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N uptake, soil retention and loss of soil-applied $^{15}NH_4{}^{15}NO_3$ in young Fuji/M.26 apple trees with different N status

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SUMMARY
Nitrogen (N) uptake, soil retention and loss of soil-applied N were studied in young apple trees with different N backgrounds. Bench-graft potted Fuji/M.26 (*Malus domestica* Borkh) trees were fertigated with 5, 10 or 20 mM N twice a week from June to August, and the trees were removed from soil and bare-root stored in a 2°C cold room in December of the first season. In April of the second season, the trees were washed and replanted in containers with a N-free medium (perlite:vermiculite=l:l v), and received 500 ml Hoagland's nutrient solution without N weekly through the experiment. The trees received $^{15}NH_4{}^{15}NO_3$ at 1g per plant or no N on June 21. Four trees from each treatment were harvested at one, two and four weeks after ^{15}N application, and ^{15}N and total N in plant tissues and soil were analysed. N fertigation rates during the first growing season increased tree growth and N reserve levels, and N content in trees in the second year. New shoot and leaf growth in the following season was positively related to reserve N. ^{15}N uptake increased during the four weeks after ^{15}N application while soil ^{15}N retention decreased. There was no significant difference in the total ^{15}N uptake per tree. However, trees with the lowest N contents at the end of the first growing season had the highest rate of ^{15}N uptake per unit root dry weight. Four weeks after application of ^{15}N, tree uptake of N accounted for about 60% of applied ^{15}N, while about 20% of the N still remained in the soil, and another 20% of the N was lost. Our results suggested that trees with lower N status are more efficient in N uptake from soil.

Nitrogen (N) is essential for plant growth and development, and largely determines crop productivity (Faust, 1989; Marschner, 1995). Soil N is frequently supplemented by N fertilizer applications to sustain crop production. In the past half century, use of commercial N fertilizer in agriculture has increased as production of chemical fertilizers has increased due to technology development and great fertilizer demand (Dinnes *et al*., 2002). As more N fertilizer is applied to farmlands, public concern regarding N movement from agricultural lands to contaminate water resources has increased. Balancing the amount of N needed for optimum plant growth while minimizing the loss of NO_3^- to surface and ground waters remains a major challenge to improve agricultural nutrient use efficiency (Dinnes *et al*., 2002).

Nitrogen is the most heavily used fertilizer in orchards, and is often used as an "insurance policy" to achieve maximum productivity (Sanchez *et al*., 1995). As a result, N use efficiency is generally low in orchards. When fertilizer is applied to soil as nitrate in the fall, approximately 16% of the N is recovered (Hill-Cottingham and Lloyd-Jones, 1975). During early spring growth, the recovery rate of soil-applied N can be less than 20% in young apple trees (Dong *et al*., 2001a). Others have reported recovery rates of soil-applied N between 25–35% (Khemira, 1995). In tree-fruit production, the fate of N from soil-applied fertilizers that is not recovered in trees has not been well documented. It is not known what proportion of the N that is not taken up by the trees is actually lost into the environment and what proportion still remains in soil which may subsequently become available for plants.

The objectives of this study were to determine (1) the fate of soil applied N to young apple trees, and (2) the influence of tree N status on N uptake by young apple trees.

MATERIALS AND METHODS

Bench-grafted Fuji/M.26 apple trees (*Malus domestica* Borkh) were planted in 4 l plastic pots containing a mix of peat moss, perlite and loam soil of 1:1:1 by volume at Oregon State University in Corvallis, Oregon. USA. Trees were grown in a lathhouse until early June, and then uniform trees were selected based on height (about 65 cm) and diameter (0.6±0.13 cm) and moved outside. The selected trees were divided randomly into three groups with 30 trees per group in a random design, and fertigated, respectively, with 5, 10 or 20 mM N in a 20–10–20 (N:P:K) formula twice a week from June to August. All trees were well watered and allowed free drainage. After natural defoliation in December of the first growing season, trees were removed from pots and stored bare-root in a 2°C cold room. Five trees from each

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group were destructively sampled for roots, shank and stems for baseline analysis of total N content.

In early April of the second season, trees were removed from cold storage, and their root systems were washed to remove all soil particles. Trees were replanted into new 4 l plastic pots in a N-free medium (perlite : vermiculite =1:1 by volume), and placed on a flat sand bed in a random design. Each tree received 500 ml Hoagland's solution without N weekly until harvest. Half of the trees from each fertigation treatment received $^{15}NH_4{}^{15}NO_3$ (0.05% 15N atom depleted, ISOTEC, Inc., Miamisburg, OH, USA) at 1 g per tree on June 21 (73 d after transplanting), and the other half received no N. Irrigation water was supplied daily to each tree as required and no water was allowed to leach after ^{15}N application. Four trees from each treatment were sampled at one, two and four weeks after ^{15}N application, and separated into new shoots and leaves, old stem and shank, and roots. Soil in each pot was weighed after harvest, and a sub-sample was taken for ^{15}N analysis to determine the N remaining in the soil. All samples were immediately frozen in an –80°C freezer, and then freeze dried. Samples were first ground with a Wiley mill (20 mesh) and reground with a cyclone mill (60 mesh) prior to analyses.

Total N was determined through Kjedahl analysis (Schuman *et al.*, 1973) by the Central Analysis Laboratory of Oregon State University. The amount of ^{15}N in samples was determined from the gas evolved from combustion of powdered tissue in an elemental analyser coupled with a mass spectrometer by the laboratory of Isotope Services, Inc. (329 Potrillo Dr., Los Alamos, NM, USA). The percentage of N derived from fertilizer (NDFF %) in each tissue/soil was calculated as:

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

Concentration of ^{15}N was calculated from NDFF% and total N concentration. The amount of ^{15}N in each tissue/soil was calculated by multiplying ^{15}N concentration by the dry weight. Total ^{15}N uptake per plant was calculated by pooling the ^{15}N content in different tissues. The average ^{15}N absorption rate at each harvest was calculated from total ^{15}N uptake per plant divided by root dry weight (RDW) and time of uptake (day). Nitrogen use efficiency (recovery) was calculated as percentage of ^{15}N absorbed by plant to total ^{15}N applied. ^{15}N loss was calculated by the following equation:

^{15}N loss = total ^{15}N applied -^{15}N recovered in plants -^{15}N remained in soil.

The experiment was a completely randomized design with 90 trees randomly divided into three groups for fertigation, and trees in each group were further divided into three sub-groups for different N uptake period treatments with or without ^{15}N (four replicates for each treatment at each harvest date). All data were subjected to a two-factor (N fertigation treatment and sample date) analysis of variance (ANOVA) to determine differences among different fertigation treatments over time. All statistical analyses were performed with NCSS Statistical System Software (NCSS Statistical Analysis Software, Kaysville, UT, USA).

RESULTS

Plant growth and nitrogen background in the first season

Plant growth in the first growing season increased with increasing N supply from fertigation. Root and stem biomasses were significantly lower in the lower N than in the higher fertigation treatments (P<0.0001), but no significant differences were found in shank biomass (P=0.35) (Figure 1). N content of stems, shanks and roots also increased with increasing N concentrations in fertigation solution (P<0.0001) (Figure 2). Trees receiving the highest N fertigation concentration (20 mM) had significantly higher N contents in all tissues than trees receiving the lower N fertigation rates (5 and 10 mM) at the end of the first growing season.

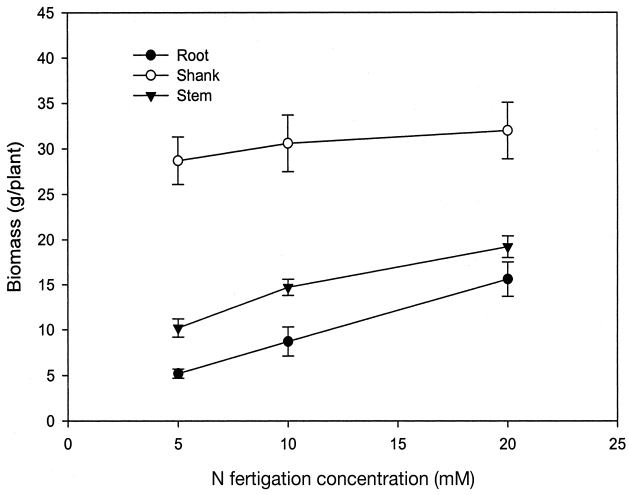

FIG. 1
Root (closed circle), shank (open circle) and stem (closed triangle) biomass of Fuji/M.26 apple trees at the end of the first growing season fertigated with different N concentrations twice a week from June to August. Bars on each data point represent standard errors of mean of five replicates.

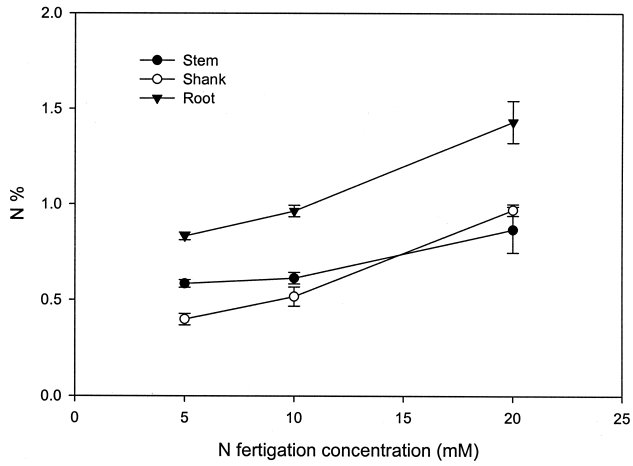

FIG. 2
Nitrogen concentration in stem (closed circle), shank (open circle) and root (closed triangle) at dormant stage of Fuji/M.26 apple trees fertigated with different N concentrations twice a week from June to August. Bars on each data point represent standard errors of the mean of five replicates.

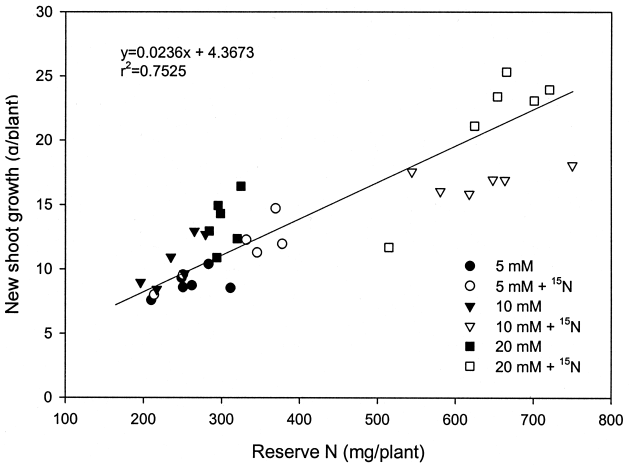

FIG. 3
Relationship between new shoot growth and N reserve levels of Fuji/M.26 apple trees when dormant. Trees were fertigated with 5 (circle), 10 (triangle), and 20 (square) mM N twice a week from June to August in previous year and applied with (open) or without (closed) ^{15}N in the second year. The new shoot growth occurred from April to July in the following season.

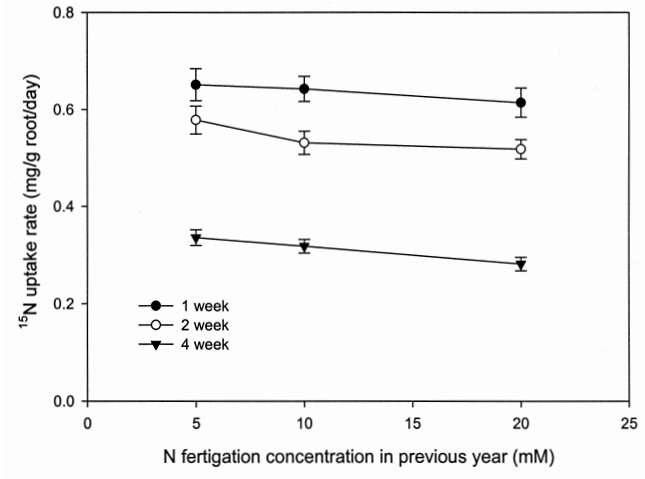

FIG. 5
Average ^{15}N uptake rate (mg/g DW root/day) at 1 week (closed circle), 2 weeks (open circle) and 4 weeks (closed triangle) following $^{15}NH_4{}^{15}NO_3$ application on June 21 in Fuji/M.26 apple trees fertigated with different N concentration twice a week from June to August in previous year. The ^{15}N uptake occurred from 21 June to 19 July in the following season. Bars on each data point represent standard errors of the mean of four replicates.

Regrowth performance in the second season

New shoot and leaf growth in the second season was highly correlated with reserve N levels at the end of the first growing season (r^2=0.7525) (Figure 3). Application of ^{15}N (as $^{15}NH_4{}^{15}NO_3$) in the second growing season did not change this relationship between N reserves and new shoot and leaf growth, but increased new growth biomass.

^{15}N uptake

Uptake of ^{15}N occurred throughout the four-week period following application of $^{15}NH_4{}^{15}NO_3$ and there was no significant difference in total ^{15}N uptake between trees with different N backgrounds due to different rates of N fertigation during the prior year ($P_{sample\ date}$<0.00001, $P_{fertigation}$=0.14, $P_{sample\ date\ x\ fertigation}$=0.58) (Figure 4). The ^{15}N uptake rate was significantly decreased with time after application ($P_{sample\ date}$<0.00001, $P_{fertigation}$=0.0006, $P_{sample\ date\ x\ fertigation}$=0.07). The average rate of ^{15}N uptake across all fertigation treatments was approximately 0.61±0.11 mg N g^{-1} root dry weight d^{-1} in the first week after N application, 0.55±0.06 mg N g^{-1} root dry weight d^{-1} during the second week after N application and 0.3l±0.03 mg N g^{-1} root dry weight d^{-1} four weeks after N application (Figure 5). Trees that received the lowest rates of N fertigation during the previous growing season had the highest rates of ^{15}N uptake for all three harvest dates, but only at the second and third dates (two and four weeks after ^{15}N application) were differences significantly higher relative to the highest rate of N fertigation. Trees recovered between 21–30% of applied ^{15}N during the first week after ^{15}N application and between 62–69% by four weeks

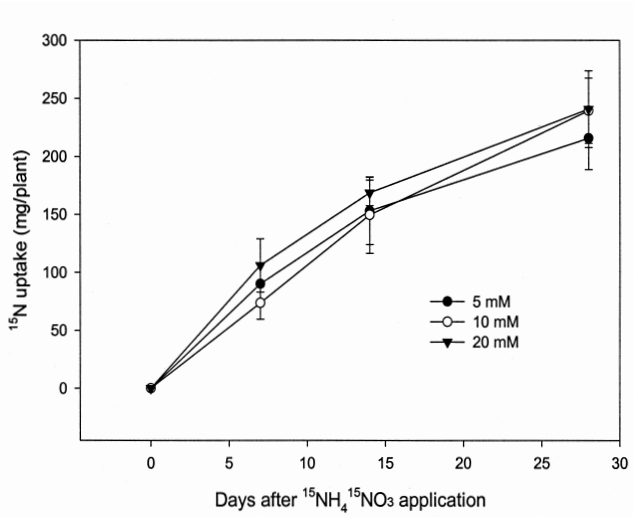

FIG. 4
Total ^{15}N uptake following $^{15}NH_4{}^{15}NO_3$ application on 21 June in Fuji/M.26 apple trees. Trees were fertigated with 5 (closed circle), 10 (open circle), and 20 (closed triangle) mM N twice a week from June to August in previous year. The ^{15}N uptake occurred from June 21 to 19 July in the following season. Bars on each data point represent standard errors of the means of four replicates.

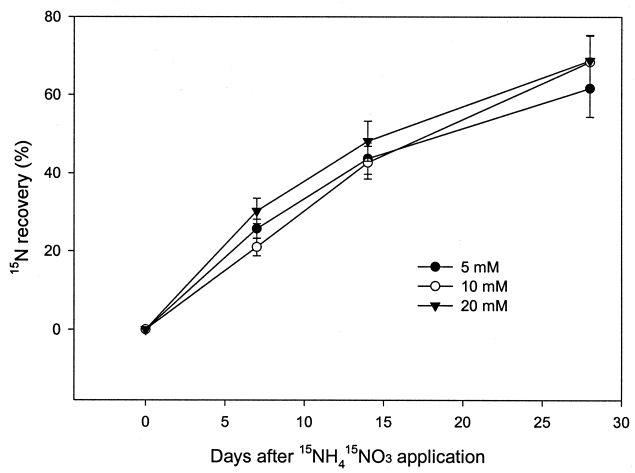

FIG. 6
^{15}N recovery by plant following $^{15}NH_4{}^{15}NO_3$ application on June 21 in Fuji/M.26 apple trees. Trees were fertigate with 5 (closed circle), 10 (open circle), and 20 (closed triangle) mM N twice a week from June to August in previous year. The ^{15}N uptake occurred from 21 June to 19 July in the following season. Bars on each data point represent standard errors of the means of four replicates.

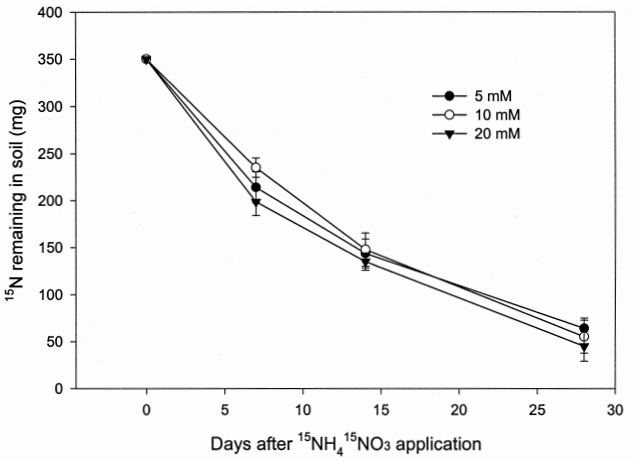

FIG. 7
^{15}N remaining in soil following $^{15}NH_4{}^{15}NO_3$ application on 21 June in Fuji/M.26 apple trees. Trees were fertigated with 5 (closed circle), 10 (open circle), and 20 (closed triangle) mM N twice a week from June to August in previous year. Bars on each data point represent standard errors of the means of four replicates.

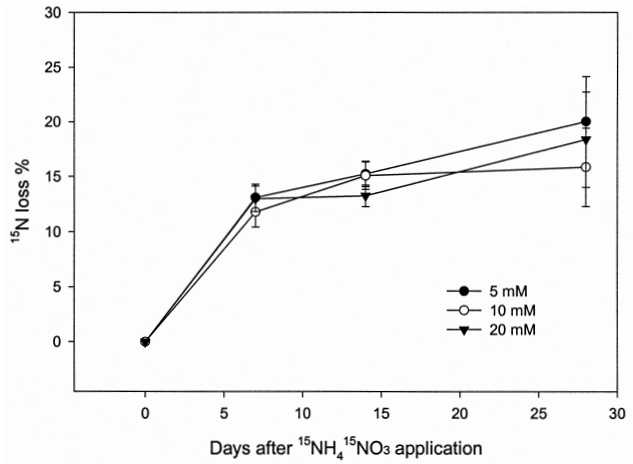

FIG. 8
^{15}N loss following $^{15}NH_4{}^{15}NO_3$ application on 21 June in Fuji/M.26 apple trees. Trees were fertigated with 5 (closed circle), 10 (open circle), and 20 (closed triangle) mM N twice a week from June to August in previous year. Bars on each data point represent standard errors of the means of four replicates.

after ^{15}N application (Figure 6). N fertigation rates from the previous growing season did not significantly affect the recovery rate of ^{15}N.

^{15}N in soil

The amount of ^{15}N in the soil decreased by approximately 19.2±2.8 mg d^{-1} during the first week after N application, 14.9±0.9 mg d^{-1} during the second week after N application, and 10.5±0.4 mg d^{-1} during the remainder of the experiment. About 20% of the applied N was still in the soil four weeks after application regardless of the N rate of N fertigation given to trees during the previous growing season ($P_{\text{sample date}}$<0.00001, $P_{\text{fertigation}}$=0.03, $P_{\text{sample date x fertigation}}$=0.07) (Figure 7).

^{15}N loss

Approximately 12% of the ^{15}N applied to soil was lost during the first week after application, and by four weeks after application, approximately 20% of the applied ^{15}N was unaccounted for (Figure 8). There was no significant difference in N loss among trees subjected to different fertigation treatments during the previous season ($P_{\text{sample date}}$<0.00001, $P_{\text{fertigation}}$=0.84, $P_{\text{sample date x fertigation}}$=0.97).

DISCUSSION

Nitrogen is one of the most important elements affecting plant growth and production. Our data showed that the biomass, tissue N content, and the amount of reserve N of young apple trees were positively related to N supplied in the fertigation solution during the growing season. This is consistent with the findings of numerous other experiments that a close relationship exists between plant growth and N availability (Alcoz *et al.*, 1993; Cheng *et al*, 2002; Dong *et al.*, 2001b; Neilsen *et al.*, 1997).

Apple trees store nutrients at the end of the growing season, and remobilize these stored nutrients to support the new growth in the following spring (Millard, 1996; Millard and Neilsen, 1989; Titus and Kang, 1982). We found that increasing N supply to young apple trees during the first growing season increases the amount of N reserves in trees and results in an increase in the growth of new shoots and leaves the following year, similar to the response observed by Cheng and Fuchigami (2002), in which they concluded that initial growth in the spring was determined by the amount of reserve N and not limited by reserve carbohydrates in young apple trees.

Young apple trees with low N status are more efficient in absorbing and mobilizing N from foliar-applied urea in autumn than those with high N status (Cheng *et al.*, 2002). In our experiment, we found that trees with different nitrogen status absorbed similar amounts of N from soil during the second growing season. Trees with a low N content at the end of the first growing season had smaller root systems than trees with a higher N content. Thus, when the uptake rate for N is expressed on the basis of root biomass, trees with lower N contents and smaller root systems have higher rates of N uptake than trees with higher N contents. The less efficient use of available N in the soil by high N trees may suggest a feedback mechanism in regulating N uptake as high N trees have higher levels of free amino acids and protein amino acids than low N trees (Cheng *et al.*, 2003).

As availability of ^{15}N in the soil decreased, the rate of ^{15}N uptake also decreased, while ^{15}N loss increased. By the end of the experiment, about 20% of the applied ^{15}N was lost and 20 % remained in soil. Some of the N that remains in the soil may continue to be available for subsequent uptake by the plants (Harris *et al.*, 1994; Ranells and Wagger, 1997; Shipley *et al.*, 1992; Varco *et al.*, 1989), while some may be immobilized by microbes and organic residues and become unavailable to plants (Allison, 1966). The concentration of N remaining in the soil decreases as roots absorb N and may fall below the minimum concentration for root uptake, and therefore it becomes unavailable to plants (Marschner, 1995). Unfortunately, we did not test the proportion of the 20% soil-remaining ^{15}N that was available for further plant uptake in this experiment.

Under our experimental conditions, about 60% of the N applied was recovered in trees four weeks after application. This percentage may be higher than other reported recovery rates (25–35%) (Khemira, 1995) since our study was performed with young trees in pots where experimental conditions were much better controlled than in field experiments. For example, we controlled irrigation water application to minimize N losses resulting from leaching. N losses due to leaching is reportedly one of the major reasons for low efficiency of N use in agronomic systems (Bilderback, 2002; Dinnes *et al*., 2002; Heckman, 2002; Neilsen and Neilsen 2002). In addition, our high ^{15}N recovery may be a result of timing of ^{15}N application. We applied ^{15}N in late June, a time of high N demand and high uptake of the young trees (Dong *et al*., 2001a; Faust, 1989; Neilsen and Neilsen, 2002; Shu, 1993). High root density in small pots (Lu, 1998) may also contribute to the high N recovery in this experiment. Even with good control of the leaching in the experiment, there was still about 20% of the applied N lost into the environment, possible due to soil denitrification or NH_3/NH_4^+ volatilization.

In summary, N uptake of nitrogen by young apple trees is related to their background N status. Trees with lower N status are more efficient in taking up nitrogen than those with high N status on a root-biomass basis. However, there is not much difference in total N uptake on a whole-tree basis as low N trees have a smaller root system than high N trees. A significant proportion (approximately 20%) of applied N is lost into environment within four weeks after N application even when measures are taken to minimize the loss and high root uptake efficiency is observed.

REFERENCES

ALCOZ, M. M., HONS, F. M. and HABY, V. A. (1993). Nitrogen fertilization timing effects on wheat production, nitrogen uptake efficiency, and residual soil nitrogen. *Agronomy Journal*, **85**, 1198–203.

ALLISON, F. E. (1966). The fate of nitrogen applied to soils. *Advances in Agronomy*, **18**, 219–58.

BILDERBACK, T. E. (2002). Water management is key in reducing nutrient runoff from container nurseries. *HortTechnology*, **12**, 541–4.

CHENG, L. and FUCHIGAMI, L. H. (2002). Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiology*, **22**, 1297–303.

CHENG, L., DONG, S. and FUCHIGAMI, L. H. (2002). Urea uptake and nitrogen mobilization by apple leaves in relation to tree nitrogen status in autumn. *Journal of Horticultural Science & Biotechnology*, **77**, 13–8.

CHENG, L., MA, F. and RANWALA, D. (2004). Nitrogen storage and its interaction with carbohydrates of young apple trees in response to nitrogen supply. *Tree Physiology*, **24**, 91-8.

DINNES, D. L., KARLEN, D. L., JAYNES, D. B., KASPAR, T. C., HATFIELD, J. L., COLVIN, T. S. and CAMBARDELLA, C. A. (2002). Nitrogen management strategies to reduce nitrate leaching in tile-drained Midwestern soils. *Agronomy Journal*, **94**, 153–71.

DONG, S., SCAGEL, C. F., CHENG, L., FUCHIGAMI, L. H. and RYGIEWICZ, P. T. (2001a). Soil temperature and plant growth stage influence nitrogen uptake and amino acid concentration of apple during early spring growth. *Tree Physiology*, **21**, 541–7.

DONG, S., CHENG, L. and FUCHIGAMI, L. H. (2001b). New root growth in relation to nitrogen reserves of young Gala/M26 apple trees. *Acta Horticulturae*, **564**, 365–70.

FAUST, F. (1989). *Physiology of temperate zone fruit trees*. John Wiley & Sons., London and New York.

HARRIS, G. H., HESTERMAN, O. B., PAUL, E. A., PETERS, S. E. and JAHNKE, R. R. (1994). Fate of legume and fertilizer nitrogen-15 in a long-term cropping systems experiment. *Agronomy Journal*, **86**, 910–5.

HECKMAN, J. R. (2002). In-season nitrate testing as a guide to nitrogen management for annual crops. *HortTechnology*, **12**, 706–10.

HILL-COTTINGHAM, D. G. and LLOYD-JONES, C. P. (1975). Nitrogen-15 in apple nutrition investigations. *Journal of the Science of Food and Agriculture*, **26**, 165–73.

KHEMIRA, H. (1995). *Nitrogen partitioning and remobilization in field-grown apple trees*. Thesis, Oregon State University. Corvallis, OR, USA.

LU, D. (1998). *Studies on the structures and functions of the roots of apple trees within different soil layers and the effects of media on them*. Thesis, Shandong Agricultural University, Taian, Shandong, China.

MARSCHNER, H. (1995). *Mineral nutrition of higher plants.*, (second edition). Academic Press, London and New York.

MILLARD, P. (1996). Ecophysiology of the internal cycling of nitrogen for tree growth. *Journal of Plant Nutrition and Soil Science*, **159**, 1–10.

MILLARD, P. and NEILSEN, G. H. (1989). The influence of nitrogen supply on uptake and remobilization of stored N for the seasonal growth of apple trees. *Annals of Botany*, **63**, 301–9.

NEILSEN, D., MILLARD, P., NEILSEN, G. H. and HOGUE. E. J. (1997). Sources of N for leaf growth in high-density apple (*Malus domestica*) orchard irrigated with ammonium nitrate solution. *Tree Physiology*, **17**, 733–9.

NEILSEN, D. and NEILSEN, G. H. (2002). Efficient use of nitrogen and water in high-density apple orchards. *HortTechnology*, **12**, 19–25.

RANELLS, N. N. and WAGGER, M. G. (1997). Nitrogen-15 recovery and release by rye and crimson clover cover crops. *Soil Science Society of America Journal*, **61**, 943–8.

SANCHEZ, E. E., KHEMIRA, H., SUGAR, D. and RIGHETI, T. L. (1995) Nitrogen management in orchards. In: *Nitrogen fertilization in the environment*. (Bacon, P. E., Ed.). Marcel Dekker, New York, USA. 327–80

SCHUMAN, G. E., STANLEY, M. A. and KNUDSEN, D. (1973). Automated total nitrogen analysis of soil and plant samples. *Soil Science Society of America Proceedings*, **37**, 480–1.

SHIPLEY, P. R., MEISINGER, J. J. and DECKER, A. M. (1992). Conserving residual corn fertilizer nitrogen with winter cover crops. *Agronomy Journal*, **84**, 869–76.

SHU, H. (1993). *Physiology of fruit trees*. China Agricultural Press, Beijing, China.

TITUS, J. S. and KANG, S. (1982). Nitrogen metabolism, translocation and recycling in apple trees. *Horticultural Reviews*, **4**, 204–46.

VARCO, J. J., FRYE, W. W., SMITH, M. S. and MACKOWN, C. T. (1989). Tillage effects on nitrogen recovery by corn from a nitrogen-15 labelled legume cover crop. *Soil Science Society of America Journal*, **53**, 822–7.