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Changes in carbohydrates and mineral elements in *Citrus* leaves during flowering and fruit set

A. Sanz, C. Monerri, J. González-Ferrer and J. L. Guardiola

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Mineral elements and metabolizable carbohydrates in *Citrus* leaves [*Citrus sinensis* (L.) Osbeck cv. Washington navel] have been determined from bud sprouting until the end of the June drop and related to fruitlet growth and abscission. Mineral elements in old leaves decreased during the spring flush of growth and reached minimum values at flower opening, coinciding with a peak in abscission. This was followed by a rapid recovery in potassium and nitrogen to the initial values, with little change afterwards. Old leaves accumulate carbohydrates until flowering, and lose them during post-anthesis at a constant rate for more than 4 months; this rate of export is unaltered by the presence of a nearby growing fruit. Inflorescence leaves accumulate carbohydrates and mineral elements during post-anthesis; during the June drop there is an interruption in the accumulation of nitrogen and a net loss of phosphorus, potassium and carbohydrates from these leaves, coinciding with the attainment of the maximum growth rate of the fruit.

The two main periods of abscission coincide with minima in the amount of reserves in leaves, suggesting that a limitation in metabolite supply may be the primary cause of drop. There is a closer relationship of the fruit with inflorescence leaves than with old mature ones; however, the regulation of carbohydrate levels in the inflorescence leaves cannot be simply explained in terms of source-sink relationships with the nearby growing fruit, and the smaller size of inflorescence leaves vs. vegetative ones is not due to the presence of the flower during leaf development.

Additional key words – Fruit growth, leaf growth, orange trees, starch, sucrose.

A. Sanz (permanent address), Cátedra de Fisiología Vegetal, Facultad de Ciencias, Univ. de Valencia, Burjasot, E-46100 Valencia, Spain; C. Monerri, J. González-Ferrer and J. L. Guardiola (reprint requests), Cátedra de Fisiología Vegetal, Univ. Politécnica, E-46020 Valencia, Spain.

Introduction

Orange trees flower profusely, and in most cases fruit set and not flower number is the factor that determines yield (Goldschmidt and Monselise 1977, Agustí et al. 1982). Abscission of reproductive structures may occur at anthesis (flower abscission) or several weeks later, when the developing fruitlets have enlarged significantly (June drop); the relative importance of these two processes depends upon the level of flowering, the proportion of ovaries reaching the fruitlet stage decreases as the number of flowers increases (Agustí et al. 1982). In addition, when the number of flower buds is high, a significant proportion may abscise before anthesis (Erickson and Brannaman 1960, Agustí et al. 1982). Competition for nutrients among the developing organs is among the factors that determine abscission (Guardiola et al. 1984).

Based on differences in composition of the ovaries from persisting and abscising flowers, the abscission of flowers has been related to the availability of mineral nutrients under conditions of intense competition (Guardiola et al. 1984); however, the final set of fruit was not related to this factor. The possibility that fruit set is limited by carbohydrate supply may be inferred from the effect of inflorescence leaves on set (Sauer

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1954) and has recently received support (Goldschmidt and Golomb 1982, Borrás et al. 1984, Schaffer et al. 1985, González-Ferrer et al. 1987). However, the moment at which this supply may become insufficient has not been determined, and Jones et al. (1974) could not find any correlation between the carbohydrate content of the leaves during the June drop period and fruit set.

If fruit set is determined by nutrient supply it should be feasible to demonstrate that fruit abscission coincides with minima in the nutrient levels; further, a direct relationship between nutrient levels and set must exist. The first of these aspects has been studied in the present paper, in which we describe changes in carbohydrates and mineral elements in leaves during flowering and fruit set and relate them to fruit growth and abscission. In addition, the competing capacity of the developing fruitlets has been determined through the effect of their elimination at two stages of development on leaf growth and composition.

Materials and methods

Samples were taken from nine orchards of Washington navel orange trees [*Citrus sinensis* (L.) Osbeck], 25 years old, grafted on sour orange. Fertilizers were applied at the end of February, early April and the end of July at an annual rate of 1.2 kg N, 0.10 kg P and 0.25 kg K per tree. At the end of September the percentage of mineral elements in the spring-flush leaves from fruit-bearing terminals were on a dry matter basis %N, 2.42 ± 0.07; %P, 0.105 ± 0.02; %K, 0.68 ± 0.05; %Ca, 4.7 ± 0.15; %Mg, 0.25 ± 0.012; which, according to published standards (Chapman 1968), is considered as non-limiting.

Fruit and leaf samples were picked at fortnightly intervals in each orchard from bud sprouting until the end of June drop. Fruits were sampled from among the biggest, on single flowered leafy inflorescences. Two types of leaves were sampled separately: leaves from the leafy inflorescences formed during the spring flush cycle studied (young leaves), and leaves developed during the summer flush the previous year, which were 5 months old at the beginning of the samplings (old leaves). Old leaves were sampled from bud sprouting (mid-February); young leaves and fruitlets from flowering (early May). At this time, the young leaves had completed their expansion, the leaf area averaging 11.1 ± 0.3 cm^{-2}, smaller than for old leaves (19.6 ± 0.2 cm^{-2}). Each sample consisted of a minimum of 100 organs taken from 20 trees; the same trees were used for sampling for the whole experiment.

To determine the influence of the developing fruit on leaf growth and composition, a sufficient number of inflorescences were tagged and the fruit removed from half of them, either at the time of June drop (defruiting experiment) or, in a separate experiment, before anthesis at the flower bud stage (deflowering experiment). Leaf development was compared in fruiting and defruited inflorescences as well as on leaves from vegetative sprouts formed during the same flush of growth. Each experiment was repeated in a different year and orchard with similar results.

To determine the influence of fruit on composition of old leaves, all fruits were removed from twigs of about 2 cm diameter. One twig was defruited per tree, so the amount of fruits removed was less than 1% the total amount of fruits in the tree. The composition of old leaves was compared in defruited and control twigs of the same trees, two composite samples being taken from twenty trees.

The analyses of mineral elements were performed as described previously (Guardiola et al. 1984). Total nitrogen was determined by a micro-Kjeldahl, and total phosphorus and potassium after digestion of the samples with an acid mixture. Total soluble sugars and starch were extracted and determined as described by McCready et al. (1950). Free sugars were separated through HPLC using a 0.4×25 cm 5 µm Lichrosorb-NH_2 column eluted with acetonitrile: water (3:1 v/v) at a rate of 2.5 ml min^{-1}, and sucrose quantified using a refractive index detector. Results are expressed on a leaf area basis to compensate for the differences in weight and size of the leaves; leaf area was determined with a Li-Cor Portable Area Meter (Lincoln, NE, USA). The results were subjected to an analysis of variance when appropiate as an aid to interpretation.

Results

Changes in mineral elements from sprouting to June drop

From bud sprouting until full bloom there was a net loss of nitrogen, phosphorus and potassium from the old leaves (Fig. 1). Relative to the initial contents, this loss was bigger for phosphorus (46%) and potassium (31%) than for nitrogen (6.7%). One month after full bloom, both nitrogen and potassium had recovered to the initial values. No further change was detectable in nitrogen contents, whilst a transient drop in potassium was found during June drop. There was a steady increase in phosphorus for 3 months after bloom, but the final contents were lower than at sprouting.

Young leaves accumulated mineral elements until the beginning of the June drop. For two weeks there was then a transient interruption in the accumulation of nitrogen, and afterwards an increase to the value found in the old leaves. There was a net loss of phosphorus and potassium from the young leaves during the June drop. No significant increase in their levels was detectable for 6 weeks after the end of drop; the final value for phosphorus was higher, and for potassium lower, than in old leaves at the end of the test period.

The increase in dry weight of the fruit was small in absolute terms (ca 2 mg day^{-1}) for 20 days after bloom (Fig. 2). Fruitlet drop started on 5 June, 40 days after bloom, coinciding with increasing growth rate of the

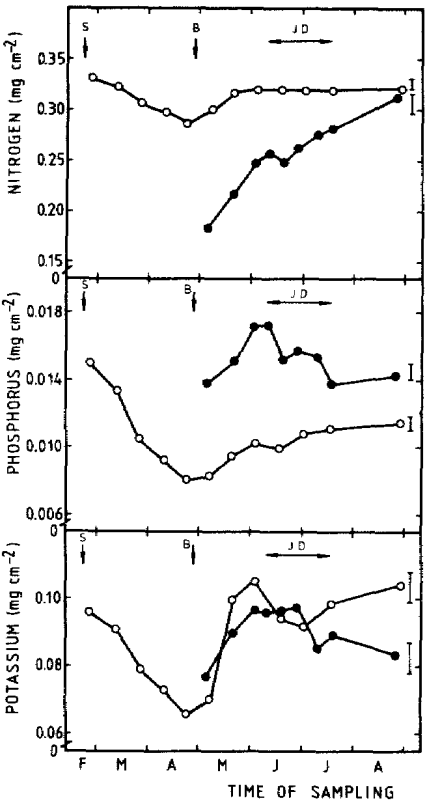

Fig. 1. Total mineral element contents in old (open symbols) and young (closed symbols) leaves from bud sprouting until the end of June drop. Each value is the mean from nine orchards. The lowest significant difference ($P = 0.95$) between two samples is given as vertical bars. S, bud sprouting; B, bloom; JD, June drop.

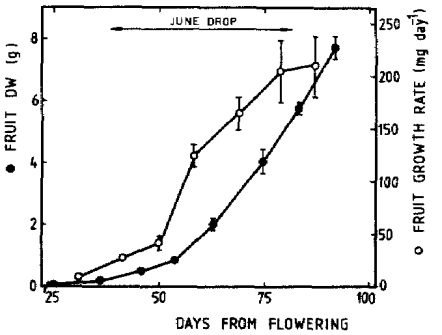

Fig. 2. Fruit dry weight (closed symbols) and growth rate (open symbols) from the end of flowering until the end of the June drop. Data for the fruits from single flowered leafy inflorescences. Each value is the mean from nine orchards ± SE.

fruit, and ended by 15 July when the fruit growth rate reached its maximal value. During this time, the accumulation of mineral elements in the fruit is linearly and directly related to the accumulation of dry matter (Guardiola et al. 1984, González-Ferrer et al. 1987).

Carbohydrate changes in the leaves

Metabolizable carbohydrates accumulated in the old leaves from sprouting until bloom, and then decreased at a constant rate until mid-August, when drop had ended (Fig. 3). This pattern of change reflects the change in starch levels of the leaves; ethanol-soluble sugars and sucrose in the old leaves remained fairly constant throughout the experimental period, except for the slightly higher levels of ethanol-soluble sugars before bloom.

Both starch and soluble sugars accumulated in the young leaves until the beginning of drop (Fig. 3). During the June drop there was a rapid and transient decrease in their contents followed by a recovery, after which they decreased to the same levels as in the old leaves at the end of the test period. The accumulation of starch before the June drop did not take place in the

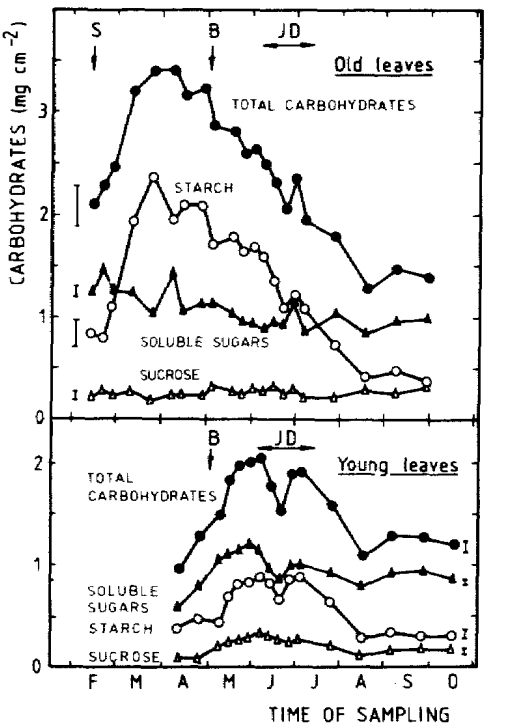

Fig. 3. Changes in starch (○), ethanol-soluble sugars (▲), sucrose (△) and total metabolizable carbohydrates (starch + soluble sugars (●) in old and young leaves from sprouting until the end of drop. Otherwise as for Fig. 1.

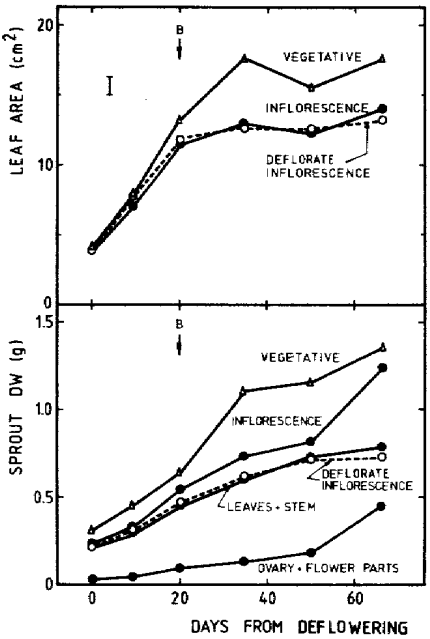

Fig. 4. The influence of deflowering on the total leaf surface area and on the accumulation of dry matter in the inflorescence as compared to vegetative shoots. Flower buds removed on 18 April, twenty days before bloom (Arrow B). Data for vegetative shoots (△), deflorate (○) and intact inflorescences (●). For intact inflorescences the distribution of the dry matter between the developing fruitlet + flower parts and leaves + stem is also given. The lowest significant difference ($P = 0.95$) is given as vertical bar.

leaves from vegetative shoots, which had a lower starch content than leaves from inflorescences during this time (data not shown). The sucrose changes paralleled the changes in soluble sugars, with maximal values at the beginning of the June drop and then a decrease at the end of July to a value lower than in old leaves (Fig. 3).

The influence of deflowering on leaf growth and composition

Deflowering the inflorescence 20 days before anthesis did not affect leaf growth as measured by the surface area, nor the increase in dry weight of the non-reproductive organs of the inflorescence (Fig. 4). Leaves from vegetative shoots grew faster and their growth period lasted 2 weeks longer than in leaves from inflorescences, whether the latter had been deflowered or not. Differences in leaf composition due to deflowering became significant only 30 days later, coinciding with the increase in growth rate of the fruit. The leaves from deflorate inflorescences were higher than the intact ones in nitrogen, phosphorus and potassium, which were close to the values found in leaves from vegetative shoots (Fig. 5). In contrast, the increase in growth rate

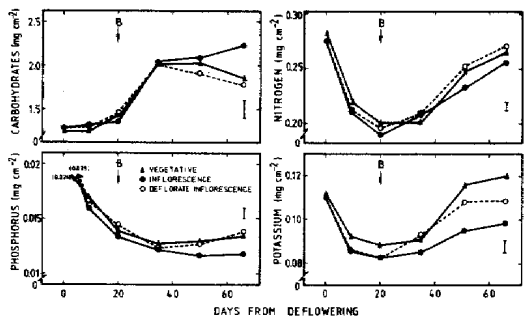

Fig. 5. The influence of deflowering on the composition of inflorescence leaves. Data from young vegetative leaves given as a comparison. See Fig. 4 for further details.

of the fruit brought about an accumulation of metabolizable carbohydrates in the inflorescence leaves (Fig. 5) above the levels found in deflorate inflorescences and vegetative shoots. These differences were due to differences in the accumulation of starch, since the soluble sugars were similar in the three types of leaves (0.95 ± 0.05 mg cm^{-2}).

The influence of fruit removal on leaf composition

Removal of fruit during the June drop resulted in a rapid accumulation of nitrogen and potassium in leaves from defruited inflorescences, which was completed after 7 days. Accumulation of phosphorus was more gradual and lasted for at least 3 weeks (Fig. 6).

Defruiting the inflorescence accelerated starch loss

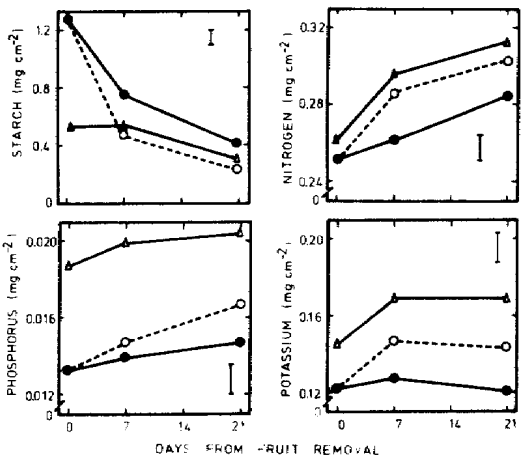

Fig. 6. The influence of fruitlet removal on the composition of inflorescence leaves. Inflorescences defruited 25 June, 50 days after bloom, at the end of the June drop. Data from young vegetative leaves given as a comparison. Otherwise as for Fig. 5.

from the leaves, which after 7 days had a starch content similar to leaves from vegetative shoots and significantly lower than in control inflorescences (Fig. 6). This faster starch breakdown was paralleled by an increase in amylolytic activity to the level found in leaves from vegetative shoots (data not shown). It was not accompanied by an accumulation of ethanol-soluble sugars, which 1 week after the removal of the fruit had decreased from an initial value of 0.99 ± 0.01 mg cm^{-2} to 0.73 ± 0.02 mg cm^{-2}, a value significantly lower than in control inflorescences (0.95 ± 0.03 mg cm^{-2}).

Removing all the fruits from a branch affected neither the rate of starch breakdown nor the contents of soluble sugars in old leaves as compared with equivalent leaves from control branches of the same tree (data not shown).

Discussion

Flower opening coincides with minima in the mineral nutrient contents in old leaves (Fig. 1); and June drop with a transient decrease in metabolizable carbohydrates (Fig. 3) as well as in some mineral nutrients (Fig. 1) in young inflorescence leaves. The two main periods of drop of reproductive structures thus coincide with a minimum in the amount of reserves, either organic or mineral, in vegetative organs of the tree, which supports the assumption that a limitation in metabolite supply may be the primary cause of drop (Guardiola et al. 1984).

From bud sprouting to flowering, photosynthesis exceeds carbohydrate consumption as shown by the accumulation of non-structural carbohydrates both in leaves (Fig. 3) and other tree parts (data not shown). Thus, the suggestion of Goldschmidt and Golomb (1982) that carbohydrate supply may be a limiting factor in flower emergence seems unlikely from our results. However, mineral nutrient uptake by the roots is low during this period of time (Chapman and Parker 1942, García-Martínez and Martí 1974), and the growth of the developing organs strongly depends on the retranslocation of reserves. Phosphorus and potassium are retranslocated more readily than is nitrogen (Fig. 1), and the amount of those elements that were retranslocated from the old leaves may represent up to 70% of the total amount used in the formation of new organs until flower opening (González-Ferrer et al. 1987); while retranslocated nitrogen represents less than 40% of the total nitrogen needs. Through manipulation of the number of developing flowers it has been shown that this limitation in the supply of mineral elements affects the initial fruit set, and that this effect is accompanied by changes in the mineral composition of the ovaries and developing fruitlets (Guardiola et al. 1984). Under the conditions of ample supply in our experiment, this competition may last for only a short time after flower opening, since the mineral contents again increase in the old leaves (Fig. 1). However, Moreno and García-Martínez (1984) found an increase in the nitrogen content in old leaves only after the end of the June drop, which could be due to a lower availability of soil nitrogen in their experiments.

During the June drop period the rate of dry matter accumulation in the developing fruitlets (Fig. 2) is higher than the potential amount of carbohydrates formed as calculated from the published values for CO_2 fixation in *Citrus* leaves (Kriedemann 1968, Moss et al. 1972, Akao et al. 1981) and the mean leaf surface in the tree per developing fruitlet (1.6 dm^2 of old leaves and 0.6 dm^2 of young leaves by day 65 from flowering). Carbohydrate reserves are mobilized from the leaves (Fig. 3) and other tree organs (data not shown), but the rate of mobilization is different for old and inflorescence leaves. Old leaves lost carbohydrates from flower opening until the end of August at a constant rate irrespective of fruit growth rate, and elimination of all fruits from the branch had no effect on the rate of transport from these leaves, which is largely independent on the presence of nearby growing fruits. This conclusion is supported by the observations of Kriedemann (1970), who showed that old lemon leaves translocate recently synthesized assimilates basipetally rather than towards the terminal fruit. It is then not surprising that the amount of carbohydrates in old leaves appears unrelated to fruit set (Jones et al. 1970, 1974).

On the contrary, carbohydrate levels in inflorescence leaves are closely related to fruit growth. Inflorescence leaves accumulate carbohydrates until the beginning of June drop (Fig. 3). At this moment the mean CO_2 fixation in the inflorescences is not sufficient to meet the requirements of a single fruit (Moss et al. 1972), and there is a sudden drop in carbohydrate in the inflorescence leaves (Fig. 3). The supply of carbohydrates may thus limit fruit growth in some inflorescences and cause fruit abscission. A direct relationship between carbohydrate availability and fruit abscission has, however, not been proven by our work, since inflorescences with the biggest fruitlets on the tree were selected for the samplings, and these fruitlets have the maximum probability of survival (Zucconi et al. 1978, Agustí et al. 1982). A comparison of carbohydrate levels in inflorescences with a different probability of fruit survival would be necessary. The competition between the fruitlets and the leaves for the supply of mineral elements (Figs 1 and 5) is unrelated to fruit set under conditions of ample supply (Guardiola et al. 1984).

The developing fruitlets compete with inflorescence leaves for metabolites, and the increase in mineral levels in the leaves following fruit elimination (Figs 5 and 6) is easily explained in terms of competition. However, the transient accumulation of carbohydrates in inflorescence leaves as compared to young leaves from vegetative sprouts, and which depends critically on the presence of the fruit during both accumulation (Fig. 5) and depletion (Fig. 6), is not easily explainable in this way. The possibility of a hormonal regulation as suggested

for other plant systems (Daie 1985) should be taken into account. The sink strength of the fruit becomes significant only at the beginning of the June drop, when the growth rate of the fruit increases and the differences in composition between the leaves from deflorate and intact inflorescences appear (Fig. 5). The smaller size of inflorescence leaves as compared to young vegetative leaves is not due to the presence of the fruit and, accordingly, is not increased by blossom removal (Fig. 4). A condition of the inflorescence other than the presence of the flower limits leaf growth. Flower buds have a lower endogenous gibberellin level than vegetative buds (Goldschmidt and Monselise 1972). The application of exogenous gibberellins increases leaf size in the inflorescence to a value close to vegetative sprouts (data not shown), so it is likely that the differences in endogenous gibberellins are responsible for these differences in size.

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