Response of evergreen perennial tree crops to gibberellic acid is crop load-dependent: II. GA_3 increases yield and fruit size of 'Hass' avocado only in the on-crop year of an alternate bearing orchard

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A B S T R A C T

Despite problems of low fruit set, small fruit size and alternate bearing, the Hass cultivar dominates commercial avocado production worldwide. To increase yield and fruit size, gibberellic acid (GA_3) (25 mg L^{-1}) was applied at different stages of 'Hass' avocado tree phenology: (*i*) mid–late April (flower abscission), end of June–beginning of July (fruit abscission and beginning of the exponential phase of fruit growth), and mid-January (beginning of pre-harvest fruit drop); (*ii*) end of June–beginning of July; and (*iii*) mid-September (near the end of the major fruit abscission period; period of exponential fruit growth). In both years of the research, applications of GA_3 in April and June–July were within the periods of intense flower and fruit abscission, respectively; fruit abscission was low in September and January. Maximum air temperature was not related to flower or fruit abscission. In the on-crop year (391 fruit per untreated control tree), a single application of GA_3 at the end of June–beginning of July significantly increased total yield (kilograms only) and yield of commercially valuable fruit (178–325 g/fruit) (as kilograms and number per tree) compared with the control ($P < 0.0001$). GA_3 applied in September increased total yield (kilograms only) and yield of commercially valuable fruit (kilograms and number per tree) to values intermediate to and not significantly different from all other treatments, except trees receiving multiple applications of GA_3. This treatment reduced total yield and yield of commercially valuable fruit (kilograms and number per tree) relative to all treatments ($P \leq 0.0002$). In contrast, during the off-crop year (32 fruit per control tree), no GA_3 treatment had a significant effect on yield or fruit size compared with the control and all other GA_3 treatments. For 'Hass' avocado, there was no negative effect from applying GA_3 at the end of June–beginning of July in both the off- and on-crop years; 2-year cumulative total yield and yield of commercially valuable fruit were increased by 27 kg (128 fruit) and 22 kg (101 fruit) per tree, respectively, above the yield of untreated control trees ($P < 0.0001$).

1. Introduction

The 'Hass' avocado (*Persea americana* Mill.) dominates worldwide avocado production (>80%) (http://www.avocadosource.com), with California among the top four largest producers in terms of 'Hass' bearing hectares (26,125 ha in 2008–2009) and production (79,150 metric tons in 2008–2009) (http://www.avocado.org). Despite its popularity, this cultivar is problematic with regard to fruit set, fruit size and alternate bearing. 'Hass', like other avocado cultivars, has extremely low fruit set (<0.1%) due to excessive abscission of flowers and developing fruit even in healthy, well-managed orchards (Cameron et al., 1952; Garner and Lovatt, 2008; Lahav and Zamet, 1975; Slabbert, 1981; Whiley and Schaffer, 1994). The percentage of small fruit harvested each year varies from 20% to 60%, depending on climate, tree health, cultural practices and crop load (Cutting, 1993; Moore-Gordon et al., 1998; Zilkah and Klein, 1987). In an alternate bearing orchard, the number of young fruit that abscise and the number of small fruit that are harvested both increase during the on-crop year. For example, in California, maximum fruit abscission reached ~280 fruit per day for the on-crop year, but only ~50 fruit per day in the off-crop year (Garner and Lovatt, 2008). Similarly, 44% and 34% of the harvested crop by number and mass, respectively, were characterized as small (≤177 g/fruit) in the on-crop year compared with only 3% and 2% of the harvested crop by number and mass in the off-crop year (Lovatt, unpublished data).

Gibberellic acid (GA_3) is registered for use to increase fruit set and fruit size of numerous vine and fruit crops: grape (*Vitis vinifera*), citrus (*Citrus* spp.), banana (*Musa* spp.), currant (*Ribes aureum*), pineapple (*Ananas comosus*) and sweet cherry (*Prunus* *avium*) (Valent BioSciences Corp., 2006, 2009). Research testing the efficacy of GA_3 to increase yield and fruit size of avocado has been minimal. Application of GA_3 at full bloom dramatically increased the number of seedless fruit, known as "cukes" to a value 40 times greater than that of the untreated control with no effect of the yield of normal seeded fruit (Loupasssaki et al., 1995). In a 2-year study to mitigate the problem of low fruit retention, Köhne (1989) applied GA_3 (500 mg L^{-1}) shortly after flowering to the canopy of 'Fuerte' avocado trees. The rate of fruit abscission was decreased, with the effect still evident 2–3 months after treatment. At harvest, yield was slightly greater than that of the untreated control as number of fruit per tree but not as kilograms per tree, due to a positive effect on fruit retention and a negative effect on individual fruit mass. The GA_3 treatment did not increase the yield of seedless fruit. Later application of GA_3 to the 'Hass' avocado at the beginning of fruit abscission and again 3 weeks later had no significant effect on total yield but reduced the number of very small fruit (<133 g/fruit) by 50% and increased the yield of export size fruit by 17.2% (Zilkah and Klein, 1987; Zilkah et al., 1995). GA_3 effects on yield and fruit size were determined in a 2-year study using GA_3 to manipulate the floral intensity of an alternate bearing 'Hass' avocado orchard (Salazar-García and Lovatt, 2000). September and January applications of GA_3 (25 mg L^{-1}) had a positive effect on yield (kg/tree) in the on- and off-crop years, respectively. The September GA_3 application significantly increased the yield of commercially desirable fruit (213–269 g/fruit) in the off-crop year. It was observed that fruit treated with GA_3 had delayed color break and blackening of the exocarp, with no delay in on-tree maturation or post-harvest ripening.

In many avocado-growing areas, the number of indeterminate floral shoots greatly exceeds the number of determinate floral shoots. Schroeder (1944) reported that for most avocado cultivars grown in California, indeterminate floral shoots accounted for 80–95% of the total floral shoots produced at spring bloom. For the 'Hass' cultivar grown in Australia (Thorp et al., 1994) and California (Salazar-García and Lovatt, 1998), indeterminate floral shoots constituted 65% and 90%, respectively, of the total floral shoots. Indeterminate floral shoots have a lower percent fruit set (0.05%) than determinate floral shoots (0.17%) (Salazar-García and Lovatt, 1998) as a result of competition between the elongating vegetative shoot apex of the indeterminate floral shoot and the setting fruit (Bower and Cutting, 1992; Cutting and Bower, 1990; Whiley, 1990; Zilkah and Klein, 1987). Thus, Kalmer and Lahav (1976) and Kotzé (1982) cautioned against application of nitrogen fertilizer or other treatments during flowering and fruit set that would stimulate the growth of the vegetative shoot apex of indeterminate floral shoots, predicting this would reduce fruit set and yield. Contrary to this prediction, applying GA_3 (25 mg L^{-1}) at the cauliflower stage of inflorescence development (before full bloom) caused precocious development of the vegetative shoot apex of indeterminate floral shoots and increased yield and fruit size (Salazar-García and Lovatt, 2000). Furthermore, supplying nitrogen (56 kg ha^{-1}) to the soil during the period of flower opening and early fruit set when elongation of the vegetative shoot apex of indeterminate floral shoots would be initiated (mid-April) increased 4-year cumulative yield (39%) as kilograms per tree, with more than 70% of the net increase in yield commercially valuable size fruit (178–325 g/fruit), and reduced the severity of alternate bearing compared with control trees receiving no nitrogen during this period (Lovatt, 2001). Additionally, Köhne (1989) observed that GA_3 applied shortly after flowering increased fruit retention, despite stimulating the growth of the vegetative shoot apex of indeterminate floral shoots. Taken together, these results indicate that the period from early flowering (cauliflower stage) to shortly after flowering responds well to treatments designed to increase 'Hass' avocado yield.

Worldwide, 'Hass' avocado yields averaged 8.7 tons per hectare for the period 1993–2003 (http://www.avocadosource.com), well below the estimated theoretical yield of 32.5 tons per hectare (Wolstenholme, 1986). Average yield of the 'Hass' avocado in California has been considerably lower (6.6 metric tons per hectare) over the past decade (http://www.avocado.org). Thus, the research reported herein was undertaken to test the efficacy of GA_3 (25 mg L^{-1}) applied at different stages of 'Hass' avocado tree phenology to increase fruit set and/or fruit size in an alternate bearing orchard.

2. Materials and methods

2.1. Plant material

This experiment used 7-year-old 'Hass' avocado trees on a Mexican race rootstock in a commercial orchard in Carpinteria, CA (34.39°N, 119.51°W). The experiment was initiated in spring of an off-crop bloom.

2.2. GA_3 treatments

The experiment included three GA_3 treatments and an untreated control, each replicated on 20 individual trees per treatment in a randomized complete block design. There were buffer trees between treated trees within a row and buffer rows between treated rows. GA_3 was applied at 25 mg L^{-1} at the time specified in each treatment (the objective of each treatment is given in parentheses): (1) mid–late April with N at 56 kg ha^{-1} as NH_4NO_3, end of June–beginning of July, and again in mid-January (to increase fruit retention during early fruit drop, June drop and pre-harvest fruit drop); (2) end of June–beginning of July (to increase fruit retention and fruit size); and (3) mid-September (to increase fruit size). GA_3 treatments were prepared from ProGibb (4% GA_3, Valent BioSciences, Corp.) and contained the surfactant Silwet L-77 (General Electric Co.) at a final concentration of 0.05%. All treatments were applied in 1869 L of water per hectare with a 2758 kPa handgun sprayer. Treatments were applied according to tree phenology (calendar dates for years 1 and 2, respectively, are given in parentheses): (*i*) mid–late April – beginning of the intense flower abscission period, initiation of elongation of the vegetative shoot apex of indeterminate floral shoots, and early fruit set (19 and 30 April); (*ii*) end of June–beginning of July – during the period of intense fruit abscission and beginning of the exponential phase of fruit growth (6 July and 26 June); (*iii*) mid-September – near the end of the major fruit abscission period; during the period of exponential fruit growth (15 and 16 September); and (*iv*) mid-January – beginning of pre-harvest fruit drop (17 and 16 January). The objective of treatment 1 was to increase total yield. To this end, soil-applied N (56 kg ha^{-1}) in mid–late April was included in treatment 1 based on a demonstrated increase in yield in response to N applied at this stage of 'Hass' avocado tree phenology (Lovatt, 2001). A mid-January GA_3 application was also included in treatment 1 as one of three applications to reduce early fruit drop, June drop and pre-harvest fruit drop, respectively.

Nets were placed under 10 untreated trees within the orchard. Contents of the nets were collected starting in January on a bi-weekly basis and then weekly from April through August when the rate of abscission of reproductive structures was high. Net samples were collected bi-weekly from September through February. The samples collected were used to determine the intense periods of flower and fruit abscission and their relationship to maximum air temperature and GA_3 application times.

All fruit were harvested in October each year. Total yield was determined as kilograms per tree. At harvest, a randomly selected sample of 100–150 fruit per tree, representing ~30–100% of the mean total number of fruit on a tree for each year of the experiment, was collected for each data tree and the mass of each fruit in the subsample was determined. These data were used to calculate pack-out, i.e., the kilograms of fruit of each packing carton size per tree and to estimate the total number fruit and number of fruit in each packing carton size category per tree. The following packing carton fruit sizes (grams per fruit) were used: size 84 (99–134 g), size 70 (135–177 g), size 60 (178–212 g), size 48 (213–269 g), size 40 (270–325 g), size 36 (326–354 g), and size 32 (355–397 g). In addition, at harvest, two fruit were selected randomly per tree and allowed to ripen to "eating soft" in a controlled temperature chamber at 18–21 °C. When ripe, external and internal fruit quality was evaluated for abnormalities and discoloration. Vascularization (presence of vascular bundles and associated fibers) of the mesocarp was also determined. The above fruit quality parameters were rated on a scale from 0 (normal) to 4 (high incidence of abnormalities, discoloration, or vascularization). Exocarp color was rated on a scale from 1 (100% of the exocarp was green) to 5 (with 2 indicating 25% and 5 indicating 100% of the exocarp was black, respectively).

To determine treatment effects on the severity of alternate bearing, the alternate bearing index (ABI) was calculated for each data tree for the two harvests using the following equation: ABI = (year 1 yield minus year 2 yield)/(sum of year 1 yield and year 2 yield) in which yield was defined as total kilograms of fruit per tree. ABI ranges from 0 (no alternate bearing) to 1 (complete alternate bearing) (Pearce and Dobersek-Urbanc, 1967).

2.3. Temperature data

Maximum average air temperatures for the 2 years of the research were downloaded from the California Irrigation Management Information System (CIMIS) website (California Department of Water Resources, 2009) for the closest coastal station, Santa Barbara #107 (34.26°N, 119.44°W, elevation 76 m).

2.4. Leaf nutrient analysis

In September of each year, 20 spring flush leaves from non-fruiting terminals were collected uniformly around each data tree at a height of 1.5 m above ground. Leaves were washed with soapy water and rinsed thoroughly with distilled water, oven dried at 60 °C for 72 h, and ground in a Wiley mill to pass through a 40-mesh (0.635-mm) screen (Embleton et al., 1973). The ground samples were sent to Albion Laboratories, Clearfield, UT, for mineral nutrient analysis. Samples were combusted at 1050 °C and nitrogen (N) and sulfur (S) concentration were determined by thermal conductivity (Leco Corp., St. Joseph, MI). Sample concentrations of phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), iron (Fe), manganese (Mn), boron (B), copper (Cu) and zinc (Zn) were determined using inductively coupled plasma (ICP) emission spectroscopy (Accuris; Beverly, MA).

2.5. Statistical analysis

Repeated measure analysis was used to test treatment effects on yield parameters with year as the repeated measure factor. This analysis was performed using the General Linear Models procedure of SAS (version 6.12; SAS Institute, Cary, NC). Analysis of variance was used to test treatment effects on fruit quality parameters, leaf nutrient analyses, and on all yield parameters for a specific year and for 2-year cumulative yield. Linear regression analysis was used to test the effect of total yield on fruit size. Means were separated using Duncan's Multiple Range Test at $P = 0.05$.

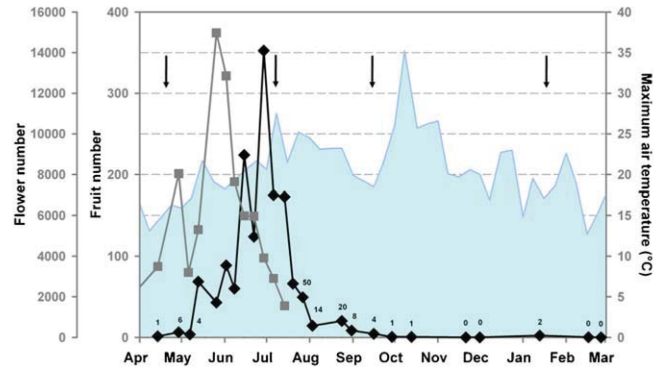

Fig. 1. Maximum air temperature (blue area plot) and the mean total number of flowers (-■-) and fruit (-◆-) that abscised from 10 untreated trees within the orchard per week in April through August and bi-weekly in September through February during the off-crop year of 'Hass' avocado trees in a commercial orchard in Carpinteria, CA. Arrows indicate gibberellic acid (GA_3) application dates: 19 April, 6 July, 15 September and 17 January. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

3. Results

3.1. Flower and fruit abscission and relationship to air temperature and gibberellic acid applications

Year 1 was characterized by the low floral intensity of an off-crop year. The mean number of flowers that abscised during the entire bloom period (14 February to 17 July) was 75,950 (data not shown). It is of interest that 98% of all flowers that abscised did so between 14 April and 29 June (Fig. 1). Fruit abscission began 14 April, with collection of abscised fruit reported herein through 26 February of the following year (Fig. 1). The total number of fruit that abscised over this period was 1489, with 79% abscising between 8 June and 20 July. In contrast, for year 2, during the bloom of the on-crop year (13 January through 20 June), 339,570 flowers abscised, a 4.5-fold greater number of abscised flowers than the previous year (data not shown). Approximately 88% of the abscised flowers were collected from 6 April to 20 June during the on-crop year (Fig. 2). Collection of abscised fruit for year 2 began 6 April and continued through 21 February the following year. During this period 15,678 fruit abscised, 10.5-fold more fruit than in the off-crop year. The period of intense fruit abscission started earlier in the on-crop year (4 May to 27 July) compared with the off-crop year (8 June to 20 July). During this period, 14,373 fruit abscised during the on-crop

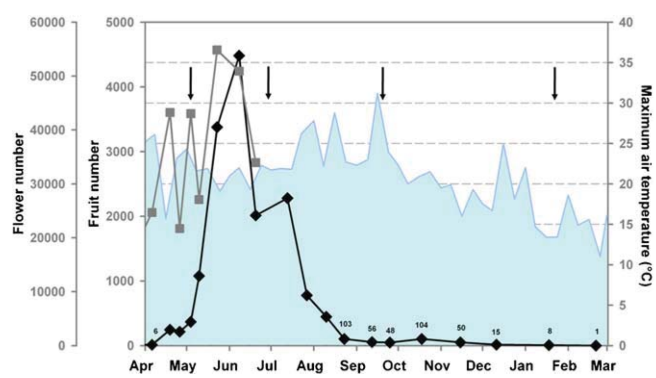

Fig. 2. Maximum air temperature (blue area plot) and the mean total number of flowers (-■-) and fruit (-◆-) that abscised from 10 untreated trees within the orchard per week in April through August and bi-weekly in September through February during the on-crop year of 'Hass' avocado trees in a commercial orchard in Carpinteria, CA. Arrows indicate gibberellic acid (GA_3) application dates: 30 April, 26 June, 16 September and 16 January. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Table 1
Effect of gibberellic acid (GA_3) applied at different stages of 'Hass' avocado tree phenology during an off-crop year on total yield and fruit size distribution (based on individual fruit mass) as kilograms and number of fruit per tree.

GA_3 applications times[y]	Total		Fruit packing carton size[z]					
	kg	no.	84 + 70		60 + 48 + 40		≥60	
			kg	no.	kg	no.	kg	no.
Mid–late April[x] + end of June–beginning of July + mid-January	6.5 a[w]	28 a	0.6 a	4 a	5.4 a	22 a	5.9 a	24 a
End of June–beginning of July	12.4 a	59 a	2.3 a	15 a	9.7 a	43 a	10.1 a	44 a
Mid-September	7.8 a	34 a	0.7 a	4 a	6.9 a	29 a	7.1 a	30 a
Control–untreated	7.5 a	32 a	0.5 a	3 a	6.6 a	27 a	7.0 a	28 a
P-value	.3547	.2852	.1394	.1415	.5340	.4914	.5289	.4851

[w] Values in a vertical column followed by different letters are significantly different at specified *P* levels by Duncan's Multiple Range Test.
[x] In conjunction with application of GA_3 in April, N at 56 kg ha^{-1} as NH_4NO_3 was applied to the soil.
[y] Mid–late April – flower abscission period (19 April); end of June–beginning of July – fruit abscission period and beginning of the exponential phase of fruit growth (6 July); mid-September – near the end of the fruit abscission period (15 September); and mid-January – prior to pre-harvest fruit drop (17 January). GA_3 was applied at 25 mg L^{-1} in 1869 L of water per hectare.
[z] Packing carton fruit sizes (grams per fruit): 84 (99–134 g); 70 (135–177 g); 60 (178–212 g); 48 (213–269 g); 40 (270–325 g); 36 (326–354 g); size 32 (355–397 g).

year compared with only 1175 fruit during the analogous period in the off-crop year. Air temperature did not exceed 33 °C in the orchard until October of each year of the research. High temperatures in October appeared to have no effect on fruit retention (Figs. 1 and 2).

In year 1, the off-crop year, application of GA_3 on 19 April was just after the start of the intense period of flower abscission (14 April) (Fig. 1). The 6 July application of GA_3 was near the end of flower abscission (14 July) and within the period of intense fruit abscission (8 June to 20 July). The applications of GA_3 on 15 September and 17 January were during periods characterized by low numbers of abscised fruit. Similarly, in year 2, the on-crop year, the 30 April GA_3 application was after the start of the period of intense flower abscission (6 April) and the 26 June application was within the intense fruit abscission period (4 May to 27 July) (Fig. 2). There were still approximately 50 fruit abscising per week when the 16 September GA_3 treatment was applied; however, fruit abscission was low by 16 January.

3.2. *Effect of gibberellic acid on yield, fruit size and fruit quality*

In the off-crop year, no GA_3 treatment had a statistically significant positive or negative effect on total yield or fruit size as kilograms or number of fruit per tree (Table 1). Yield was extremely low in the off-crop year, averaging just 7.5 kg (32 fruit) per untreated control tree.

In the on-crop year, yield was more than 10-fold greater than in the off-crop year, averaging 83.3 kg (391 fruit) per untreated control tree. GA_3 applied at the end of June–beginning of July significantly increased total yield and yield of commercially valuable fruit (packing carton sizes 60 + 48 + 40, 178–325 g/fruit) as kilograms per tree compared with all other treatments, except trees treated with GA_3 in mid-September (P = 0.0001) (Table 2). Trees treated with GA_3 in mid-September had a mean total yield and yield of large size fruit intermediate to and not significantly different from trees treated with GA_3 at the end of June–beginning of July and untreated control trees. Application of GA_3 in mid–late April with soil-applied N, end of June–beginning of July, and again in mid-January reduced total yield and yield of the combined pool of fruit of packing carton sizes 60 + 48 + 40 as kilograms per tree compared with trees in all other treatments (P = 0.0001). The mean total yield for trees in this treatment was 37% less than the untreated control trees and 50% less than trees treated with GA_3 at the end of June–beginning of July, with a reduction in the yield of large size fruit proportionate to the reduction in total yield. For trees treated with GA_3 at the end of June–beginning of July 92% of the net increase in total yield (22 kg/tree) above that of the untreated control trees was commercially valuable large fruit (packing carton sizes 60 + 48 + 40).

The increase in total kilograms of fruit per tree attained with GA_3 applied at the end of June–beginning of July was due to a greater number of commercially valuable fruit (178–325 g/fruit) per tree (P = 0.0001) not an increase in the total number of fruit compared with the untreated control trees (Table 2). Thus, GA_3 applied at this time stimulated fruit growth rather than reducing fruit abscission.

The degree of alternate bearing in the orchard for the 2 years of the experiment was severe. The ABI for the untreated control trees was 0.8. An ABI of 1.0 is complete alternate bearing, with crop 1 year and no crop the other year. GA_3 treatments did not significantly reduce the severity of alternate bearing in the orchard. Trees treated with GA_3 in mid–late April with soil-applied N, end of June–beginning of July, and again in mid-January had an ABI of 0.7, trees treated with GA_3 at the end of June–beginning of

Table 2
Effect of gibberellic acid (GA_3) applied at different stages of 'Hass' avocado tree phenology during an on-crop year on total yield and fruit size distribution (based on individual fruit mass) in kilograms and number of fruit per tree.

GA_3 applications times[y]	Total		Fruit packing carton size[z]					
	kg	no.	84 + 70		60 + 48 + 40		≥60	
			kg	no.	kg	no.	kg	no.
Mid–late April[x] + end of June–beginning of July + mid-January	52.7 c[w]	244 b	7.3 a	48 a	44.3 c	192 c	45.4 c	196 c
End of June–beginning of July	105.5 a	491 a	12.6 a	83 a	91.9 a	406 a	92.9 a	409 a
Mid-September	93.7 ab	436 a	11.4 a	74 a	81.6 ab	359 ab	82.3 ab	361 ab
Control–untreated	83.3 b	391 a	11.9 a	77 a	70.9 b	313 b	71.3 b	314 b
P-value	<0.0001	0.0002	0.4381	<0.4576	<0.0001	<0.0001	<0.0001	<0.0001

[w] Values in a vertical column followed by different letters are significantly different at specified *P* levels by Duncan's Multiple Range Test.
[x] In conjunction with application of GA_3 in April, N at 56 kg ha^{-1} as NH_4NO_3 was applied to the soil.
[y] Mid–late April – flower abscission period (30 April); end of June–beginning of July – fruit abscission period and beginning of the exponential phase of fruit growth (26 June); mid-September – near the end of the fruit abscission period (16 September); and mid-January – prior to pre-harvest fruit drop (16 January). GA_3 was applied at 25 mg L^{-1} in 1869 L of water per hectare.
[z] Packing carton fruit sizes (grams per fruit): 84 (99–134 g); 70 (135–177 g); 60 (178–212 g); 48 (213–269 g); 40 (270–325 g); 36 (326–354 g); size 32 (355–397 g).

Table 3
Effect of gibberellic acid (GA_3) applied at different stages of 'Hass' avocado tree phenology on total yield and fruit size distribution (based on individual fruit mass) as kilograms per tree averaged across the off- and on-crop years of the experiment.

GA_3 applications times[y]	Total (kg fruit/tree)	Fruit packing carton size[z]							
		84 (kg fruit/tree)	70 (kg fruit/tree)	84 + 70 (kg fruit/tree)	60 (kg fruit/tree)	48 (kg fruit/tree)	40 (kg fruit/tree)	60 + 48 + 40 (kg fruit/tree)	≥60 (kg fruit/tree)
Mid–late April[x] + end of June–beginning of July + mid-January	29.6 c[w]	0.3 a	3.7 a	4.0 a	7.4 c	13.6 c	3.8 a	24.8 c	25.6 c
End of June–beginning of July	59.0 a	0.5 a	7.0 a	7.4 a	18.4 a	26.6 a	5.8 a	50.8 a	51.5 a
Mid-September	50.8 ab	0.3 a	5.8 a	6.1 a	15.5 ab	23.1 ab	5.6 a	44.3 ab	44.7 ab
Control – untreated	45.4 b	0.2 a	6.0 a	6.2 a	13.4 b	20.5 b	5.0 a	38.8 b	39.2 b
Year									
Off-crop year	8.5 b	0.1 b	1.0 b	1.0 b	2.0 b	3.7 b	1.5 b	7.2 b	7.5 b
On-crop year	83.8 a	0.6 a	10.3 a	10.8 a	25.4 a	38.2 a	8.6 a	72.2 a	73.0 a
P-value									
Treatment (T)	<0.0001	0.3945	0.2088	0.2465	0.0001	0.0002	0.5373	<0.0001	<0.0001
Year (Y)	<0.0001	0.0003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
T × Y	0.0066	0.8872	0.6361	0.6899	0.0449	0.0074	0.4813	0.0019	0.0023

[w] Values in a vertical column followed by different letters are significantly different at specified *P* levels by Duncan's Multiple Range Test.
[x] In conjunction with application of GA_3 in April, N at 56 kg ha^{-1} as NH_4NO_3 was applied to the soil.
[y] Mid–late April – flower abscission period (19 and 30 April); end of June–beginning of July – fruit abscission period and beginning of the exponential phase of fruit growth (6 July and 26 June); mid-September – near the end of the fruit abscission period (15 and 16 September); and mid-January – prior to pre-harvest fruit drop (17 and 16 January). GA_3 was applied at 25 mg L^{-1} in 1869 L of water per hectare.
[z] Packing carton fruit sizes (grams per fruit): 84 (99–134 g); 70 (135–177 g); 60 (178–212 g); 48 (213–269 g); 40 (270–325 g); 36 (326–354 g); size 32 (355–397 g).

Table 4
Effect of gibberellic acid (GA_3) applied at different stages of 'Hass' avocado tree phenology on 2-year cumulative total yield and fruit size distribution (based on individual fruit mass) in kilograms and number of fruit per tree.

GA_3 applications times[y]	Total		Fruit packing carton size[z]					
	kg	no.	84 + 70		60 + 48 + 40		≥60	
			kg	no.	kg	no.	kg	no.
Mid–late April[x] + end of June–beginning of July + mid-January	59.2 c[w]	273 c	8.5 a	56 a	49.1 c	213 c	50.7 c	217 c
End of June–beginning of July	117.9 a	548 a	15.8 a	103 a	100.1 a	439 a	102.2 a	445 a
Mid-September	101.5 ab	466 ab	11.7 a	76 a	88.2 ab	386 ab	89.8 ab	390 ab
Control–untreated	90.8 b	420 b	12.0 a	78 a	77.4 b	338 b	78.8 b	342 b
P-value	<0.0001	<0.0001	0.1746	0.1822	<0.0001	<0.0001	<0.0001	<0.0001

[w] Values in a vertical column followed by different letters are significantly different at specified *P* levels by Duncan's Multiple Range Test.
[x] In conjunction with application of GA_3 in April, N at 56 kg ha^{-1} as NH_4NO_3 was applied to the soil.
[y] Mid–late April – flower abscission period (19 and 30 April); end of June–beginning of July – fruit abscission period and beginning of the exponential phase of fruit growth (6 July and 26 June); mid-September – near the end of the fruit abscission period (15 and 16 September); and mid-January – prior to pre-harvest fruit drop (17 and 16 January). GA_3 was applied at 25 mg L^{-1} in 1869 L of water per hectare.
[z] Packing carton fruit sizes (grams per fruit): 84 (99–134 g); 70 (135–177 g); 60 (178–212 g); 48 (213–269 g); 40 (270–325 g); 36 (326–354 g); size 32 (355–397 g).

July had an ABI of 0.8, and trees receiving GA_3 in mid-September had an ABI of 0.8 ($P = 0.4472$). Year had a strong effect on total yield and the yield of fruit in all size categories as kilograms (and number of fruit) per tree averaged across all treatments, with the kilograms (and number of fruit) of all size categories significantly greater in the on-crop year (Table 3). Note that when averaged across the 2 years of the experiment, all treatments produced analogous results whether expressed as kilograms or number of fruit per tree with identical levels of statistical significance. Thus, yield data expressed as number of fruit per tree are not shown. Low yield did not increase the proportion of fruit ≥ packing carton size 60: compare 88% in the off-crop year to 87% in the on-crop year (based on kg/tree). Regression analysis indicated a very weak relationship between total kilograms per tree and the number of small (packing carton sizes 70 + 84) and large (packing carton sizes 60 + 48 + 40) fruit per tree for the 2 years of the research ($r^2 = 0.38$ and 0.46, respectively). However, there was a strong positive relationship between total yield and yield of large fruit (packing carton sizes 60 + 48 + 40) per tree in the off-crop year ($r^2 = 0.78$), but not in the on-crop year ($r^2 = 0.07$). Additionally, there were significant year by treatment interactions on total yield and yield of fruit of packing carton size 60, packing carton size 48, the combined pool of fruit of packing carton sizes 60 + 48 + 40, and the combined pool of fruit ≥ packing carton size 60 as kilograms (and number of fruit) per tree ($P \leq 0.0449$) (Table 3). Comparison of the yields (kg/tree) of fruit ≥ packing carton size 60 with those for the combined pool of fruit of packing carton sizes 60 + 48 + 40 (Table 3) confirmed that the GA_3 treatments did not dramatically increase the yield of fruit larger than packing carton size 40 for the 2 years of the research. Averaged over the 2 years of the experiment, GA_3 applied at the end of June–beginning of July not only significantly increased total yield and yield of commercially valuable size fruit (packing carton sizes 60 + 48 + 40, 178–325 g/fruit), but also yield of fruit of packing carton size 60 and packing carton size 48 compared with all other treatments, except trees treated with GA_3 in mid-September ($P = 0.0002$) (Table 3). Similarly, GA_3 applied in mid–late April with soil-applied N, end of June–beginning of July, and again in mid-January reduced total yield and yield of fruit of packing carton size 60, packing carton size 48, the combined pool of fruit of packing carton sizes 60 + 48 + 40, and fruit ≥ packing carton size 60 as kilograms per tree compared with trees in all other treatments ($P = 0.0002$).

There were no significant treatment effects on external or internal abnormalities or discoloration, or on vascularization of the mesocarp in years 1 and 2 or averaged across both years of the experiment (data not shown). The proportion of fruit with black exocarp at harvest was slightly greater for trees treated with GA_3 in mid–late April, along with soil-applied N at 56 kg ha^{-1}, end of June–beginning of July, and again in mid-January compared with all other treatments ($P = 0.0975$) (data not shown).

Table 5
Effect of gibberellic acid (GA_3) applied at different stages of 'Hass' avocado tree phenology on 2-year cumulative crop value (US$/tree). Mean US$ per kg of fruit of each size category was: 84, $1.168; 70, $1.565; 60, $2.028; 48, $2.315; 40, $2.359; 36, $2.205; and 32, $2.205.[z]

GA_3 applications times[x]	Total (US$/tree)	Fruit packing carton size[y]							
		84 (US$/tree)	70 (US$/tree)	84 + 70 (US$/tree)	60 (US$/tree)	48 (US$/tree)	40 (US$/tree)	60 + 48 + 40 (US$/tree)	≥60 (US$/tree)
Mid–late April[w] + end of June–beginning of July + mid-January	126.27 c[v]	0.80 a	12.17 a	12.97 a	30.50 b	61.40 c	17.85 a	109.74 c	113.30 c
End of June–beginning of July	251.23 a	1.23 a	23.04 a	24.27 a	69.63 a	122.20 a	30.58 a	222.41 a	226.96 a
Mid-September	217.81 ab	0.55 a	17.54 a	18.09 a	60.65 a	106.65 ab	28.81 a	196.11 ab	199.72 ab
Control–untreated	193.89 b	0.46 a	18.21 a	18.68 a	52.71 a	92.64 b	26.96 a	172.32 b	175.21 b
P-value	<0.0001	0.2511	0.1498	0.1686	0.0003	0.0001	0.2063	<0.0001	<0.0001

[v] Values in a vertical column followed by different letters are significantly different at specified *P* levels by Duncan's Multiple Range Test.
[w] In conjunction with application of GA_3 in April, N at 56 kg ha^{-1} as NH_4NO_3 was applied to the soil.
[x] Mid–late April – flower abscission period (19 and 30 April); end of June–beginning of July – fruit abscission period and beginning of the exponential phase of fruit growth (6 July and 26 June); mid-September – near the end of the fruit abscission period (15 and 16 September); and mid-January – prior to pre-harvest fruit drop (17 and 16 January). GA_3 was applied at 25 mg L^{-1} in 1869 L of water per hectare.
[y] Packing carton fruit sizes (grams per fruit): 84 (99–134 g); 70 (135–177 g); 60 (178–212 g); 48 (213–269 g); 40 (270–325 g); 36 (326–354 g); size 32 (355–397 g).
[z] Dollar values are based on the 5-year mean retail price per kilogram calculated from data supplied by the California Avocado Commission; dollar amounts per kilogram reflect the cost of packing a carton, $3.75, which was subtracted from the retail price per carton.

3.3. Tree nutrient status

There were no treatment effects on leaf concentrations of P, K, Mg, Ca, Fe, Mn, B, Cu and Zn in either year of the experiment ($P = 0.10$) (data not shown). Nutrient concentrations were each within the range considered optimal for 'Hass' avocado with the exception of N. For both years of the research, leaf N concentrations for trees in each treatment were above the 2.2% (dry mass) recommended by the California Avocado Commission (Lovatt and Witney, 2001). Leaf N (2.55%) was highest for trees treated with GA_3 applied in mid-September compared with trees treated with GA_3 in mid–late April, along with soil-applied N at 56 kg ha^{-1}, end of June–beginning of July, and again in mid-January (2.38% N), at the end of June–beginning of July (2.35% N) and the untreated control (2.30% N) ($P = 0.0940$). Leaf nutrient concentrations, including N, were not significantly different in year 1 versus year 2 ($P = 0.10$).

3.4. Economic impact of gibberellic acid treatment

Application of GA_3 at the end of June–beginning of July during both the off- and on-crop years of the experiment significantly increased 2-year cumulative total yield by 27 kg (128 fruit) per tree, yield of commercially valuable large size fruit (packing carton sizes 60 + 48 + 40) by 23 kg (101 fruit) per tree and the value of the crop by US$57 per tree compared with untreated control trees ($P = 0.0001$) (Tables 4 and 5). Thus, there was no negative effect from applying GA_3 at this stage of tree phenology during consecutive years despite differences in crop load.

4. Discussion

During both years of the research, maximum air temperatures during the periods of flower and fruit abscission were above 20 °C and below 30 °C, the optimal temperature range for pollen germination, fertilization and fruit set (Sedgley, 1977; Sedgley and Annells, 1981), with the exception of approximately 7 days when maximum air temperature was below 20 °C but above 15 °C in early April (year 1) and mid-April (year 2). Maximum air temperature did not exceed 30 °C until the beginning of October (year 1) or the end of September (year 2), at which time it had little to no effect on fruit abscission (Figs. 1 and 2). During the 2 years of this research, periods of intense flower and fruit abscission (when ~80% of flowers and fruit abscised, respectively) appeared to be influenced by the off- or on-crop potential (floral intensity) of the trees at bloom rather than air temperature. No temperature extremes occurred during the periods of intense flower or fruit abscission in either year of the research. In addition to 4.5-fold more flowers and 10.5-fold more fruit abscising during the on-crop year (year 2) compared with the off-crop year (year 1), each abscission period started 1 month earlier in the on-crop year than off-crop year: compare 6 April to 6 May for the start of the period of intense flower abscission, respectively, and 4 May and 8 June for the start of the period of intense fruit abscission, respectively. Inflorescences have been observed previously to develop faster during the heavy bloom that produces the on-crop (Salazar-García et al., 1998). Despite similar and mild climatic conditions during the periods of flower and fruit abscission in both years of the study and the significantly lower number of flowers and fruit that abscised in year 1 compared with year 2, the resulting year 1 yield was an extremely low off-crop (7.5 kg/tree for the untreated control) compared with year 2 (83 kg/tree for the untreated control). These results are consistent with previous findings demonstrating that yield is dependent largely on the initial number of flowers at bloom (Hanke et al., 2007; Salazar-García et al., 1998).

Based on the number of flowers and fruit collected in the nets under untreated trees within the orchard, in both years of the study the April application of GA_3 was within the period of intense flower abscission and the application of GA_3 at the end of June–beginning of July was within the period of intense fruit abscission. The dramatically different responses to GA_3 application in years 1 and 2 were, thus, likely due to the differences in crop potential of the off- and on-crop trees. Application of GA_3 in mid-September of the off-crop year was during a period of little to no fruit drop, whereas in the on-crop year approximately 50 fruit were abscising per week in September. As a result, this treatment had a positive effect on yield and fruit size in the on-crop year, but no effect in the off-crop year. For both the off- and on-crop years, there were few to no fruit abscising at the time the mid-January GA_3 treatment was applied and it had no effect in either year. Thus, the failure of any GA_3 treatment to have a statistically significant positive or negative effect on yield or fruit size as kilograms or number of fruit per tree in the off-crop year was likely because flower number and, hence, the yield potential of the trees was too low. Thus, three applications of GA_3 (mid–late April, with soil-applied N, end of June–beginning of July and mid-January) only reduced total yield 14% and yield of large size fruit (178–325 g/fruit) 12% compared with untreated control trees in the off-crop year, but significantly reduced total yield 37% and yield of large size fruit 38% compared with untreated control trees in the on-crop year ($P \leq 0.0001$).

The negative effect of multiple applications of GA_3 might have offset the potential yield benefit that has previously been obtained by applying N (56 kg ha^{-1}) to the soil during the period of intense flower abscission, initiation of elongation of the vegetative shoot apex of indeterminate floral shoots, and early fruit set (~April) (Lovatt, 2001). This higher rate of N increased total yield, yield of commercially valuable fruit (≥178–325 g/fruit) and reduced the ABI of the orchard, whereas supplying half as much N during this period provided no yield benefit. The yield results obtained in response to applying N with GA_3 applications compared with the other GA_3 treatments do not support the idea that nutrient resources (fertilizer) should be supplied with PGR applications in order to obtain a greater increase in yield. The physical effect of multiple sprays might have resulted in a cumulative increase in flower and fruit drop that had significant impact on yield in the on-crop year. The negative effect of multiple sprays is consistent with previous results demonstrating that foliar sprays remove flowers and young fruit (Chao et al., in press). It is unlikely that multiple applications of GA_3 at 25 mg L^{-1} as widely separated in time as April, end of June–beginning of July and mid-January were phytotoxic. In previous research, foliar-applied GA_3 at 100 mg L^{-1} to 'Hass' avocado trees had no negative effects on flower morphology or development and showed no signs of phytotoxicity (Salazar-García and Lovatt, 1998). Even a single application of GA_3 at 1000 mg L^{-1} did not cause phytotoxicity, although it caused floral shoots to be highly elongated and too weak to support developing fruit. In this experiment, the possibility that GA_3 applied in April stimulated the growth of the vegetative shoot apex of indeterminate floral shoots, which increased fruit abscission and reduced yield, cannot be ruled out (Bower and Cutting, 1992; Cutting and Bower, 1990; Kalmer and Lahav, 1976; Kotzé, 1982; Whiley, 1990; Zilkah and Klein, 1987). However, during the on-crop year, a single application of GA_3 at the end of June–beginning of July, one of the stages of tree phenology included in the multiple GA_3 application treatment, significantly increased total yield as kilograms of fruit per tree (27%) and the yield of commercially valuable fruit of packing carton sizes 60 + 48 + 40 as both kilograms and number per tree (30%) compared with the untreated control ($P \leq 0.0002$). This treatment had no significant effect on the total number of fruit per tree in the on-crop year, implying that GA_3 had a greater effect on fruit growth than fruit retention, which is consistent with targeting the GA_3 application to the beginning of exponential fruit growth and with the role of GA_3 in increasing fruit sink strength (Köhne, 1989; Salazar-García et al., 2007).

Despite total yield being approximately 10-fold greater in the on-crop year than off-crop year, the number of small size fruit ($\leq$177 g/fruit) harvested was only 20% of the total on-crop and only 9% greater than in the off-crop year. The proportion of small fruit harvested was within the range of 20–60% reported previously (Cutting, 1993; Moore-Gordon et al., 1998; Zilkah and Klein, 1987), but considerably lower than the previously observed 44% for an on-crop 'Hass' orchard in California (Lovatt, unpublished). It is noteworthy that when GA_3 was applied at the end of June–beginning of July, 63% and 92% of the numerical and statistically significant increases in total yield in the off- and on-crop years, respectively, were commercially valuable large size fruit (packing carton sizes 60 + 48 + 40).

The results of this research provide strong evidence that the efficacy of GA_3 in this experiment was crop load-dependent, making alternate bearing a factor in the yield response of 'Hass' avocado to GA_3. However, the interaction between GA_3 and crop load produced a different outcome for 'Hass' avocado than for 'Nules' Clementine mandarin. GA_3 treatment increased yield and fruit size in the off-crop year of the alternate bearing cycle of 'Nules' Clementine mandarin, but not when averaged across the off- and on-crop years of the research (Chao et al., in press). In contrast, the positive effect of GA_3 on 'Hass' avocado yield and fruit size occurred in the on-crop year and was statistically significant averaged across the off- and on-crop years of the alternate bearing cycle. It should be noted that for both the 'Hass' avocado and 'Nules' Clementine mandarin, multiple applications of GA_3 during the on-crop year reduced total yield and yield of commercially valuable fruit. Failure of GA_3 treatments to have a significant effect on the yield of 'Hass' avocado trees setting an off-crop is not unique to GA_3. Several plant growth regulators (PGRs) highly efficacious in increasing yield and fruit size in the on-crop year of 'Hass' avocado trees have proven ineffective in the off-crop year (Lovatt, 2007). In this experiment, applying GA_3 at the end of June–beginning of July during 2 consecutive years had a significant positive effect on 2-year average yield and 2-year cumulative yield and on fruit size. Applying this treatment in the off-crop year added US$5231 per hectare (based on ~273 trees/ha) to the 2-year cumulative crop value of $15,558 per hectare. However, the potential economic benefit of the off-crop year application will depend largely on the cost of application to individual growers. Thus, crop load is a factor that should be taken into consideration when GA_3 is to be used to increase avocado fruit set or fruit size.

Ever-increasing production costs (e.g., labor, water, and fertilizer) dictate that 'Hass' avocado growers worldwide increase revenue per hectare by increasing not only production per hectare, but also by increasing fruit size. A single properly timed GA_3 application in the on-crop year may prove to be a viable strategy to achieve this goal with the 'Hass' avocado.

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