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Effects of Trunk-shaker Harvester and Ethephon on Plant Water Status, Leaf Gas Exchange, and Yield of Citrus Cultivated under Mediterranean Conditions

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***Abstract*. This work was aimed to study whether the application of ethephon as an abscission agent and mechanical harvest using a trunk shaker have any effect on plant water status, leaf gas exchange, and yield of mandarin and orange trees cultivated under Mediterranean conditions. The experiment was performed from 2008 to 2011 in five commercial orchards where parameters related to the plant water status and leaf gas exchange were measured before the application of ethephon, at harvest time and at different occasions after harvest. In addition, the effects of ethephon dose on yield in the current and subsequent seasons were also evaluated. Results showed that ethephon applications and mechanical harvest did not detrimentally affect plant water status in any of the cultivars studied. Furthermore, either had no effect or had a short temporal decrease effect on leaf gas exchange depending on the cultivar studied although with no consequences for the fruit yield obtained during the current season. Increasing ethephon doses led to fruit yield reductions in the mandarin 'Orogrande' trees in subsequent seasons. When trunk-shaker and ethephon applications were combined, however, yields from the late-maturing orange significantly decline in subsequent seasons. Overall, results show that using a trunk shaker is a viable technique to mechanically harvest citrus trees destined to both fresh and industry market and can be considered as an alternative to the traditional manual harvest usually performed under Mediterranean conditions. However, its use cannot be recommended for late-maturing oranges, such as the 'Navel Lane Late' in which mature fruit and fruitlets coexist in the tree at the time of harvest.**

Citrus is one of the most important fruit crops in the world with an annual production over 131 million tons. Spain is the sixth citrus producer and a leading exporter of fresh citrus worldwide (FAO, 2012). Despite its economic and social importance, farmers' incomes are suffering large economic declines due to the constant increase in production costs meanwhile the prices received remained virtually constant since 1985. One way for farmers to increase their income level is by decreasing production costs. In Spain, citrus production costs are higher than those of competitor countries (Juste et al., 2000). Hand-labor operations represent the highest percentage of the citrus production costs, with harvest costs accounting for 35% to 45% of the total (Sanders, 2005). This is particularly important in those areas where citrus production is mainly oriented toward the fresh fruit market and therefore fruit have to be picked carefully to meet the quality standards. Mechanization of this labor could reduce the total costs in 30% to 35% (Juste et al., 2000).

Mechanical harvest with continuous canopy or trunk shakers has been used in citrus areas of Florida for years (Roka et al., 2014a, 2014b), where 95% of the orange crop is destined to juice production (NASS, 2015). The efficiency of these machines depends on fruit variety, tree characteristics, and operating conditions. In the case of trunk shakers, detachment rates between 57% and 90% have been obtained in 'Hamlin' (*Citrus sinensis* L. Osbeck cv. Hamlin) and 'Valencia' oranges (*C. sinensis* L. Osbeck cv. Valencia) under Florida agroclimatic conditions (Whitney et al., 1986, 2000; Whitney and Wheaton, 1987). To improve the efficiency of these technologies, abscission agents have been studied, and their use has been promoted in citrus areas of Florida (Burns, 2002; Burns et al., 2003; Hartmond et al., 2000; Whitney et al., 1986).

Studies performed recently under Mediterranean conditions to analyze the efficiency of trunk shakers in orange (*C. sinensis*) and mandarins (*Citrus reticulata* L.) trees have reported fruit detachment percentages ranging between 52% and 85% (Moreno et al., 2015; Torregrosa et al., 2009), with a percentage of fruit without calyx (important loss of quality for fresh consumption) between 0.6% and 9.0%. These results showed that harvesting with a trunk shaker may be a feasible solution for Spanish citrus for fresh market. The use of ethephon as an abscission agent increased fruit detachment as its dose increased, but its use also increased the percentage of fruit without calyx, so it should be recommended only for citrus destined to industry (Moreno et al., 2015).

Mechanical harvesting with trunk shakers produces an apparent violent shaking of the trees, which, depending on the machine, operators, and orchard conditions, can cause visible physical injuries to the trees such as shedding of leaves, flowers, and young fruit and breaking of branches and/or scuffing of bark. These observations fuel grower concerns about long-term tree health over using trunk shakers, alone or combined with abscission agents, to harvest fruit. As a result, there is a low widespread adoption of mechanical harvesting systems among Spanish citrus growers. For this reason, several field trials were conducted in Florida between 1970 and 2005 to investigate whether trunk shakers adversely affected fruit yield and long-term tree health. Except for the case of the late-season 'Valencia' oranges, the results of these field trials showed no short- or long-term adverse effects. Instead, the research suggested that trees that were well-nourished before and after mechanical harvesting fully recovered from all harvest related stresses (Hedden et al., 1984; Li and Syversten, 2005; Whitney, 2003). A more recent study analyzed grower yield data between 1998 and 2008 obtained from hand-picked and mechanically harvested orchards. It showed no evidence of shortened tree life or reduced yields caused by mechanical harvest (Roka et al., 2014c). However, no studies have been conducted to assess the effects of mechanical harvesting and the use of abscission agents on citrus tree physiology and yield under Mediterranean conditions. Citrus cultivation is different in Florida than in the Mediterranean regions because of different soil and environmental conditions, irrigation techniques employed, and citrus varieties cultivated. For example, in Florida, soils are predominantly sandy, whereas in the southeastern Spain, where citrus is the most important crop, soils are more calcareous and often with high clay content. In addition, Florida citriculture normally employs micro-jet sprinkler wetting most part of the orchard floor, whereas in Spanish citriculture, drip irrigation is used, and as a consequence in Spanish orchards the root system is more concentrated below the drippers. Under these conditions it could be that the trunk shaker could be more harmful because of the more concentrated root system close to the tree trunk. Before attempting to recommend any practice regarding mechanical harvesting to Mediterranean citrus growers, more research on the most common mandarin varieties cultivated should be conducted.

The present work aimed to assess the physiological and fruit yield responses of four mandarin cultivars (Orogrande, Marisol, Clemenules, and Fortune) and one orange variety ('Navel Lane Late'), all of them mechanically harvested, with and without ethephon applications, under Mediterranean conditions. Yield effects were monitored in both the current and subsequent seasons.

Materials and Methods

Experimental orchards and treatments. The study was performed in parallel with the work presented by Moreno et al. (2015) and was carried out in the same five drip-irrigated commercial citrus orchards and seasons: four mandarin orchards ['Orogrande' A (seasons 2008–09, 2009–10, and 2010–11) 'Orogrande' B (seasons 2009–10 and 2010–11), 'Marisol' (seasons 2009–10 and 2010–11), 'Clemenules' (season 2009–10), and 'Fortune' (season 2009–10)] and one late maturing orange orchard ['Navel Lane Late' (seasons 2009–10 and 2010–11)]. The characteristics of each orchard are shown in Table 1.

The experimental design and treatments (applications of ethephon and harvesting) are described in detail in the work of Moreno et al. (2015). In summary, five treatments were carried out in each test: one control (water) and four different doses of ethephon (Ethrel 48; Numarf España, S.A., Barcelona, Spain) resulting from the combination of two concentrations (600 and 1200 ppm) and two spray volumes, one higher, which was defined

Table 1. Characteristics of the orchards: rootstock, location, tree age, planting framework, canopy volume, harvest time, and days from application to harvest.

Orchard	Rootstock	Location	Tree age (yr)	Planting framework (m^2)	Canopy vol[z] (m^3/tree)	Observations and harvest time	Treatments date: Ethephon spray	Treatments date: Days between spray and vibration
'Orogrande' [*Citrus reticulata*, Blanco (orchard A and B in the same field)]	'Carrizo' citrange	39°55′ 55.40″ N 0°22′ 14.71″ W	13–14	6 × 2	11.22	Mid-late clementine mandarin (October–January)	5 Nov. 2008 (orchard A) 30 Oct. 2009 2 Nov. 2010	6 12 10
'Marisol' (*C. reticulata*, Blanco)	'Carrizo' citrange	39°39′ 9.08″ N 0°18′ 39.74″ W	28	5 × 3.80	8.66	Early clementine mandarin (September–October)	5 Nov. 2008 30 Oct. 2009	6 12
'Navel Lane Late' (*C. sinensis* (L.) Osbeck)	'Carrizo' citrange	39°36′ 0.07″ N 0°21′ 48.65″ W	21	5.4 × 2.4	9.5	Mid-late orange (January–May)	15 Mar. 2010 22 Mar. 2011	8 8
'Clemenules' (*C. reticulata*, Blanco)	'Carrizo' citrange	39°28′ 57.52″ N 0°36′ 53.51″ W	13	6 × 4	18.8	Mid-late clementine (November–January)	23 Nov. 2009	10
'Fortune' (*Citrus clementina*, Hort, ex Tan. × *C. tangerina* Hort, ex Tan.)	'Carrizo' citrange	39°36′ 49.22″ N 0°21′ 12.79″ W	23	5.8 × 4	18.8	Late hybrid (January–April)	31 Mar. 2010	7

'Carrizo' citrange = *Citrus sinensis* (L.) Osbeck × *Poncirus trifoliata* (L.) Raf.

[z]Canopy volume was calculated as the mean of three replicates considering citrus canopy as an ellipsoid with the tree dimensions of height, diameters 1 and 2.

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as the volume of liquid until the runoff point, and one lower, which was defined as a 40% reduction of the higher volume. Runoff volume varied as function of the average canopy volume of each orchard, so volumes of application varied across orchards. The lower volume 40% of runoff was chosen because in previous test enough coverage (between 30% and 50%) was obtained over water-sensitive paper distributed in the canopy. A reduction in the spray volume would reduce the risks of runoff and drift and the time and cost of treatments, therefore, it could optimize the application. Treatments were assessed in 50 random trees from each orchard (10 trees per treatment).

Six to twelve days after the applications, five trees from each treatment (25 trees per orchard) were hand-picked while the other five were mechanically harvested using a commercial trunk shaker (Topavi, brazo soporte vibrador; Maquinaria Garrido S.L., Autol, Spain). The frequency in the different tests ranged between 14.1 and 15.5 Hz and the amplitude between 15 and 35 mm (Ortiz and Torregrosa, 2013). The duration of vibration was 5 s. Taking into account the ethephon doses and harvest technique employed, 10 treatments were used in the experiment (Table 2).

Table 2. Treatments carried out in the experiment.

Treatment	Harvest technique	Spray vol	Ethephon concn (ppm)
NTHP	Hand-pick	Runoff	0
NTMH	Mechanical harvest		
ET1HP	Hand-pick	–40%	600
ET1MH	Mechanical harvest		
ET2HP	Hand-pick	Runoff	
ET2MH	Mechanical harvest		
ET3HP	Hand-pick	–40%	1,200
ET3MH	Mechanical harvest		
ET4HP	Hand-pick	Runoff	
ET4MH	Mechanical harvest		

ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees; NT = nontreated trees (sprayed with water + adjuvant); NTHP = nonethephon-treated and hand-picked trees; NTMH = nonethephon-treated and mechanically harvested trees.

Trials were made in a completely randomized experimental design. The experimental unit was one tree, and each treatment was repeated five times, with a total of 50 trees per test. Between each experimental unit tree a barrier tree was left.

Plant water status and leaf gas exchange determinations. Plant water status and leaf gas exchange were monitored in trees sprayed with water plus adjuvant and those sprayed with the highest dose of ethephon. Thus, the following four treatments were compared: nonethephon-treated and hand-picked trees (NTHP), non ethephon-treated and mechanically harvested trees (NTMH), highest dose of ethephon-treated and hand-picked trees (ET4HP), and highest dose of ethephon-treated and mechanically harvested trees (ET4HP).

Plant water status was determined by measuring the midday stem water potential (Ψ_{stem}, MPa). Measurements were taken at solar noon with a Scholander pressure chamber (Model 600; PMS Instrument Company, Albany, OR) in two mature and homogeneous leaves per tree bagged in silver foil at least 1 h before the measurements, following the recommendations of Turner (1981). Concurrently to Ψ_{stem} measurements, stomatal conductance (g_s, mmol $CO_2/m^2/s$), net assimilation of CO_2 (A_{CO2}, μmol $CO_2/m^2/s$), and leaf transpiration (E_l, mmol $H_2O/m^2/s$) were also determined in three sunny-mature leaves per tree (a total of 30 leaves per treatment) with a portable photosynthesis measurement system (ADC LCiPro+; ADC Bioscientific, Great Amwell, Herts, UK). In each season, trees were monitored some days before ethephon applications, some days after ethephon application, and some days after harvesting. Because the measurements should be taken at sunny days, it was not possible to plan a fixed previous schedule to take the measurements. The dates of measurements in each orchard are shown in Figs. 1–4.

Yield. Yield was determined at harvest time in both mechanically and manually harvested treatments. In the mechanically harvested trees, the yield was calculated by adding fruit mechanically removed during shaking and fruit remaining after shaking that was manually picked.

Statistical analysis. To study the effect of ethephon, trunk shaker, and their interaction on Ψ_{stem}, g_s, A_{CO2}, and E_l, a temporal evolution of the average ± 95% confidence interval for each variety was plotted. Previously, the residual data for each variable were analyzed by normal probability plot to identify possible outliers. After preliminary study of the temporal evolution data, on the dates when visual differences were observed, analyses of variance were conducted to study its significance. In the case of finding significant differences, least square difference test was used for mean comparisons. In this study, the assumption of normal distribution of data were assessed using the normal probability plot of the residuals, and the assumption of homoscedasticity using the Levene's test (Levene, 1960). In all the analyses a confidence level of 95% was considered.

The factor *harvest technique* cannot affect the yield of the same season in which it is being applied. However, the factor *ethephon dose* could affect yield of the same season in which it is sprayed because it could cause fruit drop before harvesting. For this reason, the effect of *ethephon dose* over the yield remained in the tree at harvest in the current season per orchard was studied using the data of mechanically harvested trees in the first season (NTMH, ET1MH, ET2MH, ET3MH, and ET4MH) by linear regression analysis.

Both factors *ethephon dose* and *harvest technique* could affect yield obtained in the following seasons. To study their effect and their interactions on yield in the subsequent season, multiple linear regression (MLR) analyses per orchard and season were carried out except in the case of 'Clemenules' and 'Fortune' orchards where there was only one experimental season. MLR analysis followed an iterative process, which started by including the *ethephon dose* as independent variable. To test if the relationship between *ethephon dose* and yield was affected by the factor *harvest technique*, an indicator variable was included in the regression models (Suits, 1957). The indicator variable was *Harvest technique = Mechanical harvest*. It took the value 1 for the data obtained with mechanical harvest and 0 for the data obtained with hand-picking harvest. Its single effect and its interaction with the independent variable were also included in the model. The variable with the highest, nonsignificant P value ($\alpha > 0.05$) was eliminated, and the model was recalculated until all variables present in the model had significant coefficients.

In all fitted models, the presence of possible outliers and all the assumptions of linear regression were checked.

Results

Ethephon and trunk shaking effects on plant water status

All the trees within each orchard had similar plant water status at the beginning of the experiment (Fig. 1). Throughout the seasons, Ψ_{stem} values registered in 'Orogrande' A, 'Orogrande' B, 'Marisol', and 'Clemenules' orchards ranged from –0.71 to –2.23 MPa whereas in the 'Fortune' and the 'Navel Lane Late' orchards, Ψ_{stem} was less negative, ranging from –0.46 to –1.56 MPa.

Ethephon applications had no detrimental effects on tree plant water status in any of the varieties studied (Fig. 1). In the 'Orogrande' A, 'Orogrande' B, and 'Clemenules' trees no significant differences in Ψ_{stem} were found between treatments after the ethephon applications (Table 3). However in the 'Marisol', 'Navel Lane Late', and 'Fortune' orchards ethephon-treated trees showed higher Ψ_{stem} than nontreated trees just after the ethephon applications (Fig. 1). Statistically significant differences in Ψ_{stem} were observed in the 'Marisol' orchard between ethephon-treated and the NTMH treatments on 1 Oct. 2009 (7 d after the ethephon application), in the 'Navel Lane Late' orchard between the ET4MH treatment and the rest of the treatments on 16 Mar. 2010 (just 1 d after the ethephon application), and in the 'Fortune' orchard between the ET4HP and the NTHP treatment on 6 Apr. 2010 (days after the ethephon application) (Table 3).

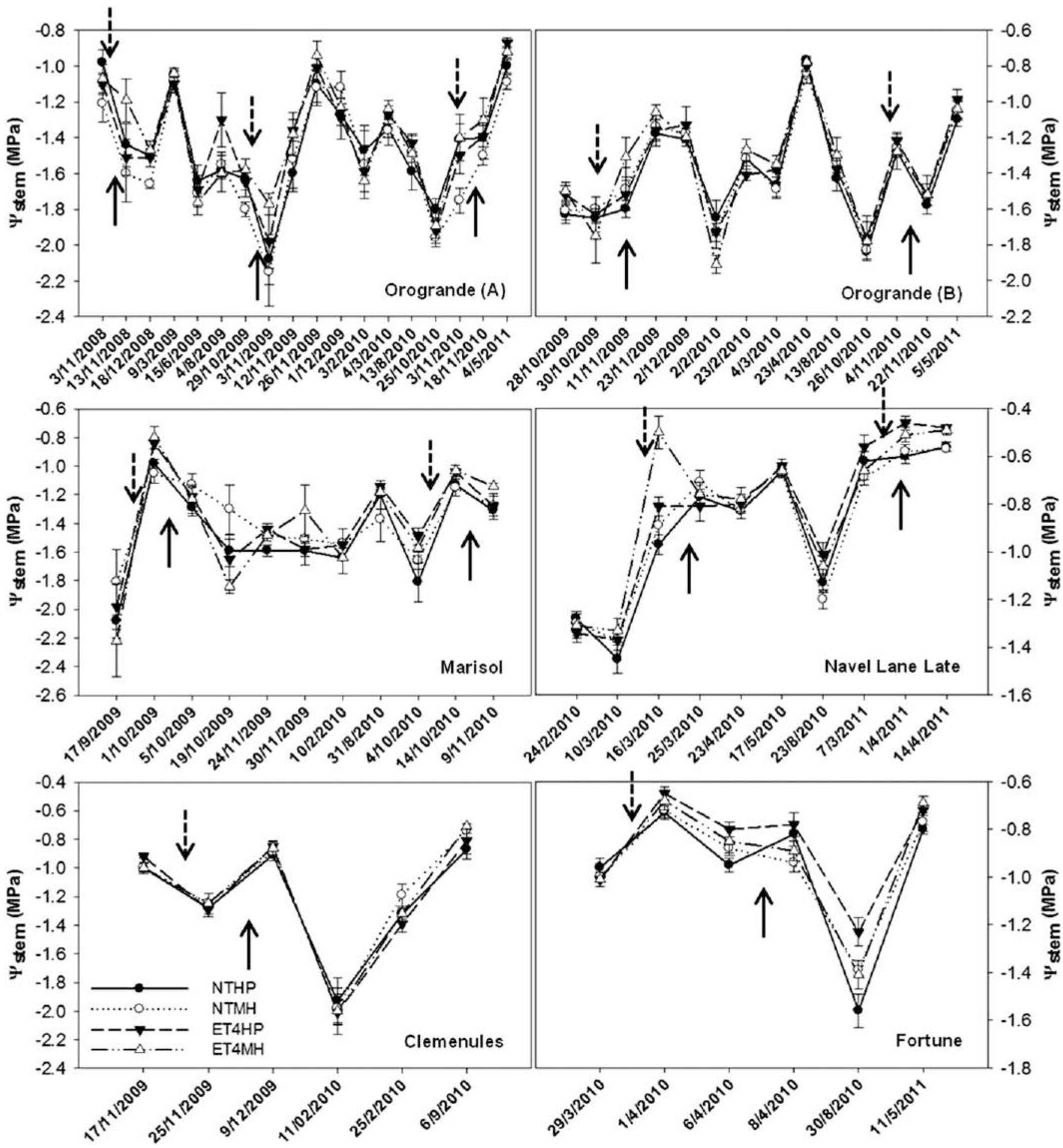

Fig. 1. Stem water potential (Ψ_{stem}, MPa) evolution in the nonethephon-treated hand-picked (NTHP), nonethephon-treated mechanically harvested (NTMH), ethephon-treated hand-picked (ET4HP), and ethephon-treated mechanically harvested (ET4MH) treatments within each of the citrus cultivars studied. Vertical bars show the SE. Downward arrows depict the date of ethephon applications. Upward arrows indicate the date of harvest.

No significant differences in Ψ_{stem} were found after harvest in the 'Orogrande' A, 'Orogrande' B, 'Clemenules', 'Navel Lane Late', and 'Fortune' orchards between hand-picked and mechanically harvested trees with a trunk shaker (Fig. 1). However, in the 'Marisol' orchard, NTMH trees had significantly higher Ψ_{stem} than NTHP, ET4HP, and ET4MH trees on 19 Oct. 2009 (17 d after harvest) (Table 3).

Ethephon and trunk shaking effects on leaf gas exchange

Effects on g_s. All the trees within each orchard had in general similar g_s values at the beginning of the experiment (Fig. 2). Once ethephon applications and mechanical harvest took place, no effects were observed during the experiment on g_s in the 'Fortune' and 'Orogrande' B orchards (Table 4). In 'Orogrande' A and 'Clemenules' orchards, statistically significant differences were observed between ethephon-treated and nontreated trees, and between NTMH and NTHP or ET4MH treatments, respectively, in punctual days (9 Mar. 2009 in the 'Orogrande' A orchard and 25 Nov. 2009 in the 'Clemenules' orchard) during their second experimental seasons but not immediately after the ethephon application or harvest (Table 4).

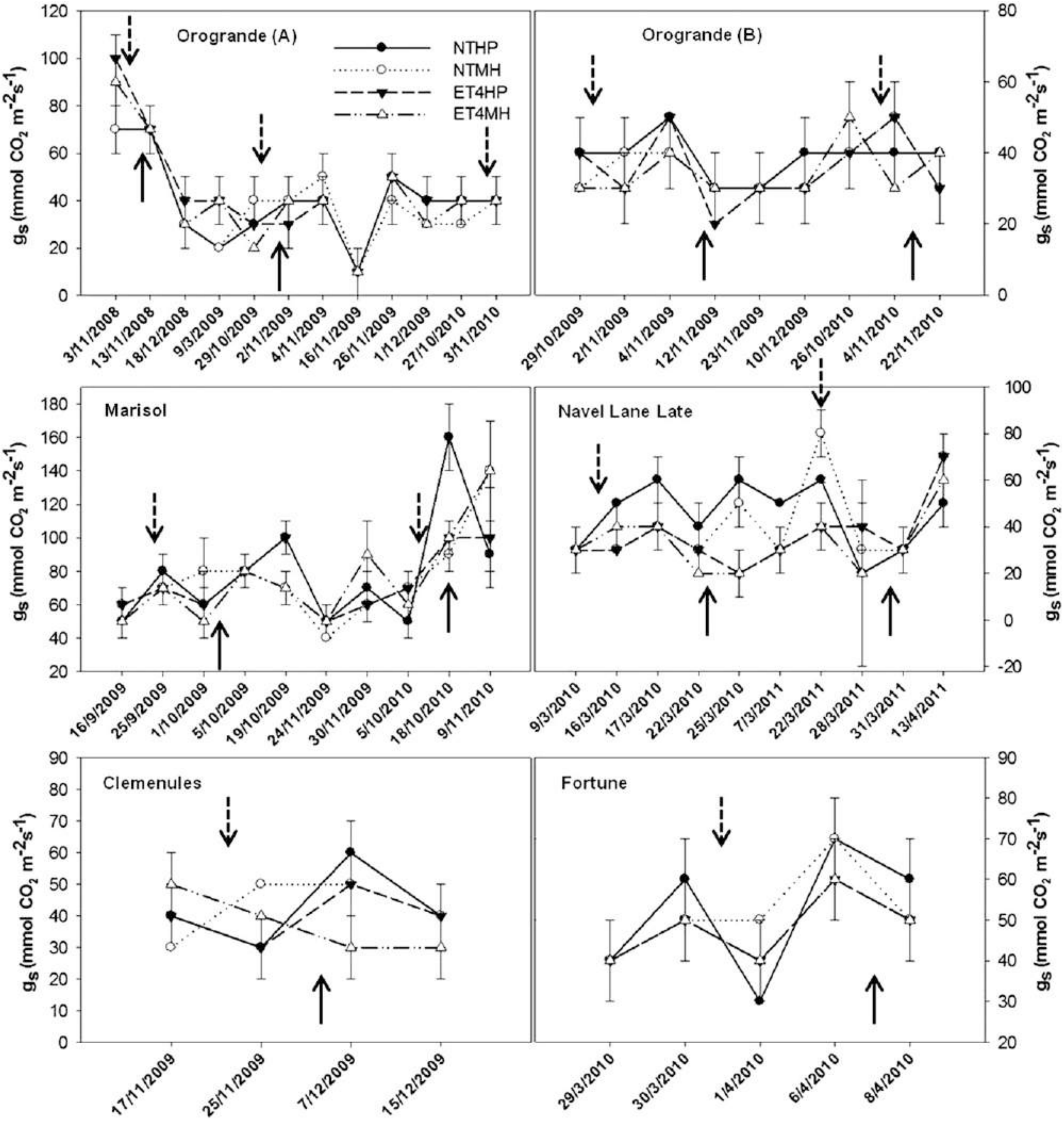

Fig. 2. Evolution of the stomatal conductance (g_s, mmol $CO_2/m^2/s$) in the nonethephon-treated hand-picked (NTHP), nonethephon-treated mechanically harvested (NTMH), ethephon-treated hand-picked (ET4HP), and ethephon-treated mechanically harvested (ET4MH) treatments within each of the citrus cultivars studied. Vertical bars show the SE. Downward arrows depict the date of ethephon applications. Upward arrows indicate the date of harvest.

Higher values of g_s were measured in 'Marisol' manually harvested trees during the first experimental season on 19 Oct. (2 weeks after harvest; Fig. 2). Nevertheless, these differences in g_s between hand-picked and mechanically harvested trees only were statistically significant in the case of the ET4MH treatment (Table 4). In the same orchard in 2010, control trees (NTHP treatment) showed statistically significant higher g_s values than the rest of the treatments just 1 d after harvest. In both experimental seasons, differences observed between treatments disappeared in the subsequent date of measurements around a month later (Fig. 2).

The results obtained in the late-maturing orange 'Navel Lane Late' show that control trees had in general higher g_s values than the rest of the treatments during the first experimental season (Fig. 2). Statistically significant differences in g_s were obtained between NTHP and the rest of treatments 2 d after the ethephon applications on 17 Mar. Five days later, no significant differences were found between treatments (Table 4). Moreover, measurements performed just after harvest during 2010 and 1 d after the ethephon applications in 2011 showed that nontreated trees (NTHP and NTMH treatments) had significantly higher g_s than ethephon-treated trees.

Effects on A_{CO2}. As reported for the g_s measurements, A_{CO2} values were similar between all the trees within each orchard at the beginning of the experiment (Fig. 3). Ethephon applications and mechanical harvest did not have a significant decreasing effect on A_{CO2} in 'Clemenules' and 'Fortune' orchards (Table 5). There was no clear effect of ethephon and using a trunk shaker on A_{CO2} in the other mandarin cultivars studied. In the

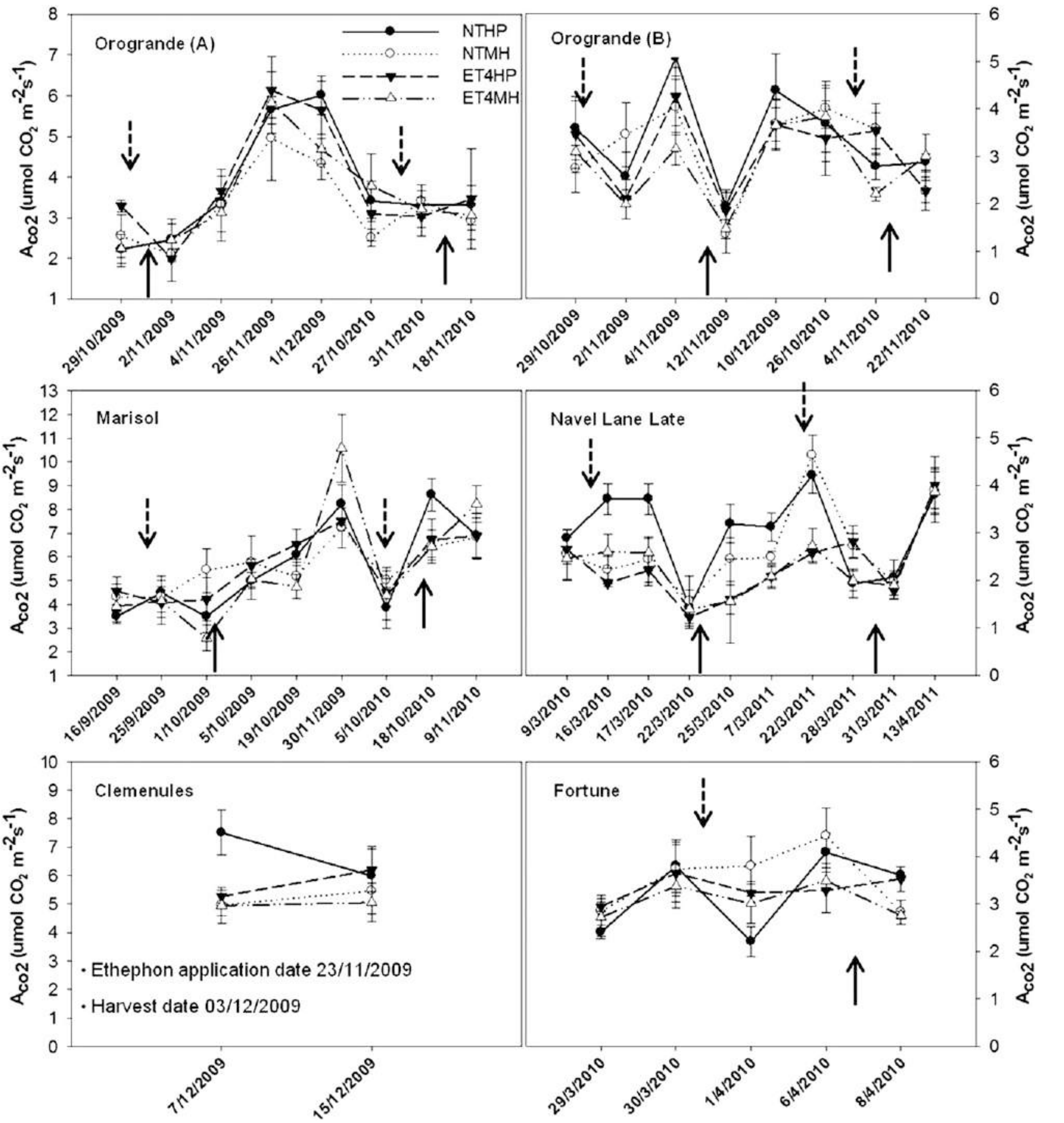

Fig. 3. Net assimilation of CO_2 (A_{CO2}, μmol $CO_2/m^2/s$) in the nonethephon-treated hand-picked (NTHP), nonethephon-treated mechanically harvested (NTMH), ethephon-treated hand-picked (ET4HP), and ethephon-treated mechanically harvested (ET4MH) treatments within each of the citrus cultivars studied. Vertical bars show the SE. Downward arrows depict the date of ethephon applications. Upward arrows indicate the date of harvest.

'Orogrande' A orchard, hand-picked trees had significantly higher A_{CO2} values than mechanically harvested trees on 1 Dec. 2009 (Table 5). No differences, however, were observed during the three previous measurements after the harvest. In the 'Orogrande' B orchard, ET4MH trees had the lowest A_{CO2} values just after the ethephon application in 2010 while the other treatment trees sprayed with ethephon (ET4HP) had the highest (Fig. 3). The opposite was observed after harvest (11 Nov. 2010), when NTMH and ET4HP showed the lowest A_{CO2} values and NTHP and ET4MH the highest. Similar results were obtained during the first experimental season in the 'Marisol' orchard, where NTMH trees had the highest A_{CO2} values, ET4MH trees had the lowest values, and NTHP and ET4HP had similar values. In 2010, on the other hand, control trees (NTHP) had significantly higher values than the other treatments 1 d after harvest. These differences were not evident in the subsequent measurement 3 weeks later (Table 5).

Contrary to the results obtained in the mandarin orchards, ethephon applications had a reducing effect of A_{CO2} in 'Navel Lane Late' trees (Fig. 3). In 2010, the NTHP treatment had significantly higher A_{CO2} values than the rest of the treatments after the ethephon applications (Table 5). When harvest took place, the differences were more evident between ethephon-treated and non-treated trees since both NTHP and NTMH trees showed statistically significant differences with the ET4MH treatment. The leaf gas exchange measurements taken in this orchard after the ethephon applications during the second experimental season also

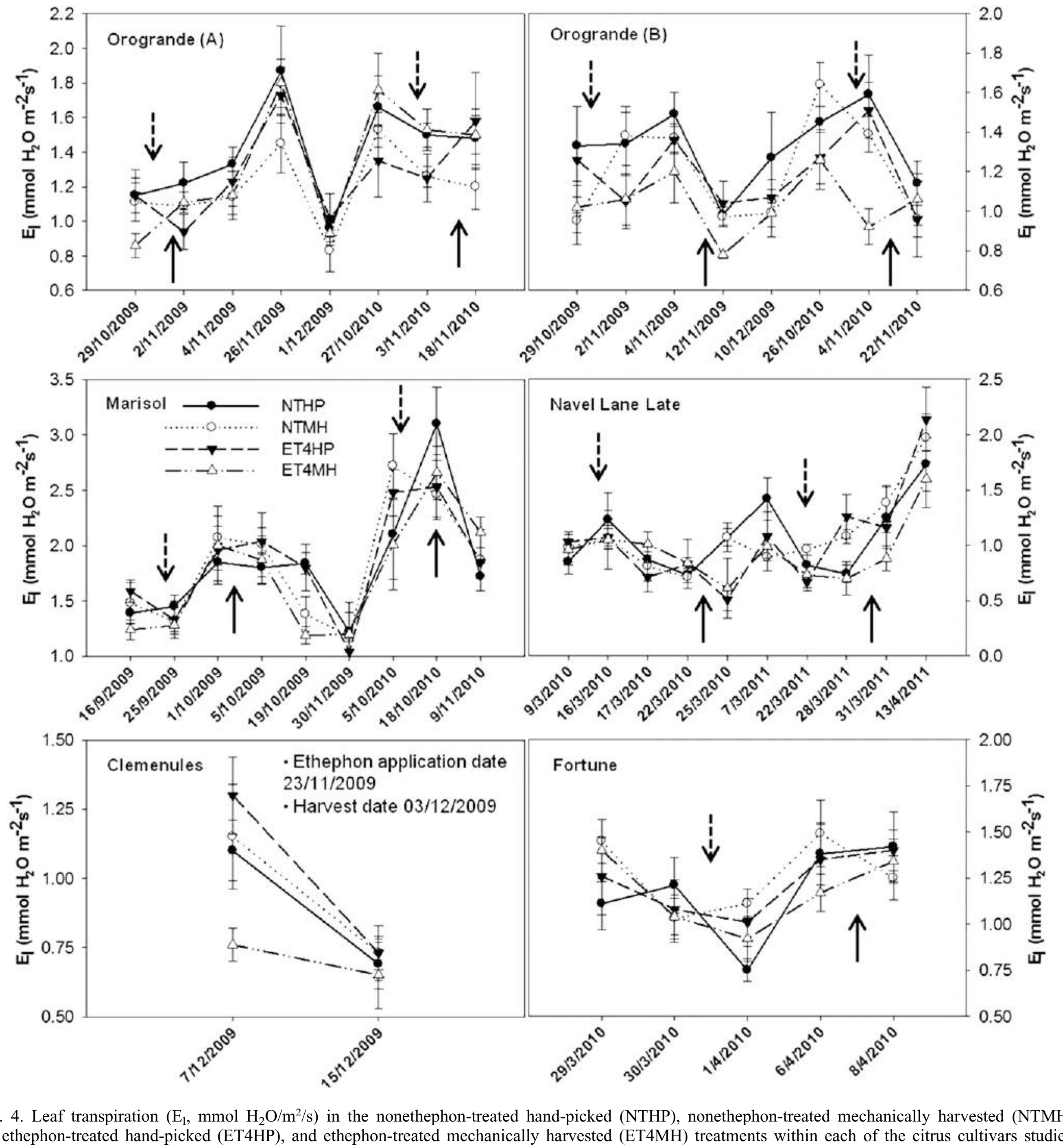

Fig. 4. Leaf transpiration (E_l, mmol $H_2O/m^2/s$) in the nonethephon-treated hand-picked (NTHP), nonethephon-treated mechanically harvested (NTMH), ethephon-treated hand-picked (ET4HP), and ethephon-treated mechanically harvested (ET4MH) treatments within each of the citrus cultivars studied. Vertical bars show the SE. Downward arrows depict the date of ethephon applications. Upward arrows indicate the date of harvest.

revealed statistically significant differences in A_{CO2} between ethephon-treated trees (on average 2.65 μmol $CO_2/m^2/s$) and nontreated trees (4.42 μmol $CO_2/m^2/s$). No differences were observed after harvest.

Effects on E_l. Concerning E_l, neither ethephon treatment nor the harvest method had any effect on this parameter in the 'Clemenules' and 'Fortune' orchards (Fig. 4; Table 6). No clear effects were observed in the 'Orogrande' A orchard, where ET4MH trees had significant lower E_l values than the other treatments even before the ethephon applications. Once trees were sprayed with the ethephon, the NTHP treatment had the highest E_l values with statistically significant differences even with the NTMH treatment. Similarly, no clear effects on E_l were observed in the 'Marisol' orchard, where statistically significant differences between hand-picked and mechanically harvested trees were only observed in a punctual day (19 Oct. 2009) but not just after harvest or in the subsequent measurement.

In the 'Orogrande' B orchard, ethephon applications and mechanical harvest had a decreasing effect on E_l during 2009. Three days after the ethephon applications, ethephon-treated trees (ET4HP and ET4MH) had significant lower values of E_l than nontreated trees. After harvest in 2009 and after the ethephon applications in 2010, ET4MH trees had significantly lower values than the other treatments (Fig. 4; Table 6).

No differences in E_l were obtained between treatments when 'Navel Lane Late' trees were sprayed with ethephon in 2010, although statistically significant differences arose between ethephon-treated and nontreated trees when harvest took place. In 2011, ET4HP had significantly higher E_l values than NTHP trees but also than the ET4MH treatment (Table 6).

Table 3. Results of analyses of variance conducted for the studies of the effect of treatment on the stem water potential (Ψ_{stem}) in 'Orogrande' A, 'Orogrande' B, 'Marisol', 'Clemenules', 'Fortune', and 'Navel Lane Late' orchards at the dates when visual differences were previously observed.

							Ψ_{stem} (MPa)			
Orchard	Date	DAE	DAH	F	df	*P* value	NTHP	NTMH	ET4HP	ET4MH
Orogrande A	13 Nov. 2008	8	2	1.78	3, 19	0.1912				
	4 Aug. 2009	—	—	2.07	3, 19	0.1453				
	3 Nov. 2009	3	—	1.18	3, 17	0.3524				
	3 Nov. 2010	1	—	2.61	3, 19	0.0872				
	18 Nov. 2010	16	6	0.72	3, 19	0.5533				
Orogrande B	11 Nov. 2009	11	0	1.77	3, 19	0.1933				
	2 Feb. 2010	—	—	1.98	3, 17	0.1630				
Marisol	1 Oct. 2009	6	—	3.42	3, 18	0.0446	–0.98 ± 0.03 ab	–1.05 ± 0.07 b	–0.84 ± 0.04 a	–0.80 ± 0.08 a
	19 Oct. 2009	25	17	9.30	3, 16	0.0015	–1.59 ± 0.11 b	–1.16 ± 0.15 a	–1.79 ± 0.03 b	–1.84 ± 0.05 b
	9 Nov. 2010	34	46	1.33	3, 19	0.3003				
Clemenules	NVD			NVD	NVD	NVD				
Fortune	6 Apr. 2010	6	—	4.12	3, 18	0.0256	–0.95 ± 0.03 b	–0.88 ± 0.03 ab	–0.80 ± 0.03 a	–0.85 ± 0.05 a
	30 Aug. 2010	—	—	5.12	3, 16	0.0148	–1.56 ± 0.07 b	–1.39 ± 0.02 ab	–1.24 ± 0.06 a	–1.41 ± 0.06 ab
Navel Lane Late	16 Mar. 2010	1	—	12.60	3, 17	0.0004	–0.97 ± 0.04 b	–0.89 ± 0.08 b	–0.81 ± 0.04 b	–0.50 ± 0.07 a

In the orchards where no visual differences were observed, it is indicated as NVD (no visual differences).
DAE = days after ethephon application; DAH = days after harvest; ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees; NTHP = nonethephon-treated and hand-picked trees; NTMH = nonethephon-treated and mechanically harvested trees.
[z]Mean ± SE followed by different letter in the same date were significantly different at $P < 0.05$ for least square difference test.

Table 4. Results of analyses of variance conducted for the studies of the effect of treatment on stomatal conductance (g_s) in 'Orogrande' A, 'Orogrande' B, 'Marisol', 'Clemenules', 'Fortune', and 'Navel Lane Late' orchards at the dates when visual differences were previously observed.

							g_s (mmol $CO_2/m^2/s$)[z]			
Orchard	Date	DAE	DAH	F	df	*P* value	NTHP	NTMH	ET4HP	ET4MH
Orogrande A	3 Nov. 2008	—	—	0.75	3, 19	0.5359				
	9 Mar. 2009	—	—	5.04	3, 19	0.0120	0.02 ± 0.00 b	0.02 ± 0.00 b	0.04 ± 0.00 a	0.04 ± 0.01 a
	29 Oct. 2009	—	—	0.98	3, 18	0.4276				
Orogrande B	NVD			NVD	NVD	NVD				
Marisol	19 Oct. 2009	25	17	3.54	3, 17	0.0427	0.10 ± 0.01 a	0.07 ± 0.01 ab	0.09 ± 0.01 a	0.06 ± 0.01 b
	19 Oct. 2010	13	1	3.42	3, 18	0.0447	0.16 ± 0.02 a	0.09 ± 0.01 b	0.10 ± 0.23 b	0.10 ± 0.01 b
	9 Nov. 2010	33	21	0.43	3, 19	0.7333				
Clemenules	17 Nov. 2009	—	—	2.89	3, 18	0.0704				
	25 Nov. 2009	2	—	4.88	3, 18	0.0145	0.03 ± 0.00 b	0.05 ± 0.00 a	0.03 ± 0.01 ab	0.04 ± 0.00 b
	7 Dec. 2009	14	4	0.65	3, 14	0.5978				
Fortune	1 Apr. 2010	1	—	1.65	3, 19	0.2174				
Navel Lane Late	16 Mar. 2010	1	—	2.57	3, 19	0.0902				
	17 Mar. 2010	2	—	3.62	3, 18	0.0380	0.07 ± 0.01 a	0.04 ± 0.00 b	0.04 ± 0.01 b	0.04 ± 0.05 b
	22 Mar. 2010	7	—	0.16	3, 10	0.9212				
	25 Mar. 2010	10	2	6.74	3, 17	0.048	0.06 ± 0.01 a	0.05 ± 0.01 a	0.02 ± 0.00 b	0.02 ± 0.01 b
	7 Mar. 2011	—	—	1.85	3, 19	0.1783				
	23 Mar. 2011	1	—	8.13	3, 19	0.0016	0.06 ± 0.01 a	0.08 ± 0.01 a	0.04 ± 0.00 b	0.04 ± 0.01 b

In the orchards where no visual differences were observed, it is indicated as NVD (no visual differences).
DAH = days after harvest; DAE = days after ethephon application; ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees; NTHP = nonethephon-treated and hand-picked trees; NTMH = nonethephon-treated and mechanically harvested trees.
[z]Mean ± SE followed by different letter in the same date were significantly different at $P < 0.05$.

Effects of ethephon dosage on yield from the current season and ethephon combined with trunk shaking on yield from the subsequent season

No significant differences on yield remained on the tree were observed during the first experimental season between ethephon-treated trees (ET1MH, ET2MH, ET3MH, and ET4MH) and nontreated trees (NTMH) at any of the dosage evaluated in any of the varieties studied (Table 7).

Regarding the effect of ethephon applications and trunk shaking on yield from the subsequent season in the 'Marisol' orchard no significant effects were observed regardless of the harvest technique used (Tables 8 and 9). In the 'Orogrande' A orchard, increasing doses of ethephon applied during the first experimental season had a statistically significant decreasing effect on yield from the subsequent season regardless of the harvest technique used. However, ethephon applications during the 2009–10 season had no effect on yield from the third and last experimental season (Tables 8 and 9). Similar results were obtained in the 'Orogrande' B orchard in which only increasing doses of ethephon applications during the 2010–11 season had a significant decreasing effect on yield obtained in mechanically harvested trees during the 2011–12 season.

In the case of the 'Navel Lane Late' orchard, a quadratic relation was obtained between dose of ethephon and yield obtained during the second experimental season (2010–11). Ethephon dose had a significant decreasing effect on yield, this reduction being dependent on the harvest technique employed (Tables 8 and 9).

Yield losses were estimated using the regressions coefficients, and a short economic assessment of these yield losses was carried out and the results are shown in Table 10.

Discussion

The generally lower Ψ_{stem} values recorded in the 'Orogrande' A, 'Orogrande' B, 'Marisol', and 'Clemenules' orchards in comparison with those obtained in the 'Fortune' and 'Navel Lane Late' orchards were most likely because most of the measurements performed in these latter orchards were taken during winter time, when harvest takes place for these cultivars and when a decrease in the water status of citrus trees is often observed (Intrigliolo et al., 2008) as a consequence of a decrease in the soil temperature (Barkataki et al., 2013). In the case of the orchards planted with 'Orogrande' (A and B), low Ψ_{stem} values were also recorded during summer, around –1.4 MPa, which could be considered as a value indicative of some moderate water stress in citrus trees (Ballester et al., 2014).

Inappropriate operational conditions during mechanical harvest may provoke serious

Table 5. Results of analyses of variance conducted for the studies of the effect of treatment on net assimilation of CO_2 (A_{CO2}) in ‘Orogrande’ A, ‘Orogrande’ B, ‘Marisol’, ‘Clemenules’, ‘Fortune’, and ‘Navel Lane Late’ orchards at the dates when visual differences were previously observed.

							A_{CO2} (μmol $CO_2/m^2/s$)[z]			
Orchard	Date	DAE	DAH	F	df	*P* value	NTHP	NTMH	ET4HP	ET4MH
Orogrande A	29 Oct. 2009	—	—	1.41	3, 18	0.2778				
	1 Dec. 2009	30	19	4.09	3, 17	0.0280	6.01 ± 0.48 ab	4.33 ± 0.38 c	6.45 ± 0.89 a	4.68 ± 0.37 bc
	27 Oct. 2010	—	—	0.85	3, 19	0.4871				
Orogrande B	2 Oct. 2009	2	—	1.89	3, 19	0.1717				
	4 Nov. 2009	4	—	2.56	3, 19	0.0915				
	4 Nov. 2010	2	—	4.85	3, 18	0.0149	2.79 ± 0.28 ab	3.05 ± 0.16 a	3.54 ± 0.36 a	2.21 ± 0.14 b
	22 Nov. 2010	20	10	3.44	3, 17	0.0462	2.88 ± 0.14 a	2.25 ± 0.23 b	2.89 ± 0.09 a	2.66 ± 0.385 ab
Marisol	1 Oct. 2009	7	—	3.66	3, 17	0.0390	3.49 ± 0.67 b	6.16 ± 0.74 a	4.21 ± 0.91 ab	2.58 ± 0.55 b
	19 Oct. 2009	25	17	2.22	3, 19	0.1249				
	30 Nov. 2009	66	58	0.54	3, 19	0.6603				
	19 Oct. 2010	13	1	3.79	3, 17	0.0351	8.61 ± 0.67 a	6.43 ± 0.46 b	5.91 ± 0.45 b	6.43 ± 0.70 b
Clemenules	7 Dec. 2009	14	4	3.04	3, 12	0.0852				
Fortune	1 Apr. 2010	1	—	2.39	3, 19	0.1072				
	8 Apr. 2010	8	1	2.75	3, 17	0.0823				
Navel Lane Late	16 Mar. 2010	1	—	7.50	3, 19	0.0024	3.71 ± 0.32 a	2.21 ± 0.29 b	1.94 ± 0.08 b	2.60 ± 0.36 b
	17 Mar. 2010	2	—	3.45	3, 19	0.0417	3.71 ± 0.32 a	2.43 ± 0.48 b	2.20 ± 0.32 b	2.58 ± 0.30 b
	25 Mar. 2010	10	2	5.28	3, 16	0.0133	3.19 ± 0.40 a	2.44 ± 0.46 ab	1.60 ± 0.31 bc	0.69 ± 0.26 c
	7 Mar. 2011	—	—	4.81	3, 19	0.0142	3.12 ± 0.30 a	2.48 ± 0.12 ab	2.08 ± 0.21 b	2.07 ± 0.24 b
	23 Mar. 2011	1	—	9.05	3, 19	0.0010	4.21 ± 0.37 a	4.63 ± 0.42 a	2.57 ± 0.18 b	2.72 ± 0.37 b
	28 Mar. 2011	6	—	4.92	3, 18	0.0141	1.64 ± 0.05 c	2.72 ± 0.26 ab	2.81 ± 0.34 a	1.98 ± 0.21 bc

DAH = days after harvest; DAE = days after ethephon application; ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees; NTHP = nonethephon-treated and hand-picked trees; NTMH = nonethephon-treated and mechanically harvested trees.
[z]Mean ± SE followed by different letter in the same date were significantly different at $P < 0.05$.

Table 6. Results of analyses of variance conducted for the studies of the effect of treatment on the leaf transpiration (E_l) in ‘Orogrande’ A, ‘Orogrande’ B, ‘Marisol’, ‘Clemenules’, ‘Fortune’, and ‘Navel Lane Late’ orchards at the dates when visual differences were previously observed.

							E_l (mmol $H_2O/m^2/s$)[z]			
Orchard	Date	DAE	DAH	F	df	*P* value	NTHP	NTMH	ET4HP	ET4MH
Orogrande A	29 Oct. 2009	—	—	4.61	3, 17	0.0191	1.29 ± 0.08 a	1.10 ± 0.11 ab	1.15 ± 0.10 a	0.86 ± 0.07 b
	2 Nov. 2009	3	—	9.26	3, 17	0.0013	1.32 ± 0.09 a	1.09 ± 0.02 b	0.85 ± 0.04 c	1.11 ± 0.06 b
	26 Nov. 2009	27	15	0.94	3, 19	0.4430				
	27 Oct. 2010	—	—	0.92	3, 19	0.4528				
	3 Nov. 2010	1	—	2.18	3, 19	0.1304				
Orogrande B	29 Oct. 2009	—	—	1.07	3, 17	0.3949				
	2 Nov. 2009	3	—	3.49	3, 16	0.0470	1.53 ± 0.20 a	1.51 ± 0.10 a	1.05 ± 0.14 b	1.06 ± 0.13 b
	12 Nov. 2009	13	1	7.73	3, 8	0.0252	0.98 ± 0.06 b	0.97 ± 0.05 b	1.25 ± 0.00 a	0.78 ± 0.02 c
	26 Oct. 2010	—	—	4.44	3, 17	0.0216	1.29 ± 0.12 b	1.64 ± 0.11 a	1.27 ± 0.13 b	1.11 ± 0.01 b
	4 Nov. 2010	2	—	5.67	3, 17	0.0093	1.59 ± 0.17 a	1.39 ± 0.09 a	1.51 ± 0.14 a	0.92 ± 0.09 b
Marisol	16 Sept. 2009	—	—	3.04	3, 17	0.0643				
	19 Oct. 2009	25	17	6.10	3, 18	0.0064	1.84 ± 0.10 a	1.38 ± 0.16 bc	1.60 ± 0.10 ab	1.19 ± 0.08 c
Clemenules	7 Dec. 2009	14	4	2.22	3, 14	0.1429				
Fortune	29 Mar. 2010	—	—	0.84	3, 19	0.4932				
	1 Apr. 2010	1	—	2.34	3, 19	0.1120				
	6 Apr. 2010	6	—	0.64	3, 19	0.5986				
Navel Lane Late	25 Mar. 2010	10	2	5.36	3, 17	0.0114	1.61 ± 0.08 a	1.07 ± 0.13 a	0.51 ± 0.10 b	0.61 ± 0.27 b
	7 Mar. 2011	—	—	1.85	3, 19	0.1733				
	28 Mar. 2011	6	—	4.09	3, 19	0.0248	0.74 ± 0.08 b	1.08 ± 0.06 ab	1.26 ± 0.20 a	0.70 ± 0.15 b
	31 Mar. 2011	9	1	2.55	3, 17	0.0973				

DAH = days after harvest; DAE = days after ethephon application; ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees; NTHP = nonethephon-treated and hand-picked trees; NTMH = nonethephon-treated and mechanically harvested trees.
[z]Mean ± SE followed by different letter in the same date are significantly different at $P < 0.05$.

canopy or root damages depending on the method employed, which can directly affect some physiological functions of the trees. Studies performed in Florida on ‘Hamlin’ and ‘Valencia’ orange trees by Li and Syvertsen (2005) detected a decrease in Ψ_{stem} in trees harvested with an excessive shaking time. In the present experiment, Ψ_{stem} was not detrimentally affected by shaking the trees in any of the varieties studied (Fig. 1; Table 3), which suggests that there was no critical root damage, and that the operating characteristics used for the trunk shaker in each orchard could be considered as appropriate.

Ethephon applications did not impair the plant water status of either the ‘Navel Lane Late’ trees or any of the mandarin cultivars studied. These results are in agreement with those reported by Li et al. (2006) in a study performed in Florida with ‘Hamlin’ orange trees, in which ethephon applications did not decrease Ψ_{stem} but improved it. Indeed, a short temporal increase in Ψ_{stem} was recorded in ethephon-treated trees in the present study after the ethephon applications in the ‘Marisol’, ‘Navel Lane Late’, and ‘Fortune’ orchards (Fig. 1). This short temporal improvement in Ψ_{stem} was probably related with the defoliation experienced by these trees as a consequence of the ethephon applications, which was recently reported in the work of Moreno et al. (2015), where detachment fruit and defoliation of the same trees used in this experiment were studied.

The g_s, A_{CO2}, and E_l evolution in each of the treatments assessed did not follow a similar trend for all the mandarin and the orange cultivars studied. Ethephon applications and mechanical harvest had not a clear effect on g_s, A_{CO2}, and E_l in the ‘Fortune’, ‘Clemenules’, and ‘Orogrande’ A orchards. In the ‘Orogrande’ B orchard, however, both treatments had a reducing effect on E_l although no effect was observed on g_s and A_{CO2}. Finally, a short temporal decreasing trend of these parameters was observed in the ‘Marisol’ and ‘Navel Lane Late’ trees as a consequence of both ethephon applications and trunk shaking. Control treatment (NTHP) in ‘Navel

Table 7. Linear regression significance for yield (kg/tree) as a function of *ethephon dose* (mg/tree) in mechanically harvested trees in 'Orogrande' A, 'Orogrande' B, 'Marisol', 'Clemenules', 'Fortune', and 'Navel Lane Late' orchards in the first season of the trial. Yield (Mean ± SE) obtained for each ethephon dose in each orchard.

			Yield (kg/tree)				
Orchard	Season	Model significance[z]	NTMH	ET1MH	ET2MH	ET3MH	ET4MH
Orogrande A	2008–09	F = 0.41; df = 1, 23; *P* = 0.5304	65.86 ± 3.53	65.70 ± 9.97	59.96 ± 4.55	62.97 ± 4.83	61.42 ± 4.06
Orogrande B	2009–10	F = 0.51; df = 1, 24; *P* = 0.4812	56.19 ± 5.91	43.49 ± 6.91	55.68 ± 5.78	47.74 ± 4.35	47.89 ± 3.09
Marisol	2009–10	F = 0.56; df = 1, 24; *P* = 0.4622	64.90 ± 17.20	72.27 ± 12.85	58.46 ± 5.62	51.18 ± 13.65	58.26 ± 7.47
Clemenules	2009–10	F = 0.80; df = 1, 24; *P* = 0.3809	37.75 ± 8.43	26.04 ± 6.42	23.53 ± 3.46	33.50 ± 4.59	43.54 ± 7.33
Fortune	2009–10	F = 0.05; df = 1, 24; *P* = 0.8310	33.70 ± 11.05	43.50 ± 7.86	41.89 ± 7.76	50.55 ± 11.52	35.27 ± 6.71
Navel Lane Late	2009–10	F = 0.03; df = 1, 24; *P* = 0.8561	61.69 ± 5.49	68.75 ± 5.86	56.54 ± 8.66	67.04 ± 6.34	61.53 ± 8.03

ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees; NTHP = nonethephon-treated and hand-picked trees; NTMH = non ethephon-treated and mechanically harvested trees.

[z]When $P > 0.05$, there is no significant linear relationship between yield (in the first year of application) and ethephon dose.

Table 8. Yield (kg/tree) (Mean ± SE) obtained for the different treatments in 'Orogrande' A, 'Orogrande' B, 'Marisol', and 'Navel Lane Late' orchards in the next season from the applications.

			Yield (kg/tree)				
Orchard	Season	Harvest technique	NT	ET1	ET2	ET3	ET4
Orogrande A	2009–10	HP	57.77 ± 7.41	58.86 ± 7.92	45.85 ± 8.50	42.33 ± 6.39	30.52 ± 6.39
		MH	42.78 ± 2.52	46.72 ± 10.73	35.92 ± 7.79	31.51 ± 7.97	30.96 ± 6.66
	2010–11	HP	73.11 ± 8.90	86.98 ± 10.32	74.85 ± 13.91	62.93 ± 8.14	64.91 ± 7.09
		MH	64.99 ± 3.78	74.39 ± 11.37	64.13 ± 7.35	69.65 ± 7.63	70.16 ± 8.39
Orogrande B	2010–11	HP	73.46 ± 9.07	51.83 ± 7.67	69.19 ± 14.85	72.80 ± 9.33	66.86 ± 12.71
		MH	77.98 ± 10.98	59.72 ± 8.59	79.50 ± 9.92	63.04 ± 6.30	59.01 ± 9.43
	2011–12	HP	69.01 ± 4.96	51.36 ± 4.55	57.19 ± 10.91	68.47 ± 12.00	58.34 ± 8.25
		MH	68.13 ± 3.79	55.13 ± 4.92	61.91 ± 3.99	55.96 ± 10.49	38.77 ± 5.13
Marisol	2010–11	HP	70.85 ± 13.11	91.68 ± 19.27	74.70 ± 15.75	84.97 ± 10.85	81.27 ± 7.22
		MH	93.26 ± 19.80	87.54 ± 18.38	60.65 ± 14.22	82.18 ± 19.10	62.98 ± 9.57
Navel Lane Late	2010–11	HP	110.79 ± 5.07	50.80 ± 6.53	43.08 ± 11.85	34.27 ± 6.45	15.59 ± 4.67
		MH	73.03 ± 7.54	41.68 ± 6.65	46.74 ± 1.12	24.28 ± 8.74	18.57 ± 4.87

ET = ethephon-treated trees; HP = hand-picked trees; MH = mechanically harvested trees.

Table 9. Results obtained from the multiple linear regressions (MLR): model significance and regression coefficients for yield (kg/tree) (Mean ± SE) as a function of *ethephon dose* (mg/tree) and *harvest technique* in 'Orogrande' A, 'Orogrande' B, 'Marisol', and 'Navel Lane Late' orchards in the next season of the trial.

			Regression coefficients			
Orchard	Season	Model significance[z]	Parameter	Estimate	*t* statistic	*P* value
Orogrande A	2009–10	F = 10.02; df = 1, 48; *P* = 0.0027	Constant	52.65	12.96	<0.0001
			Dose	–0.0026	–3.17	0.0027
	2010–11	F = 0.49; df =1, 48; *P* = 0.4869	—	—	—	—
Orogrande B	2010–11	F = 0.43; df =1, 48; *P* = 0.5153	—	—	—	—
	2011–12	F = 6.70; df =1, 48; *P* = 0.0127	Constant	62.76	21.70	<0.0001
			Dose × (Harvest technique = mechanical harvest)[y]	–0.0022	–2.59	0.0127
Marisol	2010–11	F = 1.88; df = 1, 49; *P* = 0.1767	—	—	—	—
Navel Lane Late	2010–11	F = 32.01; df = 0, 49; *P* < 0.0001	Constant	103.39	16.19	<0.0001
			Dose	–0.0221	–7.09	<0.0001
			$Dose^2$	0.0000013	3.46	0.0012
			(Harvest technique = mechanical harvest)[y]	–27.56	–3.42	0.0013
			Dose × (Harvest technique = Mechanical harvest)[y]	0.0051	2.65	0.0111

[z]F = F-ratio, df = degrees of freedom, *P* = *P* value. When $P > 0.05$, there is no significant linear relationship between yield (of the subsequent year) and ethephon dose. In this case, regression coefficients are not shown.

[y](Harvest technique = mechanical harvest) = 1 for data obtained with mechanical harvest, 0 otherwise.

Lane Late' trees had higher g_s and A_{CO2} values than the rest of the treatments during most of the experiment. This result could be explained by the high crop load of 'Navel Lane Late' trees in comparison with the other cultivars studied in which ethephon applications and mechanical harvest promoted fruit detachment (Moreno et al., 2015). The higher crop load in this cultivar could stimulate leaf gas exchange because of the higher photoassimilates demand (Nebauer et al., 2011).

Notwithstanding the temporal decrease observed in the physiological parameters studied in some of the mandarin orchards, fruit yield from the current season was not detrimentally affected by mechanical harvest in any case. No consistent results were obtained within mandarin cultivars regarding the effect of the ethephon dosage on yield from the subsequent seasons. Effects of ethephon treatments on citrus trees may be highly variable and temperature dependent (Yuan and Burns, 2004). Different responses depending on the harvest technique employed can be attributed not only to the intrinsic physiological response of each variety but also to the differences in the orchard characteristics, that is the reason why treatments were compared with a control (NTHP) in each of the varieties studied.

Similarly to what was observed in all the mandarin orchards, mechanical harvest combined with ethephon as an abscission agent in the 'Navel Lane Late' orchard did not decrease fruit yield during the first experimental season. Different results were obtained in the subsequent seasons when yield of 'Navel Lane Late' trees significantly decreased due to the ethephon applications and mechanical harvest. This higher sensitivity of 'Navel Lane Late' orange cultivar than all the mandarin cultivars to the ethephon and mechanical harvest treatments was expected since, as a late-maturing orange cultivar, at the moment of harvest, mature fruit from the

Table 10. Yield losses estimated using the regressions coefficients and a short economic assessment of these yield losses.

		Ethephon dose (mg/tree)				
		0	*2,400*	*4,200*	*4,800*	*8,400*
Orogrande A[x] season 2009–10	Yield loss (kg/ha)[z]	0	2,496	4,368	4,992	8,736
	Economic loss (€/ha)[y]	0	6,864	12,012	13,728	24,024
Orogrande B[w] season 2011–12	Yield loss (kg/ha)[z]	0	2,112	3,696	4,224	7,392
	Economic loss (€/ha)[y]	0	5,808	10,164	11,616	20,328
Navel Lane Late[v] season 2010–11		*0*	*2,100*	*3,600*	*4,200*	*7,200*
	Yield loss (kg/ha)[z]	11,024	119,868	177,408	193,872	220,032
	Economic loss (€/ha)[y]	272,2928	29,607,396	43,819,776	47,886,384	54,347,904

[z]For the estimation, the number of trees per hectare was considered 400.
[y]For the estimation of economic loss, the price for fresh 'Orogrande' was considered 0.275 €/kg and for fresh 'Navel Lane Late' 0.247 €/kg.
[x]The yield and economic loss only affect trees treated with ethephon.
[w]The yield and economic loss only affect trees treated with ethephon and harvested mechanically.
[v]The yield and economic loss is due to both ethephon and harvesting with trunk shaker.

current season usually coexists with fruitlets of the subsequent season. The use of ethephon as an abscission agent and a trunk shaker for harvesting in the 'Navel Lane Late' trees led to an increase of the number of fruitlet dropped and consequently to a decrease of yield in the subsequent season.

Yield losses have been reported in other studies related to the assessment of mechanical harvest machines in late-maturing orange trees (Hedden et al., 1984; Roka et al., 2005). In subtropical humid climates like the one characteristic of Florida, where the citrus flowering may be triggered by rainfall or irrigation events after a dry period, drought stress strategies applied in winter can be used to delay flowering. A delay of 3 to 4 weeks in flowering in late-maturing orange trees has been shown as an effective strategy to reduce the size of fruitlet at the moment of harvest, which significantly decrease fruit drop, avoiding any negative effect of mechanical harvest on the subsequent season yield (Melgar et al., 2010). In Mediterranean climatic conditions, however, drought stress strategies are not useful to mechanical harvest of late-maturing orange trees since flowering in dry subtropical regions is mainly induced by variations in the temperature.

Conclusions

Although similar studies have been performed in the agroclimatic conditions of Florida, this study is the first to provide evidence that ethephon applications and mechanical harvest with a trunk shaker did not detrimentally affect plant water status of citrus trees under Mediterranean conditions. These treatments either had no effect or had a short temporal decrease effect on leaf gas exchange depending on the cultivar studied, with no consequences for the fruit yield during the current season. The use of ethephon as an abscission agent, however, significantly decreased fruit yield in the subsequent season in late-maturing oranges like the Navel Lane Late cultivar studied here and may lead to reductions in fruit yield in the subsequent season in some mandarin cultivars when applied at high doses, as observed for the 'Orogrande' orchards.

Taking into account the results obtained from this experiment and those reported in the work of Moreno et al. (2015) regarding the effect of ethephon applications and mechanical harvest on fruit detachment and defoliation, authors are confident to recommend the use of trunk shakers to mechanically harvest citrus trees destined to both fresh and industry market under Mediterranean conditions, with the exception of late-maturing oranges in which mature fruits coexist with fruitlets at the time of harvest, and thus this technique lead to a significant reduction of fruit yield in the subsequent season. On the other hand, the use of ethephon could be recommended only for citrus destined to industry, with the exception of cultivars Clemenules and Fortune, because ethephon does not increase the fruit detachment (Moreno et al., 2015), and 'Navel Lane late' or others late-maturing oranges, due to its effect in the reduction of yield in the subsequent years. A research of collateral consequences of ethephon applications on the internal and external fruit quality is envisaged, which enables authors to do recommendations to the growers about the use of ethephon.

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