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PROHEXADIONE-CALCIUM AFFECTS SHOOT GROWTH AND YIELD OF LEMON, ORANGE AND AVOCADO DIFFERENTLY

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ABSTRACT

Lemon (*Citrus limon* L.) and navel orange (*C. sinensis* L.) trees were pruned in May and prohexadione-calcium (P-Ca) was applied to the canopy 24 h later. Prohexadione-calcium (250 mg L^{-1}) delayed lemon shoot regrowth by ~2 weeks, but had no effect on the final amount of regrowth or on the 2-year average number of fruit per tree. P-Ca (500 mg L^{-1}) had no effect on lemon shoot regrowth but reduced the 2-year average number of large fruit (≥ 6.1 cm transverse diameter) per tree, reducing 2-year average total fruit number per tree relative to untreated control trees ($P < 0.05$). P-Ca (250 and 500 mg L^{-1}) had no significant effect on navel orange shoot regrowth, but P-Ca (500 mg L^{-1}) consistently stimulated navel orange shoot regrowth during the 2 years of research. Both P-Ca concentrations significantly reduced the 2-year average number of small fruit per tree (≤ 68.9 mm transverse diameter) to significantly reduce 2-year average total number of fruit per tree relative to untreated control trees. Three foliar applications of P-Ca (250 mg L^{-1}) to 'Hass' avocado (*Persea americana* Mill.) trees during bloom (March, April, May) reduced vegetative shoot length in April and May compared to the untreated control when averaged across the 2 years of the study ($P < 0.05$). P-Ca (125 mg L^{-1}) applied in March and April increased the 2-year average number of large fruit (≥ 270 g per fruit) per tree compared to untreated control trees ($P < 0.09$). The varied responses to P-Ca among lemon, orange and avocado are of physiological interest.

INTRODUCTION

Prohexadione-calcium (P-Ca) is a multifunctional plant growth regulator (PGR). For apple and pear, P-Ca has been shown to inhibit the biosynthesis of GA_1 and ethylene (Rademacher *et al*., 2006). These multiple effects are due to the structural similarities between P-Ca and 2-oxoglutaric acid, which is required by GA_{20} 3β-hydroxylase, and between P-Ca and ascorbic acid, which is required by aminocyclopropanecarboxylic acid oxidase (Rademacher et al., 2006). Through these effects on plant biochemistry, P-Ca has proven a reliable tool for reducing apple shoot elongation and increasing yield (Greene, 1999; Costa *et al*., 2006; Ramirez *et al*., 2006). Relative to deciduous fruit tree crops, there are few reports on the PGR effects of P-Ca on subtropical perennial fruit tree crops (Lovatt, 2001; Mandemaker *et al*., 2005). Here we report the efficacy of foliar-applied P-Ca to reduce vegetative shoot regrowth (the sum of the length of all shoots) of ‘Eureka’ lemon and ‘Washington’ navel orange after spring pruning or to decrease the elongation of vegetative shoots of the ‘Hass’ avocado during spring bloom. In all cases, the goal was to reduce competition to achieve a corresponding increase in fruit retention (fruit number) with no reduction in fruit size.

MATERIAL AND METHODS

The research was conducted in commercial orchards of 16-year-old 'Eureka' lemon trees on *C. volkemariana* rootstock and 21-year-old 'Washington' navel orange trees on 'Troyer citrange' rootstock (*C. sinensis* 'Washington' navel x *Poncirus trifoliata* L. Raf.) at the Citrus Research Center and Agricultural Experiment Station, University of California, Riverside (33.57° N, 117.23° W) and 7-year-old (experiment 1) and 8-year-old (experiment 2) 'Hass' avocado trees on Mexican race rootstocks (*P. americana* var. *drymifolia*) located in Irvine, California (33.40° N, 117.49° W). Citrus trees were topped and hedged in mid-May and P-Ca (250 or 500 mg L^{-1}) was applied 24 h later. Avocado trees in experiment 1 received P-Ca (250 mg L^{-1}) at the cauliflower stage of inflorescence development (March), full bloom (April) and early fruit abscission (May). In experiment 2, P-Ca (125 mg L^{-1}) was applied at the cauliflower stage of inflorescence development (March) and again at full bloom (April). In all cases, P-Ca was applied in water (2337 L ha^{-1} for citrus and 1869 L ha^{-1} for avocado, containing 0.05% Silwet L77® surfactant) to the entire canopy using a 2758 KPa handgun sprayer. The experimental design was a randomized complete block with 16 (citrus) and 25 (avocado) individual tree replicates per treatment, including an untreated control, in each experiment. For citrus, the number and length of shoots that developed within 12 cm of the pruning cut were determined weekly through July and every two weeks thereafter. Total regrowth was calculated as the sum of the length of all shoots that developed in response to pruning and expressed as a percent of the regrowth of the untreated control. At harvest, fruit number and kilograms per tree and fruit size distribution (pack out as number of fruit of each commercial packing size) was determined, using a portable, commercial in-field fruit sizer. The following packing carton sizes (based on fruit transverse diameter in mm) were used: lemon = 235 (47.75-51.05), 200 (51.054-54.10), 165 (54.11-56.90), 140 (56.90-61.21), and 115 (61.21-65.28); and navel orange = < 113 (< 63.5), 113 (63.5-68.9), 88 (69.0-74.9), 72 (75.0-80.9), and 56 (81.0-88.0).). For avocado, the length of 10 developing spring flush vegetative shoots and the apical vegetative shoot of 10 indeterminate floral shoots was measured monthly from April through July. At harvest, total yield was determined as number of fruit per tree. A randomly selected subsample of 100 to 150 fruit per tree was collected and the weight of individual fruit determined to calculate pack out, i.e., number of fruit of each commercial packing carton size per tree. The following packing carton fruit sizes (g per fruit) were used: 84 (99-134); 70 (135-177); 60 (178-212); 48 (213-269); 40 (270-325); 36 (326-354) and 32 (355-397). All data were analyzed using the General Linear Model procedure of the SAS 9.2 statistical program (SAS Inst., Inc., Cary, NC). Repeated measure analysis with year as the repeated measure was used to test for treatment effects averaged across the 2 years of the study. For individual years, means were separated using Fisher's Protected LSD Test at $P \leq 0.05$. Pair-wise comparisons were preformed using Dunnett's Test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Crop load (either as total fruit number or kilograms) per tree was significantly different between the two years of the study in all orchards (Tables 1-3). With exception of lemon, annual differences in fruit size distribution and yield of commercially valuable large fruit were related to crop load. Crop load also significantly affected shoot elongation of navel orange and avocado, but not lemon (Data not shown).

A single application of P-Ca (250 mg L^{-1}) 24 h after pruning delayed lemon shoot regrowth by ~ 2 weeks for the 2 years of the study ($P < 0.001$) (Fig. 1A), but P-Ca (250 and 500 mg L^{-1}) had no significant effect on the amount of vegetative shoot regrowth that developed in response to pruning (mid-May) for any sampling date, largely due to the fact that P-Ca had no effect on, or in some cases increased, the number of shoots that sprouted near the cut. P-Ca was effective in reducing shoot length through July (500 mg L^{-1}) and August (250 mg L^{-1}) (Garner *et al.*, in review). At commercial harvest in January, P-Ca (250 mg L^{-1}) had no effect on the 2-year average total number of lemon fruit per tree or the 2-year average number of fruit in any size category and, hence, had no effect on the 2-year average total kilograms of fruit per tree compared to trees treated with P-Ca (250 mg L^{-1}) but not the untreated control trees (Table 1). In contrast, P-Ca (500 mg L^{-1}) reduced the 2-year average total number of lemon fruit per tree. P-Ca (500 mg L^{-1}) also reduced the 2-year average number of commercially valuable large fruit (61.21-65.28 mm transverse diameter) per tree compared to trees treated with P-Ca (250 mg L^{-1}), which reduced the 2-year average total kilograms of fruit per tree compared to trees treated with P-Ca (250 mg L^{-1}), but not the untreated control trees (Table 1).

Contrasting results were obtained with the use of P-Ca on 'Washington' navel orange trees. P-Ca (250 and 500 mg L^{-1}) had no significant effect on regrowth after pruning (Fig. 1B). However, P-Ca (500 mg L^{-1}) tended to increase regrowth by increasing shoot elongation, but not shoot number (Data not shown). This resulted in an increase in the total amount of navel orange regrowth by the end of August ($P = 0.0592$) (Fig. 1B). P-Ca (250 and 500 mg L^{-1}) reduced the 2-year average total number of navel orange fruit per tree by reducing the 2-year average number of small fruit (≤ 68.9 mm transverse diameter) compared to untreated control trees (Table 2). This resulted in a reduction in the 2-year average kilograms of fruit per tree compared to the untreated control (P = 0.0532).

For avocado trees in experiment 1, P-Ca (250 mg L^{-1}) applied three times during spring bloom (March, April, and May) significantly reduced the 2-year average length of spring flush vegetative shoots from the beginning of April through the end of May compared to the untreated control trees (Fig 1C). Averaged across the 2-years of the study, the effect of P-Ca (250 mg L^{-1}) on elongation of the apical vegetative shoot of indeterminate floral shoots was significant only in June (Fig. 1D). For both shoot types, P-Ca was more effective in reducing shoot elongation when trees were setting a heavy crop (Garner *et al.*, in review), a result noted in an earlier study of P-Ca-treated 'Hass' avocado trees (Mandemaker et al., 2005). In experiment 2, P-Ca (125 mg L^{-1}) applied twice during bloom (March and April) increased the 2-year average number of large fruit in the combined pool of packing carton sizes 36, 32 and 28 (> packing carton size 40; > 326 g per fruit) per tree ($P = 0.0819$), without reducing the 2-year average number of fruit in other fruit size categories or the total number of fruit per tree (Table 3). Despite this, P-Ca treatment did not translate into an increase in the 2-year average kilograms of fruit per tree compared to the untreated control (Table 3). The positive effect of P-Ca on the yield of large avocado fruit was dramatic during the year the trees flowered profusely and set a large number of fruit (Data not shown), but not in the light off-bloom, off-crop year. When averaged across the high-yield and low-yield years of the study, the effect of P-Ca on fruit size was less pronounced. However, when the yield data were analyzed as 2-year cumulative yield, the capacity of P-Ca to increase the yield of large fruit (> 326 g per fruit) was significant as both number and kilograms of fruit per tree compared to the untreated control ($P < 0.04$) (Garner *et al*., in review).

P-Ca reduced the 2-year average number of lemon (500 mg L^{-1}) and navel orange (250 or 500 mg L^{-1}) fruit per tree, providing evidence that P-Ca reduces fruit set of citrus. Since P-Ca did not significantly reduce the regrowth of lemon or navel orange shoots in response to pruning, it appears for citrus that fruit set is more sensitive to P-Ca treatment than vegetative shoot growth. Regrowth in this study was a function of the number of new shoots that sprouted (bud break) and their length (shoot elongation). For lemon, shoot elongation was reduced by P-Ca treatment, but for navel orange, bud break decreased (Garner *et al*., in review). The reduction in lemon and navel orange fruit number per tree (2-year average) resulting from P-Ca treatment provides evidence consistent with inhibition of GA biosynthesis by P-Ca in citrus. In contrast, our results do not support an inhibitory effect of P-Ca on ethylene biosynthesis in citrus. Foliar-applied aminoethoxyvinylglycine (AVG), a known inhibitor of ethylene biosynthesis, has been shown to increase fruit set early in the season through harvest (January) of the ‘Washington’ navel orange (Gonzalez and Lovatt, 2007). However, for avocado, the increased yield of large fruit (≥ 326 g per fruit), without a reduction in total yield per tree in response to P-Ca applied during bloom is consistent with the inhibition of ethylene biosynthesis. Application of AVG to ‘Hass’ avocado trees at bloom has been shown to significantly increase the 3-year cumulative yield of commercially valuable large size fruit without reducing total yield (Lovatt, unpublished). Not only were the results obtained with foliar application of P-Ca to citrus and avocado different, they were different from results achieved with use of P-Ca on apple. For apple, P-Ca applied at concentrations similar to those used in the present research (175 to 250 mg L^{-1}) significantly reduced shoot elongation and increased total yield (Ramirez et al., 2006), which was not achieved with the use of P-Ca on citrus or avocado. The differences in the physiological responses of citrus, avocado and apple to P-Ca appears to be dependent on the capacity of P-Ca to inhibit GA and/or ethylene biosynthesis in these tree fruit crops.

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Table 1. Effect of prohexadione-calcium applied to the canopy of 'Eureka' lemon trees 24 h after topping and hedging (mid-May) on 2-year average yield and fruit size.

			Packing carton size based on fruit transverse diameter (mm)					
Treatment	Concentration (mg L^{-1})	Total no.	235 47.75-51.05	200 51.06-54.10	165 54.11-56.90	140 56.91-61.20	115 61.21-65.28	Total wt.
		no. of fruit/tree						*kg/tree*
Prohexadione-Ca	250	2249 a[z]	125 a	193 a	490 a	445 a	509 a	260.5 a
Prohexadione-Ca	500	1999 b	118 a	175 a	433 a	403 a	410 b	231.3 b
Control		2123 ab	130 a	196 a	439 a	417 a	496 a	246.9 ab
Date								
Year 1		2105 a	141 a	270 a	434 b	460 a	356 b	231.1 b
Year 2		2142 a	108 b	106 b	474 a	383 b	588 a	261.4 a
P-value								
Treatment		0.0253	0.6958	0.5599	0.1094	0.4111	0.0408	0.0705
Date		0.5416	0.0361	<0.0001	0.0911	0.0004	<0.0001	<0.0001
T x D		0.8734	0.5127	0.7191	0.6824	0.8446	0.9501	0.9730

[z] Values in a vertical column followed by different letters are significantly different at *P*-value specified by Fisher's Protected LSD Test.

Table 2. Effect of prohexadione-calcium applied to the canopy of 'Washington' navel orange trees 24 h after topping and hedging (mid-May) on 2-year average yield and fruit size.

Treatment	Concentration (mg L^{-1})	Total no.	Packing carton size based on fruit transverse diameter (mm) < 113 (< 63.5)	113 (63.6-68.9)	88 (69.0-74.9)	72 (75.0-80.9)	56 (81.0-88.0)	Total wt.
		---------- *no. of fruit/tree* ----------						-- kg/*tree* --
Prohexadione-Ca	250	267 b[z]	23 b	47 b	103 a	44 a	37 a	69.1 b
Prohexadione-Ca	500	267 b	37 b	54 b	86 a	36 a	41 a	67.4 b
Control		342 a	69 a	91 a	97 a	34 a	37 a	82.4 a
Date								
Year 1		251 b	20 b	21 b	49 b	60 a	74 a	69.2 a
Year 2		333 a	65 a	106 a	142 a	16 b	3 b	76.7 a
P-value								
Treatment		0.0116	0.0034	0.0010	0.4858	0.1407	0.6285	0.0532
Date		0.0234	0.0038	<0.0001	<0.0001	<0.0001	<0.0001	0.3059
T x D		0.1707	0.1062	0.1275	0.0778	0.1584	0.4759	0.1452

[z] Values in a vertical column followed by different letters are significantly different at *P*-value specified by Fisher's Protected LSD Test.

Table 3. Effect of prohexadione-calcium (125 mg L^{-1}) applied to the canopy of 'Hass' avocado trees at the cauliflower stage of inflorescence development and full bloom on 2-year-average yield and fruit size.

		Packing carton size based on individual fruit mass (g)						
Treatment	Total no.	84 99-134	70 135-177	60 178-212	48 213-269	40 270-325	> 40 > 325	Total wt.
	------ *no. of fruit/tree* ------							-- kg/*tree* --
Prohexadione-Ca	96 a[z]	4 a	16 a	27 a	36 a	11 a	14 a	21.1 a
Control	72 a	5 a	16 a	21 a	22 a	7 a	9 a	14.1 a
Date								
Year 1	110 a	7 a	27 a	37 a	33 a	7 b	7 b	22.1 a
Year 2	58 b	2 b	5 b	11 b	25 a	12 a	16 a	14.0 a
P-value								
Treatment	0.1907	0.7388	0.5505	0.2812	0.1989	0.1339	0.0819	0.1465
Date	0.0644	0.0543	0.0035	0.0043	0.4486	0.0764	0.0107	0.1586
T x D	0.4260	0.0953	0.2952	0.5688	0.6747	0.7468	0.8415	0.4750

[z] Values in a vertical column followed by different letters are significantly different at *P*-value specified by Fisher's Protected LSD Test.

Figures

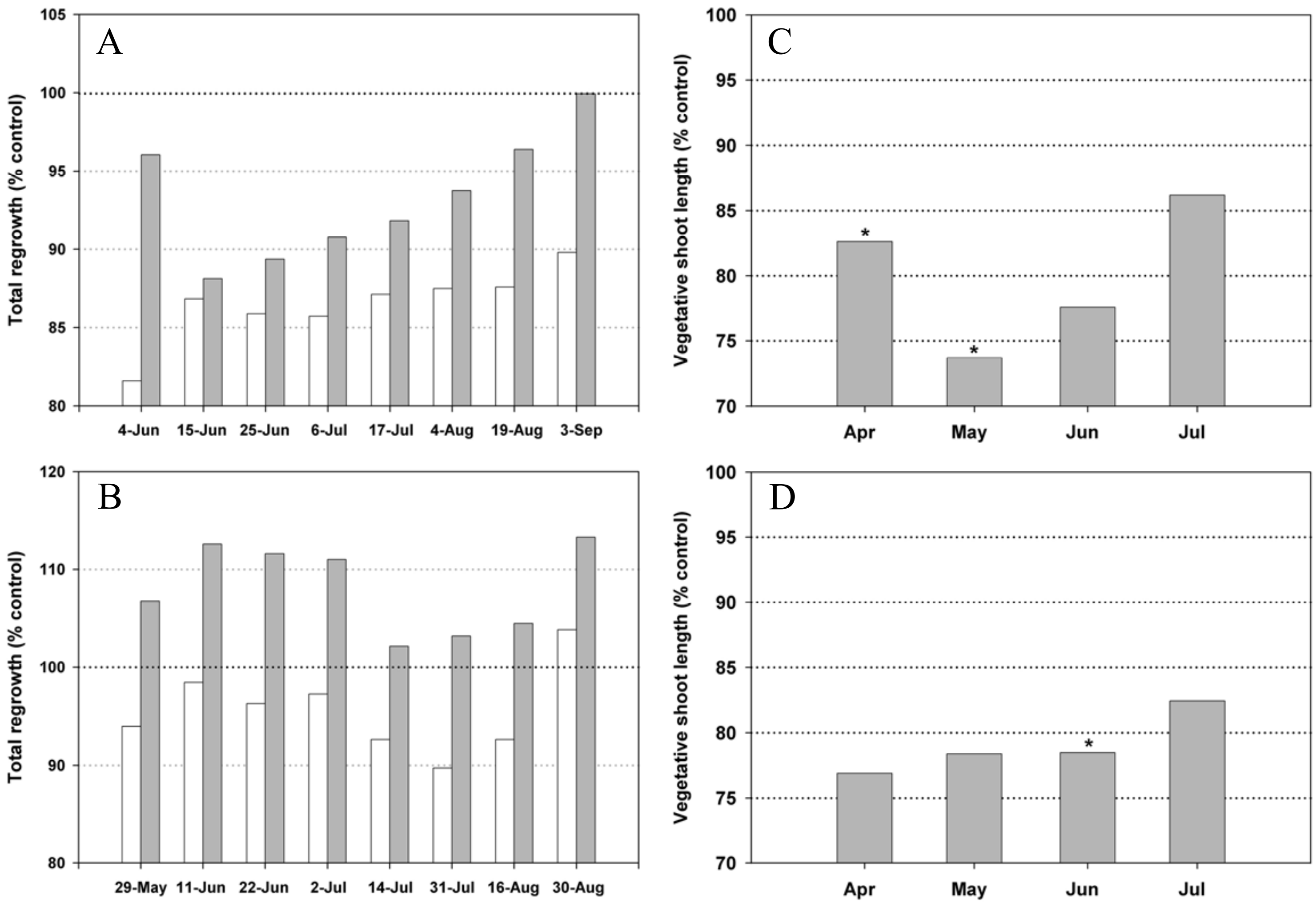
A
Total regrowth (% control)
105
100
95
90
85
80
4-Jun
15-Jun
25-Jun
6-Jul
17-Jul
4-Aug
19-Aug
3-Sep
C
Vegetative shoot length (% control)
100
95
90
85
80
75
70
*
*
Apr
May
Jun
Jul
B
Total regrowth (% control)
120
110
100
90
80
29-May
11-Jun
22-Jun
2-Jul
14-Jul
31-Jul
16-Aug
30-Aug
D
Vegetative shoot length (% control)
100
95
90
85
80
75
70
*
Apr
May
Jun
Jul

Figure 1. 'Eureka' lemon (A) and 'Washington' navel orange (B) - effect of prohexadione-calcium (P-Ca) at 250 mg L^{-1} (□) or 500 mg L^{-1} (■) on 2-year average total regrowth of vegetative shoots in response to topping and hedging (mid-May) expressed as a percent of the untreated control. 'Hass' avocado - effect of P-Ca (250 mg L^{-1}) applied at the cauliflower stage of inflorescence development (March), full bloom (April) and during fruit set (May) on the 2-year average length of spring flush vegetative shoots expressed as a percent of the untreated control (C) and on the 2-year average length of the apical vegetative shoot of indeterminate floral shoots expressed as a percent of the untreated control (D) * Significantly different by Dunnett's Test at $\alpha \leq 0.05$.