Uptake of ^{15}N -nitrate by Citrus Trees in Winter and Repartitioning in Spring

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Summary

The amount of ^{15}N -nitrate taken up by a satsuma mandarin tree (*Citrus unshiu* Marc.) in the coldest season (minimum temp. -3°C , mean temp. 3°C) was about one tenth of that taken up in summer (maximum temp. 28°C , mean temp. 23°C). In gravel culture, 90% or more of total nitrogen taken up during the cold season was kept in the roots, especially in the fine roots. A large portion of it began to translocate upwards after late February/early March and was used, preferentially, to build up new organs, such as flower, leaves and twigs. This accounted for 40% of the total nitrogen taken up during the cold season; another 36% was partitioned to the existing organs in the top and 24% to the roots. This partitioning pattern is close to that in soil culture. One-year-old leaves acted as a sink for ^{15}N even at the developing stage of flushed shoots. Data on ^{15}N abundances in the amino acids and nitrate indicate that nitrate, which is taken up in winter, translocate upward mainly as nitrate and asparagine in the following spring.

Introduction

Nitrogen was generally applied to satsuma mandarin trees in spring, summer and autumn. According to our laboratory's data on the uptake and partitioning of spring, summer and autumn nitrogen by them, Spring (9) and summer(6,10) nitrogen was used mainly to develop new shoots and fruit. In contrast, a portion of autumn nitrogen (8) translocated to the top, especially to the leaves. The remaining portion (63%) was stored in the roots, especially in the fine roots, and used for the development of new organs in the following spring(1). This suggests that most of the winter-nitrogen taken up remains in the root parts in winter and then, in spring, a portion of it is translocated upward and used to build up new organs.

To confirm this suggestion, the fate of labelled nitrate absorbed in winter was examined.

Materials and Methods

1. Gravel culture experiments

The experiments were carried out in 1976. A four-year-old satsuma mandarin tree (*Citrus unshiu* Marc.) grown in a 15-liter pot and a 6-year-old tree in a 180-liter pot (each containing gravel) were used. These trees had been fed with a ^{14}N -nutrient solution composed of 2 mM KNO_3 , 1 mM NH_4NO_3 , 1 mM $\text{Ca}(\text{NO}_3)_2$, 1 mM KH_2PO_4 , 1 mM MgSO_4 , 1.5 μM CuSO_4 , 2.3 μM ZnSO_4 , 0.2 μM MnSO_4 , 10 μM $\text{FeC}_6\text{H}_5\text{O}_7$, 50 μM H_2BO_3 , 0.25 μM H_2MoO_4 and tap water. After ^{14}N -nitrogen in gravel had been removed by leaching with a large volume of tap water on January 19, ^{15}N -feeding experiments began on January 21. The ^{15}N -nutrient solution contained 3.5 mM $\text{Ca}(\text{NO}_3)_2$, 2 mM KH_2PO_4 and 1 mM MgSO_4 in tap water. ^{15}N concentration of nitrate was 10 atom %. For the 4-year-old tree 1 l of ^{15}N -solution was circulated through the gravel bed at 10 a.m. every day for 10

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days. The tree was harvested at 10 a. m. on January 31. For the 6-year-old tree 9 l of ^{15}N -solution was supplied from January 21 to February 20 in the same manner. ^{15}N -nitrate was removed by passing a large volume of tap water through the bed on February 20. The tree was fed with tap water only from February 21 to March 14 and then given the 10 l of ^{14}N -solution mentioned above until harvest on June 2. Samples of the leaves and fine roots were taken on February 20, March 4 and April 2 and the tree was harvested on June 2. Both trees were divided into the parts shown in Table 1. All samples were temporarily stored in a freezer at -20°C .

2. Soil culture experiments

This experiment was designed to provide data for comparison with the gravel culture experiments. A 12-year-old tree grown in a 180-liter pot containing soil was used. The tree had been supplied with 65 g of N, 40 g of P_2O_5 , and 40 g of K_2O per year for several years. In order to remove ^{14}N -fertilizer from the soil bed, 40 l of tap water was passed through the bed every day for 10 days from January 7. Two liters of ^{15}N - $\text{Ca}(\text{NO}_3)_2$ solution containing 5 g of nitrogen was applied to the soil surface on January 21. Subsequently 2 l of ^{15}N - $\text{Ca}(\text{NO}_3)_2$ solution containing 4.5 g of nitrogen was applied on February 5. ^{15}N uptake was allowed until February 20. To remove the ^{15}N -nitrate from the soil bed 40 l of tap water was passed through the bed on February 20. This procedure was repeated 4 more times. On March 15, spring fertilizer (N, 14.4 g; P_2O_5 , 8.8 g; K_2O , 8.8 g) was applied. The tree was harvested on June 2. Soil samples were taken on February 20 and June 2.

The tree fed with summer- ^{15}N , which was mentioned in another paper(7), was assayed for comparison with partitioning of winter- ^{15}N . Diagrams showing ^{15}N -treatments for the four trees are given in Figure 1.

3. Assays of nitrogenous compounds and their isotopic abundances

A portion of the plant samples were homogenized with 70% ethylalcohol containing 100 mM KCl. The alcohol-soluble fractions were

extracted and used to determine nitrate and amino acid concentrations and their isotopic abundances. Another portion of the plant samples was treated so as to determine total nitrogen content and isotopic abundances. The methods used are given in detail previously(3,4). Soil samples were treated with 10% KCl(W/V). Inorganic forms of nitrogen were extracted, distilled by microdiffusion using MgO and Devaldas alloy(2), and collected in separate vessels; one for quantitative determination and the other for isotopic determination (3).

Results and Discussion

1. Uptake and partitioning of ^{15}N -nitrate

Air temperatures during the experiments are shown in Figure 1. The minimum temperature (-3°C) was observed in late January. The dry weights and nitrogen levels of the plant parts at the harvest time are shown in Table 1. The two 4-year-old trees and the 12-year-old tree show normal levels of nitro-

Fig. 1. Air temperatures in 1976 and ^{15}N treatments for the four trees. A=4-year-old tree in gravel culture, summer- ^{15}N uptake and partitioning; B=12-year-old tree in soil culture, winter- ^{15}N uptake and repartitioning in spring; C=6-year-old tree in gravel culture, winter- ^{15}N uptake and repartitioning in spring; D=4-year-old tree in gravel culture, winter- ^{15}N uptake and partitioning. (---): application of ^{14}N . (---): application of tap water only. (—): application of ^{15}N . →: harvest. The brackets show the year in which the experiments were carried out.

Table 1. Dry weights and N contents in the plant parts of the four trees used. These values are for the harvest time shown in Figure 1. A, B, C and D as in Figure 1.

Part of the tree	Short-period experiment				Long-period experiment			
	D Winter- ^{15}N 4-year-old tree (gravel-culture)		A Summer- ^{15}N 4-year-old tree (gravel-culture)		B Winter- ^{15}N 12-year-old tree (soil culture)		C Winter- ^{15}N 6-year-old tree (gravel-culture)	
	dry weight (g)	Total N (%)	dry weight (g)	Total N (%)	dry weight (g)	Total N (%)	dry weight (g)	Total N (%)
Fruit	—	—	15.0	4.12	149	2.23	—	—
Current leaves	—	—	33.5	3.39	516	2.73	34.3	2.45
Current twigs	—	—	2.0	1.89	43	1.87	16.7	1.45
1-year-old leaves	42.1	3.24	6.3	2.74	361	2.67	29.8	2.44
1-year-old twigs	7.3	1.90	36.1	1.28	265	1.20	42.0	0.91
2-year-old twigs	25.4	1.55						
3~4-year-old branches	—	—	—	—	280	1.13	64.0	0.66
5~10-year-old branches	—	—	—	—	635	1.08	—	—
trunk	19.9	1.17	22.4	1.26	306	0.97	71.5	0.70
Crown roots	77.7	1.45	40.0	1.21	369	0.98	108	0.71
big roots					640	1.20	22.5	0.72
medium roots	24.7	2.00	45.0	1.73	485	1.25	10.8	1.05
fine roots	21.8	2.35	20.8	2.48	524	1.90	46.8	1.78
Sum	218.8	—	221.1	—	4573	—	446.2	—

Fig. 2. Percentage distribution of total nitrogen (TN) and fertilizer-N (FN) for the measurement of winter- ^{15}N (left two columns) on January 31, and summer- ^{15}N (right two columns) on June 22. Values in brackets show the total amount of nitrogen (g) or fertilizer-N (mg) for each tree. A and D shown in Figure 1. Fig. 3. Repartitioning of ^{15}N -fertilizer taken up in the coldest season at the stage of new shoot development. % distribution of total nitrogen (TN, left column) and ^{15}N -fertilizer (FN, right column) on June 2. Values in the brackets show the amount (g) of total nitrogen or Fertilizer-N in each tree. B and C shown in Figure 1.

gen. But the 6-year-old tree showed lower levels.

The amount of nitrate taken up in 10 days

in the coldest season was 28.4 mg as nitrogen, about one tenth of the summer figure (Figure 2). More than 90% of it was found in the roots especially in the fine roots. On the other hand, in summer, 55% of the absorbed nitrogen translocated upwards and most of it was found in the developing new shoots.

2. Repartitioning of winter-absorbed nitrogen in spring

Most of the winter- ^{15}N was temporarily stored in the roots. This nitrogen was repartitioned in the following spring as shown in Figure 3. In the gravel culture, 24% of the total ^{15}N remained in the roots at the end of the experiment (June 2) and the other 76% was partitioned to the top; 40% of the residue was found in the new organs and 36% in the old organs. Though the portion of the winter-nitrogen used for the development of new organs is not small, it is clearly less than in summer (10).

In the soil culture, it was impossible to

Table 2. Residual amount of ^{15}N -fertilizer in soil after leaching with tap water.

Depth (cm)	Feb. 20		June 2	
	^{15}N (excess %)	Residual Source N (mg/Pot)	^{15}N (excess %)	Residual Source N (mg/Pot)
0~10	2.02	75.7	0.23	11.0
10~20	1.78	76.5	0.22	17.4
20~	1.19	29.0	0.18	8.3
Sum	—	181.2	—	36.7
Residual(%)	—	1.9	—	0.4

completely remove ^{15}N -nitrate from the soil bed by leaching with water. However, only 1.9% of the applied ^{15}N remained in the bed just after leaching on February 20 and 0.4% at the end of the experiment (Table 2). These values mean that, at most, 1.5% of the applied ^{15}N was taken up by the tree between February 20 and the end of the experiment. This quantity corresponds to 8% of the total nitrogen taken up in 30 days in the coldest season. Nevertheless the partitioning of ^{15}N was close to that found for

Fig. 4. Changes in ^{15}N abundances in each part of the tree; (○): fine roots. (⊙): small roots. (★): medium roots. (□): 1-year-old twigs. (▲): 1-year-old leaves. (△): alcohol-soluble fraction of 1-year-old leaves. (⊙): alcohol-insoluble fraction of 1-year-old leaves. (○): current leaves. (●): flowers. The tree was fed with ^{15}N -nitrate from Jan. 21 to Feb. 20(A), then with tap water only until Mar. 15(B), and thereafter with ^{15}N -nitrate until harvest on Jun. 2.

Table 3. Isotopic abundances of the main amino acids and nitrates in fine roots after cutting off the supply of ^{15}N . Samples from the 6-year-old tree fed with ^{15}N from Jan. 21 to Feb. 20, then with tap water only until Mar. 15, and thereafter with ^{14}N until harvest. Amount of source N was calculated as follows :

$$\text{Source N} = \frac{^{15}\text{N atom } \% \text{ excess}}{10 - 0.37} \times \text{pool size of N-compound.}$$

N-compound	^{15}N excess %					Source N $\mu\text{g/g f. w.}$				
	Feb. 20	Mar. 4	Apr. 2	May 1	June 2	Feb. 20	Mar. 4	Apr. 2	May 1	June 2
Aspartate	3.47	2.98	1.25	0.78	0.35	6.96	6.24	3.89	1.34	0.19
Glutamate	3.04	2.10	0.81	0.53	0.21	3.18	2.44	0.38	0.62	0.13
Asparagine	3.66	4.14	1.71	1.34	0.44	47.7	35.2	11.7	2.77	0.64
Alanine	2.99	1.59	0.41	0.17	0.15	5.69	3.05	0.61	0.09	0.16
Proline	0.62	0.74	0.59	0.46	0.35	7.10	9.94	5.70	1.98	3.23
γ -Aminobutyric acid	3.19	2.30	0.58	0.46	0.22	8.25	2.98	1.41	0.32	0.30
Arginine	1.74	1.34	1.05	1.03	0.80	3.54	2.42	2.75	1.50	0.46
NO_3^-	7.00	5.38	1.08	0.69	0.16	125	71	22.5	32.9	7.10

gravel culture. This suggests that partitioning of ^{15}N absorbed on and after February 20 is similar to that in the coldest season. This suggestion is supported by the fact that the partitioning patterns for winter-nitrogen in late spring were very similar to that for spring-nitrogen for an 8-year-old tree(9).

3. Major forms of nitrogen translocated upwards

The translocation of ^{15}N in spring is illustrated in Figure 4. Fine roots had the highest isotopic abundance at the final stage of ^{15}N -feeding, whereas top parts, leaves and 1-year-old twigs showed low isotopic abundances. After February 20 small roots and 1-year-old twigs showed rapid increases and reached maximum abundances in early March. In contrast, in the fine roots a concomitant decrease occurred. These patterns indicate rapid translocation from the fine roots to the top parts.

As is shown in the isotopic abundances of the new organs the winter-nitrogen was preferentially used to develop flowers and new leaves. Isotopic abundance in 1-year-old leaves increased during the period of new organ development, indicating that one-year-old leaves act as a sink for winter-nitrogen. Similar results have been obtained for autumn (1) and spring-nitrogen (9).

The changes with time of isotopic abundances in alcohol-soluble and -insoluble nitrogens in 1-year-old leaves showed that most of the winter-nitrogen was present in

low molecular compounds until late February, but thereafter the incorporation of the winter-nitrogen into protein proceeded actively.

Nitrate and asparagine were the main compounds with high isotopic abundances and had large pool sizes of ^{15}N in fine roots(Table 3). Both pool sizes decreased rapidly after the supply of ^{15}N was stopped. This pattern agrees with that of ^{15}N in fine roots(Figure 4), indicating that the decrease of ^{15}N in fine roots is caused mainly by the translocation of labelled nitrate and asparagine and that the nitrate nitrogen taken up in winter translocates upwards mainly in the form of nitrate and asparagine in spring, as seen in summer season (5).

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ウンシュウミカンによる冬季吸収窒素 (^{15}N) の分配と春季における再配分

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摘 要

冬季に吸収された窒素の温州ミカンにおける分布と春季における再配分を明らかにするため、 ^{15}N -硝酸を使って 1976 年に実験を行なった。

厳寒期における窒素の吸収量は初夏（6月上旬）の約 1/10 であった。また吸収された窒素の 90% 以上が根部に留まり、地上部へ移動した量は 10% に満たなかった。根部にあってはとくに細根に多く分布した。春季にはこ

れら窒素のかなりの部分が地上部へ移動し、吸収 ^{15}N の分布は根部 24%、地上部旧器官 36%、地上部新生器官 40% となった。地上部新生器官のなかにおいて花器が最も高い重窒素濃度を示した。新生器官の旺盛な伸長期にあっても 1 年生葉は ^{15}N のシンクとして働いた。

冬季吸収窒素が地上部へ移動する際の主要窒素化合物は硝酸およびアスパラギンであると推測された。