase distinguished by sexual reproduction (Jones, 1999). Phase change is defined as the events that occur when a young plant passes from the juvenile phase through a transitional phase to the adult phase (Brink, 1962). The end of the juvenile phase is identified only by the production of flowers so that the transition period is commonly considered to be a part of the juvenile period (Visser, 1965; Zimmerman, 1972; Brown, 1975; Faust, 1989b).

2.3.1 Developmental Basis of Phase Change

There is considerable evidence that seedlings must attain a certain size before they can flower (Hackett, 1983), and therefore the most vigorous seedlings within a progeny population are likely to attain flowering size in the shortest time. There is a significant negative correlation between the length of the juvenile phase of a seedling and its vigor (Visser 1964). Vigorous growth, as measured by stem diameter of two and

three year-old apple and pear seedlings, is a reliable characteristic for the more precocious seedlings and it is possible to use stem diameter as a physiological marker for selection of precocity (Visser 1964, 1970).

The transition from the juvenile to adult stages is more closely correlated with tree size and node number than with the chronological age (Zimmerman 1972; Hackett, 1983; Poethig, 1990). Early fruiting of seedlings is correlated with large seedling size (Way, 1971), with the tallest plants flowering first (Jonkers 1971). Environmental conditions, weather, soil and cultural practices, which promote growth, tend to shorten the juvenile phase according to their influence on the time required to attain a certain minimum size (Visser, 1964). On crabapple seedlings, increasing the atmospheric concentration of carbon dioxide to 3000 ppm increased the height, number of nodes, as well as the number and the length of lateral shoots (Zimmerman et al., 1970). Growing seedlings in conditions that allow continuous growth (such as a greenhouse) greatly shortened the length of the juvenile phase. Apple seedlings were induced to flower 16-20 months after germination under optimum continuous growth conditions in the greenhouse, otherwise 3-8 years was needed in the field (Zimmerman, 1971). Zimmerman (1977) found that vigor alone did not always predict early flowering in pear seedlings as plants with shorter juvenile periods were smaller at time of flowering than plants with longer juvenile period. This suggests that rapid growth is not the only factor contributing to a short juvenile period (Hackett, 1985).

2.3.2 Phase Change and Cold Hardiness

The cold hardiness of woody trees varies during the phase change from juvenile phase to physiologically mature phase. Lapins (1961) studied the cold hardiness of two

groups of apple trees propagated from the juvenile zone and the adult zone of the same seedlings onto 1- and 2-year-old ‘Jonathan’ seedling rootstock. Those results showed that trees propagated from the juvenile zone of the seedlings were hardier than those from the adult zone of the same seedlings. There are contradicting literatures on cold hardiness during phase change. Lim et al. (1999) compared mature ortets with juvenile cuttings in *Rhododendron* and found that freezing tolerance increased with both chronological age and developmental stage. Studies done on forest trees indicated that young seedlings were more prone to cold injury than older trees as young seedlings tended to continue growing longer and delayed in development of cold acclimation (Li and Adams, 1993). The variation of the cold hardiness during phase change may be due to the difference on the timing of cold acclimation. The age of a tree can modify the time of growth cessation of its shoots and thus affecting the timing of cold acclimation of the tree (Faust, 1989a; Guak and Fuchigami, 2001), consequently, it can affect the cold hardiness of the tree. The higher position of shoots in mature trees also has colder air temperature and earlier cold acclimation and hence greater cold hardiness than the shoots of young trees that are closer to the ground and have warmer temperatures and delayed cold acclimation (Sakai and Larcher,1987).

2.4 Grafting

Grafting is the technique of placing a scion onto a rootstock so that they grow together. Grafting is used to propagate plants, to substitute one part of a plant for another, to join plants each of which are selected for disease resistance or adaptability to special conditions of soil or climate, to repair damage, or to elucidate problems of structure, growth, and disease (Garner, 1993). The success in forming a permanent graft

union between plant parts depends on compatibility and cambial contact (Garner, 1993). The cambium is the secondary meristematic tissue that produces the vascular network, phloem and xylem. Graft incompatibility is identified by the breakage at the point of union, where the break is complete, smooth, and unsplintered. Garner (1993) proposed that unequal growth rates, the lack of essential metabolic substances such as enzymes or hormones, or the lack of interlocking fibers were potential causes of graft failure. Successful grafting is most likely to occur between closely related species or the same species. Even if the plant parts assembled are compatible, intimate cambial contact remains essential to success of the graft. It is simple to place the cambia in contact when the stems of the rootstock and scion are of similar size. More care is required to achieve cambial contact between stems of different size.

2.4.1 Grafting Techniques

Numerous grafting techniques have been described in the literature, but all existing grafting methods fall into two categories: approach grafting and detached scion grafting (Garner, 1993). Approach grafting is where the scion and the rootstock are not totally severed from the parent plant until a union is formed, such as inarching and bridge grafting. Detached scion grafting involves complete severance of the scions before the union is formed, such as cleft grafting, whip-and-tongue grafting, and bud grafting (budding). The simple, highly efficient and most widely used grafting techniques in woody fruit trees are cleft grafting, whip-and-tongue grafting; T-budding, and chip budding (Wertheim and Webster, 2003).

In cleft grafting, a tree limb is cut across and the scion with two slope cuts is inserted in the slit with cambium layer contact. This technique is commonly used with

large rootstocks. For whip-and-tongue grafting, two cuts are made on both the rootstock and the scion. The first one is a long sloping cut and the second cut is down the centre of the rootstock and the scion. To complete the graft the rootstock and the scion are pushed together to create an overlapping joint with cambium layer contact. The whip-and-tongue grafting method is suited to scions and stocks less than 25 mm in diameter and of equal size (Garner, 1993). The scions for cleft grafting and whip-and-tongue grafting are from wood taken in the previous season, and they must be in a dormant condition when grafted.

In T-budding, a T-shaped incision is cut into the bark of the recipient rootstock. The bark is peeled back and a shield of donor tissue with a single bud is affixed within the incision in the rootstock. This technique is employed during the growing season when the bark peels readily from the wood, where the portion of the stock to be budded are one or two years old, and where the bark is thin enough that manipulative difficulties do not occur. The scion-buds for T-budding are usually taken from shoots collected in the current season, and they exist in the axils of the leaves. Chip budding (Jones or Dry budding) replaces an oval shield shape piece of bark from the rootstock with a similarly shaped piece of scion containing a single bud. This technique requires particular attention to alignment of cambia. Chip budding does not require peeling bark at the time of budding, and is successful most of the year and in a wide range of moisture conditions (Garner, 1993).

Grafting is usually done outdoors in spring or late summer but it can be done indoors on benches using dormant stocks. This is called bench grafting, regardless of the grafting techniques (Garner, 1993). Bench grafting is done from December to April

when the severe winter prevents outdoor operation. The bench grafts are stratified in boxes containing moist peat and stored at approximately 5^{o}C until field planting in spring (Garner, 1993; Wertheim and Webster, 2003).

Grafting can be conducted on both young rootstocks and established mature trees. For young rootstocks, grafting is performed on the base of the rootstocks, 10-15 cm above the soil. For established trees, grafting is performed on the top of the trees, either grafting one or more new scion cultivars onto the established limbs or grafting many scions onto the tips of one- or two-year-old branches of established trees. The former method is usually called top grafting or top working (Wertheim and Webster, 2003), and the latter was used by Burbank (1921) and is being called tip grafting in this thesis.

2.4.2 Use of Tip Grafting for Space-Saving

In fruit breeding programs, 1000's to 10,000's of plants are often assessed to develop cultivars with improved traits. In the apple breeding program at the University of Saskatchewan, since the mid 1960s, 30,297 seedlings from controlled crosses have been evaluated. Only 8717 of these seedlings were selected for field planting (Bors, 2003). Patterson (1936) recommended 20 x 20 feet spacing for apple trees, which represents 109 trees per acre. For 8717 seedlings on their own roots, 80 acres are required for approximately 10 years since the juvenile period is approximately 10 years.

Grafting onto dwarfing rootstocks has been used in handling seedling populations in fruit breeding programs (Zimmerman, 1972). The breeding program at the University of Saskatchewan has been evaluating its selections on 'Ottawa 3' rootstock, which has similar vigor to M.9 (Domoto, 2003), which dwarfs trees to

approximately one fourth the size of trees on seedling rootstocks. 20 x 5 feet spacing was used for apple selections grafted onto 'Ottawa 3' rootstocks. If the 8717 seedlings were grafted onto 'Ottawa 3' rootstocks, only 20 acres would be required.

Tip grafting onto mature trees has been used to save space in handling seedling populations in fruit tree breeding programs. Burbank (1921) grafted more than 500 cherry seedlings onto the tips of branches of mature trees to simultaneously test all the seedlings. A similar method has been used in pecan breeding programs (Sherman and Lyrene, 1983). Crabapple varieties developed in Canada are very cold hardy and could be an alternative choice for saving space in the breeding program if multiple grafts are done on each tree. The University of Saskatchewan has a collection of ten year old crabapple trees. Each of these trees has hundreds of small branches that were suitable for grafting. If 50 seedlings were grafted on one mature crabapple tree, for the 8717 seedlings, approximately 175 trees would be needed. If the crabapple trees are planted in the field at 20 x 20 feet spacing, less than two acres would be required. Obviously, tip grafting has the advantage of space-saving over grafting onto dwarfing 'Ottawa 3' rootstocks and growing as self-rooted seedlings.

2.4.3 Effect of Rootstock on Cold Hardiness of Scions

Significant rootstock-induced effects have been noted in peach trees. Rootstocks may influence trunk cross-sectional area, cumulative tree height and spread, winter injury, cold hardiness, and tree survival (Layne, 1994; Layne and Jui, 1994). It has been reported that rootstocks influenced the cold hardiness of scions in cherry (Palonen and Buszard, 1997) and apple trees (Rieger, 1989; Hiirsalmi and Sako, 1991). However, Stuart (1941) reported in their studies that the hardy apple rootstocks did not prevent the

tender varieties from injury while Ormrod and Layne (1974) found only a small rootstock influence on scion acclimation in peach trees.

Graft transmission from rootstock to scion has been reported for a range of characteristics in many plants. Traits transferred across the graft union include: branching in poinsettia (Dole and Wilkins, 1992); virus resistance in tobacco (Smironv et al., 1997) and in sweet potato (Okada et al., 2001); phytochrome-sensitive flowering in peas (Weller et al., 1997); and phloem protein transportation in heterografts of Cucurbitaceae (Golecki et al., 1998). Studies by Fuchigami et al. (1970) on *Cornus stolonifera* Michx using genotypes differing in their ability to cold acclimate showed the enhanced acclimation of a less hardy scion after being grafted onto a hardier genotype. Although a translocatable hardiness promoting factor has not been identified, it is believed to be a growth inhibitor which indirectly influences hardiness by stopping growth, or a regulatory substance which regulates the metabolic pathway responsible for the first stage of acclimation (Weiser, 1970).

Raese (1983) found hardiness was enhanced by treatments which promoted growth cessation in plants which otherwise tended to continue growing in the fall. Coleman & Estabrooks (1988, 1992) and Coleman et al. (1992) found that the plant growth regulator EL-500®, a growth retardant, increased cold hardiness levels in ‘Spur McIntosh’ apple trees than untreated controls. Cultural practices that promote timely growth cessation leading to earlier maturity can trigger cold acclimation, thus increasing cold hardiness (Westwood, 1970). The mature rootstocks on which tip grafting is performed may result in early growth cessation and influence the cold acclimation process of scions, thus affecting cold hardiness of the scions.

2.4.4 Effect of Rootstock on Phase Change

Grafting seedlings onto dwarf rootstocks can considerably shorten the juvenile phase (Sherman and Lyrene, 1983; Brown, 1975; Zimmerman, 1972). The weak apple rootstock 'MIX' has been demonstrated to accelerate the onset of flowering of apple seedlings by 1.5 years (Campbell 1961; Visser 1964). Similarly, the average juvenile period was shortened by 1.25 years by top grafting onto 10-year-old established trees having 'MIX' rootstock (Way 1971). Hackett (1985) indicated that seedling scions grafted onto certain clonal apple dwarf rootstocks ('M9' and 'M27') flowered 2-4 years earlier than the seedlings from which they were taken. The ability of the rootstocks to induce early flowering of scions is determined by their specific precocity and not all dwarf rootstocks are capable of promoting early flowering (Visser 1973).

Tip grafting of young seedlings onto mature trees has been recommended as a method to shorten the juvenile period (Burbank, 1921; Zimmerman, 1972), but experimental evidence was not provided to substantiate their claims, nor were experiments conducted to elucidate how mature trees affect the juvenile period of tip-grafted seedlings. It is possible that tip grafting allowed the scions to transfer from a juvenile phase to an adult phase due to graft transmission. However, many studies showed that the duration of the juvenile phase of seedlings is related to their vegetative growth, as measures which promote the vegetative growth also reduce the juvenile period (Smeets 1956; Visser 1964; Zimmerman et al. 1970; Zimmerman 1971; Way 1971; Jonkers 1971; Aldwinckle 1975; Hackett 1985). Conversely, those methods that restrict growth in young seedlings do not shorten but extend the duration of the juvenile

phase, resulting in delayed fruiting (Spinks, 1925; Brown, 1975; Sherman and Lyrene, 1983).

3. EXPERIMENTAL SITE AND WEATHER CONDITIONS

The experiments for this thesis were conducted at the Horticulture Field Laboratory of the University of Saskatchewan in Saskatoon (52°07' North, 106°41' West), Saskatchewan, Canada. Soil type at the field site is a Dark Brown Chernozem clay loam. This study was started in May 2000 and was completed by May 2003. Daily maximum and minimum air temperatures and rainfall during the study period from May 2000 to April 2003 were obtained from University of Saskatchewan, Kernen Research Weather Station located approximately 1.5 km east of the experimental site. Daily snowfall during the study period was obtained from Saskatoon International Airport weather station (Environment Canada, 2003) located approximately 5 km northwest of the Horticulture Field Laboratory. Daily maximum and minimum temperatures are presented in Figure 3.1. Mean monthly maximum and minimum air temperatures were calculated (Table 3.1) along with total monthly rainfall and snowfall (Table 3.2).

The first frost occurred on September 23, September 12, and September 24 in 2000, 2001, and 2002, respectively. The lowest temperatures were –35^{o}C in February, –35 ^{o}C in January, and –38^{o}C in March for the years 2001, 2002, and 2003, respectively (Figure 3.1). In the experiment year 2001-2002, both the maximum and minimum temperatures in March and April were well below the long-term averages (Table 3.1).

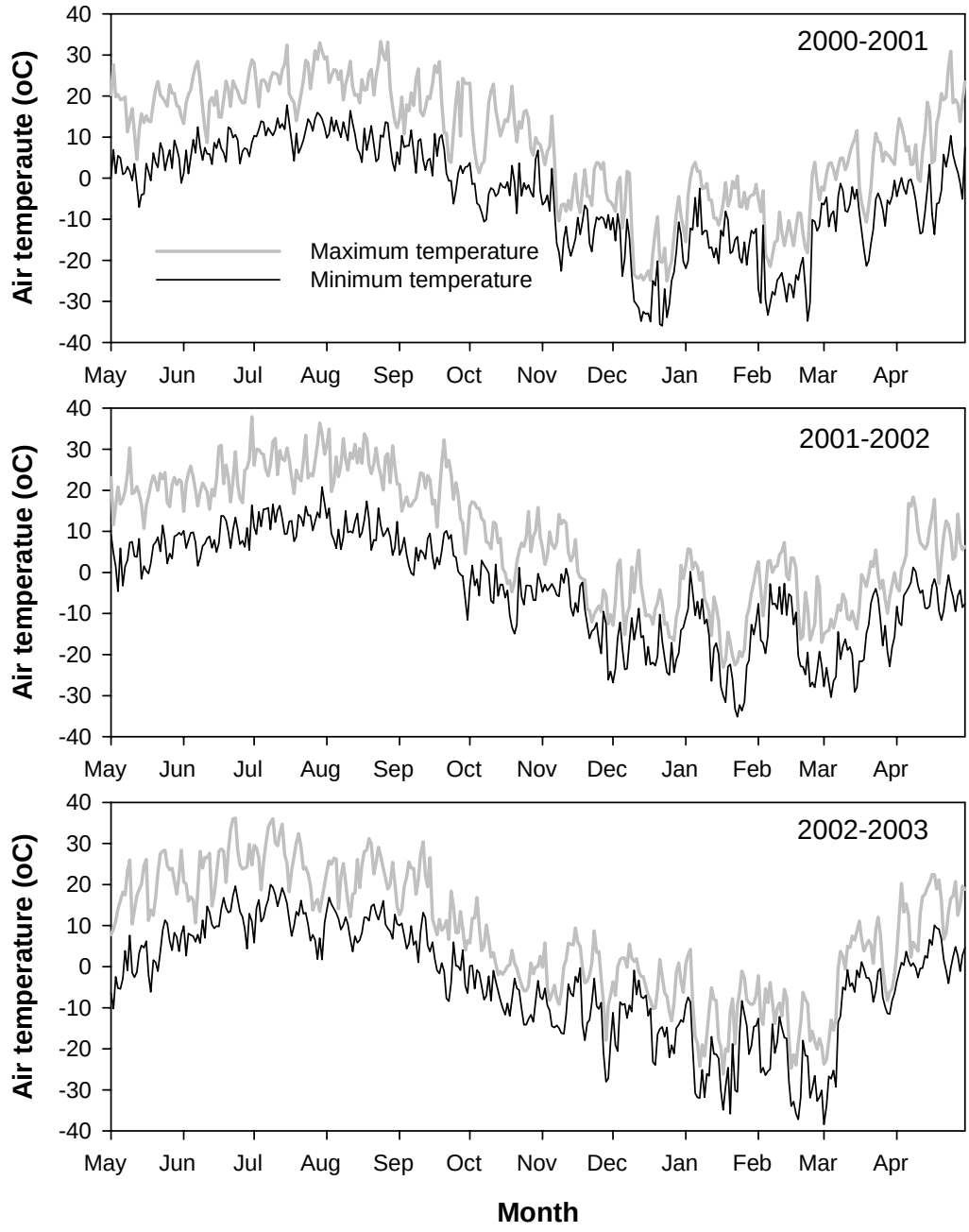

Figure 3.1 Maximum and minimum daily air temperatures from May 1st to April 30th during the study period in Saskatoon, SK, Canada.

Table 3.1 Mean monthly maximum and minimum air temperature during the study period from May 2000 to April 2003 and long-term average at Saskatoon[z].

	Experiment year							
	2000-2001		2001-2002		2002-2003		30-yr average[z]	
Month	Max	Min	Max	Min	Max	Min	Max	Min
May	16.8	2.1	19.6	3.5	13.2	-2.9	18.4	4.5
June	19.4	6.6	21.8	8.2	22.6	8.1	22.6	9.4
July	23.1	10.3	26.5	12.0	28.3	13.9	24.9	11.4
Aug.	25.3	11.0	27.9	11.4	21.8	9.9	24.4	10.2
Sep.	18.9	6.4	21.7	5.7	20.9	8.3	18.0	4.4
Oct.	14.3	-2.4	8.6	-4.0	6.6	-4.2	10.8	-1.9
Nov.	1.1	-9.3	4.0	-6.0	-0.8	-9.5	-1.5	-10.9
Dec.	-11.9	-21.5	-7.3	-18.6	-3.0	-13.3	-9.2	-19.3
Jan.	-4.1	-14.8	-9.7	-18.9	-11.3	-22.6	-11.8	-22.3
Feb.	-11.0	-23.6	-3.1	-13.9	-10.5	-21.4	-7.8	-18.2
Mar.	2.8	-8.1	-7.8	-20.0	-4.1	-15.0	-0.7	-10.9
Apr.	12.0	-2.9	4.7	-8.5	9.0	-0.8	10.6	-1.9

[z] Long-term (1971-2000) average air temperature at Saskatoon International Airport (Environment Canada, 2003).

Table 3.2 Total monthly rainfall and snowfall during the study period from May 2000 to April 2003 and long-term average at Saskatoon[z].

	Experiment year							
	2000-2001		2001-2002		2002-2003		30-yr average	
Month	Rain (mm)	Snow (cm)	Rain (mm)	Snow (cm)	Rain (mm)	Snow (cm)	Rain (mm)	Snow (cm)
May	18.0	0.0	26.4	0.0	2.8	0.8	46.8	2.2
June	54.8	0.0	35.6	0.0	50.2	0.0	61.1	0.0
July	75.2	0.0	53.6	0.0	68.2	0.0	60.1	0.0
Aug.	40.4	0.0	11.4	0.0	85.4	0.0	38.8	0.0
Sep.	21.2	1.4	11.2	0.0	37.8	0.2	29.0	1.5
Oct.	1.2	0.0	7.6	6.4	39.6	13.0	8.6	7.7
Nov.	1.0	13.6	4.4	8.0	6.2	5.0	2.0	13.4
Dec.	3.4	23.8	2.0	11.2	1.4	22.0	0.9	18.5
Jan.	1.8	4.4	0.4	7.8	2.0	9.6	0.6	17.9
Feb.	0.6	6.0	0.4	8.9	2.6	16.8	0.5	12.3
Mar.	4.2	3.6	2.0	13.8	4.8	7.6	2.3	14.1
Apr.	32.2	2.8	9.4	6.6	15.8	17.6	14.4	9.7

[z] Total monthly snowfall and long-term (1971-2000) average rainfall and snowfall at Saskatoon International Airport (Environment Canada, 2003).

Total rainfall from June to September in the study year 2001-2002 was much lower than the long-term average (Table 3.2). The 30 year average total rainfall is 265.2 mm. The total rainfall was 254.0, 164.4, and 316.8 mm for the experiment year 2000-2001, 2001-2002 and 2002-2003, respectively.

The 30 year average snowfall is 97.2 cm. Although the snowfall in 2000-2001 and 2001-2002 study years was lower than the average, the snowfall was heavy in December in those years and did not melt, so the snow cover was relatively uniform for the three experiment years.

4. FEASIBILITY OF A TIP GRAFTING SYSTEM FOR APPLE BREEDING

4.1 Introduction

Grafting onto mature trees (Sherman and Lyrene, 1983) or dwarfing rootstocks (Zimmerman, 1972) has been used to save space in handling seedling populations in fruit tree breeding programs. Burbank (1921) grafted more than 500 cherry seedlings onto the tips of branches on mature trees to simultaneously test all the seedlings. A similar method has been used in pecan breeding programs (Sherman and Lyrene, 1983). 'M.9', the most popular dwarfing rootstocks for apple, is sensitive to winter cold (Webster and Wertheim, 2003). The apple breeding program at the University of Saskatchewan has been evaluating its selections on more cold hardy 'Ottawa 3' rootstock. 'Ottawa 3' has similar vigor to 'M.9' (Domoto, 2003), which dwarfs trees to approximately one fourth the size of trees on seedling rootstocks. 'Ottawa 3' is currently the most widely used rootstocks on the prairies, but is it is considered only moderately hardy in Saskatchewan. Crabapple varieties developed in Canada are very cold hardy and could be an alternative choice as rootstock particularly if multiple grafts are done on each tree.

Graft transmission from rootstock to scion has been reported in many plants for a variety of traits including: branching in poinsettia (Dole and Wilkins, 1992); virus resistance in tobacco (Smironv et al., 1997); phytochrome-sensitive flowering in peas

(Weller et al., 1997); and phloem protein transportation in heterografts of Cucurbitaceae (Golecki et al., 1998). Cold hardiness could also be graft transmitted from rootstocks to apple scions. Reports attributing enhanced cold hardiness of grafts to the use of cold hardy rootstocks are found for cherry (Palonen and Buszard, 1997), peach (Layne, 1994; Layne and Jui, 1994) and apple trees (Westwood, 1970).

The enhanced cold hardiness of the scions grafted onto the cold hardy rootstocks is probably related to cold acclimation. The first stage of cold acclimation in woody deciduous perennials involves growth cessation and formation of terminal buds. The transition from the first to the second stage of cold acclimation is triggered by low temperatures and occurs during leaf drop (Weiser, 1970). Terminal growth cessation has been used as an indicator of cold acclimation and cold hardiness (Proebsting, 1978; Guak and Fuchigami, 2001; O'Neill et al., 2000). Leaf retention has also been correlated with winter injury (Van Adrichem, 1970; Wood and Reilly, 2001). The ability of grafting systems to cause cessation in vegetative growth of scions before early frost and induce timely leaf drop is unknown and may be an important index of cold hardiness.

A natural or artificial screening system for cold hardiness is required in apple breeding programs located in northern latitudes. In this study, two grafting systems were proposed for apple breeding: the first, tip grafting system, involved tip grafting scions onto the tips of branches of mature crabapple trees and the second, traditional grafting system, involved the more traditional approach of grafting the scions onto the

base of young ‘Ottawa 3’ rootstocks. The effect of these systems on growth and cold hardiness of scions is unknown.

The objectives of this study were to determine if these two grafting systems influenced winter survival of the apple scions, and to determine if either system could be used for screening cold hardiness of apple scions. In addition, the relationship of winter survival to growth cessation, leaf drop, and vegetative growth was also evaluated.

4.2 Materials and Method

4.2.1 Plant Materials

Two trees of each of four crabapple cultivars: ‘Dauphin’, ‘Garnet’, ‘Trailman’ and ‘Fushia Girl’, were used as rootstocks for tip grafts. These eight trees which were all on Siberian crabapple seedling rootstocks were ten years old. They showed no signs of winter damage, were of similar size, and were located in the same row in the orchard. These crabapple trees were used to represent cold hardy rootstocks for tip grafting while one-year-old ‘Ottawa 3’ was used as the rootstock for traditional grafting. ‘Ottawa 3’ is a dwarf rootstock developed in Ontario, Canada, and is considered less cold hardy than crabapple.

‘Golden Delicious’, ‘McIntosh’, and ‘SK Prairie Sun’ were chosen to represent apple cultivar scions with a range of inherent cold hardiness. ‘SK Prairie Sun’, a cultivar developed at the University of Saskatchewan, was the cold hardiest cultivar investigated. ‘McIntosh’, originally selected in Ontario, Canada, represented the intermediate cold hardy cultivar. ‘Golden Delicious’, originally selected in West Virginia, USA, was the least cold hardy cultivar studied. ‘Ottawa 3’ and the crabapples were self and reciprocally grafted as controls.

In February 2001, budwood of ‘Golden Delicious’ and ‘McIntosh’ was obtained from the Canadian Clonal Genebank, Harrow, Ontario. Budwood of ‘SK Prairie Sun’, ‘Ottawa 3’ and crabapple was collected in February 2001 from the University of Saskatchewan Orchard. The budwood was wrapped with moist paper towels covered with polyplastic film and stored at 5°C in a cold room until used in grafting. ‘Ottawa 3’ rootstocks were obtained from Treeco Nurseries (Oregon, USA) in February 2001. The rootstocks were mixed with moist sawdust and stored at 5^{o}C until grafting. At the end of April, buds were chip-budded onto ‘Ottawa 3’ rootstocks and returned to the cold room. These were planted in mid-May with the basal grafted buds facing North. In early May, buds were chip budded onto the tips of the branches of crabapple trees, with the tip grafted buds facing up. Budding was done by hand using Parafilm® (American National Can™, Chicago, IL) to hold buds in place and retain moisture. The stem above the graft union was cut in both basal and tip grafts during the 3rd week of May. In 2001, the basal grafted trees were irrigated once a week until late August. The crabapple trees were watered twice in July and August. In 2002, the basal and tip grafted trees were watered twice in July and August.

4.2.2 Data Collection

Grafts that were successfully established during the initial growing season were recorded on August 17, 2001 (Appendix 1-1). Non-established grafts were considered missing data in the analysis.

Data were collected on apical shoots over a two-year period after grafting, except for shoot diameter which was measured as a cumulative variable in 2002. Winter survival was calculated as a percentage of the previous season’s growth that had bud

break in late-May. Cumulative percentage of terminal growth cessation in each experimental unit was recorded in mid-August and again in mid-September, the latter being around the first frost. Terminal growth cessation was defined as the point where 'apical meristematic tissues were free of visually-recognized leaves' (Guak and Fuchigami, 2001). In 2002, the stage of terminal bud development was also assessed using a numerical rating system: 0 = apical meristematic tissues visible with new leaves being formed; 1 = apical meristematic tissues with no new leaves being formed; 2 = green bud visible, the terminal two leaves fully expanded; 3 = bud tip turned brown; 4 = bud scales visible; 5 = bud scales totally formed and brown (Figure 4.1). Under this new classification system, terminal growth cessation as defined by Guak and Fuchigami (2001) could be classified as stage 1. Leaf number was counted in mid-September after growth cessation and before leaf drop. The number of leaves retained on the shoots was determined in both early and late November. Percent leaf drop was calculated from total leaf number and leaves retained on the shoots. Shoot length and shoot diameter were measured in mid-November. In 2001, shoot length was measured from the grafted bud scale scar to the shoot tip using a rubber ruler. In 2002, shoot length was measured from the previous year's terminal bud scale scar to the shoot tip. Shoot diameter was measured at the grafted bud scale scar using an electronic digital caliper. Leaf number to shoot length ratio was calculated.

4.2.3 Statistical Analysis

The experimental design was a split-plot design with four replications, two grafting systems as main plots and five scions as sub-plots. Crabapple cultivars were nested in different replicates, so differences between the crabapple cultivars were not

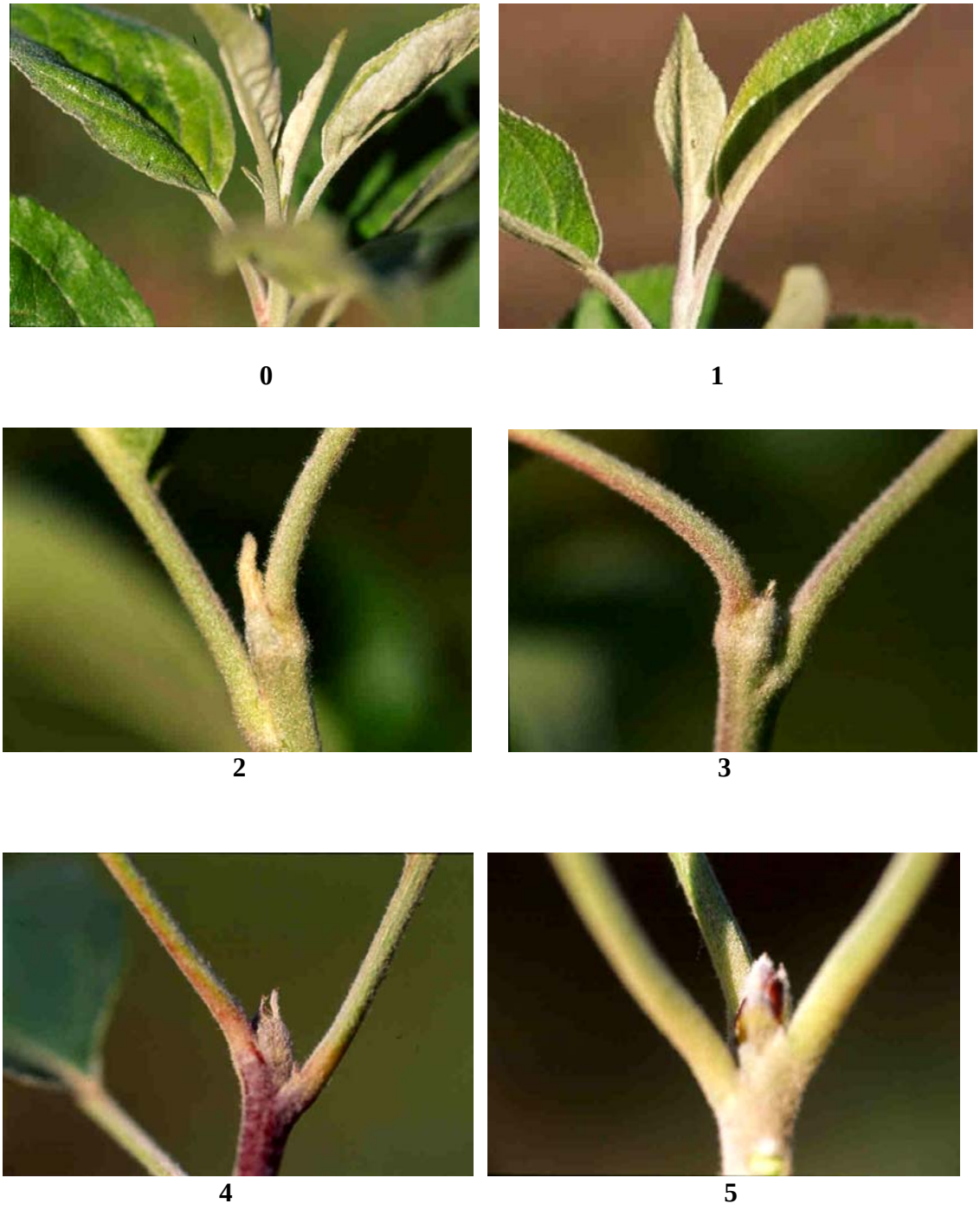

Figure 4.1 Terminal bud stage of apple grafts. 0 = apical meristematic tissues visible with new leaves being formed; 1 = apical meristematic tissues with no new leaves being formed; 2 = green bud visible, the terminal two leaves fully expanded; 3 = bud tip turned brown; 4 = bud scales visible; 5 = bud scales totally formed and brown.

analyzed. For each treatment within a replication, 20 buds were taken alternately from the budwood and chip budded onto either crabapple rootstocks or ‘Ottawa 3’ rootstocks.

The SAS program (SAS Institute, 1999) was used for statistical analysis. Analysis of variance (ANOVA) was conducted on winter survival and vegetative growth using a split-split-plot model, adding year as a source of variation, in the General Linear Model (GLM) program. When the year effect was significant, data were analyzed separately for each year, using a split-plot model. ANOVA on terminal growth cessation and leaf drop was conducted using a split-split-plot model, adding year as a source of variation, using the data from each observation time as a separate variable. Means of treatments were separated using a least square means (lsmeans) multiple comparison procedure. Percentages of terminal growth cessation and leaf drop were subjected to square root arc sine transformation before data analysis as the percent was between 0 and 100%. This transformation did not alter the significance of the variable affects in AVOVA, nor did it alter the means separation groupings, therefore, the untransformed original data were presented in tables.

Linear regression analysis was done using percentage of winter survival as a dependent variable and vegetative growth, terminal growth cessation, terminal bud stage, or leaf drop as an independent variable. Vegetative growth was based on leaf number, shoot length, leaf number to shoot length ratio, and shoot diameter. Regression analysis was done separately for each year using the means of each experimental unit.

4.3 Results

4.3.1 Winter Survival

The years in which the data were collected did not significantly affect winter survival. Differences between scions, grafting systems, and the interaction between scions and grafting systems were significant for winter survival (Appendix 1-2). Replications were not significantly different for winter survival.

Autografts of crabapple (crabapple scions onto tip grafting system) had 27.6% higher winter survival than autografts of ‘Ottawa 3’ (‘Ottawa 3’ scions onto traditional grafting system) (Table 4.1). The tip grafting system significantly increased winter survival of apple scions over the traditional grafting system, especially for the cold susceptible cultivars. On average, the tip grafting system had 37.1% greater winter survival than the traditional grafting system (Table 4.1). With the traditional grafting system, winter survival of apple scions was significantly different between cold hardy and cold sensitive scions. The cultivar ranking for winter survival within the traditional grafting system was: ‘SK Prairie Sun’ > ‘McIntosh’ > ‘Golden Delicious’. By contrast, under the tip grafting system, scions of ‘Golden Delicious’ had significantly lower winter survival than the other scions (Table 4.1). The tip and traditional grafting systems were only moderately correlated for winter survival ($r = 0.52$, $P = 0.0001$, $n = 40$).

Table 4.1 Winter survival (%) of five apple scion cultivars as affected by the tip and traditional grafting systems on apical shoots over two years (2001 and 2002) at Saskatoon.

Grafting system	Scion				
	Crabapple	Ottawa 3	Prairie Sun	McIntosh	Golden Delicious
Tip grafting system	95.5 a[z]	94.9 a	97.5 a	95.0 a	76.4 bc
Traditional grafting system	84.8 b	67.9 c	76.2 bc	34.6 d	10.5 d

[z] Mean separation by lsmeans multiple comparison procedure at P = 0.05 level. Numbers followed by different letters were significantly different.

4.3.2 Cold Acclimation Factors

Terminal growth cessation was measured twice. Repeated measures analysis showed that the pattern of terminal growth cessation for apple cultivars was significantly affected by the types of grafting systems over time. There was also a significant difference between the results from 2001 and 2002 (Appendix 1-3). Within each year, terminal growth cessation differed for the two observation times. Grafting systems, scions, and their interaction were significant for terminal growth cessation but replication was not a significant factor (Appendix 1-4).

The terminal growth cessation of scions in the traditional grafting system occurred earlier in 2002 than in 2001. In both years, the tip grafting system resulted in earlier terminal growth cessation than the traditional grafting system. By mid-August of both years, terminal growth cessation had occurred in more than 96% of the test samples with the tip grafting system, versus only 25.9% and 53.3% using the traditional grafting system in 2001 and 2002, respectively (Figure 4.2). By mid-September, more than 98% terminal growth cessation was achieved on scions in the tip grafting system, versus 53.8% and 90.3% on scions in the traditional grafting system in 2001 and 2002,

respectively. The terminal growth cessation was not significantly different between different scions in the tip grafting system. Within the traditional grafting system, 'Golden Delicious', the most tender cultivar, consistently had the lowest percentage of terminal growth cessation in the fall relative to other cultivars; 'McIntosh', the second most cold sensitive cultivar, had the second lowest percentage of terminal growth cessation in both years (Figure 4.2).

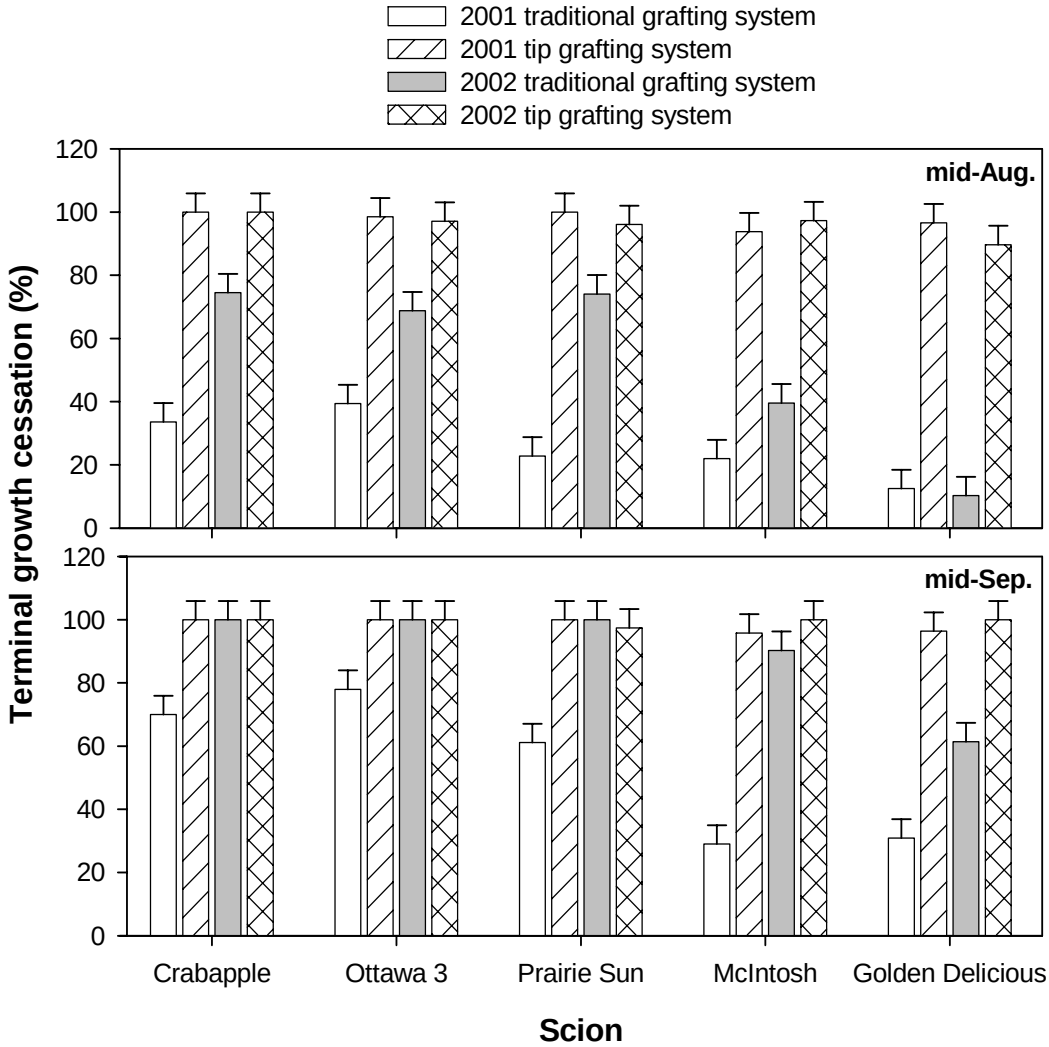

Figure 4.2 Terminal growth cessation (%) of apical shoots of scions for five apple cultivars as affected by the tip and traditional grafting systems in mid-August and mid-September in 2001 and 2002 at Saskatoon. Vertical bars indicate SE.

Terminal bud stage was also significantly different for the two observation times. Grafting system, scion, and their interaction all showed significant differences, but replication was not significant for terminal bud stage (Appendix 1-4). During the evaluation period, the tip grafting system induced more advanced terminal bud development compared with the traditional grafting system for all five scions (Figure 4.3). At the end of evaluation period, the tender cultivar 'Golden Delicious' had the least number of developed terminal buds in both systems. Within the traditional grafting system, 'Golden Delicious' had the least, 'McIntosh' had the second least, and 'SK Prairie Sun' had the most developed terminal buds.

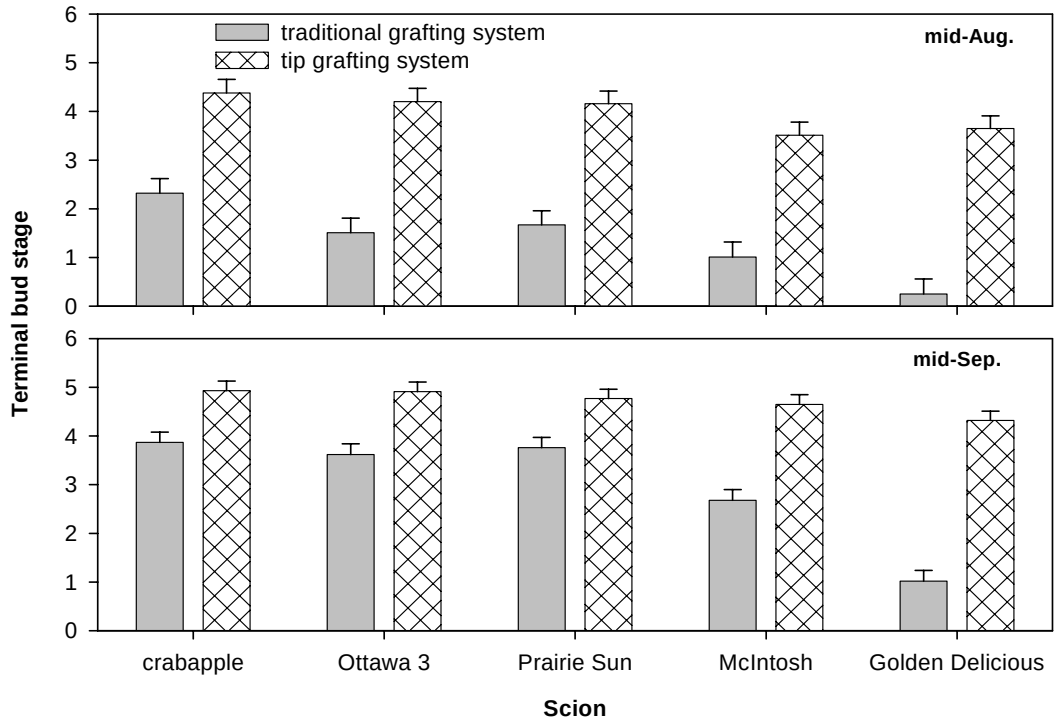

Figure 4.3 Terminal bud stage in apical shoots of scions of five apple cultivars as affected by the tip and traditional grafting systems in mid-August and mid-September in 2002 at Saskatoon. Vertical bars indicate SE. Terminal bud stage: 0 = apical meristematic tissues visible with new leaves being formed; 1 = apical meristematic tissues with no new leaves being formed; 2 = green bud visible, the terminal two leaves fully expanded; 3 = bud tip turned brown; 4 = bud scales visible; 5 = bud scales totally formed and brown (Figure 4.1).

Percentage of terminal growth cessation was positively correlated with winter survival at both observation times and in both years, but the correlation in mid-September of 2001 was higher than at the other sampling dates (Table 4.2). In 2002, terminal bud stage was positively correlated with winter survival at both sampling times but with a higher correlation in mid-September (r=0.71 and 0.85 in mid-August and mid-September, respectively).

Table 4.2 Estimated correlation (r) between winter survival (%) and various independent variables of scions of five apple cultivars in the tip and traditional grafting systems in 2001 and 2002 at Saskatoon[z].

Independent variable	Observation time	Year	
		2001	2002
Terminal growth cessation (%)	mid-Aug.	0.76 ***	0.76 ***
	mid-Sep.	0.90 ***	0.75 ***
Terminal bud stage	mid-Aug.	--------	0.71 ***
	mid-Sep.	--------	0.85 ***
Leaf drop (%)	early-Nov.	0.43 **	0.19 ns
	late-Nov.	0.54 ***	0.43 **
Leaf no.	mid-Sep.	-0.73 ***	-0.63 ***
Shoot length (cm)	late-Nov.	-0.73 ***	-0.72 ***
Leaf no./shoot length	mid-Sep./late Nov.	0.66 ***	0.51 ***
Cumulative shoot diameter (mm)	late-Nov.	--------	-0.45 **

[z] Estimated correlation was obtained with regression analysis done separately for each year using the means of each experimental unit. Data were sampled in the apical shoots of grafted scions except for cumulative shoot diameter which was sampled at the grafted bud scale scar.
ns, **, and *** not significant at P = 0.05 level, and significant at P = 0.01, 0.001 level, respectively.

Percentage of leaf drop was significantly different for both years and observation times (Appendix 1-5). When analyzed separately for each year, cultivar scion had a significant influence on leaf drop both in 2001 and in 2002. The influence

of grafting system on leaf drop was significant in 2001 but not in 2002. The interaction between grafting system and scion was significant only in 2001. Replication was not significant in 2001 but was significant in 2002 (Appendix 1-6).

Leaf drop of scions was greater in the tip grafting system than the traditional grafting system in 2001, but in 2002, leaf drop in these two grafting systems was similar (Figure 4.4). Crabapple scions had the highest leaf drop within each grafting system in both years. Within the tip grafting system, 'Golden Delicious' and 'McIntosh' had the least leaf drop; in contrast, within the traditional grafting system, 'Ottawa 3' and 'McIntosh' had the least leaf drop (Figure 4.4).

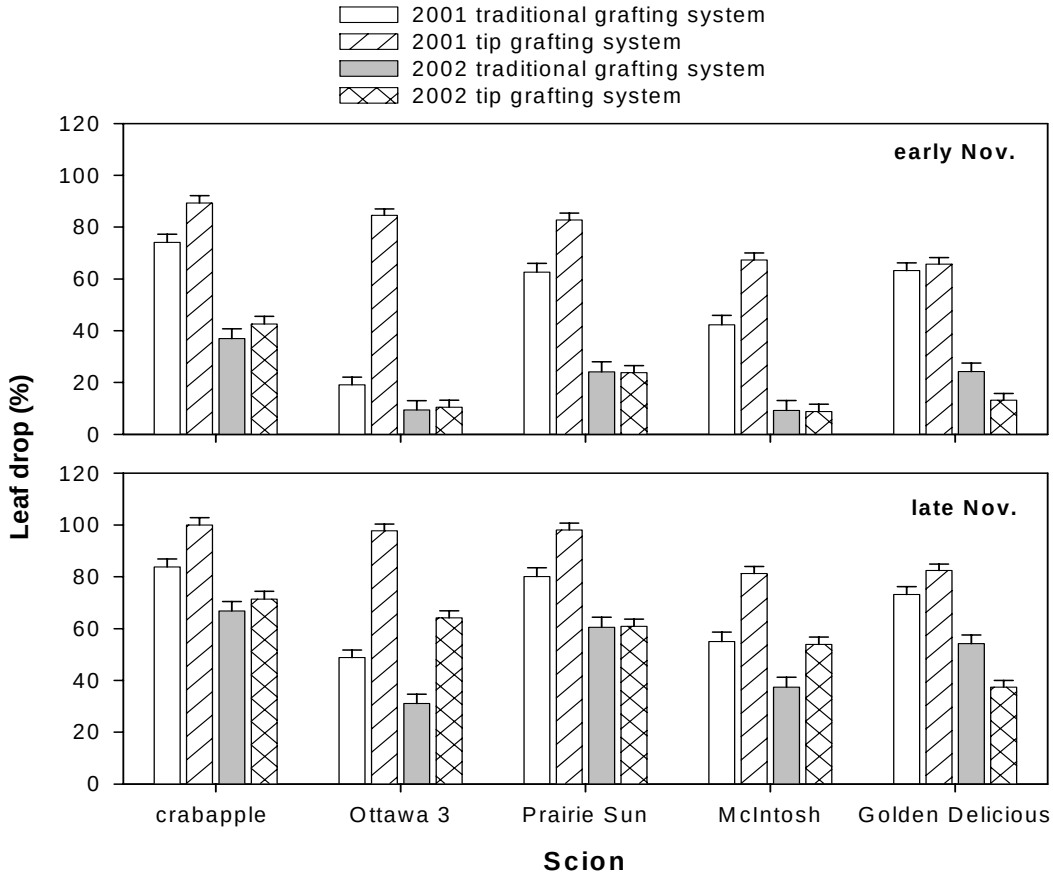

Figure 4.4 Leaf drop (%) of apical shoots of scions for five apple cultivars as affected by the tip and traditional grafting systems in early and late November in 2001 and 2002 at Saskatoon. Vertical bars indicate SE.

The correlation between winter survival and leaf drop was only moderate (Table 4.2). In 2001, leaf drop was positively correlated with winter survival, with a higher correlation in late November (r = 0.54) than in early November (r = 0.43) (Table 4.2). In 2002, leaf drop was positively correlated with winter survival only in late November (r = 0.43).

4.3.3 Growth Factors

Significant sources of variation for leaf number, shoot length, and leaf number to shoot length ratio are summarized in Appendix 1-7. Significant sources of variation for cumulative shoot diameter are presented in Appendix 1-8.

Rootstocks influenced growth responses of the scions (Table 4.3). Shoots in the tip grafting system averaged 15 cm shorter than those in the traditional grafting system. Leaf numbers were lower for tip grafts by approximately 10 leaves. Within the traditional grafting system, ‘Golden Delicious’ and ‘McIntosh’ had the longest shoot lengths and highest leaf numbers; in contrast, within the tip grafting system, leaf numbers were not significantly different among the cultivar scions (Table 4.3).

Although the tip grafting system decreased both shoot length and leaf number of the apple scions, the mean leaf number to shoot length ratio under this system was 3.2 times greater than in the traditional grafting system. Within the tip grafting system, ‘Ottawa 3’ scions had the highest leaf number to shoot length ratio (6.7) and crabapple had the lowest (3.4). There was no significant difference in leaf number to shoot length ratio within the traditional grafting system (Table 4.3). Scions of crabapple in the tip grafting system and ‘Ottawa 3’ in the traditional grafting system had the smallest and

largest shoot diameter, respectively. The tip grafting system decreased shoot diameter by 50% over two years (4.59 mm vs. 9.09 mm).

Leaf number, shoot length, and shoot diameter were negatively and leaf number to shoot length ratio was positively correlated with winter survival in both years, but the correlation was low for leaf number, leaf number to shoot length ratio and shoot diameter (Table 4.2).

Table 4.3 Leaf number, shoot length, leaf number/shoot length and cumulative shoot diameter of scions of five apple cultivars as affected by the tip and traditional grafting systems over two years (2001 and 2002) at Saskatoon[z].

Grafting system	Scion				
	Crabapple	Ottawa 3	Prairie Sun	McIntosh	Golden Delicious
	----------Shoot length (cm)----------				
Tip grafting system	7.3 e[y]	4.0 fg	3.3 g	3.6 fg	5.6 ef
Traditional grafting system	19.7 c	17.9 cd	15.6 d	25.8 b	35.3 a
	----------Leaf number----------				
Tip grafting system	10.0 e[y]	10.0 e	9.2 e	8.5 e	9.7 e
Traditional grafting system	15.3 d	18.8 bc	17.4 cd	20.9 ab	23.6 a
	----------Leaf number/shoot length ----------				
Tip grafting system	3.4 c[y]	6.7 a	5.5 b	5.3 b	3.8 c
Traditional grafting system	1.3 d	1.5 d	1.7 d	1.2 d	0.8 d
	----------Cumulative shoot diameter (mm)----------				
Tip grafting system	3.80 e[y]	4.70 de	4.86 d	4.67 de	4.93 d
Traditional grafting system	8.88 bc	11.04 a	8.41 bc	7.93 c	9.20 b

[z] Data were sampled in apical shoots and pooled over two years, except for cumulative shoot diameter which was sampled at the grafted bud scale scar only in 2002.
[y]Mean separation by lsmeans multiple comparison procedure at P = 0.05 level. Numbers followed by different letters were significantly different.

4.4 Discussion

4.4.1 Winter Survival

The winter survival of scions agreed with expectations of the inherent differences in the relative hardiness ranking of the three cultivars studied: ‘SK Prairie Sun’ > ‘McIntosh’ > ‘Golden Delicious’, with the differences more profound in the traditional grafting system than the tip grafting system. This study found that the average winter survival of the apple scions was greatly increased by the tip grafting system (91.9%) as compared with the traditional grafting system (54.8%). As shown in Table 4.1, the increase tended to obscure differences in the inherent cold hardiness of the scions. Tip grafting improved winter survival of scions of ‘McIntosh’ from 34.6% to 95.0%, reaching a level with no detectable difference from ‘SK Prairie Sun’ (97.5%). The results indicated that selection for inherent cold hardiness of scions cannot be done based on the tip grafting system using hardy crabapple trees as rootstocks.

Unexpectedly, tip grafted ‘Golden Delicious’ had an average of 76% winter survival over two years compared to the traditionally grafted ‘Golden Delicious’ that had only 10.5% winter survival. There are no previous reports that indicated ‘Golden Delicious’ had survived -38^{o}C as had occurred in 2002 although ‘Golden Delicious’ was found to have a T_{10} of -33^{o}C when properly acclimated (Chilton et al., 1994). These results suggested that tip grafting onto crabapple trees could be a valuable method for maintaining non-hardy germplasm for breeding programs. It could also be used to maintain hybrids that are the results of hardy parents crossed to non-hardy parents, thus assisting in the introgression of genes into hardy germplasm.

The results agreed with other studies that cold hardiness of scions can be affected by rootstocks (Layne, 1994; Lu and Bors, 2004; Westwood, 1970), but the exact cause of enhanced winter survival is a complicated issue. Some of the differences between the two systems were likely due to inherent cold hardiness differences between 'Ottawa 3' and crabapples, which were demonstrated by data from autografts and reciprocal grafts in this study. Another difference is the relative sizes of scions to rootstocks. With the traditional grafting system, scions and rootstocks have similar biomass, but with the tip grafting system the scions make up less than 1% of the tree's biomass. Under the tip grafting system, chemicals being freely translocated between rootstocks and scions would be primarily from the rootstocks, with less influence from the scions. If cold hardiness can be so dramatically altered by tip grafting, perhaps it can be a useful tool for studying other translocated factors and signal transduction. While dramatically improved, the tip grafting system did not increase the survival of 'Golden Delicious' to the level of the other cultivars, which suggests that not all factors involved in cold hardiness are translocated.

There are also different microclimates for scions under the two systems. The traditional grafts are located close to the ground and would be more influenced by ground temperatures, radiant heat and snow cover, whereas the tip grafts are more influenced by air temperatures. Air temperatures are much colder than soil temperatures in winter and the tip-grafts would be more exposed to wind and subsequent desiccation. These later factors should have contributed to decreased winter survival of tip grafts but, evidently, other factors had a greater influence on winter survival.

4.4.2 Cold Acclimation Factors Related to Winter Survival

Tip grafting onto crabapple trees induced early termination of growth and advanced terminal bud development for all the scions (Figure 4.2, Figure 4.3). In contrast, terminal growth cessation and bud development under the traditional grafting system varied according to the inherent cold hardiness of the scions. Cold sensitive scions had delayed terminal growth cessation, while cold hardy scions expressed early terminal growth cessation. 'Golden Delicious', a cold sensitive cultivar, ceased vegetative growth late in the season. 'McIntosh', a more cold hardy cultivar, had an intermediate terminal growth cessation. 'SK Prairie Sun', a cold hardy cultivar developed in the high latitudes, stopped growing early. A similar phenomenon for terminal growth cessation was observed with Douglas firs obtained from various latitudes (Hurme et al., 1997).

By mid-September in 2002, 99.5% of scions under the tip grafting system had terminal growth cessation, but the developmental stages were different. For example, the tip grafts of 'McIntosh' and 'Golden Delicious' had 100% terminal growth cessation, but the terminal bud stages were less advanced compared with those scions of other cultivars. The terminal bud stage had a higher degree of correlation with winter survival ($r = 0.85$) than the percentage of terminal growth cessation ($r = 0.75$). Therefore, the terminal bud stage was a more accurate indicator of winter survival. In this study, an advanced terminal bud stage in mid-August and mid-September was closely related to increased winter survival. The first frost usually occurs in early September in the study area, so terminal bud stage around the first frost would appear useful for predicting winter survival.

Leaf drop evaluated in late November had only a moderate positive correlation with cold hardiness, and early November observations had even lower correlations. In raspberries, researchers have had conflicting results correlating leaf drop with winter hardiness (van Adrichem, 1970; Zatylny et al., 1996). The results in the current study indicated that leaf drop in apple is not as reliable as terminal growth cessation and terminal bud stage for predicting winter survival. The less cold hardy cultivar 'Golden Delicious' had higher leaf drop than 'McIntosh' in this study.

For practical applications in breeding, terminal bud developmental stage is much easier to use than leaf drop; it can be done earlier in the season than leaf drop and it requires only one observation at the time of frost. Conversely, leaf drop data requires two observations; to establish a baseline and later to determine leaves remaining.

4.4.3 Growth Factors Related to Winter Survival

Both the tip grafting system using crabapple trees and the traditional grafting system using 'Ottawa 3' rootstocks resulted in the growth factors of the scions, such as shoot length, leaf number, leaf number to shoot length, and shoot diameter, approximating the growth responses of their respective rootstocks. This result was in agreement with that found on sour cherry and saskatoon berry, in which the vegetative growth of scions approximated the rootstocks (Lu and Bors, 2004). Dole and Wilkins (1991, 1992) found the free branching characteristic in poinsettia was altered by the rootstocks, and a similar result was found on leaf morphology changes induced by the rootstock in tomato (Kim et al., 2001). It is safe to conclude that the growth factors were influenced by the rootstocks regardless of the types of grafting system used.

Tip grafting shortened shoot length, which may due to the induced early terminal growth cessation. Shoot length is also related to dwarfing characteristics. Crabapple had longer shoot length but higher winter survival than ‘Ottawa 3’. The other growth factors, such as leaf number to shoot length ratio and shoot diameter were only moderately correlated with winter survival. For example, basal grafts of ‘Ottawa 3’ were not the cold hardiest, but they had the highest leaf number to shoot length ratio and shoot diameter. In contrast, scions on the tip system on crabapple rootstocks had the highest winter survival, but they had the smallest shoot diameter. Overall, the growth factors are not as feasible as terminal bud stage as indicators for cold hardiness, because it is more difficult to measure the growth factors than terminal bud stage and the correlation was lower.

4.4.4 Graft Transmission of Cold Hardiness

The results from this study indicated that cold hardiness can be graft transmitted from rootstocks to scions. The rootstocks were 10-year-old crabapple and 1-year-old ‘Ottawa 3’ in the tip grafting system and traditional grafting system, respectively. Compared to autografts, the less cold hardy ‘Ottawa 3’ scions grafted onto the cold hardy mature crabapple trees (cold hardy rootstock) showed increased winter survival. On the other hand, the cold hardy crabapple scions grafted onto the less cold hardy young ‘Ottawa 3’ rootstocks (less cold hardy rootstock) showed decreased winter survival. Graft transmission also influenced terminal growth cessation and terminal bud stages, cold acclimation factors that are closely related to winter survival.

It has long been known that viruses use the phloem to establish a systemic infection. Long-distance transmission of phytohormones (Jackson, 1997), mRNA (Kim

et al., 2001), and structural phloem proteins (Golecki et al., 1998) was observed through the phloem in reciprocal grafts. Molecular studies have identified many genes that are induced or upregulated by cold acclimation. A generic pathway for the transduction of cold acclimation signals in plants starts with signal perception, followed by the generation of secondary messengers, and finally targets proteins directly involved in transcription factors controlling specific sets of stress-regulated genes (Xiong et al., 2002). Heterografting systems indicated transmission of mRNA signals in plants (Kim et al., 2001; Haywood et al., 2002). It is reasonable to speculate that in a grafting system the alteration of cold hardiness of a scion by a rootstock is through the transduction of cold acclimation signals.

4.5 Conclusions

Tip grafting onto crabapple trees increased winter survival of apple scions significantly compared to the traditional grafting system of grafting scions onto 'Ottawa 3' rootstocks. Regardless of the types of grafting system, cold hardiness was graft transmitted from rootstocks to scions. The tip grafting system could be used to conserve less cold hardy parental materials and handle seedling populations in breeding programs. The traditional grafting system of grafting onto 'Ottawa 3' rootstocks was better able to differentiate between varieties for cold sensitivity and may be a better screening tool than tip grafting onto crabapple trees for predicting cold hardiness of scions in a breeding program.

The tip grafting system accelerated terminal growth cessation and induced early leaf drop of scions relative to the traditional grafting system. This likely resulted in earlier onset of cold acclimation and enhanced winter survival. Terminal growth

cessation and terminal bud developmental stage were positively correlated with winter survival in this study compared with leaf drop and vegetative growth factors. This indicated that under natural conditions, cold acclimation factors, especially terminal bud developmental stage, can be used to predict the initiation and extent of cold acclimation and winter survival.

5. EFFECT OF A TIP GRAFTING SYSTEM ON JUVENILITY AND COLD HARDINESS IN SOUR CHERRY HYBRIDS AND SASKATOON BERRY SEEDLINGS

5.1 Introduction

To develop new fruit cultivars, breeders need to evaluate fruit and tree characteristics as early as possible. A long juvenile period represents a major impediment to rapid screening. Shortening the juvenile phase can shorten the selection cycle and thus decrease the costs of a breeding program. Burbank's (1921) success with tip grafting cherry seedlings onto mature trees presents a method which may promote early transition from the juvenile to the adult phase, but his study did not mention details such as seedling age or size, nor were proper controls utilized. Vegetative growth is closely related to reproductive development (Buban and Faust, 1982) and the vigor of vegetative growth as measured by trunk diameter is negatively correlated with the juvenile period of apple and pear seedlings (Visser, 1964). The vegetative growth of the scions can be affected by the rootstocks, thereby influencing the juvenility of the scions. In tip grafting systems, the effects of rootstocks on vegetative growth and juvenility of scions are unknown.

In most woody perennials, cold hardiness is developed through cold acclimation. The cessation of vegetative growth is a prerequisite for cold acclimation in woody perennials and maximum hardiness occurs after leaf abscission (Weiser, 1970; Fuchigami et al., 1971; Arora et al., 1992). Consequently, terminal bud formation and

leaf drop could be used as indicators of cold acclimation and cold hardiness (Proebsting, 1978; Guak and Fuchigami. 2001; Wood and Reilly, 2001).

Sour cherry and saskatoon berry were selected for this study because they are important bush fruit in Saskatchewan, the former being intensively tested as a new crop and the latter having the largest acreage in Saskatchewan. Both sour cherry and saskatoon berry plants have relatively short juvenile periods that make them suitable models to study the effect of a tip grafting system on juvenility. Saskatoon berry plants are native and widely distributed in western North America. They are well adapted to cold northern climates. Sour cherry is not native to Saskatchewan. Through breeding it had been adapted to this area, but it represents the northern most limit of where sour cherry is grown in North America. The cold hardy nature and cold acclimation patterns of these two species make them useful to study the phenological development related to cold hardiness.

The objectives of this study were to determine the effect of a tip grafting system on juvenility and cold hardiness of scions of sour cherry and saskatoon berry seedlings. The response of tip grafted scions associated with juvenility and cold hardiness were also evaluated.

5.2 Materials and Methods

5.2.1 Plant Materials and Experimental Design

Two types of sour cherry hybrid seedlings were chosen as scion donors and rootstocks, respectively (Figure 5.1). In Figure 5.1 it can be observed that the seedling used for rootstocks has a higher percentage of ancestors from cold regions and it is expected that these rootstocks would be more cold hardy than the scion donors. ‘Kerr’s

Easy Pick' is considered the hardiest as it was selected in Prince Albert, Saskatchewan after particularly cold winters in the 50's and has the hardy species *P. fruticosa* in its linkage. 'Cacanski Rubin' may be the next most hardy genotype as it was selected in Poland followed by 'Northstar and lastly 'Erdi Jubileum'.

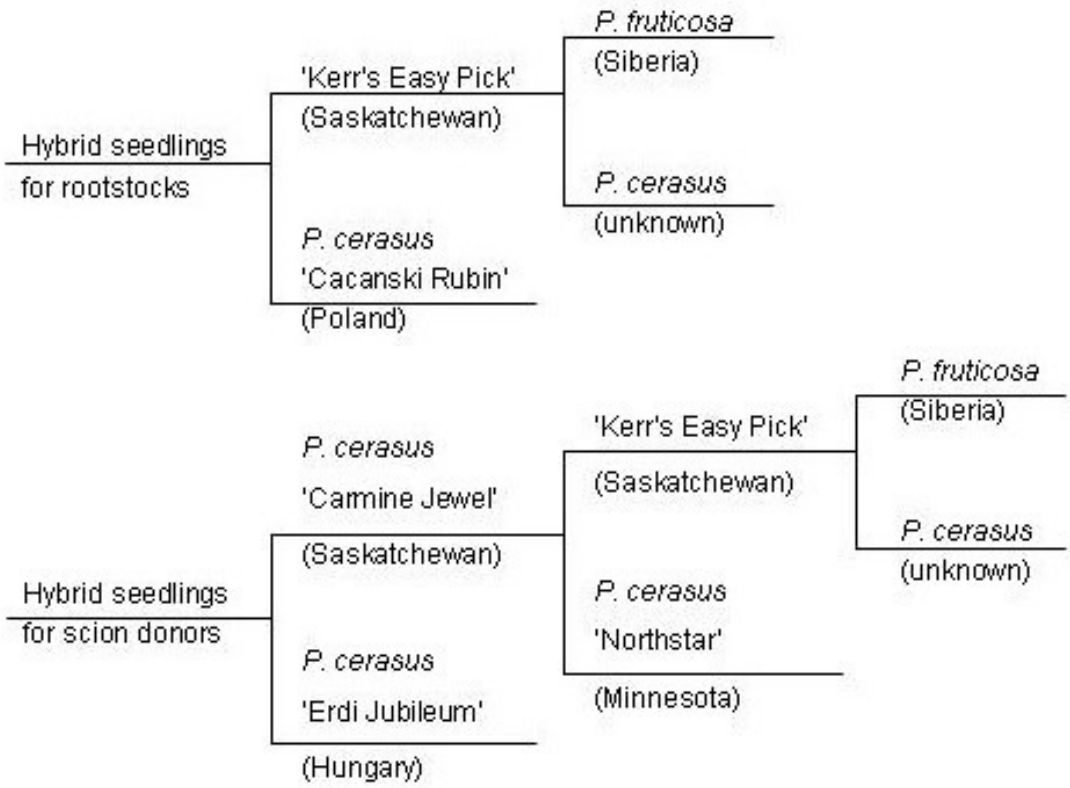

Figure 5.1 Pedigree of the two types of sour cherry hybrid seedlings used as rootstocks and scion donors in this study. These plants were bred at the University of Saskatchewan. Names in parentheses indicate origin.

In spring 2000, three reproductively mature eight-year-old rootstock seedlings on their own roots that displayed uniform vegetative growth and no winter kill symptoms were chosen to receive tip grafts. The juvenile seedlings on their own roots

(scion donor) were grown in the field with a spacing of 0.33 m between plants within rows. Thirty one-year-old, juvenile scion donor seedlings were randomly chosen for collecting scions. Three buds from each scion donor were individually tip grafted onto the three rootstocks in early May 2000 (tip grafts).

In spring 2000, the scion donors for saskatoon berry plants were the open pollinated progeny of the variety 'Nelson'. This variety is a multi-stemmed, compact shrub that grows to 1.5 m. The one-year-old, juvenile seedlings were grown in 210mm x 210 mm sized pots and 40 seedlings were randomly selected as scion donors. Three reproductively mature six-year-old trees of the saskatoon berry cultivar 'Pembina', on their own roots, were chosen as rootstocks based on their uniform vegetative growth. Both 'Nelson' and 'Pembina' are native to Saskatchewan, widely grown by growers, and considered to be very cold hardy in this location. Three buds from each juvenile seedling were individually tip grafted onto the three mature rootstocks in early May 2000 (tip grafts). After grafting, the juvenile seedlings on their own roots (scion donors) were transplanted into the field with a spacing of 0.33 m between plants within rows.

For both sour cherry and saskatoon berry, chip budding was done by hand using Parafilm® to hold buds in place and retain moisture. The stems above the graft unions were cut off immediately after budding. All the scion donors and rootstocks with tip grafts were irrigated when needed.

A completely random design was used with three sampling populations: rootstocks, tip grafts, and scion donors. One shoot on each juvenile seedling on its own roots that had similar vigor to the shoot from which the three buds were taken was sampled for each scion donor. In scion cherry plants, one non-grafted shoot at a similar

height and vigor to the shoot on which the scion was grafted was sampled corresponded to one tip graft. Of the 90 scions grafted in sour cherry, only 41 were established including 26 scion donors. Therefore, 26 scion donors, 41 tip grafts, and 41 non-grafted shoots in rootstocks (rootstocks) were evaluated. In saskatoon berry plants, six non-grafted shoots on each rootstock at a similar height and vigor to the shoots on which the scions were grafted were chosen to gather data for each rootstock. There were 31 scion donors, 40 tip grafts, and 18 non-grafted shoots in rootstocks (rootstocks) evaluated.

5.2.2 Data Collection

Data were gathered from the three sampling populations two growing seasons after grafting. In the spring of 2002, flowering and winter dieback were evaluated. Flowering was assessed when flower buds were at approximately the full bloom stage. The shoots were scored as either one (with more than one flower) or zero (with no flowers) and the flower number was also recorded. Winter shoot dieback in sour cherry was assessed as the percentage of shoot length that had dieback. In saskatoon berry plants, since the winter dieback was present only in terminal buds, terminal bud survival was assessed on apical shoots as dead (one) or alive (zero). This binomial system was also used on the sour cherry plants.

Terminal bud stage was assessed on apical shoots in mid-September for sour cherry and in mid-July for saskatoon berry in 2001 by scoring the buds using the following six stages, as shown in Figure 4.1:

0 = apical meristematic tissues visible with new leaves being formed;

1 = apical meristematic tissues with no new leaves being formed;

2 = green bud visible, the terminal two leaves fully expanded;

3 = bud tip turned brown;

4 = bud scale visible;

5 = bud scale totally formed and brown.

Total leaf number was counted in September after the terminal buds had completely formed. Leaf retention was quantified by the percentage of leaves retained on the shoots in late October. Percent leaf drop was calculated from leaf retention.

Vegetative growth was assessed by measuring shoot length and shoot diameter in November 2001 after leaf fall. Shoot length was measured on apical shoots from the bud scale scar to the shoot tip from each growing season and was added up for total shoot length of two growing seasons. Shoot diameter was measured at the bud scale scar of two-year-old shoots from 2000.

5.2.3 Statistical Analysis

SAS program (SAS institute, 1999) was used for statistical analysis. All binomial data were analyzed using the GENMOD procedure. Other data, e.g. vegetative growth, terminal bud stage and leaf drop were analyzed using the GLM procedure. Means were separated using the lsmeans multiple comparison procedure.

5.3 Results

5.3.1 Flowering and Winter Dieback

Tip grafting significantly affected flowering in sour cherry (Appendix 2-1, Appendix 2-2) and saskatoon berry (Appendix 2-3, Appendix 2-4). All the non-grafted shoots on the mature rootstocks flowered in both sour cherry and saskatoon berry

(Table 5.1). In sour cherry, four times more shoots with flowers were found on the scion donors compared to the tip grafts (42.3% vs. 9.8%). The flower number for sour cherry was also much higher on the scion donors (1.4) than on tip grafts (0.2). In contrast, in saskatoon berry the tip grafts had more shoots with flowers compared to scion donors (70.0% vs. 22.6%). The number of flowers produced was much higher on the tip grafts (10.2) than the scion donors (2.0).

Table 5.1 Flowering[z] of rootstocks, tip grafts and scion donors of sour cherry and saskatoon berry plants two growing seasons after grafting in the spring of 2002 at Saskatoon.

	Sour cherry			Saskatoon berry		
Sample populations	n	Shoots with flowers (%)	Flower no.	n	Shoots with flowers (%)	Flower no.
Rootstocks	41	100 a[y]	2.5 a	18	100 a	6.3 a
Tip grafts	41	9.8 c	0.2 c	40	70.0 b	10.2 a
Scion donors	26	42.3 b	1.4 b	31	22.6 c	2.0 b

[z] Data were sampled on shoots over two growing seasons.
[y] Numbers followed by different letters within a column were significantly different at P = 0.05 level.

In sour cherry plants, the tip grafting system significantly reduced winter dieback as measured by shoot length dieback (Appendix 2-1) and terminal bud death (Appendix 2-2). No shoot winter dieback symptoms were found on the mature rootstocks and there was no significant difference in winter dieback between tip grafts and rootstocks (Table 5.2). Significantly fewer terminal buds died in the tip grafts than the scion donors (4.9% vs. 69.2%). The percent dieback of individual shoots was also lower in tip grafts relative to scion donors (0.5% vs. 3.4%).

In saskatoon berry, the tip grafting system had no significant effect on cold hardiness as measured by percent shoots with dead terminal buds (Appendix 2-4). There was no terminal bud death on the mature rootstocks, and the percentage of terminal bud death was similar between the tip grafts and scion donors, at only approximately 12% (Table 5.2).

Table 5.2 Winter dieback[z] of rootstocks, tip grafts and scion donors of sour cherry and saskatoon berry plants two growing seasons after grafting in the spring of 2002 at Saskatoon.

	Sour cherry			Saskatoon berry	
Sample populations	n	Terminal bud death (%)	Shoot dieback (%)	n	Terminal bud death (%)
Rootstocks	41	0.0 b[y]	0.0 b	18	0.0 a
Tip grafts	41	4.9 b	0.5 b	40	12.4 a
Scion donors	26	69.2 a	3.4 a	31	12.9 a

[z] Data were sampled on one-year-old apical shoots.
[y] Numbers followed by different letters within a column were significantly different at P = 0.05 level.

5.3.2 Terminal Bud Stage and Leaf Drop

In sour cherry plants, the effects of tip grafting on terminal bud stage and leaf drop were significant (Appendix 2-1). The tip grafting system enhanced terminal bud development (Table 5.3). The scion donors continued to grow into September, while the terminal buds on the rootstocks and tip-grafts had formed by mid-August (data not shown). Data collected in mid-September indicated that the terminal buds of rootstocks and tip grafts were more advanced than those of scion donors.

In saskatoon berry plants, tip grafting had no significant effect on terminal bud stage but leaf drop was significantly different among the three sampling populations (Appendix 2-3). The tip grafting system appeared to delay terminal bud development in

saskatoon berry seedlings, although this effect was not significant at P = 0.05 level (Table 5.3). The terminal buds of the shoots from the three sample populations had all formed by the end of July.

The tip grafting systems on both sour cherry and saskatoon berry plants enhanced leaf drop over scion donors. In general, leaf drop of rootstocks > tip grafts > scion donors (Table 5.3).

Table 5.3 Terminal bud stage[z] and leaf drop of rootstocks, tip grafts and scion donors of sour cherry and saskatoon berry plants on one-year-old apical shoots two growing seasons after grafting in the fall of 2001 at Saskatoon.

	Sour cherry			Saskatoon berry		
Sample populations	n	Terminal bud stage (14-Sep)	Leaf drop (%) (31-Oct)	n	Terminal bud stage (14-July)	Leaf drop (%) (19-Oct)
Rootstocks	41	5.0 a[y]	96.6 a	18	4.0 a	100 a
Tip grafts	41	4.7 a	78.7 b	40	3.8 a	42.9 b
Scion donors	26	2.7 b	47.8 c	31	4.2 a	31.4 c

[z] Terminal bud stage: 0 = apical meristematic tissues visible with new leaves being formed; 1 = apical meristematic tissues with no new leaves being formed; 2 = green bud visible, the terminal two leaves fully expanded; 3 = bud tip turned brown; 4 = bud scales visible; 5 = bud scales totally formed and brown (Figure 4.1).

[y] Numbers followed by different letters within a column were significantly different at P = 0.05 level.

5.3.3 Vegetative Growth

The tip grafting system significantly affected the vegetative growth in both sour cherry (Appendix 2-1) and saskatoon berry plants (Appendix 2-3). The vegetative growth of tip grafts corresponded with the bushes onto which they were grafted (Table 5.4). In sour cherry plants, tip grafts had an 84% decrease in shoot length and a 76% decrease in shoot diameter relative to the scion donors. In saskatoon berry plants, the tip

grafting system increased shoot length by 257% and shoot diameter by 42% compared with scion donors.

Table 5.4 Shoot length and shoot diameter[z] of rootstocks, tip grafts and scion donors of sour cherry and saskatoon berry plants two growing seasons after grafting in the fall of 2001 at Saskatoon.

	Sour cherry			Saskatoon berry		
Sample populations	n	Shoot length (cm)	Shoot diameter (mm)	n	Shoot length (cm)	Shoot diameter (mm)
Rootstock	41	13.1 b[y]	2.48 b	18	30.2 a	4.02 b
Tip grafts	41	12.4 b	3.01 b	40	34.7 a	4.62 a
Scion donors	26	101.6 a	12.53 a	31	9.7 b	3.26 c

[z] Data were sampled on apical shoots over two growing seasons for total shoot length and at the base of two-year-old shoots for accumulative shoot diameter.
[y] Numbers followed by different letters within a column were significantly different at P = 0.05 level.

5.4 Discussion

5.4.1 Effect of Tip Grafting System on Cold Hardiness

In sour cherry plants, the rootstocks used in the tip grafting system were very cold hardy and the cold hardiness of tip grafts was significantly increased by the rootstocks relative to the scion donors. This finding was in agreement with that of Chapter 4 that regardless of grafting systems used, rootstocks affected the cold hardiness of scions. The effect of the tip grafting system on enhancing cold hardiness could be due to early growth cessation and leaf drop. Terminal bud stage and leaf drop were negatively correlated with winter shoot dieback (r = -0.50 and -0.40, respectively, p = 0.0001, n= 108). Earlier growth cessation and leaf drop lead to an earlier initiation of cold acclimation and decrease the risk of cold injury (Weiser, 1970), because the

most severe low temperature injury to fruit trees usually occurs in late fall or early winter. Under such conditions cold acclimation is of prime importance (Westwood, 1970). Since tip grafting increased the cold hardiness of sour cherry seedlings, it can be used for conservation of non-hardy germplasm, but it would not be useful for screening of cold hardiness of scions.

In saskatoon berry plants, the tip grafting system had no significant influence on winter dieback. In both the tip grafts and scion donors, approximately 12% of the shoots had terminal bud death. The fact that tip grafting had little effect on cold hardiness of saskatoon berry plants is likely due to the inherent cold hardy nature of this species. Saskatoon berry plants, native to the Canadian prairies, are well adapted to our climate. Terminal buds had formed by early July in both the tip grafts and scion donors (Table 5.3). Kaurin et al. (1984) also found that the time of onset of vegetative maturity (the stage of development at which removal of leaves will no longer stimulate lateral bud break (Seibel and Fuchigami, 1978)) in the saskatoon berry cultivar 'Smoky' occurred very early in summer (29 May) and the initiation of cold acclimation was correlated with the cessation of growth. In 2001, the first frost occurred on September 12; therefore, there was enough time for cold acclimation development in the shoots from all the three sample populations in saskatoon berry plants. This early cessation of terminal growth results in the adaptation to cold environmental stress of this native species.

5.4.2 Effect of Tip Grafting System on Juvenility

The rootstocks in both tip grafting systems all flowered, but the flowering responses of tip grafts were crop-dependent. In sour cherry plants, the tip grafting

system reduced flowering by 77%, decreased shoot length by 84% and shoot diameter by 76% compared to scion donors. In saskatoon berry plants, the tip grafting system enhanced flowering by 68%, increased shoot length by 257% and shoot diameter by 42% relative to the scion donors. These results indicated that the effect of the tip grafting systems on flowering may not be through graft transmission of mature status but rather though graft transmission of vigor from the rootstock to scions. For young seedlings, the flower bud induction may be closely related to vegetative growth. Any reduction in vegetative growth may restrict flower bud induction, while improved vegetative growth enhances flower bud induction. Visser (1964) also concluded that there was a significantly negative correlation between the juvenile period and the vigor of the seedling (as measured by trunk diameter). The finding that the vigor of young seedlings influenced flowering of the young seedlings is in agreement with results in other species, such as apples and pears (Visser, 1964; 1965; Visser et al., 1976; Zimmerman et al., 1970; Zimmerman, 1971, 1977). In young seedlings, the juvenile period could be shortened by promoting their vegetative growth. If the young seedlings show good vigor of vegetative growth then tip grafting is not necessary, but if they lack vigor, tip grafting might be beneficial.

5.5 Conclusions

In the sour cherry experiment, the tip grafting system reduced the vegetative growth of the scions and accelerated growth cessation when compared with scion donors. The shoots were delayed in attaining the minimum size for flower initiation, and consequently, floral bud development was retarded. It is known that earlier growth cessation leads to an earlier onset of dormancy and decreases the risk of cold injury.

While the tip grafting system did not enhance flowering, it did increase cold hardiness of the sour cherry seedlings. Thus tip grafting is not suitable for the purpose of early evaluation of fruit quality but is beneficial in conservation of non-hardy germplasm in the sour cherry breeding program.

Tip grafting of saskatoon berry increased vegetative growth compared with scion donors and consequently enhanced flower bud induction. The tip grafting system on saskatoon berry plants provided fruit one year earlier compared to the scion donors. The tip grafting system appeared to have no effect on cold hardiness of scions as the scions and rootstocks had similar cold hardiness. The very cold hardy characteristic of this species is likely due to the very early growth cessation.

6. GENERAL DISCUSSION AND FUTURE RESEARCH

6.1 General Discussion and Conclusions

When breeding woody fruit species, early selection for precocity, cold hardiness, and fruit quality is very important due to the long juvenile period and large plant size. This study was initiated to test the feasibility of a tip grafting system for evaluation of cold hardiness of scions for apple breeding and to study the effect of tip grafting on juvenility and cold hardiness of scions in sour cherry hybrids and saskatoon berry seedlings. It was also intended to determine the correlation of cold hardiness with vegetative growth and cold acclimation factors in an effort to identify the physiological markers for cold hardiness.

The experiments indicated that regardless of types of grafting systems used, rootstocks can affect the cold hardiness and juvenility of scions of all three species tested in this study. The effects of rootstocks on cold hardiness and juvenility of scions are probably due to the induction of early cessation of vegetative growth associated with the timing of cold acclimation and influence of vegetative growth on precocity.

In Chapter 4, two grafting systems (tip grafting onto mature crabapple trees and traditional grafting onto dwarf ‘Ottawa 3’ rootstocks) were tested for screening cold hardiness of apple scions varying in low temperature sensitivity. In both grafting systems, the rootstocks affected the winter survival of scions. The crabapple rootstocks

used in the tip grafting system were very cold hardy and the ‘Ottawa 3’ rootstocks used in the traditional grafting system were only moderately cold hardy (Table 4.1). As a result, the grafts in the traditional grafting system represented the inherent cold hardiness levels of the cultivar scions, so the traditional grafting system would be feasible for screening cold hardiness of scions. Compared with the traditional grafting system, the cold hardiness of scions was improved significantly by the rootstocks used in the tip grafting system, so the tip grafting system can be used for conservation of sensitive breeding materials. Due to very cold hardy characteristics of crabapple rootstocks, the tip grafting system improved the cold hardiness of scions to a similar level. The correlation on winter survival between the two systems was only moderate ($r = 0.52$, $P = 0.0001$, $n = 40$). Therefore, the tip grafting system tested is not feasible for evaluation of cold hardiness of scions in apple breeding programs.

In the sour cherry experiment, the rootstocks were more cold hardy than the scion donor seedlings. As a result, the tip grafting system in sour cherry increased the cold hardiness of scions compared to the self-rooted scion donor seedlings. This result is similar to that in apple trees in which the cold hardiness of scions was improved by the cold hardy rootstocks.

The tip grafting system in saskatoon berry plants had little influence on winter survival of scions, likely due to the very cold hardy nature of this species. The cultivars released are naturally selected from superior native genotypes with most cultivars originating from the Canadian prairies (Kaurin et al., 1984; Steeves and Steeves, 1990). Most cultivars are cold hardy and the rootstocks and scions in this study have similar

cold hardiness, so the cold hardiness of scions cannot be changed very much by the rootstocks.

The tip grafting system affected timing of cold acclimation. This may be the major factor involved in enhancing the cold hardiness of the scions. Earlier cessation of terminal growth leads to an earlier onset of dormancy and decreases the risk of cold injury. In all three species, early termination of bud development improved winter survival. The tip grafting system induced earlier terminal bud development than the traditional grafting system in apple trees (Chapter 4). Tip grafting sour cherry accelerated terminal bud formation which was associated with an 85.3% decrease in winter dieback (Chapter 5). The very cold hardy nature of saskatoon berry may be also due to the fact that the terminal bud formation in saskatoon berry was fulfilled by July.

Terminal bud development can be used as an indicator of the initiation of cold acclimation and potential for winter survival. Leaf drop was not consistently related to winter survival in apple (Chapter 4), sour cherry and saskatoon berry plants (Chapter 5), so it is not recommended as an indicator of cold hardiness.

The tip grafting system could affect the juvenility of scions depending on how the vegetative growth was influenced by the rootstocks used in the systems. Vegetative growth of scions approximated that of the rootstocks on which the scions were tip grafted, which is in agreement with the result for vegetative growth in Chapter 4. In sour cherry plants, rootstocks were less vigorous as measured as shoot length and diameter than the scion donors, and consequently the tip grafts had decreased vegetative growth compared with the scion donors. In contrast, in saskatoon berry plants, rootstocks were more vigorous than the scion donors and the tip grafts consequently had

increased vegetative growth relative to the scion donors. For young seedlings, the reduced vegetative growth has a restriction for flower bud induction, while the improved vegetative growth enhances flower bud induction.

6.2 Future Research

The apple cultivar comparisons (Chapter 4) indicate that cold hardiness is graft transmitted from the rootstocks to the scions. The rootstock effect was not diluted after two growing seasons, as the cold hardiness of the less cold hardy scions was maintained on the cold hardy rootstocks. In the future it would be valuable to test the stability of the enhanced cold hardiness of tip-grafted ‘Golden Delicious’ and ‘McIntosh’.

It is likely that the mature trees used as rootstocks can cause maturation of the tip grafted scions in all three species. The mature rootstocks can modify the time of cessation of growth of scions. The earlier growth cessation induced by the mature rootstocks would enhance the development of cold acclimation. If cold hardiness can be so dramatically altered by tip grafting, perhaps it can be a useful tool for studying other translocated factors and signal transduction, thereby providing a better understanding of the physiological mechanisms of graft transmission of cold hardiness from rootstocks to scions. In the future, grafting test should be done on mature and young rootstocks using the same genotype to test the effect of tip grafting on the cold hardiness of the scions.

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APPENDICES

Appendix 1-1 Grafting survival rate (%) of scions for five apple cultivars as affected by the tip and traditional grafting system in 2001 at Saskatoon[z].

Grafting system	Scion				
	crabapple	Ottawa 3	Prairie Sun	McIntosh	Golden Delicious
Traditional grafting system	67.5	50.0	75.0	61.3	76.3
Tip grafting system	81.3	92.5	100	96.3	98.8

[z] Means were based on 20 buds for each experimental unit within each treatment, which was replicated four times.

Appendix 1-2 Analysis of variance for the effect of grafting systems and scion cultivars on the winter survival of apple grafts over two years (2001 and 2002) at Saskatoon.

Source	DF	Mean Square	F value
Replication (R)	3	369	1.29 ns
Grafting system (G)	**1**	**27421**	**95.79 ****
Error a	3	286	
Scion (S)	**4**	**5979**	**73.24 *****
G*S	**4**	**2420**	**29.64 *****
Error b	24	82	
Year (Y)	**1**	**378**	**2.48 ns**
Error c	6	95	
Y*G	4	36	2.60 ns
Y*S	1	396	0.23 ns
Y*G*S	4	232	1.52 ns
Error	24	152	

ns, *, **, *** not significant at P = 0.05 level, significant at P = 0.05, 0.01, 0.001 level, respectively.

Appendix 1-3 Analysis of variance for the effects of grafting systems and scion cultivars on the terminal growth cessation (%)[z] of apple grafts for two observation times (mid-August and mid-September) over two years (2001 and 2002) at Saskatoon.

Between subjects effects			Within subjects effects		
Source	DF	Mean square	Source	DF	Mean square
			Year (Y)	**3**	**9972*** **
Replication (R)	3	262 ns	Y*B	1	118 ns
Grafting system (G)	**1**	**70639 ****	Y*G	3	10439 ***
Error a	3	567	Y*R*G	4	95 ns
Scion (S)	**4**	**3220 *** **	Y*S	4	331 ns
G*S	**4**	**2251 *** **	Y*G*S	24	293 ns
Error b	24	118	Y*R*G*S	1	131 ns
Time (T)	**1**	**11813 *** **	Y*T	6	359 ns
Error c	6	248	Y*T*R*G	1	325 ns
T*G	1	9464 ***	Y*T*G	4	102 ns
T*S	4	30 ns	Y*T*S	4	425 ns
T*G*S	4	11 ns	Y*T*G*S	24	315 ns
Error	24	99	Error(Y)	24	157

ns, * , ** and *** not significant at P = 0.05 level, significant at P = 0.05, 0.01 and 0.001 level, respectively.

[z] percent of terminal growth cessation as apical meristem visually free.

Appendix 1-4 Analysis of variance for the effects of grafting systems and scion cultivars on the terminal growth cessation (%) and terminal bud stage of apple grafts for two observation times (mid-August and mid-September) in 2001 and 2002.

Source	DF	Mean squares		
		2001	2002	
		Terminal growth cessation	Terminal growth cessation	Terminal bud stage
Replication (R)	3	119 ns	261 ns	0.29 ns
Grafting system (G)	**1**	**67695 *** **	**13384 *** **	**95.92 ** **
Error a	3	376	287	0.90
Scion (S)	**4**	**1312 *** **	**2239 *** **	**6.27 *** **
G*S	**4**	**811 ** **	**1734 *** **	**2.02 ** **
Error b	24	84	165	0.36
Time (T)	**1**	**4028 ** **	**8143 *** **	**28.32 *** **
Error c	6	322	251	0.14
T*G	1	3668 ***	5598 ***	4.14 ***
T*S	4	194 *	261 ns	0.34 *
T*G*S	4	218 *	109 ns	0.34 *
Error	24	69	187	0.12

ns, * , ** and *** not significant at P = 0.05 level, significant at P = 0.05, 0.01 and 0.001 level, respectively.

Appendix 1-5 Analysis of variance for the effects of grafting systems and scion cultivars on the leaf drop (%) of apple grafts for two observation times (early and late November) over two years (2001 and 2002) at Saskatoon.

Between subjects effects			Within subjects effects		
Source	DF	Mean square	Source	DF	Mean square
			Year (Y)	**1**	**50194 *****
Replication (R)	3	2832 ns	Y*B	3	536 *
Grafting system (G)	**1**	**6947 ***	Y*G	1	4496 ***
Error a	3	455	Y*R*G	3	8 ns
Scion (S)	**4**	**4155 *****	Y*S	4	66 ns
G*S	**4**	**1571 ***	Y*G*S	4	270 ns
Error b	24	378	Y*R*G*S	24	223 ***
Time (T)	**1**	**21811 *****	Y*T	1	3184 **
Error c	6	88	Y*T*R*G	6	166 **
T*G	1	253 ***	Y*T*G	1	420 ns
T*S	4	122**	Y*T*S	4	28 ns
T*G*S	4	86ns	Y*T*G*S	4	238 **
Error	24	26	Error(Y)	24	39

ns, * , ** and *** not significant at P = 0.05 level, significant at P = 0.05, 0.01 and 0.001 level, respectively.

Appendix 1-6 Analysis of variance for the effects of grafting systems and scion cultivars on the leaf drop (%) of apple grafts over two observation times (early- and late-November) in 2001 and 2002 at Saskatoon.

Source	DF	Mean Square	
		2001	2002
Replication (R)	3	674 ns	2693 *
Grafting system (G)	**1**	**11310 ** **	**133 ns**
Error a	3	223	240
Scion (S)	**4**	**2066 *****	**2154 ** **
G*S	**4**	**1416 ** **	**425ns**
Error b	24	234	366
Time (T)	**1**	**4164 *****	**20830 *****
Error c	6	104	150
T*G	1	11 ns	662 ***
T*S	4	30 ns	121 **
T*G*S	4	53 ns	272 **
Error	24	31	35

ns, * , ** and *** not significant at P = 0.05 level, significant at P = 0.05, 0.01 and 0.001 level, respectively.

Appendix 1-7 Analysis of variance for the effects of grafting systems and scion cultivars on the apical shoot length, leaf number and leaf number to shoot length ratio of apple grafts over two years (2001 and 2002) at Saskatoon.

Source	DF	Mean square		
		Leaf no.	Shoot length	Leaf no./shoot length
Replication (R)	3	28.4 ns	90 ns	1.77 ns
Grafting system (G)	**1**	**1978.3 ****	**6755 ****	**270.92 *****
Error a	3	67.0	216	2.00
Scion (S)	**4**	**34.2 ****	**281 *****	**9.65 *****
G*S	**4**	**44.1 ****	**214 *****	**5.42 *****
Error b	24	7.7	33	0.76
Year (Y)	**1**	**1.4 ns**	**83 ns**	**2.02 ns**
Error c	6	8.6	19	0.61
Y*G	1	67.3 ns	118 **	0.60 ns
Y*S	4	2.5 ns	9 ns	0.43 ns
Y*G*S	4	2.2 ns	9 ns	0.32 ns
Error	24	8.5	16	0.49

ns, * , ** and *** not significant at P = 0.05 level, significant at P = 0.05, 0.01 and 0.001 level, respectively.

Appendix 1-8 Analysis of variance for the effects of grafting systems and scion cultivars on the cumulative shoot diameter of apple grafts two growing seasons after grafting in 2002 at Saskatoon.

Source	DF	Mean Square	F value
Replication (R)	3	1.11	0.34 ns
Grafting system (G)	**1**	**202.60**	**62.58 ****
Error a	3	3.24	
Scion (S)	**4**	**3.41**	**6.61 *****
G*S	**4**	**3.12**	**6.06 ****
Error	24	0.52	

ns, ** and *** not significant at P = 0.05 level, significant at P = 0.01 and 0.001 level, respectively.

Appendix 2-1 Analysis of variance for the effects of tip grafting system on flower number, shoot dieback, terminal growth cessation, leaf drop, and vegetative growth of sour cherry hybrid seedlings two growing seasons after grafting at Saskatoon[z].

Variable	Source	DF	Mean Square	F value
Flower number	Sampling population	2	46.2	39.00 ***
	Error	105	1.2	
Shoot dieback	Sampling population	2	4.218	53.28 ***
	Error	105	0.079	
Terminal bud stage	Sampling population	2	28.50	62.14 ***
	Error	105	0.46	
Leaf drop	Sampling population	2	1.690	24.47 ***
	Error	105	0.069	
Shoot length	Sampling population	2	75234	369.28 ***
	Error	105	279	
Shoot diameter	Sampling population	2	905.6	205.83 ***
	Error	105	4.4	

ns, ***, not significant, significant at P = 0.001 level, respectively.
[z] Data were based on 41 tip grafts, 26 scion donors, and 41 non-grafted shoots in rootstocks.

Appendix 2-2 Chi-square tests for the effects of tip grafting system on shoots with flowers and shoots with dieback of sour cherry hybrid seedlings two growing seasons after grafting at Saskatoon[z].

Variable	Source	DF	Chi-squre	Pr > ChiSq
Shoots with flowers	Sampling population	2	85.85	< .0001 ***
Shoots with dieback	Sampling population	2	29.78	< .0001 ***

***, significant at P = 0.001 level, respectively.
[z] Data were based on 41 tip grafts, 26 scion donors, and 41 non-grafted shoots in rootstocks.

Appendix 2-3 Analysis of variance for the effects of tip grafting system on flower number, shoot dieback, terminal growth cessation, leaf drop, and vegetative growth of saskatoon berry seedlings two growing seasons after grafting at Saskatoon[z].

Variable	Source	DF	Mean Square	F value
Flower number	Sampling population	2	583	9.31 ***
	Error	86	62	
Terminal bud stage	Sampling population	2	1.31	1.84 ns
	Error	86	0.71	
Leaf drop	Sampling population	2	2.886	104.51 ***
	Error	86	0.028	
Shoot length	Sampling population	2	5741	65.93 ***
	Error	86	87	
Shoot diameter	Sampling population	2	16.37	19.41 ***
	Error	86	0.84	

ns, ***, not significant, significant at P = 0.001 level, respectively.
[z] Data were based on 40 tip grafts, 31 scion donors, and 18 non-grafted shoots in rootstocks.

Appendix 2-4 Chi-square tests for the effects of tip grafting system on shoots with flowers and shoots with dieback of saskatoon berry seedlings two growing seasons after grafting at Saskatoon [z].

Variable	Source	DF	Chi-squre	Pr > ChiSq
Shoots with flowers	Sampling population	2	39.08	< .0001 ***
Shoots with dieback	Sampling population	2	2.58	0.2755 ns

ns, ***, not significant at P = 0.05 level, significant at P = 0.001 level, respectively.
[z] Data were based on 40 tip grafts, 31 scion donors, and 18 non-grafted shoots in rootstocks.