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Plant Growth Regulators for Avocado Production

Background

Plant growth regulators (PGRs) are the most powerful tools for manipulating tree growth and yield in an existing orchard. A quick review of current commercial uses of PGRs in apple, citrus, stone fruit, nut, kiwi, and grape production provides insight into the physiological processes that can be influenced to the economic benefit of growers.

PGRs are used successfully to manipulate flowering. Benzyladenine (6-benzylaminopurine, BA) causes early bud break in numerous tree crops and increases floral bud retention of pistachio (Lovatt and Ferguson, 1999). Gibberellic acid (GA_3) inhibits or delays flowering in deciduous tree crops (Sedgley, 1990) and citrus if applied before irreversible commitment to flowering (Lord and Eckard, 1987). After this developmental stage, GA_3 is without effect on many crops including coffee (Schuch et al., 1990) and mango (Kachru et al., 1972).

PGRs are used to manipulate fruiting. The powerful synthetic diphenylurea-type cytokinin 1-(2-chloro-4-pyridynl)-3-phenyluea (forchlorfenuron, CPPU), the synthetic auxin 2,4-dichlorooxyacetic acid (2,4-D) and GA_3 all have the capacity to stimulate parthenocarpic fruit development in various crops. GA_3, BA, CPPU, 2,4-D and the powerful synthetic auxin 3,5,6-trichloropyridyloxyacetic acid (3,5,6-TPA) stimulate fruit growth directly to increase fruit size, but with the caveat that a PGR may be more effective on some crops than on others. GA_3, 2,4-D and aminoethoxyvinylglycine (AVG) reduce early drop, which occurs during the initial fruit set period, June drop and preharvest drop of mature fruit. In contrast, ethylene-releasing compounds, such as 2-chloroethyl phosphonic acid (ethephon, CEPA), the auxin naphthalene acetic acid (NAA), and even BA at high concentration are used to induce flower or fruit abscission to reduce fruit number and indirectly increase fruit size.

PGRs are used to manipulate fruit quality both in the orchard and in the packinghouse. Ethephon can be used preharvest to advance color development and fruit maturation (ripening), whereas GA_3 delays color development and maturity in tree crops, including citrus. Ethephon is also used to "loosen" fruit to increase the efficiency of mechanical or hand harvesting of nut crops, olives, cherries, plums and citrus (Jaumien and Rejman, 1978; Knapp, 1981; Metzidakis, 1999). The PGRs abscisic acid (ABA), 2,3,5-triiodobenzoic acid (TIBA) and methyljasmonate have also been shown to increase fruit loosening in citrus (Burns et al., 2003; Kender et al., 2001; Hartmond et al., 2000). Ethylene-releasing compounds are also used postharvest to enhance color development and ripening.

PGRs can also be used to manipulate vegetative shoot development, with GA_3 stimulating vegetative shoot growth and prohexadione-calcium, paclobutrazol and uniconazol restricting canopy growth. The latter two are used in avocado production in Australia to retard vegetative regrowth after pruning.

Plant Growth Regulators and the Avocado

The avocado is a relatively new crop compared to apples, citrus and grapes. As a result, avocado PGR research is less advanced. Notably, a number of factors have further delayed progress towards commercialization of PGR strategies for avocado production in California. With the limited amount of avocado acreage, manufacturers are reluctant to make the financial investment in the research and development necessary to register a PGR for use on avocados. Moreover, simple adoption of PGR strategies from other crops is precluded because the avocado frequently responds differently to PGRs than other crops. PGR responses unique to the avocado include: (1) GA_3 stimulated precocious floral shoot development; (2) BA increased fruit set when applied in April or May, but when applied at the same concentration in June, BA increased fruit abscission; (3) AVG increased fruit set when applied in April or May, but was without effect in June; (4) AVG stimulated vegetative shoot growth, a novel response to AVG; (5) prohexadione-calcium inhibited growth of the vegetative shoot apex of indeterminate floral shoots, but not the growth of vegetative shoots; and (6) prohexadione-calcium applied three times at 250 mg/L increased the length to width ratio of fruit from 1.4 for the untreated control to 2.1 (Garner, 2004). In addition, due to poor uptake by avocado leaves, higher PGR concentrations are required to elicit a response than those typically used for other crops. Thus, the nature of the avocado necessitated research to determine such basics as which PGR to apply, at what concentration and when. In addition, obtaining consistent results is difficult due to alternate bearing and the presence of two crops on the tree for increasing lengths of time — up to 6 months.

Plant Growth Regulator Registration

Joseph Vandepeute, Ph.D., Associate Environmental Research Scientist of the California Environmental Protection Agency (EPA), Department of Pesticide Regulation (DPR), Division of Registration and Health Evaluation, was kind enough to provide specifics regarding the efficacy data required for registration of a PGR for use on avocado. Results from research using individual tree replications in a randomized complete block design collected over 2 to 4 years are acceptable. Results from large acre-block trials are not required. Treatment effects that are statistically significant each year of the study, when averaged across all years of the study or on a cumulative basis (to compensate for alternate bearing) are acceptable.

A PGR treatment can be specified for use in the on-crop or the off-crop year. However, for the specified use the "label rate" needs to be efficacious *consistently*. Since we are only dealing with one avocado cultivar, the rate for each use specified on the label will likely have a narrow range. This must be confirmed experimentally by demonstration of a "dose response", whereby the desired response to a PGR treatment increases incrementally with increasing dose (PGR application rate). The response obtained at the label rate must be statistically significantly different from the control, but the other doses do not have to elicit responses that are significantly different from the control or the label rate.

For PGRs with a non-toxic mode of action, a low toxicity profile, or a low application rate such that the use of the PGR will not increase intake above normal, the Federal EPA issues an exemption from the requirement of a tolerance limit. Currently, the Federal EPA has exempted the gibberellins GA_3, GA_4, and GA_7, the cytokinins in aqueous seaweed extracts and kinetin, the auxins indole-3-acetic acid and indole-3-butyric acid, ethylene, and pelargonic acid. The Federal EPA may extend exemption to other PGR active ingredients. Under review are naphthalene acetic acid (NAA), BA for apple and pistachio, CPPU for kiwi, grapes, apple, blueberry, fig, cranberry, olive, pear and citrus, and prohexadione-calcium for peanut. In general, the California DPR exempts the same compounds as the Federal EPA.

Current Investigations of PRGs for Avocado

Our research results provide evidence of several promising PGR strategies for avocado. GA_3 (25 mg/L) applied at the cauliflower stage of inflorescence development (~March) increased total yield in both kilograms and number of fruit per tree and increased the yield of commercially valuable large size fruit (packing carton sizes 60, 48, and 40; fruit weighing 178-212 g/fruit, 213-269 g/fruit, and 270-325 g/fruit, respectively) in both kilograms and number per tree. Statistically, the yield effects of GA_3 were only significant for the on crop-year, but due to positive numerical increases in both yield parameters in the off-crop year, the treatment had a statistically significant effect on total yield and packout of large size fruit when averaged across the 2 years of the study or as 2-year cumulative yield (Table 1). For the growers of the 'Hass' avocado in California, this GA_3 treatment translates to a 2-year cumulative net increase of 3,771 lbs more fruit per acre (110 trees per acre) than the untreated control. Moreover, 68% of the net increase in yield was large size fruit. This GA_3 treatment resulted in a 2-year cumulative net increase of 2,571 lbs per acre of fruit of packing carton sizes 60, 48 and 40 over that of the control.

In a second study, GA_3 (25 mg/L) applied at the end of June - beginning of July increased total yield as both kilograms and number of fruit per tree and also yield of commercially valuable large size fruit (packing carton sizes 60, 48, and 40) as kilograms and number per tree. As in the previous experiment, both GA_3 effects were only statistically significant in the on-crop year, but were statistically significant when averaged across both the on- and off-crop years and as 2-year cumulative yield (Table 2). Per acre, this second GA_3 treatment resulted in a 2-year cumulative net increase of 6,579 lbs more fruit than the untreated control, of which 83% were large size fruit. This GA_3 application resulted in a 2-year net increase of 5,490 lbs more fruit of packing carton sizes 60, 48 and 40 per acre than the control. This application time was selected because it is prior to the periods of "June" drop for the current crop (Garner, 2004), exponential increase in fruit size for the current crop and abscission of mature fruit (Garner, 2004), and development of the summer vegetative flush (Salazar-García et al., 1998), which we now know is critical to the floral intensity of the return bloom (Lopez-Jimenez and Lovatt, personal communication), but also sufficiently before inflorescence initiation for next year's crop, which occurs at the end of July - beginning of August, to not interfere with phase transition (Salazar-García et al., 1998).

In addition to GA_3, in a third experiment BA (25 mg/L) applied at anthesis and a combined treatment of GA_3 (25 mg/L) applied in mid-July followed by application of prohexadione-calcium (125 mg/L) 30 days later significantly increased the kilograms and number of large size fruit of packing carton sizes 40 (270-325 g/fruit) and 36 (326-354 g/fruit) per tree and the combined pool of fruit of packing carton sizes 40, 36 and 32 (270-397 g/fruit) per tree averaged across the 3 years of the study (Table 3) and as 3-year cumulative yield. The net increase in yield of fruit of packing carton sizes 40, 36 and 32 was 1,317 and 1,232 lbs per acre per year for 3 consecutive years for BA and GA_3 followed by prohexadione-calcium, respectively.

It should be noted that for all experiments reported here, no PGR had a negative effect on any fruit quality parameter evaluated. We routinely quantified the effect of each PGR treatment on the number of days to ripen at 22 ± 2 °C. When ripe, we measured seed length and width and flesh width from seed to peel. In addition, external and internal fruit quality was evaluated for abnormalities, discoloration and decay. Vascularization (presence or absence of vascular bundles and associated fibers) of the flesh was also determined. The above fruit quality parameters were rated on a scale from 0 (normal) to 4 (high incidence of abnormalities, discoloration, decay or vascularization; all four quadrants of the fruit affected).

To meet the requirements of the state DRP for proof of efficacy, we must successfully reproduce the results of the four PGR strategies in a second study in a new 'Hass' orchard in a different avocado growing-area of California than our previous studies. For each strategy we also need to demonstrate that total yield and yield of large size fruit increase with increasing PGR concentration. Thus, in all cases, PGR effects on yield, fruit size distribution and fruit quality must be determined. Since GA_3 is exempt from tolerance by the Federal EPA, once we have satisfied the efficacy data requirements of the state DPR, we are in a position to pursue having a manufacturer add the use of GA_3 on avocado to an existing GA_3 label. Similarly, since an exemption for use of BA on apple and pistachio is under consideration, it may be possible to include avocado once efficacy requirements are met. Additionally, manufacturers of GA_3 and BA are showing interest in our results but obviously must wait for the efficacy data demonstrating a dose response and reproducibility in a second orchard. Within 3 to 4 years one or more PGR should be available for use in commercial avocado production in California.

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Our increased understanding of the phenology and physiology of the 'Hass' avocado has been critical to selecting the correct plant growth regulator (PGR) initially, designing the PGR strategy, and properly timing its application annually to consistently elicit the desired result. I would like to acknowledge the following members of my laboratory whose efforts contributed to this research: Drs. Isa Bertling, Samuel Salazar-García, Langtao Xiao, Lauren Garner, Jaime Salvo, Anwar Ali, Alfredo Lopez-Jimenez, and Larry Summers, Anita Weng, Yusheng Zheng, Elias Serna, Toan Khuong and Grant Klein. I would also like to thank our collaborators, Corona Foothill, Inc., Corona, CA, Jess Ruiz of the Irvine Company, Irvine, CA, and Rick Shade of AgRx, Goleta, CA, for use of the orchards and assistance with harvests. This research was supported in part by the Citrus Research Center and Agricultural Experiment Station of the University of California, Riverside, and the California Avocado Commission.

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Table 1. Effect of GA_3 (25 mg/L) applied at the cauliflower stage on 'Hass' avocado yield.

	2-year average		2-year cumulative	
Treatment	Total yield	$\Sigma_{60+48+40}$	Total yield	$\Sigma_{60+48+40}$
	------------ *kg fruit/tree* ------------			
GA_3	22.91 a[y]	17.07 a	45.83 a	34.01 a
Control	15.14 b	11.65 b	30.28 b	23.41 b
P-value				
Treatment (T)	0.0105	0.0184	0.0105	0.0212
Year (Y)[z]	<0.0001	<0.0001	–	–
T x Y	0.0026	0.0029	–	–

[y] Values in a vertical column followed by different letters are significantly different by Fisher's protected LSD test.

[z] On- or off-crop year.

Table 2. Effect of GA_3 (25 mg/L) applied at the end of June – beginning of July on 'Hass' avocado yield.

Treatment	2-year average Total yield	2-year average $\Sigma_{60+48+40}$	2-year cumulative Total yield	2-year cumulative $\Sigma_{60+48+40}$
	kg fruit/tree			
GA_3	58.96 a[y]	50.79 a	117.92 a	100.08 a
Control	45.39 b	38.76 b	90.79 b	77.44 b
P-value				
Treatment (T)	0.0299	0.0208	0.0299	0.0309
Tree status (S)[z]	<0.0001	<0.0001	–	–
T x S	0.2462	0.1473	–	–

[y] Values in a vertical column followed by different letters are significantly different by Duncan's Multiple Range Test.

[z] On- or off-crop.

Table 3. Effect of PGR treatments on 'Hass' avocado yield averaged across the 3 years of the study.

Treatment	Total	Packing carton size				
		60	48	40	Σ60+48+40	> 40
GA_3	21.14	5.89	7.06	3.31 c[y]	16.25	4.18 c
Prohexadione-Ca	26.61	4.66	10.37	6.59 ab	21.62	8.99 ab
6-benzyladenine	26.29	3.64	9.81	8.13 a	21.57	10.82 a
CPPU	23.08	4.45	9.01	4.73 bc	18.19	6.37 bc
GA_3+Prohexadione-Ca	25.77	3.65	9.55	8.15 a	21.36	10.50 a
Control	25.63	5.72	10.53	4.38 bc	20.63	5.42 bc
P-value						
Treatment (T)	0.8148	0.1814	0.7197	0.0033	0.7072	0.0029
Year (Y)[z]	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T x Y	<0.0001	<0.0001	0.0047	0.0274	0.0008	0.0139

[y] Values in a vertical column followed by different letters are significantly different by Fisher's protected LSD test.

[z] On- or off-crop year.