Effects of Low Temperature in Autumn on the Uptake, Assimilation and Partitioning of Nitrogen in Citrus Trees¹

Tadashi KATO and Shuji KUBOTA²

Shikoku Agricultural Experiment Station, Zentsuji, Kagawa 765

Summary

A 3-year-old satsuma mandarin tree (*Citrus unshiu* Marc. cv. Sugiyama) on *Poncirus trifoliata* rootstock was grown in a 15-liter pot containing soil. Immediately after fed with 7.1g of ¹⁵N-ammonium sulfate, it was kept in a refrigerator for one month from mid-September to mid-October. Daily mean temperature in the refrigerator was gradually decreased from 20°C (corresponding to the daily mean temperatures in mid-September) to 10°C (corresponding to the daily mean temperatures in late November) during the first 12 days, and then kept at 10°C for 18 days. The diurnal range of temperature was also held between 12°C and 5°C. Another tree, as a control, was kept in a box at the ambient temperature.

Low temperature decreased uptake of nitrogen, its translocation to the top and its partitioning to the leaves and fruit. In the leaves, the decrease in incoming ¹⁵N led to a concomitant decrease in labelled free amino acids, except for proline. Low temperature had no influence on the labelling of proline. This indicates that proline is formed rather actively in leaves in a low temperature regime. There was no effect of low temperature on the labelling pattern of amino acids in the organs other than leaves. Distribution patterns of labelled amino acids in each part of the tree showed that proline decreased at lower parts; being higher in the leaves than the trunk and small roots, whereas asparagine, arginine and γ -aminobutyrate decreased at upper parts; being lower in the leaves than the fine roots.

Introduction

In many perennials, there are changes in nitrogen metabolism during the season of progressively decreasing temperatures (3, 14). In the concrete, there are increases in soluble protein (9, 12) and in free amino acids, especially arginine (11, 12) and proline (1, 2, 8, 15).

Tromp and Ovaas (13) reported that in winter the bark of a young apple tree stored a special protein with a markedly high level of arginine. Kubota *et al.* (8) showed that there was an accumulation of proline and arginine in citrus leaves and twigs, and of proline and asparagine in fine roots, in winter. Yelenosky (15) reported proline accu-

mulation in the leaves of orange and grapefruit trees on a wide range of citrus rootstocks during cold hardening. Such accumulation varied with the leaf age and light conditions. Information on proline accumulation in organs other than leaves is limited.

Low temperature seriously influences partitioning of nitrogen uptake. Kubota *et al.* (7) showed that the nitrate translocated upward amounted to 47% of the total uptake in October, but decreased to 37% in November. There are no data on the partitioning of ammonium nitrogen.

To learn more about the utilization of nitrogen during the season of decreasing temperatures, partitioning of ¹⁵N-ammonium uptake and the accumulation of labelled free amino acids, especially arginine and proline, in various parts of the tree were examined.

¹ Received for publication September 7, 1981

² Present address: National Institute of Agricultural Sciences, Tsukuba, Ibaraki, 305

Materials and Methods

Two 3-year-old satsuma mandarin trees on *Poncirus trifoliata* rootstock grown in 15-liter pots containing soil were used. At 8.00 hr on September 18, 1976, each tree was supplied with 200ml of nutrient solution containing 7.1g of $(^{15}\text{NH}_4)_2\text{SO}_4$ (5.5 atom%), 2.2g of KH_2PO_4 , 2.0g of $\text{CaCl}_2 \cdot \text{H}_2\text{O}$ and 0.5g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$. Then, one of the trees was immediately put in a refrigerator (70cm in height, 45cm in depth, and 60cm in width) with a glazed front door, which was always slightly opened. The other tree, as a control, was put in a box of the same size, and its glass door was set at a distance 10cm apart from the front face so as not to raise the temperature. The trees received only natural light through the door. The temperatures at various places in the tree crown were measured in the refrigerator but not in the control box. The temperature difference among the various places of the tree crown was less than 6°C at 13.00 hr. This difference decreased to less than 3°C after sunset. Temperatures at the centers of both tree crowns were recorded by a maximum-minimum thermometer. Mean day temperatures in the refrigerator was progressively lowered from 20°C to 10°C over 12 days and then kept around 10°C for the following 18 days with a diurnal range of temperature within 12°C. The control tree, however, was kept at ambient temperatures. One to two hundreds ml of tap water was supplied to each pot every day. The refrigerator and the control box were rotated slightly at 8.00, 10.00, 12.00, 14.00 and 16.00 hr so that they faced the sun at 9.00, 11.00, 13.00, 15.00 and 17.00 hr, respectively. The trees were harvested at 13.00 hr on October 19 and were divided into several parts shown in Table 1. Samples were kept in a freezer (-20°C) until assay.

Assays of nitrogenous components: A portion of the samples was digested by the modified Kjeldahl method (salicylic acid-thio-sulfate method). Distilled ammonium was collected in separate vessels, one containing an indicator solution to estimate nitrogen

content and the other containing a small volume of 1 N HCl for ^{15}N determination (4). Another portion of the samples was homogenized and extracted with 70% ethyl alcohol containing 100mM KCl. The extract was treated with activated charcoal to remove plant pigments and then passed through an Amberlite IR 120 (H^+)-resin column. Absorbed components were eluted with 2M NH_4OH and concentrated to a small volume. Amino acids in the concentrated solution were analyzed with an autoanalyzer. The major free-amino acids were separated by two dimensional paper chromatography and combusted to N_2 gas by the Dumas method (5). ^{15}N abundances were assayed emission-spectrometrically.

Results and Discussion

Partitioning of nitrogen uptaken

At 12.00 hr on October 22, the maximum light intensities in the empty refrigerator and the control box were 74% and 76%, respectively, of the solar radiation (700W/

Fig. 1. Air temperatures in the refrigerator and the control box during the experiment.

Table 1. Influence of the low temperature on the partitioning of ^{15}N -ammonium uptaken.

Plant part	Fresh weight (g)	Total N (mg)	Percentage distribution of N (B)	Source ^{15}N mg/part	Percentage distribution of ^{15}N (A)	(A) (B)
Control tree						
Fruit	395	1128	25.3	33.1	12.9	0.51
{ Peel	99	719	16.1	19.6	7.6	0.47
Flesh	296	410	9.2	13.5	5.3	0.58
Current leaves	67	1126	25.2	70.1	27.2	1.08
Current twigs	7	77	1.7	4.3	1.7	1.00
1-year-old leaves	30	407	9.1	22.2	8.6	0.95
1-year-old twigs	41	313	7.0	17.7	6.9	0.98
Trunk	18	114	2.5	6.7	2.6	1.04
{ Bark	5	43	1.0	2.4	0.9	0.97
Wood	13	71	1.6	4.3	1.7	1.05
Crown root & large roots ²	44	354	7.9	18.0	7.0	0.89
{ Bark	14	165	3.7	8.0	3.1	0.84
Wood	30	189	4.2	10.0	3.9	0.92
Medium roots	42	373	8.4	20.7	8.0	0.95
{ Bark	15	167	3.7	9.4	3.6	0.98
Wood	27	206	4.6	11.3	4.4	0.95
Small roots	13	126	2.8	8.1	3.1	1.11
Fine roots	69	448	10.0	56.6	22.0	2.20
Sum	726	4466	99.9	257.5	100.0	1.00
Cooled tree						
Fruit	238	463	19.3	7.1	9.1	0.47
{ Peel	61	248	10.3	2.9	3.7	0.36
Flesh	177	215	9.0	4.2	5.4	0.60
Current leaves	44	616	25.7	14.4	18.5	0.72
Current twigs	3	30	1.3	1.1	1.4	1.08
1-year-old leaves	23	274	11.4	4.3	5.5	0.48
1-year-old twigs	16	108	4.5	3.6	4.6	1.03
Trunk	18	115	4.8	3.0	3.8	0.79
{ Bark	6	52	2.2	1.3	1.7	0.77
Wood	12	63	2.6	1.7	2.2	0.85
Crown root & large roots ²	49	300	12.5	6.2	7.9	0.63
{ Bark	15	121	5.0	2.4	3.1	0.62
Wood	34	179	7.5	3.8	4.9	0.65
Medium roots	27	148	6.1	2.8	3.6	0.59
{ Bark	8	55	2.3	1.2	1.5	0.68
Wood	19	93	3.9	1.6	2.1	0.54
Small roots	11	92	3.8	3.6	4.6	1.21
Fine roots	47	256	10.7	31.9	41.0	3.83
Sum	476	2402	100.1	78.0	100.0	1.00

² Roots were classified based on thickness; large roots, 1.0~2.0 cm; medium roots, 0.5~1.0 cm; small roots, 0.2~0.5 cm; fine roots, below 0.2 cm.

m²). When the refrigerator contained a tree, the light intensity on the back surface of the tree crown was 12%. Under such conditions, a few leaves inside the tree crown received the minimum light of 20W/m² at 12.00 hr, which was twice the compensation point for light intensity(10). Thus, for the tree as a whole, the decrease in the

light intensity was not so marked in the refrigerator nor in the control box that nitrogen metabolism was not probably affected differently from in natural light conditions.

Temperatures in the refrigerator and the control box during the experiment are shown in Figure 1. In the control box, mean day

temperature declined from 20°C to 18°C, whereas, in the refrigerator, it decreased from 20°C to 10°C, which corresponded to the mean day temperature in late November. Temperatures in the soil beds were close to the mean day temperatures $\pm 1^\circ\text{C}$.

The partitioning of the nitrogen uptaken is shown in Table 1. Amounts of nitrogen uptaken were 258mg and 78mg in the control and in the cooled tree, respectively, showing that the low temperature markedly decreased the absorption of nitrogen. Nitrogen partitioned to the top parts amounted to 59.9% and 42.9% of total nitrogen uptaken in the control and in the cooled tree, respectively. The upward translocation was reduced by the low temperature. Most of the ^{15}N remaining in the underground parts was found in the fine roots. Furthermore, partitioning to the leaves was also limited by the low temperature, as shown in the distribution of source nitrogen. The ratios of the percentage distribution of $^{15}\text{N}(\text{A})$ to that of total nitrogen (B) in both old and new leaves were shown to be lower, while those in the spring twigs to be higher, in the cooled tree than in the control one. This means that nitrogen translocation from the twigs to leaves is disturbed by the cold treatment. However, in a tree fed with nitrate, this phenomenon was not found(7). Kato (6) has indicated that the nitrogen compounds were transported upward mostly as amides, and particularly as asparagine, in a citrus tree fed with ammonium nitrogen. Therefore, amide translocation might be affected more severely in the petiole than nitrate translocation in a low temperature regime.

The ratios of A/B showed that partitioning to the fruit, especially to the peel, was also limited by the cooling treatment and that, in the trunk and crown root, partitioning to the bark was less than that to the wood, while the reverse was true in the medium roots, irrespective of the cooling treatment. We consider that this is because medium roots can take up ammonium ions in the soil. At least in the trunk, more nitrogen taken up as ammonium form is partitioned

to the wood than to the bark in autumn.

Formation and accumulation of labelled amino acids

Total content of free amino acids in the leaves of both trees slightly increased during the month of the experiment. However, no amino acids showed a marked change in quantity except for proline, which increased

Table 2. Influence of the low temperature on the pool size of free amino acids in the current leaves ($\mu\text{mol/g fw}$).

Free amino acid	Control tree		Cooled tree	
	At the start	After 31 days	At the start	After 31 days
Aspartate	0.72	1.52	1.44	0.98
Threonine	1.61	0.82	1.14	0.97
Serine	5.82	5.92	4.34	3.46
Asparagine	2.00	2.40	2.05	2.09
Glutamate	0.35	0.35	0.62	0.36
Glutamine	0.10	0.13	0.13	0.08
Proline	26.00	28.50	27.50	38.40
Glycine	0.40	0.25	0.51	0.45
Alanine	2.76	1.43	3.20	2.94
Valine	0.16	0.11	0.17	0.37
Methionine	0.03	0.02	0.04	0.04
Isoleucine	0.12	0.08	0.11	0.19
Leucine	0.06	0.07	0.06	0.20
Tyrosine	0.05	0.03	0.04	0.07
Phenylalanine	0.03	0.03	0.04	0.10
γ -Aminobutyrate	3.38	4.65	3.65	4.12
Lysine	0.17	0.34	0.24	0.18
Histidine	0.14	0.10	0.11	0.13
Arginine	2.44	4.10	4.91	2.16
Sum	46.3	50.9	50.3	57.3

Table 3. Influence of the low temperature on the synthesis of free amino acids in the current leaves.

Free amino acid	Control tree		Cooled tree	
	Amount ^z	%	Amount ^z	%
Aspartate	0.49	5.5	0.14	3.0
Threonine	0.23	3.0	0.09	2.0
Serine	2.48	32.3	0.69	14.7
Asparagine	0.86	11.2	0.17	3.6
Glutamate	0.07	0.9	0.05	1.1
Proline	3.01	39.2	3.14	67.1
Glycine	0.08	1.0	0.04	0.9
Alanine	0.23	3.0	0.20	4.3
γ -Aminobutyrate	0.13	1.7	0.10	2.1
Lysine	0.02	0.3	0.01	0.2
Arginine	0.14	1.8	0.04	0.9
Others	0.01	0.1	0.01	0.2
Sum	7.75	100	4.68	100

^z Values are expressed as amounts of respective amino acid newly synthesized with source nitrogen and pooled.

remarkably in the cooled tree (Table 2). The composition of the free amino acids formed from ^{15}N in the leaves is shown in Table 3. Major labelled amino acids in the control tree were serine(32%), proline(39%) and asparagine(11%), instead of serine(15%) and proline (68%) in the cooled tree. The decrease in serine in the cooled tree seems to be caused by decreased photosynthesis, since serine synthesis is related to photore-spiration of leaves. The decrease in labelled asparagine in the leaves of cooled tree is, we consider, connected with the above suggestion that amide translocation is limited more severely in the petiole than nitrate translocation by low temperatures. Isotopic abundances of proteine in the leaves were 0.56 and 0.42 atom% excess in the control and in the cooled tree, respectively. On the other hand, isotopic abundances of total

nitrogen were 0.32% and 0.12%, respectively. Comparison of these values indicates that ^{15}N concentration of proline in the cooled tree is relatively high, as might expected if proline formation in the leaves was hardly suppressed by the cooling treatment.

Proline accumulation in citrus leaves has been reported by Kubota *et al.* (8) and Yelenosky (15), but its mechanisms are unknown. Proline accumulation is a function of its synthesis, its formation by protein degradation and proline breakdown. Increases in protein content during the autumn season are observed in many plants (9, 12) and unpublished data from our laboratory also show increases of protein in citrus leaves. Proline synthesis is not severely suppressed by the cooling treatment and it is unlikely that proline accumulation is caused by protein degradation. Therefore, proline accumulation

Table 4. Influence of the coolant treatment on the pool size of free amino acids in the current twigs, trunk, small roots and fine roots ($\mu\text{mol/g fw}$).

Free amino acid	Current twigs		Trunk		Small roots		Fine roots	
	CT ^z	C ^z	CT	C	CT	C	CT	C
Aspartate	1.65	1.32	1.57	1.62	5.23	4.40	2.05	2.59
Glutamate	0.04	0.04	0.34	0.20	0.35	0.39	0.39	0.48
Serine	4.57	4.61	3.92	3.48	10.7	16.1	5.41	4.90
Alanine	1.46	0.99	1.36	1.12	3.08	2.52	1.44	1.76
γ -Aminobutyrate	2.77	2.27	3.32	2.61	4.21	3.90	2.31	2.38
Proline	19.7	19.4	16.8	16.3	18.6	20.9	6.59	9.48
Arginine	15.6	16.8	12.9	14.8	9.28	10.8	5.27	5.92
Asparagine	6.99	8.90	5.13	7.41	18.2	43.3	17.3	18.5
Others	1.70	1.76	1.71	1.78	3.44	3.73	2.66	4.40
Sum	54.5	56.1	47.1	49.3	73.1	106	43.4	50.4

^z CT : cooled tree, C : control tree.

Table 5. Influence of the cooling treatment on the composition of labelled free amino acids in the current twigs, trunk, small roots and fine roots (on molar basis).

Free amino acid	Current twigs		Trunk		Small roots		Fine roots	
	CT ^z	C ^z	CT	C	CT	C	CT	C
Aspartate	6.1	4.5	5.3	5.4	8.9	4.4	5.3	5.1
Glutamate	0.2	0.2	0.9	0.6	0.5	0.4	0.8	1.0
Serine	13.3	12.3	8.4	6.8	8.7	6.1	10.1	7.9
Alanine	3.9	2.5	3.7	3.5	6.9	5.4	5.0	4.6
γ -Aminobutyrate	5.7	5.9	9.4	9.9	9.4	5.6	7.2	6.1
Proline	25.1	28.7	18.1	24.7	18.2	12.1	11.6	11.1
Arginine	6.8	5.7	10.3	9.5	6.4	5.8	4.3	7.2
Asparagine	36.0	37.0	40.5	36.2	37.5	57.5	51.4	51.2
Others	2.9	3.3	3.4	3.5	3.5	2.8	4.4	5.8
Sum	100	100	100	100	100	100	100	100

^z CT : cooled tree, C : control tree.

seems to be caused by suppression of its degradation.

As shown in Table 4, the pool size of serine and asparagine was decreased only in the small roots but that of proline and arginine was not affected in any plant parts by the cooling treatment. According to the unpublished data from our laboratory, increases of proline and arginine have been observed in twigs, stems and other woody parts in winter. Probably the accumulation of these amino acids in woody parts proceeds after late November.

Molar composition of labelled free amino acids in the spring twigs, trunk, small roots and fine roots is shown in Table 5. The major amino acids found were asparagine, proline, independent of the treatment or plant parts, and these three amino acids accounted for 64-78% of the total labelled amino acids. The cooling treatment was suppressive on the labelling of proline in the trunk, asparagine in the small roots and arginine in the fine roots, while stimulative on the labelling of asparagine in the trunk, and aspartate and proline in the small roots. No information is yet available to show how low temperature affects the nitrogen metabolism in woody parts.

Fig. 2. Labelled aspartate, glutamate, serine and alanine in the current leaves (L), current twigs (CT), trunk (T), small roots (SR) and fine roots (FR). Values are expressed as amounts of each amino acid newly synthesized with source nitrogen and pooled. Solid bars : the cooled tree. Open bars : the control tree.

Fig. 3. Labelled γ -aminobutyrate, proline, arginine and asparagine in the current leaves (L), current twigs (CT), trunk (T), small roots (SR) and fine roots (FR). Solid bars : the cooled tree. Open bars : the control tree.

Amount of labelled free amino acids in each part are shown in Figure 2 and 3. Aspartate, glutamate, serine and alanine were high in the leaves and fine roots, while low in the trunk, irrespective of the treatment. These patterns were relatively similar to that of total ^{15}N (Table 1). These amino acids are located at an important position in the nitrogen assimilation pathway and so are formed actively, which might, therefore, result in their distribution patterns being similar to that of total ^{15}N . The proline pattern is characterized by the high amount found in the leaves. On the other hand, asparagine, arginine and γ -aminobutyrate accumulated to the greatest extent in fine roots and decreased at the upper parts. The asparagine pattern is explained by the fact that asparagine is the main form of nitrogen translocated upward from roots fed with ammonium (6). From the same view point, arginine and γ -aminobutyrate seem also to be able to translocate upward from roots. Furthermore, proline accumulated in the woody parts might originate from the leaves.

Literature Cited

1. CHU, T. M., M. JUSAITIS, D. ASPINALL and L. P. PALEG. 1978. Accumulation of free proline at low temperature. *Physiol. Plant.* 43: 254-260.
2. DURZAN, D. J. 1968. Nitrogen metabolism of *Picea glauca*. 1. Seasonal changes of free

- amino acids in buds, shoot apices, and the metabolism of uniformly labelled ^{14}C -L-arginine by buds during the onset of dormancy. Can. J. Bot. 46 : 909—919.
3. DURZAN, D. J. 1973. Nitrogen metabolism of *Picea glauca*. V. Metabolism of uniformly labelled ^{14}C -L-proline and ^{14}C -L-glutamine by dormant buds in late fall. Can. J. Bot. 51 : 359—369.
 4. KATO, T. 1974. Determination of N-15 abundance in ammonium, nitrates and amino acids in plant extracts. Bull. Shikoku Agric. Exp. Stn (Zentsuji, Japan). 28 : 151—157.
 5. KATO, T. 1975. Determination of ^{15}N abundance in amino acids separated by paper chromatography. J. Sci. Soil Manure (Japan) 46 : 66—67.
 6. KATO, T. 1981. Major nitrogen compounds transported in xylem vessels from roots to top in citrus tree. Physiol. Plant. 52 : 275—279.
 7. KUBOTA, S., S. AKAO and H. FUKUI. 1972. ^{15}N absorption and translocation by satsuma mandarin trees. II. Behavior of nitrogen supplied in autumn. Bull. Shikoku Agric. Exp. Stn (Zentsuji, Japan) 25 : 105—118.
 8. KUBOTA, S., H. FUKUI and S. AKAO. 1974. Studies on nitrogen metabolism in satsuma mandarin trees. Part 3. Seasonal changes with composition of amino acids under different nitrogen nutrition supplies. Bull. Shikoku Agric. Exp. Stn (Zentsuji, Japan). 28 : 133—150.
 9. LI, P. H. and C. J. WEISER. 1969. influence of photoperiod and temperature on potato foliage protein and 4S RNA. Plant Cell Physiol. 10 : 929—934.
 10. ONO, S., K. KUDO and H. DAITO. 1978. Studies on photosynthesis and productive structure of satsuma mandarins (*Citrus unshiu* Marc.). Part 1. Effect of environmental factors on photosynthetic activity. Bull. Shikoku Agric. Exp. Stn (Zentsuji, Japan). 31 : 147—158.
 11. SAGISAKA, S. 1974. Effect of low temperature on amino acid metabolism in wintering poplar : Arginine-glutamine relationships. Plant physiol. 53 : 319—322.
 12. TROMP, J. 1970. Storage and mobilization of nitrogenous compounds in apple trees with special reference to arginine. p. 143—159. In : L. C. Luckwill and C. V. Cutting (eds.) Physiology of tree crops. Academic Press, London and New York.
 13. TROMP, J. and J. C. OVAA. 1973. Spring mobilization of protein nitrogen in apple bark. Physiol. Plant. 29 : 1—5.
 14. WEISSER, C. J. 1970. Cold resistance and injury in woody plants. Science 169 : 1269—1278.
 15. YELENOSKY, G. 1979. Accumulation of free proline in citrus leaves during cold hardening of young trees in controlled temperature regimes. Plant Physiol. 64 : 425—427.

ウンシュウミカンの窒素の吸収・代謝および分配に対する秋季低温の影響

加 藤 忠 司・久保田 収 治

四国農業試験場 765 香川県善通寺市

摘 要

^{15}N -硫酸を与えた3年生ウンシュウミカンを1976年9月中旬より10月中旬の1か月間、ガラス戸の付いた冷蔵庫内で生育させ、窒素の吸収、代謝および分配に対する低温の影響を調べた。冷蔵庫内の温度ははじめの12日間に9月中旬の温度(日平均20°C)から11月下旬のそれ(同10°C)に徐々に低下させ、その後18日間は10°Cに保った。なお、冷蔵庫と同容積で前面に10cm離してガラス戸を付けた木箱内で生育させた樹を対照とした。

この低温処理によって、 ^{15}N の吸収量は対照の約1/3に減少し、地上部への分配も60%から43%に低下した。さらに地上部へ移動した ^{15}N の葉への分配が低下し、低温は枝から葉への移動を抑制した。

窒素代謝に対する影響をみると、葉では流入した重窒素量が低温によって約1/4に減少したにもかかわらず、標識プロリンの量は減少せず、他のアミノ酸の著しい減少と対照的であった。このことはプロリンの生成・集積が低温の影響をあまり受けないことを意味する。葉以外

の部位においては顕著な影響は認められなかった。

根から葉に至る各部位の標識アミノ酸の集積量をみると、アスパラギン、アルギニンおよびガンマーアミノ酪

酸は細根で多く、葉に近い部位ほど低下した。一方、プロリンは逆の集積パターンを示した。