FACTORS AFFECTING 'HASS' AVOCADO FLOWER NECTAR AND THEIR IMPACT ON POLLINATION AND FRUIT SET.

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ABSTRACT

To determine if inconsistent yields are due to a lack of effective pollination under the climatic conditions in New Zealand, avocado flowering opening, nectar volume and sugar concentration, bee activity, orchard and tree factors were followed in detail. Hass avocado flowering followed the typical progression through the female and male sex phases as described for sub-tropical or Mediterranean climates. Flowers in the male stages of opening had increased nectar volume and sugar concentration the longer they were open while the middle female flower stage had the most nectar and greatest sugar concentration. Flowers had similar nectar sugar and volume at each gender. This suggests avocado flowers in the female or male phase would be equally attractive to bees. Nectar sugar concentration, volume and bee numbers per tree decreased as the percentage of bloom increased. By 85% bloom bee numbers per tree had dropped below five bees per tree; considered the minimum number of bees for effective pollination. Bees had a preference for avocado flowers with the highest nectar sugar concentration and volume. Pre-bloom starch was correlated to the number of fruit remaining after the December fruit drop but not nectar sugar or volume. The authors contend that effective pollination and fruit drop are separate processes. We conclude from this study that 'Hass' avocado flowering and pollination, with regard to bee numbers per tree as well as to flower nectar sugar concentration and volume, is not the main factor limiting fruit set in the Western Bay of Plenty, New Zealand. Therefore, the yield of 'Hass' avocado trees should be as great in the cooler New Zealand climate as in the warmer Mediterranean and sub-tropical climates elsewhere in the world.

Keywords: *bees, starch, fruit drop*

INTRODUCTION

Avocado trees have a complex flowering biology where although the flowers contain both male and female parts they become functionally female or male at different times in a co-ordinated manner (Gazit and Degani, 2002). The avocado flower opens and closes twice over a two day period going through changes in functional gender in readily identifiable phases (Dixon and Sher, 2002) that have been described in detail (Ish Am and Eisikowitch, 1991; Soriano *et al*., 2003). This opening in the different gender phases appears to be a mechanism to facilitate pollination by pollen from a different flower but not necessarily from a different cultivar or tree. The overlap period between flowers open in the male phase and flowers open in the female phase is considered to be important for accomplishing both cross-pollination, overlap between different cultivars, and close pollination, overlap within the tree or within the cultivar (Ish Am and Eisikowitch, 1991; 1995). Past research has established that the pattern of flower opening in the different sex phases is largely dependent on temperature (Ish Am and Eisikowitch, 1991; Sedgley, 1977; Sedgley and Annells, 1981). The factors relating to numbers of flowers open on each day are poorly understood, however.

Avocado pollination is considered by most researchers to require an insect vector; most commonly in commercial avocado orchards this is by honey bees (Ish-Am and Eisikowitch, 1993; Ish-Am and Eisikowitch, 1998a; Robbertse *et al*., 1998). High numbers of bees primarily gathering nectar and actively visiting flowers in both gender phases are needed to ensure effective pollination

of the female phase flowers, as pollination when the flowers are in the male phase has been shown to be ineffective (Shoval, 1987). Effective pollination is where pollination leads to a new fruit developing from the flower but that fruit is not necessarily retained until harvest. An efficient honeybee activity on the avocado flowers is necessary to deposit (> 20) pollen grains onto the stigma for effective pollination; therefore multiple visits by bees are required (Ish-Am, 1994).

Avocado flower nectar sugar content and volume has been studied on a range of avocado cultivars, including 'Hass', in Israel (Ish-Am and Eisikowitch, 1998a) a Mediterranean climate warmer and more arid than New Zealand. The concentration of nectar sugar reached a peak when about 50% bloom, with bloom defined as the percentage of total flowers that have opened, and fell to low amounts near the end of the flowering season before peaking again. Bee numbers were low over the course of the flowering season but peaked near the end of bloom. In Israel the bees were not attracted to avocado flowers when citrus and wild flowers were flowering at the same time and vicinity. Therefore, low bee numbers over the peak of flowering were considered to be a major reason for low avocado yields in Israel. The sugar content and volume of nectar from flowers of flowering plants, in general, may be influenced by a number of factors, including: tree carbohydrate status, soil moisture deficit, daily temperature and relative humidity (Corbet, 1990). Bee numbers on an avocado tree when flowering and effective pollination may be related to the quantity and sugar content of nectar which in turn may be influenced by soil moisture deficit and by the proportion of flowers that have opened.

To determine if the cause of irregular bearing is related to effective pollination being insufficient to set commercially viable avocado crops under the climatic conditions in New Zealand, avocado flowering opening, nectar volume and sugar concentration, honeybee activity on avocado flowers and orchard environmental conditions were followed in detail to determine the relationship between nectar volume and sugar concentration, tree and field factors and both honeybee numbers and fruit set.

MATERIALS AND METHODS

Nectar measurements

Volume and sugar content

One flowering branch exposed to full sun with at least six flowering buds (panicles) was selected from each of ten healthy trees. The branches were located between 1 m and 2 m from ground level. At least twice in each season, five individual flowers that had visibly damp nectaries, from each branch were sampled for nectar. At each sampling time a total of 50 flowers per orchard were measured for their nectar content. The date and time of day nectar samples were collected were recorded along with the stage of flower opening. The nectar was collected using graduated 5 µl microcapillary pipettes (Ish-Am and Eisikowitch, 1998a; Corbett, 2003) and the volume read to the nearest 0.25 µl. The sugar concentration (ºBrix) was measured using a low volume field refractometer (Stanley and Bellingham, UK) calibrated against standard sucrose solutions. The flowers produced less than 1 µl of free nectar. Collecting sufficient amounts of nectar from individual flowers was impossible therefore it was necessary to wash the nectar off the nectaries using 5 µl of distilled water added to each flower 1.5 to 3 min before sampling. The amount of sugar in a flower, expressed in milligram per flower, was calculated as the product of the volume recovered and the ºBrix reading on the refractometer which converted from percent w/w to percent w/v. To establish an average ºBrix value of raw nectar 0.5, 1.0 and 1.0 µl samples of raw nectar was collected in a 5 µl capillary pipette from 4 Hass flowers each in the female phase on 17/11/2004 at 12:00 to 12:27 h. The average raw nectar ºBrix value was 43. The average volume of the raw nectar in a flower was calculated as the product of the sample ºBrix value divided by the raw nectar average ºBrix times the diluted nectar volume.

Ten flowering branches exposed to full sun, from a seven year old 'Hass' avocado tree grafted onto

'Zutano' seedling rootstock were selected for flower nectar sampling. Each branch, comprising of 6 to 7 flowering buds (panicles), were covered with a brown paper bag (250 mm wide, 580 mm long and 140 mm deep) at 12:30 h on the 10/11/2004 and at 8:30 h on the 11/11/2004. The temperature inside the paper bags was measured using thermocouples connected to a digital thermometer and found to average 0.3°C higher than outside the bags. Bags were used to exclude bees from the flowers, in order to prevent loss of nectar due to bee foraging. Yet, they should not affect the nectar content of the flowers (Wyatt *et al.*, 1992). During the course of the afternoon, when flowers were fully open, at least five flowers per branch were sampled for nectar. These flowers were tagged and not sampled again.

Orchard factors

To compare nectar production between orchards, nectar was sampled from flowers of 'Hass' avocado trees grafted onto 'Zutano' seedling rootstocks, ranging in age from seven to twelve years from four orchards in the Western Bay of Plenty, New Zealand. Soil moisture matrix potential, shade temperature and relative humidity (RH) within the orchard were measured at the same time as the nectar was sampled. Soil moisture matrix potential was recorded using 30 cm and 60 cm tensiometers (Irrometer, Riverside, California) installed inside the drip line of one representative tree within the block being sampled. Shade air temperature and relative humidity at the time of sampling was recorded using a data logger placed within a shaded area of the canopy (TinyTag Ultra Gemini, Hamilton, New Zealand). To account for differences in cropping history of trees within orchards, nectar was collected during flowering in an 'on' alternate bearing year (2004) and an 'off' alternate bearing year (2005).

Honeybee numbers

Bee numbers on each tree was estimated as described by Ish-Am and Eisikowitch (1998a).

Fruit set

Fruit numbers on each branch where flowers were sampled for nectar were counted on all panicles in the first 0.5 m from the terminal end of the branch towards the main trunk of the tree.

Percentage bloom

The percentage of bloom, defined as the percentage of the total number of flowers on a panicle that have opened up to the time of sampling, was subjectively assessed on a scale of 0 to 100.

Starch

Each tree was sampled for trunk starch content using a cordless drill equipped with an 8 mm bit for drilling wood. The bark was removed and discarded; wood shavings were collected from a hole up to 25 mm deep from each of three main limbs within 0.5 m of the main trunk. Wood samples for starch analysis were collected pre-flowering and post flowering. The wood shavings were stored in sealed polyethylene bags at -18°C before drying at 55°C and ground to a fine powder for analysis of starch according to the total starch assay Megazyme AACC method 76.13 (Megazyme International Ireland Limited) as described by McCleary *et al*. (1997).

Statistical analysis

Comparisons of flower nectar sugar content, as well as nectar volume were made using a One-way analysis of variance using Tukey's family error rate of 5%. Where appropriate, measurements were transformed to meet the assumptions of analysis of variance. The values reported in tables are untransformed means. Linear regression analysis was used to describe the relationships between tree factors and flowers. Statistical calculations were conducted using MINITAB version 13.31.

RESULTS

'Hass' avocado flowering in NZ when closely followed over two days had the same progression through the female and male sex gender as described in Israel (Ish-Am and Eisikowitch, 1991) and New Zealand (Dixon and Lamond, 2004). There were flowers in the male or female gender at the same time on the same tree although the

majority of flowers were either in the male or female gender at any one point in time. The typical sequence of opening for individual 'Hass' flowers was to open in the female gender about mid-day of the first day then to close mid-afternoon and to open in the male gender the following mid-afternoon. The patterns of flower opening were essentially the same as reported for 'Hass' flowers in a sub-tropical or Mediterranean climate.

The secretion of nectar, visible as wet nectaries, occurred as described by Ish-Am and Eisikowitch (1991). The amount of nectar within an individual flower was small and only visible as a wet sheen on the nectaries. This was observed to be sufficient to attract honey bees to visit the flower where they licked each nectary in the flower they were visiting before moving to another flower.

Nectar, tree factors and fruit set

Numbers of bees per tree, nectar sugar content and volume per flower averaged across all flower stages decreased across all trees in the orchards used in this study as the bloom progressed (Figure

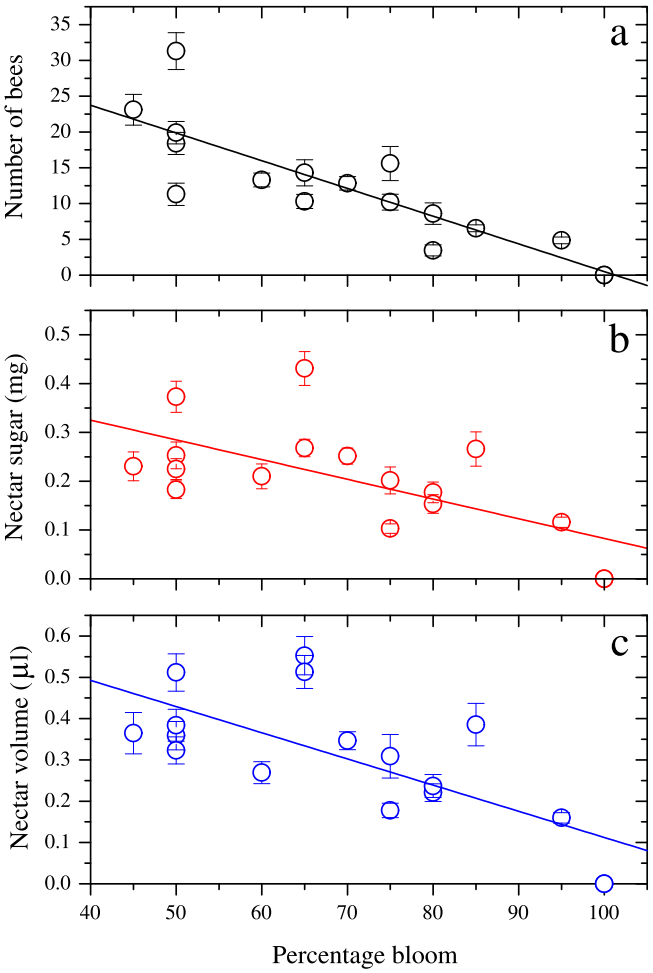

Figure 1. Relationship between a) average bee numbers per tree, b) average nectar sugar concentration per flower across all gender stages and c) average nectar volume per flower across all gender stages and the percentage bloom of 'Hass' avocado trees on four orchards located in the Bay of Plenty, New Zealand. Measurements were made from 50% bloom only. Vertical bars represent the standard error of the mean for 10 trees.

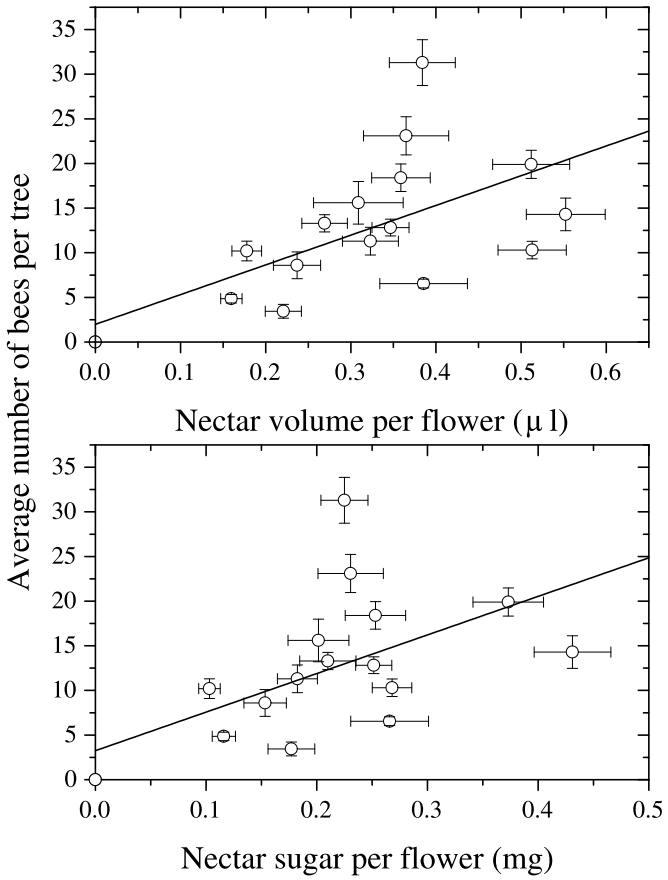

Figure 2. Relationship between nectar sugar concentration per flower or nectar volume per flower and average bee numbers per tree during the bloom of 'Hass' avocado trees on four orchards located in the Bay of Plenty, New Zealand. Vertical bars and horizontal bars represent the standard error of the mean.

1). Linear regression equations were for bee numbers = 39.22 + -0.387 x percentage bloom (r^2 = 0.733, p<0.01), nectar sugar concentration per flower = 0.487 + -0.004 x percentage bloom (r^2 = 0.434, p<0.01) and nectar volume per flower = 0.746 + -0.006 x percentage bloom (r^2 = 0.523, p<0.01). Bee numbers increased as nectar sugar and volume per flower increased (Figure 2). Linear regression equations were for bee numbers = 3.25 + 43.215 x nectar sugar per flower (r^2 = 0.344, p=0.013) and bee numbers = 1.98 + 33.301 x nectar volume per flower (r^2 = 0.416, p=0.005).

When comparing between orchards, at the time of sampling, nectar sugar concentration or volume per flower was not related to soil moisture matrix potential at 30 cm or 60 cm, shade air temperature or relative humidity. Average nectar sugar concentration per flower and average bee numbers per tree was not related to fruit set one month after flowering had finished or after the December fruit drop (Table 1). The pre-bloom starch content of the wood of main limbs was not related to the nectar sugar concentration per flower. However, pre-bloom starch was correlated to the number of fruit remaining after the December fruit drop (r = 0.785, p=0.036).

DISCUSSION

A major influence on the nectar sugar concentration and volume of 'Hass' avocado flowers was when the flower opened in relation to the time course of bloom. This study measured flowers from about 50% bloom onwards. This was strongly related to nectar sugar concentration and volume yet only explained between 43% and 52% of the data variation as aspects of the microclimate such as temperature, relative humidity, time of day and flowering intensity (Corbet, 1990) may explain the remaining variation. The decrease in nectar sugar concentration and bee numbers per tree was such that by 85% through the bloom honeybee numbers per tree had dropped below an average of

Table 1. Summary of avocado flowering parameters of four orchards in the Bay of Plenty and fruit counts in 2004 and 2005

Year	Orchard	Bloom[1] (%)	Bee numbers[2]	Nectar sugar[3] (mg)	Starch[4] (%)	Fruit number pre drop[5,6]	Fruit number post drop[7]
2004	A	60	15.1	0.32	4.4	52.8	1.9
	B	60	14.3	0.43	-	26.9	2.1
	C	95	9.6	0.12	4.0	104.4	6.3
	D	75	12.8	0.25	1.2	8.7	0.0
2005	A	70	9.8	0.17	5.3	16.0	5.9
	B	60	16.7	0.17	6.4	30.4	10.0
	C	60	15.9	0.23	3.7	145.1	3.0
	D	65	23.5	0.22	2.1	22.3	4.2

[1]Percentage of all flowers that have opened; [2]Number of bees observed to be foraging around a tree in 90 seconds; [3]Average concentration of nectar of 5 flowers per branch from each of 10 trees (50 flowers in total); [4]Average percentage of starch in main limbs from each of 10 trees; [5]Average number of fruit of 10 flowering branches; [6]Fruit were counted after flowering was more than 90% complete before the natural drop; [7]Fruit were counted after the natural drop in December.

five bees per tree. Since five bees per tree were considered by Ish-Am and Eisikowitch (1995) as the minimum number of honeybees required for effective pollination we assume the last 15% of bloom was unlikely to have contributed to fruit set. It is commercial practice in NZ avocado orchards to introduce managed beehives into avocado orchards at 10% to 15% bloom. The above reasoning would suggest this practice is unlikely to affect fruit set. We suggest there is a need to have large numbers of bees from early in the bloom through to at least 85% bloom for a good fruit set.

The orchard and tree factors measured in relation to flowering and fruit set had little influence on the flower nectar sugar concentration or volume. The range of temperatures, relative humidity's and soil moisture matrix potentials measured in this study was small and it is possible that greater extremes of temperature, for example, may have a greater influence on flower opening and nectar variables. The greatest influence was on fruit retention where the pre-bloom starch content in the wood of the main limbs, which was correlated to the number of fruit remaining after the December drop. Honeybees had a preference for avocado flowers that had the highest nectar sugar concentration and volume, although other independent factors such as the weather, can have a significant influence on bee behaviour (Ish-Am and Eisikowitch, 1998b). Initial fruit set in this study could not be related to tree or orchard factors but it seems reasonable that fruit set was influenced by the number of bees foraging on the flowers (Ish-Am and Eisikowitch, 1995) and the duration of the flower open stages in each gender phase. Fruit drop was correlated to the pre-bloom starch levels in the tree suggesting that effective pollination and fruit drop may be separate processes. The relationship between fruit drop and starch content merits further research to increase our understanding of the fruit drop process.

CONCLUSIONS

'Hass' avocado flowering and pollination, with regard to bee numbers per tree as well as to flower nectar sugar concentration and volume, is not the main factor limiting fruit set in the Western Bay of Plenty, New Zealand. Therefore, the yield of 'Hass' avocado trees should be as great in the cooler New Zealand climate as in the warmer Mediterranean and sub-tropical climates elsewhere in the world.

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