AN ABSTRACT OF THE THESIS OF

Habib Khemira for the degree of Doctor of Philosophy in Horticulture presented on December 1, 1995. Title: Nitrogen Partitioning and Remobilization in Field-grown Apple Trees.

Abstract approved: _____
Timothy L. Righetti

_____
Anita N. Azarenko

Uptake and cycling of nitrogen (N) in mature trees are not well understood. Fertilizer-N uptake, partitioning, and use-efficiency were studied in standard strain 'Topred Delicious' and spur strain 'Redspur Delicious' mature apple trees (*Malus domestica* Borck) on 'Malling 7A' (M.7A) rootstocks. The treatments consisted of a ground application in spring, a preharvest ground application in August, a foliar spray in fall, or a combination of each of the last two treatments with the first. When soil-applied in spring, labeled N (^{15}N) was allocated preferentially to above ground tissues and to a lesser extent to roots of both strains. The amount of newly absorbed soil-N allocated to above ground tissues decreased as the season progressed. little ^{15}N from pre-harvest ground applications reached the leaves, fruit, buds, or branches, while roots were heavily labeled.

Total fertilizer-N recovery in the trees was similar regardless of the time of fertilizer application. However, losses of ^{15}N to fruit removal, leaf fall, and pruning were most severe when N was applied in spring and minimal for the pre-harvest timing compared to all others. About a third of the variability in recovery was due to variation

in tree size. When recoveries were adjusted to account for size differences, spur-type trees tended to be more efficient at utilizing fertilizer-N.

When ^{15}N-urea was applied in April to young apple leaves, the label was not exported. Labeled N from fall urea sprays was exported from the leaves to the buds, but was restricted to the treated spurs and branches. Foliar urea sprays immediately after harvest contributed more N to the buds than later applications.

Mobilization of ^{15}N from storage in various tree parts was assessed. In moderately vigorous trees, N stored in aerial parts of the tree was mobilized first, followed by simultaneous mobilization of root and soil N. Utilization of root reserves depended on the N status of the tree. When the buds were low in N but the roots had adequate N reserves, root to shoot N transport started early in winter.

Nitrogen Partitioning and Remobilization in Field-grown Apple Trees

by

Habib Khemira

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APPROVED:

Co-Major Professor, representing Horticulture

Co-Major Professor, representing Horticulture

Head of Department of Horticulture

Dean of Graduate School

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

2/7/1996

Habib Khemira, Author

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DEDICATION

This thesis is dedicated with deepest love and respect to my mother, Saïda.

NITROGEN PARTITIONING AND REMOBILIZATION IN FIELD-GROWN APPLE TREES

CHAPTER 1

INTRODUCTION

Nitrogen is an indispensable elementary constituent of numerous organic compounds of general importance (amino acids, proteins, nucleic acids) (Beevers, 1976), and constitutes about 2% to 4% of plant dry matter (Mengel and Kirkby, 1987). Numerous field experiments indicate that for most soils, N is the most important growth limiting factor. Consequently, N fertilizer application has had the most significant effect in increasing crop production (Mengel and Kirkby, 1987).

Since the discovery of the Haber-Bosch process (1910) in which N_2 and H_2 react together under high temperature and pressure conditions to yield ammonia, the production and use of N fertilizer has increased dramatically throughout the world. Today, industrial fixed dinitrogen amounts to 73 x 10^6t N/year, which is about 60% of the dinitrogen fixed biologically (Mengel, 1992). Most of this industrially-fixed dinitrogen is returned to the environment in the form of fertilizers. This process significantly alters the global nitrogen cycle. Nitrate released into soils modifies agricultural ecosystems, can eventually reach aquifers and oceans, and at high concentrations seriously endangers the safety of humans and animals.

Over-fertilization is a common practice among high-value fruit crops, since nitrogen fertilizer accounts for a small portion of production costs. Orchardists, in effect, use fertilizers as insurance against a relatively small chance of nutrient deficiency (Sanchez et al., 1995). The problem is made worse by the low N use efficiency of fruit trees. The percentage of fertilizer-N utilized by crop plants is estimated to be only 15% to 20% for pears (Sanchez et al., 1991), but 37% for grapes (Miller and Smith, 1976), and between 30% and 80% for many field crops, depending on a variety of environmental, genetic, and cultural factors (Craswell and Godwin, 1984).

The efficiency with which plants utilize fertilizer-N depends on the form and amount of N available, the availability of soil water, and the distribution and sink strength of the plant root system (Craswell and Godwin, 1984). Societal concerns about groundwater contamination in recent years have prompted many studies with the objective of improving N fertilizer management and ultimately reducing the overall use of fertilizers (Embleton and Jones, 1978; Weinbaum et al., 1978; Craswell and Godwin, 1984; Bronson et al., 1991; Löhnertz, 1991).

Nitrogen budget studies (Magness et al., 1948; Hill-Cottingham and Cooper, 1970), and, more recently, tracer techniques (Hill-Cottingham and Lloyd-Jones, 1975; Weinbaum et al., 1980 and 1984; Kato et al., 1982; Sanchez et al., 1992; Muñoz et al., 1993) showed that fertilizer-N is partitioned differently among tree parts depending on when and how it was applied. In pear, nitrogen applied in spring is preferentially allocated to fruit and vegetative growth (Sanchez et al., 1992), while late season N is partitioned mainly to the roots and stock with very little reaching the developing buds

(Sanchez, 1990). Postharvest sprays of N-containing solutions is the only practical way to provide N to the bud late in the growing season (Oland, 1960; Sanchez et al., 1990).

Deciduous fruit trees conserve N that would otherwise be lost by the abscission green leaves. This involves the autumnal mobilization of leaf N into woody tissues, where it is available for growth in the following season. Accurate evaluation of the contribution of this internal cycling to the total N budget of the tree should be made if N fertilizer-use efficiency in our orchards is to be improved. Assessing the importance of various nitrogen reserve pools in the tree and their contribution to next year's growth is of paramount importance in evaluating fertilizer programs. A better appreciation of the internal cycling, coupled with more efficient utilization of fertilizer-N, may eventually lead to a reduction in the overall fertilizer input to the orchard. The benefit from such reduction comes not only from lowering the production cost and improving the public perception of modern agriculture, but also from a better fruit quality, which can translate into higher returns for the grower. There are numerous reports where high N fruit leads to less desirable color development (Weeks et al., 1952; Reuther et al., 1958), greater susceptibility to storage disorders (Bramlage et al., 1980), and increased incidence of decay (Sugar et al., 1992). Excessive N use has both direct and indirect effects on fruit quality (Sanchez et al., 1995).

Nitrogen fluxes in a tree can be followed directly using an isotopically-labeled N carrier. However, the experimental use of N isotopes with tree species has often been restricted to young seedlings and rooted cuttings in pot culture (Taylor and May, 1967; Hill-Cottingham and Lloyd-Jones, 1975; Weinbaum et al., 1978; Muñoz et al., 1993). Results from these experiments may not be applicable to mature trees under orchard

conditions (Hill-Cottingham, 1967). Nevertheless, there are a number of studies where tracer techniques were used to investigate how management alters N partitioning in mature trees (Weinbaum et al., 1980, 1984, 1987; Sanchez et al., 1990, 1992). It may be misleading to extrapolate data from these studies to other tree species with different growth and fruiting habits. Furthermore, since a total N budget requires destructively sampling entire trees, most of these researchers used concentration differences to describe N pools and fluxes rather than total amounts. Unfortunately, changes in nutrient concentrations do not necessarily reflect changes in total amounts of a nutrient within a tissue, nor can they be interpreted as changes in the uptake of nutrients from the soil (Righetti, 1986). A change in concentration of N, for instance, can be brought about either by a change in the amount of N or in dry matter content of the tissue (Taylor, 1967). Therefore, elemental concentration data should be interpreted cautiously (Sanchez, 1990).

In summary, previous studies dealing with N uptake, utilization, and cycling in plants are numerous. However, most of them used conventional methods (unlabeled N), whereby it was impossible to distinguish between newly absorbed and reserve N. In many cases where stable or radioactive isotopes of N were used, the results were reported as concentrations rather than total amounts. Furthermore, the plant material used in these studies often consisted of herbaceous species or young seedlings and rooted cuttings of woody species with a limited capacity to absorb, use, and store nutrients. It is my belief that extrapolating the results of such studies to mature field-grown apple trees will lead to erroneous interpretations.

The main hypothesis of this study is that N reserve accumulation and mobilization is much more localized in large trees than the literature on young trees suggests. Several more specific hypotheses can be derived from this general statement:

- Partitioning of N in large trees can be readily altered by changing the time, form, and mode of application (ground applications vs. foliar sprays) of the N carrier.
- Relative partitioning of N among different reserve pools will also depend on tree growth habit.
- Availability of N reserves within a tree for early spring growth is more dependent on storage sites in large bearing trees.
- The fundamental difference between small and large trees in terms of partitioning and remobilization of storage N is that the latter have a bigger buffering capacity due to their larger N reserve pools.
- Managing N reserves in mature trees has important practical applications. We should be able to ensure adequate N supply for developing flower buds while maximizing fertilizer use efficiency and minimizing fruit N content.

My approach was to thoroughly evaluate N cycling with labeled N in mature apple trees. Destructive sampling of entire trees was used when necessary. The objectives of this study were:

1) Assess the effect of the time, form, and mode of N-fertilizer application on N partitioning in standard- and spur-type mature apple trees.

2) Determine the best timing of N application for standard- and spur-type apple trees to maximize uptake and optimize utilization.

3) Investigate the distribution of N derived from urea sprayed onto apple trees in spring and autumn.

4) Determine the order of spring mobilization of tree canopy and root N storage pools and how it is affected by tree N status.

5) Characterize N pools in mature dormant apple trees.

CHAPTER 2

LITERATURE REVIEW

1. Overview:

Nitrogen (N) has long been considered the dominant nutritional factor in the growth and development of plants. Although N is a rather small constituent of dry plant material (2% to 4% N compared to about 40% carbon), it is an indispensable component of numerous organic compounds of great importance (amino acids, proteins, nucleic acids).

Crop yields depend much on the supply of inorganic N and are generally increased by the application of N fertilizers. Without N fertilizers it would be impossible to feed the world's population (Mengel, 1992). Unfortunately, the low cost of N fertilizers in comparison with other inputs and the necessity to achieve early fruit production has led to widespread over-fertilization which, in turn, can cause a variety of ecological problems.

Pollution from agricultural cropland generally is due to chemical and sediment transport in runoff and leaching of soil chemicals by percolation. Although not much publicized, fruit orchards may have a significant impact on the environment (Sanchez et al., 1995). A major concern is the movement of fertilizer-derived nitrates into ground water. Furthermore, excessive N fertilizer application may lead to high NO_3^- concentrations in plant tissues. Nitrate taken up in food or drinking water is not noxious to humans. However, when reduced to NO_2^- in the human body, it can cause methemoglobinemia especially in babies (Mengel, 1992). In California, an estimated 311,000 tons of N are leached annually from irrigated land (Pratt, 1984). In Washington,

continuous application of ammonium fertilizers in some areas has also reduced the soil pH to levels not compatible with fruit production. Excess N in the tree favors vegetative growth rather than fruiting and can lead to undesirable fruit quality (Sanchez et al., 1995).

Deciduous fruit trees are generally less demanding of N than other crops (Sanchez et al., 1991; Weinbaum et al., 1992). They evolved to conserve N that would otherwise be lost by leaf abscission. This involves the autumnal mobilization of leaf N into woody tissues, where it is available for growth in the following season (Habib, 1984). As the days become shorter and air temperature decreases in autumn, leaf proteins undergo a massive breakdown (Titus and Kang, 1982). The resulting amino acids are translocated back into the woody tissues of the tree where they are stored either as amino acids or more commonly as proteins. In fact, there is a growing body of evidence suggesting that the main form of N storage in dormant tree tissues is proteins. Upon regrowth, these proteins are degraded to provide N for blossom development and early vegetative growth (Millard, 1995). The importance of reserve N is obvious since budbreak takes place at a time when root uptake is minimal (Titus and Kang, 1982).

Accurate evaluation of the contribution of internal cycling to the total N budget of the tree should be made if N fertilizer-use efficiency in the orchard is to be improved. Assessing the importance of N reserves in the tree and their contribution to next year's growth is of paramount importance in evaluating fertilizer programs.

Selecting nutrient efficient germplasm may be important, but the choice of scion and rootstock are often dictated by other cultural and economical considerations. Therefore, altering timing, rate, and method of fertilizer application may be the only practical way to improve fertilization efficiency. Improving fertilizer-N recovery by the

tree could allow growers to substantially reduce fertilizer-application rates while providing the tree with the N it needs.

2. Nitrogen Fertilization Practices:

Fertilizers are commonly applied to the soil. However, foliar fertilization is gaining popularity among growers because plants often respond faster when nutrients are applied directly to the leaves. Furthermore, foliar applications of nutrients are thought to be less polluting than soil applications.

2.1 Soil fertilization:

2.1.1 *Spring fertilization:*

Orchardists have traditionally applied fertilizer N during winter, when deciduous trees are dormant. This practice was developed when growers depended on winter rains to carry the applied fertilizer-N to the root zone. Since irrigation is more common now, winter fertilization has become less favored, and growers generally apply N in the spring.

Part of the rationale for spring applications was to provide N for the early spring growth. However, it appears that spring-applied N does not reach the bud or flowers although exceptions may occur on sandy soils under warm spring conditions (Sanchez et al., 1990). Several ^{15}N-studies on a variety of fruit tree species show that flower development and early growth of vegetative tissues are almost entirely dependent on stored N (Weinbaum et al., 1978, 1980, 1984; Sanchez et al., 1990, 1992; Muñoz et al., 1993).

While there is little benefit to current year blossoms, spring fertilization provides ample N for late spring and early summer periods when maximum vegetative growth occurs (Sanchez et al., 1995). This practice often allows high fertilizer recovery and ample N reserves for the developing buds. However, excessive spring fertilization can lead to high tree vigor adversely affecting yield and fruit quality. Furthermore, excessive vigor predisposes tree to fire blight (*Erwinia amylovora*) infection (Zwet et al., 1979). In general, a level of N nutrition high enough to ensure maximum fruit yields usually produces a high proportion of poorly colored fruit (Weeks et al., 1952; Mori et al., 1963). This is especially true for apple. Early studies demonstrated that red surface color and yellow background color development is inversely related to the N level of the tree (Shaw and Southwick, 1936; Magness et al., 1940; Boynton, 1954).

Fruit high in N at harvest tend to be larger, greener, softer, more subject to pre-harvest drop, and more likely to be affected by cork spot and bitter pit then low-N fruit (Bramlage et al., 1980). Following storage, high-N fruit develop more scald, bitter pit, internal browning, and internal breakdown . Terblanche et al. (1980) found a positive relationship between fruit N and incidence of bitter pit in apple. However, the role of N in bitter pit development, is not clearly understood. Some workers reported an increasing incidence of this disorder with an increasing level of N supply, while others were unable to find any effect on bitter pit incidence from heavy N application (Sharples, 1973). Other researchers explained the susceptibility of the fruit to disorders and pests in relation to both its N and Ca contents (Sugar et al., 1992). Pear fruit with a low Ca:N ratio show higher incidence of mold during cold storage compared to high Ca:N fruit (Sugar et al., 1994).

2.1.2 *Summer fertilization:*

In N timing experiments on mature almond trees, Weinbaum et al. (1984) showed that the later N fertilizer is applied during the season, the less fertilizer N is recovered in the current year's fruit and leaves, and the greater its contribution to these organs is the following year. Sanchez (1990) reported similar results with 'Comice' pear. Nitrogen applied during the second half of the summer is particularly beneficial for next year's blossom (Williams, 1965). Such applications advances bloom, extends the effective pollination period of the flowers, and increases fruit set (Hill-Cottingham, 1963; Williams, 1965). Although earliness is not desirable in areas where spring frost can occur, the precocity of leaf expansion may be desirable since spur leaves play a vital role in the early stages of fruit growth (Llewelyn, 1966).

2.1.3 *Autumn fertilization:*

Root activity of temperate zone trees continue well into the autumn. Apple roots reportedly grow and absorb nutrients as long as the soil temperature is above a minimum of 4 to 6.2 °C (Kolesnikov, 1971 reported by Faust, 1989; Rogers, 1939). In pear, N absorbed in autumn stays in the roots (Sanchez et al., 1992), while N applied before harvest causes a build up of storage N in the roots, trunk, and large branches but it does not reach the leaves, fruit, or buds (Sanchez, 1990).

The main objection to applying N fertilizer in autumn has been the suspicion that late application would stimulate tree growth and delay leaf senescence and wood maturity thus predisposing the trees to winter injury. Although, there is evidence that a large dose of N applied late in the autumn may increase the susceptibility of both the twigs and bark

to freezing (Edgerton, 1957), it is generally accepted that a moderate application of N fertilizer does not affect the ability of the tree to cold acclimate (Pellett and Carter, 1981).

2.2 Foliar feeding:

The use of foliar sprays as a N source for fruit trees started early this century in the western United States. Ballard and Volck (1914), in California, and Lewis (1915), in Oregon, successfully used sodium nitrate sprays applied during dormancy to increase the yield of apple trees. The practice became more popular in commercial fruit growing during the 1940s. In addition to N, other nutrients were applied foliarly to control deficiencies and promote growth. Solutions of Fe, Zn, Cu, or B salts were used effectively to control deficiency symptoms of these elements in apple, citrus, banana, and pineapple trees (see Boynton, 1954).

Hamilton et al. (1943) were the first to recognize that apple leaves may absorb N in appreciable quantities. This finding was followed by extensive research on the use of foliar urea sprays on apple and other fruit trees. During the next decade, Boynton (1954) and Fisher (1952) attempted to supply the total N needs of apple trees by spring and summer urea sprays but had difficulty achieving this goal.

Oland (1960) was the first to recommend late season urea sprays for commercial fruit tree production. During the next two decades several studies used ^{14}C and ^{15}N labeled N sources to provide valuable information on the uptake and metabolism of foliarly applied N (Shim et al., 1972, 1973; Titus, 1976; Weinbaum and Neumann, 1977; Titus and Kang, 1982). Urea N is translocated from the leaves as amino acids or urea (Shim et al., 1973). Urea metabolism involves hydrolysis of urea and incorporation of ammonium

into amino acids. Some amino acids may be directly exported (Shim et al., 1973). Transamination, protein synthesis, and eventual protein breakdown and export of resulting amino acids can also occur.

Urease (E.C.3.5.1.5. urea amidohydrolase), which hydrolyzes urea to ammonia and carbon dioxide, is widely distributed in animal and plant tissues. It was found in fresh extracts of young leaves of peach, apricot, and cherry, whereas no activity was found in similar extracts from grape and pear (Dilley and Walker, 1961). In apple, urease was found in leaves, roots, and bark, with actively growing tissues containing more activity than senescing tissues (Shim et al., 1973). In senescing apple leaves, urea sprays induce a rapid *de novo* synthesis of urease (Shim et al., 1973).

Fall foliar urea sprays are more effective than spring applications mainly because higher concentrations can be used. Once the fruit are harvested, more tree damage can be tolerated. High concentrations early in the season may damage the fruit and the young leaves. Sanchez et al. (1990) showed that a single postharvest urea spray at 5% or 10%, increases bud and shoot N concentrations substantially. However, N does not reach the roots. Nevertheless, such practice was used successfully to extend the longevity of the ovules in the low setting 'Comice' pear (Khemira, 1991).

2.2.1 *Factors affecting efficiency of foliar sprays:*

The first barrier to mineral nutrient uptake by leaves is the external wall of the epidermal cell (Kannan, 1986). This wall is covered by a layer of wax and cutin (a condensation product of C18 hydroxy fatty acids with semihydrophobic properties) and certain pectins, hemicellulose and cellulose (Marshner, 1986).

Leece (1978) suggested that the seasonal build-up and development of secondary wax structure on the abaxial surface of plum leaves is related to increasing light intensities as the season progresses. Thus, he concluded, foliar absorption should be maximal in the spring when abaxial waxes are not fully developed. Similarly, the amount of cuticle, cutin matrix, and cuticular wax was reported to be greater in high than in low light intensities in *Brassica oleracea*, *Eucalyptus*, and cereal crops (Tribe et al., 1968; Macey, 1970; Hallam, 1970). Contrary to that, light intensity did not affect the amount or chemical composition of epicuticular waxes in apple leaves (Darnell and Ferree, 1983).

Temperature affects both air relative humidity and solution concentration. Most reports seem to agree that high temperatures or low relative humidity decrease leaf absorption of sprayed nutrients (Cook and Boynton, 1952; Lidster et al., 1977).

Cook and Boynton (1952) showed that terminal leaves of 'McIntosh' apple absorb almost two fold more urea than do basal leaves within a 2-hour period. Similarly, P absorption by young apple leaves was greater than absorption by old leaves (Fisher and Walker, 1955). The difference between young and old leaves is related to differences in both their physiological age and the environmental conditions during their development.

Lower surfaces of leaves absorb urea more rapidly than upper surfaces in apple (Cook and Boynton, 1952), plum (Leece, 1978), and banana (Freiberg and Payne, 1957). Absorption by the lower surface of the apple leaf is very rapid within the first 24 hours and then plateaus, whereas the upper surface absorbs urea more steadily. Within 7 days after urea application, total absorption by both surfaces may not differ greatly (Boynton et al., 1953).

Cook and Boynton (1952) reported that apple leaves high in N absorb more urea than do those low in N. They did not see, however, any effect of the differences in carbohydrate levels in the trees receiving various light intensities.

Among fruit tree species, *Prunus* leaves are regarded as less efficient at absorbing foliar sprayed nutrients than are apple or citrus leaves (Norton and Childers, 1954; Leece, 1978).

2.2.2 *Practical considerations:*

Foliar feeding of plants at critical growth periods to remedy transient or unexpected nutrient unbalances in the plant is advocated throughout fruit growing areas. Foliar fertilization is also proposed as a means to reduce ground-water pollution through excessive fertilizer application (Kannan, 1986). This practice has proved successful with micronutrients, whereas reports on N foliar feeding are inconsistent. Since N is a macronutrient required in relatively large amounts, supplying all of a plant's N requirements through foliar sprays is difficult.

Forshey (1963) reported a rather limited translocation of foliarly absorbed urea-N. His study showed less transport of N to permanent structures of apple trees when this element was supplied via the leaves as compared to soil application. Supplying N exclusively via sprays resulted in low levels of N in the bark but maintained adequate levels of N in the leaves. He suggested that the low vigor and productivity of trees exclusively supplied with N via sprays is caused this particular N distribution.

Postharvest urea sprays at concentrations as high as 10% (w/v) were used effectively to increase N reserves in the above ground plant tissues (Oland, 1960; Sanchez

et al., 1990). This N is readily available for blossom and early spring vegetative growth (Shim et al., 1972).

Urea sprays appear to be an effective supplement, or even a substitute at times, for soil applications of nitrogen fertilizers (Oland, 1963). Other nutrients such as B and Zn can be added to the spray solution making the operation more economical. Urea also increases the absorption of some solutes but it is incompatible with several others such as the pesticides Karathane, Dikar, and Omite (Leffingwell, 1981).

Despite half a century of research and trials, commercial use of urea is still limited. Several reasons can be cited. In apple, urea sprays in summer are often blamed for the poor red color or persistence of green color in red and green varieties, respectively (Swietlik and Faust, 1984). Late fall applications can cause leaves to remain green longer than usual, increasing the possibility that they may be killed by frost before N is translocated to the perennial parts of the tree (Swietlik and Faust, 1984). A more serious limitation is the presence of biuret, a potentially phytotoxic contaminant of fertilizer grade urea formed during the manufacturing process at high temperatures (Sanford et al., 1954). Commercially used ureas contain from 1% to 2.5% (w/w) biuret (Sanford et al., 1954; Poole et al., 1983). Biuret added to urea and sprayed onto pineapple plants increased the degree of dieback and chlorosis of the leaves (Sanford et al., 1954). Similar results were reported by Jones (1954) with citrus. It is believed that biuret interferes with protein synthesis in the leaf.

Leaf damage after high nutrient concentration sprays is also the result of local imbalance that can be created in the leaf tissue (Marshner, 1986). Several techniques were used to alleviate the severe damage that can be caused by high urea concentrations.

Simultaneous spraying with sucrose, despite the further increase in the osmotic potential of the spray solution, can help. However, its effect is mainly due to less uptake of urea by the leaves (Cook and Boynton, 1952). Lowering the solution pH (Neumann, 1983) and adding silicon-based surfactants seem to decrease leaf burn, in addition to increasing uptake, particularly in leaves with cuticles (Horesh and Levy, 1981).

3. Nitrogen Utilization Efficiency:

Exploiting the genetic diversity of plants and the advances in fertilizer technology and farm management for enhanced productivity with less industrial inputs is a desirable, if not an essential, goal in order to secure a steady and safe food supply for an increasing world population. The ability of plants to acquire and utilize nutrients from the environment has been the subject of many reviews (Chapin, 1988; Duncan and Baligar, 1990; Clark, 1990; Sauerbeck and Helal, 1990). The term 'nutrient efficiency' has been used widely as a measure of the capacity of a plant to acquire and utilize nutrients for production of timber, crops, and forages. However, its exact meaning varies widely depending on the context in which it was used and the plant for which it was measured.

3.1 Definition:

The broad definition of efficiency is output divided by input. Researchers use various definitions depending on the crop they are dealing with and the specific objectives of their research. Craswell and Godwin (1984) used three definitions that relate plant productivity to nutrient supply:

$$\text{Agronomic efficiency} = \frac{\text{grain yield}_F - \text{grain yield}_C}{\text{fertilizer N applied}} \quad \text{kg kg}^{-1}$$

$$\text{Apparent nitrogen recovery} = \frac{\text{N uptake}_F - \text{N uptake}_C}{\text{fertilizer applied}} \times 100\ (\%)$$

$$\text{Physiological efficiency} = \frac{\text{grain yield}_F - \text{grain yield}_C}{\text{N uptake}_F - \text{N uptake}_C} \quad \text{kg kg-1}$$

where F = fertilized crop and C = unfertilized control. The apparent recovery is normally based on measurements of N uptake in the above-ground plant parts, hence the term "apparent". Both physiological and agronomic efficiencies are based on grain yield rather than total dry matter yields. The apparent N recovery reflects the efficiency of the crop in obtaining fertilizer N from the soil, while the physiological efficiency and apparent recovery represent the efficiency of utilization of N by the plant to produce grains. Weinbaum et al. (1978) studied N utilization efficiency in plum during nine 10-day periods. They defined efficiency as the ratio of the total fertilizer N absorbed per tree in 10 days and the total fertilizer N applied per tree during the same period. Other researchers have used 'nitrogen uptake efficiency' (NUE), defined as nutrient uptake per unit root length, surface area, or weight (Gourley et al., 1994). Using such a definition to study the efficiency of mature trees would be challenging since it is often difficult to recover all the roots of a large plant.

Efficiency values may not be comparable for different soils and genotypes especially in field studies. A genotype will react differently to the environment in terms of growth and N absorption. Similarly, the soil affects both nutrient availability and root activity (Marschner, 1986; Sanchez, 1990).

3.2 Factors affecting efficiency:

Crops have differing capabilities to utilize applied N fertilizers. Estimates of the percentage of N from applied fertilizer which is removed from the field in the harvested portion of crops are lowest for fruit trees (<20%) (Sanchez et al., 1991); followed by grapes (37%), vegetable crops (≈55%), and hay (>70%) (Miller and Smith, 1976).

Plant size, amount of fertilizer applied, and weather conditions are all important factors in N recovery. In general, larger plants recover more fertilizer N and the larger the doze of fertilizer applied the smaller the proportion recovered in the tree (Sanchez et al., 1995). Applying N fertilizers during the rainy season results not only in considerable amounts of nitrate being leached beyond the reach of crop roots, but also in high amounts of N being lost from the soil by denitrification (Mengel and Kirkby, 1987). Woldendorp (1968) reported 10% to 40% loss of the N fertilizer applied to grassland. Denitrification may be particularly high if rainfall follows the application of N fertilizer (Webster and Dowdell, 1982). In flooded soils, reducing conditions prevail and therefore these soils are highly susceptible to denitrification (Mengel and Kirkby, 1987).

The relative amount that a plant can recover from the available N pool will depend on its sink strength in relation to loss mechanisms (Craswell and Godwin, 1984). Nye and Tinker (1969) have introduced the concept of a "root demand coefficient" in an attempt to quantify the strength of this sink. They also reviewed factors that affect root demand coefficient (Nye and Tinker, 1977). Of these factors, climate has a major effect on the sink strength of the crop through its impact on crop growth processes.

Efficiency of N recovery is influenced by climate both through its effect on losses, and hence the availability of N, and through the effect on crop growth (Craswell and

Godwin, 1984). Various climatic factors affect the amount of volatile nitrogen loss from the soil and plant tops (Wetselaar and Farquhar, 1980) and the amount of N lost via root exudation (Russel, 1977). These losses, together with losses through environmentally determined senescence of plant parts, affect both recovery efficiency and physiological efficiency (Crasswell and Godwin, 1984). Environmental stresses are important determinants of the plant metabolic activity and the partitioning of plant dry matter between fruit, leaves, canopy, and roots and thus affect physiological efficiency.

Soil is equally important in determining uptake efficiency of fertilizer N. Various soils have different capacities to retain nutrients (Mengel and Kirkby, 1987). Often loss of fertilizer by leaching is more pronounced in sandy soils and soils poor in organic matter. The soil also profoundly affects the rate of denitrification and plant growth.

4. Internal Cycling of Nitrogen in Deciduous Trees:

Plants economically utilize N. High xylem and phloem mobility allows redistribution throughout the life cycle of the plant (Feller, 1990). In deciduous trees, this redistribution involves the autumnal mobilization of leaf N into woody tissues where it is stored mainly as proteins and partly as free amino acids (Titus and Kang, 1982; Millard, 1995). Stored N is used in the following spring to support early growth. Further shoot and fruit growth in summer is dependent on root uptake of soil-N. The leaves are the main sink for N taken up in spring and summer (Sanchez et al., 1992).

4.1 Nitrogen distribution in summer:

Although ribulose-1, 5-biphosphate carboxylase (RuBPCase) is responsible for CO_2 fixation and hence dry matter accumulation, it also plays a fundamental role in the N economy of plants. This is because of its high concentration in plant tissues, usually in the range of 6 to 33 mg g-1 fresh weight, which can represent up to 50% of the leaf soluble protein (Peterson and Huffaker, 1975). In the middle of summer, leaves can contain as much as half the total N in a tree. Batjer et al. (1952) presented data indicating that a 30-year-old 'Delicious' apple tree contained 43% of its total N in the leaves at mid-season. Forshey (1963) also reported that, depending on previous N fertilization, 40% to 50% of the total N in an apple tree was present in late August leaves.

During late summer, little or no net increase in leaf N occurs (Sanchez et al., 1990). This time of the year, newly absorbed N is largely allocated to the roots, trunk, and large branches (Sanchez et al., 1992). When shoot growth ceases in late summer, N concentrations of tree woody tissues increase gradually (Mason and Whitfield, 1960). Taylor and May (1967) showed that in young peach trees the accumulation of N in the woody tissues begins as soon as the rate of shoot extension slows in early summer.

4.2 Leaf senescence and nitrogen remobilization:

Most plants have a remarkable ability to reutilize N from their senescing parts to replenish their living parts. In his review of the literature on N remobilization during senescence, Feller (1990) stated that senescence is important for an efficient use of soil N. Most of the literature available on the subject deals with annual plants especially cereals (Thimann, 1980; Feller, 1983, 1990). However, senescence that is associated with

the onset of autumn in deciduous perennials may not be the same as that caused by anthesis in annual plants or stress induced states of senescence, the sequence of events ultimately leading to the natural death of plant organs is largely common to most types. One of the first indications of senescence in apple leaves, for instance, is the decline in leaf protein, which begins when daylength declines to less than 14 h (Spencer and Titus, 1972). The leaves, however, maintain their ability to synthesize proteins; therefore, the decline in the protein content is the result of an imbalance between synthesis and degradation. The amino acids produced during protein hydrolysis are translocated to N sinks within the plant (Feller, 1990). When day-length decreases to less than 12 h, the activities of RNase, polyphenol oxidase, and malate dehydrogenase enzymes increase dramatically, while chlorophyll, DNA, and RNA in the leaf begin to decline. Along with chlorophyll disappearance, photosynthesis decreases sharply (Thimann, 1980). The two phenomena are not closely connected. Although most enzymes lose their activity during senescence, some of them remain active or even increase in activity late in the life of the leaf. For instance, the cytosolic glutamine synthetase and alanine aminotransferase in wheat (*Triticum aestivum* L.) (Tobin et al., 1985) and *Lolium temulentum* (Thomas, 1975), respectively, retain their activity until the late stages of leaf senescence. Feller (1990) hypothesized that these enzymes might be involved in the formation of transport compounds from breakdown products of protein and chlorophyll catabolism. The liberated amino acids are secondarily converted to asparagine and/or glutamine and partly deaminated to ammonia (Thimann, 1980). If the leaf is attached to the tree, the amino acids are transported out to the stem where they are stored for future use.

4.3 Nitrogen storage:

The presence of N-storing compounds was suggested when it was observed that N content of woody tissues in deciduous trees such as apple undergoes seasonal fluctuation (Murneek, 1930). After cessation of shoot growth in late summer, N contents of root, frame, and shoot tissues start increasing to reach a maximum in winter before decreasing again upon regrowth in spring (Murneek, 1942; Mason and Whitfield, 1960; Wetzel et al., 1989; Coleman et al., 1990).

The importance of reserve N for deciduous trees is obvious since bud-break in the spring takes place at a time when conditions for root uptake are not always optimal. It is well established that as plants grow and their capacity of storage increases, internal cycling of N becomes increasingly important to their overall N economy (Millard, 1993). Sanchez et al. (1991) found that some 45% of N used for annual growth in five-year-old pear trees came from storage. Similarly, in mature almond trees, it is estimated that N reserve contributes about half the N needed for annual growth (Weinbaum et al., 1987).

4.3.1 *Sites of storage:*

The early work of Murneek (1930) and Murneek and Logan (1932) indicated that in apple trees N is stored mainly in the old wood and in the roots. Later, Taylor and May (1967) agreed somewhat with Murneek's conclusion in that they found that between 60% and 80% of the storage N in dormant two-year-old peach trees was present in the root tissues, irrespective of the previous N treatments. It is usual, however, to find a higher concentration of total N in the bark than in the wood, at least in the above ground parts of fruit trees. Since the concentration of total N in the bark often falls sharply during the

growing season it has been suggested that most of the reserve N of trees is held in bark tissues (Mason and Whitfield, 1960). As pointed out by Taylor (1967) and by Titus and Kang (1982), this is a matter of expression and interpretation of data, i.e., the question of absolute amount per tree or tissue or concentration per unit weight. Mason and Whitfield (1960) looked at the seasonal changes in N in a whole apple tree and concluded that, after shoot extension has ceased, a gradual increase of N in wood and bark of all parts of the tree including roots occurs. However, the N needed at the beginning of leaf development is derived mainly from bark of branches and stems, mostly from the points nearest to leaf formation. Root N drops appreciably only between the end of April and July, suggesting that most of the reserve N in the apple tree is contained in bark tissues.

In the bark of dormant young apple trees, proteins account for as much as 90% of total extractable N (Kang et al., 1982). These proteins decline considerably during spring growth, while amino acids increase. It appears that the immediate requirement of N at the early stage of growth (up to silver-tip) is met by the transport of soluble N present in adjacent bark and that redistributed from the wood. The large requirement of nitrogen after the silver-tip stage of growth is then met by massive breakdown of storage proteins (Kang et al., 1982). However, these conclusions were based only on concentrations of proteins and amino acids in the bark of one-year-old shoots. Similarly, in poplar (*Populus spp.*), wood and bark tissues of stems and roots are thought to be the main sites of N storage (Sauter et al., 1989; Pregitzer et al., 1990). Sauter et al. (1989) reported higher protein concentrations in one-year-old shoots than in two-year-old roots. Bark and wood tissues of shoots averaged 12.4 and 31.9 μg protein mg^{-1} DW, respectively, and 12.7 and 8.8 μg mg^{-1} DW for roots. These proteins were found to

accumulate in special vacuoles in the bark parenchyma and xylem ray cells (Wetzel et al., 1989; Greenwood et al., 1990).

4.3.2 *Chemical nature of storage compounds:*

Free arginine is the principal constituent of extracts of woody storage tissues of dormant apple (Oland, 1954), peach (Taylor and May, 1967), plum and cherry trees (Romanovskaya, 1963, reported by Taylor, 1967). All these species belong to the family Rosaceae. Glutamine and asparagine are also important constituents of the storage N of dormant apple trees (Oland, 1954), but this is not the case with peach trees (Taylor and May, 1967). The molecular structure of arginine explains why it is theoretically the most efficient form of storage N. It contains 4 N for 6 C atoms resulting in a high N/C ratio (Titus and Kang, 1982). In a study of the nitrogenous reserves in apple trees, Oland (1959) argued that the statement that the free amino acids have a main function as nitrogenous reserves, is supported by the fact that they accumulate in considerable quantities, and by the lack of evidence of any quantitative demand for amino acids even where the metabolic activity is supposed to be vigorous. However, the protocol he used to extract the various nitrogenous compounds from the plant materials did not deal with the problem of proteases found in the tissues. To the contrary, the proteolytic activity could have been aggravated by leaving the tissues at room temperature for an extended period of time and subsequently heat drying them. It is possible that some of the amino acids recovered in this manner are actually the product of protein breakdown during handling.

4.3.3 *Vegetative storage proteins:*

There is a growing body of evidence suggesting that the main form of storage of N in dormant tree tissues is proteins (Titus, 1982; Kang et al., 1982; Wetzel et al., 1989; Coleman et al., 1991). These proteins were considered to be vegetative storage proteins (VSP). They were found to accumulate in the vacuoles of bark parenchyma and xylem ray cells (Wetzel et al., 1989). The turnover of plant proteins releases amino acids for reutilization, often after the amino acids have been translocated to new organs. Vegetative storage proteins have not received as much attention as seed storage proteins mainly because the latter have a far greater value for humans. However, VSPs are extremely important in the tree N economy.

The definition of storage proteins differs depending on the organ in which they are present. In seeds, they are defined as proteins that accumulate in significant quantities during the later stages of development of the seed, then upon germination they are rapidly hydrolyzed to provide a source of reduced N for the early stages of growth of the seedling (Higgins, 1984). In deciduous woody perennials, proteins are considered to store N if they accumulate to high levels in the tree during autumn and winter, decline in spring, and are absent during the summer (Titus and Kang, 1982). Although all proteins may be considered to "store" amino acids, the accumulation of most proteins is governed by the need for their enzymatic or other metabolic activity. Thus, proteins usually have limited flexibility in their storage capacity. In contrast, the pattern of VSP accumulation and loss correlates well with the changing need for reserve storage in vegetative tissues during plant development (Staswick, 1990).

Kang et al. (1982) monitored the seasonal changes in peptide composition of 'Golden Delicious' apple bark extracts. They found little evidence of net breakdown in the majority of proteins during the early growth period. They noticed, however, that peptides of molecular weights less than 20 kD fluctuated throughout the of the study, whereas two polypeptides, 38 and 56 kD in size, were present up until mid-April and disappeared in May. However, the overall 60% decline in total protein could not be accounted for by the loss of these two polypeptides. More recently, Lang and Tao (1992) reported the presence of a 31-kD polypeptide that may act as a storage protein in various apple tissues.

In poplar, the accumulation of the VSP is preceded by a dramatic but transient appearance of the corresponding mRNA (Clausen and Apel, 1991; Coleman et al., 1991; van Cleve and Apel, 1993). The signals responsible for inducing the massive and sudden increase in the mRNA content are not yet known. It has been suggested that a change in photoperiod may directly regulate the accumulation of the storage protein(s) (Coleman et al., 1992) and that phytochrome may be involved in this control (Langheinrichand Tischner, 1991). However, recently van Cleve and Apel (1993) demonstrated that large amounts of storage protein accumulate not only under short days but also under long-day conditions when poplar trees were exposed to low temperatures or fed with an excess of nitrogen. Woody species may modulate the synthesis of their storage protein(s) in a manner similar to that of herbaceous plants. In soybean and potato, the deposition of VSPs has been shown to be dependent on the sink/source conditions within the plant (Paiva et al., 1983; Staswick, 1990).

CHAPTER 3

NITROGEN PARTITIONING IN APPLE IS AFFECTED BY TIMING AND TREE GROWTH HABIT

1. Abstract:

Nine-year-old standard- (Std) 'Topred Delicious' and spur-type 'Redspur Delicious' apple trees (*Malus domestica* Borkh.) on Malling 7A (M.7A) rootstocks were fertilized with ground-applied ammonium nitrate or foliarly-applied urea depleted in ^{15}N on various dates. The treatments consisted of a ground application in spring (March) (SG), a pre-harvest ground application in August (PHG), a foliar spray in fall (September) (FF), or a combination of each of the last two treatments with SG (SG/PHG and SG/FF). All trees received a total of 120 g N each with the exception of FF trees, which received half the dose. Labeled N from the SG application was allocated preferentially to the fruit, leaves, shoots, and branches and to a lesser extent to the roots. Leaves on young shoots had higher concentrations of ^{15}N label than those on mature shoots or spurs, indicating an increasing dependence of the tree on soil N from spring to summer. The amount of N allocated to above ground tissues decreased as the season progressed. Very little ^{15}N from the PHG application reached the leaves, fruit, buds, or branches, but the roots were heavily labeled and substantial amounts of labeled N were found in the following season's fruit, leaves, and shoots. Losses of fertilizer-^{15}N from the PHG application to fruit removal, leaf fall, and pruning were minimal, suggesting a higher utilization efficiency of absorbed N. The percentage of recovered N derived from the FF spray was low in all tissues. However, this treatment resulted in ^{15}N reaching the buds and shoots. Spur-type tree tissues tended to have higher N and ^{15}N concentrations

compared to those of Std-type trees. These differences suggest that the smaller spur-type trees are more manipulatable with N management, and may be more subject to the adverse effects of over-fertilization. The subtle differences in N partitioning between tree types may cause spur-type trees to recycle N more efficiently.

2. Introduction:

Increasing public concern about ground water contamination has fueled interest in alternative fertilization strategies that can minimize the amount of N applied in the orchard without compromising yield and quality. Applying N during the time of maximum root absorption efficiency, i.e. late spring and early summer (Weinbaum et al., 1978; Muñoz et al., 1993), can maintain the same level of N supply to the growing tissues with less fertilizer applied. However, fertilization when the capacity of the tree to absorb NO_3^- is maximal, need not necessarily lead to an optimal partitioning of N between reproductive and vegetative organs (Weinbaum et al., 1980). The goal should be to promote flowering and fruit set, thus assuming good yield, and also produce reasonably vigorous trees with low-N fruit (Sanchez et al., 1992). The orchardist should be more concerned with providing adequate nutrients to specific organs in addition to optimizing the over-all nutrition of the tree.

Distribution of newly absorbed or reserve N in a tree can be followed directly using an isotopically labeled N carrier. However, the experimental use of N isotopes with tree species has been mostly restricted to immature plants in pot culture (Taylor and May, 1967; Hill-Cottingham and Lloyd-Jones, 1975; Weinbaum et al., 1978; Muñoz et al., 1993). Results from these experiments may not be applicable to mature trees under

orchard conditions (Hill-Cottingham, 1968). Among the few instances where tracer techniques were used to investigate how management alters N partitioning in mature deciduous fruit trees are the papers of Weinbaum et al. (1980, 1984,1987) and Sanchez et al. (1990, 1992). However, it may be misleading to extrapolate data from these studies to fruit trees with different growth and fruiting habits.

Using ^{15}N-fertilizers, my objectives were to a) verify previous reports where spring applications to fruit trees result in large amounts of fertilizer-N being partitioned into fruit and vegetative growth while late season applications favor the roots and frame, b) evaluate the potential use of timing, form, and mode of application of N to target specific nitrogen reserve pools in the tree, and to determine the impact of these practices on bud and fruit N content, and c) study how tree growth habit (standard vs. spur) alters N partitioning patterns in mature apple trees.

3. Materials and Methods:

Two adjacent rows of nine-year-old 'Redspur Delicious', a spur-type, and 'Topred Delicious', a standard-type (Std), apple trees on M.7A rootstock growing in a silty clay loam soil at the Lewis-Brown Research Farm near Corvallis, Ore. were used in this study. The trees were trained to a modified central leader and spaced 3.5 x 5.5 m. A 1.5 m herbicide strip was maintained with red fescue between the rows. In 1992, three single replicate trees, randomly selected within each row (strain), were given either a spring ground (SG) on 20 Mar., a pre-harvest ground (PHG) on 27 Aug., a fall foliar (FF) on 23 Sept., a SG and a PHG (SG/PHG), or a SG and a FF (SG/FF) N fertilizer application. Ammonium nitrate and urea, both depleted in ^{15}N (0.09 atom % ^{15}N), were

used for ground and foliar applications, respectively. Application rates are summarized in Table 3.1. On 1992, full bloom was during the first week of April.

Table 3.1. Amounts of actual N applied (g/tree) for each treatment. Urea and ammonium nitrate were used for foliar sprays and ground applications, respectively. Both fertilizers were depleted in ^{15}N (0.09 atom %). All treatments were applied in 1992.

	Date of fertilizer application		
Treatment	20 Mar.	27 Aug.	23 Sept.
Spring ground (SG)	120	0	0
Pre-harvest ground (PHG)	0	120	0
Fall foliar (FF)	0	0	60
Split:1/2SG + 1/2PHG (SG/PHG)	60	60	0
Split:1/2SG + FF (SG/FF)	60	0	60

Since the foliar treatments were applied after fruit and leaves were sampled (in 1992), the experimental design enabled us to determine a dosage response of the two tree types. Towards this purpose, N and ^{15}N concentrations were determined for a variety of leaf and fruit tissues from the SG and SG/FF treatments. On 9 June 1992, spur leaves, old and new shoot-leaves, and fruit were sampled. On 27 Aug., mid-shoot leaves were sampled and on 17 Sept. fruit and a mixed sample of leaves were collected. The samples were randomly selected from around the tree.

In winter, the trees were moderately pruned and about twenty 5-10 cm portions of new growth (1992 shoots) and branches (more than one year old) were collected from each tree. Small roots (diameter $<$ 1 cm), bark, and buds were sampled on 27 Dec. Mature fruits were freeze-dried; all other samples were oven-dried at 60 °C.

All biomass losses, i.e. harvested fruit, senescent leaves (in fall), and prunings (in January 1993), were recorded for each tree. A net was placed over each tree after harvest to collect falling leaves. Five fruit and about 30 leaves were randomly selected from each tree on 12 Oct. 1993 and after complete leaf senescence (mid-December), respectively.

In 1994, the trees were cut off at the graft union. The above-ground structure was fractionated into new growth (1993 shoots), branches (diameter 4 cm), and frame. Stumps were pulled out using a tractor and the roots were recovered by hand while carefully shoveling soil from a 2 x 2 x 1 m hole around the main root. Since only few roots of small caliper extended outside the excavation zone, root losses were considered minimal. Soil was washed from the roots with a high-pressure water stream. Roots were classified as either small (diameter $<$ 1 cm) or large (diameter $>$ 1 cm). Each tree portion was immediately weighed and sub-samples were collected to determine dry matter content. Dry weight data were used in the analyses. New growth, branches, and small roots were sampled by collecting about twenty 4-6 cm portions from the middle of several tissue pieces. A composite frame sample was constructed by mixing sawdust samples from trunk and large branches based on their relative weights (Sanchez et al., 1992). Large roots were sampled in a similar manner.

After drying, the samples were ground to pass a 40-mesh screen. Nitrogen was determined colorimetrically with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973). Sub-samples were analyzed for ^{15}N by mass spectrometry at Isotope Services (Los Alamos, N.M.). Atom percentage values were converted to nitrogen

derived from the labeled fertilizer (NDFF) using the following equation (adapted from Hauck and Bremner (1976)):

$$NDFF = \frac{(^{15}N_{\text{natural abundance}}) - (\text{atom } \%\,^{15}N)_{\text{tissue}}}{(^{15}N_{\text{natural abundance}}) - (\text{atom } \%\,^{15}N)_{\text{fertilizer}}} \quad ; \text{ where}$$

^{15}N abundance in the soil was considered equal to 0.37 atom percent (Handbook of Chemistry and Physics, 1969). The data were tested using Analysis of Variance (ANOVA) for a factorial design. Means were separated by LSD.

4. Results and Discussion:

There were few differences in N concentration between either strains or fertilizer doses (Table 3.2). The plot used in this study was typical of many commercial orchards with a history of high N applications. Under these conditions it is often difficult to show N responses or treatment differences in a single year due to ample tree reserves and naturally fertile soil (Sanchez et al., 1995). However, treatment effects could be detected by evaluating ^{15}N labeling even though the maximum percentage of NDFF in any of the tissues analyzed was only 11.7% (Table 3.3). The low contribution of fertilizer-N confirms the importance of other N sources (tree reserves and soil) for established trees (Sanchez et al., 1992). At the first sampling in June, new shoot-leaves derived a larger proportion of their N from the fertilizer compared to spur and old-shoot leaves. This suggests an increasing dependence of the trees on newly absorbed soil N for their growth as the season progressed. Our results are consistent with earlier reports by Sanchez et

Table 3.2. Effect of spring application fertilizer dose (60 vs. 120 g N/tree) on N concentration in leaves and fruit of standard- (Std) 'Topred' and spur-type 'Redspur' apple trees[z].

	Sampling date					
	9 June 1992				27 Aug. 92	23 Sept. 92
	Tissue					
	Spur leaves	Old shoot-leaves	New shoot-leaves	Fruit	Shoot-leaves	Leaves
N dose	N concentration (% dry wt)					
60	2.62a[y]	2.72a	2.76a	0.66a	2.08a	2.06a
120	2.52a	2.75a	2.69a	0.72a	2.02a	1.85b
Significance	NS	NS	NS	NS	NS	*
Tree type						
Std	2.48a	2.80a	2.76a	0.66a	2.06a	1.72b
Spur	2.66a	2.67a	2.69a	0.72a	2.04a	2.15a
Significance	NS	NS	NS	NS	NS	***
Dose x type						
Significance	NS[x]	NS	NS	NS	NS	NS

[z]The fertilizer was soil-applied on 20 Mar. 1992.

NS, *, ***Nonsignificant or significant at $P = 0.05$ or 0.001, respectively.

[y]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

[x]Treatment effects were significant at $P = 0.1$.

al. (1991, 1992), which showed that the ability of fully expanded pear (*Pyrus communis* L.) spur leaves to acquire N is limited. It has been demonstrated, however, that leaves are capable of accumulating N if it is provided to them in abundance (Staswick, 1990).

Doubling the fertilizer dose increased the percentage of N and NDFF in spur-type fruit, but not in Std-type fruit as is evident statistically as a significant interaction (Tables 3.2 and 3.3). High N fruit can be a serious management problem. Excessively vigorous trees typically have an undesirably high N concentration in the fruit which can lead to an increased susceptibility to disorders and decay (Bramlage et al., 1980; Embleton and Jones, 1978; Sanchez et al., 1995; Sugar et al., 1992).

In 1992, June fruit, September leaves, and new growth of the spur strain had higher N and NDFF percentages than those of the Std strain (Tables 3.2, 3.3, 3.4, and Fig. 3.1B). These differences were more apparent with the SG treatment. Therefore, spur-type trees may be more manipulatable with N management and deleterious effects of high N applications could be more severe.

There may be several explanations why leaves and fruit of the spur strain appear more responsive to ground applications of nitrogen. Having less branch and frame biomass, spur-type trees could have less reserves to draw upon and therefore be more dependent on soil derived N. Also, if total uptake was comparable for both strains, similar amounts of labeled N would be allocated to the two types of trees. Since the tissues of spur-type trees were smaller (less bulky) and contained less N (g/tree) (Fig. 3.2A), similar uptake would result in higher percentages of NDFF than the Std strain tissues (Table 3.3, 3.5, Fig. 3.1B). Lastly, N could be partitioned differently among tissues depending on the strain.

Standard-type trees had 58% more dry matter, but only 50% more N than the spur-type (Fig. 3.2A). This may partially explain why several spur-type tissues were

Table 3.3. Effect of spring application fertilizer dose (60 vs. 120 g N/tree) on the percentage of N derived from the labeled fertilizer (% NDFF) in leaves and fruit of standard-(Std) 'Topred' and spur-type 'Redspur' apple trees[z].

	Sampling date					
	9 June 1992				27 Aug. 92	23 Sept. 92
	Tissue					
	Spur leaves	Old shoot-leaves	New shoot-leaves	Fruit	Shoot-leaves	Leaves
N dose	% NDFF					
60	4.5a[y]	4.9a	9.1a	3.9b	7.0a	5.4b
120	4.3a	5.5a	11.7a	6.1a	11.3a	8.7a
Significance	NS	NS	NS	*	NS[x]	*
Tree type						
Std	4.6a	4.1a	9.4a	3.8b	7.1a	3.6b
Spur	4.2a	6.2a	11.4a	6.2a	11.2a	6.7a
Significance	NS	NS	NS	*	NS[x]	**
Dose x type						
Significance	NS	NS	NS	*	NS	NS

[z]The fertilizer was soil-applied on 20 Mar. 1992.

NS, *, **Nonsignificant or significant at $P = 0.05$ or 0.01, respectively.

[y]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

[x]Treatment effects were significant at $P = 0.1$.

more concentrated in N (Table 3.4). Trees of both strains allocated similar amounts of fertilizer derived-N to their branches, frames, and large roots (Fig. 3.2A). With the exception of small roots, distribution of total N was similar for both tree types (Fig. 3.2B). Since total uptake was similar (Fig. 3.2A), the 120 g N applied in spring 1992 were a relatively more important (larger) supply for the spur strain.

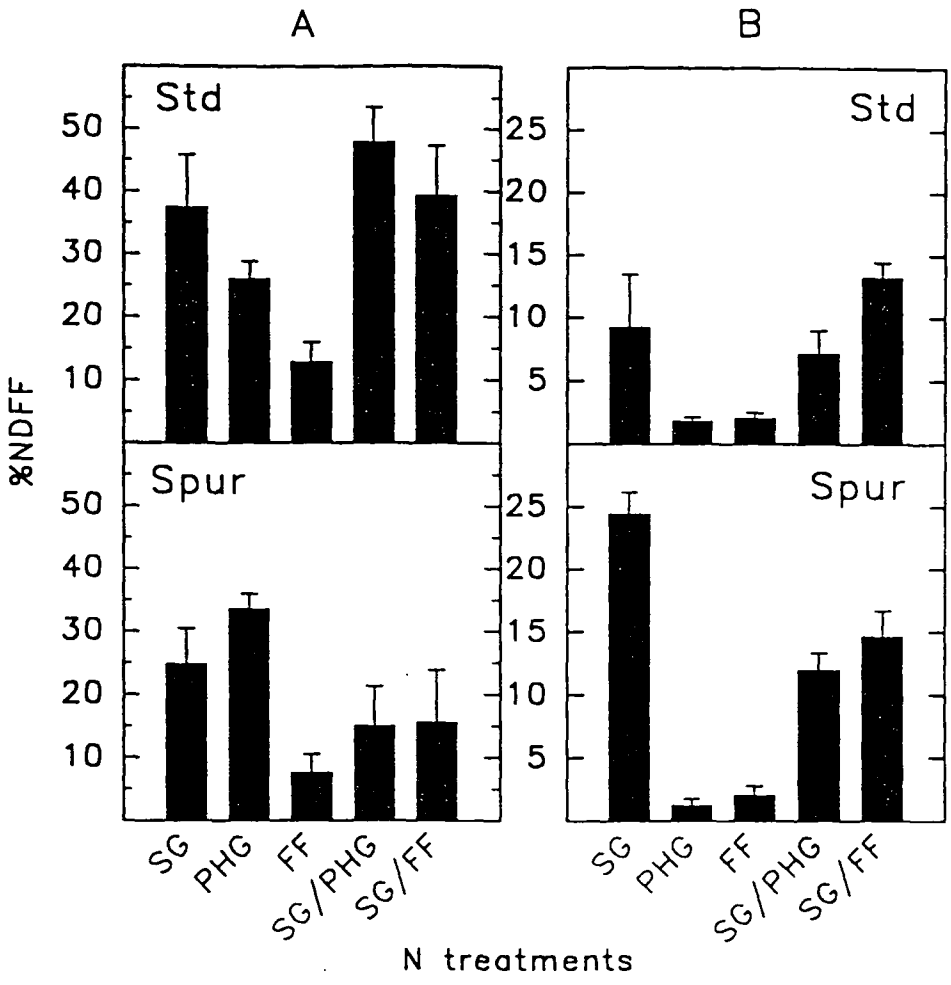

Fig. 3.1. **Percent of the total N derived from the labeled fertilizer (%NDFF) in small roots (A) and shoots (B) of 'Topred Delicious', a standard-type (Std) apple variety, and 'Redspur Delicious', a spur-type variety. The tissues were sampled on 15 Jan. 1993 and 27 Dec. 1992, respectively. Each bar represents the mean of three replicates. The treatments were a 120 g N spring ground-applied in Mar. (SG), a similar pre-harvest application in Aug. (PHG), a 60 g urea-N as fall foliar spray (FF), a split (SG/PHG): half (SG) plus half (PHG), and a split (SG/FF): half (SG) plus (FF).**

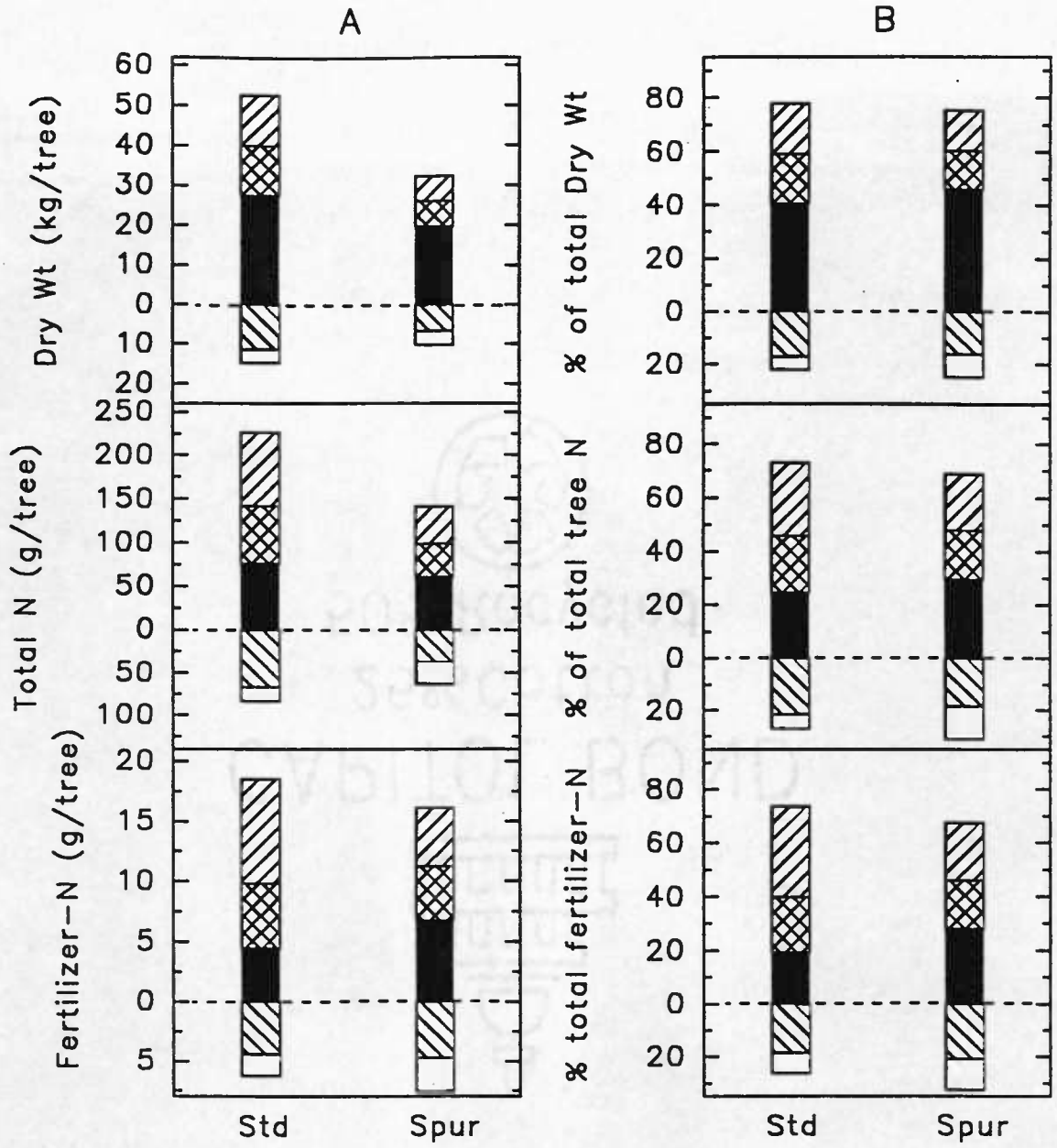

Fig. 3.2. **Total (A) and relative (B) contribution of various tissues to whole tree dry matter, N and fertilizer-derived-N (fertilizer-N) contents for Standard- (Std) 'Topred Delicious' and spur-type 'Redspur Delicious' apple trees at the end of 1993. The labeled fertilizers were applied in 1992. The trees were divided into senescent leaves plus fruit plus shoots (▨), branches (▩), frame (■), large roots (more than 1 cm in diameter) (▧), and small roots (less than 1cm in diameter) (□). Each bar is an average of 15 trees. The graft union position is indicated by the horizontal dashed line.**

Table 3.4. Effect of time of fertilizer application on N concentration in different components of standard- (Std) 'Topred' and spur-type 'Redspur' trees.

	23 Sept. 92[x] leaves	17 Sept. 92 fruit	27 Dec. 92 bark	15 Jan. 93 new growth[w]
N treatment[z]	N concentration (% dry wt)			
SG	1.85bc[y]	0.21a	1.54a	0.80a
PHG	1.74c	0.21a	1.44a	0.93a
FF	1.97abc	0.18a	1.40a	0.87a
SG/PHG	2.26a	0.20a	1.44a	0.75a
SG/FF	2.06ab	0.20a	1.48a	0.93a
Significance	*	NS	NS	NS
Tree type				
Std	1.72b	0.16b	1.38b	0.74b
Spur	2.15a	0.24a	1.53a	0.97a
Significance	***	***	**	***
Treatment x type				
Significance	NS	NS	NS	NS

[z]The treatments were SG= 120 g N soil-applied on 20 Mar. 1992, PHG= 120 g N soil- applied on 27 Aug. 1992, FF= 60 g urea-N sprayed on 23 Sept. 1992, SG/PHG= 1/2 SG + 1/2PHG, SG/FF= 1/2SG + FF.

NS, *, **, ***Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

[y]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

[x]Sampling date of the tissue.

[w]Shoots of 1992, used also to obtain bark samples.

Table 3.5. Effect of time of fertilizer application on the percent of the N derived from the labeled (% NDFF) fertilizer in different components of standard- (Std) 'Topred' and spur-type 'Redspur' trees.

	23 Sept. 92[y] leaves	17 Sept. 92 fruit	27 Dec. 92 buds	15 Jan. 93 branches
N treatment[z]	% NDFF			
SG	8.7a[x]	9.8a	10.1a	15.1a
PHG	0.3b	0.5c	0.6b	2.0d
FF	ND	ND	1.7b	4.4dc
SG/PHG	6.3a	4.7b	7.5a	9.3bc
SG/FF	5.4a	5.3b	9.8a	14.8ab
Significance	**	***	**	***
Tree type				
Std	3.6b	2.8b	5.3a	7.8a
Spur	6.7a	7.4a	6.2a	10.4a
Significance	*	**	NS	NS
Treatment x type				
Significance	NS	NS	NS	NS

[z]The treatments were SG= 120 g N soil-applied on 20 Mar. 1992, PHG= 120 g N soil-applied on 27 Aug. 1992, FF= 60 g urea-N sprayed on 23 Sept. 1992, SG/PHG= 1/2 SG + 1/2PHG, SG/FF= 1/2SG + FF.

NS, *, **, *** Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

[y]Sample date of the tissue.

[x]Values within the same column that are followed by the same letter are not significantly different at P = 0.05.

[ND]Not determined because ^{15}N-urea was not applied yet at that time.

The difference in the relative size of the fertilizer N supply would explain the higher %NDFF in the shoot leaves and fruit (June 1992) of the spur strain compared with those of the Std strain (Table 3.3). Spur-type trees appear to be more responsive to spring N-fertilization because smaller size was accompanied by similar uptake.

Small roots were an exception. Spring ground-applications of ^{15}N resulted in higher %NDFF in small roots (Dec. 1992) of Std-type trees compared to those of spur-type trees (Fig. 3.1A). Spur- and Std-type trees were similar for the FF and PHG treatments. Since the small-root systems of the two strains were similar in size and N content (on a dry weight basis), the differences observed in the %NDFF could be due to differences in partitioning of the N absorbed in spring and early summer.

Differences between treatments for N concentration were insignificant for most of the tissues sampled late in 1992 (Table 3.4). Differences were more apparent for the proportion of NDFF (Table 3.5). Nitrogen applied in spring led to the highest percentage of NDFF in fruit and leaves sampled in Sept. 1992. Since both tissues had little label from PHG application and the FF spray was applied after sampling, it is reasonable to assume that most of the ^{15}N recovered in the fruit and leaves of trees that received the split treatments came from the spring application. Labeled N applied in the spring was also heavily partitioned into branches and roots (Table 3.5 and Fig. 3.1A). The split treatments gave the next highest percentage of NDFF in Sept. 1992 fruit and leaves and Jan. 1993 branches. In contrast, PHG applied N was preferentially allocated to the roots. Leaves sampled 25 days after the application of PHG treatment had only traces of ^{15}N even though they appeared healthy. The treatments had no effect on the time of leaf abscission. These results are in agreement with previous reports on pear (Sanchez et al., 1992), peach (Muñoz et al., 1993), and apricot (Weinbaum et al., 1980).

The differences in partitioning between N application timings are related to the strength of each tree part as a N sink when the labeled N is being absorbed. Early in the growing season, fruit, leaves, and shoots are the strongest sinks (Sanchez et al.,

1992; Muñoz et al., 1993). Later, when shoot growth stops, roots are most active and the bulk of N and photosynthates is diverted to them (Pregitzer et al., 1990). Some of the treatment-growth habit interactions (Fig. 3.1) might be due to differences in the dates of cessation of shoot growth and the onset of intensive root activity.

The percentage of NDFF in trees that received only a fall foliar spray was low in most tissues analyzed (Table 3.5 and Fig. 3.1). This suggests that the urea solution used was too dilute [1% (w/v)] compared to the 5% (w/v) concentration often used commercially. The amount of fertilizer-N applied was also small. The concentration of ^{15}N in leaves was not determined immediately after urea spray; therefore, urea uptake could not be measured. However, the buds, new growth, and small branches collected in winter of 1992 had a substantial amount of urea-^{15}N (Table 3.5 and Fig. 3.1), whereas senescent leaves had only 2.6% of their N from the sprayed urea (data not shown). These results agree with earlier reports in which fall foliar-urea-sprays were used to deliver N to the buds (Oland, 1960; Sanchez et al., 1990). It is our belief that the labeled N detected in the roots (FF treatment) originated from direct root uptake of urea that dripped onto the ground during spraying rather than translocation from the leaves. Contrary to the findings of Shim et al. (1973) with rooted *Malus* cuttings, N from foliar urea sprayed onto foliage in fall has a limited mobility during the year of its application for mature pear and apple trees (Sanchez et al., 1990; Chapter 5).

In 1993, the proportion of N derived from 1992 ^{15}N-applications in the fruit, senescent leaves, branches, new growth and small roots was highest for PHG and lowest for FF treatment. The split treatments also contributed substantially to the N found in these tissues, while the SG was intermediate compared to FF and PHG treatments (Table

3.6). These results were predictable from the partitioning patterns of newly absorbed N in 1992 (Table 3.5 and Fig. 3.1). Nitrogen derived from the PHG application was heavily allocated to the roots (1992); therefore, it escaped losses by fruit removal, leaf senescence and pruning (Chapter 4). Ground-applied N-fertilizers may thus be used more efficiently if applied in the late summer.

Table 3.6. Percentage of total 1993 N which was obtained from 1992 N-treatments in standard- (Std) 'Topred' and spur-type 'Redspur' apple trees. The 1993 shoots were called new growth.

	12 Oct. 93[y] fruit	Senescent leaves	Feb. 94 branches	Feb. 94 new growth	Feb. 94 small root
N treatment[z]	1992 fertilizer-N contribution (%)				
SG	11.1b[x]	9.9bc	8.2bc	6.3a	10.8ab
PHG	21.4a	16.8a	15.8a	18.0a	15.1a
FF	4.8c	5.5c	3.7c	4.8a	4.5c
SG/PHG	16.4ab	13.2ab	10.3ab	14.5a	14.1a
SG/FF	14.8ab	12.5ab	10.6ab	9.0a	9.8b
Significance	***	**	*	NS[w]	**
Tree type					
Std	13.6a	9.6b	8.2a	9.8a	10.1a
Spur	13.8a	13.6a	11.2a	11.2a	11.5a
Significance	NS	*	NS	NS	NS
Treatment x type					
Significance	NS	NS	NS	NS	NS

[z]The treatments were SG= 120 g N soil-applied on 20 Mar. 1992, PHG= 120 g N soil-applied on 27 Aug. 1992, FF= 60 g urea-N sprayed on 23 Sept. 1992, SG/PHG= 1/2 SG + 1/2PHG, SG/FF= 1/2SG + FF.

NS, *, **, *** Nonsignificant or significant at P = 0.05, 0.01, or 0.001, respectively.

[y]Sampling date of the tissue.

[x]Values within the same column that are followed by the same letter are not significantly different at P = 0.05.

[w]Treatment effects were significant at P = 0.1.

Spur-type trees had higher percentages of NDFF in the senescent leaves of 1993. This may indicate that spur-type trees could have had a higher portion of the previous year's ^{15}N available for spring growth. Since both strains recovered similar amounts of N from the applied fertilizer (Fig. 3.2 and Chapter 4), this difference in reserve-^{15}N is likely due to the higher losses of labeled N in 1992 fruit, senescent leaves, and prunings for Std-type trees (Chapter 4).

By the end of the second year, Std-type trees had allocated a larger proportion of their fertilizer-derived-N to the fruit, leaves, and shoots (Fig. 3.2B). Since most of the N in these tissues is removed from the system, spur-type trees may have superior N recycling efficiency. It was possible to estimate leaf biomass at the end of Aug. 1993 using the weights of senescent leaves and specific leaf weights recorded from August to mid-December, time of total leaf fall. We assumed that the total leaf area per tree did not increase after 27 Aug. It was estimated that the leaves recycled 59% of the N accumulated up to 27 Aug. 1993, assuming that all the N not accounted for in the litter re-entered the perennial parts of the tree. It has long been recognized that prior to their abscission, leaves re-export some 40% to 50% of their total N (Murneek, 1930; Castagnoli et al., 1990). This figure can be as high as 70% in some cases (Shim et al., 1972; Chapin and Kedrowski, 1983; Sanchez et al., 1990). Differences between closely related species and years are mainly due to fall weather conditions (Sanchez et al., 1990). During long warm autumns (conditions common to western Oregon), leaves recycle a larger proportion of their N compared to what can be recycled in colder climates. This recycled leaf-N accounted for about 20% of the total N in dormant trees, regardless of fertilizer treatment and tree growth habit (strain).

Uptake of soil N by field grown mature deciduous fruit trees is continuous throughout the year in mild climates with relatively high peaks in late spring and mid-summer (Grasmanis and Nicholas, 1971; Titus and Kang, 1982; Habib, 1984; Muñoz et al., 1993). In the present study, new growing tissues in spring drew heavily on reserves, while subsequent growth depended more on new uptake. It appears that N from spring applications was available for uptake during the period of high root activity. Labeled N absorbed in late spring was allocated preferentially to fruit and vegetative growth and to a lesser extent to storage tissues. The concentration of ^{15}N derived from PHG applications was low in Sept. 1992 leaves and fruit and in winter buds and branches. However, 1993 tissues derived a high proportion of their N from this late summer application. In autumn, N can be delivered to the buds and shoot tissues by foliar sprays. However, the concentration of the urea spray solution should be increased from what was used in this trial especially since substantial leaf burn can be tolerated late in the season.

Our results indicated that by altering N timing, amount, and method of application (foliar urea vs. ground), trees can be managed for both optimum vigor and N concentration of various tissues. This is especially true for spur-type trees which seem to be more dependent on newly absorbed N and more manipulatable with N management. Subtle differences in the partitioning of total N and recently obtained N, may cause spur-type trees to recycle N more efficiently. However, they may be more subject to the adverse effects of over-fertilization (Sanchez et al., 1995).

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CHAPTER 4

NITROGEN UPTAKE AND RECYCLING EFFICIENCY IN STANDARD- AND SPUR-TYPE APPLE TREES

1. Abstract:

Nine-year-old standard- (Std) 'Topred Delicious' and spur-type 'Redspur Delicious' apple trees (*Malus domestica* Borkh.) on Malling 7A (M.7A) rootstocks were fertilized with ground-applied ammonium nitrate or foliarly-applied urea depleted in ^{15}N on various dates in 1992. The treatments consisted of ground applications in spring (20 Mar.) (SG) or pre-harvest (27 Aug.) (PHG), a fall foliar spray (23 Sept.) (FF), or a combination of each of the last two treatments with SG (SG/PHG and SG/FF). The application rates were 60 g N per tree for FF and 120 g for all other treatments. The trees were dug in Feb. 1994 and their dry matter, total N, and ^{15}N contents were determined. Nitrogen uptake efficiency (NUE) was calculated as the ratio of the total amount of fertilizer-N recovered in a tree and amount applied. There was no treatment effect on NUE although Std-type trees tended ($P = 0.098$) to recover more fertilizer-N than spur-type trees if differences in N losses and tree size are not accounted for. About a third of the variation in NUE was due to tree size. Larger trees recovered more applied N. When NUE was adjusted for N that was lost in 1992 fruit, senescent leaves and prunings (termed actual-NUE) and tree size used as a covariate, differences between treatments became more apparent. The PHG application had a higher actual-NUE compared to all other treatments.

2. **Introduction:**

Over-fertilization is a common practice among high-value fruit crops. In fruit orchards, nitrogen fertilizer accounts for a small portion of production cost. Growers use fertilizers as insurance against a relatively small chance of nutrient deficiency (Sanchez et al., 1995). Excessive N fertilizer application may lead directly to the leaching of nitrate into deep soil layers where eventually it may reach the ground water and aquifers which feed drinking-water wells (Pilbeam and Mengel, 1992). The problem is made worst by the low N use efficiency of fruit trees. The percentage of fertilizer-N removed from the field in the harvested portion of plants is estimated to be 15-20% for pears (Sanchez et al., 1991), 37% for grapes (Miller and Smith, 1976), and between 30 and 80% in annual crops depending on a variety of environmental, genetic, and cultural factors (Craswell and Godwin, 1984). The efficiency with which the plant utilizes the fertilizer-N in the available N pool depends on the form and amount of N available, the availability of soil water, and the distribution and sink strength of the plant root system (Craswell and Godwin, 1984).

Societal concerns about groundwater contamination in recent years have prompted many studies with the objective of improving the efficiency of N fertilizer management and ultimately reducing the over-all use of fertilizers in the production system (Embleton and Jones, 1978; Weinbaum et al., 1978; Craswell and Godwin, 1984; Löhnertz, 1991). The benefit from such reduction comes not only from lowering the production cost, and improving the public perception of modern agriculture, but also from a better fruit quality which can translate into higher returns.

Mineral nutrient efficiency has little meaning unless specifically defined (Clark, 1990). The broad definition of efficiency is output divided by input. In the context of this study, efficiency refers to fertilizer-N uptake efficiency (NUE) defined as the amount of fertilizer-N taken into the tree compared to the amount applied (modified from Weinbaum et al., 1978; Hauck, 1978; and Moll et al., 1982). When determining NUE, it is important to consider the amount of N lost in crop, senescent leaves, and prunings. Both N uptake and recycling efficiency may be important when evaluating germplasm or management options.

Several methods can be used to determine the recovery of applied nitrogen by plants, but use of the stable isotope ^{15}N remains the method of choice (Hauck, 1978). Complete destructive harvest can accurately assess N recovery, but less time consuming and expensive methods are required. In this study we used N fertilizers depleted in ^{15}N to a) investigate the potential use of timing and mode of fertilizer application to improve N use efficiency, b) compare N recoveries of Std- and spur-type apple trees, and c) evaluate approaches to estimate fertilizer-N recovery without complete destructive harvests.

3. Materials and Methods:

Two adjacent rows of nine-year-old spur-type 'Redspur Delicious', and standard (Std) 'Redtop Delicious', apple trees on M.7A rootstock growing on a silty clay loam soil at the Lewis-Brown Research Farm near Corvallis, Ore., and spaced 3.5 x 5.5 m were used in this study. The trees were trained to a modified central leader. A 1.5 m herbicide strip was maintained with red fescue between the tree rows. In 1992, three single

replicate trees, randomly selected within each row (strain), were given either a spring ground (SG), pre-harvest ground (PHG), fall foliar (FF), SG and PHG (SG/PHG), or SG and FF (SG/FF) N-fertilizer application. All trees received a total of 120g N each with the exception of FF trees, which received half the dose. Ammonium nitrate and urea, both depleted in ^{15}N (0.09 atom % ^{15}N), were used for ground and foliar applications, respectively (see Chapter 3 for more details). Full bloom was during the first week of Apr. 1992.

Fruit were harvested on 17 Sept. 1992. Yield was recorded for each tree and five fruit were randomly selected for dry matter and N content determination. After harvest, a net was placed over each tree to collect senescent leaves. These nets were removed after complete leaf fall (mid-December) and samples of leaves were taken for dry matter and N content determination. In winter, the trees were moderately pruned and about twenty 5-10 cm portions of new growth (1992 shoots) and branches (more than one year old) were collected from each tree. The prunings were divided into the two above categories, oven-dried to a constant dry weight, and their weights were recorded. The trees received no fertilizer in 1993. Harvest was on 12 Oct. 1993. Fruit and fallen leaves were handled as in 1992.

In Feb. 1994, the trees were cut off at the graft union. The aboveground structure was fractionated into new growth (shoots of 1993), branches (diameter < 4 cm), and frame. Stumps were pulled out using a tractor and the roots were recovered by hand while carefully shoveling soil from a 2 x 2 x 1 m hole around the main root. Only few roots of small caliper were extending outside the excavation zone, therefore root losses were minimal. Soil was washed from the roots with a high-pressure water stream. Roots

were classified as either small (diameter <1 cm) or large (diameter >1 cm). Each tree portion was immediately weighed and sub-samples collected for dry matter and N determination (see Chapter 3 for more details). Mature fruit were freeze-dried; other tree components were oven-dried at 60 °C. Dry weight data were used in the analysis.

After drying, the samples were ground to pass a 40 mesh screen. Nitrogen was determined colorimetrically with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973). Sub-samples were analyzed for ^{15}N by mass spectrometry at Isotope Services (Los Alamos, N.M.). Atom percentage values were converted to nitrogen derived from the labeled fertilizer (NDFF) using the following formula (adapted from Hauck and Bremner, 1976).

$$NDFF = \frac{(^{15}N_{\text{natural abundance}}) - (\text{atom \% } ^{15}N)_{\text{tissue}}}{(^{15}N_{\text{natural abundance}}) - (\text{atom \% } ^{15}N)_{\text{fertilizer}}} \quad ; \quad \text{where}$$

^{15}N natural abundance in the soil was considered equal to 0.37 atom percent (Handbook of Chemistry and Physics, 1969). Fertilizer-N uptake efficiency (NUE) was defined as:

$$NUE = \frac{\text{fertilizer-N uptake in entire tree}}{\text{fertilizer-N applied}}$$

The N removed during 1992 in the harvested fruit, fallen leaves and prunings can be considered lost at least in the short run. Much of this N is returned to the system if leaves and prunings are incorporated into the soil, but only a small portion is likely to be returned to the tree. In our experiments these sources of labeled N were purposely removed from the orchard to estimate an actual NUE (ANUE) assuming most of the ^{15}N

added back into the system would either be slowly available from the soil organic N pool, or lost from the system during the heavy winter rains. We defined ANUE as:

$$\text{ANUE} = \frac{\text{(total fertilizer-N uptake)} - (L1 + L2 + L3)}{\text{fertilizer-N applied}}$$

where L1, L2, and L3 represent, respectively, the total amounts of fertilizer-N lost in the fruit, senescent leaves, and prunings during autumn and winter of 1992. The value of L2 and L3 will depend on whether their corresponding tree components are incorporated into the soil or not.

The data were tested using Analysis of Variance (ANOVA) for a factorial design in SAS (SAS Institute Inc., 1987). The means were separated by LSD.

The soil was tested for any detectable residual labeled fertilizer-N by planting barley in place of the removed trees after the displaced soil was put back in the holes. The percentage of labeled fertilizer in the barley plants was determined the following summer.

4. Results and Discussion:

The percentage of ^{15}N in barley plants was not different from natural abundance. Therefore, we concluded that after two years, there was little residual fertilizer-N left in the soil profile explored by the roots. However, N can be immobilized in the soil organic matter and subsequently re-mineralized, thus our determination of fertilizer uptake may underestimate the actual N recovery (Hauck, 1978).

Recovery of the fertilizer-N varied among trees between 10% and 45% for the Std-type and between 6% and 31% for spur-type, but there was no fertilizer treatment or

strain type effect (Table 4.1). Although, spur-type trees tended (P = 0.098) to absorb less N (Table 4.1). The subtle difference between tree types could be because of their difference in size, since NUE increased with tree size (Fig. 4.1). Our results agree with previous reports on pear (Sanchez et al., 1995) where considerable variability in NUE was not related to application method, amount, or timing of the fertilizer. Instead, 31% of this variability could be explained by differences in tree size (P = 0.002). Tree size may also influence foliar fertilizer efficiency since FF spur-type trees recovered only 14.2% of the urea-N applied in contrast to 26.5% for Std-type trees. This may be due to the 40% lower leaf area on spur-type trees compared to that of Std-type trees (on a leaf area basis). Furthermore, spur-type trees have a denser canopy because of their more compact growth and upright-branch characteristic (Warrington et al., 1990) making it difficult to wet the entire interior of the canopy during spraying. Recovery of ground-applied N was similar for both strains (data not shown).

Because of their reduced size, but similar N uptake, spur-type trees accumulated more (Chapter 3) and exported about twice as much fertilizer derived N to their 1992 leaves and fruit as Std-type trees (Table 4.1). However, these differences were largely offset by the bigger N losses to pruning by the more vigorous Std strain. Losses were calculated by dividing the proportion of the fertilizer-N removed from trees in 1992 in fruit, senescent leaves, and prunings by NUE (Table 4.1). On average, Std trees exported 20.8% of the fertilizer-N they absorbed in two years to 1992 fruit, senescent leaves, and prunings, vs. 12.6% for spur-type trees. Treatments that involved a SG application had the highest losses. Approximately a quarter of the N absorbed in SG and SG/FF treatments and about 15% for SG/PHG were lost from the trees. Differences between

treatments were related to the preferential allocation of SG-N to fruit and vegetative growth (Chapter 3).

Table 4.1. Effect of timing and mode of application on the percentage of the fertilizer-N recovered in 1992 and 1993 tissues and in the total biomass (N uptake efficiency (NUE) and actual NUE (ANUE)) of standard- (Std) and spur-type apple trees.

	Fertilizer recovery (%)				
	1992 fruit and senescent leaves	1992 prunings	1993 fruit and senescent leaves	NUE	ANUE
N treatment[z]					
SG	1.2a	6.1a[y]	4.0a	27.4a	20.2a
PHG	0.1b	0.9c	8.5a	30.1a	29.2a
FF	0.6ab	2.1b	5.1a	20.4a	17.7a
SG/PHG	0.7a	3.3b	6.6a	27.6a	23.6a
SG/FF	0.8a	6.5a	6.3a	28.4a	21.1a
Significance	*	**	NS[x]	NS	NS[x]
Tree type					
Std	0.5b	5.4a	7.8a	28.3a	22.5a
Spur	0.9a	2.1b	4.4a	23.8a	21.0a
Significance	*	*	NS[x]	NS[x]	NS
Trt x type					
Significance	NS	NS	NS	NS	NS

[z]The treatments were SG = 120 g N soil-applied on 20 Mar. 1992, PHG = 120 g N soil-applied on 27 Aug. 1992, FF = 60 g urea-N sprayed on 23 Sept. 1992, SG/PHG = 1/2 SG + 1/2 PHG, SG/FF = 1/2 SG + FF.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

[y]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

[x]Treatment effects were significant at $P = 0.1$.

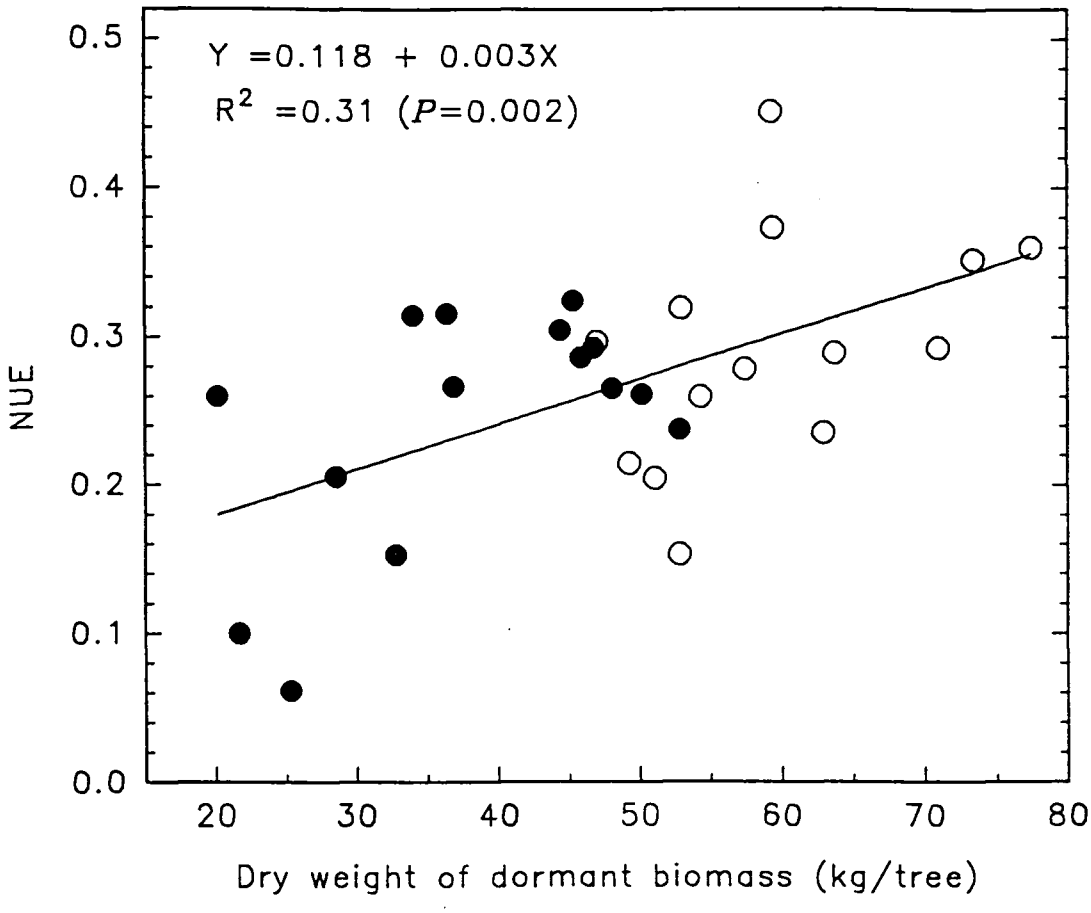

Fig. 4.1. **Relationship between tree size and fertilizer-N uptake efficiency (NUE) by standard- (○) and spur-type (●) apple trees. The trees were fed ^{15}N-depleted fertilizer in 1992 and harvested in February 1994.**

Management approaches, that allow high fertilizer-N recovery (or NUE), though beneficial for the purpose of reducing the quantities of N leached into the water table, do not necessarily lead to maximum N retention, or an optimal partitioning of N between reproductive and vegetative organs (Weinbaum et al., 1980). The amount of uptake, the

status of N reserve pools, and removal of N in fallen leaves, fruit, and prunings are important since N applied in one year often benefits next year's crop (Sanchez et al., 1992).

Since spur-type trees need little pruning, their N losses in 1992 were minimal and their ANUE was not different from that of Std-type trees (Table 4.1). Actual NUE values revealed a treatment pattern similar to NUE, and differences were also not significant.

Since tree size is an important factor in N recovery (Sanchez et al. 1995), dormant biomass as measured in Feb. 1994 was introduced into the analysis as a covariate. There were statistical differences between treatments or tree types for NUE when values were adjusted to account for differences in tree size (Table 4.2). Covariate adjusted NUE was greater for spur-type trees. This further supports the view that possibly greater NUE ($P = 0.098$) apparent in the raw data for the Std trees was due to their larger size rather than directly related to their growth type. With covariate analysis, the PHG treatment tended to have a higher ANUE ($P = 0.07$) than all other treatments. With dormant tree biomass as a covariate, spur-type trees also had significantly higher ANUE.

If efficiency is expressed as total fertilizer-N uptake per unit weight of dormant biomass or retained fertilizer-N in the dormant biomass, results are similar to covariate adjusted NUE or covariate adjusted ANUE, respectively (Table 4.2). Although, fertilizer treatment and tree-type interactions were significant, the mean squares of the main effects were much larger than that of the interaction; therefore the levels of the main factors were compared. The PHG treatment was superior to all others when losses to leaf fall,

Table 4.2. Effect of timing and mode of fertilizer application on covariate adjusted (dormant biomass as covariate) N-uptake efficiency (NUE) and actual NUE (ANUE) of standard- (Std) and spur-type apple trees. Efficiency is also expressed as total fertilizer-N uptake per unit weight of dormant biomass and retained fertilizer N per unit weight of dormant biomass.

	Covariate adjusted NUE (%)	Covariate adjusted ANUE (%)	Total fertilizer-N uptake/dormant biomass (g N kg^{-1})	Retained fertilizer-N/dormant biomass (g N kg^{-1})
N treatment[z]				
SG	0.221a	0.160a[y]	0.582b	0.438b
PHG	0.286a	0.272a	0.780a	0.756a
FF	0.223a	0.190a	ND	ND
SG/PHG	0.200a	0.163a	0.516b	0.438b
SG/FF	0.249a	0.186a	0.618b	0.456b
Significance	NS	NS[x]	*	**
Tree type				
Std	0.192b	0.153b	0.516b	0.348b
Spur	0.280a	0.235a	0.696a	0.504a
Significance	*	*	**	***
Trt x type				
Significance	NS	NS	*	*

NDNo data presented because FF treatment consisted of half the fertilizer dose and is not directly comparable to the other treatments.

[z]The treatments were SG = 120 g N soil-applied on 20 Mar. 1992, PHG = 120 g N soil-applied on 27 Aug. 1992, FF = 60 g urea-N sprayed on 23 Sept. 1992, SG/PHG = 1/2 SG + 1/2 PHG, SG/FF = 1/2 SG + FF.

NS, *, **, *** Nonsignificant or significant at $P = 0.05$, 0.01, or 0.001, respectively.

[y]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

[x]Treatment effects were significant at $P = 0.1$.

pruning, and fruit removal are considered. The enhanced ANUE for PHG compared to SG treatments is a result of both higher PHG uptake (especially for spur-type trees) and much greater N losses for the SG treatment. Losses suggest that SG treatment is less

efficient at recycling N. The greater losses from the SG treatment are not simply due to the fact that the earlier applied N of the spring treatment moves through the plant system sooner. Although the PHG treatment would have greater losses in subsequent years its recycled N is not as specifically partitioned to high loss tissues as the SG applied fertilizer (Chapter 3). The PHG treatment would have to lose 37% to 44% of its total N for Std- and spur- type trees, respectively, to have the same amount of fertilizer N per dormant biomass as the SG treatment.

Although spur-type trees take up N more efficiently on a per dormant biomass basis (Table 4.2), a greater N recycling efficiency (Chapter 3) is partly responsible for the higher ANUE. Although we calculated ANUE based only on first year N losses to fruit, fallen leaves, and prunings, second year fruit and leaf N losses (Table 4.1) make long term ANUE differences between tree types even greater (data not shown; derived from Fig. 3.2 Chapter 3).

In addition to investing less in shoot growth, therefore losing less N to pruning, spur-type trees have a higher uptake capacity. Trees of this type took up the same amount of fertilizer N yet were smaller (Table 4.2 and Chapter 3). To ascertain whether this higher uptake capacity is simply related to tree size and not genotype, the relationship between tree size within a genotype and fertilizer-N uptake per unit weight of dormant biomass was determined. Smaller tree size was not related to a higher fertilizer uptake per unit weight of dormant biomass. The coefficients of determination for Std- and spur-type trees was $R^2 = 0.001$ and 0.018, respectively.

Differences in dry matter partitioning and root architecture (Chapter 3) could be responsible for the higher spur-type efficiency. Indeed, spur-type trees have more small

roots than Std-type trees (Chapter 3). In many cases, plants with a larger or finer root system have a competitive advantage (Clark, 1990). However, nitrate is a mobile anion and moves in the soil primarily by mass-flow (Barber, 1984). Thus root length and extensiveness may not be as important for N availability at root surfaces, provided adequate amounts of N are in the soil and adequate moisture is available to move N to the root surface (Clark, 1990).

The ANUE could be approximated from NDFF percentages in 1993 senescent leaves, branches, and large roots, or from fertilizer-N content of senescent leaves (Table 4.3). The coefficients of determination values were 0.32, 0.27, 0.36, and 0.57 for the four tissues, respectively (Table 4.3). The low R-squared values for the three NDFF percentages suggest that comparative statistics on how partitioning differences alter recovery can only be crudely evaluated without a destructive harvest of the tree. However, it is possible to make a fair estimate of ANUE by using the fertilizer-N content of captured fallen leaves without a destructive harvest.

Table 4.3 Regression equations, coefficients of determination (R^2), and model significance probabilities ($P > F$) describing the relationship between percentage of nitrogen derived from the fertilizer (NDFF) in senescent leaves (1993), branches (Feb. 1994), and large roots (Feb. 1994), and total fertilizer-derived N (gm/tree) in leaves and actual nitrogen uptake efficiency (ANUE) (%) for nine-year-old apple trees. Thirty trees were fed ^{15}N-fertilizers in 1992 and harvested in Feb. 1994.

Independent variable	Equation	R^2	$P > F$
Leaf %NDFF	Y = 10.78 + 0.73X	0.32	0.001
Branch %NDFF	Y = 12.92 + 0.65X	0.27	0.004
Root %NDFF	Y = 12.04 + 0.92X	0.36	0.002
Leaf fertilizer-N	Y = 04.45 + 0.06X	0.57	0.001

Unless differences in N losses and tree size are accounted for, NUE did not differ significantly between N treatments and tree types. However, spur-type trees tended to be less efficient than Std-type trees. The PHG treatment had a higher NUE when differences in tree size were accounted for. Since 31% of the variability in NUE was due to differences in tree size (Fig. 4.1), an analysis with tree size as a covariate or expressing recoveries relative to tree size was required to completely interpret the data. Partitioning differences created by different N application timings can also alter recoveries if one considers losses from crop removal, leaf fall, and prunings. If these losses are considered, rather than being less efficient, spur-type trees tended to have higher NUE and ANUE. Actual NUE recoveries could only be crudely predicted by second season NDFF in leaves, branches, and roots suggesting that recoveries can not be estimated without complete destructive harvests of the trees. Although not as precise as complete destructive harvest, N content of second year fallen leaves can be used to estimate ANUE. Tree size must be considered when studying nutrient use efficiency. Tree size also has practical implications. Unless within orchard variability in tree size and consequent N demand is addressed, it will be difficult to improve N use efficiency (Sanchez et al., 1995).

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CHAPTER 5

DISTRIBUTION OF UREA-DERIVED NITROGEN SUPPLIED TO APPLE LEAVES

1. Abstract:

Different fruit tree responses to foliar urea sprays have been reported. We hypothesized that variable responses are a function of changes in leaf capacity to mobilize urea-N to other tree parts after urea is absorbed by leaves. Two experiments were conducted to study the distribution of urea-derived N in shoots and branches of apple (*Malus domestica* Borkh.) trees. Urea labeled with ^{15}N was applied to young expanding leaves in spring and to senescing spur leaves in fall. At the low concentrations used [0.5%, 1%, and 2% (w/v)], very little spring-applied labeled N was found in tree tissues other than the treated leaf. Fall-applied urea-^{15}N, however, was detected in high concentrations in dormant buds and bark of the spurs to which the treated leaves were attached. Almost no N was exported to neighboring tissues. The following spring, there was some redistribution of labeled N to adjacent buds. The effects of application time in the fall on export of urea-N to buds were also evaluated. Foliar urea sprays applied immediately after harvest contributed the most to bud N. Less urea-N was exported to the buds with later fall applications.

2. Introduction:

The results of spring-applied foliar urea sprays for fruit trees are inconsistent. Some researchers have claimed that spring foliar urea is equally or more effective than soil N applications in improving fruit set, fruit size, and yield (Fisher and Cook, 1950;

Blasberg, 1953; Boynton, 1954). Others have found that the effects of this practice are largely confined to sprayed leaves and do not affect fruiting or the N status of the entire tree (Weinberger et al., 1949; Forshey, 1963). We believe that lack of within-plant mobility of urea-derived N when urea is applied at low concentrations, so as not to injure tender tissues early in the season, is one of the main factors behind this inconsistency. Sanchez et al. (1990) argued that N moving into a pear leaf early in its development is used to build cell structure rather than being incorporated into enzymes or storage compounds, and it may be most difficult to remobilize, while N that accumulates in the leaf later in the season is preferentially exported. This is supported by data from apple experiments where senescent leaves have less ^{15}N in the year of spring ground or fall foliar applications of labeled N fertilizer than they do one year later (Chapter 4). Most of the second year leaf-^{15}N likely came from reserve sources mobilized early in the season. We hypothesized that if urea is spring applied at low concentrations to young expanding leaves, most of the absorbed N will remain in these leaves and little, if any, will be exported to other organs. To investigate this hypothesis, the distribution of N derived from urea sprayed onto apple leaves in spring and autumn was studied.

Since Oland (1960) first introduced the use of autumn urea sprays on apple trees to increase their N reserves, a considerable amount of literature has accumulated on the subject (Oland, 1960, 1963; Williams, 1965; Shim et al., 1972, 1973a and b; O'Kennedy et al., 1975; Sanchez et al., 1990). However, the relationship between time of urea application in the fall and translocation efficiency of the resulting N has received little attention. Since leaves may be injured and photosynthetic capacity of the tree decreased after spraying, a late application of urea may be more favorable than an early

one. However, the N from a late fall application may not have enough time to move into the tree before the leaf's abscission zone forms and the vascular connections are severed (Hill-Cottingham, 1968). We hypothesize that late fall urea sprays would be least efficient at providing N to the bud. Therefore, a timing experiment was also initiated to study the effect of the time of urea application in the fall on N movement from the leaves to the buds.

3. Materials and Methods:

Mobility of urea-derived N from young leaves: Four weeks after bloom (28 Apr. 1994), ^{15}N-depleted urea (0.01 atom % ^{15}N) (Isotec Inc., Miamisburg, Ohio) at 0.5%, 1%, or 2% (w/v) concentration was painted with a brush onto both sides of young expanding leaves (about 2 cm long and 30% the final size) of 10 four-year-old 'Golden Delicious' apple trees on 'Malling 7A' (M.7A) rootstock. Each treatment was applied to three randomly selected trees in the plot. One month later, the following plant material was collected from each shoot with a treated leaf: 1) the treated leaf, 2) the older leaf immediately below, 3) a younger leaf from the shoot-tip (about 8 cm away), 4) a 2 cm long stem section 1 cm below the treated leaf, and 5) a similar section 1 cm above the treated leaf. The section of the shoot bearing the treated leaf was discarded to preclude any direct contamination with ^{15}N from run-off during application. This sampling was repeated a month and a half later.

The sampled tissues were carefully washed with 0.1% Alconox detergent (Alconox Inc., New York) and thoroughly rinsed once with warm tap water and once with deionized water to remove residual urea from external surfaces. The samples were

then oven dried at 60 °C for at least 3 days, then ground to pass a 40-mesh screen before the isotopic composition was determined by mass spectrometry at Isotope Services (Los Alamos, N.M.). Atom percentage values were converted to nitrogen derived from the fertilizer (NDFF) using the following formula (adapted from Hauck and Bremner, 1976):

$$NDFF = \frac{(^{15}N_{\text{natural abundance}}) - (\text{atom \% } ^{15}N)_{\text{tissue}}}{(^{15}N_{\text{natural abundance}}) - (\text{atom \% } ^{15}N)_{\text{urea}}}$$

, where ^{15}N natural abundance was considered equal to 0.37 atom percent (Handbook of Chemistry and Physics, 1969). The data were analyzed as a completely randomized design using the general linear model procedure in SAS (SAS Institute Inc., 1987).

Mobility of urea-derived N from fall foliar applications: In 1992, shortly after harvest (7 Oct.), 15 spurs of comparable size and one branch were selected on each of three single replicate eight-year-old 'Golden Delicious' apple trees on M.7A rootstock. The leaves were still green. The spurs and branches were sprayed individually with a 7.5% (w/v) aqueous solution of urea enriched in ^{15}N (1.346 atom % ^{15}N). The solution contained 0.1% (v/v) non-ionic surfactant Triton X100. Care was taken not to contaminate the rest of the tree and the ground with labeled urea. The spurs and branches were then covered with plastic bags and the whole trees sprayed to run-off with a 7.5% solution of unlabeled urea. Senescing leaves were removed from under the trees to preclude any ground contamination with ^{15}N. On 23 Dec., five buds and surrounding bark from the individually treated spurs and five others from the treated branches were collected from each tree. Additionally, buds and bark 10 cm below (proximal 1) or above (distal 1) and those 20 cm below (proximal 2) or above (distal 2) a treated spur were sampled. Buds and bark were also sampled from an adjacent and a distant branch.

This sampling was repeated at full bloom (12 Apr. 1993) and the following winter (4 Jan. 1994). The buds and bark strips were washed and processed and the data analyzed as in the first experiment.

Effect of timing of fall application on urea-derived N export: On 10 Oct. 1994, the leaves of eight spurs on each of four single replicate mature 'Red Delicious' apple trees were dipped in a 5% (w/v) aqueous solution of urea enriched in ^{15}N (10 atom % ^{15}N). The same treatment was repeated on 1 and 18 Nov. 1994. Leaf fall started on 1 Nov. By 18 Nov., the trees had lost 50% of their leaves. A new set of spurs on the same trees was used each time. All three treatments were applied in the mornings of sunny days, but the average day temperature was lower on 18 Nov. compared to the earlier dates. Buds from the treated spurs were collected on 3 Jan. 1995. They were promptly washed, oven dried, and analyzed as in the previous experiments.

4. Results and Discussion:

Mobility of urea-derived N from young leaves: One month after application of ^{15}N-urea, treated leaves had reached their final size. Overall, ^{15}N was largely restricted, for the duration of the experiment, to the leaf where it was applied (Table 5.1). Similar results were obtained 75 days after urea application (data not shown). Little ^{15}N label was recovered in leaves sprayed with the 0.5% urea solution, but in the 1% treatment, treated leaves showed 5.0% NDFF and this increased to 10.1% when the treatment concentration was doubled. It seems that the 0.5% urea solution was too dilute for any substantial absorption to take place, as it is well established that uptake of solutes across the leaf cuticle is directly proportional to the spray concentration (Marschner, 1986;

Kannan, 1986). In pear (*Pyrus communis* L.), there was no yield response to spring foliar urea using concentrations ranging from 0.2% to 0.5% (Proebsting, 1957; Franke, 1967). Repeated low concentration urea sprays are sometimes effective in improving yield. Blasberg (1953) reported higher yields for 'McIntosh' apple with 4 sprays of a 0.6% urea solution in spring, but the response was less with 3 sprays.

Table 5.1. Effect of post-bloom ^{15}N-urea sprays at different concentrations on the enrichment of the treated leaves and adjacent younger and older leaves and bark. Urea was brush applied to the leaves when they were young and still expanding. The samples were taken one month later.

	% N Derived from fertilizer		
Tissue	Concentration of urea solution (% w/v)		
	0.5	1	2
Younger leaf	0.1a[z]	0.7b	0.9b
Treated leaf	0.1a	5.0a	10.1a
Older leaf	0.2a	0.3b	0.3b
Bark	0.3a	0.2b	0.2b

[z]Mean of 3 replicates.
[y]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

The leaves immediately below or above and the bark 1 cm away from the treated leaves had only small percentages of NDFF (Table 5.1). These results are in agreement with several earlier reports on spring N sprays (Freiberg and Payne, 1957; Forshey, 1963). This lack of mobility is not limited to urea-N. Weaver and Morris (1983) followed the faith of $(^{15}NH_4)_2SO_4$ applied onto the uppermost leaves of young soybean plants. They found that 18 h after application, translocation of ^{15}N away from the sprayed leaves was limited to 15% of the applied N. However, in a similar experiment

in 1982 using older plants, the same workers found that translocation of 15N starts immediately upon pod initiation and continues at nearly a linear rate through pod development. Whether spring-applied urea-derived N is mobile or immobile likely depends on the physiological age of sprayed leaves. The lack of mobility of urea-derived N in our study may be an extreme case compared to what is reported elsewhere. Unlike other studies, we used only young shoot-leaves ($\approx$30% their final size). Our study did not address whether urea sprayed on more mature leaves is mobile early in the season. However, Sanchez and Righetti (1990) found that little of the soil-derived leaf N is exported out of the leaf until late in the season. They also showed that the net efflux of endogenous N out of pear leaves does not start until onset of senescence in October under Medford, Ore. conditions.

Our data suggest that concentrations less than 0.5% may lead to inadequate uptake by apple leaves. Some reports of ineffective urea treatments may be due to low concentrations limiting uptake rather than poor translocation out of treated leaves. Differences in urea mobility due to differences in leaf age could also explain inconsistent responses to spring foliar sprays.

Mobility of urea-derived N from fall foliar applications: Two and a half months after urea application (23 Dec.), dormant flower buds from both individually sprayed spurs and branches had a substantial fraction of their N derived from the applied urea (16.1% and 38.5% NDFF, respectively) (Table 5.2). However, very little labeled N was found in buds on spurs either above or below those treated. Similarly, buds from branches adjacent to the ^{15}N sprayed branch showed negligible amounts of label (Table 5.2). The treated branches had higher percentages of NDFF than the individually treated

spurs because they were sprayed more liberally. With the spurs, we were careful not to contaminate the leaves of adjacent spurs and applied a lower volume of treatment solution (data not shown).

Our data clearly indicated limited movement of urea-derived N beyond the tissues where it was applied. Similar results were reported for 'Comice' pear (Sanchez et al., 1990). However, the bark near the treated bud had 3.5% of its NDFF (urea) (Table 5.2). Although we did not sample roots, data from the above-ground structure indicate that movement of ^{15}N from the labeled urea was localized. This is in contrast to previous reports by Shim et al. (1973) and Swietlik and Faust (1984). In both studies, there was some translocation of foliarly-applied urea or its metabolites to the roots within a few days after application. However, they used young apple seedlings or rooted cuttings which have limited storage capacity. We believe that older bearing trees, with larger N storage sites, behave differently than much smaller plants. It may be inappropriate to extrapolate data of N transport and distribution from small to mature trees. Indeed, even in annual plants, translocation of foliarly-applied N to roots is limited (Morris and Weaver, 1983).

By spring 1993, the percentage of ^{15}N in the treated buds had decreased considerably, though the NDFF values were still higher than neighboring buds (Table 5.2). This decrease could be a dilution effect due to mobilization of unlabeled storage N from surrounding bark and wood or a sharing of the label with adjacent buds. The NDFF values for treated buds declined further by the following winter (Jan. 1994). Although urea-N remains near the site of its absorption during the first fall and winter after application, it is likely redistributed throughout the apple tree in subsequent years,

as is the case for pears (Sanchez et al., 1990). Declining NDFF was expected, since labeled N was likely exported in the harvested fruit and senescent leaves, incorporated into the perennial parts of the tree and/or diluted with N from unlabeled sources (Chapter 3).

Table 5.2 Effect of postharvest ^{15}N-urea at 7.5% on the enrichment of bud and bark in proximal and distal spurs and in treated, adjacent, and distant spurs and branches. Urea was applied on 7 Oct. 1992 and samples were taken on 23 Dec. 1992, 12 Apr. 1993, and Jan. 1994.

		% N derived from fertilizer[z]			
		Buds			Bark
Sprayed part	Position of spur or branch	23 Dec. 92	12 Apr. 93	4 Jan. 94	23 Dec. 92
Spur					
	proximal 2[y]	1.1b[x]	1.9b	0.4b	0.6b
	proximal 1	1.3b	1.2b	0.4b	0.9b
	treated	16.1a	3.5a	1.3a	3.6a
	distal 1	1.2b	1.4b	0.4b	0.9b
	distal 2	0.8b	1.0b	0.3b	0.4b
Branch					
	treated	38.5a	13.6a	3.6a	24.1a
	adjacent	0.9b	1.4b	1.0b	0.2b
	distant	0.3b	0.4b	0.3b	0.1b

[z]Mean of 3 replicates.
[y]Proximal 1 and proximal 2 = 10 and 20 cm from the treated spur toward the shoot tip, respectively. Distal 1 and distal 2 = 10 and 20 cm from the treated spur toward the shoot base, respectively.
[x]Values within the same column that are followed by the same letter are not significantly different at $P = 0.05$.

Postharvest urea sprays may provide a valuable method to target buds and their surrounding tissues. This practice makes it possible to increase N reserves available for the buds without the excessive vigor often associated with large early-season, ground-applications of N fertilizers.

Effect of timing of fall application on urea-derived N export: Of the three urea treatment dates, 10 Oct., 1 Nov., and 18 Nov., the 10 Oct. spray was the most effective in providing N for the buds. The NDFF of winter buds of the spurs sprayed on this date was about five times higher (26.4%) than comparable values (6.7% and 4.8%) on subsequent treatment dates. Since all buds were similar in size and total N concentration (data not shown), a higher percentage of NDFF translates into a higher urea-derived N content. Oland (1963) found no difference in N content of dormant buds from 'Gravenstein' apple trees that received a foliar application of 4% urea either in September or October. However, the two applications were applied earlier in the season, separated by only two weeks, and the more sensitive ^{15}N tracer was not used. As leaves senesce (in November), they likely become less efficient at absorbing and exporting urea (or its metabolites) (Cook and Boynton, 1952). Since foliar sprays may damage fruit, preharvest applications are not recommended. However, foliar applications should be made as soon after harvest as possible to maximize uptake and transport. Differences in uptake and translocation efficiency may be due to the multitude of internal and environmental factors controlling the initiation and duration of natural leaf senescence. The most important among these factors are genotype, photoperiod, temperature, and plant vigor (Doering and Gericke, 1986).

It appears that a postharvest urea application at the beginning of leaf senescence would be most efficient in providing N to the developing flower buds. At the low concentrations [up to 2% (w/v)] that can be safely used in spring, little urea-N is transported from young leaves. Fall-applied urea-N, however, was detected in high concentrations in dormant buds and bark of treated spurs, but almost no N was exported to neighboring tissues. Subsequently there was some redistribution of labeled N to adjacent buds the following spring. Fall foliar urea sprays should be applied soon after harvest as urea absorption and export become less efficient as leaf senescence advances.

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CHAPTER 6

REMOBILIZATION OF N FROM STORAGE POOLS OF MATURE APPLE TREES DEPENDS ON N STATUS

1. Abstract:

Remobilization of reserve N and uptake of soil N in winter and spring were assessed in relation to the N status of trees. Ten-year-old 'Newtown Pippin' apple trees on 'Malling 7A' (M.7A) rootstock were fertilized to create moderately vigorous (MV) trees, trees with above ground portions (tops) and roots relatively low in N (L/L), tops high in N and roots low in N (H/L), both tops and roots high in N (H/H), or tops low in N and roots high in N (L/H). Labeled (^{15}N) fertilizers were used to tag the soil and frame and root N pools in the MV trees prior to winter and spring remobilization. The level of ^{15}N in the buds and new growth was monitored throughout winter and spring. Nitrogen stored in above ground tree parts was first to be remobilized to meet N requirements of the developing buds. Labeled N from the roots and soil arrived at the buds almost simultaneously. Trees of the L/H treatment transported labeled N upward to the bud as soon as 9 Feb. even though average air temperature was close to 7 °C, whereas L/L trees did not send any root-^{15}N to the buds until two-and-a-half months later. When trees received an abundance of N in the fall (H/H and L/H), their buds grew faster in the spring and they bloomed earlier compared with L/L and H/L trees. It appears that not only the bud needs to be low in N for the root-to-shoot N translocation to start (in winter) but the roots have to have adequate of N reserves.

2. Introduction:

Nitrogen budget studies (Magness et al., 1948; Hill-Cottingham and Cooper, 1970), and more recently, tracer techniques (Hill-Cottingham and Lloyd-Jones, 1975; Weinbaum et al., 1980 and 1984; Kato et al., 1982; Sanchez et al., 1992; Muñoz et al., 1993) have shown that, depending on when and how it was applied, fertilizer-N is partitioned differently among tree parts. Nitrogen applied in spring is heavily allocated to fruit and vegetative growth and contributes effectively to the overwintering N reserves in the perennial parts of the tree, especially in the current year's growth (Weinbaum et al., 1984; Sanchez et al., 1992; Muñoz et al., 1993; Chapter 3). The amount of soil-N allocated to above ground tissues decreases as the season progresses. In a study using nine-year-old apple trees of two 'Delicious' strains (Chapter 3), it was found that little N reached the buds and shoots when it was applied at the end of August. The roots, however, derived large proportions of their N from this fertilizer application. Nitrogen from fall ground applications is often confined to the roots and stock (Hill-Cottingham and Cooper, 1970; Sanchez, 1990), so foliar sprays are the only practical way to deliver N to the buds and shoots late in the growing season (Oland, 1960; Sanchez et al., 1990; Chapter 5).

By changing the time and mode of application of N-fertilizers one could deliver N to certain parts of the tree and not to others. It appears possible to select which part of the dormant tree would be the main N storage site. However, changing N storage distribution may be less important if all overwintering N is equally accessible early in spring.

Maximum root activity (nutrient and water uptake) in deciduous trees coincides with peak production of so-called white roots (new roots) in late spring and summer (Head, 1967; Muñoz et al., 1993). However, unlike buds and shoots of trees, roots do not have rest periods (Faust, 1989); and their growth and activity depend mainly on temperature. In apple trees, little or no root activity has been found at soil temperatures below 7°C (Head, 1967; Atkinson, 1974). In the temperate zone, such temperatures are common in early spring. Under these conditions, fruit trees depend largely on the utilization of N stored in the older tissues (Harley et al., 1958; Weinbaum et al., 1987; Deng et al., 1989; Millard and Neilsen, 1989; Sanchez et al., 1991). This period of dependence can extend until two to three weeks after bloom. However, even minor root N-uptake activity could be important in meeting N needs of the developing buds.

The amount of N remobilized depends upon the size of the N store and is unaffected by the current N supply (Millard and Thomson, 1989; Millard and Neilsen, 1989). However, little is known about the order of mobilization of frame and root N pools in relation to their relative size. The subject is one of considerable practical and economical interest. For example, recommendations regarding estimation of nutrient status of trees and the timing of fertilizer applications would differ if the nitrogen supply stored within tree roots is not used during early spring, regardless of the status of N storage pools in the above ground structure of the tree.

The goal of the present study was to determine the order of remobilization of frame (trunk plus branches), root, and soil N for early spring bud-growth. Our objectives were to determine: a) the order of appearance in the bud of N from the above ground structure, root, and soil N pools and b) the effect of N status of the roots and

above ground structure on remobilization of root-N for spring growth in mature apple trees.

3. Materials and Methods:

The trees used in this study were ten-year-old 'Newtown Pippin' apple trees on M.7A rootstock growing in a silty clay loam soil at the Lewis-Brown Research Farm near Corvallis, Ore., and spaced 3.5 x 5.5 m. They were trained to a modified central leader and received routine horticultural care suitable for commercial fruit production.

Order of appearance in the bud of frame, root, and soil N:

Eight trees were given a ground application of 300 g/tree of NH_4NO_3 on 17 May 1994 in order to maintain them moderately vigorous (MV). Thereafter, treatments were applied to label the frame, the roots, or the soil. A first group of 3 trees had their above-ground structure ^{15}N-labeled by trunk injection of 33 ml of a 5% (w/v) solution of ammonium sulfate enriched in ^{15}N (60.5 atom % ^{15}N) on 26 Jan. 1995. The labeled fertilizer was dissolved in a 5.0 mM KCl and 0.4 mM malic acid buffer and injected through a 5 mm-deep hole drilled in the trunk (Horwath, 1992). A second set of 3 trees was given a soil application of 15 g per tree of urea enriched in ^{15}N (10 atom % ^{15}N) on 2 Nov. 1994 in order to label the roots without substantially altering their N status. Urea was evenly spread in a trench 5 cm deep and 20 cm wide that was dug around the tree, 0.5 m away from the trunk. The trench was refilled with soil to minimize ammonia volatilization. The third set of two trees received a dressing of 150 g/tree of NH_4NO_3 depleted in ^{15}N (0.01 atom % ^{15}N) on 26 Jan. 1995. Appearance of labeled N in the

buds of trees in this treatment would indicate uptake and translocation of soil N during winter and spring of 1995.

Effect of tree N status on remobilization of reserve N in the canopy and roots:

Twelve similar trees were selected and divided into 4 groups of 3 trees each, and treated to establish four N status categories. The categories were defined as: a) low N above-ground portion of the tree (top), low N roots (L/L); b) high N top, low N roots (H/L); c) high N top, high N roots (H/H); and d) low N top, high N roots (L/H). Labeled N was applied in late fall of 1994 to label the roots while leaving the rest of the tree unlabeled (Sanchez et al., 1992; Sanchez, 1990; Chapters 3 and 5). Details are presented below.

The L/L group received no N in spring 1994 then the roots were labeled while only mildly altering their N status. On 2 Nov. 1994, 15 g of urea enriched in ^{15}N (10 atom % ^{15}N) were soil applied to each L/L tree as described in the previous section. Trees of the H/L group were given no N in spring then sprayed with a total of 400 g of urea on 11 and 24 Oct. 1994. The ground under the tree canopies was covered with plastic sheets to prevent the spray solution from dripping and reaching the ground. In order to label the roots with a minimum effect on their N status, 15 g of urea enriched in ^{15}N (10 atom % ^{15}N) was soil-applied to each tree as described previously on 2 Nov. 1994. The H/H group was given 3 ground applications: 360 g unlabeled NH_4NO_3 on 17 May 1994, 180 g unlabeled NH_4NO_3 on 11 Oct. 1994, and 180 g NH_4NO_3 depleted in ^{15}N (0.01 atom % ^{15}N), to label the roots, on 24 Oct. 1994, and two foliar sprays with a 5% (w/v) urea solution ($\approx$ 400 g of urea per tree) on 11 and 24 Oct. 1994. Trees of the remaining group (L/H) were not fertilized in spring, but received two ground applica-

tions of fertilizer: 180 g NH_4NO_3 depleted in ^{15}N (0.01 atom % ^{15}N) on 24 Oct. 1994 to label the roots and 145 g unlabeled NH_4NO_3 on 2 Nov. 1994 to further increase their N content.

Sampling and analysis:

Shoot leaves were sampled from all treatments on 15 Aug. 1994. To assess both the tree N status and the distribution of ^{15}N label, shoots and roots were sampled on 19 and 26 Jan. 1995, respectively. About 20 4-6 cm portions of shoots and roots were collected from each tree. On various dates from Jan. to May 1995, flower bud development was assessed and bud samples were taken for dry weight, total N, and ^{15}N determination. All tissues were dried at 60 °C for several days, then ground to pass a 40-mesh screen. Nitrogen was determined colorimetrically with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973). Sub-samples were analyzed for ^{15}N by mass spectrometry at Isotope Services (Los Alamos, N.M.). Atom percentage values were converted to nitrogen derived from the fertilizer (NDFF) using the following formula (adapted from Hauck and Bremner, 1976):

$$NDFF = \frac{(^{15}N_{\text{natural abundance}}) - (\text{atom \% } ^{15}N)_{\text{tissue}}}{(^{15}N_{\text{natural abundance}}) - (\text{atom \% } ^{15}N)_{\text{fertilizer}}} \text{ ; where}$$

^{15}N natural abundance in the soil was considered equal to 0.37 atom percent (Handbook of Chemistry and Physics, 1969).

The data were analyzed using Analysis of Variance (ANOVA) for a completely randomized design using the general linear model procedure in SAS (SAS Institute, Inc., 1987).

4. Results and Discussion:

Order of appearance in the bud of frame, root, and soil N:

Overall, the trees used in this trial (MV) were average in vigor when compared with those used in the second experiment. Mid-August leaves had 1.57 % N (on a dry weight basis). Although this concentration is lower than expected for moderately vigorous trees (Shear and Faust, 1980), it was higher than that of the L/L treatment trees and lower than that of the H/H treatment trees (Table 6.1). The low leaf N concentrations could have been caused by a mite infestation that started during the month of July. Roots sampled on 19 Jan. 1995 had 0.86 % N. This concentration is intermediate compared with L/L and H/L treatments (Table 6.1).

Table 6.1 Effect of differential N supply to the canopies and roots of ten-year-old 'Newtown Pippin'/'Malling 7A' apple trees on N concentrations in August leaves and January bud, shoot, and root tissues. The trees were treated to establish four N status categories: low N top, low N roots (L/L); high N top, low N roots (H/L); high N top, high N roots (H/H); and low N top, high N roots (L/H).

Treatment	15 Aug. 94 Leaves	19 Jan. 95 Buds	19 Jan. 95 Shoots	19 Jan. 95 Roots
L/L	1.46b[z]	1.35b	0.89a	0.48c
H/L	1.45b	1.67a	0.88b	0.89b
H/H	1.77a	1.76a	1.09a	1.67a
L/H	1.79a	1.36b	0.96a	1.09b
Significance	**	**	NS	*

[z]Value within the same column that are followed by the same letter are not significant at $P = 0.05$.
NS, *, ** Nonsignificant or significant at $P = 0.05$, 0.01, respectively.

Trunk and shoot tissues sampled 16 d after the injection of ^{15}N-ammonium sulfate into the trunk had 15.8% and 0% of their total N derived from the labeled fertilizer, respectively. Therefore, the labeling of the frame (trunk and branches) N pool was considered successful. Trees that received a soil application of ^{15}N-urea in November had no labeled N in their shoots on 9 Feb. 1995, but roots had 4.2% to 7.7% of their N derived from the fertilizer.

Labeled N injected into the tree frame started appearing in the buds as early as 9 Feb. (Fig. 6.1 A1) but accounted for only a small fraction of the total N in these tissues. The highest percentage of NDFF (2.6%) in buds was found on 24 Mar. These buds had reached the 'tight cluster' developmental stage.

Trace amounts of labeled N from the roots and soil were first detected in the buds at the second sampling (24 Mar. 1995) with 3 and 5 μg fertilizer-derived N per bud, respectively (Fig. 6.1 A1). The contribution of root and soil derived N to bud growth increased rapidly after first bloom (10 Apr. 1995) in a pattern similar to bud growth (Fig. 6.1 A2). Since storage pools were unequally labeled among treatments, the differences in patterns of N mobilization among treatments are more important than the actual amounts of ^{15}N. By petal fall, N derived from the fertilizer applied in January (soil pool) accounted for 5.3% of N in the blossom and leaves (data not shown). Undoubtedly, the contribution of soil N could be much larger since the labeled N represented only a fraction of the total soil N available for uptake.

Fairly warm weather during the second and third weeks of March (Fig. 6.1 A3) could be responsible for the apparent increase in root activity. The daily average soil and air temperatures were above 7°C during this entire period. Such temperatures are

favorable for root uptake of N and its translocation to tree tops (Batjer et al., 1943). Nightingale (1934) showed that dormant apple trees absorb N, and that organic nitrogen compounds are formed at a temperature of 8.9°C. Similarly, in a controlled experiment, Kato and Kubota (1982) showed that 4-year-old 'Satsuma' mandarin trees (*Citrus unshiu* Marc.) are capable of nitrate-uptake and reduction in a low temperature regime (minimum temperature -4°C and mean temperature 2.5°C for both air and gravel bed). Kato et al. (1982) also found that the bulk of the N absorbed in winter did not translocate upward until late February when average air temperature exceeded 7°C. In the present study, we could not determine when uptake of winter-applied N took place. Therefore, the lag between uptake and translocation of winter N could not be verified.

The performance of the root system is essentially governed by the same factors as growth; both are dependent on root metabolism, which in turn is clearly dependent on temperature. However, since the upward translocation of nitrogenous compounds occurs mainly in the transpiration stream (Titus and Kang, 1982), this process is more temperature sensitive than is N-uptake.

The data suggest that N stored in the above ground parts of MV trees was mobilized much earlier than N from the roots or soil. It is possible, however, that injected N remained in a chemical form different from the N storage compounds naturally occurring in dormant apple trees, and are more readily mobilizable.

From the results of this experiment, we conclude that N stored in the tree canopy is the first to be remobilized to meet the N needs of the growing buds in early spring.

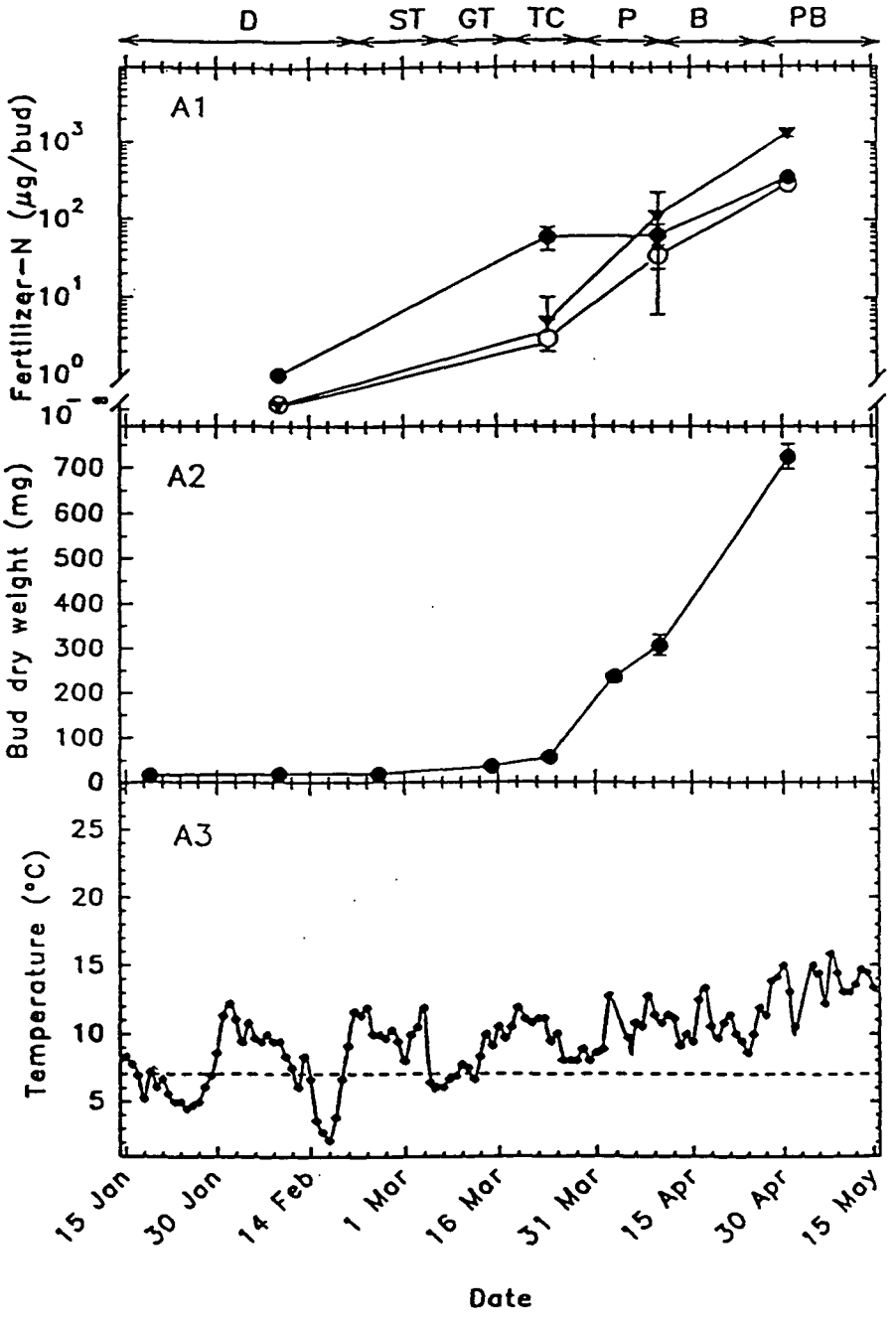

Fig. 6.1. Appearance in the buds of N derived from fertilizers previously applied to the trunk on 26 Jan. 1995 (•) or to the soil either on 2 Nov. 1994 (o) or on 26 Jan. 1995 (▾) (A1). The first two treatments were applied to 3 replicate trees, the third was applied to two. Average bud-dry-weight (A2) and soil temperature at 10 cm depth (A3) during the first five months of 1995 are presented. Developmental stages of the flower buds are presented on the top of the graph (D = dormant, ST = silver tip, GT = green tip, TC = tight cluster, P = pink, B = bloom, PB = post-bloom). The trees were 'Newtown Pippin' apple on 'Malling 7A' rootstock.

Subsequently, as soil temperatures rise and N demand increases sharply after bloom, N is mobilized upward from the roots and soil.

Effect of tree N status on remobilization of reserve N in the canopy and roots:

Nitrogen concentrations in August leaves and January shoot, root, and bud tissues are presented in Table 6.1. In mid-August, leaves from the H/H and L/H treatments were higher in N than those from L/L and H/L treatments. However, leaf N concentrations were generally low. Unexpectedly, leaves from the L/H treatment were as high in N as those from the H/H treatment even though no fertilizer was applied to the trees of the former treatment up to the date of sampling. This inconsistency could be due to the confounding effects of such factors as mite population, tree vigor, crop load, and natural variability in soil fertility. Vigorous trees often have low leaf N concentrations (Righetti, 1986), while heavy crop loads are associated with high leaf N concentrations (Sadowski et al., 1995). In the present study, no differences in yield between treatments were found (data not shown). However, the effect of other factors could not be verified. Variation in soil fertility would not be expected to have a major effect on leaf N because the soil at the experimental site is fairly uniform.

Urea sprays onto H/L trees resulted in considerable leaf damage and shoot dieback. This damage could be caused by biuret in the commercial grade urea used, and possibly worsened by the reduced vigor of these trees. In fact, similar sprays onto H/H trees were far less toxic, as little leaf burn was noticed.

Shoot and trunk tissues showed no difference in N concentrations between treatments (data not shown). Nitrogen in January buds reflected the differential N treatments. Buds from the H/H and H/L treatments had markedly higher N concen-

trations than buds from the other treatments (Table 6.1). Given the small and similar size of the dormant buds (Fig. 6.2 A4), their N concentration could be less sensitive to differences in biomass and may be a better indicator of N status of tree canopy.

The H/H and L/L treatments resulted in the highest and lowest root N concentrations, respectively (Table 6.1). Considering the bud and root N results, it appears that the desired tree N distributions were at least partially created.

Buds from all treatments started 'silver tip' developmental stage during the second and third weeks of February (Fig. 6.2 A1). Thereafter, buds of trees that received ample soil N in fall (H/H and L/H) developed more rapidly than those of the other treatments. Bloom was at least one week earlier in H/H trees compared to L/L trees. Our results are in agreement with Hill-Cottingham and Williams (1967) where they found that flower buds of 2-year-old 'Lord Lambourne'/M.2 apple trees fertilized in August or at the end of October continue to differentiate at a steady rate throughout fall and winter, while those from trees given no N, stop differentiating during the winter.

Transport of labeled N to the buds was related to the N status of the roots and above ground structure (Fig. 6.2 A2). As was the case in the previous experiment, unequal labeling of storage pools makes patterns more important than actual amounts. However, L/L and H/L or H/H and L/H treatments were similarly labeled and can be directly compared. In the L/H trees, labeled N moved upward from the roots to the buds as soon as 9 Feb. even though average air temperature was lower or only a few degrees higher than 7°C (Fig. 6.2 A3), the temperature considered by many as the lower limit for any root to shoot translocation (Head, 1967; Atkinson, 1974; Kato et al., 1982).

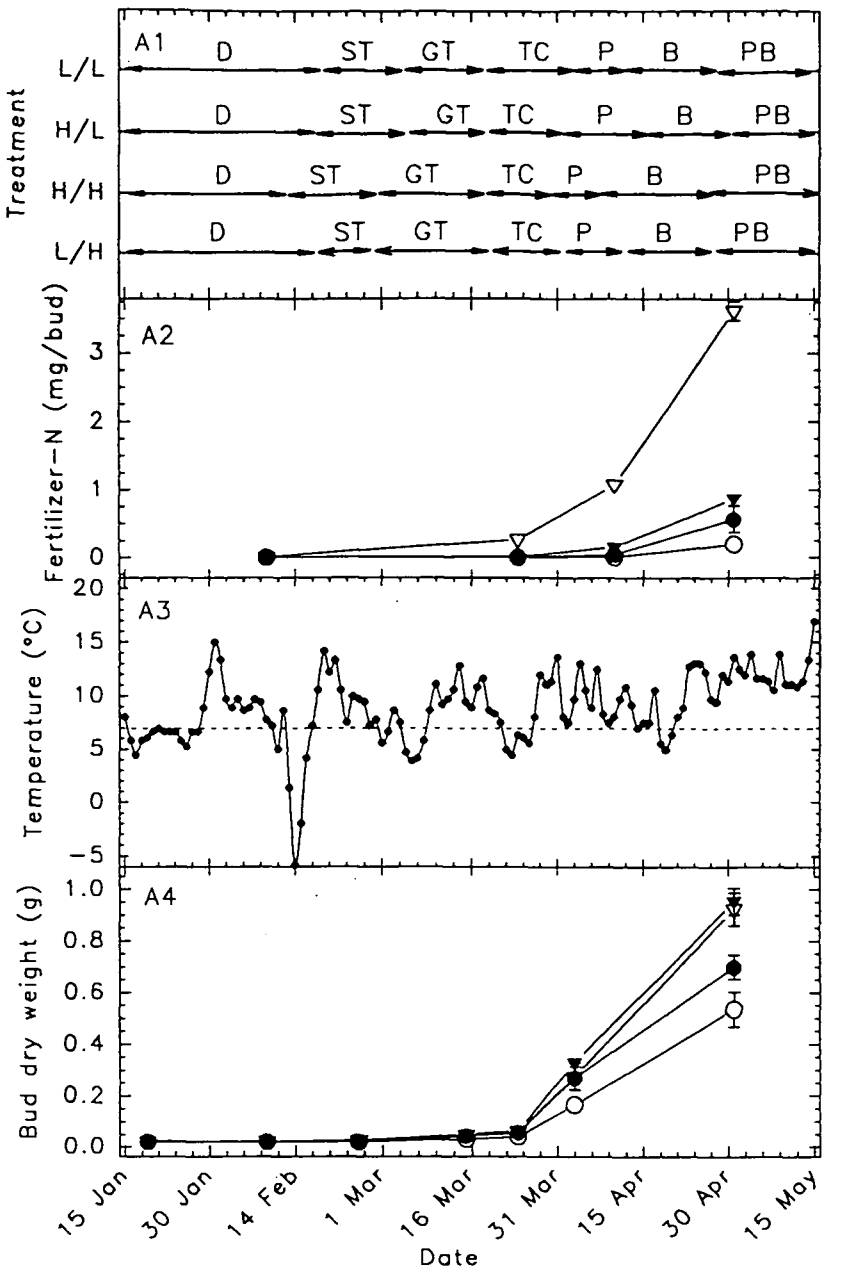

Fig. 6.2. **Effect of differential N supply to tree tops and roots on bud development (A1), enrichment in N derived from the applied fertilizers (A2), average air temperature near Corvallis, Ore. is also presented (A3) (the dashed line indicates the 7°C temperature) and dry weight (A4). Ten-year-old 'Newtown Pippin'/'Malling 7A' apple trees were treated to establish four N status categories: low N top, low N roots (L/L) (●); high N top, low N roots (H/L) (○); high N top, high N roots (H/H) (▾); and low N top, high N roots (L/H) (▽). Each treatment was applied to 3 single replicate trees. Seven stages of flower bud development were identified: dormant (D), silver tip (ST), green tip (GT), tight cluster (TC), pink (P), bloom (B), and post-bloom (PB).**

It is possible that when bud N requirements cannot be met by remobilizing N stored locally in the canopy, the tree has a special mechanism to transport N upward from its store in the roots even under relatively unfavorable temperatures. It is notable that unlike L/H trees, L/L trees did not translocate any labeled N from the roots to the buds during January, February, and the first half of March (Fig. 6.2 A2), even though January buds of these trees were low in N (Table 6.1). The L/H were heavily fertilized in autumn, whereas, L/L received only a small dose of N. Similarly, Millard and Thomson (1989) showed that the provision of an autumnal N supply to 2-year-old M.26 apple rootstock trees significantly increases the amount of stored N remobilized for spring leaf growth. However, they did not determine when this remobilization started. It appears that when the buds are low in N and the roots have adequate N-reserves, root-to-shoot transport of N starts early in winter. Any signal involved in a possible bud-root communication must be complex since the N status of both the sink (bud) and the source (roots) are important.

During April, buds of the H/H and L/H trees grew faster than those of the other two treatments (Fig. 6.2 A4). Since rapid mobilization of root N reserves and root uptake of N (Fig. 6.1 A1, Fig. 6.2 A2) occurred during this period, the shortage of N in the roots and soil of L/L and H/L trees likely caused the growth reduction. However, the pattern of growth of H/L buds may be more difficult to interpret because of urea phytotoxicity to the trees. Despite their high N content (Table 6.1), these buds grew less than in all other treatments. Urea could have affected the buds directly by partially burning the flower and leaf primordia upon application causing them to grow less in spring, or indirectly by hastening leaf abscision thus reducing carbohydrate and N supply

to the storage tissues in the tree. Early defoliation or shortage of N supply in autumn has been shown to reduce new growth of young apple and plum (*Prunus salicina* Lindl.) trees in the following spring (Millard and Thomson, 1989; Castagnoli et al., 1989).

Although temperature has long been recognized as the overriding factor governing root activity and translocation of nutrients to the aerial parts of trees during winter and early spring (Wiersum, 1980), our data suggest the distribution and magnitude of tree N reserves may play a role in triggering these processes.

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CHAPTER 7

CONCLUSION

Fertilizer-N uptake, partitioning, and use-efficiency were studied in standard strain 'Topred Delicious' and spur strain 'Redspur Delicious' mature apple trees (*Malus domestica* Borck) on 'Malling 7A' (M.7A) rootstocks. Total N, and ^{15}N evaluations revealed that when applied in spring, labeled N was allocated preferentially to fruit and vegetative tissues and to a lesser extent to roots of both strains. When sampled in mid-season, leaves on young shoots had higher concentrations of the spring applied ^{15}N than those on more mature shoots or spurs. This indicates an increasing dependence of the tree on soil N from spring to summer. The amount of newly absorbed soil-N allocated to above ground tissues decreased as the season progressed. Very little ^{15}N from pre-harvest ground applications reached the leaves, fruit, buds, or branches, while roots were heavily labeled. The percentage of ^{15}N derived from foliar urea sprays was low. However, urea-N did appear in the dormant buds.

Total fertilizer-N recovery was similar regardless of the time of fertilizer application. However, losses of ^{15}N to fruit removal, leaf fall, and pruning were most severe when N was applied in spring and minimal for the pre-harvest timing. About a third of the variability in recovery was due to variation in tree size with large trees absorbing more fertilizer-N than small trees. When recoveries are adjusted to account for size differences, spur-type trees tended to be more efficient at utilizing fertilizer-N. Recoveries correlated positively with second-year ^{15}N concentrations in senescent leaves, fruit, and branches. Combining spring ground applications with summer ground or fall foliar applications resulted in ^{15}N distributions that had the characteristics of both

treatments and average recoveries (compared to the three treatments).

When ^{15}N-urea was applied in April to young expanding apple leaves, the label was not exported elsewhere. At higher concentrations, urea-^{15}N applied to leaves in fall was exported to the buds, but was restricted to spurs and branches to which urea was applied. Foliar urea sprays immediately after harvest contributed the most to bud N compared with later applications.

In moderately vigorous trees, N stored in aerial parts of the tree was mobilized first, followed by simultaneous use of root and soil N. Utilization of root reserves depended on the N status of the tree. When tree canopy was low in N but the roots had adequate N reserves, root-^{15}N was transferred to the buds two months before bloom. In contrast, such root-to-shoot transport of N did not start until two and a half months later in unfertilized (low N) trees. High N trees used their canopy-N first then root-N when N demand increased after bloom. It appears that when the buds are low in N but the roots have adequate N reserves, root-N is transported upward to the buds early in winter.

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APPENDICES

APPENDIX A

BIURET TOXICITY TO APPLE FOLIAGE SPRAYED WITH UREA

1. Abstract:

The effect of urea and biuret on leaf burn was investigated. Urea and biuret were combined at the rates of 0%, 1%, and 2% (w/v) and 0%, 0.05%, 0.1%, and 0.15% (w/v), respectively, for a greenhouse trial in late April using 2-year-old 'Braeburn'/'Malling-Merton 111' (MM.111) apple (*Malus domestica* Borkh.) trees. The experiment was repeated in the field in late May and in August using 11-year-old 'Golden Delicious'/'Malling 7A' (M.7A). In August, one more concentration level was added to urea [4% (w/v)] and biuret [0.4% (w/v)]. Biuret alone did not cause any leaf damage. Within the range of concentrations used, urea was largely responsible for leaf burn and chlorosis following foliar sprays. The injury was increased when both chemicals were sprayed at the same time.

2. Introduction:

Urea sprays appear to be an effective supplement, or even a substitute at times, for soil applications of nitrogen fertilizers (Oland, 1963). Unfortunately, its use is often limited by the presence of biuret, a potentially phytotoxic contaminant of fertilizer grade urea formed during the manufacturing process at high temperatures (Sanford et al., 1954). Commercially used ureas contain from 1 to 2.5% (w/w) biuret (Sanford et al., 1954; Poole et al., 1983). Biuret added to urea and sprayed onto pineapple plants increased the degree of dieback and chlorosis of the leaves (Sanford et al., 1954).

Similar results were reported by Jones (1954) with citrus. However, in both cases the urea used was not pure but of fertilizer grade. Furthermore, both investigators noticed that more chlorosis resulted from the combination of urea with biuret than from biuret alone. More recently, Poole et al. (1983) found no reduction in soybean [*Glycine max* (L.) Merr.] yield from addition of biuret to foliar fertilizers. Because of this uncertainty regarding the effect of biuret, we conducted an experiment to assess the contributions of biuret and urea to the phytotoxic effect of fertilizer-grade urea and to determine if leaf susceptibility to urea spray phytotoxicity depends on time of application.

3. Materials and Methods:

An experiment was carried out in a greenhouse with a set of 2-year-old 'Braeburn'/MM.111 apple trees in full bloom (30 Apr. 1995), then repeated in the field on 24 May and 22 Aug. using 11-year-old 'Golden Delicious'/M.7A apple trees. Each treatment was applied to three branches, each on a separate tree. Spray solutions contained 0%, 1%, or 2% (w/v) of enzyme-grade pure urea (LIFE Technologies, Inc., Gaithersburg, Ohio) combined with 0%, 0.05%, 0.1%, or 0.15% (w/v) biuret (ICN Biochemical, Inc., Cleveland, Ohio). On 22 Aug., a 4% and a 0.4% concentrations were added to the previous concentrations of urea and biuret, respectively. Phytotoxicity was visually assessed on the foliage 10 days after spraying. Each branch was rated for leaf burn and/or chlorosis (yellowing) on a scale from 0 to 4. Branches with leaves looking similar to those from water treated branches were given a score of 0; those with more than 50% of their leaf area burned or chlorotic were given a score of 4. The data were analyzed by Analysis of Variance (ANOVA) for a factorial experiment using a

general linear model and a multiple regression procedures in SAS (SAS Institute Inc., 1987)

4. Results and Discussion:

Leaf injury was observed in some urea/biuret treatments 10 days after spraying. The injury consisted of leaf-tip burn (or necrosis), which extended down the leaf margins toward the base in the more severe cases. Some chlorosis (yellowing) was noticed, especially on interior leaves at high concentrations of urea. This chlorosis was not seen when urea was combined with a high concentration of biuret, although this combination did increase leaf burn (Fig. A.1 and Table A.1). At all dates biuret, alone caused little, if any, damage (Fig. A.1), even at the highest concentration of 0.4% (w/v). This concentration is at least three times the biuret concentration in a 5% solution of low grade commercial urea [2.5% (w/w) biuret]. Within the range of concentrations used, most of the variance in leaf burn was due to urea (Table A.1). Leaf damage was increased when biuret was added to the urea solution. This was true for all three dates. The synergy suggested by the significant urea x biuret interaction could be indirect, such a urea-enhanced uptake of biuret. Urea has been shown to improve absorption of other nutrients such as boron by plant foliage (Doering and Gericke, 1986). Unfortunately, the rate of absorption of biuret by the leaves was not measured. Injury was most severe in the greenhouse (in April) and least for July field application (Fig. A.1). However, differences between the two varieties used in this experiment could have had a confounding effect.

Our results are contrary to the common belief that biuret is the toxic contaminant in fertilizer-grade urea and that urea by itself is not toxic (Sanford et al., 1954). In an experiment with pineapple plant, Sanford et al. (1954) argued that biuret is responsible for leaf burn following urea sprays. However, even in their study some chlorosis occurred after spraying pineapple leaves with an almost biuret-free urea solution. Furthermore, for the same urea concentration, doubling biuret concentration increased leaf injury only slightly (Sanford et al., 1954). In a similar trial on soybeans, Poole et al., (1953) found no effect of biuret on yield and suggested that a biuret contamination of 1% to 1.5% in fertilizer-grade urea should be acceptable in the formulation of foliar fertilizer.

Within the range of concentrations used in our experiment, urea was the primary cause of leaf burn and chlorosis following foliar sprays. However, when we fit a linear regression model to leaf injury as affected by urea and biuret sprays we found that the coefficient for the second factor (biuret) is always higher than that of urea (Table A.2). This analysis suggests that at similar concentrations biuret would be more toxic to the leaves than urea; but each one of them was toxic by itself.

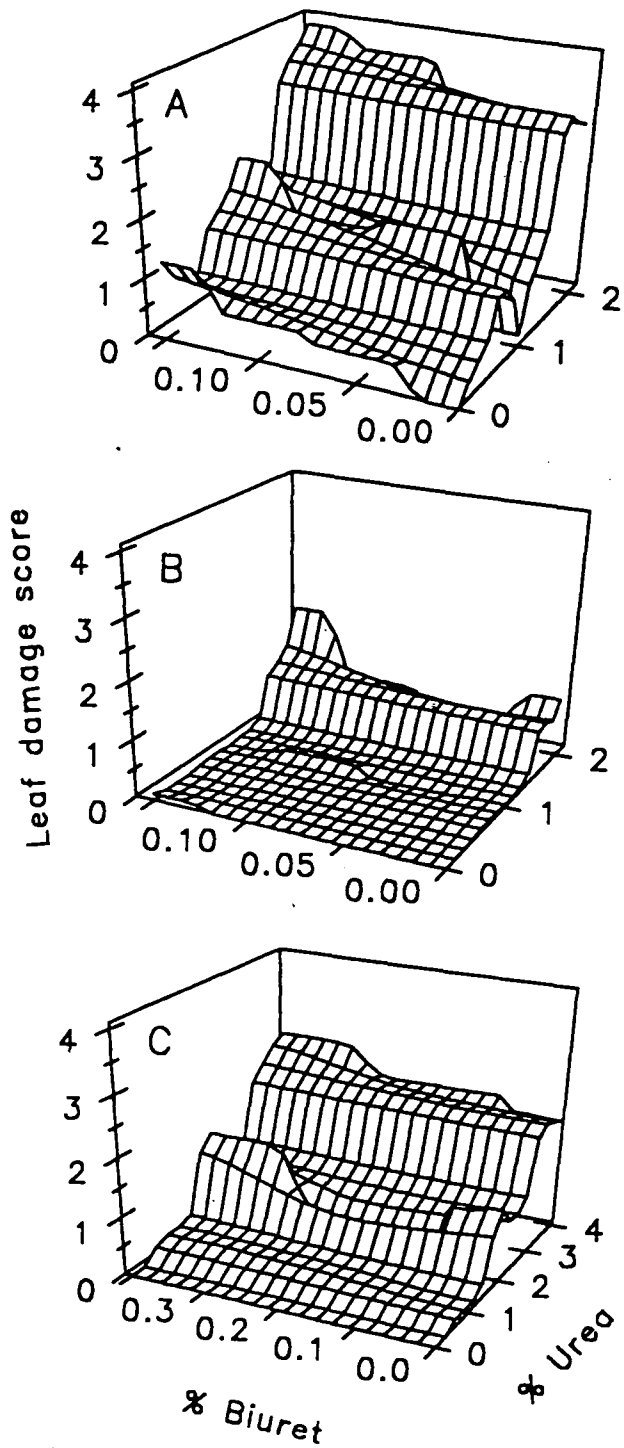

Fig. A.1. Phytotoxicity of urea and biuret mixes sprayed onto apple (*Malus domestica* Borkh.) leaves in April (A) to 2-year-old 'Braeburn'/MM.111 growing in a greenhouse or in July (B), and August (C) to 11-year-old 'Golden Delicious'/M.7A trees growing in the field. Leaf injury was rated from 0 to 4; 0 for no damage and 4 for more than 50% burn or chlorosis. Phytotoxicity was assessed 10 days after spraying.

Table A.1. **Mean squares (MS) and significance probabilities (P > F) of main effects and interactions for phytotoxicity due to foliar sprays of urea with different levels of biuret onto apple leaves at different times during the growing season. Data from each date were analyzed separately as a factorial design.**

	Date of application					
	Greenhouse		Field			
	30 Apr.		24 May		22 Aug.	
Treatments	MS	P > F	MS	P > F	MS	P > F
Urea	24.84	0.000	3.34	0.000	14.55	0.000
Biuret	3.45	0.000	0.53	0.009	0.39	0.014
Urea x biuret	0.34	0.037	0.41	0.009	0.24	0.036

Table A.2. **Regression equations, coefficients of determination (R^2), and model significance probabilities ($P > F$) of the degree of leaf burn and chlorosis caused by urea (U) and biuret (B) sprays. Combinations of three concentrations of U [0%, 1%, and 2% (w/v)] and four concentrations of B [0%, 0.05%, 0.10%, 0.15%, and 0.4% (w/v)] were used for April and August sprays. In August, one more concentration level was added to the levels used previously [4% and 0.4% (w/v) for U and B, respectively].**

Time of application	Equation	R^2	$P > F$
30 Apr. (greenhouse[z])	$Y = -0.285 + 1.396\ U + 9.444\ B$	0.87	0.0001
24 May (field)	$Y = -0.292 + 0.467\ U + 2.389\ B$	0.44	0.0001
22 Aug. (field)	$Y = -0.297 + 0.569\ U + 0.948\ B$	0.83	0.0001

[z]Location of the trees.

APPENDIX B

CHARACTERIZATION OF NITROGEN POOLS IN MATURE APPLE TREES AND THEIR CONTRIBUTION TO SPRING GROWTH

1. Materials and Methods:

Bark protein characterization:

Seasonal protein fluctuations:

Plant material. A block of 11-year-old 'Topred Delicious' apple trees on M.7A rootstock growing on a silty clay loam soil at the Lewis-Brown Research Farm near Corvallis, Ore. were used in this study. Shoots were randomly collected monthly from Sept. 1992 to May 1993. Bark was peeled off from mid-shoot sections, pooled, and quickly frozen in liquid nitrogen before being transferred to a -70°C freezer. When needed, the samples were removed from the freezer and immediately lyophilized for at least 60 h then stored at -20°C for subsequent analysis.

Protein extraction. Soluble proteins were extracted as described by Coleman et al. (1991) with some changes. Replicate samples of the lyophilized ground (40-mesh) tissue (0.5 to 1 g dry weight) were further ground into a fine powder in liquid nitrogen in a prechilled mortar and pestle. The resulting powder was suspended in 10 ml of extraction buffer (50 mM sodium borate, 50 mM ascorbic acid, 1% ß-mercaptoethanol, 1mM PMSF added just before homogenizing; pH 9.0) then homogenized at 4°C with a Tekmar Tissummizer for 45 s at maximum speed. Insoluble polyvinyl-polypyrrolidone (PVP) pre-equilibrated in the same buffer was added to the homogenate and mixed in gently (Loomis, 1969). The slurry was then squeezed through 8 layers of cheese cloth. The filtrate was centrifuged for 30 min at 4°C and 35,000x *g*. Aliquots were taken from

the supernatant for protein determination by the bicinchoninic acid method (Brown et al., 1989) using bovin serum albumin as a standard. Five volumes of 0.1 M ammonium acetate in -20°C methanol was added to the rest of the supernatant and held overnight at -20°C to precipitate the proteins. The precipitate was collected by centrifugation at 12,000x *g* for 20 min at 4°C. The protein pellet was washed three times with cold (-20°C) 0.1 M ammonium acetate in methanol and once with -20°C acetone then air dried. The pellet was resuspended in Laemmli (Laemmli, 1970) lysis buffer, held in boiling water for 5 min, and cooled to room temperature.

Protein analysis. Proteins were electrophoresed at 24 mA using 12.5% polyacrylamide gels with Tris buffer (pH 8.3) containing 0.1% (w/v) SDS (Garfin, 1990). Gels were stained with 0.1% (w/v) Coomassie blue R-250 in 40% (v/v) methanol and 10% acetic acid (v/v) and destained in the same solution. Relative protein band intensity was determined for the dried gels using a densitometer (Molecular Dynamics, Sunnyvale, Calif.).

Proteins from SDS-PAGE were electro-blotted onto nitrocellulose (Mini Trans-blot, Bio-Rad, Richmond, Calif.) in 25 mM Tris, 192 mM glycine, and 5% methanol. Blotting efficiency was determined by restaining the polyacrylamide gels and by monitoring the extent of transfer of rainbow molecular weight markers (Bio-Rad). The protein blots were blocked and incubated overnight with a 1:5000 dilution of a polyclonal antibody raised against the 32 kD-bark storage protein (BSP) of poplar (*Populus deltoides* Bartr. ex Marsh.) (Coleman et al., 1990). The blots were washed then incubated with a 1:3000 dilution of goat anti-rabbit alkaline phosphotase-conjugated IgG (Bio-Rad, Richmond, Calif.). Cross-reacting proteins were visualized with nitroblue tetrazolium and

5-bromo-4-chloro-3-indolyl phosphate. A positive control (poplar bark protein extract) was loaded on the same gel as apple bark extracts.

In order to verify which polypeptides will be most extensively degraded to provide N for new shoot growth in the absence of an exogenous N source, shoots were collected on 16 Feb. 1994 and on 2 Apr. 1994 (10 days before first bloom) from mature 'Topred Delicious' apple trees. The first set of shoots was taken immediately to the laboratory where bark was removed and stored at -70°C. The second set of shoots was induced to grow in the laboratory. The shoots were placed in a Hoagland's nutritive solution that lacked N but contained 10 mM of sucrose. The solution was changed twice a week. When typical symptoms of N deficiency appeared on the foliage and shoot tips (yellowing and lack of growth, respectively), the old bark (1993 growth) was peeled off, frozen, and stored as described previously. Bark samples from both sets of shoots were extracted for protein and the extracts were analyzed by SDS-PAGE as described in the first experiment.

Characterization of N pools in apple trees:

Plant materials. Three trees from the same block described above were used in this study. These trees received a ground application (120 g N/tree) of ammonium nitrate in Spring 1992 and no fertilizer in 1993. In Feb. 1994, the trees were cut off at the graft union. The above-ground structure was fractionated into shoots (1993 growth), branches (diameter $<$ 4 cm), and frame (branches more than 4 cm in diameter plus trunk). Stumps were pulled out using a tractor and the roots were recovered by hand while carefully shoveling soil from a 2 x 2 x 1 m hole around the main root. Since only few roots of small caliper extended outside the excavation zone, root losses were

considered minimal. Soil was washed from the roots with a high-pressure water stream. For the sake of simplicity roots were classified as either small (diameter < 1 cm) or large (diameter > 1 cm). Each tree portion was immediately weighed, and sub-samples were collected for dry matter and total N determination. An other set of samples was placed in a plastic bag and taken immediately to the laboratory. Shoots, branches, and small roots were sampled by collecting about twenty 20-30 cm long portions. A composite frame sample was constructed by mixing large cross-sectional pieces from the trunk and large branches based on their relative weights. Large roots were sampled in a similar manner. Once in the laboratory, the wounded ends of the tissues were discarded and the rest was cut to smaller pieces and quickly frozen in liquid nitrogen before being transferred to a -70°C freezer. When needed the samples were removed from the freezer and immediately lyophilized for at least 60 h. The dry samples were then ground to pass a 40-mesh screen and stored at -20°C for subsequent analysis (Khanizadeh et al., 1989).

Total N, protein-N, amino acid-N, NO_3^--N, NH_4^+-N determination: Total N was determined colorimetrically with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973).

For protein-N determination, the samples were extracted as above but PVP was not added. After the first centrifugation, aliquots of the supernatants were taken for micro-Kjeldahl digestion and N was then determined colorimetrically with an autoanalyzer.

The amino acids in each tissue (lyophilized and ground) were extracted with 5 volumes (w/v) of 80% (v/v) boiling ethanol with two additional sets of 5 volumes used as washes. The extracts were combined and the ninhydrin-positive compounds were

determined by the method of Yemm and Cocking (1955). Standards were prepared from L-leucine. Amino acid-N was estimated by dividing the amount of amino acids by 6.25 (Beevers, 1976).

For ammonium and nitrate determination, half a gram of dried tissue was extracted with 5 ml of 2% acetic acid (v/v). Samples were shaken for 12 h at room temperature and filtered through an in-tube serum filter (Plasma/serum separator, Karlan Chem. Corp., Torrence, Calif.). Nitrate concentration was determined by reflectometry using a Reflectoquant (EM Science, Gibbstown, N.J.). Ammonium concentration was determined with an Ammonium Analyzer (Wescan Ammonium Analyzer Model 360, Alltech., Inc./Wescan Instruments, San Jose, Calif.).

All data were converted to N equivalents and expressed on a dry weight basis.

Protein extraction. Soluble proteins were extracted and separated by SDS-PAGE as described above from the same lyophilized samples used for N determination.

Monitoring of current-year uptake of soil N:

In order to know when soil N reaches the new tissues, 225 g ammonium nitrate depleted in ^{15}N (0.01 atom % ^{15}N) was applied 11 days before full bloom to four trees in the same block described above. The fertilizer was applied evenly under the tree canopy at 5 cm depth. The treated ground was immediately covered with soil to minimize ammonia volatilization. New growth was sampled weekly, thereafter. The sampled tissue was dried, ground to pass a 40-mesh screen, and sent to Isotope Services (Los Alamos, N.M.) for isotopic composition determination by mass spectrometry. Atom percentage values were converted to N derived from the fertilizer (NDFF) using the following formula (adapted from Hauck and Bremner, 1976):

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

^{15}N natural abundance in the soil was considered equal to 0.366% (Handbook of Chemistry and Physics, 1969).

Estimation of N needed for new growth by a mature apple tree:

Three 11-year-old 'Topred Delicious' apple trees on M.7A rootstock growing in the same block described above were used in this experiment. They were trained to modified central leader. They received a ground application (120 g N/tree) of ammonium nitrate in Spring 1992 and no fertilizer in 1993. Buds were collected from each tree at various dates during February, March, and April. At full bloom (19 Apr. 1994) all flower cluster, shoots, and spurs on each tree were counted. On 18 May all new growth of each tree (fruit, spurs, shoots) was harvested, weighed, and sampled for dry weight and N determination. The trees were then cut off at the graft union. The fresh weight of the above ground structure of each tree was recorded and a composite sample was taken for dry weight determination. Throughout this manuscript the term "new growth" refers to the visible tissues (blossom, fruitlets, spurs, shoots) that developed during Spring 1994.

We used data from Chapter 3 to develop a relationship between the dry weight of the above ground biomass (X) and that of the total biomass (Y) of a dormant 11-year-old 'Topred Delicious' apple tree ($Y=3.686 + 1.243X$, $R^2=0.99$). The total weight of such a tree consists of 7.7% as shoots, 21.1% as branches, 46.3% as frame, 19.4% as large roots, and 5.5% as small roots (Chapter 3). For tree dry weight distribution

estimation we used average percentages of 15 trees, while for total N distribution we used only data from three trees fertilized in a similar manner as the trees used in this study. Our logic was that dry matter partitioning would be less sensitive to a one year difference in N fertilization than partitioning of N. Assuming that the above ground tree biomass (new growth excluded) changed little in dry weight from February to May, we used the above relationships to estimate the size and distribution of the total dormant biomass (TDB) and total N of the harvested trees. Data were normalized by dividing by the estimated dry weight (kg) of the TDB.

2. Results and Discussion:

Bark protein characterization:

Seasonal protein fluctuations:

From early September to mid-February, N and extractable protein contents of shoot bark increased by 82% and 167%, respectively (Fig. B.1). If we assume that the proportion of bark N in the form of protein is fairly constant throughout the year, the large increase in extractable protein level indicates a preferential accumulation of this fraction of the proteins as the tree becomes dormant.

Both extractable protein and N concentration of the apple bark started to decline during the second half of February (Fig. B1). By 17 May, the two had decreased by 52% and 66%, respectively, compared to mid-February values. These proportions are comparable to what was reported by Kang et al. (1982), although, they used a different apple cultivar. The range of fluctuation of extractable protein levels was wider than that of N content suggesting an abundance of possible storage proteins in the former fraction.

Extractable protein values were converted to N units by dividing by 6.25. Total protein N content per gram of dry bark increased by 7.5 mg from 9 Sept. 1992 to 16 Feb. 1993. Nitrogen in the extractable protein fraction accounted for only 30% of this increase. This fact suggests either other non-protein nitrogenous compounds accumulated during the same period or the method of extraction used failed to extract the bulk of the proteins contained in the tissue. Kang et al. (1982) were faced with the same problem of low yield when extracting proteins from shoot bark of 'Golden Delicious' apple trees using a phosphate-citrate buffer (pH 6.0). They estimated the amount of N found in proteins and amino acids to about 5 mg/g dry bark; this is less than one forth of what is commonly reported (O'Kennedy et al., 1975). Hill-Cottingham and Cooper (1970) studied the distribution and identity of the nitrogenous constituents of young apple trees. They found that proteins represent 84% to 96% of the total N in the shoots depending on previous N fertilization. However, shoots contained only 6% to 8% of all tree-N.

The soluble protein pool, as discerned by one-dimensional SDS-PAGE, underwent seasonal changes in several polypeptides (Fig. B.2). Although there is no precise definition of storage proteins in woody plants, it is generally accepted that such a protein should accumulate during the dormant season and disappear (or at least decrease) as growth resumes (O'Kennedy and Titus, 1979; Kang et al., 1982; Wetzel et al., 1989). Three polypeptides of an estimated MW of 16, 17, and 30 kD, respectively, appeared to satisfy both criteria mentioned above (Fig. B.2). These proteins accounted for about 16% of the all the proteins extracted form the apple bark. A 30-kD protein was found to accumulate in shoot bark of 'Ana' apple (Lang and Tao, 1992).

The poplar BSP antiserum did not cross-react with the 30-kD polypeptide or any other protein present in the apple bark extract (data not shown) suggesting that any possible apple BSP may be markedly different from that found in poplar (Coleman et al., 1991).

The 66% decline in bark extractable protein during spring (Fig. B.1) could not be accounted for by the loss of the three polypeptides. If they are the main BSP, the peptides are only partially extracted with the method used or other unidentified proteins are the storage form of N in apple bark. Oland (1959) suggested that the total soluble N (in 70% ethanol) consisting of amino acids and amides should be considered as the main reserve N in apple tissues. Later reports by Taylor and his colleagues (1967, 1969, 1971) concluded that arginine and to a lesser extent the amides in the ethanol soluble N fraction are the main form of storage N in young and mature peach trees. A growing body of evidence, however, points to proteins as the main form of storage N in bark and wood of a wide range of woody species (Tromp and Ovaa, 1973; O'Kennedy et al., 1975; Kang and Titus, 1980; Kang et al., 1982; Wetzel et al., 1989; Coleman et al., 1991). Therefore, the main N storage compound(s) in apple bark is likely to be a protein that would require more vigorous methods to be extracted.

The examination by SDS-PAGE of soluble protein extracts obtained from the shoots collected in mid-February revealed the presence of the relatively abundant 30 kD polypeptide as well as the less abundant polypeptides of 16 and 17 kD MW (Fig. B.3). After 3 weeks at room temperature in a N free nutrient solution, similar shoots collected in early April showed evident signs of N deficiency (shoot growth ceased and leaves turned yellow) after substantial growth took place. The three polypeptides decreased to

about 17% of their mid-February levels. This result is agreement with the first experiment.

Characterization of N pools in an apple tree:

The 11-year-old trees averaged 62.58 kg in dry weight and 280.72 g N in February 1994. The large roots, frame, and branches contained about 77% of all the tree N. Ammonium, nitrate, and amino acids, and soluble proteins accounted for only 32.3% of tree N (Table B.1). On average the trees had less than 0.25% of their N in the form of ammonium and nitrate.

Monitoring of current-year uptake of soil N:

Labeled N was detected in the flower buds around full bloom (18 Apr. 1994) (Fig. B.4). By this date, the buds had 1.2% of their NDFF. This proportion increased steadily thereafter to reach 6.2% one month later. When expressed on a dry weight basis, the contribution of labeled fertilizer-N to new growth was very small. Although, not all the N in the soil came from the fertilizer, however, given the qualities applied, this exogenous source of N should account for high proportion of the N available to the tree. Therefore, abundance of labeled N in the new tissues would reflect the contribution of the soil.

Estimation of N needed for new growth by a mature apple tree:

Dry weight and N content (mg N/bud) of the buds changed only slightly during February and the first half of March (through silver-tip stage) then they increased sharply until full bloom on 18 Apr. 1994 (Fig. B.5). From 1 Feb. to full bloom, the buds increased 16-fold in dry weight and 23-fold in N content. Upon petal fall, the blossom dry weight and N content decreased temporarily. Nitrogen

concentration in the buds followed the same pattern as N content. It increased up to full pink stage (8 Apr.) then decreased steadily thereafter until full bloom (Fig. B.4). This decrease was apparently due to a dilution effect caused by a faster accumulation of dry matter compared to N.

On a kilogram of TDB basis, bud dry weight increased from less than 1 mg on 1 Feb. to 10 mg by mid-March, then to 23 g by mid-April (first-pink stage) (Fig. B.4A). Nitrogen content of the buds was less than 1 mg in mid-March then increased to 0.67 g by mid-April (Fig. B.4B). New growth increased sharply in dry weight and N content from 18 Apr. (full bloom) to 18 May with a temporary decrease during petal fall period (25 Apr.).

Nitrogen mobilization toward the buds started one month before full bloom as indicated by the increase in bud N content (Fig. B.5). By full bloom, the tree had used 0.88 g N per kilogram TDB to build its new tissues. This figure increased to 2.81 g when the trees were harvested on 18 May.

It appears that the bulk of this N came from N reserves within the tree since the contribution of the pre-bloom fertilizer application amounted to only 6.2% of all N in the new growth. Although the contribution of new root uptake is likely larger than the 6.2% of the N found in the new growth since not all soil N originated from the labeled fertilizer, it is evident that reserve N accounts for the bulk of the N mobilized in the spring. It is well established that deciduous trees rely heavily on their N reserves for early spring growth (Murneek, 1930; Taylor, 1967; Tromp and Ovaa, 1971; Titus and Kang, 1982; Habib, 1984; Deng et al., 1989).

ACKNOWLEDGEMENT

We would like to thank G. Coleman for providing the poplar 32-kD BSP antibody used in this study.

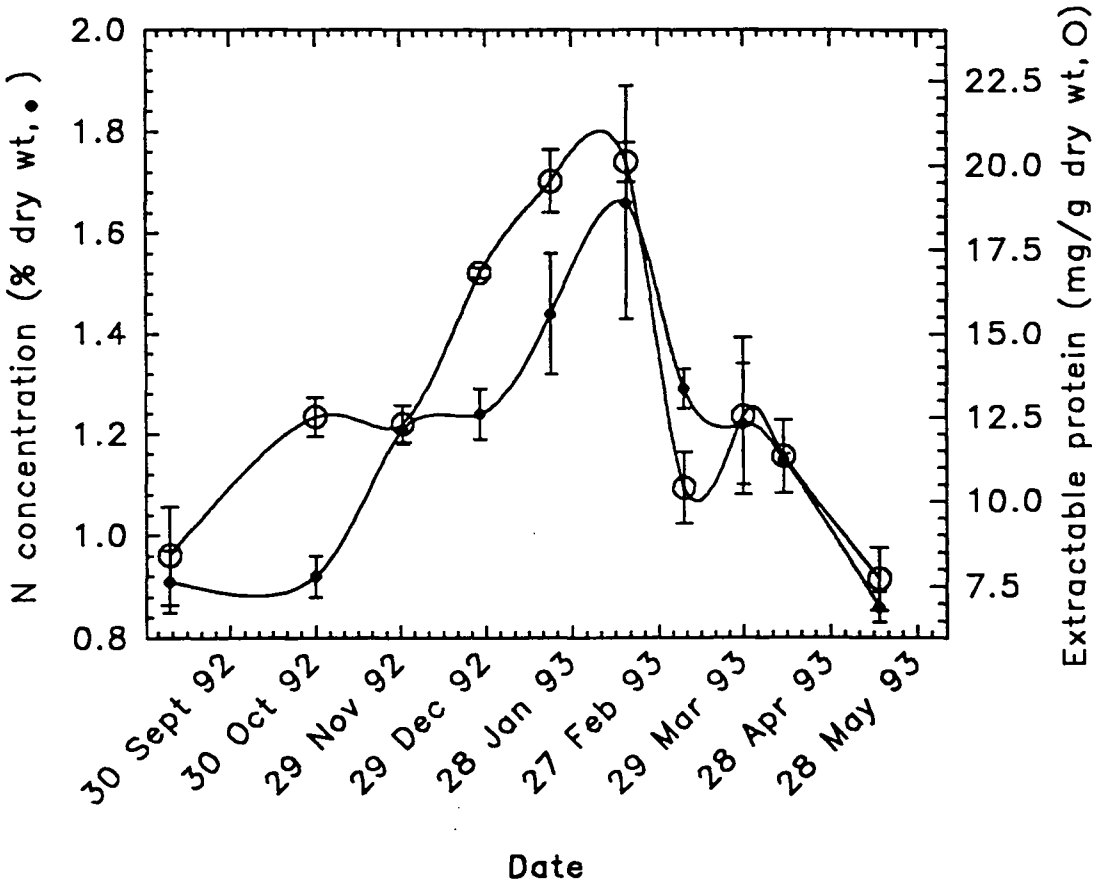

Fig. B.1. Seasonal changes in bark N concentration (•) and extractable protein levels (o) in 'Topred Delicious' apple trees. Extractable protein represent that found in the 35,000x *g* supernatant.

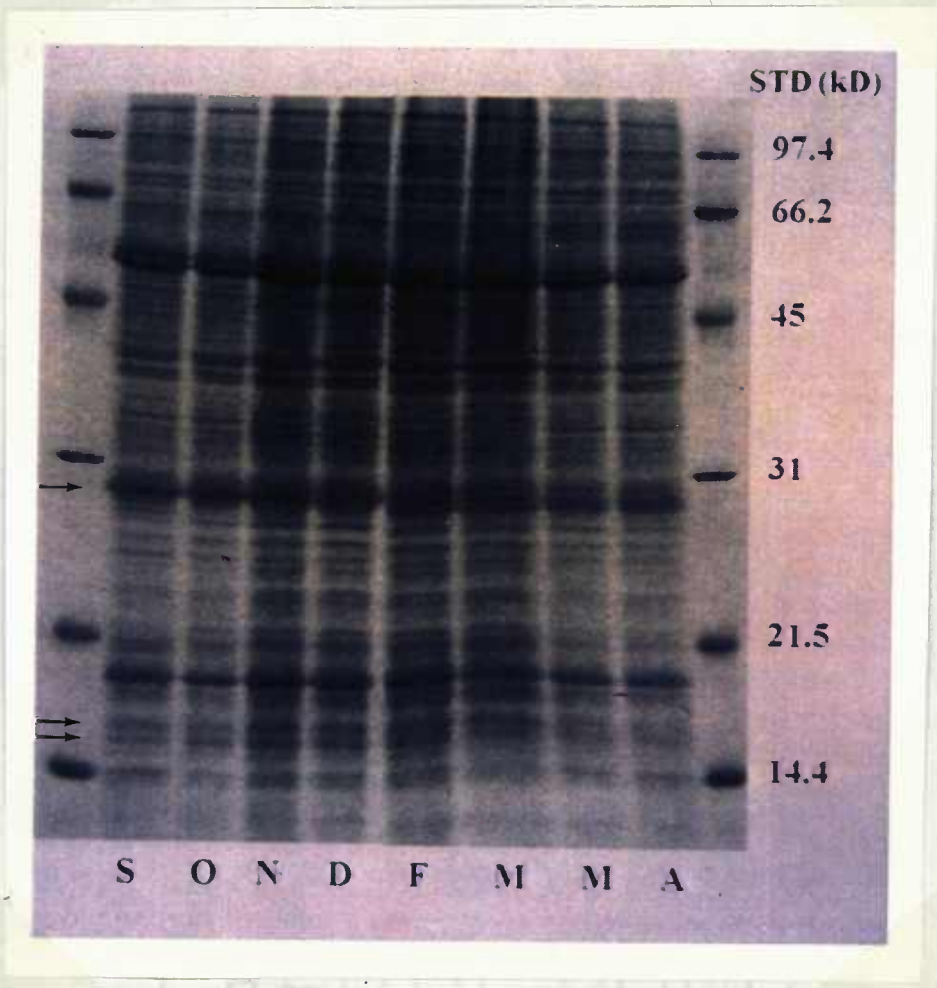

Fig. B.2 Seasonal changes in polypeptide composition of 'Topred Delicious' apple bark. SDS-PAGE of soluble bark proteins extracted from shoots collected at various times during the year (S= 9 Sept. 1992; O= 30 Oct. 1992; N= 30 Nov. 1992; D= 27 Dec. 1992; F= 16 Feb. 1993; M= 8 Mar. 1993; M= 19 Mar. 1993; A= 12 Apr. 1993). The molecular weights of the protein standards are shown on the right side of the gel. The arrows indicate the position of the 16, 17 and 30-kD polypeptides.

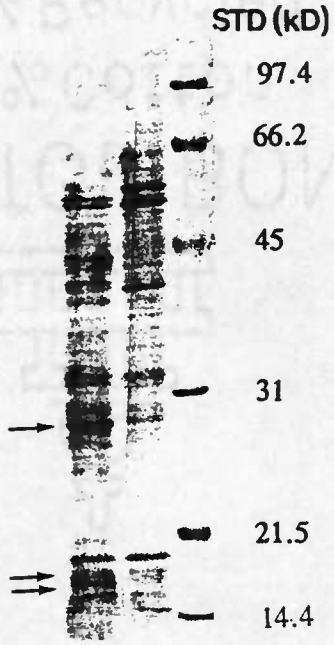

Fig. B.3 SDS-PAGE analysis of the composition of soluble bark-proteins extract from 'Topred Delicious' shoots either immediately after collection from the field on 16 Feb. or on 23 Apr. 1994 after 3 weeks culture in a N free nutrient solution. First bloom in the field was on 12 Apr. The molecular weights of the protein standards are shown on the left side of the gel. The arrows indicate the position of the 16, 17 and 30-kD polypeptides.

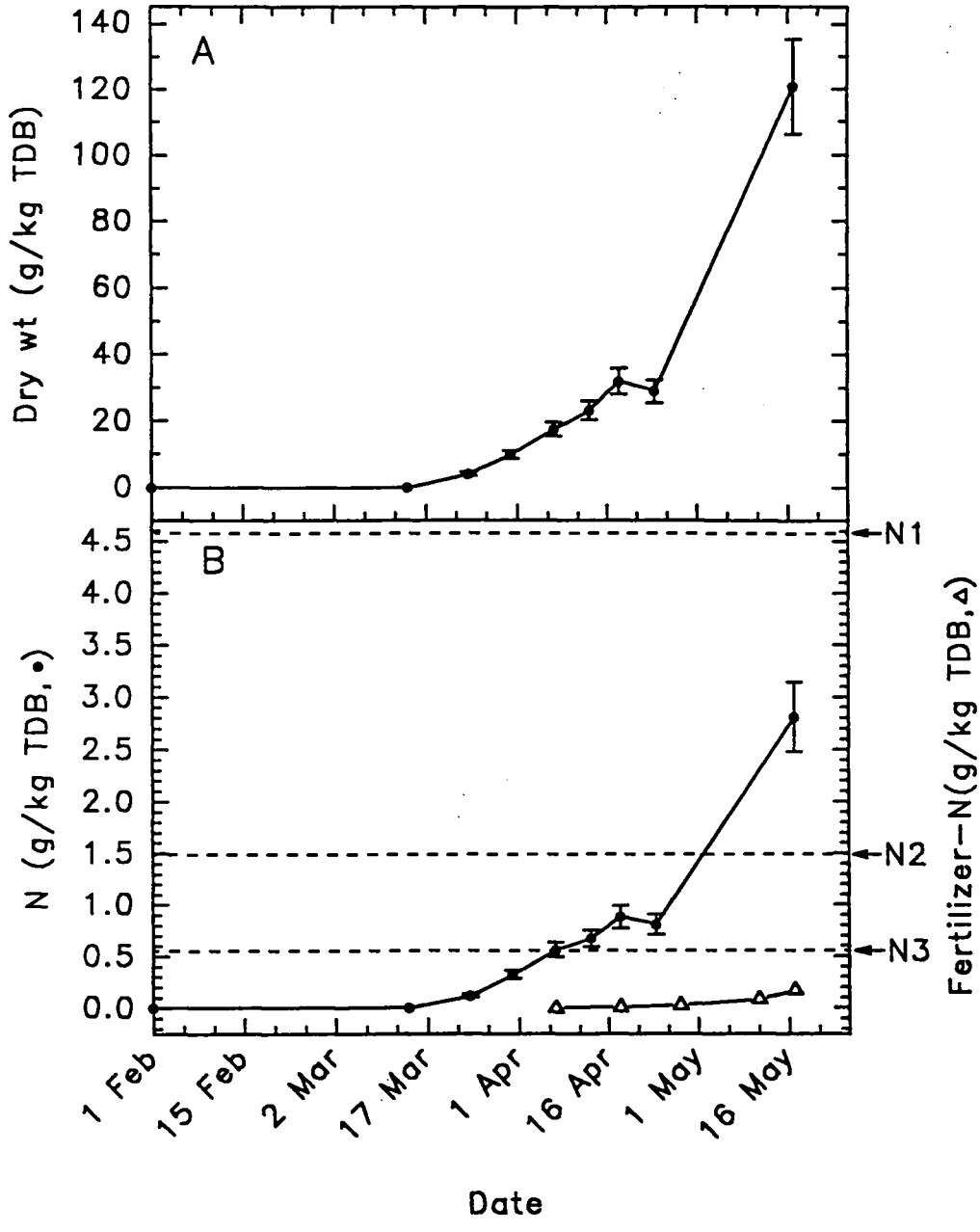

Fig. B.4 Dry weight (A) and total N (•) and labeled fertilizer-N (Δ) (B) accumulation in buds and new growth [g N in the buds or new growth per kg total dormant tree biomass (TDB)] in 'Topred Delicious' apple trees between 1 Feb. 1994 and 18 May 1994. The arrows indicate the estimated amounts of total N (N1), extractable N (nitrate-N + ammonium-N + amino-N + extractable protein-N) (N2), and "reserve" N (nitrate-N + ammonium-N + amino-N + 30-kD peptide-N) per unit dry weight of TDB.

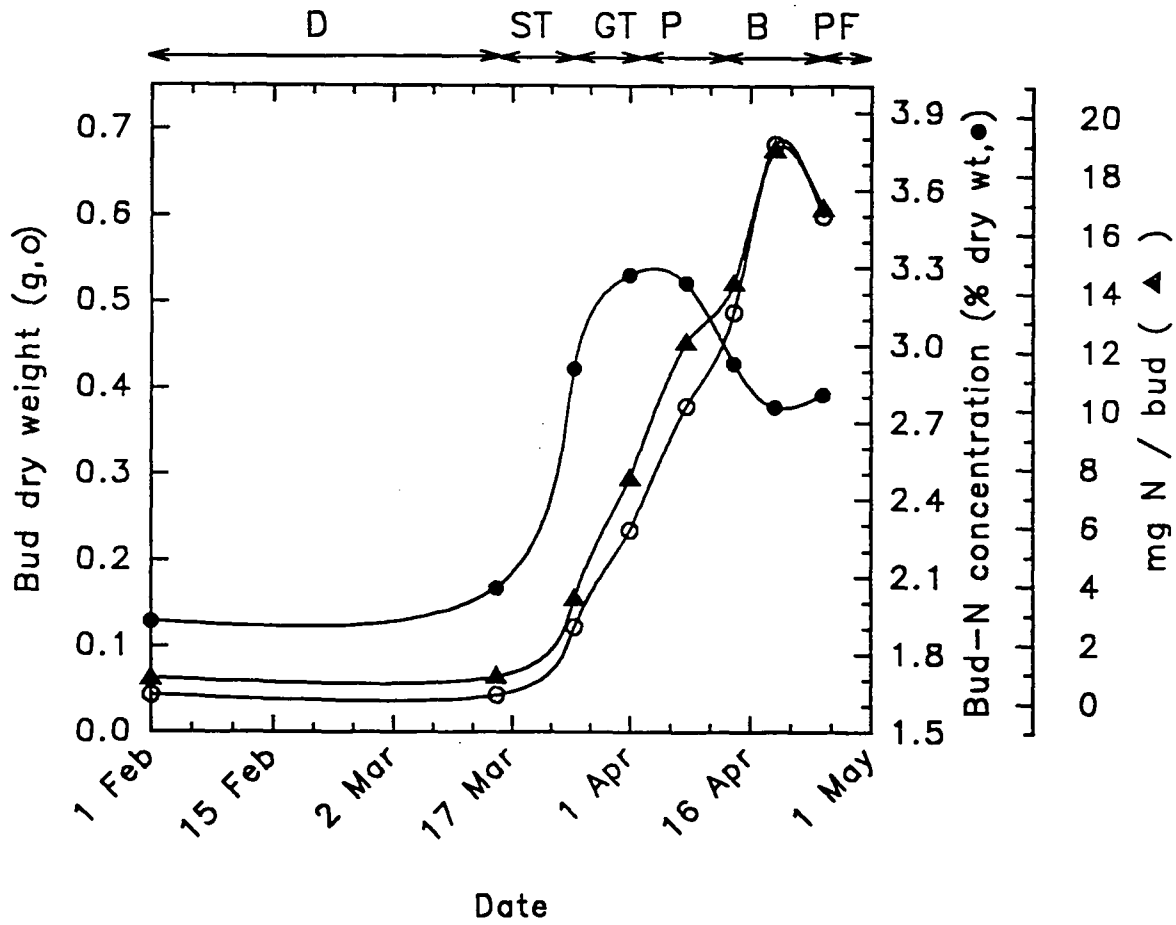

Fig. B.5 Changes in bud weight (●), N concentration (o), and N content during the months (▲) of February, March, and April. The buds were collected from 'Topred Delicious' apple trees growing in the field. Developmental stages of the flower buds are presented on the top of the graph (D = dormant, ST = silver tip, GT = green tip, P = pink, B = bloom, PB = post-bloom).

Table B.1. Total nitrogen (TN) concentration and distribution (as percentages of total N) in various components of 11-year old 'Topred Delicious' apple trees on Malling 7A rootstock. The trees were harvested in February 1994.

	Tree component				
N fraction	Shoots	Branches	Frame	Big roots	Small Roots
TN (% dry wt)	0.65±0.02	0.39±0.03	0.40±0.02	0.56±0.06	0.57±0.08
Extractable proteins (% TN)	19.1±3.1	30.8±7.5	37.0±1.6	12.5±0.8	15.8±1.4
Amino acids (% TN)	4.5±0.8	10.1±2.9	13.1±2.4	4.7±1.2	5.5±1.5
Nitrate (% TN)	0.2±0.0	0.1±0.0	0.1±0.0	0.0±0.0	0.2±0.0
Ammonium (% TN)	0.1±0.0	0.1±0.0	0.1±0.0	0.2±0.0	0.2±0.0