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Physiological Study of Ethephon- and ACC-Induced Fruitlet and Leaf Abscission in Peach Trees Under Different Conditions of Temperature

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Abstract

The effect of temperature on the ability of 2-chloroethylphosphonic acid (ethephon, ETH) and the 1-aminocyclopropane carboxylic acid (ACC) to induce ethylene production in fruitlets and abscission of fruitlets and leaves when applied at postbloom (~15 mm fruit diameter) was studied using 5-year-old 'Sweet Lady'/Rootpac-20 peach [*Prunus persica* (L.) Batsch] trees kept in pots in environment-controlled growth rooms. ETH at 150 mg L^{-1} and ACC at 500 mg L^{-1} effectively thinned peaches. Increasing the temperature from 10 to 20 °C pronounced the ETH-induced fruit and leaf abscission and the ACC-induced leaf abscission and decreased stomatal conductance in leaves. However, the ACC thinning effect was not affected by increasing temperature, as well as ETH- and ACC-induced ethylene production in fruitlets and in the quantum yield of PSII photochemistry. Changes in ethylene peak in fruitlets and stomatal conductance in leaves were related to the abscission response.

Keywords Thinning · Ethylene · Stomatal conductance · Photosynthesis · Crop load · *Prunus persica* · Plant growth regulator · Plant hormone

Introduction

Chemical thinning is a critical management practice to optimize orchard performance of commercial fruits. Tree physiology and environmental conditions are the two major factors affecting the response of most chemical thinners. Application timing is another important factor. Although bloom thinning is a common practice in early-ripening peach cultivars characterized by smaller fruits as its effect on final fruit size is very significant, most of growers prefer fruit thinning around 40–60 days after full bloom, when physiological fruit drop is already occurring, and the risk of late frosts has passed. Nevertheless, there is a lack of post bloom chemical thinners for use in peach fruit, despite many who have searched for suitable products (Costa and Botton 2022). Some hormonal products, such as naphthaleneacetic acid, 6-benzyladenine and gibberellins, and the photosynthesis inhibitor metamitron have been investigated in peach trees as chemical thinners but with erratic and inconsistent results (Costa and Botton 2022; Costa and Vizzotto 2000). Hence, a key global challenge in peach research is to find new products for thinning.

The common plant growth bioregulator 2-chloroethylphosphonic acid (ethephon, ETH) is a product with several pomological applications which has been used for fruit thinning with some success in several crops (Hagemann et al. 2015; Torres and Asín 2022; Torres et al. 2021; Wertheim 2000). ETH is an ethylene-releasing molecule which is stable in a low pH solution, but it hydrolyses in the higher pH of plant tissues releasing ethylene (Ferrara et al. 2016). Hence, exogenously applied ETH stimulates endogenous ethylene production and triggers ethylene-dependent processes such as flower or fruit abscission (Wertheim 2000).

Another ethylene-releasing plant regulator is the 1-aminocyclopropane carboxylic acid (ACC), recently registered in the United States as early chemical thinner for peaches and that can be used to extend the chemical thinning window in apples when king fruit is sized 15 to 20 mm (https://www.valentbiosciences.com/). ACC is an ethylene precursor that increases the corresponding rate of ethylene production (Adams and Yang 1979; Yoshii and Imaseki 1981). The

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ethylene biosynthesis pathway consists of two steps: in a first reaction, S-adenosyl-L-methionine (SAM) is converted into ACC by ACC-synthase; then, in a second reaction, ACC is converted into ethylene by ACC-oxidase (Houben and Van de Poel, 2019). Research on different crops has demonstrated that ACC is an effective fruitlet thinner for apples (McArtney and Obermiller, 2012; Schupp et al. 2012), pears (Wertheim 2000), Japanese plums (Theron et al. 2017), and peaches (Cline et al. 2021; Theron et al. 2020; Torres and Asín 2022).

Results from our own previous research indicated that either ETH or ACC could be used commercially in peaches to induce both flower and fruitlet abscission (Torres and Asín 2022; Torres et al. 2021). However, one of the main concerns about the use of these plant growth regulators deals with the fact that its effectiveness is tightly linked to both endogenous and exogenous factors, such as the metabolic activity and weather conditions (Costa and Botton 2022). Hence, its practical application in peach orchards is still limited, and more research is needed to better understand the effectiveness of these products under different conditions.

Some authors have reported that temperature following ETH application is the most important environmental factor affecting fruit abscission response to ETH in cherries (Flore and Bukovac 1982; Olien 1978; Wittenbach and Bukovac 1973), apples (Jones and Koen 1985; Yuan 2007), and oranges (Yuan and Burns 2004). Low temperatures following ETH application can result in poor fruit abscission whereas high temperature may lead to over-thinning or excessive leaf abscission (Jones and Koen 1985; Olien 1978; Yuan and Burns 2004). As far as we know, there is no information about the effects of temperature on fruit and leaf abscission following ACC application. Exogenously applied ACC increases ethylene levels in plants; therefore, our initial hypothesis was that a similar response as with ETH could be expected with ACC application (Theron et al. 2020). But the ETH- and ACC-induced ethylene biosynthesis are produced by different pathways; therefore, we could also expect different responses. Besides fruit and leaf abscission, ethylene plays a regulatory role in other physiological parameters associated with regulation of photosynthesis such as stomatal conductance (G_s), chlorophyll content, light reactions, carboxylation events, carbohydrate partitioning and senescence (Ceusters and Van de Poel, 2018). This effect of ethylene on photosynthesis-related parameters could also indirectly influence the abscission of organs like fruitlets and leaves.

Few studies have investigated the effect of temperature on the rate of endogenous ethylene in higher plants and its relation to physiological parameters related with photosynthesis such as G_s and efficiency of photosystem II (PSII) in leaves. The present study explores the relationship between ETH- and ACC-induced ethylene, physiological parameters (G_s and PSII), and fruit and leaf abscission in peach trees subjected to different temperatures, testing the hypothesis that higher temperatures just after ETH and ACC application are involved in elevated ethylene levels and ETH- and ACC-induced fruit thinning. The main objectives of the study were (1) to study how peach trees respond to the application of ETH and ACC at different air temperatures and (2) to explore the impact of applying ETH and ACC on ethylene development patterns in fruitlets and G_s and PSII in leaves. A better understanding of these topics will be able to find ways to improve the effectiveness of these products as chemical thinners.

Material and Methods

Plant Material and Environmental Conditions

Five-year-old 'Sweet Lady' peach trees grafted on 'Rootpac-20' rootstock growing in 25-L containers were used for this experiment. Peach trees had an average of 1.4 m in canopy height and 1.5 m in canopy diameter. Twenty-seven 'Sweet Lady' trees were selected and randomly assigned into environment-controlled growth rooms of nine trees each. Just after the different precursors were applied, the selected trees were kept in growth chambers under different temperature regimes (see Sect. Treatments) for 10 days. Relative humidity in the chambers was kept constant at 75% (±5%). Irradiance (photosynthetic photon flux density) was 400 µmol m^{-2} s^{-1} at upper leaf level, provided with high-pressure sodium lamp (Sunlight Supply Inc., USA) from 07:30 to 18:30 HR and completely dark from 18:31 to 07:29 HR.

Treatments

When fruit averaged 15.3 mm in diameter (~40 days after full bloom), three of the nine trees from each environment-controlled growth room were sprayed with ETH (Ethrel, Bayer CropScience Inc) at 150 mg L^{-1} and other three trees with ACC (liquid formulation at 10% w/w from Valent BioSciences Corp.) at 500 mg L^{-1}, using a low-pressure hand-wand sprayer. Spray treatments were applied to the canopy until runoff. The remaining three trees from each environment-controlled growth room were not sprayed and served as untreated control (UTC). The environment-controlled growth rooms were set at day (07:30 to 18:30 HR)/night (from 18:31 to 07:29 HR) with a constant air temperature of 10, 15 or 20 °C (±0.5 °C). Finally, we evaluated nine different treatments from the application of the two ethylene precursors (ETH and ACC) and UTC, and the three

environment-controlled growth room temperatures (10, 15, or 20 °C).

Fruitlet and Leaf Abcission

Fruit abscission was determined by tagging two primary scaffold limbs on each tree and counting the number of fruitlets 20 days before and after ETH and ACC treatments, when the physiological fruit drop was over, and no more fruit abscission occurred from untreated control trees and ETH- or ACC-treated trees. Rate of abscised fruitlet was calculated as number of remaining fruitlets per number of fruitlets before the treatments. Leaf abscission was evaluated on whole tree as percentage of drop leaves at the end of the period in the environment-controlled growth room.

Ethylene Evolution Pattern

Time course of ethylene evolution of fruitlets in situ was measured 0, 2, 5, 7 and 20 days after treatment as described in Yuan (2007). On each sampling date, five fruitlets per tree from non-tagged primary scaffold limbs were enclosed in a 100 mL round wide-mouth plastic jars. After closing the plastic lid, the containers with attached fruit inside were incubated for 2 h on the tree. Then, a 1-ml air sample was withdrawn for ethylene measurement from the sealed container through a rubber septum affixed to the lid. A gas chromatography 6890 Agilent (Agilent 154 Technologies, Wilmington, Germany) equipped with a flame ionization detector and a 155-alumina column was used for quantifying ethylene concentrations.

Stomatal Conductance and Chlorophyll Fluorescence

Four measurements per tree of G_s and chlorophyll fluorescence were made 2, 5 and 7 days after treatment on fully expanded leaves between 10:00 and 13:00 HR. Instantaneous determinations of leaf G_s were done using a steady-state leaf porometer (SC-1, Decagon Devices, WA, USA). Chlorophyll fluorescence measurements were carried after 20–30 min dark adaptation with a handheld portable fluorometer (FluorPen FP100, Photon Systems Instruments, Czech Republic) to provide an indication of the effects of treatments on the maximum potential quantum efficiency of PSII (Fv/Fm).

Statistical Analysis

The data were analysed using analysis of variance (ANOVA) and mixed model procedures in SAS software (SAS Institute Inc., Cary, NC). A two-way ANOVA was performed to test main effects of treatment (ETH, ACC, UTC) and temperature (10, 15, 20 °C), and their interaction, on the response variables fruitlet and leaf abscission. Differences between treatment means were assessed by Duncan's multiple range test at the 0.05 P level for these response variables. Two-way repeated measures ANOVA was performed on ethylene production, G_s and Fv/Fm data with the proc MIXED command specifying a compound symmetry covariance structure.

Results

Fruitlet and Leaf Abscission

Fruitlet Abscission

The temperature in the environment-controlled growth room affected fruitlet abscission in different ways depending on the treatment (Table 1). Increasing the temperature from 10 to 20 °C caused a significant increase of fruitlet abscission in ETH- and ACC-treated trees but not in untreated trees (Fig. 1A). No significant differences were observed between trees treated with ACC and ETH within a same environment-controlled growth room. However, they differed with UTC trees depending on the temperature in the chamber. ACC-induced fruitlet abscission was less temperature dependent and a significant fruitlet abscission respect

Table 1 *P* values from mixed model two-way ANOVA of fruitlet and leaf abscission in 'Sweet Lady' peach trees, treated with ethephon (ETH), 1-aminocyclopropanecarboxylic acid (ACC), and untreated control (UTC), and kept during 7 days in environment-controlled growth room at three different temperatures (10, 15 and 20 °C)

Source of variation	df	Pr > F	Temperature effect (Pr > F)		Treatment effect (Pr > F)	
Fruitlet abscission						
Treatment	2	0.0003	ETH	0.0277	10 °C	n. s
Temperature	2	0.0366	ACC	n. s	15 °C	0.001
Treatment × temperature	4	0.0462	UTC	n. s	20 °C	0.006
Leaf abscission						
Treatment	2	< .0001	ETH	0.0787	10 °C	0.0003
Temperature	2	0.0002	ACC	0.0024	15 °C	< .0001
Treatment × temperature	4	0.0032	UTC	n. s	20 °C	< .0001

n. s. non-significant differences

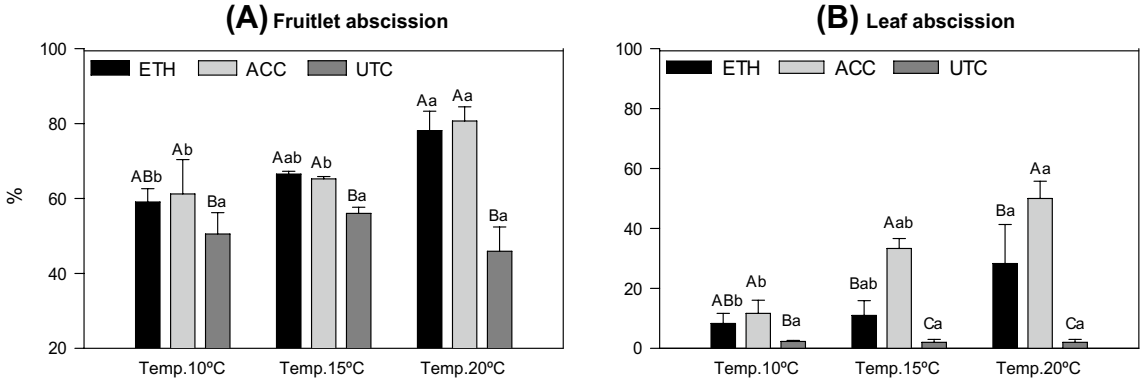


Fig. 1 Percentage of abscission of fruitlet **A** and leaves **B** in 'Sweet Lady' peach trees treated with ethephon (ETH), 1-aminocyclopropanecarboxylic acid (ACC) and untreated control (UTC), after 7 days in environment-controlled growth room at different temperatures (10, 15 and 20 °C) and 13 days outside the chamber at non-controlled conditions. Error bars represent the standard error. Different upper-case letters within a same temperature indicate significant difference between treatments according to Duncan's Multiple Range test ($P < 0.05$). Different lower-case letters for a same treatment indicate significant differences between temperatures according to Duncan's Multiple Range test ($P < 0.05$)

to UTC trees was observed at air temperatures of 10 °C or higher. On the other hand, ETH-treated trees had a significant abscission compared to UTC at air temperatures of 15 °C or higher (Fig. 1A). The greatest differences between treated and untreated trees, regardless the precursor applied, were observed at 20 °C (78–81% vs. 46% fruitlet abscission, respectively).

Leaf Abscission

The temperature in the environment-controlled growth room influenced leaf abscission in different ways, depending on the treatment (Table 1). There were significant differences between either ETH or ACC treatments and UTC, regardless of temperature in the environment-controlled growth room. The ETH- and ACC-induced leaf abscission increased with the increase of air temperature, while temperature did not have a significant effect on the untreated trees (Figs. 1B and 2). Unlike fruitlet abscission, the application of ACC caused a significantly greater leaf abscission than the ETH treatment, especially in the environment-controlled growth room at 15 °C (11% vs. 33%) and 20 °C (28% vs. 50%). No increase of leaf abscission was observed after removing the trees from the growth chamber and the trees recovered their normal appearance within the next few weeks.

Ethylene Evolution Pattern

The temperature in the environment-controlled growth room did not have a significant influence on fruit ethylene evolution. However, treatments and time of measurement (days after applications, DAA) had a significant effect on fruitlet ethylene evolution (Table 2). The endogenous ethylene production of untreated fruitlets was constant and without significant differences between dates during the period in the environment-controlled growth room (average of 0.75 μL kg^{-1} h^{-1}). However, the ETH and ACC treatments significantly increased the endogenous ethylene production of fruitlets compared to UTC, starting just after the application (Fig. 3). Both ETH- and ACC-treated fruitlets reached the ethylene peak 2 days after application. The peak in ACC-treated fruitlets was significantly greater than in ETH-treated fruitlets (15.4 vs. 8.3 μL kg^{-1} h^{-1}). Then, the endogenous ethylene production in the ACC-treated fruitlets rapidly decreased until reaching levels significantly lower than in the ETH-treated fruitlets but significantly higher than in the untreated fruitlets, while ETH-treated fruitlets showed a slower decrease from the peak (Fig. 3).

After 10 days at ambient temperature, ETH- and ACC-treated fruitlets showed a significant decrease of ethylene production compared to the days after the application within the environment-controlled growth room. However, ETH-treated fruitlets continued to produce higher ethylene levels than UTC (0.66 vs. 0.12 μL kg^{-1} h^{-1}), and even than ACC-treated fruitlets which obtained ethylene levels like UTC. On the other hand, there was no significant differences between the UTC and ACC treatments at the end of the experiment (Fig. 3).

Stomatal Conductance and Chlorophyll Fluorescence

Stomatal conductance was significantly affected by the treatments and days in the environment-controlled growth room but not by air temperature in the environment-controlled growth room (Table 3). Regardless of treatment, there was a significant increase of G_s from 2 to 7 days in the environment-controlled growth room (143–220 to



Fig. 2 View of 'Sweet Lady' peach trees treated with ethephon (ETH), 1-aminocyclopropanecarboxylic acid (ACC) and untreated control (UTC), after 7 days in environment-controlled growth room at different temperatures (10, 15 and 20 °C)

275–365 mmol m^{-2} s^{-1}). During this period, G_s in the ETH- and ACC-treated trees was significantly lower than in the UTC trees (Fig. 4). Besides, ACC-treated trees showed a significantly lower G_s than ETH-treated trees 5 days after their application (i.e. 5 days in the environment-controlled growth room), but there were no significant differences between both treatments at 2 and 7 days after the applications.

Table 2 *P* values from mixed model three-way ANOVA of ethylene production in 'Sweet Lady' peach fruitlets treated with ethephon (ETH), 1-aminocyclopropanecarboxylic acid (ACC) and untreated control (UTC), at 0, 2, 5 and 20 days after application (DAA) and in environment-controlled growth room at different temperatures (10, 15 and 20 °C)

Source of variation	df	Pr > F	Treatment effect (Pr > F)		DAA effect (Pr > F)	
Treatment	2	< .0001	ETH	< .0001	0	n. s
Temperature	2	n. s	ACC	< .0001	2	0.0002
Days after application (DAA)	3	< .0001	UTC	0.0027	5	< .0001
Treatment × temperature	4	n. s			7	< .0001
Treatment × DAA	6	< .0001			20	< .0001
Temperature × DAA	6	n. s				
Treatment × temperature × DAA	12	n. s				

n.s. non-significant differences

The temperature in the environment-controlled growth room significantly affected to the Fv/Fm ratio (Table 3). Fv/Fm values were significantly inferior at 10 and 20 °C in the environment-controlled growth room than those at 15 °C (0.812 vs. 0.825), regardless the treatment and the days in the environment-controlled growth room or after the applications (Fig. 4). On the other hand, the treatments, as well as the days after the applications, did not have a significant influence on Fv/Fm ratio. In all cases (treatments × temperature), the Fv/Fm values were higher than 0.80 which indicate plants were not stressed (Murchie and Lawson 2013).

Discussion

ETH and ACC treatments have been proposed as chemical thinners in peaches by different authors (Costa and Botton 2022; Theron et al. 2020); however, weather conditions during and just after the application can influence their efficacies (lack or over-thinning) and/or induce side effects such as defoliation or gummosis. It had been previously reported that fruit and leaf abscission response to ETH are temperature-dependant in cherries (Olien 1978), oranges (Yuan and Burns 2004), and apples (Jones and Koen 1985; Yuan 2007), but there was no study that focused on the ETH- and ACC-treated peach trees response to temperature.

According to our results, ETH-induced fruit abscission in peach trees had a significant response to increase the temperature from 10 to 20 °C. Yuan (2007), in an experiment under temperature-controlled conditions, found that the thinning effect of ETH applied on 'Golden Delicious' apples at 20 mm stage of fruit development was not affected by increasing day/night temperature from 21.1/10 to 32.2/21.1 °C. Jones and Koen (1985), using Cox's Orange Pippin × Democrat apples, reported that ETH applied at petal fall had no effect on fruit thinning when air temperature was equal or lower than 8.8 °C, whereas fruit thinning increased linearly with the increase in air temperature from 12 to 24.8 °C (day and night temperature was constant). These discrepancies between ETH studies could be attributed (i) to the difference in the stage of fruit development, which determines the sensitivity of fruitlets to ethylene, (ii) to the genetic differences in ethylene sensitivity in each study and (iii) to the nutritional and/or water status of plants (i. e. stress level). To our knowledge, there was no previous reported studies about the temperature dependence of ACC-induced abscission in higher plants. The effect of both products on fruit and leaf abscissions have been associated to capacity to trigger endogenous ethylene production in plants, hence a similar response to ETH would be expected (Theron et al. 2020). From our results, ACC-induced fruit abscission was not significantly temperature dependent when air temperature increased from 10 to 20 °C, unlike ETH-induced fruit abscission, and a significant effect respect to UTC trees could be observed under either at a temperature of 10, 15, or 20 °C. Hence, these results suggest that ACC treatments could be more effective than ETH to thinning fruitlets in less favourable weather conditions under temperatures inferior to 15 °C.

Nevertheless, we must warn that leaf abscission was significantly more pronounced in the ACC treatments, although an excessive leaf abscission response to ACC occurred only when the room temperature was 20 °C. In general, leaf abscission response to ETH and ACC application resulted linearly temperature dependent for both treatments, but the responses to ETH treatments were significantly less pronounced. Previous studies had reported that ETH can cause excessive leaf abscission in cherries (Bukovac et al. 1969; Olien and Bukovac, 1978) and citrus (Yuan and Bruns 2004) but only when temperature at the time of application is above 20 °C. In works under field conditions in 'Flatbeuty' peach, we did not observe significant ETH and ACC response to leaf abscission using doses similar to the ones in this study (Torres and Asín 2022; Torres et al. 2021). These differences may be due to the stress to which potted trees grown under constant light and temperature in an environment-controlled

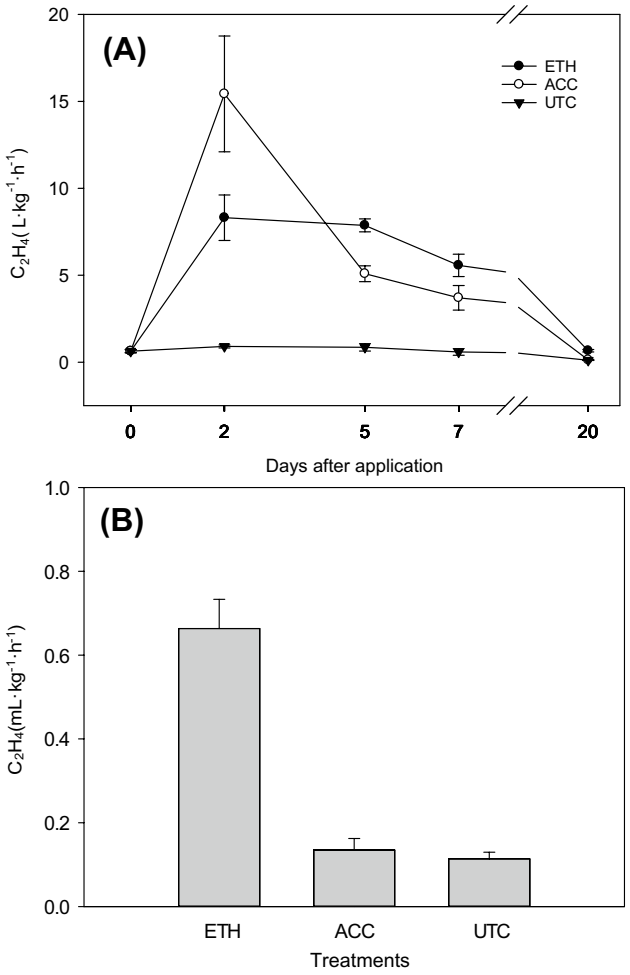


Fig. 3 **A** Dynamic of fruitlet ethylene production in response to the ethephon (ETH) and 1-aminocyclopropanecarboxylic acid (ACC) applications and of untreated control (UTC), in 'Sweet Lady' peach trees in environment-controlled growth rooms from 0 to 7 days after application, and at ambient conditions from 10 to 20 days after application. **B** Ethylene production after 10 days in environment-controlled growth rooms and 10 days at ambient conditions. Each value corresponds to the mean of three replications and three environment-controlled growth rooms (no significant differences between environment-controlled growth rooms at different temperatures: 10, 15 and 20 °C, see Table 2). Error bars indicate standard error ($n = 9$)

Table 3 *P* values from mixed model two-way repeated measures ANOVA of stomatal conductance (G_s) and the maximum quantum yield of photosystem II photochemistry (Fv/Fm) in 'Sweet Lady' peach trees, treated with ethephon (ETH), 1-aminocyclopropanecarboxylic acid (ACC) and untreated control (UTC), and kept during 7 days in environment-controlled growth room at three different temperatures (10, 15 and 20 °C)

Source of variation	df	G_s (Pr > F)	Fv/Fm (Pr > F)
Treatment	2	< .0001	n. s
Temperature	2	n. s	0.0002
Days after application (DAA)	2	< .0001	n. s
Treatment × temperature	4	n. s	n. s
Treatment × DAA	4	n. s	n. s
Temperature × DAA	4	n. s	n. s
Treatment × temperature × DAA	8	n. s	n. s

n.s. non-significant differences

growth room were subjected and/or to the genetic differences in ethylene sensitivity.

The differences between ETH and ACC in the fruit and leaf abscission were related to the differences in the peak of ethylene in fruitlets and G_s in leafs. We observed a relationship between the intensity of fruit abscission and the peak of ethylene in fruitlets after applications. The fruitlet ethylene production was not significantly temperature-dependent, unlike ETH-induced fruitlet abscission. Hence, the effect of ETH treatment on fruitlet abscission could be a combination among ethylene production capacity and other ETH-induced factors related to auxin signalling and carbohydrate deficiency, as other authors have previously suggested for other fruit crops (Goldental-Cohen et al. 2017; Hagemann et al. 2015; Xie et al. 2013). Hagemann et al. (2015) postulated that the ETH-induced abscission process in mangos begins with a reduction of the polar auxin transport capacity in the pedicel, followed by an upregulation of ethylene receptors, and finally a decrease of the sucrose concentration in the fruitlets. Goldental-Cohen et al. (2017) found that ETH treatment in olives enhanced pectinase activity and induction of several genes encoding for auxin and abscisic acid (ABA), as well as for ethylene and several cell wall degrading enzymes.

Our results suggest that alterations in G_s could also be a symptomatic cause associated with ETH- and/or ACC-induced abscissions in peach trees. Both ETH and ACC treatments maintained their G_s values significantly lower than those in the UTC, and these differences were not temperature-dependent unlike leaf abscission. Hence, the ETH- and ACC-induced ethylene could trigger the decrease of G_s. Different studies have investigated the role of ethylene on net photosynthesis and/or G_s in different crop species. According to the revision carried out by Ceusters and Van de Poel (2018), ethylene inhibited photosynthesis and/or decreased G_s in most of studied herbaceous crops such as *Arachis hypogaea* (Pallas Jr and Kays, 1982; Squier et al. 1985), *Nicotiana tabacum* (Squier et al. 1985), *Glycine max* (Gunderson and Taylor Jr, 1991; Squier et al. 1985; Taylor Jr and Gunderson, 1986, 1988), *Gossypium hirsutum* (Pettigrew et al. 1993; Taylor Jr and Gunderson, 1986), *Solanum tuberosum* (Dueck et al. 2003; Govindarajan and Poovaiah 1982; Taylor Jr and Gunderson, 1986), and *Phaseolus vulgaris* (Taylor Jr and Gunderson, 1986; Vitagliano and Hoad, 1978). According to our results, ETH- and ACC-induced ethylene also decreased G_s in peach trees. Nevertheless, G_s significantly increased 2 to 7 days after the application, even

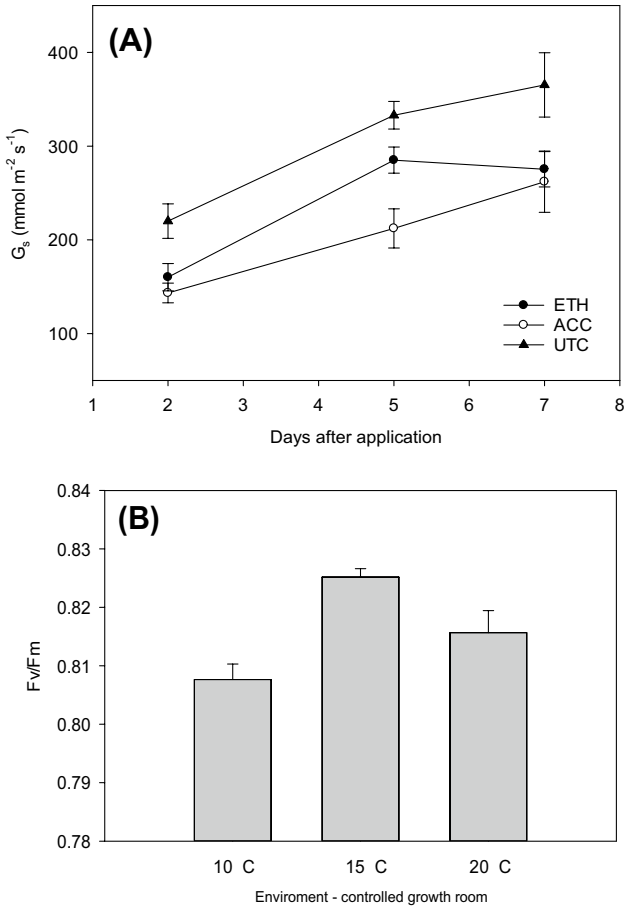


Fig. 4 **A** Dynamic of stomatal conductance (G_s) and **B** the maximum quantum yield of photosystem II photochemistry (Fv/Fm) in peach leaves in response to ethephon (ETH) and 1-aminocyclopropanecarboxylic acid (ACC), and of the untreated control (UTC), in 'Sweet Lady' peach trees in environment-controlled growth rooms (from 2 to 7 days). Each G_s value corresponds to the mean of three replications and three environment-controlled growth rooms (no significant differences between environment-controlled growth rooms at different temperatures: 10, 15 and 20 °C, see Table 3). Each Fv/Fm value corresponds to the mean of three replications, three treatments (no significant differences between treatments ETH, ACC and UTC, see Table 3) and three times (no significant differences between times, see Table 3). Error bars indicate standard error ($n_{Gs}=9$; $n_{Fv/Fm}=27$)

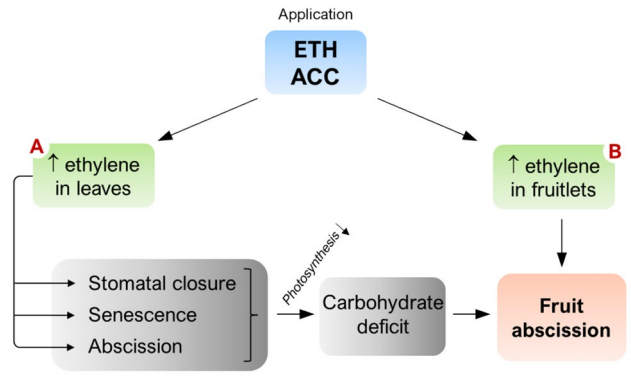


Fig. 5 Schematic representation of the two proposed fruitlet abscission pathways **A** and **B** that occur in peach trees sprayed with ethephon (ETH) or 1-aminocyclopropanecarboxylic acid (ACC)

in the untreated trees and regardless of air temperature, possibly due to an acclimation effect. During this period, G_s in ETH- and ACC-treated trees was significantly lower than in untreated trees, especially in the ACC-treated trees which showed a significantly lower G_s than ETH-treated trees after 5 days, which could be related to the differences in the leaf abscission between both treatments.

Photosynthesis inhibition and/or stomatal closure may lead to carbohydrate deficit, which also promotes fruit abscission (Cheng et al. 2009; Gonzalez et al. 2020; Li et al. 2001). Hence, ETH- and ACC-induced leaf stress is suggested to be another symptomatic cause of fruitlet abscission (Fig. 5). Stomatal conductance may play a key role in regulation of photosynthesis by limiting the photochemical efficiency of the PSII reaction centre under high crop load (Cheng et al. 2009); however, we did not observed significant differences between treatments (ETH, ACC and UTC) for the maximum potential quantum efficiency of PSII (Fv/Fm). A decline in Fv/Fm indicates a decline in the quantum yield of PSII photochemistry and disturbances or damage in the photosynthetic apparatus. Unlike G_s, Fv/Fm was affected by temperature but not by treatments or days in the environment-controlled growth room. Specifically, Fv/Fm at 10 and 20 °C were significantly inferior to those at 15 °C. These differences between environment-controlled growth rooms could be related to changes in leaf temperature. Increased leaf temperature following a reduction in stomatal aperture has been proposed as a critical factor in regulating photosynthesis in fruit trees and reduction in Fv/Fm values (Jifon and Syvertsen 2003; Li and Li 2005). However, we must note that the Fv/Fm values were higher than 0.80 in all cases, being the most frequently values of Fv/Fm in non-stressed plants around 0.74–0.85 (Lichtenthaler et al. 2005).

In conclusion, an increase of temperature from 10 to 20 °C increased the ETH- and ACC-induced leaf abscission and the ETH-induced fruit abscission. The quantum yield of PSII photochemistry was also affected by temperature, being higher at 15 °C than at 10 and 20 °C. On the other hand, changes in the temperature did not have a significant feedback effect on the ACC-induced fruitlet abscission, ETH- and ACC-induced ethylene production in fruitlets and G_s in leaves. These changes in ethylene peak in fruitlets and G_s in leaves were related to the ETH- and ACC-induced abscission response. Hence, ETH- and ACC-induced abscission process could be associated with an effect on stomatal closure, besides ethylene production. Future experiments should focus on the mechanisms of alteration of stomatal movement triggered by ETH and ACC for understanding their

effects on fruit and leaf abscission. Also, the possibility that ETH and ACC may down-regulate photosynthesis in peach leaves and activate/deactivate some of the key enzymes and hormones, such as auxin and ABA, should be studied in the future in order to improve knowledge and understanding of action mode of these plant growth regulators as chemical thinners.

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Declarations

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

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