AN ABSTRACT OF THE THESIS OF

Enrique Eduardo Sanchez for the degree of Doctor of Philosophy

Title: Nitrogen Dynamics in Field Grown Comice Pears

Abstract approved: ____________________________

Timothy L. Righetti

The dynamics of N was studied in field grown Comice/Provence quince BA29 pears in Medford, Oregon. Total tree biomass, N content, and \(^{15}\)N evaluations suggest that young pears require little N (48 kg N ha\(^{-1}\) yr\(^{-1}\)). About 45% of total tree N present in dormant trees was remobilized into new growth the following season. Main storage organs were roots, frame and one-year wood. Before leaf fall, peripheral, medium and interior canopy leaves exported to storage tissues 71, 61 and 52% of their total N, respectively. The export of N was influenced more by leaf position in the canopy than the nutritional status of the tree.

Shoot and fruit growth were dependent on newly absorbed N. A heavy crop load caused more stored N to be diverted into fruits at the expense of other tree components. Early spring application of N resulted in a buildup of tree reserves for developing buds, but produced excessive growth and resulted in fruits with undesirable high concentration of N. From harvest until leaf fall very little N was partitioned into the aerial portion of the tree. In order to increase substantially N reserves in the aboveground structure of the
tree, and avoid excessive shoot growth and high N fruits. N should be applied 3-6 weeks before harvest. When N was applied at or after harvest but before leaf fall, roots were primarily the site of N storage. At that time 5 or 10% postharvest urea spray was the only effective way to obtain labelled N in flower buds.

Early spring growth normally depended on N reserves. However when temperature around bloom was warmer than the long term average newly absorbed N was translocated to the flowers. During the first 3-4 weeks after bloom newly absorbed N was partitioned to spur leaves while shoot leaves were more dependent on stored N. Once spur leaves reached full expansion N was diverted into shoot leaves and fruits.

Fruits from the same tree varied considerably in N concentration especially when fertilizer N was applied after bloom. Trees with high N status discriminated in the allocation of N to fruit in different canopy positions but trees with low N status did not. Large number of fruits in any specific location lessened N concentrations. The location of the fruit in the canopy only partially explains N variability. Similar sized fruits only a few centimeters apart may have a two fold concentration range.
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Enrique E. Sanchez and Viki Freeman
Typed by
DEDICATION

This thesis is dedicated to my children, Juan Andres and Francisco. They are the driving force for me to improve myself as a father, husband, person and scientist.
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These years have been good ones for the family. The experience of living in another culture and especially in beautiful Oregon was worthwhile. My wife Nora, studied and received her MS. People that have been in the same situation, especially with two kids can perfectly understand all the problems involved. Fortunately everything worked well and here we are, ready to restart our life in Argentina.

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NINETY-FOUR percent of the pears in the U.S. are grown in the western coastal states: Washington, Oregon, and California. Commercial pear cultivars in the U.S. number nearly 40, but 'Bartlett', 'Anjou', 'Bosc', and 'Comice' make up 99% of the total production. In Oregon, the industry is concentrated in the Rogue and Hood River Valleys and, to a lesser extent, along the Willamette River.

The present study involved 'Comice' on Quince BA29 rootstock grown at the Southern Oregon Experiment Station near Medford. 'Comice' is considered the best eating winter pear cultivar because of its juicy, buttery texture and well balanced flavor. Although alternate bearing is a problem in the young trees, Quince rootstock is still preferred for 'Comice' trees because this combination is the most precocious (Lombard, 1986).

Next to water, nitrogen (N) is the most critical input for tree production. Since the optimum range for available soil N is narrower than for P and K, careful N management is vital for optimal yield and crop quality. Unfortunately, the economic necessity of achieving early production has led to widespread over-application of N. The low cost of N fertilizer in comparison with other inputs has also encouraged excess use. Even after N application is reduced with the initiation of cropping, excessively high levels may persist in the soil, grass sod, and tree (Sharple, 1980).
Deciduous fruit crops are less demanding of N than other crops (Greenham, 1980), but blossom quality, ovule longevity, and fruit set are greatly influenced by N nutrition (Williams, 1965). Thus, in spite of the low N requirement for fruit trees, the first stage of growth is a period of high N demand. Management is complicated because high levels of N in the tree favor vegetative growth rather than fruiting.

Efficient use of N fertilizers is not an economic necessity for high value crops, but excess N use creates potential ecological problems. In California, an estimated 311,000 tons of N is annually leached from irrigated land (Pratt, 1984). In Washington, continuous application of ammonium fertilizers have reduced the soil pH to levels not compatible with fruit production. In addition to these ecological problems, excess N negatively affects fruit quality and production (Bramlage et al., 1980).

Determining appropriate nitrogen fertilization rates and application times requires considerable expertise. Specific recommendations to maximize application efficiency of N fertilizers, avoid N excess, and still meet the requirements of the crop are not available. Workable guidelines are based on nutrient concentrations in leaf tissue, but interpretation is complex.

Unfortunately, changes in nutrient concentrations do not necessarily correspond to altered 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 the concentration of N in a tissue may be brought about by a change either in N or in dry matter content of the tissue (Taylor, 1967). According to
Titus and Kang (1982), all the data reported on a concentration basis are unsatisfactory and should be changed in the future. This may be especially true for N because leaf values vary over a relatively narrow range. Tissue analyses certainly need to be interpreted cautiously, because elemental concentration can be misleading.

Deciduous fruit trees 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. Assessing the importance of reserve N and its contribution to next year's growth is of paramount importance in evaluating fertilizer programs. Understanding the dynamics of N in the tree is essential to properly interpret nutrient levels in leaf tissue.

Some aspects of storage and mobilization of nitrogenous compounds in fruit trees have been reviewed by Taylor (1967), Tromp (1970), and Titus and Kang (1982). These studies further emphasize the difficulties in interpreting data dealing only with concentration rather than both concentration and total amounts. Although it is possible to express the data from leaves on both parameters, results on spurs, branches, and roots cannot be assessed in this manner unless the entire tree is taken for analysis.

The partitioning and recycling of N within mature trees can be measured directly using an isotopically labeled N carrier. However, the experimental use of the N isotope with deciduous tree fruit species has been restricted primarily to study the uptake and translocation of fertilizer in immature trees in pot culture (Grasmanis and Nicholas, 1971; Hill-Cottingham and Lloyd-Jones, 1975; Weinbaum
et al., 1978, 1980). Results from these experiments may not be applicable to mature trees under orchard conditions. The use of mature trees represents a notable exception (Atkinson et al., 1978, 1980; Weinbaum et al., 1984a,b). The only antecedent in the literature about recycling of N using tracer techniques are the papers of Weinbaum et al. (1984a,b, 1986, 1987) on almond trees. However, the dilution of labeled N was assessed indirectly using concentrations in leaves and fruits rather than total amounts in all plant parts. Furthermore, data from those studies cannot be extrapolated to pome fruits because of the difference in N demand among crops.

In summary, previous investigations involving N recycling have usually utilized conventional methods (unlabeled N) whereby it was impossible to distinguish between currently absorbed and reserve N. Most studies also interpret concentrations rather than total amounts. In the case of pome fruits, the relative importance of pools of reserve N accumulated in previous seasons has not yet been determined.

The objectives of our study were:

1) Assess the relative contributions of the total N in the tree from the N assimilated in previous years (stored N) and the N derived annually from the soil or fertilizer.

2) Evaluate the relationship between the timing of isotopically labelled N fertilizer application and the availability of fertilizer N to reproductive and vegetative organs.

3) Evaluate the potential use of postharvest N sprays in increasing N reserves in the tree and fruit set.
4) Determine the influence of soil texture on fertilizer N availability.

5) Determine best timing of N application for pears to maximize uptake efficiency minimizing fruit N concentrations, and still insure that adequate N is available to developing buds.
CHAPTER 2

LITERATURE REVIEW

Nitrogen Fertilizer Requirements

In 1936, Macy introduced the concept of critical nutrient concentrations in leaf dry matter. He held that, for any given plant, a minimum leaf concentration of a given element was required to produce a good crop (Macy, 1936). In 1948, Ulrich defined critical nutrient levels as that range of concentrations below which growth of the plant is restricted when compared to plants at a higher nutrient level (Ulrich, 1948). He showed that plants with widely different nutrient composition gave similar yields as long as these nutrient concentrations were well above the critical level.

Shortly after initial studies, leaf analysis began to be used to evaluate plant nutritional status. According to Bould (1966), the method of leaf analysis is based on the principle that the leaf is the major site of plant metabolism, that changes in nutrient supply are reflected in the composition of the leaf, that these changes are more pronounced at certain stages of development than at others, and that the concentrations of nutrients in the leaf at specific growth stages are related to the performance of the tree.

In the 40's, 50's, and 60's, optimum leaf nutrient standards and sampling approaches were established in apples and pears (Boyton and Compton, 1945; Kenworthy, 1950; Smith and Taylor, 1952; Bollard et al., 1962). These studies suggest that analyzing August leaf samples can be helpful in determining nitrogen needs and adjusting nitrogen
application rates. However, tissue analysis needs to be interpreted cautiously. Understanding the dynamics of nitrogen in the tree is essential to properly interpret nutrient levels. Elemental concentrations in themselves can be misleading. This is especially true for nitrogen because leaf values vary over a relatively narrow range. A ten percent difference (2.0 to 2.2%) is enough to radically change one’s interpretation (Righetti, 1986). Unfortunately, these small changes in nutrient concentration do not necessarily correspond to altered total amounts of nitrogen within a tissue, nor can they be interpreted as changes in the uptake of nitrogen from the soil (Jarrell and Beverly, 1981; Righetti, 1986). Liberal fertilizer practices combined with irrigation often result in vigorous tree growth, and require heavy pruning, which promotes even more growth. Frequently, trees with above average growth are diagnosed as nitrogen deficient when dilution produces a below normal concentration. In cases where little or no growth occurs, nutrients are often concentrated and deficiencies may not be apparent. Since vigor is related to cropping and other management factors, growth and crop load often affect the interpretation of tissue analysis. From a practical perspective, nitrogen concentrations can be interpreted if growth and vigor are considered. Dry matter partitioning between leaves and fruit can also drastically affect element composition (Smith, 1962; Hansen, 1980).

The standard approach to interpreting leaf analysis is to compare observed concentrations in leaves to reference values (critical concentration). Defining critical concentrations involves many years of field data over a range of orchards with different soil
types, tree size, density, training systems, and varieties. Thus, judgment rather than vigorous testing is often the basis of the critical ranges (Righetti, 1986). Current standards for pome fruits have been developed in most fruit producing areas, but the sufficiency ranges often differ even for the same species. As an example, Oregon State University has sufficiency ranges for 'Anjou' and 'Bartlett' pears depending upon whether the samples are from Hood River or Medford. It is not clear if these regional differences are justified.

Recently, the idea of nutrient balance has been applied to fruit trees. Several recent reports described the use of Beaufils' Diagnosis and Recommendation Integrated System (DRIS) (Beaufils, 1971). DRIS is a diagnostic approach that uses nutrient concentration ratios rather than concentrations themselves. In some cases, it can provide better interpretations of mineral analyses than the conventional sufficiency range for both field crops (Beaufils and Sumner, 1976; Beaufils and Sumner, 1977) and perennial trees (Beverly et al., 1984; Davee et al., 1986, Alkoshab et al., 1987). The principal advantage is that DRIS provides a measure of nutritional imbalance rather than evaluating only a single deficiency or excess at a time. DRIS may also minimize the effects of a general dilution or concentration due to dry matter and age factors and better evaluate possible nutritional interactions (Sumner, 1977).

Since all DRIS evaluations have relative deficiencies and excesses, it is important to determine if these relative values are diagnostically important. A nutritional imbalance index (NII) is
calculated by adding the values of DRIS indices irrespective of the sign (Sumner, 1977). The larger the NII, the greater the intensity of imbalance among nutrients and the more likely relative excesses or deficiencies are diagnostically important. Despite its usefulness, recent studies suggest that DRIS will not detect all deficiencies or excesses, at least in fruit trees (Alkoshab et al., 1987; Righetti et al., 1988a, 1988b). Thus, DRIS is best viewed as a supplement to sufficiency range diagnoses which provides additional information when severe imbalances are detected (Righetti et al., 1988b). Tissue analysis has to be viewed as a useful tool rather than a means of making rigid diagnoses. Answers to the question, "How much fertilizer should I apply?" are often philosophical rather than scientific. Regardless of whether using DRIS or critical concentrations, there is no simple way to determine how much nitrogen fertilizer to apply in the orchard.

**Nutrient Cycling and Orchard Budgets**

An understanding of the movement of nutrients in the soil-plant system is essential to determine appropriate rates and timing of fertilizer addition. Among the soil components, rate of leaching and mineralization of the organic matter are probably the most relevant ones. The study of the plant component has been a horticultural concern since the beginning of this century. Early estimates of mineral requirements were based on the concept of replacing nutrients which trees remove from the soil. Nutrient removed was estimated by determining the minerals contained in the tree and the amount of nutrients removed by the harvest (Magness and Regeimbal, 1938; Bajter
et al., 1952). These studies suggest that fruit crops are less nitrogen demanding than other crops. Cooke (1970) further demonstrated the usefulness of crop nutrient balance sheets as indicators of fertilizer requirements. However, an understanding of the nitrogen requirements for fruit trees is far from complete. The idea of applying whatever the crop removes at harvest does not consider the optimum nitrogen fruit status, the balance between shoot growth and fruit production, or the contribution of stored nitrogen to the next year's crop.

Knowledge of the portions of nitrogen that new growth obtains from tree reserves is critical in evaluating nitrogen fertilizer programs. The use of the stable isotope $^{15}$N allows differentiation of both pools. Unfortunately, early studies dealing with nitrogen recycling used conventional methods (unlabeled N), where it was impossible to isolate the contribution of the endogenous (storage) and exogenous (new) nitrogen.

It is important to express tree N in total amounts rather than N concentration when developing N budgets. Although it is possible to express leaf data as both concentration and total amounts, results on spurs, branches, and roots can only be estimated in this manner if the entire tree is taken for analyses. The only antecedent literature regarding recycling of N using tracer techniques in mature deciduous fruit trees is the work of Weinbaum et al. (1984a, 1984b, 1987) on almond trees. Nevertheless, the dilution of labelled N was assessed indirectly using concentrations in leaves and fruits rather than total amounts in all new developing tissues.
The importance of reserve N is obvious since budbreak in the spring takes place at a time when conditions for root uptake are not always optimal. Ironically, blossom quality, ovule longevity, and fruit set are greatly influenced for nitrogen nutrition (Williams, 1965), in spite of the low nitrogen requirement for fruit trees. The first stage of growth is a period of high nitrogen demand. Thus, nitrogen recycling not only plays an important role in the economy of nitrogen use by the tree, but also is an important yield determining factor.

**Nitrogen Accumulation in the Leaves**

The leaf tissue is a major reservoir of nitrogenous compounds in deciduous fruit trees. The major single portion of plant leaf protein is present as the photosynthetic enzyme ribulose-1,5-biphosphate carboxylase, which accounts for up to 50% of the total leaf proteins (Kawashima and Wildman, 1970). One of the earliest reports that quantitatively showed the distribution of N in apple trees was reported by Murneek (1942). He estimated that the amount of N required for different parts of the average size of 18- to 20-year-old bearing trees were as follows, in kg/N per tree: 0.18 each for the fruit crop and the abscised leaves, 0.16 for root and top growth, 0.05 removed by pruning, and 0.03 removed by abscised flowers and fruits. Thus, the abscised leaves contained nearly 30% of the total tree nitrogen. Bajter 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 upon the methods of N application, 40 to 50% of the
total N in an apple tree was present in leaves in late August.

Nitrogen Storage

The N concentration of woody tissues in deciduous fruit trees increases with the cessation of shoot growth in late summer and continues until winter (Murneek, 1942; Oland, 1959; Mason and Whitfield, 1960; Taylor, 1967). Mason and Whitfield (1960) looked at the seasonal changes in N in the whole tree of the apple 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, occurred. However, Taylor and May (1967) showed that, when values are expressed on an absolute basis, N storage in woody tissues of young trees begins as soon as the rate of shoot extension growth slows down in early summer. The increase of storage N in tree tissues was usually proportional to the current N supply. However, the rate of dry matter accumulation exceeds the rate of N accumulation, thus N concentration increases may not occur.

In the fall, tree roots are growing and are highly active (Heinicke, 1935). Both field trials and pot experiments suggest that fruit trees take up and accumulate N during autumn and winter, provided the soil temperature is above freezing (Smith and Murneek, 1938; Oland, 1959; Hill-Cottingham and Williams, 1965). The N which is taken up in the fall and winter is stored in the larger roots or stock wood (Smith and Murneek, 1936; Bajter et al., 1943). According to Bajter et al. (1943), this stored N is not translocated to tree tops if the air temperature is less than 40 to 45°F).
Nitrogen also accumulates in woody storage tissues of deciduous trees during late fall as a large part of the N migrates from the leaves prior to leaf fall (Murneek and Logan, 1932; Oland, 1959; Oland, 1963). In apple trees, the translocation of N from leaves to wood shoots commences three to four weeks prior to abscission (Oland, 1963). Since up to one-half of tree N content at the end of the growing season is present in the leaves (Murneek, 1942), this migration is a most important factor in the efficient use of N by the tree.

The percentage of N lost from the apple leaves during senescence varies from 23 to 50% (Murneek, 1930; Murneek and Logan, 1932; Oland, 1963; Spencer and Titus, 1972; O'Kennedy et al., 1975a; Hennerty and Morgan, 1977). In an extreme case, where leaf senescence was induced in a growth chamber, as much as 70% of the initial N had been lost (Shim et al., 1972).

Sites of Nitrogen Storage

The early work of Murneek and Logan (1932) indicated that the leaf N initially migrated into spurs and branches but was eventually translocated to the older wood and root system. This has important consequences with regard to time of pruning. They recommend delaying pruning until late winter or early spring when N movement to the more proximal parts of the tree is complete.

Harley et al. (1958), using a multiple bark-ringing technique in mature apple trees, demonstrated that early spring growth depends primarily on nutrients stored in the aboveground structure. Yokomizo et al. (1964) found that most of the N which was mobilized for growth
in nitrogen-rich apple trees came from the roots, whereas in low nitrogen trees, most of the mobilized N came from old shoot tissues. In contrast, Taylor and May (1967) found that between 60 and 80% of the storage N in dormant, two-year-old peach trees was present in root tissues, irrespective of previous N treatment. Hill-Cottingham and Cooper (1970) also reported the accumulation of asparagine and arginine, especially in the roots of young apple trees with the autumn application of N. These observations may well be a reflection of N conservation in the roots.

The autumn application of N (end of October through the beginning of November) increased the N level mainly in the roots during the late autumn and winter (Tromp, 1970). However, the N migrating from senescing leaves benefited primarily the aerial parts of the tree. The aerial parts of the tree may be a more important source of N for early growth than the roots. The rationale for this consideration may be in the proximity of the aerial tissues to developing buds in spring. It is usual to find a higher concentration of total N in bark than in the wood, at least in the aboveground parts of fruit trees. Since the concentration of total N in the bark falls sharply during the growing season, it has been suggested that most of the reserve N of trees is held in bark tissues (Mochizuki and Hanada, 1956; Mason and Withfield, 1960). However, this suggestion needs checking since such results must be expressed on an absolute basis rather than as N concentration. The increase in N supplied by post-harvest urea sprays was observed in stem and shoot bark, and in the roots. O'Kennedy et al. (1975b) found a higher percentage of N in
the bark, but they did not exclude the role of wood as N storage
tissue, especially for the storage of soluble N. The controversy on
major storage locations is further complicated by a report by Shim et
al. (1973) which did not clearly indicate the predominant role of any
specific parts of the tree for N storage.

The major forms of storage N in fruit trees is controversial.
Oland (1954, 1959) concluded that N is stored in the apple tree
mainly as a soluble. Taylor and May (1967) report similar results
for young peach trees and Taylor and Van den Ende (1960) confirmed
this finding for bearing peach trees. Tromp (1970) and Tromp and
Ovaa (1971, 1973) argued that protein N is the most important stored
form in the bark of apple trees and that hydrolysis starts before
buds break. Recent work confirms that proteins in apple shoot bark
are of prime importance in supplying N for early spring growth
(O'Kennedy and Titus, 1979; Titus and Kang, 1982; Millard and
Neilsen, 1988). It is clear that both protein N and soluble N play a
role as N reserves, but it appears protein may be more important in
pome fruit.

Mobilization of Nitrogen in the Spring

Between bud swell and flowering, the concentration of total and
soluble N constituents, especially non-protein N, markedly increases
in developing buds and the youngest shoots (Murneek, 1942; Taylor,
1967). This increase is accompanied by a sharp decline in the total
N concentration of the older shoots and branches, suggesting that
protein hydrolysis releases soluble N for translocation to the develop-
ning meristems (Tromp, 1970; Tromp and Ovaa, 1971a, 1971b; Kang et
Several studies, in which results have been expressed on an absolute basis, clearly show that N is exported from roots and old shoot tissues to the new shoots (Yokomizo et al., 1964; Taylor and May, 1967). Marley et al. (1958) in apple, Taylor (1967) and Taylor and May (1967) in peach, and Taylor et al. (1975) in pear have demonstrated a positive correlation between the level of N storage and the extent of new shoot growth the following spring.

Millard and Neilsen (1988) studied N recycling in two-year-old M26 apple rootstocks in sand culture. They supplied the plants with three levels of N. In well fertilized plants, there was a greater proportion of N in leaves and less in the roots and stems at the final harvest than in unfertilized plants. In the following season, the N remobilized from the stems into the new tissues was not affected by the previous N supply and the amount of N from the reserves allocated to the new growth was similar for the three N levels.

Grasmanis and Nicholas (1971), however, indicated the importance of currently absorbed N for early spring growth, since the total N of other tissues such as bark, wood, and roots were not significantly depleted, N increased in newly developing tissues. Furthermore, a considerable amount of newly absorbed 15N was translocated to the growing tissues. Their unusually high winter and early spring temperatures (26 and 9°C for maximum and minimum, respectively) may explain the uptake of N early in the season. Tromp and Ovaa (1976, 1979) also concluded that the new growth was dependent on newly
absorbed N, as total N in the roots and bark decreased little or remained unchanged in the spring. Even if there was a supply of newly absorbed N, the hydrolysis of the bark was not affected (Tromp and Ovaa, 1973).

Weinbaum et al. (1987) quantified the proportion of current N an almond tree obtains from tree reserves. They defined the percent annual depletion of the label (PAD) as the percentage decrease of tissue labeling between consecutive years. Mature trees had a PAD of 50%, which represents the percent annual influx of the total tree N coming from the soil N pool. Therefore, reserve N assimilated in previous years contributed the remaining 50%.

Effect of Nitrogen on Fruit Quality

Fruit color is an important quality component. Factors affecting skin pigmentation have received widespread attention. In general, a level of N nutrition high enough to insure maximum fruit yields usually produces a high proportion of poorly colored fruit (Weeks et al., 1952; Reuther et al., 1958). This is especially true for apples. Early apple studies demonstrated that red surface color and yellow ground color development is inversely related to the nitrogen level of the tree (Shaw and Southwick, 1936; Magness et al., 1940; Fisher et al., 1948; Shear and Horsfall, 1952; Beattie, 1954; Boyton, 1954). Pear color is similarly affected by N nutrition (Overholser and Claypool, 1935).

Flesh firmness is an important quality and maturity index for some fruits. Boyton (1954) cites some examples where an increase in the N rate is sometimes associated with softer fruit and shorter
storage life. In some cases, fruit from high N apple trees were less firm than fruits from low N trees (Weeks, 1952; Southwick, 1954). Nitrogen level in peaches usually does not influence flesh firmness (Proebsting et al., 1957) as it does in pome fruits.

Many wasteful physiological disorders of stored fruit are related to fruit mineral content. In apple, mineral related disorders such as bitter pit and senescent breakdown cause major storage losses (Kidson et al., 1963; Shear, 1972; Boon, 1980; Bramlage et al., 1980; Fallahi et al., 1985; Marmo et al., 1985; Perring et al., 1985; Autio et al., 1986). With the pear, particularly 'Anjou', the disorders known as cork spot, black-end, and alfalfa greening have been related to fruit mineral levels (Woodbridge, 1971; Al-Aní, 1978; Vas, 1984; Brun et al., 1985).

Many disorders are related to excessive N levels in the tree (Bramlage et al., 1980). Fruits high in N at harvest tend to be larger, softer, and more likely to have cork spot and bitter pit (Boyton and Oberly, 1966; Bramlage et al., 1980; Martin et al., 1964; Sharples, 1964; Martin et al., 1970; Sharples and Little, 1970; Shear, 1971; Shear and Faust, 1971; Bangerth et al., 1972; Shear, 1972). High N fruits develop even greater amounts of bitter pit and internal breakdown following storage (Boyton and Oberly, 1966). Pitted tissues have lower Ca and higher N (Martin et al., 1964; Martin et al., 1970; Sharples and Little, 1970; Bramlage et al., 1980). Richardson and Al-Aní (1982) found that N:Ca ratio was positively related to cork spot in 'd'Anjou' pear fruits at harvest and after storage. However, they reported a weak correlation for N
along with the disorder. Other studies demonstrated that N:Ca ratio is a good indicator for potential storage disorders (Al-Ani, 1978; Vas, 1984; Brun et al., 1985; Curtis, 1988).

High N can indirectly affect fruit Ca concentrations by increasing fruit weight, thereby diluting Ca, or by inducing excessive growth which competes with the fruit for Ca moving in the transpiration stream (Faust and Shear, 1968; Shear and Faust, 1971).

The form of N fertilization (NH4, NO3, or urea) is sometimes related to bitter pit incidence (Kenworthy, 1965; Wilcox et al., 1973; Phill and Lambeth, 1977; Bramlage et al., 1980) and affects fruit Ca concentration and Ca distribution within the plant. Ammonium-N fertilizers can aggravate Ca deficiency in apples (Shear, 1971). Ludders (1979) demonstrated that the use of ammonium rather than nitrate N substantially increased K:Ca ratios in apples by reducing Ca accumulation, resulting in greater incidence of bitter pit.

Apple susceptibility to bitter pit in response to high levels of N fertilization depends on the cultivar. Link (1980) reported that high N rates increased the incidence of bitter pit in 'Gravenstein’, but not in ‘Cox’s Orange Pippin’. Richardson and Al-Ani (1982) found that N:Ca ratio was positively related to cork spot in 'd’Anjou' pear fruits at harvest and after storage. However, they reported a weak correlation for N alone with the disorder. Other studies demonstrated that N:Ca ratio is a good indicator for potential storage disorders (Al-Ani, 1978; Vas, 1984; Brun et al., 1985; Curtis, 1988).
Role of Postharvest Urea Sprays

Most of the work involving N sprays has been devoted to apples, which readily respond to foliar-applied urea (Cook and Boyton, 1952; Fisher, 1952; Oland, 1960; Shim et al., 1972; O'Kennedy, 1975). In contrast, stone fruits and pears are regarded as less efficient in foliar absorption than either apples or citrus (Leece and Dirou, 1977; Swietlik and Stowick, 1981; Swietlik and Faust, 1984). The poor foliar absorption in Prunus species is caused by epicuticular waxes impeding penetration (Leece and Kenworthy, 1972). In pears, Norris and Bukovac (1968) found similar compounds in the outer surface of the cutin matrix that may also limit penetration.

In apples, foliar urea applications early in the growing season can supplement soil N applications (Cook and Boyton, 1952; Boyton, 1954). However, applying urea in the fall has the advantage that N is not diverted into vegetative or fruit growth. Furthermore, phytotoxicity to buds, flowers, or developing fruits is not a problem with postharvest application while much more leaf damage is tolerable provided leaves still senesce.

In pears, inefficient utilization of spring foliar urea applications using concentrations ranging from 0.2 to 0.5% (Proebsting, 1957; Franke, 1967) may not be a good indication of foliar urea potential. These concentrations are much lower than can be used in postharvest sprays, which range from 2 to 10%.

Positive responses to urea sprays in pears are rare. Ystaas (1980) reported that 6% postharvest urea spray on Molke pear immediately increased the N concentration of leaves and flower buds, and
this difference was maintained throughout autumn and winter, but did not affect yield. Khattab et al. (1981) sprayed 2 and 6% urea on 'Leconte' pear trees in the fall and found increased number of flowers per spur. Fruit set did not change, but was measured in only 75 spurs per treatment. This number is lower than the optimum recommended (Lombard et al., 1988). Furthermore, none of these studies used tracer techniques.

Timing of Nitrogen Application

It is difficult to optimize timing of nitrogen fertilizer application to pome orchards. Problems arise because N promotes fruiting while also stimulating excessive and competing vegetative growth (Titus and Kang, 1982). Furthermore, many experiments may be inconclusive because of existing reserves of nitrogen in the soil organic matter (Greenham, 1965) or in the tree structure (Greenham, 1980).

Mineralization of soil organic matter increases during summer. This fluctuation of available nitrogen is superimposed on any differences due to timing treatments (Delap, 1967). Sand culture was used by many researchers to provide more precise environmental control and study how altered timings of nitrogen supply affected growth, flower bud formation, and nitrogen uptake (Hill-Cottingham, 1963; Mori et al., 1963; Hill-Cottingham and Williams, 1967; Delap, 1967).

In Japan, Mori et al. (1963) studied the effects of nitrogen timing on tree growth and fruit quality in 4-year-old 'Rails Janet' apple trees. A high level of N applied in May-June optimized tree growth and fruit production, but fruit quality was seriously impaired. When N was applied in July-August, tree growth and fruit
production declined, but fruit color development was better and bitter pit did not occur. Nitrogen applied in September-October decreased tree growth and fruit production, but red color was best and bitter pit occurrence was negligible.

In 'Cox's Orange Pippin', summer N (May-July) applications produced more N uptake and leaf and shoot growth than either spring N (January-March) or autumn N (September-November) applications. Autumn treatments did not increase growth, but N concentrations uptake were higher than both the controls and spring N applications. Flowers were strongest and had better set when N was autumn-applied, but blossoming and leaf development were a few days earlier (Delap, 1967). Earlier flowering has been observed in fruit trees having high N status (Bould and Jarrett, 1962; Hill-Cottingham, 1963; Williams, 1965). Although earliness is not desirable in areas where spring frosts 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).

Hill-Cottingham and Cooper (1970) concluded that N applied in either spring, summer, or autumn rapidly increases the total N content of most tree tissues, although autumn-applied N remained mainly in the tree roots through the winter. Grasmanis and Nicholas (1971), using 15-N on apple trees, reported that either ammonia or nitrate uptake is continuous through the year with a relative high peak in the summer and low values in the winter. However, the average winter maximum and minimum temperatures were 26 and 9°C, respectively, which obviously favored nutrient uptake.
Recently, Weinbaum et al. (1984a), using mature almond trees, found that the later the fertilizer was applied during the season, the less was recovered in the fruit and leaves that year, but the greater was its N contribution to those organs the following year. However, this marked effect on N partitioning had little effect on the total recovery of the fertilizer which was almost the same regardless of the timing of N application.

As mentioned earlier, N nutrition has great impact on flower initiation and development (Tukey, 1985). Autumn application of N increased ovule viability and, therefore, had a positive effect in extending the effective pollination period (Williams, 1965). However, growers are reluctant to apply N in the fall because some reports suggested that high N trees are more susceptible to frost damage.

Effect of Nitrogen on Cold Hardiness

It is a general belief that high N status predisposes trees to winter injury by inducing late season growth and delaying natural maturity (Childers, 1969). Fall N applications are not recommended in areas where early frosts or injury due to cold temperatures occur (Pellett and Carter, 1981).

Pellett and Carter (1981) reviewed past methodology and reevaluated relationships between cold hardiness and plant nutrition. They concluded that only excessive fertility levels retard cold acclimation. Thus, most fruit trees at nutrient levels (especially N) that promote good growth and fruit quality will cold acclimate well.

Some past observations lack statistical verification. Sudds and
Marsh (1943) reported severe frost damage in 8-year-old apples after an N fertilizer application of 0.34 kg of sodium nitrate/tree the first week of November. However, the whole block was fertilized and there was no means to prove that the N was responsible for the damage. The authors argued that in a nearby non-fertilized block of similar aged trees no negative symptoms were observed.

The methodology used also influences reported responses. Edgerton (1957) has concluded that October and early November application of N to apples may increase the susceptibility of both twigs and bark to freezing. He applied 0.45 kg N per tree during 3 years and collected samples for laboratory freezing evaluations using conductivity techniques. The interpretation of the data can be misleading since it is difficult to know what level of conductivity would parallel death or severe injury in the field. This is especially true if the conductivity analysis is used in conjunction with freezing at only one temperature (Pellet and Carter, 1981). In cold hardiness research, the borderline between death and life is usually a few degrees. Although differences in injury can be observed in lab experiments, the magnitude of hardiness differences and their importance under field situations are not reported in most studies (Pellet and Carter, 1981).

Cold hardiness experiments in fruit trees where fertilizer N was applied are inconclusive. For instance, negative effects were found in apples (Sudds and Marsh, 1943; Way, 1954; Edgerton, 1957), but positive effects were reported in peaches (Proebsting, 1961), citrus (Smith and Rasmussen, 1958), and pecans (Smith and Cotten, 1985).
A major constraint in all field studies is that the environment cannot be consistently manipulated. However, laboratory freezing tests can provide reliable data on cold tolerance if samples are frozen at temperatures over a realistic range (Pellett and Carter, 1981).
CHAPTER 3

RECYCLING OF NITROGEN IN FIELD-GROWN COMICE PEARS

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Abstract

Five-year-old Comice/Provence quince BA29 pear trees growing in a Central Point sandy loam soil near Medford, Oregon were fertilized with ammonium nitrate depleted in $^{15}\text{N}$ to study the capacity of the tree to use reserve N. Eight dormant trees were removed each year for three years and partitioning of biomass, total N, and depleted $^{15}\text{N}$ derived from the fertilizer were evaluated for various tree components. Losses of N due to harvested fruits, leaf dehiscence, and pruning were measured. New shoots and fruits were dependent on newly absorbed N. About 45% of the reserve N was partitioned to new growth in 1989. Leaves, fruits, and one-year-old wood consumed 28 and 34% of the total N reserve pool in 1988 and 1989, respectively. The main tissues exporting reserve N were branches, trunk, and roots. Partitioning of reserve N to new tissues was affected by a heavy crop load in 1989 with more N diverted into fruits at the expense of other tree components. The data support the contention that pear trees recycle a substantial portion of their N content, and therefore, require little supplemental N.
Introduction

An accurate assessment of the total annual N requirement for bearing pear trees is critical in evaluating fertilizer programs. Knowledge of the relative importance of N reserves and newly absorbed N for various tissues is also valuable in developing management strategies.

The portion of tree N that is annually remobilized has not been established for bearing pear trees. Early studies dealing with storage and remobilization of N used conventional methods (unlabelled N), with which it was impossible to isolate the contribution of the endogenous (storage), and exogenous (new) N (Oland, 1959; Taylor and May, 1967; Taylor and van den Ende, 1969). The use of the stable isotope $^{15}$N allows the differentiation of N pools. The only antecedent literature regarding recycling of N using tracer techniques in mature deciduous fruit trees is the work of Weinbaum et al. (1984; 1987) on almond trees. They estimated that approximately one-half of the total N in new growth was newly absorbed N and one-half came from tree reserves. Nevertheless, the portion of tree N that was annually remobilized was indirectly assessed by monitoring the decline of labelled N over several seasons in spur leaves, blossoms, and embryos (Weinbaum et al., 1987). This approach assumes that tree biomass does not change and that changes in the tissues sampled reflect changes occurring in the entire tree.

A quantitative assessment of annual N requirements and a complete evaluation of N reserves can be calculated if the whole tree is
sampled and all losses recorded. The objectives of this study were to: 1) determine the contribution that stored N makes to new growth, 2) determine which tissues are most dependent on newly absorbed (soil derived) N, 3) determine which organs provide N to new growth, and 4) estimate the annual N requirement of young bearing pear trees.
Materials and Methods

General: Five-year-old Comice/Provence quince BA29 pear trees growing in a Central Point sandy loam soil near Medford, Oregon and spaced 2.3 x 5.4 m were used in this study. In May 1987, one month after full bloom, twenty-four individual trees, randomly selected among those with similar growth and trunk cross-sectional area, were fertilized with 180 g N per tree applied as ammonium nitrate depleted in $^{15}$N (0.01 atom % $^{15}$N). The fertilizer was dissolved in 20 l of water and applied evenly under the tree canopy at 5 cm depth. The treated ground was immediately covered with soil to preclude ammonia volatilization. Shoot growth was excessive and yields were poor after the first year, thus the trees received no fertilization in 1988. One hundred fifty grams per tree of unlabelled ammonium sulfate was applied in 1989, 6 weeks after bloom. The plot was irrigated by overhead sprinklers all three seasons.

All biomass losses, i.e. fruits (at harvest), senescent leaves (at leaf fall), and prunings, were recorded for each tree (24 in 1987, 16 in 1988, and 8 in 1989). To collect senescent leaves, a net was placed over each tree after harvest. In December of each of the three years, eight trees were sawed off at the graft union. The aboveground structure was divided into the following tree components: one-year shoots, two-year shoots, trunk, and branches. After digging around the periphery of the trees, stumps were pulled out using a tractor. Additional roots were recovered by hand while carefully shoveling soil surrounding the removed tree until no additional roots could be found. Roots were found within the 2 m herbicide strip and
primarily in the top 40 cm. Soil was washed from the root masses with a high-pressure water stream. Roots were classified as small (diameter <1 cm) or large (diameter >1 cm) to facilitate sampling and N analysis.

Each tree portion was immediately weighed and subsamples collected to determine moisture content. Biomass was evaluated on a dry weight basis.

**Evaluating residual effects**: Our intent was to supply the trees with labelled N during the first year and observe the decline in labelled N over time. Excessive irrigation in the sandy soil was utilized to minimize residual N carryover in the soil. Residual effects of the labelled fertilizer (applied in 1987) were evaluated in 1988 with two approaches: 1) barley seedlings were planted under the tree canopies of the remaining 16 trees and the proportion of labelled fertilizer in the barley was determined the following summer, and 2) after removing the first eight trees, new replacement trees were placed in the same spots. Mid-terminal shoot leaves of these trees were sampled in August and analyzed for $^{15}$N.

**Sampling for analysis**: One- and two-year-old shoots were sampled by collecting ten to fifteen 2-5 cm portions from the middle of the shoots. In 1987, branches and larger roots were sampled by collecting 5 portions of wood. The procedure was improved in the 1988 and 1989 evaluations by sampling the entire set of branches and large roots. A cylindrical portion of the trunk was also saved for analysis. Small roots were sampled by taking twenty root pieces about 10 cm long. Two and six fruits per tree (where available) were sampled in 1987 and 1988, respectively, but six fruits per tree from
the remaining eight trees were sampled in 1989. Two hundred senescent leaves per tree were randomly collected from the nets. All tissue samples except fruits were dried at 60°C for 72 hours. Fruits were freeze-dried.

**Nitrogen analysis:** After dry weights were determined, samples were ground to 20 mesh. Nitrogen was colorimetrically determined with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al, 1973). Aliquots of the digest containing 0.5-2 mg N were used for separation of ammonium for isotopic analysis following the diffusion technique described by MacKown et al. (1987). Samples were diffused at room temperature for 3 weeks before the isotopic composition was determined by mass spectrometry at Isotope Services (Los Alamos, NM). Samples having less than 0.2 mg N/ml were concentrated using an aluminum block digester at 90°C for 15 hours. Atom percentage values were converted to nitrogen derived from the labelled fertilizer (NFF) using standard conversions (Hauck and Bremner, 1976).

**Evaluating N dynamics:** Total N and total nitrogen derived from the labelled fertilizer (total NFF) for each tree component, and ultimately per tree, were determined for all three years. The portion of newly absorbed N (net total N increase between years) that was allocated to various tree parts was determined for both 1988 and 1989.

Since a small residual carryover was detected in 1988, it was not possible to determine if the label was soil- or reserve-derived. Therefore, the calculation for the portion of tree N that is annually remobilized was limited to the 1988-1989 seasons. By determining the
total amount of labelled N present in December 1988, and accounting for losses due to removal of senescent leaves, fruits, and prunings, we could predict the amount of label expected in 1989. Thus, the accuracy of our N accounting could be verified.

The portion of labelled N in new growth in both 1988 and 1989 was calculated by adding the total NFF in senescent leaves, fruits, and one-year-old wood. To estimate the portion of tree reserves consumed by increase in tree structure, a ring sample from the previous year's growth was taken from the trunks and analyzed for N concentration and NFF. It was assumed that new growth of existing branches and shoots was the same as the trunk ring sample. The increase in biomass for trunk, branches, and roots was calculated from 1988 and 1989 data.

Results are reported as mean ± SE for all components. For simplicity, the two-year wood, branches, and trunk were pooled for each tree and are subsequently referred to as "frame".
Results and Discussion

The study trees increased substantially in dry weight from 1988 to 1989 (Table 3.1). A similar percentage of total dry weight increase occurred in 1987-1988 (data not shown). The dry weight increment was predominately due to increases in the aboveground structure and fruits. The 1989 growing season produced above-average tree yields in the Medford area. The study plot yielded 32 t.ha⁻¹, an excellent crop for 7-year-old trees. Roots did not increase in dry weight. This can be explained by the preference of the tree in allocating carbohydrates to fruit growth rather than root growth (Hansen, 1980; Faust, 1989). Roots accounted for 23 and 16% of the total tree biomass in 1988 and 1989, respectively.

Based on representative specific leaf weights measured from August through November (Sanchez and Righetti, 1990), N concentration in August, and the biomass of senescent leaves, we were able to estimate the percentage of total tree N present in August leaves. Leaf biomass in August contained 37 and 38% of the total N in the tree for 1988 and 1989, respectively. This is slightly less than values reported in apple (Batjer et al., 1952).

Fruits are a strong sink for stored nitrogen. The heavy crop load in 1989 took as much N from the reserve pool as senescent leaves, but much more than shoot growth (Table 3.1). However, low fruiting in 1988 (only 5 t. ha⁻¹) resulted in N reserves being allocated preferentially to one-year shoots rather than fruits. Comparing the percentage of the total labelled nitrogen partitioned into senescent leaves, fruits, and one-year-old shoots revealed
significant differences between years for fruits and one-year-old shoots (Table 3.2). The presence of a larger number of fruits decreased the partitioning of reserve N to shoot growth. Interestingly, senescent leaves removed the same proportion of N from the reserve pool in both years. In 1989 the total percentage of reserves utilized by leaves, fruits and one-year old shoots was larger than in 1988. The difference is mostly explained by the large portion of reserve N allocated into fruits.

Nitrogen concentrations changed slightly for the different tree components during the three years with the exception of senescent leaves (Figure 3.1). A long warm fall in 1988 may have allowed the leaves to recycle more N than in 1989. The generally consistent N concentrations despite large differences in biomass of various components between years further demonstrates flexibility in the allocation of newly absorbed N. Increases in biomass in various components appear to create N demands that are met with a combination of newly acquired and reserve N, with the former pool being more responsive to increased demand.

The NFF varied with tissue and decreased after the 1987 application in all tree components (Figure 3.2). The sharp decrease in NFF is attributable to increases in tree growth structure and the annual export of N reserves into senescent leaves, prunings, and fruits. From Figure 3.1, it is apparent that leaves, one-year shoots and fruits are strongly dependent on newly absorbed N. These tissues have high NFF percentages in 1987, indicating their dependence on newly absorbed N. In 1989, these same tissues are low in NFF, indicating that reserve sources of N are less important. The 1988
results are more difficult to interpret because of the uptake of residual fertilizer.

NFF values for senescent leaves (spur and shoot leaves) were similar in the first two years. The 1987 value for senescent leaves was lower than might have been expected based on the values for August leaves, since the fertilizer was applied after spur leaves were expanded and shoot growth began. Nitrogen arriving in leaves later in the season is also more likely to be recycled than early N that may be incorporated in leaf structure. There is a strong negative relationship between the NFF of the different tissues in 1987 and 1989 (r² = 0.75, excluding senescent leaves). This suggests that the dependence of the tissues on newly-acquired N can be predicted by either the first year uptake of labelled fertilizer or the persistence of labelled N in later years.

The calculation of the total N recycled was made using the 1988 and 1989 data. Although it was possible to use 1987 data and estimate the recycling in two consecutive years, this was avoided since the effect of residual fertilizer would confound interpretation of the data.

Nitrogen losses from the reserve pool accounted for by prunings, senescent leaves, and fruits in 1988 for trees pulled out in 1989 were considered in the balance sheet (Table 3.3). Thus, the value of 13.29 g of total NFF for 1989 includes the 1989 harvest (9.26 g, Table 3.1) plus the 1988 losses (4.03 g, Table 3.3). Total recovery of the labelled fertilizer in 1988 and 1989 was similar (14.01 g vs. 13.29 g).
In order to calculate the total amount of N in the new growth that came from the reserve pool in 1989, senescent leaves, fruits, one-year-old shoots, and the portion of the reserve N used in increasing the structure of the tree must be considered. Biomass increases were mainly in the aboveground structure (frame) since roots changed little (Table 3.1). To measure the amount of reserve N allocated in the last year growth, tree rings of the trunk were analyzed with the assumption that the main branches behaved similarly (Table 3.4). Since the increase in total N in the frame was 11.71 g (57.85 - 46.14, Table 3.1) and the NFF of the last year growth was 8.7% (Table 3.5), a total of 1.02 g was fertilizer-derived. Thus, 4.15 g from a total of 9.26 g of depleted fertilizer in the tree was allocated to new growth in 1989 (Table 3.5). That amount is equal to 45% of the reserve N pool. Weinbaum et al. (1987) estimated a 50% turnover of N in mature almond trees by measuring the annual dilution of the fertilizer in spur leaves, blossoms, and embryos based on the assumption that these tissues reflected the turnover of N within the tree as a whole. In our case, it is not valid to use a dilution approach and calculate percent turnover from the data of Figure 3.2 since we worked with actively-growing trees where some of the N reserves were allocated into new structural growth.

By the use of the destructive technique we were able to evaluate which tissues function as storage organs. The frame increased in dry matter by 22% from 1988 to 1989 but the total NFF decreased 40% (Table 3.1). The biomass of the roots remained constant but the total NFF decreased 37%. Therefore, roots and frame were important as storage organs. Millard and Neilsen (1989) found that the stems
were the most important organs in remobilizing N to new growth in one-year-old M26 apple rootstocks. However, in mature trees the roots play an important role in the storage and remobilization of N.

The reserve N pool may be considered as a buffer in deciduous fruit trees. The large capacity for storing N is used both to supplement soil N during the growing season and to supply the majority of N for early spring growth, (Tromp and Ovaa, 1973; Titus and Kang, 1982; Weinbaum et al., 1984). Our study demonstrates that pears do not require large amounts of N and supports the concept that fruit trees are less N demanding than field crops (Greenham, 1980). High-yielding trees demand only 23 g N·tree⁻¹ to support fruit growth (Table 3.1). This represents 18.5 kg N·ha⁻¹ in pears that yielded 32 T·ha⁻¹. Even when the total N requirement for fruits and the net increase in new growth is combined, nitrogen demand was approximately 60 g N·tree⁻¹ (48 kg N·ha⁻¹). Even if we assume only half of the N requirement is supplied by the soil, exogenous N needs for high-yielding pear trees would only be 24 kg N·ha⁻¹.

Nitrogen negatively influences fruit quality (Bramlage et al., 1980; Raese and Staiff, 1983), and fertilizer applied in spring or mid-summer is partitioned into the fruits (Sanchez, 1990). If the storage pool of N can be increased without affecting fruit N and shoot growth, it would be possible to fulfill most of the N requirement of the tree with a late-season (near harvest) N application along with natural mineralization of the soil organic matter. Postharvest soil applications resulted in N partitioned to the roots but not to flower buds and frame (Sanchez, 1990; Sanchez et
al., 1990b).
Fig. 3.1: Nitrogen concentration in different tree components. AL = August leaves; SL = senescent leaves; FR = fruits (sampled at harvest); 1 = one-year-old shoots; 2 = two-year-old shoots; B = branches T = trunk; R1 = roots < 1 cm diameter; R2 = roots > 1 cm diameter.
Fig. 3.2: Percent of the total nitrogen derived from the labelled fertilizer (NFF) in different tree components. AL = August leaves; SL = senescent leaves; FR = fruits (sampled at harvest); 1 = one-year-old shoots; 2 = two-year-old shoots; B = branches; T = trunk; R1 = roots < 1 cm diameter; R2 = roots > 1 cm diameter.
Table 3.1: Dry matter, total nitrogen (Total N) and total nitrogen derived from depleted $^{15}$N fertilizer (Total NFF) in various tree components for trees removed in 1988 and 1989. Labelled fertilizer was applied in 1987.

<table>
<thead>
<tr>
<th>Grams</th>
<th>Leaves</th>
<th>Fruits</th>
<th>1-year shoots</th>
<th>Frame</th>
<th>Roots</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1988</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>1585±117</td>
<td>905±199</td>
<td>850±104</td>
<td>11082±690</td>
<td>4423±149</td>
<td>18843±1076</td>
</tr>
<tr>
<td>Total N</td>
<td>10.94±0.86</td>
<td>2.90±0.69</td>
<td>6.29±0.73</td>
<td>46.14±4.48</td>
<td>19.98±0.71</td>
<td>86.25±4.63</td>
</tr>
<tr>
<td>Total NFF</td>
<td>2.35±0.18</td>
<td>0.49±0.11</td>
<td>1.13±0.14</td>
<td>7.22±0.36</td>
<td>2.82±0.10</td>
<td>14.01±0.66</td>
</tr>
<tr>
<td><strong>1989</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry matter</td>
<td>2146±98</td>
<td>6467±268</td>
<td>969±132</td>
<td>13519±978</td>
<td>4492±174</td>
<td>27894±1305</td>
</tr>
<tr>
<td>Total N</td>
<td>20.39±0.60</td>
<td>23.01±0.91</td>
<td>7.09±0.88</td>
<td>57.85±4.15</td>
<td>20.17±0.75</td>
<td>128.50±4.58</td>
</tr>
<tr>
<td>Total NFF</td>
<td>1.55±0.11</td>
<td>1.24±0.08</td>
<td>0.34±0.03</td>
<td>4.36±0.31</td>
<td>1.78±0.11</td>
<td>9.26±0.47</td>
</tr>
</tbody>
</table>

*Mean ± SE of 8 replicates.
Table 3.2: Percentages of the total labelled tree N partitioned into senescent leaves, fruits, and one-year-old shoots in 1988 and 1989. Labelled fertilizer was applied in 1987.

<table>
<thead>
<tr>
<th>Year</th>
<th>Senescent leaves</th>
<th>1-year shoots</th>
<th>Fruits</th>
<th>New growth total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1988</td>
<td>16.8 a</td>
<td>7.7 a</td>
<td>3.4 b</td>
<td>27.8 b</td>
</tr>
<tr>
<td>1989</td>
<td>16.7 a</td>
<td>3.6 b</td>
<td>13.5 a</td>
<td>33.9 a</td>
</tr>
</tbody>
</table>

Means under the same column followed by the same letter are not significantly different (t-test P < 0.05).
Table 3.3: Losses of dry matter, total N and total labelled N (total NFF) in 1988 from leaves, fruits, and pruning on trees removed in 1989.

<table>
<thead>
<tr>
<th></th>
<th>Leaves</th>
<th>Fruits</th>
<th>Pruning</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>1704±77</td>
<td>610±251</td>
<td>1051±109</td>
<td>3365±256</td>
</tr>
<tr>
<td>Total N</td>
<td>11.40±0.67</td>
<td>2.16±0.78</td>
<td>7.36±0.78</td>
<td>20.92±1.19</td>
</tr>
<tr>
<td>Total NFF</td>
<td>2.45±0.15</td>
<td>0.37±0.13</td>
<td>1.21±0.13</td>
<td>4.03±0.25</td>
</tr>
</tbody>
</table>

^Mean ± SE of 8 replicates.
Table 3.4: Proportion of the nitrogen derived from depleted $^{15}$N fertilizer (NFF) in annual tree rings.

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>1987</th>
<th>1988</th>
<th>1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>1987</td>
<td>32.2±2.2</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1988</td>
<td>21.5±1.18</td>
<td>16.0±1.4</td>
<td>—</td>
</tr>
<tr>
<td>1989</td>
<td>16.5±1.1</td>
<td>8.9±0.6</td>
<td>8.7±0.6</td>
</tr>
</tbody>
</table>

2Mean ± SE of 8 replicates.
Table 3.5: Total nitrogen derived from depleted $^{15}\text{N}$ fertilizer (Total NFF) applied in 1987 partitioned into the new growth in 1989.

<table>
<thead>
<tr>
<th>Total NFF (g)</th>
<th>Senescent leaves</th>
<th>Fruits</th>
<th>1-year shoots</th>
<th>Increase in frame</th>
<th>Subtotal</th>
<th>Tree Total</th>
<th>Percent Recycled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1.55</td>
<td>1.24</td>
<td>0.34</td>
<td>1.02</td>
<td>4.15</td>
<td>9.26</td>
<td>44.8</td>
</tr>
</tbody>
</table>
Literature Cited


CHAPTER 4

EFFECT OF TREE NITROGEN STATUS AND LEAF CANOPY POSITION ON POSTHARVEST NITROGEN ACCUMULATION AND EFFLUX FROM PEAR LEAVES

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Abstract

'Comice' pear trees (Pyrus communis L.) were fertilized with ammonium nitrate depleted in $^{15}$N in the spring of 1987 and 1988. In August, October, and November 1988, mid-leaves on current season shoots were sampled at three positions from the periphery to the center of the canopy. Total $N \text{cm}^{-2}$ of leaf area remained almost constant through October even though $\% N$ declined as specific leaf weight increased. Furthermore, there was no substantial net change in either labelled or unlabelled $N$ in either treatment until senescence began in October. Peripheral leaves contained higher levels of both reserve and newly acquired $N$ than did less exposed leaves. Despite large differences in $N \text{cm}^{-2}$ for October samples, by November leaves from both high and low $N$ trees exported similar percentages of their total $N$. The average $N$ export to storage tissues irrespective to tree $N$ status was 71, 61, and 52% for peripheral, medium, and interior leaves, respectively. The export of $N$ was influenced more by the leaf position in the plant canopy than the nutritional status of the tree.
Introduction

Early spring growth in fruit trees is heavily dependent on tree N reserves (Oland, 1959; Taylor, 1967; Titus and Kang, 1982). However, the dynamics of how this reserve N is translocated from leaves to storage tissues in late summer and early fall is not fully understood. After 'Comice' pears are harvested, typically during the first week of September, leaves transpire and remain photosynthetically active before senescence begins in October. Nitrogen accumulation and remobilization during this postharvest period has important physiological consequences in the tree. Castagnoli et al., (1990) demonstrated in peach and nectarine that leaf N remobilization ranged from 45% to 50% irrespective of N status, but little is known about how both N status and canopy position affect N dynamics.

The distribution of light within fruit trees clearly influences leaf physiology (Barden, 1974; Barden, 1977; DeJong, 1982; DeJong and Doyle, 1985; Jackson, 1980; Marini and Marini, 1983; Porpiglia and Barden, 1980). Previous research has addressed the effect of light on photosynthesis (DeJong, 1982; DeJong, 1983; Marini and Barden, 1982), dark respiration, (Barden, 1974; Barden, 1977; Porpiglia and Barden, 1980), chlorophyll content (Kappel and Flore, 1983; Marini and Marini, 1983), and specific leaf weight (Barden, 1974; Barden, 1977; Marini and Barden, 1982; Wooge and Barden, 1987). However, light influences the distribution of N as well, and there is a strong positive relationship between leaf N.cm\(^{-2}\) leaf area and photosynthetic capacity (DeJong, 1982; DeJong and Doyle, 1985). In peach and
other Prunus species, both photosynthetic CO₂ assimilation and mesophyll conductance are linearly related to N.cm⁻² (DeJong, 1982; DeJong and Doyle, 1985). In view of the association between leaf position in the canopy with leaf N.cm⁻² and photosynthetic capacity, we suspected that light exposure might also affect net N accumulation and efflux.

Shading reduces both N.cm⁻² and N % dry weight (DeJong et al., 1989). Weinbaum et al., (1989) reported that mineral weight per unit of leaf area increased with increasing photosynthetic photon flux, but leaf nutrient concentration expressed as percent dry matter did not. However, most information has been gathered with stone fruits, and pome fruits need evaluation. Our objectives were to 1) determine the extent of N accumulation in pear leaves during the late fruit maturation and postharvest period and 2) evaluate how tree N status and canopy position affects accumulation and efflux of N from leaves.
Materials and Methods

Six-year-old 'Comice' pear trees on BA-29 quince rootstock trained to a central leader were used in this study. Trees were spaced 2.3 x 5.4 m with rows oriented east to west on a Central Point sandy loam soil in Medford, Oregon. In May 1987, thirteen individual trees were fertilized with 180 g N, applied in the form of ammonium nitrate depleted in 15-N (0.01 atom % $^{15}$N). Eight of those thirteen trees were removed at the end of the first season and new trees were replanted at the same location. Isotopic analysis of these young trees and barley seedlings planted adjacent to the trunks of five other similarly treated trees did not reveal significant labelled N from the previous season. The remaining five trees were not refertilized in 1988. Thus, despite the large application in 1987, the N status of these young growing trees was low in 1988.

In 1988, one month before bloom, another set of five trees was fertilized with 120 g of ammonium nitrate-N, similarly depleted in $^{15}$N. Since the plot was frost protected with overhead sprinklers before and during bloom, an additional 70 g of non-labeled N was broadcasted under the tree canopy the week after bloom to assure high levels of soil N and, therefore, high N status. In the text, the former treatment (1987) is referred to as Low N (LN) and the second is referred to as High N (HN). We were more concerned with establishing different N status than attempting to label soil and within tree storage pools. Although there is unequal labelling, we have a clear case where we can compare labelled trees growing in low N
conditions (without additional label) with high N trees obtaining labeled N during the current season.

On August 20, October 10, and November 18, 1988, mid-shoot leaves on current season shoots were sampled from all tree sides at a height between 1.5 and 2.0 m aboveground in three positions of the canopy from the periphery to the center of the tree. Light was measured at midday in August at all three positions of the canopy with a quantum sensor (LI-188B, LI-COR Inc., Lincoln, Nebraska, USA). Leaves received an average of 68, 41, and 22% of full sunlight for peripheral, medium, and interior leaves, respectively. Ten leaf discs (1.03 cm$^2$ each) were punched with a sharp cork borer from vein-free regions of 5 leaves at each position on 5 single trees per treatment. All samples were collected at the same time of day. The leaf discs were dried at 60°C for 24 hours and weighed to permit calculation of specific leaf weight (SLW). Current year shoots were also sampled in August and October from the peripheral canopy of LN trees and divided into bark, wood, and leaves from the upper and lower portion of the shoot. Adjacent branches of similar size were used for the two sampling times.

Nitrogen content was measured using a Technicon Autoanalyzer after micro-Kjeldahl digestion in an aluminum block. Aliquots of the digest containing at least 1 mg of N were used for ammonium separation following the diffusion technique described by MacKown et al., (1987). Samples were diffused at room temperature for 3 weeks before the isotopic composition was determined by mass spectrometry at Isotope Services, Los Alamos, New Mexico. Atom % values were
converted to nitrogen derived from the fertilizer (NFF) using standard conversions (Hauck and Bremner, 1976).

Each treatment was applied to randomly selected trees in the orchard. The data were analyzed as both a split split-plot and completely randomized experiment with a factorial arrangements of treatments. When treated as a split split-plot, we had nitrogen treatments as main plots (factor A). Therefore, the levels of factor B (time) are randomized within each treatment and the levels of factor C (canopy position) are randomized within each time. To deal with the theoretical problems with split split-plot in time, we modified the analysis to pull out the time x block interaction instead of pulling it into error b. Significant main effects and interactions do not change regardless of the statistical approach. Therefore, only the results of the completely randomized statistical evaluation are shown.
Results and Discussion

Uptake of labelled fertilizer N in the HN treatment did not persist throughout the entire season. The percentage of N that was labelled in leaves increased rapidly early in the season, reached a peak of 19% 2 weeks after bloom, and then declined steadily until August (Sanchez et al., 1990). This suggests that non-labelled soil N was the major N source after the first month, and HN trees accumulated almost all of their label early in the season. The HN treatment was a pulse of labelled N rather than a continuous supply.

Since leaf area remains constant near harvest (Cain, 1973), SLW and N.cm⁻² are good indicators of biomass and N changes in the leaves. Specific leaf weight in both treatments was greatest in October (Fig. 4.1 and 4.2). Apple SLW increased throughout the season (Brown et al., 1985; Wooge and Barden, 1987). Castagnoli et al. (1990) recently reported a general trend of increasing SLW from mid-season until late in the season in peach and nectarine. Increases in leaf carbohydrates, especially starch, results from low demand by other parts of the tree during late season and likely explain differences in SLW (Brown et al., 1985). Patterns for HN and LN trees were similar. However, SLW values for LN were higher than for HN, particularly for peripheral leaves (Fig. 4.1 and 4.2). Increasing SLW with decreasing N availability has been observed in other species (DeJong, 1986; Gulmon and Chu, 1981) and may relate to increases in the cell wall fraction (Radin and Parker, 1979). It should be stressed that LN trees were not N deficient. Shoot growth was normal and 'Comice' on quince rootstock have low N content with
values as low as of 1.7% N from high yielding trees (Lombard and Sanchez, unpublished), and the long-term average for 'Comice' is 1.80% (Plant Analysis Laboratory, Oregon State University).

Position x time interactions are significant (Table 4.1), suggesting that increases in SLW occur to a greater degree in the more exposed, and presumably more photosynthetically active, leaves. Although total N.cm⁻² remained almost constant between August and October (Fig. 4.1 and 4.2), N concentration decreased substantially during this period (Table 4.2). This decrease is not attributable to N export but to increases in SLW. DeJong (1986) reported similar results in peach. Total labelled N per unit of area (TLN.cm⁻²) remained almost constant for all leaf positions between August and October (Fig. 4.1 and 4.2). TLN.cm⁻² was significantly greater in peripheral leaves than in medium and interior leaves (Fig. 4.1). The percentage labelled N in bark and wood (LN treatment) did not vary with position in well exposed peripheral shoots (Table 4.3). However, leaves significantly differed in their percentage of labelled N (LN treatment), suggesting that distal leaves (younger) were more dependent on newly acquired N than the proximal. No net change in N occurred until senescence began in October. Although leaves are transpiring and physiologically active, there is not a substantial change in either labelled or unlabelled N accumulation or efflux. If there is N uptake during the postharvest period, it does not appear to be translocated to the leaves (Weinbaum et al., 1984; Sanchez, 1990), even though the label can be found in the roots during the dormant season (Sanchez, 1990).
Once export of N begins, all three canopy positions decrease to a similar level of N.cm$^{-2}$, with peripheral leaves having only a slightly higher N value when expressed on an area basis (Fig. 4.1 and 4.2). On a percentage dry weight basis, peripheral and medium leaves are clearly lower in N than the interior leaves in November (Table 4.2). Despite differing in SLW, senescent leaves from different parts of the canopy are much closer with respect to N.cm$^{-2}$. Differences in nitrogen status of senescent leaves are greater when expressed on a dry matter basis (Table 4.2) than when expressed on an area basis (Fig. 4.1 and 4.2).

Nitrogen derived from fertilizer (NFF) reveals how canopy position affects leaf partitioning of stored vs. soil derived N (Table 4.4). Values for the LN treatment are easier to interpret because all of the label was acquired the previous year and differences in leaf values represent differences between the utilization of stored and soil N pools. The HN values (not shown) are harder to interpret because lower values can either mean more tree reserve utilization early in the uptake period or a greater uptake of unlabelled soil N late in the uptake period.

In August, LN trees had the highest percent NFF for interior and medium leaves and the lowest for peripheral ones (Table 4.4). This suggests that peripheral leaves are more dependent on newly acquired N, leaving them less enriched in the labelled N that come from reserves.

Since the first N coming to leaf tissues is structural rather than the photosynthetically functional, the N which enters the leaf earliest during leaf development may be most difficult to remobilize.
Previous studies on almonds (Weinbaum et al., 1984) and our own evaluations on pear (Sanchez et al., 1990) suggest that leaves are dependent on reserve N for their initial N accumulation. If leaf N accumulated later in the season is preferentially derived from the soil, we would expect the leaf to preferentially export the soil-derived N and retain the N accumulated earliest, i.e. from tree reserves.

Retranslocation of N to storage tissues presumably occurs after the first week of October (i.e. coincident with leaf N remobilization). Leaf remobilization varied among the different canopy positions. The average N efflux per unit of area for both treatments was 71, 61, and 52% for peripheral, medium, and interior leaves, respectively (Fig. 4.1 and 4.2). Also, peripheral and medium leaves in both treatments exported exactly the same proportion of their total N (71 and 61%) in spite of the differential N status. The export from interior leaves differed slightly with treatment (48% vs. 56% for LN and HN treatments, respectively). This suggests that the export of N is more influenced by the light exposure than the nutritional status of the tree. The differences observed in % N in November between LN and HN are due again to differences in SLW since the total N per unit of area is quite similar. This study does not agree with results reported in peach by DeJong (1986), who concluded that the amount of N partitioned to leaves receiving low amounts of light is the same regardless of tree N status. However, the results do agree with more recent studies where N fertilized trees have substantially more N.cm$^{-2}$ in similar canopy positions than unfertilized trees (DeJong et
al., 1989). In our study, interior leaves from HN trees have 26% more N than interior leaves of the LN treatment (Fig. 4.1 and 4.2).

In summary, regardless of how the N was delivered to the leaves, via tree reserves or from the soil, efflux patterns are similar for both low and high N conditions. No net change in N content or isotopic composition occurs until senescence begins. Nitrogen does not increase in any of the leaves regardless of canopy position or tree N status. Interestingly, peripheral and middle leaves in both treatments exported exactly the same proportion of their total N in spite of differential N status. This suggests that the export of N is more influenced by light exposure than nutritional status of the tree.
Fig. 4.1. Seasonal changes in specific leaf weight (SLW), total N cm$^{-2}$, and total labelled N (TLN) cm$^{-2}$ for pear leaves as influenced by canopy position. Trees were fertilized with depleted $^{15}$N in May 1987 (LN treatment), therefore all label is from tree reserves. Mean separation by LSD ($P < 0.05$).
Fig. 4.2. Seasonal changes in specific leaf weight (SLW), total N cm\(^{-2}\), and total labelled N (TLN) cm\(^{-2}\) for pear leaves as influenced by canopy position. Trees were fertilized with depleted \(^{15}\)N in March 1988 (HN treatment), therefore all label is from the uptake of current application. Mean separation by LSD (\(P < 0.05\)).
Table 4.1. Main effects and interactions for specific leaf weight (SLW), nitrogen concentration, total nitrogen per unit of leaf area (TN.cm\(^{-2}\)), and total labelled nitrogen per unit of leaf area (TLN.cm\(^{-2}\)). Data were analyzed as a completely randomized design.

<table>
<thead>
<tr>
<th></th>
<th>SLW</th>
<th>N (% dw)</th>
<th>TN.cm(^{-2})</th>
<th>TLN.cm(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Treatment</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Position</td>
<td>***</td>
<td>***</td>
<td>***</td>
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</tr>
<tr>
<td>Time x Tmt</td>
<td>NS</td>
<td>***</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>Time x Pos</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Tmt x Pos</td>
<td>**</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>T x Pos x Tmt</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS Not significant; * P < 0.05; ** P < 0.01; *** P < 0.001.
Table 4.2. Nitrogen concentration (% dry matter) within the canopy in mid-shoot leaves of trees receiving labelled nitrogen in 1987 (LN) and 1988 (HN), respectively.

<table>
<thead>
<tr>
<th>Position</th>
<th>August LN</th>
<th></th>
<th>October LN</th>
<th></th>
<th>November LN</th>
<th></th>
<th>LN</th>
<th></th>
<th>HN</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Interior</td>
<td>1.40b</td>
<td>1.64b</td>
<td>1.14b</td>
<td>1.33c</td>
<td>0.69a</td>
<td>0.80a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium</td>
<td>1.40b</td>
<td>1.64b</td>
<td>1.19b</td>
<td>1.39b</td>
<td>0.63b</td>
<td>0.73b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral</td>
<td>1.69a</td>
<td>2.00a</td>
<td>1.36a</td>
<td>1.74a</td>
<td>0.59b</td>
<td>0.69b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers within a column followed by the same letter are not significantly different (LSD, $P < 0.05; n = 5$).
Table 4.3. Partitioning of $^{15}\text{N}$ depleted fertilizer in bark, wood, and leaves from the low nitrogen treatment in August and October for upper and bottom portions of current season peripheral shoots.

<table>
<thead>
<tr>
<th>% N derived from $^{15}\text{N}$ fertilizer</th>
<th>Bark</th>
<th>Wood</th>
<th>Leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper</td>
<td>16.2a</td>
<td>15.4a</td>
<td>16.4a</td>
</tr>
<tr>
<td></td>
<td>16.0a</td>
<td>15.8a</td>
<td>16.8a</td>
</tr>
<tr>
<td>Bottom</td>
<td>17.8a</td>
<td>14.0a</td>
<td>20.6b</td>
</tr>
<tr>
<td></td>
<td>16.6a</td>
<td>15.5a</td>
<td>20.8b</td>
</tr>
</tbody>
</table>

Numbers followed with the same letter for an individual tissue are not significantly different (LSD, $P < 0.05$; $n = 5$).
Table 4.4. Nitrogen derived from $^{15}$N depleted fertilizer within the canopy in mid-shoot leaves of trees receiving labelled nitrogen in 1987 (LN).

<table>
<thead>
<tr>
<th>Position</th>
<th>August</th>
<th>October</th>
<th>November</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interior</td>
<td>22.8a</td>
<td>24.0a</td>
<td>22.6a</td>
</tr>
<tr>
<td>Medium</td>
<td>21.4a</td>
<td>21.3b</td>
<td>21.0a</td>
</tr>
<tr>
<td>Peripheral</td>
<td>15.8b</td>
<td>17.0c</td>
<td>18.3b</td>
</tr>
</tbody>
</table>

Numbers within a column followed by the same letter are not significantly different (LSD, $P < 0.05$; $n = 5$). Time and Time x Position are not significant.
Literature Cited


DeJong, T. M. and J. F. Doyle. 1985. Seasonal relationships between leaf nitrogen content (photosynthetic capacity) and leaf canopy light exposure in peach (Prunus persica). Plant Cell and Env. 8:701-706.


CHAPTER 5

NITROGEN VARIABILITY AMONG 'COMICE' PEAR FRUITS FROM TREES HAVING HIGH AND LOW NITROGEN STATUS

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Abstract

Nitrogen variability among pear fruits within trees having high (HN) and low nitrogen (LN) status was evaluated in 'Comice'/Quince pears. Nitrogen concentration in dry matter and the percent N derived from the labelled fertilizer (NFF) for peels were strongly correlated to cortex and core values. Therefore, peel data accurately reflected the N status of individual fruit. Variability was high, especially in the HN treatment with fruit peel N concentration ranging from 0.38 to 0.94 percent. The NFF ranged from 5.7 to 23.5 percent. No relation was found between peel N and fruit fresh weight or number of seeds. HN trees discriminated in their allocation of N to fruit in different canopy locations, but LN trees did not. Fruit in west and south quadrants had more N than north and east locations. Fruits from the top and middle canopy levels had more N than fruit from the bottom level. A large number of fruits in any specific location lessened N concentrations. Nitrogen applied in early spring had less influence on fruit N at harvest than N applied after bloom. Fertilizer N available after bloom produced high N fruits with considerable variability. In our trials, fruit depended more on
newly absorbed N than N from reserves. Fruit orientation, level, and crop load can partially explain the N variability. However, we conclude that other factors are also important. For example, fruits of the same size had almost twice as much N as similar fruit a few centimeters away.
Introduction

Nitrogen nutrition greatly influences fruit quality of pome fruits. In apples, fruits from high N trees have delayed red color development and are less firm (Weeks et al., 1952). Many physiological disorders are related to the mineral content of fruits, especially calcium and nitrogen (Bramlage et al., 1980; Raese, 1986). A high incidence of bitter pit in apples has been associated with high N/Ca ratio (Shear, 1974). In 'Anjou' pears, high N and N/Ca ratio is associated with cork spot (Richardson and Al-Ani, 1982; Curtis, 1988; Fallahi et al., 1988; Bevacqua, 1989) and alfalfa greening (Raese, 1988).

In view of the clear impact minerals have on the quality of stored pears in the Pacific Northwest, fruit testing programs have been proposed (Al-Ani, 1978; Vaz, 1984). Although storage quality and fruit disorders can sometimes be predicted (Al-Ani, 1978; Vaz, 1984), relationships are often weak, especially when the incidence of fruit disorders are low (Curtis, 1988; Fallahi et al., 1988). Sampling difficulties probably explain some inconsistencies (Raese, personal communication). Testing programs generally use composite samples to estimate mean concentration of nutrients, but this procedure provides no information about orchard variability. The amount of fruit that is low or high in a specific element is more important than a mean concentration for an orchard. There is some evidence that mean concentrations may not clearly predict the actual number of fruit with either high or low mineral concentrations (Curtis, 1988; Curtis et al., 1990).
There is a dearth of information on fruit variability. Wilkinson and Perring (1961) sampled twenty-five fruits from a thirty-year-old 'Cox's Orange Pippin' apple tree and found a two-fold range in any of the major elements among the sample. We could find no references on how variability among pear or apple fruits is affected by the N status of the tree. Understanding the nature and causes of fruit N variability could assist in the development of management techniques to limit the number of high N fruit and of sampling procedures to accurately quantify them. Since pome fruits are dependent on both tree reserves and currently absorbed N, the isolation of these two pools using $^{15}$N is important. The objectives of this study were to 1) assess the variability in N concentration among fruits in trees having low and high N status, 2) investigate possible cause(s) of variability, and 3) determine the relative contributions of reserve and newly absorbed N to pear fruit.
Materials and Methods

The study was initiated in 1987 in Medford, Oregon on 5-year-old Comice/Provence quince BA29 pear trees spaced 2.3 x 5.4 m and trained to a multiple leader. In 1987, four weeks after full bloom, five randomly selected trees growing on a Central Point sandy loam soil were fertilized with 180 g of N per tree applied as ammonium nitrate depleted in $^{15}$N (double labelled, 0.01 atom percentage $^{15}$N). The fertilizer was dissolved in 20 l of water and evenly applied to the root zone (5 cm depth) under the tree canopy and immediately covered with a layer of soil. This treatment is referred to as Low N (LN) in 1988 harvest comparisons since these young growing trees did not receive additional N throughout the experiment. Despite the large application in 1987, no residual effect of the fertilizer was detected in the soil in 1988 (Sanchez and Righetti, 1990).

In 1988, four weeks before full bloom, another set of five trees were fertilized with 120 g of N per tree of the same labelled fertilizer as described above. Since the plot was frost protected with overhead sprinklers before and during bloom, an additional 70 g N per tree of nonlabelled N in the form of ammonium sulfate was broadcast under the tree canopy the week after full bloom to assure high levels of soil N and, therefore, trees with high N status. This treatment is referred to as High N (HN) in the 1988 fruit comparisons.

At harvest, in 1987, a total of fourteen fruits of similar size were sampled from the $^{15}$N-treated trees and analyzed for total N and $^{15}$N in the peel, cortex, and core. Since all parts were correlated to each other ($r = 0.98$ for peel:cortex, peel:core, and $r = 0.97$ for
cortex:core for atom percentage $^{15}\text{N}$), further analyses were performed only on peels. In 1988, all fruits from two of the LN and HN trees were harvested and the position in the canopy of each individual fruit was recorded. The level of the branch bearing each fruit was placed into three categories (bottom, middle, and top third of the main trunk). The fruit position on each branch was also placed into three categories (proximal, middle, and distal third). The distance to the nearest adjacent fruit on the same branch and whether more than one fruit were borne on the same spur was recorded. The entire procedure was initiated on each of the four quadrants (N, S, E, W) of each tree. All fruits were analyzed for N concentration in the peel but only twelve fruits of each tree were analyzed for $^{15}\text{N}$. In this case, four samples were taken from high, medium, and low N fruits.

In 1989, thirty individual fruits from a tree fertilized one month after bloom with 130 g N per tree of labelled ammonium nitrate were also evaluated at harvest for seed number, fresh weight, and peels analyzed for total N and $^{15}\text{N}$.

Total N was colorimetrically determined with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973). Aliquots of the digest containing at least 1 mg of N were used for separation of ammonium following the diffusion technique described by MacKown et al. (1987). Isotopic composition was determined by mass spectrometry at Isotope Services, Los Alamos, New Mexico. Atom percentage values were converted to nitrogen derived from the fertilizer (NFF) using standard conversions (Hauck and Bremner, 1976).
Results and Discussion

Although trees and fruit sampled were similar in size and the fertilizer was evenly applied to the soil, there was tremendous variability in both N concentration and in the percentage N derived from the fertilizer (NFF) among fruits (Fig. 5.1). Total N varied from 0.35 to 0.85%. Some fruits had 22% of NFF, whereas others had 51% of NFF. Fruits having low percent N generally also have low NFF. The preferential movement of soil N into some fruit but not others explains some of the variability observed.

Peel N was strongly related to cortex N (Fig. 5.2), suggesting that the allocation of N within the fruit is the same for all tissues. This was further confirmed with $^{15}$N analysis of the peel and cortex (Fig. 5.3). Although data from the core is not presented, this portion correlated well with the rest of the fruit ($r = 0.98$ for NFF).

Although our initial intent was to have the labelled fertilizer in the HN treatment in 1988 present for the entire season as it was in 1987, this did not occur. The percent of fertilizer-derived N in leaves rapidly increased early in the season, reached a peak of 19% two weeks after bloom, and steadily declined until August (Sanchez and Righetti, 1990). This suggests that non-labelled N was the major source after the first month and HN trees accumulated almost all of their label early in the season. Mid-terminal August leaves had 1.69 and 2.00% N for LN and HN trees, respectively.

Both the labelled N derived from the 1988 fertilization and the labelled N from tree reserves (1987 application) behave somewhat
similarly. Unlike the pattern observed in the soil-labelled 1987 trees, decreasing values of NFF represent greater uptake of soil and fertilizer N (unlabelled) during the 1988 season (Table 5.1). In LN trees, there was a significant negative correlation between percent peel N and percent NFF. Since NFF in the LN trees came from the storage pool (reserves), this provides additional evidence that fruit N depends more on newly absorbed N than N from reserves (Table 5.1).

Although trends were similar in HN trees, the regression between peel N and percent NFF was not significant, suggesting that the contribution of the N available early in the season was less important than N applied after bloom. The HN trees had higher N concentration in the fruits than LN trees due to the application of both labelled and non-labelled N (Table 5.1). Although mean fruit N concentration was an average of 37% higher in the HN trees, NFF contributed at most 15% (Table 5.1). This indicates that unlabelled N available after bloom probably moved into fruits to a greater extent than the labelled prebloom N. We do not rule out the impact of prebloom N. In our trial, prebloom N was inefficient due to frost control irrigations. If available throughout the season, prebloom N may greatly influence fruit N.

Differences in mean fruit N concentration between trees of the same treatment were not significant, but the mean NFF between trees differed (Table 5.1). For both LN and HN treatments, individual trees having the smaller percentage N also had higher mean NFF. This suggests that non-labelled N diluted the N reserves in the LN treatment and also diluted the labeled N in the HN treatment. The data also indicate that as the mean N concentration increased so did the
standard deviation. Furthermore, the lower end of the ranges in percentage N varied much less than the high ends. This can be explained if one imagines N preferentially enters some but not all fruits as it becomes more available to the tree, thus increasing the range and variability of its fruits.

Frequency distribution of N concentration of all fruits in both treatments is shown in Figure 5.4. Variability was high, especially in the HN condition. More than 80% of the fruits had N concentration below 0.5% in the LN trees, but around 70% of the fruits in the HN treatment had N levels above that value. Furthermore, a portion of HN pears had very high N content that suggest the possibility of developing physiological disorders in storage. Although variability also existed in the LN trees, the concentration range was much narrower.

Knowing the location and weight of each fruit in the tree allowed us to evaluate fruits of each treatment by orientation, level, or position, and relate N concentration to fruit weight and distance from neighboring fruit. The correlation of fruit weight or distance to adjacent fruit with percent N was not significant. There were significant differences between LN and HN trees for all orientations, levels, and positions (Fig. 5.5). Orientation was the only main effect that significantly influenced N concentrations, however orientation x N status and level x N status interactions were also significant (Fig. 5.5). The LN trees did not discriminate in allocating N to different locations of the canopy, but HN trees did. West and south fruits had the highest N levels, whereas eastern
located fruits had the least. This difference was related to the number of fruits in each orientation (Fig. 5.5). The east side of the trees have the largest number of fruits and competition among them for N may be the cause of the low N concentration. On the other hand, only 20% of the fruits were located on the west and south sides (Fig. 5.5). Similar results can be seen regarding level. Most of the fruits were located in the bottom of the canopy and corresponded to fruits having low N concentration in comparison with fruits from the middle and top. No significant relationship was apparent when analyzing position of the fruit within the branch (Fig. 5.5) but in general most of the fruits having low N were distally located on the branch. This position also corresponded with the highest crop density (Fig. 5.5). In 'Anjou' pears, Brun et al., (1985) also found that fruits in the top of the canopy have high peel N values. Jackson et al., (1971) found higher N levels in the lower portion of apple trees, but unlike our example they found fewer fruit at low levels. These differing results suggest that fruit density in a specific area rather than other physiological factors affect N concentration. A densely-cropped limb may have less variability but high N fruits if the overall tree N status is high.

Even though regions with high and low N fruits can be identified, the variability existing within each region is also large. This is illustrated in Table 5.2 for the HN treatment.

The 1989 experiment confirmed earlier results where no relationship existed between fruit weight and peel N. Seed number correlated with neither peel N nor peel NFF. Again, variability was high, with ranges of 0.37 to 0.69 and 8.8 to 38.4 for percent peel N and percent
NFF, respectively. As was the case in 1987, the regression of peel N concentration versus NFF was significant ($r = 0.55$).

In conclusion, fruit from trees receiving newly absorbed N had greater concentration of N than fruit from trees which were more dependent on N reserves. It is also clear that fertilizer N available after bloom produces trees having high N fruits (Fig. 5.1, Table 5.1) and considerable variability (Fig. 5.1 and 5.4). The partitioning of N among fruits is related to N availability. High N trees differentially partition newly absorbed N more than LN trees. Our study shows that fruit more densely distributed within the canopy have less N in comparison with fruits less densely distributed. Although the N concentration trends (orientation and level) can partially explain the N variability found within the tree, we conclude that other factors are also important. There must be important physiological reasons why a fruit may have almost twice as much N as a similar fruit 20 cm away.
Fig. 5.1. Regression between the percentage nitrogen derived from the labelled fertilizer (NFF) and nitrogen concentration in the peel of 'Cornice' pears harvested in 1987. Regression equation $y = 0.12 + 0.0114x$. 

$$r = 0.66$$
Fig. 5.2. Regression between peel and cortex nitrogen in 'Comice' pears harvested in 1987. Regression equation $y = -7.29 E^{-3} + 0.606x$. 

$r = 0.94$
Fig. 5.3. Regression between the nitrogen derived from the labelled fertilizer (NFF) in peel and cortex in 'Comice' pears harvested in 1987. Regression equation $y = -1.448 + 1.015x$. 

$r = 0.98$
Fig. 5.4. Relative frequency of peel concentrations in 1988 in 'Comice' pears from Low Nitrogen (trees 1 and 2) and High Nitrogen trees (trees 3 and 4). Total number of fruits: Tree 1, n = 46; tree 2, n = 42; tree 3, n = 72; tree 4, n = 69.
Fig. 5.5. Effects of fruit position, level, and orientation on nitrogen concentration of 'Comice' pears harvested in 1988. Numbers in parentheses correspond to the percent of the total number of fruits on the trees that were associated with each orientation, level, or position for Low Nitrogen and High Nitrogen trees, respectively. Values followed by the same letter are not significantly different ($P < 0.05$) when comparing orientation, levels, or positions. Codes are as follows: W = west, S = south, N = north, E = east; M = middle, T = top, B = bottom; P = proximal, D = distal.
Table 5.1. Percent nitrogen (N), and means of nitrogen derived from the labelled fertilizer (NFF) in fruit peels of High Nitrogen (HN) and Low Nitrogen (LN) 'Comice' trees in 1988.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tree number</th>
<th>Mean N (%)</th>
<th>SD</th>
<th>Range (%)</th>
<th>Mean NFF (%)</th>
<th>Range (%)</th>
<th>Regression of Peel N with Peel NFF</th>
<th>Equation</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Nitrogen</td>
<td>1</td>
<td>0.437</td>
<td>0.087</td>
<td>0.35-0.68</td>
<td>12.0</td>
<td>6.7-19.5</td>
<td>y = 20.8-19.3x</td>
<td>-0.58*</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0.404</td>
<td>0.046</td>
<td>0.31-0.57</td>
<td>23.8</td>
<td>19.6-28.8</td>
<td>y = 34.6-26.4x</td>
<td>-0.50*</td>
<td>-</td>
</tr>
<tr>
<td>High Nitrogen</td>
<td>3</td>
<td>0.594</td>
<td>0.116</td>
<td>0.38-0.94</td>
<td>7.7</td>
<td>4.1-13.1</td>
<td>y = 18.8-6.8x</td>
<td>-0.18ns</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>0.561</td>
<td>0.093</td>
<td>0.36-0.82</td>
<td>14.7</td>
<td>5.7-23.5</td>
<td>y = 20.7-14.1x</td>
<td>-0.38ns</td>
<td>-</td>
</tr>
</tbody>
</table>

* - significant at $P < 0.05$, ns - not significant.
Table 5.2. Percent nitrogen ranges in fruit peels of 'Comice' pears from High Nitrogen trees in different canopy positions. Numbers in parenthesis denote number of fruit in each category.
Table 5.2.

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Prox.</th>
<th>Middle</th>
<th>Distal</th>
<th>Prox.</th>
<th>Middle</th>
<th>Distal</th>
<th>Prox.</th>
<th>Middle</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tree 3</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>East</td>
<td></td>
<td>0.44-0.75</td>
<td>0.38-0.66</td>
<td></td>
<td>0.46</td>
<td></td>
<td></td>
<td>0.45-0.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>West</td>
<td>0.55</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.63-0.82</td>
<td>0.54-0.94</td>
<td>---</td>
<td>0.45-0.75</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(4)</td>
<td>(10)</td>
<td></td>
<td>(7)</td>
<td></td>
</tr>
<tr>
<td>North</td>
<td></td>
<td>---</td>
<td>0.47-0.81</td>
<td>0.50</td>
<td>0.51-0.77</td>
<td>0.44-0.66</td>
<td>0.58</td>
<td>0.69</td>
<td>0.53-0.62</td>
</tr>
<tr>
<td></td>
<td>(5)</td>
<td>(3)</td>
<td>(6)</td>
<td></td>
<td></td>
<td></td>
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<td>(2)</td>
<td></td>
</tr>
<tr>
<td>South</td>
<td>0.77</td>
<td>---</td>
<td>0.51-0.64</td>
<td>0.67-0.83</td>
<td>---</td>
<td>0.66</td>
<td></td>
<td>---</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(7)</td>
<td></td>
<td>(4)</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td><strong>Tree 4</strong></td>
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<tr>
<td>East</td>
<td></td>
<td>0.42-0.63</td>
<td>0.36-0.74</td>
<td>0.50-0.55</td>
<td>0.37-0.61</td>
<td>0.45-0.61</td>
<td></td>
<td>---</td>
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</tr>
<tr>
<td></td>
<td>(8)</td>
<td>(18)</td>
<td>(3)</td>
<td>(7)</td>
<td>(5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>West</td>
<td></td>
<td>0.48-0.68</td>
<td>0.49-0.58</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0.65</td>
<td>0.56-0.58</td>
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<tr>
<td></td>
<td>(3)</td>
<td>(4)</td>
<td></td>
<td></td>
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<td>(4)</td>
<td></td>
</tr>
<tr>
<td>North</td>
<td></td>
<td>---</td>
<td>0.52-0.53</td>
<td>0.82</td>
<td>0.63-0.70</td>
<td>0.53-0.65</td>
<td></td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>(3)</td>
<td>(4)</td>
<td>(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>South</td>
<td></td>
<td>0.41</td>
<td>---</td>
<td>0.70-0.75</td>
<td>---</td>
<td>0.52-0.55</td>
<td></td>
<td>---</td>
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<tr>
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<td>(2)</td>
<td>(2)</td>
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</table>
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Al-Ani, A. M. 1978. Postharvest physiology of 'Anjou' pear fruit: Relations between mineral nutrition and cork spot, respiration, and ethylene evolution. Thesis, Oregon State University, Corvallis, USA.


CHAPTER 6

RESPONSE OF 'COMICE' PEAR TREES TO POSTHARVEST UREA SPRAY

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Abstract

A single postharvest urea spray at 5 and 10% increased nitrogen (N) content in one-year-old bark and wood and in the flower buds and blossoms in 1987. In 1988, there was also an increase in N in one-year-old bark and wood but not in the N content of flower buds or blossoms. Fruit set was not significantly increased either year. Control trees were much higher in N in 1988 than 1987 since additional soil N was applied. Urea labelled with $^{15}$N was applied to branches and individual spurs immediately after harvest. When the $^{15}$N-urea was applied to branches, the next season's flower buds had 12% of their N derived from the fertilizer (NFF), but when urea was applied to individual spurs only 8% NFF was detected. Remobilization of labelled N from treated to adjacent spurs resulted in less NFF than would occur if all spurs on the same branch received labelled N. Flower clusters apetal and basal to the spurs treated with labelled $^{15}$N showed considerable amounts (>60% of sprayed spur) of N from the labelled spray. However, harvest analysis of adjacent and distant spur leaves and fruits from treated spurs and branches revealed that urea was only locally mobilized in the tree. Nitrogen status can be altered with postharvest urea sprays but the response varies with the
N status of the tree. Postharvest soil application of labelled N showed N movement into blossoms but not in the flower buds.
Introduction

Most of the work involving N sprays has been devoted to apples, which readily respond to foliar-applied urea (Cook and Boyton, 1952; Fisher, 1952; Oland, 1960; Shim et al., 1972; O'Kennedy, 1975). In contrast, stone fruits and pears are regarded as less efficient in foliar absorption than either apples or citrus (Leece and Dirou, 1977; Swietlik and Stowick, 1981; Swietlik and Faust, 1984). The poor foliar absorption in Prunus species is caused by epicuticular waxes impeding penetration (Leece and Kenworthy, 1972). In pears, Norris and Bukovac (1968) found similar compounds in the outer surface of the cutin matrix that may also limit penetration.

In apples, foliar urea applications early in the growing season can supplement soil N applications (Cook and Boyton, 1952; Boyton, 1954). However, applying urea in the fall has the advantage that N is not diverted into vegetative or fruit growth. Furthermore, phyto-toxicity to buds, flowers, or developing fruits is not a problem with postharvest application while much more leaf damage is tolerable provided leaves still senesce.

In pears, inefficient utilization of spring foliar urea applications using concentrations ranging from 0.2 to 0.5% (Proebsting, 1957; Franke, 1967) may not be a good indication of foliar urea potential. These concentrations are much lower than can be used in postharvest sprays, which range from 2 to 10%.

Positive responses to urea sprays in pears are rare. Ystaas (1980) reported that 6% postharvest urea spray on 'Molke' pear
immediately increased the N concentration of leaves and flower buds, and this difference was maintained throughout autumn and winter but did not affect yield. Khattab et al. (1981) sprayed 2 and 6% urea on 'Leconte' pear trees in the fall and found an increased number of flowers per spur. Fruit set did not change, but was measured in only 75 spurs per treatment. This number is lower than the optimum recommended (Lombard et al., 1988). Furthermore, none of these studies used tracer techniques.

Our objectives were to: a) determine the usefulness of post-harvest urea spray to increase tree N reserves, b) assess the contribution of N from the spray to newly developing tissues during the following season, and c) determine the mobility of foliarly-applied urea in fully developed, field-grown trees.
Materials and Methods

The study was conducted on six-year-old 'Comice' trees on Provence quince BA 29 pear rootstock spaced 2.3 x 5.4 m and trained to a multiple leader. Trees received no fertilizer N in 1987 but were fertilized with 70 g N per tree in the form of ammonium sulfate in 1988 the week after bloom.

**Experiment 1:** In 1987 and 1988, immediately after harvest (September 14), urea was sprayed at 5 or 10% (w/v) containing 0.1% (v/v) of the non-ionic surfactant Activator 90 (Alkyl polyoxyethylene ether) to 10 single-tree replicates. A third treatment included a control, sprayed with water. Each treatment was applied to randomly selected trees in the orchard. Following the 1987 spray, ten mid-terminal shoot leaves per tree were sampled periodically until leaf fall and analyzed for total N. Leaves were carefully washed with 0.1% Liqui-nox (ALCONOX, Inc, New York, USA), and rinsed twice in deionized water to remove residual urea from leaf surfaces. Five samples of one-year-old shoots (separated into bark and wood), and ten flower buds per tree were taken in February (dormant) in all treatments. Similarly, ten blossoms were collected at bloom. Fruit set was measured at harvest on five of the ten replicates in 1987 but all replicates were used in 1988, totaling at least 3,000 flower clusters per treatment. The data were analyzed as a completely randomized experiment.

**Experiment 2:** In 1988, labelled urea (10.2 atom % $^{15}$N) was applied at harvest to either spurs or branches. Twenty-five two- to three-year-old non-fruited spurs were selected from each of five
trees and covered with plastic bags prior to spraying tree canopies with 5% of non-labelled urea. Healthy-appearing spurs which we suspected would bear fruit the following year were chosen. Selected spurs were then uncovered and sprayed with the labelled urea. The soil and adjacent spurs were covered during spraying to preclude any contamination. Similarly, single branches located in the lower portion of the trees where the majority of fruit was produced were sprayed with the tracer for three different trees. In this case, unlabelled urea was not sprayed on remaining branches. Five trees were soil-fertilized the same day with 250 g per tree of double-labelled ammonium nitrate depleted in $^{15}$N (0.01 atom % $^{15}$N) to compare the effectiveness of spray versus ground application.

During the dormant season, five flower buds per tree were randomly collected from foliar-treated spurs and branches. Similar samples were collected from the soil-applied treatment. Individual flower clusters (flowers plus spur leaves) were collected at bloom. Spurs located adjacent to treated ones were also sampled to investigate whether or not urea was mobilized. One month after bloom, leaves and fruit samples (average of two per tree) were taken from all treatments. At harvest in 1989, fruit and spur leaf samples were again collected from all treated spurs which bore fruit, adjacent spurs located within 20-30 cm of the treated spurs, and spurs that were on the opposite side of the tree. The entire sprayed branch, an adjacent branch, and a distant branch were also sampled. Although the actual amount of remobilized urea was expected to be small, using 10.2 atom % $^{15}$N assured that even tiny amounts of remobilized N could be detected in unsprayed tissues.
Total N was colorimetrically determined with an autoanalyzer after micro-Kjeldahl digestion (Schuman et al., 1973). Aliquots of the digest containing at least 1 mg of N were used for separation of ammonium for isotopic analysis following the diffusion technique described by MacKown et al. (1987). Samples were diffused at room temperature for 3 weeks before the isotopic composition was determined by mass spectrometry at Isotope Services, Los Alamos, New Mexico. Atom percentage values were converted to nitrogen derived from the fertilizer (NFF) using standard conversions (Hauck and Bremner, 1976).
Results and Discussion

Experiment 1: Samples of sprayed leaves collected the day after spraying contained significantly more N than leaves of non-treated trees (Table 6.1). The difference remained until leaf fall in November. These results clearly differ from other reports using more dilute solutions (Proebsting, 1957). Although the cuticle presents a barrier to foliar uptake, by increasing the concentration gradient it is possible to facilitate the penetration of compounds that otherwise are difficult to absorb. Urea applied at 10% reached its greatest value 7 days after treatment, suggesting that more time is required to completely absorb greater amounts. A change in N concentration in the control is evident one month after treatment (middle of October). Seasonal patterns were similar and difference among treatments decreased with time, suggesting that applied urea was being remobilized out of the leaves and urea sprays do not severely alter normal senescence. We observed some tip burn with 5% urea sprays and marginal necrosis in the 10% treatment. However, damage did not cause earlier leaf fall or affect tree blossoming the following season. The higher concentrations of leaf N at senescence in the urea treatments may partially result from phytotoxicity, but the difference of N content shortly after the application of urea is much greater than the small difference at leaf fall.

In 1987, urea sprays increased N in the bark and wood of one-year-old shoots (Table 6.2). There was also a positive response in the N concentration of dormant flower buds and blossoms. However, differences in fruit set were not significant, possibly because of
insufficient sampling and large variability. In 1988, significant differences were found only for bark and wood samples. The N status of the trees differed between years. The 1988 concentrations in the bark and wood samples from unsprayed controls during dormancy clearly shows that trees were higher in N than in 1987. Furthermore, the N content of flower buds and blossoms in control trees were also higher in the second year. The data confirm results reported by other researchers where the response to urea foliar spray is better in trees with low N status (Delap, 1967). The N concentration in bark was close to the wood N levels in 1988 even though they clearly differed in 1987. The bark is considered to be the primary tissue for N storage in fruit trees (Titus and Kang, 1982), however under high N nutrition the wood is an important sink for N reserves.

Taylor and May (1967) indicated that young peach trees accumulate N in their woody tissues in proportion to fertilizer supply. High N concentration in blossoms following the 1988 treatment could be related to the warmer weather near bloom in 1989. In another experiment in the same orchard where labelled N was applied to the soil before bloom, N uptake was unusually high. Nitrogen from the fertilizer accounted for 20% of the total N in flower clusters at full bloom (Sanchez et al., 1990).

Fruit set was not influenced by urea either year (Table 6.2). Although differences in N status among treatments for the 1988 application were not significant, suggesting a fruit set response is unlikely, the data from the 1987 application suggest a positive fruit set response may occur under certain conditions. However, large variability in fruit set within treatments likely limited statistical
significance. The apparent trends between bark, wood, or blossom N concentrations and fruit set (Table 6.2) suggest that N nutrition may be related to fruit set. Williams (1965) found that N can affect ovule longevity and Lombard et al., (1971) reported a very short effective pollination period (EPP) in 'Comice' pears. Therefore, good N nutrition at the beginning of the season might improve fruit set by increasing EPP.

**Experiment 2**: Dormant flower buds from both individually sprayed spurs and treated branches sampled during the post-treatment dormant season contained N from the labelled urea (Table 6.3). In spurs, the recovery was lower, suggesting that urea was remobilized to other tree parts and, therefore, diluted. At full bloom, flower clusters from treated spurs and sprayed branches had less percent N derived from spray than they had earlier (flower buds). This was likely due to soil uptake of N that diluted the tracer (Sanchez et al., 1990).

The mobility of foliar-sprayed N in fruit trees is debatable. Swietlik and Faust (1984) reported that considerable spray-derived N was translocated to the roots. Forshey (1964) reported in apples rather limited movement of urea-N after foliar spray. We sampled flower clusters either apetal or basal from the treated spurs and found considerable amounts of tracer N (Table 6.3), indicating movement of the spray-applied urea-N. To evaluate the degree of mobility, additional samples of fruit and spur leaves were taken at harvest. As expected, the label was diluted with distance from treated spurs and branches but was still detectable in distant leaves.
and fruits (Table 6.4). However, our data clearly indicate that the translocation of the urea-N was over short distances. A ten-fold decrease in the amount of $^{15}\text{N}$ was found in untreated adjacent branches. The results from treated spurs are similar but less apparent. Since we labelled twenty-five spurs evenly distributed in the tree canopy, it is reasonable to find smaller differences between sprayed and unsprayed spurs than between sprayed and unsprayed branches. Urea-N was translocated from treated spurs in all directions. Consequently, any untreated adjacent spur was also close to other treated spurs. This was not the case in the branch where translocation to an adjacent one was not influenced by other treated branches.

Although we did not sample roots, the data from the aboveground structure indicate that the translocation is rather local. Thus, urea sprayed at postharvest can provide a ready source of N to developing reproductive tissues. Postharvest soil applications may not be as available to new growth. The five treated postharvest soil N showed only 2% of N from the fertilizer in the flower buds, whereas 18.5% of the N found in the roots during the dormant season was fertilizer-derived. This is consistent with other studies where very little postharvest soil-applied N was detected in dormant flower buds (Sanchez, 1990).

Fruits at harvest clearly contained more newly absorbed N (less label from reserve N) than spur leaves (Table 6.4). However, partitioning of the $^{15}\text{N}$ between spur and fruit from tree reserves was almost equal early in the season (Table 6.3). This is consistent with other studies where N applied in mid-season was diverted to
shoot and fruit growth (Sanchez, 1990).

Pears respond to urea sprays which can complement soil N applications. As expected, trees with low N status benefited the most. Foliar-applied urea can reach flower buds and it is more readily mobilized at bloom than postharvest soil-applied N. A buildup of N reserves may play a crucial role the first weeks after bloom when N demand is high and soil N supply is insufficient. The higher temperatures at full bloom in 1989 may have negated some effects of foliar-applied urea since newly absorbed N was found in the flowers at bloom (Sanchez et al., 1990). However, early season availability of soil-applied N is an exception rather than a rule.
Table 6.1. Leaf nitrogen levels (% dry matter) following postharvest urea treatments on September 14, 1987.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>1</th>
<th>7</th>
<th>14</th>
<th>32</th>
<th>45</th>
<th>62</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.64</td>
<td>1.61</td>
<td>1.67</td>
<td>1.37</td>
<td>0.94</td>
<td>0.80</td>
</tr>
<tr>
<td>Urea 5%</td>
<td>2.06</td>
<td>1.95</td>
<td>1.92</td>
<td>1.68</td>
<td>1.13</td>
<td>0.92</td>
</tr>
<tr>
<td>Urea 10%</td>
<td>2.21</td>
<td>2.36</td>
<td>2.17</td>
<td>1.72</td>
<td>1.51</td>
<td>0.98</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>0.12</td>
<td>0.15</td>
<td>0.09</td>
<td>0.10</td>
<td>0.17</td>
<td>0.11</td>
</tr>
</tbody>
</table>
Table 6.2. Nitrogen concentration in one-year-old bark and wood, flower buds, and blossoms, and percent of fruit set following a postharvest foliar application of urea in 1987 and 1988.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Bark (%)</th>
<th>Wood (%)</th>
<th>Flower buds (%)</th>
<th>Blossoms (%)</th>
<th>Fruit set/100 clusters (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1987 application</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.04</td>
<td>0.58</td>
<td>1.46</td>
<td>3.01</td>
<td>21.4</td>
</tr>
<tr>
<td>Urea 5%</td>
<td>1.14</td>
<td>0.75</td>
<td>1.66</td>
<td>3.29</td>
<td>23.8</td>
</tr>
<tr>
<td>Urea 10%</td>
<td>1.19</td>
<td>0.80</td>
<td>1.66</td>
<td>3.33</td>
<td>26.8</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>0.08</td>
<td>0.11</td>
<td>0.15</td>
<td>0.19</td>
<td>7.3</td>
</tr>
<tr>
<td><strong>1988 application</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.17</td>
<td>1.04</td>
<td>1.59</td>
<td>3.72</td>
<td>33.2</td>
</tr>
<tr>
<td>Urea 5%</td>
<td>1.24</td>
<td>1.07</td>
<td>1.71</td>
<td>3.74</td>
<td>33.1</td>
</tr>
<tr>
<td>Urea 10%</td>
<td>1.28</td>
<td>1.26</td>
<td>1.71</td>
<td>3.80</td>
<td>32.8</td>
</tr>
<tr>
<td>LSD (P &lt; 0.05)</td>
<td>0.09</td>
<td>0.08</td>
<td>0.13</td>
<td>0.17</td>
<td>3.0</td>
</tr>
</tbody>
</table>
Table 6.3. Nitrogen derived from postharvest 5\% foliar 15N-Urea ± standard deviation on treated branches and spurs in flower buds, flower clusters, spur leaves, and fruits.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flower buds(^z)</th>
<th>Flower clusters(^y)</th>
<th>Spur leaves(^x)</th>
<th>Fruits(^x)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branches</td>
<td>11.6 ± 0.9</td>
<td>7.5 ± 1.3</td>
<td>4.0 ± 1.1</td>
<td>4.0 ± 0.4</td>
</tr>
<tr>
<td>Spurs</td>
<td>7.5 ± 1.3</td>
<td>2.9 ± 1.8</td>
<td>1.5 ± 0.5</td>
<td>1.7 ± 0.5</td>
</tr>
<tr>
<td>Apetal from treated area</td>
<td></td>
<td></td>
<td>2.2 ± 0.4</td>
<td></td>
</tr>
<tr>
<td>Basal from treated area</td>
<td></td>
<td></td>
<td>1.8 ± 0.7</td>
<td></td>
</tr>
</tbody>
</table>

\(^z\)Sampled two months before bloom.
\(^y\)Sampled at bloom.
\(^x\)Sampled one month after bloom.
Table 6.4. Effect of postharvest 15N-urea spray at 5% on the enrichment of spur leaves and fruits in treated, adjacent, and distant spurs and branches. Samples were taken at harvest, one year after the application.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Atom % excess*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Treated</td>
</tr>
<tr>
<td></td>
<td>Leaf</td>
</tr>
<tr>
<td>Spurs</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
<tr>
<td>Branches</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td>SD</td>
</tr>
</tbody>
</table>

*Atom % excess = atom % $^{15}$N in sample - atom % $^{15}$N in controls.
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CHAPTER 7

SEASONAL DIFFERENCES AND SOIL TEXTURE ALTER UPTAKE OF NEWLY ABSORBED NITROGEN IN FIELD-GROWN PEAR TREES

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Abstract

The early season availability of newly applied nitrogen was evaluated in 'Comice' pear trees grown in sandy loam and clay soils in Medford, Oregon. In 1988, labelled N applied to the sandy soil four weeks before full bloom (FB) appeared considerably in developing tissues two weeks after FB. However, during 1989 labelled N from pre-bloom fertilizer was apparent in the flower clusters and early growth. Temperatures during the weeks before and after bloom were higher in 1989 than in 1988 or the corresponding long-term average. In 1989, labelled N was also applied before bloom to the clay soil. At FB, 20% of the N in the flower clusters had come from the labelled fertilizer in the sandy soil, but only a tiny amount was present in the clay soil until four weeks after FB. During the first weeks after FB, newly absorbed N was preferentially partitioned to spur leaves regardless of soil type or seasons, while shoot leaves were more dependent on stored N.
Introduction

Early spring growth of deciduous fruit trees depends largely on the utilization of reserve materials stored in older tissues (Oland, 1959; Taylor, 1967; Titus and Kang, 1982; Weinbaum et al., 1984a and 1984b). Weinbaum et al. (1980, 1984b) reported that N must be absorbed before leaf fall to reach the new developing tissues in the spring. Similarly, Tromp and Ovaa (1976) have shown that developing tissues are strongly dependent on stored N early in the season but gradually become more dependent on soil-absorbed N. However, in young apple trees grown in water culture, Grasmanis and Nicholas (1971) indicated the importance of currently absorbed N for early spring growth. This suggests that generalizations may not be appropriate.

In a series of field studies on pear trees, different climatic conditions before bloom in 1988 and 1989 and similar planting on different soil textures allowed us to study early season availability and partitioning of newly applied nitrogen under different conditions.
Materials and Methods

The study was initiated in Medford, Oregon on 6-year-old 'Comice'/Provence quince BA 29 pear trees spaced 2.3 x 5.4 m. In 1988, four weeks before full bloom, five randomly selected trees growing on a Central Point sandy loam soil were fertilized with 120 g of N per tree applied as ammonium nitrate depleted in $^{15}$N (double labelled, 0.01 atom % $^{15}$N) plus 70 g of non-labelled N in the week after bloom. The fertilizer was dissolved in 20 l of water and evenly applied in the root zone (5 cm depth) under the tree canopy and immediately covered with a layer of soil. In 1989, five weeks before full bloom, four new randomly selected trees were fertilized with the same labelled fertilizer at the rate of 130 g N per tree, but this time the nitrogen was naturally incorporated into the soil by rain during and after the application.

In 1988, the plot was frost protected with overhead sprinklers before and after bloom for a total of 70 mm of water, but this was unnecessary in 1989. At a nearby location in 1989, 9-year-old 'Comice'/Provence quince BA29 pear trees growing on a Carney clay soil were also fertilized with 130 g N per tree of labelled ammonium nitrate depleted in $^{15}$N and applied as described above.

Starting at full bloom, four samples were collected at weekly intervals from various tissues and analyzed for total N. Flower clusters (flowers plus developing spur leaves) were sampled from bloom to two weeks later. Shoot and spur leaves were also sampled at weekly intervals beginning one week after bloom. However, spur leaves were insufficient to adequately sample one week after bloom in
1988. Leaf samples were collected at the end of the season to determine the proportion of N from the labelled fertilizer (NFF) when applied under three different conditions.

Total N was determined colorimetrically with an autoanalyzer after Microkjeldahl digestion (Schuman et al., 1973). Aliquots of the digest containing at least 1 mg of N were used for separation of ammonium for isotopic analysis following the diffusion technique described by McKown et al. (1987). Isotopic composition was determined by mass spectrometry at Isotope Services, Los Alamos, New Mexico. Atom percentage values were converted to nitrogen derived from the fertilizer (NFF) using standard conversions (Hauck and Bremner, 1976). Daily air temperatures of Medford were provided by the Climatic Research Institute of Oregon State University.
Results and Discussion

The most striking difference between the 1988 and 1989 seasons was the April temperatures. Average daily temperatures in March of 1988 and 1989 were very similar to the long-term average for Medford of 7.5°C. However, daily average temperatures in April were variable (Fig. 7.1). In 1988, the temperatures during the week after bloom were considerably higher than the long-term average recorded for that particular week, but close to the average at full bloom. In 1989, temperatures were also higher than the long-term average for the weeks before, during, and after full bloom.

In 1988, only a small fraction of N in flower clusters at bloom was fertilizer-derived. Labelled fertilizer N (9% of total) appeared in the flower clusters the week after full bloom, while the maximum labelled N was recorded in spur leaves seven days later (Fig. 7.2.). However, by full bloom in 1989, the flower clusters had 20.3% of the total N coming from the fertilizer (Fig. 7.3). The high proportion of new N (20%) in the beginning of the season is a substantial input, considering that the N from the spring-applied fertilizer probably constitutes less than half of the soil-derived N in pear trees (Sanchez, 1990). As tissues developed, the NFF also increased, suggesting a very active uptake of soil N.

Results for early season uptake on experiments conducted on the clay soil differed radically (Fig. 7.4). Uptake was generally much less, with a large increase in NFF not occurring until four weeks after bloom. Soil-applied N is less available than in the sandy soil, especially early in the season. Texture alters many physical
and chemical soil characteristics such as aeration, denitrification, leaching, temperature, and nutritional status, so the cause of the observed differences is not clearly apparent. However, it is clear that texturally induced differences can be as great or greater than seasonal-induced changes.

On the sandy soil, N concentrations were higher in 1989 than in 1988 for all tissue samples (Table 7.1). Assuming similar growth rates during the first week in both years, concentration differences are explained by the uptake of newly absorbed N. Furthermore, early N concentrations in the 1989 flower clusters from the clay soil are similar to those from the sandy loam soil in 1988. Little new N was available early in the season in both cases (Fig. 7.4). Nitrogen percentage is a function of both nutrient allocation and dilution from leaf growth. It is probably not valid to assume equal growth later.

The comparison of early and late season NFF for spur and mid-shoot leaves, respectively (Table 7.2), reveals further differences between the three conditions. In the clay soil, availability of the labelled N fertilizer increased with season and N was diverted towards shoot growth. In the sandy soil, the availability of the soil-applied fertilizer in the mid-season differed between years. In 1988, the portion of N from the fertilizer was smaller than in 1989 (Fig. 7.2 and 7.3 and Table 7.2), probably a result of early spring irrigations for frost control. Barley seedlings grown under the tree canopy in June 1988 contained a negligible amount of N derived from the labelled fertilizer. This observation and low proportion of NFF in shoot leaves in August 1988 indicated that the main source of N
for the new growth was non-labelled N (Table 7.2). On the contrary in 1989, there was a continual accumulation of labelled N throughout the season.

Our data suggest that under moderate spring temperatures of around 10°C, newly acquired N generally becomes important after fruit set, while stored compounds are the primary early N source, as has been reported (Oland, 1959; Taylor, 1967; Tromp, 1970; Titus and Kang, 1982; Weinbaum et al., 1984a,b). However, under warmer conditions newly absorbed N may be available at bloom from sandy soils. This supports the work of Grasmanis and Nicholas (1971), where soil uptake nitrogenous compounds were the primary source of N for new growth in glasshouse studies conducted at high temperatures. Although temperatures were not reported for the periods before, during, and after blooming, they stated that the average maximum and minimum temperatures in winter were 26 and 9°C, respectively. Therefore, under elevated soil/air temperatures, soil N can be effectively absorbed and translocated to growing tissues.

Our data indicate that fertilizer N from the soil is partitioned preferentially into spur leaves during the three or four weeks after bloom, while shoot leaves are more dependent on stored N (Figures 7.2, 7.3, and 7.4). This occurred regardless of season or soil texture. However, once the spur leaves are fully expanded, fertilizer N is diverted to shoot growth (Table 7.2). Late-season partitioning of N preferentially to shoot leaves has also been verified in earlier experiments (data not shown).
We conclude that under average spring temperatures \((10^\circ C)\), fruit trees are strongly dependent on stored N during the first weeks after bloom, but afterwards, soil N is the primary source for tree growth. However, under the 1989 conditions the availability of N applied before bloom was substantial. Even so, applying large rates of N early in the season to ensure increased delivery to developing tissues is less efficient. An earlier study on the same site suggests that recovery of prebloom N application was less than 15\% (Sanchez, 1990). Weinbaum et al. (1978) reported that N uptake is efficient during the rapid phase of shoot elongation, but we have also observed that soil-applied N at this time is also diverted to fruits (Sanchez, 1990). Excessive fruit N is not desirable in pears or apples (Bramlage et al., 1980; Raese and Staiff, 1983). Furthermore, pome fruits require little N for current growth and reproductive tissue based on their concentration and biomass (Greenham, 1980). Large amounts of mid-season N are not required. However, the period from bloom to fruit set is strongly influenced by N nutrition (Williams, 1965). The fact that autumn application of N was necessary for affecting fruit set in the spring supports the contention that spring-applied N is generally unavailable to developing flowers. Thus, a key for N management in pome fruits is to maintain high concentration in the aboveground tree structure before growth starts, but low levels of soil-applied N during the period of rapid shoot and fruit growth.
Fig. 7.1. Daily maximum, average, and long-term average (LTA) air temperatures during the week before and after full bloom (FB) in 1988 and 1989.
Fig. 7.2. Percent of the total nitrogen derived from the labelled fertilizer (NFF) in flower clusters, spur, and shoot leaves after full bloom from the sandy loam soil in 1988. Mean separation between spur and shoot leaves analyzed by paired t test. Different letters denote significant differences at $P < 0.05$. 
Fig. 7.3. Percent of the total nitrogen derived from the labelled fertilizer (NFF) in flower clusters, spur, and shoot leaves after full bloom from the sandy loam soil in 1989. Mean separation between spur and shoot leaves analyzed by paired t test. Different letters denote significant differences at $P < 0.05$. 
Fig. 7.4. Percent of the total nitrogen derived from the labelled fertilizer (NFF) in flower clusters, spur, and shoot leaves after full bloom from the clay soil in 1989. Mean separation between spur and shoot leaves analyzed by paired t test. Different letters denote significant differences at $P < 0.05$. 
Table 7.1. Nitrogen concentration as percent of dry matter in flower buds, spur, and shoot leaves after bloom. Labelled ammonium nitrate depleted in 15N was applied one month before full bloom in the sandy loam soil in 1988 and in the sandy loam and clay soil in 1989.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sampling period (weeks after full bloom)</th>
<th>Sandy loam 1988</th>
<th>Sandy loam 1989</th>
<th>Clay 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Flower cluster</td>
<td>3.37</td>
<td>3.69b</td>
<td>3.81b</td>
<td></td>
</tr>
<tr>
<td>Spur leaves</td>
<td>--</td>
<td>--</td>
<td>4.06a</td>
<td>3.51a</td>
</tr>
<tr>
<td>Shoot leaves</td>
<td>--</td>
<td>4.28a</td>
<td>4.04a</td>
<td>3.69a</td>
</tr>
<tr>
<td>Flower cluster</td>
<td>4.01</td>
<td>4.86b</td>
<td>4.73b</td>
<td></td>
</tr>
<tr>
<td>Spur leaves</td>
<td>--</td>
<td>5.22a</td>
<td>5.04a</td>
<td>--</td>
</tr>
<tr>
<td>Shoot leaves</td>
<td>--</td>
<td>5.08a</td>
<td>4.28c</td>
<td>--</td>
</tr>
<tr>
<td>Flower cluster</td>
<td>3.55</td>
<td>3.65a</td>
<td>3.22a</td>
<td></td>
</tr>
<tr>
<td>Spur leaves</td>
<td>--</td>
<td>3.64a</td>
<td>3.24a</td>
<td>--</td>
</tr>
<tr>
<td>Shoot leaves</td>
<td>--</td>
<td>3.60a</td>
<td>2.86b</td>
<td>--</td>
</tr>
</tbody>
</table>

Numbers within a column followed by the same letter are not significantly different at $P < 0.05$. Mean of five trees.
Table 7.2. Nitrogen derived from $^{15}$N fertilizer (%) in August shoot and spur leaves in trees receiving labelled nitrogen in 1988 (sandy loam soil) and 1989 (sandy loam and clay soil). Mean of five trees.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Sandy loam 1988</th>
<th>Sandy loam 1989</th>
<th>Clay 1989</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spur leaves</td>
<td>18.1a</td>
<td>31.0a</td>
<td>10.3b</td>
</tr>
<tr>
<td>Shoot leaves</td>
<td>13.9b</td>
<td>33.1a</td>
<td>14.3a</td>
</tr>
</tbody>
</table>

Numbers within a column followed by the same letter are not significantly different ($P < 0.05$).
Literature Cited


CHAPTER 8

TIMING OF NITROGEN APPLICATION AFFECTS NITROGEN PARTITIONING
BETWEEN FRUITS, SPUR AND SHOOT LEAVES OF MATURE 'COMICE' PEARS

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Abstract

Seven- and nine-year-old Comice/Provence quince BA29 pear trees
grown on sandy loam and clay soils, respectively, were fertilized
with $^{15}$N-depleted ammonium nitrate one month before bloom (BB), one
month after bloom (AB) or at harvest (HT). Nitrogen applied BB
partitioned almost equally among spur leaves, shoot leaves, and
fruits, but N applied AB clearly was partitioned preferentially to
shoot leaves and fruits. Nitrogen applied at HT remained in the
roots during the dormant season. Flower buds sampled in December
were high in labelled fertilizer when applied BB and AB but not at
HT. Fertilizer applied at HT was available to flowers, probably as a
result of translocation from the roots. Soil texture did not influ-
ence N partitioning between spur leaves, shoot leaves, and fruits,
but fertilizer applied BB in clay soil contributed less total N than
similar applications in sandy loam.
Introduction

It is difficult to optimize the timing of nitrogen fertilizer application in pome orchards. Problems arise because N application promotes fruiting (Titus and Kang, 1982) but stimulates shoot growth and results in fruits with undesirable high N concentration (Weeks et al., 1952; Bramlage et al., 1980; Raese, 1986; Fallahi et al., 1988; Bevacqua, 1989; Sanchez et al., 1990b). Therefore, it is crucial to determine how timing of N application affects the allocation of N to fruits, leaves, and reproductive buds.

Previous fertilizer timing studies in fruit trees have used young potted plants and $^{14}$N (Delap, 1967; Hill-Cottingham, 1970; Hill-Cottingham and Cooper, 1970; Grasmanis and Nicholas, 1971; Taylor et al., 1975). Studies with labelled $^{15}$N were carried out almost exclusively in young-potted trees (Grasmanis and Nicholas, 1971; Hill-Cottingham and Lloyd-Jones, 1975; Weinbaum et al., 1978). Although these approaches can provide information on nutrient uptake, it is difficult to translate the results to the complex field situation. Furthermore, the use of young non-bearing trees provides no information on N partitioning to fruits and may underestimate the role of stored and remobilized N in the tree. Weinbaum et al. (1984) used mature almond trees to show that the later fertilizer was applied during the season, the less it was recovered in the fruit and leaves. However, late applications resulted in greater contribution of N to those organs in the following year. Almond fruits were more dependent on storage reserves than newly absorbed soil or fertilizer N. Sanchez et al. (1990b) reported that fruit from well-fertilized
'Comice' pear trees derived up to 50% of their total N from fertilizer applied during the growing season, suggesting that pome fruits respond differently than stone fruits.

Soil texture can also influence the availability of fertilizer (Sanchez et al., 1990a) and may alter N partitioning between spur and shoot leaves and fruits. It is important to determine if timing effects are texture-dependent. In view of the impact that N nutrition has on pome fruit quality and orchard management, we attempted to study the effect of N fertilizer timing on the allocation of N to leaves and fruits for similar plantings in two soil textures.
Materials and Methods

Comice/Provence quince BA29 pear trees were grown near Medford, Oregon in two different soil types: a Central Point sandy loam (coarse-loamy, mesic Pachic Haploxeroll), and a Carney clay (very-fine, montmorillonitic, mesic Typic Chromoxerent). Trees were seven and nine years old in the sandy loam and clay soil, respectively. In 1989, three different N timing treatments were imposed on four single tree replicates randomly selected in the plots. One month before the average date for full bloom in 'Comice' (BB), one month after bloom (AB) or at harvest (HT), 130 g of N per tree was applied as ammonium nitrate depleted in $^{15}$N (double labelled, 0.01 atom % $^{15}$N). In the AB and HT treatments the fertilizer was dissolved in 20 l of water and evenly applied in the root zone (5 cm depth) under the tree canopy and immediately covered with a layer of soil. In the BB treatment the fertilizer was naturally incorporated in the soil by rain during and the day after application.

Ten well-exposed spur and mid-terminal shoot leaves per tree were collected periodically during the season until harvest (September 6, 1989) for the BB and AB treatments. At harvest, spur and shoot leaves of the HT treatment were sampled as a control. Six fruits from each trees were collected at harvest and individually analyzed. Root samples and ten flower buds per tree were sampled from the dormant trees on December 6, 1989. Ten flower clusters per tree were collected at full bloom on April 3, 1990. All samples were analyzed for total N (microkjeldahl) and $^{15}$N. The proportion of the N derived from the labelled fertilizer (NFF) was determined as
described previously (Sanchez et al., 1990a). Variability of the data about the mean is expressed as standard deviation.
Results and Discussion

Fertilizer applied BB was more available from the sandy loam than the clay soil, but availability from both soils was similar in the AB treatment (Table 8.1). Texture alters many physical and chemical soil characteristics such as aeration, denitrification, leaching, temperature, and nutritional status, so the cause of the observed differences is not apparent.

NFF partitioning between spur and shoot leaves differed markedly with timing, irrespective of soil texture. Values of NFF from the BB treatment were similar for spur and shoot leaves. However, after May, newly absorbed N was preferentially partitioned towards shoot leaves in all cases. Differences were more apparent for the clay soil. The subtle differences in the BB treatments became more apparent in the AB values. After bloom, newly-absorbed N was partitioned preferentially to growing shoots with less allocation to spur leaves (Table 8.1). Nitrogen fertilizer in the AB treatment resulted in a larger increase in N concentration in shoot leaves but not in spur leaves in the August and September samples, irrespective of soil texture (Table 8.2). Spur leaves had N concentrations similar to BB levels. Our results are consistent with Sanchez et al. (1990a), where newly-absorbed N was partitioned preferentially into shoot leaves several weeks after bloom. This occurs even though shoot leaves are more dependent on stored N (reserve pool) immediately after bloom (Sanchez et al., 1990a).

Since we sampled mid-terminal shoot leaves, it would be reasonable to expect terminal (i.e. younger) leaves to be more dependent on
newly absorbed $N$ than fully expanded spur leaves. In almonds, Weinbaum et al. (1984) reported a linear rate of accumulation of isotopic $N$ by spur leaves between April and June. Our data indicate that, for pears, once spur leaves reach full expansion the acquisition of $N$ is rather limited. On the other hand, shoot leaves and fruits are strong sinks for newly absorbed $N$ (Table 8.1).

The NFF for fruits sampled at harvest for the BB treatment were similar to shoot leaves but had slightly more NFF than spur leaves irrespective of soil type (Table 8.1). However, when fertilizer was applied AB, fruits had slightly less NFF than shoot leaves but much more NFF than spur leaves. These results do not agree with the work of Weinbaum et al. (1984) who sampled only spur leaves in almond. Their data suggest greater relative availability of fertilizer $N$ to vegetative (spur leaves) than fruit tissue for a wide range of fertilization timings. Pears and almonds appear to partition $N$ differently.

The poor contribution of the labelled fertilizer in clay soil for the BB treatment was reflected by the low percentage of $N$ in spur and shoot leaves, with values similar to the control (HT) treatment (Table 8.2). Despite the large contribution of NFF in the AB treatment and the normal shoot growth, $N$ concentration was always lower in clay soil treatments.

Allocation of labelled fertilizer into fruits differed markedly with timing and soil type. The proportion of labelled $N$ was lower for clay soil, especially when $N$ was applied BB. It should be emphasized that, despite two months' difference in time of $N$ application between the two treatments, $N$ applied AB was very effective in reach-
ing fruits. Either BB or AB applications led to large amounts of N partitioned to fruit and shoot leaves. The relationship between percent peel N and NFF (data not presented) was weak \( r = 0.52 \), as previously reported (Sanchez et al., 1990b), but variability for NFF was high (Table 8.3). Interestingly, variability in concentration values were always much less than the variability in NFF, especially in the clay soil for the AB treatment (Tables 8.1 and 8.2).

Nitrogen applied at harvest concentrated the label in the roots and only a tiny portion was found in dormant flower buds (Table 8.4). To increase N levels in flower buds at this time of the year, only foliar urea spray was effective in pears (Sanchez et al., 1990c).

In the following spring, NFF in the flowers made an important contribution to the total N of the tissue when the fertilizer was applied BB and AB (Table 8.4). This was less apparent in the clay soil for the BB treatment due to low efficiency in uptake of the fertilizer. Unlike flower buds, flowers had more NFF for the HT treatment (Table 8.4), suggesting that either N was diverted from the roots or from residual fertilizer. Sanchez et al. (1990a) reported that newly absorbed N can be available to flowers and developing leaves in a warm spring with average air temperature of 17.2°C the week before bloom. Average air temperature in the present study was 12.6°C. Therefore, it was assumed that the increase in NFF for flowers in the HT treatment was mainly due to remobilization of stored N from the roots. Sanchez (1990) found that both roots and the aerial portion of the tree are important in delivering N reserves.
We conclude that N needs to be incorporated into the aboveground structure prior to harvest in order to substantially affect flower buds the year of application. Flowers and leaves developing at bloom can then benefit from N applied at the previous harvest.

Pears require relatively little N for optimum yield and fruit quality (Sanchez, 1990), but early spring growth requires N and is dependent upon reserves. Nitrogen applied before bloom has been inefficient in reaching developing organs in early spring (Sanchez et al., 1990a). Nitrogen applied during spring (i.e. April-May) was partitioned towards shoot leaves and fruits. Currently we are studying the effect of N fertilizer applications before, at, and after harvest in the N allocation to fruits (in the case of pre-harvest application), leaves, buds, and storage organs and its contribution in supplying N to developing tissues in early spring. Since early spring growth is dependent on reserves, and pears require little N, our goal is to supply the aboveground structure with N while minimizing N allocation into fruits and shoots in the period of rapid growth.
Table 8.1. Effect of time of fertilizer application and soil texture on the proportion of nitrogen derived from labelled fertilizer in spurs leaves, shoot leaves, and fruits at various sampling periods.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Trees on sandy loam soil</th>
<th>Trees on clay soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spur leaves</td>
<td>shoot leaves</td>
</tr>
<tr>
<td>May 12</td>
<td>28.1±4.3$^z$</td>
<td>26.4±5.7</td>
</tr>
<tr>
<td>June 10</td>
<td>30.4±5.8</td>
<td>34.9±6.7</td>
</tr>
<tr>
<td>Aug. 2</td>
<td>31.1±4.5</td>
<td>33.1±5.0</td>
</tr>
<tr>
<td>Sept. 6</td>
<td>30.1±4.5</td>
<td>32.2±2.0</td>
</tr>
</tbody>
</table>

$^z$Mean ± SD. Sample size: Leaves (n = 4), fruits (n = 24). ND (not determined).
Table 8.2. Effect of time of fertilizer application and soil texture on the concentration of nitrogen in spur leaves, shoot leaves, and fruits at various sampling periods.

<table>
<thead>
<tr>
<th>Sampling date</th>
<th>Trees on sandy loam soil</th>
<th>Trees on clay soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>spur leaves</td>
<td>shoot leaves</td>
</tr>
<tr>
<td>N application prior to bloom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 12</td>
<td>3.41±0.16&lt;sup&gt;2&lt;/sup&gt;</td>
<td>2.78±0.10</td>
</tr>
<tr>
<td>June 10</td>
<td>2.58±0.13</td>
<td>2.20±0.14</td>
</tr>
<tr>
<td>Aug. 2</td>
<td>2.19±0.09</td>
<td>2.12±0.20</td>
</tr>
<tr>
<td>Sept. 6</td>
<td>1.87±0.10</td>
<td>1.99±0.17</td>
</tr>
<tr>
<td>N application after bloom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 10</td>
<td>2.42±0.24</td>
<td>2.29±0.13</td>
</tr>
<tr>
<td>Aug. 2</td>
<td>2.21±0.13</td>
<td>2.34±0.11</td>
</tr>
<tr>
<td>Sept. 6</td>
<td>1.85±0.12</td>
<td>2.13±0.13</td>
</tr>
<tr>
<td>N application at harvest</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sept. 6</td>
<td>1.72±0.15</td>
<td>1.90±0.13</td>
</tr>
</tbody>
</table>

<sup>2</sup>Mean ± SD. Sample size: Leaves (n = 4), fruits (n = 24). ND (not determined).
Table 8.3. Summary statistics for 'Comice' pears sampled at harvest in the sandy loam and clay soils when fertilizer was applied one month before bloom (BB) or one month after bloom. Sample size for each treatment $n = 24$. Values indicate the proportion of the nitrogen derived from the labelled fertilizer.

<table>
<thead>
<tr>
<th></th>
<th>Trees on sandy loam soil</th>
<th>Trees on clay soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB</td>
<td>AB</td>
</tr>
<tr>
<td><strong>Percent of N derived</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>from the labelled fertilizer</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td>32.9</td>
<td>28.4</td>
</tr>
<tr>
<td><strong>Median</strong></td>
<td>33.8</td>
<td>28.1</td>
</tr>
<tr>
<td><strong>Std. dev.</strong></td>
<td>4.7</td>
<td>7.1</td>
</tr>
<tr>
<td><strong>Minimum</strong></td>
<td>23.4</td>
<td>17.6</td>
</tr>
<tr>
<td><strong>Maximum</strong></td>
<td>40.9</td>
<td>39.8</td>
</tr>
</tbody>
</table>
Table 8.4. Effect of time of fertilizer application and soil texture on the proportion of the nitrogen derived from the labelled fertilizer in flower buds, roots, and flowers. Values are the mean of 4 replicates.

<table>
<thead>
<tr>
<th>Tissue sampled</th>
<th>Trees on sandy loam soil</th>
<th>Trees on clay soil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BB&lt;sup&gt;x&lt;/sup&gt;</td>
<td>AB</td>
</tr>
<tr>
<td>Flower buds&lt;sup&gt;y&lt;/sup&gt;</td>
<td>31.7±3.2&lt;sup&gt;z&lt;/sup&gt;</td>
<td>27.6±4.7</td>
</tr>
<tr>
<td>Roots&lt;sup&gt;y&lt;/sup&gt;</td>
<td>15.6±3.1</td>
<td>15.8±4.6</td>
</tr>
<tr>
<td>Flowers&lt;sup&gt;w&lt;/sup&gt;</td>
<td>27.9±3.5</td>
<td>23.8±5.8</td>
</tr>
</tbody>
</table>

<sup>z</sup>Mean ± SD.
<sup>y</sup>Sampled on December 6, 1989.
<sup>w</sup>Sampled on April 3, 1990.
<sup>x</sup>Fertilizer applied: BB (before bloom); AB (after bloom); HT (harvest).
Literature Cited


CHAPTER 9

NITROGEN APPLIED NEAR HARVEST PARTITIONS PREFERENTIALLY INTO FLOWER BUDS AND STORAGE ORGANS RATHER THAN LEAVES AND FRUITS IN MATURE 'COMICE' Pears

E. E. Sanchez, T. L. Righetti, D. Sugar, and P. B. Lombard
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Corvallis, OR 97331 U.S.A.

Abstract

Since conventional bloom or prebloom applications of N result in a large allocation of N to fruits and may lead to excessive vegetative growth, near harvest fertilizer timings were evaluated. Mature pear (Pyrus communis L. cv. 'Comice') trees were fertilized with ammonium nitrate depleted in $^{15}\text{N}$ at 5 various dates from 6 weeks before harvest to 6 weeks after harvest to assess the distribution of absorbed N in various organs of the tree. N allocation was strikingly different from previous studies with spring applications. Even the earliest treatment had less labelled N in leaves, fruits, and shoots than flower buds, frame (branches plus trunk), and roots. Roots always had the highest percentage of fertilizer-derived N. However, when N was applied at or after harvest, the amount of N allocated to the frame decreased and became negligible at later dates. In the following spring, flowers from trees fertilized before or at harvest had more nitrogen derived from the fertilizer than trees fertilized after harvest, suggesting that reserves built up with the former treatments were more available to the developing reproductive tissue. These results were confirmed in a second study.
on Bosc/Old Home x Farmingdale trees. Nitrogen fertilizer applied 3-6 weeks before harvest may be more efficient than later applications, assure adequate tree N reserves, avoid excessive shoot growth, and prevent high N fruits.
Introduction

It is difficult to optimize the time of N application in pome orchards. Nitrogen fertilization promotes fruiting (Titus and Kang, 1982) but stimulates shoot growth and results in fruits with undesirable high N concentration (Weeks et al., 1952; Bramlage et al., 1980; Raese, 1986; Fallahi et al., 1988; Bevacqua, 1989; Sanchez et al., 1990a). In addition to optimizing the overall nutrition of the tree, there is increasing concern in fertilizing specific organs (Faust, 1980). The goal should be to promote flowering and fruit set, thus assuring good yield, but also produce non-vigorous trees with low N fruits.

In studies of 'Comice' pears using $^{15}$N, Sanchez et al. (1990a) found that N applied either before or after bloom is allocated preferentially into leaves and fruits. Trees fertilized after bloom had the highest N concentration in fruits. Another study (Sanchez et al., 1990b) suggested that, regardless of canopy position, little or no net N increase occurs in leaves after harvest even though leaves remain physiologically active. This suggests that any N uptake that occurs results in a build-up of storage N. When N is applied at harvest, most of the N remains in the roots and only a small portion is found in flower buds and aboveground structure, making N less available for early spring growth (Sanchez et al., 1990c).

Previous reports indicated that fruit trees use early summer N very efficiently (Delap, 1967; Hill-Cottingham and Williams, 1967; Grasmanis and Nicholas, 1971; Taylor et al., 1975; Weinbaum et al., 1978), but the most widespread objections to applying N at that time
are due to its unfavorable effect on fruit color and storage quality (Hill-Cottingham, 1968). In the present study, we focused on the timing of N application from the period of late fruit growth until the beginning of leaf senescence to assess a) N allocation in leaves, fruits, flower buds, and storage tissues and b) the effectiveness of the stored N in allocating N into the flowers the following spring. Our goal was to find the optimum timing to build tree reserves, avoid excessive growth, and minimize N concentration in fruits.
Materials and Methods

Experiment 1: Fertilizer N was applied at five different times in 1989 to three single-tree replicates of seven-year-old Comice/Provence quince BA29 pear trees growing on Central Point sandy loam soil near Medford, Oregon. Trees were randomly selected in the orchard. Labelled ammonium nitrate (double labelled, 0.01% Atom percent $^{15}$N) was applied at the rate of 200 g/tree either six weeks before the average harvest date for 'Comice' (6BH), three weeks before harvest (3BH), at harvest (H), three weeks after harvest (3AH), or six weeks after harvest (6AH). Harvest was on September 6, 1989. The 6AH treatment was applied when about 50% of the leaves were yellow. The fertilizer was dissolved in water and applied evenly in the root zone (5 cm depth) under the tree canopy. The area fertilized was immediately covered with a layer of soil. The plot was irrigated with overhead sprinklers periodically up to one month after harvest. On February 6, 1990, after a heavy rain, all trees were irrigated with the equivalent of 10 cm of precipitation to leach residual labelled N from the root zone. Soil temperature was recorded at 10 cm depth from August, 1989 to January, 1990. At harvest, twenty spur and shoot leaves and six fruits from each tree were sampled from the first two treatments. Samples were also taken from trees fertilized at harvest as controls. Four weeks after leaf fall, samples were taken from roots (<1 cm diameter), flower buds (10 per tree), and bark from the trunk and branches (a single 4 cm square section) from all treatments. Ten flowers per tree were collected at full bloom on April 3, 1990. The experiment was analyzed as a
completely randomized design when comparing tissues in common for all treatments. Leaves and fruits from 6BH and 3BH were analyzed by a t-test.

**Experiment 2:** Fertilizer nitrogen was applied to five-year-old Bosc/Old Home x Farmingdale 333 trees. Trees were grown in Corvallis, Oregon in 1 m x 1 m x 0.4 m plastic containers placed in the field. Labelled ammonium nitrate was supplied to three single-tree replicates either six weeks before harvest (BH) or at harvest (H) at the rate of 150 g/tree. BH trees were removed at harvest and H trees were removed one month after harvest. Trees were divided into spur leaves, shoot leaves, one-year-old wood, frame (trunk and branches), roots, flower buds, and fruits (only the BH treatment) to assure a representative sampling of all tissues.

**Nitrogen analysis:** Tissue samples from experiments 1 and 2 were analyzed for total N and depleted $^{15}$N as described previously (Sanchez et al., 1990b).
Results and Discussion

Experiment 1: During the late period of fruit growth little N was partitioned to leaves and fruits (Table 9.1). However, the distribution of N was not uniform in all fruits as reported previously (Sanchez et al., 1990a). The proportion of N derived from the labelled fertilizer (NFF) ranged from 0 to 9%. Analysis of variance showed no significant differences in N concentration between fruits of the 6BH, 3BH, and H treatments, respectively (data not presented). In addition, these trees had fruits with lower N concentration than trees fertilized before or after bloom (Sanchez, 1990). Since fruits are highly dependent on newly acquired N (Sanchez, 1990), there may be some advantages to forcing trees to rely on their reserves and soil N from bloom to almost harvest.

The partitioning of NFF in the different organs differed markedly between treatments (Table 9.1). Aboveground storage organs had high concentrations of labelled N when applied before harvest, but not at or after harvest. N levels in flower buds confirmed an earlier finding that soil-applied N at harvest was ineffective in reaching flower buds. After harvest, only foliar-applied urea resulted in buds containing fertilizer-derived N (Sanchez et al., 1990c).

Bark from branches and trunk had N distribution patterns similar to flower buds but values of NFF were lower. A possible explanation is that the sampling technique used included some dead tissue (the cork) that diluted NFF in the phloem. What is relevant is the absence of labelled N in treatments after harvest. It is apparent
that autumn applications do not allocate N into the aerial portion of the tree.

Roots behaved differently than the frame. The proportion of labelled N in roots increased from 6BH to H and decreased afterward (Table 9.1). There was some N uptake when applied 6AH (end of October) even though trees had 50% of yellow leaves present and soil temperature was near 12°C (Figure 9.1).

The NFF stored in the structure contributed considerably to the total N in the flowers at bloom when the fertilizer was applied before or at harvest. Since we leached residual labelled N we assumed that this N was derived from tree reserves. If the labelled N in flowers was derived from soil reserves rather than tree reserves, we would not expect the NFF to decline with later application times. We suggest that the stored N is derived mainly from the frame and roots since roots and flowers had labelled N in the harvest and postharvest treatments (Table 9.1).

**Experiment 2:** Although this study was conducted with a different cultivar, rootstock, and in a different region, the results were similar to Experiment 1. Leaves and fruits were the least-labelled tissues when fertilizer was applied BH (Figure 9.2). Interestingly, one-year-old shoots had more NFF than corresponding shoot leaves. A possible explanation is the preference of the tree in allocating N into reserves late in the season rather than into leaves. Flower buds had 15% of their total N derived from the fertilizer applied prior to harvest, a similar value to Experiment 1. The frame had twice as much NFF as leaves. Even higher NFF values could be expected if samples included only the last year growth rather than
the entire structural biomass. Fine roots stored considerable amounts of N, but on a biomass basis larger roots accumulated more N than finer ones (data not presented). With the exception of large roots, NFF was significantly different for the organs in common for the two treatments (Figure 9.2).

We conclude that fertilizer N can be successfully applied approximately a month before harvest without severely altering fruit N status. At that time, trees allocate N into branches, trunk, roots, and flower buds. At or after harvest, the allocation of N progressively favors the roots. Nitrogen applied BH was more effective in reaching the flowers than N applied AH. Reserve N was derived from both the frame and the roots for early spring growth.

Acknowledgement

The authors want to thank Dr. Robert Stebbins for providing the trees used in Experiment 2.
Fig. 9.1. Average soil temperature at 10 cm depth from August, 1989 through January, 1990 (Experiment 1).
Fig. 9.2. Percent of the total N derived from the labelled fertilizer (NFF) in different tree components of 'Bosc' pears when applied one month before harvest or at harvest. Sp.L. = spur leaves; Sh.L = shoot leaves; F = fruits; SH = one-year-old shoots; FB = flower buds; FR = frame; R1 = roots <1 cm diameter; R2 = roots >1 cm diameter (Experiment 2). Uncommon letters between treatments for the same tissue indicate significant difference (t-test at P=0.05), mean of 3 trees.
Table 9.1. Effect of the time of fertilizer application on the proportion of the nitrogen derived from the labelled fertilizer in various tissues of 'Comice' pear trees (Experiment 1).

<table>
<thead>
<tr>
<th>Tissue</th>
<th>6BH&lt;sup&gt;Z&lt;/sup&gt;</th>
<th>3BH</th>
<th>H</th>
<th>3AH</th>
<th>6AH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spur leaves</td>
<td>1.2 a&lt;sup&gt;Y&lt;/sup&gt;</td>
<td>1.7 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shoot leaves</td>
<td>1.9 a</td>
<td>1.5 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fruits</td>
<td>2.2 a</td>
<td>2.8 a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flower buds</td>
<td>8.8 a</td>
<td>12.0 a</td>
<td>4.7 b</td>
<td>0.3 c</td>
<td>0.3 c</td>
</tr>
<tr>
<td>Roots</td>
<td>20.4 b</td>
<td>23.1 ab</td>
<td>28.3 a</td>
<td>17.4 bc</td>
<td>12.2 c</td>
</tr>
<tr>
<td>Bark trunk</td>
<td>9.4 a</td>
<td>9.8 a</td>
<td>6.2 a</td>
<td>0.0 b</td>
<td>0.0 b</td>
</tr>
<tr>
<td>Bark branch</td>
<td>9.9 a</td>
<td>11.0 a</td>
<td>6.7 a</td>
<td>0.0 b</td>
<td>0.0 b</td>
</tr>
<tr>
<td>Flowers</td>
<td>14.4 a</td>
<td>18.5 a</td>
<td>14.1 a</td>
<td>5.8 b</td>
<td>3.2 b</td>
</tr>
</tbody>
</table>

<sup>Y</sup>Numbers within a row followed by the same letter are not significantly different (P < 0.05).

<sup>Z</sup>6BH = 6 weeks before harvest; 3BH = 3 weeks before harvest; H = harvest; 3AH = 3 weeks after harvest; 6AH = 6 weeks after harvest.
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DESCRIPTION OF THE DIFFUSION TECHNIQUE USED IN THE THESIS

Although the diffusion technique has been described previously (MacKown et al., 1987), some modifications were necessary for analyzing tissues of low N concentration such as fruits and wood. In addition, total N was analyzed using an Automated Autoanalyzer (Technicon Instruments Corp., New York) which uses 0.4 g of dry tissue and 8 ml of concentrated sulfuric acid. This amount of sulfuric acid is excessive for the diffusion technique since the amount of aliquot to be diffused releases heat when reacting with sodium hydroxide. The heat interferes with the seal of the lid and ammonia gas is lost. To overcome this disadvantage, the following procedure was developed:

**Determination of total N:** In all cases, between 0.4 and 0.5 g of dry tissue was used. Nitrogen content was determined following a micro-Kjeldahl digest using 0.8 g of catalyst and 4.5 ml of concentrated sulfuric acid. The time of digestion was 80 minutes at 120°C and 200 minutes at 370°C. The digest was then diluted to 75 ml with distilled water. An aliquot of this solution was used for total N determination in the Autoanalyzer.

**General diffusion procedure.** Depending upon the concentration of N in the Kjeldahl digest, aliquots of different volume were used for diffusion. If the percent of N was less than 1.5, 25 ml of the digest was transferred to the disposable specimen containers. Between 15 and 20 ml were used for samples having more than 1.5% N. This separation allowed faster processing of samples, since the time for complete diffusion at room temperature was shortened from 3 weeks
(when 25 ml was used) to 2 weeks. However, preliminary tests demonstrated that the diffusion is almost complete (>95%) after 2 weeks at room temperature (usually 20°C). When 10 or 15 ml of the Kjeldahl digest were used, the time needed for diffusion was 7 and 10 days, respectively. When timing was not critical, 25 ml was used regardless of the N concentration of the tissues.

Diffusions were conducted with 128 ml disposable polypropylene specimen containers (Fisher and VWR Scientific). Containers from Fisher were better because the screw cap remained completely sealed after the addition of sodium hydroxide. The ones from VWR had an overlapping thread design but required careful attention. After the addition of sodium hydroxide, they had to be resealed because the lid became very loose when more than 20 ml of Kjeldahl digest was added.

To increase the pH above 10, sufficient amounts of 19 M sodium hydroxide was added. Ammonia was trapped in a 12 by 75 mm disposable glass tube containing 5 ml of dilute sulfuric acid. The concentration of sulfuric acid depended upon the concentration of N in the tissue. When less than 1.5%, the acid trap solution had a concentration of 1 ml of sulfuric acid per liter, but when the N concentration was above 1.5%, 2 ml was preferred to assure an acid pH after the diffusion.

At the end of the diffusion, the test tube trap was removed and the outside of each tube was rinsed with tap and distilled water. Trapping solutions were concentrated in a hood when the concentration of N in the tissues was lower than 0.5%. This was done to meet the specifications of Isotope Services, Inc. (ISI) of a minimum concent-
tration of 0.17 mg of N per ml. The atom percent $^{15}\text{N}$ was determined by the ISI Automated Mass Spectrometer developed at the Los Alamos National Laboratory.

Concentrations were performed by placing the tubes in an Al block heated at $95^\circ\text{C}$ under the hood. Samples were sent to Los Alamos, New Mexico in 4 ml borosilicate glass vials with rubber-lined screw caps. Other vials were tested but did not seal properly. Since Los Alamos is at high altitude, the quality of the vials was critical. Samples had to be shipped at most in three days to avoid leaking of the vials.