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Techniques for Reducing the Abundance of Spring–Summer Flush Shoots in Southern Spanish Orange Orchards

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Abstract: The Mediterranean Basin is the second highest citrus growing region in the world behind China. Citrus trees are known to produce several flush shoots per year, particularly during the spring–summer season. Farmers endeavor to reduce the growth of summer shoots by means of hand pruning, especially those located at the top of the tree, as most of these shoots become vigorous, nutrient consuming, non-productive, and attractive to several pests. Furthermore, hand pruning substantially increases the costs of citrus orchards production. This research was therefore intended to study new different treatments to control spring–summer flush shoots and thus reduce growers' investments in citrus production. Six different treatments were applied in two experimental and high density orange orchards over two consecutive years: (1) control; (2) topping (mechanical pruning); (3) dichlorprop-p; (4) triclopyr; (5) topping + dichlorprop-p; and (6) topping + triclopyr. The treatment of dichlorprop-p alone reduced the number of summer young shoots in both years. Moreover, these applications did not negatively affect yield or fruit quality. These mechanical methodologies help citrus growers manage the density of flush shoots and reduce hand labor costs in citrus orchards.

Keywords: auxins; citrus; high density orchards; topping

1. Introduction

Citrus are fruits crops that are widely grown in Mediterranean Basin countries, this region is the second highest citrus producing region worldwide behind China. According to the Food and Agriculture Organization (FAO), Spain, with a production higher than six millions of tons, it is the sixth citrus-producing country and the top exporter of fresh citrus [1].

In addition, the human population has grown from 1 billion to more than 7 billion nowadays, and it is expected to exceed 9 billion by 2050 [2]. This circumstance leads to urban expansion and reduction of agricultural land; agricultural production thus needs to increase in lower cropped areas [3]. As such, sustainable crop intensification is a positive resource under high density plantation by increasing yield in several fruit crops, such as citrus. High density citrus orchards require an initial high investment, which can be recovered with higher yield and lower costs of mechanical operations [4].

Citrus trees can produce several young shoots (three or four times a year), but spring–summer flush shoots are the most numerous [5]. In Spain, flush shoots abundance was higher during spring, totaling 51% to 96% for sweet oranges and mandarins, respectively [6]. This shoot incidence is usually synchronized with all trees at the same time. However, young trees can exhibit less synchronized periods of flush shoots growth than older trees, and additionally the flushing period of is more continuous for younger trees [7].

The application of auxins in citrus crops has previously been described as fruit development regulators [8]. In addition, these compounds can inhibit shoot growth by citrus trees [9,10]. Conventionally, citrus growers have used hand pruning to reduce the amount of young vegetative and non-productive vigorous shoots.

Further, new trends involving higher density production systems are leading to the implementation of mechanical pruning in citrus crops, which is reported to be a more leasable technique than traditional hand pruning [11,12]. Mechanical pruning contributes to reducing the costs compared to conventional hand labor in citrus orchards.

On the other hand, certain citrus insect pests are exclusively developed and fed during young shoot flushing period, and they can even spread and/or induce citrus disease pathogens. Thus, the wounds caused by citrus leafminer (*Phyllocnistis citrella* Stainton) can increase the damage of citrus canker (*Xanthomonas axonopodis* pv. *citri* Hasse) [13–15]. Additionally, aphids such as spirea aphid (*Aphis spiraecola* Patch) and brown citrus aphid (*Toxopera citrida* Kirkaldy) are the main vectors of citrus tristeza virus [13,16]. *Trioza erytreae* is one of the main insect vectors of *Candidatus* Liberibacter spp., which is the causal agent of Huanglongbing or citrus greening disease [17,18]. These pests are clear examples of exclusive feeding of young shoots. Thus, different prior studies have assessed the influence of irrigation treatments on summer flush shoots as a control method against pests [19,20].

The aim of this work was to research new different treatments for reducing spring-summer flush shoots and helping growers reduce hand pruning in citrus production.

2. Materials and Methods

2.1. Plant Material and Experimental Design

The experiments were carried out in two experimental orange orchards covering an area of 3550 m^2 each, located in the Andalusian Institute of Agricultural and Fisheries Research and Training in the "Las Torres" Center, in the municipality of Alcalá del Río, Seville, Spain (37°30'49.3'' N; 5°57'53.0'' W). One orchard is comprised of the 'Lane Late' cultivar grafted on rootstock CIVAC19 ('Cleopatra' mandarin × *Poncirus trifoliata*) (Lane Late/CIVAC19), while the other orchard consists of the 'Valencia Delta Seedless' cultivar grafted on Forner-Alcaide no. 517 ('King' mandarin × *Poncirus trifoliata*) (Valencia DS/FA517). FA517 was obtained and registered under the number 20010062 by Instituto Valenciano de Investigaciones Agrarias (IVIA) [21]; whereas CIVAC19 was obtained and registered under the number US 2020/028864 P1 by Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia, Spain) and Agromillora Group nursery (Subirats, Barcelona, Spain) [22]. 'Lane Late' and 'Valencia Delta Seedless' cultivars are registered in the Spanish office of plant cultivars (Spanish Ministry of Agriculture, Fishery and Food; Madrid, Spain) under the numbers 11960036 and 19990349, respectively [21].

Both orchards were established under a high-density plantation system (2200 plants/ha) in April 2015, spaced at 3.5 m × 1.25 m, with an overall tree volume of 2.30 m^3, and recording the first bearing in 2018 for both orchards. Soil characteristics from both orchards are loam texture (19% clay, 39% sand and 42% silt), 0.77% organic matter, electrical conductivity (1:5 soil water extract) 0.167 dS/m, 5.31% active CaCO$_3$ and 8.7 soil pH (25 °C 1:5). Mediterranean climate (17.16 °C average temperature and 781.60 mm rainfall for the 2018 season; 17.99 °C average temperature and 265.20 mm rainfall for the 2019 season).

Crop management was followed by new recommended practices for high density systems [4], including drip irrigation, black net mulching, and mechanical pruning. Water requirements were calculated using evapotranspiration values (ET$_0$) and the citrus crop coefficient (K$_c$) [23]. Crop fertilization was conducted using a fertigation system program following the instructions reported by Quiñones et al. [24].

This work was carried out over two consecutive years (2018 and 2019). The design of each experimental orange orchards consisted of three randomized blocks (experimental plots) per treatment. Each block was composed of a 25-tree experimental plot.

2.2. Treatment Application and Shoot Evaluation

Six different treatments per each orchard were applied. Each treatment was applied in three randomized experimental plots, for a total of 75 trees per treatment. The treatments consisted of topping (mechanical pruning from the top of the tree), two individual synthetic auxins applications, such as dichlorprop-p (Dichlorprop-p 2.5% ec, Nufarm Spain S.A.; Barcelona, Spain), and triclopyr (Triclopyr 10% tb, Arysta LifeScience Benelux Sprl.; Ougrée, Liège, Belgium), and a combination of topping with each auxin compound and control treatment (neither topping nor auxin application). Dichlorprop-p (2,4-dichlorophenoxyacetic acid) is a phytoregulator that delays enzymatic activity in orange, mandarin, and lemon crops, and confers a protective effect upon the fall of fruits, which is registered in the official register of phytosanitary products (Spanish Ministry of Agriculture, Fishery and Food; Madrid, Spain) under the number 18926 and commercial name Clementgros Plus [21,25]. Triclopyr (3,5,6-trichloro-2-pyridinyloxyacetic acid) is a synthetic auxin to prevent pre-harvest drop and increase fruit size, which is registered in the official register of phytosanitary products (Spanish Ministry of Agriculture, Fishery and Food; Madrid, Spain) under the number 19684 and commercial name Maxim [21,26].

All treatments were applied in the first week of June per assayed year following the fruit harvest season. Synthetic auxins and topping treatments were applied using a mechanical sprayer and a mechanical pruner, respectively. Auxin treatments were carried out with a 150 mL/100 L concentration and a 1.5 tablet (15 g)/100 L for dichlorprop-p and triclopyr, respectively, at a volume rate of 2000 L/ha per auxin treatment.

Two shoot evaluations were carried out in July and September per year assayed (2018 and 2019) to gauge the inhibition capacity of different treatments on spring–summer and summer-autumn flush shoots, respectively. The quantification process was performed using a hoop of 56 cm in diameter, which was placed in the center (upper area), north and south of the tree canopy, with the new shoots in development (up to 50 cm in length, approximately) being counted in the area covered by the hoop in each of the three tree locations. In each experimental orchard, two trees were randomly selected and evaluated per experimental plot, which resulted in a total of six trees evaluated per treatment and evaluation period.

2.3. Fruit Production and Quality in 2020

Fruit sampling was carried out in February 2020 after two years of treatment application and nearly five years since tree plantation. A total of 12 trees were selected, with all fruits per tree being harvested and weighed using an industrial scale (Acculab Ltd., SVI-200F, Royal Road, Nouvelle-France, Mauritius) to obtain total production per tree in each treatment within each experimental orange orchard. Three samples, comprised of 14 fruits per sample, were collected to obtain the quality parameters: equatorial diameter (mm), height (mm), peel thickness (mm), weight (g), juice content (%; w/w), total soluble solids (TSS; °Brix), titratable acidity (TA; g/100 cm^3), and maturity index (MI = TSS/TA), following the procedure described by Hervalejo et al. [27].

2.4. Statistical Analysis

The raw values obtained from shoot evaluation were analyzed by two-way ANOVA and LSD-Fisher test ($p < 0.05$) [28] using free software R version 4.0.2 [29] through the "agricolae" package [30]. In addition, the raw values of production and quality parameters were subjected to one-way ANOVA and LSD-Fisher test ($p < 0.05$) [28] using the same software and package described above.

3. Results

3.1. Effect of Treatments on Shoot Flushing in 2018

During the first evaluation (July 2018), the topping treatment displayed the highest response for shoots in both experimental orange orchards, without significant differences between both orchards. On the contrary, the auxin treatment of dichlorprop-p reported the

lowest statistical response for shoots in both orchards compared with the topping treatment, and with control in the case of Lane Late/CIVAC19, but without statistical differences between orchards, neither in control treatment nor in the treatment of dichlorprop-p. The treatment control and topping + dichlorprop-p showed the second highest outcome for Lane Late/CIVAC19 and Valencia DS/FA517, respectively, without significant differences compared with the topping treatment. These two treatments were followed by triclopyr, topping + dichlorprop-p and topping + triclopyr in the case of Lane Late/CIVAC19, and by topping + triclopyr, triclopyr and control in the case of Valencia DS/FA517, with significant differences compared with the topping treatments (Table 1).

Table 1. Shoot number ± standard error (SE) under the effect of six different treatments (control, topping, dichlorprop-p, triclopyr, topping + dichlorprop-p and topping + triclopyr) in two experimental orchards with different rootstocks (CIVAC19 and Forner-Alcaide no. 517) and cultivars ('Lane Late' and 'Valencia Delta Seedless') for the evaluation of 2018.

Treatment	July		September	
	Lane Late/CIVAC19	Valencia DS/FA517	Lane Late/CIVAC19	Valencia DS/FA517
Control	4.33 ± 0.33 bcd	3.50 ± 0.22 cdef	7.50 ± 0.76 a	5.50 ± 0.34 abc
Topping	5.00 ± 1.00 abc	6.50 ± 0.72 a	3.17 ± 1.25 cd	4.00 ± 1.03 bcd
Dichlorprop-p	1.83 ± 0.40 f	2.17 ± 0.60 f	4.50 ± 1.26 bcd	2.60 ± 0.60 d
Triclopyr	3.00 ± 0.37 def	4.00 ± 0.68 cde	5.00 ± 0.89 bcd	7.50 ± 1.15 a
Topping + dichlorprop-p	2.83 ± 0.40 def	6.00 ± 0.86 ab	5.67 ± 0.76 ab	2.83 ± 0.48 d
Topping + triclopyr	2.50 ± 0.62 ef	4.33 ± 0.67 bcd	3.67 ± 0.49 bcd	2.67 ± 0.33 d
p value (Treatment)	<0.001		<0.001	
p value (Orchard)	0.002		0.001	
p value (Treatment: Orchard)	0.04		0.02	

Values with different letters are significantly different among treatments and between the orchards assayed according to the LSD-Fisher test ($p < 0.05$). Valencia DS: 'Valencia Delta Seedless'; FA517: Forner-Alcaide no. 517. *p* value from the last row (Treatment: Orchard) is the interaction between Treatment and Orchard.

In the September 2018 evaluation process, the highest value of shoots was reached with the control treatment and triclopyr for Lane Late/CIVAC19 and Valencia DS/FA517, respectively, without significant differences between both these treatments. A similar response was obtained from the control treatment and topping + dichlorprop-p in Valencia DS/FA517 and Lane Late/CIVAC19, respectively, without statistical differences between both these treatments and the highest shoot values.

This incidence was followed by the treatments triclopyr, dichlorprop-p and topping + triclopyr in Lane Late/CIVAC19, and by topping, topping + dichlorprop-p and topping + triclopyr in Valencia DS/FA517, with significant differences compared with the highest values of shoots. Therefore, topping and dichlorprop-p reported the lowest statistical value of shoots, compared with the highest rate for Lane Late/CIVAC19 and Valencia DS/FA517, respectively, without significant differences between both treatments (Table 1).

3.2. Effect of Treatments in Shoot Flushing in 2019

In the first 2019 evaluation (July), topping and control treatments showed the highest shoots rate for Lane Late/CIVAC19 and Valencia DS/FA517, respectively, without significant differences between both these treatments. This incidence was similar to the result obtained for topping + dichlorprop-p. On the other hand, the lowest shoot value was achieved with the auxin treatment of dichlorprop-p alone for both orchards, without significant differences between them, but with statistical differences compared with the highest shoot rate obtained. This incidence was followed by the triclopyr, control and topping + triclopyr treatments in the case of Lane Late/CIVAC19, and by the topping + triclopyr, topping + dichlorprop-p, triclopyr and topping treatments in the case of Valencia DS/FA517, without significant differences compared with the treatment of dichlorprop-p, but with statistical differences compared with the highest shoot rate (Table 2).

Table 2. Shoot number ± standard error (SE) under the effect of six different treatments (control, topping, dichlorprop-p, triclopyr, topping + dichlorprop-p and topping + triclopyr) in the two experimental orchards with different rootstocks (CIVAC19 and Forner-Alcaide no. 517) and cultivars ('Lane Late' and 'Valencia Delta Seedless') for the evaluations of 2019.

Treatment	July		September	
	Lane Late/CIVAC19	Valencia DS/FA517	Lane Late/CIVAC19	Valencia DS/FA517
Control	3.33 ± 0.71 bcd	6.29 ± 1.80 ab	1.60 ± 1.03 ab	0.33 ± 0.21 bc
Topping	7.00 ± 1.72 a	2.89 ± 0.75 cd	0.40 ± 0.24 bc	0.00 ± 0.00 c
Dichlorprop-p	1.33 ± 0.53 d	1.44 ± 0.80 d	0.20 ± 0.20 bc	2.17 ± 0.79 a
Triclopyr	2.00 ± 0.55 cd	1.67 ± 0.65 cd	2.60 ± 1.03 a	0.17 ± 0.17 c
Topping + dichlorprop-p	4.44 ± 1.76 abc	1.67 ± 0.69 cd	0.00 ± 0.00 c	0.33 ± 0.21 bc
Topping + triclopyr	3.89 ± 0.99 bcd	1.56 ± 0.47 d	0.40 ± 0.24 bc	0.33 ± 0.21 bc
p value (Treatment)	0.003		0.01	
p value (Orchard)	0.04		0.03	
p value (Treatment: Orchard)	0.02		0.001	

Values with different letters are significantly different among treatments and between the orchards assayed according to LSD-Fisher test ($p < 0.05$). Valencia DS: 'Valencia Delta Seedless'; FA517: Forner-Alcaide no. 517. *p* value from the last row (Treatment: Orchard) is the interaction between Treatment and Orchard.

Lastly, the auxin treatments of triclopyr and dichlorprop-p showed the highest shoot values in Lane Late/CIVAC19 and Valencia DS/FA517, respectively, in September, without significant differences between these treatments in both rootstocks. Similarly, the control treatment reported a high shoot rate in Lane Late/CIVAC19, without statistical differences compared with the highest values obtained. These high shoot values were followed by the topping, topping + triclopyr and dichlorprop-p treatments, with significant differences compared with triclopyr for Lane Late/CIVAC19, and by the control, topping + dichlorprop-p, topping + triclopyr and triclopyr treatments, with statistical differences compared with dichlorprop-p for Valencia DS/FA517. Hence, the lowest statistical response was achieved with the treatments of topping + dichlorprop-p and topping for Lane Late/CIVAC19 and Valencia DS/FA517, respectively, compared with the highest shoot rate obtained (Table 2).

3.3. Fruit Production and Quality in 2020

Following two years of treatment application, fruit production showed different responses among the different treatments according to orchard conditions. Hence, fruit production results were similar without significant differences for Lane Late/CIVAC19 (*p* value = 0.205), in which the highest value was obtained with the topping + triclopyr treatment, and the lowest one with the dichlorprop-p treatment. On the contrary, Valencia DS/FA517 displayed values with statistical differences among the treatments assayed (*p* value = 0.007), thus the treatment of topping + dichlorprop-p reported the highest fruit production rate. A similar response was obtained from the treatment of topping + triclopyr, without significant differences compared with the highest fruit production value. These results were followed by the treatments triclopyr, dichlorprop-p, control and topping, which were not statistically different, but showed significant differences compared with the treatment of topping + dichlorprop-p, with the lowest fruit production result being reached with the pruning treatment (Figure 1). The treatments carried out did not significantly alter the fruit quality parameters analyzed of each orchard (Table S1).
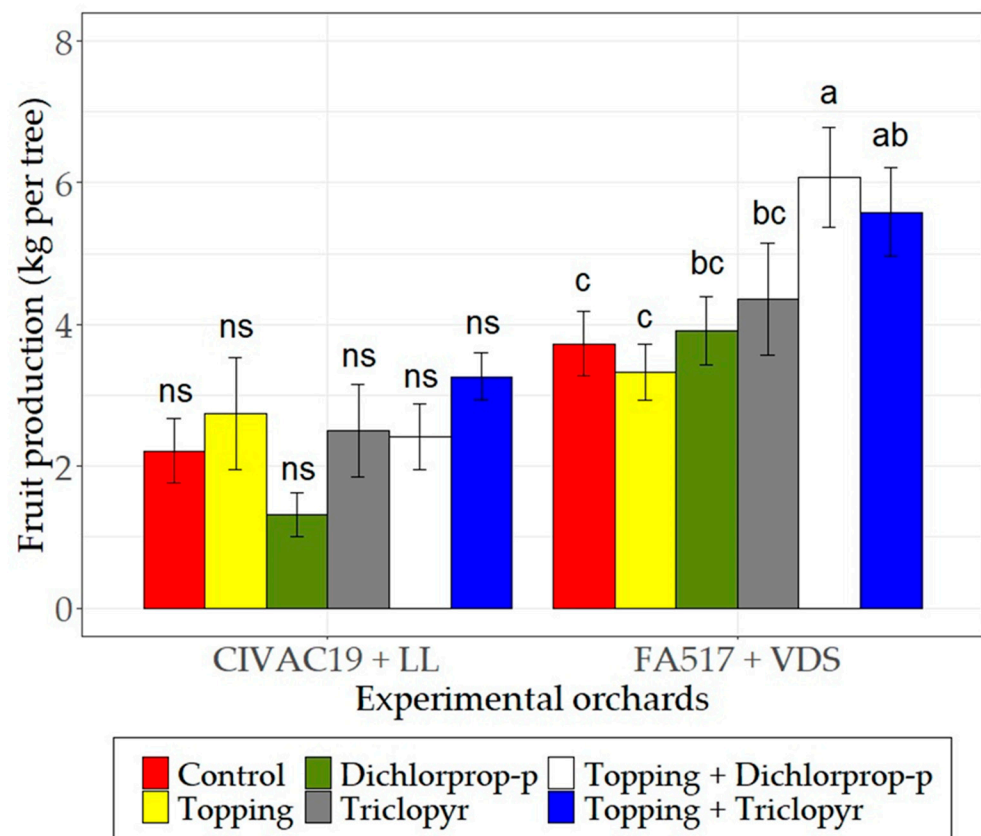
Figure 1. Fruit production ± standard error (SE) under the effect of six different treatments (control, topping, dichlorprop-p, triclopyr, topping + dichlorprop-p and topping + triclopyr) in the two experimental orchards with different rootstocks (CIVAC19 and Forner-Alcaide no. 517) and cultivars ('Lane Late' and 'Valencia Delta Seedless') from the 2020 harvest season. Values of columns with different letters were significantly different among treatments for each orchard according to LSD-Fisher test ($p < 0.05$). LL: 'Lane Late', FA517: Forner-Alcaide no. 517, VDS: 'Valencia Delta Seedless', ns: not significant.

4. Discussion

In this study, we have carried out different efficient techniques for reducing flush shoots abundance in citrus orchards. Thus, the application of auxin compounds can reduce shoot flushing in citrus crops to save hand-pruning work [9,10]. In our research, the spring-summer shoot flushing was reduced using dichlorprop-p treatments for both experimental orchards (Lane Late/CIVAC19 and Valencia DS/FA517) and years (2018 and 2019) assayed (Table S2). On the contrary, the topping treatment induced a higher growth of young shoots in both orchards during 2018 and in Lane Late/CIVAC19 during 2019. According to Velázquez and Fernández [11], mechanical pruning is a non-selective method of branch and shoot cutting, which can induce the growth of new shoots during the spring–summer season given that plant production is more intensive than in another period [5]. Most of these shoots appear near the recent cuts of pruned branches and are an excessive plant mass for cultivation practices.

On the other hand, autumn shoot flushing results differed between both years and experimental orchards assayed (Table S2). Thus, the number of shoots in the autumn evaluation was reduced with the topping and topping + dichlorprop-p treatments in the orchard comprised of Lane Late/CIVAC19 during 2018 and 2019, respectively. On the other hand, the dichlorprop-p and topping treatments showed the lowest shoots values in the orchard comprised of Valencia DS/FA517 during 2018 and 2019, respectively. Overall, shoot growth was similar throughout the months and years assayed; however, this growth

rate was lower in September 2019 (after four years of tree plantation) than others months assayed (Table S2).

In addition, auxin treatments are recommended for fruit production regulation [8]. In particular, the application of the compound dichlorprop-p and triclopyr contributes to increase fruit weight and prevents the pre-harvest drop of mature citrus fruits [31]. In our case, the application of each auxin (dichlorprop-p and triclopyr) alone, or combined with topping, increased fruit production in the orchard comprised of Valencia DS/FA517, but the application of dichlorprop-p did not increase production in the Lane Late/CIVAC19 orchard. Furthermore, these cultural practices did not influence the quality parameters of harvested fruits products. Therefore, according to our quality results, the orange fruit managed to achieve the minimum diameter and maturation requirements established by the European Union authorities on the commercial maturity of citrus fruits (R (UE) no. 543/2011 of European Commission, of 7 June 2011) [32].

On the other hand, the rootstocks assayed in this work are reported as dwarfing citrus rootstocks. Thus, they confer lower vigor to the cultivar than other conventional citrus rootstocks for a better adaptation to the high density plantation system [33–35].

Nowadays, environmental concerns have prompted a reduction in insecticides to control agricultural pests [36]. Thus, European Union authorities have restricted or even banned several active ingredients belonging to different action groups in recent decades [37–39]. Nevertheless, preventive agricultural practices can be a helpful substitute and/or supplement for citrus growers in integrated crop management, in order to reduce the use of chemical pest control. In addition, the decline of shoot flushing will reduce farmer spray programs for specific feeding flush insects, which are less effective if they are based on calendar dates than on the flush presence. Furthermore, farmers should avoid agricultural practices that encourage the growth of new plant tissues but fail to increase production, as these conditions are suitable for pest development, especially during the spring–summer season [40], because flushing in this crop period is highly abundant [7].

In this work, we successively applied and evaluated different treatments for reducing flush shoots in citrus orchards as a replacement of hand prune methodology. The treatment of dichlorprop-p could be an interesting methodology for reducing shoot growth with suitable fruit production and quality. Similarly, these tools are more efficient for farmers nowadays, because they can include mechanical methods for controlling the flush shoots density and reduce the hand labor costs in citrus orchards. Furthermore, the treatments performed did not negatively alter the fruit production and quality parameters.

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