REPLENISHMENT OF NECTAR REMOVED FROM 'HASS' AVOCADO FLOWERS DURING THE FEMALE PHASE.

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ABSTRACT

To achieve good pollination bees are required to actively forage as many flowers in both genders as possible before returning to the hive. To facilitate multiple visits of bees to the same flower it would be expected that the nectar would be replenished to maintain the food reward value of the flower. 'Hass' avocado flowers were sampled after visits by bees and after a period of bagging to determine if the flowers replenish their nectar following honey bee foraging. The nectar sugar content and volume of flowers in the female gender were sampled after visits from foraging bees and compared to nectar samples from the same flowers two and a half hours later and the nectar content of flowers not visited by bees or sampled by micropipette. Bees foraging on avocado flowers were calculated to have removed an average of two thirds of the nectar present. Whether nectar was removed by a foraging bee or by micropipette the nectar sugar content or volume did not replenish to the original level in 'Hass' avocado flowers in the female gender. The stage of flower opening appeared to influence the nectar sugar content and volume with the female F2f stage flowers having the most nectar sugar and volume. The lack of nectar replenishment in New Zealand 'Hass' avocado flowers would indicate that multiple bee visits to the flowers is not based solely on the food reward of the flowers.

Keywords: *bees, pollination*

INTRODUCTION

Effective pollination of avocado flowers in Israel has been determined to rely on good honeybee activity (Ish-Am and Eisikowitch, 1993; 1998) identified by large numbers of bees working flowers. 'Hass' avocado flowers open in a sequence of female and male gender stages through a two day period (Dixon and Sher, 2002) as described by Soriano *et al*. (2003). The female gender flowers are believed to have the most chance of setting fruit if pollinated with sufficient pollen grains and the temperature during the day remains high enough to support rapid pollen tube growth to the ovary (Ish-Am and Eisikowitch, 1993). To achieve good pollination bees are required to actively forage as many flowers in both genders as possible before returning to the hive (Ish-Am and Eisikowitch, 1998). In Israel a single visit by a bee to an avocado flower is thought to deposit 5 pollen grains on average (Ish-Am PhD Thesis, 1994). To get large numbers (>20) pollen grains deposited onto the stigma multiple visits by bees are therefore required. An important aspect of honeybee activity on avocado flowers may be the food reward of the nectar in the flowers being foraged. To facilitate multiple visits of bees to the same flower it would be expected that the nectar would be replenished to maintain the food reward value of the flower. 'Hass' avocado flowers were sampled after visits by bees and after a period of bagging to determine if the flowers replenish their nectar following honey bee foraging.

MATERIALS AND METHODS

Three flowering branches exposed to full sun from a seven year old 'Hass' grafted onto 'Zutano' seedling rootstock avocado tree were selected for nectar sampling of flowers. Each branch, comprising of 6 to 7 flower buds (panicles), was covered with a brown paper bag (250mm wide, 580mm long and 140mm deep) at 11:15am on the 16/11/2004. The bags were used to control which flowers were exposed to foraging honey bees or sampled for nectar collection. During the course of the afternoon, when the flowers opened in the

female phase, at least five individual flowers per inflorescence were sampled for nectar. After the branches had been in bags for at least 2 hours the bags were removed and tagged flowers were sampled for nectar. Then the branches were re-bagged for a further 2 hours before the same flowers sampled previously were re-sampled for nectar (Figure 1). Care was taken to prevent bees visiting these flowers when sampling for nectar. While the bags were removed bees foraging on flowers of the inflorescences under study were carefully followed. After each flower had been visited by a bee the flower was tagged and sampled immediately for residual nectar then the inflorescence re-bagged for at least 2 hours before these tagged flowers were re-sampled for nectar (Figure 1). In addition, flowers that were neither visited by bees nor sampled for nectar collection were sampled for nectar after at least 5 hours, as a check on total nectar accumulation during the time the inflorescences were under study. To calculate the amount of nectar sugar removed from each flower following a visit from a bee, the average values for residual nectar and volume for flowers visited by bees were subtracted from the values of nectar sugar and volume for flowers bagged and sampled with micropipettes.

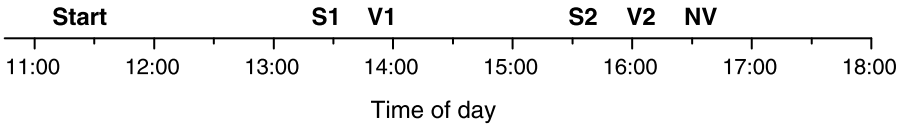
The time and floral stage (Soriano *et al*., 2003; Dixon and Lamond, 2004) were recorded at sampling. Sampling for nectar was conducted as described previously (Dixon and Lamond, 2004). Bee numbers were estimated by counting honeybees on the whole tree in 90 seconds while walking around the tree (Ish-Am and Eisikowitch, 1998).

The results for flowers were grouped into when the flowers were sampled and analysed using MINITAB version 13.31.

RESULTS

The nectar volume and sugar content measured in 'Hass' avocado flowers in the female gender were similar to those measured in female flowers 6 days earlier on 10/11/2004 (Table 1; Dixon and Lamond, 2004). Bee counts on the whole tree ranged from 17 to 22 during the time the flowers were in bags and were sampled. The average shade temperature within the canopy over the measurement period was 18.1°C ± 0.7°C. This temperature was about 2°C lower than 6 days earlier and may account for the lower bee counts compared to the 10/11/2004. The lower daytime temperature did not appear to have affected the nectar sugar content or nectar volume, however.

Bees foraging on avocado flowers were calculated to have removed an average of two thirds of the nectar present (Table 1). Both bees and micropipettes appeared to remove similar amounts of nectar as the nectar sugar and volume were not different after a further 2 and a half to 2 and three quarters hours in bags before re-sampling with micropipettes (Table 1).



Start = time when the inflorescences were bagged
S1 = first samples by micropipette then re-bagged
V1 = unsampled flower visited by a bee then bagged
S2 = S1 flowers re-sampled by micropipette
V2 = V1 flowers re-sampled by micropipette
NV = flowers never visited by bees but bagged for the whole time

Figure 1. Timeline of flower sampling.

The sugar content and nectar volume of first sampled and bee visited flowers did not recover to the same levels as when the first sampled flowers were initially sampled (Table 1). It is not known if the reduced nectar sugar or volume continued through to the male gender phase of the same flowers. That there was a low amount of sugar detected suggests that there may have been further secretion of nectar or the micropipette sampling method did not remove all of the diluted nectar from the flower. That never visited flowers had similar nectar sugar content but a lower nectar volume to first sampled flowers suggests that more nectar was not produced once the flower was sampled or visited by bees.

The stage through the flowering phase influenced the nectar sugar content and volume in flowers. The flowers in the F2f stage had the greatest nectar sugar and volume (Table 2).

Table 1. Average sugar content in the nectar and nectar volume of individual 'Hass' avocado flowers sampled in the female phase after visits by bees or sampling by micropipette then bagged for more than 2 hours or bagged for more than 5 hours before sampling.

Treatment	Sugar in nectar mg/flower	Nectar volume µL	Time in bag Hours:minutes
Never visited	0.35a[1]	0.33a	5:24
Sampled by micropipette	0.45a	0.62b	2:27
Re-sampled by micropipette	0.12b	0.13c	5:10
Sampled immediately after a bee visit	0.15b	0.21ac	2:46
Re-sampled by micropipette	0.10b	0.10c	5:11

[1]Values within the same column with the same letter are not different according to a One-way analysis of variance using Tukey's family error rate of 5%.

Table 2. Average sugar content in the nectar and nectar volume of individual 'Hass' avocado flowers at different stages of opening in the female phase.

Flower stage	Sugar in nectar mg per flower	Nectar volume µL
F1f[1,2]		
F2f	0.34a[3]	0.46a
F3f	0.22ab	0.21b
F1c	0.18b	0.20b

[1]Flower stage according to Soriano et al (2003); [2]Only 2 flowers were sampled at this stage; [3]Values within the same column with the same letter are not different according to a One-way analysis of variance using Tukey's family error rate of 5%.

DISCUSSION

Pollination of avocado flowers in New Zealand may be limited by the nectar sugar content not recovering in a flower once visited by a bee. Flowers that have already been visited by bees have less nectar than unvisited flowers and would therefore be expected to be less attractive to bees, due to the reduced food reward of a lower nectar sugar content. Multiple visits by bees to the same female gender flower are desirable to ensure sufficient pollen grains are deposited onto the stimga to ensure effective pollination (Ish-Am and Eiskowich, 1993). If the number of bees foraging is low the removal of nectar from an individual flower by a foraging bee could be expected to reduce effective pollination as that flower would not be re-

visited. Nectar collecting bees visit both male and female gender flowers for 2-10 s per visit (Ish-Am and Eisikowitch, 1993). This implies that 50 bees could potentially visit at least 18,000 individual flowers in an hour while 10 bees would only visit about 3,600 flowers in an hour. Therefore, it is important to exceed a certain number of bees to ensure that the number of bees active on a tree are enough to visit each open flower several times. This would explain the observations by Ish-Am and Eiskowich (1998) that the effective pollination and fruit set success was related to the number of bees actively foraging on trees. The recovery of nectar after foraging by bees was examined on flowers in the female phase of opening. It is unknown if the reduced nectar sugar content of the flower is carried over into the male opening phase. However, excess nectar appears to be absorbed in the final stages of female gender opening and nectar is secreted from another set of nectaries when the flower is in the male gender phases (Gazit and Degani, 2002). This suggests that the nectar sugar content of male gender flowers may be unaffected by removal of nectar in the female gender. It is suggested that future research could examine whether removal of nectar from 'Hass' flowers in the female gender lowers the quantity and/or quality of nectar in the same flower in the male gender with respect to bee activity and fruit set.

CONCLUSIONS

Whether nectar was removed by a foraging bee or by micropipette the nectar sugar content or volume did not replenish to the original level in 'Hass' avocado flowers in the female phase. The stage of flower opening appears to influence the nectar sugar content and volume with F2f stage flowers having the most nectar sugar and volume. The lack of nectar replenishment in New Zealand 'Hass' avocado flowers would indicate that multiple bee visits to the flowers is not based solely on the food reward of the flowers.

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