UPTAKE EFFICIENCY AND INTERNAL ALLOCATION OF NITROGEN IN APPLE TREES

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Abstract

Improved nitrogen (N) uptake efficiency (NUE) can lead to better economic and environmental outcomes. The physiological processes of N storage and remobilisation within deciduous fruit trees are relatively well understood. However, much can be gained through better understanding of management and environmental factors on these processes. This study aimed to determine the influence of pre- and post-harvest N application on NUE and the partitioning of N within the tree determined at winter dormancy. Fifteen-year-old potted 'Jonagold' trees, grafted on M26 rootstock were allocated to either pre- or post-harvest 5% ^{15}N enriched calcium nitrate application. Fruit was harvested at commercial harvest. At winter dormancy, each tree was destructively harvested with plant material allocated to either roots, stem or branches. ^{15}N recovery from dried plant samples was determined using Isotope Ratio Mass Spectroscopy. Nitrogen uptake efficiency was significantly greater with pre-harvest N application at 64.5% of applied N compared to post-harvest applied N at 50.5%. Timing of N application significantly influenced N distribution throughout the tree. Over 30% of pre-harvest N was recovered from fruit. The proportion of recovered N allocated to roots, stem and branches was significantly greater for post – harvest N application with leading to increased N storage reserves. This study shows that greater uptake efficiency can be achieved with pre-harvest N application, but that the majority of this was allocated to the fruit. This may present some risk to fruit quality outcomes as high fruit N has been shown to delay maturity, limit red colouration and lead to softer fruit that stores relatively poorly. Results from this study improve the current understanding of the influence of timing of N application on allocation and storage within deciduous fruit trees.

Keywords: ^{15}N, partition, stable isotope, calcium nitrate, pre- and post-harvest harvest

INTRODUCTION

Fertilizer use efficiency is a critical aspect of orchard management that can improve both yield and fruit quality. A constituent of many essential aspects of growth including nucleic acids, proteins, hormones and chlorophyll (Reuter and Robinson, 1997), nitrogen (N) is one of the most abundant elemental nutrients by dry weight found in plants. As a result, N typically contributes one of the largest portions of fertilizer expense within apple orchard production. Improving nitrogen uptake efficiency (NUE) offers potential for significant savings on the cost of production and greater and NUE improvements result in reduced environmental leaching and subsequent unintended movement off site.

The significance of N remobilization to growth in deciduous fruit trees is well known (Cheng et al., 2004; Tagliavini and Millard, 2005). During early season growth, remobilization of stored N from the previous season supplies actively growing regions until the production of small fruit occurs (Tagliavini and Millard, 2005). As the quantity of stored N is depleted, the tree relies on root uptake to meet the N demand of vegetative and fruit growth (Cheng and Raba, 2009b). As the winter dormant phase approaches, N is withdrawn from the leaves

and actively partitioned into storage organs (Tagliavini et al., 1999). The resorption of N from leaves prior to senescence has been found to contribute significantly to total stored N (Castagnoli et al., 1990; Muñoz et al., 1993; Tagliavini et al., 1998) to a range of specific proteins within the bark, branch and root material of the tree until remobilization the following season (Tromp, 1983).

In order to isolate and analyse current season N application from previously supplied N, the stable isotope ^{15}N can be used. As the less abundant of the two stable isotopes of N, ^{15}N contributes with 0.366% to total N in the atmosphere. Due to the differing molecular weights the two forms can be determined in high precision mass spectrometers, a technique known as Isotope Ratio Mass Spectrometry (IRMS). The aim of this study was to use the ^{15}N isotope dilution method as a tool to investigate how altering the timing of N application influences NUE over the course of a growing season and the quantity of N allocated to storage at dormancy.

MATERIALS AND METHODS

Plant material

The trial utilised fifteen-year-old 'Jonagold' trees, grafted on M26 dwarfing rootstock which were maintained in 45 L pots and pruned to a central leader. The trees selected were of uniform size, shape and general health prior to the experiment. Prior to the treatment application, the majority of the existing potting mix was removed and replaced with well drained low nutrient potting mix (90% composted pine bark, 5% cocoa peat, 5% sand, 3kg per ½ cubic meter of lime). The N content of the pots was analysed in order to determine the background N content prior to treatment application. All pots were fitted with catch trays to allow leachate to be reapplied to the pot. Watering was supplied uniformly to all trees as required for the length of the trial. All trees were covered using 20 mm netting to prevent wildlife damage.

Fertiliser treatments

The plant-available soil N was artificially enriched making use of ^{15}N-labelled fertiliser. Apple trees were randomly assigned to one of three ^{15}N treatments, which consisted of: pre-harvest application; post-harvest application; and a control in which no ^{15}N was applied. Harvest was determined when fruit would normally be removed for commercial harvest. ^{15}N application was made via 5% enriched calcium nitrate supplied by Cambridge Isotope Laboratories. Five weekly applications of 4.8 g calcium nitrate/tree in 1 L of water were made, totalling 24 g calcium nitrate per tree for both the pre-harvest and post-harvest treatments, initiated on the 28/10/2014 and 17/3/2015 respectively. At treatment application, control trees received 1 L of water. Treatments were applied to four trees, each designated as a replicate.

Destructive harvest

Fruit from all trees were removed on the 25/2/2015 aligning with commercial harvest. Fruit wet weights were recorded before sub samples were dried for analysis. At winter dormancy all trees were destructively harvested to determine recovery of ^{15}N. Destructive harvest consisted of removing the entire tree from the pot ensuring all biomass was retrieved. The upper and lower halves of the tree structure were separated at the graft join. Using water at low-pressure, the root system was thoroughly cleaned to remove the vast majority of soil. Remaining soil was removed by soaking the root systems overnight in individual water baths before air-drying.

The soil from each pot was collected as it was removed from the root system. Soil was mixed thoroughly before being quartered. Three quarters were discarded and the quartering process was repeated until a sample of approximately 100 g remained. This 100 g sample was then air dried for a two-week period in the laboratory. Once dried the dry weight equivalence of the soil was determined by initially heating to 40 ^{0}C for 24 hr followed by heating to 105 ^{0}C

for 24 hr. Sub samples were taken for ^{15}N content analysis as described in detail below. Calculating the total soil mass to estimate overall ^{15}N recovery from the soil was completed by determining the bulk density of the soil and scaling this value to the volume of the pot.

Trees were separated into the stem (the whole trunk including below the soil line to the primary roots), branches (including buds), roots (all roots branching off the primary root), fruit and soil. Care was taken to ensure there was no contamination across the varying categories. Total weight of the tree was recorded as the sum of the two parts (above and below the graft) before separating different components. The wet weight of each biomass category was recorded before random sub-samples were taken for oven drying and analysis.

Laboratory and data analysis

All samples were oven dried at 60 °C, then ground using a Retsch MM200 ball mill at an oscillation frequency of 30 Hz for 3 min. Generally, between 0.4 to 0.7 mg of sample were weighed in tin capsules and nitrogen stable isotopes analyzed using flash combustion isotope ratio mass spectrometry (varioPYRO cube coupled to Isoprime100 mass spectrometer) at the Central Science Laboratory, University of Tasmania. Stable isotope abundances are reported in delta (∂) values as the deviations from conventional standards in parts per mil (‰) from the following equation:

$$\partial\ ^{15}N\ (‰) = [(^{15}N/^{14}N\ \text{sample}/^{15}N/^{14}N\ \text{standard} - 1) \times 1000]$$

$\delta^{15}N$ values are reported respective atmospheric air. As recommended by IUPAC (Coplen et al., 1992), the value of 272 was employed for $^{14}N/^{15}N$ of N_2 in air for the calculation of atom fraction ^{15}N from measured $\delta^{15}N$ values; the applied formula (Hauck, 1982), is valid for low enrichments (<5%).

Enriched laboratory standards were prepared from mixtures of enriched and natural abundance fertilizer and calibrated against International Reference standard IAEA311 (Danso et al., 1983). The analytical performance of the instrumentation, drift correction and linearity performance were calculated from the repetitive analysis of these standards. Precision was 0.06 % at the highest enrichment level.

Current season N was determined as the ^{15}N component of the sample calculated using a modified equation from Millard and Nielsen (1989) as follows: A = (B*C)/D, where A = Total ^{15}N from fertilizer (g), B = atom ^{15}N excess in tissue, C = total N content of tissue (g), D = atom % ^{15}N excess in fertilizer. B was corrected for background ^{15}N enrichment using the value of 0.366 atom percent as natural abundance. Calculating values for D were also corrected for natural abundance of ^{15}N with the value of 0.366 atom percent (Danso et al., 1983).

Nitrogen uptake efficiency was determined as the percentage of ^{15}N recovered from the applied and was compared statistically using an independent sample T-test in SPSS statistical package 22, IBM, USA. Unrecovered ^{15}N was calculated as the proportion of ^{15}N not recovered in the plant or soil from the applied. Allocation of ^{15}N to storage was determined by comparing differences in ^{15}N recovery from storage organs including trunk, branches primary root and secondary roots. Fertiliser recovery was standardised for tree size using dry weight measurements.

RESULTS AND DISCUSSION

Nitrogen uptake efficiency

At winter dormancy, an average of 64.5% of the pre-harvest applied ^{15}N was recovered from the plant organs (including harvested fruit) when destructively harvested (Figure 1). This was significantly greater than the 50.5% of the ^{15}N applied post-harvest. There was no difference in the recovery of ^{15}N remaining in the soil from the pots at the time of destructive harvest. Whilst recovery of ^{15}N from the plant and the soil accounted for all the ^{15}N that was applied pre-harvest, approximately 16% of the post-harvest applied ^{15}N was unrecovered.

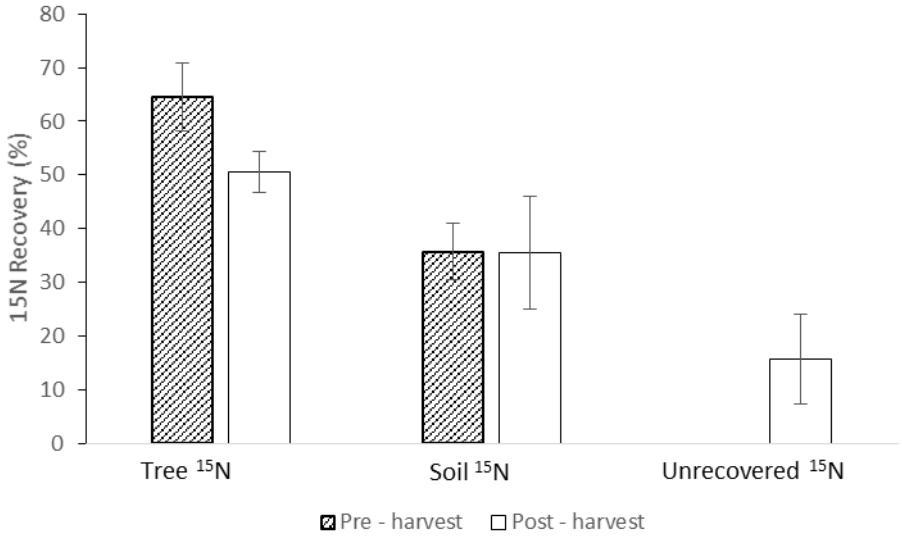

Figure 1. Comparison of nitrogen uptake efficiency (NUE) between pre- and post-harvest ^{15}N treatments as demonstrated by the proportion of recovered N from the plant and soil. Error bars denote standard error and letters above bars indicate significant differences between treatments.

Nitrogen uptake efficiency for apples and other deciduous tree fruit crops using this technique has been reported in a few studies. Our results are slightly higher than previously reported (20 – 30%) in field studies of young apple trees by Khemira et al., (1998) and Neilsen et al., (2001) but very similar to those reported for seven year-old cherry trees where NUE was significantly higher with spring rather than summer application (65.7% vs. 37.44%) of labelled ammonium nitrate (San-Martino et al., 2010). It is likely that pot trials such as ours will have higher rates of recovery given the ability to control for fertiliser losses and as such may overestimate NUE that would occur in orchard conditions.

Nevertheless, our results indicate there is strong demand for pre-harvest N during the active growing period. The influence of remobilization of stored N is well known to contribute the majority of N to early season growth (Cheng and Raba, 2009a; Grassi et al., 2002; Millard, 1996). Grassi et al., (2002) showed that remobilization solely supplied N to actively growing organs for the first 25-30 days of spring growth in sweet cherry, *Prunus avium* L. Following this period, uptake of soil N increasingly contributed N supply until approximately 40 to 60 days after initial growth where stored reserves are typically depleted. Considering the lengthy period of remobilisation and relatively high N uptake found in pre-harvest N application, it can be inferred that there is strong sink demand at this stage. This is likely explained by the growth of fruit and possibly leaves, consistent with previous studies (Munoz, 1993, Tromp, 1983). Aiding N uptake, increasing soil temperature facilitates the absorption of soil N as the season progresses (Dong et al., 2001), as well as increased metabolic rate and root permeability (Bowen, 1991). These findings indicate that pre-harvest N application gives potential for significantly higher overall NUE when compared with the typically cooler (and sometimes higher rainfall) climatic conditions post apple harvest.

As the pre-harvest fertiliser treatment commenced in late October, there was a considerably longer period of time for N uptake to occur. Within this longer time period there is greater potential for N loss through either leaching or gaseous loss of N as N_2 or N_2O (Swarts et al., 2016). Thus it is important to interpret our results in the context of leachates being re-used in contrast to the field situation of leachates being lost, once below the root zone. Indeed, our data shows that we could account for 100% of the pre-harvest applied N. The unrecovered

component of the post-harvest applied N was unexpected and difficult to explain, but most likely due to overflow of leachate from a high rainfall event and/or volatilisation.

Nitrogen partitioning and storage

The proportion of recovered pre-harvest applied ^{15}N following destructive harvest at dormancy was greatest in the fruit at 30.1%, followed by the roots at 29.5%, stem at 26.1% and branches at 14.3% (Figure 2). The pattern of ^{15}N recovery from tree organs receiving post - harvest applied N was substantially different, with 40.7% of the recovered ^{15}N found in the roots, 31.9% in the stem and 27.3% in the branches. For the roots, stem and branches, recovery proportions were significantly higher with post-harvest fertiliser application than pre-harvest, where almost a third of the recovered fertiliser was partitioned to the fruit.

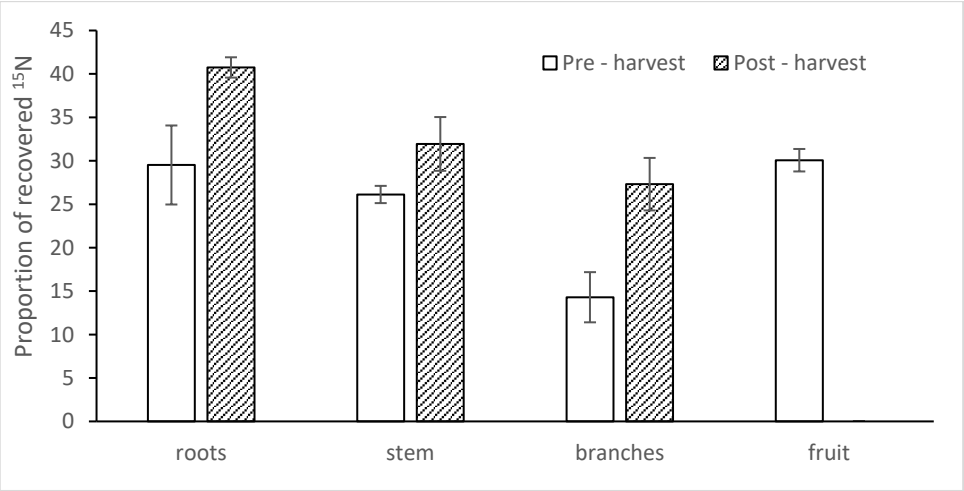

Figure 2. Proportion of recovered ^{15}N from pre and post-harvest application partitioned to roots, stem, branches and fruit of 15-year-old 'Jonagold' apple trees.

These findings show that the timing of application influences the relative allocation of N to different regions of the tree. The canopy (branches and fruit) of the pre-harvest fertiliser applied trees accumulated more than half of the total ^{15}N recovered, in comparison with post-harvest application where just over a quarter of recovered ^{15}N was found in the canopy. This demonstrates the greater sink strength of the fruit and leaves in the pre-harvest treatment. It is well established that fruit development accumulates a large fraction of available N within the tree (Cheng and Raba, 2009a; Guak et al., 2003; Muñoz et al., 1993; Neilsen et al., 2006).

The branches, stem and roots of deciduous trees are typically known as storage organs to which N is withdrawn prior to autumn leaf senescence. As a greater proportion of recovered N was found in these organs following post-harvest fertiliser application, it is likely that this fertiliser strategy may result in greater N availability for the following season's early spring growth (Cheng and Raba, 2009a; Guak et al., 2003; Muñoz et al., 1993; Neilsen et al., 2006). However, the capacity to transport soil accumulated N into the canopy in a relatively short time from fertiliser application is demonstrated, given that over 25% of recovered ^{15}N was found in the branches of post-harvest applied trees. We speculate that withdrawal of N into storage organs would have commenced in late April, only a week or so after the final treatment application.

CONCLUSIONS

Post-harvest N application resulted in a greater proportion of applied N allocated to storage. In contrast, whilst higher NUE was found from pre-harvest application, a significant portion of this N is utilised for fruit development and thus removed during harvest. In this trial we were not attempting to achieve an optimised fertiliser strategy, but rather to compare

the effects of pre- or post-harvest N application on NUE and N allocation during distinct ontogenetic stages of the tree. Our data suggests that to meet tree demand, a greater proportion of total fertiliser should be applied pre-harvest with the balance applied post-harvest to facilitate storage for the following season. Future research using ^{15}N should take a multi-season approach to study the benefits to yield and fruit quality of increased storage associated with post-harvest applied fertiliser.

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