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Responses of Ethylene Emission, Abscission, and Fruit Quality to the Application of ACC as a Chemical Thinner in 'Flatbeauti' Peach

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Abstract

Peach (*Prunus persica* (L.) Batsch) trees are prone to heavy cropping, but crop load management options are limited. 1-aminocyclopropane-1-carboxylic acid (ACC) has been suggested to reduce crop load and improve fruit quality in peaches, but many questions remain concerning the role of endogenous ethylene in the abscission response and other side effects. Here, the use of ACC as a chemical thinner in peach trees was studied at different rates (350, 500, and 750 mg L^{-1}) and timings [at full bloom (FB) and after petal fall (AP) when the fruit was approximately 15–20 mm in diameter] by comparing the results to those of an untreated control and a hand-thinning treatment as a reference. The abscission response and ethylene emission were related to the ACC concentration. ACC-induced ethylene production, as well as some degree of defoliation, was time-dependent, with the highest ethylene emission peaks and the lowest defoliation degree occurring when ACC was applied at FB. On the other hand, the intra-annual differences in the abscission response between the FBs and APs varied depending on the season. AP-treated fruits produced more endogenous ethylene than did untreated fruits up to harvest, which could have influenced fruit color. Finally, our results indicate that ACC in the range of 500 and 350 mg L^{-1} can be used in 'Flatbeauti' peaches at FB and AP, respectively, to induce adequate levels of fruit crop load without or with minor undesired effects.

Keywords Thinning · Crop load · *Prunus persica* · Plant growth regulator · Plant hormone

Introduction

Flower and/or fruit thinning is indispensable in peach orchards because it reduces competition among fruits and, consequently, improves fruit size and other fruit quality parameters. This labor is usually carried out by hand, which is an expensive and intensive practice (Assirelli et al. 2018). Hence, different chemical thinning approaches, such as GA-induced inhibition of floral buds, caustic products to damage flowers, or plant hormones to promote abscission, have been previously evaluated in peach trees, but few have produced reliable results (Costa and Botton 2022). With respect to these different approaches, the preferred method by farmers is the application of plant hormones to promote abscission after a bloom when the danger of frost has passed. However, early bloom thinning can also increase final fruit size in early-ripening cultivars, which are more sensitive to excess load than late-ripening cultivars (Miranda and Royo 2002).

The ethylene signaling pathway can have practical applications and lead to substantial improvements in fruit production (Chang 2016). This knowledge has great potential for the crop load regulation of fruit trees using ethylene precursors. Ethylene accelerates the rate of abscission in many plants by inducing the synthesis of wall hydrolases in the abscission zone (Meir et al. 2019). Hence, the application of ethylene precursors can increase the amount of ethylene produced by developing fruits, leading to a decrease in the number of fruits that ultimately mature (Torres and Asín 2022a, b; Torres et al. 2021). Aminocyclopropane-1-carboxylic acid (ACC) is a natural ethylene precursor plant hormone that has been studied as a potential tool for fruit thinning. Plants synthesize ethylene using a two-step biochemical pathway starting from S-adenosyl-L-methionine (SAM), which is converted to ACC by the enzyme ACC synthase (ACS). Afterward, ACC can be converted to ethylene by ACC oxidase (ACO) in the presence of oxygen (Adams and Yang 1979; Yoshii and Imaseki 1981). Large amounts

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of ACO are present in plants; thus, the step between ACC and ethylene is not rate limiting when ACC is present (Cline et al. 2021). The use of ACC for fruit thinning has been previously studied in different fruit crops, such as apples (Fallahi and McArtney 2022; Schupp et al. 2012), Japanese plums (Theron et al. 2017), and even peaches (Theron et al. 2020; Torres and Asín 2022a). In fact, an ACC formulation was recently identified in the United States as a chemical bloom thinner for stone fruit (Fallahi and McArtney 2022). Nevertheless, bloom thinning may represent an excessive risk since spring frosts occurring thereafter might cause an unexpected reduction in fruit set, thus further decreasing yield and the value of production (Costa and Botton 2022). Climate change is expected to lead to an increase in the chance of spring frost. Indeed, in the 2021 and 2022 seasons, spring frost damaged the flowers and fruitlets of peach trees, which severely affected the overall peach crop output across northern Spain and other southern European regions. Hence, delaying chemical thinning until the risk of frost damage to flowers and fruitlets has already passed could be a better option in regions with chances of spring frost.

It is important to note that ACC is still a relatively new technology for fruit thinning, and more research is needed to fully understand its effects on fruit quality, yield, and tree health. Many questions remain concerning the role of endogenous ethylene evolution during the early stages of fruit development and whether it is involved in the response to ethylene-releasing or -precursor compounds, such as ACC. Blanpied (1972) reported that the ethylene content in fruitlets was correlated with the abscission of early fruit drops in cherry trees but not in apple trees. Similar results were observed by Walsh and Solomos (1987) for 'Golden Delicious' apples. Germani et al. (2022) observed a relative ethephon dose-dependent increase in ethylene emission and abscission in 'Montmorency' sour cherry, but the magnitude of this increase depended on the phenological stage and ambient temperature. In a previous study in which we used ethephon to promote ethylene-induced abscission of peach flowers and fruitlets, we observed that the thinning effect was greater when endogenous ethylene production at the time of application was also greater; hence, we hypothesized that differences in endogenous ethylene production at the time of application could explain different responses to ethephon (Torres et al. 2021). Exogenously applied ethephon increases ethylene levels in plants; therefore, a similar response to that of ethephon is expected to occur with ACC application (Theron et al. 2020).

One of the primary concerns over the efficacy of plant hormonal products, such as chemical thinning in fruit trees, is their inconsistency and dependency on internal (genotype, growth stage, plant health, etc.) and external (climate, irrigation, etc.) factors. The species *Prunus persica* (L. Batsch) encompasses many economically important peach cultivars, such as round and flat peaches. Although flat peaches require greater thinning (Costa and Botton 2022), most studies published to date on chemical thinning have been carried out on round peach cultivars. Additionally, a possible inconvenience of using inducers or precursors of endogenous ethylene is that they can influence other side effects or developmental processes, such as leaf senescence (Germani et al. 2022; Torres and Asín, 2022a), fruit ripening (Torres et al. 2021), and/or the formation of gummosis in the genus *Prunus* L. (Germani et al. 2022). New field-crop research with ACC will help to improve the knowledge and thus maximize its potential as a chemical thinner while minimizing the risk associated with its use. Taken together, the objectives of this work were to study the potential of using ACC as a chemical thinner in 'Flatbeauti' peach trees at different rates and phenological stages, as well as its effect on fruit quality parameters and other side effects and its relationship to the ethylene evolution pattern throughout peach fruit growth.

Materials and Methods

Experimental Site and Plant Material

The study was conducted at the experimental orchard of the *Institut de Recerca i Tecnologia Agroalimentàries* (IRTA) in Gimenells, NE Spain (41° 39′ 20.50″ N latitude, 0° 23′ 22.33″ E longitude) during three subsequent seasons. Every season, mature 'Flatbeauti' peach trees (*Prunus persica* L. var. *platycarpa*) on GF-677 rootstock were carefully selected for uniformity in terms of tree size and flower intensity. The trees were spaced at 5 × 3 m (667 trees ha^{-1}) and trained to a vase system.

Experimental Layout

For each treatment, two trees per replicate were arranged in completely randomized blocks. Within each replicate, a single tree was used for destructive flower or fruit sampling (ethylene measurement), and the remaining tree was used for the assessments of thinning efficacy, fruit yield, and quality parameters. The experimental units were separated by at least one guard tree to minimize spray drift.

Three different rates (350, 500, 750 mg L^{-1}) of ACC (liquid formulation of 10% w/w ACC from Valent BioSciences) were tested at two stages: full bloom (FB) and after petal fall (AP) when the fruit was approximately 15–20 mm in diameter. A high-pressure handgun sprayer (25 atm) was used at a rate of ~ 1000 L ha^{-1} for the ACC treatments. These ACC treatments were compared to an untreated control and a reference treatment (hand thinning). All the ACC treatments were sprayed very early in the morning, when the air temperature was below 24 °C. The third season recorded the highest

temperatures during the applications at FB (~ 11 °C) and AP (~ 11 °C) followed by the first and second years (Fig. 1). In the second year, there were three days with temperatures below 0 °C (from − 0.2 to − 1.4 °C) during the first 5 days after the application at FB. This, together with other weather events (rainy, cloudy, and windy days) during the fruit set period, resulted in a decrease in fruit set compared to what was expected. Hand thinning was carried out at 45–55 days after FB by spacing the fruits approximately 15–20 cm apart. The timing of the applications and the evolution of temperatures for each season are presented in Fig. 1.

Data Collection

Fruit Set

Before treatment, at the balloon stage (phenological growth stage 57–59 according to the BBCH and stage D according to Baggiolini), two homogenous primary scaffold limbs on each tree, with similar number of flowers (average of 335 flowers per scaffold limbs), were tagged and the number of flowers was counted. The number of peaches was counted after treatment and physiological fruit dropping. The fruit set ratio was calculated as the number of remaining fruits per 100 flowers.

Fruit Yield and External Quality Parameters

At harvest, all fruits were separately hand-picked from each tree with a single pick to eliminate the variability caused by the decisions of fruit pickers, which could influence fruit quality results. Fruit weight, diameter, color, and total fruit yield (kg and number of fruits per tree) were recorded by automatic fruit sorting equipment (Maf Roda Agrobotic, Cedismafrut, Lleida, Spain). The trunk cross-sectional area (TCSA) was determined each year by measuring the trunk circumference at harvest 30 cm above the ground. The fruit crop load per tree was calculated as the number of fruits per TCSA (fruit cm^{-2}).

Fruit Internal Quality Parameters

Thirty randomly selected peaches per replicate were selected for measurement of fruit firmness, total soluble solid concentration (TSS), and titratable acidity. Fruit flesh firmness was measured at two opposite sides on the fruit equator using a digital firmness tester (Penefel®; Ctifl, France) with an 8-mm (diameter) tip. The TSS content (Brix) of the sampled fruit juice was determined by a digital temperature-compensated refractometer (model PR–101, Atago Co., Tokyo, Japan). The titratable acidity (malic acid g L^{-1}) was determined by titrating 10 mL of freshly prepared juice from the whole sample with 1.0 M NaOH to pH 8.2.

Other Side Effects

Leaf abscission and other side effects, such as the presence or absence of gummosis on the tree trunk and branches, were noted for each tree one week and one month after the last application. The reduction in leaf area was rated from visual observations using a percentage scale.

Return Bloom

Bloom intensity the year following application was rated using a linear 9-point scale (1 = no flower present; 9 = abundant flowering).

Ethylene Evolution Pattern

Ethylene evolution after application was measured in the last two years of the study according to the methodology described by Torres et al. (2021). In brief, just before application and every 2–25 days (the sampling frequency pregressively decreased with the time), according to the fruit growth stage at sampling time, 20 flowers, 10 fruitlets, or 5–3 fruits from each replicate were enclosed in jars (0.1 to 0.5 L depending on fruit size) sealed with a rubber cap. The rubber septum consisted of a disk of silicone installed along the inside of the cap of each jar for the purpose of piercing with a syringe needle. The sealed jars with the samples inside were kept in the dark at 20 °C. After 2 h, the ethylene production of each jar was determined from a 1-mL air sample. An Agilent 6890 gas chromatograph (Agilent Technologies, Wilmington, Germany) equipped with a flame ionization detector and an F1 80/100 alumina column (2 m × 1/8 × 2.1, Tecknokroma, Barcelona, Spain) was used for quantifying the ethylene concentration. The oven temperature was 140 °C, while the injector and detector were kept at 180 °C and 280 °C, respectively.

Statistical Analysis

The experimental design of the trial was a randomized complete block with four blocks and eight experimental units per block. Two-way ANOVA was performed with the GLM procedure to test the main effects of season and treatment and their interaction on the analyzed parameters. Analyses were performed in SAS Enterprise Guide version 7.1 (SAS. Institute, Inc., Cary, NC, USA). Duncan's multiple range tests were used for the mean separation of significant effects if the preharvest treatment effect from ANOVA models was significant ($P < 0.05$).

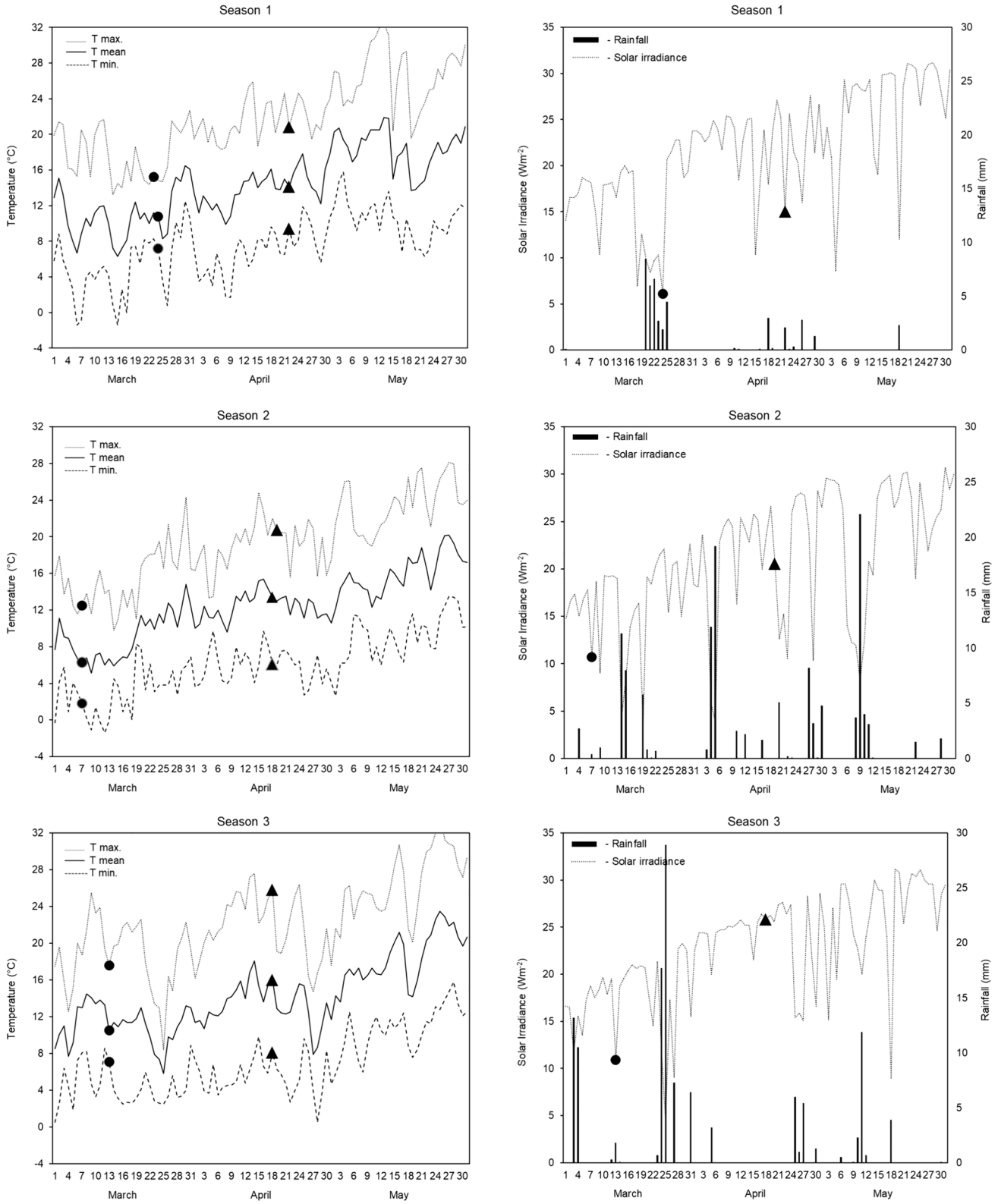


Fig. 1 Weather conditions and time of application. Mean temperature during the ACC application period (from March to May) for the three seasons. The circles indicate applications at 100% full bloom (FB,) and the triangles indicate applications after petal fall (AP, ~ 40 days after FB)

Results

Fruit Set and Fruit Yield Parameters

All tested ACC rates had an effect on the reduction in fruit set at some point in the study. Increasing the ACC rate produced a greater response, although not always significantly (Fig. 2). In general, increasing the ACC rate from 350 to 750 mg L^{-1} was associated with a decrease in fruit set from 34.9 to 62.7% less than that of UTC. The differences between the application of FBs and AP depended on the season. The ACC application at AP had a greater effect than that applied at FB in two of the three seasons (seasons 1 and 3), with significant fruit set reductions compared to those at UTC of 25–33% (season 1) and 27–50% (season 3). In contrast, in season 2, the ACC application at FB, with significant reductions in fruit set of 10–16% compared to that at UTC, had a greater effect than did the application of AP. We must note that the natural fruit set, crop load, and yield levels in season 2 were inferior to those in seasons 1 and 3. Therefore, the hand-thinning treatment in season 2 was applied only to improve the fruit distribution but not to reduce the fruit crop load; hence, no significant differences were detected between the hand-thinning treatment and the UTC treatment (Fig. 2).

When comparing the fruit crop load (fruit cm^{-2} of TCSA, Fig. 2) of the ACC treatments with that of the HT treatment, we found that the treatments with 500 mg L^{-1} ACC had results more similar to those of the HT treatment. However, they produced a crop load level significantly lower than that of the HT treatment on two occasions (season 1 AP and season 2 at FB), whereas the highest ACC rates of 750 mg L^{-1} occurred on three occasions (seasons 1 and 3, both AP, and season 2 at FB). Conversely, the lowest ACC rate, 350 mg L^{-1}, was significantly greater than that of HT in four cases (seasons 1 and 3, regardless of the time of application), and the ACC rate of 500 mg L^{-1} occurred on only one occasion (season 3 at FB). In the remaining cases, the fruit crop load values of the ACC treatments were statistically comparable to those of the HT treatment.

Significant reductions in fruit yield (kg of fruit, Fig. 2) occurred in two of the three seasons (seasons 1 and 2) when ACC was applied at 500 and 750 mg L^{-1}, particularly when it was applied at FB. However, we considered overthinning to occur when the fruit yield was lower than that obtained by the HT treatment. This occurred more frequently when ACC was applied at 750 mg L^{-1} AP (seasons 1 and 3), whereas the ACC rate of 500 mg L^{-1} resulted in a fruit yield significantly lower than that of HT only once when it was applied at AP (season 1). In the remaining cases, the fruit yields were comparable to those obtained with the HT treatment.

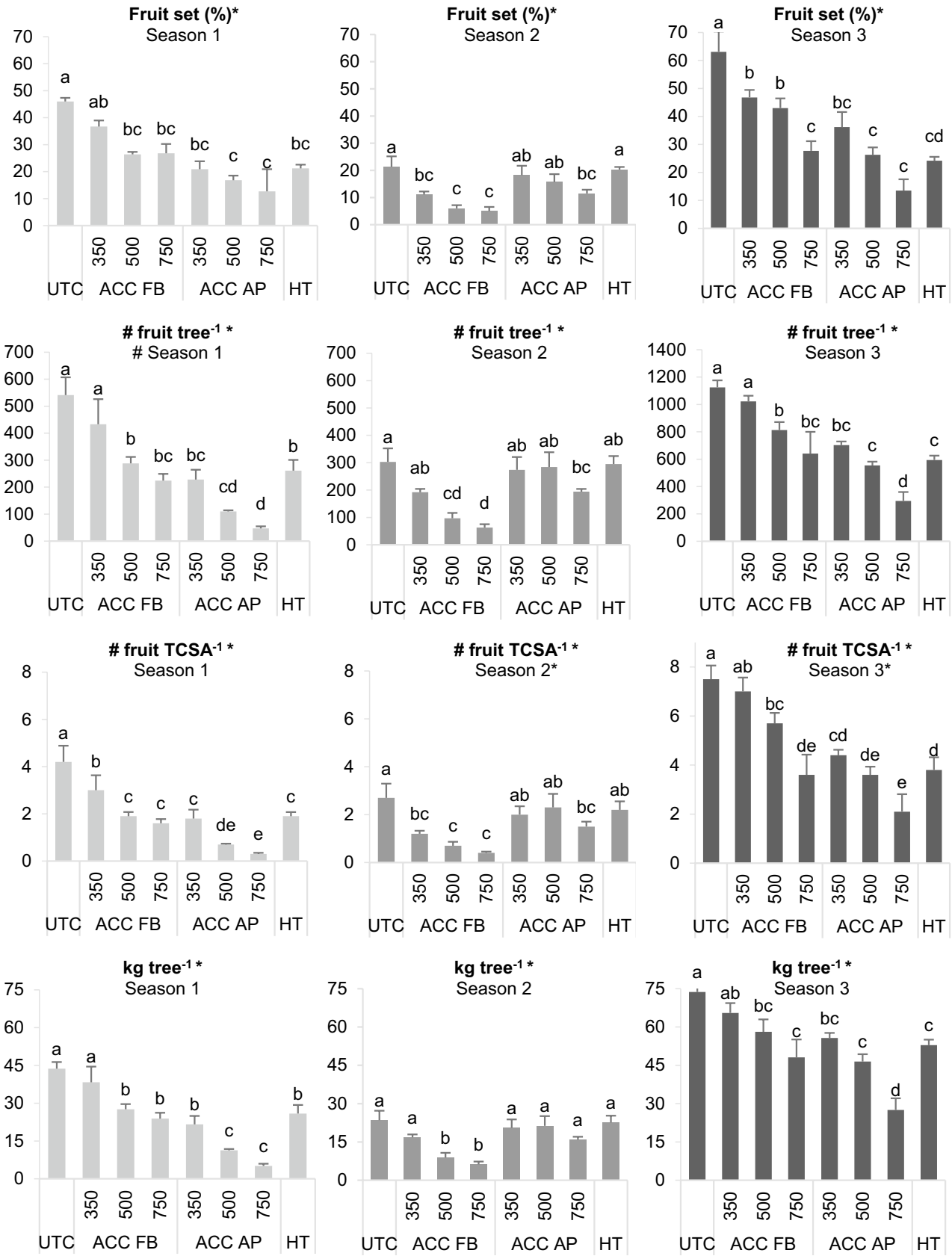
External and Internal Quality Parameters

Fruit thinning had a positive effect on fruit size. In general, a greater thinning effect correlated with greater fruit size (Table 1). It is known that reducing fruit crop load can have a positive effect on the color of fruit peel. We found that an increase in the red of the fruit color was also related to the late application of ACC. In seasons 1 and 3, most of the ACC treatments in which AP was applied recorded a percentage of red-colored surface that was significantly greater than that in the UTC treatment, and this effect was greater with increasing AP dose. In contrast, the ACC treatments applied at FB, as well as the HT treatment, did not significantly differ from those applied at UTC (Table 1).

No significant differences between treatments were observed for the internal fruit quality parameters. However, although fruit firmness did not significantly differ between the earlier treatments at FB and the later treatments at which AP was applied, the latter treatments showed a nonsignificant tendency to reduce fruit firmness as the dose increased (Table 2). No other relevant effects of the treatments were observed on the TSS content or acidity of the fruit.

Effects on Leaf Area Reduction and Other Side Effects

ACC application did not result in gummosis either on the trunk or on the main scaffold branches, but reductions in leaf area (defoliation) were observed, especially in response to AP and increasing the dose (Table 3). This ACC-induced decrease in leaf area was due to the occurrence of burning buds or necrosis in one-year-old shoots when ACC was sprayed on FB plants and leaf drop when it was sprayed on AP plants. The intensity of symptoms and differences between treatments depended on the year. The lowest ACC rate of 350 mg L^{-1} showed slight leaf area reductions throughout the whole study, with significant differences compared to that in the UTC treatment in only one season (season 1). An ACC rate of 500 mg L^{-1} resulted in a significant reduction in leaf area in one season (season 1) when it was applied at FB (30%) and in three seasons when it was applied at AP (13–49%). The highest ACC rate of 750 mg L^{-1} resulted in a significant reduction in leaf area compared to that in the UTC treatment in all cases; in most of the seasons, the effect was greater when AP was applied (up to 54% in one of the three seasons). In all treatments, the symptoms were transient, and the trees presented a healthy appearance at harvest, without apparent long-term effects on their health.


Fruit set (%)*
Season 1
Fruit set (%)*
Season 2
Fruit set (%)*
Season 3
fruit tree-1 *
Season 1
fruit tree-1 *
Season 2
fruit tree-1 *
Season 3
fruit TCSA-1 *
Season 1
fruit TCSA-1 *
Season 2*
fruit TCSA-1 *
Season 3*
kg tree-1 *
Season 1
kg tree-1 *
Season 2
kg tree-1 *
Season 3
UTC
ACC FB
ACC AP
HT
350
500
750

◂**Fig. 2** Yield parameters [fruit set (fruit per 100 flowers), number of fruit and kg per tree and crop load as fruit per trunk cross-section area (fruit cm^{-2})] for each treatment [untreated control (UTC), ACC 350, 500, 750 mg L^{-1} tested at full bloom (FB) and after petal fall (AP) and hand thinning (HT)]. *P values (season, treatment, and its interaction) < 0.001 for a two-way ANOVA; treatments with different letters are significantly different according to Duncan's test ($P < 0.05$). The error bars indicate the standard errors ($n = 4$)

Return Bloom

The ACC and HT treatments had no effect on the return bloom. No variation in the flowering date due to the treatments was observed (Table 3).

Ethylene Production

The ethylene emissions from ACC-treated flowers and fruitlets were rate- and application timing-dependent. In season 2 (Fig. 3A), the UTC and ACC treatments resulted in the highest ethylene production at FB (9.0–19.6 µL C_2H_4 kg^{-1} h^{-1}). At 350 and 750 mg L^{-1}, the ACC reached its peak 2 days after application, at which point approximately 7 and 8 times more ethylene were produced, respectively, than in the case of UTC. The ACC at 500 mg L^{-1} reached the peak level 4 days after FB application, with approximately 14 times more ethylene than UTC. Thereafter, ethylene production in the treated fruitlets decreased rapidly, especially in the ACC-treated fruitlets at 350 mg L^{-1}, whose ethylene production rates were significantly lower than those in the ACC-treated fruitlets at 500 and 750 mg L^{-1}. However, a significant difference persisted in the three ACC treatments compared to that in the UTC treatment up to 25 days after application, when no significant differences between treatments were observed.

For the AP treatments applied in season 2 (Fig. 3B), the ethylene emissions of the non-treated fruitlets rarely exceeded 1 µL C_2H_4 kg^{-1} h^{-1}. The AP-induced ethylene production peaks were lower than those at FB (7.7–12.4 µL C_2H_4 kg^{-1} h^{-1}). However, the three ACC treatments significantly increased ethylene production in fruitlets throughout the whole period under consideration (until 21 days after application). These values reached their peak 3 days after application, when treated fruitlets produced approximately 28, 38, and 46 times more ethylene, respectively, than non-treated fruitlets (0.27 µL C_2H_4 kg^{-1} h^{-1}). After that point, ethylene production decreased rapidly in the three ACC treatments but produced approximately 3 times more ethylene than did UTC until 13 days after application. In the last measurement, 21 days after application, no significant differences between treatments were observed; however, fruitlets treated with the highest dose of 750 ppm tended to produce more ethylene than did the untreated fruitlets (0.20 vs. 0.10 µL C_2H_4 kg^{-1} h^{-1}).

In the following season (season 3), we measured ethylene evolution throughout the whole fruit growth period. The ethylene emission peak in non-treated flowers was approximately 2.5 times less than that in season 2, while in ACC-treated flowers, it was greater at the same rates (Fig. 4A). As in season 2, ACC application at FB induced the highest ethylene production (15.4–32.3 µL C_2H_4 kg^{-1} h^{-1}). All treatments, even the UTC treatment, reached the ethylene peak 4 days after FB. The ethylene peaks in the ACC application at FB were between 5 and 10 times greater than those at UTC (15.4–32.3 vs. 3.5 µL C_2H_4 kg^{-1} h^{-1}). The ACC-induced increase in ethylene production lasted 19 days for the lowest ACC rate of 350 mg L^{-1}, 28 days for the ACC mid-rate of 500 mg L^{-1}, and 96 days for the highest ACC rate of 750 mg L^{-1}. At harvest, fruits treated with the highest ACC dose at FB, tended to produce more ethylene, but the difference was not significant.

When the trees were treated AP in season 3 (Fig. 4B), the peak level was reached between 2 and 6 days after application (2.0–9.1 µL C_2H_4 kg^{-1} h^{-1}). The major ACC-induced increase in ethylene production for the three ACC rates lasted for 38 days, which was up to 46 days before harvest. During this period, the highest ACC dose, 750 mg L^{-1}, produced approximately 98 and 210 times more ethylene than did UTC (9.6–9.1 vs. 0.10–0.04 µL C_2H_4 kg^{-1} h^{-1}), respectively, and approximately 4–5 times more ethylene than did the other ACC treatments (1.7–2.5 µL C_2H_4 kg^{-1} h^{-1}). After that point, ethylene production decreased rapidly, and significant differences persisted only for ACC applied at 500 and 750 mg L^{-1}, with ethylene production approximately 19–1.1 times greater than that of UTC up to harvest, while ACC-treated fruit at 350 mg L^{-1} did not show a significant difference compared to that of UTC. At harvest, there was a rise in ethylene emission, especially in ACC-treated fruits. At 750 mg L^{-1}, ACC-treated fruits produced approximately five times more ethylene than did UTC and ACC at 350 mg L^{-1} (1.03 vs. 0.22 µL C_2H_4 kg^{-1} h^{-1}) and twice as much ethylene as did ACC at 500 mg L^{-1} (0.45 µL C_2H_4 kg^{-1} h^{-1}).

Discussion

Here, we assessed the use of ACC as a chemical thinner in peach trees at different rates and timings by comparing the results to those of a UTC and a hand-thinning treatment as a reference. Our results support the hypothesis that ACC can be used in peach trees to induce floral and/or fruitlet abscission early in fruit development, resulting in crop load ratios statistically comparable to those for hand thinning. In general, the ACC rate had a greater influence on thinning intensity than did application timing. The rate of 500 mg L^{-1} ACC was the best treatment to ensure that the results were statistically significant compared to those of the UTC

Table 1 External fruit quality parameters [fruit weight (g), fruit diameter (mm), and red-colored fruit surface (%)] for each treatment [untreated control (UTC), ACC 350, 500, 750 mg L^{-1} tested at full bloom (FB) and after petal fall (AP) and hand thinning (HT)], and *P* values (Ss.: season; Tr.: treatment; and its interaction) for two-way ANOVA

Treatment	Fruit weight ± standar error (g)			Fruit diameter ± standar error (mm)			Fruit color ± standar error (%)		
	Season 1*	Season 2*	Season 3*	Season 1*	Season 2*	Season 3*	Season 1*	Season 2 ns	Season 3*
UTC	84.9 ± 5.0 d	77.6 ± 1.6 c	68.4 ± 2.0 d	63.9 ± 1.4 c	62.7 ± 0.4 d	59.1 ± 0.6 d	62.1 ± 1.3 d	45.7 ± 1.5	33.8 ± 2.4 d
ACC 350 FB	93.5 ± 5.7 cd	87.4 ± 2.4 b	67.3 ± 2.6 d	66.3 ± 1.5 bc	65.0 ± 0.6 bc	58.8 ± 0.8 d	64.5 ± 2.2 d	46.9 ± 0.9	34.2 ± 2.4 d
ACC 500 FB	96.7 ± 3.0 bc	91.5 ± 2.1 ab	74.4 ± 1.9 cd	67.2 ± 0.5 b	65.8 ± 0.5 b	60.8 ± 0.4 cd	66.0 ± 2.4 cd	47.5 ± 0.9	37.8 ± 2.5 cd
ACC 750 FB	107.6 ± 3.8 a	100.1 ± 3.7 a	85.4 ± 8.5 bc	70.0 ± 0.5 a	68.0 ± 0.9 a	63.5 ± 2.2 bc	68.5 ± 2.6 bcd	46.5 ± 2.3	40.9 ± 2.3 bcd
ACC 350 AP	95.2 ± 2.4 bc	75.3 ± 3.3 c	84.3 ± 4.4 bc	65.8 ± 0.5 bc	62.0 ± 0.7 d	63.5 ± 1.2 bc	72.9 ± 3.1 bc	48.9 ± 0.9	42.1 ± 2.5 bcd
ACC 500 AP	104.3 ± 2.8 ab	76.0 ± 3.9 c	87.5 ± 1.6 bc	67.6 ± 0.4 ab	62.1 ± 0.9 d	64.3 ± 0.6 bc	74.6 ± 2.9 b	49.8 ± 1.5	47.7 ± 2.9 abc
ACC 750 AP	107.8 ± 1.1 a	82.1 ± 3.3 bc	104.0 ± 6.8 a	68.4 ± 0.7 ab	63.5 ± 0.6 cd	68.0 ± 1.2 a	83.4 ± 3.9 a	49.4 ± 1.3	55.0 ± 4.3 a
HT	100.8 ± 4.4 abc	76.6 ± 3.5 c	92.0 ± 1.9 ab	67.8 ± 1.0 ab	62.3 ± 0.9 d	65.5 ± 0.6 ab	64.6 ± 2.9 d	47.9 ± 2.8	39.1 ± 4.6 cd
Season	***			***			***		
Treatment	***			***			***		
Season × treat	***			***			**		

*Treatments with different letters are significantly different according to Duncan's test ($P < 0.05$); *ns* not significant

**$P < 0.005$

***$P < 0.0001$

Table 2 Internal fruit quality parameters [fruit firmness (kg), solid soluble content (brix), and acidity (g L^{-1} malic acid)] for each treatment [untreated control (UTC), ACC 350, 500, 750 mg L^{-1} tested at full bloom (FB) and after petal fall (AP) and hand thinning (HT)], and P values for the two-way ANOVA (season, treatment, and its interaction)

Treatment	Fruit firmness ± standar error (kg)			Solid soluble content ± standar error (Brix)			Acidity ± standar error (g L^{-1} malic acid)		
	Season 1	Season 2	Season 3	Season 1	Season 2	Season 3	Season 1	Season 2	Season 3
UTC	5.8 ± 0.1	6.2 ± 0.2	5.9 ± 0.3	10.4 ± 0.1	11.3 ± 0.3	9.4 ± 0.3	4.6 ± 0.1	5.1 ± 0.1	5.0 ± 0.5
ACC 350 FB	6.2 ± 0.1	6.5 ± 0.2	5.9 ± 0.0	11.0 ± 0.2	11.7 ± 0.3	9.8 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	5.2 ± 0.3
ACC 500 FB	6.3 ± 0.2	6.4 ± 0.2	6.1 ± 0.2	10.7 ± 0.2	12.5 ± 0.4	9.7 ± 0.3	4.8 ± 0.1	4.8 ± 0.2	4.8 ± 0.5
ACC 750 FB	6.4 ± 0.1	6.6 ± 0.1	6.2 ± 0.2	11.2 ± 0.1	12.4 ± 0.3	9.7 ± 0.1	5.2 ± 0.3	5.0 ± 0.4	5.3 ± 0.5
ACC 350 AP	6.0 ± 0.1	6.0 ± 0.2	6.0 ± 0.1	10.8 ± 0.2	11.8 ± 0.4	9.8 ± 0.3	4.9 ± 0.2	5.1 ± 0.4	4.8 ± 0.4
ACC 500 AP	5.9 ± 0.1	5.8 ± 0.2	6.0 ± 0.3	11.0 ± 0.2	12.1 ± 0.4	10.0 ± 0.3	4.9 ± 0.2	4.9 ± 0.4	5.0 ± 0.3
ACC 750 AP	5.8 ± 0.3	6.1 ± 0.2	5.6 ± 0.3	10.9 ± 0.2	11.8 ± 0.2	10.0 ± 0.4	5.0 ± 0.1	4.9 ± 0.2	4.9 ± 0.2
HT	5.7 ± 0.1	6.2 ± 0.2	6.2 ± 0.2	10.2 ± 0.3	11.4 ± 0.3	9.5 ± 0.2	4.5 ± 0.2	5.3 ± 0.1	5.5 ± 0.4
Season	***			***			***		
Treatment	ns			ns			ns		
Season × Treatment	ns			ns			ns		

nsNot significant

***$P < 0.0001$

treatment and were similar to those achieved with the HT treatment. The lowest rate of 350 mg L^{-1} ACC obtained crop load and fruit yield levels statistically equivalent to those of hand thinning in the three seasons when AP was applied, but its effect was not significant compared to that of UTC in most cases when it was applied at FB. On the other hand, the highest rate of 750 mg L^{-1} ACC showed a greater tendency to reduce the crop load and fruit yield below the levels of the hand-thinning treatment (in 3 of 6 cases, these values were statistically significant). These results were similar to those obtained by Theron et al. (2020) who suggested a rate of 400 µL L^{-1} ACC for the peach 'Keisie.' In contrast,

Table 3 Defoliation by leaf drop and/or necrosed buds at 7 and 30 days after the applications (DAA) and return bloom the following year of applications for each treatment [untreated control (UTC), ACC 350, 500, 750 mg L^{-1} tested at full bloom (FB) and after petal fall (AP) and hand thinning (HT)], and *P* values for the two-way ANOVA (season, treatment, and its interaction)

Treatment	Leaf area reduction by necrosis and/or leaf drop (%)						Return bloom (1–9)
	Season 1		Season 2		Season 3		
	7 DAA*	30 DAA*	7 DAA*	30 DAA*	7 DAA*	30 DAA*	
UTC	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	6 ± 0.4
ACC 350 FB	12 ± 4 bc	26 ± 3 b	0 ± 0 c	0 ± 0 c	9 ± 3 bc	0 ± 0 c	7 ± 0.4
ACC 500 FB	25 ± 3 b	30 ± 3 ab	9 ± 3bc	9 ± 3 bc	11 ± 3 bc	3 ± 2 bc	6 ± 0.6
ACC 750 FB	50 ± 5 a	39 ± 3 ab	19 ± 3 a	20 ± 0 a	18 ± 3 b	18 ± 4 a	6 ± 0.5
ACC 350 AP	20 ± 3 b	27 ± 3 b	0 ± 0 c	5 ± 0 bc	12 ± 2 bc	9 ± 4 abc	7 ± 0.4
ACC 500 AP	30 ± 3 b	49 ± 5 ab	13 ± 4ab	13 ± 4 b	30 ± 3 ab	19 ± 4 a	6 ± 0.4
ACC 750 AP	46 ± 3 ab	54 ± 3 a	25 ± 3 a	25 ± 3 a	48 ± 4 a	21 ± 4 a	6 ± 0.5
HT	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	0 ± 0 c	7 ± 0.3
Season	**						n. s
Treatment	**						n. s
Season × treat	**						n. s

n.s. no significant differences

*Within the same season, treatments with different letters are significantly different according to Duncan's test ($P < 0.05$)

**$P < 0.005$

Cline et al. (2021) and Ceccarelli et al. (2016) obtained poorer dose–response relationships than our results. Cline et al. (2021) did not observe significant differences between 300 and 600 mg L^{-1} in 2 years of research on 'Redhaven' peaches, and the highest dose of 600 mg L^{-1} was the only dose that significantly reduced the fruit set ratio compared to that in the UTC. Ceccarelli et al. (2016) did not observe a significant thinning effect at 350, 500, or 750 mg L^{-1} ACC in the 'Stark Red Gold' nectarine or at 350 or 500 mg L^{-1} ACC in the 'Flamina' peach. These differences between studies on the relationship between ACC concentration and the observed thinning response in peach trees suggest strong cultivar- and/or environment-dependent variance.

Early flower thinning in peaches is generally more effective at increasing fruit size than late fruit thinning, especially for early-ripening cultivars in which the fruit development cycle is shorter. Here, 'Flatbeauti' is a medium-maturiting variety and its effect on fruit size was more influenced by thinning intensity than by the time of application. Flower thinning may represent a risk since spring frosts occurring thereafter might cause an unexpected reduction in yield and value of production (Costa and Botton 2022). Hence, fruit thinning carried out when the fruitlet diameter is approximately 15–20 mm is the preferred method by many growers for medium- or late-maturing varieties. We found a wide window from bloom to 15–20 mm of fruit diameter in which ACC could be applied as a chemical thinner. However, the time–response relationship of the ACC varied depending on the season: the ACC application at FB had a greater effect than did the application AP in season 2, while the application AP had a greater effect in seasons 1 and 3. These results seem to indicate that late applications are more effective at reducing crop load, provided that the expected fruit set ratio is not affected during the bloom period, as in season 2. Cline et al. (2021) proposed that this inconsistent timing response for ACC application may be related to differences in interfruit competition between years or differences in carbohydrate reserves in trees. According to the authors, when the overall fruit set is lower, fruit growth is more active early in the onset of fruitlet development, causing fruitlets to abscise more easily during this period (Cline et al. 2021). Additionally, the final number of set fruit would be reduced, and consequently, the interfruit competition after petal fall would likely be lower, causing fruit to be more resistant to physiological abscission and therefore more difficult to thin during the AP period. Both hypotheses are not contradictory to each other; indeed, they may complement each other. The present results, along with published results (Ceccarelli et al. 2016; Cline et al. 2021; Theron et al. 2020; Torres and Asín, 2022a), seem to be in line with this approach.

Abiotic stress conditions during the period just before or after application could also have a direct effect on the response to ACC. A richbody of literature indicates that abiotic stress has an impact on the course of most components of ethylene signaling and responses, which could influence the response to ACC as a chemical thinner. Plants respond to stress by modulating the levels of various hormones, and one of the most common plant hormones that mediates the response to stressors is ethylene. Temperatures below 0 °C (between 2 and 6 days after FB, the minimum temperatures ranged from − 0.2 to − 1.4 °C) were recorded during the application period at FB in season 2 (Fig. 1). It has been demonstrated that cold stress promotes ethylene production in various perennial

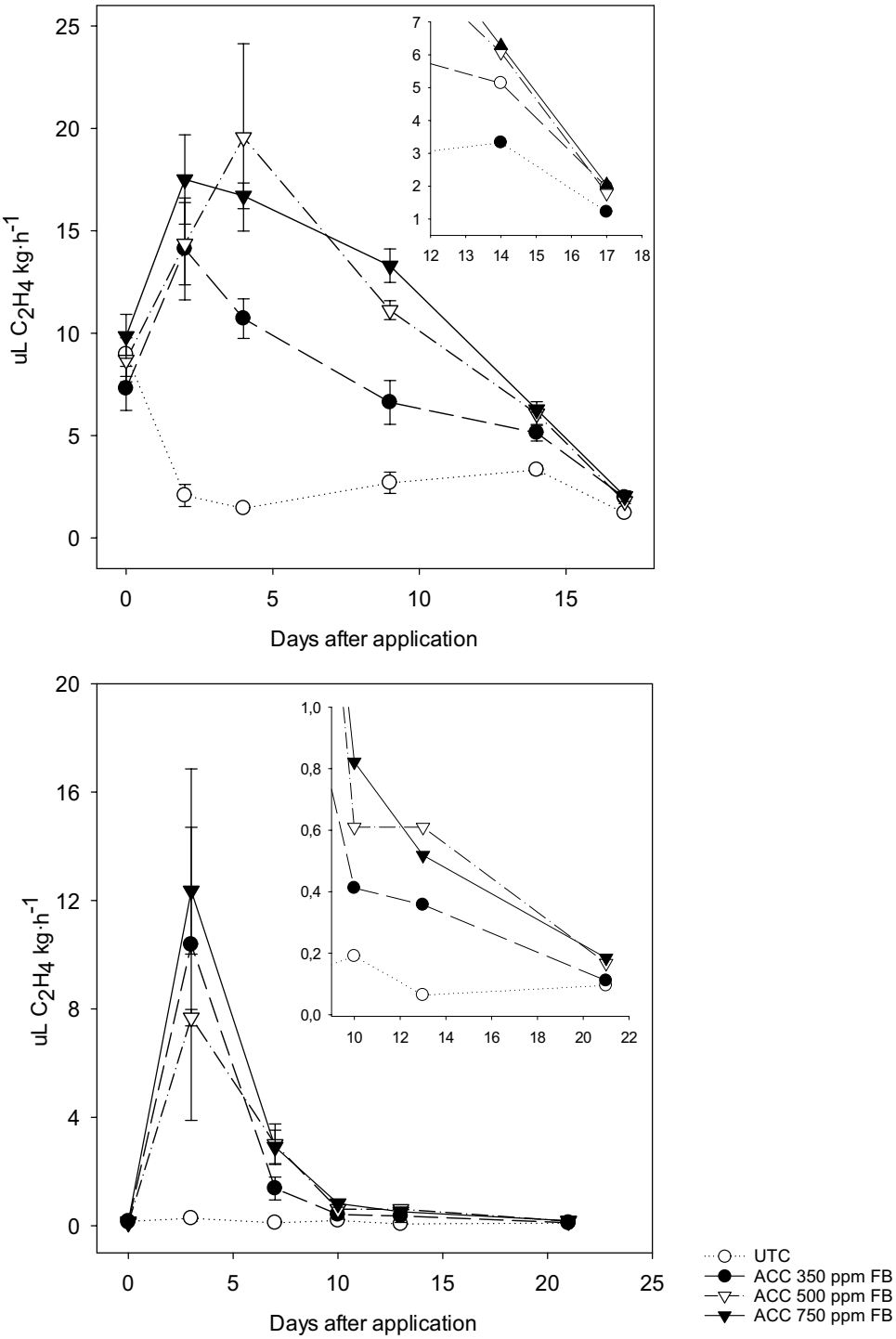


Fig. 3 Dynamics of ethylene evolution during season 2. Above: dynamics of flower/fruit ethylene production in response to the different ACC treatments (350, 500, and 750 mg L^{-1}) at full bloom (FB) and of the untreated control (UTC) from just before the applications (Day 0) to 20 days later. Below: dynamics of fruit ethylene production in response to the different ACC treatments (350, 500, and 750 mg L^{-1}) applied after petal fall (AP, 40 days after FB) and of the UTC from just before the applications and up to 22 days after. The error bars indicate the standard errors ($n=4$)

fruit crops, such as avocado, grapevine, and apple (Huang et al. 2023). Anyway, increased ethylene biosynthesis following cold stress occurs in a species-dependent manner in response to physiological conditions, and no data are available for peach. We observed that, in untreated flowers, ethylene production was greater in season 2 than in season 3. These differences in the endogenous ethylene level before the application could be related to the differences in the time–response relationship of ACC between the two seasons. However, it is important to note that this previous increase in the level of endogenous ethylene did not result in increased ethylene emission after ACC application. We observed a relationship between the ethylene peaks and the abscission response within the same season and timing of application. Nevertheless, we did not observe a relationship between the abscission response to ACC and the

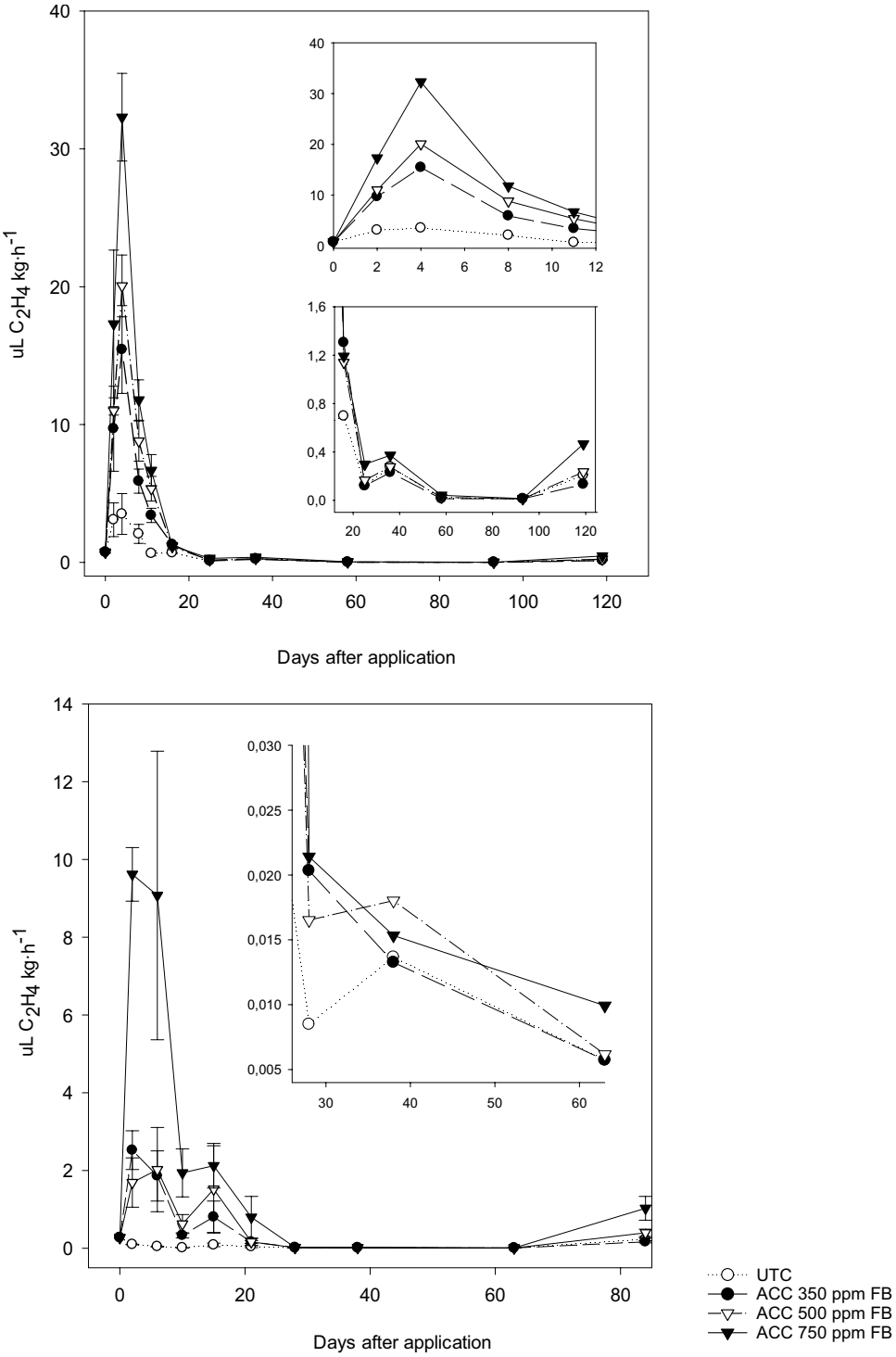


Fig. 4 Dynamics of ethylene evolution during season 3. Left: dynamics of flower/fruit ethylene production in response to the different ACC treatments (350, 500, and 750 mg L^{-1}) at full bloom (FB) and of the untreated control (UTC) from just before the applications (Day 0) up to the time of harvest (120 days later). Right: dynamics of fruit ethylene production in response to the different ACC treatments (350, 500, and 750 mg L^{-1}) applied after petal fall (AP, 40 days after FB) and of the UTC from just before the applications up to harvest (85 days later). The error bars indicate the standard errors ($n=4$)

ethylene emission peaks to explain the interannual differences. Unlike the abscission response, our results showed a clear time-dependent response to ethylene emission. ACC application at FB induced the highest ethylene production, and ACC application at the AP stage resulted in lower ethylene production. These timing-dependent responses to ethylene emission have also been observed using ethephon as a chemical thinener in peach (Torres et al. 2021) and cherry (Germani et al. 2022) plants.

The process by which some fruitlets fall and others remain is still not understood. We suggest that ACC-induced ethylene causes premature senescence of flowers and fruitlets and, consequently, their abscission. Additionally, in a previous paper (Torres and Asín, 2022b), we hypothesized

that an ACC-induced carbohydrate deficit could also be related to this process. We proposed that ACC-induced stomatal closure and/or premature ethylene-induced senescence in leaves could cause carbohydrate stress, which could also promote fruit abscission (Gonzalez et al. 2019). These two proposed fruitlet abscission pathways ('ACC-induced senescence triggered' abscission model and 'ACC-induced carbohydrate stress' model) could interact at the same time and induce different sensitivity levels. From our point of view, the 'ACC-induced carbohydrate stress' model places more weight on fruitlet abscission when leaves are in full development than on flower abscission when no leaves are present in peach. This would explain why the ACC applications at the AP stage, even with lower ethylene emission peaks, had a greater abscission response than did the ACC applications at FB under no stress conditions. Overall, more studies are needed to better understand ACC-induced abscission and its relationship with ethylene.

The use of ethylene-releasing compounds in fruit crops can have other effects on crops in addition to the desired effect. When more ethylene is produced than its threshold level, ethylene stress can lead to several adverse processes for plant development, such as early defoliation (Singh et al. 2015). We observed a positive intra-annual but not interannual relationship between the ACC rate, ethylene emission peak, and defoliation level for the same timing of application. Other authors have also observed defoliation or leaf yellowing as a consequence of spraying ACC as a chemical thinner. Cline et al. (2021) observed leaf yellowing and a decrease in response to foliar sprays in one of two years of study and, in the other year, a moderate decrease in leaf growth when ACC (~ 20 mm of fruit diameter) was applied after petal fall at the highest dose of 600 mg L^{-1}. Ceccarelli et al. (2016) also indicated that high concentrations (750 mg L^{-1} ACC) at petal fall unfortunately caused leaf phytotoxicity. Theron et al. (2020) detected leaf drops resulting from the application of 800 µL L^{-1} ACC at the AP stage. In all these cases, trees were affected only by application rates that were much greater than the suggested use rates. In addition to these dose-related differences, we observed a time-dependent response to defoliation, contrary to the ethylene emission peak. Indeed, the ACC applications at FB, despite producing higher ethylene emission peaks, showed lower defoliation levels than did the AP applications. This may be due to differences in the capacity to convert ACC ethylene, differences in sensitivity to ethylene or greater direct uptake in leaves. Even so, we observed different response levels between years for the same time of application. These interannual differences for the same time of application may be a consequence of differences in the weather conditions just before or after the application time. In this regard, the greatest ACC-induced leaf area reduction at FB was observed in the first season, which could be related to cold temperatures below 0 °C just before the application period at FB. This response could be involved in the induction of endogenous cold-induced ethylene mentioned above. Wang et al. (2021) reported that an increase in the expression of genes involved in the ethylene signaling pathway in apple seedlings incubated at 4 °C (vs. 24 °C) likely promoted cold tolerance. On the other hand, greater defoliation in response to ACC application at the AP stage could be related to warm temperatures after the application. In line with this, seasons 1 and 3 were the years with the highest defoliation levels and were the two seasons with the highest temperatures after application (Fig. 1). The maximum temperatures in seasons 1 and 3 during the 7–10 days after application were 22–24 °C, whereas in season 2, the maximum temperatures for that days were equal to or lower than 20 °C. In a previous study in peach trees under a controlled environment, the response of leaf abscission to AP application via ACC was linearly dependent on temperature after the application from 10 to 20 °C (Torres and Asín 2022b). Research in different plant species has demonstrated that temperature modulates ethylene levels to activate the ethylene signaling pathway and promote higher stress temperature tolerance in plants (Huang et al. 2023). We hypothesize that exogenous ACC application under these conditions could result in the overexpression of ethylene-associated genes and, consequently, a greater level of phytotoxicity symptoms. However, the temperature sensitivity of ethylene appears to vary among plant species and experimental conditions, and information about this phenomenon in peach trees is limited. Future investigations are necessary to deepen our understanding of the role of ACC and ethylene in peach trees under different environmental conditions.

A positive effect on fruit color related to ACC application was observed. A reduction in the number of fruits can enhance the fruit color, growth and development, promoting maturation (Wang et al. 2023). Taheri et al. (2012) observed a similar response when using ethephon for peach thinning and they thought this influence on fruit maturity was more likely attributed to a reduced crop load effect than a direct effect of ethephon. In our case, the time of application had a greater influence on the fruit color response than did the ACC rate. Thus, the percentage of red-colored fruits on the fruit surface increased when ACC was applied later in the season. This effect was significant in seasons 1 and 3, where most of the ACC treatments that applied AP recorded a percentage of red-colored surface that was significantly greater than that in the UTC, and this effect was greater when the dose was increased. Unlike the AP treatments, the FB treatments, as well as the HT treatment, did not significantly differ from those of UTC. Torres et al. (2021) observed a similar response when they used ethephon (other ethylene-related compound) for fruit thinning in peaches. This ACC-induced increase in fruit color could be caused by the effect

of ethylene on pigment compounds. The primary pigment responsible for red coloration in peaches is cyanidin, one of the most common anthocyanin pigments in fruits. The accumulation of anthocyanins varies during fruit growth and ripening. Ravaglia et al. (2013) detected two peaks of anthocyanin accumulation in the peel of 'Stark Red Gold' nectarines during peach fruit growth—one early in development (50 days after FB) and the other at the end of fruit growth (135 days after FB)—and the concentrations of anthocyanins in the middle stages of development were very low, similar to the production of ACC-induced ethylene observed for AP period. Ethylene treatment has been reported to influence the composition of anthocyanin pigments (Cheng et al. 2016), and these findings suggest that ACC-induced ethylene production at early (~40 to 50 days after FB) fruit development stages can also affect pigment composition and, consequently, the final hue of the fruit.

In conclusion, ACC can be an effective tool for fruit thinning when used appropriately, but it should be carefully managed to avoid negative impacts on tree health and fruit yield. Overall, a rate of 500 mg L^{-1} ACC at FB and AP would ensure significant effectiveness with a low risk of reduction in leaf area and without apparent long-term effects on the health of the trees. However, considering its effectiveness and defoliation, 350 mg L^{-1} ACC could also be an option for the late application after petal fall. It is worth noting that the studies published thus far suggest that the thinning response of peaches to ACC may vary by cultivar and/or environmental conditions.

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Declarations

Conflict of interest On behalf of all the authors, the corresponding author states that there are no conflicts of interest.

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