Sources of N for leaf growth in a high-density apple *Malus domestica*) orchard irrigated with ammonium nitrate solution

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Summary Elstar apple trees (*Malus domestica* Borkh.) on M.9 rootstock received either 5 or 35 g N tree⁻¹ year⁻¹ during the first two growing seasons after planting, applied as Ca(NO₃)₂ on a daily basis for nine weeks through a drip irrigation system. During the third growing season (1994), all trees were treated with 20 g N tree⁻¹ year⁻¹ as ½NH₄NO₃ with applications starting on April 22 and continuing for 10 weeks. Soil solution nitrate-N and ammonium-N were monitored weekly with suction lysimeters located 30 cm beneath the drip emitters. Spur and shoot leaves were sampled intensively from full bloom to the end of rapid shoot growth. During the period of nitrogen application, soil solution nitrate-N and ammonium-N were relatively constant, at about 24 and 1.0 mg l⁻¹ respectively. Growth of the spur leaves was completed by one week after full bloom (May 12), whereas biomass of the shoot leaves increased until mid-June. Nitrogen for growth of the spur leaves was supplied mainly from remobilization, which was dependent on previous N supply. Accumulation of fertilizer N in spur leaves was independent of previous N treatments and continued until the end of the monitoring period (June 24), but contributed only 13% to total spur leaf N. Nitrogen for shoot leaf growth was independent of previous N treatments and was initially supplied primarily by remobilization, but by the end of extension growth, fertilizer N contributed 48% to total shoot leaf N. Linear increases in leaf N uptake throughout the period of rapid shoot growth and the large contribution of fertilizer N to total shoot leaf N were attributed to the constant supply of N available in the root zone through daily N fertilization.

Keywords: biomass, fertilization, nitrogen, nitrogen remobilization, nutrient cycling, partitioning, root uptake, shoot leaf, spur leaf.

Introduction Early season growth of deciduous trees is supported by N remobilized from woody tissue (Titus and Kang 1982, Weinbaum et al. 1984, Millard and Neilsen 1989). The ability of trees to remobilize N in the spring appears to be related to the previous year’s N supply and to be independent of N supplied by root uptake in the current season (Millard 1996). Most N remobilization usually occurs before there is much root uptake (Millard and Neilsen 1989, Millard and Proe 1991), but root uptake of N may become increasingly important as the season progresses (Tromp and Ovaa 1976).

The relative contribution of stored and root-supplied N to annual tree growth is dependent on soil fertility (Millard 1996), timing of fertilizer applications (Weinbaum et al. 1978, 1984, Sanchez et al. 1992) and tree age (Miller and Miller 1987). Weinbaum et al. (1978, 1984) found that, for nonbearing prune (*Prunus domestica* L.) trees, N supplied any time in the growing season after the onset of rapid shoot growth contributed more to shoot N than N supplied earlier in the season. For mature almond (*Prunus dulcis* (Mill.) D.A. Webb), the later in the growing season that N was applied the smaller the amount of fertilizer N recovered in the leaves and fruit that year. Investigations with small, immature trees, often in solution or sand culture, indicate that fertilizer may supply up to 50% of total shoot N (Grasmanis and Nicholas 1971) and up to 82% of leaf N (Millard 1996). In mature trees, fertilizer N makes a much smaller contribution to total leaf N content than stored N (Weinbaum et al. 1984, Sanchez et al. 1990, 1992). Possible explanations for this difference between immature and mature trees are the presence of a larger pool of stored N in mature trees than in young trees and the existence of a fruit sink that competes with shoots for N (Weinbaum et al. 1984, 1987).

Recently adopted management practices in apple orchards have combined the use of high density plantings of precocious, dwarf trees with micro-irrigation systems through which nutrients can be delivered in solution. These changes are widespread and have occurred both in regions with an annual water deficit (Haynes 1985) and in humid climates (Kipp 1992). In the southern interior of British Columbia, many such orchards are located on coarse textured soils with low organic matter content. This offers the opportunity to conduct nutrition studies in a field environment with fruiting trees, under the controlled conditions usually found in pot experiments.

In the present study, labeled N was supplied to apple trees daily through a drip irrigation system, over a 10-week period starting just before full bloom. The trees had received either a low or high amount of N in the previous season. The aim of the research was to determine the relative importance of N remobilization and root uptake of N for leaf growth and to measure...
the impact of the amount of N supplied the previous year. We hypothesized that field-grown, dwarf apple trees supplied daily with N may be more dependent on root uptake of N to support leaf growth than has previously been reported for large apple trees.

Materials and methods

Experimental design

Elista apple trees (*Malus domestica* Borkh.) on M.9 rootstock were planted in May 1992 in a drip-irrigated orchard on a Skaha loamy sand soil (Wittneben 1986). Nitrogen-treated trees received 5 (low-N) or 35 (high-N) g N tree⁻¹ year⁻¹ as Ca(NO₃)₂ applied over a 9-week period starting May 17 in 1992 and May 19 in 1993. Fertilizer N was introduced to the irrigation system through a Mazzei Injector-Model 283 (Mazzei Injector Corp., Bakersfield, CA) and delivered in two 1-h applications through a single 4 l h⁻¹ Hardie pressure compensating dripper (Hardie Irrigation, El Cajon, CA) giving 8 l of irrigation water tree⁻¹ day⁻¹. Single-tree plots were replicated five times in a randomized complete block design. Trees were planted at a spacing of 1.5 m within rows and 4 m between rows and subjected to normal orchard management practice for insect and weed control. Trees were not allowed to crop in 1992 and 1993. In 1994, the four most uniform plots in each treatment were chosen for study. Thus, there were four single-tree replicates of each treatment. A new irrigation system was installed in which N fertilizer and 8 l of water were supplied daily by a 30-min application through two 8 l h⁻¹ dippers (Hardie Irrigation) to each tree. Nitrogen was supplied as ¹⁵NH₄¹⁵NO₃ (2.1 atom % ¹⁵N) at the rate of 20 g N tree⁻¹ year⁻¹ over a 10-week period starting April 22, 1994.

Soil solution monitoring

Soil suction lysimeters with 2.0-cm diameter porous ceramic cups (Irrometer Co. Inc., Riverside, CA) were installed in the soil on an angle so that the cup was located beneath one of the drip emitters at 30-cm depth, which was considered to represent the zone of maximum root activity in drip-irrigated trees (Levin et al. 1979). Suction was applied within 10 min after the end of the irrigation cycle and the sample was collected within 2 h. Samples were either analyzed immediately or stored at 4 °C until analyzed. Nitrate-N was measured with an ion-specific electrode (Cardy meter, Horiba Ltd., Kyoto, Japan). Ammonium was measured colorimetrically as an ammoniumsulfate complex by standard autoanalyzer procedures (Industrial Method No. 329-74W/A, Technicon Industrial Systems, Tarrytown, NY). A pre-fertilization sample was collected on April 21 and samples were collected weekly starting on May 2.

Plant measurement and sampling

Trunk diameter was measured in spring 1994, leaf number per tree was counted on July 29, and fruit number and weight were determined at harvest (August 30). Leaves were subdivided into two categories: spur and shoot. Spur leaves are borne on short reproductive shoots that may or may not have fruit, and the number of spur leaves is usually determined in the previous season (Faust 1989). Shoots are free-growing and the number of shoot leaves is determined in the current season (Kramer and Kozlowski 1979). Shoot leaves were sampled 16 times between April 25 and June 23. Macroporous shoot growth was not initiated until May 7–8 and shoot leaves were sampled 10 times between May 9 and June 23. Samples of five, randomly selected leaves were collected from each tree based on a system where each branch was numbered and subdivided into quarters so that all parts of the tree had an equal chance to be represented in the sample. All samples were weighed, dried for 24 h at 65 °C, reweighed and milled before analysis.

Analysis of samples

Total N and ¹⁵N concentrations in samples were determined with an ANA-SIRA mass spectrometer (VG Isogas, Middlewich, Cheshire, U.K.). The amounts of fertilizer N and N remobilized from plant storage were calculated as follows:

\[ A = CD/E, \]
\[ B = (1 - C/E)D, \]

where \( A \) = fertilizer N in tissue (g), \( B \) = remobilized N in tissue (g), \( C = \) atom ¹⁵N excess in tissue, \( D = \) N content of tissue (g) and \( E = \) atom % ¹⁵N excess in fertilizer (Millard and Neilson 1989). For values of \( E \), the natural abundance of ¹⁵N was taken to be 0.37 atom % (International Atomic Energy Agency 1983).

Statistical analysis

Effects of the previous season’s (1993) N treatments and time were analyzed by repeated measures analysis of variance using SAS procedures (SAS Institute Inc., Cary, NC, 1985). The count data (numbers of leaves and fruits) were log-transformed before analysis.

Results and discussion

Soil solution monitoring of N

The concentration of nitrate-N in the root-zone soil solution at 30-cm depth was controlled, for the most part, by the application of fertilizer with the irrigation water. Before the onset of fertilization, soil solution nitrate-N concentration was less than 5 mg l⁻¹ but rose to 20 mg l⁻¹ after fertilization was initiated and was maintained between 20 and 30 mg l⁻¹ until fertilization ended (Figure 1). Thus, throughout the period of N fertilization, which included the period of intensive plant monitoring, N availability was relatively constant. This is consistent with findings for drip-irrigated tomatoes (Bar-Yosef and Sagiv 1982) and drip-irrigated apples (Klein and Spieler 1987). The average concentration of nitrate-N derived from NH₄NO₃ applied at 286 mg day⁻¹ in 8 l of irrigation water was approximately 18 mg l⁻¹. Because soil solution nitrate-N concentration beneath the drip emitter is probably similar to that of the inflowing fertilizer solution (Bar-Yosef and Sheikso-
lami 1976), this suggests that some nitrate-N from mineralization and nitrification of fertilizer ammonium was also present. The gradual decrease in nitrate-N after application of NH\(_4\)NO\(_3\) solution ceased contrasts with the sharp declines measured in similar soils when N was derived solely from a nitrate source (Neilsen et al. 1995), providing further evidence for nitrification of the fertilizer ammonium. Ammonium-N concentration was constant throughout the period of N fertilization and averaged 1.0 mg l\(^{-1}\), therefore monitoring was discontinued after Julian day (JD) 195.

**Leaf growth**

Spur and shoot leaves exhibited distinct patterns of growth (Figure 2). With the exception of two peaks on JD 124 and JD 133, which were probably the result of experimental variation, spur leaf biomass increased until the end of the full bloom period (JD 126) and, thereafter, remained relatively constant (Figure 2a). In contrast, the biomass of the shoot leaves increased throughout the monitoring period. These differing patterns of dry matter accumulation in individual leaves probably reflect the pattern of development in the whole canopy. In contrast to many deciduous trees in which the total canopy develops in response to increased shoot growth in spring and early summer, fruit tree species that carry spurs may have a considerable portion of their total leaf area, i.e., spur leaves, expressed early in the growing season. Cain (1973) found that 50% of the total (spur + shoot) canopy leaf area was attributable to spur leaves in ‘McIntosh’ apple trees and this portion of the canopy had developed by the end of petal fall.

Biomass of individual spur and shoot leaves was not affected by N supplied the previous year. This corroborates previous findings for other tree species in which the biomass of individual leaves was related to the current season’s rather than the previous season’s N supply (Millard 1993). Because the spur-leaf canopy was fully developed early in the season, and the numbers of flower buds, spurs and hence spur leaves are determined during the previous season (Faust 1989), it might be expected that the mass of the spur leaves would have been affected by the previous season’s N supply. The higher N supply in the previous season did increase growth (trunk cross-sectional area) and fruit number (Table 1). It is possible that in the high-N treatment the greater number of developing fruits increased carbohydrate sink strength making carbohydrates less available for leaf growth than in the low-N treatment; however, the N treatment effect did not persist until harvest, and final fruit yields were not significantly different between N treatments.

**Source of N for leaf growth**

In spur leaves, the pattern of accumulation of N remobilized from storage (Figure 3a) was similar to that of dry matter accumulation (Figure 2a) reaching approximately 2.5 mg leaf\(^{-1}\) by 7 days after full-bloom (JD 132) and remaining relatively constant thereafter. The amount of stored N remobilized for growth of spur leaves in trees that had received the low-N treatment was less than that remobilized in trees that had received the high-N treatment. In contrast, the uptake of labeled fertilizer N into spur leaves was unaffected by the 1993 N treatments and was gradual throughout the sampling period, supplying approximately 13% of spur leaf N by JD 175 (calculated on the basis of data in Figure 3b). Thus, growth of the spur leaves was dependent primarily on N from storage. This may explain why effects of N fertilizer on apple production are frequently not expressed in the season of application, because spur leaves play an important role in fruit growth. Before midsummer, when shoot leaves may contribute more than spur leaves (Rom and Ferree 1986), most of the carbohydrates for fruit growth come from proximate leaves (Forshey and Elfving 1989) so that there may be nearly total transfer of carbon assimilates from spur leaves to fruit (Hansen 1970). It should also be noted that fertilizer N, much of which was absorbed after leaf growth ceased, was probably more mobile than N from storage, which had been absorbed earlier and probably fixed into tissue constituents. Thus, the measured value of labeled N in the spur leaves may reflect the net difference between N import and export to fruit and other tissues.

The amount of stored N remobilized for growth of shoot leaves was relatively constant over time and averaged 3.2 mg
leaf\(^{-1}\) (Figure 4a). There were no effects of previous fertilizer treatments on the amount of N remobilized into shoot leaves. The first shoot-leaf sample was taken when shoots had extended between 1.5 and 2.5 cm in length, approximately 2–3 days after extension growth had started. At that time, shoot leaves contained about 0.5 mg N leaf\(^{-1}\) derived from fertilizer (Figure 4b) indicating that N taken up from the soil since the start of N application was already partitioned to shoot leaves, but was of less importance than remobilized N for early spring growth, as previously indicated by Millard and Neilson (1989) and Sanchez et al. (1992). However, by the end of the extension growth period (JD 175), when shoot lengths were between 20 and 36 cm, the amount of fertilizer N in shoot leaves had increased linearly to 4.0 mg leaf\(^{-1}\). Therefore, in shoot leaves, remobilization provided more than half of the total N used for leaf growth. This pattern of N remobilization followed by root uptake is typical of that reported for several species of young trees in sand culture (Millard 1996). In contrast, only 20–30%
of shoot leaf N was supplied by spring-applied fertilizer for mature pear trees (Sanchez et al. 1990) and mature almond trees (Weinbaum et al. 1984).

Large, mature trees contain larger reserves of N and carry a much heavier fruit load than the three-year-old dwarf apple trees studied here, thus providing a greater source of stored N for leaf growth and a greater fruit sink for fertilizer N. Additionally, in many studies (Weinbaum et al. 1984, Sanchez et al. 1990, 1992), N fertilizer is applied as a surface dressing in single doses and watered in with either rainfall or irrigation, thus the availability of soil N during the leaf sampling period (post full bloom) is unknown and probably less consistent than in the current experiment. In the present study, N was available for uptake over the entire period of leaf sampling (Figure 1), which is similar to procedures used for sand culture experiments (Millard and Neilsen 1989, Millard and Proe 1991).

The findings of this study have implications for the efficient use of N fertilizer in irrigated fruit trees. Providing that irrigation is supplied to meet the water requirements of the tree, thus minimizing leaching, it appears that N uptake by growing shoots can be readily supplied by maintaining a relatively low, constant concentration of N in the soil solution.

Our results do not support the idea that N taken up by the roots is preferentially partitioned to spur leaves immediately after bloom (Sanchez et al. 1990). Spur leaves started to take up N about 4 days after N application was initiated, which was about 7 days before the onset of shoot growth and 10 days before the first shoot leaf samples were taken. At that time (5 days after full bloom), shoot leaves already contained a much larger amount of fertilizer N on a per leaf basis than spur leaves (Figures 3b and 4b). However, more fertilizer N was probably partitioned to the almost fully developed total spur-leaf canopy than to the shoot-leaf canopy, which was only about 8% developed (based on extension growth) at JD 129 and which continued to develop until the end of June. Because the number of spur leaves in July was similar to that in May (Cain 1973), by multiplying the average number of spur leaves (Table 1) and the average spur leaf fertilizer N content at JD 129 (Figure 3b) together, the total fertilizer N in the spur-leaf canopy at 5 days after full bloom can be approximated as 103 mg N tree$^{-1}$ and by the end of June as 319 mg N tree$^{-1}$. By comparison, because few shoot leaves were opened, the total fertilizer N in the shoot-leaf canopy would have been much lower on JD 129, but at the end of shoot growth (JD 175) it was
approximately 1591 mg N tree\(^{-1}\). Thus, on a per leaf basis, fertilizer N appeared to be preferentially allocated to shoot leaves, because although the greater spur-leaf canopy provided a larger sink for fertilizer N initially, this changed as the shoot-leaf canopy expanded.

**Conclusions**

The apparent size of the storage N pool and remobilization of N to apple tree spur leaves were affected by the N regime of the previous season. Spur leaves were fully expanded and the remobilized N content reached a plateau by the end of petal fall, which was about 16 days after the initiation of N application. Uptake of fertilizer N by spur leaves was unaffected by previous N treatments and continued after growth of the spur leaves stopped, but the amount of fertilizer N in spur leaves relative to total spur leaf N was small (approximately 13\%). Thus, growth of the spur leaves depended mainly on stored N. In contrast, shoot leaf growth and N content were unaffected by previous N treatments. Remobilized N in shoot leaves was constant throughout the measurement period. Uptake of fertilizer N by shoot leaves, which increased linearly over the period of rapid shoot growth, contributed slightly more to total shoot leaf N than did storage N. The relatively high contribution of fertilizer N to current-season leaf N content is more typical of studies with solution and sand culture than of field studies with single applications of surface applied fertilizer. This difference reflects the continuous and controlled availability of fertilizer nutrients that is achievable with a low-N fertilizer rate (20 g N tree\(^{-1}\) year\(^{-1}\)) and high frequency (daily) application of fertilizer with irrigation water. The difference also underscores the effect of the method of nutrient supply on plant responses because differences in N availability over time may influence the patterns of plant uptake and partitioning of N.

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