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Agronomic practices and bio-stimulants for persian lime (*Citrus latifolia* Tan.) production in Mexico

Ambriz-Cervantes, R.[1]; **Ariza-Flores, Rafael**[2*]; **Alia-Tejacal, Iran**[3]; **Gálvez-Marroquín, L.A.**[2]; **Avendaño-Arrazate, Carlos Hugo**[4]

[1] Instituto Nacional de Investigación Forestales Agrícolas y Pecuarias (INIFAP)-Campo Experimental Zacatepec. Hidalgo, Morelos, México, C. P. 62780.
[2] INIFAP-Campo Experimental Valles Centrales de Oaxaca. Oaxaca, Oaxaca, México, C. P. 68200.
[3] Universidad Autónoma del Estado de Morelos-Facultad de Ciencias Agropecuarias. Cuernavaca, Morelos, México. C. P. 62210.
[4] INIFAP-Campo Experimental Rosario Izapa, km 18, Carretera Tapachula-Cacahoatán, Tuxtla chico, Chiapas, México C.P. 30870.
* Correspondence: avendano.carlos@inifap.gob.mx

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ABSTRACT

Objective: To evaluate biostimulants and cultural practices for floral induction in Persian lime (*Citrus* × *latifolia* Tanaka ex Q. Jiménez) to obtain production during the winter season, when the highest price in the market is offered with.

Design/methodology/approach: From July to September period, biostimulants and cultural practices were applied to six-year-old trees with different treatments as follows: 1. Pruning only, 2. Pruning + urea, 3. Pruning + urea + ringing, 4. Pruning + biofol® + ringing. Additionally, during September, two more treatments were included: 5. Pruning + calcium prohexadione, and 6. Pruning + Citomax®. The design used was randomized blocks with four repetitions. The experimental unit was one lime tree. Flowering, yield, physical-chemical quality and cost-benefit ratio were evaluated.

Results: The outstanding effect of pruning and the effect of nitrogen were confirmed with the foliar application of granulated urea (6.0 kg ha^{-1}) during the period from July to September, obtaining an average yield of 23 t ha^{-1} during the harvest from December to March, with a B/C 2.5 ratio. The quality of the fruit was kept within the NMX-FF-077-1996 Mexican standard. September applied Pruning + Citomax® (cytokinins) showed a yield of 30 t ha^{-1}, with a B/C 3.1 ratio.

Findings/conclusions: Pruning + nitrogen, and pruning + cytokinins induce flowering and produce Persian lime with the best winter yields.

Keywords: quality fruit, pruning, phytohormones, productivity.

INTRODUCTION

In Mexico, Persian lime (*Citrus* × *latifolia* Tanaka ex Q. Jimenez) is produced between May and September, when supply is greater and prices are low, making production unprofitable; winter production has better prices due to their scarce supply. Through

pruning practices, banding and application of biostimulants from July to September, high yields are obtained during winter, with better market prices, increasing the productivity and income competitiveness of Mexican lime producers (Ariza *et al.*, 2004; Ariza *et al.*, 2015), so these practices were applied to other citrus fruits.

In Mexico, Persian lime is grown in over 72 thousand ha, with an average annual yield of 13.5 t ha^{-1}, the state of Morelos being the fourteenth in surface area with 349 ha and ninth in average yield with 11.4 t ha^{-1} of limes (SIAP, 2019). Technologies relating biostimulants application (Ariza *et al.*, 2015) or cultural practices on plants such as banding, total fruit harvest, water stress and pruning (Ariza *et al.*, 2004) or combined have been recently evaluated (Ambriz *et al.*, 2013; Ambriz *et al.*, 2013 a; Ambriz *et al.*, 2018). There is a necessity to explore new technologies to avoid plant wear in a sustainable and maintained way, to contribute to the productivity and competitiveness of lime plantations. By improving the technological level of orchard care and researching the application timing of agronomic practices and biostimulants for Persian lime production and competitiveness during winter. Subsequently making these results accessible to producers and with it offer an alternative to improve their production and income.

The objective of this research was to evaluate and generate technologies for flowering and to increase the production and profitability of Persian lime, along with the evaluation of the effects of agronomic practices and biostimulants on fruit quality.

MATERIALS AND METHODS

Trials were established at Ahuahuetzingo, Puente de Ixtla, state of Morelos, Mexico, to evaluate biostimulants and cultural practices on six-year-old Persian lime trees, with a density of 357 trees per ha, managed following directions by Alia *et al.* (2011).

Five treatments were established throughout July, August and September 2018: a) pruning, b) pruning + 46% granular urea (6.0 kg ha^{-1} foliar application), c) pruning + 46% granular urea (6.0 kg ha^{-1} foliar application) + branch banding, d) pruning + biofol® (3 L ha^{-1} foliar application). Two additional treatments were added during September: e) pruning + calcium prohexadione (3.0 kg ha^{-1} foliar application), f) pruning + cytokinins (Citomaxx® at 3 L ha^{-1} foliar application). The employed assessment had a randomized complete block design with four replications, one tree as experimental unit.

Variables were obtained from four branches to quantify the number of flowers and marble-like fruits ($<$1.0 cm in diameter); from each tree, the number of fruits per individual, the fruit weight per tree was determined with a clock scale and expressed in kg (Ohaus® brand), and the yield was calculated as kg ha^{-1}. From 10 fruit samples, fruit quality was determined as fruit mass, polar and equatorial diameters were assessed with a Vernier (Mitutoyo® brand), total soluble solids determined with a refractometer (Hanna® brand, model HI96801) and expressed as °Brix and juice content in mm (Alia *et al.*, 2009). Profitability was also determined for competitiveness with the production costs and production value in Mexican pesos through by the benefit/cost ratio (B/C).

Five fruit harvests were carried out on the following dates: November 27-30, January 15-17, February 9-11, March 5-6 and March 25-26, to obtain the total sum of the sum of each of the evaluated treatments on the plants.

With the results of the three experiments and analysis of variance (ANOVA) was performed according to the experimental design and the DMS mean comparison tests in the SAS statistical software (Anonymous, 2015).

RESULTS AND DISCUSSION

During the trial conducted in July 2018, the number of flowers, number of fruits, weight of fruits per tree and total yield were significantly higher in the Persian lime trees in which pruning + urea or pruning + urea + banding was applied, compared to the rest of the treatments (Table 1). Pruning promoted new shoots formation, both vegetative and reproductive, which were part of the flowering and production (Ariza *et al.*, 2004); the effect of urea was to convert ammonium, reduce ethylene production and induce flowering (Lovat *et al.*, 1988). Similar results were reported by Ambriz *et al.* (2018); they concluded pruning, urea application and banding in September to promote flower budding and fruit setting in Persian limes for winter production. No significant differences attributed to the treatments were detected in the fruit weight variables, polar and equatorial diameter, juice percentage and total soluble solids content (Table 1).

During the August 2018 assessment, the largest number of tree flower was recorded in the pruning + urea treatment (Table 2); the highest fruit number, fruit weight and yield, were determined, on the trees with the pruning + urea + banding performance (Table 2). Trees which were only pruned showed the lowest values of yield components (Table 2).

In the September 2018 evaluation, the results indicate significant differences in the number of flowers, number of fruits, weight and kilograms per hectare yield (kg ha^{-1}); it is also observed that in the pruning + cytokinins treatment differences on the polar diameter quality variable existed, this did not occur for the rest of the evaluated treatments (Table 3 and Figure 1). There are no reports about the effect of cytokinins on flowering and fruit production in citrus plants, but there are in cv. Golden Delicious/MM111 apple, that indicate that cytokinins individually applied or combined with calcium

Table 1. July experiment results for flowering variables, yields and fruits quality using biostimulants and agronomic practices for floral induction and production of Persian lime during winter at the state of Morelos, Mexico. July 2018/2019 period.

Treatment	FloA (Number)	FruA (Number)	PesA (kg)	Yield (t ha^{-1})	MF (g)	PD (mm)	ED (mm)	J (%)	TSS °Brix
Pruning	48 C	389 bc	43.7 bc	15.6 ab	132	73.4	59.7	46	9.5
Pruning + urea	101 a	572 a	60.9 a	21.7 a	136	74.3	60.4	44	9.4
Pruning + urea + ringed	83 ab	509 ab	56.9 ab	20.3 a	133	72.8	61.2	48	9.4
Pruning + Biofol® + ringed	65 bc	309 c	37.0 c	12.4 b	131	71.9	60.7	45	9.4
D.M.S.	32.8	126	17.2	6.2	22	4.9	3.7	5.2	0.9
C.V.	27.7	18.3	22.4	23.0	10.7	4.3	4.0	7.4	6.6
Significance	*	*	*	*	n.s.	n.s.	n.s	n.s.	n.s.

Means with different letters in the direction of the columns indicate statistically significant differences according to the least significant difference test (DMS≤0.05); C.V.=coefficient of variation; DMS=significant >0.05, >0.01, >0.0001 (*, **, ***), n.s.=not significant. FloA=Flowers per tree, FruA=Fruit per tree, PesA=Fruit weight per tree, MF=Fruit mass, PD=Polar diameter, ED=Equatorial diameter, J=juice, TSS=Total soluble solids.

Table 2. Results of the August assessment on flowering, yield and fruit quality variables when using biostimulants and agronomic practices for floral induction and production of Persian lime during winter at the state of Morelos. August period 2018/2019.

Treatment	FloA (Number)	FruA (Number)	PesA (kg)	Yield (t ha^{-1})	MF (g)	PD (mm)	ED (mm)	J (%)	TSS °Brix
Pruning	61 ab	429 b	48.9 b	17.5 b	135	73.0	60.7	46	9.1
Pruning + urea	91 a	615 ab	72.2 ab	25.8 ab	136	73.4	61.3	46	9.0
Pruning + urea + ringed	56 b	789 a	84.4 a	30.1 a	139	74.4	62.1	47	9.3
Pruning + Biofol® + ringed	78 ab	476 b	55.6 ab	20.1 ab	136	74.0	61.0	45	9.6
D.M.S.	30.5	236	28.7	10.2	26.8	5.9	2.9	6.7	0.9
C.V.	27.7	26.6	28.5	28.5	12.7	5.1	3.1	9.6	6.6
Significance	*	*	*	*	n.s.	n.s.	n.s.	n.s.	n.s

Averages with different letters in the direction of the columns indicate statistically significant differences according to the least significant difference test (DMS≤0.05); C.V.=coefficient of variation; DMS=significant >0.05, >0.01, >0.0001 (*, **, ***), n.s.=not significant. FloA=Flowers per tree, FruA=Fruit per tree, PesA=Fruit weight per tree, MF=Fruit mass, PD=Polar diameter, ED=Equatorial diameter, J=juice, TSS=Total soluble solids.

prohexadione (P-Ca) produced heavier fruits, increased the carotenoids and vitamin C concentration and increased the induction of flower buds (Ramírez *et al.*, 2017), calcium prohexadione stimulated flowering and production in cv. Red Delicius apples (Kiessling *et al.*, 2008). Palmer *et al.* (2015) also demonstrated the cytokinins potential to induce flower bud development in cv. Scifresh/M9 apple trees, concurring with this experiment. Corbesier *et al.* (2003) reported cytokinins accumulation in leaves, leaf exudates and apical meristems, all of which correlate with early events of the flowering transition in *Arabidopsis thaliana* (L.).

There were highly significant differences, mainly for yield (t ha^{-1}), due to the applied practices and biostimulants. If only pruning is performed, the average yield is 16.8 t ha^{-1}

Table 3. Results of flowering, yield and fruit quality variables during the September assessment using biostimulants and agronomic practices for floral induction for Persian lime production during winter at the state of Morelos, Mexico. September 2018/2019 period.

Treatment	FloA (Number)	FruA (Number)	PesA (kg)	Yield (t ha^{-1})	MF (g)	PD (mm)	ED (mm)	J (%)	TSS °Brix
Pruning	45 ab	465 bc	48.9 bc	17.5 bc	127	70.1ab	60.2	48	9.3
Pruning + urea	47 ab	592 ab	63.3 b	22.6 b	138	72.6ab	60.5	47	9.2
Pruning + urea + ringed	45 ab	442 bc	51.8 bc	18.5 bc	143	72.7ab	61.9	47	9.3
Pruning + Biofol® + ringed	33 b	361 c	42.2 c	15.0 c	141	75.2a	60.9	46	9.4
Pruning + Prohexadione calcium	53 a	442 bc	49.5 bc	17.6 bc	140	71.9ab	61.5	44	9.3
Pruning + cytokinins	50 a	694 a	85.0 a	30.3 a	134	66.6b	60.7	45	9.6
D.M.S.	17.7	192.7	19.9	7.13	22.2	4.5	2.7	7.3	1.0
C.V.	26.8	25.9	23.6	23.7	10.9	4.0	2.9	10.7	7.3
Significance	*	**	**	*	n.s.	*.	n.s.	n.s.	n.s.

Averages with different letters in the direction of the columns indicate statistically significant differences according to the least significant difference test (DMS≤0.05); C.V.=coefficient of variation; DMS=significant >0.05, >0.01, >0.0001 (*, **, ***), n.s.=not significant. FloA=Flowers per tree, FruA=Fruit per tree, PesA=Fruit weight per tree, MF=Fruit mass, PD=Polar diameter, ED=Equatorial diameter, J=juice, TSS=Total soluble solids.

Figure 1. Appareance of Persa lemon fruits by pruning branches treatment with cytokinins (a) and pruning without biostimulants (b).

and if pruning plus urea is applied, the yield increases to 23.3 t ha^{-1}, equivalent to 38.7% more production. Banding branches is optional, since it is a potential pathogen entry point and can significantly affect the plants, for instance, gummosis and death caused by the *Lasiodiplodia theobramae* (Pat.) fungus (Valle *et al.*, 2019).

Regarding fruit quality, all the evaluated treatments were within the MX-FF-077-1996 quality norm parameters, such as equatorial diameters between 59.73 and 62.10 mm, juice percentages between 44 and 48% and total soluble solids between 9.0 and 9.6 °Brix. Statistical analyses of the fruit quality showed no significant differences between treatments, so biostimulants and practices applied to produce winter Persian lime had no effects on fruit quality (Table 1, 2 and 3); only significant differences between treatments were found for the polar diameter variable in the September experiment.

Biofol® is not a good option, as it increases production costs for flower induction, as well as calcium prohexadione application due to its high cost which does not represent a viable option; instead, the cytokinin-based product Citomax® showed the highest yield (30.3 t ha^{-1}) in Persian lime winter production, as reflected in the benefit-cost ratio economic analysis (Table 4).

CONCLUSIONS

Using biostimulants and agronomic practices to induce flowering and yield during winter in Persian limes at the state of Morelos, Mexico, were effective during July and September. Pruning and foliar urea application (6.0 kg/ha) had the best effects on winter Persian lime yield and their cost-benefit ratio. Cytokinin application (Citomax®) is a favorable option to achieve the best yields of Persian lime during winter. Fruit quality is not affected by the application of biostimulants and agronomic practices in the production of Persian lime during winter.

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Table 4. Economic analysis of experiment treatments using biostimulant and agronomic practices for floral induction and production of Persian lemon during the winter at the state of Morelos, Mexico, 2018/2019 (Taking a $10.00 /kg average rural price of Persian limes).

Treatment	CC	Yield	IB	IN	B/C	Rc
Pruning in july	85,000	15.6	156,000	71,000	1.8	0.8
Pruning in august	85,000	17.5	175,000	90,000	2	1
Pruning in september	85,000	17.5	175,000	90,000	2	1
Average pruning	85,000	16.8	168,000	83,000	1.9	0.9
Pruning + urea in july	95,000	21.7	217,000	122,000	2.3	1.3
Pruning + urea in august	95,000	25.8	258,000	163,000	2.7	1.7
Pruning + urea in september	95,000	22.6	226,000	131,000	2.4	1.4
Average pruning + urea	95,000	23.3	233,000	138,000	2.5	1.5
Pruning + urea + ringed in july	100,000	20.3	203,000	103,000	2	1
Pruning + urea + ringed in august	100,000	30.1	301,000	201,000	3	2
Pruning + urea + ringed in september	100,000	18.5	185,000	85,000	1.8	0.8
Average pruning + urea + ringed	100,000	22.9	229,000	129,000	2.3	1.3
Pruning + biofol® + ringed in july	97,000	12.4	124,000	27,000	1.3	0.3
Pruning + biofol® + ringed in august	97,000	19.9	199,000	102,000	2	1
Pruning + biofol® + ringed in september	97,000	15	150,000	53,000	1.5	0.5
Average pruning + biofol® + ringed	97,000	15.7	157,000	60,000	1.6	0.6
Pruning + Prohexadione calcium in september	167,000	17.6	176,000	9,000	1	0
Pruning + Citomax® in september	97,000	30.3	303,000	206,000	3.1	2.1

CC=Crop Costs, Yield, IB=Gross Index, IN=Net Index, B/C=Benefit (Cost) Ratio, Rc=Return on Capital.

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