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STORAGE NITROGEN MANIPULATIONS IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING ROOTSTOCKS

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STORAGE NITROGEN MANIPULATIONS IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING ROOTSTOCKS

By

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ABSTRACT

STORAGE NITROGEN MANIPULATIONS IN SWEET CHERRY (*Prunus avium* L.) ON DWARFING ROOTSTOCKS

By

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Management of storage nitrogen (N) is of critical importance to maintain optimum growth of sweet cherry (*Prunus avium* L.). Sweet cherries on dwarfing Gisela® rootstocks tend to produce large crops but smaller fruit when crop load is not balanced with adequate leaf area. Study objectives were to 1) characterize the effect of N remobilization during fall on storage N reserves, 2) characterize the effect of storage N remobilization during winter and early spring on spur leaf formation, 3) determine the effect of fall foliar urea applications on N levels in flowering spurs, and 4) determine the effect of foliar-applied urea during fall on the development of cold hardiness. During fall, total N decreased in leaves by up to 51 % (dry weight, d.w.) and increased in perennial tissues, such as flower spurs, by up to 27 % (d.w.). The N concentration in flower spurs increased further in spring by up to 150 % (d.w.). Fall foliar applications of urea increased storage N levels in flowering spurs, shoot tips, and bark. New spur leaf size in the spring was associated with storage N levels; fall foliar urea treatments increased spur leaf area by up to 25 %. Foliar urea applications increased flower spur N levels most when applied in late summer to early fall. Such applications also had positive effects on cold acclimation of shoots; those treated with urea were up to 4.25˚C more hardy than untreated shoots during fall.

DEDICATION

To my parents and my brother

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CHAPTER 1

LITERATURE REVIEW

Introduction

Sweet cherries (*Prunus avium* L.) are one of the most profitable commercially grown fruit species (Lang, 2000). In 2007, total production of sweet cherries in the US was 293,656 metric tons (323,700 tons) on 33,734 ha (83,550 acres) (USDA, 2007a). Production value for the 2007 crop totaled $583.7 million (USDA, 2007b). In Michigan, sweet cherry production was 24,766 metric tons (27,300 tons) on 2,914 ha (7,200 acres) (USDA, 2007a).

During the last 15 years or so, dwarfing and semi-dwarfing rootstocks have become available for cherries. Although trees on the new rootstocks produce high yields, the problem is that fruit often are too small (Andersen et al., 1999; Whiting and Lang, 2001). Since the vigor of the rootstock affects orchard costs (pruning, harvesting, spraying), selecting the best rootstock is important. The standard rootstocks ['Mazzard' (*P. avium* L.) and 'Mahaleb' (*P. mahaleb* L.)] are vigorous but not precocious trees, with disadvantages like large tree size and a long establishment period before fruiting (Lang, 2000). These characteristics are unwanted because of low initial yields and inefficiency of orchard operations, like pruning, pest management and harvest (Whiting, 2001). Consequently, sweet cherry growers have begun to use dwarfing and semi-dwarfing rootstocks. The advantages of these rootstocks include smaller tree sizes and greater precocity in yield (Weber, 2001), making them a good choice for high density systems. Trees on standard rootstocks are too vigorous and tend not to flower until the 6th or the 7th year, but trees on dwarfing rootstocks are less vigorous and can flower

in the 2nd or 3rd year (Lang, 2000). Some of the most promising precocious rootstocks are the Gisela (GI) series, especially GI5 (148/2) and GI6 (148/1) (Webster and Lucas, 1997; Andersen et al., 1999; Lang 2000; Webster, 2001). The problem with these dwarfing and semi-dwarfing rootstocks is that they produce high yields, but small fruits (Andersen et al., 1989; Whiting and Lang, 2001); this can reduce returns to growers, since large fruit is highly prized.

Nitrogen (N) fertilizer is usually applied to orchards in the spring since N needs to be available for bud break and spring growth. Recent studies with ^{15}N, though, have shown that soil N is not taken up by sweet cherry until after bud break (Zavalloni, 2004). Therefore, trees use N from storage for spring growth and bud break. Soil N influences new shoot leaf size (Ayala, 2004), but spur leaves develop only from bud break through two to four weeks after bud break, when soil N uptake is low (Zavalloni, 2004). Consequently, spur leaf size may be limited by storage N availability; that is, it may be driven by storage N rather soil N. Ayala (2004) showed that spur leaves supply the majority of photosynthetic carbon for fruit growth, so spur leaf size is very important for optimal fruit development.

Little is known about the relative importance of N sources for shoot and fruit development in dwarfing cherry trees. The reproductive and vegetative growth can become unbalanced in trees on dwarfing rootstocks without intensive orchard management (Ayala, 2004). Manipulations of N sources and/or seasonal availability may provide a strategy to alter the leaf area-to-fruit (LA:F) ratio of dwarf cherry trees.

Nitrogen storage reserves and nitrogen remobilization during fall and spring

Nitrogen in the plant

Nitrogen is one of the most important macronutrients for trees. Nitrogen is a component of chlorophyll and other organic compounds like amino acids, proteins, and nucleic acids (Titus and Kang, 1982). In periods of rapid growth, N is of great importance and trees require it in large amounts (Epstein and Bloom, 2005; Taiz and Zeiger, 2006). The major forms of inorganic N present in the soil are nitrate (NO_3^-), ammonium (NH_4^+), and N_2 (of little importance in fruit trees), and organic nitrogen. Fruit trees take up mostly NO_3^- and NH_4^+, depending on the availability of these two forms and transformation of N in the soil, as well as tree species characteristics and environmental conditions (Titus and Kang, 1982). Marschner (1995) reported that N absorption by fruit trees is influenced by soil pH, temperature and mineral composition and carbohydrate supply to the roots; it is also affected by light (Frith and Nichols, 1975). Metabolic energy in the form of ATP is required for uptake and assimilation of NO_3^- and NH_4^+. Two ATPs are required per NH_4^+ absorbed and 12 ATPs for NO_3^- (Bloom, 1997). Urea and several amino acids are forms of organic N taken up by plants; Shim et al. (1973a) found that urea was mostly (80%) absorbed within two hours after application in growth chambers. The hydrolysis of urea into ammonia and carbon

dioxide is catalyzed by the enzyme urease. Shim et al. (1973b) found urease in apple (*Malus pumila* Rehd.) leaves, bark, and roots.

Assimilation and translocation of nitrate and ammonium

For NO_3^- to be assimilated, it has to be reduced to NO_2^- via nitrate reductase, which is localized in the cytoplasm, and then NO_2^- is reduced to NH_4^+ via nitrite reductase, which is localized in chloroplasts in leaves and in proplastids in roots (Marschner, 1995). Nitrate reduction occurs in the roots of apple (Frith, 1972) and when there is high availability of NO_3^-, nitrate reductase is saturated and NO_3^- is translocated to shoots and leaves through the xylem (Titus and Kang, 1982). Nitrate reductase also has been found in sweet cherry (*P. avium*), sour cherry (*P. cerasus*), plum (*P. domestica*), and apricot (*P. armeniaca*) (Leece et al., 1972). Ammonium is assimilated only in roots because of its high toxicity, even in low concentrations. Ammonium absorption often is carbon limited since NH_4^+ assimilation in roots has a large requirement for carbon skeletons (Lawlor, 2002). Ammonium is converted into amino acids by the GS/GOGAT (glutamine synthetase/glutamate synthase) enzymes (Marschner, 1995); the first product of NH_4^+ assimilation is glutamine, synthesized by GS, and then GOGAT synthesizes glutamate (Titus and Kang, 1982).

Movement and sites of storage of nitrogenous compounds

Absorbed mineral elements move upward via the xylem and the organic forms move downward through the phloem (Titus and Kang, 1982). Shim et al. (1973b) indicated that urea and possibly amino acids move radially, probably through ray cells and cambium. Nitrogenous compounds in fruit trees move upwards via the xylem (Tromp and Ovaa, 1976). The major amino acids found in the xylem sap are aspartate and glutamate, their amides and arginine (Tromp and Ovaa, 1967; Titus and Kang, 1982). The prevalence of these amino acids depends on the source of N that is taken up by roots or hydrolysis of stored proteins (Titus and Kang, 1982).

Nitrate is translocated from the roots to the shoots through the xylem and accumulates in the leaves (Titus and Kang, 1982), specifically in the vacuole (Smirnoff and Stewart, 1985). Leaf tissue is considered to be a very important reservoir of nitrogenous compounds. Amino acids present in the leaves undergo transformation during growth and proteins are synthesized. Therefore, N assimilated from roots as well as stored nitrogen becomes part of proteins in the leaves (Raven and Smith, 1976). The major photosynthetic enzyme in leaves is ribulose-1,5-biphosphate (RuBP) carboxylase (RUBISCO) (Kawashima and Wildman, 1970; Stoddart and Thomas, 1982). RUBISCO can comprise more than 90% of the soluble protein that is removed from apple leaves during senescence (Titus and Kang, 1982), and accounted for 32 to 48% of the N then remobilized the next year to support leaf growth, depending on the N supply (Millard and Thomson, 1989). The amount of amino acids in the leaves

decreases and the amount of the proteins increases as growth occurs (Spencer and Titus, 1972).

Particular storage proteins that were present in great amounts during winter and absent during summer were isolated from bark by Wetzel et al. (1989) as well as from ray parenchyma cells by Harms and Sauter (1992). During fall and winter, N is stored in the form of amino acids in many species (Sagisaka, 1993). Tromp (1983) reported that roots of young trees also store N. Arginine (a free amino acid) can be stored in roots of apple (Tromp, 1983), but in sycamore (*Acer pseudoplatanus*) seedlings having a big tap root, N was stored in arginine-rich proteins (Millard and Proe, 1991). Millard et al. (2006) found that, due to N remobilization and dilution of sap, the concentrations of the amino compounds were highest in the xylem sap soon after bud burst in cherry and poplar [*Populus trichocharpa* Torr. & Gray ex Hook var. Hastata (Dode) A. Henry x *Populus balsamifera* L. var. Michauxii (Dode) Farwell]. In cherry, glutamine and asparagine were the most two abundant amino N compounds that were recovered in the xylem sap.

Nitrogen demand during tree growth can be met either by uptake of external sources such as NH_4^+, NO_3^-, and organic N (Gessler et al., 1998) or by remobilization of internal stores (Millard, 1996). Remobilization can provide the majority of N required for growth each year by a range of tree species (Millard and Proe, 1991; Neilsen et al, 1997; Weinbaum and van Kessel, 1998; Dyckmans and Flessa, 2001).

Deciduous trees store N in perennial tissues at the end of the growing season (Titus and Kang, 1982; Millard, 1996). This is an important characteristic because the quantity of reserve N will affect tree growth and fruiting the following season (Titus and Kang, 1982; Roubelakis-Angelakis and Kliewer, 1992). Remobilization of N reserves will support the initial growth of fruit trees in the spring. There is a positive correlation between spring growth and the amount of N reserves in apple (*Malus domestica* Borkh, Cheng et al., 2002), nectarine (*Prunus persica*, Tagliavini et al., 1997), and cherry (*P. avium*, Grassi et al., 2002).

Nitrogen remobilization

N reserves are essential for tree survival during winter and for growth during the spring. Deciduous trees store nutrients, which are remobilized to support early season growth (Titus and Kang, 1982; Weinbaum et al., 1984; Millard and Neilsen, 1989; Dong et al., 2001). They have the ability to remobilize N in the spring using the reserves from the previous year's supply, so that they are not wholly dependent on N supplied the current year by root uptake (Millard, 1996). The remobilization of most N usually occurs before there is significant root uptake (Millard and Neilsen, 1989), although root uptake of N becomes more substantial as the season progresses (Tromp and Ovaa, 1976).

Annual tree growth is correlated with both stored and root supplied N. The relative contributions of these N sources are dependent on soil fertility (Millard,

1996), timing of fertilizer applications (Weinbaum, 1978; Weinbaum et al., 1984; Sanchez et al., 1992) and tree age (Miller and Miller, 1987). For nonbearing prune (*P. domestica*) trees, the N supplied any time in the growing season after initial shoot growth resulted in more N in the shoot than when N was supplied earlier in the season. Weinbaum et al. (1984) also found that for mature almond (*P. dulcis*) trees, the later that N was applied during the growing season, the smaller the quantity of N fertilizer that was recovered in leaves and fruits that year.

It was suggested from studies in peach trees that N uptake occurs after shoot development and water uptake in spring, then decreases during fruit growth and increases again after harvest (Soing and Mandrin, 1993). After primary shoot development has stopped, late summer seems to be a good time to apply N to increase N storage and remobilization the next year (Sanchez et al., 1990, 1991).

Tree age has an effect on N remobilization. There is a difference between immature and mature trees concerning N fertilizer and the amount of N that is supplied to shoots and leaves. Grasmanis and Nicholas (1971) found that in small and immature trees, fertilizer can supply up to 50% of total shoot N. Millard (1996) found up to 82% of leaf N was supplied from fertilizer for immature trees. In contrast, the contribution of N fertilizer to leaf N content in mature trees was much smaller than stored N (Weinbaum et al., 1984; Sanchez et al., 1990, 1992). Weinbaum et al. (1984, 1987) explained this difference by saying that mature trees have a greater pool of stored N compared to immature trees or because fruit sinks compete with shoots for N.

Fate of leaf nitrogen and foliar urea applications during fall senescence

The most efficient internal cycling of N during fall results when maximum N is remobilized from senescing leaves prior to abscission. N reaching the leaves later in the season is more likely to be recycled than N applied earlier because N applied to the soil in the fall may be retained in the roots rather moved to the leaves (Sanchez et al., 1991, 1992). An alternative to soil fertilizer applications in the fall is foliar urea sprays. This has been shown to increase N content in storage tissues (Oland, 1960; Han et al., 1989.; Sanchez et al., 1990).

Foliar urea applications increase the amount of N in the leaves and promote shoot growth (Hill Cottingham and Lloyd Jones, 1975; Klein and Weinbaum, 1984; Swietlik and Faust, 1984; Rosecrance et al., 1998a,b; Tagliavini et al., 1998; Bondada et al., 2001). Urea sprays can be used any time in the growing season; however, autumn applications seem to be the most efficient for deciduous tree fruit crops because large concentrations of urea can be applied with little concern about phytotoxicity (Johnson et al., 2001). Moreover, urea application during fall can increase N reserves and consequently enhance flowering, fruit set and shoot growth the following year (Oland, 1963; Shim et al., 1972; Sanchez et al., 1990; Khemira et al., 1998; Cheng et al., 2002).

Leaves have the ability to readily absorb mineral nutrients; foliar applications have been used extensively as a method of fertilization (Bondada et al., 2001; Johnson et al., 2001). Foliar urea sprays in the fall have shown positive responses in deciduous fruit trees (Titus and Kang, 1982). Urea is the most

common and appropriate form of N for foliar applications because of its rapid absorption, low phytotoxicity and high solubility (Yamada et al., 1965; Bondada et al., 2001). Rosecrance et al. (1998a,b) reported that nectarine and peach leaves absorb urea rapidly, regardless of the application time. Dong et al. (2002) found that apple leaves also absorbed urea rapidly during fall.

Many studies have examined the effect of urea sprays on growth and N status, but few have focused on the parameters needed to predict the results of spraying urea, like N absorption rate relative to leaf area, N assimilation and N translocation (Dong et al., 2002). Compared to N applied in the soil, N use efficiency (N recovered as a percent of the N applied) is higher when N is applied foliarly. Weinbaum (1988) found that N recovery was approximately 60% after the foliar application. In potted apple trees, Hill-Cottingham and Lloyd-Jones (1975) reported that 16% of the soil-applied ^{15}N (potassium nitrate) and 47% of the foliarly-applied ^{15}N (urea) were recovered. Rosecrance et al. (1998a,b) observed 48 to 58% recovery of foliar urea N in peach trees and Tagliavini et al. (1998) reported similar results in young potted nectarine trees. Shim et al. (1972) reported that 70% of applied urea was absorbed from senescing leaves within 8 hours.

Dong et al. (2002) found that after spraying ^{15}N urea in the fall, the leaves quickly absorbed ^{15}N. The rate of ^{15}N absorption was highest during the first two days after the application; the rate decreased by the seventh day and after that ^{15}N absorption was insignificant. Considering the translocation of the N, they found that the amount of amino acids in the leaves increased quickly the first two

days and peaked by the fourth day after the urea application. The concentrations of amino acids also increased in the bark (peaking by the fourth day after application) and in the roots where concentrations increased during the first ten days. Amino acid concentrations were higher in the roots, bark and leaves than in the xylem and shank. During their experiment, ^{15}N export from the leaves occurred at a decreasing rate with time. Three weeks after the urea application, 63.6% of the absorbed ^{15}N was exported from the leaves (Dong et al., 2002).

Urea is hydrolyzed by urease to NH_4^+ and carbon dioxide. Urea N can be translocated as urea in the storage tissues and then urea is metabolized and incorporated into amino acids (Freiberg and Payne, 1957; Titus and Catlin, 1964; Shim et al., 1972, 1973c; Dong et al. 2002). However, some of the urea N can be incorporated first into amino acids and proteins in the leaf tissue and then translocated to the storage tissues as amino acids, resulting in a decline in leaf protein (Dilley and Walker, 1961; Spencer and Titus, 1972; Shim et al., 1972, 1973c; Swietlik and Faust, 1984).

Shim et al. (1973c) found that almost 70% of the initial N in the leaves was transported to shoots, stems and roots during senescence. More N accumulated in the shoot (Marschner et al., 1996) than in the stem; furthermore, both shoot and stem bark tissue accumulated more N than wood tissue. Roots also accumulated a significant portion of N (Shim et al., 1973c; Titus and Kang, 1982). Protein in the leaves can decrease by ~50% during fall senescence (Spencer and Titus, 1972, 1973; Shim et al., 1972). However, amino acids do not accumulate in the leaves while proteins decline, suggesting that they are quickly

translocated into woody tissues (Titus and Kang, 1982). After urea spray applications, the amino acid content is likely to increase in the leaves, bark tissue of shoots and stems, and roots until leaf abscission. The protein content increases in the leaves and shoots during the first days after application and then declines steadily, similarly to trees which do not receive urea treatments. Shim et al. (1973c) reported that the protein content in shoot wood was higher two weeks after the urea application but after that, the protein content was similar to that of untreated trees; shoot bark protein was enhanced three weeks after treatment. The stem bark protein was higher during the first two weeks after treatment and negligible after that; stem wood protein was not affected from the urea applications. Generally, more total N is found in bark than wood tissues (Shim et al., 1973c).

Nitrogen and timing of foliar urea applications

Timing of foliar urea sprays affects in the distribution of N. Hill-Cottingham and Lloyd-Jones (1975) reported that N absorbed after a foliar urea application in June was maintained primarily in the leaves of potted apples, while N absorbed after foliar application in fall was quickly transported to perennial tissues (Titus and Kang, 1982; Rosecrance et al., 1998a,b; Tagliavini et al., 1998). Varying the date of the foliar urea application from September to November did not alter the percentage of N recovered from urea in peach trees, but did affect the partitioning of absorbed N. Less N was recovered in abscised leaves and more in

the perennial parts following foliar urea application in September or October, compared with November (Rosecrance et al., 1998a,b). Dong et al. (2002) reported that about two-thirds of the N absorbed from urea applications to the leaves was delivered to the bark and roots, with little to the xylem.

When Dong et al. (2005) compared three different times (May, July, September) of foliar applied urea on young apple trees, roots absorbed more ^{15}N than leaves during July and leaves absorbed more ^{15}N than roots in the beginning of May and later in September. Leaf N uptake rate was similar in May and July, but higher (60% increase) in September, and root N uptake rate was highest in July and lowest in May. A similar tendency was shown for the recovery of ^{15}N. In May, July and September, 26, 29 and 48% of the foliar N application was recovered by trees. At the same time, 11, 37 and 17% of the soil N application was recovered by trees. More than 70% of ^{15}N absorbed from the urea applied foliarly in May, July and September was translocated to new shoot parts (Dong et al., 2005).

Soil and foliar applications

Fertilizers usually are applied to the soil because plants absorb nutrients and water from the soil through roots (Mengel, 2002). Soil applications are a big source of nutrients for enhancing plant production, but this practice also leads to a great concern about possible environmental contamination from nitrate leaching (Dinnes et al., 2002). In several agricultural regions, nitrate leaching

from soil N applications is a possible source of groundwater contamination (Mackay and Smith, 1990; van der Voet et al., 1996). As discussed before, an alternative solution to soil applications is foliar applications (Khemira et al., 1998), which can lessen the risk of N leaching in many agricultural areas (Bondada et al., 2001). Swietlik and Faust (1984) considered urea as the most appropriate form of foliar N to be sprayed because of its quick absorption, low phytotoxicity, and high solubility in water and oil (Wittwer et al., 1963; Yamada et al., 1965; Knoche et al., 1994). Previous work in fruit trees (Weinbaum, 1988) has shown that foliar applications of N have higher N use efficiency than those for soil. Some newer work has shown that nitrate leaching loss was lower in the foliar application when compared to the soil application (Dong et al., 2004).

It is suggested that foliar applications can be a very good alternative to conventional soil applications (Bondada et al., 2001). Johnson et al. (2001) reported that sufficient quantities of N in buds, shoots and roots can be supplied by foliar applications to peach. However, three to six foliar N applications were needed to supply the same amount of N as one annual soil N application. In red raspberries (*Rubus idaeus* L.), Reickenberg and Pritts (1996) reported that there was important uptake of foliar N and K; nonetheless, the absolute quantity from one foliar application was minor. Consequently, to maintain sufficient N levels in the leaves, several foliar applications were needed. Dong et al. (2004) demonstrated that to preserve tree N levels and productivity, many (seven) sprays of urea were required during the growing season. The cost of urea sprays can be high, but urea can be mixed with other chemicals (Yamada et al., 1965;

Weinbaum, 1988), allowing growers to combine it with other scheduled sprays. The best fertilizer treatment may be a combination of soil and foliar applications (Embleton et al., 1986), which can decrease the amount of N leaching from fertilizer applications and reduce groundwater pollution, while preserving tree productivity and fruit quality.

Quantifying nitrogen remobilization

To quantify N remobilization in the spring, nitrogen budget studies and isotopes have been used. A limitation of budget studies is that they are not precise enough and rarely allow the processes of internal cycling to be quantified immediately (Millard and Proe 1991, Millard, 1995). A different approach to quantify N remobilization is the use of isotopes. Fertilizer ^{15}N and its uptake have been studied by many researchers (Hill-Cottingham and Lloyd-Jones, 1975; Sanchez et al., 1992), but fewer studies have been done with ^{15}N to measure internal cycling (Weinbaum et al., 1984; Sanchez et al., 1991).
Weinbaum et al. (1984) and Sanchez et al. (1991) applied ^{15}N enriched fertilizers to the soil and measured the amount of ^{15}N in the new growth over the next few years. This kind of study, though, may undervalue remobilization because roots will take up native soil N as well as ^{15}N labeled fertilizers (Millard, 1995).

An alternative method is the use of sand culture to allow precise control of the nutrient supply and remobilization. In addition, the uptake by roots can be measured accurately using isotopes (Millard and Neilsen, 1989; Millard and

Thomson, 1989; Millard and Proe, 1991). Studies with ^{15}N have revealed that the current supply of N from the soil does not affect the amount of N remobilized during spring, which depends upon stored N. This has been shown by experiments in which trees are grown with a large or reduced ^{15}N supply for a year and then supplied with N the following year. The recovery of the ^{15}N in the leaves grown the second year was dependent upon the N supply of the first year and not on the current N supply, and that remobilization of N in the spring furnished N for leaf growth before root N uptake occurred (Millard, 1993, 1995). This means that fertilizing trees with N will definitely have a positive effect on tree growth and N storage, but it will have limited or no impact on the effectiveness of N remobilization for spring growth (Millard, 1993). These results also illustrate the common view that fruit trees respond best to pre- or post- harvest fertilizer applications, rather than in spring (Taylor, 1967; Sanchez et al., 1992).

Such studies have allowed researchers to measure N remobilization for leaf growth in the spring in field grown trees. ^{15}N was applied to the soil just before bud burst in sycamore and leaf samples were analyzed to estimate when soil N uptake occurred (Millard, 1994). As a direct estimate of N remobilized, the leaf N content before root uptake of N was used. Millard also suggested that one-third of the leaf N was remobilized from storage.

During remobilization in evergreen trees, N usually is stored in the leaves and transported from old to new leaves (Millard et al., 2001), but in deciduous trees, N is stored in the roots and bark (Millard and Proe, 1991) and then translocated in the xylem. Since remobilization is a source driven process, the

sand culture techniques for quantifying remobilization in deciduous trees is likely to be successful for application in the field, specifically for measuring stored N in trees (Millard et al., 2001); nonetheless, it remains to be verified whether, during remobilization, the N translocation patterns in young trees also are established in mature trees.

Cold hardiness and metabolic changes during fall senescence

Cold hardiness

Plants are exposed to different kinds of environmental stress throughout their life cycle, such as high and low temperatures, drought, wind, flooding, and pollution. Low temperature is one of the most important factors limiting plant growth, distribution and production of fruit crops in many areas in the world (Alberdi and and Corcuera, 1991; Larcher and Bauer, 1981; Palonen and Buszard, 1997). Cold hardiness is defined as the ability of a plant or plant part to survive or resist freezing conditions (Fuchigami, 1996). Improving cold hardiness has been a major objective in physiological and breeding research efforts (Palonen and Buszard, 1997).

Cold hardiness of trees is a complex phenomenon affected by external factors, such as temperature and daylength, as well as internal factors like physiological age of the plant, developmental stage, water content, nutritional status, and genotype (Stushnoff, 1972; Weiser, 1970b). Generally, adult plants

are more resistant than juveniles and seedlings (Levitt, 1980). Even within the same plant, cold hardiness can be substantially different among proximate tissues or even positions in the same tissue or organ (Weiser, 1970a,b; Sakai and Weiser, 1973). Understanding the nature and responses of individual species and their commercially important varieties can be important (Chandler, 1954).

The two main types of low temperature stress are chilling and freezing. At temperatures between -1 and 10 °C, tropical and subtropical plants are exposed to chilling stress and below -1 °C they are exposed to freezing stress. Extremely low temperatures at any time of the year can cause damage to fruit trees. In January 1994, the minimum temperature in Amasa, Michigan was –47 °C (the lowest recorded in the last 100 years) and the maximum temperature on that date was –15 °C (Michigan Agricultural Statistics, 1994). This caused severe damage to fruit trees and the collective economic loss exceeded $22 million (Michigan Agricultural Statistics, 1995). Even when such low temperatures do not occur in the winter, trees may be injured by temperature fluctuations during fall and spring, transitional periods of cold acclimation and deacclimation.

The majority of temperate woody perennials, including sweet cherries, have the ability to tolerate severe winters by changing their cold hardiness level through the year. During dormancy, there are three stages: 1) cold acclimation (CA, hardening), a significant increase in cold hardiness beginning in early fall; 2) mid-winter (MW), the attainment and preservation of the maximum cold

hardiness; 3) deacclimation, (DCA, dehardening), the decrease in cold hardiness in spring (Proebsting, 1970; Wolf and Cook, 1992; Howell, 1994).

Cold acclimation in most woody perennials develops in two stages (van Hyustee et al., 1967; Howell and Weiser, 1970b; Weiser, 1970a,b; Lu and Rieger, 1990). During the first stage, there is an increase in cold hardiness from a few degrees below 0 °C to approximately – 10 to –20 °C. The first stage mainly is triggered by short days (long nights) and other factors like low temperatures under long days or high temperatures under short days (Howell and Weiser, 1970b; Weiser, 1970a,b). When the second stage occurs, cold hardiness increases significantly to a maximum level for each species. The second stage is triggered by low temperatures, usually by the first frost (Weiser, 1970a, b).

As cold acclimation progresses during the coldest season (MW, late December to late February depending on the species and environmental conditions), cold hardiness reaches a maximum, which is the lowest killing temperature for each plant. After MW, cold hardiness decreases as deacclimation occurs. The main factor controlling cold hardiness appears to be air temperature, which affects tissue temperatures (Edgerton, 1954; Proebsting, 1963; Howell and Weiser, 1970a; Bittenbender and Howell, 1975).

The maximum intensity of cold hardiness is not usually crucial for survival, but factors like timing and rate of cold acclimation, the preservation of hardiness, the timing and rate of deacclimation, and ability to re-acclimate all contribute to winter hardiness and survival of plants (Palonen and Buszard, 1997). Researchers usually study only one aspect of cold hardiness, often mid-winter

hardiness, but early and late stages of cold acclimation also are significant. These factors make the evaluation of cold hardiness complex.

The mechanisms of plant injury by freezing temperatures

Chen (1994) stated that freezing injury occurs when ice forms in tissues of actively growing plants, no matter what the temperature is when freezing begins. However, resistant plants can tolerate ice formation to some extent in their tissues after acclimation in the fall (Weiser, 1970a,b). Levitt (1980) classified freezing injury into three types: 1) primary direct injury due to intracellular freezing, 2) secondary, freeze-induced dehydration injury due to extracellular freezing, and 3) injury to other secondary or tertiary freeze-induced stresses.

Levitt (1980) reported that rapid freezing (changes of 5 to 20 °C per hour) can lead to ice formation within cells that ruptures the plasmalemma and kills the cell. The mechanical destruction of the membrane system is the reason that intracellular ice formation causes cell death (Sakai, 1973; Burke et al., 1976; Levitt, 1980). However, when freezing is extremely quick (>100 °C per sec), some cells are able to survive intracellular freezing. At that time, only fine or no crystals are formed (called vitrification, Levitt, 1980). Also, if the process of thawing is extremely fast, icy crystals cannot grow enough to cause injury; this is important for the method of cryopreservation (Levitt, 1980; Chen et al., 1995). At very low temperatures, some non-hardy cells also can survive vitrification

(Weiser, 1970a,b). Therefore, death is caused by ice crystal formation rather than low temperature.

When water in the extracellular spaces freezes first, it produces a lower water potential and leads to slow freezing (<5 °C per hour), something that happens often in the field. As temperatures decrease, the intracellular water moves across the plasma membrane to extracellular ice nucleation points (Levitt, 1980; Sakai, 1982). When this process occurs slowly, the cytoplasm volume decreases gradually and the cell sap dehydrates. The dehydration reduces the freezing point of the intracellular water (Burke et al., 1976) and favors the ability to supercool; supercooling is the avoidance of ice formation at its theoretical freezing point (Ishikawa and Sakai, 1981). When the water potential of the cell water is equal with that of the extracellular ice at a given temperature, then equilibrium is reached.

There are few studies on the impact of freezing duration on plant survival (Levitt, 1980). Once equilibrium is reached, injury appears to be independent of the freezing duration for short periods of time (2 to 24 hr), but for longer periods (1 to 30 d), injury seems to increase with duration (Levitt, 1980). Nonetheless, in chambers with temperatures below zero, cold hardiness increases with time (Howell and Weiser, 1970a).

Thawing can raise the killing temperature (deacclimation) rapidly (15 °C) within 24 hr. Thus, when freezing and thawing is repeated, the temperature fluctuation can be more harmful than a single freezing episode (Howell and Weiser, 1970a). When warm temperature follows a freezing episode, the

deacclimated tissues can be killed more easily; the sunscalding of tree trunks during winter follows this kind of mechanism.

Weiser (1970a,b) stated that freezing injury ($\leq$-30 ^{o}C) is not a continuous process in the stems of woody plants, but is related with an apparent freezing point. To explain how freezing stress causes cell death, two hypotheses were suggested: a second supercooling point (Tumanov and Krasavtzev, 1959) and a vital water exotherm (Weiser, 1970b). In the first hypothesis, a point is reached when the protoplast or the plasma membrane limits the free movement of water out of the cell; this results in intracellular ice formation and eventually the cell dies. In the second hypothesis, during freezing a point is reached when all the water, which is readily available, freezes extracellularly and only the 'vital' water stays in the protoplasm. This vital water moves from the protoplasm and freezes extracellularly when the temperature decreases, finally resulting in death. Extracellular freezing is not lethal, but ice formation can lead to dehydration (drought stress) and possible injury when tissues are exposed to temperatures below a critical point, or if they are frozen for too long at temperatures slightly above the killing point (Chen et al., 1995). Burke and Stushnoff (1979) reported that temperature-dependent structural transitions of cell substances (protein aggregation and membrane phase transition) can result from dehydration.

Mechanisms of plant survival after freezing stress

The two main types of resistance to freezing stress are avoidance and tolerance (Levitt, 1980). Although annuals and some herbaceous plants end the life cycle of the above ground tissues to avoid ice formation, woody perennials can tolerate ice formation in their tissues (Levitt, 1980). Considering the above injury mechanisms, hardy plants can survive freezing stress either by avoiding ice formation (supercooling or deep supercooling) or by tolerating ice formation in their tissues (Levitt, 1980).

Taiz and Zeiger (1998) define supercooling as the process by which a solution remains in the liquid state several degrees below its theoretical freezing point. It can be expressed as either supercooling or deep supercooling. In the case of supercooling, ice formation can be averted until -10 to -15 ^{o}C (Burke et al., 1976). On the other hand, deep supercooling can be as low as –38 to – 40 ^{o}C (Burke et al., 1976; Becwar et al., 1981; Quamme, 1995). The homogeneous nucleation point represents a temperature at which pure water can crystallize automatically while nucleators are absent (Burke et al., 1976). Nucleators are particles that act as initiators for ice to form (Burke et al., 1976).

Supercooling was reported for the first time on cherry flower buds (Tumanov et al., 1969), although Graham (1971) first showed that the killing temperature for azalea buds (*Rhododendron sp.*) was correlated with the tissue supercooling temperature. Since then, supercooling has been studied in buds and stems of various species (Burke et al., 1976; Graham and Mullin, 1976a,b; Quamme, 1978; Proebsting et al., 1980; Andrews and Proebsting, 1987; Quamme et al.,

1982a,b; Brown and Blackburn, 1987; Ketchie and Kammereck, 1987; Quamme, 1991, 1995).

Deep supercooling was first reported in flower primordia, vegetative buds, and xylem ray parenchyma cells in various shrubs and fruit trees (George et al, 1974; Rajashekar and Burke, 1978; Quamme, 1991). Rajashekar et al. (1982) also reported deep supercooling in xylem and bark tissues of pears (various *Pyrus spp*); Pierquet and Stushnoff (1980) found deep supercooling in the phloem, xylem and vegetative buds of grapes (*Vitis* sp.). It also has been found in the xylem of apples and raspberries (*Rubus ideaus* L.), as well as in the xylem and flower buds of plums, sweet cherries, and blueberries (*Vaccinium corymbosum* L.) (Quamme, 1991). The majority of these crops exhibit supercooling in the xylem tissues and many in flower buds. These tissues can bear low temperature to a point, below which the deep supercooled water in the tissue freezes, causing a lethal injury. The freezing of the water releases some heat which causes a low temperature exotherm (LTE), detectable by differential thermal analysis (DTA), one of the methods used in cold hardiness experiments (Quamme, 1991; Palonen and Buszard, 1997).

When a liquid changes a phase to a solid, heat is released and this increase in temperature is known as an exotherm. Tissues that deep supercool demonstrate one high temperature exotherm (HTE) around –5 to –10 °C and many LTEs around –20 to –48 °C. The HTE is not lethal to woody perennials and is a result of ice formation in the apoplast (Graham and Mullin, 1976a; Ishikawa and Sakai, 1981). A single LTE, though, is caused by ice formation within the

primordium of the tissue (bud), which equates with the lethal temperature of that tissue (Andrews and Proebsting, 1987; George et al., 1974; Graham and Mullin, 1976a; Pierquet and Stushnoff, 1980; Ishikawa and Sakai, 1981; Quamme, 1991, 1995). The lowest LTE depends on the species. For instance, peach flower buds exhibit a LTE ranging from –20 to –30 °C (Quamme, 1978, 1991; Quamme et al., 1982b) and apple flower buds from –30 to –40 °C (Quamme et al., 1982b; Ketchie and Kammereck, 1987).

These responses seem to depend on a mechanism involving loss of membrane function during chilling and freezing (Taiz and Zeiger, 2006). It has been shown that loss of solutes to the water in chilling sensitive conch apple (*Passiflora maliformis*) reflects to damage in the plasma membrane and probably to the tonoplast; then, in turn, photosynthesis and respiration are inhibited, reflecting injury to chloroplast and mitochondrial membranes (Taiz and Zeiger, 2006).

Membranes are clearly affected by low temperatures. Plant membranes consist of a lipid bilayer dispersed with proteins and sterols. Lipids influence the activities of the integral membrane proteins (e.g. H^+-ATPases), that regulate ion and solute transportation, as well as enzyme transportation on which metabolism depends. The lipids in the bilayer have saturated fatty acid chains and membranes with this kind of composition tend to solidify into a semi-crystalline state at chilling and freezing temperatures. As the membranes become less liquefied, their proteins are unable to function normally, resulting in inhibition of H^+-ATPase activity, of solute transportation and of enzyme-dependent

metabolism. All these cause significant damage to the photosynthetic machinery (Taiz and Zeiger, 2006).

The plant parts that supercool to survive low temperatures (buds and woody tissues) must lose their excess free water during cold acclimation in fall, so that they avoid fatal intracellular freezing during the subsequent winter (Levitt, 1980). The formation of ice crystals occurs first in the intercellular spaces and in the xylem vessels. During rapid freezing, ice crystals are too small to cause mechanical damage; however, rapid warming of the frozen tissue is required to prevent the growth of the small ice crystals to bigger ones that can cause lethal damage. The water in the cell remains in a liquid form at temperatures several degrees below its theoretical freezing point. Several water molecules are needed for an ice crystal to begin forming, in a process called ice nucleation. Proteins and some polysaccharides facilitate ice crystal formation and they are called ice nucleators. When plants are exposed to freezing temperatures for a long period of time, extracellular ice crystals are being formed resulting in the movement of liquid water from the protoplast to the extracellular ice; therefore, excessive dehydration occurs which leads to cell death (Taiz and Zeiger, 2006). Many specialized proteins can help limit the growth of ice crystals by a noncolligative mechanism, which does not depend on the lowering of the freezing point of water by the presence of solutes. These antifreeze proteins are induced by cold temperatures and bind to the surfaces of the ice crystals to stop or slow more crystal growth (Taiz and Zeiger, 2006).

Research Techniques

Different methods can be used to study and evaluate injury after artificial freezing. The methods used most frequently (Stushnoff, 1972) are: 1) regrowth tests, 2) visual rating of injury, 3) electrical conductivity (EC) of diffused electrolytes, 4) color reaction tests like tetrazolium chloride (TTC), 5) impedance measurement of intact tissues, and 6) exotherm analysis including DTA. A seventh method, chlorophyll fluorescence, also has been reported by Brennan and Jefferies (1990).

The reliability of these methods depends on experimental conditions and the plant species. The methods usually give, to some extent, different lethal temperature estimates (LT_{50}, temperature at which 50% of the samples are dead) (Bittenbender and Howell, 1974); LT_{50} is modified from LD_{50} in toxicology, which is the insecticide dosage that is lethal to 50% of the population (Proebsting and Fogle, 1956). The temperature at which a plant or a tissue is injured beyond recovery is called a killing point. The same tissue or organ of the same cultivar may exhibit different killing points or T_{50} values during different periods of the dormant season. T_{50} is used worldwide as a statistically valid cold hardiness index.

The most frequently used methods for freeze injury estimation are visual rating of injury and EC. The TTC method is not used often (Palonen and Buszard, 1997). Electrical impedance seems promising because it can be used without freezing the plant material (Coleman, 1989; Prive and Zhang, 1996), and

chlorophyll fluorescence provides a screening method for frost hardiness (Brennan and Jefferies, 1990; Jiang et al., 1999, Jiang and Howell, 2002). Stergios and Howell (1973) showed that the tissue browning viability test is suitable for cherries.

Cold hardiness in sweet cherries

The most susceptible tissues (flower buds and xylem) of sweet cherries (as well as all *Prunus* species) avoid freezing injury by deep supercooling. The temperatures that can cause injury are correlated with the average minimum temperatures at the northern limit of their distribution (Quamme, 1974, 1978; Ashworth, 1982; Quamme et al., 1982b). The level of deep supercooling can be a limiting factor in the commercial production of sweet cherries.

Andrews and Proebsting (1987) reported that, in sweet cherries, the ability to deep supercool was related closely to the water content of flower primordia and to a previous minimum air temperature. When sour cherries where exposed to deacclimating conditions, the LTEs of the flower primordia increased (Callan, 1990). Proebsting and Mills (1976) reported that ethephon applied during late summer to mid-fall (late July to late October) effectively increased bud hardiness and delayed bloom in sweet cherry; they concluded that survival of spring frosts was improved and yields were higher in the year following treatment. When rootstocks were tested, Howell and Perry (1990) reported that sweet cherry scion

cultivars are more hardy on either Mazzard or MxM 39 (Mazzard x Mahaleb) rootstock than on 'Colt' (*Prunus avium* x *pseudocerasus*).

Guak et al. (2005) made the only attempt to correlate foliar applications of urea and cold hardiness. They concluded that urea (applied on 25 Sept and 2 Oct) had no effect on cold hardiness of flower buds of sweet cherries in early winter and early spring (assessed by DTA); they also found no effect on shoot hardiness in early winter (evaluated by visual injury).

OBJECTIVES

The main objectives of this study were to:

a) characterize the effect of nitrogen remobilization during fall on N storage reserves.
b) characterize N remobilization during winter and early spring on spur leaf formation.
c) determine the effect of fall foliarly urea applications on N levels in flowering spurs.
d) determine the effect of foliarly applied urea during fall on the development of cold hardiness.

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CHAPTER 2

NITROGEN DISTRIBUTION AND REMOBILIZATION DURING FALL AND SPRING IN SWEET CHERRIES, AND THE EFFECT OF FALL FOLIAR APPLICATIONS OF UREA ON N LEVELS IN SELECTED TISSUES

Introduction

Optimum management of nitrogen (N) is critical to maintain growth and high production in tree fruits (Titus and Kang, 1982). Sweet cherries (*Prunus avium* L.) on dwarfing Gisela (Gi) rootstocks (e.g., Gi5) tend to produce large crops, but small-sized fruit when crop load is not balanced with adequate leaf area (Andersen et al., 1999; Whiting and Lang, 2000). Limited availability of N early in spring, when root uptake is minimal, may exacerbate the development of unbalanced leaf area-to-fruit (LA:F) ratios (Andersen et al., 1999).

Trees remobilize N in the spring by using reserves from the previous year's supply, so that they do not wholly depend on N supplied by current year root uptake (Millard, 1996). Sweet cherries absorb little soil N before bud break (Zavalloni, 2004); so storage N drives initial spring growth. Spur leaves are important for supplying the majority of the photosynthetic carbon for cherry fruit growth (Ayala, 2004). Nitrogen also affects leaf, bud and fruit development. Spur leaf size probably is most affected by storage N rather than soil N (Zavalloni, 2004).

Foliar urea sprays in the fall have had positive effects in deciduous fruit trees (Titus and Kang, 1982). Urea is the most common form of N for foliar applications due to its rapid absorption, low phytotoxicity and high solubility (Yamada et al., 1965; Bondada et al., 2001). Sufficient quantities of N in buds, shoots and roots can be supplied by foliar applications in deciduous trees (Johnson, 2001). Increasing the amount of stored N available for initial spring growth of leaves, buds, and shoots is important for cherries. Sweet cherry flowering usually occurs

before full expansion of leaves; therefore, the early stages of flowering and fruiting, as well as initial vegetative growth, depend on the storage reserves from the previous season (McCammant, 1988; Keller and Loescher, 1989). Other deciduous trees, like apples (*Malus domestica* Borkh.), may be less dependent on storage reserves because a portion of their leaf population may expand before anthesis (promoting earlier N uptake by roots) and/or their fruit development period may be longer (meaning a greater proportion of fruit development occurs during active current season carbon and nitrogen uptake), (Keller and Loescher, 1989). Since sweet cherries bloom early and have a short fruit development period, a better understanding of storage N partitioning may lead to better management strategies for improving fruit quality on high-yielding, vigor-reducing rootstocks such as Gisela 5.

We hypothesized that:

- N remobilization from senescing leaves in fall is a major source of stored N for flowering spurs.
- Storage N levels in flowering spurs increase between fall leaf drop and spring bud swell.
- Premature defoliation in fall decreases storage N levels in flowering spurs, shoot tips, and bark
- Foliar urea applications in fall increase storage N levels in flowering spurs, shoot tips, and bark.
- Storage N levels affect leaf length and fruit size.

Materials and Methods

To better understand the role and extent of N remobilization in the fall and spring with respect to fruiting spur N levels and sweet cherry growth potential, three experiments were conducted at Michigan State University's Clarksville Horticultural Experiment Station (CHES) (latitude = 42.842 N, longitude = 85.242 W).

Experiment 1. Nitrogen remobilization from leaves during fall senescence was characterized in six-year-old 'Sandra Rose'/Gi5 and 'Rainier'/Gi5 sweet cherry trees. Five single tree replications per cultivar were used. These trees received standard cultural practices (i.e., pruning, fertilization, pesticide applications) through the years. In fall 2005, 10 shoot leaves, 10 flowering spur leaves, and 10 flowering spurs with buds were sampled from each tree on 18, 24, and 28 Oct (start of leaf color change), and on 1, 4 (start of leaf drop), and 8 Nov. The samples were placed in zipper-lock bags in a cooler for transport from the farm to the laboratory; immediately upon arrival, they were frozen and stored at -60 °C for later analysis of total N. Leave abscission was completed by 21 Nov.

Experiment 2. The potential to manipulate the levels of storage N in overwintering tissues of 'Hedelfinger'/Gi5 sweet cherry trees was examined. A total of 36 trees were used for this experiment. Five fall treatments were imposed in 2005:

- C = untreated control (8 single tree replications),
- T1 = complete manual defoliation on 11 Oct (4 single tree replications), T2 = a single application (1X) delivered as 5% urea (10 lb/25 gal or 4.5 kg/95 L) on 7 Oct (8 single tree replications),
- T3 = a double application (2X) delivered as 5% urea on 7 Oct + 4% urea (8 lb/25 gal or 3.6 kg/95 L) on 11 Oct (8 single tree replications),
- T4 = a triple application (3X) delivered as 5% urea on 7 Oct + 4% urea on 11 Oct + 3% urea (6 lb/25 gal or 2.7 kg/95 L) on 18 Oct (8 single tree replications).

Foliar urea was applied to T1, T2, T3, and T4 at a rate comparable to 250 gal/acre (2,340 L/ha). A handgun was used to apply urea carefully to drip and to avoid drifting.

One bark disk (2 cm in diameter) from the trunk of the tree, 10 shoot apices (2 cm in length), and 10 flowering spurs per tree were sampled on 21 Nov 2005 (post-leaf drop) and on 31 March 2006 (bud swell). Samples were transported, frozen, and stored as above for later analysis of total N.

From each treatment, all leaves from ten fruiting spurs per tree were sampled randomly on 9 June 2006. The number of leaves per spur, the length (cm) of the largest spur leaf, and total leaf area (cm^2) per spur (measured with LI-300 Area Meter, by LI-COR, Lincoln, Nebraska, USA) were measured. A total of 100 fruit per tree were sampled on 3 July 2006 to determine average fruit weights.

Experiment 3. The natural distribution and remobilization of N in selected sweet cherry canopy tissues (shoot leaves, spur leaves, and spur buds) from fall 2006 to spring 2007 was characterized. Five six-year-old 'Hedelfinger'/Gi5 sweet cherry trees were used. Ten flower spurs were sampled from each tree on 26 Oct, 2 and 9 Nov, and 9 Dec of 2006, as well as on 9 Jan, 9 Feb, 9 Mar, 9 April, and 28 Apr of 2007. Samples were transported, frozen, and stored for later analysis of total N as above.

Total N Analysis. All samples were removed from the freezer and dried for at least three days in a drying oven (Blue M, by Blue Electrical, Blue Island, Illinois, USA) at 60 °C. The dried samples, which were not weighed individually before preparation, were ground with a Wiley mill (40 mm mesh size) and stored in boxes with a dessicant (anhydrous calcium sulfate, size 8 mesh) until further preparation for Kjeldahl analysis (Bradstreet, 1965).

For each sample, 0.15 g of finely ground material was placed into a digestion tube, after which 3.5 ml of concentrated sulfuric acid was added. After gently swirling to mix, one digestion tablet (Kjeltab) was added to each tube. The tubes were placed into a preheated (400 °C) aluminum heating block for digestion for 30 min after clearing (disappearance) of carbon. The tubes were then removed from the heating block and allowed to cool for ~10 min. Deionized (DI) water (10 to 20 ml) was added to the digestion tubes while still warm. A vortex mixer was used to dissolve any crystals present. Each tube was diluted to the 50 ml mark

with DI water and mixed, then the contents were stored in small bottles (23ml) in a refrigerator at 4 °C until further analysis.

Colorimetric determination of ammonium content was made at 660 nm using a QuickChem 8500 (Lachat Instruments, Rapid City, South Dakota, USA). All samples and reagents were brought to room temperature, and the samples were poured into 5 ml plastic sampler cups, rinsing each cup with a small amount of sample digest before filling. DI water was pumped through all the manifold tubing (lines) for 5 to 10 min before placement of all reagent lines. The reagents used are Stock Sodium Hydroxide (NaOH) solution, Stock Sodium Potassium Tartrate solution, Stock Buffer solution 0.5M Sodium Phosphate (NaH_2PO_4), Working Buffer solution, Sulfuric acid/Sodium Chloride (H_2SO_4/NaCl) solution, Sodium Salicylate/Sodium Nitroprusside solution, Sodium Hypochlorite solution, and sampler wash solution. These reagent lines are placed in their respective reagent bottles, with the exception of the sodium salicylate line. When the reagents had been pumping for at least 5 min, the salicylate line was placed in its container and the system was allowed to equilibrate for 10 min before beginning sample analysis (Cope, 1916; Bradstreet, 1965; Bowman et al., 1988; AOAC, 1990). The total N data were expressed on a dry weight (d.w.) basis rather than absolute N content per organ, since individual sample weights were not recorded.

Statistical analysis. The data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Analysis of variance was conducted with proc ANOVA and mean separation by LSMEANS. For the urea and defoliation treatments, each

treatment was paired with the control for statistical significance of total N content and effect on spring leaf and fruit development.

Results

Experiment 1. During fall 2006, N levels in shoot and spur leaves of both 'Sandra Rose' and 'Rainier' sweet cherries decreased (Fig 2-1). There was a concomitant increase in N content in flower spurs from the beginning of Oct until the beginning of Nov (leaf drop). In 'Sandra Rose', there was a tremendous decrease in shoot leaf and spur leaf N concentration (-39 and -49%, respectively) and an important increase (+27%) in N bud content (Fig. 2-1). In 'Rainier', there was also a tremendous decrease in shoot leaf and spur leaf content (-36 and -51%, respectively), though the overall change in bud N content was not significant. Nitrogen content decreased the most in spur leaves.

Experiment 2. During fall 2005 (21 Nov), N content increased in most of the tissues treated with urea (Table 2-1). The N content in bark did not change significantly. Shoot tip N content increased (+20%) at the highest rate of urea application, but the flower spurs (leaf and bud primordia) had the highest concentrations and the biggest relative increase (+40%). During spring 2006, the same tissues were sampled. In every situation, the tissue N content was more than in the fall (Table 2-1). Across all treatments, the relative N content of the bark increased the most (25%) from fall to spring; the increase in the flower spurs and shoot tips was about 17%. The amount of N in the flower spurs was

significantly more (+25%) for the highest urea treatment and was lowest for the defoliated treatment.

There was little difference between the treatments for leaf number per spur, though the higher urea treatments were slightly but significantly higher compared to the control (Table 2-2). However, leaf area per spur and size of the largest leaf per spur was influenced to a greater extent by treatment. The greatest leaf area per spur occurred in T3 and T4, the highest rates of urea application. When largest leaf per spur sizes were compared, there was only a statistical difference between C and T3 or T4. Fruit size variability was high between the treatments, resulting in no statistically significant differences.

Experiment 3. Flower spur N concentration did not change between leaf drop and bud swell (i.e., during the winter period) (Fig. 2-2). As budswell and development began in April, N content increased dramatically (up to 150%) compared to N content during the winter.

Discussion

In the 2005 study, total N in leaves of 'Sandra Rose' and 'Rainier' sweet cherry trees decreased during senescence while N in flower spurs increased. This translocation of N from leaves to storage tissues like flowering spurs before leaf abscission is an important source of N for spring growth, just as if a nitrogen fertilizer were applied directly to flower spurs (as well as other tissues to which N may be remobilized for storage rather than immediate growth). This natural N

enrichment of flower spurs in the fall suggested that orchard fertilization strategies to preferentially increase leaf N during senescence may increase storage N levels in such tissues without stimulating new episodic canopy growth activity.

Foliar urea applications in deciduous fruit trees during fall have been used extensively to increase the amount of N in storage tissues (Johnson et al., 2001), though little has been documented in sweet cherry. Some researchers have stated that foliar urea applications to stone fruits are ineffective (Peryea and Willemsen, 2000). In our second fall 2005 study, urea applications increased total N levels at the beginning of dormancy by up to 40% in flower buds, 20% in the shoot tips, and 13% in the bark (Table 2-1). This indicates that flower buds and shoot tips are sinks for remobilized N taken up from foliar urea sprays in sweet cherries. Bark tissue showed a big increase in total N content between fall and spring for all treatments. Total N concentration in the shoot tips also increased between fall and spring, although the smallest impact occurred with the higher rates of urea. Flower spur N concentration increased between fall and spring, but rather variably across treatments. The treatment with the lowest N values, the defoliated trees, increased from fall to spring but the levels were still much lower than for any of the other treatments, indicating a long-lasting negative effect that persisted at least until budswell. The urea concentrations used in this study (3 to 5%), caused slight marginal leaf browning, presumably due to pooling of spray at the leaf margin where it became more concentrated during drying.

Dong et al. (2002) found that N from urea was converted to amino acids in leaves after foliar application in the fall in apples (*Malus domestica*), with roots and bark being the main sinks for N. They noted that only 35% of the N applied as urea was absorbed by leaves and 63.6% of absorbed N was translocated out of leaves. Tagliavini et al. (1997) found that pear (*Pyrus communis* L.) leaves absorbed 58 to 69% of the N applied as urea and concluded that more N derived from root uptake was used for remobilization in the spring than from foliar N. Rosecrance et al. (1998a,b) found that 48 to 58% of foliar-applied urea was recovered in peach leaves and perennial organs, similar to results of Tagliavini et al. (1997). When applied in September and October, most of the urea N (38%) was recovered in roots and when applied on November (prior to leaf senescence in California), 45% remained in the current year wood. In our studies, we applied urea mainly in mid-October, just prior to leaf senescence, since export and translocation of the foliar-applied urea N likely would have been reduced at the final stages of leaf senescence in early November.

This experiment showed that total N concentration in flower spurs can affect sweet cherry spur leaf development (Table 2-2). Lower N concentration can lead to smaller leaves and thus be a negative factor in attempts to achieve balanced leaf-area-to-fruit ratios suitable for promoting good fruit size. Trees with the highest N levels developed larger spur leaves in the spring and leaf area per spur increased with foliar urea application. The number of leaves per spur was not affected significantly. Fruit size was smallest on the defoliated trees, but crop

loads were highly variable, resulting in statistically insignificant fruit size data across the urea treatment trees.

These results do not imply that foliar applications of urea may provide adequate N for all phases of sweet cherry growth. When Johnson et al. (2001) applied only foliar urea to peach and no soil N across a full season, they found a significant reduction in weight per fruit and a tendency toward reduced tree yields compared to the soil applications. However, a combination of foliar urea in October and soil fertilization in summer maintained productivity and fruit weight compared to treatments with N applied only via the soil. Embleton et al. (1986) also suggested that the best practice in citrus (*Citrus limon*) may be a combination of foliar and soil applications of N. From an environmental perspective, Dong et al. (2004) reported that multiple foliar N applications can reduce the risk of nitrate leaching in apple orchards, without reducing yield on fruit quality. Foliar fertilization can improve N use efficiency relative to soil applied N, and therefore can lead to reduced leaching of nitrates in the groundwater (Embleton et al., 1986).

Our previous experiments had shown an increase in flower spur N concentration between leaf drop and just before bloom, but whether N was actively remobilized throughout the winter period or just before budbreak was not known. When sampled on a monthly basis during this period, it was clear that little remobilization occurred from December to the end of March. Soon thereafter, the N concentration showed a tremendous increase (150%) in April, during budswell and budbreak. This also indicated that N is required in high

amounts during the early stages of spring growth in sweet cherries (Fig. 2-2), and that N remobilized from other storage sites within the tree is in high demand by reproductive meristems like flower spurs for both spur leaf development and bloom.

Table 2-1. Fall (2005) foliar treatment effects on tissue nitrogen levels after leaf drop (dormant, 21 Nov 2005) and prior to budbreak (bud swell, 31 March 2006) in 'Hedelfinger' sweet cherries. C: Control, T1: Defoliated on 11 Oct, T2: 5% urea on 7 Oct, T3: 5% urea on 7 Oct + 4% urea on 11 Oct, T4: 5% urea on 7 Oct + 4% urea on 11 Oct + 3% urea on 18 Oct.

Treatment		Tissue					
		Flower spurs		Shoot tips		Bark	
		%N	% change from C	%N	% change from C	%N	% change from C
21 Nov 2005	C	1.61	-	1.62	-	0.97	-
	T1	1.15 *	(-29%)	1.29 *	(-20%)	0.74 n.s.	(-24%)
	T2	2.19 *	(+36%)	1.73 n.s.	(+7%)	1.07 n.s.	(+10%)
	T3	2.06 n.s.	(+28%)	1.93 n.s.	(+19%)	1.03 n.s.	(+6%)
	T4	2.25 *	(+40%)	1.95 *	(+20%)	1.10 n.s.	(+13%)
31 Mar 2006	C	2.19	-	2.20	-	1.17	-
	T1	1.52 *	(-30%)	1.64 *	(-25%)	0.92 *	(-21%)
	T2	2.28 n.s.	(+4%)	2.05 n.s.	(-7%)	1.31 n.s.	(+12%)
	T3	2.10 n.s.	(-4%)	2.05 n.s.	(-7%)	1.23 n.s.	(+5%)
	T4	2.74 *	(+25%)	2.08 n.s.	(-5%)	1.51 n.s.	(+29%)

Treatment values are significantly (*) or not significantly (n.s.) different from the control at a=0.05.

+/- % represents difference from C

Table 2-2. Fall foliar treatment effects on subsequent season leaf (9 June 2006) and fruit (3 July 2006) parameters in 'Hedelfinger' sweet cherries. C: Control, T1: Defoliated on 11 Oct, T2: 5% urea on 7 Oct, T3: 5% urea on 7 Oct + 4% urea on 11 Oct, T4: 5% urea on 7 Oct + 4% urea on 11 Oct + 3% urea on 18 Oct.

Treatments	Leaves/spur (no.)	Leaf area/ spur (cm^2)	Area of largest leaf (cm^2)	Fruit size (g)
C- Control	6.3	147	39	9.3
T1- Defoliated	6.3 n.s.	153 n.s.	39 n.s.	8.2 n.s.
T2- 5% urea	6.5 n.s.	165 *	42 n.s.	9.0 n.s.
T3- 5%+4% urea	6.7 *	183 *	44 *	8.7 n.s.
T4- 5%+4%+3% urea	6.6 *	183 *	45 *	8.9 n.s.

Treatment values are significantly (*) or not significantly (n.s.) different from the control at a=0.05.

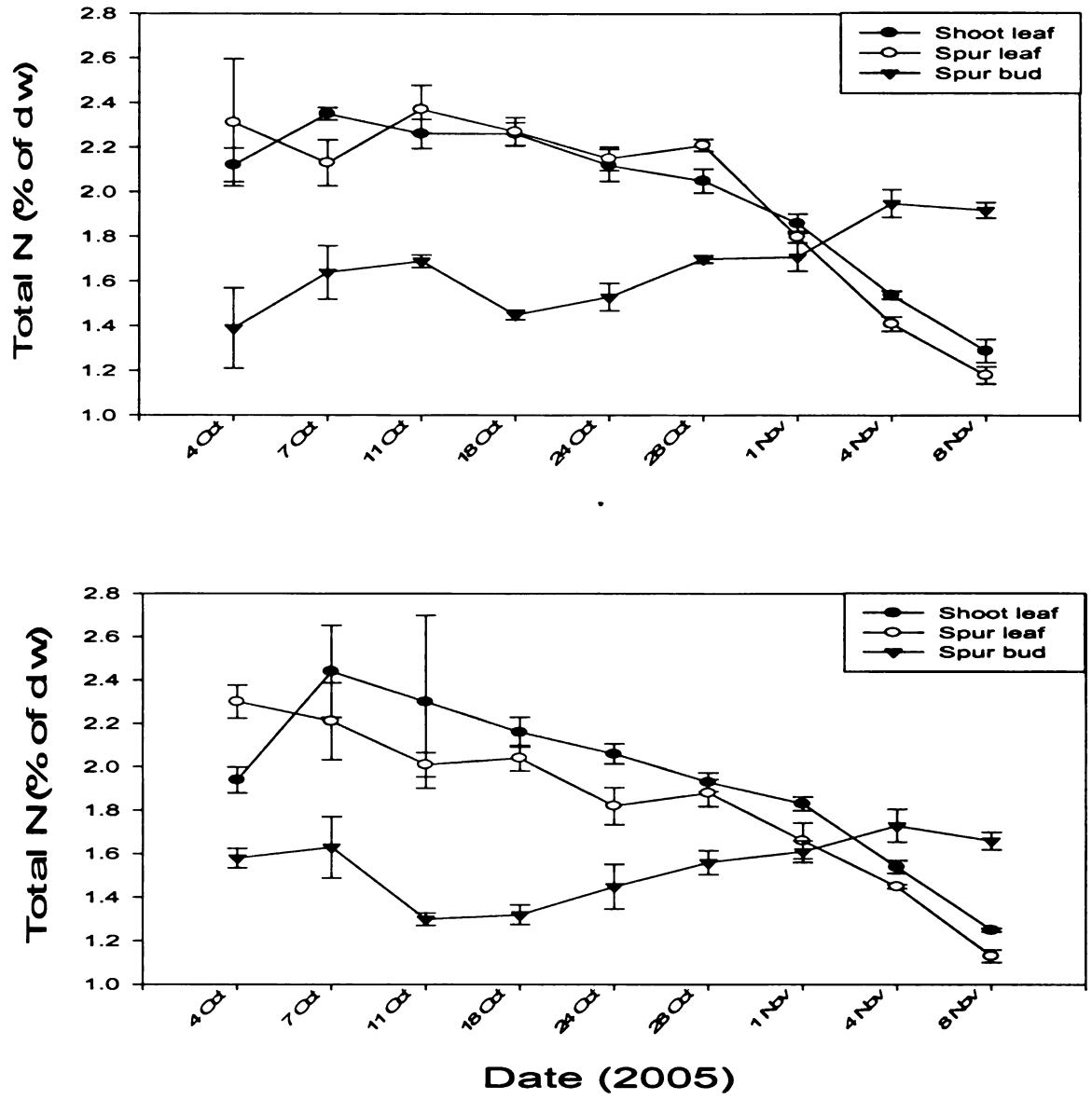

Figure 2-1. Changes in total nitrogen content (dry wt. basis) in 'Sandra Rose' (A) and 'Rainier' (B) sweet cherry shoot leaves, spur leaves, and flower spurs during fall 2006. Data are means of five replications. Error bars are standard errors from the mean data.

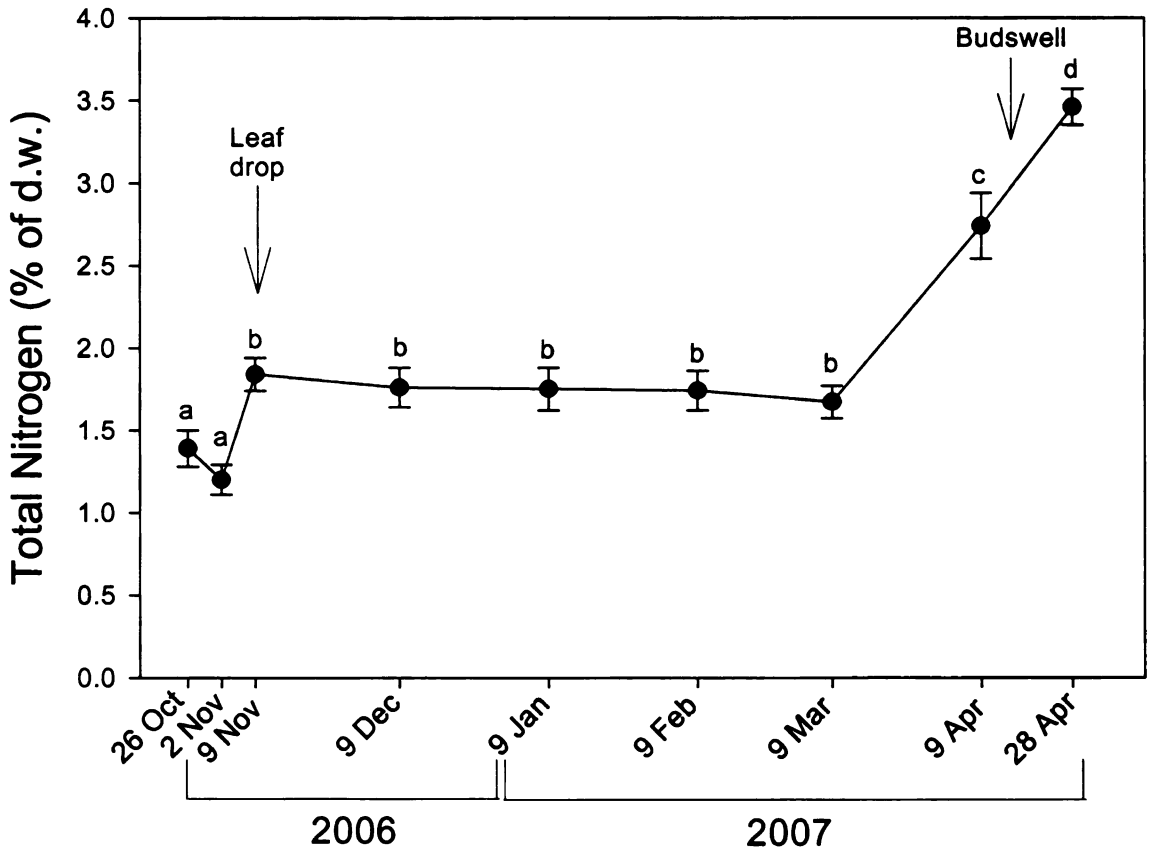

Figure 2-2. Flowering spur N levels in 'Hedelfinger' sweet cherries from fall 2006 to spring 2007. Data presented are the averages of five replications. Error bars are standard errors from the mean data. The same letter following values indicates values that are not significantly different.

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CHAPTER 3

THE EFFECT OF FALL-APPLIED UREA SPRAYS ON COLD ACCLIMATION OF SWEET CHERRY

Introduction

Trees are exposed to cold and freezing temperatures throughout their life cycles. To certain degrees, woody perennials can tolerate low temperature damage in their tissues (Levitt, 1980), which is termed cold hardiness. Cold-hardy plants can survive cold temperatures by avoiding ice formation (supercooling or deep supercooling) or tolerating ice formation in their tissues.

Little is know about the physiological relationship between nutrients and cold tolerance mechanisms, though high levels of nutrient fertility, particularly soil–applied nitrogen (N) in mid-to-late summer, can stimulate prolonged growth that is susceptible to damage by low temperatures in fall or winter. The use of foliar applied nutrients has been limited for alteration of tree responses to stress. Some foliar-applied nutrients can help promote acclimation and stress resistance, or enhance repair mechanisms after damage (Raese, 1996; Stover et al., 1999). In sweet cherry, urea has been used experimentally to increase nitrogen reserves in the fall (Chapter 2); the effect of such treatments on susceptibility to freezing injury is unknown. To examine the effect of fall foliar urea treatments on cold acclimation, a controlled freeze/tissue browning viability test has been reported to be suitable for cherries (Stergios and Howell, 1973).

Therefore, we hypothesized that:

- fall foliar urea applications fall affect shoot cold hardiness.
- apical and basal parts of the shoot differ in cold hardiness development (cold acclimation).
- foliar damage by Japanese beetles affect shoot cold hardiness.

Materials and Methods

The study was conducted at Michigan State University's Clarksville Horticultural Experiment Station (CHES) (latitude = 42.842 N, longitude = -85.242 W) in fall 2007. Thirty (30) 'Ulster'/Gisela 6 (Gi6) sweet cherry trees (9 years old) were selected randomly. The trees received standard cultural practices over the years (i.e., pruning, fertilization, pesticide applications). Six were used as controls (no treatment); the remaining 24 received foliar applications of 3.5% urea [3.2 kg in 102 L of water (7 lbs in 25 gal)] at various times (six trees per regime) during the fall (Table 3-1). A handgun was used to apply urea carefully to drip and to avoid drifting.

Cold hardiness sampling. For each treatment, shoots were sampled for cold hardiness evaluation at 1 and 3 weeks after the second urea application plus after leaf drop in late fall. On each sampling date, 24 current year shoots were collected, 12 were for the control and 12 for the urea treatment to be compared. Four 50 cm shoots of the current year's growth were sampled per treatment, two each from the west and east sides of each tree. The west side (-JB) had good insecticide spray coverage for control of Japanese Beetle, whereas the east side (+JB) had poor spray coverage and increased foliar damage due to beetle feeding. Harvested shoots were placed in plastic bags, and kept in the covered bed of a pick-up truck for transportation between the experimental station and the campus laboratory. They were stored overnight in a 4 °C cold room until set-up for controlled freezing tests the next day. Air temperatures at the research site during the sampling period were acquired from the Michigan Automated Weather

Station (http://www.agweather.geo.msu.edu/mawn/). Treatment and sampling dates for all treatments are shown in Table 3-1.

Nitrogen sampling. During fall, ten flowering spurs from two-year-old shoots were harvested from each tree, transported and stored for later analysis of total nitrogen (N) as described previously (Chapter 2). Control samples were harvested on 31 Aug, 12 and 28 Sep, 12 Oct, and 12 Dec; urea treatment samples were harvested just before the second application for each treatment (T1 on 8 Sep, T2 on 21 Sep, T3 on 6 Oct, and T4 on 20 Oct), as well as on 12 Dec (fully dormant, after leaf drop).

Cold hardiness analysis. The shoot samples were prepared based on the procedure described by Howell and Weiser (1970) and McKenzie and Weiser (1975). Shoots were cut into three sections (apical, middle and basal). The apical and basal sections also were cut into two pieces. One piece per treatment was positioned vertically on masking tape (sticky side up) at 4 cm intervals. A total of four shoot pieces per replication were positioned on the masking tape, two representing +JB and two representing –JB. Another length of tape on top secured the twigs. Four replications were done for each target temperature and a control. Each strip was then centered on top of moistened gauze over aluminum foil strips, with the basal end of each shoot piece at the lengthwise fold. A copper-constantan thermocouple was inserted into the apical-end pith of a central twig from each strip and secured by tape to the foil. The lower half of the

foil was folded up to pocket the strips inside. The whole unit was then rolled into a loose bundle.

The freezer temperature was allowed to equilibrate at 0 °C for one hour followed by an incremental temperature drop of 4 °C/hr for the samples before 12 Dec and 2 °C/hr for the samples from 12 Dec. For the shoots sampled before 12 Dec, the freezer temperatures used were -3, -7, -11, and -15 °C; for the last sampling on 12 Dec, the temperatures used were -11, -13, -15, -17, -19, and -21 °C. When the target temperature was reached, four bundles (replications) were removed from the freezer and placed in a 4 °C storage room to slowly thaw overnight. The following day, bundles were removed from the 4 °C storage room, unrolled, and placed in a 25 °C room temperature humidity chamber. Damage was determined 4 to 7 days later by slicing the wood with a razor blade and rating the phloem/cambial/xylem tissue for visual browning. Shoot pieces with less than 50% browning were considered alive and those with more than 50% browning were considered dead.

The temperature causing 50% damage (LT_{50}) was calculated by the Spearman-Karber formula (Bittenbender and Howell, 1974):

$$LT_{50} = T_L - \tfrac{1}{2} d + [d (\Sigma i) / n]$$

where T_L = Lethal temperature or temperature below which no twigs are alive

d = Temperature intervals

Σi = Number of dead twigs

n = Number of replications

Statistical analysis. The data were analyzed using SAS software (SAS Institute Inc., Cary, NC). Analysis of variance was conducted with proc ANOVA and mean separation by LSMEANS.

Results

Cold acclimation. In fall 2007, cold acclimation of untreated 'Ulster' sweet cherry shoots increased from a moderately hardy condition in early September to about an 8°C more hardy condition by early December (Fig. 3-1). The increase in cold hardiness was actually lost in early October, during a period of unusually warm temperatures when daily highs were near or exceeded 30°C (Fig. 3-4); it took 3 to 4 weeks to return to a stage of cold hardiness similar to that of mid-September.

Each foliar urea treatment was compared with the untreated control at about one week and 3.5 weeks following the second application for each treatment series (Fig. 3-1). In every treatment comparison date except the first comparison for T2, the urea-treated shoots were significantly more cold-hardy, with LT_{50} values ranging from 1.0 to 4.25°C lower. The first T2 sampling occurred during a period of warmer temperatures in which highs again were near or exceeded 30°C (Fig. 3-4), and the difference between the control and the treatment was only about 1°C.

Canopy orientation (west and east side of the tree) was examined. The west side of the trees received direct pesticide sprays and adequate control of

Japanese beetles (*Popillia japonica*) while the east side received only indirect sprays and thus suffered significant leaf damage/defoliation and complete premature defoliation by early October. However, there was no statistical difference (a=0.05) between cold hardiness of twigs from the east and west side of the trees (Fig. 3-2).

Likewise, the hardiness of the apical and basal parts of the current year's shoot growth were compared to determine whether sampling position influenced cold acclimation values. For three of the five treatments (T1, T3, and T4), there were no significant differences in the level of cold hardiness for a=0.05 (Fig. 3-3). For the controls, and for T2, the basal shoot segments were significantly more hardy, by about two °C, than the apical segment.

By early December, trees had defoliated completely and were fully endodormant. On 12 Dec, the LT_{50} values for all of the urea treatments were significantly lower (about 4 °C) than the untreated controls (Fig. 3-5). There were no significant differences between the urea treatments.

Nitrogen status. For each urea treatment series, flower spur total N concentrations about 8 days after the first application were significantly higher than those in control shoots sampled before or after the urea treatment samples (Fig. 3-6). When fully dormant (12 Dec sampling), total N concentrations were still significantly higher for the T1 and T2 urea treatments (Table 3-2), although T3 and T4 were not different from the control. Foliar damage by Japanese beetles affected total N concentrations in flower spurs for samples taken during

the first three weeks of September (C, T1, and T2), with lower N concentrations in spurs on beetle-damaged shoots for each case (Table 3-3). However, from late September onwards, there were no significant differences in total N content for the controls or the later urea treatments (T3 and T4).

Discussion

Andrews and Proebsting (1987) found a significant correlation in fall and winter between the previous night's minimum air temperature and LT_{50} values for sweet cherry flower buds. The shoot acclimation trends in our experiment likewise were influenced by periods of warm temperatures that interrupted, and briefly reversed, acclimation.

Our results indicate that foliar urea applications in late summer through mid-fall increase reserve N in sweet cherry flower spurs. These results are in agreement with those of Guak et al. (2005) who applied foliar urea to sweet cherries in early fall. They measured N concentration on 5 Oct after the foliar urea applications (0, 2, 4, and 6%) on 25 Sep and 2 Oct. The found that the 6% treatment was the most effective and increased the N concentration in flower buds by 24%. The 4% treatment was also effective, but N concentration in flower buds increased by 12% and the 2% treatment increased N concentration by 9%. In the cold hardiness portion of our study, T3 (sprayed on 28 Sep and 6 Oct) twigs had a lower early winter LT_{50} (~3.2 °C) than the controls. Guak et al. (2005) found no effect on cold hardiness from repeat treatments in mid-fall (on 25 Sept and 2 Oct). We used 3.5% urea and 9-year-old 'Ulster' sweet cherries on clonal

Gisela 6 rootstock, while Guak et al. (2005) used 2, 4 or 6% urea and 4-year-old 'Lapins' trees on seedling Mazzard rootstock. Different tree ages, varieties, rootstocks, locations, and/or amount of urea may be responsible for the different impacts on cold hardiness.

The shoots of sweet cherries supercool and deep supercool, characteristic of dormant *Prunus* species (Burke and Stushnoff, 1979). The difference between the LT_{50} values of the treatments and the controls was greater in early fall, indicating that as fall progressed and lower temperatures were experienced, the untreated trees acclimated better than at more moderate temperatures. The urea treatments generally had a positive effect on cold resistance suggesting that perhaps higher storage N levels facilitate more rapid acclimation at moderate temperatures.

Surprisingly, there was no statistical difference in cold hardiness between the shoots having minor and severe foliar damage by Japanese beetles. Similarly, there were few differences in cold hardiness between apical and basal shoot segments. Similar studies in peaches (J.Flore, personal communication), showed that basal portion of the shoots were more hardy than apical parts. A similar result also was found in grape (*Vitis labruscana*) by Jiang (2001).

The total N concentration of the flower spurs increased more through the fall for the urea treatments than for the controls. Interestingly, in Dec 2007, the flower spur N concentrations from the October urea applications (T3 and T4) were the same as the controls, suggesting that the late-applied urea N was more prone to translocation away from the spurs than when urea was applied earlier in fall.

Our results support the idea that urea N can improve cold hardiness of sweet cherries during fall. In early winter, the LT_{50}s of the shoots on trees treated with urea were as much as 4 ^{o}C more hardy than the untreated trees.

Table 3-1. Treatment and sampling dates of foliar urea treatments applied to 'Ulster' sweet cherry trees during fall 2007.

Treatments	Treatment dates	Cold hardiness sample dates (current season shoot growth)	Tissue nitrogen sample dates (flower spurs)
C - Control	No urea	12 and 25 Sep; 2,9,17, 24, and 30 Oct; 13 Nov; 12 Dec	30 Aug, 12 and 28 Sep; 12 Oct; and 12 Dec
T1	31 Aug and 8 Sep	12 Sep, 2 Oct, and 12 Dec	8 Sep and 12 Dec
T2	14 Sep and 21 Sept	25 Sep, 17 Oct, and 12 Dec	21 Sep and 12 Dec
T3	28 Sep and 6 Oct	9 and 30 Oct, and 12 Dec	6 Oct and 12 Dec
T4	12 Oct and 20 Oct	24 Oct, 13 Nov, and 12 Dec	20 Oct and 12 Dec

Table 3-2. Total nitrogen levels in dormant 'Ulster' sweet cherry flower spurs after leaf drop (12 Dec 2007) following fall applications of urea (3.5%) on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4).

Treatments	Total N (%)
Control	1.7 a[z]
T1	2.2 b
T2	2.1 b
T3	1.7 a
T4	1.6 a

[z] The same letter following values indicates that respective values are not significantly different within the column.

Table 3-3. Total nitrogen levels in 'Ulster' sweet cherry flower spurs after the first fall (2007) application of 3.5% urea in each treatment series, separated into samples from portions of the canopy with significant (+) and minimal (-) damage by Japanese beetles. Foliar urea (3.5%) was applied on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4).

Treatment and application date prior to sampling	Sampling date	Total N (%) -JB	Total N (%) +JB
Control	30 Aug	0.60 a[z]	0.60 a
Control	12 Sep	0.75 a	0.53 a
Control	28 Sep	1.00 a	0.94 a
Control	12 Oct	1.48 a	1.43 a
T1 – 31 Aug	8 Sep	1.47 a	1.15 a
T2 – 14 Sep	21 Sep	1.31 a	1.18 a
T3 – 28 Sep	6 Oct	1.57 a	1.63 a
T4 – 12 Oct	20 Oct	1.78 a	1.72 a

[z] The same letter following values indicates that respective values are not significantly different within a single row. The comparisons made are between + and – values at each date.

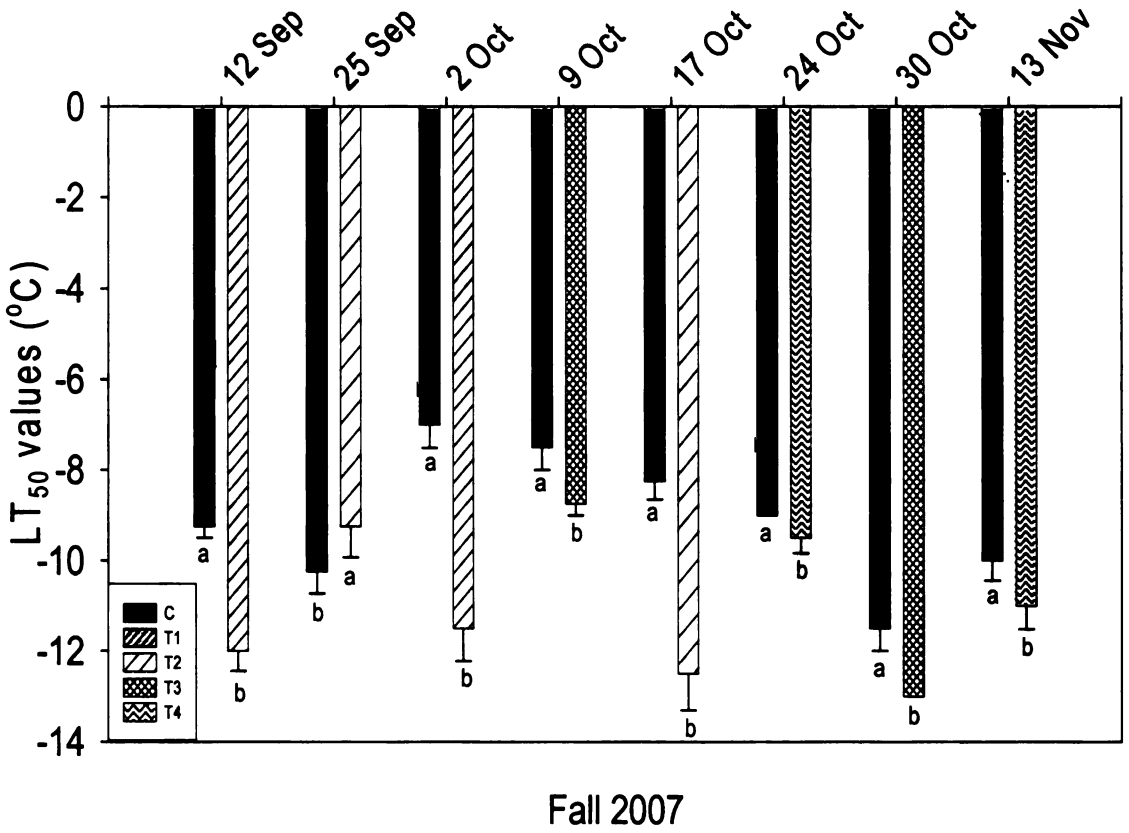

Figure 3-1. Effect of fall foliar urea applications on LT_{50} values for 'Ulster' sweet cherry shoots during cold acclimation in fall 2007. Foliar urea (3.5%) was applied on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4); C represents the untreated control. Error bars are standard errors from the original mean data. The same letter following paired values (control and treatment) for each sampling time indicates that they are not significantly different.

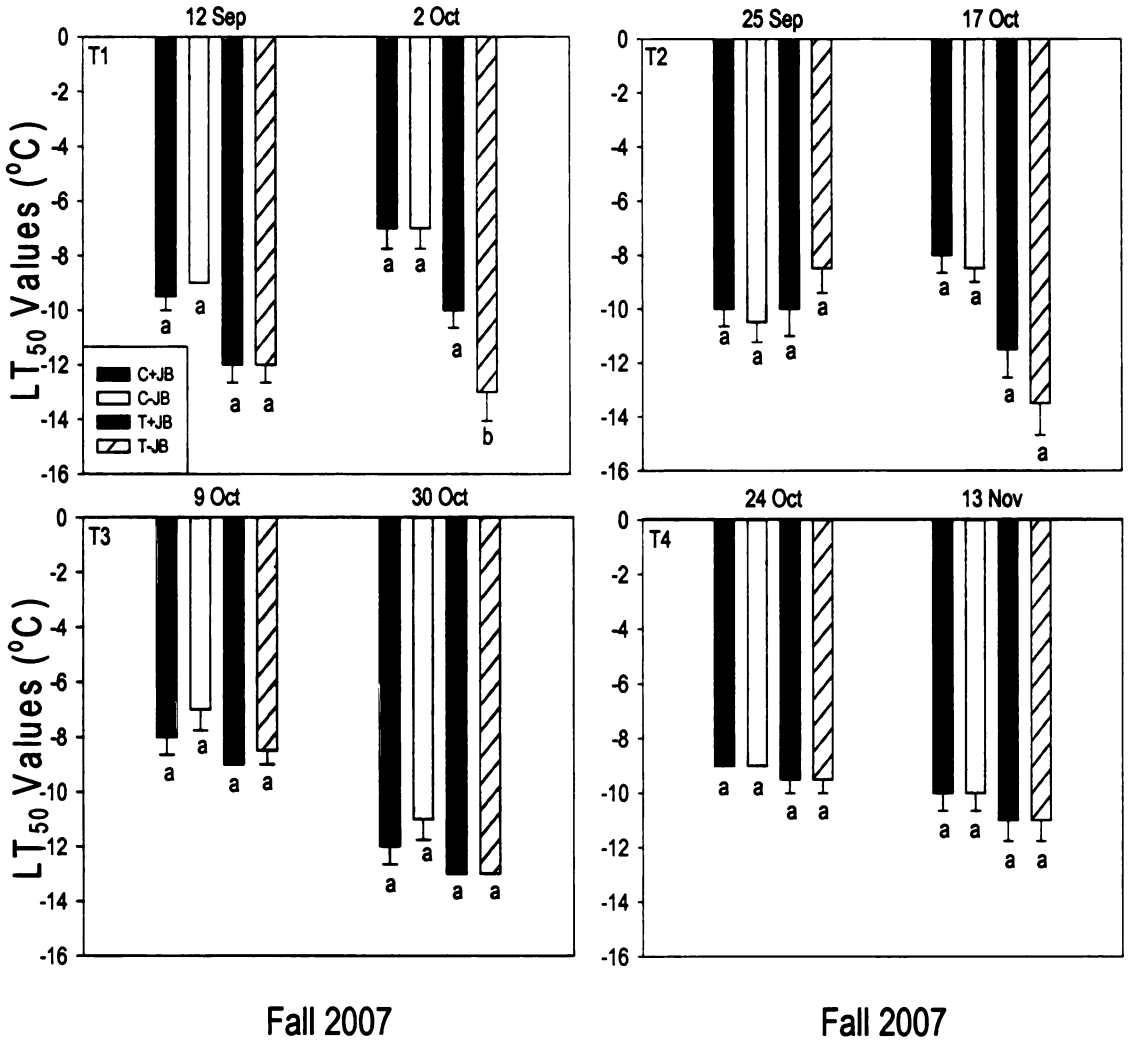

Figure 3-2. Average LT_{50} values for treated (T) and control (C) 'Ulster' sweet cherry shoots, separated into east (significant damage by Japanese beetles, +JB) and west (minimal damage, -JB) sides of the tree canopy. Averages represent all sampling dates except post-leaf drop (12 Dec). Foliar urea (3.5%) was applied on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4). The same letter following paired values (control and treatment) for each sampling time indicates that they are not significantly different.

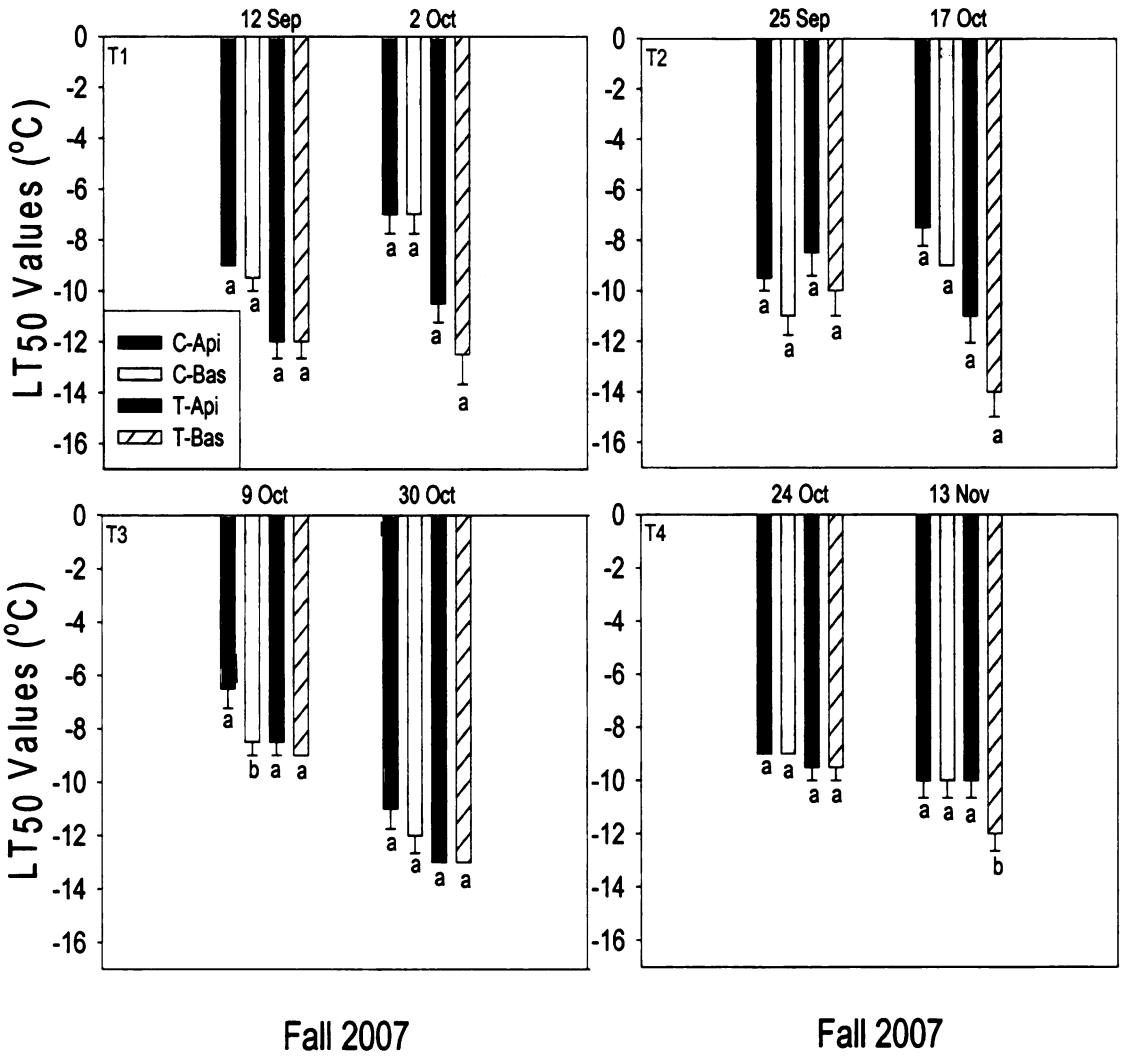

Figure 3-3. Average LT_{50} values for treated (T) and control (C) 'Ulster' sweet cherry shoots, separated into apical (Api) and basal (Bas) shoot segments. Averages represent all sampling dates except post-leaf drop (12 Dec). Foliar urea (3.5%) was applied on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4). The same letter following paired values (control and treatment) for each sampling time indicates that they are not significantly different.

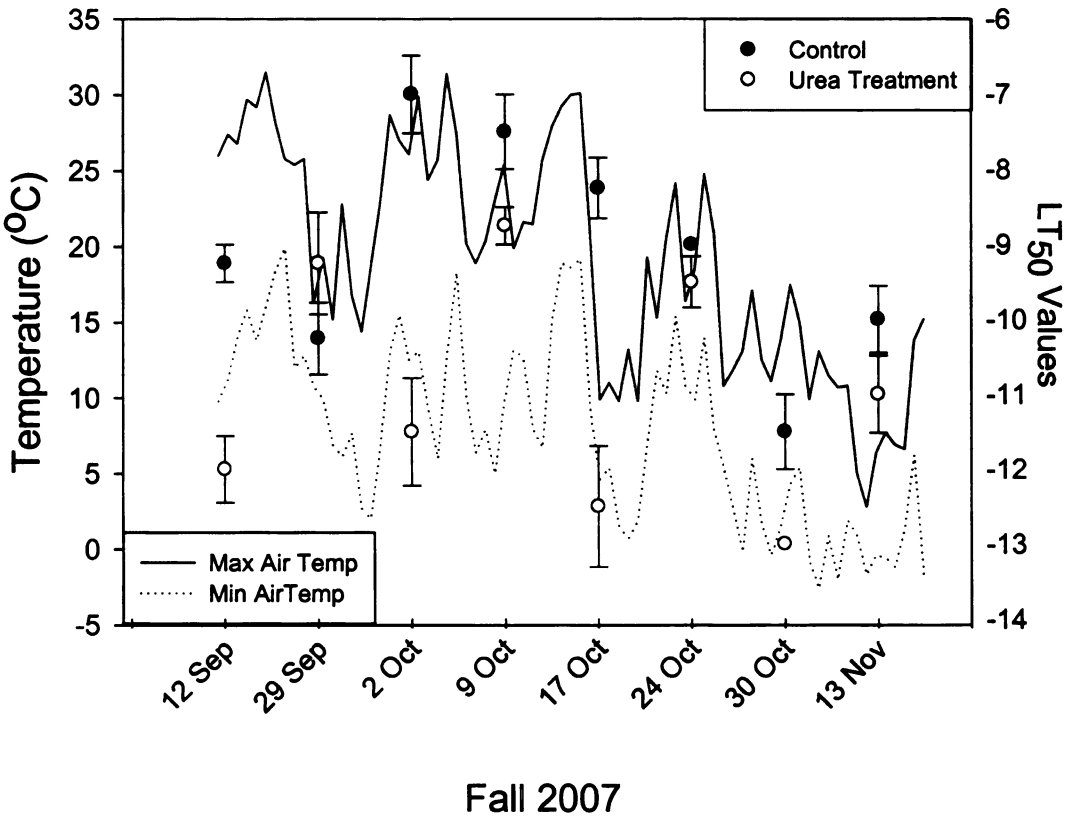

Figure 3-4. Changes in air temperature and LT_{50} values for control and urea-treated 'Ulster' sweet cherry shoots during cold acclimation in fall 2007. Error bars are standard errors from the original mean data. T1 was sampled on 12 and 25 Sep, T2 on 25 Sep and 17 Oct, T3 on 9 and 30 Oct, and T4 on 24 Oct and 13 Nov.

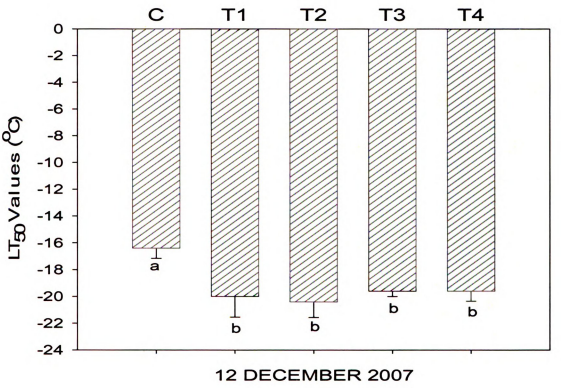

Figure 3-5. Effect of fall foliar urea treatments on LT_{50} values for 'Ulster' sweet cherry shoots on 12 Dec 2007. Foliar urea (3.5%) was applied on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4). Error bars are standard errors from the original mean data. Different letters indicate significant difference between the treatments.

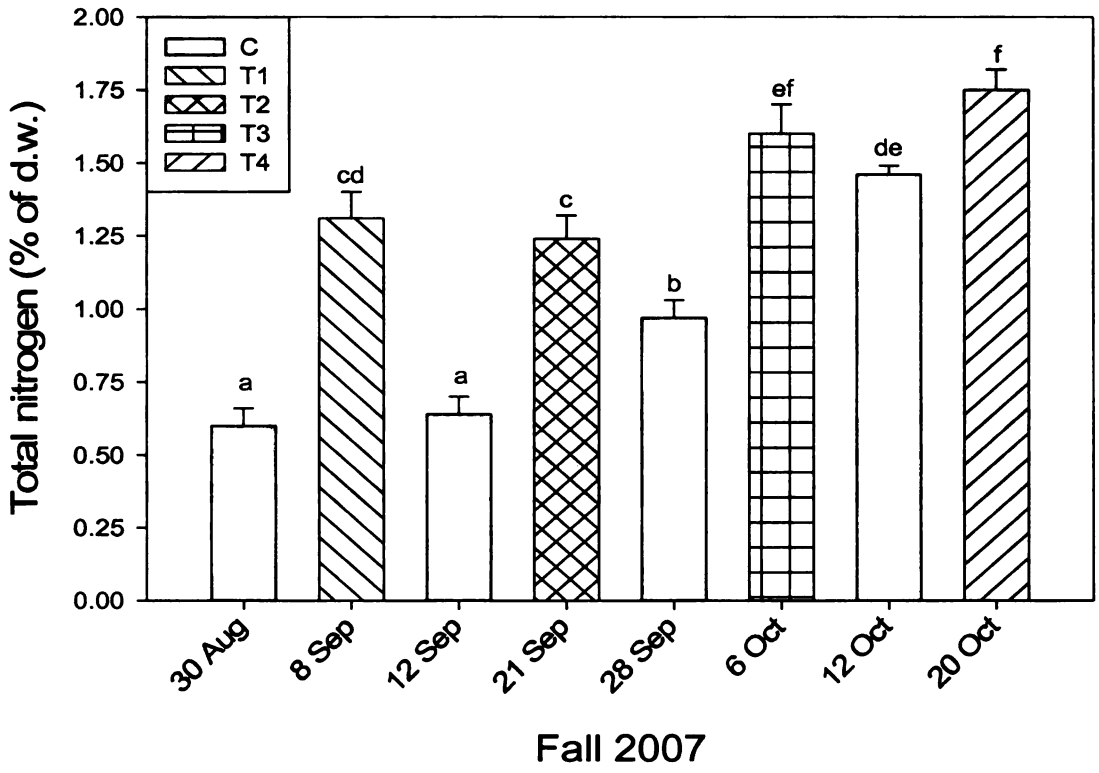

Figure 3-6. Effect of fall foliar urea treatments on total nitrogen content in 'Ulster' sweet cherry fruiting spurs. Foliar urea (3.5%) was applied on 31 Aug and 8 Sep (T1), on 14 Sep and 21 Sep (T2), on 28 Sep and 6 Oct (T3), and on 12 Oct and 20 Oct (T4); C represents the untreated control. Error bars are standard errors from the original mean data. The same letter following values indicates that respective values are not significantly different.

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Chapter 4

Conclusions

Conclusions

Foliar applications of urea applied in the fall increase storage N levels in flowering spurs, shoot tips, and bark of sweet cherry. Flowering spurs show the biggest increase in N. Tissue N concentrations in the spring were greater than in fall, indicating remobilization from other tissues. Differences in storage N levels were associated (the more N applied the bigger the leaf size) with subsequent differences in spur leaf size and total leaf area per spur in spring.

Foliar urea applications were most effective at increasing total N in flowering spurs if applied in late summer to early fall (late August to early September). Such applications also had positive effects on sweet cherry cold acclimation in fall. Shoots treated with urea were up to 4.25˚C more hardy than untreated shoots during fall. Periods of warm temperatures during fall reversed shoot cold acclimation.

Further investigation with N manipulations in different storage tissues in sweet cherries is needed to understand the impact of N on leaf and fruit size, since large fruit is highly prized. The impact of N on cold hardiness of sweet cherries should also be examined during winter and spring, due to incidents like extremely low temperatures during the winter and spring frosts.

APPENDIX

Table A-1. Percentage of 'Ulster' sweet cherry shoot samples with live cambial tissue after controlled freezing tests following fall (2007) foliar applications of 3.5% urea on Aug 31 and Sep 8 (T1), on Sep 14 and Sep 21 (T2), on Sep 28 and Oct 6 (T3), and on Oct 12 and Oct 20 (T4).

Sample Date	**Temperature (°C)**	**Live cambial tissue (%)**	
		Control	**T1**
Sep 12	-3	100	100
	-7	100	100
	-11	100	75
	-15	6.25	0
		Control	**T1**
Oct 9	-3	100	100
	-7	50	100
	-11	0	25
	-15	0	37.5
		Control	**T2**
Sep 25	-3	100	100
	-7	100	68.75
	-11	31.25	31.25
	-15	68.75	6.25
		Control	**T2**
Oct 17	-3	100	100
	-7	81.25	100
	-11	0	50
	-15	0	0

		Control	T3
Oct 10	-3	100	100
	-7	62.5	93.75
	-11	0	0
	-15	0	0
		Control	**T3**
Oct 30	-3	100	100
	-7	100	100
	-11	56.25	100
	-15	0	0
		Control	**T4**
Oct 24	-3	100	100
	-7	100	100
	-11	0	12.5
	-15	0	0
		Control	**T4**
Nov 13	-3	100	100
	-7	100	100
	-11	25	50
	-15	0	0

Table A-2. Percentage of 'Ulster' sweet cherry shoot samples with live cambial tissue after controlled freezing tests following fall (2007) foliar applications of 3.5% urea on Aug 31 and Sep 8 (T1), on Sep 14 and Sep 21 (T2), on Sep 28 and Oct 6 (T3), and on Oct 12 and Oct 20 (T4); samples were divided into those with significant (+JB) and minor (-JB) damage by Japanese beetles due to poor or adequate insecticide coverage, respectively.

Sample Date	**Temperature (°C)**	**Live cambial tissue (%)**			
		Control (+JB)	**Control (-JB)**	**T1 (+JB)**	**T1 (- JB)**
Sep 12	-3	100	100	100	100
	-7	100	100	100	100
	-11	0	0	75	75
	-15	12.5	0	0	0
		Control (+JB)	**Control (-JB)**	**T1 (+JB)**	**T1 (- JB)**
Oct 9	-3	100	100	100	100
	-7	50	50	100	100
	-11	0	0	12.5	37.5
	-15	0	0	12.5	50
		Control (+JB)	**Control (-JB)**	**T2 (+JB)**	**T2 (- JB)**
Sep 25	-3	100	100	100	100
	-7	100	100	75	62.5
	-11	25	37.5	37.5	25
	-15	0	0	12.5	0

		Control (+JB)	Control (-JB)	T2 (+JB)	T2 (- JB)
Oct 17	-3	100	100	100	100
	-7	75	87.5	100	100
	-11	0	0	50	50
	-15	0	0	0	0
		Control (+JB)	**Control (-JB)**	**T3 (+JB)**	**T3 (- JB)**
Oct 10	-3	100	100	100	100
	-7	75	50	100	87.5
	-11	0	0	0	0
	-15	0	0	0	0
		Control (+JB)	**Control (-JB)**	**T3 (+JB)**	**T3 (- JB)**
Oct 30	-3	100	100	100	100
	-7	100	100	100	100
	-11	62.5	50	100	100
	-15	0	0	0	0

		Control (+JB)	Control (-JB)	T4 (+JB)	T4 (- JB)
Oct 24	-3	100	100	100	100
	-7	100	100	100	100
	-11	0	0	12.5	12.5
	-15	0	0	0	0
		Control (+JB)	**Control (-JB)**	**T4 (+JB)**	**T4 (- JB)**
Nov 13	-3	100	100	100	100
	-7	100	100	100	100
	-11	25	25	50	50
	-15	0	0	0	0

Table A-3. Total nitrogen levels in 'Ulster' sweet cherry flower spurs and episodic rainfall after the initial foliar application of 3.5% urea in each treatment series during fall 2007.

Treatment and application date prior to sampling	Sampling date	Total N (%)	Total rainfall (mm) between urea application and sampling date
Control	30 Aug	0.60 a[z]	-
Control	12 Sep	0.64 a	-
Control	28 Sep	0.97 a	-
Control	12 Oct	1.46 b	-
T1 – 31 Aug	8 Sep	1.31 b	18.0
T2 – 14 Sept	21 Sept	1.24 b	0
T3 – 28 Sept	6 Oct	1.60 c	15.7
T4 – 12 Oct	20 Oct	1.75 c	4.6

[z] The same letter following values indicates that respective values are not significantly different within the column.