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Spring growth of almond nursery trees depends upon nitrogen from both plant reserves and spring fertilizer application

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SUMMARY

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

Nitrogen (N) is required for the initial growth of deciduous trees in the spring. The ability to store N the previous year and utilize it during the following growing season is a characteristic of fruit trees (Taylor and May, 1967; Titus and Kang, 1982; Tromp, 1983; Millard, 1995). In certain species, the amount of N remobilized depends upon the total amount of N in reserve, and is not affected by the uptake of N from spring applications of fertilizers (Millard and Neilsen, 1989; Millard and Thompson, 1989; Millard and Proe, 1992; Tagliavini *et al.*, 1998; Cheng *et al.*, 2001; Neilsen *et al.*, 2001a, b).

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

MATERIALS AND METHODS

Experimental design

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

Fifteen plants from each N-fertigation level were randomly selected and sprayed with 3% urea on 10 and 20 October (F+U treatment). The remaining plants were sprayed with water as our control (F treatment). All the plants were then barerooted and harvested in late November following natural leaf fall, and were stored at 2°C. Afterward, five plants from each treat-

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ment were destructively sampled and divided into stem and root portions that were washed with double-distilled (DD) water to remove any urea residue from their surfaces. All samples were immediately put into a –80°C freezer, freeze-dried, then ground with a Wiley mill (20 mesh) and reground with a cyclone mill (60 mesh) for analysis.

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

Analysis of samples

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

$$NDFF\% = \frac{(atom\%^{15}N)_{natural\ abundance} - (atom\%^{15}N)_{tissue}}{(atom\%^{15}N)_{natural\ abundance} - (atom\%^{15}N)_{fertilizer}} \times 100\%$$

The ^{15}N content in each tissue type was calculated from NDFF% and tissue total N content. For trees that did not receive any supplemental N during regrowth, the total N content in new shoots and leaves was taken as the amount of reserve N remobilized from storage tissues for new growth. Here, we assumed that N from other sources was negligible. For trees supplied with depleted ^{15}N during regrowth, the remobilized reserve N was estimated as the difference between the total and the labelled ^{15}N content in new shoots and leaves.

Statistical analysis

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

RESULTS AND DISCUSSION

Nitrogen concentrations and contents in dormant almond trees

Concentrations of N in the stems and roots of fertigated almond trees (treatment F) increased with increasing N fertigation concentrations (Figure 1A, B). Trees treated with foliar urea had significantly higher N concentrations in their stems and roots than did those receiving only fertigation (Figure 1A, B: F+U v F treatments at each N-fertigation concentration, $P<0.05$). This effect of foliar urea on the root nitrogen concentration was greater when plants were fertigated with low N concentrations than in those fertigated with high N concentrations (Figure 1B: N fertigation concentration × Urea treatment interaction: $P = 0.03$). N fertigation had no effect on N concentrations in stems (Figure 1A: $P = 0.5317$) and roots (Figure 1B: $P = 0.1393$) of trees

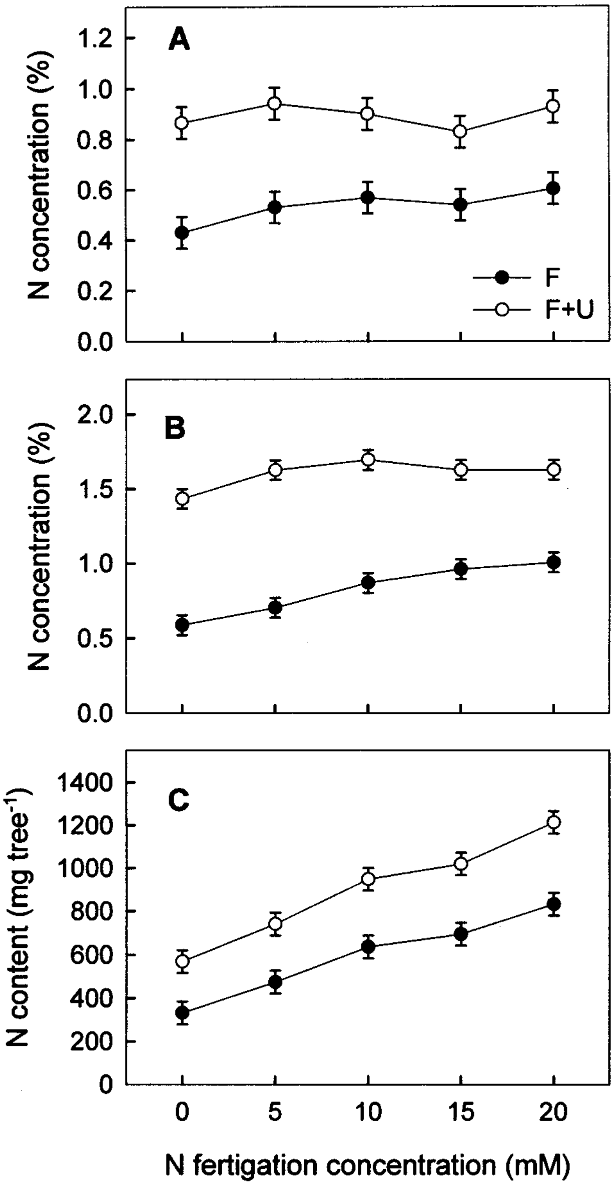

Fig. 1
Effects of N fertigation rate during the 1999 growing season and foliar urea applications in the fall of 1999 on (A) stem and (B) root N concentrations and (C) total N content of almond nursery trees. Each value is the mean of five replicates. Vertical bars represent standard errors. Treatments: N fertigation (F), N fertigation and foliar urea (F+U).

treated with urea. The total amount of tree nitrogen increased with increasing N-fertigation concentrations (Figure 1C). Plants given fall foliar applications of urea (F+U) had significantly higher levels of N ($P<0.05$) than did those receiving only fertigation (F), with total-N contents increasing by 237 to 382 mg per tree, depending on the N-fertigation concentration.

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

Tree growth

Regardless of spring N application, total leaf areas and lengths of new shoots increased with increasing N-fertigation concentrations from the previous year (Figure 2). At any given N concentration, trees sprayed with urea in the fall (F+U–N) had significantly greater leaf areas and longer new shoots ($P<0.05$) than trees receiving no urea (F–N). These data show that new shoot and leaf growth in the spring is closely related to reserve-N levels, thereby supporting the results reported from previous studies of deciduous fruit trees (Taylor and May, 1967; Titus and Kang, 1982; Tromp, 1983; Millard, 1995; Cheng and Fuchigami, 2002). Therefore, we believe that a tree's N reserves can be enhanced either by N fertigation during the growing season or through foliar urea applications in the fall, both treatments resulting in increased new growth the following spring.

Spring N fertilizer application increased new growth regardless of the amount of N reserves in the plant. Spring application of N significantly increased leaf area and new shoot growth of fertigated trees (Figure 2A, B: F+N v F–N treatments at each N-fertigation concentration, $P<0.05$) and urea-treated trees (Figure 2A, B: F+U+N v F+U–N treatments at each N-fertigation concentration, $P<0.05$). Nonetheless, plants treated with either foliar urea in the fall or N fertilizer in the following spring had the same total leaf areas and new shoot growth at each N-fertigation concentration from the previous year (Figure 2A, B: F+U–N v F+N treatments, $P>0.05$). Our data suggest that spring N applications can be as effective as fall-applied foliar urea in increasing total leaf areas and new shoot lengths.

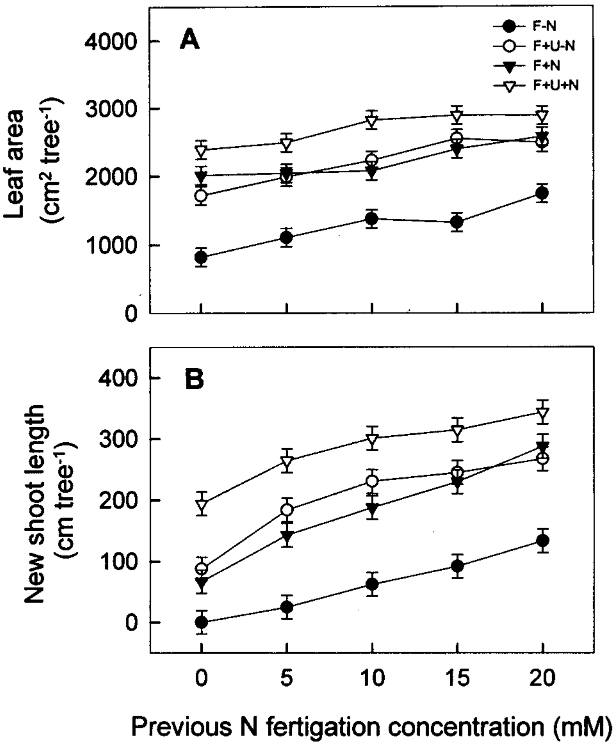

Fig. 2

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

Use of nitrogen reserves

The amount of reserve N used for new shoot and leaf growth increased with increasing N-fertigation concentrations in the previous season, with or without the use of spring fertilizers (Figure 3A). Trees receiving foliar urea (F+U–N and F+U+N) used significantly more reserve N ($P<0.05$) for producing new shoots and leaves than did trees gaining N only from fertigation (F–N and F+N). Although foliar urea application increased the amount of N remobilized for new growth, trees fertigated with low concentrations of N were more responsive to urea application than trees fertigated with high concentrations of N (Figure 3B, Fertigation concentration × Urea treatment interaction $P<0.05$). There was no significant difference (Figure 3A, B: at each N-fertigation concentration, $P>0.05$) in the amount or proportion of N used for new growth between trees receiving N or no N in spring (F+N v F–N; F+U+N v F+U–N). This indicates that the level of N remobilization depends on the amount of nitrogen stored in the plant during the previous year, and is not affected by current-year N fertilization (Millard, 1996; Neilsen *et al.*, 2001b; Cheng and Fuchigami, 2002). Generally, trees with higher N reserves used more of that stored N for new growth than did trees with less accumulated nitrogen (Figure 3C). Enhancing the level of N reserves via either N fertigation during the growing season or foliar urea applications in the fall of that season increased the amount of N remobilized for new growth the following spring.

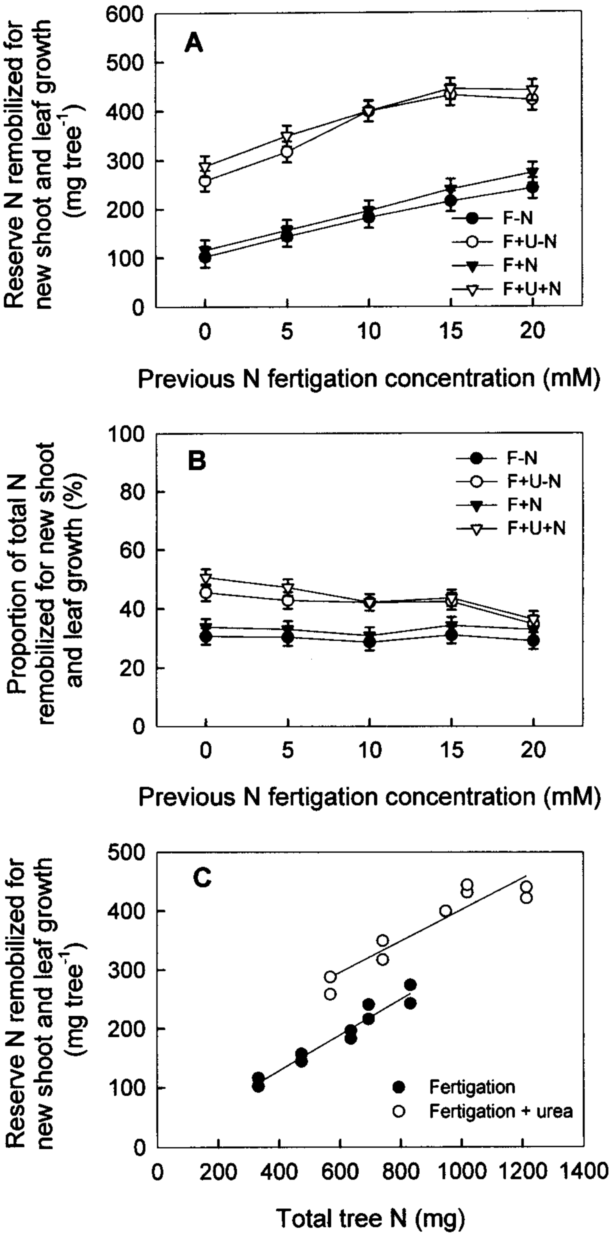

FIG. 3

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

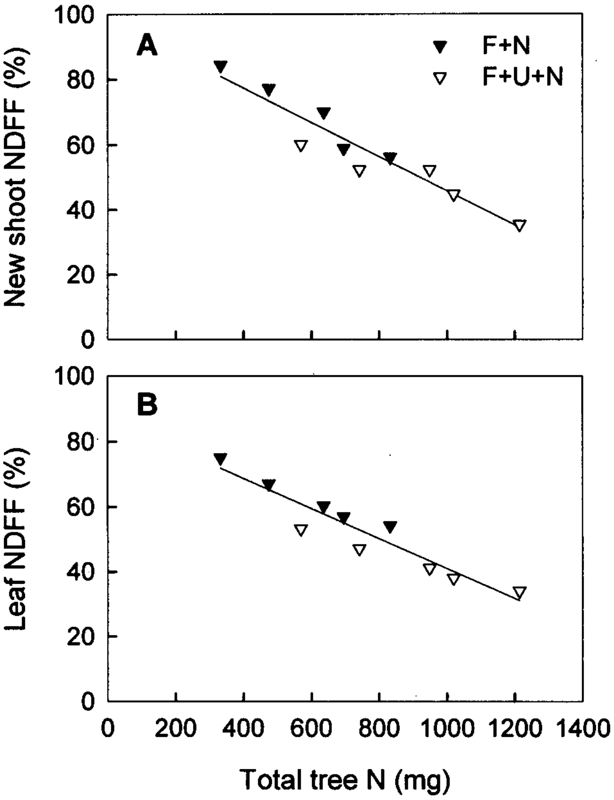

FIG. 4

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

For several deciduous tree species, nitrogen taken up late in the season contributes more to storage and subsequent remobilization for new growth in the following spring than does N taken up earlier in the season (Weinbaum *et al.*, 1984b; Millard, 1996; Tagliavini *et al.*, 1999). In our experiment, almond nursery trees sprayed with fall foliar urea used significantly more N from reserves for new growth than did those having the same total amount of N but which had been treated only with N fertigation (Figure 3C). One possible explanation for this phenomenon is that the increase in plant growth associated with early-season nitrogen fertigation may have resulted from a greater proportion of N being used for structural growth. This would have meant less N in storage and, consequently, less remobilization for new growth the following spring. In contrast, the N received from foliar urea applied after terminal bud set may not have been used for building the tree's structure, but rather was stored in a non-structural, more readily available form of nitrogen. We have found that a greater proportion of N was accumulated as free amino acids in trees sprayed with fall foliar urea compared with those receiving only N fertigation (Bi *et al.*, unpublished).

Uptake and use of N from fertilizer

The percentage of N in the new shoots and leaves derived from ^{15}N-NH$_4$NO$_3$ applications (N derived from fertilizer, or NDFF) in the spring decreased with the increasing levels of nitrogen accumulated in plants from the previous year (Figure 4A, B). For every 100 mg increase in total N in a plant, the NDFF in new shoots and leaves decreased by approximately 5%. This

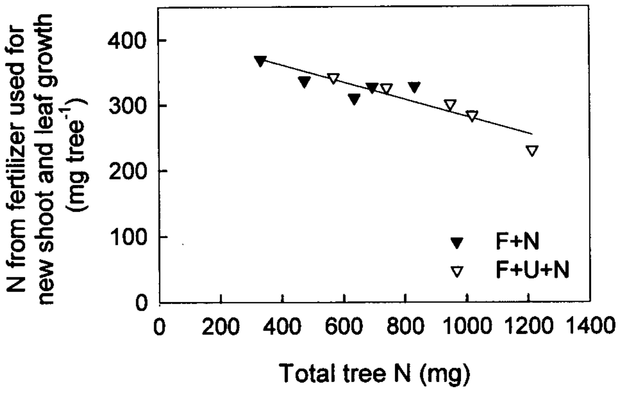

Fig. 5

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

indicates that the N status of the tree affected N uptake; trees with lower N reserves took up more nitrogen from the current-year fertilizer, similar to the results described for mature citrus trees by Feigenbaum *et al.* (1987). Likewise, the amount of N derived from spring fertilizer that was used for new growth declined with increasing reserve-N levels in the plants (Figure 5). For every 100 mg decrease in total N accumulated during the previous year, trees compensated by taking up 13 mg of nitrogen for new shoot and leaf growth.

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

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

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