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Influence of Crop Load on the Composition of Free Amino Acids in Organs of Mature 'Valencia' Late [*C. Sinensis* (L.) Osbeck] Trees During the Growth Cycle

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Abstract

Concentration changes of free amino acids in trees with low and high crop load, grown under trickle irrigation were determined at flowering, fruit set, fruit growth, second flush and dormancy.

In low crop load trees, only fibrous roots concentrated significantly more amino acids in all stages. In other tree parts the results were inconsistent, although the higher accumulations were more frequent.

The results of the three main amino acids of the low versus high crop load trees throughout the cycle were as follows: bark and wood tissues of branches (4-13 mm 0) and fibrous roots accumulated more proline. The reverse was observed in tissues of lateral roots; high asparagine values appeared in flowers and developing fruits, bark and wood tissues (spring flush twigs and lateral roots) and fibrous roots; arginine levels were higher in lateral root tissues and fibrous roots, and lower in reproductive organs, leaves and tissues of branches (<4 mm 0).

The bark of woody organs in trees with both crop loads concentrated more proline than the wood in all stages and the same was observed for asparagine in branches (4–13 mm 0) and lateral roots, whereas the arginine accumulation was lower in the bark of twigs and branches.

The highest relative concentrations were as follows: proline 78% in old leaves at dormancy, asparagine 57% in flowers, and arginine 29% in branch wood at flowering.

Introduction

Earlier and recent reports on nitrogen nutrition in citrus trees have described a decrease on the leaf N concentration during spring flush and initial fruit growth (2, 10, 12, 18), because the low N uptake by roots in these stages (5, 7, 13, 14) cannot supply the high N demand for the development of new organs and citrus need to mobilize the N stored in the reserve organs (9, 11, 14, 16). Kato (6) in 1986 reported that the N storage forms are proteins and amino acids. A few studies have been done on storage and mobilization of protein N (1, 8, 17). However, more information is available about the role of the tree major free amino acids on storage and translocation of nitrogen compounds (6, 10, 16, 19, 20).

The purpose of this work was to determine whether the crop load had some influence on the concentration and translocation of the free amino acids in different organs of 'Valencia' oranges at significant physiological stages of their growth cycle.

Materials and Methods

Trees with alternate bearing were chosen. An orchard of mature 'Valencia' trees on Troyer citrange rootstock with a low crop load (eight tonnes per hectare) was used. These trees can be considered as off-trees. They came from an on-season, because the previous year were overloaded. The soil type was red, deep, sandy clay loam texture and contained a very low amount of calcium carbonate. Another orchard with trees of the same variety also mature and grafted on sour orange and a high crop load (forty seven tonnes per hectare) was used. These trees were considered as on-trees and the previous year had a standard crop load. The alluvial soil was brown, deep, of sandy loam texture and with a moderate concentration of calcium carbonate. In both orchards similar N rates and the same sort of nitrogen fertilizers were supplied through a drip irrigation system.

Three trees from each orchard were randomly selected. They were sampled at five significant physiological stages of the growth cycles (Table 1). In each tree the following parts were fractionated: reproductive organs (flowers, young fruits, immature fruits), young leaves (of vegetative and fruiting shoots), old leaves (leaves from previous year), bark and wood tissues (of young twigs, branches thinner than 4 mm, branches of 4 up to 13 mm and lateral roots from 3 up to 14 mm diameter) and fibrous roots with a diameter smaller than 3 mm.

The method for analysis of free amino acids was described previously (19).

Results and Discussion

Changes in the concentrations of free amino acids in organs of citrus trees with different crop loads

Table 2 shows the comparison between the concentrations of total free amino acids in various organs of 'Valencia' orange trees with high and low crop load at the main stages of growth. To make the analysis of results easier, we are comparing here the data of trees with low crop load versus high load trees.

Table 1: Stages and sampling dates for 'Valencia' oranges[z]

Growth cycle stages at time of sampling		Date
Flowering	End of flowering	May, 12 1986
Fruit set	Total petal fall	June, 5 1986
Fruit growth	Average diameteter (3.5 mm)	July, 23 1986
Second flush	Average diameter (5.2 mm)	September, 3 1986
Dormancy	End of dormancy	February, 18 1987

z = three trees with low and high crop load were sampled.

y = fully expanded second flush.

The reproductive organs generally reached higher concentration of amino acids along their development. The differences between concentrations were statistically significant at flowering and second flush, probably due to the high accumulation reached by asparagine (Fig. 2). The influence of crop load on the level changes in leaves may be dependent on their age. The spring flush leaves showed similar behaviour to that of young fruits. However, old leaves accumulated significantly less amino acids in two stages and in another two the levels were also lower.

Differences were not meaningful in the bark and wood tissues of spring flush twigs of both trees (different crop loads), although the higher levels appeared more often in trees with low crop. Similar results were found in tissues of branches of any diameter, but some stages the differences were significant (Table 2).

Fibrous roots accumulated significantly more amino acids in all stages studied. A similar trend was observed in the wood of lateral roots, but the differences were not significant, whereas in the bark the higher values appeared only in two stages.

Twenty-two free amino acids were detected in all organs. The same number was found in other studies (19, 20). We are reporting the results only of the three most important free amino acids. The comparison between the concentrations of proline, asparagine and arginine in organs of 'Valencia' trees with different crop loads throughout the year is shown in Figs. 1 to 9.

Reproductive organs. Fruits concentrated less proline and arginine during initial fruit growth. Nevertheless, towards dormancy the concentrations were slightly higher and both amino acids increased (20). Asparagine reached markedly high values during flowering and second flush (Figs. 1 and 2).

Young and old leaves. Both leaves accumulated less arginine in all stages, the values were significant at fruit set and second flush. Lower levels were also found in relation to proline and asparagine until fruit set. Then the concentrations were higher in young leaves. In old leaves the proline level reached a significantly lower value at dormancy, whereas the asparagine concentration was significantly higher during fruit growth. These amino acids except for arginine presented similar values during the second flush in both trees.

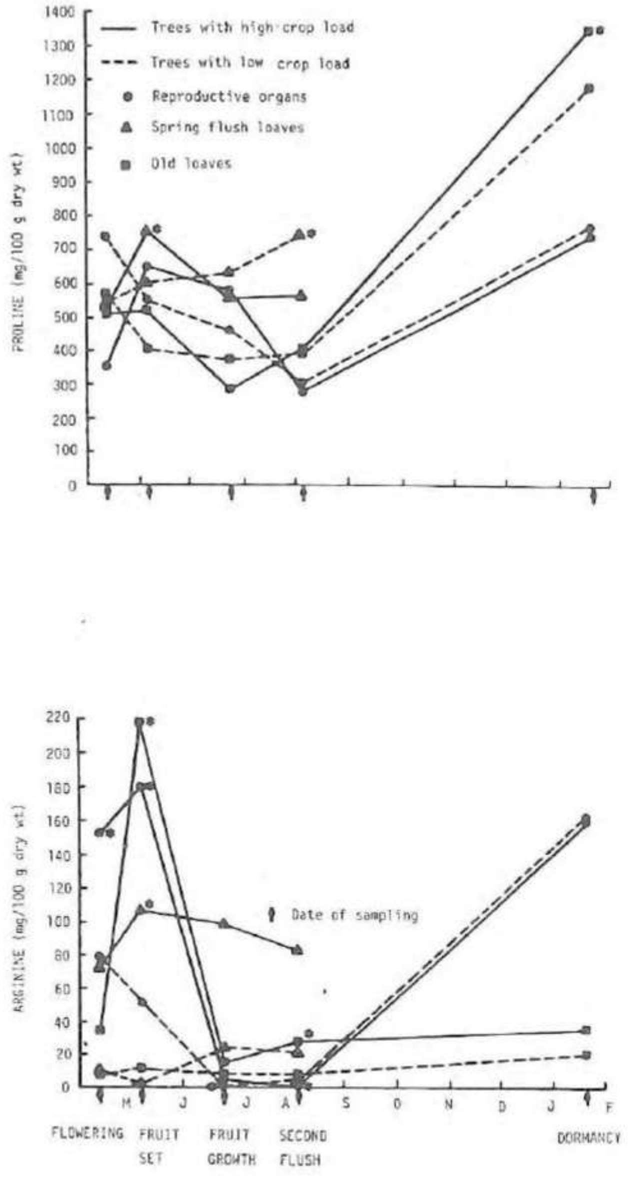

Figure 1: Comparison of free amino acid concentrations in 'Valencia' oranges with different crop loads throughout the year. * = significant at 5% level.

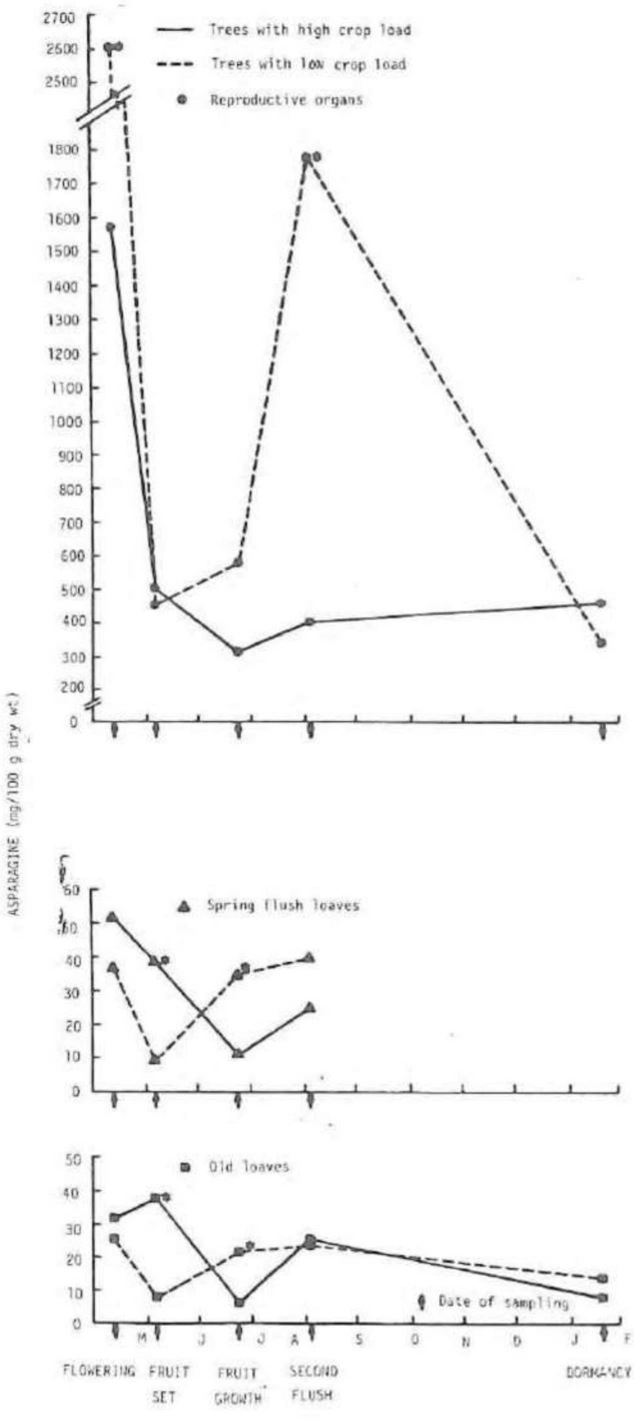

Figure 2: Comparison of free amino acid concentrations in 'Valencia' oranges with different crop loads throughout the year. * = significant at 5% level.

Table 2. Comparison of total free amino acids[y] in different organs of 'Valencia' orange trees[y] throughout the year

	Flowering		Fruit set		Fruit growth		Second flush		Dormancy	
	I	II	I	II	I	II	I	II	I	II
Flowers	2780±324	4552±304*								
Young fruits			1836±255	1597±33	1373±195	1592±9	1178±117	3261±153		
Fruit rind									966±44	1077±80
Endocarp									3267±119	2891±50
Spring flush leaves	1067±53	1077±9	1318±31	1005±37*	1093±23	1181±78	1153±25	1338±10*		
Old leaves	873±60	848±22	1017±9	653±51*	489±31	743±114	806±50	778±47	1746±25	1547±1*
Spring flush twig bark	797±31	862±16	1026±54	1122±59	1110±8	1098±71	1074±62	1011±23	748±27	801±69
Spring flush twig wood	479±20	670+233	679±59	638±20	680±20	667±50	802±50	822±35	444±28	551±69
Branch bark (<4 mm Ø)	800±51	871±3	958±11	858±166	765±46	898±156	792±53	714±7	1582±139	1917±15
Branch wood (<4 mm Ø)	610±64	485±14	657±23	539±115	576±35	579±59	641±5	474±23*	754±51	949±89
Branch bark (4–13 mm Ø)	465±36	776±46*	515±61	587±110	601±42	826±106	682±80	657±28	1022±43	1224±47*
Branch wood (4–13 mm Ø)	344±14	333±12	455±5	298±47	294±17	403±5*	416±30	382±49	505±7	539±5*
Lateral root bark (4–13 mm Ø)	1567±147	958±30*	1546±98	1424±4	1291±173	1571±140	1701±323	1946±7	1762±203	1419±134
Lateral root wood (4–13 mm Ø)	701±70	771±66	695±6	593±59	557±12	655±140	639±73	809±12	657±16	701±87
Fibrous roots (<3 mm Ø)	697±13	781±16*	792±67	1170±55*	668±8	998±7*	534±9	1195±37*	696±32	1070±47±*

z = each value is the mean ± SE of 3 trees (expressed as mg/100 g dry wt). y = I: tees with high crop load and II: trees with low crop load.
* = significant at 5% level.

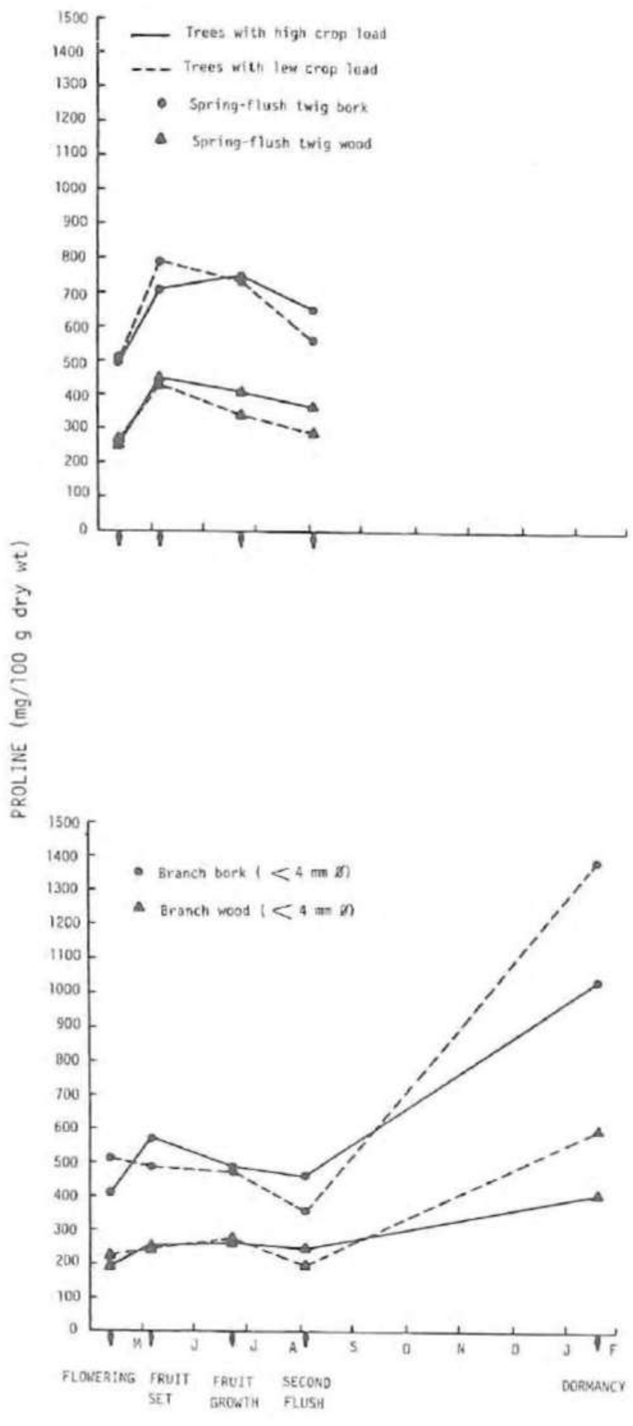

Figure 3: Comparison of free amino acid concentrations in 'Valencia oranges with different crop loads throughout the year. * = significant at 5% level.

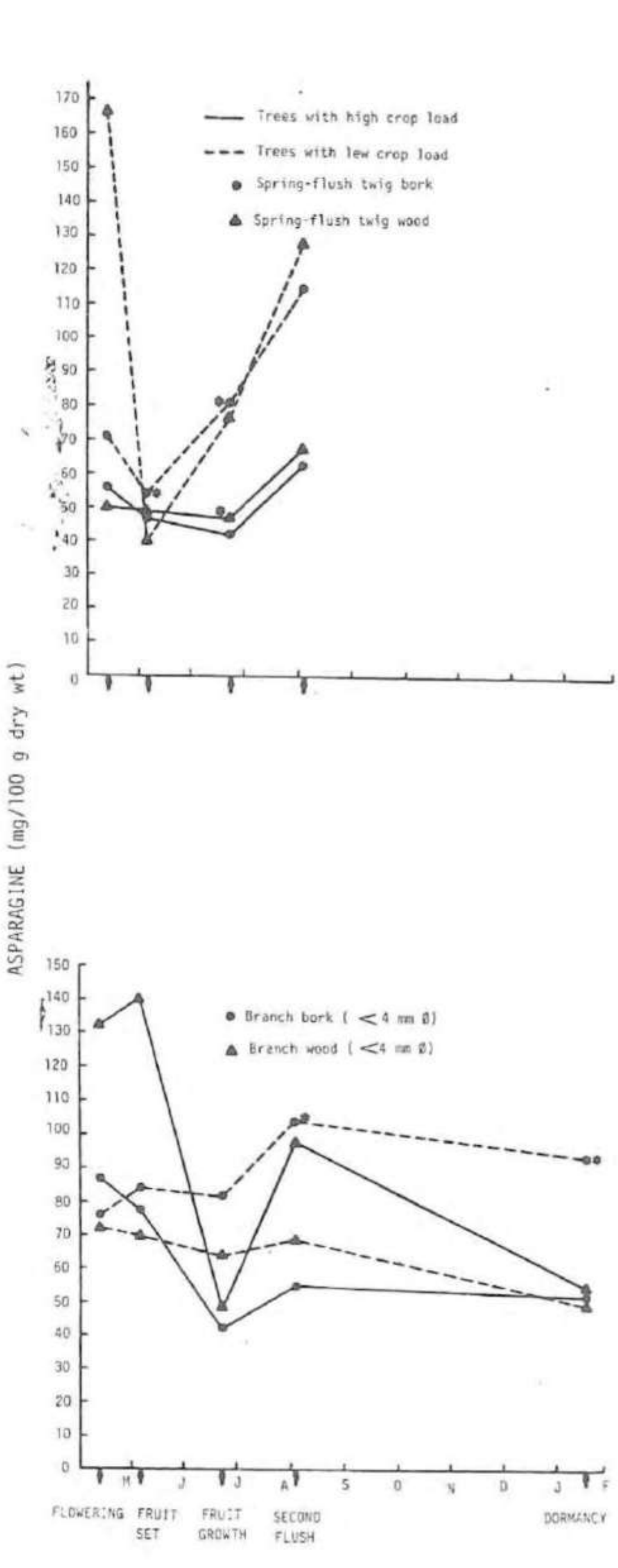

Figure 4. Comparison of free amino acid concentrations in 'Valencia' oranges with different crop loads throughout the year. * = significant at 5% level.

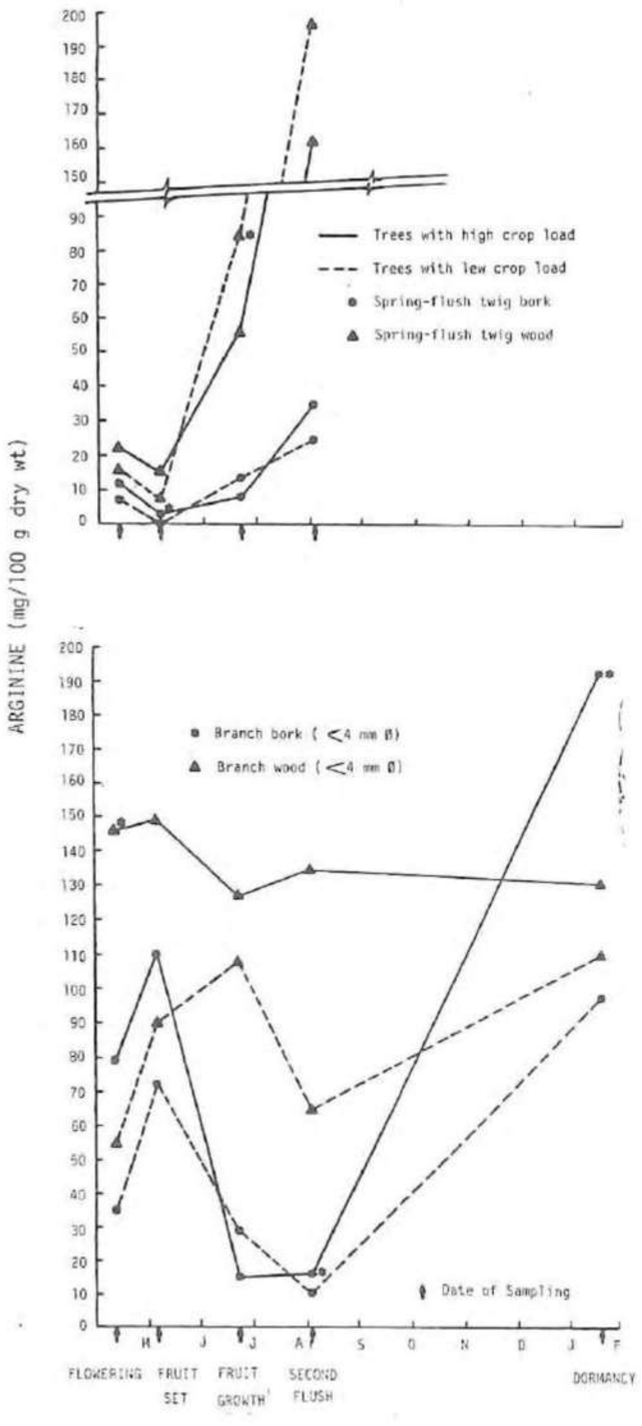

Figure 5: Comparison of free amino acid concentrations in 'Valencia' oranges with crop loads throughout the year. *= significant at 5% level.

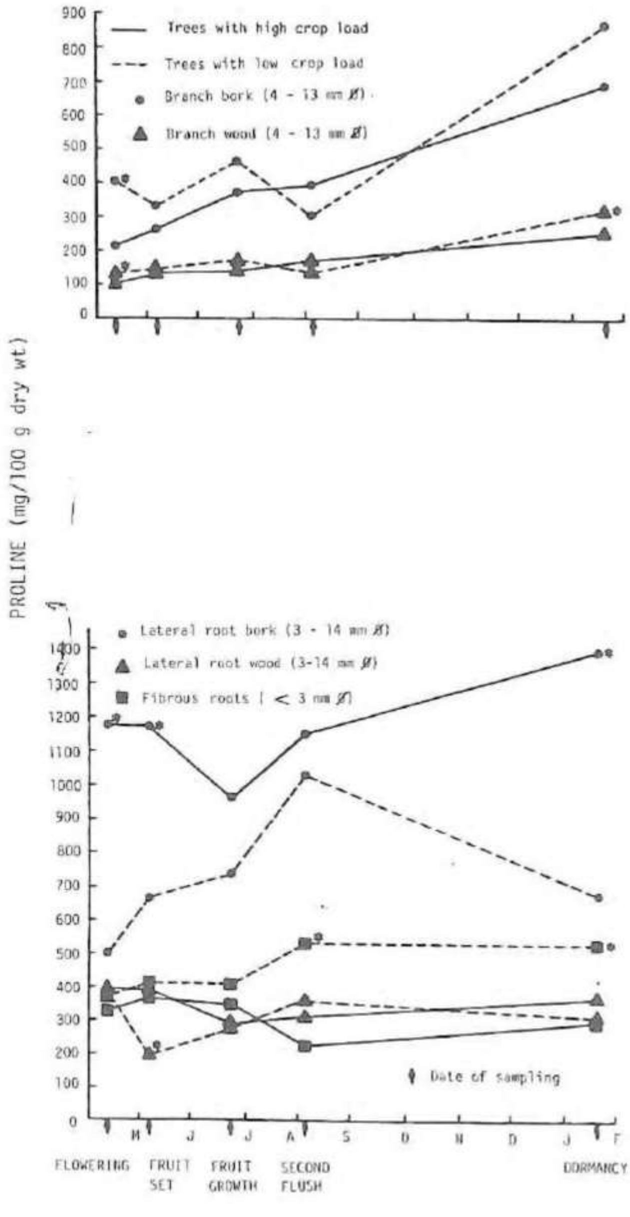

Figure 6. Comparison of free amino acid concentrations in 'Valencia' oranges with different crop loads throughout the year. *= significant at 5% level/

Table 3. Comparison of relative concentrations of free amino acids[z] (proline, asparagine and arginine) in different organs of 'Valencia' orange trees[y] throughout the year

Organs		Flowering			Fruit set			Fruit growth			Second flush			Dormancy		
		PRO	ASP	ARG	PRO	ASP	ARG	PRO	ASP	ARG	PRO	ASP	ARG	PRO	ASP	ARG
Flowers	I	13	55.0	5.8												
	II	16	57.0	1.8												
Young fruits	I				35	28.0	9.4	43	22.0	0.4	21	34	tr			
	II				35	28.0	3.2	29	36.0	tr	9	55	0.2			
Fruit rind														50	10.0	3.0
														53	11.0	4.2
Endocarp														31	25.0	9.1
														34	20.0	9.7
Spring flush leaves	I	50	4.8	6.6	57	3.7	8.2	51	1.0	9.1	49	2.2	7.2			
	II	51	3.5	1.0	60	0.9	0.2	57	3.0	2.0	56	3.0	1.6			
Old leaves	I	59	3.7	4.6	52	3.7	21.0	58	1.4	3.2	50	3.3	3.5	78	0.5	2.1
	II	67	3.0	0.9	62	1.2	2.6	50	3.0	1.0	51	3.0	1.0	77	0.9	1.3
Spring flush twig bark I		63	7.0	1.5	69	4.6	0.3	70	3.8	0.7	61	6.0	3.3			
	II	58	8.2	0.8	70	4.9	tr	67	7.4	1.3	56	11.5	2.5			
Spring flush twig wood	I	54	10.0	4.7	66	7.2	2.2	60	7.0	8.2	45	8.5	20.0			
	II	43	21.0	2.2	67	6.2	1.0	51	12.0	13.0	35	15.0	24.0			
Branch bark (<4 mm Ø)	I	51	12.0	9.7	60	8.1	11.0	64	5.4	2.0	58	6.9	2.0	65	3.3	12.0
	II	59	8.8	4.0	58	9.2	7.4	58	9.2	3.6	49	14.0	1.4	72	4.9	5.1
Branch wood (<4 mm Ø)	I	31	21.0	24.0	38	21.0	23.0	46	8.4	22.0	38	15.0	21.0	54	7.4	18.0
	II	46	15.0	11.0	48	12.0	16.0	45	11.0	20.0	41	15.0	14.0	64	5.2	11.0
Branch bark (4–13 mm Ø)	I	46	15	7.7	51	19	7.1	63	11	1.1	57	11	2.5	68	4.3	7.2
	II	52	16	5.2	55	11	3.1	56	18	1.5	47	22	1.3	71	5.9	2.8
Branch wood (4–13 mm Ø)	I	39	15	29.0	30	23	25.0	48	12.0	16.0	42	12.0	21.0	50	8.0	20.0
	II	39	13	25.0	50	10.0	13.0	44	14.0	20.0	37	17.0	21.0	60	6.6	12.0
Lateral root bark (3–14 mmØ)	I	75	7.4	2.2	75	9.7	0.9	75	10.0	1.1	69	15.0	1.4	75	9.0	1.5
	II	53	10.0	16.0	47	22.0	17.0	47	23.0	13.0	53	26.0	6.5	48	17.0	14.0
Lateral root wood (3–14 mm Ø)	I	57	5.9	20.0	56	8.6	15.0	52	7	21	49	9.7	19.0	57	4.8	16.0
	II	49	8.4	22.0	33	26.0	24.0	41	18	22.0	45	14.0	23.0	45	16.0	16.0
Fibrous roots (<3 mm Ø)	I	47	20.0	5.2	46	19.0	7.2	52	13.0	5.6	42	17.0	5.9	44	20.0	4.4
	II	48	19.0	5.6	35	38.0	4.6	41	28.0	6.9	45	24.0	10.0	50	20.0	6.2

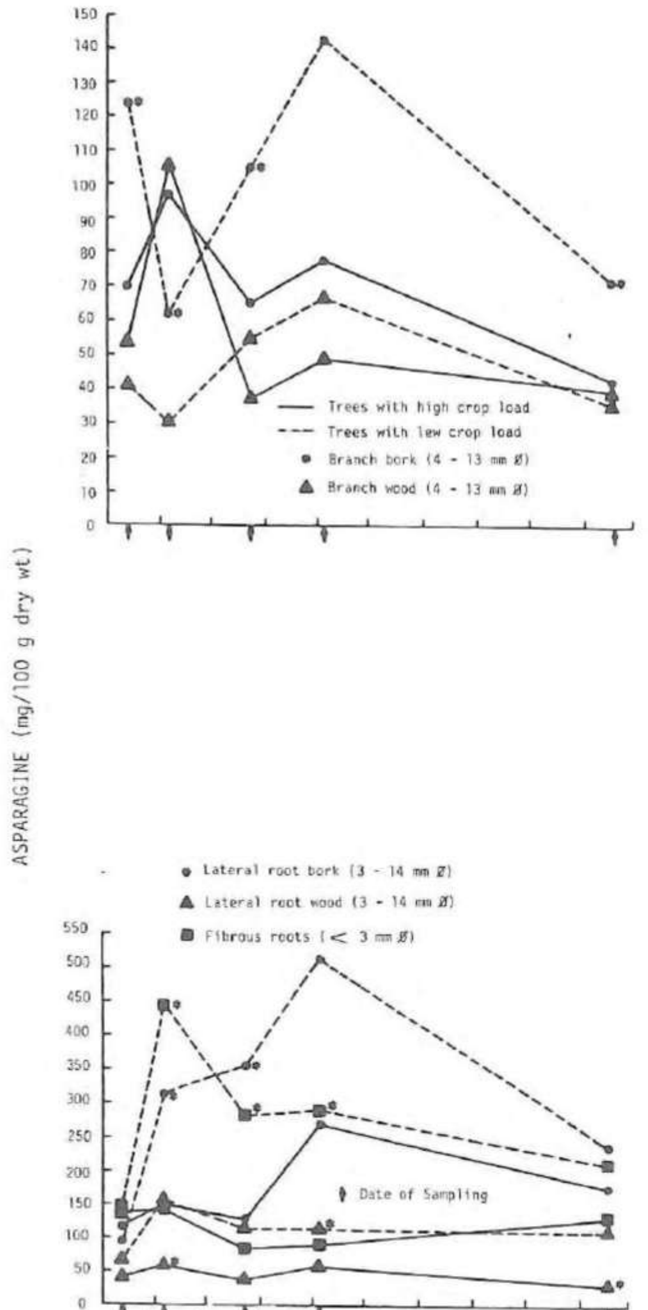

Figure 7: Comparison of free amino acid concentrations in 'Valencia' oranges with different crop loads throughout the year. * = significant at 5% level.

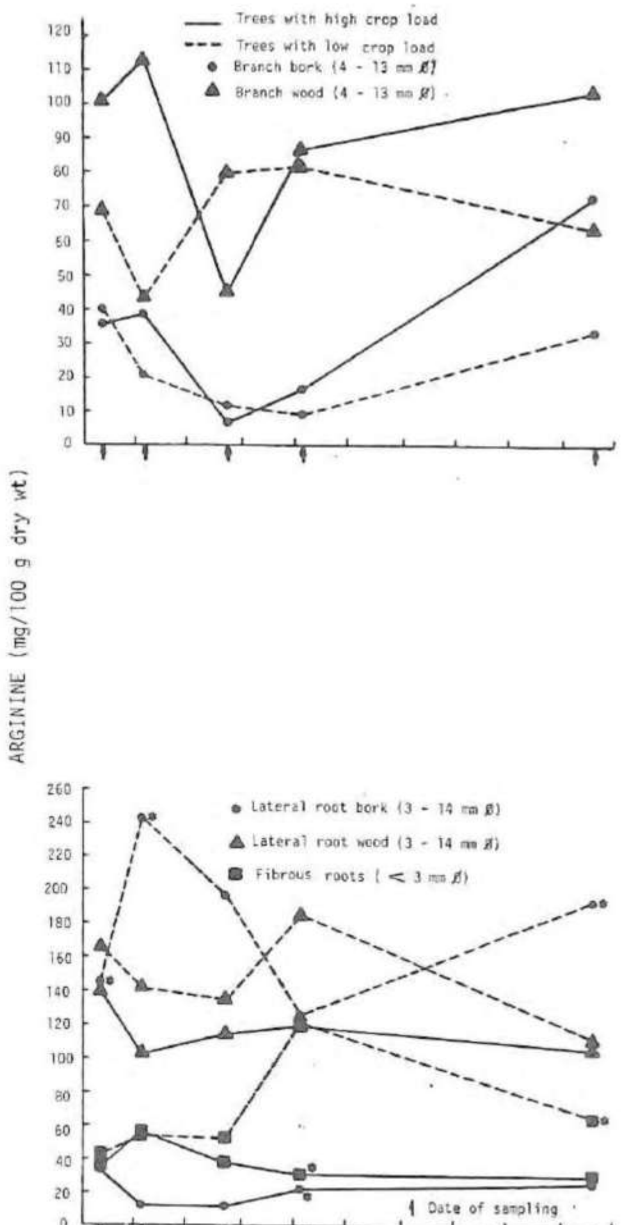

Figure 8. Comparison of free amino acid concentrations in 'Valencia' oranges with different crop loads throughout the year. * = significant at 5% level.

The active proline synthesis may have taken place in the leaves at low-temperature conditions (6). Proline concentration reached the highest values in the top old organs (fruits, leaves, bark and wood of branches) of both trees at dormancy. The same behaviour was reported in other studies (3, 4, 17, 19, 20).

Bark and wood of twigs and branches. The crop load scarcely influenced the proline concentration in the bark and wood tissues of spring flush twigs. However, the trees with low load showed higher levels of asparagine in both tissues. Arginine accumulations were lower until fruit set, and later higher values were found in wood, while in bark the values were erratic (Figs 3, 4 and 5).

As in twigs, the differences between proline concentrations were inconsistent in the tissues of branches (< 4 mm diameter). The trees with low yield accumulated more asparagine in the bark since flowering, whereas the amounts were lower in the wood, except during fruit growth. The arginine values were lower in both tissues at all stages studied.

The bark and wood of branches (4–13 mm diameter) concentrated more proline in the stages sampled, except during the second flush. The asparagine level was lower for both tissues at fruit set. The bark showed higher accumulations in the rest of stages and the wood only during fruit growth and second flush. The arginine differences were inconsistent in both tissues (Figs. 6, 7 and 8).

Roots. The proline amount accumulated was lower in both tissues of lateral roots. The asparagine and arginine showed superior values in both tissues, being statistically significant, mainly in the bark.

Consistent results appeared in fibrous roots, because concentrations of the three amino acids were higher in all stages, showing significant values since fruit set.

The most important differences between accumulation of proline, asparagine and arginine were mainly found in the lateral and fibrous roots of both trees. Probably because roots store these amino acids from fall to winter, they synthesize partially arginine and especially asparagine, and proline is transported out of the leaves to the roots (6).

To compare the concentrations between bark and wood. We have found the following values in both trees: the proline levels were always higher in the bark than those in the wood in twigs, branches and roots. The same trend was observed in relation to asparagine in lateral roots and branches with diameter of more than 4 mm, but in twigs and branches, of less than 4 mm, the concentration differences between these tissues were erratic. Arginine reached lower levels in the bark of aerial woody organs. The differences were erratic in the tissues of lateral roots.

The comparison between the percentages of relative concentrations of proline, asparagine and arginine are given in Table 3. The differences of relative concentrations of each of these amino acids between any organ and stage in low versus high crop trees showed, generally, similar trends to the differences of concentrations shown in Figs. 1 to 9. Different trends were observed in some organs and stages, because the relative concentration of each amino acid is influenced by the concentrations of the other amino acids. The percentages of the sum of proline, asparagine and arginine between both trees gave erratic values for any part of the trees. Although the organs with higher percentages were more frequent in low crop trees. Only in fibrous roots the sum of relative percentages was always greater in trees with low crop. The sum of relative percentages of these amino acids in both trees were very similar to those reported by Tadeo et al. (19) in 1984 on young 'Valencia' trees and also agreed with the results obtained by Culianez et al. (4) in leaves and young fruits on mature 'Navelate' and 'Washington' navel orange trees. This sum represented an average range of 55–81 % for fruits and leaves and of 64–86 % for twigs, branches and roots of the total free amino acids found in both trees.

The findings on synthesis, storage and translocation of the major free amino acids reviewed by Kato (6) are very useful to explain the variations of these amino acids throughout the year. Nevertheless, it is very difficult to discuss the amino acid differences found between some organs of trees with different crop loads, because in addition to the crop load other factors might have had an influence on these differences. Monselise and Goldschmidt (15) in 1981 reported different contents in nitrogen compounds to compare on-and-off-trees of 'Wilking' mandarin. The capacity of the rootstock to concentrate amino acids has been reported only in relation to proline (21). The total dry matter contents of new organs might be different in our study, because the low load trees had a light flowering and heavy flushes. The opposite was observed in trees with high load.

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