

## PLANT GROWTH REGULATORS FOR AVOCADO PRODUCTION

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### ABSTRACT

Plant growth regulators (PGRs) are the most powerful tools available for manipulating tree growth and yield in an established orchard. Understanding the phenology and physiology of the avocado (*Persea americana* Mill.) is critical for selecting the correct PGR and for properly timing the application annually to consistently elicit the desired response. Results from two recent multi-year PGR experiments have provided evidence of promising PGR strategies for increasing ‘Hass’ avocado yield and fruit size. Gibberellic acid (GA<sub>3</sub>) (25 mg/L) applied at the cauliflower stage of inflorescence development or applied at the end of June-beginning of July significantly increased total yield and yield of large size fruit (packing carton sizes 60-40, 178-325 g/fruit) in kilograms per tree as 2-year average and 2-year cumulative yield ( $P \leq 0.03$ ). Benzyladenine (BA) (25 mg/L) applied at anthesis or GA<sub>3</sub> (25 mg/L) applied in mid-July followed by prohexadione calcium (ProCa) (125 mg/L) 30 days later significantly increased the yield of larger size fruit (packing carton sizes 40-28, 270-446 g/fruit) in kilograms per tree as 2-year average yield ( $P = 0.0069$  and  $P = 0.0564$ , respectively) and 2-year cumulative yield ( $P = 0.0120$  and  $P = 0.0624$ , respectively) without reducing total yield or yield of fruit of packing carton sizes 60, 48 and 40. These strategies will be tested in additional orchards.

### INTRODUCTION

Plant growth regulators (PGRs) are the most powerful tools available for manipulating tree growth and yield to solve production problems in an established orchard. Current commercial uses of foliar-applied PGRs on apple, citrus, kiwi and grape provide excellent examples of what can be accomplished. *Flowering* – PGRs can be used to cause early bud break and early flowering, resulting in early harvest, or to increase floral bud and flower retention, which increases floral intensity and yield. Conversely, there are PGRs that delay or reduce flowering. *Fruit number* – Several PGRs reduce early drop, June drop and preharvest fruit drop, and, thus, increase yield. *Fruit size* – There are PGRs that stimulate fruit growth to increase fruit size directly and others that increase fruit size indirectly by inducing flower or fruit abscission to reduce the competition for resources among the remaining fruit. *Fruit quality* – PGRs can advance or delay color development, maturation and ripening and influence sugar to acid ratio to provide high quality fruit for a specific marketing period. *Harvest* – PGRs can be used to “loosen” fruit for faster, more efficient hand or mechanical harvesting or to “hold” fruit on the tree and delay fruit senescence to extend the harvest period.

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Avocado is a relatively new commercial fruit crop compared to apple, citrus and grape. As a result, avocado PGR research is less advanced. However, 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 avocado. Moreover, simple adoption of PGR strategies used in the production of other tree fruit

crops is not possible. Due to poor uptake by avocado leaves, higher PGR concentrations than those typically used with other crops are required to elicit a response. As a further complication, the PGR responses elicited from avocado are frequently different from those obtained when using the same PGR on other tree 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, achieving consistent results from year to year and orchard to orchard is difficult due to alternate bearing and the presence of two crops on the tree for varying lengths of time up to 6 months.

Results of research undertaken by the Lovatt lab over the past 20 years have increased our understanding of the phenology and physiology of the ‘Hass’ avocado. This knowledge has been critical to selecting the correct PGR initially, designing the PGR strategy, and properly timing the application of the PGR annually to consistently attain the desired production goal. As a result, two recent multi-year PGR experiments have provided evidence of promising PGR strategies for increasing ‘Hass’ avocado yield and fruit size.

## **MATERIALS AND METHODS**

### **Plant material**

The efficacy of GA<sub>3</sub> applied at the end of June-beginning of July was determined in a commercially bearing orchard of 6-year-old ‘Hass’ avocado trees on Mexican race rootstocks in Carpinteria, Calif. (lat. 34°40’N). The remaining PGR strategies were tested using 10-year-old ‘Hass’ avocado trees on ‘Duke 7’ clonal rootstock in a commercial orchard located in Irvine, Calif. (lat. 33°67’N). All trees used in the experiment were selected for uniform health, size and vigor. Trees were maintained by regular pruning.

### ***PGR treatments***

The following PGRs applied at key phenological stages (see below) were tested: (1) GA<sub>3</sub> (25 mg ai/L) from ProGibb<sup>®</sup> (4% GA<sub>3</sub>, Abbott Laboratories, North Chicago, Ill.) applied at S-8, cauliflower stage; (2) GA<sub>3</sub> (25 mg ai/L) applied at the end of June-beginning of July; (3) 6-benzyladenine (BA) (25 mg ai/L) from MaxCel<sup>®</sup> (1.9% BA, Valent BioSciences Corp., Libertyville, Ill.) applied at S-11, anthesis; (4) GA<sub>3</sub> (25 mg ai/L) applied in mid-July [prior to S-2 (transition from vegetative to reproductive growth)] followed by prohexadione calcium (ProCa) (125 mg ai/L) from Apogee<sup>®</sup> (27.5% calcium 3-oxido-5-oxo-4-propionylcyclohex-3-enecarboxylate, BASF Corp., Research Triangle Park, N.C.) 30 days later (mid-August); and (5) control trees receiving no PGRs. All PGR

solutions contained 0.05% Silwet L-77 (Loveland Industries, Inc., Greeley, Colo.), a non-ionic surfactant, and were adjusted to pH 5.5 with citric acid before application at the rate of  $10 \pm 1$  L per tree with a handgun sprayer at 2.76 MPa between 600 and 1000 HR. PGR application times were based on the following phenological events: S-8, cauliflower stage of inflorescence development – obvious elongation of secondary axes, all flower parts are present, meiosis has occurred and microspores are present, and integuments are forming on the ovule (March) (Salazar-García et al., 1998); S-11, anthesis, fruit set and initiation of the spring vegetative flush (April) (Salazar-García et al., 1998); prior to the periods of June drop for the current crop (Garner, 2004), exponential increase in fruit size for the current crop (Garner, 2004), abscission of mature fruit (Garner, 2004) and development of the summer vegetative flush (Salazar-García et al., 1998) (end of June-beginning of July); and during inflorescence initiation for next year's crop (mid-August) (Salazar-García et al., 1998).

Fruit were harvested from March to October, depending on the site and year. Total yield was determined as kilograms fruit per tree. A randomly selected subsample of 100 to 150 fruit per tree, representing approximately 20% to 100% of the fruit per tree, was collected for each data tree. The weight of each fruit in the subsample was determined. These data were used to determine pack-out, i.e., kilograms of fruit of each packing carton size per tree, and to estimate the total number of fruit per tree. The following packing carton fruit sizes (grams per fruit) were used: 84 (99-134 g), 70 (135-177 g), 60 (178-212 g), 48 (213-269 g), 40 (270-325 g), 36 (326-354 g), 32 (355-397 g) and 28 (398-446 g). In addition, at harvest, two fruit were selected randomly per tree and allowed to ripen at  $22 \pm 2$  °C. The number of days required for fruit to ripen was recorded. When ripe, fruit length and width, seed width and flesh width were measured and external and internal fruit quality was evaluated for decay, abnormalities, discoloration, and seed germination. Vascularization (presence or absence of vascular bundles and associated fibers) of the flesh was also determined. Fruit quality parameters were rated on a scale from 0 (normal) to 4 (high incidence of decay, abnormalities, discoloration, or vascularization, i.e., occurring in all four quadrants of a fruit).

### ***Experimental design and statistical analyses***

The experimental design, with 20 individual tree replications per treatment, was a randomized complete block design. Repeated measure analysis was used to test for treatment effects on yield parameters with year or crop status (on or off) as the repeated measure factor. This analysis was performed using the General Linear Model procedures of the SAS statistical program (SAS Inst., Inc., Cary, N.C.). Analysis of variance was used to test for treatment effects on cumulative yield parameters. Means were separated using Duncan's multiple range test or Fisher's protected LSD test at  $P = 0.05$  as indicated in the tables. Note that for cumulative yields, a missing datum point for a tree in any year excluded all data for that tree from the statistical analysis.

## RESULTS AND DISCUSSION

### GA<sub>3</sub> increases yield and fruit size

GA<sub>3</sub> (25 mg/L) applied at the cauliflower stage of inflorescence development (March) significantly increased the 2-year average yield in kilograms fruit per tree ( $P = 0.0105$ ) and increased the 2-year average yield of commercially valuable large size fruit (combined pool of fruit of packing carton sizes 60, 48 and 40; fruit weighing 178-325 g/fruit) in kilograms per tree ( $P = 0.0184$ ) (Table 1). The effects of GA<sub>3</sub> applied at the cauliflower stage on 2-year average total yield and yield of commercially valuable large size fruit were also statistically significant as number of fruit per tree ( $P = 0.0085$  and  $P = 0.0094$ , respectively) (data not shown), providing evidence that the treatment increased both fruit retention and fruit growth, not just the weight of individual fruit.

Due to alternate bearing, year had a significant effect on the 2-year average total yield and yield of commercially valuable large size fruit (packing carton sizes 60, 48 and 40) as kilograms per tree ( $P < 0.0001$ ) (Table 1). There was a significant treatment by year interaction on 2-year average total yield and yield of large size fruit of packing carton sizes 60, 48 and 40 as kilograms per tree ( $P = 0.0026$  and  $P = 0.0029$ , respectively) (Table 1).

Application of GA<sub>3</sub> at the cauliflower stage of inflorescence development also significantly increased the 2-year cumulative total yield ( $P = 0.0105$ ) and 2-year cumulative yield of commercially valuable large size fruit (178 g to 325 g/fruit) ( $P = 0.0212$ ) as kilograms per tree (Table 1). The treatment significantly increased the total number of fruit per tree ( $P = 0.0074$ ) and the number of large size fruit in the combined pool of fruit of packing carton sizes 60, 48 and 40 ( $P = 0.0107$ ) (data not shown). The treatment resulted in a 2-year cumulative net increase of 4,227 kilograms more fruit per ha than the untreated control. Moreover, 68% of the net increase in yield was large size fruit. The 2-year cumulative net increase of fruit of packing carton sizes 60, 48 and 40 was 2,882 kilograms per ha over that of the control. In previous studies, the cauliflower stage of inflorescence development proved responsive to treatments designed to increase fruit set and yield (Jaganath and Lovatt, 1998; Lovatt, 1999), including GA<sub>3</sub> (Salazar-García and Lovatt, 2000).

Table 1. Effect of GA<sub>3</sub> (25 mg/L) applied at the cauliflower stage on ‘Hass’ avocado yield in Irvine, Calif.

Treatment	2-year average		2-year cumulative	
	Total yield	Σ60+48+40	Total yield	Σ60+48+40
	----- <i>kg fruit/tree</i> -----			
GA <sub>3</sub>	22.91 a <sup>z</sup>	17.07 a	45.83 a	34.01 a
Control	15.14 b	11.65 b	30.28 b	23.41 b
<i>P</i> -value				
Treatment (T)	0.0105	0.0184	0.0105	0.0212
Year (Y) <sup>y</sup>	<0.0001	<0.0001	–	–
T x Y	0.0026	0.0029	–	–

<sup>z</sup> Values in a vertical column followed by different letters are significantly different by Fisher’s protected LSD test.

<sup>y</sup> On- or off-crop year.

In a second study, GA<sub>3</sub> (25 mg/L) applied at the end of June-beginning of July increased total yield as kilograms of fruit per tree ( $P = 0.0299$ ) and also yield of commercially valuable large size fruit (packing carton sizes 60, 48, and 40) as kilograms per tree ( $P = 0.0208$ ) when averaged over the on- and off-crop status of the trees (Table 2). The GA<sub>3</sub> effects on total yield ( $P = 0.0411$ ) and yield of commercially valuable large size fruit (178-375 g/fruit) ( $P = 0.0148$ ) were also statistically significant as number of fruit per tree when averaged across the on- and off-crop status of the trees (data not shown).

The on- or off-crop status of the tree had a significant effect on the 2-year average total yield and yield of commercially valuable large size fruit of packing carton sizes 60, 48 and 40 per tree ( $P < 0.0001$ ) (Table 2). The effect of GA<sub>3</sub> on both yield parameters was only statistically significant when the trees were carrying an on crop. There was no significant treatment by crop status interaction effect on any yield parameter (Table 2).

GA<sub>3</sub> applied at the end of June-beginning of July statistically significantly increased the 2-year cumulative total yield ( $P = 0.0299$ ) and 2-year cumulative yield of large size fruit (packing carton sizes 60, 48, and 40) ( $P = 0.0309$ ) in kilograms per tree (Table 2). This second GA<sub>3</sub> treatment resulted in a 2-year cumulative net increase of 7,374 kilograms per ha more fruit than the untreated control, of which 83% were large size fruit. The 2-year net increase of fruit of packing carton sizes 60, 48 and 40 was 6,153 kilograms more per ha than the control.

This application time was selected because it is just prior to June drop and exponential fruit growth for the current crop and preharvest abscission of the 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, unpublished). Moreover, this application time is 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).

Table 2. Effect of GA<sub>3</sub> (25 mg/L) applied at the end of June – beginning of July on 'Hass' avocado yield in Carpinteria, Calif.

Treatment	2-year average		2-year cumulative	
	Total yield	Σ60+48+40	Total yield	Σ60+48+40
	----- <i>kg fruit/tree</i> -----			
GA <sub>3</sub>	58.96 a <sup>z</sup>	50.79 a	117.92 a	100.08 a
Control	45.39 b	38.76 b	90.79 b	77.44 b
<i>P</i> -value				
Treatment (T)	0.0299	0.0208	0.0299	0.0309
Tree status	<0.0001	<0.0001	–	–
(S) <sup>y</sup>				
T x S	0.2462	0.1473	–	–

<sup>z</sup> Values in a vertical column followed by different letters are significantly different by Duncan's Multiple Range Test.

<sup>y</sup> On- or off-crop.

### PGR strategies that increase fruit size

*BA.* Foliar-application of BA (25 mg/L) at anthesis significantly increased the kilograms and number of large fruit of packing carton sizes 40 (270-325 g/fruit) ( $P = 0.0092$ ) and 36 (326-354 g/fruit) per tree ( $P = 0.0257$ ) and the combined pool of fruit of packing carton sizes 40, 36, 32 and 28 (270-446 g/fruit) per tree (as kg  $P = 0.0069$  and as fruit number  $P = 0.0071$ ) averaged across 2 years of the experiment (Table 3). Notably, the increased yield of large size fruit was not at the expense of total yield. The 2-year average total yield per tree and yield of fruit of packing carton sizes 60, 48 and 40 as both kilograms and number of fruit per tree were numerically but not statistically greater than untreated control (Table 3). Thus, the BA treatment had a positive effect on both fruit retention and fruit growth.

Year, predominantly due to alternate bearing, had a significant effect on the 2-year average total yield as kilograms per tree ( $P = 0.0482$ ), an insignificant effect on the 2-year average yield of commercially valuable large size fruit in the pool of fruit of packing carton sizes 60, 48 and 40 in kilograms per tree ( $P = 0.0806$ ), and a highly significant effect on the 2-year average yield of larger size fruit in the pool of fruit of packing carton size 40, 36, 32 and 28 in kilograms per tree ( $P = 0.0016$ ) (Table 3). A marginal treatment by yield interaction was obtained for only the latter yield parameter ( $P = 0.0993$ ).

Foliar application of BA at anthesis also increased the 2-year cumulative yield of fruit of packing carton size 40 ( $P = 0.0156$ ), packing carton size 36 ( $P = 0.0373$ ) (data not

shown) and the combined pool of fruit of packing carton sizes 40, 36, 32 and 28 ( $P \leq 0.0124$ ) as both kilograms (Table 3) and number of fruit (data not shown) per tree compared to the untreated control. The treatment numerically, but not significantly, increased the 2-year cumulative total yield and yield of large size fruit of packing carton sizes 60, 48 and 40 as both kilograms and number of fruit per tree (Table 3) and significantly reduced the yield of small size fruit (packing carton sizes 84 and 70, 99-177 g/fruit) as both kilograms and number of fruit per tree compared to the control ( $P \leq 0.0459$ ) (data not shown). The BA treatment resulted in a 2-year cumulative net increase of 1,653 kilograms per ha of large fruit of packing carton size 40, but a net increase of only 363 kilograms per ha of fruit of packing carton size 36 over that of the control. The 2-year cumulative net increase of fruit in the combined pool of fruit of packing carton sizes 40, 36, 32 and 28 was 2,218 kilograms per ha more than the control.

BA is used as a fruit-thinning agent in apples. In addition to increasing fruit size indirectly by reducing fruit number and competition among the remaining fruit, BA also increases fruit size by stimulating cell division. For the avocado fruit, cell division continues through all stages of fruit development. Since the 2-year average and 2-year cumulative total number of fruit per tree was numerically greater than the control, it is unlikely that BA increased fruit size of the ‘Hass’ avocado in this experiment through a fruit thinning effect.

Table 3. Effect of BA (25 mg/L) applied at anthesis on ‘Hass’ avocado yield in Irvine, Calif.

Treatment	2-year average			2-year cumulative		
	Total yield	$\Sigma 60+48+40$	$\Sigma 40+36+32+28$	Total yield	$\Sigma 60+48+40$	$\Sigma 40+36+32+28$
	----- kg fruit/tree -----					
BA	19.91	16.54	6.86 a <sup>z</sup>	39.82	33.03	14.01 a
Control	15.14	11.65	2.66 b	30.28	23.41	5.85 b
<i>P</i> -value						
Treatment (T)	0.1686	0.1195	0.0069	0.1686	0.1271	0.0120
Year (Y) <sup>y</sup>	0.0482	0.0806	0.0016	–	–	–
T x Y	0.3932	0.7043	0.0993	–	–	–

<sup>z</sup> Values in a vertical column followed by different letters are significantly different by Fisher’s protected LSD test.

<sup>y</sup> On- or off-crop year.

*GA<sub>3</sub> with ProCa.* GA<sub>3</sub> (25 mg/L) applied in mid-July followed by ProCa (125 mg/L) 30 days later in mid-August produced yield responses similar to those of the BA treatment, but that were just under significance at  $P = 0.05$  (Table 4). The treatment increased the kilograms and number of large fruit of packing carton sizes 40 (270-325 g/fruit) ( $P = 0.0633$ ) and 36 (326-354 g/fruit) per tree ( $P = 0.0562$ ) (data not shown) and the combined

pool of fruit of packing carton sizes 40, 36, 32 and 28 (270-446 g/fruit) per tree (as kg  $P = 0.0564$  and as fruit number  $P = 0.0572$ ) averaged across 2 years of the experiment without reducing total yield or yield of large size fruit of packing carton sizes 60, 48 and 40 as either kilograms or number of fruit per tree (Table 4).

Year had a significant effect only on the 2-year average yield of larger size fruit in the combined pool of fruit of packing carton sizes 40, 36, 32 and 28 in kilograms per tree ( $P = 0.0021$ ) Table 4). A treatment by year interaction only marginally influenced the 2-year average total yield in kilograms per tree ( $P = 0.0953$ ), had no influence on the 2-year average yield of fruit of packing carton size 60, 48 and 40 in kilograms per tree, but significantly affected the 2-year average yield of larger size fruit in the combined pool of packing carton sizes 40, 36, 32 and 28 in kilograms per tree ( $P = 0.0492$ ) (Table 4).

GA<sub>3</sub> applied in mid-July followed by ProCa 30 days later in mid-August had a positive, almost significant effect at  $P = 0.05$  on the 2-year cumulative yield of fruit of packing carton size 40 ( $P = 0.0665$ ), packing carton size 36 ( $P = 0.0773$ ) (data not shown) and the combined pool of fruit of packing carton sizes 40, 36, 32 and 28 ( $P \leq 0.0627$ ) as both kilograms (Table 4) and number of fruit (data not shown) per tree compared to the untreated control. The treatment numerically, but not significantly, increased the 2-year cumulative total yield and yield of large size fruit in the combined pool of packing carton sizes 60, 48 and 40 as both kilograms (Table 4) and number of fruit per tree (data not shown). Thus, this PGR strategy had a positive effect on fruit retention and fruit growth.

Application of GA<sub>3</sub> in mid-July was a strategy to stimulate the summer vegetative shoot flush to increase the number of shoots on which to bear inflorescences the next spring and to simultaneously take advantage of GA<sub>3</sub>'s ability to reduce fruit drop and increase fruit size during this period. Application of ProCa 30 days later was a strategy to stop summer vegetative shoot growth in order to increase the number of shoot apical meristems that transitioned from vegetative to reproductive (floral) development in response to environmental cues present at this time (Salazar-García et al., 1998). Since total yield as number of fruit per tree was not significantly increased, this strategy likely did not work as anticipated. The mechanism by which this strategy caused a shift to large size fruit ( $\geq$  packing carton size 40) is not presently understood. The fruit size distribution (pack out) obtained by application of BA at anthesis or GA<sub>3</sub> in mid-July followed by ProCa 30 days later was considered commercially valuable by most California 'Hass' avocado growers.



Table 4. Effect of GA<sub>3</sub> (25 mg/L) applied in mid-July followed by ProCa (125 mg/L) 30 days later (25 mg/L) on ‘Hass’ avocado yield in Irvine, Calif.

Treatment	2-year average			2-year cumulative		
	Total yield	Σ60+48+40	Σ40+36+ 32+28	Total yield	Σ60+48+40	Σ40+36+ 32+28
----- <i>kg fruit/tree</i> -----						
GA <sub>3</sub> + ProCa	18.62	15.11	7.37 a	37.23	30.35	15.05 a
Control	15.14	11.65	2.66 a	30.28	23.41	5.85 a
<i>P</i> -value						
Treatment (T)	0.4454	0.3830	0.0564	0.4454	0.3811	0.0621
Year (Y) <sup>y</sup>	0.3795	0.6280	0.0021	–	–	–
T x Y	0.0953	0.1390	0.0492	–	–	–

<sup>z</sup> Values in a vertical column followed by different letters are significantly different by Fisher’s protected LSD test.

<sup>y</sup> On- or off-crop year.

### Postharvest fruit quality

It should be noted that for all experiments reported here, no PGR had a negative effect on any fruit quality parameter evaluated (data not shown). Salazar-García (unpublished) recently discovered that GA<sub>3</sub> (50 mg/L) applied 4 months before normal harvest advanced fruit maturity (21.5% dry mass) 36 days in Nayarit, México, with no negative effects on yield or fruit size of the current crop or return crop. Early fruit maturity is of significant economic benefit to ‘Hass’ avocado growers in Nayarit. Effects of the GA<sub>3</sub> strategies reported here on avocado fruit dry matter accumulation were not quantified. However, it is important to know whether these promising strategies also influence the rate at which ‘Hass’ avocado fruit in California reach legal maturity (21.6% dry mass) since this would impact when growers could harvest and market their fruit. Thus, future research will evaluate the effects of PGR strategies using GA<sub>3</sub> (and those using the GA biosynthesis inhibitor ProCa) on the rate of fruit dry matter accumulation and date on which fruit reach legal maturity in California ‘Hass’ avocado orchards.

### CONCLUSIONS

The results reported herein provide evidence of the potential of properly timed foliar applications of BA and GA<sub>3</sub>, alone or in combination with a subsequent application of ProCa, to increase yield and/or yield of commercially valuable large size fruit. To meet the requirements of the California Department of Pesticide Regulation (DPR), the results for each PGR need to be successfully reproduced in an additional experiment at a new orchard in a different avocado-growing area of the state than the ones used in the experiments presented here. Due to alternate bearing, PGR treatments that produce statistically significant results averaged across on- and off-crop years, as cumulative yield

or in only in the on- or off-crop year (with use restricted to only the on- or off-crop year, respectively) are acceptable to the DPR. In addition, the DPR requires demonstration that PGR-induced yield responses are dose dependent. Thus, total yield or yield of large size fruit must increase incrementally with increasing PGR concentration. We intend to undertake the additional experiments necessary to meet the requirements of the state DPR, with the goal of making one or more PGR strategy available for use in commercial 'Hass' avocado production in California within the next 3 to 4 years.

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