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Synthetic Auxin 3,5,6-TPA Provokes *Citrus clementina* (Hort. *ex* Tan) Fruitlet Abscission by Reducing Photosynthate Availability

Carlos Mesejo · Salvatore Rosito · Carmina Reig · Amparo Martínez-Fuentes · Manuel Agustí

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Abstract The aim of this study was to determine the effects of the synthetic auxin 3,5,6-trichloro-2-pirydiloxyacetic acid (3,5,6-TPA) on photosynthetic activity, photosynthate transport to the fruit, and fruitlet abscission to further explain the physiological basis of auxin-mediated citrus fruit thinning. Applying 15 mg l^{-1} 3,5,6-TPA to trees during the fruit cell division stage significantly increased fruitlet abscission of Clementine mandarin. On treated trees, abnormal foliar development and photosynthetic damage were observed at the same time as 3,5,6-TPA reduced fruitlet growth rate. Briefly, treatment reduced chlorophyll and carotenoid concentrations and modified chlorophyll *a* fluorescence parameters, that is, reduced the quantum yield (ΦPSII) of the noncyclic electron transport rate, diminished the capacity to reduce the quinone pool (photochemical quenching; q_p), and increased nonphotochemical quenching (q_N), thereby preventing the dissipation of excess excitation energy. In addition, the net photosynthetic flux (μmol CO_2 m^{-2} s^{-1}) and leaf photosynthate content decreased in treated trees. As a result, the 3,5,6-TPA treatment significantly reduced the photosynthate accumulation in fruit from day 3 to day 8 after treatment, thus reducing fruitlet growth rate. Hence, treated fruitlets significantly increased ethylene production and abscised. Twenty days after treatment, chlorophyll *a* fluorescence parameters and fruitlet growth rate were reestablished. Accordingly, the thinning effect of 3,5,6-TPA may be due to a temporarily induced photosynthetic disorder that leads to reduction in photosynthate production and fruitlet uptake that temporarily slows its growth, triggering ethylene production and fruitlet abscission. Afterward, the remaining treated fruit overcame this effect, increased growth rate, and reached a larger size than control fruit.

Keywords Auxins · Carbohydrates · Citrus · Photosynthesis · Thinning

C. Mesejo (✉) · C. Reig · A. Martínez-Fuentes · M. Agustí
Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Camino de Vera s/n, Valencia 46022, Spain
e-mail: carmeco@prv.upv.es

S. Rosito
Facoltá di Agraria, Università degli studi di Bari, Piazza Umberto I, 1, 70121 Bari, Italy

Introduction

In *Citrus*, reduced fruit size is a major constraint to the efficient production of some mandarin cultivars for the fresh market. Therefore, synthetic auxins are commonly used to increase fruit size of Satsuma mandarins (*Citrus unshiu* Marc), hybrid-like mandarins (that is, 'Fortune' mandarin), and Clementine mandarins (*Citrus clementina* Hort *ex* Tan) (Agustí and others 1995, 2002, 2007; Aznar and others 1995; El-Otmani and others 2000).

The application of synthetic auxins at the onset of the cell enlargement stage increases final fruit size by stimulating carbohydrate accumulation and fruit growth (Agustí and others 2002). But when applied during the cell division stage, they thin fruitlets, reducing the competition for carbohydrates between the remaining fruitlets and thus increasing final fruit size (Agustí and others 1995). In both cases, the magnitude of the response depends on the type of auxin, the concentration applied, the climatic region, and the species and cultivar (El-Otmani and others 2000).

The manner in which synthetic auxins thin developing fruitlets is not fully understood. The application of 1-naphthalene acetic acid (NAA) to sweet orange trees (*Citrus sinensis* (L.) Osb.) during the fruit cell division

stage reduced fruit growth rate and inhibited ^{14}C-photoassimilate transport from leaf to fruit (Mauk and others 1986). In Satsuma mandarin, the application of 3,5,6-trichloro-2-pirydiloxyacetic acid (3,5,6-TPA) at the same phenological growth stage induced leaf chlorosis that significantly correlated with ethylene production and fruit abscission (Agustí and others 2007).

The increase in ethylene production found during the days after treatment might be a consequence of the abscission process triggered by the decline in fruit growth rate caused by reduced photosynthesis and photoassimilate transport. This hypothesis is supported by the fact that (1) in *Citrus*, a shortage of photosynthates, caused by tree defoliation or pedicel girdling, increases stress-sensitive signals that trigger ethylene production and fruitlet abscission (Gómez-Cadenas and others 2000; Iglesias and others 2006), and (2) in pea (*Pisum sativum* L.), high concentrations of synthetic auxins damage the mesophyll cells, interfering with their photosynthetic rate (Romero-Puertas and others 2004).

To better understand the manner in which synthetic auxins thin citrus fruitlets, we investigated the effects of the synthetic auxin 3,5,6-TPA on Clementine mandarin photosynthesis, photosynthate transport to the fruit, fruit growth rate, ethylene production, and fruitlet abscission.

Materials and Methods

Plant Material and Treatments

Clementine mandarin trees cvs. 'Marisol' and 'Clemenules' (*Citrus clementina* Hort *ex* Tan), grafted onto Carrizo citrange rootstock (*Poncirus trifoliata* Raff × *Citrus sinensis* (L.) Osb.), were used in the experiments. Trees were grown in a commercial orchard located in Llíria, Spain, using standard cultivation practices.

Two sets of 12 trees were selected for the experiments. For the first experiment, the isopropyl ester of 3,5,6-TPA (Maxim, Dow Chemical Ibérica SL, Madrid, Spain) was applied as a foliar spray (5 l per tree) to 8-year-old 'Marisol' Clementine mandarin trees at concentrations of 0 and 15 mg l^{-1} (acid equivalent) during the cell division stage. The diameters of remaining fruitlets and fruitlet drop were periodically recorded for populations of at least 150 fruits on four tagged branches per tree. The quantum yield of PSII (ΦPSII) of old leaves and young leaves from mixed and vegetative shoots was measured at 3–4-day intervals during the 3 weeks following treatment. Leaf and fruitlet carbohydrate contents were also determined at 0, 3, and 8 days after treatment (DAT). Fruitlet ethylene production was determined 14 DAT at the maximum fruitlet abscission rate. The experiment was conducted as a randomized complete-block design, with two-tree plots and six replications.

For the second experiment, the isopropyl ester of 3,5,6-TPA was applied as a foliar spray (5 l per tree) to 5-year-old 'Clemenules' Clementine mandarin trees at concentrations of 0, 5, 10, and 15 mg l^{-1} (acid equivalent) during the cell division stage. The phytotoxic effect of the auxin on the leaf was characterized 10 DAT by measuring (1) SPAD units as a leaf chlorosis indicator; (2) chlorophyll (Chl) *a* and *b* and total carotenoid concentrations; (3) Chl *a* fluorescence parameters ΦPSII, photochemical quenching (q_p), and nonphotochemical quenching (q_N); and (4) the net photosynthetic flux (P_n, μmol CO_2 m^{-2} s^{-1}). A randomized complete-block design with four-tree plots and three replications was performed.

All treatments were applied to the point of runoff with a high-pressure handgun sprayer at 30 atm. Average fruitlet diameter at the moment of the treatments was 8.0 ± 0.2 mm. A nonionic wetting agent (alkyl polyglycol ether, 20% w/v) was added to the spray solution at a concentration of 0.025% v/v.

Leaf Chlorosis Measurement

Leaf chlorosis was measured in 25 leaves randomly selected per tree using a SPAD-502 Chlorophyll Meter (Minolta Corp., NJ, USA) and expressed in relative units (SPAD readings). Average leaf SPAD readings were used to choose leaves for sampling and measurements.

Chlorophyll and Carotenoid Extraction and Quantification

Chlorophyll and carotenoids were extracted from six average leaves per tree, as previously described by Rodrigo and others (2003). Briefly, Chls *a* and *b* and total (*a* + *b*) contents were determined by measuring the absorbance at 644 and 662 nm using a spectrophotometer Lambda 25 UV/Vis (PerkinElmer Corp., Waltham, MA, USA). The pigment ethereal solution was dried and saponified using 10% methanolic:KOH solution. Carotenoids were subsequently re-extracted with diethyl ether until the hypophase was colorless. An aliquot of this extract was used for total carotenoid content quantification by measuring the absorbance at 450 nm and using the extinction coefficient of β-carotene, $E^{1\%} = 2{,}500$ (Davies 1976).

Chlorophyll *a* Fluorescence Measurements

Chlorophyll *a* fluorescence was measured in two leaves per shoot and two shoots per tree using a portable fluorometer (PAM-2000, Walz, Effeltrich, Germany), as previously described by Calatayud and others (2006). Specifically,

leaves were darkened for 30 min prior to measuring. The minimum (dark) fluorescence (F_0) was obtained upon excitation of leaves with a weak beam from a light-emitting diode. The maximum fluorescence (F_m) was determined following a 600-ms pulse of saturating white radiation. The yield of variable fluorescence (F_v) was calculated as $F_m - F_0$. Following 2 min of dark re-adaptation, actinic white radiation [230 μmol (photon) m^{-2} s^{-1}] was switched on and saturating pulses [8,000 μmol (photon) m^{-2} s^{-1}] were applied at 60-s intervals for 10 min to determine maximum fluorescence yield during actinic irradiation (F'_m), the level of modulated fluorescence during a brief interruption of actinic irradiation in the presence of far-red radiation (F'_0), and the Chl fluorescence yield during actinic radiation (F_s). Calculation of quenching due to nonphotochemical dissipation of absorbed photon energy (NPQ) was determined for each saturating pulse according to the equation: $NPQ = (F_m - F'_m)/F'_m$ (Bilger and Björkman 1991). The coefficient for photochemical quenching (q_p) was calculated as $(F'_m - F_s)/(F'_m - F'_0)$ (Schreiber and others 1986). The ΦPSII was estimated from $(F'_m - F_s)/F'_m$ (Genty and others 1989).

Gas Exchange Measurements

Net photosynthetic rate was determined in ten average leaves per tree by using a LI-COR-6400 IRGA (LI-COR Biosciences, Lincoln, NE, USA), as previously described by Iglesias and others (2002). The system was equipped with a leaf chamber that carries a gallium arsenide phosphide (GaAsP) photosynthetically active radiation (PAR) sensor. Measurements were performed under an airflow rate of 500 μmol s^{-1}, at environmental humidity and CO_2. Within the chamber, relative humidity, average temperature, and leaf-to-air vapor pressure deficit did not differ significantly between measurements. To ensure similar exposure to natural irradiance, leaves were selected from the external southeast side of the canopy at a height of about 1.5 m, and measurements were performed before midday (09:00–10:30 h). Regarding stability conditions and measurements taken, the LI-6400 automatically measures and computes P_n, providing continuously a coefficient of variation of the last ten monitored values. Photosynthesis values were taken into account only when the coefficient of variation for each measurement was lower than 1%.

Carbohydrate Analysis

Carbohydrate contents were determined in fruitlets and leaves. Ten fruitlets and five average leaves per tree and date were collected. Samples were frozen immediately in liquid nitrogen, lyophilized, and stored as powder at −28°C. The procedure for carbohydrate determination was done as described previously by Martínez-Fuentes and others (2010). Specifically, 100 mg powdered samples were extracted with 1 ml of 800 ml l^{-1} ethanol and purified sequentially using cation and anion exchange columns. The eluates were then passed through a C18 Sep-Pak cartridge (Waters-Millipore, Billerica, MA, USA) and analyzed in a Spectra HPLC System (Spectra, San Jose, CA, USA) equipped with a vacuum pump (Spectra P2000) and a differential refractometer (Spectra R150). Sucrose, glucose, and fructose were identified according to their retention times. Results were expressed as mg g^{-1} dry weight (DW).

Ethylene Analysis

Ethylene production was determined for fruitlets with clear symptoms of imminent abscission from trees treated with 0 and 15 mg l^{-1} of 3,5,6-TPA, at the maximum fruitlet abscission rate (14 DAT). Fruits were manually detached from the tree by pulling them, distinguishing between those released from abscission zone A (AZ-A), in the pedicel, and abscission zone C (AZ-C), in the calix. Four replicates of 15 fruits were incubated in 200-ml jars. After 2 h of incubation at 20°C, a 1-ml air sample from the jar headspace was withdrawn with a hypodermic syringe and injected into a gas chromatograph Finnigan Trace GC Ultra (Thermo Fisher Scientific Inc., Wilmington, MA, USA) equipped with a flame ionization detector and an alumina column.

Statistical Analysis

Variance and regression analyses were performed on the data and means were separated using the Student–Newman–Keuls' multiple-range test.

Results

3,5,6-TPA Temporarily Decreased Fruitlet Growth

An immediate effect of fruitlet growth reduction was observed after applying 15 mg l^{-1} of 3,5,6-TPA. Three DAT a significant reduced fruitlet growth rate was registered in treated trees. Five days later (8 DAT) the diameter of fruitlets on treated trees was 20% smaller on average than that of fruitlets on control trees ($P < 0.05$; Fig. 1a). Nevertheless, 16 DAT treated fruitlets overcame this depressive effect and grew significantly faster than untreated fruits (Fig. 1b) up to the end of the experiment,

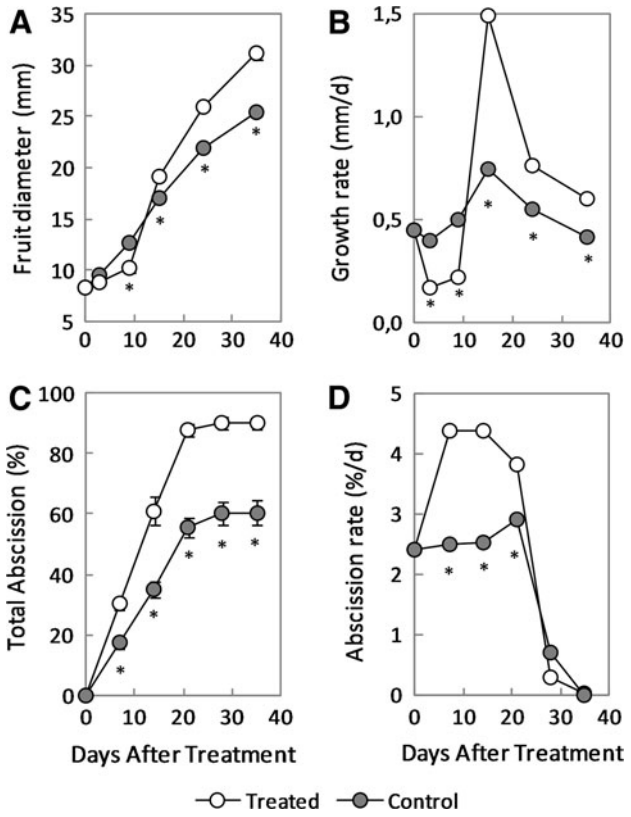

Fig. 1 Effect of 15 mg l^{-1} 3,5,6-TPA (*open circle*) on the time course of fruit diameter (**a**), fruit growth rate (**b**), total fruit abscission (**c**), and fruit abscission rate (**d**). Treatments were applied to 8-year old 'Marisol' Clementine mandarin trees at the fruit cell division stage. Control trees (*filled circle*) were not treated. Data are the mean ± SE of five trees and four tagged branches per tree. * indicates significant differences at $P < 0.05$. In some cases, SE bars were smaller than symbol size

reaching a larger final size [65.9 ± 0.9 vs. 57.8 ± 0.1 mm, respectively ($P < 0.05$)].

The auxin also increased fruitlet abscission. Thus, whereas natural fall reached 60% in control trees, the treatment significantly increased fruitlet abscission up to 90% in treated trees (Fig. 1c). Eight DAT the abscission rate increased significantly, from 2.4 fruitlets day^{-1} in control trees to 4.3 fruitlets day^{-1} in treated trees, and significantly higher up to the end of the fruitlet drop period at 28 DAT (Fig. 1d).

3,5,6-TPA Induced Photosynthetic Damage

The application of 3,5,6-TPA induced leaf chlorosis, the intensity depending on the concentration applied. Compared to the control, 10 DAT 5 mg l^{-1} had no significant effect, but 10 and 15 mg l^{-1} reduced leaf SPAD readings by 14% (not significantly) and 32%, respectively; Chl *a* concentration by 18 and 23%, respectively; Chl *b* concentration by 62 and 63%, respectively; and carotenoid concentrations by 21% (not significantly) and 49%, respectively (Table 1). Twenty DAT leaves showed symptoms of regreening, although differences between 0 and 15 mg l^{-1} persisted at 30 days (data not shown). In addition, 3,5,6-TPA induced abnormal foliar development in treated leaves. Fifty DAT significant differences in leaf weight and leaf area were found between treatments (Table 1); besides, leaves had typical curling on the 15 mg l^{-1}-treated trees (data not shown).

The Chl *a* fluorescence analysis revealed that the quantum yield efficiency of photosystem II (ΦPSII) paralleled fruit growth rate. Thus, from 3 to 13 DAT, ΦPSII was significantly reduced by 3,5,6-TPA in leaves from mixed and vegetative shoots and in old leaves, with differences reaching a maximum at 8–13 DAT (Fig. 2). This reducing effect on ΦPSII was overcome 20 DAT, when ΦPSII of leaves on treated trees caught up with leaves on control trees. This moment coincided with the highest fruit growth rate and cessation of fruitlet abscission (Fig. 1).

The steady state for ΦPSII and q_p in leaves from control and treated trees was reached after 3 min of actinic illumination 10 DAT (Fig. 3). The application of 3,5,6-TPA significantly reduced both the ΦPSII and q_p highest level, with the magnitude of the response depending on the concentration applied (Fig. 3a, b). Nonphotochemical quenching (q_N) rose rapidly after actinic irradiation, reaching the maximum value (1.5–1.7) within 1 min. Thereafter, q_N declined sharply (down to 0.6–0.9) and

Table 1 Effect of 3,5,6-TPA concentration on leaf SPAD readings, chlorophyll and carotenoid contents, and leaf weight and area

3,5,6-TPA (mg l^{-1})	Leaf chlorosis[A] (SPAD units)	Chl *a*[A] (mg g^{-1})	Chl *b*[A] (mg g^{-1})	Carotenoid[A] (mg g^{-1})	Leaf weight[B] (mg)	Leaf area[B] (mm^2)
0	30.2 ± 1.0 (100) b	298 ± 5 (100) b	274 ± 4 (100) c	4.96 ± 0.06 (100) b	509 ± 36 (100) c	1367 ± 102 (100) c
5	27.0 ± 2.2 (89) b	286 ± 3 (96) b	172 ± 6 (63) b	4.94 ± 0.55 (99) b	404 ± 46 (79) b	1150 ± 82 (84) bc
10	25.9 ± 2.0 (86) ab	245 ± 9 (82) a	106 ± 17 (38) a	3.90 ± 0.11 (79) ab	393 ± 29 (77) b	1019 ± 68 (75) b
15	20.7 ± 2.5 (68) a	231 ± 2 (77) a	103 ± 3 (37) a	2.49 ± 0.02 (50) a	239 ± 18 (46) a	562 ± 43 (41) a
P value	0.048	0.0029	0.0007	0.0001	0.0001	0.0001

Treatments applied to 5-year-old 'Clemenules' Clementine mandarin trees at the fruit cell division stage. Young leaves were harvested [A] 10 and [B] 50 days after treatment. Different letters show statistically significant differences among means using the Student–Newman–Keuls test. Numbers in parenthesis: percent of control

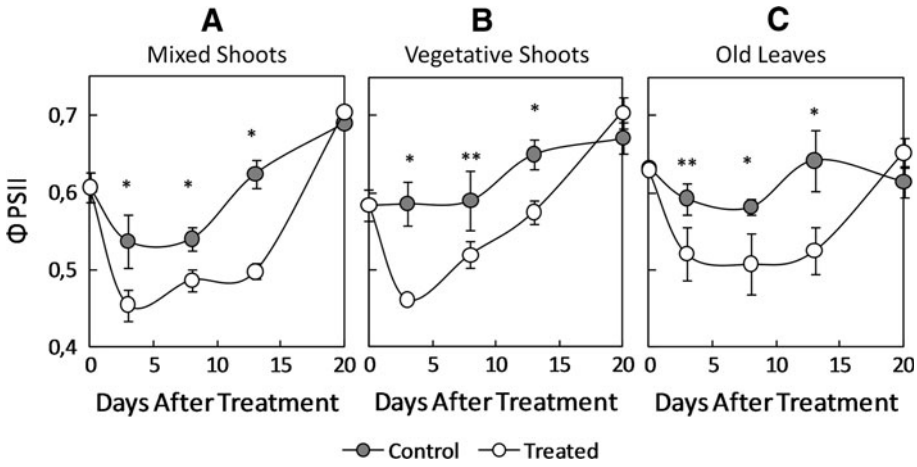

Fig. 2 Effect of 15 mg l^{-1} 3,5,6-TPA (*open circle*) on the time course of quantum yield of PSII (ΦPSII) in leaves of mixed shoots (**a**), vegetative shoots (**b**), and old leaves (**c**). Treatments were applied to 8-year-old 'Marisol' Clementine mandarin trees at the fruit cell division stage. Control trees (*filled circle*) were not treated. Data are the mean ± SE of five trees and two shoots per tree. * indicates significant differences at $P < 0.05$

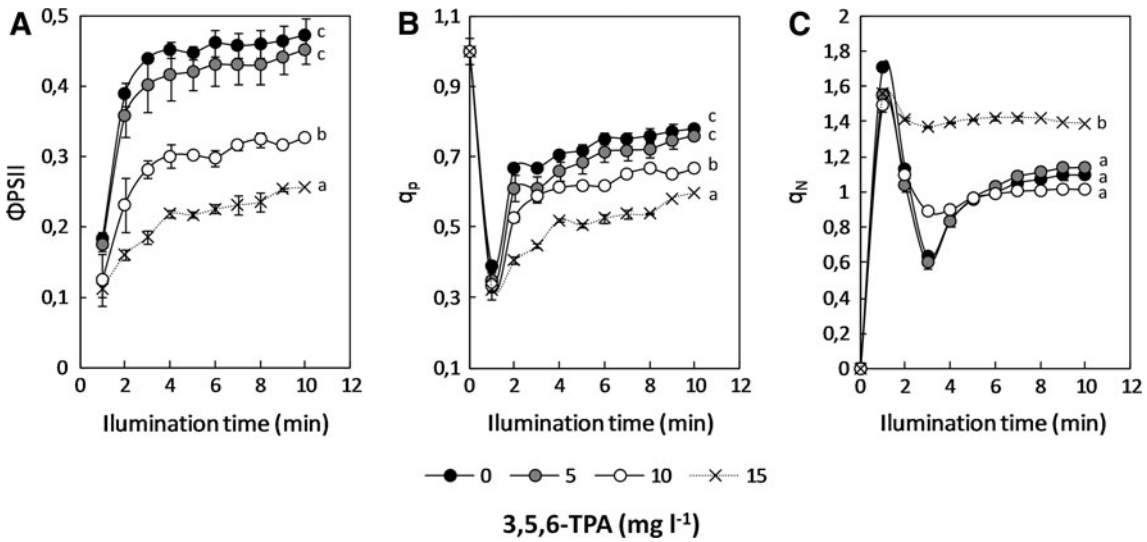

Fig. 3 Effect of 3,5,6-TPA concentration applied on the kinetics of chlorophyll *a* fluorescence parameters. **a** Quantum yield of PSII (ΦPSII). **b** Photochemical quenching (q_p). **c** Nonphotochemical quenching (q_N). Treatments were applied to 5-year-old 'Clemenules' Clementine mandarin trees at the fruit cell division stage. Values correspond to 10 days after treatment. Data are the mean ± SE of five trees and two average leaves per tree. Different letters indicate significant differences at $P < 0.05$

progressively achieved steady-state values (1.0–1.1) after 7 min of actinic irradiation in control trees and in trees treated with 5 and 10 mg l^{-1} 3,5,6-TPA. However, for 15 mg l^{-1}-treated trees, q_N did not decline and achieved the steady state close to the maximum value of 1.4 (Fig. 3c). In addition, leaves from control trees yielded a net photosynthetic flux (P_n) of 5.4 μmol CO_2 m^{-2} s^{-1}; 5 mg l^{-1} 3,5,6-TPA had no significant effect on P_n (4.7 μmol CO_2 m^{-2} s^{-1}), but 15 mg l^{-1} 3,5,6-TPA significantly reduced P_n by 43% on average (3.1 μmol CO_2 m^{-2} s^{-1}).

3,5,6-TPA Temporarily Reduces Photosynthate Leaf Production and Fruitlet Uptake

Trees treated with 15 mg l^{-1} 3,5,6-TPA significantly reduced the sucrose concentration of leaves. The effect was noticeable for old leaves and leaves from vegetative shoots 3 DAT, but not for leaves from mixed shoots, which showed significantly higher values for leaves from treated trees than for leaves from control trees (Fig. 4). However, 5 days later (8 DAT), at the same time as 3,5,6-TPA induced photosynthetic damage, all leaves from treated trees exhibited a significantly lower concentration of sucrose than leaves from control trees. As a result, treatment caused a significant reduction in the carbohydrate pool (sucrose, fructose, and glucose) of developing fruitlets of treated trees (Fig. 5), coinciding with the period of reduced fruitlet growth rate brought about by 3,5,6-TPA (Fig. 1).

Fourteen DAT, on-tree fruitlets with symptoms of abscission showed that auxin treatment significantly increased ethylene production in fruitlets abscising either by the AZ-A or by the AZ-C abscission zone (Fig. 6).

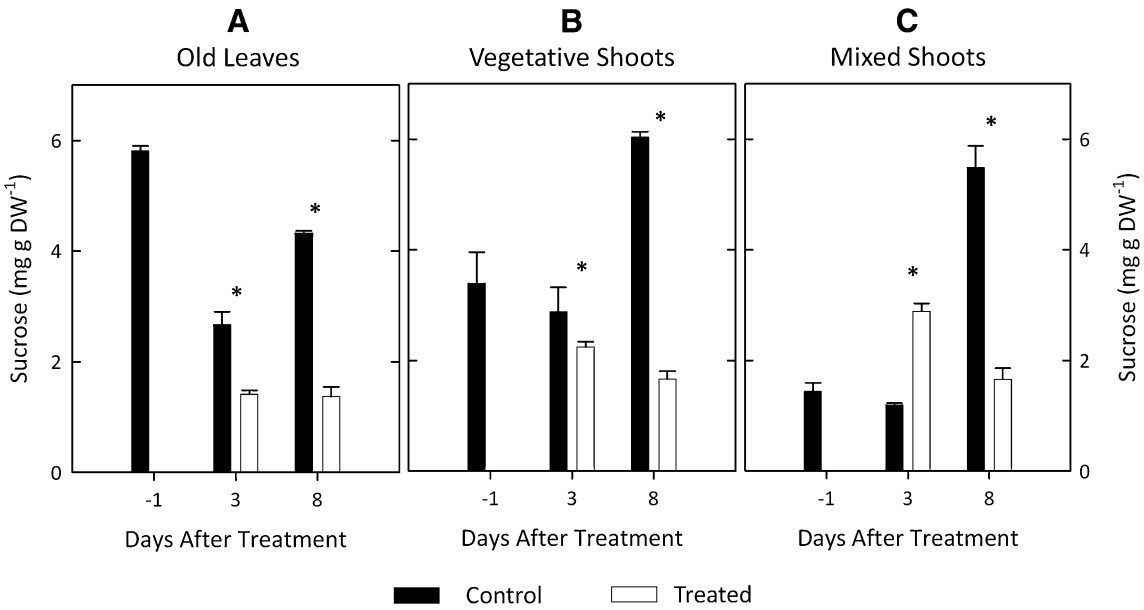

Fig. 4 Effect of 15 mg l^{-1} 3,5,6-TPA (*white bars*) on leaf photosynthate concentration. Treatments were applied to 8-year-old 'Marisol' Clementine mandarin trees at the fruit cell division stage. Control trees (*shaded bars*) were not treated. Data are the mean ± SE of five trees and five leaves from two shoots per tree. * indicates significant differences at $P < 0.05$

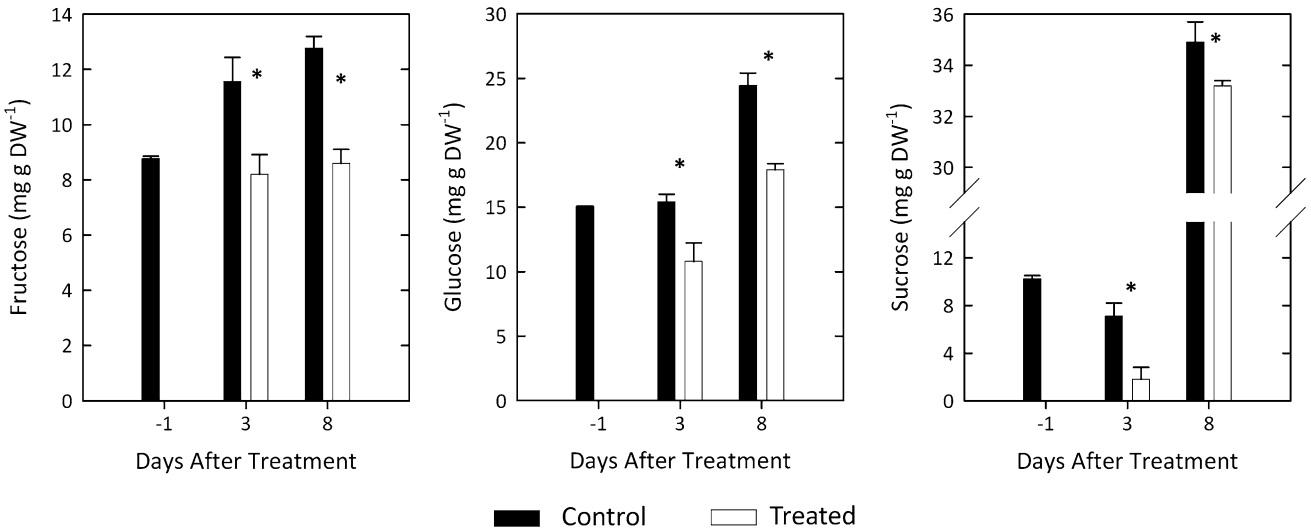

Fig. 5 Effect of 15 mg l^{-1} 3,5,6-TPA (*white bars*) on fruitlet CHs concentration. Treatments were applied to 8-year-old 'Marisol' Clementine mandarin trees at the fruit cell division stage. Control trees (*shaded bars*) were not treated. Data are the mean ± SE of five trees and ten fruitlets per tree. * indicates significant differences at $P < 0.05$

Discussion

Synthetic auxins applied at the cell division stage give rise to a transient reduction in daily fruitlet growth rate in citrus, which is probably the reason for the concomitant increase in fruitlet abscission rate and, therefore, fruit thinning (Mauk and others 1986; Agustí and others 1995, 2007). Our results using 3,5,6-TPA in Clementine mandarin agree with this hypothesis and reveal that the depressive effect on fruit growth rate is produced a few hours after the treatment (72 h in our experiments). This effect is the reason why 3,5,6-TPA is commonly used to thin developing mandarin fruitlets to reduce the number of undersized fruit for fresh market.

The manner in which synthetic auxins thin developing fruitlets has been correlated to an increase in fruitlet ethylene production (Iwahori and Oohata 1976; Agustí and others 2007), owing to reduced basipetal indol-3-acetic acid (IAA) transport through the pedicel (Okuda and Hirabayashi 1998; Bangerth 2000). IAA is an essential stimulation element in vascular tissue differentiation (Aloni 2010), and a direct effect of the synthetic auxins

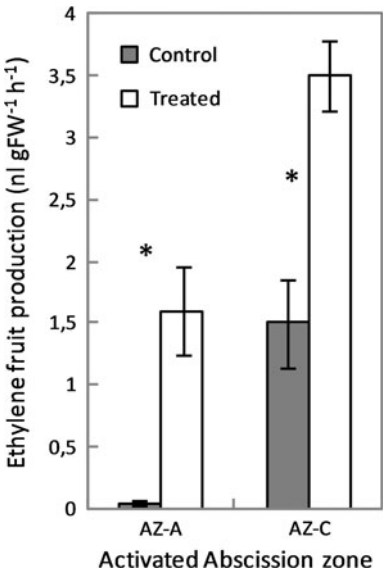

Fig. 6 Effect of 15 mg l^{-1} 3,5,6-TPA (*white bars*) on ethylene fruitlet production. Treatments were applied to 8-year-old 'Marisol' Clementine mandarin trees at the fruit cell division stage. Control trees (*shaded bars*) were not treated. Fruitlets showing clear symptoms of abscission were collected and distinguished between the activated abscission zone (AZ), AZ-A, and AZ-C. Values corresponding to 14 days after treatment. Data are the mean ± SE of five trees. * indicates significant differences at $P < 0.05$

promoting the development of peduncle vascular tissue has been demonstrated by applying the auxin locally to the peduncle (Mesejo and others 2003). IAA also reduces the sensitivity of cells to ethylene in the abscission layers (Van Doorn and Stead 1997; Paterson 2001; Meir and others 2006).

However, ethylene production might also be a consequence of the process triggered by the decline in fruit growth rate. In this study, we have tested the hypothesis that 3,5,6-TPA applied during the fruit cell division stage thins fruits by provoking a temporary phytotoxic effect in leaves. Photosynthetic activity is impaired and, hence, photosynthate transport to the fruit and fruit growth rate are reduced. As a consequence, the fruitlet triggers ethylene production and, finally, abscises.

This hypothesis is supported by results for several species. In pea (*Pisum sativum* L.), high doses of 2,4-dichlorophenoxyacetic acid (2,4-D) induced structural damage and oxidative stress in the mesophyll cells and chloroplasts of leaves, interfering with their photosynthetic rate (Romero-Puertas and others 2004). In apple (*Malus domestica* Borkh.), Untiedt and Blanke (2001) reported that the application of naphthalene acetic acid (NAA) reduced CO_2 exchange and increased dark respiration. These authors also proposed that reducing leaf photosynthesis might be a prerequisite for successful fruit thinning. In citrus, Agustí and others (2007) found leaf chlorosis induced by 3,5,6-TPA and a high fruitlet abscission rate when the treatment was locally applied to the leaves, suggesting that there is interference with photosynthesis and carbohydrate transport to the fruit. Our results reveal that 15 mg l^{-1} 3,5,6-TPA induces photosynthetic damage by means of leaf chlorosis, reduces Chl *a*, Chl *b*, and carotenoid concentrations, reduces ΦPSII and q_p, increases q_N, and reduces CO_2 assimilation. Chlorophyll *a* concentration significantly correlated to ΦPSII ($\Phi_{PSII} = -0.468 + 0.003$ Chl_a; $r = -0.994$; $P = 0.005$) and q_p ($q_p = -0.028 + 0.003$ Chl_a; $r = -0.988$; $P = 0.011$). Results suggest that 3,5,6-TPA provokes an incomplete reoxidation of the Q_A acceptor and an increase in closed PSII centers, thus reducing the possibility of electron transport to photosystem I (Calatayud and others 2004). In addition, the incomplete reoxidation of the Q_A acceptor leads to an increase in nonphotochemical quenching, as shown by our results for q_N values. The reduction in carotenoid concentration deals with increasing q_N values because xanthophylls are related to the ability to safely dissipate excess energy and, hence, nonphotochemical quenching (Demming-Adams and Adams 1996). Furthermore, as a consequence of all these effects, reduced leaf sucrose concentration was detected in treated trees, regardless of the type of leaf, both mature and young, except for mixed shoots 3 DAT. The possibility that this latter effect may be caused by 3,5,6-TPA disturbing the homeostatic endogenous hormone system, thus activating the abscission process and blocking sugar supply to the fruit resulting in feedback inhibition of photosynthesis (Iglesias and others 2002), cannot be discounted, but it seems unlikely because the 8-DAT level of control leaves was twice that of treated leaves and no further fruitlet abscission was measured in control trees, with sucrose concentration being similar in both control and treated fruits.

Much more important, however, is the fact that in our experiments the photosynthetic damage coincided with the reduced fruitlet growth period induced by the auxin. Thus, from 3 to 8 DAT, the interference in photosynthesis reduced the photosynthate production and supply to the fruit, as shown by the fact that the treatment caused a significant reduction in the carbohydrate pool (sucrose, fructose, and glucose) and the diameter of developing fruitlets. Thereafter, fruitlet ethylene production significantly increased in treated trees, increasing the abscission rate, and confirming that *Citrus* adjusts fruit load to the ability of the tree to supply metabolites (Goldschmidt and Monselise 1977).

Because ethylene was measured on fruits that showed clear symptoms of abscission, it might be possible that the ethylene measured was the result of the abscission process, not the cause. However, fruits from control trees also produced ethylene, but at significantly lower levels than treated fruits and dropped to a lesser extent, suggesting an

additional abscising effect of 3,5,6-TPA over that of natural abscission. On the other hand, Gómez-Cadenas and others (2000) showed that during the cell division stage, a shortage of photosynthates triggers specific hormonal responses, inducing sequential increases in abscisic acid and 1-aminocyclopropane-1-carboxylic acid (ACC) that activate fruitlet abscission. On the other hand, girdling delays fruitlet abscission by increasing fruitlet photosynthate availability (Rivas and others 2006), and it increases leafy shoots fruit set by increasing ΦPSII (Rivas and others 2007). In our experiments, 3,5,6-TPA reduced both carbohydrate uptake and fruitlet growth rate when applied during the fruit cell division stage, and, afterward, an increase of ethylene production was measured, suggesting that ethylene is the cause of the abscission process, not the result.

In conclusion, synthetic auxin 3,5,6-TPA applied during the fruit cell division stage increased fruitlet abscission of Clementine mandarin by means of temporary photosynthetic damage, suggesting that this phytotoxic effect reduced photosynthate production and transport to the fruitlet and, thus, its growth rate, which triggered ethylene production and fruitlet abscission.

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