AN ABSTRACT OF THE DISSERTATION OF

Guihong Bi for the degree of Doctor of Philosophy in Horticulture presented on April 22, 2004.
Title: Nitrogen, Defoliation and New Growth in Almond (*Prunus dulcis* (Mill) D. A. Webb) Nursery Plants.

Abstract Approved:

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The relationships between nitrogen (N), defoliation, and plant growth in nursery plants were studied using almond (*Prunus dulcis* (Mill) D. A. Webb) trees. In several experiments the effects of N source and availability on tree growth, N uptake, N remobilization, and nonstructural carbohydrates (TNC) were investigated to determine the most efficient and effective methods and timing for N fertilization. The influence of chemical defoliants (CuEDTA and $ZnSO_4$) and foliar urea applications on defoliation, N reserves, and new growth performance was also assessed to determine optimal methods for promoting early defoliation without negative effects on tree growth and quality.

Nitrogen from both reserves within the plant and fertilizer applications in the spring was found to be important for enhancing new growth of almond nursery trees during establishment after transplanting. N fertigation during the growing season or foliar urea applications in the fall increased the N reserves in trees, resulting in increased new growth the following spring. Applications of N fertilizer in the spring increased new growth regardless of the amount of N reserves in the plant. Young

almond trees were found to take up N from the soil as early as two weeks after transplanting. Maximum N uptake occurred during the period of rapid new shoot growth. For trees with low N reserves, spring-applied nitrogen fertilizer was found to be particularly important for promoting new growth.

Almond trees accumulated N dynamically in the form of amino acids and protein. Increasing N availability increased concentrations of both free and total amino acids prior to winter storage. However, protein was the main form of N stored in almond nursery trees. The synthesis of amino acids and proteins occurred at the expense of carbohydrates, and the amount of TNC used to assimilate N increased as the N availability increased.

Both CuEDTA and $ZnSO_4$ were found safe and effective for promoting early defoliation in almond nursery trees, however, CuEDTA was more effective than $ZnSO_4$. Foliar urea application prior to or with defoliant treatments was found to promote early defoliation and improve N reserves, without affecting the new growth of almond nursery trees.

Nitrogen, Defoliation and New Growth in Almond (*Prunus dulcis* (Mill) D. A. Webb) Nursery Plants

by
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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Guihong Bi, Author

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CONTRIBUTION OF AUTHORS

Dr. Leslie H. Fuchigami was involved in overseeing this work and in the discussion of experiments, and proofreading and critical editing each of manuscript. Dr. Carolyn F. Scagel contributed her knowledge in the design of experiments, data interpretation, writing and critical editing of each manuscript. Dr. Lailiang Cheng assisted in experimental design and editing of each manuscript. Dr. Shufu Dong assisted with data collection (Chapter 3).

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CHAPTER 1

INTRODUCTION

Nitrogen (N) is an essential macronutrient required for plant growth and development. It is the elementary constituent of numerous important organic compounds such as amino acids, proteins, and nucleic acids. Although nitrogen constitutes only about 2-4% of plant dry matter, much less than carbohydrates which constitute about 20-35% of the total dry matter, nitrogen is considered the most limiting factor in supporting plant growth and production (Cheng et al., 2001).

Knowing the importance of nitrogen for plant growth, nurserymen commonly over-fertilize with N to decrease the potential for N deficiency to limit growth. However, compared to many field crops, deciduous trees generally are less efficient in their uptake of soil applied N (Weinbaum et al., 1992). The percentage of N from fertilizer used by many fruit trees is estimated to be only less than 20% (Hill-Cottingham and Lloyd-Jones, 1975; Sanchez et al., 1991), while for vegetable crops, hay, wheat and other small grains it is estimated that over 50% of fertilizer-N is used

by the crop (Sanchez et al., 1995). The leaching of nitrate from the soil as resulting of over-fertilization can result in ground water contamination.

In deciduous trees, nitrogen used for new growth in the spring may come from two sources: N stored from the previous year and N uptake during the current year. Deciduous trees have the ability to store N from the previous year and utilize it during the following growing season (Taylor and May, 1967; Titus and Kang, 1982; Tromp, 1983; Millard, 1995). The internal cycling of nitrogen provides N needed for early growth in the spring (Titus et al. 1982; Tromp 1983; Millard 1988, 1996; Millard and Neilsen 1989; Neilsen et al. 1997, 2001a; Tagliavini et al. 1998; Dong et al. 2001). It has been known that a positive relationship exists between N reserves and spring growth in many species (Oland, 1959; Taylor, 1967; Taylor and May, 1967; Taylor et al., 1975; Cheng et al., 2001; Cheng and Fuchigami, 2002). N uptake by roots is regulated by N demand of the plant, although it may also be affected by environmental factors such as temperature, soil texture, etc. (Weinbaum et al. 1984b, 1987; Tagliavini et al. 1996; Neilsen et al. 1998, 2001a, 2001b; Youssefi et al. 2000 ; Dong et al. 2001).

Nitrogen uptake, utilization and cycling in deciduous trees have been studied extensively. However, there is, at present, little research on N dynamics during growth of almond nursery trees. Almond is an early foliating species (Weinbaum et al., 1984a). Studies with mature almond trees (Weinbaum et al., 1987) have shown that stored N in the plant can supply 50% of the nitrogen used for annual growth. Compared with mature almond trees, young almond nursery trees have only a small

capacity for storing N. Therefore, they may be more dependent on uptake of N from soil during nursery production and establishment after transplanting into orchards.

Efficient management of N fertilization in nurseries requires practices to meet plant needs and improve performance, as well as minimize fertilizer losses and groundwater contamination. A better understanding of how trees use N from reserves and N from fertilizers for growth, will aid in the development of cultural strategies that use optimal rates of N fertilizer, and methods and timing of N application. This knowledge is important for improving plant quality and the efficiency of N fertilization, and will help to reduce overall fertilizer inputs.

Natural defoliation of most deciduous nursery plants in the U.S. Pacific Northwest occurs in late autumn or early winter. To aid harvesting procedures, growers of bareroot deciduous nursery plants rely on defoliants to promote early defoliation. However, some chemical defoliants are known to cause either physical damage to plant tissues (Larsen, 1977), and/or physiological problems associated with storage or establishment (Abusrewil and Larsen, 1981; Larsen et al., 1984). There is no defoliant that can be recommended to defoliate a wide range of species because the responses of plant species and cultivars to defoliants vary considerably in efficiency of defoliation and susceptibility to plant damage (Larsen, 1973). Effective and safe chemical defoliation of bareroot deciduous nursery plants prior to harvest has been a major challenge for researchers and growers.

N mobilized from senescing leaves makes an important contribution to whole tree N economy (Taylor and May, 1967; Chapin and Kedrowski, 1983). Early

defoliation may decrease the amount of N mobilized from leaves back to plants for storage. Foliar application of urea in the autumn has been found to be an effective way to increase reserve N in deciduous fruit trees, and consequently, improve new growth the following spring (Oland, 1960, 1963; Shim et al., 1972, 1973; Rosecrance et al., 1998; Tagliavini et al., 1998; Millard, 1996; Reickenberg and Pritts, 1996). If the goal of nurserymen is to promote early defoliation without reducing the N reserves of nursery trees, foliar urea application(s) prior to or with a defoliant treatment, theoretically, could be a good strategy to reach this goal.

The objectives of the research described in this thesis were to 1) determine which source of N (i.e., reserves or spring fertilizer applications) has the greater effect on new growth of almond nursery trees; 2) determine whether soil N application alters N remobilization; 3) quantify the effects of soil N availability in the spring on N uptake, distribution and new growth; 4) determine the most efficient time for spring soil N application; 5) determine the chemical composition of nitrogen and non-structural carbohydrates in response to different N supply; and 6) look at the effects of chemical defoliants (CuEDTA and $ZnSO_4$) with/without urea on defoliation, N reserves, and new growth performance of almond nursery plants.

CHAPTER 2

LITERATURE REVIEW

Nitrogen (N) is one of the most important macronutrients required for plant growth and development. Nitrogen used for deciduous tree growth can come from two main sources: external N and N stored within the tree. Sources of external N include fertilizers, N in soil, and N in the atmosphere. Stored N in stems and roots during winter can be remobilized for new growth the following spring.

2.1 N fertilization

Fertilizer N that is used for deciduous tree growth can be applied to soil or to foliage. Efficient management of N fertilization for deciduous tree production requires fertilization practices to meet plant needs and improve tree performance, as well as minimize fertilizer losses and groundwater contamination caused by nitrate leaching.

2.1.1 Soil N fertilization

2.1.1.1 N uptake from soil and plant assimilation

Nitrogen is taken up from the soil by plants mainly as nitrate (NO_3^-) or ammonium (NH_4^+). Roots of deciduous tress also absorb organic forms of N such as urea (CH_4N_2O) (Shim et al. 1973). N from fertilizer that is taken up by trees is

assimilated into carbon containing compounds such as amino acids, amides, and related compounds. Amino acids are the basic compounds from which proteins, nucleic acids and the nitrogen compounds of secondary metabolism are synthesized. These compounds are important starting materials for building cells. In a leaf, typically, 70-80% of the organic N is in proteins, 10% in nucleic acids, 5-10% in chlorophyll and lipoproteins, and the remainder in free amino acids (Chapin and Kedrowski, 1983).

Under most conditions, when nitrate is taken up by deciduous trees, it must first be reduced to ammonium before being assimilated into amino acids. This reduction takes place in two steps. The first step is the reduction of nitrate to nitrite, catalyzed by nitrate reductase (NR). The second step is the reduction of nitrite to ammonium, catalyzed by nitrite reductase. Ammonium taken up by tree roots or produced from NO_3^- reduction can be toxic at high concentrations and must be assimilated into organic compounds such as amino acids, amides, and related compounds. In deciduous trees glutamine and glutamate are considered to be the primary products of ammonium assimilation, with the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT) acting as catalysts in a cycle in which glutamate is both substrate and end product (Titus and Kang, 1982).

Among all the enzymes involved in nitrogen assimilation, NR is considered the key regulatory enzyme. NR exists as an enzyme complex present in the cytosol. It is substrate inducible (Frith, 1972; Leece et al., 1972; Perez and Kliewer, 1978), and can be synthesized rapidly when needed. It is inactivated by end product (NH_3) inhibition

and can be promoted by increasing supplies of NO_3^- (substrate induction). Because of its very high turnover rate, NR is able to play a regulating role in NO_3^- reduction. In most plant species, both roots and shoots have the capacity to reduce nitrate. Several factors, including the nitrate level supplied to the roots and the plant species, can affect the relative extent to which nitrate is reduced in the roots or in the leaves. It has been found that in herbaceous species the greatest activity of NR is usually in the leaves (Radin, 1978). When perennial species grow at low external NO_3^- concentrations, most NO_3^- assimilation occurs in the roots. As external NO_3^- concentrations increase, shoot NO_3^- assimilation becomes increasingly important (Pate, 1980). In fruit trees, as in many other tree species, NO_3^- reduction via NR mainly occurs in the root system. Some studies found that in rosaceous fruit trees NO_3^- absorbed from the soil is entirely reduced in the roots (Pate, 1973). However, under high NO_3^- conditions, once the root system is saturated with NO_3^-, excess NO_3^- is transported in the xylem and nitrate reduction may occur in the leaves. Nitrate reductase activity has been reported in the leaves of several species of fruit trees including apricot, cherry, pear, walnut, and plum (Leece et al., 1972; Perez and Kliewer, 1978; Oliveira and Priestley, 1988).

2.1.1.2 Timing of soil N fertilization

Uptake of N by roots is regulated by N demand in the plant, as well as environmental factors such as temperature, soil texture, etc. (Weinbaum et al., 1984b, 1987; Tagliavini et al., 1996; Neilsen et al., 1998, 2001a, 2001b; Youssefi et al., 2000; Dong et al., 2001). Deciduous fruit trees are able to take up N at any time of year,

provided that the soil temperature remains above freezing. Although trees are capable of taking up N throughout the year, there appears to be specific times when N uptakes optimize tree growth. Studies with peach (Lobit et al., 2001) showed that uptake of N by roots follows shoot development in spring, peaks during the period of maximum vegetative growth, and then decreases during autumn. In apple, a combination of plant developmental stage and soil temperature influences soil N uptake and use (Dong et al., 2001) and the timing of demand for root-supplied N may depend on whether flowering occurs (Nielsen et al., 2001a). In mature almond trees, N demands resulting from the presence of fruits may also play a role in the regulation of N uptake by roots (Weinbaum, 1984b). Timing of N application for optimal tree growth depends on several factors including N uptake, N status of the tree, and developmental stage of the tree. The timing of N uptake by trees during the growing season will influence growth during the current year and also the amount of N remobilized for new growth in the following year (Neilsen et al., 2001a).

Applications of nitrogen fertilizer to soil in the early spring may be inefficient because early growth in several deciduous plant species is mainly supported by N remobilization from storage tissues, and root uptake from soil is limited due to low soil temperature (Hogue and Neilsen, 1986; Westwood, 1988; Tagliavini et al., 1991; Toselli et al., 1999). In apple trees, root uptake of N from soil was not detectable until 3 weeks after bud break in the spring (Cheng and Fuchigami, 1997), and demand for N from root uptake was minimal until 11 weeks after transplanting (Neilsen et al., 2001a). However, application of N to soil in the spring increases flower bud size in

apple trees (Faust, 1989) and early applications of N fertilizer to soil during the first year of growth increases the amount of flowers, spur leaves and shoots in the following year (Nielsen et al., 2001a). When marginally N-deficient peach trees are not supplied with N fertilizer in the autumn and are fertilized with N the following spring, trees exhibit comparable vegetative growth, fruit size and yield to trees receiving soil applications of N fertilizer in the autumn of the previous year (Niederholzer et al., 2001).

N availability during rapid shoot growth has a large impact on plant growth (Cheng et al., 2001). High N fertilizer application rates in late spring and early summer can be more effective in stimulating vegetative growth of fruit trees than fertilization with N in the spring and autumn (Taylor et al., 1975; Sanchez et al., 1995). However, excess fertilization in late spring and early summer can also cause highly vigorous vegetative growth and affect the quality of fruit (Bramlage et al., 1980; Sugar et al., 1992; Sanchez et al., 1995).

Nitrogen uptake by roots can be high during late summer and autumn, therefore applying N fertilizer to the soil during this time can potentially increase N reserves, resulting in the remobilization of more N for new growth the following year (Weinbaum et al., 1984b; Millard and Thomson, 1989; Sanchez, 1990; Tagliavini et al., 1999; Neilsen et al., 2001a). Many studies have shown that application of N fertilizer to the soil late in the season had better effects on the following year's tree fruit production than soil applications of N fertilizer at other times of the year (Hill-Cottingham, 1963; Taylor, 1967b; Sanchez et al., 1992). However, trees fertigated

with high N concentrations throughout late summer and autumn tend to keep growing late into the autumn (Cheng et al., 1999). Late applications or high rates of N fertilizers during this time may delay dormancy, and increase the susceptibility of plants to environmental stresses such as freezing (Bramlage et al., 1980; Millard, 1995).

Uptake of N during different times of the year alters N partitioning within trees. In apple, when N was supplied in the spring, most of the N was partitioned to fruit and leaves, and only a small amount was detected in the roots, whereas, when N was supplied in late summer or autumn, most N was partitioned to roots and 2- to 4-year old wood (Tromp, 1970; Sanchez, 1990; Sanchez et al., 1992). By using ^{15}N Munoz et al. (1993) demonstrated that the uptake of N by peach trees from fertilizers applied to the soil in autumn increased the partitioning of N to roots compared with N taken up earlier in the year. This increased N availability in the autumn increased N storage, and resulted in more N for internal cycling. Similar results were found with mature 'Comice' pear trees (Sanchez et al., 1992).

The availability of soil-applied N from fertilizer varies greatly and is affected by soil texture, microorganisms, pH, temperature, leaching, aeration, moisture, etc. (Weinbaum, 1988). Deciduous fruit trees generally are less efficient than field crops in their uptake of soil applied N (Weinbaum et al., 1992). Using ^{15}N-labeled fertilizer, the annual recovery of nitrogen from soil-applied fertilizer was 15-20% for pears (Sanchez et al., 1991), 16% in potted apple trees (Hill-Cottingham and Lloyd-Jones, 1975), and less than 20% in mature almond trees (Weinbaum et al., 1984b). In

contrast, the reported annual recoveries of nitrogen from soil-applied fertilization for wheat and other small grains are 50% and greater (Sanchez et al., 1995).

2.1.2 Foliar N fertilization

2.1.2.1 Foliar uptake of N and assimilation

Nitrogen can be taken up by the leaves in the form of inorganic and organic N sources. Urea (CH_4N_2O) is commonly used for foliar fertilizer applications because of its non-polarity, high solubility, and rapid and efficient absorption by leaves (Wittwer et al., 1963; Yamada et al., 1965; Shim et al., 1972; Swietlik and Faust, 1984; Sanchez et al., 1990; Knoche et al., 1994; Bondada et al., 2001). Once urea is absorbed by the leaves, it is hydrolyzed by urease to ammonia and carbon dioxide. Urease is found in leaves, roots, and bark of fruit trees (Shim et al. 1973). Urease activity can be rapidly induced by the application of urea to plant tissues and inhibited by high concentrations of ammonia (product inhibition). Foliar application of urea to leaves can increase urease activity in the leaves and bark of apple trees (Shim et al. 1973). Actively growing tissues contain more urease activity than senescing tissues, and although urease activity in leaves declines steadily during leaf senescence, abscised leaves still contain about half of their initial urease activity. Ammonia released by urease is converted to ammonium, and incorporated into amino acids by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT).

2.1.2.2 Timing of foliar N fertilization

Foliar application of N fertilizers can improve leaf color and shoot growth, and increase N content of trees (Shim et al., 1972; Hill-Cottingham and Lloyd-Jones,

1975; Klein and Weinbaum, 1984; Rosecrance et al., 1998; Johnson et al., 2001; Cheng et al., 2002; Cheng and Fuchigami, 2002; Dong et al., 2002). Foliar uptake of N by deciduous fruit trees can occur any time of the year when leaves are present. Uptake of N by leaves is proportional to the concentration of N applied (Klein and Weinbaum, 1985; Oland, 1960). However, the concentration of N that can effectively be used in foliar applications is limited by the tolerance of the foliage and varies with species and leaf phenology. For example, foliar application of urea in the early postbloom period to apple at concentrations greater than 0.5% may cause phytotoxicity and damage to fruit and young leaves (Weinbaum, 1988). In contrast, foliar application of urea before leaf senescence at concentrations about 4% may be sprayed on the same trees with greatly reduced phytotoxicity.

2.1.2.2.1 Foliar application of urea in fall

Foliar application of urea in the autumn is the most common method of foliar N fertilization used in deciduous tree production. Oland (1960, 1963) was the first to advocate foliar applications of urea as a method to increase N storage and therefore improve flowering, fruit set and growth in the following season. Since then, many researchers have addressed the theoretical basis regulating tree responses to foliar urea applications and the practical use of foliar urea sprays in fruit tree production (Shim et al., 1972, 1973; Rosecrance et al., 1998; Tagliavini et al., 1998; Millard, 1996; Reickenberg and Pritts, 1996).

Several studies have shown that plant leaves rapidly absorb a majority of the urea from foliar applied applications in autumn, even during leaf senescence, and

translocate the absorbed N from the leaves into storage tissues (Oland, 1960, 1963; Shim et al., 1972, 1973; Hill-Cottingham and Lloyd-Jones, 1975; O'Kennedy et al., 1975; Klein and Weinbaum, 1984, 1985; Rosecrance et al., 1998; Tagliavini et al., 1998; Johnson et al., 2001; Cheng et al., 2002; Dong et al., 2002). By using a 4% foliar urea spray in mid-October on leaves of apple after fruit harvesting, Oland (1960) increased organic N content of the leaves by 51% within 2 days, and increased organic N content in spur buds 31% by leaf fall. Increased organic N content consequently correlated with increased fruit yield the following year. About 70-80% of the urea from foliar application was absorbed by leaves within 24 to 48 hours in olive (Klein and Weinbaum, 1984), mature almond (Klein and Weinbaum, 1985) and apple (Shim et al., 1972), and approximately 80-90% of the urea-N absorbed can be mobilized back to the storage tissues in young apple trees (Cheng et al., 2002). Peach and nectarine leaves can also rapidly absorb urea from foliar applications, and about 48-58% of urea-N absorbed by the canopy was recovered in peach trees (Rosecrance et al., 1998) and in potted nectarine trees (Tagliavini et al., 1998).

The concentration of amino acids can increase quickly in leaves following foliar application of urea to apple trees in the autumn. This indicates that the absorbed urea-N is assimilated within leaves (Dilley and Walker, 1961; Dong et al., 2002). These amino acids are subsequently translocated to storage tissues of the tree (Dilley and Walker, 1961; Swietlik and Faust, 1984). Roots and bark appear to be the main sinks for N from urea applied to apple leaves (Dong et al., 2002). However, there are conflicting reports on the location of urea hydrolysis and assimilation following foliar

application of urea. Shim et al. (1972) found that no amino acids accumulated in senescencing apple leaves following foliar urea application in the autumn, and the soluble N in leaves was present as urea rather than amino acids, suggesting that leaves may not be the location of urea hydrolysis.

The response of trees to foliar urea application in the autumn is influenced by the N content (status) of the tree. An inverse relationship between tree N status and the likelihood of response to foliar urea applications has been suggested (Weinbaum, 1988). Mature apple trees with a high N status show no response to postharvest foliar applications of urea (Little et al., 1966; Wilson, 1966; Delap, 1967). The response of pear trees to a postharvest foliar application of urea was also related to tree N status (Sanchez et al., 1990). In apple nursery trees, foliar urea application after terminal budset can increase the amount of stored N (reserve N), however, trees with low N status are more efficient in absorbing and translocating N from foliar urea applications than those with higher N status (Cheng et al., 2002).

2.1.2.2.2 Foliar application of urea in spring

Besides foliar urea application in the autumn, some studies have investigated the effect of foliar urea applications during the spring. Application of urea in the spring is largely confined to the leaves and does not affect the N status of the whole tree (Cook and Boynton, 1952; Boynton, 1954). Yang and Luo (1991) found that the branches of apple had the ability to absorb ^{15}N-urea (0.5% and 1% urea) before bud break in the spring, and approximately 20% of the applied ^{15}N-urea can be absorbed. About 40 to 50% of the ^{15}N-urea absorbed by branches can be translocated to new

organs as bud break and leaf growth progresses. This consequently improved the quality of leaves (such as leaf area, specific leaf weight and chlorophyll content) that emerged in the early spring. Application of urea in the spring may be a useful method for increasing N in trees with low N reserves. Foliar urea application at bloom during the period between the exhaustion of stored N and significant uptake of N by roots in spring may be able to correct a transient N deficiency, and improve fruit set, especially in immature fruit trees because the buffering capacity of the endogenous pool of storage N is limited (Hill-Cottingham, 1963; Weinbaum, 1988). However, the potential amount of N absorbed following foliar urea application is restricted by the limited leaf area in early spring (Weinbaum, 1988), and also the N concentration that can safely be used is low because the foliage is more susceptible to phytotoxicity during early development. Numerous urea sprays in the spring would be required to meet total plant N requirements and would not be cost effective.

2.1.2.3 Dependence on foliar-applied urea nitrogen

Foliar urea applications can increase leaf N and fruit N concentrations, and sometimes fruit yields (Marks and Clarke, 1995). However, using only foliar urea application to supply N to an early maturing peach tree can reduce mean fruit weights and cause a tendency to reduce tree yield compared to trees receiving N from soil fertilization (applied as ammonium nitrate) (Marks and Clarke, 1995). In contrast, a combination of soil N application in late summer and foliar urea in October maintained tree productivity comparable to trees receiving only soil N applications, and a mixture of soil and foliar N fertilization controlled excessive vegetative growth.

The best fertilizer treatment for young peach appears to be a combination of foliar urea and soil applied N (Embleton et al., 1986; Johnson et al., 2001). Soil-applied N may be needed to support root proliferation and associated processes (Johnson et al., 2001). It appears unlikely that foliar application of macronutrients in deciduous fruit trees could completely replace the need for soil-derived nutrients (Weinbaum, 1988).

2.1.2.4 Efficiency of soil vs. foliar N fertilization

In the autumn, foliar application of N is considered an alternative to supplying N to the soil. Foliar N fertilization does not stimulate new growth late in the season and can also decrease the potential of groundwater contamination from leaching of nitrate from the soil (Cheng et al., 1999; Embleton et al., 1986; Khemira, 1995). Compared to plants receiving only soil N, plants that receive foliar N fertilization usually have more rapid and efficient uptake of N (Oland, 1960; Shim et al., 1972; Millard, 1996; Reickenberg and Pritts, 1996; Dong et al., 2002). This results in a higher efficiency of N mobilization from foliar N fertilization compared to soil N fertilization. N recovery (percentage recovery of the applied N) is typically 50-70% following foliar urea application (Weinbaum, 1988; Rosecrance et al., 1998; Tagliavini et al., 1998). With apple the percentage recovery of N from foliar fertilization is three to four times higher than the recovery of N from soil fertilization (Shim et al., 1972). N recovery in potted apple trees following foliar urea application was 47%, whereas recovery was only 16% following application of N to soil, as potassium nitrate, in October (Hill-Cottingham and Lloyd-Jones, 1975).

2.2 Reserve N and carbohydrates

Deciduous trees have a characteristic feature common to most perennial plants accumulate reserves in the previous year and remobilize these stored compounds for new growth during the following growing season. Among the reserves, nitrogen and carbohydrates play the most important roles as they provide structural components and energy needed for new growth when conditions are not optimal for root uptake and no photosynthetic surface is available for carbon production (Oland, 1959; Taylor, 1967a; Taylor and May, 1967; Taylor et al., 1975; Titus, 1976, 1981; Tromp, 1970, 1983; Titus and Kang, 1982; Millard, 1988, 1995, 1996; Oliveira and Priestley, 1988; Millard and Neilsen, 1989; Millard and Thomson, 1989; Loescher et al., 1990; Neilsen et al., 1997, 2001a; Tagliavini et al., 1998; Dong et al., 2001). Carbohydrate reserves constitute 20-35% of the total dry matter in apple trees (Priestley, 1970; Kandiah, 1979a; Oliveira and Priestley, 1988). In contrast, nitrogen only accounts for less than 2% of dry matter in apple trees in the winter. However, reserve nitrogen, not reserve carbohydrates, is considered the most limiting factor in supporting initial new growth in the spring (Cheng et al., 2001).

2.2.1 Reserve N

N is considered in reserve if 'it can be mobilized from one tissue and subsequently reused for growth or maintenance of another' (Millard and Catt, 1988). Storage of N allows trees to make the most efficient use of the available nutrient resources (Millard, 1995).

Early in the growing season, N is remobilized from storage tissues to support

new growth, and no N accumulation occurs during this time. Later in the growing season, N begins to increase under conditions of high external N or low light, primarily as specialized storage proteins, amino acids, and nitrate, depending on the species (Titus and Kang, 1982; Chapin and Shaver, 1989). Some of the ribulose 1,5-bisphosphate carboxylase/oxygenase (rubisco), which accumulates in leaves under high external nitrogen in summer, is inactive and therefore should be considered stored N (Millard, 1988; Cheng, 1999). During leaf senescence, N is withdrawn from leaves and mobilized back to the storage tissue. There are two main periods of N storage in deciduous tree fruit: during the transition from late autumn to winter, and during the summer.

2.2.1.1 Autumn and winter storage

Deciduous trees store N primarily in the late autumn and winter (Taylor 1967b; Tromp, 1970; Titus and Kang, 1982; Sanchez et al., 1992; Munoz et al., 1993; Millard, 1995). The N content of root and stem tissues begins to increase after the cessation of shoot growth if there is an adequate external N supply. Wood and bark of all parts of the tree including roots can serve as storage tissues. However, there is some controversy in the literature about the main organs that store N in deciduous trees (Mason and Whitfield, 1960). Some studies found that roots represented the main storage site for N in winter, especially for young trees (Taylor and May, 1967; Hill-Cottingham and Cooper, 1970; Hill-Cottingham and Lloyd-Jones, 1975; Tromp, 1983; Wendler and Millard, 1996; Khemira et al., 1998). For instance, approximately 60-80% of the storage N in dormant 2-year-old peach trees was in root tissues,

irrespective of the nitrogen treatment (Taylor and May, 1967). However, the significance of roots as storage sites for N in larger trees has not been determined (Millard, 1995). The above ground tree part, especially the bark tissues of twigs and trunk, has been considered the predominant regions for N storage during winter (Mason and Whitfield, 1960; O'Kenney et al., 1975; Titus and Kang, 1982). As pointed out by Taylor (1967b) and Titus and Kang (1982), this seemingly conflicting data is a result of how data is expressed and interpreted, i.e., the question of the absolute amount per tree or tissue or the concentration per unit weight.

N reserves in deciduous fruit trees during winter are composed of a soluble fraction including amino acids and amides, mainly arginine and asparagine, and an insoluble protein fraction (Taylor, 1967a, b; Hill-Cottingham and Cooper, 1970; Tromp, 1970; Oliveira and Priestley, 1988). In general, N is stored in roots as amino acids or proteins (Tromp, 1983; Millard and Pore, 1991) and in bark as proteins (Titus and Kang, 1982).

There is some controversy on whether proteins or free amino acids are the main forms of storage nitrogen in deciduous trees. Some previous findings with apple (Oland, 1959), peach (Taylor and May, 1967), and pear (Taylor et al., 1975) showed that storage organs contain a high proportion of their N in soluble compounds, mainly free amino acids, and a low proportion in insoluble compounds, and the percentage of nitrogen in the form of amino acids increases with increasing concentrations of N reserves. At very high concentrations of N reserves, up to 50 % of the total N may exist as amino acids. Because only part of the protein-N is remobilized in the spring,

these authors concluded that free amino acids are the main form of storage N. However, there is an increasing amount of evidence suggesting that protein may be the primary form of N storage, especially in the bark (Tromp, 1970; Shim et al., 1973; O'Kenney et al., 1975; Kang and Titus, 1980; Kang et al., 1982; Titus and Kang, 1982; Coleman et al., 1991). For instance, in the bark of one-year-old shoots of 'Golden Delicious' apple trees, proteins accounted for 90% of the total N in late November (Kang and Titus, 1980).

O'Kenney and Titus (1979) set up two criteria for the definition of storage proteins in apple shoot bark. Storage proteins are proteins that: (1) are prominent in dormant shoots and may contain a high proportion of high-N amino acids, and (2) disappear as growth is resumed. They separated total proteins extracted from apple bark tissues into three working groups of proteins, designated as peak I, II and III proteins. Proteins designated as peak I and II were associated with neutral sugars and proteins categorized as peak III proteins contained a high percent of arginine. Kang and Titus (1980) found that the predominant accumulation of peak III proteins occurs only in the later stages of leaf senescence and their accumulation appears to be temperature dependent.

Several proteins, including proteins with a molecular mass of 16, 17, 30, 38, 56kD, have been identified in apple bark that meet the criteria set by O'Kenney and Titus (1979), and may play a role in nitrogen storage of apple trees (Kang et al., 1982; Khemira, 1995). In contrast, one bark storage protein with a molecular mass of 32kD

has been found to account for a large proportion of the protein-N in poplar (*Populus spp.*) trees (Coleman et al., 1991).

Stored proteins are hydrolyzed in spring, starting a few weeks before budbreak, resulting in a decrease in protein in woody tissues and a rapid increase in the soluble N levels for use in new growth (Tromp 1970; Tromp and Ovaa 1971, 1973; O'Kenney et al., 1975; Kang et al., 1982). The closer it gets to budbreak, a higher the proportion of free amino acids is present in the total nitrogen pool (Oland, 1959; Tromp, 1970; O'Kenney et al., 1975).

Arginine is considered, theoretically, the most efficient form for N storage because of its low C/N ratio. It contains maximum N at the least expense of carbon: 4 N with 6 carbon (C) atoms (Titus and Kang, 1982). It has been well documented that free arginine is the principal constituent of the soluble N fraction in dormant apple (Oland, 1959; Tromp, 1970) and peach trees (Taylor and May, 1967). Tromp and Ovaa (1973) found that arginine is also the predominant amino acid in the proteins of apple trees with high concentrations of stored N, but not in the trees with low-N storage. It is suggested that trees with high concentrations of stored N posses a special storage protein characterized by high arginine content (Tromp and Ovaa, 1973). Glutamine and asparagines are also important constituents of the stored N in dormant apple trees (Oland, 1959), but not in peach trees (Taylor and May, 1967). The level of arginine in woody tissues of the dormant trees is considered the most sensitive indicator of the N status of the tree (Taylor and May, 1967).

2.2.1.2 Summer storage

Leaf tissue is considered the major sink for nitrogenous compounds among all the developing tissues of fruit trees (Titus and Kang, 1982; Feigenbaum et al., 1987; Deng et al., 1989; Millard and Neilsen, 1989). About 40 to 50% of the total N in apple trees is present in leaves in late August (Forshey, 1963). Much of the N assimilated in the roots is translocated upward in the form of amino acids, and becomes part of the proteins in leaves (Raven and Smith, 1976). Ribulose 1, 5-bisphosphate carboxylase/oxygenase (rubisco), an important enzyme involved in photosynthetic carbon reduction and photorespiratory carbon oxidation, is the most abundant protein in C3-plants (Kawashima and Wildman, 1970; Stoddart and Thomas, 1982). For instance, up to 54% of the total protein of apple leaves in mid-July is rubisco (Kang and Titus, 1980).

Rubisco exists in either active or inactive form. Before rubisco can regulate carboxylation and oxygenation, it must be activated (Portis, 1990, 1992; Cheng, 1999). This involves the reversible reaction of a carbon dioxide (CO_2) molecule with a lysine residue at the active site to form a carbamate, followed by the rapid binding with a magnesium ion (Mg^{2+}) to create an active ternary complex. Activation of rubisco *in vivo* is catalyzed by rubisco activase (Portis, 1990). Several environmental factors, particularly light intensity, have been shown to modulate the activity of this enzyme. The proportions of active and inactive forms are also determined by pH and concentrations of CO_2 and Mg^{2+}.

Because of the very high N cost for rubisco, N availability to the plant affects the amount and activities of rubisco. Rubisco content and total activity increases linearly with increasing leaf N (Evans, 1983, 1989; Evans and Seemann, 1984; Sage et al., 1987; Makino et al., 1992, 1997; Cheng, 1999). The ratio of rubisco to total leaf N varies among species. It increases with increasing leaf N content in rice (Makino et al., 1992, 1997), spinach (Makino et al., 1992), pea (Makino et al., 1992), apple (Cheng, 1999) and other species (Evans, 1989; Makino et al., 1992), but is constant in wheat regardless of leaf N content (Evans, 1983; Makino et al., 1992). The activation state of rubisco (the ratio of initial activity to total activity) decreases with increasing N supply in wheat (Lawlor et al., 1987; Mächler et al., 1988) and apple trees (Cheng, 1999). Cheng (1999) calculated that about 40% of the rubisco was activated in apple leaves at high N concentrations and saturating light conditions, whereas 90% of the rubisco in apple leaves with low N concentrations was activated.

It has been suggested that the inactive rubisco may serve as a storage protein in leaves during the summer (Titus and Kang, 1982, Millard, 1988a, 1996; Cheng, 1999), then break down and translocate to reproductive organs in herbaceous plants (Millard, 1988) or to bark and root tissues in woody perennials during leaf senescence (Titus and Kang, 1982; Millard and Thomson, 1989). Accumulating an excessive amount of rubisco, under high N supply conditions, may be beneficial for plant resource acquisition and reutilization (Cheng, 1999). For example, in apple, rubisco is the major protein broken down during leaf senescence in the fall, with 80-90% of the decline in total leaf protein content attributable to the hydrolysis of rubisco. The N

from rubisco hydrolysis subsequently contributes from 32-48% of the N remobilized to support leaf growth in next spring (Kang and Titus, 1980; Millard and Thomson, 1989).

2.2.2 Mobilization of N for storage during leaf senescence

Deciduous trees have the ability to withdraw and mobilize significant amounts of N from the leaves to the storage tissues during leaf senescence (Titus and Kang, 1982). The period at which leaf N begins to decline may start from the onset of leaf senescence (Hennerty and Morgan, 1977) or from the time when active shoot growth ceases (Taylor, 1967a), and may vary depending upon the availability of nutrients, crop load, and climate conditions, especially temperatures (Titus and Kang, 1982).

The N mobilized from senescing leaves is an important contributor of whole tree N economy (Taylor and May, 1967; Chapin and Kedrowski, 1983). For instance, in apple, about 40-50% of the total tree N is present in leaves in late summer and early fall (Forshey, 1963). A rapid decrease in leaf N content begins 3 to 4 weeks prior to abscission (Oland, 1963), and leaf protein content decreases 50-70% during senescence with no accumulation of amino acids in the leaves (Shim et al., 1972; Spencer and Titus, 1972; Kang and Titus, 1980). This suggests that amino acids from the hydrolyzed proteins are quickly translocated into the woody tissues (Oland, 1963; Shim et al., 1972; Kang and Titus, 1980; Millard and Thomson, 1989). The N withdrawn during leaf senescence constitutes approximately 25% of the total N in the tree. Therefore, it is important to achieve the maximum mobilization and withdrawal

of N from senescing leaves before abscission (Millard, 1995). Maintaining the leaves in the fall is important for the maximum mobilization of leaf N to storage tissues.

The efficiency of N mobilization and translocation during senescence is reduced in trees with high N concentrations (Boerner, 1984; Millard and Pore, 1991). This is consistent with the observation that the onset of leaf senescence can be delayed by an increase in N supply to plants in the autumn (Millard and Thomson, 1989). Sanchez et al. (1991) also showed that N arriving in the leaves later in the season is more likely to be translocated to storage than N in leaves from earlier in the season. However, N fertilization to the soil late in the growing season can often cause continued growth, and delay dormancy and cold acclimation (Cheng et al., 1998).

In addition to the mobilization of existing N in leaves, leaves can absorb urea in the fall and the N derived from foliar urea can be translocated rapidly out of leaves and into storage tissue. N from urea is quickly converted into amino acids in leaves after foliar application in autumn (Dong et al., 2002). Amino acids derived from foliar urea may be directly translocated out of leaves (Shim et al., 1973), while, in contrast, the existing proteins in the leaves can only be translocated to storage after they are broken down to amino acids.

2.2.3 Reserve carbohydrates

Deciduous trees contain high amounts of carbohydrates, 70-75% of total dry matter, both as structural components and non-structural carbohydrate reserves (Oliveira and Priestley, 1988). Non-structural carbohydrates make up 20 to 35% of the total dry matter of a dormant apple tree and include an insoluble starch fraction

and a soluble sugar fraction comprises mainly of sucrose, glucose, fructose, and sorbitol (Oliveira and Priestley, 1988), (Priestley, 1970; Kandiah, 1979a; Oliveira and Priestley, 1988).

Starch is considered to be the main storage form of non-structural carbohydrates in fruit trees (Taylor et al., 1975; Kavakli, et al., 2000). Large amounts of starch often exist in wood ray parenchyma in shoots, trunk and especially roots of woody plants (Keller and Loescher, 1989). Of the soluble carbohydrates, sucrose is the major photosynthetic product, the main form of carbon translocated, and the main storage sugar in many plants (Rees, 1984), but its presence is limited in woody roots (Loescher et al., 1990). However, in the rosaceae, sorbitol (D-glucitol) may play a physiological role similar to that of sucrose in other plants (Titus and Kang, 1982). Sorbitol may be the principal photosynthetic product, the major transport carbohydrate, and an important storage compound in the soluble fraction of carbohydrate reserves (Chong and Taper, 1971; Tromp, 1983; Yamaki and Ishikawa, 1986; Loescher, 1987). It has been suggested an inverse relationship between starch and sorbitol concentrations exists which may indicate starch synthesis from the sorbitol pool. Fructose and glucose are commonly present in roots at higher concentrations than sucrose, but lower concentrations in above-ground parts (Loescher et al., 1990).

Although all perennial organs of woody plants may serve a storage function, roots usually contains the highest concentrations of nonstructural carbohydrates, and therefore have been considered the main site of carbohydrate storage (Priestley, 1960,

1964; Chong and Taper, 1971; Lockwood and Sparks, 1978; Abusrewil et al., 1983; Loescher et al., 1990).

Seasonal changes of carbohydrate reserves have been studied extensively in fruit trees (Priestley, 1960, 1964, 1981; Hansen, 1967; Hennerty and Forshey, 1971; Hansen and Grausland, 1973; Keller and Loescher, 1989). Carbohydrate reserves decrease rapidly to support new vegetative and reproductive development in early spring before any photosynthesis occurs, reaching a minimum at about 4 to 6 weeks after budbreak. Replenishment starts before current season's extension growth begins, but, the most rapid accumulation of reserves will not take place until after fruit harvest. The amount of carbohydrate reserves in fruit trees peaks at the time of leaf fall. Carbohydrate reserves remain unchanged or slowly decrease during the winter, after which the cycle repeats. Most authors consider fluctuations in concentration over time as the main feature of a reserve compound (Oliveira and Priestley, 1988).

Similar to N reserves, carbohydrate reserves also play essential roles in deciduous tree fruits growth and productivity. Carbohydrate reserves are important for tree winter survival and necessary for maintenance respiration and bud growth and development. New leaf and shoot growth in early spring depends on carbohydrate reserves. However, deciduous tree fruit species, where flowering occurs before canopy development, (e.g. stone fruits and pecans) (Westwood, 1988), may be more dependent on carbohydrate reserves than other deciduous fruits such as apple in which leaves on fruiting spurs are nearly fully expanded before anthesis. Carbohydrate reserves may critically affect stone fruit productivity (Keller and Loescher, 1989). In

contrast, in young apple rootstock, less than 20% of ^{14}C-labeled reserves assimilated in fall was fixed in new growth the following spring (Hansen, 1967; Kandiah, 1979b), suggesting that most of the carbohydrate reserves in apple are used in respiration rather than for new growth (Hansen and Grausland, 1973; Tromp, 1983).

2.2.4 The interaction between reserve N and carbohydrates

Nitrogen and carbon metabolism are interrelated in all phases of plant growth and development (Titus and Kang, 1982). N assimilation depends on carbohydrates for carbon skeleton and energy supply, and carbon assimilation requires N metabolism to provide the photosynthetic machinery. It has been found that high levels of light and carbohydrates favor N assimilation into carbon-rich compounds that will participate in the synthesis of new plant materials. In contrast, low levels of light and carbohydrates favor N assimilation into N-rich compound.

A negative relationship was reported between N and the concentration of non-structural carbohydrates (TNC) in apple and pear nursery trees (Cheng et al, 2001; Cheng and Fuchigami, 2002). TNC concentration decreased with increased N supply from either soil or foliar applications, because N assimilation uses carbohydrates for carbon skeleton and energy supply. However, others have reported soil N fertilization causes no measurable short-term or long-term changes in carbohydrate concentrations, though the concentrations of soluble N increased (Priestly, 1972; Priestley and Catlin, 1974; Catlin and Priestley, 1976). This could be due to the large amount of carbohydrates that exist in the plants resulting in carbohydrate changes caused by N assimilation being undetectable (Priestley and Catlin, 1974).

Manual defoliation early in the fall decreases reserve carbohydrates and N. In apple nursery trees, early defoliation caused a 33% reduction in total non-structural carbohydrate content, which was associated with reduced new growth during establishment the following year (Abusrewil and Larsen, 1981). Tustin et al. (1997) found that complete defoliation of Gala/ M9 trees (10 days after harvesting) caused delayed budbreak and reduced fruit set, spur leaf development, and trunk growth the following season. Experiments on cherry (McCamant, 1988; Loescher et al., 1990), pistachio (Nzima et al., 1999) and pecan (Worley, 1979) also found that early defoliation resulted in reduced new growth the following spring. Defoliation decreases reserve carbohydrates by diminishing the normal, rapid accumulation of reserve carbohydrates that takes place during the postharvest period for bearing trees and after cessation of shoot growth for nursery trees. Poor tree performance in the following spring due to early defoliation in the fall demonstrates the importance of leaf retention in the fall for the growth and fruiting the next spring. It is hard to establish a causal relationship between the changes in carbohydrate reserves and new growth performance the following spring (Cheng et al., 2002a) because early defoliation can also stop N mobilization and decrease the amount of N withdrawn from leaves for storage (Faby and Naumann, 1986; Guak et al., 2001). Some studies in young apple and pear trees found that the initial growth in the spring was primarily determined by the amount of reserve N, not limited by reserve carbohydrates (Cheng et al., 2001, 2002a).

2.3 Remobilization of nitrogen in spring for new growth

Deciduous trees have the ability to remobilize N in the spring for new growth. N remobilization in the spring provides N for both vegetative and reproductive growth of trees (Titus and Kang, 1982; Weinbaum et al., 1984a; Millard, 1996; Tagliavini et al., 1998). From bud swell to flowering, the concentration of total and soluble N constituents, especially non-protein N, increases markedly in developing buds and the youngest shoots. The N concentration in apple leaves is highest soon after leaf emergence (Taylor and May, 1967). This increase is accompanied by a progressive decrease in N content in all woody structures including the older shoots, branches and roots. This suggests that protein hydrolysis occurs and that soluble N is translocated from these tissues to the developing flower buds and growing points. There is a significant positive correlation between the level of storage N in tree tissues and fruit tree growth in early spring (Oland, 1959; Taylor, 1967a; Taylor and May, 1967; Taylor et al., 1975; Cheng et al., 2001; Cheng and Fuchigami, 2002). For example, the new shoot growth in the spring is highly correlated with reserve nitrogen levels in peach (Taylor and May, 1967; Taylor and van den Ende, 1969) and pear (Taylor et al., 1975).

In general, N remobilization in spring occurs before any significant root uptake of N takes place (Titus and Kang, 1982; Weinbaum et al., 1984a; Millard, 1996; Tagliavini et al., 1998). In apple trees, this N remobilization can provide 50% of the N for leaf growth and the majority of N needed for spur leaf growth (Neilsen et al., 1997). Approximately 50-55% of N in apple trees at planting is subsequently

remobilized (Neilsen et al., 2001a; Cheng and Fuchigami, 2002). In 3 year old peach trees, 93% of the N used for growth during flowering and fruit set can come from N reserves (Munoz et al. 1993). In mature almond trees, approximately 50% of N used for annual growth can come from N remobilization (Weinbaum et al., 1987), 45% in five–year-old pear trees (Sanchez et al., 1991), and 84% in mature citrus trees (Feigenbaum et al., 1987).

The amount of N remobilized in the spring depends upon the amount of N in reserves, which is related to the N supply in the previous year, but may not be affected by the amount and timing of N supply during the spring of the current year (Millard and Neilsen, 1989; Millard and Proe, 1991, 1992; Millard, 1995, 1996; Neilsen et al., 1997; Tagliavini et al., 1998; Cheng et al., 2001; Cheng and Fuchigami, 2002; Neilsen et al., 2001a, 2001b). Millard (1995) concluded that fertilizing trees in the current year will increase growth and storage, but has little or no effect on the efficiency of N remobilization for spring growth.

2.4 Defoliation and nitrogen

Bareroot deciduous tree nurseries comprise one of the largest segments of the nursery industry in the Pacific Northwest. The relatively mild climate of the Northwest and the long, bright sunny days and cool nights during the summer is ideal for production of many deciduous plant species. Natural defoliation of deciduous nursery plants is often in late autumn and early winter. Growers of bareroot deciduous nursery trees would like to harvest plants before natural defoliation occurs, to avoid the problems associated with harvesting plants during the rainy season. Harvesting

plants during wet conditions can increase plant injury, increase harvest time and costs, and increase disease problems. Therefore, early defoliation of deciduous nursery stock is essential for efficient nursery management. Hand-stripping is most commonly used in nurseries to remove leaves prematurely. But it is time consuming, expensive, and can result in damage to shoot bark and buds (Dozier et al., 1987). There is an obvious need for an effective and safe method for early defoliation of deciduous nursery stock.

The use of chemicals to promote leaf abscission of nursery stock dates back to at least 1940, when Milbrath et al. advocated the use of ethylene gas for defoliation of roses in storage. Since then, chemical defoliants have received attention from researchers and growers. A wide range of chemicals such as ethylene gas, Dupont WK surfactant (DWK), ethephon or combinations of both, harvade, Harvade+DWK, KI, Bromodine, CEPA, CGA-15281, FeEDTA, N252, ABA, CuEDTA have been tested on various types of deciduous plant material (Fuchigami, 1977; Larsen and Lowell, 1977; Knight 1979, 1983; Insley and Boswell, 1980; Abusrewil and Larsen, 1981; Larsen et al., 1984; Larsen and Fritts 1986; Dozier et al., 1987). Some of these chemicals have been found to be effective in defoliating various plant species. However, certain chemical defoliants can cause either physical damage to plant tissues, including bark and/or bud damage (Larsen and Lowell, 1977), and/or physiological problems associated with storage or establishment, such as shoot dieback and delayed budbreak (Abusrewil and Larsen, 1981; Larsen et al., 1984); and certain material, e.g. ABA, are impractical to be used for commercial application due

to its present cost (Larsen, 1973). Although there has been considerable research on chemical defoliants, so far, no chemical can be recommended to defoliate a broad range of species. The response of plants to defoliants is affected by many variables such as temperature, humidity and precipitation, species and cultivar, timing, chemical concentrations, soil moisture, nutrition, age of plant, spray pressure and droplet size, adjuvants, water pH (Larsen, 1973). The responses of different plant species to defoliants can also vary considerably in susceptibility to defoliation and plant damage. Therefore, promoting earlier defoliation and maintaining good new growth performance during establishment have become the goals of bareroot deciduous nursery management and a major challenge for researchers and growers.

Defoliation prior to natural leaf abscission can ensure earlier harvest of plants, easier and faster harvesting, and lower harvest costs. However, earlier defoliation can also decrease the mobilization of nutrients from leaves into stems and roots for storage. The initial growth of deciduous plants in the spring is mainly supported by N reserves, and there is a positive relationship between N reserves and spring growth in many species (Oland, 1959; Taylor and May, 1967; Cheng et al., 2001; Cheng and Fuchigami, 2002). The N mobilized from senescencing leaves makes an important contribution to whole tree N economy (Taylor and May, 1967; Chapin and Kedrowski, 1983). It is important to achieve maximum mobilization and withdrawal of N from senescing leaves before abscission (Millard, 1995). Studies with abscisic acid (ABA) have found that ABA can not only promote leaf defoliation but also induce leaf senescence and N mobilization from leaves. These findings suggest that leaf

senescence prior to abscission is important for protein breakdown and N mobilization (Guak and Fuchigami, 1997; Larsen and Higgins, 1998). Earlier defoliation caused by other defoliants, e.g. Copper chelate, promotes leaf abscission, but does not appear to enhance leaf-senescence as does natural defoliation or the use of ABA, thus preventing the mobilization of N from leaves to storage tissues. This can cause a decrease in N reserves that could potentially result in poor regrowth performance.

Foliar urea application in the fall is an effective way to increase N reserves in deciduous trees, (Oland, 1960, 1963; Shim et al., 1972, 1973; Millard, 1996; Reickenberg and Pritts, 1996; Rosecrance et al., 1998; Tagliavini et al., 1998). Some plant leaves, such as olive (Klein and Weinbaum, 1984), mature almond (Klein and Weinbaum, 1985) and apple (Shim et al., 1972), can absorb approximately 70-80% of the urea from foliar application within 24 to 48 hours. N derived from foliar urea sprays can be rapidly converted to amino acids in leaves, and the high concentrations of amino acids in leaves and bark can occur within 4 days after application (Dong et al., 2002). About 50% or more of the urea-N absorbed can be quickly mobilized back to the storage tissues (Rosecrance et al., 1998; Tagliavini et al., 1998; Cheng et al., 2002; Dong et al., 2002). Thus, foliar application of urea can bypass the need for protein breakdown during leaf senescense to increase N movement to storage tissues. Foliar urea spray prior to or with a defoliant treatment, theoretically, could be a good strategy to reach the goal of promoting early defoliation without reducing N reserves of the nursery stock.

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CHAPTER 3

SPRING GROWTH OF ALMOND NURSERY TREES DEPENDS UPON NITROGEN FROM BOTH PLANT RESERVES AND SPRING FERTILIZER APPLICATION

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3.1 Abstract

June-budded 'Nonpareil/Nemaguard' almond (*Prunus dulcis* (Mill) D. A. Webb) trees were fertigated with one of five nitrogen (N) concentrations (0, 5, 10, 15, or 20 mM) from July to September. The trees were sprayed with either water or 3% urea in October, then harvested bareroot after natural leaf fall, and stored at 2°C. One set of trees was destructively sampled for total N content; the remaining trees were transplanted into N-free media in the spring after cold storage. After budbreak, these trees were supplied for 70 d with either N-free Hoagland's solution or Hoagland's solution containing ^{15}N-NH_4NO_3. Nitrogen concentrations in both stem and root tissues were positively correlated with the N- fertigation concentration. Foliar urea applications increased levels of stem and root N regardless of the N-fertigation concentration. During the first 70 d of spring growth, the trees utilized nitrogen from both their reserves and spring fertilizer applications. The amount of N reserves used for growth of new shoots and leaves was proportional to the total amount of reserves. Trees with low N reserves relied primarily on the spring fertilizer as their source of nitrogen. We conclude, therefore, that both reserve N and spring-applied N fertilizers are important for enhancing the regrowth of bareroot almond nursery trees during establishment after transplanting. Nitrogen fertilization in the spring can especially improve the performance of trees with low N reserves.

3.2 Introduction

Nitrogen (N) is required for the initial growth of deciduous trees in the spring. The ability to store N the previous year and utilize it during the following growing

season is a characteristic of fruit trees (Taylor and May, 1967; Titus and Kang, 1982; Tromp, 1983; Millard, 1995). In certain species, the amount of N remobilized depends upon the total amount of N in reserve, and is not affected by the uptake of N from spring applications of fertilizers (Millard and Neilsen, 1989; Millard and Thompson, 1989; Millard and Proe, 1992; Tagliavini et al., 1998; Cheng et al., 2001; Neilsen et al., 2001a, b).

Almond is an early foliating species (Weinbaum et al., 1984a). Studies with mature trees (Weinbaum et al., 1987) have shown that storage N can supply 50% of the nitrogen used for annual growth. Compared with mature almond trees, young almond nursery trees have only a small capacity for storing N. Therefore, their current uptake of N may be more important to overall N economy during nursery production and establishment after transplanting into orchards. Understanding the relative contribution of reserve N and N from spring fertilizer to plant development has direct, practical implications. If new growth is mainly affected by levels of N reserves, nursery cultural practices should be optimized to improve a tree's reserves. However, if N from fertilizer applications in spring is the primary influence, management strategies directed at optimizing N uptake in spring would improve regrowth performance. The objective of this study was to determine which source of N (i.e., reserves or spring fertilizer applications) has the greater effect on new growth of almond nursery trees. Here, we used labeled ^{15}N to distinguish between N reserves and N derived from spring fertilizer applications.

3.3 Materials and Methods

3.3.1 Experimental design

June-budded 'Nonpareil' almond (*Prunus dulcis* (Mill) D. A. Webb) trees on 'Nemaguard' rootstocks were planted in 7.6 l pots containing a 1:2:1 (v/v/v) mix of peat moss, pumice, and sandy loam soil. Trees were grown outdoors under natural conditions in Corvallis, Oregon (44° 30' N, 123° 17' W), USA. On 1 July, 1999, uniform plants was selected for our experimental treatments. Thirty plants were randomly assigned to one of five groups. Trees in each group were then fertigated (300 ml each) with one of five N concentrations (0, 5, 10, 15 or 20 mM N from NH_4NO_3), using a modified Hoagland's solution (Hoagland and Arnon, 1950; Cheng and Fuchigami, 2002). These treatments were conducted twice weekly, from 1 July to 1 September.

Fifteen plants from each N-fertigation level were randomly selected and sprayed with 3% urea on 10 and 20 October (F+U treatment). The remaining plants were sprayed with water as our control (F treatment). All the plants were then barerooted and harvested in late November following natural leaf fall, and were stored at 2°C. Afterward, five plants from each treatment were destructively sampled and divided into stem and root portions that were washed with double-distilled (DD) water to remove any urea residue from their surfaces. All samples were immediately put into a –80°C freezer, freeze-dried, then ground with a Wiley mill (20 mesh) and reground with a cyclone mill (60 mesh) for analysis.

In the following spring (2000), the remaining ten trees from each treatment (F or F+U) were transplanted into N-free perlite and vermiculite media (1:1 v/v) in 7.6 l pots, and were grown outdoors under natural conditions. After budbreak, the trees in each treatment (F or F+U) were divided equally between two groups. Half of the plants were supplied with 400 ml of N-free modified Hoagland's solution (designated as F-N or F+U-N) twice a week. The remaining half received, twice weekly, 400 ml of modified Hoagland solution with 10 mM ^{15}N-depleted NH_4NO_3 (0.03% ^{15}N abundance; ISOTEC, Miamisburg, OH) (F+N or F+U+N). After 70 d, we measured leaf areas and the lengths of the new shoots. Trees were harvested and separated into leaves, new shoots, stems, and roots. All samples were washed in DD H_2O, immediately placed in a –80°C freezer for pre-freezing, and freeze-dried. They were then ground with a Wiley mill (20 mesh) and reground with a cyclone mill (60 mesh) for analysis. The dry weights were recorded for each tissue type.

3.3.2 Analysis of samples

Total N was assessed via Kjeldahl analysis (Schuman et al., 1973). The atom% ^{15}N in the samples was determined from the gas evolved from combustion of powdered tissue in an elemental analyzer coupled with a mass spectrometer at Isotope Services (Los Alamos, NM, USA). The percentage of nitrogen derived from the labeled fertilizer (NDFF%) was calculated as described by Khemira et al. (1998):

$$NDFF\% = \frac{(atom\%^{15}N)_{natural.abundance} - (atom\%^{15}N)_{tissue}}{(atom\%^{15}N)_{natural.abundance} - (atom\%^{15}N)_{fertilizer}} x100\%$$

The ^{15}N content in each tissue type was calculated from NDFF% and tissue total N content. For trees that did not receive any supplemental N during regrowth, the total

N content in new shoots and leaves was taken as the amount of reserve N remobilized from storage tissues for new growth. Here, we assumed that N from other sources was negligible. For trees that were supplied with depleted ^{15}N during regrowth, the remobilized reserve N was estimated as the difference between the total and the labeled ^{15}N content in new shoots and leaves.

3.3.3 Statistical analysis

The experiment was a completely randomized design, with five replicates per treatment. The effect of N status and new growth were evaluated by analysis of variance (ANOVA), and comparisons among treatment means were performed by contrasts (significance level $p<0.05$). Effects of reserve N and N uptake from fertilizer were determined through linear regression analysis. All statistical analyses were performed with SAS (SAS Inst. Inc., Cary, N.C., USA).

3.4 Results and Discussion

3.4.1 N concentrations and contents in dormant almond trees

Concentrations of N in the stems and roots of fertigated almond trees (treatment F) increased with increasing N fertigation concentrations (Figure 3.1A, B). Trees treated with foliar urea had significantly higher N concentrations in their stems and roots than did those receiving only fertigation (Figure 3.1A, B: F+U v F treatments at each N-fertigation concentration, $p<0.05$). This effect of foliar urea on the root nitrogen concentration was greater when plants were fertigated with low N concentrations than in those fertigated with high N concentrations (Figure 3.1B: N fertigation concentration x Urea treatment interaction: $p=0.03$). N fertigation had no

effect on N concentrations in stems (Figure 3.1A: $p=0.5317$) and roots (Figure 3.1B: $p=0.1393$) of trees treated with urea. The total amount of tree nitrogen increased with increasing N-fertigation concentrations (Figure 3.1C). Plants given fall foliar applications of urea (F+U) had significantly higher levels of N ($p<0.05$) than did those receiving only fertigation (F), with total-N contents increasing by 237 to 382 mg per tree, depending on the N-fertigation concentration.

Our data clearly demonstrate that foliar applications of urea later in the season, prior to leaf senescence, improved N reserve status of container-grown almond trees. This result is consistent with those reported for apples (Oland, 1960, 1963; Han et al., 1989; Cheng et al., 2002), nectarines (Rosecrance et al., 1998; Tagliavini et al., 1998), peaches (Rosecrance et al., 1998; Johnson et al., 2001), and pears (Sanchez et al., 1990). An inverse relationship has been suggested between tree N-status and the response to foliar urea (Delap, 1967; Weinbaum, 1988; Sanchez et al., 1990; Cheng et al., 2002). In our experiment, foliar urea applications brought N concentrations in plants to a similar level across all N-fertigation concentrations. This indicates that trees with lower concentrations of N are more responsive to foliar urea than trees with higher N concentrations. There may also be an optimum level of tree reserve N concentration under our experimental conditions, above which trees may not be able to store more reserves.

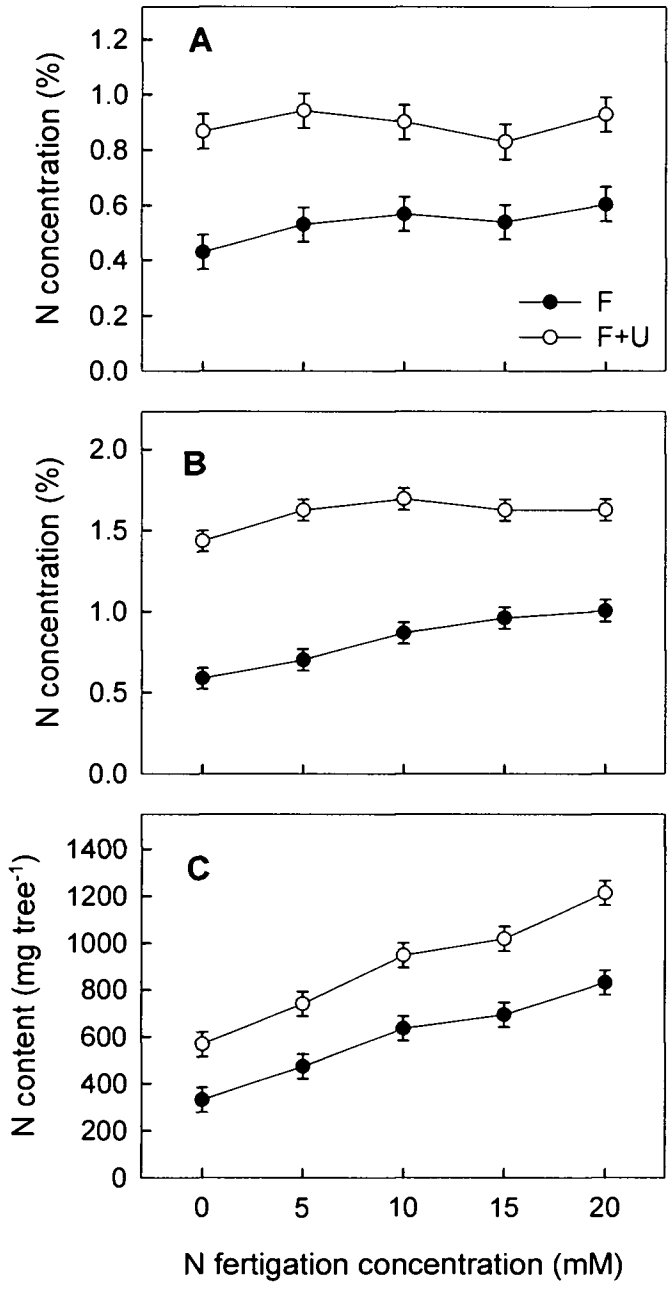

FIG. 3.1. Effects of N fertigation rate during the 1999 growing season and foliar urea applications in the fall of 1999 on (A) stem and (B) root N concentrations and (C) total N content of dormant almond nursery trees. Each value is the mean of five replicates. Vertical bars represent standard errors. Treatments: N fertigation (F), N fertigation and foliar urea (F+U).

3.4.2 Tree growth

Regardless of spring N application, total leaf areas and lengths of new shoots increased with increasing N-fertigation concentrations from the previous year (Figure 3.2). At any given N concentration, trees sprayed with urea in the fall (F+U-N) had significantly greater leaf areas and longer new shoots ($p<0.05$) than trees receiving no urea (F-N). These data show that new shoot and leaf growth in the spring is closely related to reserve-N levels, thereby supporting the results reported from previous studies of deciduous fruit trees (Taylor and May, 1967; Titus and Kang, 1982; Tromp, 1983; Millard, 1995; Cheng and Fuchigami, 2002). Therefore, we believe that a tree's N reserves can be enhanced either by N fertigation during the growing season or through foliar urea applications in the fall, both treatments resulting in increased new growth the following spring.

Spring N fertilizer application increased new growth regardless of the amount of N reserves in the plant. Spring application of N significantly increased leaf area and new shoot growth of fertigated trees (Figure 3.2A, B: F+N v F-N treatments at each N-fertigation concentration, $p<0.05$) and urea-treated trees (Figure 3.2A, B: F+U+N v F+U-N treatments at each N-fertigation concentration, $p<0.05$). Nonetheless, plants treated with either foliar urea in the fall or N fertilizer in the following spring had the same total leaf areas and new shoot growth at each N-fertigation concentration from the previous year (Figure 3.2A, B: F+U-N v F+N treatments, $p>0.05$). Our data suggest that spring N applications can be as effective as fall-applied foliar urea in increasing total leaf areas and new shoot lengths.

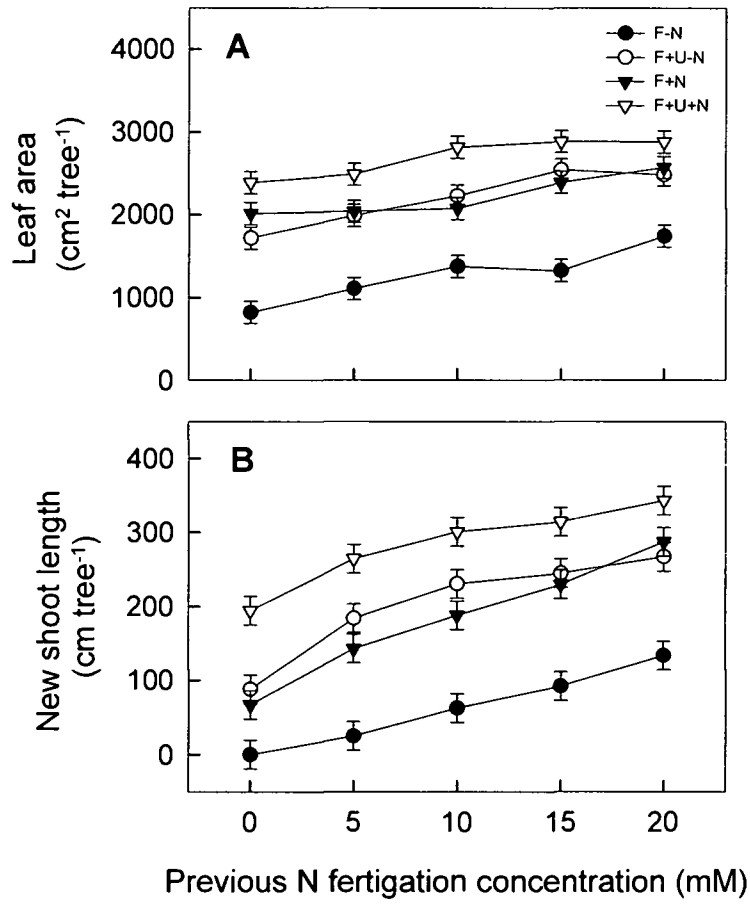

FIG. 3.2. Total (A) leaf area and (B) lengths of new shoots for almond trees receiving no N fertigation (-N) or N fertigation (+N) in spring 2000 in relation to previous N fertigation (F) and foliar urea (F+U) treatments (1999). Each value is the mean of five replicates. Vertical bars represent standard errors. Treatments: N fertigation (F-N), foliar urea (F+U-N), spring-applied ^{15}N to fertigated trees (F+ N), and spring-applied ^{15}N to fertigated trees that received urea (F+U+N).

3.4.3 Use of N reserves

The amount of reserve N used for new shoot and leaf growth increased with increasing N-fertigation concentrations in the previous season, with or without the use of spring fertilizers (Figure 3.3A). Trees receiving foliar urea (F+U-N and F+U+N) used significantly more reserve N ($p<0.05$) for producing new shoots and leaves than did trees gaining N only from fertigation (F-N and F+N). Although foliar urea application increased the amount of N remobilized for new growth, trees fertigated with low concentrations of N were more responsive to urea application than trees fertigated with high concentrations of N (Figure 3.3B, Fertigation concentration x Urea treatment interaction $p<0.05$). There was no significant difference (Figure 3.3A, B: at each N-fertigation concentration, $p>0.05$) in the amount or proportion of N used for new growth between trees receiving N or no N in spring (F+N v F-N; F+U+N v F+U-N). This indicates that the level of N remobilization depends on the amount of nitrogen stored in the plant during the previous year, and is not affected by current-year N fertilization (Millard, 1996; Neilsen et al., 2001b; Cheng and Fuchigami, 2002). Generally, trees with higher N reserves used more of that stored N for new growth than did trees with less accumulated nitrogen (Figure 3.3C). Enhancing the level of N reserves via either N fertigation during the growing season or foliar urea applications in the fall of that season increased the amount of N remobilized for new growth the following spring.

For several deciduous tree species, nitrogen taken up late in the season contributes more to storage and subsequent remobilization for new growth in the

following spring than does N taken up earlier in the season (Weinbaum et al., 1984b; Millard, 1996; Tagliavini et al., 1999). In our experiment, almond nursery trees sprayed with fall foliar urea used significantly more N from reserves for new growth than did those having the same total amount of N but which had been treated only with N fertigation (Figure 3.3C). One possible explanation for this phenomenon is that the increase in plant growth associated with early-season nitrogen fertigation may have resulted from a greater proportion of N being used for structural growth. This would have meant less N in storage and, consequently, less remobilization for new growth the following spring. In contrast, the N received from foliar urea applied after terminal bud set may not have been used for building the tree's structure, but rather was stored in non-structural more readily available form of nitrogen. We have found that a greater proportion of N was accumulated as free amino acids in trees sprayed with fall foliar urea compared with those receiving only N fertigation (Bi et al., unpublished).

3.4.4 Uptake and use of N from fertilizer

The percentage of N in the new shoots and leaves that was derived from ^{15}N-NH_4NO_3 applications (N derived from fertilizer, or NDFF) in the spring decreased with the increasing levels of nitrogen accumulated in plants from the previous year (Figure 3.4A, B). For every 100mg increase in total N in a plant, the NDFF in new shoots and leaves decreased by approximately 5%. This indicates that the N status of the tree affected N uptake; trees with lower N reserves took up more nitrogen from the current-year fertilizer, similar to the results described for mature citrus trees by

Feigenbaum et al. (1987). Likewise, the amount of N derived from spring fertilizer that was used for new growth declined with increasing reserve-N levels in the plants (Figure 3.5). For every 100 mg decrease in total N accumulated during the previous year, trees compensated by taking up 13 mg of nitrogen for new shoot and leaf growth.

In our experiment, almond trees with low N reserves use nitrogen primarily from spring N fertilization to promote shoot and leaf development. This observation differs from that reported in a study on pears (Cheng et al., 2001), in which new growth of young pear trees during the first 70 d after transplanting depended mainly upon N reserves, rather than current uptake from available N sources in the soil. This variation between pears and almonds may result because the latter have more vigorous vegetative growth in early spring.

In conclusion, new growth on almond nursery trees in the spring is affected both by their levels of N reserves and by spring N fertilization. Efforts to increase fall N reserves as well as supplying plants with nitrogen in the spring can improve the growth of almond nursery trees. Moreover, for trees with low N reserves, spring-applied nitrogen fertilizer is particularly important for promoting new growth.

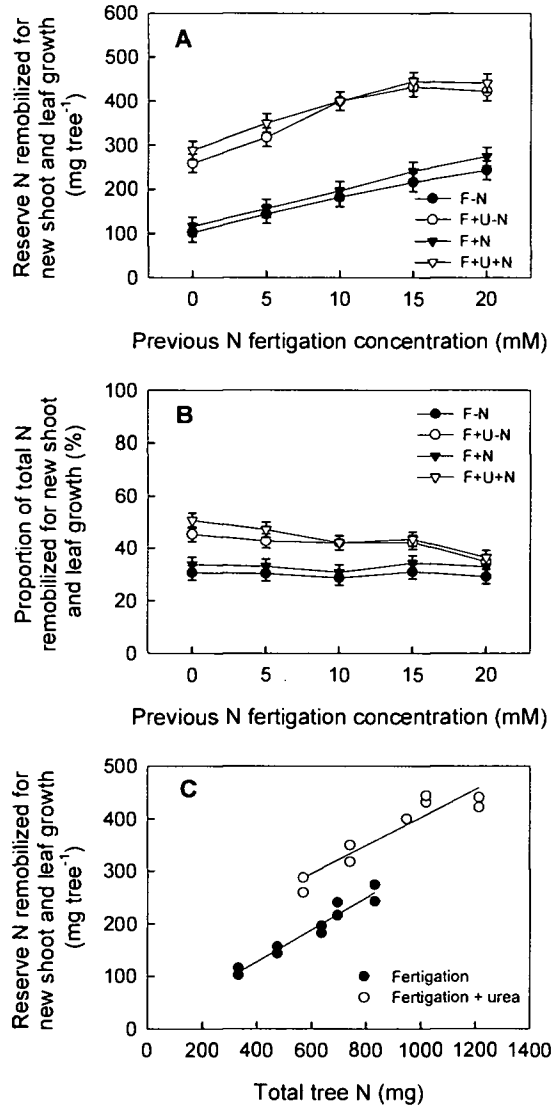

FIG. 3.3. (A) Reserve nitrogen remobilized for new shoot and leaf growth in 2000 and (B) proportion of total N accumulated during previous growing season (1999) that was remobilized for new shoot and leaf growth in 2000 in relation to previous N fertigation and foliar urea treatments. (C) Reserve nitrogen remobilized for new shoot and leaf growth in 2000 in relation to total-tree N accumulated during the previous growing season (1999). Regression equations: Fertigation + urea (Trees treated with fertigation and fall foliar urea in 1999, receiving N or no N in Spring 2000): $Y= 135.4 + 0.267x$, $r^2 = 0.871$; Fertigation (Trees fertigated in 1999, receiving N or no N in Spring 2000): $y = 5.75 + 0.306x$, $r^2 = 0.941$. Each value is the mean of five replicates. Vertical bars represent standard errors. Treatments: N fertigation (F-N), foliar urea (F+U-N), spring-applied ^{15}N to fertigated trees (F+ N), and spring-applied ^{15}N to fertigated trees that received urea (F+U+N).

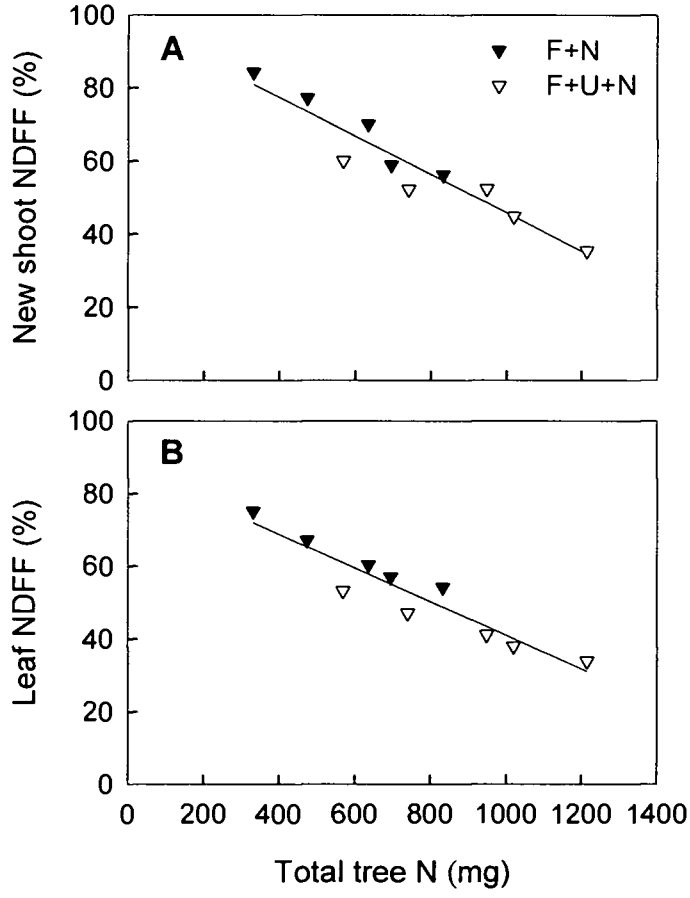

FIG. 3.4. Nitrogen derived from fertilizer (NDFF%) in (A) new shoots and (B) leaves in 2000 in relation to total N accumulated in the tree during the previous growing season (1999). Each value is the mean of five replicates. Regression equations: (A) $Y = 98.40 - 0.053x$, $r^2 = 0.899$; (B) $Y = 87.27 - 0.046x$, $r^2 = 0.893$. Treatments: spring-applied ^{15}N to fertigated trees (F+ N), and spring-applied ^{15}N to fertigated trees that received urea (F+U+N).

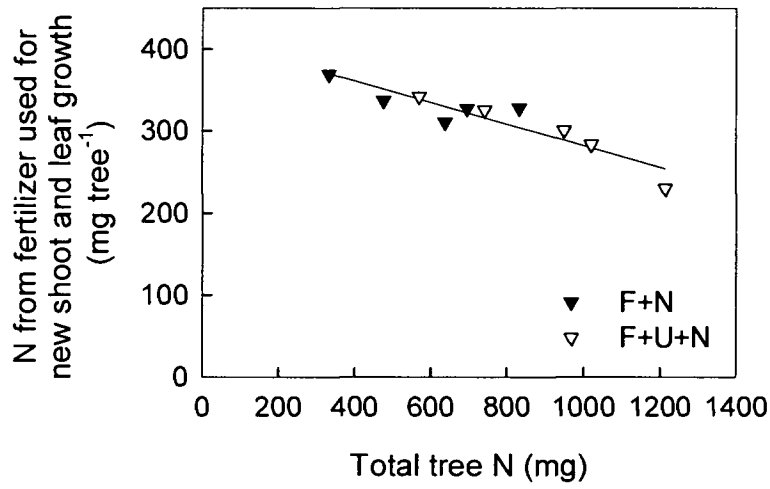

FIG. 3.5. Uptake of ^{15}N from fertilizer used for new shoot and leaf growth in 2000 in relation to total amount of N accumulated in the tree during the previous growing season (1999). Each value is the mean of five replicates. Regression equation: $Y = 414.1 - 0.132x$, $r^2 = 0.845$. Treatments: spring-applied ^{15}N to fertigated trees (F+ N), and spring-applied ^{15}N to fertigated trees that received urea (F+U+N).

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CHAPTER 4

EFFECTS OF SPRING SOIL NITROGEN APPLICATION ON NITROGEN REMOBILIZATION, UPTAKE, AND PARTITIONING FOR NEW GROWTH IN ALMOND NURSERY PLANTS

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4.1 Abstract

One-year-old 'Nonpareil' almond (*Prunus dulcis* (Mill) D. A. Webb) trees on 'Lovell' rootstocks were used to evaluate the effects of soil nitrogen (N) availability in the spring on N remobilization, uptake, partitioning, and tree growth. After being transplanted to an N-free medium, the trees received a modified Hoagland solution, with or without N from ^{15}N-depleted NH_4NO_3, twice a week for 12 weeks. During the first four weeks, the N used for new shoot and leaf growth mainly came from the nitrogen that had accumulated in storage tissues. No significant differences were seen in the amount and duration of N remobilization between N-fertilized trees and those that received no N. However, trees that were fertilized in the spring had significantly more new shoot and leaf growth. Uptake of ^{15}N by the roots began two weeks after transplanting. Nitrogen was rapidly taken up from the soil during the period of greatest shoot and leaf growth; leaves were the major sink for N from both root uptake and remobilization. Six weeks after transplanting, the whole-tree N content was significantly higher in fertilized trees than in unfertilized trees. We conclude that the remobilization of N for spring new growth takes place irrespective of the current-year external N supply. However, the new growth in young almond trees is highly dependent on soil N availability, which demonstrates the importance of spring N fertilizer applications following transplantation.

4.2 Introduction

In deciduous fruit trees, the nitrogen (N) used for new growth in the spring may come from two sources: N stored the previous year and that which is taken up

from the soil during the current growing season. The internal remobilization from storage tissues provides the nitrogen needed for early new growth before significant root uptake occurs (Taylor and May, 1967; Titus and Kang, 1982; Millard and Neilsen, 1989). However, as the season progresses, root uptake of nitrogen plays a more important role in satisfying the tree N demand (Weinbaum et al., 1984a; Sanchez et al., 1990; Rufat and DeJong, 2001). N uptake by roots is also affected by environmental factors such as temperature, soil texture, etc. (Weinbaum et al., 1987; Neilsen et al., 2001a; Dong et al., 2001). For example, in apple trees, a combination of soil temperature and plant developmental stage influences the uptake and use of soil nitrogen in the spring (Dong et al., 2001). The timing of demand for root-supplied N may depend on whether flowering occurs (Neilsen et al., 2001b). In mature almond trees, the need for nitrogen that is triggered by the presence of fruits may also be involved in regulating its uptake (Weinbaum et al., 1984b).

Spring applications of N can help to satisfy the tree's demand, thereby improving growth and development. Faust (1989) has reported that this practice may enhance flower-bud sizes in apple trees, while Neilsen et al. (2001b) have shown that early applications of N during the first year of growth increases the amount of flowers, spur leaves, and bourse shoots in the following year. Moreover, when marginally N-deficient peach trees are supplied with N in early April, without having been treated with N fertilizer in the fall of the previous year, they exhibit vegetative growth, fruit size, and yield comparable to those trees that are supplied with soil N the previous fall (Niederholzer et al., 2001).

Compared with mature trees, nursery trees may be more dependent on the uptake of N from the soil because of their smaller size, limited storage reservoirs, and vigorous vegetative growth. Exogenous application of nitrogen to young peach trees early in the growing season has been shown to enhance vegetative growth (Taylor and May, 1967); the dry matter of their new shoot and leaves is greater than that measured from non-fertilized trees (Niederholzer et al., 2001). However, for the pear, supplying plants with N in spring only slightly increases new shoot and leaf growth for the first 70 d after budbreak (Cheng et al., 2001).

Seasonal N uptake, demand, utilization and cycling in fruit trees have been studied extensively. However, there is at present little research on almond nursery trees. Understanding the effects of the soil-N supply in the spring on N remobilization, uptake, and vegetative growth in young almond trees is important for optimizing the timing of fertilizer applications to meet tree uptake and demand. Therefore, the objectives of this study on one-year old almond trees were to (1) determine whether soil N application alters N remobilization; (2) quantify the effects of soil N availability in the spring on N uptake, distribution, and new growth; and (3) determine the most efficient time for spring soil N applications.

4.3 Materials and Methods

4.3.1 Experimental design

One year old 'Nonpareil' almond (*Prunus dulcis* (Mill) D. A. Webb) trees on 'Lovell' rootstocks were removed from cold storage and transplanted on 20 April, 2001, into 7.6 l polyethylene pots containing a 1:2 (v:v) mix of perlite and vermiculite.

Before transplanting, five trees had been randomly selected and divided into root and stem portions to measure their N contents and biomass. The transplanted trees were grown outdoors under natural conditions in Corvallis, Oregon, USA. Uniform trees were selected for our experimental treatments based on height and stem-diameter measurements, and 30 trees were randomly assigned to one of two groups. Beginning the second day after transplanting, the trees were fertigated twice a week for 12 weeks. One group was supplied with 400 ml of an N-free modified Hoagland solution (-N treatment); the other received 400 ml of a modified Hoagland solution containing 10mM ^{15}N-depleted NH_4NO_3 (0.03% ^{15}N abundance; ISOTEC, Miamisburg, OH) (+N treatment). Five trees from each treatment were then randomly selected and harvested every two weeks during the experimental period. Buds started to open 10 d after transplanting. Some leaves were visible but no measurable new shoot growth was present on either the first or second harvest date. The trees sampled on those two dates were separated into leaf, stem, and root portions. For the remainder of the harvest dates, the samples were divided into leaves, new shoots, stems, and roots. All samples were washed in DD water and oven dried. The dry weight was recorded for each tissue. The samples were then ground to pass a 20-mesh screen in a Wiley mill and reground to pass a 60 mesh screen in a cyclone mill for determination of total N and ^{15}N.

4.3.2 Analysis of samples

Total-N concentrations were determined with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973). The atom% ^{15}N in the samples was

determined by mass spectrometry, and the percentage of nitrogen derived from the labeled fertilizer (NDFF%) was calculated as:

$$NDFF\% = \frac{(atom\%^{15}N)_{natural.abundance} - (atom\%^{15}N)_{tissue}}{(atom\%^{15}N)_{natural.abundance} - (atom\%^{15}N)_{fertilizer}} x100\%$$

The ^{15}N content in each tissue was calculated from NDFF% and tissue total-N content. The total amount of ^{15}N taken up per tree was calculated by pooling the ^{15}N contents calculated for each tissue. The total N content in the new shoots and leaves from non-N fertigated trees was regarded as the amount of N remobilized from their storage tissues. The difference between total- and labeled- ^{15}N content of the new shoots and leaves from N-fertigated trees was used to estimate the amount of nitrogen they had remobilized.

4.3.3 Statistical analysis

The experimental design was completely randomized, with five replicates per treatment per harvest date. Data for dry weights and N contents were analyzed using a two-factor ANOVA model, with the main effects being time after planting and N treatment (+N and –N). Comparisons of the means at different harvest dates with the means measured at the beginning of the experiment were performed using t-tests, adjusting for multiple comparisons via Dunnett's method. Comparisons of means at individual dates between fertilized and non-fertilized plants were performed with t-tests, adjusting for multiple comparisons using Tukey's method. All statistical analyses were conducted with SAS (SAS Institute Inc., Cary, N.C.).

4.4 Results and Discussion

4.4.1 Plant growth

The seasonal patterns of growth were similar in N-fertilized (+N) and non-fertilized (-N) trees (Figure 4.1). Total-tree dry weights increased slowly during the first four weeks after transplanting (Figure 4.1A). Significant increases ($p<0.0001$) in total-tree dry weights were not detectable until six weeks after transplanting for both fertilized and non-fertilized trees. Thereafter, increases were rapid until the end of the experiment. Significant differences ($p<0.0001$) in total-tree dry weights between fertilized and non-fertilized trees were detectable eight weeks after transplanting. The rate of dry-weight accumulation varied between +N and –N trees over time (Figure 4.1B), with amounts accumulated in the first 12 weeks being 48% greater in the former (1.15 mg versus 0.77 mg $tree^{-1}$ day^{-1}). This suggests that, starting four weeks after transplanting, the fertilized trees not only accumulated more dry weight, but also at a faster rate.

Dry weights of leaves (Figure 4.2A), new shoots (Figure 4.2B), roots (Figure 4.2C), and stems (Figure 4.2D) also varied with time for the fertilized and non-fertilized trees. New leaves grew more rapidly during 6-10 weeks after transplanting in the +N compared to -N trees. The new shoot growth was more rapid in the +N than -N trees between 8-10 weeks after transplanting. Supplying plants with N in the spring increased the growth of new shoots, leaves, and stems, as seen in the 34% greater dry weights measured from fertilized trees by the end of the experimental period. This demonstrated relationship between growth and current-year N supply is

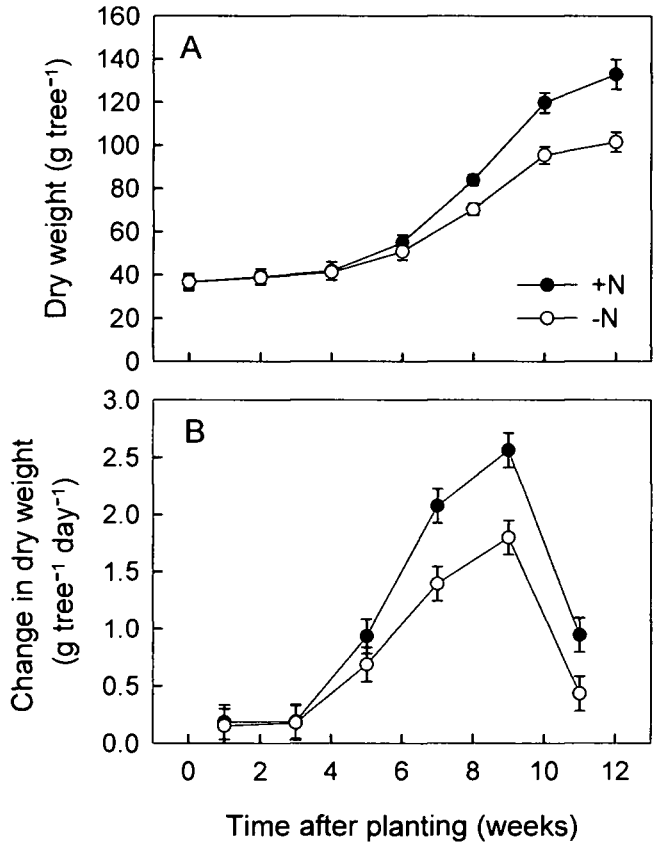

FIG. 4.1. Total tree dry weight (A) and change in total tree dry weight (B) of young almond trees fertigated with nitrogen (N) (+N) or without N (-N) in the spring. Each data point represents the least squares mean; error bars are standard errors of the mean of five replicates.

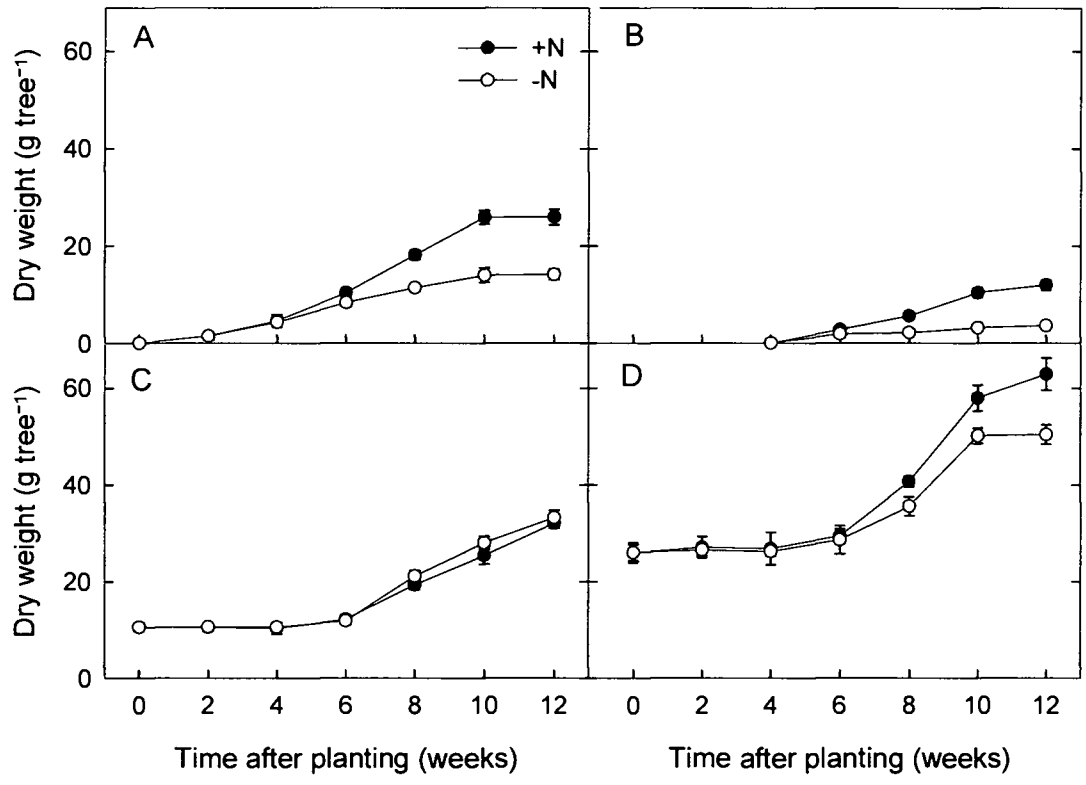

FIG. 4.2. Dry weight of leaves (A), new shoots (B), roots (C), and stems (D) of young almond trees fertigated with nitrogen (N) (+N) or without N (-N) in the spring. Each data point represents the least squares mean; error bars are standard errors of the mean of five replicates.

consistent with results reported for peaches and other tree fruit species (Oland, 1959; Niederholzer et al., 2001; Rufat and DeJong, 2001).

Compared with the performance of above-ground tissues, root growth showed differential responses to N fertilization (Figure 4.2C), with dry weights from fertilized trees being slightly, but non-significantly ($p>0.05$), lower than those of the roots from the controls. Trees that were treated with N had comparable root systems, but supported a larger above-ground biomass than did trees grown without N. Similar results have been reported with apple (Millard and Neilsen, 1989) and pear (Cheng et al., 1998). This partitioning of dry matter may be a mechanism used by plants to optimize the available resources. When ample soil N is available and other factors do not restrict its uptake, a relatively small root system is sufficient. However, when nitrogen levels are low, trees tend to allocate more biomass to the root systems in order to maximize the uptake of available nutrients.

4.4.2 Uptake and translocation of ^{15}N

The appearance of ^{15}N in tree tissues was used to determine when nitrogen uptake from fertilizer started. ^{15}N was detected in the roots two weeks after transplanting (Table 4.1). Significant amount of ^{15}N was measured in plant tissues four weeks after transplanting, indicating that significant uptake of N from the soil began 2-4 weeks after nitrogen was applied. This timing in young almond trees is slightly earlier than that reported for young apple, in which roots begin their uptake approximately three weeks after budbreak (Cheng and Fuchigami, 1997). The presence of ^{15}N in the stems and leaves at two weeks post-transplanting (Table 4.1)

TABLE 4.1. Percentage of nitrogen derived from ^{15}N fertilizer (NDFF%) in leaf, stem, root and new shoot tissues following spring applications of ^{15}N fertilizer to soil of young almond trees.

Weeks after planting	NDFF (%) in tissues			
	Leaves	Stems	Roots	New Shoots[z]
2	0.37±0.19[y]	0.51±0.19	1.01±0.19	
4	15.95±3.09	6.86±1.82	10.44±2.95	
6	39.57±3.29	18.61±1.28	33.56±1.31	45.76±1.91
8	57.36±1.92	34.03±1.52	58.00±3.90	66.70±1.19
10	65.73±3.13	49.96±3.37	70.37±4.68	72.88±3.06
12	69.04±5.32	55.43±4.02	75.85±4.35	78.05±3.41

[z]Shoot tissue from 2001 growing season.
[y]Mean ± the standard error of 5 replicates.

indicates that translocation was rapid to the above-ground portions. Similar results have been found in mature almond trees, where isotopic N is detected in both developing fruit and leaves within two weeks following ^{15}N -fertilizer application to the soil in March (Weinbaum et al., 1984a).

During the first four weeks after transplanting, prior to rapid shoot expansion, the rate of ^{15}N uptake was low. This may have been due to a combination of factors: (1) Low demand for nitrogen because of slower new growth (Figure 4.1 and Figure 4.2); (2) N remobilization from storage tissues, which plays a key role in supporting the growth of deciduous tree fruits in spring (Titus and Kang, 1982; Tromp, 1983; Millard, 1995); (3) root damage due to transplanting (Dong et al., 2003); and (4) environmental factors, such as low soil temperature in early spring decreased rates of N uptake by the roots (Hogue and Neilson, 1986; McMichael and Burke, 1998; Dong et al., 2001).

^{15}N was taken up quickly between four and ten weeks after transplanting, coinciding with a period of rapid growth by the leaves and new shoots. This suggests that the uptake of nitrogen is correlated with the rate of spring new growth. Reports on apple, peach, and mature almonds have also shown that uptake can be affected by high demands for N during the period of vigorous spring vegetative growth (Weinbaum et al., 1984b, 1987; Munoz et al., 1993; Neilsen et al., 2001b).

4.4.3 Distribution of ^{15}N

At two weeks after transplanting, when uptake of ^{15}N was very low, 64% of the ^{15}N taken up by the roots was present in the root tissues; 35% in the stems (Figure

4.3). By six weeks post-transplanting, however, the new shoots had started to rapidly expand, and 57% of the N that had been taken up by the roots was now detected in the leaves, 13% in stems, 22% in roots, and 8% in shoots (Figure 4.3). Leaves, as a major sink, accumulated the highest amount of translocated ^{15}N, an observation that has also been reported with other tree fruit species, e.g., pecan (Kraimer et al., 2001), apple (Neilsen et al., 2001a, 2001b), and peach (Niederholzer et al., 2001). However, for mature fruit trees, the fruit may become a more important sink for N partitioning later in the growing season (Neilsen et al., 2001a; Niederholzer et al., 2001).

4.4.4 Tree N content

Six weeks after transplanting, the total N content from fertilized trees was significantly higher ($p<0.0001$) than from those that received no N (Figure 4.4), and continued to increase rapidly between six and 12 weeks after planting. By the end of the experiment, the total amount of nitrogen was about three times higher in the fertilized trees. Fertilization with N in the spring significantly increased almond nursery tree N content similar to results reported for mature peach trees, applying N in the spring (200 kg N ha^{-1} in April) results in a total tree N content that is nearly double that measured in non-fertilized trees (Rufat and DeJong, 2001).

Leaf-N content began increasing in both treated and control trees between two and four weeks after transplanting (Figure 4.5A). Although the fertilized trees started accumulating N from both reserves and soil for new leaf growth during that early period (Figure 4.5A), new shoots did not start their accumulation until four to six weeks after transplanting (Figure 4.5B). In contrast, the leaves and new shoots of

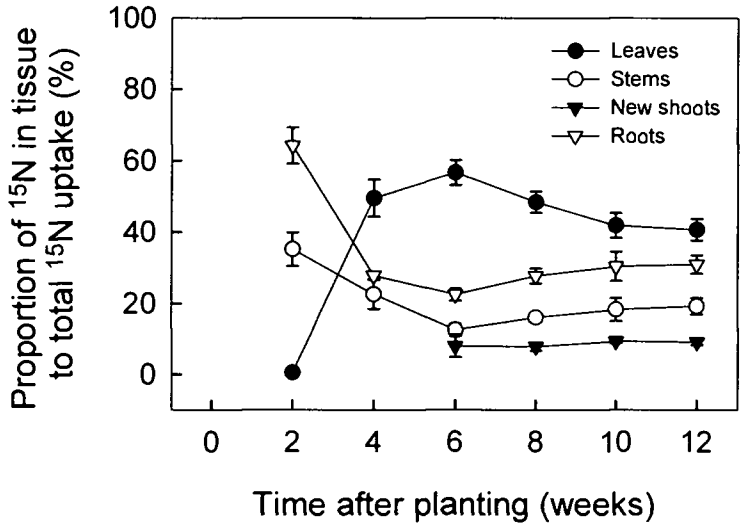

FIG. 4.3. Distribution of ^{15}N in leaves, stems, new shoots and roots following ^{15}N fertigation of young almond trees in the spring. Each data point represents the least squares mean; error bars are standard errors of the mean of five replicates.

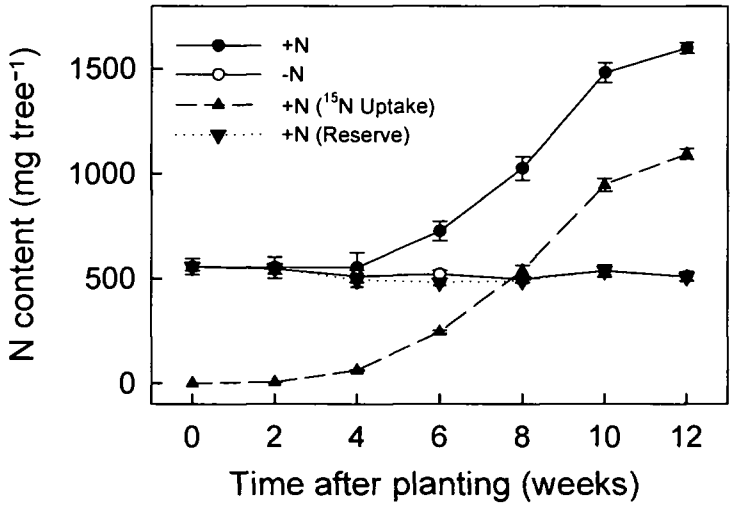

FIG. 4.4. Total-tree N content of young almond trees fertigated with nitrogen (N) (+N) or without N (-N) in the spring. Each data point represents the least squares mean; error bars are standard errors of the mean of five replicates. +N (^{15}N uptake) = total-^{15}N content in trees that were fertigated with N. +N (Reserve) = difference between the total-N and labeled-^{15}N contents in trees that were fertigated with N (= reserve N remobilized in N-fertigated plants).

non-fertilized trees had to depend entirely on N reserves. Six weeks post-transplanting, when rapid shoot elongation occurred, the amount of N used for shoots was higher in the fertilized trees (Figure 4.5B). In the control plants, a higher proportion of reserve N was used to promote new leaf growth, coming at the expense of shoot development.

In the first 2-6 weeks, N content in the roots (Figure 4.5C) and stems (Figure 4.5D) decreased, prior to rapid shoot elongation. Remobilized nitrogen from storage in the roots and stems was used primarily in the new leaves. Six to 12 weeks after transplanting, the N content in the roots and stems of fertilized trees increased in conjunction with an increased uptake rate from the soil. In contrast, the N content for stems of our control trees remained relatively constant during that time when no N was being supplied externally and little was being remobilized for new growth. However, in the same period, the N content in roots of trees receiving no fertilizer was slightly higher. For them, some of the nitrogen that had been remobilized from the roots to support new above-ground growth may have been mobilized back to the roots to stimulate their development. Similar observations have been made with one-year-old apple (Millard and Neilsen, 1989), in which N-deficient plants withdraw nitrogen from their leaves to reinvest to the roots after the initial phase of leaf growth. This appears to be a plant response mechanism in N-deficient environments.

4.4.5 N remobilization

During the first four weeks after transplanting, the nitrogen used for new growth by our almond trees mainly came from the N that had been stored in the roots

and stems (Figure 4.5). As uptake increased, the release of stored N decreased and ended approximately six weeks after transplanting. By this point, rapid shoot elongation had commenced. In all, about half the total tree N detected at transplanting time was remobilized for new growth (primarily leaves). The total amount and the duration of N remobilization did not differ significantly between the +N and -N fertigated trees (Figure 4.4). Stored N was utilized for new growth irrespective of the externally available nitrogen. Moreover, the amount that was remobilized depended mainly on the amount that had been stored, and was unaffected by the current N supply. This result is similar to the pattern of spring N remobilization reported for other tree fruit species (Millard and Neilsen, 1989; Millard, 1996; Neilsen et al., 1997).

Numerous studies have described the remobilization of nitrogen in deciduous tree fruits. Newly planted apple trees mainly use stored N for new growth for 35 to 55 d after planting (Neilsen et al., 2001b). Approximately 50 to 55% of the nitrogen in dormant young apple trees is used to support new growth (Neilsen et al., 2001b; Cheng and Fuchigami, 2002). The remobilization of N in young, fruiting apple trees ends by five days after full bloom (Neilsen et al., 1997). In peach trees, all N used for new growth during the first 25 to 30 d of the growing season came from storage; remobilization continues until about 75 d post-anthesis (Rufat and DeJong, 2001). With young almond trees, we found that the amount of stored N was limited due to the small tree size, and was insufficient to achieve maximum new growth. Therefore, the supply of readily accessible stored N was exhausted by the end of six weeks after

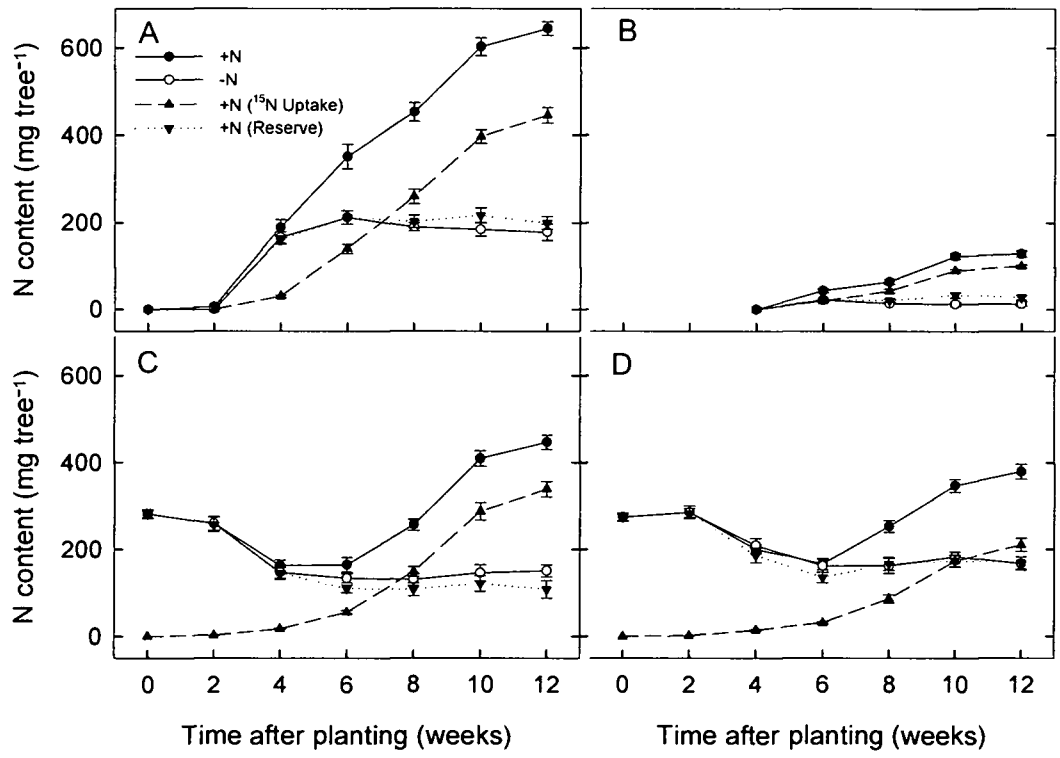

FIG. 4.5. N content of leaves (A), new shoots (B), roots (C), and stems (D) of young almond trees fertigated with nitrogen (N) (+N) or without N (-N) in the spring. Each data point represents the least squares mean; error bars are standard errors of the mean of five replicates. +N (^{15}N uptake) = total-^{15}N content in trees that were fertigated with N. +N (Reserve) = difference between the total-N and labeled-^{15}N contents in trees that were fertigated with N (= reserve N remobilized in N-fertigated plants).

transplanting, when the rate of new shoot elongation was greatest. Although that earliest growth was related to the level of nitrogen reserves, development after those first six weeks was highly dependent on external N. When an exogenous nitrogen source was not available after the stored-N supply was nearly exhausted, the new growth was affected. This was manifested by typical N-deficiency symptoms, which were observed later in the growing season.

Compared with other tree fruit species, almond is a heavy consumer of nitrogen (Weinbaum et al., 1987), with an estimated 50% of the tree N being replaced annually from the soil in mature almond trees (Weinbaum et al., 1987). Despite the presence of an endogenous pool of previously assimilated N, mature almond trees appear to be highly dependent on the annual availability of soil N (Weinbaum et al., 1984a). Our data for young almond trees support this observation, and can be used to emphasize the importance of having nitrogen available to promote plant new growth in this species.

In conclusion, spring soil application of nitrogen fertilizer significantly enhances new shoot and leaf growth in young almond trees. Uptake of N from the soil begins two weeks after its application, and is correlated with the rate of new growth. Maximum uptake occurs during the period of rapid new growth. Leaves are the major sink for nitrogen from both stored N and that taken up by the roots, and shoot growth is severely inhibited when nitrogen fertilizer is not provided. Trees that are fertilized in the spring have N contents that are approximately three times higher than that measured in untreated trees. Therefore, our results suggest that applying N fertilizer in

the spring, during the period of rapid new growth, can significantly improve vegetative growth and the N status in young almond trees.

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CHAPTER 5

SOIL AND FOLIAR NITROGEN SUPPLY AFFECTS THE COMPOSITION OF NITROGEN AND CARBOHYDRATES IN YOUNG ALMOND TREES

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5.1 Abstract

June-budded 'Nonpareil/Nemaguard' almond (*Prunus dulcis* (Mill) D. A. Webb) trees were fertigated with one of five nitrogen (N) concentrations (0, 5, 10, 15, or 20 mM) in a modified Hoagland's solution from July to September. In October, the trees were sprayed twice with either water or 3% urea, then harvested after natural leaf fall and stored at 2°C. Trees were destructively sampled during winter storage to determine their concentrations of amino acids, protein, and non-structural carbohydrates (TNC). Increasing N supply either via N fertigation during the growing season or with foliar urea applications in the fall increased the concentrations of both free and total amino acids, but decreased their C/N ratios. Moreover, as the N supply increased, the proportion of nitrogen stored as free amino acids also increased. However, protein was still the main form of N used for storage. The predominant amino acid in both the free and the total amino-acid pools was arginine. Arginin N accounted for an increasing proportion of the total N in both the free and the total amino acids as the nitrogen supply was increased. However, the proportion of arginine N was higher in the free amino acids than in the total amino acids. A negative relationship was found between total amino acid and non-structural carbohydrate concentrations, suggesting that TNC is increasingly used for N assimilation as the supply of nitrogen increases. Urea applications decreased the concentrations of glucose, fructose, and sucrose, but had little influence on concentrations of sorbitol and starch. We conclude that protein is the primary form of storage N, and that arginine is the predominant amino acid. Furthermore, the

synthesis of amino acids and proteins comes at the expense of non-structural carbohydrates.

5.2 Introduction

Deciduous fruit trees store nitrogen and carbohydrates in the previous year, and then remobilize these accumulated compounds for new growth in the following growing season. This internal cycling provides the structural components and energy needed for early new growth (Taylor and May, 1967; Titus and Kang, 1982; Tromp, 1983; Oliveira and Priestley, 1988; Millard and Thomson, 1989; Loescher et al., 1990; Millard, 1995, 1996; Neilsen et al., 2001; Cheng and Fuchigami, 2002; Bi et al., 2003).

Non-structural carbohydrates include an insoluble starch fraction and a soluble sugar fraction, i.e., sucrose, glucose, fructose, and sorbitol (Oliveira and Priestley, 1988). Similar to stored carbohydrates, N reserves are composed of a soluble fraction that includes amino acids and amides, as well as an insoluble protein fraction (Taylor, 1967; Oliveira and Priestley, 1988). It is somewhat controversial as to whether proteins or free amino acids serve as the main storage form for nitrogen. Previous research with apple (Oland, 1959), peach (Taylor and May, 1967), and pear (Taylor et al., 1975) has shown that storage organs and dormant vegetative tissues contain a high proportion of their N in soluble forms, mainly free amino acids, with only a low proportion in proteins. Based on those studies, free amino acids would appear to be the primary source of nitrogenous reserves. However, more evidence suggests that protein may be the main form of N storage in the dormant tissues of apple and poplar

trees (Tromp, 1970; Tromp and Ovaa, 1971; Shim et al., 1973; O'Kennedy et al., 1975; Kang and Titus, 1980; Titus and Kang, 1982, Kang et al., 1982; Coleman et al., 1991). Nevertheless, no tests had yet been reported on identifying the main form of storage N (protein v free amino acid) in young almond trees.

Arginine is, theoretically, the most efficient form of storage N because of its low C/N ratio (Titus and Kang, 1982). Free arginine has been found to be the principal constituent of extracts from dormant apple (Oland, 1959; O'Kennedy et al., 1975), peach (Taylor and May, 1967; Taylor and van den Ende, 1969), and poplar (Sagisaka, 1974). It is also the predominant amino acid in the proteins of apple trees with high levels of stored nitrogen (Tromp and Ovaa, 1973). Glutamine and asparagine are other important N constituents measured in dormant apple (Oland, 1959), but not in peach (Taylor and May, 1967). Although the level of arginine in woody tissues of dormant trees is considered the most sensitive indicator of tree-N status (Taylor and May, 1967), it is not known how the pool of amino acids in storage responds to different nitrogen supply in young almond trees. Therefore, the objectives of this study were to determine (1) the chemical composition of nitrogen and non-structural carbohydrates in response to different N supplies, and (2) the interaction between storage N and non-structural carbohydrates in young almond trees.

5.3 Materials and Methods

5.3.1 Experimental design

June-budded 'Nonpareil' almond (*Prunus dulcis* (Mill) D. A. Webb) trees, on 'Nemaguard' rootstocks, were planted in 7.6 l pots containing a 1:2:1 (v/v/v) mix of

peat moss, pumice, and sandy loam soil. Trees were then grown under natural conditions in Corvallis, Oregon (44° 30' N, 123° 17' W). Starting from budbreak, the trees were fertigated every two weeks with 150 mg l^{-1} N, using Plantex® 20N-10P_2O_5-20K_2O water-soluble fertilizer with micronutrients (Plantex Corp., Ontario, Canada). On 1 July, the trees were selected for uniformity. Thirty plants were randomly assigned to one of five groups. From 1 July to 1 September, each group was fertigated twice weekly (300 ml per pot) with one of five N concentrations (0, 5, 10, 15, or 20 mM N from NH_4NO_3), using a modified Hoagland's solution (Hoagland and Arnon, 1950).

Fifteen plants from each N fertigation concentration were randomly selected and sprayed with 3% urea on10 and 20 October, (F+U treatment). The remaining plants were sprayed only with water, as our control (F treatment). After natural leaf fall, all the trees were harvested bare-root in December and stored in a cold room at 2°C. Five plants from each treatment were destructively sampled and their stems and root systems were washed with double distilled water to remove any urea residue. All the samples were immediately put into a –80°C freezer for pre-freezing, then freeze-dried and ground to pass a 40-mesh screen.

5.3.2 Carbohydrates and nitrogen analysis

The composition and concentration of non-structural carbohydrates was determined via high performance liquid chromatography. Tissue samples (50 mg) were weighed and extracted three times at 70°C with 3 ml 80% ethanol. Xylitol was added as an internal standard, and the suspensions were centrifuged at 4000 g for 10

min. The extract was passed through ion exchange columns consisting of 1 ml Amberlite IRA-67 (acetate form) (Sigma) and 1 ml Dowex 50W (hydrogen form) (Sigma), then evaporated to dryness at 55°C, and dissolved in 10 ml water. After the appropriate dilution, 25 µl of the extract was injected into a Dionex DX-500 series chromatograph that was equipped with a Carbopac PA-1 column, a pulsed amperometric detector, and a gold electrode (Dionex, Sunnyvale, CA, USA). Carbohydrates were eluted at a flow rate of 1.0 ml min^{-1} with 200 mM NaOH for 15 min. The peak area and the calibration curve derived from the corresponding standard authentic sugar were used to determine individual sugar concentrations. Tissue residue, used for measuring starch content, was dried and digested with amyloglucosidase at 55°C overnight to convert starch to glucose. The concentration of glucose was quantified via the Dionex chromatograph.

To determine the concentration of total amino acids in our samples, 100 mg of tissue was weighed and hydrolyzed in 10 ml 6M hydrochloric acid at 110°C for 22 h (Tromp and Ovaa, 1973). Standard amino acids were added to the sample at the beginning. After cooling and filtration, the volume was brought to 25 ml, an aliquot of which was taken to remove HCl, then dissolved in a citrate buffer (pH 2.2). After proper dilution, 50 µl of the extract was injected into a Beckman 121 automatic amino-acid analyzer, equipped with an FR-10 spherical cation exchange resin column (Beckman Instruments, Inc., Fullerton, CA, USA). To assess the composition and concentration of free amino acids, 200 mg of tissue was weighed and extracted with 20 ml 80% ethanol at room temperature for 24 h. Standard amino acids were added to

the sample before the extraction began. The extract was evaporated at 75°C to dryness after filtration, and was dissolved in a citrate buffer (pH 2.2). The analysis then continued as described above.

Total amino acids were defined as the amino acids present in the samples after protein hydrolysis, and included both free amino acids and protein amino acids. The nitrogen in free amino acids or total amino acids was the sum of N from each individual amino acid. Furthermore, carbon in the free or the total amino acids was the sum of C from each individual amino acid. Carbon in the non-structural carbohydrates was the total of carbon in glucose, fructose, sucrose, sorbitol and starch. The sum of the soluble sugars and starch was considered the amount of total non-structural carbohydrates (TNC).

5.3.3 Statistical analysis

This experiment was a completely randomized design, with five replicates in each treatment. The amino-acid and carbohydrate data were analyzed using analysis of variance (ANOVA). Comparisons of means among treatments were performed by contrasts, adjusting for multiple comparisons using Tukey's method. The relationship between total amino acid and TNC concentrations was determined by linear regression analysis. All statistical analyses were conducted with SAS (SAS Inst. Inc., Cary, N.C., USA).

5.4 Results and Discussion

5.4.1 Free amino acids and total amino acids

The concentration of free amino acids in fertigated almond trees (F) increased with increasing N-fertigation concentration (Figure 5.1A). This was also true for the concentration of total amino acids (Figure 5.1B). Applying foliar urea in the fall significantly increased ($p<0.0001$) the concentrations of both free and total amino acids at each given N-fertigation concentration. However, the concentrations of free amino acids and total amino acids in plants fertigated with lower N concentrations showed a greater response to urea treatment than those fertigated with higher amounts of nitrogen. Foliar urea applications increased the concentrations of free amino acids by 2.139 (20 mM) to 2.916 mg g^{-1}DW (0 mM), as well as the concentrations of total amino acids by 12.720 (20 mM) to 18.530 mg g^{-1}DW (0 mM). The ratio of protein N to free amino-acid N decreased with increasing N-fertigation concentrations when trees did not receive foliar urea (Figure 5.1C). Foliar urea application in the fall significantly decreased ($p<0.0001$) the N ratio at each given fertigation concentration, with trees that received less nitrogen being more responsive. All the trees treated with foliar urea had N ratios of approximately 7.5.

Our data clearly demonstrate that the amino acid concentrations in young almond trees are closely related to N supply. Increasing N supply by N- fertigation during the growing season or applying foliar urea in the fall increased the concentrations of both free and total amino acids during winter storage. The proportion of N stored as free amino acids was also increased when the nitrogen supply was increased by either fertigation or urea. However, protein was still the main form of stored N in our almond trees (Figure 5.1C). These results agree with those

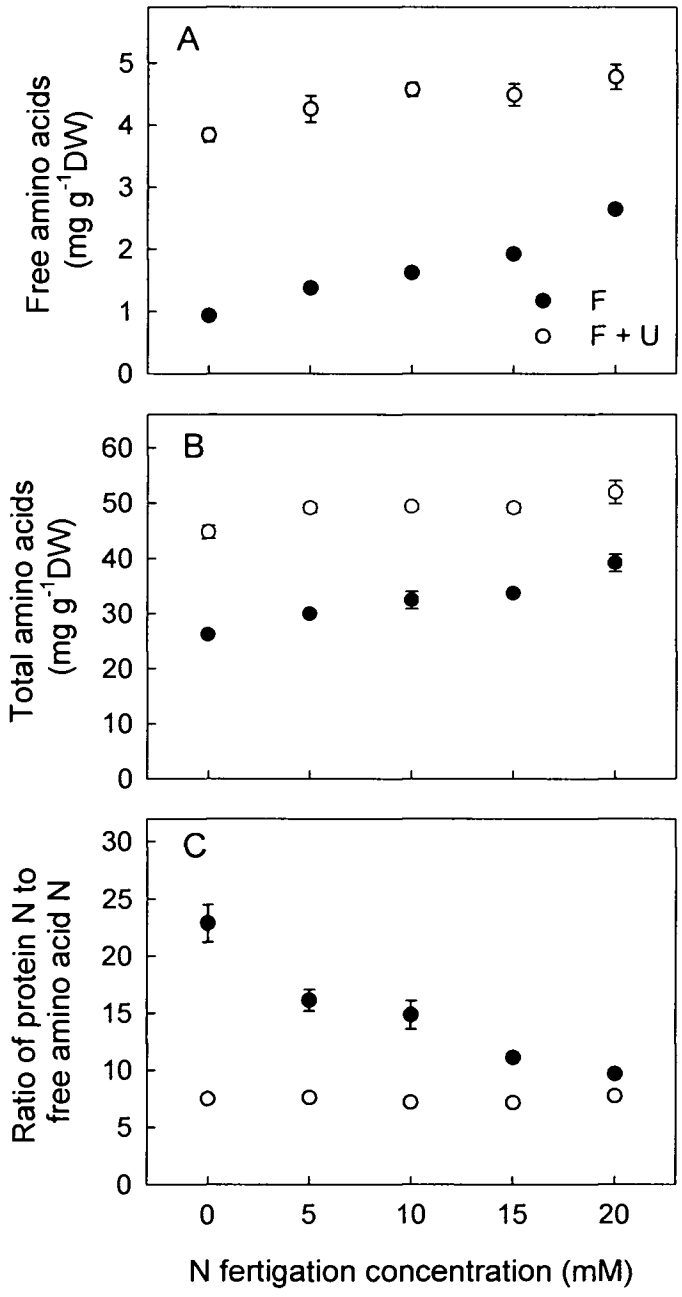

FIG. 5.1. Effects of nitrogen [N] fertigation during the growing season and foliar urea application in the fall on concentrations of free (A) and total amino acids (B), and the ratio of protein N to free amino acid N (C) of young almond trees. Each value is the mean of five replicates. Error bars represent standard errors of the mean for each treatment. F = N fertigation, and F + U = N fertigation and foliar urea treatments.

reported for apple (Tromp, 1970; O'Kennedy et al., 1975; Kang and Titus, 1980) and poplar (Coleman et al., 1991). In contrast, other research has shown that soluble compounds, including amino acids, are the main storage form of N for apple (Oland, 1959), peach (Taylor and May, 1967), and pear (Taylor et al., 1975). This difference may be a consequence of different sampling times and protocols used to extract the various nitrogenous reserves. Protein hydrolysis begins a few weeks before budbreak (Oland, 1959; Hill-Cottingham, 1968; Tromp, 1970; O'Kennedy et al., 1975), resulting in a rapid increase in the level of soluble nitrogen available for plant growth (Kang et al., 1982). In addition, if the samples were collected close to budbreak, the concentration of non-protein N could increase markedly because of protein hydrolysis.

5.4.2 Individual amino acids

On a whole-tree basis, arginine (Arg), glutamate (Glu), proline (Pro), aspartate (Asp), serine (Ser), alanine (Ala), and lysine (Lys) were identified as the main constituents of storage nitrogen in both free and total amino acids; their concentrations increased with the increasing N-fertigation concentrations (Tables 5.1, 5.2). Foliar urea applications generally increased the concentrations of individual amino acids, especially for Arg (Tables 5.1, 5.2), which was the predominant amino acid in both free amino-acid and total amino-acid pools.

The ratio of N in free Arg to that in the free amino acids increased with increasing N-fertigation concentration in trees not receiving foliar urea (Figure 5.2A). Foliar urea application significantly increased ($p<0.01$) the N ratio of free Arg to free amino acids in trees fertigated with 0 and 5 mM N concentrations. All trees treated

with foliar urea had N ratios of approximately 0.7. The ratio of N in total Arg to that in total amino acids also increased with increasing N-fertigation concentrations in trees not receiving the fall urea application (Figure 5.2B). Foliar urea significantly increased ($p<0.0001$) the N ratio at each given level of fertigation, but trees fertigated at low-N concentrations responded more to urea treatment than those receiving higher nitrogen concentrations. All trees treated with foliar urea had N ratios of approximately 0.4.

Our results demonstrate that, in dormant almond trees, Arg is the main amino acid in both the free and the total amino-acid pools. The N found in Arg accounted for an increasing proportion of the nitrogen in the free and total amino acids as the N supply increased. This agrees, in part, with previous findings with apple (Oland, 1959; Tromp and Ovaa, 1973; O'Kennedy et al., 1975) and peach (Taylor and May, 1967; Taylor and van den Ende, 1969). The proportion of arginine N in the free amino acids was also higher than that measured in the total amino acids. We also noted that Glu and Asp served as important constituents of stored N.

5.4.3 C/N ratio

The C/N ratios of free amino acids and total amino acids decreased with increasing N-fertigation concentrations when trees did not receive foliar urea in the fall (Figure 5.3A, B). As the supply of nitrogen increased, N-rich amino acids, e.g., Arg, accounted for an increasing proportion of the free and the total amino acids (Tables I, II; see also Tromp and Ovaa, 1973). This resulted in lower C/N ratios for both free and total amino-acid pools.

TABLE 5.1. Effects of nitrogen (N) fertigation during the growing season and foliar urea application in the fall on the concentration of free amino acids in young almond trees

Amino acid	Free amino acid concentrations (mg g^{-1} DW)									
	Fertigation concentration (mM)									
	0		5		10		15		20	
	F[z]	F+U	F	F+U	F	F+U	F	F+U	F	F+U
Arg	0.279[y]	1.910	0.534	2.132	0.735	2.279	0.887	2.289	1.409	2.323
Glu	0.202	0.270	0.252	0.369	0.209	0.372	0.259	0.400	0.307	0.377
Pro	0.144	0.438	0.203	0.498	0.218	0.523	0.249	0.474	0.309	0.573
Asp	0.078	0.202	0.094	0.205	0.108	0.215	0.099	0.203	0.145	0.206
Ser	0.056	0.548	0.083	0.556	0.101	0.589	0.093	0.598	0.127	0.586
Ala	0.049	0.096	0.062	0.141	0.066	0.139	0.083	0.137	0.084	0.148
Phe	0.024	0.035	0.025	0.033	0.026	0.033	0.027	0.031	0.021	0.040
Met	0.020	0.016	0.025	0.019	0.024	0.016	0.045	0.020	0.033	0.031
Lys	0.016	0.142	0.032	0.144	0.056	0.170	0.071	0.240	0.099	0.261
Tyr	0.015	0.047	0.015	0.056	0.021	0.031	0.025	0.050	0.022	0.074
Ile	0.011	0.011	0.013	0.021	0.019	0.028	0.023	0.053	0.019	0.089
Val	0.008	0.005	0.012	0.014	0.009	0.006	0.011	0.011	0.014	0.024
His	0.006	0.027	0.011	0.039	0.015	0.053	0.018	0.048	0.032	0.052
Leu	0.006	0.028	0.009	0.034	0.012	0.033	0.017	0.033	0.015	0.045
Gly	0.006	0.009	0.007	0.011	0.007	0.011	0.009	0.013	0.009	0.014
Cyr	0.005	0.005	0.006	0.004	0.006	0.004	0.006	0.006	0.006	0.008

[z]F = N fertigation and F+U = N fertigation and foliar urea treatments.
[y]Each value is the mean of five replicates.

TABLE 5.2. Effects of nitrogen (N) fertigation during the growing season and foliar urea application in the fall on the concentration of total amino acids in young almond trees

Amino acid	Total amino acid concentrations (mg g^{-1} DW)									
	Fertigation concentration (mM)									
	0		5		10		15		20	
	F[z]	F+U	F	F+U	F	F+U	F	F+U	F	F+U
Arg	2.082[y]	10.106	3.188	11.802	3.911	12.983	4.469	11.988	6.399	11.880
Glu	3.530	4.766	3.920	5.075	4.058	5.027	4.052	5.081	4.614	5.797
Pro	1.648	2.332	1.883	2.657	1.902	2.662	1.954	2.484	2.153	3.153
Asp	2.950	6.681	3.261	6.568	3.381	6.679	3.310	6.825	3.947	6.800
Ser	1.514	1.836	1.621	2.065	1.659	2.039	1.635	2.106	1.893	2.176
Ala	1.495	1.854	1.602	2.070	1.625	2.006	1.661	2.031	1.809	2.663
Phe	1.204	1.684	1.410	1.835	1.406	1.688	1.479	1.802	1.609	1.898
Met	0.451	0.487	0.453	0.514	0.456	0.512	0.525	0.562	0.431	0.625
Lys	1.995	2.835	2.154	3.082	2.269	2.995	2.273	3.042	2.698	3.117
Tyr	0.776	1.135	0.917	1.236	0.959	1.129	1.028	1.238	1.121	1.314
Ile	1.220	1.497	1.345	1.643	1.342	1.544	1.347	1.577	1.467	1.738
Val	1.628	2.003	1.746	2.318	1.813	2.193	1.916	2.208	1.919	2.481
His	0.912	1.331	1.029	1.441	1.109	1.414	1.108	1.485	1.399	1.534
Leu	2.099	2.709	2.351	2.939	2.404	2.793	2.433	2.957	2.716	3.163
Gly	1.372	1.709	1.507	1.904	1.533	1.812	1.558	1.863	1.754	2.169
Cyr	0.054	0.095	0.078	0.081	0.098	0.109	0.104	0.113	0.116	0.134

[z]F = N fertigation and F+U = N fertigation and foliar urea treatments.
[y]Each value is the mean of five replicates.

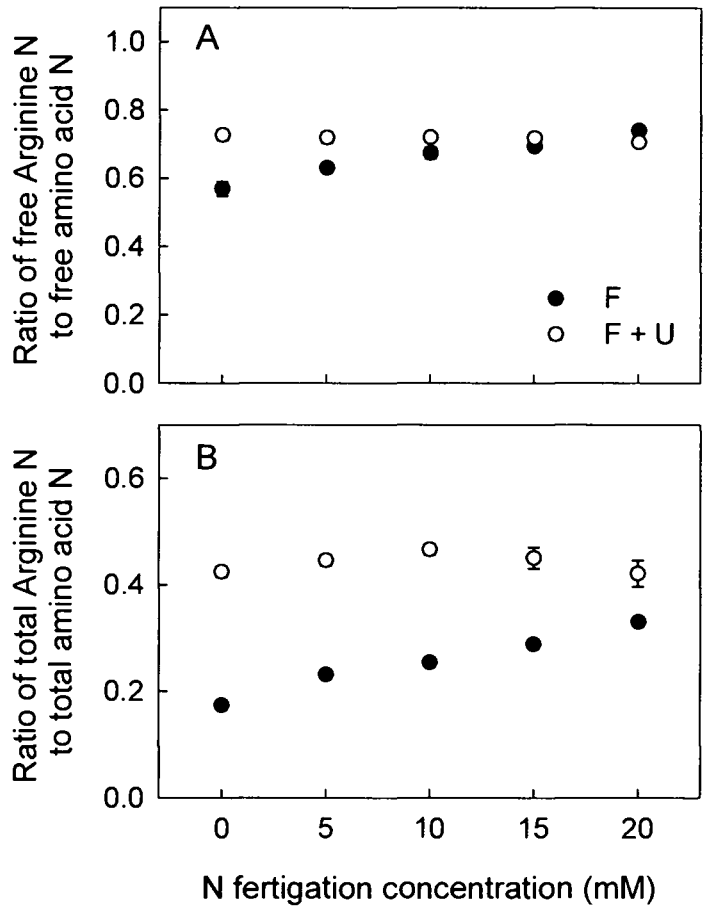

FIG. 5.2. Effects of nitrogen [N] fertigation during the growing season and foliar urea application in the fall on the ratio of nitrogen in free arginine to that in free amino acids (A), and the ratio of nitrogen in total arginine to that in total amino acids (B) of young almond trees. Each value is the mean of five replicates. Error bars represent the standard errors of the mean for each treatment. F = N fertigation, and F+U = N fertigation and foliar urea treatments.

Applying foliar urea in the fall significantly decreased ($p<0.0001$) the C/N ratio of free amino acids in trees fertigated with 0 mM to 15 mM N concentrations, but not the highest nitrogen concentration (20 mM). The C/N ratio in free amino acids was approximately 2.2 for all our urea-treated trees.

Fall applications of foliar urea also significantly decreased ($p<0.0001$) the C/N ratio in total amino acids at each given fertigation concentration. Compared with the ratio determined for the total amino acids (i.e., 3.0), free amino acids had a lower C/N ratio at each given fertigation concentration. This suggests that the proportion of N-rich amino acids, such as Arg, is higher in the free amino-acid pool than in the total amino-acid pool. That lower C/N ratio makes N storage more efficient in terms of carbon investment (Titus and Kang, 1982).

5.4.4 Non-structural carbohydrates

Concentrations of glucose, fructose, and sucrose generally decreased with increasing N-fertigation concentrations, up to 15 mM N (Figure 5.4A, B, C). Foliar urea applications also significantly decreased ($p<0.0001$) their concentrations at all fertigation concentrations. The influence of urea was largest in trees that received the lowest concentration of nitrogen. In contrast, urea treatment significantly decreased ($p<0.01$) the amount of sorbitol only at the two lowest fertigation concentrations (0 and 5 mM N; Figure 5.4D). Starch concentration was slightly higher in trees fertigated at the lowest N concentrations (Figure 5.4E). Foliar urea also significantly decreased ($p<0.0001$) starch concentration at each given fertigation concentration.

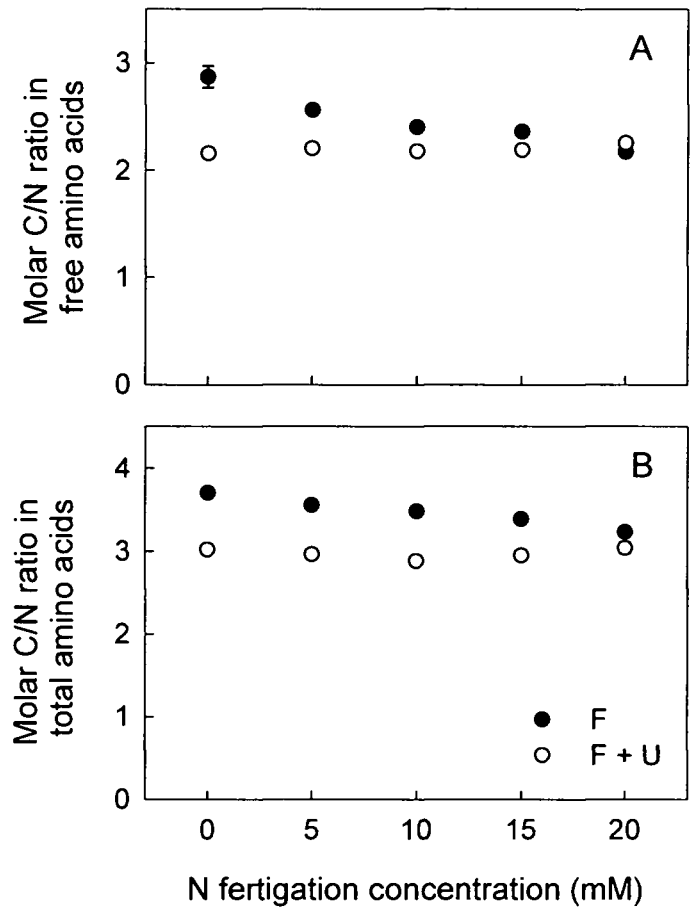

FIG. 5.3. Effects of nitrogen [N] fertigation during the growing season and foliar urea application in the fall on molar Carbon/Nitrogen [C/N] ratio in free (A) and total amino acids (B) from young almond trees. Each value is the mean of five replicates. Error bars represent the standard errors of the mean for each treatment. F = N fertigation, and F+U = N fertigation and foliar urea treatments.

Total non-structural carbohydrates (TNC) showed a response similar to that of starch with the N-fertigation treatments (Figure 5.4F). Foliar urea application in the fall significantly decreased ($p<0.0001$) TNC concentration at each fertigation concentration. These carbohydrates provide energy as well as carbon skeleton for N assimilation and the synthesis of amino acids and proteins (Oliveira and Priestley, 1988). In our study, the concentrations of all TNC components decreased when the N supply was augmented through foliar urea applications. This indicates an increased use of TNC for nitrogen assimilation as the N supply increases. The greater decrease in glucose, fructose, and sucrose concentrations (compared with sorbitol and starch) in response to urea suggests that those first three components are more readily available for N assimilation than the latter two. This can be explained in part by the fact that sorbitol and starch must first be converted to fructose or glucose before being usable.

5.4.5 Interaction between nitrogen and carbohydrates

The concentration of carbon (C) in the total amino acids increased with increasing fertigation concentrations (Figure 5.5A). In contrast, the C in TNC generally decreased as the amount of nitrogen supplied from fertigation was enhanced (Figure 5.5B). Applying foliar urea significantly increased ($p<0.0001$) the C concentration in the total amino acids at each given fertigation concentration, but significantly decreased ($p<0.0001$) the carbon concentration in TNC to a similar level across all fertigation treatments (Figure 5.5A, B). In addition, the concentration of total amino acids was negatively related to TNC concentration (Figure 5.6), with a 1 mg g^{-1} increase in amino acid concentration resulting in a decrease of approximately

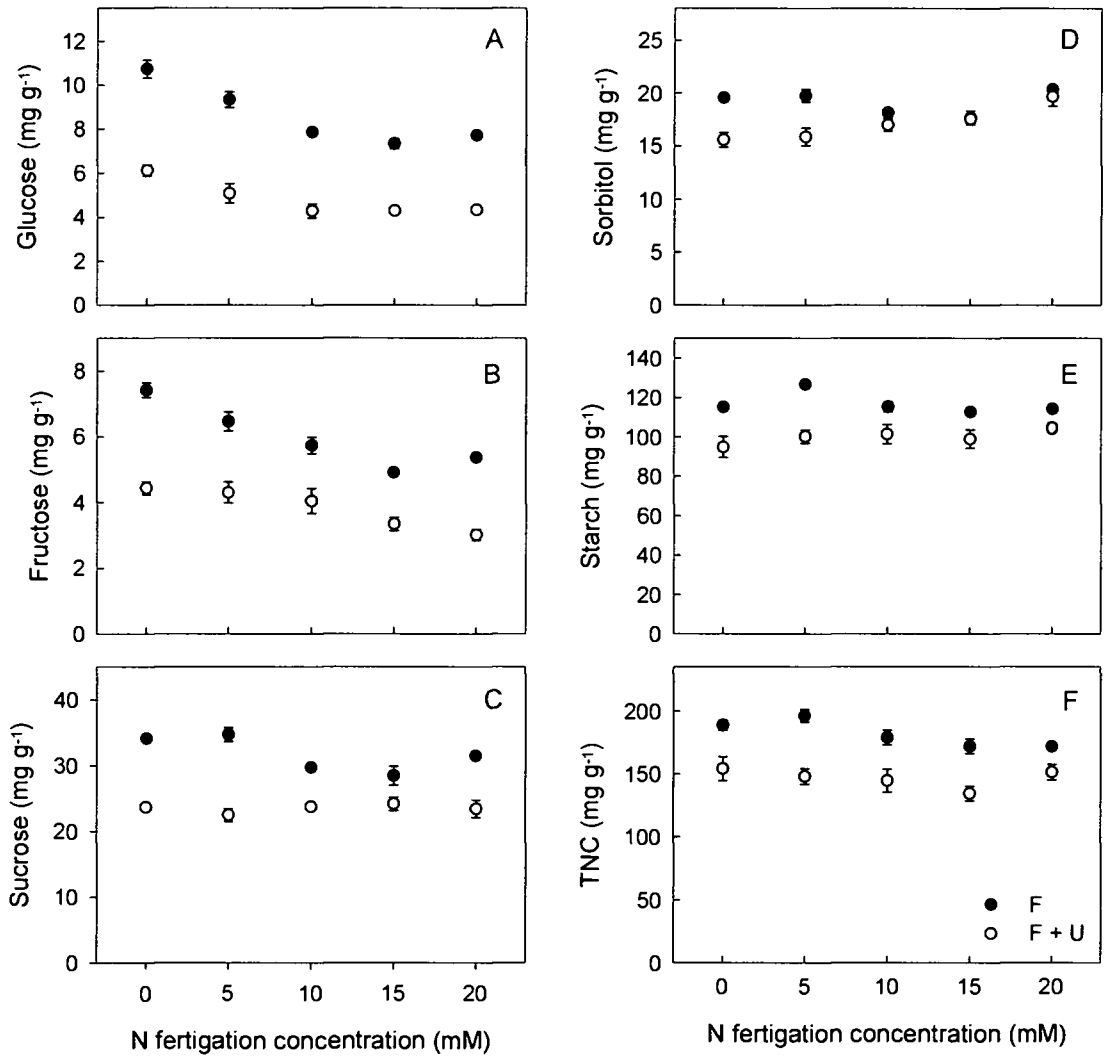

FIG. 5.4. Effects of nitrogen [N] fertigation during the growing season and foliar urea application in the fall on the concentrations (mg g^{-1}DW) of glucose (A), fructose (B), sucrose (C), sorbitol (D), starch (E), and TNC [total non-structural carbohydrates] (F) from young almond trees. Each value is the mean of five replicates. Error bars represent the standard errors of the mean for each treatment. F = N fertigation, and F+U = N fertigation and foliar urea treatments.

2 mg g^{-1} of TNC. These results, therefore, demonstrate that the synthesis of amino acids and proteins for storage in almond trees occurs at the expense of carbohydrates.

In conclusion, young almond trees accumulate nitrogen dynamically in both the amino-acid and protein forms, which are closely related to N supply. Enhancing the amount of available nitrogen through either fertigation during the growing season or foliar urea applications in the fall increases the concentrations of both free and total amino acids in storage. Although the proportion of nitrogen stored as free amino acids increases as the N supply increases, we believe that protein is still the primary form of stored N in dormant young almond trees. Arginine, the predominant amino acid in both free and total amino-acid pools, accounts for an increasing proportion of the pool as the nitrogen supply increased. However, the proportion of arginine N in the free amino acids is higher than that in the total amino acids. In addition, the synthesis of amino acids and proteins occurs at the expense of carbohydrates, and the amount of TNC used for N assimilation increases as the supply of nitrogen increases.

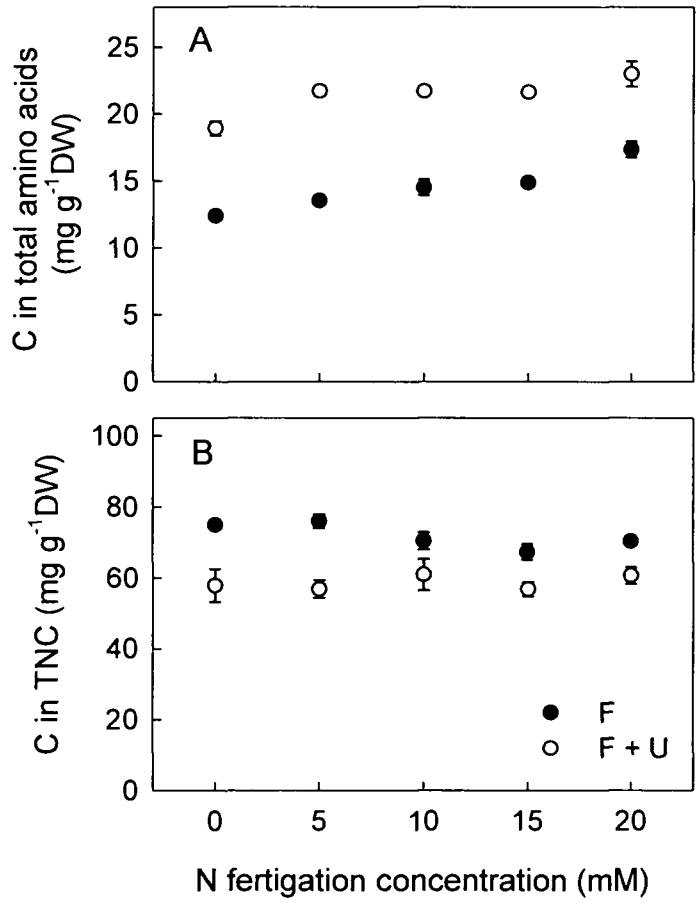

FIG. 5.5. Effects of nitrogen [N] fertigation during the growing season and foliar urea application in the fall on carbon concentrations [C] in total amino acids (A) and in total non-structural carbohydrates [TNC] (B) from young almond trees. Each value is the mean of five replicates. Error bars represent the standard errors of the mean for each treatment. F = N fertigation, and F+U = N fertigation and foliar urea treatments.

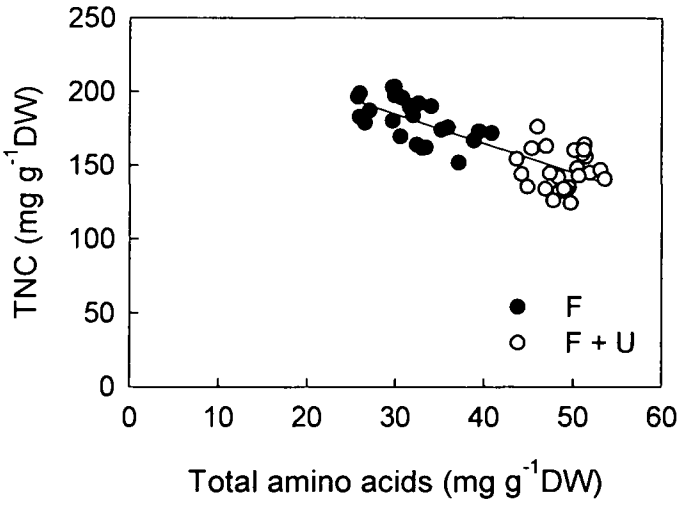

FIG. 5.6. Concentrations of non-structural carbohydrates (TNC) in relation to concentrations of total amino acids from young almond trees. Regression equation: $Y = 243.58 - 1.964X$ ($r^2 = 0.66$, $p<0.0001$). F = N fertigation, and F+U = N fertigation and foliar urea treatments.

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CHAPTER 6

EFFECTS OF DEFOLIANTS (CuEDTA AND $ZnSO_4$) AND FOLIAR UREA ON DEFOLIATION, NITROGEN RESERVES AND REGROWTH PERFORMANCE OF ALMOND NURSERY PLANTS

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6.1 Abstract

One-year-old field-grown 'Nonpareil'/'Nemaguard' almond nursery trees were used to study the effects of defoliants (CuEDTA and $ZnSO_4$) and foliar applications of urea on defoliation, nitrogen (N) reserves, and new growth the following spring in almond nursery plants. Although both chemical defoliants significantly promoted earlier defoliation, CuEDTA was more effective than $ZnSO_4$ in promoting earlier defoliation. Addition of urea to defoliant applications increased efficiency of both $ZnSO_4$ and CuEDTA in promoting earlier defoliation. Plants treated with foliar urea in the fall prior to or with chemical defoliants (e.g. the urea + defoliant treatments) had significantly higher nitrogen reserves compared to the defoliant treatments alone. N reserves were comparable in urea + defoliants treated plants to the levels found in naturally defoliated (control) trees. Compared to natural defoliation, the combination of urea and defoliants, in general, had no significant effect on bud break, or new shoot and leaf growth the following spring. Treatment with urea alone promoted greater new shoot and leaf growth. We conclude that both CuEDTA and $ZnSO_4$ are safe and effective in promoting early defoliation of almond nursery plants. Combining urea with defoliants (e.g. urea + CuEDTA) was effective in promoting early defoliation without a negative effect on subsequent growth the following spring.

6.2 Introduction

Natural defoliation of most deciduous nursery plants in the U.S. Pacific Northwest occurs in late fall or early winter. However, growers of bareroot deciduous nursery plants would like to harvest plants early, before natural leaf fall occurs. Early

harvest avoids the rainy season and problems caused by harvesting plants during wet conditions such as increased plant injury, increased harvest time and costs, and increased disease problems.

Manual stripping of leaves is a common practice in nurseries to allow for early harvesting, but it is time consuming, expensive, and may cause damage to bark and buds (Dozier et al., 1987). An alternative to manual leaf removal is chemical defoliation. The use of chemical defoliants dates back to 1940 when Milbrath et al. used ethylene gas to defoliate roses in storage. Since then, chemical defoliants have received a lot of attention from researchers and growers. A wide range of chemicals have been tested for their defoliating ability on various types of deciduous plants (Fuchigami, 1977; Larsen and Lowell, 1977; Knight 1979, 1983; Insley and Boswell, 1980; Abusrewil and Larsen, 1981; Larsen et al., 1984; Larsen and Fritts 1986; Dozier et al., 1987). Cooper et al. (1968) found that copper-chelate (CuEDTA) caused leaf abscission when used to enhance fruit abscission in citrus. Knight (1983) reported high levels of defoliation with little or no damage from the use of 2.1% spray of CuEDTA on apple and cherry rootstock. Larsen and Fritts (1986) used 0.5% CuEDTA and found about 80% leaf abscission 2 weeks after application to apple, cherry and pear seedlings. CuEDTA promises to be a useful general defoliant. However, further studies need to be done on other plant species before CuEDTA can be recommended to be used on defoliating specific plant material because the responses of different plant species or cultivars to defoliants vary considerably in susceptibility to defoliation and plant damage (Larsen, 1973).

Although chemical defoliation is widely used in many nurseries, there is some concern among nurserymen that the practice might be detrimental to the quality of the nursery stock. Some chemical defoliants are known to cause either physical damage to plant tissues, such as bark and/or bud damage (Larsen and Lowell, 1977), and/or physiological problems associated with storage or establishment, such as shoot dieback and delayed budbreak (Abusrewil and Larsen, 1981; Larsen et al., 1984). Effective and safe chemical defoliation of bareroot deciduous nursery plants prior to harvest has been a major challenge for researchers and growers.

It has been known that the initial growth of deciduous plants in the spring is mainly supported by nitrogen reserves, and there is a positive relationship between N reserves and spring growth in many species (Oland, 1959; Taylor, 1967; Taylor and May, 1967; Taylor et al., 1975; Cheng et al., 2001; Cheng and Fuchigami, 2002). The N mobilized from senescencing leaves makes an important contribution to whole tree N economy (Taylor and May, 1967; Chapin and Kedrowski, 1983). For instance, in apple, about 40-50% of the total tree N is present in leaves in late summer and early fall (Forshey, 1963). A rapid decrease in leaf N content begins 3 to 4 weeks prior to abscission (Oland, 1963). The N withdrawn during leaf senescence constitutes approximately 25% of the total tree N. Therefore, it is important to achieve the maximum mobilization and withdrawal of N from senescing leaves before abscission (Millard, 1995). Maintaining the leaves in the fall is important for maximum mobilization of leaf N to plant storage tissues. However, early defoliation reduces the time leaves stay on the tree, hence, decreasing the amount of N mobilized from leaves

back to plants. This loss of N could affect plant new growth in the following spring. Foliar urea sprays in the fall is an effective way to increase reserve N in deciduous trees and improve new growth the following spring (Oland, 1960, 1963; Shim et al., 1972, 1973; Rosecrance et al., 1998; Tagliavini et al., 1998; Millard, 1996; Reickenberg and Pritts, 1996). If the goal of the nurserymen is to promote early defoliation without reducing the N reserves of the nursery stock, using foliar urea sprays prior to or with a defoliant treatment, theoretically, could be a good strategy to reach this goal, especially for trees with low N status. This paper presents the results of two studies to assess the effects of chemical defoliants (CuEDTA and $ZnSO_4$) with/without urea on defoliation, N reserves, and subsequent spring growth of almond nursery plants.

6.3 Materials and Methods

Experiment 1

A field study was conducted at a commercial nursery in Newcastle, California (39° 03' N, 120° 97' W). One-year old rootstocks 'Nemaguard' almond (*Prunus dulcis* (Mill) D. A. Webb) were planted in the field during February 1999 and t-budded with 'Nonpareil' almond during May 1999. In October 1999, plants were selected for uniformity. Thirty plants were treated with one of seven treatments (Table 6.1). When the spray treatments were made, trees in all experiments were sprayed from the ground to the top until the leaves were slightly past the point of runoff.

The physical changes in trees, such as chemical residue and leaf color were observed, and leaf defoliation percentages were recorded periodically. All the treated

plants were barerooted and harvested after natural leaf fall, and placed in cold storage at 32-35 °F and 95-100% humidity. Twenty trees from each treatment were shipped to Oregon State University (OSU), Corvallis, Oregon in large cardboard boxes with moistened shingletoe placed around the root system. Upon arrival the trees were immediately placed in cold storage at 32-35 °F.

Ten plants from each treatment were removed from cold storage in February and each plant was divided into branch shoots, main stems and roots. All the samples were washed with double distilled (DD) water to remove urea and defoliant residues from surfaces. Tissue samples were oven-dried, and ground with a Wiley mill (20 mesh) for N analysis. The dry weight of each tissue was recorded. The nitrogen concentrations of the branch shoots, main stem and root tissues were analyzed by Kjeldahl analysis.

Ten plants from each treatment were planted in the field at the Lewis-Brown Horticulture farm at OSU (44° 30' N, 123° 17' W) and ten plants from each treatment were planted in the field at the nursery in Newcastle, California the following spring. The growth performance of the field grown trees was evaluated at both the nursery and OSU farm.

Experiment2

A field study was conducted at a commercial nursery in Yuba City, California. The plants used for this study were 'Nonpareil' almond t-budded onto 'Nemaguard' almond seedlings in May 2001. After terminal bud set was observed on most trees (31 Oct. 2001), uniform plants were selected, and 40 trees were sprayed with one of four

Table 6.1. Experimental treatments for two separate experiments assessing the effects of chemical defoliants and urea application on almond nursery trees.

Experiment	Treatment anonym	Description
1 (1999-2000)	CK	sprayed with water (control)
	Cu	0.5%CuEDTA sprayed on 11/17/99
	Zn	1.25% $ZnSO_4$ sprayed on 11/17/99
	U	3% urea sprayed twice on 10/27/99 & 11/8/99
	U+Cu	3% urea sprayed twice on 10/27/99 & 11/8/99 followed by 0.5% Cu-EDTA sprayed on 11/17/99
	U+Zn	3% urea sprayed twice on 10/27/99 & 11/8/99 followed by 1.25% $ZnSO_4$ sprayed on 11/17/99
2 (2001-2002)	CK	sprayed with water (control)
	U+Cu	3% urea sprayed on 11/10/01 followed by 1% Cu-EDTA&3%urea sprayed on 11/20/01
	2Zn	1.7% $ZnSO_4$ sprayed twice on 11/10/01 & 11/20/01
	U	3% urea sprayed twice on 11/10/01 & 11/20/01

foliar treatments (Table 6.1). Spray applications and observations on leaf color and defoliation were performed as described for Experiment 1. After trees were harvested from fields, thirty trees from each treatment were shipped to OSU. Upon arrival the trees were immediately placed in cold storage at 32-35 °F. Nitrogen analyses were performed as described for Experiment 1.

Ten plants from each treatment were planted in N-free media (1:2 (v:v) mix of perlite and vermiculite) at OSU on March 26, 2002, and grown in 6-gallon pots under natural conditions. Ten plants from each treatment were planted in the field at the Lewis-Brown Horticulture farm at OSU in early April, 2002. Both N-free media and field grown plants were analyzed separately for determination of growth performance. Ten plants from each treatment were also planted in the field at the nursery inYuba City, California, in the spring 2002 for evaluating new growth performance.

Statistical analysis

All experiments were set-up in a completely randomized design, with ten replicates per treatment. The arcsine transformations of defoliation data (percentages) were made for statistical analyses. After the analyses were completed, results were transformed back to defoliation percentages for presentation. The effect of defoliation, nitrogen and new growth were evaluated by analysis of variance (ANOVA) separately for each experiment, and comparisons among treatment means were performed by Duncan's multiple range test (significance level $p<0.05$). All statistical analyses were performed with SAS (SAS Inst. Inc., Cary, N.C.).

6.4 Results

In both experiments, some minor leaf margin and tip necrosis was observed after applying defoliants, but no visible injury to the bark or bud tissues of the plants occured. Leaves fell off the trees in green color after all defoliant treatments.

Experiment 1

Treatments of plants with defoliants, urea, or a combination of urea + defoliants promoted leaf abscission earlier than control plants (natural defoliation) (Figure 6.1). Ten days after treatment, trees sprayed with Urea + CuEDTA, CuEDTA, Urea + $ZnSO_4$, and Urea lost more leaves than control trees or trees treated only with $ZnSO_4$. Two weeks after sprays, all trees treated with defoliants or urea+defoliants lost more leaves than controls. All plants were >95% defoliated by the end of December. Compared to the controls and trees treated with $ZnSO_4$, CuEDTA significantly promoted earlier defoliation, about 80% by Nov. 27, ten days after treatment. $ZnSO_4$ promoted defoliation by approximately 50% by December 1, two weeks after defoliation treatment (Figure 6.1). Addition of urea to defoliants increased the efficiency of both $ZnSO_4$ and CuEDTA in promoting earlier defoliation. Urea + CuEDTA treatment was more effective at promoting early defoliation than with Urea + $ZnSO_4$. Among all the treatments, the combination of urea and CuEDTA caused the most rapid defoliation.

Compared to the controls, CuEDTA treatment significantly reduced N concentrations in root, stem, and branch tissues, resulting in a significantly lower total N content in the trees; while trees treated with $ZnSO_4$ had similar N concentrations or

contents as controls. Urea treatment increased plant total N content. Trees treated with the combination of urea and either defoliant had significantly greater N concentrations in root tissues than trees in all other treatments. The combination of urea and CuEDTA treatment significantly increased the N concentration in shoot tissues than CuEDTA alone. However, the combination of urea and $ZnSO_4$ had no influence on N concentrations in shoot tissues. Nevertheless, compared to all other treatments, the addition of urea to defoliant applications significantly increased tree total N contents.

At both test locations, all treated trees exhibited normal growth after transplanting in the spring (data not shown).

Experiment 2

The combination of urea and CuEDTA was the most effective in promoting early defoliation compared to other treatments. Approximately 95% of the leaves were defoliated by Dec. 1, ten days after spray (Figure 6.2). Compared to controls, $ZnSO_4$ treatment also promoted significant defoliation with 50% of the leaves being defoliated by Dec. 1. Urea alone had no significant influence on defoliation.

Compared to the natural defoliation, plants treated with urea had significantly higher N concentrations in root tissues (Table 6.3). The combination of CuEDTA and urea treated trees had similar N concentrations in roots, stems, and branches as controls. Trees receiving $ZnSO_4$ had significantly lower N concentrations in roots and stems, but similar N concentrations in branches as controls. In contrast to the control trees, the trees treated with urea contained significantly greater N (Table 6.3). Trees

treated with the combination of CuEDTA and urea had similar N content as control trees. $ZnSO_4$ treatment decreased tree total N content compared to natural defoliation.

Treatments with $ZnSO_4$, urea, or the combination of CuEDTA and urea had no influence on bud break (data not shown) and there were no obvious shoot tip dieback or delayed new growth observed on any trees. The length of new shoots and biomass of new shoots and leaves showed similar trends in response to treatments. Growth data from trees planted in the N-free media, or trees planted at the OSU farm or nursery showed similar trend, therefore, only data from N-free media are presented (Table 6.4). Trees treated with urea had significantly greater shoot and leaf growth than controls trees. Treatment of trees with $ZnSO_4$ or the combination of urea and CuEDTA had no influence on growth of shoots or leaves.

6.5 Discussion

Results from both experiments show that both CuEDTA and $ZnSO_4$ are effective for promoting early defoliation of almond nursery plants, with no obvious damage to plants. We found that CuEDTA (0.5%) was more effective than $ZnSO_4$ (1.25%) in promoting early defoliation. Larsen and Fritts (1986) investigated the effects of CuEDTA on promoting leaf abscission of apple, cherry and pear seedlings, and found that 80% or more defoliation was achieved in most instances with the 0.50% CuEDTA or higher concentration in two weeks after spray. However, they observed some bud or bark damage. Higher concentration of CuEDTA (2.1%) was also studied using apple and cherry rootstock that caused high levels of defoliation with only minor shoot tip damage (Knight, 1983). In our experiment, we found that

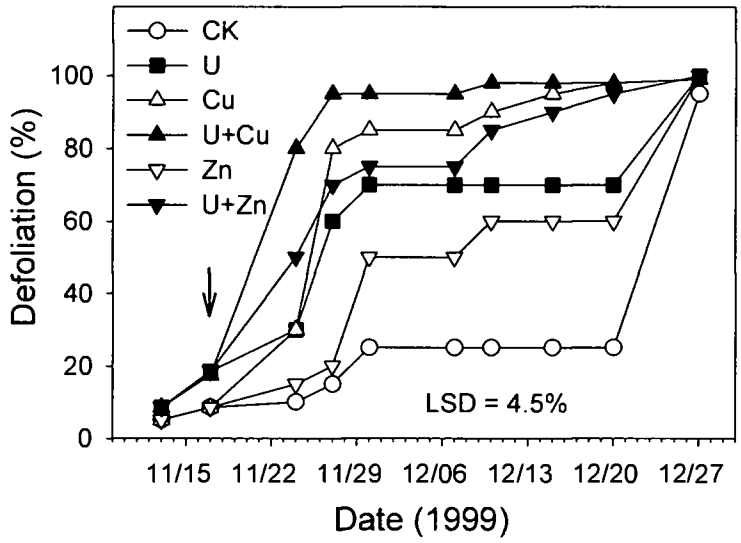

FIG. 6.1. Effects of defoliants and urea application on defoliation of 'Nonpareil'/'Nemaguard' almond trees in Experiment 1 (1999-2000). CK=control (natural defoliation), U = two applications of 3% urea (10/27/1999, 11/8/1999), Cu = one application of 0.5% CuEDTA (11/17/1999); Zn = one application of 1.25% $ZnSO_4$ (11/17/1999). U+Cu = two applications of 3% urea (10/27/1999, 11/8/1999) and one application of 0.5% CuEDTA (11/17/1999); U+Zn = two applications of 3% urea (10/27/1999, 11/8/1999) and one application of 1.25% ZnSO4 (11/17/1999). Data points represent means of 10 replicates. LSD represents least significant differences between means (Duncan's LSD $p<0.05$). Arrow indicates date of defoliant application.

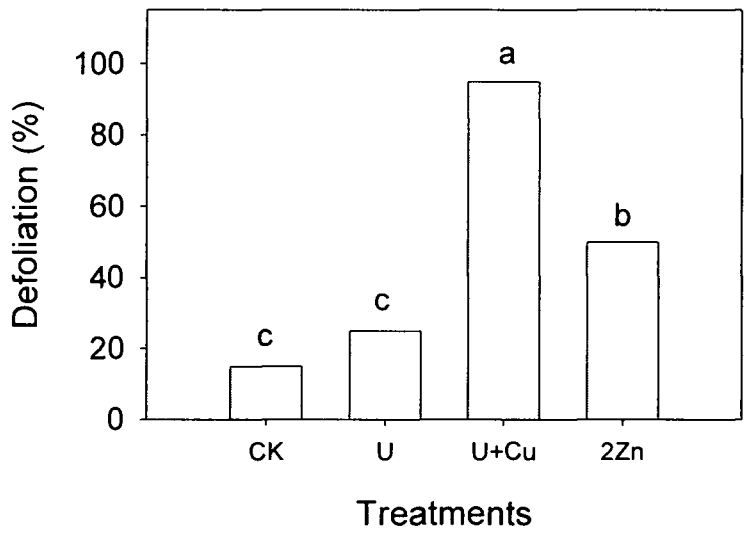

FIG. 6.2. Effects of defoliant and urea applications on defoliation of 'Nonpareil'/'Nemaguard' almond trees in Experiment 2 (2001-2002) 10 days after treatment. CK=control (natural defoliation), U = two applications of 3% urea (11/10/2001, 11/20/2001), U+Cu = 3% urea sprayed on 11/10/01 followed by 1% Cu-EDTA&3%urea sprayed on 11/20/2001, 2Zn = two applications of 1.7% $ZnSO_4$ (11/10/2001, 11/20/2001). Each value is the mean of 10 replicates. Columns with the same letter above them are not significantly different by Duncan's multiple range test ($p<0.05$).

Table 6.2. Nitrogen (N) concentration of different tissues in response to defoliant and urea treatments of 'Nonpareil'/'Nemaguard' almond trees in Experiment 1 (1999-2000).

	N (%)			Total N (mg/tree)
Treatment[z]	Roots	Stem	Branches	
CK	1.30[y]b	0.58a	1.05a	1466.4c
Cu	0.97c	0.40b	0.81b	1071.2d
Zn	1.32b	0.52a	0.93ab	1402.8c
U	1.43b	0.56a	1.15a	1568.1b
U+Cu	1.74a	0.62a	1.04a	1756.3a
U+Zn	1.75a	0.60a	1.17a	1770.6a

[z]Treatments: CK=control (natural defoliation), U = two applications of 3% urea (10/27/1999, 11/8/1999), Cu = one application of 0.5% CuEDTA (11/17/1999); Zn = one application of 1.25% ZnSO4 (11/17/1999). U+Cu = two applications of 3% urea (10/27/1999, 11/8/1999) and one application of 0.5% CuEDTA (11/17/1999); U+Zn = two applications of 3% urea (10/27/1999, 11/8/1999) and one application of 1.25% ZnSO4 (11/17/1999).

[y]Means followed by different letters are significantly different (Duncan's Multiple range test, $p<0.05$; $n=10$).

Table 6.3. N concentration and total N content in 'Nonpareil'/'Nemaguard' almond trees in response to urea and defoliant treatments in Experiment 2 (2001-2002).

	N (%)			Total N (mg/tree)
Treatment[z]	Roots	Stem	Branches	
CK	1.42[y]b	0.67a	0.95ab	1669.9b
U	1.56a	0.70a	1.05a	1819.3a
U+Cu	1.43b	0.67a	1.00a	1609.2bc
Zn	1.31c	0.59b	0.83b	1559.8c

[z]Treatments: CK=control (natural defoliation), U = two applications of 3% urea (11/10/2001, 11/20/2001), U+Cu = 3% urea sprayed on 11/10/01 followed by 1% Cu-EDTA&3%urea sprayed on 11/20/2001, 2Zn = two applications of 1.7% $ZnSO_4$ (11/10/2001, 11/20/2001).
[y]Means followed by different letters are significantly different (Duncan's Multiple range test, $p<0.05$; n=10).

Table 6.4. Length of new shoots and biomass of new shoot and leaves of 'Nonpareil'/'Nemaguard' almond trees planted in N-free media in response to urea and defoliant treatments in Experiment 2 (2001-2002).

Treatment[z]	New shoot length (cm/tree)	New shoot and leaf biomass (g dry weight /tree)
CK	354.5[y]b	39.4b
U	417.4a	48.3a
U + Cu	396.6ab	38.7b
Zn	306.6b	35.4b

[z]Treatments: CK=control (natural defoliation), U = two applications of 3% urea (11/10/2001, 11/20/2001), U+Cu = 3% urea sprayed on 11/10/01 followed by 1% Cu-EDTA&3%urea sprayed on 11/20/2001, 2Zn = two applications of 1.7% $ZnSO_4$ (11/10/2001, 11/20/2001).
[y]Means followed by different letters are significantly different (Duncan's Multiple range test, $p<0.05$; $n=10$).

combining application(s) of urea with defoliants increased the efficiency of defoliation with $ZnSO_4$ and CuEDTA. This may have been due to urea acting as a surfactant that enhanced the uptake of defoliants, and urea itself also promoted leaf abscission. In other experiment, we have looked at the effect of using one or two applications of CuEDTA (1%) or $ZnSO_4$ (1.7-2%) on promoting earlier defoliation, and found that increasing the number of defoliant applications had little effect on leaf abscission (data not shown).

Generally, we found that defoliants (e.g. CuEDTA) decreased total N in trees. This decrease in total N may be due to enhanced defoliation prior to leaf senescence and reduced mobilization of the leaf N back into the tree for storage. Studies with abscisic acid (ABA) have shown that ABA promotes leaf defoliation, but also induces leaf senescence and N mobilization from leaves (Guak and Fuchigami, 1997; Larsen and Higgins, 1998). Leaf senescence prior to abscission is important for protein breakdown and N mobilization out of leaves. However, earlier defoliation caused by CuEDTA while promoting leaf abscission does not appear to enhance the leaf-senescence process as does natural defoliation or the use of ABA, thus preventing the mobilization of N from leaves to storage tissues. This can cause a decrease in N reserves that could potentially result in poor regrowth performance.

In contrast to controls, our results showed that the urea treated trees contained significantly higher total N (Table 6.2 and 6.3). This supports the results obtained earlier on other deciduous tree species that fall foliar urea spray can be an effective way to increase N reserves (Oland, 1960; Shim et al., 1973; Rosecrance et al., 1998;

Tagliavini et al., 1998; Millard, 1996; Cheng et al., 2002; Dong et al., 2002). Plants received the combination of defoliants with urea had similar or more N than controls (Table 6.2 and 6.3), suggesting that foliar application of urea can bypass the need for protein breakdown during leaf senescence and increase N in storage tissues. Therefore, the combination of defoliant with urea treatment would be important if the goal of the nurserymen is to promote early defoliation without reducing N reserves of bare rooted nursery stock.

It has been reported that a good chemical defoliant will cause at least 50% defoliation 2-3 weeks after application, is easy to apply, inexpensive, and does not cause plant injury (Still, 1976). Our studies have shown that both CuEDTA and $ZnSO_4$ can be considered as good chemical defoliants for use on almond nursery stock; with greater than 50% defoliation occurring within 2 weeks, and no obvious plant injury after application or during plant establishment in the following spring. By combining application(s) of urea with defoliants, we can further promote earlier defoliation, increase N reserves, and improve plant growth next spring. Therefore, CuEDTA or $ZnSO_4$ in combination with urea is a good strategy for nurserymen to use for promoting early defoliation, without affecting plant N reserves and new growth in the following spring.

In conclusion, both CuEDTA and $ZnSO_4$ are safe and effective for promoting early defoliation of almond nursery plants. Combining urea with defoliant (e.g. urea + CuEDTA) was very effective in promoting early defoliation without negative effects on the growth the following spring.

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CHAPTER 7

SUMMARY AND CONCLUSIONS

Management of nitrogen (N) fertilization and defoliation in deciduous tree nurseries is complex. Efficient management of N fertilization for nursery production requires fertilization practices to meet plant needs, improve plant quality and maintain commercial viability, as well as reduce overall N fertilizer inputs and minimize N losses to surface and groundwater. Controlled defoliation is essential for the successful management of bareroot deciduous nursery plants to enhance earlier defoliation without causing plant injury or affecting growth during establishment. To improve N and defoliation management, knowledge of how trees use N from reserves and N from fertilizers for growth and how trees respond to defoliants is important. The goal of this research was to increase our understanding of the relationships between N, defoliation, and plant growth in almond nursery trees, to develop cultural strategies that use optimal rates of N fertilizer, methods and timing of N fertilizer applications, and to determine safe and effective methods to promote early defoliation.

The effects of N source and availability on tree growth, N uptake, N remobilization, and nonstructural carbohydrates (TNC) were investigated in several experiments to determine the most efficient and effective methods and timing for N

fertilization. New growth of almond nursery trees in the spring is affected both by their levels of N reserves and by N fertilization in the spring. A tree's N reserves can be enhanced either by N fertigation during the growing season or through foliar urea applications in the fall. Both methods of N fertilization can result in increased concentrations of both free and total amino acids during winter storage and can increase new growth the following spring. Protein is the main form of N stored in almond nursery trees. The synthesis of amino acids and proteins occurs at the expense of carbohydrates, and the amount of TNC used for N assimilation increases as the supply of nitrogen increases. Spring N fertilizer application increases new growth of almond nursery trees regardless of the amount of nitrogen reserves in the plant. Uptake of N from the soil in the spring begins two weeks after its application. Maximum uptake occurs during the period of rapid new growth. Trees with low N reserves use nitrogen primarily from spring N fertilization to promote shoot and leaf development. From these results we conclude:

1) The amount, timing, and method of N application has a pronounced effect on the growth, reserves (carbohydrate and N), and plant establishment in the following growing season.
2) Foliar urea application later in the season, prior to leaf senescence, is an effective way to increase N reserves and new growth in the following growing season.
3) Applying N fertilizer in the spring, during the period of rapid new growth, can significantly improve plant vegetative growth and the N status. For

trees with low N reserves, spring-applied nitrogen fertilizer is particularly important for promoting new growth.

The influence of chemical defoliants (CuEDTA and $ZnSO_4$) and foliar urea applications on defoliation, N reserves, and new growth performance was also assessed to determine optimal methods for promoting early defoliation without negative effects to tree growth and quality. Although both chemical defoliants we tested were safe and effective on promoting earlier defoliation, CuEDTA was more effective than $ZnSO_4$. Combining urea with defoliant (e.g. urea + CuEDTA) effectively promotes earlier defoliation. Plants treated with foliar urea in the fall prior to or with chemical defoliants had significantly higher contents of nitrogen reserves compared to the defoliant treatments alone, and the N reserve status was comparable to the levels found in naturally defoliated trees. Compared to natural defoliation, the combination of urea and defoliants has no significant effect on new growth during plant establishment. From these results we conclude:

1) Both CuEDTA and $ZnSO_4$ are safe and effective for promoting early defoliation in almond nursery trees, however, CuEDTA is more effective than $ZnSO_4$.
2) Foliar urea application(s) prior to or with defoliant treatments is a good strategy to effectively promote early defoliation and improve N reserves, without affecting the new growth of almond nursery trees.

Data from this research suggests that knowledge of the relationships between N, defoliation, and plant growth will enable deciduous nursery tree growers to produce

commercially competitive plants with substantially less fertilizer N inputs. The research results from the defoliation and N experiments have been instrumental in helping nurseries to safely and effectively defoliate deciduous nursery stock with chemicals and ensure high quality of the nursery plants. Nurserymen have reported that this procedure has enabled them to save millions of dollars from the improved efficiency and plant quality. However, we recognize that different plants have different N requirements and respond differently to chemical defoliants. Further studies with different plant genotypes will be required to obtain a clear understanding of N and defoliation managements on different nursery plants.

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