Thinning flat peaches with ethephon and its effect on endogenous ethylene production and fruit quality

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ABSTRACT

Peach orchards are usually hand-thinned at around 40–60 days after bloom, but this practice is labor-intensive and costly. Ethylene plays a key role in peach fruitlet abscission and foliar applications of ethephon have been reported to be effective in some cultivars to induce fruit abscission. However, results are inconsistent and there are no experiences about its application in flat peaches and/or about inducing flowers abscission. Ethephon (from 0 to 300 mg L^{-1}) was applied to 'Flatbeauti' peach trees at 30 % and 100 % of full bloom and 30, 40 and 50 DAFB to determine the best time to induce flowers or fruitlet abscission, its effect on fruit quality parameters and its relationship to the ethylene evolution pattern throughout peach fruit growth. Abscission and ethylene production were related to ethephon concentration. In general, as mean of three experiments, there was an 8–9 % reduction in fruit set, a 3–14 % increase in fruit size, and a 10–16 % reduction in yield, with each incremental increase of 75 mg L^{-1} ethephon. The late ethephon applications increased ethylene endogenous production up to harvest and this influenced fruit maturity. Finally, our results indicate that ethephon in the range of 150 mg L^{-1} can be used at 100 % full bloom and at 30–40 DAFB to induce adequate levels of fruit crop load in 'Flatbeauti' peaches without other side effects.

Keywords

Prunus persica; abscission; crop load; fruit maturation

Highlights

- Abscission and ethylene production were related to ethephon concentration
- Ethephon at 150 mg L^{-1} was commercially acceptable to induce flower and fruit abscission
- The best time range to spray was from full bloom up to 40 days after full bloom
- Late ethephon applications after 40 days after full bloom can advance fruit maturity

1. INTRODUCTION

The objective of flower or fruit thinning in fruit trees is to reduce fruit number per plant, promoting sink:source balance and reducing competition among fruit, which results in bigger fruit and the improvement of other fruit-quality parameters. In peach orchards, thinning is indispensable and usually performed by hand. However, hand thinning is an expensive, labour-intensive practice, and the skilled workforce needed to perform this operation is increasingly difficult to find (Assirelli et al., 2018; Lordan et al., 2018). Therefore, a key global challenge in peach research is to find alternative methods for regulating crop load to commercially acceptable levels to mitigate this labour (McArtney et al., 2012). An option could be chemical thinning. Although chemical thinning with plant growth regulators is an established practice in other fruit crops such as apples and pears, there are few products with hormonal action which promote abscission of flowers or fruits available to be recommended for peach cultivars. Ethephon (2-chloroethylphosphate acid) is one of the few plant growth regulators with suitable results for chemical thinning in several peach cultivars (Greene and Costa, 2013). Ethephon 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). Exogenously applied ethephon stimulates ethylene production and triggers ethylene-dependent reactions such as flower or fruit abscission (Wertheim, 2000).

Flower or fruitlet abscission as a self-regulatory-mechanism begins with the activation of specific abscission zone (Roberts et al., 2002). In this point, ethylene enhances abscission, whereas auxins (especially indole-3-acetic acid produced by seeds) reduce the sensitivity of the abscission zone to ethylene and, consequently, prevent abscission (van Doorn and Stead, 1997). Ruperti et al. (1998) demonstrated that peach fruitlet abscission is related to endogenous ethylene evolution by comparing ethylene

evolution and fruit drop in two fruit populations (with low abscission *vs*. with high abscission potential) from the same cultivar. Similar results were observed in apple by Cin et al. (2005). They both observed that the young fruit abscission is preceded by an ethylene peak which may indicate that fruitlets need a phase to gain ethylene sensitivity at the abscission zone level before being shed from the tree.

One of the primary concerns over the use of ethephon when used as a chemical thinner in fruit trees is its inconsistency (Bound, 2015). Another possible inconvenience of ethephon applications is that it can influence other developmental process such as leaf senescence, fruit ripening and/or the formation of gummosis in the genus *Prunus* L. (Saniewski et al., 2006). Some factors such as cultivar, weather conditions, or application time have been reported to affect the efficacy of ethephon in fruit thinning and/or the appearance of side effects. (Costa and Vizzotto, 2000; Drogoudi et al., 2009; Jiménez and Díaz, 2002; Meland and Kaiser, 2016; Webster and Spencer, 2000). All these aspects could be related with endogenous ethylene biosynthesis. The ethylene production in climacteric flowers and fruit is autocatalytic, which means that exposure to ethylene stimulates ethylene biosynthesis. Therefore, ethephon applications on flowers or fruitlets with higher endogenous ethylene production can result in an increase in ethephon-enhanced ethylene biosynthesis.

Although ethephon has previously been tested on peach for fruit thinning efficacy, more studies are needed to assess the its effectiveness and possible side effect. The specie *Prunus persica* (L. Batsch) encompasses a large number of economically important peach cultivars such as round peaches and flat peaches. In most of studies published up to date, ethephon had been tested on round peach cultivars and there is no experience on flat peach cultivars. Moreover, unlike in other crops, most previous ethephon research in peach trees was focused after bloom, when fruitlets were 15–20

84 mm in diameter, and few studies have been published to determine the effectiveness to
85 induce flower abscission.
86 Taking everything above into consideration, the objectives of this paper were to
87 study the efficacy of ethephon on fruit thinning in flat peach trees at different rates and
88 phenological stages, its effect on fruit quality parameters and other side effects, and its
89 relationship to the ethylene evolution pattern throughout peach fruit growth.
90
91 **2. MATERIAL AND METHODS**
92 **2.1. Plant material and location**
93 Three experiments in different seasons (2015, 2016 and 2017) were conducted at
94 the experimental orchard of *Institut de Recerca Tecnológica i Agroalimentaria* (IRTA)
95 in Gimenells, NE Spain (41° 39' 20.50" N latitude, 0° 23' 22.33" E longitude). The
96 experiments were conducted on mature 'Flatbeauti' peach trees (*Prunus persica* L. var.
97 *platycarpa*) on GF-677 rootstock. Six-year-old trees were carefully selected for
98 uniformity in tree size and flower intensity. The trees were spaced at 5 × 3 m (667 trees
99 ha^{-1}) and trained to a vase system. In 2015 (experiment 1), four single tree replicates for
100 each treatment were arranged in completely randomized blocks. In 2016 (experiment 2)
101 and 2017 (experiment 3), eight single tree replicates per treatment were arranged in
102 completely randomized blocks. Four trees were used for destructive flower or fruit
103 sampling (ethylene measurement), and the remaining four trees were assessed for
104 thinning efficacy, fruit yield and quality parameters. The experimental units were
105 separated at least by one guard tree in order to minimize spray drift.
106 **2.2. Treatments**
107 Experiment 1: three different rates (75, 150, 300 mg L^{-1}) of ethephon (2-
108 chloroethyl-phosphonic acid, Ethrel, Bayer CropScience Inc) were tested at five

different stages, specifically at 30 % full bloom (FB), 100 % FB, 30 days after full bloom (DAFB), 40 DAFB and 50 DAFB. Ethephon treatments were compared to a hand-thinning treatment and an untreated control (UTC). Experiment 2: after considering the results of the previous experiment, the three ethephon rates were tested at two stages, close to 100 % FB and 40 DAFB. Experiment 3: the three ethephon rates were tested again at 100 % FB and 40 DAFB and, in addition, two treatments of ethephon 150 mg L^{-1} at 30 % FB and 50 DAFB, respectively, were added to validate the results obtained in experiment 1.

All ethephon treatments were sprayed very early in the morning, when air temperatures were below 25 °C. A high-pressure handgun sprayer (25 atm) was used at a rate of ~1000 L ha^{-1}. Hand-thinning was carried out at 45–55 DAFB by spacing fruit ~15–20 cm apart. The application time for each treatment and year and the evolution of temperatures is presented in Figure 1.

2.3. Fruit set

Fruit set was determined by tagging two primary scaffold limbs on each tree and counting the number of flowers before treatment application and the number of peaches after physiological fruit drop. Fruit set percentage was calculated as number of remaining fruit per number of flowers.

2.4. Fruit yield parameters

Every season, all fruit were separately harvested from each tree with a single pick at commercial harvest. Fruit weight, diameter and colour and total fruit yield (kg and number of fruit per tree), were recorded by automatic fruit sorting equipment (Maf Roda Agrobotic, Cedismafrut, Lleida, Spain). Trunk circumference was measured 30 cm above the ground at harvest each year, trunk cross-sectional area (TCSA; cm^2) and fruit crop load were calculated per tree as number of fruit per TCSA (fruit cm^{-2}).

134 **2.5. Fruit quality and maturity parameters**

135 At harvest, thirty randomly selected peaches per tree free of defect were selected
136 for fruit quality determination. The parameters measured were fruit firmness, total
137 soluble solid concentration (TSS) and titratable acidity. Fruit flesh firmness was
138 measured at two opposite sides on the fruit equator using a digital firmness tester
139 (Penefel®; Ctifl, France) with an 8 mm (diameter) tip. TSS (°brix) and titratable acidity
140 (malic acid g L^{-1}) were determined using the freshly prepared juice of the whole
141 subsample. TSS was measured using a digital temperature compensated refractometer
142 (model PR–101, Atago Co. Tokyo Japan), and titratable acidity (expressed as malic
143 acid) was determined by titrating 10 mL of juice with 1.0 M NaOH to pH 8.2.

144 **2.6. Other side effects**

145 The presence or absence of gummosis on the tree trunk and branches and of other
146 effects of ethephon, such as leaf abscission, were noted for each tree one week and one
147 month after each application and again at harvest. Changes in leaf size was rated using a
148 linear 5-point scale (1 = the smallest size and 5 = the biggest size).

149 **2.7. Ethylene evolution pattern**

150 Ethylene evolution in 'Flatbeauti' peach was measured during fruit development in
151 experiments 2 and 3. Whole-flower and -fruit ethylene production of each experimental
152 plot was determined by enclosing flowers and fruitlets in jars (0.1 to 0.5 L) sealed with
153 a rubber cap and kept in the light at 25 °C. After 2 h, a 1-ml air sample was withdrawn
154 from each jar for ethylene measurement. A gas chromatography 6890 Agilent (Agilent
155 Technologies, Wilmington, Germany) equipped with a flame ionization detector and an
156 alumina column was used for quantifying ethylene concentrations.

157 **2.8. Statistical analysis**

158 The experimental design for each experiment was a randomized complete block.

159 Statistical analyses were performed in SAS 9.2 (SAS Institute Inc., 2009). A two-way
160 ANOVA was performed for each experiment with the GLM procedure to test main
161 effects of treatments on the parameters analyzed. Duncan's multiple range tests were
162 used for the mean separation of significant effects if pre-harvest treatment effect from
163 ANOVA models were significant ($P < 0.05$).
164
165 **3. RESULTS**
166 **3.1. Experiment 1**
167 *3.1.1. Fruit set and fruit yield parameters*
168 Fruit set was significantly reduced through ethephon treatments at 150 and 300 mg
169 L^{-1} applied from 30 % FB to 40 DAFB (Figure 2). The ethephon treatments at 75 mg L^{-1}
170 only reduced fruit set when applied at 30% FB and 30 DAFB, but not at the later
171 application dates. In general, the effect at bloom period was higher than after full
172 bloom, with fruit set reductions, compared to UTC, of 21–35 % at 30 % FB, 17–31 % at
173 100 % FB, 15–29 % at 30 DAFB and 13–31 % at 40 DAFB. No significant effect on
174 fruit set was observed at 50 DAFB, irrespective of the rates.
175 Generally, a trend of decreasing fruit set with increasing rate was observed except
176 at 50 DAFB. Increasing concentrations of ethephon from 150 to 300 mg L^{-1} was
177 associated with a decrease of fruit set from 11 % to 27 % less than UTC, respectively.
178 On the other hand, ethephon 75 mg L^{-1} was not enough to reduce the fruit set
179 significantly with respect to UTC, except when it was applied at 30 % FB and 30 DAFB
180 (around 15 % less than UTC in both cases).
181 All ethephon treatments and the hand-thinning treatment yielded significantly fewer
182 fruit per tree and per cm^2 of TCSA than UTC (Figure 2). Ethephon at 300 mg L^{-1} at 30
183 % FB and 100 % FB resulted in a crop load significantly lower than the hand-thinning

184 treatment (0.7–1.1 vs. 1.9 fruit cm^{-2}) whereas at 75 mg L^{-1} at 40 DAFB resulted in a
185 value significantly higher (3.0 fruit cm^{-2}). The fruit crop loads of the rest of ethephon
186 treatments were comparable to that obtained with the hand-thinning treatment.

187 Higher rates of ethephon resulted in significant reductions in yield compared tothe
188 UTC (Figure 2). On the other hand, no significant differences were observed between
189 most of ethephon treatments (except 300 mg L^{-1} applied at 30 % FB) and the hand-
190 thinning treatment. In general, all evaluated rates had a greater influence on fruit yield
191 when they were applied earlier. Ethephon 75 mg L^{-1} significantly decreased the fruit
192 yield only when it was applied at 30 % FB or 30 DAFB (29–31 kg $tree^{-1}$) and no
193 significant differences were observed at the other application times. Ethephon 150 mg
194 L^{-1} yielded fewer fruit than UTC in most cases (24–34 kg $tree^{-1}$), except the latest
195 application at 50 DAFB. Ethephon 300 mg L^{-1} resulted in a fruit yield significantly
196 lower than UTC in all cases (10–33 kg $tree^{-1}$), with a greater effect for earlier
197 applications.

198 *3.1.2. Fruit size, colour, and quality parameters*

199 Increasing the ethephon rate generally meant a higher fruit thinning effect and,
200 consequently, trees with bigger fruit (Figure 3). Most treatments provided a significant
201 increase in fruit weight compared to UTC (99–119 *vs.* 85 g). The rate of 75 mg L^{-1}, at
202 30 % FB or 100 % FB (93–94 g), and 150 mg L^{-1} at 100 % FB (96 g), recorded a non-
203 significant increase of fruit weight. The rest of ethephon treatments, even applied at 50
204 DAFB, showed a significant increase fruit size.

205 In general, fruit from trees with lower crop load were associated with increasing
206 red colour on the peel. But in this study, fruit colour seemed to be also related the
207 ethephon application. Most of the treatments applied between 30 and 50 DAFB
208 recorded a percentage of red-coloured surface significantly higher than the rest of

209 treatments (70–80 % vs. 56–63 %) (Figure 3). On the other hand, ethephon applied at
210 bloom period did not display significant differences compared to the UTC for
211 percentage of red-coloured surface.

212 The fruit firmness did not significantly differ between the earlier treatments at 30 %
213 FB to 30 DAFB and UTC (Figure 3). But later applications from 40 to 50 DAFB
214 decreased fruit firmness with values significantly lower than UTC or hand-thinning
215 treatments (2.9–3.7 *vs.* 4.7–4.8 kg). In general, the fruit firmness decrease was greater at
216 higher rates and later applications. For TSS in fruit, there were significant differences
217 between treatments. In general, the sugar concentration increased as crop load
218 decreased. Ethephon 300 mg L^{-1} at 30 % FB recorded the highest value (13.6 °brix)
219 with significant differences compared to UTC and most of ethephon treatments applied
220 from 30 DAFB (11.9–12.5 °brix) (Figure 3). No significant differences were observed
221 between treatments in terms of fruit acidity, with values between 2.6 and 3.8 g L^{-1} malic
222 acid (Figure 3).

223 *3.1.3. Return bloom*

224 Ethephon treatments had no effect on return bloom. The date of full bloom in 2016
225 season was on March 7th, around 14 days before a standard season. No variation in the
226 flowering date due to the treatments was observed (data not shown).

227 *3.1.4. Effect on leaf defoliation and other phytotoxicities*

228 Ethephon application did not result in gummosis either on the trunk or on the main
229 scaffold branches, even at 300 mg L^{-1}. Neither was leaf abscission observed, another
230 potential side effect of foliar ethephon sprays. Nevertheless, a month after first
231 application (on April 23rd, 2015), the treatments applied at 30 or 100 % FB showed a
232 reduction in leaf area due to a decrease of leaf size, especially on the oldest leaves
233 (Table 2). We observed an increase of severity at higher concentrations of ethephon

(Figure 4). In addition, there was a tendency to increase this effect when the application was carried out at 30 % FB in comparison to at 100 % FB. At 75 mg L^{-1}, the effect was non-significant and only a few replicates showed this symptom. At 150 mg L^{-1}, the effect was significant only when applied at 30 % FB. At 300 mg L^{-1}, the effect was significant compared to UTC in both bloom application times, although the effect at 30 % FB was significantly higher than at 100 % FB. No treatment applied after FB presented symptoms, and affected trees were however able to recover approximately three weeks after (mid-May).

3.2. Experiment 2

3.2.1. Fruit set and fruit yield parameters

Spring climatic conditions in 2016 decreased fruit set levels in the whole trial (21 % lower fruit set in 2016 than in 2015) and, consequently, fruit crop load was also inferior with respect to 2015. Because of this, hand-thinning treatment was applied only to improve fruit distribution, but not to reduce fruit crop load. Ethephon thinning level was also less. We observed a significant decrease of the fruit set in comparison to UTC applying ethephon at 150 mg L^{-1} and 300 mg L^{-1}, independently of the time of application, and no significant effect on fruit set was observed at 75 mg L^{-1} (Figure 5). In general, the early treatments at 100 % FB had a greater effect on fruit set than the late treatments at 40 DAFB (11 % less at 100 % FB and 7 % less at 40 DAFB).

No significant differences were observed between the fruit crop load values of ethephon 75 mg L^{-1} and UTC (2.7 fruit cm^{-2}). However, the treatments at 150 and 300 mg L^{-1}resulted a significant decrease in crop load compared to the UTC. We observed a non-significant trend to reduce the fruit crop load by increasing the rate from 150 to 300 mg L^{-1} within the same application time. Within these rates, fruit crop load was significantly lower when ethephon was applied at 100 % FB than at 40 DAFB, with

259 significant differences in comparison to the hand-thinning treatment for both rates (0.4–
260 0.8 *vs.* 1.9 fruit cm^{-2}).

261 A greater reduction of yield was observed for the early treatments at 100 % FB than
262 at 40 DAFB and with increasing rate. The differences between applying at 100 % FB
263 and 40 DAFB were significant at 150 and 300 mg L^{-1}, but not at 75 mg L^{-1} (Figure 5).
264 Ethephon 75 mg L^{-1}, independently of the application time, and ethephon 150 mg L^{-1} at
265 40 DBH did not differ significantly compared to the UTC or hand-thinning treatment
266 (19–23 kg $tree^{-1}$). On the other hand, ethephon 150 mg L^{-1} at 100 % FB and ethephon
267 300 mg L^{-1} (independently of the application time) resulted in significant reductions in
268 yield compared to UTC and the hand-thinning treatment (6–15 kg $tree^{-1}$).

269 *3.2.2. Fruit size, colour, and quality parameters*

270 Ethephon treatments, as well as the hand-thinning treatment, had a no effect on fruit
271 weight (Figure 6). Note that the crop load level in UTC was similar to the crop load in
272 the hand-thinning treatment. Ethephon treatments showed a tendency to recorded higher
273 values in comparison with the UTC and hand-thinning treatments (81–79 g vs. 77–78
274 g). The differences between treatments for the fruit colour were also non-significant
275 (Figure 6). However, the later applications (after full bloom) and the higher rates (150
276 and 300 mg L^{-1}) resulted in a tendency to increase the red-coloured fruit surface in
277 comparison with the rest of treatments (56–57 % vs. 45–48 %). Fruit firmness and TSS
278 followed the same tendency observed in experiment 1, suggesting a decrease of fruit
279 firmness (from 6.5 to 6.0 kg cm^{-2}) and TSS (from 11.8 to 11.0 °brix) when ethephon
280 was applied later, but without significant differences between treatments (Figure 6). No
281 significant differences between treatments were observed for acidity (4.8–5.3 g L^{-1}
282 malic acid).

283 *3.2.3. Return bloom*

Ethephon treatments had no effect on return bloom (data not shown). The date of full bloom in 2017 season was on March 13th. This date can be considered normal for this cultivar and region. No variation in the flowering date due to the treatments was observed.

3.2.4. Effect on leaf defoliation or other phytotoxicities

No effect on leaf defoliation or other phytotoxicity symptoms were observed in this experiment.

3.2.5. Ethylene synthesis

All ethephon treatments resulted in a dose-related increase in the ethylene production (Figure 7). UTC recorded the highest ethylene at 100 % FB (9.0 uL C_2H_4 kg^{-1} h^{-1}) and then it showed a slightly decrease of ethylene production which kept stable up to 25 DAFB (0.2–0.1 uL C_2H_4 kg^{-1} h^{-1}). The ethylene production in bloom-treated trees increased rapidly and reached the peak 2 days after full bloom, with approximately 7.5, 10 and 20 times more ethylene than UTC for the treatments at 75, 150 and 300 mg L^{-1}, respectively. Thereafter, ethylene production decreased in all ethephon treatments but keeping significantly higher levels than UTC up to 25 DAFB when no significant differences between treatments were observed.

All ethephon treatments applied 40 DAFB significantly increased the rate of ethylene throughout whole period under consideration (from 40 to 62 DAFB). The peak level was reached between 3 and 7 days after treatment, when fruit treated with ethephon at 75, 150 and 300 mg L^{-1} produced, approximately, 10, 20 and 30 times more ethylene, respectively, than non-treated fruit. After that point, ethylene production decreased in all ethephon treatments but keeping approximately 2.5 times more ethylene compared to UTC up to the end of the considered period (i.e. 21 days after the

application or 62 DAFB). No significant differences among the ethephon treatments were observed atthis point.

3.3. Experiment 3

3.3.1. Fruit set and fruit yield parameters

Fruit set was significantly reduced in comparison to UTC with most ethephon treatments (42–45 % *vs.* 63 %), except ethephon 75 mg L^{-1} at 40 DAFB and 150 mg L^{-1} at 30 % FB (Figure 8). In general, the effect of rate was stronger than the time of the application and no significant differences were found between applying at 100 % FB or 40 DAFB for a same rate. Increasing the ethephon rate from 75 to 300 mg L^{-1} resulted in a decrease of fruit set from 49.5 to 35.5 % at 100 % FB and from 58.6 to 30.6 % at 40 DAFB.

Regarding crop load, no significant differences were found between UTC and ethephon 75 mg L^{-1}, independently of the timing, and ethephon 150 mg L^{-1} at 30 % FB and 50 DAFB (7.5–6.6 fruit cm^{-2}) (Figure 8). Conversely, all ethephon treatments at 150 mg L^{-1} and ethephon 300 mg L^{-1} applied at 100 % FB and 40 DAFB, resulted in a crop load significantly lower than UTC (4.1–5.8 fruit cm^{-2}). The crop load achieved by ethephon 150 and 300 mg L^{-1}, at 100 % FB and 40 DAFB, were comparable to that obtained by the hand thinning treatment. In terms of fruit yield, only the hand-thinning treatment and ethephon 300 mg L^{-1} applied 40 DAFB resulted in significant reductions in comparison with the UTC (52–53 *vs.* 74 kg $tree^{-1}$).

3.3.2. Fruit size, colour, and quality parameters

Significant differences were found in fruit weight and colour between UTC and the late treatments after FB with 150 mg L^{-1}, as well as the hand-thinning treatment (68 g *vs*. 84–94 g and 34 % *vs*. 48–58 %) (Figure 9). The late ethephon treatments resulted in a non-significant reduction the fruit firmness in comparison to UTC or hand-thinning

333 treatments (5.0–5.6 *vs*. 5.9–6.2 kg). No significant differences were observed for the
334 TSS (9.4–10.0 °brix) or acidity (5.0–5.5 g L^{-1} malic acid) in fruit.

335 *3.3.3. Return bloom*

336 No effect on return bloom was observed due to the ethephon treatments. The date
337 of full bloom in 2018 season was also on March 13th. No variation in the flowering date
338 due to the treatments was also observed.

339 *3.3.4. Effect on leaf defoliation or other phytotoxicities*

340 No effect on leaf defoliation, gummosis or other phytotoxicity symptoms were
341 observed in this experiment.

342 *3.3.5. Ethylene synthesis*

343 After taking into consideration the results obtained in the experiment 2, we decided
344 to measure the ethylene evolution throughout whole fruit growth period (Figure 10).
345 Note that the rate of ethylene production in non-treated flowers of experiment 3 was
346 approximately 8 to 2 times less than in experiment 2.

347 The time of the ethylene peak varied depending on the treatment. The UTC reached
348 the ethylene peak 8 days after full bloom (0.2 uL C_2H_4 kg^{-1} h^{-1}). For the rates of 300
349 and 150 mg L^{-1} it was 2 days after application (i.e. 2 days after full bloom), when 22.5
350 and 15.8 times more ethylene was recorded, respectively, than UTC. For the rate 75 mg
351 L^{-1} was 4 days after application, when 7.3 times more ethylene was recorded than the
352 UTC. The ethephon-induced increase of ethylene production lasted up to 40–60 DAFB
353 for all ethephon rate and up to 60–100 DAFB for the highest rate of 300 mg L^{-1}.

354 When the trees were treated at 40 DAFB, only the 150 and 300 mg L^{-1} significantly
355 increased the rate of ethylene production. No significant difference was observed
356 between ethephon 75 mg L^{-1} and UTC (0.01 uL C_2H_4 kg^{-1} h^{-1}). The peak level for the
357 rates of 150 and 300 mg L^{-1} was reached 2 days after treatment, when they produced,

358 approximately, 13.3 and 30.6 times more ethylene, respectively, than the UTC. After
359 that point, their ethylene production decreased rapidly, however, they produced 23–1.5
360 (ethephon 150 mg L^{-1}) and 45–4 (ethephon 300 mg L^{-1}) times more ethylene than UTC
361 until harvest. At harvest, the differences with respect to UTC were 4 times more
362 ethylene for the rate of 150 mg L^{-1}, and of 8 times more for the rate of 300 mg L^{-1}.
363 We compared the values of endogenous ethylene production at the time of
364 application and the response to ethephon-enhanced ethylene biosynthesis afterward,
365 between the ethephon 150 mg L^{-1} treatments applied within a similar phenological
366 range (30 % FB *vs.* 100 % FB and 40 DAFB *vs.* 50 DAFB) (Figure 11). The
367 endogenous ethylene production at 100 % FB and 40 DAFB were around 3–4 times
368 more than at 30 % FB and 50 DAFB, respectively. Similarly, after the applications, the
369 flowers or fruitlets treated at 100 % FB and 40 DAFB produced more ethylene than
370 when they were treated at 30 % FB and 50 DAFB. It is worth pointing out that, unlike
371 experiment 1, the thinning effect at 30 % FB was inferior to at 100 % FB, although
372 without significant differences.
373
374 **4. DISCUSSION**
375 Chemical thinning with ethephon can help to improve the crop value in 'Flatbeauti'
376 peach cultivar. According to our results, a concentration of 150 mg L^{-1} can be
377 considered commercially acceptable without sacrificing excessive marketable yield. A
378 direct consequence of thinning is an increase in fruit size and weight, but also a decrease
379 in number of peaches per tree and, consequently, of total yield, as was observed in most
380 of the ethephon treatments at 300 mg L^{-1}. In general, with each incremental increase of
381 75 mg L^{-1} ethephon (from 0 to 300 mg L^{-1}), there was an 8–9 % reduction in fruit set, a
382 3–14 % increase in fruit size, and a 10–16 % reduction in yield over the three years of

383 the study. Similar results were found in Canada where the effective rate for the peach
384 cultivar ‘Redhaven’ corresponded to ethephon concentrations between 100–200 mg L^{-1}
385 (Taheri et al., 2012). Nevertheless, other effective rates have been proposed by other
386 authors for other cultivars. On the one hand, in Australia, lower rate of ethephon at 40–
387 100 mg L^{-1} were enough for successfully thin the peach cultivars ‘Golden Queen’,
388 ‘Wight’ and ‘Keimos’ (Gathercole, 1981). On the other hand, in India, higher rates of
389 ethephon at 200–300 mg L^{-1} were necessary to produce results comparable to hand-
390 thinned in ‘Redhaven’ and ‘July Elberta’ peaches (Sharma and Gautam, 1981; Sharma
391 et al., 2003).

392 Based on our findings, the best range of application time would be from bloom up
393 to 40 DAFB. In two of the three experiments, the applications at bloom trended to
394 increase the thinning efficacy. No significant differences were observed between
395 application at 30 % FB and 100 % FB within a same rate, whereas we observed a lack
396 of efficacy when ethephon was applied at 50 DAFB. Most research about ethephon-
397 induced abscission in peach trees was focused at 30–40 DAFB, when peaches were 15–
398 20 mm in diameter, and few studies tried to induce flower abscission during bloom
399 period. With respect to other species, our results are in accordance with research on
400 apples in New Zeeland (Koen and Jones, 1985), but they are contrary to those obtained
401 with plums or apricots in Nordic climate (Meland, 2007; Meland and Kaiser, 2016;
402 Webster and Spencer, 2000). These differences could be due to climatic conditions.
403 Based on past research (Knight, 1982; Meland and Kaiser, 2016), temperatures above
404 15–20 °C would be necessary to obtain a significant response, which should be more
405 easily achieved in southern than in northern Europe.

406 Bloom thinning has the distinct advantage over fruit thinning in that it can be done
407 early during fruit development and, consequently, allows competition between

developing fruitlets to be reduced at the earliest opportunity. We found that early ethephon treatments at bloom increased sugar levels in fruit in experiment 1. But this influence was more likely a reduced crop load effect than a direct effect of time of applications since the relationship between yield and sugar content in peach fruit is generally negative (Cirilli et al., 2016). Fruit firmness and colour were also influenced by the time of the applications. The fruit firmness and percentage red-coloured fruit surface decreased and increased, respectively, when ethephon $\geq$ 150 mg L^{-1} was applied later in the season (40 and 50 DAFB). Taheri et al. (2012) observed a similar response but they though this influence on fruit maturity was more likely a reduced crop load effect than a direct effect of ethephon, because they contemplated a half-life of ethephon of 48 h. Nevertheless, they could not compare their results with hand thinning or earlier applications. We observed that a reduced crop load effect due to either early-season ethephon applications or hand thinning showed levels at harvest like UTC, unlike those observed with the late-season ethephon treatments. This effect of the late treatments on fruit maturity may be a consequence of the increase in ethylene production throughout fruit development, as we detected in experiments 2 and 3. In climacteric fruit, including peach, ethylene is known to trigger the onset of ripening and to be essential for the completion of the ripening process throughout the various stages (Hiwasa et al., 2003). Exogenous ethylene applied to climacteric fruit in mature stage stimulates ethylene biosynthesis, regulated in an autocatalytic manner, resulting in fruit ripening (Yokotani et al., 2009). We must note that ethephon applications were carried out in immature stage, but even so we found an increase of endogenous ethylene in fruit at harvest. Additional research is necessary to understand the mechanism by which the autocatalytic production of ethylene is triggered after ethephon applications in pre-climateric stages.

Some authors have reported that in other crops, such as cherries and apples, high temperature led to a greater abscission and even over-thinning (Jones and Koen, 1985; Olien and Bukovac, 1978; Yuan and Burns, 2004). Our results indicate that the efficacy of ethephon to thin is significantly reduced after 40 DAFB when high temperatures are not limiting. Similar results have been observed in apple trees where ethephon has low or no thinning effect on 'Golden Delicious' apples when fruit size is 28 mm or greater in diameter (Yuan, 2007). This discrepancy between temperature and ethephon-enhanced fruit ethylene response can be attributed to environmental parameters related with degradation half-life of ethephon and its absorption, as well as to changes in fruit tissue and physiology. In the first case, temperature at the time of ethephon application, and for several days afterward, can determine both absorption and degradation of ethephon to ethylene (Olien and Bukovac, 1978; Yuan, 2007). In the second case, changes in the permeability of the cuticle can determine ethephon absorption. Hedberg and Goodwin (1980) and Nir and Lavee (1981) suggested that ethephon absorption by fruit is mainly cuticular rather than stomatal and, consequently, composition of cuticula layers could play an important role in its penetration. Endogenous auxin could be another factor that determines the sensitivity of fruit to ethephon (Bangerth, 2000; Yuan et al., 2003).

In addition to these physiological changes, differences in endogenous ethylene production at the time of application could also explain different responses for the same ethephon rate. We observed ethephon-enhanced ethylene biosynthesis for several days afterward and, consequently, the thinning effect expressed as reduction in number fruit per 100 flowers, was greater when endogenous ethylene production in the time of application was also greater. Ethephon applications on flowers or fruitlets with higher endogenous ethylene biosynthesis could result in an increase of autocatalytic ethylene

458 production and, consequently, flowers or fruitlets abscission. Many aspects such as
459 environmental parameters, bloom intensity or fruit crop load can influence endogenous
460 ethylene production. The study of these factors that promote the endogenous ethylene
461 production could help to predict the response to ethephon as thinner. Further studies
462 along this line are now in progress.

463 Pre-mature leaf yellowing and abscission as a result of ethephon treatment and high
464 temperatures has also previously been reported (Byers, 1993). In our first experiment,
465 the higher concentrations of ethephon applied at bloom showed a reduction in leaf area
466 due to a decrease in leaf size, especially of the oldest leaves. We must note that this
467 effect was observed only in one of the three experiments and it could be related to a
468 strong increase in temperatures after the applications (Figure 1). However, trees
469 developed a full canopy three weeks after application and no apparent long-term effects
470 on the health of the trees were observed. Gummosis is another concern when using
471 ethephon on *Prunus* species, particularly at higher concentrations (Olien and Bukovac,
472 1982). In our experiments, ethephon application from 75 to 300 mg L^{-1} did not resulted
473 in gummosis, either of the trunk or primary scaffold limbs. Taheri et al. (2012) observed
474 an increase in gummosis in peach trees treated with ethephon at 400 mg L^{-1}. According
475 to our results, an ethephon rate of 400 mg L^{-1} exceeds the optimum concentration for
476 thinning because of its marked negative effect on yield. Therefore, ethephon at the
477 effective rate of 150 mg L^{-1} should be without consequence when used on 'Flatbeauti'
478 peach. However, caution should be exercised before utilizing ethephon on other
479 cultivars or conditions.

480 In conclusion, these results indicate that ethephon can be used in 'Faltbeuti'
481 peaches at 150 mg L^{-1} to induce fruit thinning from full bloom to 40 days DAFB, with
482 no negative effect on the tree. Collectively, the literature suggests that the thinning

response of peaches with ethephon may vary by environmental conditions during and following application. Considering our outcomes, endogenous ethylene produced by flowers or fruitlet at the time of application could be related to the thinning response induced by ethephon.

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586 changing endogenous plant hormones. J Am Soc Hortic Sci 128, 302-308.

587

588 **TABLES**

589 **Table 1.** Leaf size assessment in the experiment 1 (2015) using a linear scoring scale
590 from 1 (severe effect on leaf size, i.e. smaller leaf size) to 5 (no effect on leaf size).

Treatment		Time of application	Leaf size (scale 1–5)	
			April 16th, 2015*	May 16th, 2015ns
UTC		-	5.0 a	-
Hand thinning		60 DAFB	5.0 a	5.0
Ethephon rate (mg L^{-1})	75	30 % FB (March 16th)	3.5 a	5.0
	150		2.3 b	5.0
	300		1.3 c	5.0
Ethephon rate (mg L^{-1})	75	100 % FB (March 23rd)	3.5 a	5.0
	150		3.0 ab	5.0
	300		2.5 b	5.0
Ethephon rate (mg L^{-1})	75	30 DAFB (April 22nd)	-	5.0
	150		-	5.0
	300		-	5.0
Ethephon rate (mg L^{-1})	75	40 DAFB (May 2nd)	-	5.0
	150		-	5.0
	300		-	5.0
Ethephon rate (mg L^{-1})	75	50 DAFB (May 12th)	-	5.0
	150		-	5.0
	300		-	5.0

591 *Values with different letters indicate significant difference by Duncan's multiple range tests at $P < 0.05$.

592 ns No significant differences between treatments (ANOVA $P < 0.05$).

FIGURES

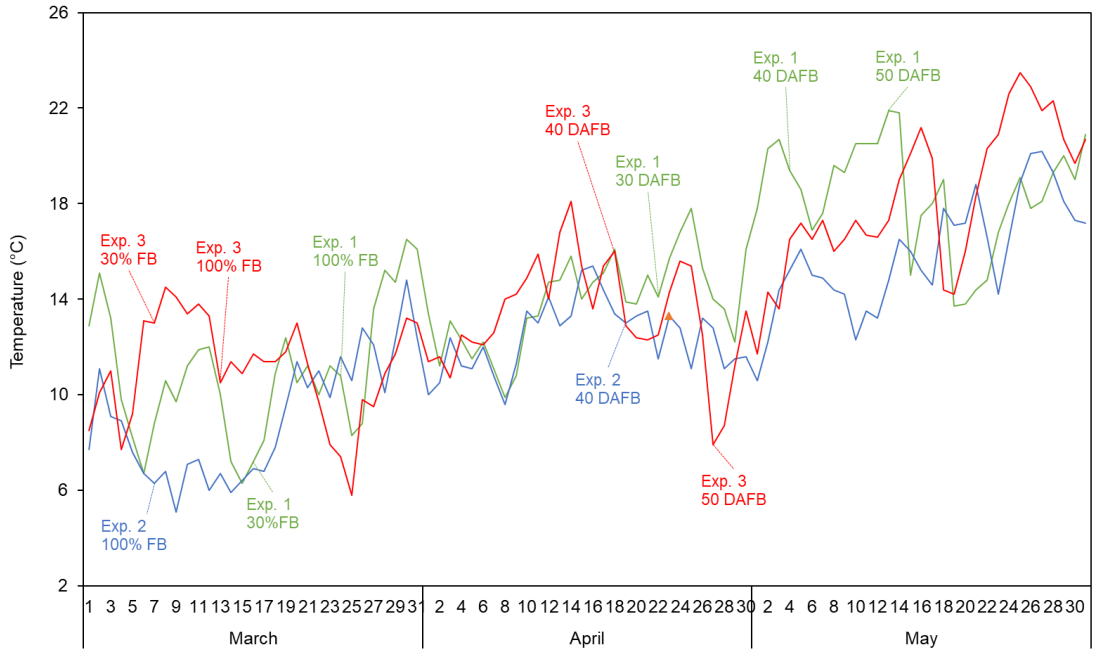

Figure 1. Mean temperature during the ethephon application period (from March to May) for the three experiments (exp. 1, exp. 2 and exp. 3). The application times at 30% full bloom (FB), 100% FB, 30 days after full bloom (DAFB), 40 DAFB and 50 DAFB are indicated for each experiment.

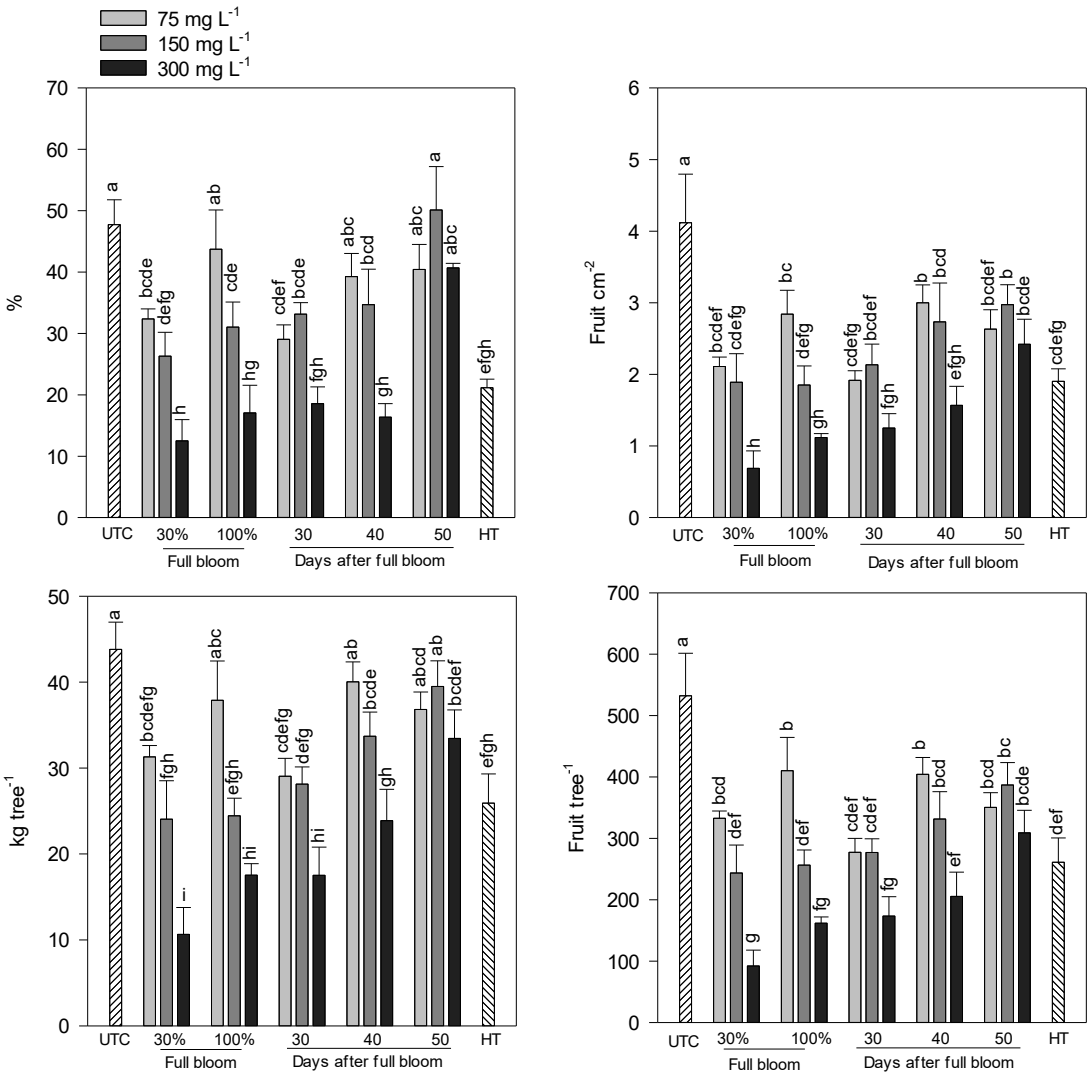

Figure 2. Fruit yield parameters experiment 1. Fruit set ratio (%), crop load (fruit cm^{-2}), yield (kg $tree^{-1}$) and number of fruit per tree (fruit $tree^{-1}$) for each treatment: untreated control (UTC), ethephon 75, 150, 300 mg L^{-1}, all them tested at 30% of full bloom (FB), 100% FB and 30, 40 and 50 days after full bloom, and hand thinning (HT). Error bars indicate standard error (n = 4). Columns with different letters indicate significant difference by Duncan's multiple range tests at $P < 0.05$.

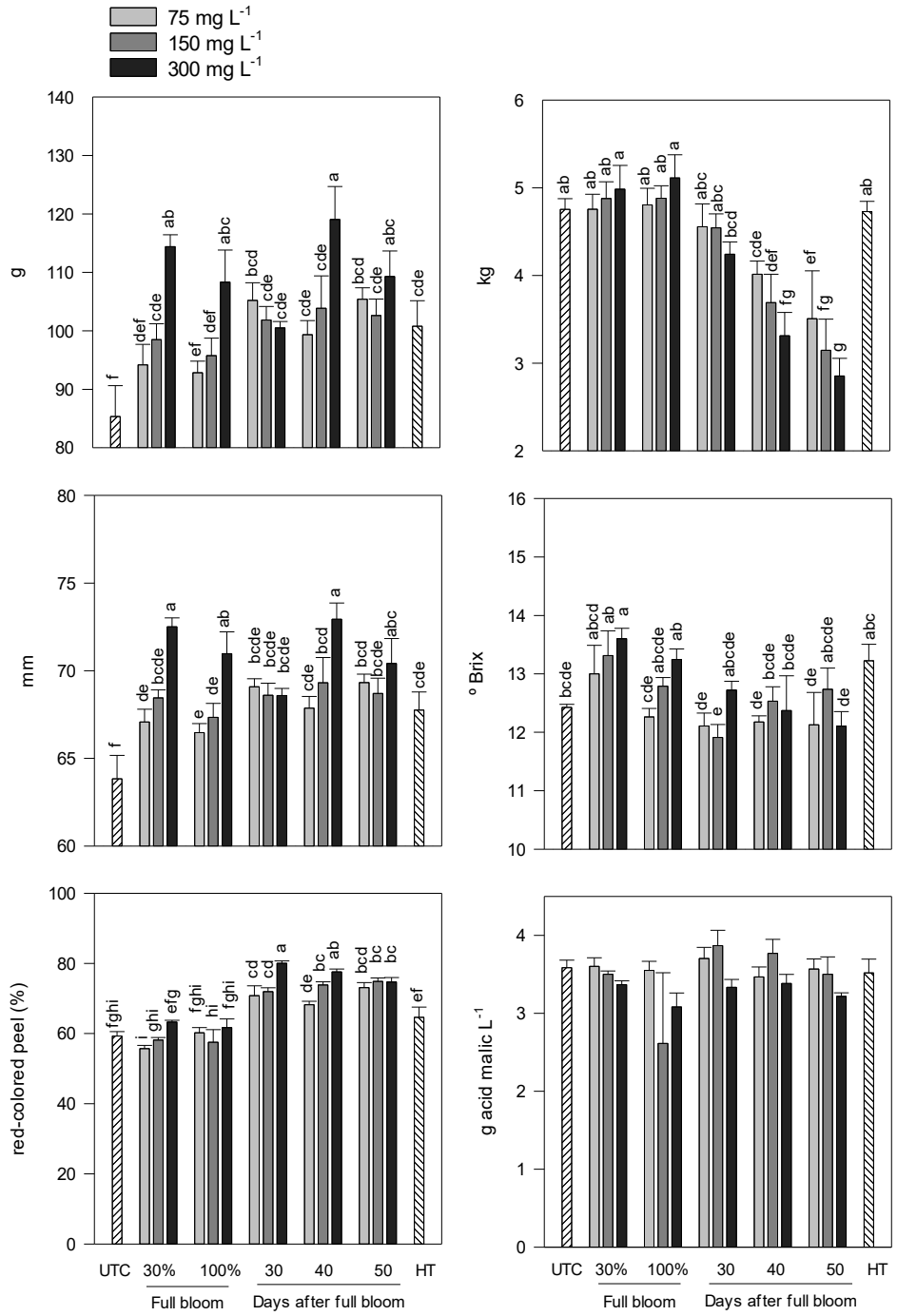

Figure 3. Fruit quality parameters experiment 1. Fruit weight (g), firmness (kg cm^{-2}), fruit diameter (mm), solid soluble content (ºbrix), red-coloured fruit surface (%), and acidity (g L^{-1} malic acid) for each treatment: untreated control (UTC), ethephon 75, 150, 300 mg L^{-1}, all them tested at 30% of full bloom, 100% FB and 30, 40 and 50 days after full bloom, and hand thinning (HT). Error bars indicate standard error (n = 4). Columns with different letters indicate significant difference by Duncan's multiple range tests at $P < 0.05$.

614

615

616 **Figure 4.** Shoots of peach leaves from the untreated control treatment (left) and from
617 the ethephon treatments applied at 30 % of full bloom at 150 mg L^{-1} (centre) and 300
618 mg L^{-1} (right). Photographs taken 30 days after the applications (April 16th, 2015).
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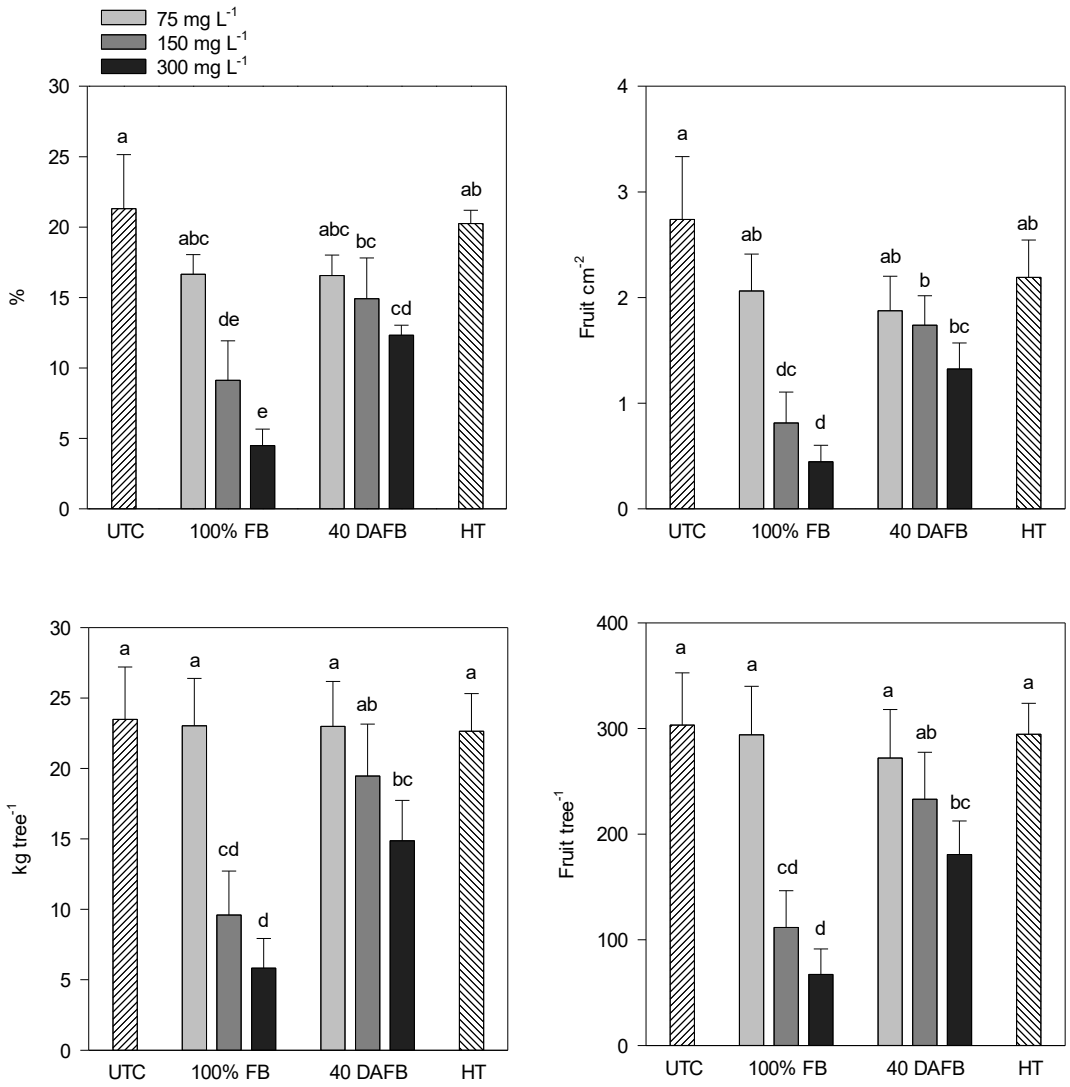

620 **Figure 5. Fruit yield parameters experiment 2.** Fruit set ratio (%), crop load (fruit
621 cm^{-2}), yield (kg $tree^{-1}$) and number of fruit per tree (fruit $tree^{-1}$) for each treatment:
622 untreated control (UTC), ethephon 75, 150, 300 mg L^{-1}, all them tested at 100% of full
623 bloom (FB) and 40 days after full bloom (DAFB), and hand thinning (HT). Error bars
624 indicate standard error (n = 4). Columns with different letters indicate significant
625 difference by Duncan's multiple range tests at $P < 0.05$.
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627

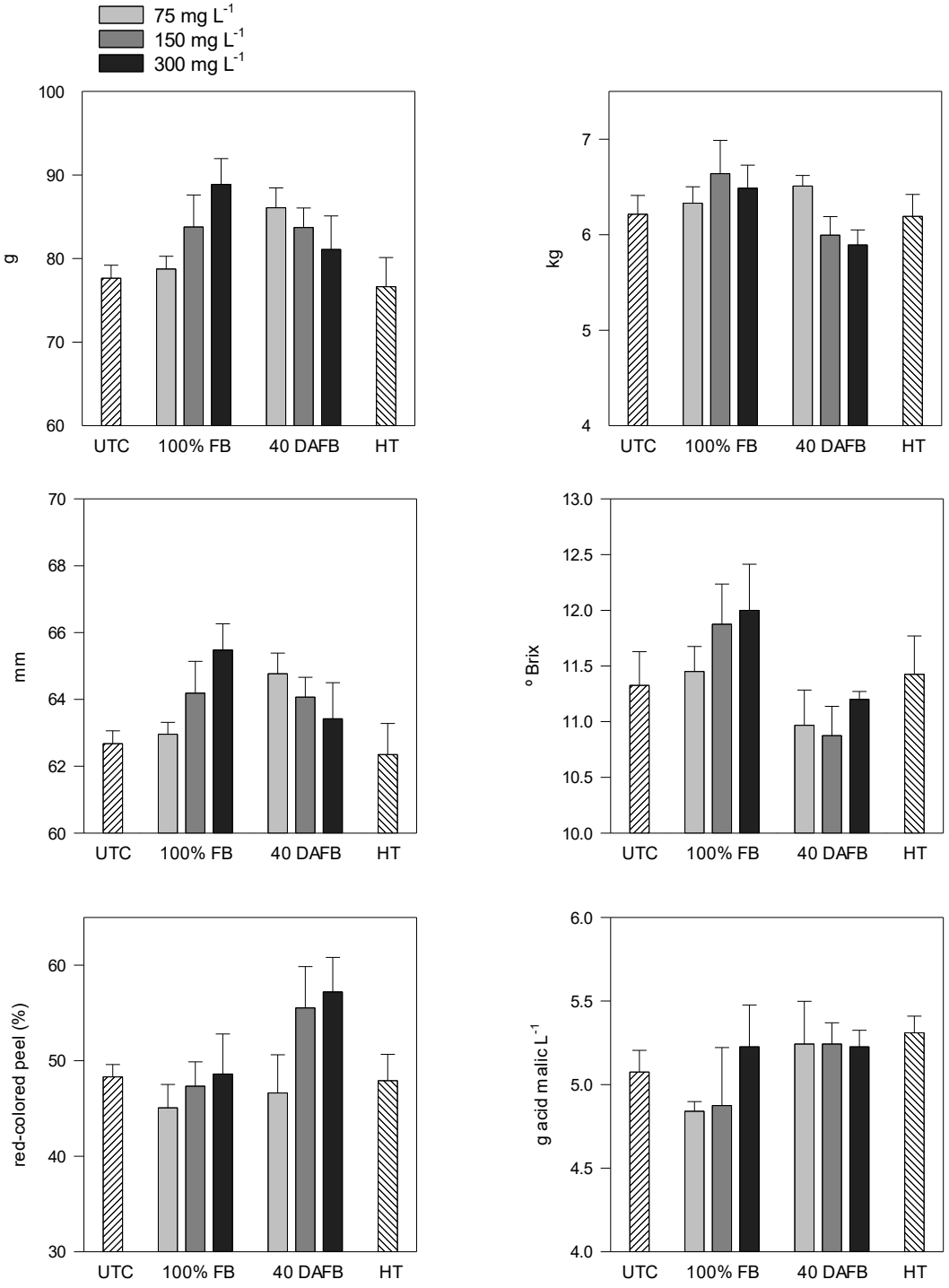

Figure 6. Fruit quality parameters experiment 2. Fruit weight (g), firmness (kg cm^{-2}), fruit diameter (mm), solid soluble content (°brix), red-coloured fruit surface (%), and acidity (g L^{-1} malic acid) for each treatment: untreated control (UTC), ethephon 75, 150, 300 mg L^{-1}, all them tested at 100% of full bloom (FB) and 40 days after full bloom (DAFB), and hand thinning (HT). Error bars indicate standard error (n = 4). No significant differences between treatments were found in all fruit quality parameters analysed (ANOVA, $P < 0.05$).

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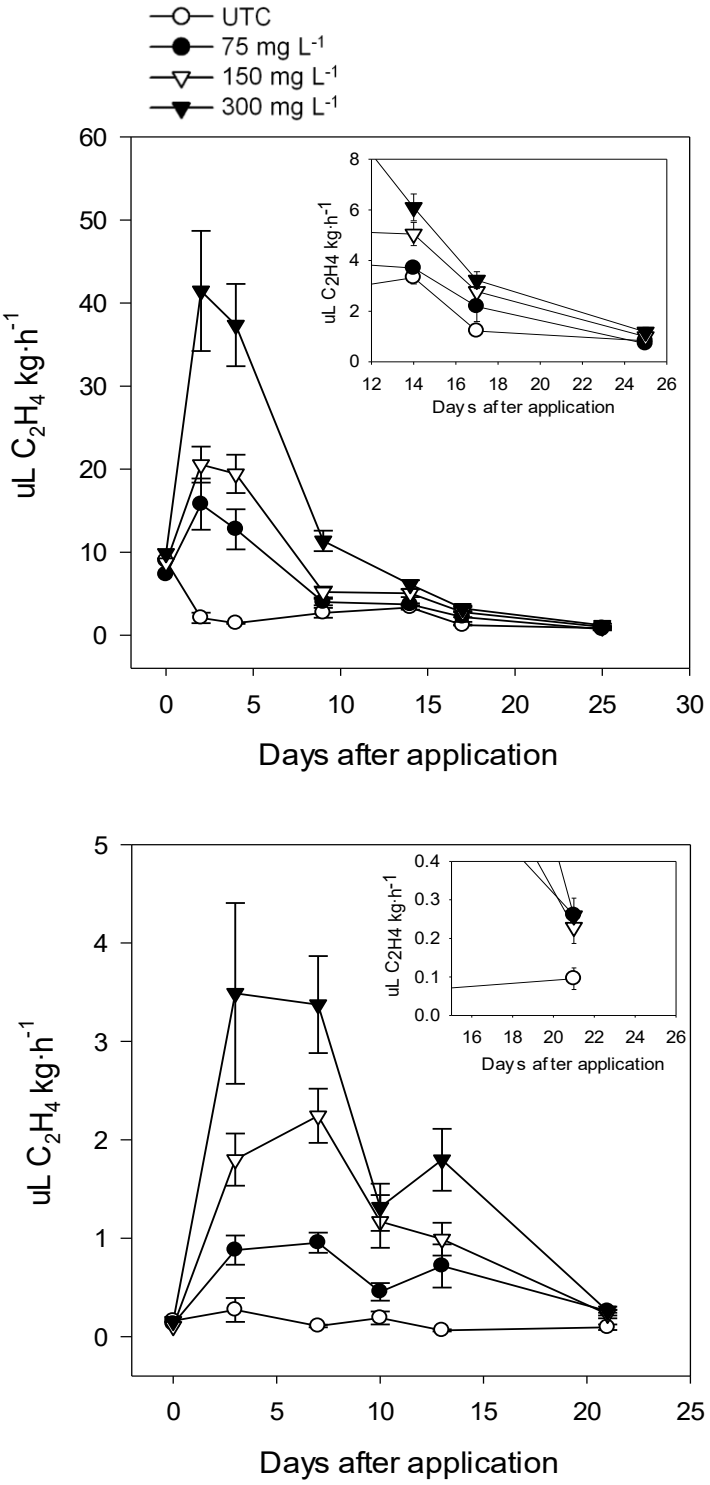

663 **Figure 7. Dynamics of ethylene evolution experiment 2**. A: dynamic of ethylene of
664 the different ethephon rates (75, 150 and 300 mg L^{-1}) of the treatments at full bloom
665 and of the untreated control (UTC) from just before the applications to 25 days later. B:
666 dynamic of ethylene of the different ethephon rates (75, 150 and 300 mg L^{-1}) of the
667 treatments at 40 days after full bloom and of the UTC from just before the applications
668 to 22 days later. Error bars indicate standard error (n = 4).

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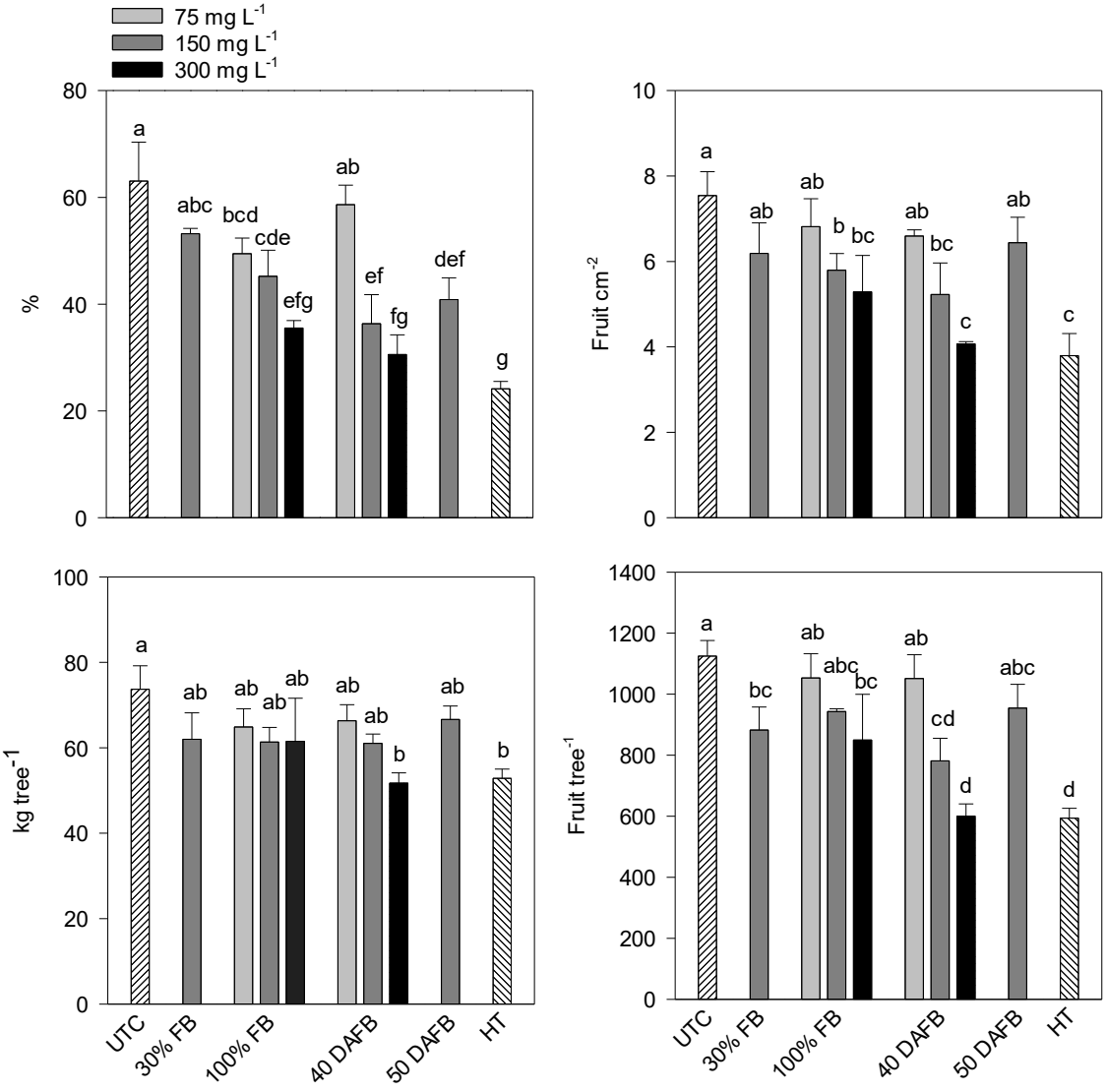

670
671 **Figure 8. Fruit yield parameters experiment 3.** Fruit set ratio (%), crop load (fruit
672 cm^{-2}), yield (kg $tree^{-1}$) and number of fruit per tree (fruit $tree^{-1}$) for each treatment:
673 untreated control (UTC), ethephon 150 mg L^{-1} at 30 % of full bloom (FB), ethephon 75,
674 150, 300 mg L^{-1}, all them tested at 100% FB and 40 days after full bloom (DAFB),
675 ethephon 150 mg L^{-1} at 50 days DAFB, and hand thinning (HT). Error bars indicate
676 standard error (n = 4). Columns with different letters indicate significant difference by
677 Duncan's multiple range tests at $P < 0.05$.

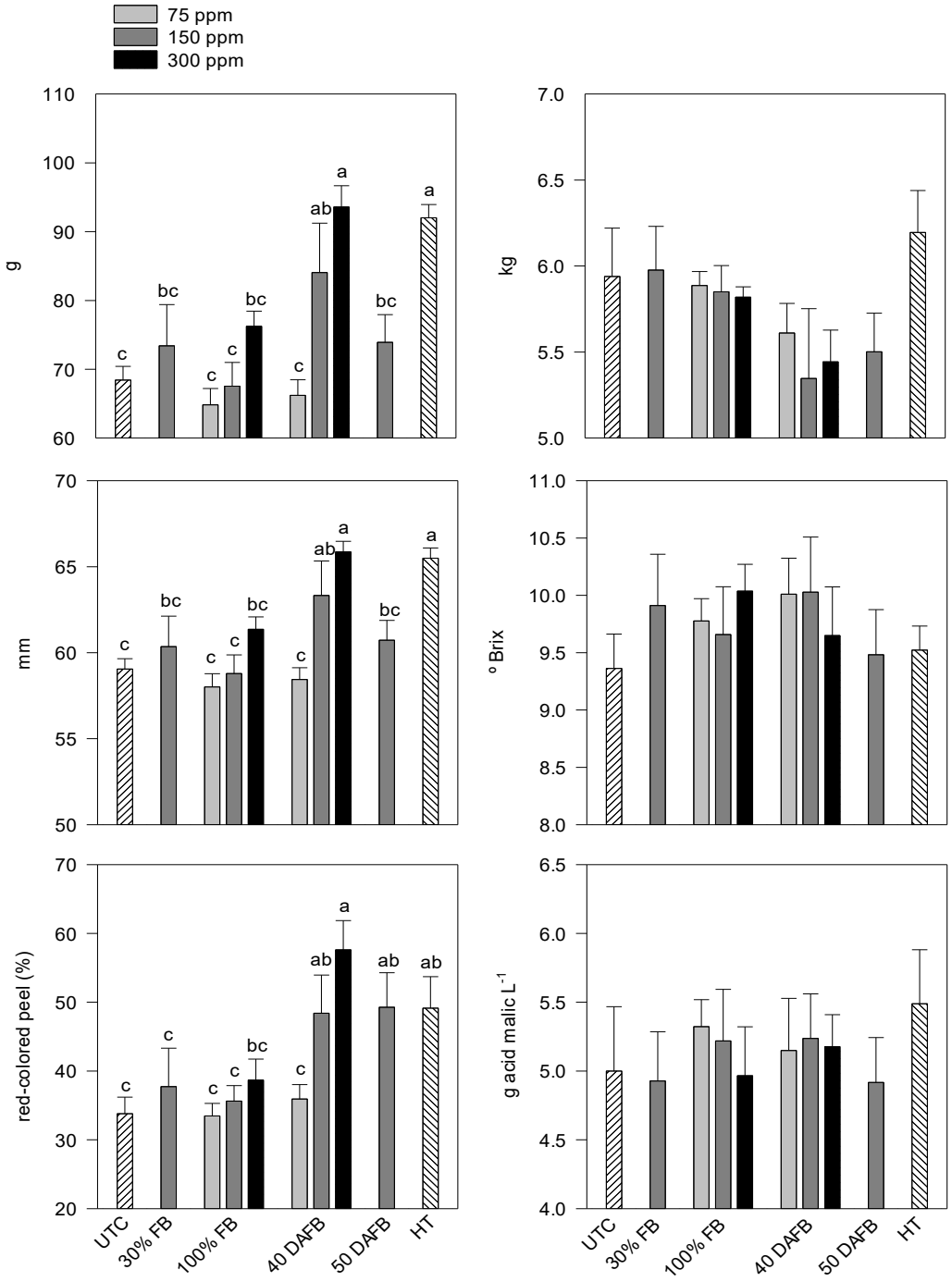

Figure 9. Fruit quality parameters experiment 3. Fruit weight (g), firmness (kg cm^{-2}), fruit diameter (mm), solid soluble content (°brix), red-coloured fruit surface (%), and acidity (g L^{-1} malic acid) for each treatment: untreated control (UTC), ethephon 150 mg L^{-1} at 30 % of full bloom (FB), ethephon 75, 150, 300 mg L^{-1}, all them tested at 100% FB and 40 days after full bloom (DAFB), ethephon 150 mg L^{-1} at 50 DAFB, and hand thinning (HT). Error bars indicate standard error (n = 4). Columns with different letters indicate significant difference by Duncan's multiple range tests at $P < 0.05$.

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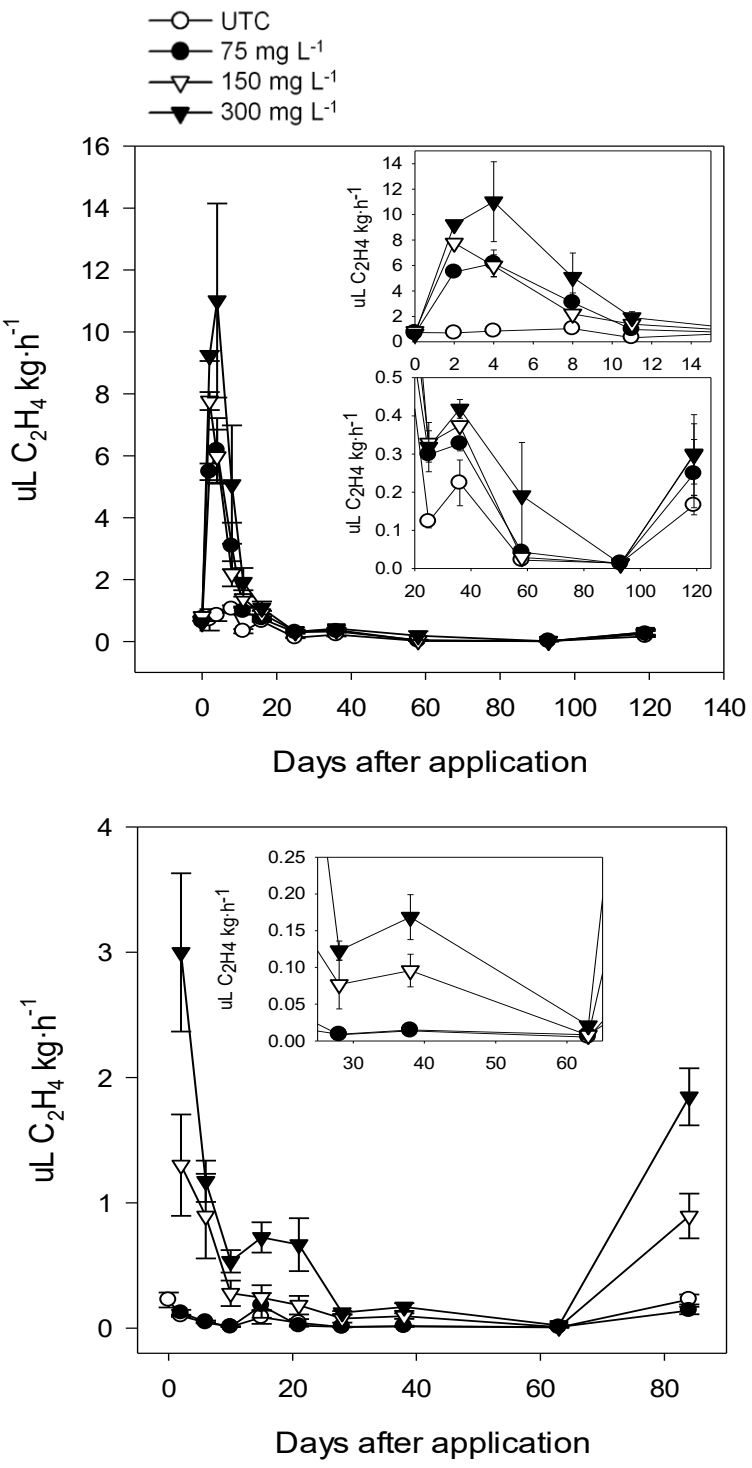

710 **Figure 10. Dynamics of ethylene evolution experiment 3**. Above: dynamic of
711 ethylene of the different rates of the early ethephon treatments (75, 150 and 300 mg L^{-1})
712 and of the untreated control (UTC) from just before the applications at full bloom up to
713 harvest time (120 days later). Below: dynamic of ethylene of the different rates of the
714 early ethephon treatments (75, 150 and 300 mg L^{-1}) and of the UTC from just before the
715 applications at 40 days after full bloom up to harvest time (85 days later). Error bars
716 indicate standard error (n = 4).

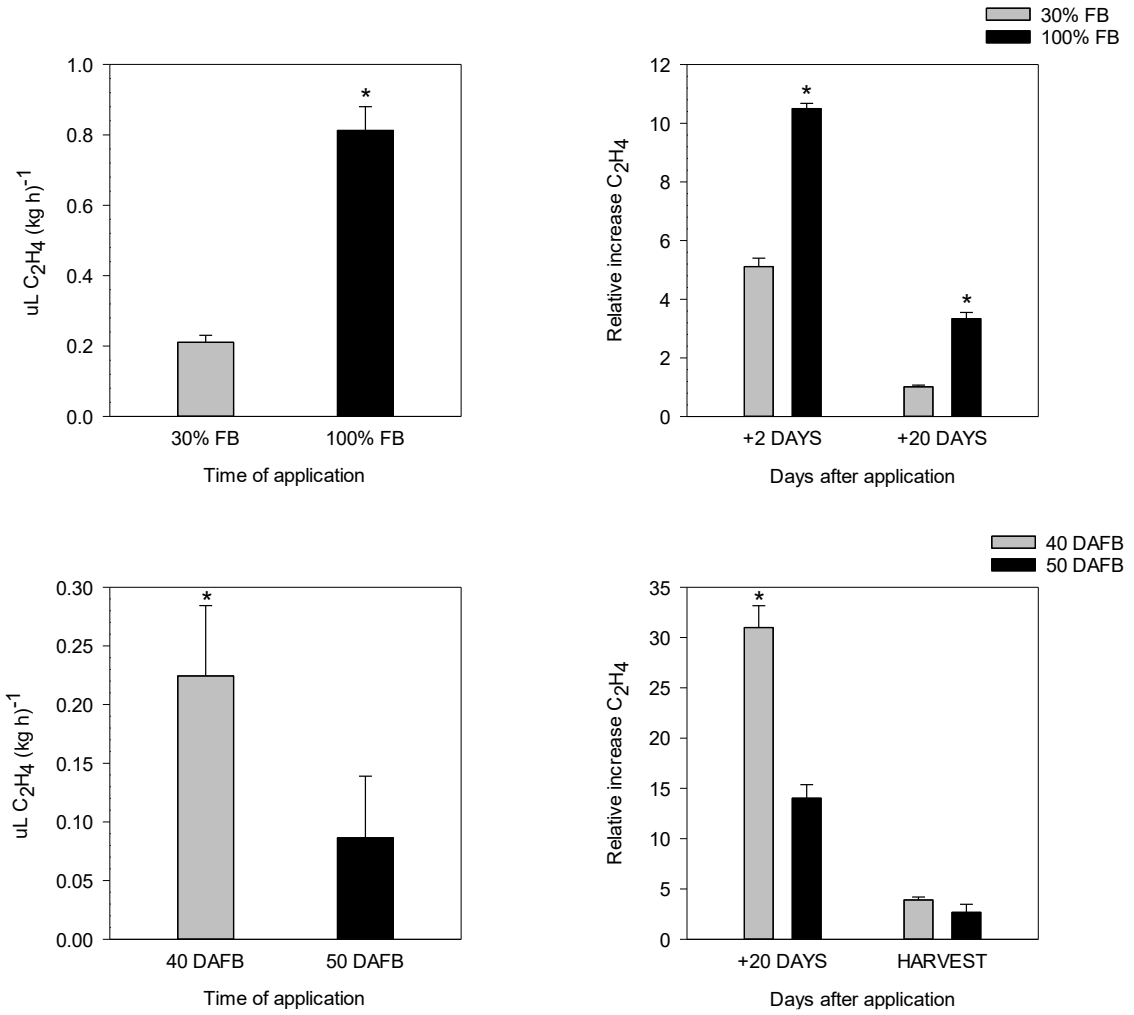

Figure 11. Endogenous ethylene production in the time of application (left) and relative increase in ethylene compared to untreated control treatment (right) for the ethephon treatments applied in the experiment 3 at 150 mg L^{-1}. Above: ethephon treatments applied at 30% and 100% full bloom (FB). Below: ethephon treatments applied at 40 and 50 days after full bloom (DAFB). Error bars indicate standard error (n = 4). Asterisk above the bars indicate statistically significant differences (P > 0.05) within the same time of evaluation.