Nitrogen Partitioning in Apple Trees as Affected by Application Time

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SUMMARY. ^{15}Nitrogen-ammonium nitrate was applied to four 'Mutsu' apple (*Malus ×domestica* Borkh.) trees 40 days before harvest of 1996 (summer supplied nitrogen, SUN) and four others at full bloom in 1997 (spring supplied nitrogen, SPN) to evaluate the effect of application timing on N partitioning in mature trees. At leaf fall the largest amount of SUN was partitioned to roots and 2- to 4-year-old wood; the largest amount of SPN was partitioned to fruit and leaves and only a small amount detected in the roots. SUN did not increase N concentration in fruit or modify fruit firmness and soluble solids concentration, although it contributed to building up N reserves in the perennial woody organs. In 1997, as a result of the different timings of N supply, two sources of labeled N were distinguished and monitored in the vegetative organs: 1) the remobilized N, taken up in summer of 1996, stored in winter and then translocated to the growing tissues; 2) the newly absorbed N, taken up and moved to the canopy after the 1997 spring supply. Both fractions of remobilized and newly uptaken labeled N contributed to leaf and fruit N. Remobilized ^{15}N was provided principally by roots which, from August to leaf fall, decreased their percentage of ^{15}N by ≈18%, replacing the labeled with unlabeled N to maintain a constant concentration of total N.

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In modern horticulture high fruit quality is one of the main challenges for fruit growers. Fruit quality depends on a correct balance between vegetative growth and fruiting to ensure a constant crop load and optimal fruit mineral composition. It is well known that the ratio between nitrogen (N) and calcium (Ca) plays an important role in apple quality (Faust, 1989). A high N to Ca ratio helps to increase postharvest fruit decay in both apple (Sharpless and Johnson, 1977) and pear (Curtis et al., 1990; Sugar et al., 1992). To reduce fruit diseases and disorders, soil N supply should never exceed tree requirements and inputs should be scheduled when N is not, or but slightly, partitioned into the fruit. In a study on 'Comice' pear (*Pyrus communis* L.) under Pacific Northwest climate conditions, Sanchez et al. (1990) found that N applied early in spring (4 weeks before bloom) does not play a significant role in flowers and developing fruit until two weeks after full bloom. Early applied N dressing preferentially sustains shoots and fruit growth (Sanchez et al., 1991). Most of the N applied at harvest remains in the roots and only a small portion of it is detectable in the flower buds and in the aboveground storage organs (Sanchez et al., 1992).

Although fruit trees use early-summer N very efficiently (Hill-Cottingham and Williams, 1967; Khemira et al., 1998; Weinbaum et al., 1978), the most widespread concerns about applying N in summer are due to its favorable effect on vegetative growth and its supposedly adverse effect on fruit color and storage quality. Nitrogen-promoted shoot growth has been related to fire blight (*Erwinia amylovora* Burr.) susceptibility in pear (Cadic et al., 1987; Van der Zwet and Keil, 1979) and to psylla (*Cacopsylla* sp.) (Rease and Staiff, 1989). Sugar et al. (1992) suggested postponing N supply in 'Comice' pear to 3 weeks before harvest, when N availability does not increase N concentration in fruit but, rather, helps to increase tree reserves and is partitioned to new buds.

The present study was designed to: 1) corroborate the assumption that summer N application in apple trees results primarily in N partitioning into roots and woody perennial organs and that spring N application results in a large amount of N being partitioned into shoots and fruit; 2) monitor the life of labeled N within a mature apple tree from stored reserves or root uptake.

Materials and methods

PLANT MATERIAL. The trial was carried out at the Michigan State University Clarksville Horticultural Experimental Station, located 30 miles (48 km) east of Lake Michigan, in 1996 and 1997. Trees were 7-year-old apple ('Mutsu' on 'Mark' rootstock) trained to modified central leader and spaced 6 × 18 ft (1.8 × 5.5 m) apart in a grass-mulched orchard. A 5-ft (1.5-m) weed-free strip was maintained along the rows and microjet irrigation ensured an adequate water supply. None of the trees received any N supply from orchard establishment. The loamy soil (sand 47%, silt 31%, clay 22%) had a low concentration of organic matter (0.7%), total N (0.06%) and NO_3^- [7 ppm ($mg \cdot kg^{-1}$)] and the following mineral concentrations ($mg \cdot kg^{-1}$): P = 45, K = 140, Ca = 1200, and Mg = 265. Before beginning the experiment (on August 27, 1996), when nutrient concentrations in leaves were stable (Diamond et al., 1998), leaf analysis performed on 20 leaf laminas (without petiole) per tree showed the following nutrient concentrations (in % dry matter): N = 2.36, P = 0.16, K = 0.97, Ca = 1.24, Mg = 0.36. Leaf analysis was performed again in July 1997 to evaluate the nutritional status of the trees under investigation.

TREATMENTS. In 1996, 40 d before harvest (28 Aug.), 4 randomly selected trees were supplied with 1.12 oz (32 g) of actual N as NH_4NO_3 in which both ammonium and nitrate were enriched with ^{15}N (5 atom %). The summer fertilizer (SUN) was dissolved in water at a concentration of 1.06 oz / gal (8 $g \cdot L^{-1}$) and applied in the morning over a circular area ≈2 ft (0.6 m) in radius around each trunk and immediately covered with a thin layer of soil. To prevent any uncontrolled ^{15}N contamination of the soil, all the leaves that naturally dropped in Fall 1996 were collected in a net and removed from the orchard along with the pruned wood. In 1997, at full bloom (20 May), a

second set of four trees was fertilized with the same amount of labeled spring N (SPN) under the same procedure.

It was assumed that the ^{15}N detected in 1997 in canopy vegetative growth (shoots, leaves, fruit and developing buds) had a different origin depending upon time of supply. In trees fed with 1996-SUN, ^{15}N was held to come from stored reserves as remobilized N while in trees fertilized with 1997-SPN, it came directly from root uptake.

MEASUREMENTS. In 1996 and 1997, at leaf abscission, the N concentration and its fraction derived from fertilizers (NFF) were evaluated in roots, 1-year-old shoots, 2- to 4-year-old wood, 2- to 4-year-old limb bark, flower buds and leaves. In mid-November, when at least one-third of leaves had naturally dropped, a 1.75 to 3.5 oz (50 to 100 g) sample of root (fresh weight) with a diameter between 0.04 to 0.24 inch (1 to 6 mm) were collected from each tree by digging at least two holes on the two sides of the tree row at a depth of 1 to 1.5 ft (0.3 to 0.45 m). Roots were then gently brushed and rinsed with deionized water to remove all the soil and the N eventually adsorbed in root free space. At the same time the apical fraction (5 to 10 inches long and 0.25 inches in diameter, 12.7 to 25.4 × 0.63 cm) of 5 shoots per tree was sampled and the apical floral bud removed when present. Samples of at least 1 lb (454 g) fresh weight of 2 to 4-year-old limbs were randomly collected from each tree at a height of 4 to 5 ft (1.2 to 1.5 m) and divided into wood, bark and floral buds. A fresh weight of 0.7 to 1.05 oz (20 to 30 g) of floral buds (mainly from spurs) was collected from each tree. Fifty naturally abscised leaves per tree were collected, measured for area, rinsed with deionized water, destemmed and weighed. At fruit harvest, N and NFF were also detected on a 3.5 to 7 oz-sample (100 to 200 g of fresh weight) randomly selected from 20 unpeeled, sliced fruit per tree.

In 1997, samples of 8 to 10 healthy, fully expanded leaves were collected monthly per tree, from May to November and analyzed for total N and ^{15}N enrichment. Samples of three to five fruit, including skin, flesh and seeds, collected from June to harvest and roots collected as already described from July to harvest were also used to determine N concentration and percentage of NFF. All samples were oven-dried at 170 °F (75 °C), ground to pass a 40-mesh screen, and analyzed for total N and ^{15}N enrichment (Harris and Paul, 1989) by mass spectrometry (Automated Carbon and Nitrogen Analyzer, Roboprep, Europe Scientific, Crewe, England). Atom enrichment values were converted to percentage of NFF according to the following equation: (atom %^{15}N tissue) – (^{15}N natural abundance) / (atom %^{15}N fertilizer) – (^{15}N natural abundance) × 100, where ^{15}N natural abundance was equal to 0.37%. Since most of the soil N was insolubilized as organic matter and nitrate concentration was only 7 $mg \cdot kg^{-1}$, it was assumed that there was a negligible dilution effect of soil N on the fertilizer N enrichment (5 atom% ^{15}N). The amount of NFF partitioned into fruit and leaves was calculated as amount of dry matter × N concentration × NFF.

FRUIT QUALITY. At 1996 and 1997 harvest, fruit firmness was measured, after removing the skin, on 20 apples per tree by a penetrometer with a 0.44 inch (11 mm) diameter tip. Soluble solids concentration was recorded after squeezing a few drops of juice from each of the 20 apples on a hand-held refractometer. In addition, total N and Ca concentrations were evaluated from the same fruit sample.

STATISTICAL ANALYSIS. Where not otherwise specified, a repeated-time, factorial experimental design was arranged with spring and summer as timings of fertilization and sampling dates indicated as days after full bloom (DAFB). Statistical analysis was performed by analysis of variance using the General Linear Model procedure (GLM) and Duncan's multiple range test ($P \leq 0.05$) separated means.

Results

Even though the orchard had not been fertilized since its establishment, the leaf analysis performed at the end of July 1997 (Table 1) showed that N, P, and Mg concentrations (as % of dry weight) were unaffected by N supplies and were in sufficient ranges for apple in North America (Jones et al., 1991). Leaf K and particularly leaf Ca were in the low range, with the latter statistically higher in unfertilized and SUN

Table 1. Effect of 1996-summer N supply (SUN) and 1997-spring N supply (SPN) on apple leaf mineral concentration (as % of dry weight) detected on 31 July 1997.[z]

Fertilizer	N	P	K	Ca	Mg
			(%)		
Not fertilized	2.61	0.17	1.31	1.15 a[y]	0.35
SUN	2.34	0.17	1.37	1.09 a	0.34
SPN	2.53	0.16	1.29	0.85 b	0.31
Significance	NS	NS	NS	*	NS

[z]Data were statistically analyzed as in a complete randomized design.
[y]Mean separation, within columns, by Duncan's multiple range test.
[NS,*]Nonsignificant or significant at $P \leq 0.05$, respectively

Table 2. Total N and fraction of N derived from fertilizer (NFF) detected at leaf fall in 1996 and 1997 in different apple organs of trees supplied with 1996-summer N (SUN) or 1997-spring N (SPN), respectively.

	SUN (1996)		SPN (1997)	
Organ	Total N (% dry wt)	NFF[z] (%)	Total N (% dry wt)	NFF (%)
Roots	1.18	18.0 a	1.23[y]	1.6 c[y]
2- to 4-year-old wood	0.47	12.9 ab	0.40	11.5 b
Bark limb	1.09	11.4 ab	0.94	10.6 b
Spurs	1.54	11.8 ab	1.37	13.4 ab
1-year-old shoots	1.26	7.6 b	0.76[x]	16.9 a[x]
Leaves	2.01	5.2 b	1.75	10.2 b
Fruit	0.21[y]	4.5 b[y]	0.23[y]	12.3 ab[y]
Significance	---	*	---	*

[z]Values in the same column were statistically analyzed as in a complete randomized design and means separated by Duncan's multiple range test.
[y]Samples collected at harvest.
[x]Samples collected on 12 Sept. 1997.

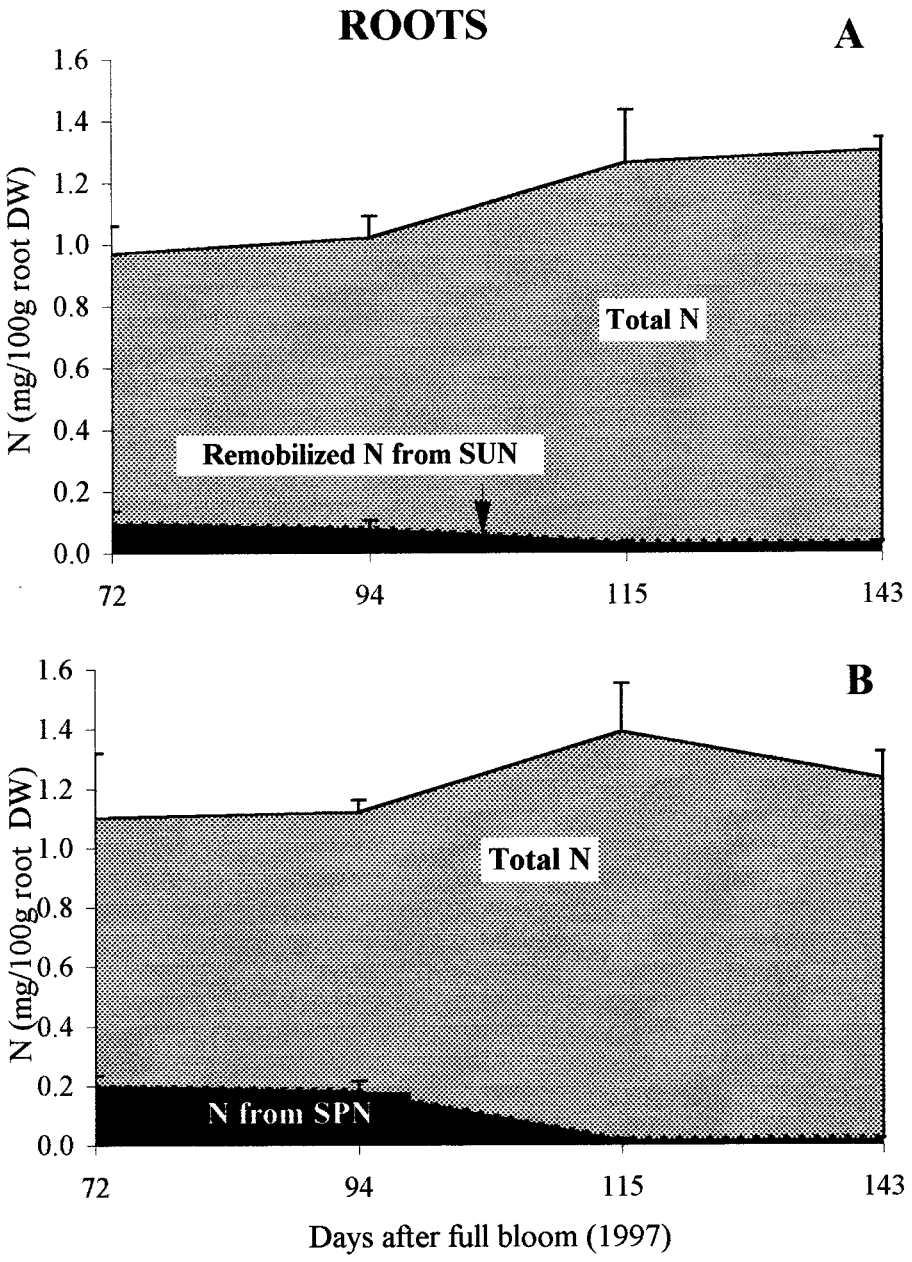

Fig. 1. Total N and N derived from fertilizer in roots of apple trees supplied with summer N (SUN) in 1996 (A) or spring N (SPN) in 1997 (B). Bars represent standard errors (n = 4). (1.0 oz = 28.35 g = 28,350 mg).

trees than in SPN ones (Table 1).

In 1996, at leaf fall, SUN preferentially accumulated in the perennial organs (roots, 2- to 4-year-old wood, spurs and limb bark) rather than 1-year-old shoots, leaves and fruit (Table 2, second column). In 1997, at leaf fall, SPN was preferentially allocated to 1-year-old shoots, spurs, fruit, 2- to 4-year-old wood, limb bark and leaves; only 1.6% of the NFF was found in roots (Table 2). Total N concentrations evaluated at leaf fall in the different organs (Table 2) were similar to the values reported by Khemhira et al. (1998) on apple 'Topred' and 'Redspur Delicious' in Oregon.

Root total N concentrations and percentages of N from summer (SUN) or spring (SPN) fertilizer, as monitored from July to October 1997, are shown in Fig. 1A and B. No interaction between timing of N supply and sampling date was detected for either total N or NFF. The former was not affected by the time of fertilization and increased over time. The percentage of NFF from 1997-SPN was statistically higher than that from the 1996-SUN (Fig. 1A and B). In both treatments the percentage of NFF decreased over time (Fig. 1A and B).

During 1997, fruit growth was not affected by N supply timing and continued over time until harvest with no interaction between timing of N supply and sampling date (Fig. 2A and B, dotted line). The accumulation trends of both total N and NFF in the fruit were not different for the two timings of fertilization (Fig. 2A and B). Total N and NFF increased over time until mid-September (115 DAFB) and remained steady in the last month before harvest. No interaction between timing of N supply and sampling date was observed.

No interaction between N supply timing and sampling date was also detected for the trend of both total N and NFF accumulation in leaves (Fig. 3A and B). Leaves of trees receiving SUN and SPN showed similar total N throughout the growing season (Fig. 3A). In both treatments leaf total N reached the maximum in September to October (115 to 143 DAFB); thereafter a sharp drop occurred during leaf senescence, when the total N content decreased from 15 to 4.3 and 5.3 mg/leaf (28,350 mg = 1.0 oz) at 178 DAFB (Fig. 3A) in SUN and in SPN trees, respectively. Leaves of the SUN trees had a higher content of NFF than the SPN ones at 8 DAFB (Fig. 3B). This difference disappeared in June, July and August (94 DAFB), but on 12 September (115 DAFB) the leaves from SPN trees showed a higher NFF than the SUN ones. At the beginning of leaf abscission a sharp decrease in NFF was observed in both sets of trees (Fig. 3B).

A complete randomized design was arranged to test the effect of SUN on fruit quality as compared to unfertilized trees, in 1996 and the effect of SPN on fruit quality as compared to unfertilized tree in 1997. In 1996, since SUN was not effective in increasing N fruit concentration or in decreasing Ca fruit concentration, the N to Ca ratio was similar in unfertilized trees (5.7) and SUN ones (5.6). Fruit quality was also unaffected by N supply: fruit firmness was 19.8 lb (88.1 N) and 20.3 lb (90.3 N) and soluble solids concentration was 13.7% and 14%, for unfertilized and SUN apple, respectively (data not tabulated). In 1997, SPN did not modify fruit N to Ca ratio which was 7.6 and 9.8 for unfertilized and SPN apple. Not was fruit quality different in unfertilized and SPN trees:

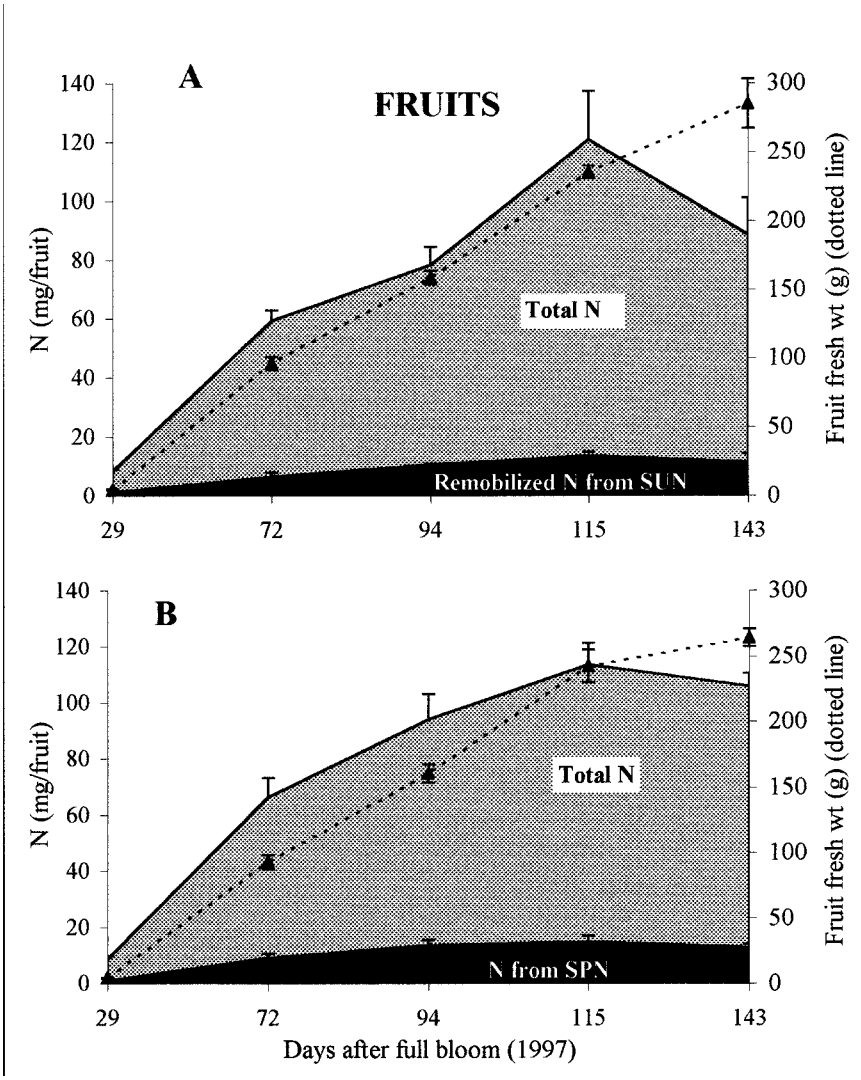

Fig. 2. Fruit fresh weight (dotted lines), total N and N derived from fertilizer in fruit (mg) of apple trees supplied with summer N (SUN) in 1996 (A) or spring N (SPN) in 1997 (B). Bars represent standard errors (n = 4). (1.0 oz = 28.35 g = 28,350 mg).

fruit firmness was 19.6 and 19.4 lb (87.2 and 86.3 N) and soluble solids concentration was 13% and 13.1%, respectively (data not tabulated). Yield was not affected by N supply, ranging between 78 lb / tree (35.4 kg / tree) in SUN, in 1996 and 93 lb / tree (42.2 kg / tree) in 1997, in SPN (data not reported).

Discussion

N accumulation in apple fruit increased constantly over the growing season until a month before harvest (which in Michigan occurs around the second week in October). This trend agree with the results obtained on 'Jonathan' grown in New Zealand (Bollard, 1971) and 'Gala' in Northern Italy (B. Marangoni, unpublished data) where accumulation of N in apple fruit increased until ≈120 DAFB. The increase of fruit weight until harvest along with the stable fruit N content made it possible a decrease of fruit N concentration (data not shown) as already reported by Fallahi et al. (1984) who observed a constant decline of fruit N concentration between bloom and harvest in 'Starkspur Golden Deliciuos' apple in Oregon. Our data add more evidence to the effectiveness of apple fruit as an active sink for N from its earliest stages of growth until ≈30 d before harvest. The N taken up by roots from mid-September (115 DAFB) to harvest (143 DAFB) does not contribute substantially to N accumulation in fruit. Therefore the N to Ca ratio is not significantly increased and fruit quality and storability are not compromised. Nitrogen applied at bloom showed a greater likelihood of increasing fruit N concentration, probably because fruit in the first stages of their growth successfully compete with other tree organs for N accumulation. Comparing Fig. 2A and B indicates that the remobilized fraction of N contributes to the total bulk of fruit N as much as the N taken up in the same season. However, it is possible that, even though soil had a light texture, a small amount of 1996-SUN remained immobilized in the soil and became available for root uptake in the spring of 1997. If so the fraction of N called remobilized may have included a small portion of newly absorbed SPN.

As already noted by Muñoz et al. (1993) in 3-year-old peach trees and by Tagliavini et al. (1997) in pear, we found that the early leaf growth in bearing apple trees mainly depends on stored rather than on newly absorbed N. In fact, a few days after full bloom the stored N was promptly remobilized from the roots to the leaves, where it supported the early stage of leaf development (Fig. 3B). Later in the season the availability of SPN in the soil induced a higher accumulation of N in the leaves of the SPN trees. Even though the incidence of remobilized N on the leaf budget in September and October was lower than the amount of newly absorbed N, the former significantly contributed to leaf growth not only at the sprouting stage but also throughout the growing season.

It was estimated from Fig. 3A that about two-thirds of leaf N (10 mg N per leaf) was remobilized from leaves to storage tissues during leaf senescence in 1997 (see the difference between total N content in leaves at 178 and 143 DAFB). However, in 1996 the leaves of the trees fertilized at the end of August recycled ≈40% of total N (5 mg of N per leaf, data not shown). These figures agree with the results reported by Sanchez et al. (1990) on pear 'Comice' and by Khemira et al. (1998) on 'Topred' and 'Redspur' apples. Our results on leaf N accumulation agree with Diamond et al. (1998), who observed on 'Fuji' a period of minimal flux of N into the leaves from 40 to 140 DAFB. In our experiment we measured leaf total N rather than N concentration (Diamond et al., 1998) and we found a lapse of time with steady leaf N content run-

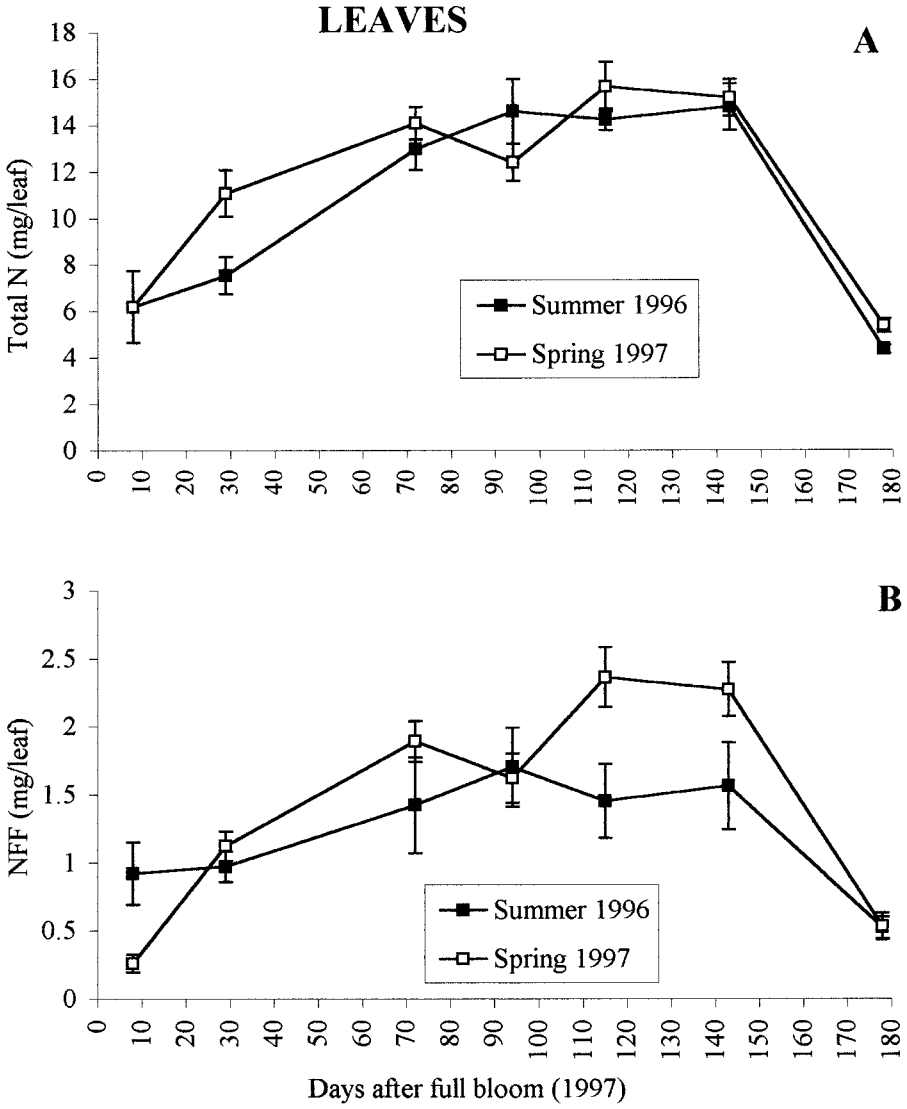

Fig. 3. Total N (A) and N derived from fertilizer (NFF) (B) in leaves (mg) of apple trees supplied with summer N (SUN) in 1996 (closed symbols) or spring N (SPN) in 1997 (open symbols). Bars represent standard errors (n = 4). (1.0 oz = 28.35 g = 28,350 mg).

ning from July (70 DAFB) to October (140 DAFB).

Starting from mid-August (94 DAFB) when shoot growth had already stopped, the fraction of root labeled N decreased from 20 to 1.6% of the total N (Fig. 1B from 94 to 143 DAFB); at the same time root total N concentration slightly increased. This means that the root labeled N taken up from bloom to August was moved somewhere else in the tree (leaves, spurs and fruit) and at the same time replaced by unlabeled N. We assume that this amount (18.4% of the total N) came only from new uptake and was stored in the roots at the end of the summer to sustain spring vegetative growth. At this time apple roots become an important sink for newly absorbed N, stressing the role of N uptake as a direct contribution to N storage late in the summer (Millard, 1996; Quartieri et al., 1998). In mature deciduous trees the internal cycling of N is a well-defined phenomenon in which almost all the N stored in the roots the previous year is remobilized to the growing organs to sustain shoot and fruit growth. When the fruit cycle is completed, newly absorbed N is stored in the roots to prepare N reserves for the following growing season.

Conclusion

In the present experimental condition, apple fruit accumulate N until ≈115 DAFB, afterwards their strength as a sink for N decreases and only a slight amount of the available N is partitioned to the fruit, where it does not impinge on fruit firmness and soluble solids concentration. Nitrogen absorbed in summer is prevailingly stored in the woody perennial organs where, together with leaf-recycled N, it constitutes reserves which trees can rely on for their vegetative growth in the subsequent early spring. Therefore, summer N supplies can become an important tool for sustaining spring vegetative growth from the first stage after sprouting even in areas (like Michigan) affected by a low soil temperature in spring and frequent rainfall that can compromise N uptake. In orchards where N is a limiting factor for correct fruit and shoot development, spring N application can improve both the leaf and fruit N concentrations more efficiently than summer supply.

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