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The Proceedings of the International Plant Nutrition Colloquium XVI

Title

EFFECT OF TIMING OF NITROGEN APPLICATION ON NITROGEN UPTAKE AND PARTITIONING IN POTTED WALNUT (JUGLANS REGIA L.) TREES

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Publication Date

2009-07-13

Peer reviewed

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Introduction

Deciduous fruit trees assimilate and store nitrogen (N) in perennial organs and remobilize it for spring growth of new tissues (Millard, 1996). Pear (Sanchez et al., 1991) and apple (Khemira et al., 1998) allocate N applied near bloom preferentially into leaves and fruits, while N applied at harvest is partitioned mostly to the roots (Toselli et al., 2000) and only a small portion to the above ground structures (Sanchez et al., 1990). Also in walnut, spring remobilization of N stored during previous years sustains new shoot growth, accounting for 54% of total N in potted walnut trees (Frak et al., 2002), 60% in a commercial, 9 year-old, cv Hartley orchard (Weinbaum and Van Kessel, 1998), and up to 95% of total leaf N in two-year-old hybrid (*J. nigra x J. regia*) (Frak et al., 2006). On the other hand the labeled fertilizer applied in February (two months before catkins maturation) was not detected in the early-spring maturing catkins, whereas, organs developing or sampled later during the season exhibited progressively greater labeling (Weinbaum and Van Kessel, 1998). The knowledge of the dynamics of N absorption, its root uptake efficiency and partitioning within tree are important to optimize orchard N management reducing N inputs and safeguarding the environment. The objectives of the present work were to evaluate the efficiency of root uptake of N applied in different times of the growing season and the subsequent contribution of these sources of N, temporary stored in perennial organs, for spring N remobilization and availability for new growth.

Materials and Methods

The investigation was conducted in 2007 and 2008 at the experiment station of the University of Bologna, in Cadriano (44°35' N, 11°27' E) on walnut (*Juglans regia*, L.) trees cv Chandler grafted on seedlings. Thirty, two-year-old trees were potted in February 2007 in 40-L pots filled with a mixture of clay loam Bathicalci Eutric Cambisols soil and sand (2:1) and were uniformly pruned to leave 2 shoots per plant. On April 21 2007, when buds were bursting (bud burst fertilization), 10 plants were addressed with 1 g N/plant dissolved in water as ammonium nitrate ($^{15}NH_4{}^{15}NO_3$) in which both ammonium and nitrate were enriched with ^{15}N (5 atom%). On May 24 (pistillate flower maturity) and on September 12 (late summer) other 2 sets of 10 plants were fertilized as above. To prevent any risk of N leaching each pot surface was protected from the atmospheric precipitations, and trees were manually irrigated. Seven days after each treatment, 5 plants were collected and separated into roots, stem above the grafting union, shoots and leaves. Samples of all tree fractions were dried, weighed and milled prior to ^{15}N analysis. On December 7 2007, the other 5 trees for each set, were carefully removed from the pot and, after washing the root system to remove all ^{15}N soil sources, two trees each group were harvested and separated in roots, stem portion above the grafting union and current year grown shoots, while the other 3 trees were planted in fresh, uncontaminated soil and arranged outdoors until harvest, scheduled after pistillate flower formation of 2008 (May 19). Trees were harvested and divided into roots, stem above the graft union, one year old twigs, shoots, leaves and developing fruits. Total N and ^{15}N enrichment were determined with an elemental analyzer EA1110, Carlo Erba (Milan, Italy) instrument coupled with a Finningan Delta Plus (Bremen, Germany) mass spectrometer. The percentage of N derived from fertilizer (NDFF) was evaluated in each organs according to the following formula:

(excess atom% in the sample - natural atom%)/(atom% in the fertilizer- natural atom%)*100,

where ^{15}N natural atom% was equal to 0.366. The amount of labeled N in the different tree organs was determined by multiplying organ mass by N concentration by the percentage of NDFF. The N uptake efficiency (NUE), 1 week after fertilization and at the end of the trial,

was evaluated dividing the total amount of labeled N found in the tree by the amount of N applied with fertilizer (1000 mg/pot). For this purpose the stem weight included also the stem portion below the graft union.

At each sampling time, data were statistically analyzed as in a factorial experimental design with 2 factors: time of fertilization (3 levels: bud burst, pistillate flower and late summer) and tree organs (3, 4 or 6 levels according to the sampling time). Data were analyzed using analysis of variance. Statistically significant ($P \leq 0.05$) differences among means were separated by Student Newman Keuls (SNK) test. When interaction between time of fertilization and tree organs was significant, the effect of time of fertilization was evaluated separately for each organ.

Results

One week after fertilizations tree dry weight (DW), similar at each sampling time (data not tabulated), was made mainly of roots, followed by leaves that were heavier than stem and shoots. The percentage of NDFF, similar in root, stem, herbaceous shoot and leaves, was higher in trees fertilized in late summer, followed by those fertilized at pistillate flower maturity and at bud burst (Tab. 1). The amount of labeled N, found preferentially in leaves and roots than in stem and shoots, was higher in organs of trees fertilized at pistillate flower maturity and late summer, than in those fertilized at bud burst (Tab. 1). The NUE was higher in trees fertilized at pistillate flower maturity than at bud burst (Tab. 1). Time of fertilization and organs interacted positively with N concentration that was higher in leaves collected 1 week after bud burst (3.10%), followed by those harvested after pistillate flower maturity (2.38%) and finally by those collected after late summer (1.67%) N application (data not tabulated).

Tab. 1. Effect of time of fertilization on the percentage of N derived from fertilizer (NDFF), amount of labeled N found in different tree organs, and N uptake efficiency (NUE) 7 days after N application

TIME OF FERTILIZATION	NDFF (%)	Labeled N (mg)	NUE[1] (%)
Bud burst	4.54c	8.06b	4.19
Pistillate flower	16.5b	39.9a	21.6
Late summer	25.6a	33.0a	n.d.
Significance	***	**	*
ORGAN			
Roots	10.7	43.7a	
Stem	14.0	3.83b	
Shoots	18.8	11.7b	
Leaves	13.0	50.3a	
Significance	*ns*	***	
Interaction	*ns*	*ns*	

n.s., *,**, ***: effect not significant or significant at $P \leq 0.05$, at $P \leq 0.01$, and at $P \leq 0.001$, respectively. Values followed by the same letter are not statistically different (at $P \leq 0.05$). [1]Stem dry weight included the portion below the grafting union that accounted for 79.8 g and 81.7 g for bud burst and pistillate flower fertilizations, respectively. n.d.: not detected.

In December, the N concentration and the percentage of NDFF were higher in trees fertilized at bud burst and late summer than at pistillate flower maturity, however they were similar in the 3 organs analyzed (Tab. 2). The amount of labeled N found in each organ was not affected by the timing of fertilization and was higher in roots than in stem and shoots (Tab. 2).

Tab. 2. Effect of time of fertilization on N concentration, percentage of N from fertilizer (NDFF) and labeled N removed by different tree organs, in December.

TIME OF FERTILIZATION	N (%)	NDFF (%)	Labeled N (mg)
Bud burst	0.924a	38.0a	128
Pistillate flower	0.667b	28.3b	75.9
Late summer	0.915a	38.4a	98.6
Significance	*	*	*ns*
ORGAN			
Roots	0.765	33.1	192a
Stem	0.772	33.4	64.6b
Shoots	0.969	38.2	45.7b
Significance	*ns*	*ns*	**
Interaction	*ns*	*ns*	*ns*

n.s., *, **: effect not significant or significant at P ≤0.05 and P ≤0.01, respectively. Values followed by the same letter are not statistically different (at P ≤0.05).

In May 2008, organ DW, percentage of NDFF and amount of labeled N removed by tree organs were not affected by time of fertilization (Tab. 3). Nitrogen concentration was higher in organ of trees fertilized in late summer followed by bud burst and finally by pistillate flower maturity (Tab. 3). Roots showed a higher DW than leaves which presented a higher DW than shoots and developing fruits (Tab. 3). The concentration of N was higher in developing fruits, followed by leaves, shoots, roots, and stem and twigs (Tab. 3). The percentage of NDFF was higher in developing fruits, leaves and shoots than in roots and stem (Tab. 3). The higher amount of labeled N was found in roots, followed by leaves and finally by twigs, stem, developing fruits and shoots (Tab. 3); NUE was similar for the 3 application times (Tab. 3).

Tab. 3. Effect of time of fertilization on dry weight (DW), N concentration, percentage of N from fertilizer (NDFF), ^{15}N removal of different tree organs, and N uptake efficiency (NUE) in May of the following year.

TIME OF FERTILIZATION	DW (g)	N (%)	NDFF (%)	Labeled N (mg)	NUE[1] (%)
Bud burst	24.5	1.29b	35.1	83.6	66.4
Pistillate flower	31.6	1.09c	35.4	76.6	60.0
Late summer	25.0	1.48a	39.7	97.9	69.1
Significance	*ns*	***	*ns*	*ns*	*ns*
ORGAN					
Roots	115a	0.873d	28.2b	272a	
Stem	12.8bc	0.554e	27.5b	18.4c	
Twigs	11.7bc	0.588e	36.0ab	25.0c	
Shoots	1.98c	1.23c	41.3a	8.73c	
Leaves	19.9b	2.10b	43.4a	177b	
Fruilets	1.40c	2.38a	44.1a	14.5c	
Significance	***	***	***	***	
Interaction	*ns*	*ns*	*ns*	*ns*	

n.s., ***: effect not significant or significant at P ≤0.001. Values followed by the same letter are not statistically different (at P ≤0.05). [1]Stem dry weight included the portion below the grafting union that accounted for 82.1 g, 123 g and 71.1 g for bud burst, pistillate flower and late summer fertilizations, respectively.

Discussion

The low percentage of NDFF (4.54%) and NUE (4.19%) calculated 7 days after bud burst N application confirm the earlier observation of Weinbaum and Van Kessel (1998) who stressed that walnut trees do not uptake N in early spring and thus rely completely on N remobilization for early shoot and flower development. In mature trees, at the time of pistillate flower maturation, Deng and co-workers (1989) calculated a ratio of storage N:current-year uptake N of 15 in flowers and <10 in leaves. Following leaf expansion, N remobilization for growth declines and the soil becomes the major N contributor (Weinbaum and Van Kessel, 1998). Also in our experiment, organs sampled after pistillate flower maturity and late summer fertilizations exhibited a progressively higher percentage of NDFF, showing an increasing ability of the use of fertilizer N as the season proceeds. One week after fertilization and at the end of the growing season (December) the percentage of NDFF was similar in the organs analyzed, no matter the time of fertilization, however the amount of ^{15}N found was positively related to the organ mass, showing a sink strength proportional to its weight. The lower N status of trees fertilized at pistillate flower maturity found in December 2007 and May 2008 compared to the other timings of fertilization, seems contradictory and consequence of a different root uptake in relation to N application time. This is not supported by the final NUE that was similar for the 3 times of fertilization and can be only in part explained considering that in December 2007, leaf and rootstock trunk labeled N were not evaluated and might have been modified by the time of N availability.

In May of the year following N fertilization, percentage of NDFF and NUE were similar for the 3 timings of fertilization, with the latter around 60-70%, which means that during the growing season, as far as N supplied remains in the soil, it is taken up by roots. Actually the NUE calculated in May is underestimated, since it did not take into consideration the ^{15}N lost with leaf fall in 2007. However, leaves collected just before leaf fall were reported to account for only 6% of the labeled N (Weinbaum and Van Kessel, 1998), consequently, if we consider that in September our leaf weight was 20.2 g and N concentration was 1.67%, we obtain a small amount of labeled N (21 mg/tree) in abscised leaves.

The repartition of N, absorbed the previous year, to the tree organs in the following spring was not affected by timing of fertilization and interested the organs of new growth (developing fruits, leaves, and shoots) almost equally with a NDFF between 41% in leaves and 44% in developing fruits. These values are lower than those found by Weinbaum and Van Kessel (1998), who found that mature fruits had 64% of the N derived from internal N sources. The discrepancies with our results may be explained considering that our data refer to the N remobilized at the end of May (beginning of fruit development); an increase of the amount of remobilized N to the fruits is expected through the season. Moreover the values of remobilized N is affected by the growing conditions, i. e. it ranged between 54% (Frak et al., 2002) and 95% (Frak et al., 2006). In general, N remobilization to leaves is expected to be greater in large than in small trees because of the larger pool of stored N (Millard, 1996).

In conclusion, 2-year-old, cv Chandler walnut trees showed a low ability to uptake N until the end of May, even with the mild temperatures (average low and high at the end of April of 10 and 20°C, respectively) of the area of investigation. For this reason, in commercial orchard, N should be applied after pistillate flower maturation, to avoid the risk of high soil moisture and low temperatures (conditions that potentially can promote NO_3^--N leaching). Summer soil N fertilization were found effective in promoting the building of reserves for the flush of growth of the following spring.

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