We studied the absorption, assimilation, translocation and distribution of nitrogen (N) from urea applied in autumn to leaves of 1-year-old potted Fuji/M26 apple (Malus domestica Borkh) trees. In early October, all leaves of each tree were painted with either 3% urea (enriched to 10 atom % with $^{15}$N) or water (control trees). Four trees were harvested before the treatment and N and amino acid contents were determined. Four trees from each treatment were harvested at 2, 4, 7, 10, 15 and 20 days after urea or water application. Total N, amino acids and $^{15}$N in leaves, bark, xylem, shank and roots were analyzed to determine uptake and mobilization of N from urea.

Most uptake of $^{15}$N by leaves occurred during the first 2 days following application of urea. The mean rate of absorption during these 2 days was 0.29 g m$^{-2}$ day$^{-1}$. Amino acids in leaves, bark and roots increased significantly after urea application compared with control values. The highest concentrations of amino acids in leaves and bark occurred 4 days after application, whereas the highest concentrations of amino acids in roots occurred 10 days after application. Total $^{15}$N content in leaves peaked 2 days after urea application and then decreased, whereas $^{15}$N content in roots and bark increased throughout the experiment. Total $^{15}$N content in xylem and shank was low. Leaves absorbed 35% of the $^{15}$N applied as urea, and 63.6% of absorbed $^{15}$N was translocated out of leaves within 20 days after urea application. We conclude that N from urea was converted to amino acids in leaves after foliar application in autumn, and roots and bark were the main sinks of N from urea applied to leaves.

Keywords: amino acids, $^{15}$N, nitrogen uptake.

Introduction

Nutrient storage in perennial tissues at the end of a growing season is an important and well-recognized characteristic of deciduous tree fruit crops (Titus and Kang 1982, Millard 1996). The amount of reserve nitrogen (N) at the end of the growing season affects tree growth and fruiting in the following season (Titus and Kang 1982, Roubelakis-Angelakis and Kliwer 1992). Initial growth of fruit trees in the spring is supported by remobilization of N reserves and there is a positive relationship between spring growth and the amount of N reserves for many species and varieties (O’Kenney et al. 1975, Millard and Neilson 1989, Neilson et al. 1997, Tagliavini et al. 1998, Cheng et al. 2001, Dong et al. 2001a). Increasing N reserves has become one of the goals of nursery and orchard management, to ensure high tree productivity.

Plant leaves readily absorb mineral nutrients and foliar application has been widely used as a method of fertilization (Swietlik and Faust 1984, Gooding and Davies 1992, Bondada et al. 2001, Johnson et al. 2001). Urea is considered the most suitable form of N for foliar application because of its non-polarity, rapid absorption, low phytotoxicity and high solubility (Wittwer et al. 1963, Yamada et al. 1965, Knoche et al. 1994, Bondada et al. 2001). Numerous reports have shown that urea applied to leaves increases the N content of tissues and improves leaf color and shoot growth (Shim et al. 1972, Hill-Cottingham and Lloyd-Jones 1975, Klein and Weimbaum 1984, Swietlik and Faust 1984, Rosecrance et al. 1998, Tagliavini et al. 1998, Bondada et al. 2001, Johnson et al. 2001). Although urea spray can be used at any time during the growing season and even during the dormant season, autumn application may be most effective for deciduous trees because high urea concentrations can be used with minimal concern about phytotoxicity (Johnson et al. 2001). Foliar application of urea in the autumn can increase N reserves and therefore improve flowering, fruit set and growth in the following season (Oland 1963, Shim et al. 1972, Han et al. 1989, Sanchez et al. 1990, Khemira et al. 1998, Rosecrance et al. 1998, Dong et al. 2001b, Cheng et al. 2002). Most studies have focused on plant N status and growth in response to urea spray, and few studies have measured the controlling parameters necessary to predict the effects of urea spray, such as N absorption rate based on leaf area, and N assimilation and translocation. Absorbed urea may be hydrolyzed within leaves (Dilley and Walker 1961) or transported out of the leaves to be hydrolyzed in other plant or-
gans (Freiberg and Payne 1957). Shim et al. (1972) found that soluble N in leaves after post-harvest urea spray was present as urea rather than amino acids, indicating that hydrolysis of urea and assimilation of the resulting N may not occur in leaves when urea is applied in autumn.

To make the most effective use of foliar applications of urea in autumn to increase tree N storage and regulate N distribution, a better understanding of how trees absorb, assimilate and translocate N among different tissues is needed. In this study, we used $^{15}$N-urea to assess uptake, translocation and distribution of N from urea after application in the autumn to leaves of apple (Malus domestica Borkh) nursery trees. Our objectives were to determine: (1) the rate at which N, applied as urea, is absorbed by leaves; (2) the location of assimilation of N from urea; (3) the rate of N translocation; and (4) the partitioning of N after translocation.

**Materials and methods**

**Plant materials**

One-year-old bench-grafted Fuji/M.26 apple (M. domestica) trees were planted in 4-dm$^3$ plastic pots containing a 1:1:1 (v/v) mix of peat moss, perlite and loam soil at Oregon State University in Corvallis, OR. The trees were grown outdoors from April to October 1998. Each tree was pruned to a single shoot and fertilized with 10 mol m$^{-3}$ of nitrogen in a 20:10:20 N,P,K formulation injected into the irrigation water once every 2 weeks from May until mid-August. In early October, uniform trees were selected based on height and stem diameter for the experimental treatments.

**Treatments and sampling**

To obtain consistent leaf status for each tree, the number of leaves on selected trees was adjusted to 22 by manually removing extra leaves (5–10) from the bottom. Trees were randomly divided into two groups of 28. On October 6, both surfaces of all leaves in one group of trees were evenly painted with 3% urea (enriched with $^{15}$N to 10 atom %; ICON, Mt. Marion, NY), and the leaves of trees in the other group were painted with water to serve as controls. Five additional trees were painted in the same way with a 3% solution of regular urea rather than amino acids, indicating that hydrolysis of urea and the resulting N may not occur in leaves when urea is applied in autumn.

Analytical methods

For each tree type at each harvest, total N, total free amino acids and $^{15}$N abundance were determined. Total N (mg kg$^{-1}$ dry mass) was determined by Kjeldahl analysis (Schuman et al. 1973) by the Central Analysis Laboratory of Oregon State University. Total free amino acids (mg kg$^{-1}$ dry mass) were determined by the ninhydrin assay (Yemm and Cocking 1955). Briefly, samples were extracted with 10% acetic acid and the extract was treated with ninhydrin reagent. Absorbance of the resulting product was measured at 580 nm (UV-160, Shimadzu, Kyoto, Japan). Absorbance values were converted to mg dm$^{-3}$ from a standard curve and then multiplied by the total extraction volume to obtain the amino acid concentration in each tissue (mg kg$^{-1}$). The amount of $^{15}$N in samples was determined by isotope ratio with a mass spectrometer (Carlo Erba NC 2500, Carlo-Erba/Fisons Instruments, Valencia, CA). The percentage of N derived from urea fertilizer in each tissue (NDFF; %) was calculated as described by Cheng et al. (2002). On a whole tree basis, NDFF was calculated as total plant $^{15}$N content divided by total plant N content. The $^{15}$N absorption rate at each harvest was calculated as the difference between $^{15}$N content at each harvest and mean $^{15}$N per plant at the previous harvest, divided by leaf area and time between the two harvests (g cm$^{-2}$ day$^{-1}$). The rate of $^{15}$N export from leaves was calculated as the percent reduction in leaf $^{15}$N content between two harvests divided by time (% day$^{-1}$). Nitrogen-use efficiency was calculated as the percentage of total $^{15}$N applied to the leaves that was absorbed by the tree.

**Statistical analyses**

The experiment was a randomized design, with 56 trees randomly divided into two treatment groups (urea and control) and four replicates per treatment at each harvest date. Total N and amino acid data were subjected to a two-factor (urea treatment and harvest date) analysis of variance (ANOVA) to determine differences between urea-treated plants and controls over time. Data for $^{15}$N and NDFF% were subjected to a one-factor (harvest date) ANOVA to determine the effect of time on measured variables. Differences between means were assessed by Fisher’s Protected LSD test. All statistical analyses were performed with NCSS 1997 Statistical System Software (NCSS Statistical Analysis Software, Kaysville, UT).

**Results**

**Uptake of N**

Leaves rapidly absorbed $^{15}$N after foliar application of $^{15}$N-urea in the autumn. The highest rate of $^{15}$N absorption occurred during the first 2 days after application. The mean absorption rate was 0.29 g m$^{-2}$ day$^{-1}$ in the first 2 days, then decreased to 0.03 g m$^{-2}$ day$^{-1}$ by Day 4 (Figure 1). The
absorption rate decreased to 0.002 g m⁻² day⁻¹ by Day 7 after application, and thereafter ¹⁵N uptake was negligible.

Plants receiving ¹⁵N-urea had a higher total N content than control plants (Figure 2A). The ¹⁵N derived from the applied urea (NDFF) accounted for about 14.5% of the total N content (Figure 2B).

Translocation of N

Concentrations of amino acids in leaves increased rapidly during the first 2 days after urea application and peaked by Day 4 (Figure 3A). The mean rate of increase in amino acid concentration in leaves was about 275 mg kg⁻¹ day⁻¹ in urea-treated plants during the first 4 days after urea application. Amino acids in the bark increased after foliar urea application, with a peak on Day 4 (Figure 3B). The mean rate of increase in amino acid concentration was lower in bark (112 mg kg⁻¹ day⁻¹) than in leaves during the first 4 days after urea application. Amino acid concentrations in roots increased during the first 10 days after urea application (Figure 3C); however, the mean rate of increase in amino acid concentration during the first 4 days after urea application was lower in roots (25 mg kg⁻¹ day⁻¹) than in leaves and bark. Amino acid concentrations were much lower in xylem and shank than in leaves, bark and roots, although there was an increase in response to the urea treatment when compared with control values (Figures 3D and 3E).

The ¹⁵N content of leaves peaked on Day 2 after urea application and then decreased, whereas ¹⁵N content of bark and roots increased throughout the 20-day experiment (Figure 4). Export of ¹⁵N from leaves occurred throughout the experiment, but the export rate decreased with time (Figure 5). Twenty days after foliar urea application, 63.6% of absorbed ¹⁵N had been exported from leaves.

Distribution of N

There was little ¹⁵N in the xylem and shank tissues. These two tissues together accounted for only about 2% of total absorbed ¹⁵N at the end of the experiment. The proportion of ¹⁵N in bark and roots increased during the experiment, whereas the proportion of ¹⁵N in leaves decreased (Figure 4). However, about 36% of the absorbed N remained in leaves at the end of the study.

Nitrogen-use efficiency

Complete painting of the abaxial and adaxial surfaces of all leaves with 3% urea solution supplied about 248 ± 20.7 mg N to each tree, and each plant recovered about 87 ± 6.8 mg of applied N during the 20-day experiment. Nitrogen-use efficiency, defined as the amount of absorbed N as a percent of applied N, was 35%.

Discussion

Most plants can rapidly absorb urea applied to leaves. Under favorable conditions, about 60–70% of applied urea is ab-
sorbed by olive leaves within 24 h (Klein and Weinbaum 1984). Peach and nectarine leaves rapidly absorb applied urea, irrespective of application date (Rosecrance et al. 1998). In this experiment, we found that apple leaves also quickly absorbed urea applied in autumn.

Numerous experiments have shown that nitrogen-use efficiency (N recovered as a percent of N applied) is higher when N is applied to the leaves than when it is applied to the soil. Weinbaum (1988) reported that N recovery was typically about 60% following foliar application. About 16% of soil-applied $^{15}$N—applied as potassium nitrate—and almost 47% of foliar-applied $^{15}$N—applied as urea—were recovered in potted apple trees (Hill-Cottingham and Lloyd-Jones 1975), whereas Shim et al. (1972) observed that senescing apple leaves absorbed 80% of applied urea within 48 h. Peach trees recovered about 48–58% of urea-N intercepted by the canopy (Rosecrance et al. 1998), and similar percentages were reported in young potted nectarine trees (Tagliavini et al. 1998). The recovery rate of N in our study was 35%, which is lower than most published values. One reason for the low recovery rate in our study may have been that the leaves were N replete (with an N concentration of 1.9% at the start of the experiment). Leaf N status has been shown to affect the efficiency of absorption of urea applied to leaves (Cheng et al. 1999, 2002). The low recovery rate may also have been a result of the lack of surfactant in the urea solution. Previous studies used various surfactants. Some of the higher published values of N recovery were estimated as the difference between applied N and residue N washed from leaf surfaces after absorption (Shim et al. 1972); however, some N loss, such as volatilization, is ignored in such estimates, and therefore the recovery rates may be overestimated. In contrast, we directly measured $^{15}$N absorbed from urea by each tissue.

Following urea application, amino acid concentrations increased quickly in leaves (Figure 3), indicating that the conversion of N from urea to amino acids occurred mainly in leaves. After applied urea was absorbed by leaves, it was broken down to $\text{NH}_4^+$ and assimilated into amino acids that were then translocated to tissues where N was needed (Dilley and Walker 1961, Swietlik and Faust 1984). Shim et al. (1972) observed no amino acid accumulation in senescing leaves after application of urea to apple trees in growth chambers. They observed parallel increases in soluble N and urea in leaves after urea application, and concluded that the bulk of the soluble N was in the form of urea (Shim et al. 1972). The difference

Figure 3. Total amino acid concentrations in leaves (A), bark (B), roots (C), xylem (D) and shank (E) tissues following autumn application of urea to leaves of Fuji/M26 apple trees. Three percent $^{15}$N-urea was painted on both surfaces of each leaf on October 6. Error bars represent standard errors of the mean of four replicates.
between our results and those of Shim et al. (1972) may be a result of different culture conditions.

Amino acid concentration peaked in bark on Day 4 after urea application and was highest in roots on Day 10, whereas 15N in these two tissues increased continuously during the experiment. This suggests that some of the amino acids in the bark and roots were transformed into other forms of N such as storage proteins. Other researchers have also found that when urea was applied to leaves in the autumn, N from urea was preferentially translocated to perennial tissues for storage because of cessation of active growth in the canopy (Swietlik and Faust 1984, Rosecrance et al. 1998, Johnson et al. 2001).

Distribution of foliar-applied N is known to change with time of application. Nitrogen from urea absorbed after a spray in June was retained mainly in leaves of potted apple trees (Hill-Cottingham and Lloyd-Jones 1975), whereas N was rapidly transported to perennial parts when urea was sprayed in autumn (Shim et al. 1972, Titus and Kang 1982, Swietlik and Faust 1984, Rosecrance et al. 1998, Tagliavini et al. 1998). Varying the date of foliar urea application from September to November did not affect percentage recovery of N from urea in peach trees, but did change the partitioning of absorbed N. More N from urea was recovered in perennial plant parts, and less in leaves that had abscised, following application in September or October compared with application in November (Rosecrance et al. 1998). We found that about two thirds of absorbed N from urea applied to leaves in autumn was nearly equally distributed in bark and roots, with little in xylem. Hill-Cottingham and Lloyd-Jones (1975) reported that fertilizer N was almost equally distributed between xylem wood and bark when sampled in February after application of 15N to either soil or foliage in the previous October. The difference between our results and those of Hill-Cottingham and Lloyd-Jones (1975) may be due to different sampling times. We took samples within 20 days after urea application, whereas they took samples about 4 months later, in February. It is possible that the distribution of 15N that they measured in February was a result of remobilization of N from roots to xylem for new growth.

We observed normal, but delayed, leaf senescence after urea application. Urea-treated leaves were greener than control leaves at the time of sampling. The urea-treated leaves may have had higher photosynthetic activity and may have produced more carbohydrates than the control leaves, which could supply more energy to the root system and keep roots more active late in the season. As a result, root N uptake might be enhanced by the foliar urea spray. We found that total N content increased by about 160 mg following the application of urea (Figure 2). About 90 mg came directly from 15N-urea, whereas the remainder may be associated with enhancement of root N uptake as a result of the application of urea to leaves in autumn.

There are conflicting reports in the literature concerning the efficiency of absorption of N from urea, the location of N assimilation, the rate of N translocation and the partitioning of N to different parts of apple trees. We found that leaves of young Fuji/M26 apple trees rapidly absorbed N from urea during the first 2 days after foliar application in the autumn. Absorbed N from urea applied to leaves in autumn was converted to amino acids in leaves, and then translocated to bark and roots. The amino acids in bark and roots were partially transformed into other N compounds such as storage proteins. Bark and roots were the primary sinks for N from urea sprayed on leaves in autumn. Root N uptake was enhanced by application of urea to leaves late in the season.
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References


