

Hormonal regulation of fruitlet abscission induced by carbohydrate shortage in citrus

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Abstract. The hormonal signals controlling fruitlet abscission induced by sugar shortage in citrus were identified in Satsuma mandarin, *Citrus unshiu* (Mak.) Marc, cv. Clausellina and cv. Okitsu. Sugar supply, hormonal responses and fruitlet abscission were manipulated through full, partial or selective leaf removals at anthesis and thereafter. In developing fruitlets, defoliations reduced soluble sugars (up to 98%), but did not induce nitrogen and water deficiencies. Defoliation-induced abscission was preceded by rises (up to 20-fold) in the levels of abscisic acid (ABA) and 1-aminocyclopropane-1-carboxylic acid (ACC) in fruitlets. Applications to defoliated plants showed that ABA increased ACC levels (2-fold) and accelerated fruitlet abscission, whereas norflurazon and 2-aminoethoxyvinyl glycine reduced ACC (up to 65%) and fruitlet abscission (up to 40%). Only the full defoliation treatment reduced endogenous gibberellin A₁ (4-fold), whereas exogenous gibberellins had no effect on abscission. The data indicate that fruitlet abscission induced by carbon shortage in citrus is regulated by ABA and ACC originating in the fruits, while gibberellins are apparently implicated in the maintenance of growth. In this system, ABA may act as a sensor of the intensity of the nutrient shortage that modulates the levels of ACC and ethylene, the activator of abscission. This proposal identifies ABA and ACC as components of the self-regulatory mechanism that adjusts fruit load to carbon supply, and offers a physiological basis for the photoassimilate competition-induced abscission occurring under natural conditions.

Key words: Abscission (fruit) – 1-Aminocyclopropane-1-carboxylic acid – Carbohydrate supply – *Citrus* (fruit abscission) – Defoliation – Phytohormone

Introduction

Like many fruit trees species, citrus trees blossom with high profusion and thereafter exhibit massive fruitlet abscission. Current evidence indicates that this process is under hormonal and metabolic regulation (Gillaspy et al. 1993). In citrus, it has been suggested that after hormonal activation of initial fruit growth subsequent development is mostly supported by nutrient supply (Talon et al. 1997). Thus, once mineral and water requirements are satisfied, competition for photoassimilates is thought to be responsible for fruit drop (Moss et al. 1972; Powell and Krezdorn 1977; Goldschmidt and Koch 1996). We have recently shown through defoliation experiments that the sucrose status of the fruitlet is a major factor in the regulatory mechanism of citrus abscission (Mehouchi et al. 1995). Similar results have previously been presented for other species, including the elegant work by Berüter and Droz (1991) in apple. However, the nature of the hormonal signals that link carbon shortage and abscission are still unknown. According to the literature, major candidates for this function in citrus are gibberellins (GAs), abscisic acid (ABA) and ethylene. Gibberellins, which are promoted by both parthenocarpic stimuli (Talon et al. 1992) and pollination events (Ben-Cheikh et al. 1997), are apparently responsible for the ovary-to-fruit transition. On the other hand, the role of ethylene in enhancing fruit abscission in citrus has been proven in explants (see Goren 1993, for review). It has also been demonstrated that ethylene is the in-vivo hormonal activator of leaf abscission (Tudela and Primo-Millo 1992). In contrast, the function of ABA in fruit abscission is not well understood. In several species, e.g. cotton (Guinn 1982), apple (Vernieri et al. 1992),

Abbreviations: ABA = abscisic acid; ACC = 1-aminocyclopropane-1-carboxylic acid; AVG = 2-aminoethoxyvinyl glycine; DAA = days after anthesis; GA_n = gibberellin A_n; GA₃ = gibberellic acid; NF = norflurazon

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and citrus (Takahashi et al. 1975; Sagee and Erner 1991; Talon et al. 1990; Zacarias et al. 1995), ABA has been implicated in the abscission of young fruit. In *Citrus*, however, exogenous ABA does not promote abscission in intact plants, although in aged or injured detached tissues it is possible to stimulate ethylene synthesis and promote abscission with high amounts of ABA (Goren 1993). In addition, recent results have demonstrated that environmental stresses can induce in-vivo ABA accumulations in intact plants, and that these rises are required for leaf abscission to occur (Gómez-Cadenas et al. 1996, 1998). Moreover, ABA in these systems appears to act as the modulator of 1-aminocyclopropane-1-carboxylic acid (ACC) levels, and therefore of ethylene.

The above observations are in agreement with the hypothesis advanced by Talon et al. (1997) that carbon shortage reduces hormonal stimulators of growth, such as GAs, and increases stress-sensitive signals, such as ABA and ACC levels. This mechanism would allow, through abscission, regulation of fruit load in accordance with the severity of the sugar deficiency. To test this proposal, several defoliation treatments were applied to citrus to manipulate sugar supply, hormonal responses, and timing and intensity of abscission. The results obtained identify the hormonal sequence that triggers citrus fruitlet abscission induced by carbohydrate shortage during the initial period of growth.

Materials and methods

Plant material. The characteristics of the Satsuma mandarin, *Citrus unshiu* (Mak.) Marc, cv. Clausellina and cv. Okitsu, used in these experiments have been described by Mehouchi et al. (1995). Briefly, both cultivars belong to the Satsuma Wase group, have similar morphology, and show moderate dwarfism. Despite the reduced size (1.5–2 m tall), fruit growth behaviour in these cultivars follows the usual pattern observed in Satsuma. All plants were 7 years old and grown in experimental fields at the Instituto Valenciano de Investigaciones Agrarias (Moncada, Valencia, Spain).

Defoliation treatments. Clausellina plants were defoliated at anthesis and 10 d after anthesis (DAA), whereas Okitsu was defoliated at 35 DAA. Treatments were as follows: no defoliation, 100% defoliation, 50% defoliation, and mature-leaf defoliation. Fruitlets attached to the plants were randomly sampled from full bloom up to the end of the fruit abscission period (66 DAA). Woody samples (branches, and pioneer and fibrous roots) were harvested in control and fully defoliated plants of Clausellina and Okitsu 10 and 7 d after defoliation, respectively. Reproductive and woody samples were lyophilised, ground and stored at -20°C until analysed. Samples of xylem fluid were collected from fully and non-defoliated Clausellina plants and immediately frozen and stored at -70°C . Fruit drop was periodically recorded on populations of 200 tagged fruitlets at 2- or 3-d intervals. Abscission rates were calculated as the percentage of fruitlets dropped each week relative to the number present at the beginning of that week.

Chemical treatments. Developing fruitlets on Clausellina plants fully defoliated at 10 DAA, were treated every 3 d with one of the following solutions: 100 μM 2-aminoethoxyvinyl glycine (AVG), 100 μM ACC, 500 μM (\pm)-ABA, 58 μM gibberellic acid (GA_3) (all from Sigma-Aldrich), 1 mM norflurazon (NF; Zorial, an 80%

NF commercial preparation from Sandoz Agro, Basel, Switzerland), 1 mM NF + 500 μM (\pm) ABA, or water. A concentration of 500 μM ABA has been found to be the lowest concentration that renders reproducible data. Applications started the same day of the defoliation treatment. All solutions contained 0.05% Tween-20, and chemicals were dissolved in 5% (v/v) aqueous ethanol. The compounds were applied by dipping fruitlets in the solutions for 15 s.

Extraction of xylem fluid. Xylem fluid was extracted using a Soilmoisture 3000 (Santa Barbara, Calif., USA) pressure chamber as described previously (Gómez-Cadenas et al. 1996, 1998). Pressures of 2.5 MPa were applied for 7 min to approximately 50-cm-long branches from fully and non-defoliated plants. Extractions were made at 9 a.m. and 0.75- to 1.50-ml aliquots were obtained from each branch.

Nitrogen analysis. Total nitrogen was determined on samples of 0.5-g DW using the semi-micro Kjeldahl method (Bremner 1965) with sulphate-selenium catalysis and steam distillation.

Carbohydrate analysis. The procedure for carbohydrate determinations was previously described (Mehouchi et al. 1995). In brief, samples were purified sequentially by cation- and anion-exchange columns, and by C_{18} -cartridges. Sucrose, glucose and fructose were analysed using a Waters-Millipore (Milford, Mass., USA) HPLC system equipped with a high-performance carbohydrate column (250 mm long, 4.6 mm i.d.; Waters-Millipore) and a R401 differential refractometer. The effectiveness of the extractions was ascertained using fucose as internal standard.

Hormone extraction and analysis. The procedures for ABA, ACC and GA analyses were previously reported in detail. Abscisic acid was purified by reverse-phase HPLC and quantified by enzyme-linked immunosorbent assay (ELISA; Gómez-Cadenas et al. 1996, 1998). The values for the endogenous ABA obtained with the ELISA were comparable to those obtained by GC with an electron-capture detector (Gómez-Cadenas et al. 1996).

Analysis of ACC was carried out by determining ethylene release as described by Tudela and Primo-Millo (1992). Gas chromatography and operating parameters were essentially as described by Gómez-Cadenas et al. (1998).

Determination of GAs involved sequential purification through adsorption, polyvinylpyrrolidone, and ion-exchange chromatography. Further clarification was obtained with C_{18} Sep-Pak cartridges and reverse-phase HPLC (Ben-Cheikh et al. 1997). The samples were quantified using GC-MS with a gas chromatograph (model 8000; Fisons, Danvers, Mass., USA) coupled to a quadrupole mass detector (800; Fisons). For quantification, different amounts of deuterated GA_{19} , GA_{20} , GA_{29} , GA_1 , and GA_8 , were used in successive extractions, following the strategy reported in Ben-Cheikh et al. (1997). Although the successive quantifications in the different experiments showed similar tendencies, only data from the last experiment are reported since, in this, GA determinations were more precise, according to the calculation method utilised.

Results

Similar effects of defoliation in the Clausellina and Okitsu cultivars. In a previous study it was demonstrated that defoliation reduced carbohydrate levels and increased abscission similarly in developing fruits of Clausellina and Okitsu (Mehouchi et al. 1995). The results presented here confirm these observations and expand that work to show further effects. Since the results were practically identical for both varieties only data for Clausellina are presented below.

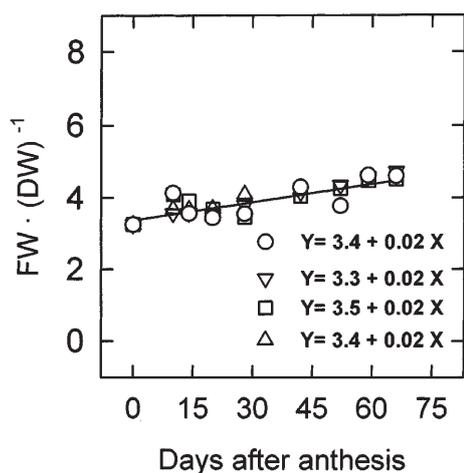


Fig. 1. Ratio of fresh (FW) to dry weight (DW) in fruitlets of Clausellina. Treatments were: ○, none; △, 100% defoliation; ▽, 50% defoliation; □, mature-leaf defoliation. All fruitlets in the full defoliation treatment abscised after 42 DAA. Defoliation treatments were carried out at anthesis. Data are means of 50 measurements and the SE bars are smaller than the symbols used. Linear regressions for all four treatments were significant at $P \leq 0.001$

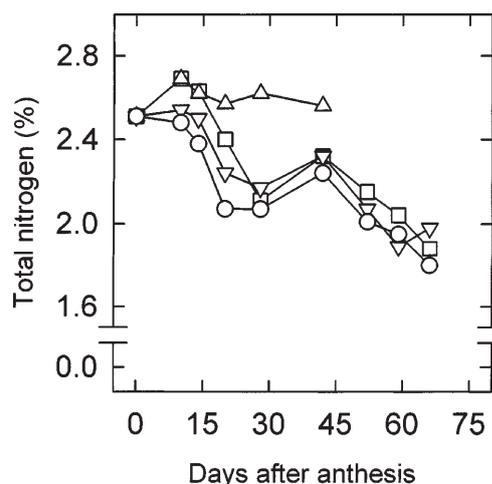


Fig. 2. Total nitrogen in fruitlets of Clausellina. Treatments were: ○, none; △, 100% defoliation; ▽, 50% defoliation; and □, mature-leaf defoliation. All fruitlets in the full defoliation treatment abscised after 42 DAA. Defoliation treatments were carried out at anthesis. Data are means of three independent measurements and the SE bars are smaller than the symbols used

Water status, nitrogen content and carbohydrate levels in fruitlets. In defoliated and non-defoliated plants of Clausellina, the ratios of fresh-to-dry weight of developing fruitlets were almost identical (Fig. 1). In control fruitlets, this ratio increased linearly according to the equation: $Y = 3.4 + 0.02 X$. Linear regressions in the defoliation treatments were very similar. The statistical analyses indicated that the four linear regressions were significant at $P \leq 0.001$. Analogous results and similar statistical significance were found in Okitsu (data not shown). Thus, the different defoliation treatments did not affect the water content of the fruitlets and the increase, in the hormone level was not due to water deficit.

In non-defoliated Clausellina plants, total nitrogen content in fruitlets decreased in two phases (Fig. 2). Nitrogen decreased from 2.51% to 2.07% during the first 20 DAA, and after a minor transient increase, levels fell again to reach 1.80% at 66 DAA. The partial defoliations did not substantially modify this pattern of change, although the absolute levels were slightly higher. Fruitlets on fully defoliated plants, however, did not show this nitrogen reduction and always had higher nitrogen levels (up to 2.62%). In Okitsu, defoliation also induced increases in total nitrogen similar to those described for Clausellina (data not shown). Total nitrogen in fruiting branches and in pioneer and fibrous roots of both cultivars was very similar in defoliated and non-defoliated plants (data not shown).

The changes in carbohydrate levels induced by defoliation in these cultivars have been previously described (Mehouachi et al. 1995). In the present work, it was confirmed that soluble sugars decreased and were low during the cell division period, or first phase of growth (until 42 DAA), and started to accumulate thereafter. Defoliations further reduced sugar depletion during the final stage of this first phase of growth and

delayed the onset of sugar accumulation in the second phase. A concise listing of the results obtained for Clausellina at 42 DAA is presented in Table 1. On a $\text{mg} \cdot (\text{g DW})^{-1}$ basis, full defoliations induced drastic reductions in the hexoses, e.g. approx. 75% for glucose, and approx. 50% for sucrose. The sugar reductions observed in partial defoliations were moderate (up to 30% as maximum). Since defoliations also promoted abscission (see below) the percentages of total soluble sugars in the whole population of fruitlets were considerably reduced (3.5% in fully, and 42.0% and 43.1% in partially defoliated plants, at 42 DAA for example). Similar trends were found in Okitsu (data not shown).

Abscission and hormonal content in fruitlets. In Clausellina, fruitlet abscission induced by mature-leaf defoliation took place mostly between 14 and 28 DAA (Fig. 3). The pattern of change in the abscission rates indicated that this fall occurred in a single wave (data not shown). During this period, accumulative abscission increased from 4% to 50%, and afterwards it was minimal. The abscission rise was preceded by transitory increases in ABA and ACC levels in fruit, that reached their highest values, 14.2 μmol and 2.3 nmol (on a per g DW basis), at 20 DAA. Subsequently, ABA and ACC levels progressively decreased to reach, at 52 DAA, values comparable to those found in fruitlets on control plants. The 50%-defoliation treatment produced two consecutive phases of fruit abscission, from 14 to 35 DAA, and from 35 to 52 DAA. These two periods, which could only be known intuitively in Fig. 4, were obvious when the abscission rates were plotted (data not shown). During the first wave, almost 40% abscission was reached, whereas accumulative abscission at 52 DAA was 71%. Abscission was minimal afterwards. Changes in ABA also followed a biphasic pattern with transitory

Table 1. Glucose and sucrose levels, and percentages of total amounts of non-reducing carbohydrates (glucose + sucrose + fructose) in the whole population of fruitlets in defoliated and non-defoliated *Clausellina* plants at 42 DAA. Defoliation treatments were carried out at anthesis. Data are means \pm SE of three independent measurements

| Treatment | Non-reducing carbohydrates [mg \cdot (g DW) ⁻¹] | | Total amounts of non-reducing carbohydrates (%) |
|-------------------------|--|----------------|---|
| | Glucose | Sucrose | |
| Control | 4.0 \pm 0.9 | 11.8 \pm 0.1 | 100.0 |
| 100% defoliation | 1.1 \pm 0.1 | 5.4 \pm 0.1 | 3.5 |
| 50% defoliation | 4.6 \pm 1.0 | 8.6 \pm 0.4 | 42.0 |
| Mature leaf defoliation | 2.8 \pm 0.2 | 9.1 \pm 0.3 | 43.1 |

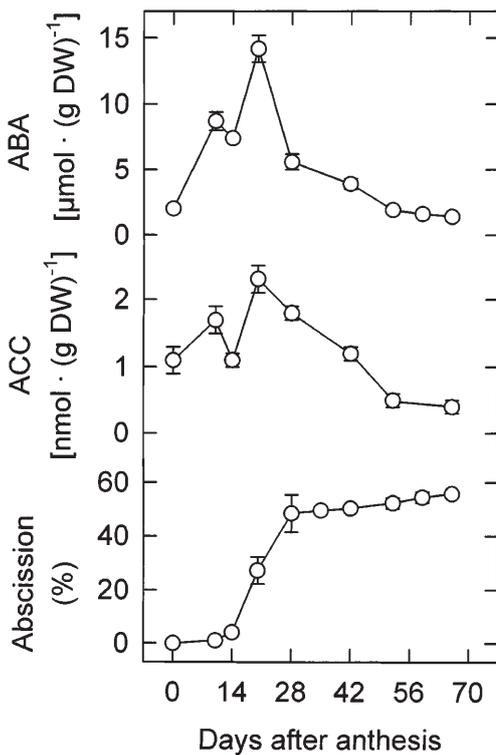


Fig. 3. Effect of mature-leaf defoliations on ABA, ACC, and abscission in developing fruitlets of *Clausellina*. Defoliations were carried out at anthesis. Values of ABA and ACC are means \pm SE of three measurements. Abscission measurements were performed on populations of 200 tagged fruitlets. When error bars are not shown they are smaller than the symbols used

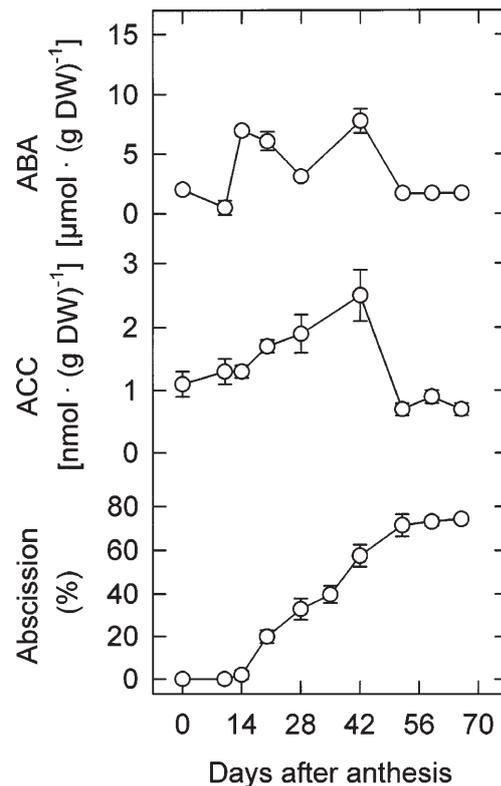


Fig. 4. Effect of 50% defoliations on ABA, ACC, and abscission in developing fruitlets of *Clausellina*. Defoliation was carried out at anthesis. Values of ABA and ACC are means \pm SE of three measurements. Abscission measurements were performed on populations of 200 tagged fruitlets. When error bars are not shown they are smaller than the symbols used

risers at 14 and 42 DAA (7.0 and 7.8 μmol , respectively), preceding both abscission waves. Thereafter, ABA decreased and was similar to the levels found in control fruitlets. For ACC, the biphasic pattern was not so evident, since ACC levels apparently increased continuously until 42 DAA (from 1.7 to 2.5 nmol). Full defoliations induced 100% fruitlet abscission at 52 DAA, through continuous fruit drop (Fig. 5). The treatment also promoted constant increases in ABA and ACC, which paralleled that of abscission. Levels of both reached 45.3 μmol and 25.0 nmol, respectively (>20 -fold increases) at 42 DAA. Similar effects to those described for *Clausellina* were found in *Okitsu*. In spite of the ABA and ACC changes in fruits, defoliations in both varieties did not modify xylematic concentrations (Fig. 6) or levels of the two metabolites in woody tissues, such as branches and roots (data not shown).

The GA analyses performed on 42-DAA *Clausellina* fruits demonstrated that, in comparison with control plants, partial defoliations reduced the levels of the GA_1 precursors, GA_{19} and GA_{20} (between 50% and 23%). These treatments did not modify GA_1 [$3.4 \text{ ng} \cdot (\text{g DW})^{-1}$] and hardly altered the 2 β -hydroxylated end-products, GA_{29} and GA_8 (Table 2). However, the full defoliation treatment showed an opposite pattern with clear-cut differences. This treatment increased GA_{19} , GA_{29} (3-fold) and GA_8 (26-fold) and reduced GA_{20} (15-fold) and GA_1 (4-fold).

Effect of exogenous chemicals on fruitlet abscission and hormonal levels. Figure 7 shows the influence of different chemicals on fruitlet abscission induced by full defoliation at 10 DAA in *Clausellina* plants. Whereas

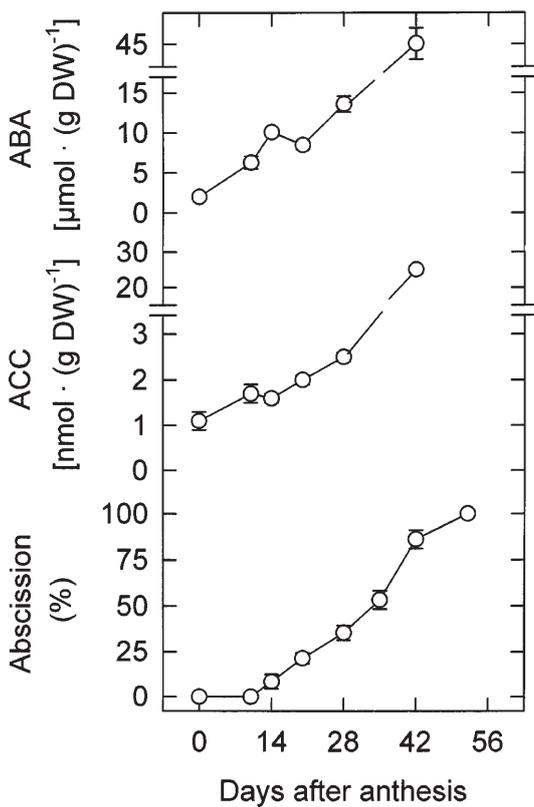


Fig. 5. Effect of full defoliations on ABA, ACC, and abscission in developing fruitlets of *Clausellina*. Defoliation was carried out at anthesis. Values of ABA and ACC are means \pm SE of three measurements. Abscission measurements were performed on populations of 200 tagged fruitlets. When error bars are not shown they are smaller than the symbols used

final fruit abscission in non-defoliated plants was 20%, full defoliation provoked 100% abscission in 35 d. The applied chemicals could be subdivided into three groups according to their effects: (i) NF (an indirect inhibitor of ABA biosynthesis) and AVG (an inhibitor of ethylene biosynthesis) reduced final abscission to 80% and 55%, respectively; (ii) GA_3 did not alter the abscission pattern; and (iii) ABA and ACC accelerated abscission rates, since both induced a 100% drop in 20 d. The simulta-

Table 2. Gibberellin content in remaining fruitlets of defoliated and non-defoliated *Clausellina* plants at 42 DAA. Defoliation treatments were carried out at anthesis. The GA quantitation was repeated in successive extractions with similar trends, although the data presented belong to the last experiment, the most precise, according to the calculation method utilised

| Treatment | Gibberellin content [$\text{ng} \cdot (\text{g DW})^{-1}$] | | | | |
|-------------------------|--|------------------|---------------|---------------|------------------|
| | GA_{19} | GA_{20} | GA_1 | GA_8 | GA_{29} |
| Control | 36.7 | 29.0 | 3.4 | 0.1 | 4.5 |
| 100% defoliation | 52.1 | 1.9 | 0.9 | 2.6 | 15.3 |
| 50% defoliation | 24.5 | 15.4 | 3.5 | 0.3 | ND ^a |
| Mature leaf defoliation | 28.2 | 21.7 | 3.4 | 0.5 | 5.2 |

^aNot determined due to sample contamination

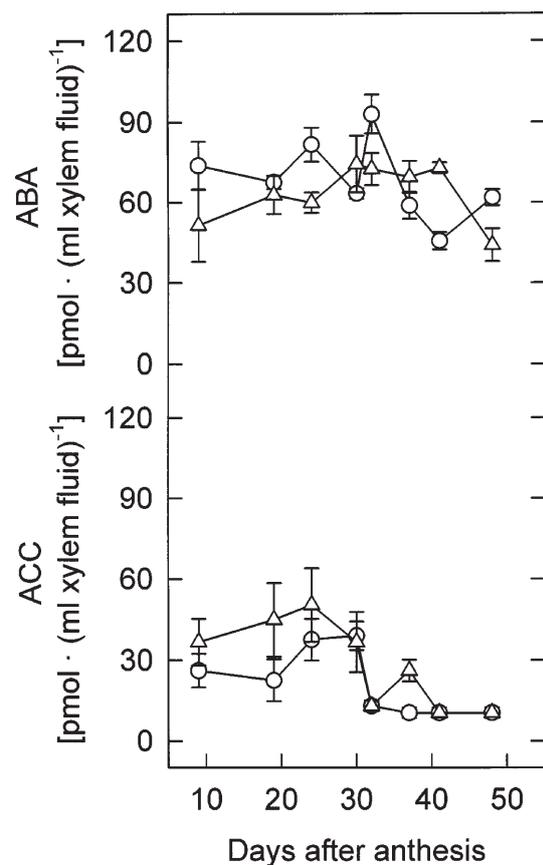


Fig. 6. Levels of ABA and ACC in the xylem fluid of *Clausellina* plants. Treatments were: ○, none; △, 100% defoliation. Defoliation was carried out at anthesis. Data are means \pm SE of three measurements. When error bars are not shown they are smaller than the symbols used. The whole experiment was repeated twice and trends were similar

neous treatment with NF and ABA had the same effect than ABA alone.

The ABA increase induced by full defoliation was notably reduced by NF applications (3-fold, after 33 DAA), whereas AVG had no effect (Table 3). On the other hand, ABA alone or in combination with NF further promoted ACC increases (almost 2-fold). In contrast, NF treatments induced substantial reductions (roughly 40%) in ACC (Table 4). The results also indicate that AVG substantially decreased ACC levels (up to 2.6-fold).

Discussion

In citrus, massive abscission of developing ovaries and fruitlets generally occurs between anthesis and the June drop during the period of cell division. Under normal conditions of growth, citrus fruits are insensitive to abscission during the following period of cell enlargement, until ripening is completed. Although fruit drop is mostly continuous during the period of cell division, different abscission waves can be distinguished. The evidence indicates that the abscission observed shortly

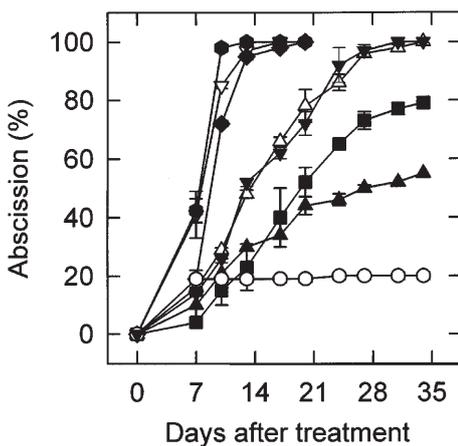


Fig. 7. Effect of applications of several chemicals on fruitlet abscission in fully defoliated *Clausellina* plants. Chemical treatments were: Δ , none; \blacktriangle , 100 μM AVG; \blacksquare , 1 mM NF; \blacklozenge , 500 μM ABA; ∇ , 100 μM ACC; \blacktriangledown , 500 μM ABA + 1 mM NF; and \circ , 50 μM GA₃. The abscission of non-defoliated plants (\circ) is also shown. Defoliation was carried out at 10 DAA and chemical applications were repeated every 3 d. Abscission measurements were made on populations of 200 tagged fruitlets

Table 3. Levels of ABA [$\mu\text{mol} \cdot (\text{g DW})^{-1}$] in *Clausellina* fruitlets from fully defoliated plants treated with either water, 1 mM NF or 100 μM AVG. Defoliation was carried out at 10 DAA and chemical applications were repeated every 3 d. Values are means \pm SE of three independent measurements

| Treatment | Days after defoliation | | |
|-----------|------------------------|----------------|----------------|
| | 10 | 16 | 23 |
| Water | 14.1 \pm 1.2 | 25.9 \pm 1.8 | 26.1 \pm 2.6 |
| NF | 9.5 \pm 1.1 | 16.3 \pm 1.9 | 9.2 \pm 0.9 |
| AVG | ND ^a | 24.4 \pm 2.2 | 25.7 \pm 2.6 |

^aNot determined

Table 4. Levels of ACC [$\text{nmol} \cdot (\text{g DW})^{-1}$] in *Clausellina* fruitlets from fully defoliated plants treated with either water, 500 μM ABA, 1 mM NF or 100 μM AVG. Defoliation was carried out at 10 DAA and chemical applications were repeated every 3 d. Values are means \pm SE of three independent measurements

| Treatment | Days after defoliation | | |
|-----------|------------------------|---------------|---------------|
| | 10 | 16 | 23 |
| Water | 2.4 \pm 0.4 | 3.7 \pm 0.2 | 4.5 \pm 0.2 |
| ABA | 6.3 \pm 0.7 | 8.1 \pm 0.2 | 7.1 \pm 0.3 |
| NF | 2.3 \pm 0.1 | 2.6 \pm 0.1 | 2.7 \pm 0.1 |
| NF + ABA | ND ^a | 6.8 \pm 0.5 | 6.6 \pm 0.1 |
| AVG | ND ^a | 2.1 \pm 0.1 | 1.7 \pm 0.1 |

^aNot determined

after anthesis, involving the transition of ovary to fruitlet, has a hormonal nature (Talon et al. 1990, 1992) and is particularly dependent upon the GA action that initiates fruit growth (Ben-Cheikh et al. 1997). It is also apparent that further development is sustained by

nutrient availability (Gillaspy et al. 1993), specially carbon supply, as has been suggested for cotton (Guinn 1982), apple (Berüter and Droz 1991), and pistachio trees (Nzima et al. 1999). In citrus, ¹⁴C-translocation experiments support the suggestion that fruit abscission is linked to carbohydrate status (Moss et al. 1972; Powell and Krezdorn 1977). Further support for this statement comes mostly from experiments manipulating the source-sink relationships, including shading, girdling, defruiting, and defoliation treatments (Goldschmidt and Koch 1996). Defoliations, for example, have clear-cut effects regarding sugar depletion and subsequent abscission (Ruan 1993; Mehouchi et al. 1995). In woody species, the effect of defoliation on fruit abortion appears to be general (Buwalda and Smith 1990; Obeso 1993; Schumacher and Stadler 1993). In addition, defoliation tends to increase nitrogen assimilation rates, nitrogen levels (Fujita et al. 1994), CO₂ assimilation, leaf pressure potential, root conductivity and water-use rates (Ruan 1993; Layne and Flore 1993; Syvertsen 1994). Using the same varieties as in the present work (*Clausellina* and *Okitsu*), we previously noted that sucrose levels in fruitlets are a major factor in the mechanism that triggers fruit abscission (Mehouchi et al. 1995). We have also proposed that carbohydrates are distributed between developing fruitlets according to source-sink relationships, and that abscission in each fruitlet is only inhibited when a certain threshold is reached (Talon et al. 1997). In the present work, the full defoliation treatment completely arrested carbon build-up and produced a continuous drop of sucrose-deficient fruitlets (Fig. 5, Table 1). The removal of mature leaves induced early sucrose shortage and, therefore, early abscission. Thus, the data indicate that as soon as the fruit load was adjusted to the carbohydrate supply, the remaining fruitlets began to regain normal sucrose levels and abscission tended to be definitively arrested (Fig. 3, Table 1). Since the 50% defoliation was more severe than the removal of mature leaves, fruit-load adjustment, sucrose levels and abscission were normalised later in this treatment (Fig. 4, Table 1).

On the other hand, the manipulation of the intensity and timing of abscission was used to study the involvement of the hormonal factors in the process. The results obtained identified ABA and ACC as regulatory signals in the mechanism of fruit abscission induced by carbohydrate shortage. It was demonstrated that the raised amounts of both hormones observed in the defoliation treatments were not induced by water (Fig. 1) or nitrogen deficiency (Fig. 2). Moreover, increase in the ABA and ACC preceded that of abscission (Figs. 3, 4, 5). Thus, exogenous ABA increased ACC levels and fruit abscission, whereas NF (Table 3) had the opposite effects (Table 4, Fig. 7). Interestingly, NF did not have a direct effect on ACC accumulation, since the simultaneous applications of ABA and NF did not suppress ACC and abscission increases. Moreover, AVG reduced ACC levels and fruit abscission (Table 4, Fig. 7), without changing ABA levels (Table 3). Also, ACC increased abscission (Fig. 7). Furthermore, the ABA and ACC implicated in carbon-shortage-induced abscis-

sion very likely originate in the fruit, and are not translocated through the xylem fluid (Fig. 6) or exported from branches and roots, as has been reported to occur in citrus under osmotic stress (Gómez-Cadenas et al. 1996, 1998; Tudela and Primo-Millo 1992). Collectively, the above observations strongly indicate that in this system ABA acts through the modulation of the levels of ACC, the precursor of ethylene.

These results are in agreement with those presented recently on the control of leaf abscission induced by water stress (Gómez-Cadenas et al. 1996) and salinity (Gómez-Cadenas et al. 1998) in citrus. The data presented here expand those observations to reproductive organs, ratifying that ethylene is the endogenous hormonal activator of fruit abscission. Therefore, we propose that, in the main, ABA is primarily a signal sensitive to stress, rather than only to water deficiency. Regarding the abscission process, ABA appears to operate as a mediator between the adverse environment and abscission.

While the above evidence favours regulatory roles for ABA and ACC, the data also indicate that GAs are not directly implicated in the control of fruit abscission induced by carbon shortage (Fig. 7). However, it is interesting to note that the levels of the active GA₁, in sucrose-deficient abscising fruitlets, are strongly reduced, through probably the increase and decrease in the 2 β -hydroxylase and GA 20-oxylase activities, respectively (Table 2). Similarly, GA₁ in persisting fruitlets of the defoliated plants is apparently kept at normal levels through increased activity of the GA 20-oxylase. These observations may indicate that GAs at this stage are implicated in fruit growth promotion rather than in the repression of abscission. The relationship between GAs and sucrose is not well understood at present, although current evidence suggests a stimulative effect of GA on sucrose synthesis (Cheikh et al. 1992; Mehouchi et al. 1996). On the other hand, several components of the sugar-induced signalling pathways have recently been reported (see Smeekens 1998 and references therein). However, clear interactions or connections between these components and phytohormones have not yet been identified (Roitsch 1999).

As a result of this work, it is suggested that the mechanisms of source-sink regulation in fruits act as regulatory elements providing a physiological link between the carbohydrate status and the severity of fruitlet abscission. The results indicate that fruitlet abscission induced by carbohydrate shortage in citrus is triggered by increases in the levels of ABA and ACC. In this process, ABA appears to act as a mediator between the nutrient deficiency and ACC, the precursor of ethylene. Although this conclusion has been obtained with artificially provoked limit drops, it probably represents the hormonal sequence that naturally regulates citrus fruitlet abscission under normal growth conditions.

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References

- Ben-Cheikh W, Perez-Botella J, Tadeo FR, Talon M, Primo-Millo E (1997) Pollination increases gibberellin levels in developing ovaries of seeded varieties of citrus. *Plant Physiol* 114: 557–564
- Berüter J, Droz P (1991) Studies on locating the signal for fruit abscission in the apple tree. *Sci Hort* 46: 201–214
- Bremner JM (1965) Total nitrogen. In: Black TC (ed) *Methods of soil analysis*. Part 2. Am Soc Agron, Madison, pp 1149–1178
- Buwalda JG, Smith GS (1990) Effects of partial defoliation at various stages of the growing season on fruit yields, root growth and return bloom of kiwifruit vines. *Sci Hort* 42: 29–44
- Cheikh N, Brenner ML, Huber JL, Huber SC (1992) Regulation of sucrose phosphate synthase by gibberellins in soybean and spinach plants. *Plant Physiol* 100: 1238–1242
- Fujita K, Furuse F, Sawada O, Bandara D (1994) Effect of defoliation and ear removal on dry matter production and inorganic element absorption in sweet corn. *Soil Sci Plant Nutr* 40: 581–591
- Gillaspy G, Ben-David H, Gruissem W (1993) Fruits: a developmental perspective. *Plant Cell* 5: 1439–1451
- Goldschmidt EE, Koch KE (1996) Citrus. In: Zamski E, Schaffer A (eds) *Photoassimilate distribution in plants and crops*. Dekker, New York Basel Hong Kong, pp 797–823
- Gómez-Cadenas A, Tadeo FR, Talon M, Primo-Millo E (1996) Leaf abscission induced by ethylene in water stressed intact seedlings of Cleopatra mandarin requires previous abscisic acid accumulation in roots. *Plant Physiol* 112: 401–408
- Gómez-Cadenas A, Tadeo FR, Primo-Millo E, Talon M (1998) Involvement of abscisic acid and ethylene in the responses of citrus seedlings to salt shock. *Physiol Plant* 103: 475–484
- Goren R (1993) Anatomical, physiological, and hormonal aspects of abscission in citrus. *Hort Rev* 15: 33–46
- Guinn G (1982) Fruit age and changes in abscisic acid content, ethylene production, and abscission rate of cotton fruits. *Plant Physiol* 69: 349–352
- Layne DR, Flore JA (1993) Physiological responses of *Prunus cerasus* to whole-plant source manipulation. Leaf gas exchange, chlorophyll fluorescence, water relations and carbohydrates concentrations. *Physiol Plant* 88: 44–51
- Mehouchi J, Serna D, Zaragoza S, Agustí M, Talon M, Primo-Millo E (1995) Defoliation increases fruit abscission and reduces carbohydrate levels in developing fruits and woody tissues of *Citrus unshiu*. *Plant Sci* 107: 189–197
- Mehouchi J, Tadeo FR, Zaragoza S, Primo-Millo E, Talon M (1996) Effects of gibberellic acid and paclobutrazol on growth and carbohydrate accumulation in shoots and roots of citrus rootstock seedlings. *J Hort Sci* 71: 747–754
- Moss GI, Steer BT, Kriedemann PE (1972) The regulatory role of inflorescence leaves in fruit-setting by sweet orange (*Citrus sinensis*). *Physiol Plant* 27: 432–438
- Nzima MDS, Martin GC, Nishijima C (1999) Effect of fall defoliation and spring shading on shoot carbohydrate and growth parameters among individual branches of alternate bearing 'Kerman' pistachio trees. *J Am Soc Hort Sci* 124: 52–60
- Obeso JR (1993) Does defoliation affect reproductive output in herbaceous perennials and woody plants in different ways? *Funct Ecol* 7: 150–155
- Powell AA, Krezdorn AH (1977) Influence of fruit-setting treatment on translocation of ¹⁴C-metabolites in citrus during flowering and fruiting. *J Am Hort Sci* 102: 709–714
- Roitsch T (1999) Source-sink regulation by sugar and stress. *Curr Opin Plant Biol* 2: 198–206
- Ruan YL (1993) Fruit set, young fruit and leaf growth of *Citrus unshiu* in relation to assimilate supply. *Sci Hort* 53: 99–107
- Sagee O, Erner Y (1991) Gibberellin and abscisic acid contents during flowering and fruit set of Shamouti orange. *Sci Hort* 48: 29–39

- Schumacher R, Stadler W (1993) Fruit set regulation and quality. *Acta Hort* 326: 49–57
- Smeekens S (1998) Sugar regulation of gene expression in plants. *Curr Opin Plant Biol* 1: 230–234
- Syvrtsen JP (1994) Partial defoliation increases net CO₂ assimilation and alters water relations of *Citrus* seedlings. *Tree Physiol* 14: 497–508
- Takahashi N, Yamaguchi I, Kono T, Igoshi M, Hirose K, Suzuki K (1975) Characterization of plant growth substances in *Citrus unshiu* and their change in fruit development. *Plant Cell Physiol* 16: 1101–1111
- Talon M, Zacarías L, Primo-Millo E (1990) Hormonal changes associated with fruit set and development in mandarins differing in their parthenocarpic ability. *Physiol Plant* 79: 400–406
- Talon M, Zacarías L, Primo-Millo E (1992) Gibberellins and parthenocarpic ability in developing ovaries of seedless mandarins. *Plant Physiol* 99: 1575–1581
- Talon M, Tadeo FR, Ben-Cheikh W, Gómez-Cadenas A, Mehouachi J, Pérez-Botella J, Primo-Millo E (1997) Hormonal regulation of fruit set and abscission in citrus: classical concepts and new evidence. *Acta Hort* 463: 209–217
- Tudela D, Primo-Millo E (1992) 1-Aminocyclopropane-1-carboxylic acid transported from roots to shoots promotes leaf abscission in Cleopatra Mandarin (*Citrus reshni* Hort. ex Tan.) seedlings rehydrated after water stress. *Plant Physiol* 100: 131–137
- Vernieri P, Tagliasacchi AM, Forino L, Lanfranchi A, Lorenzi R, Avanzi S (1992) Abscisic acid levels and cell structure in single seed tissues of shedding affected fruits of *Malus domestica* Borkh. *J Plant Physiol* 140: 699–706
- Zacarias L, Talon M, Ben-Cheikh W, Lafuente MT, Primo-Millo E (1995) Abscisic acid increases in non-growing and paclobutrazol-treated fruits of seedless mandarins. *Physiol Plant* 95: 613–619