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METABOLIC CHANGES IN FRUIT AND LEAF DURING RIPENING IN THE OLIVE*

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Key Word Index—*Olea europaea*; Oleaceae; olive; maturation; fat synthesis.

Abstract—Changes in the content of several metabolites (fat, sugars, protein nitrogen, uronic acids and Krebs cycle acids) were studied in fruits and leaves of *Olea europaea* cv. Marteño during fruit development. Fat accumulation parallels fruit development, and fat and sugar levels in both fruit and leaf follow similar patterns until maturation. Then, while the fruit fat content remains at its previous high level, the leaf fat drops to its low initial level. By contrast, the sugar content of both fruit and leaf returns to its low starting value. The total Krebs cycle acids seems to be in an inverse relationship in leaf and fruit during its development. Also a high level in uronic acids was observed in fruit during ripening. Finally, the protein nitrogen content slowly drops in the fruit during development with a small increase at maturity.

INTRODUCTION

Only a few investigations have been made concerning the content and changes in the components of olive tree leaves. Hilditch and Williams [1] studied the lipid content, Balanzard and Delphaut [2] identified malic, succinic, glycolic and lactic acids, and Mamedova *et al.* [3] showed that there were large annual changes in the soluble sugars. Even rarer are reports in which the components of fruit and leaf have been compared. Vazquez and Janer [4] found that the fatty acids of fruit and leaf glycerides are different, because of the greater unsaturation in the latter.

In previous work [5,6] we have studied the content of some components of olive fruit at different stages of development. Now we have analysed the changes that various metabolites undergo in both fruit and leaves during fruit development and maturation, and studied the relationships that exist between the different components of these two plant parts.

RESULTS

Figures 1 and 2 show the changes in the content of fat-soluble sugars and total di- and tricarboxylic acids in fruits and leaves respectively, during different stages of fruit development. Fat and sugar amounts are similar in both parts of the plant until maturation; then the fat content of the fruit continues to increase while that of the leaves drops; on the other hand, the sugar levels return to their low starting values in both fruits and leaves.

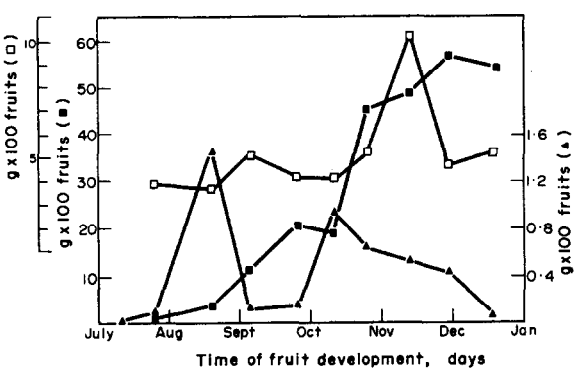

Fig. 1. Changes in the content of metabolites of olive fruit during fruit development. Krebs cycle acids (▲——▲); fat (■——■); soluble sugars (□——□).

* Part II in the series "Biochemical and Physiological Studies in the Olive Tree". For Part I see Ref. [5].

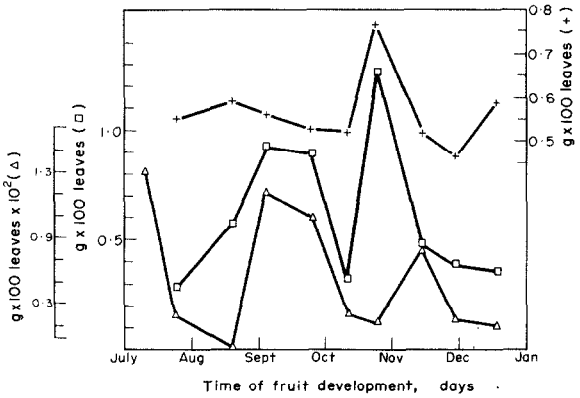

Fig. 2. Changes in the content of metabolites of the olive leaf during fruit development. Soluble sugars (+——+); fat (□——□); Krebs cycle acids (△——△).

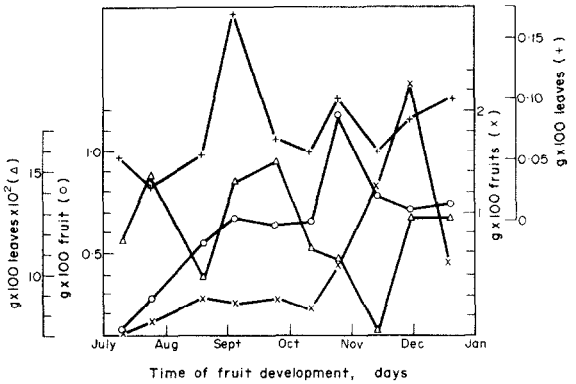

Fig. 3. Changes in the content of protein and uronic acids of olive leaf and fruit during fruit development. Leaf uronic acids (+——+); fruit uronic acids (×——×); fruit protein nitrogen (○——○); leaf protein nitrogen (△——△).

Krebs cycle acids behave similarly to fats in both organs at all stages of development. Tables 1 and 2 show the individual values for each of these acids. With regard to the malate/citrate ratio (Fig. 3) the large changes at certain stages are surely of metabolic significance. The protein nitrogen and total uronic acids for the fruits and leaves at all stages of development are shown in Fig. 4.

DISCUSSION

Hilditch and Williams [1] emphasize the possibility that fat synthesis in olive trees occurs in leaves, from where it could be transported to the fruit. This idea is in disagreement with the view that the olive fruit synthesizes its own lipids [4]. The results we have obtained show an inverse relationship between the amounts of Krebs cycle acids

Table 1. Changes in the Krebs cycle acids and dry material contents of olive fruit during fruit development

Sample	1	2	3	4	5	6	7	8	9	10
date	9.7.73	23.7.73	18.8.73	4.9.73	24.9.73	10.10.73	23.10.73	12.11.73	28.11.73	17.12.73
Dry material	5·80	19·25	56·15	71·63	89·23	94·63	107·38	125·58	131·50	133·60
Succinic acid	trace	0·58	2·45	1·34	5·01	70·35	1·16	6·81	1·03	1·82
Fumaric acid	trace	0·82	36·68	3·00	8·91	41·08	2·30	6·48	5·34	0·32
Malic acid	0·19	0·48	51·34	18·73	50·10	146·36	229·17	143·32	61·79	0·95
Aconitic acid	trace	0·20	78·25	2·66	6·86	48·69	28·37	37·56	9·46	1·59
Citric acid	2·69	26·27	809·80	128·57	297·66	587·83	356·72	241·12	295·01	45·50
Isocitric acid	trace	0·88	509·83	18·34	4·87	trace	7·60	99·33	49·33	trace

Each result is an average of four (dry material) or two (Krebs cycle acids) individual samples, and is expressed in g/100 fruits and mg/100 fruits, respectively.

Table 2. Changes in the Krebs cycle acids and dry material contents of olive leaf during fruit development

Sample	1	2	3	4	5	6	7	8	9	10
date	9.7.73	23.7.73	18.8.73	4.9.73	24.9.73	10.10.73	23.10.73	12.11.73	28.11.73	17.12.73
Dry material	6·36	6·85	7·31	7·24	7·18	7·07	7·05	7·36	7·46	7·40
Succinic acid	0·04	0·003	0·003	0·6	0·15	0·01	0·003	0·02	0·005	trace
Fumaric acid	0·06	0·0007	0·0007	0·0007	0·12	0·20	0·06	0·21	0·01	0·06
Malic acid	0·03	0·34	0·002	0·003	0·02	0·58	0·41	1·70	0·75	0·007
Aconitic acid	0·11	0·01	0·008	13·66	9·08	0·86	0·72	4·69	0·28	0·37
Citric acid	15·39	1·59	0·03	0·01	1·64	0·62	0·49	1·70	0·87	0·09
Isocitric acid	0·22	0·26	0·02	0·004	0·003	0·11	0·003	0·004	0·84	0·61

Each result is an average of four (dry material) or two (Krebs cycle acids) individual samples, and are expressed in g/100 leaves and mg/100 leaves, respectively.

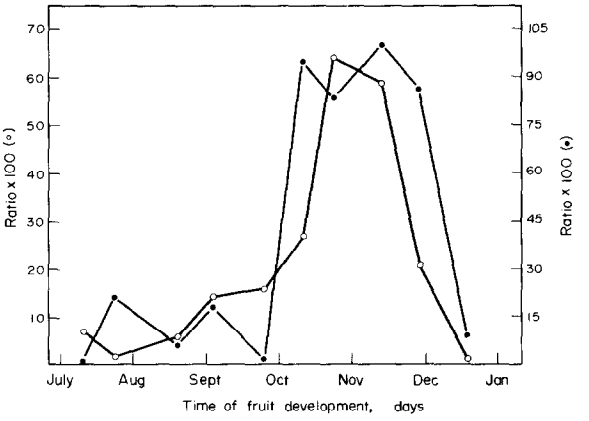

Fig. 4. Changes in the malic/citric ratio in olive leaf and fruit during fruit development. Malic/citric ratio in fruit (○——○); malic/citric ratio in leaf (●——●).

in leaves and fruits during the first stages of fruit development, suggesting a continuous migration of acids from the leaf. In accordance with this view, González *et al.* [7] found a decrease in the cation content of leaf associated with a concomitant increase in the cation content of the fruit. Subsequently the Krebs cycle acids, mainly citric and malic, decrease and at the same time there is a rapid synthesis of fat.

So, we suggest that these acids are synthesized in the leaf and then transferred to the fruit, where the fat is formed probably owing to a high level of citrate-lyase activity, which supplies acetyl-CoA. The fatty acid chains would be formed from this via the fatty acid synthetase complex, and the necessary NADPH would be supplied in the 6-phosphogluconic acid pathway and from the splitting of malate by the malic enzyme.

Later, there is a second increase in the rate of fat accumulation in fruit, with similar fluctuation in the levels of Krebs cycle acids. This second increase of fat deposition occurs just before the beginning of maturation, which is important because the ratio malate/citrate is increasing. This accumulation of malate at maturity is very common in fruits [8]. At the same time there is small increase in protein nitrogen. The marked rise in uronic acids is typical of postmaturity stages, and is due to rapid degradation of pectic material.

EXPERIMENTAL

Material. Olive trees (40–50-yr-old *Olea europaea* cv. Marteño), on reddish–brown calcareous soil, treated as reported previously [5] were used. Leaf and fruit samples were collected in the morning at random from an orchard with 16 trees, at 15-day intervals from the 1st July to 31st of Dec. Then samples were at once taken to the laboratory and any adhering dirt removed with a cloth.

Methods. All the following steps were the same for fruits and leaves. Fr. wt was calculated as an average of approx. 100 fruits or leaves; dry wt was determined by heating at 100° constant weight. Metabolic activity was arrested by grinding in a mortar with dry Na_2SO_4 (1:3 w/w), adding butylated hydroxytoluene (approx. 0·1% of the fat content) as antioxidant. Fat was determined by extracting aliquots of the dry powder 5× with 50–70° petrol ether (1:5 w/v); the extracts were combined, evaporated to dryness under vacuum, and weighed. Other portions were defatted in a Soxhlet with Et_2O, and the residue extracted twice with 80% EtOH (1:10 w/v). Both extracts were filtered through a sintered glass funnel, the residue washed with an equal vol. of ethanol. The combined extracts were passed through a column (2 × 20 cm) of a strong acidic resin (Amberlite IRA-120, H^+ form, 20–25 mesh). The eluate was then passed through a column (2 × 20 cm) of a strong basic resin (Amberlite IRA-400, CO_3^{2-} form, 20–30 mesh). Free sugars were determined in the eluent. The retained Krebs cycle acids were eluted with $(NH_4)_2CO_3$ 1·5 N and conc. at 40° in vacuum. Protein nitrogen was determined in the residue of the ethanolic extraction. This residue was again extracted 3 × with distilled water according to Salminen and Koivistoinen [9] and the pectic material determined in the combined extracts. Protein nitrogen and uronic acids are determined, the former by Kjeldahl method, the latter according to Bitter and Muir [10]. Krebs cycle acids were determined as silyl derivatives [11] by GC. (Perkin Elmer mod. F-7), and sugars were assayed by the anthrone method [12].

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