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Pollination of Hass Avocados

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ABSTRACT

Botanical horticultural and entomological aspects were studied in an attempt to compare the influence of cross-pollination *(Hass x Ettinger)* and self-pollination (Hass x Hass) as well as the role of bees on Hass yield. The experiments were carried out in two 5 year old Hass orchards bordered by rows of Ettinger trees at Mooketsi and Politsi. Semi-in *vivo* pollination experiments confirmed that Ettinger, but also Fuerte and Bacon, are effective pollen parents for Hass and slightly out-performed Hass. In an experiment where open-pollinated Hass flowers were sampled over a two- week period, it was found that weather conditions, especially low temperatures before and after female phase anthesis, gave an adverse effect on 'effective pollination'.

In night time counts in the Mooketsi orchard, powder-marked bees had visited up to 10 inflorescences out of 10 per tree sampled close to the hives, with a gradual decline to 2 at 200m distance from the hives. Day time counts of the number of foraging bees per $1m^2$ of flowering canopy (or per 20 inflorescences) were 4 near the hives (5 being considered optimal), declining to 1 at 200m distance. The six hived colonies showed an initial increase in size, brood, honey and pollen, whereafter brood and pollen production declined.

In the Politsi orchard only the inflorescences very close to the hives were powder-marked (highest count 4/10). Day time counts also revealed no bees on trees within 200m from the hives, but a high 5 bees/20 inflorescences in a far corner of the orchard, 550m from the hives. Competitive beeplants such as weeds, surrounding bush and eucalypts were possibly responsible for the absence of bees in the orchard. The 8 hived colonies, two of which also prepared to swarm, showed a gradual increase in all the measured parameters, except for a slight decline in the brood production towards the end of flowering.

Hass yields of caged trees with bees were significantly higher than those without bees. One open-pollinated block showed a decline in Hass yield with increasing distance from Ettinger pollen donor. However, in another block Hass yield was not influenced by distance from Ettinger.

OPSOMMING

Plantkundige. tuinboukundige en entomologiese aspekte is ondersoek in 'n poging om die invloed van kruisbestuiwing (Hass x Ettinger) en self, bestuiwing (Hass x Hass), asook die rol van bye op Hass opbrengs te bepaal. Die eksperiment is uitgevoer by Mooketsi en Politsi in twee 5-jaaroue Hass boorde wat deur rye Ettinger begrens is. Semi-in *vivo* bestuiwingseksperimente het bevestig dat Ettinger stuifmeel. maar ook Fuerte en Bacon stuifmeel effens beter vaar as Hass stuifmeel en daarom beter sluifmeelouers is. In 'n eksperiment waar oop-besruifde Hass-blomme oor 'n tydperk van twee weke bemonster is, is gevind dal weerstoestande, veral lae temperature voor en na die vroulike fase van anlese, nadelige gevolge het op 'effektiewe bestuiwing'.

Tydens nagtellings in die Mooketsi boord het poeier-gemerkte bye soveel as 10 bloeiwyses besoek van die 10 per boom wat naby die korwe gemonster is, met 'n geleidelike afname tot 2 op 'n afstand van 200m van die korwe. Dagtellings van die aantal bye wat wei op 1 nr blomdraende boomkroon (of die aantal bye per 20 bloeiwyses) was 4 naby die korwe (5 word as optimaal beskou), met 'n afname tot 1 per 1 m^2 blomoppervlakte op 'n afstand van 200m. Die ses bykolonies in korwe het 'n effense toename getoon in sterkte sowel as in die produksie van die broed. heuning en stuifmeel, waama broed en stuifmeelproduksie afgeneem het.

In die Politsi boord was slegs die bloeiwyses gepoeiermerk wat bale naby die korwe was (hoogste telling 4/10). Tydens dagtellings is ook geen bye gevind op blommende borne binne 'n afstand van 200m van die korwe af nie, maar wel 'n hoe telling van 5 bye/20 bloeiwyses in 'n afgelee gedeelte van die boord, 550m van die korwe af. Kompeterende byeplante soos onkruide of omliggende natuurlike bos en bloekoms was moontlik verantwoordelik vir die afwesigheid van bye in die boord. Die 8 bykolonies waarvan twee reeds voorberei het om af te swerm, het 'n geleidelike toename getoon in al die gemete parameters, behalwe vir 'n effense vermindering in broed produksie teen die einde van die blomperiode.

Die vrugopbrengs van Hass borne in hokke met bye was betekenisvol hoër as dié sonder bye. By een blok was daar 'n afname in Hass opbrengs gemeet op toenemende afstande van die Ettinger stuifmeelouer. In 'n ander blok was daar egter geen verband hissen opbrengs en afstand van die stuifmeelouer nie.

INTRODUCTION

According to Bergh (1977) adequate pollination and optimum yields in avocado can be ensured by planting two complementary cultivars in the same plot. The effect of the pollen parent on yield was, however, limited to the first row adjacent to the pollen parent. In Israel, Guil and Gazit (1992) found significantly higher yields in Hass planted close to Ettinger. Peterson (1955) showed that by caging avocado trees of single cultivars, that they were capable of setting fruit when isolated from other cultivars, if bees were present in abundance. Lesley and Bringhurst (1951) observed that an avocado tree caged with bees set fruit, while a tree without bees bore no fruit.

Pollination and fertilization of avocado are prerequisites for fruit set and fruit development respectively. Avocados apparently are not wind-pollinated and require

active transfer of pollen by insects, particularly honeybees (Free, 1993). Generally 2-3 beehives/ha are recommended for pollination (McGregor, 1976). However, literature on the number of bees per tree, number of trees per hive, honeybee flight patterns in orchards, the beeplant value of avocados, or the minimum standards of hives used for pollination, is scarce or non-existent. The project, which is running for the third consecutive year, focuses on the Hass small fruit problem in testing the effectiveness of Ettinger as a pollen donor by way of botanical, horticultural and entomological techniques.

MATERIALS AND METHODS

The trials were carried out at Mooketsi and Politsi during August-September 1997 in commercial five-year old Hass and Fuerte avocado orchards, with Ettinger pollinizer rows in one direction every 100m (figure 1). Tree spacing at Mooketsi was 5m x 5m (400 trees/h). The Politsi orchard was thinned during July 1997 to 10m x 10m (100 trees/ha). Six and eight hived honeybee colonies were placed together in the Mooketsi and Politsi orchards respectively.

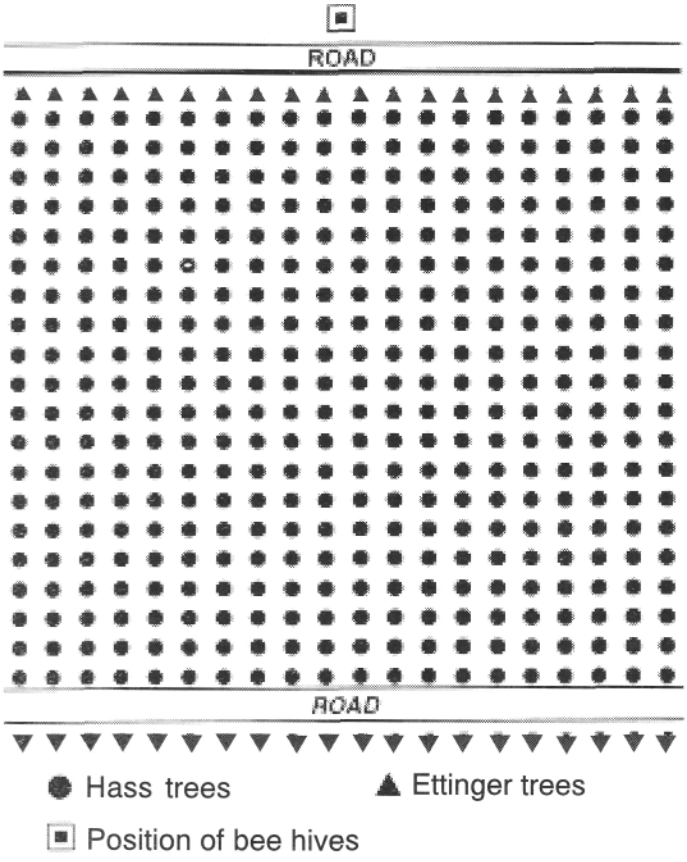

Figure 1. Schematic representation of the lay-out of one block in the orchard at Mooketsi

Botanical Aspects

Pollen performance

For comparing the pollen tube growth of Hass pollen versus Ettinger, Fuerte and Bacon pollen in Hass pistils, the same semi-in *vivo* methods were used as those described in the 1997 report (Robbertse *et al,* 1997). An additional treatment was included where boron (0.5 mg dm^2) was added to the agar medium to compare pollen tube growth in pistils from flowers kept in substrates with or without boron. The experiments were done during the period 28-08-1997 to 31-08-1997.

Effectiveness of open pollination

Two replications of 25 each, of randomly selected male phase Hass flowers were collected daily, over a two week period, 2-08-1997 to 1508-1997, from five marked trees close to the position were the bee hives were placed. These flowers were open pollinated the previous day while in the female phase. The flowers were fixed in Carnoy and prepared for fluorescence microscopy as described by Robbertse *et al.* (1997). The temperature, bee activity, wind speed and cloud cover were also recorded daily at 09h00, 13h00 and 17h00 during the same period. For the sake of comparison and reference, male phase flowers of Fuerte, which is a B-type cultivar, were also collected. The flowers were fixed in Carnoy and prepared for fluorescent microscopy.

The percentage pistils with:

a) pollen grains on the stigma,

b) pollen tubes germinated on the stigma,

c) pollen tubes in the style, and

d) pollen tubes reaching the ovule were recorded for each sample.

Horticultural Aspects

Open pollination

The yield of individual Hass trees, at increasing distance from Ettinger trees, was recorded for 3 seasons (1995-1997). Yield data were collected for 320 Hass trees in each of the two orchards.

At harvest, Hass fruit were sampled for isozyme analysis and the seeds were assayed for TPI (triose phosphate isomerase) at the Institute for Tropical and Subtropical Crops, Nelspruit, to determine the pollen parent.

Controlled pollination

Pairs of avocado trees (Ettinger and Hass; Hass and Hass) were caged with and without bees before flowering (table 2). A 40% shadecloth was used to cover the cages around the trees. After flowering, the shadecloth was removed. In cages with bees, one beehive was placed inside the cage during the flowering period. At harvest, Hass yields from caged trees were recorded and compared to those of uncaged trees.

Table 2: Caging treatments applied to Hass avocado trees in 1997

Cultivars	Pollination	Abbreviations	No. of Cages
Ettinger x Hass	With bees	E x H + b	4
Hass x Hass	With bees	H x H + b	4
Ettinger x Hass	No bees	E x H - b	1
Hass x Hass	No bees	H x H - b	1

Entomological Aspects

Distribution of marked bees

Two hives in each apiary were fitted with pollen dispensers which were filled with fluorescent powder to mark outgoing foragers. During the evening of the same day a portable UV light was used to scan inflorescences for deposited powder, i.e. visits by honeybees. Ten inflorescences per tree were randomly chosen and scanned. Hass trees at distances of 10, 20, 40, 70, 100, 150, 200, 250, 300, 350, and 400m were surveyed in three different directions from the hives in the Mooketsi orchard, (figure 10) but only up to 150m at Politsi (figure 9). In the latter orchard an additional 300m stretch was scanned alongside a road running through the middle of the orchard, with Hass trees on one side and Ettinger on the other.

Distribution of bees in the orchards

Foraging honeybees were counted at 11h00 in fine weather on flowering trees at 50m intervals across the orchards in a north-south and an east-west direction. The number of bees per square meter of flowering canopy area (or per 20 inflorescences) of a tree were counted.

Honeybee colony development

The condition of the hived honeybee colonies with regard to the number of bees, and amounts of brood, honey and pollen was assessed at the start of the flowering period and subsequently at fortnightly intervals.

RESULTS & DISCUSSION

Botanical Aspects

Pollen performance

The first day (28/08) was cloudy with a moderate wind and at 09:00 the Hass flowers were still open in the male phase of the previous day instead of in the female phase, while Ettinger and Fuerte flowers were in the female phase. Hass female phase

flowers and Fuerte and Ettinger male phase flowers only started opening at 10h00

The results are presented in table 1.

Table 1. Pollen performance - different pollen parents on Hass stigmas

% stigmas with pollen tubes reaching different levels in the pistil 24h after pollination				
Pollen donor on Hass pistils	Flowers in agar substrate + Boron		Flowers in agar substrate - Boron	
	Pt. in style	Pt. in ovule	Pt. in style	Pt. in ovule
Ettinger	96	58	95	54
Fuerte	97	58	93	64
Bacon	100	78	97	45
Hass	92	48	96	50

Pt. = pollen tube

Although there was a tendency for the Ettinger and Fuerte pollen to do better than the Hass pollen, the differences were not statistically significant. Similarly, the additional boron in the substrate did not have any significant influence on pollen performance, except for Bacon where the percentage of pistils with pollen tubes penetrating the ovule, showed a considerable increase after receiving additional boron. During the two previous years, the growth of Ettinger pollen tubes was significantly better than that of Hass pollen (Robbertse *et al,* 1996 & 1997).

Effectiveness of open pollination

The results of this experiment are shown in figures 2 to 4. Figure 2 shows the relationship between the percentage Hass pistils with pollen on the stigma (bars) and the percentage stigmas in which pollen tubes have reached the ovule (line) with a correlation coefficient of 0.55. Figure 3 shows the relation between the percentage Fuerte pistils with pollen grains that have germinated on the stigma (bars) and the percentage stigmas in which pollen tubes have reached the ovule (line) with a correlation coefficient of 0.37. The percentage pistils with pollen tubes reaching the ovule can be interpreted as **effective pollination** which is dependant on different environmental factors, such as temperature, rain, wind and bee activity.

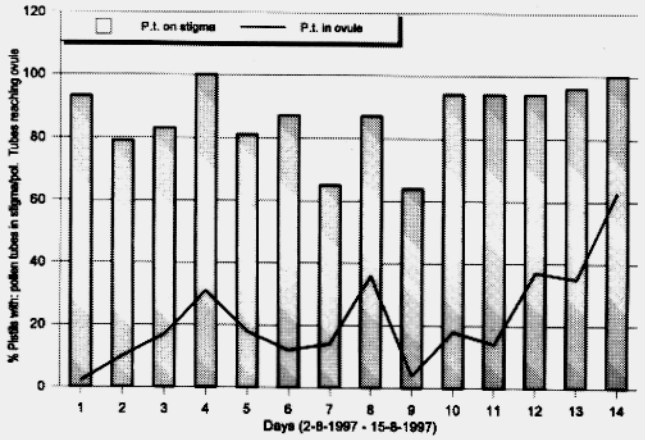

Figure 2. Relation between pollination (pollen tubes in stigma) and pollen tube growth in Hass avocado

Figure 4 shows the relation between temperature and percentage of pistils with pollen tubes reaching the ovule for Hass and Fuerte.

The performance of Ettinger as a pollen donor for Hass has now been studied for five consecutive seasons. For the first two seasons the experiments were conducted at Westfalia, Duivelskloof (Robbertse *et al.,*1994 and 1995) and for the last three seasons at Goedgelegen, Mooketsi (Robbertse *et al.,* 1996, 1997 and the present report). In all these reports, sections dealing with *in vitro* experiments, Ettinger pollen performed better than Hass pollen. In the present report, the difference between the Ettinger and Hass pollen tubes reaching the ovule was smaller than in earlier investigations, but the actual figures in both were much higher, indicating that conditions for good pollination were better than for the seasons before, and that Hass pollen was favoured by the better conditions. According to table 1, and evidence supplied in Robbertse *et al.* (1994), Fuerte and Bacon are also suitable pollen donors for Hass. The reason why only Bacon pollen reacted to the additional boron in the Hass stigmas is difficult to explain, but does indicate that more research in this regard is needed.

In figures 2 and 3, there is a tendency for the percentage pollen tubes entering the ovule, to relate to the percentage of pistils with pollen germinating on the stigma. In the case of Hass there is even a positive correlation between these figures ($r = 0.55$), indicating the importance of bee activity in affecting pollination. The lack of high correlation figures could be due to the fact that pollen tube growth is also affected by temperature and the physiology of the maternal plant.

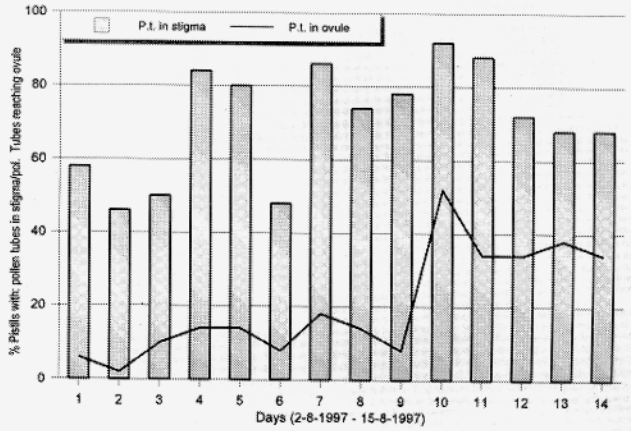

Figure 3. Relation betwee pollination (pollent ubes in stigma) and pollen tube growth in Fuerte avocado

The optimal temperature for opening of avocado flowers is 25°C (Sedgley, 1977), but during field observations at Mooketsi, it was found that they started opening at 20°C. For optimal pollen tube growth, the temperature should be between 25°C and 28°C (Sedgley, 1979), emphasising the importance of temperature during pollination and pollen tube growth as was also manifested by the results presented in figure 4. During the first week 'effective pollination' in Fuerte showed some relation to the temperature at 09h00 in the morning. Fuerte is a type B cultivar where female phase flowers start opening in the afternoon. The temperature preceding the opening of the flowers in the female phase, therefore, seems to have an effect on pollen germination and pollen tube growth. Similarly in the A cultivar Hass, the temperature 13h00 to 17h00 of the previous day show some relation to 'effective pollination'. Temperature is, however, not the only factor affecting pollination and pollen tube growth. Figure 4 also shows that 'effective pollination' during the first week when weather conditions were unfavourable, was generally lower than that of the second week when conditions were more stable. During the second week, however, the relation between 'effective pollination' and temperature was less obvious. This indicates that low temperature (below 20°C), probably is the overriding factor in effective pollination, but when temperatures rise, other factors start to interact.

Horticultural Aspects

Open pollination

Yields in the two Hass orchards differed with regard to increasing distance from the Ettinger pollen donor. In the Mooketsi orchard, Hass trees planted close to Ettinger gave high yields, and yield decreased the further away Hass trees were from the Ettinger pollen donor (figure 5).

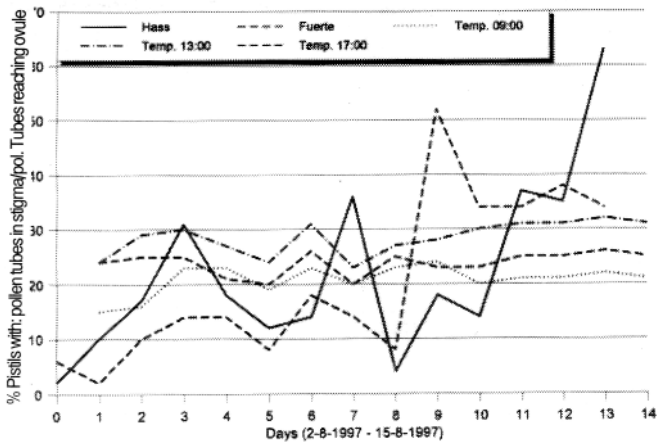

Figure 4: Relation between temperature and pollen tube growth in Hass and Fuerte

At Politsi, no such Hass yield pattern was obtained in relation to distance from the Ettinger pollen donor (figure 6)

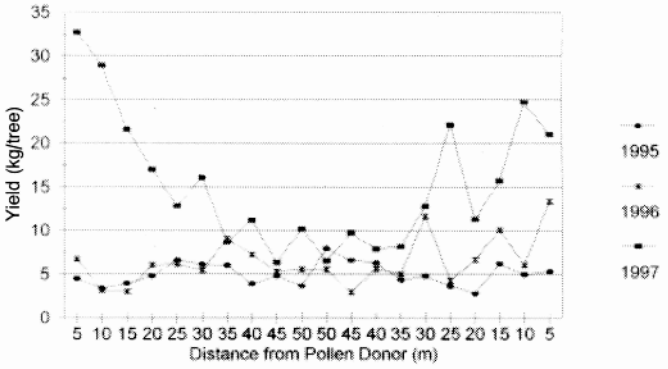

Figure 5: Hass yield in Mooketsi orchard 1, as influenced by distance from
Ettinger pollen donor (1995-1997)

The isozyme analysis results of Hass seeds harvested from trees at an increasing distance from Ettinger, showed no difference in pollen parentage. However, there was only a limited number of fruit analysed for isozymes, with no conclusive results.

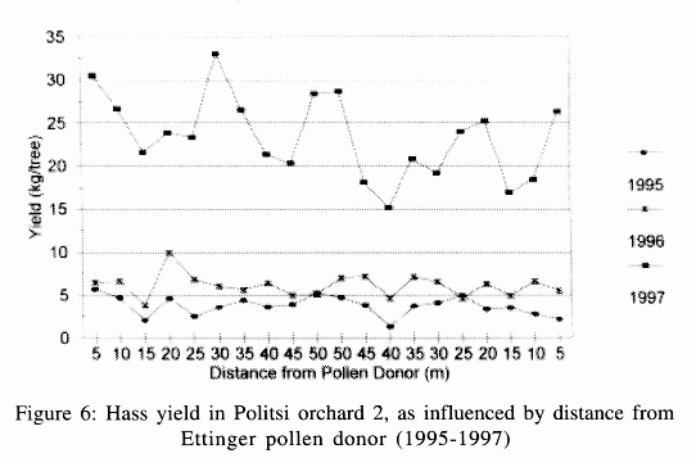

Figure 6: Hass yield in Politsi orchard 2, as influenced by distance from Ettinger pollen donor (1995-1997)

Controlled pollination

The results of the controlled pollination are shown in figure 7. Hass yields from caged trees without bees were very low. No significant yield difference was found in Hass trees caged with Ettinger or with Hass, in the absence of bees. The inclusion of bees in cages with Ettinger and Hass, or Hass and Hass, increased Hass yield considerably (figure 7). The yield of Hass trees caged with Ettinger did not differ significantly from that of Hass trees caged with Hass when bees were present. No yield difference was observed in the case of caged Hass + Hass trees with bees and open pollinated Hass trees (figure 7).

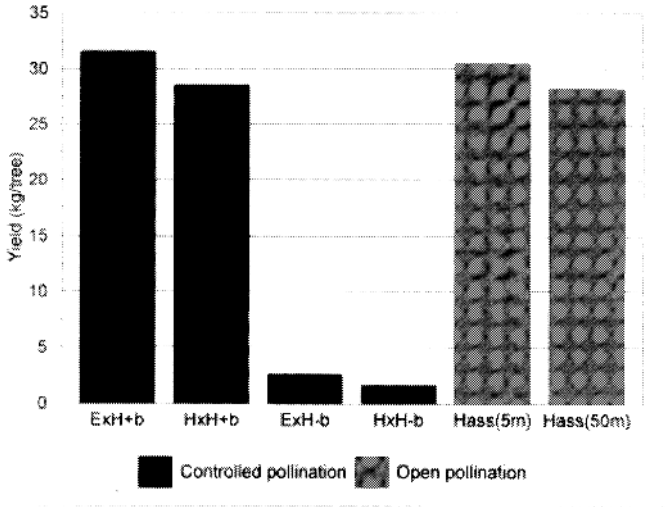

Figure 7: Hass yield as influenced by caging with Ettinger or Hass, with or without bees and open pollination (1997)

During the 1997 season, one of the Mooketsi orchards showed a decrease in yield with increasing distance from Ettinger (figure 5). Similar tendencies were not found during the two previous seasons in the same orchard, nor in the second Mooketsi orchard

(figure 6). With the exception of one season's results (1997) in one orchard (figure 5), we could not repeat the results obtained by Guil and Gazit (1992) in getting higher Hass yields closer to the pollen parent Ettinger. Bees play an important role in avocado pollination; without them fruit set is very low.

Entomological Aspects

Distribution of marked bees

The number of inflorescences marked by powder-coated bees declined with increasing distances from the hives in the Mooketsi orchard up to a distance of about 300m in two directions along the lines of Hass trees (figure 8). The survey across the rows of avocado trees and casuarina windbreaks resulted in significantly fewer marked inflorescences, the furthest along this line being only 200m from the hives.

Almost without exception only a single powder mark per inflorescence was detected with the UV scanner, presumably being the landing site of a forager before it walked to 3 or 4 different flowers and then taking off again. Even when one takes into account that the field force of only two of the six hives were powder-dusted, the number of marks per inflorescence fall far short of what can be considered adequate bee-visitation for pollination. This was particularly obvious in the Politsi orchard (figure 9) where only the trees at distances of 10m and 20m from the hives were powder-marked. The possible reason for the poor results will be discussed under the next heading.

Distribution of bees in the orchard

The highest number of bees counted at different dates and at different times of day in avocado orchards at Mooketsi, Westfalia and Politsi was 5/m^2 of canopy flower surface. This figure has provisionally been chosen as the optimal for pollination at which to aim. Ish-Am (1994) pointed out that one of the conditions for reaching an average yield potential for avocados in Israel was a bee activity of at least 20-40 bees/tree during most of the blooming period. He did not, however, equate the number of bees to the amount of flowers. Johannsmeier (1997) recommended that counts be made in fine weather between 10h00 and 12h00, and again between 14h00 and 16h00 to cover the two flower phases.

Figure 10 shows the number of bees/m^2 of flowers in different parts of the Mooketsi orchard in relation to the beehives. In the outlying parts of the orchard, i.e. away from the hives, no bees were found. This is in contrast to the conditions during the 1995 season when foraging honeybees were present in all parts of the orchard with counts of even 1 bee/m^2 of flowers in the outlying sections. This was as a result of an influx of wild honeybee swarms into the flowering orchard and surrounding bush, a phenomenon which varies much from year to year.

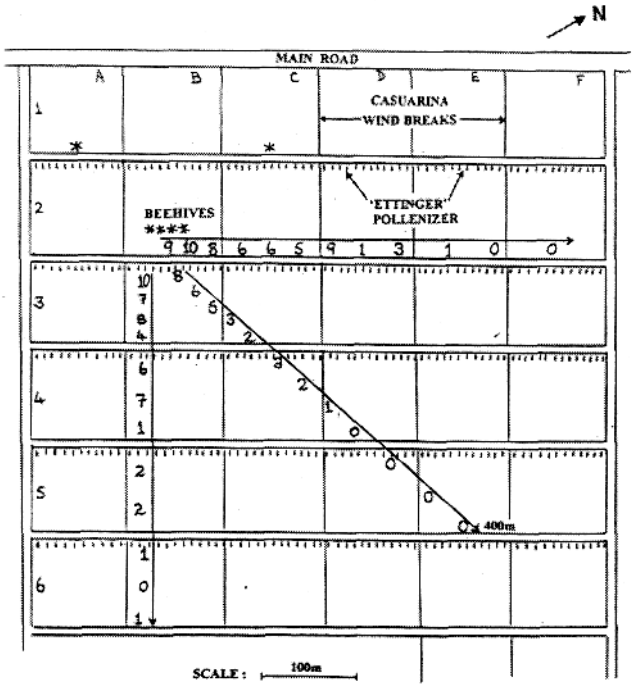

Figure 8. Number of powder-marked inflorescences out of ten per tree at different distances and in different directions from hives in a Hass avocado orchard at "Goedgelegen", Mooketsi

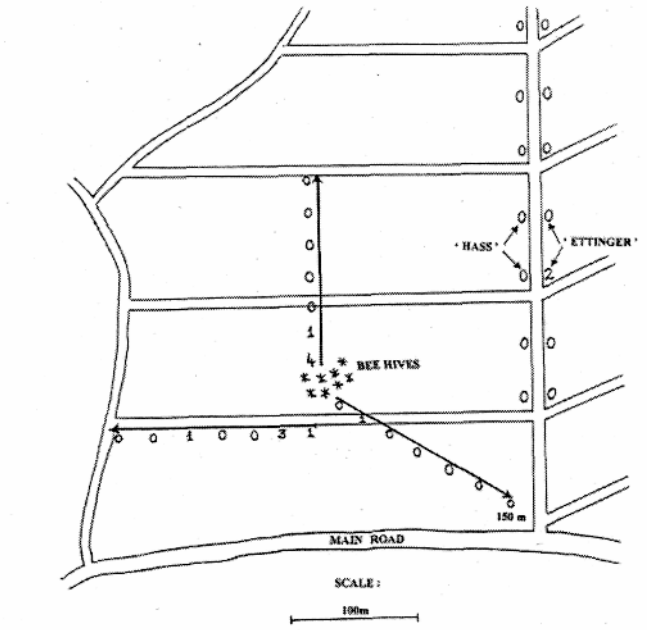

Figure 9: Number of powder-marked inflorescences out of ten per tree at different distances and in different directions from hives in a Hass avocado orchard at "Silverfontein", Politsi

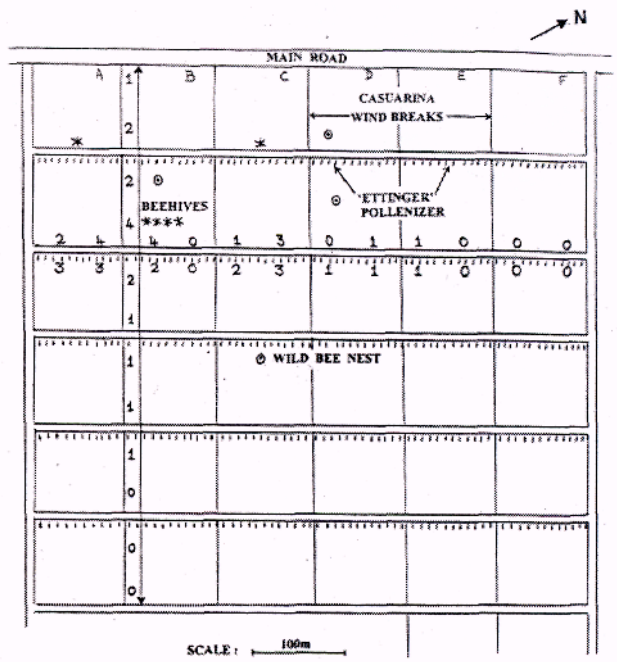

Figure 10: Number of foraging bees per 1m² of flowering tree canopy (or per 20 inflorescences at 50m intervals in an avocado orchard at "Goedgelegen", Mooketsi

As in the counts of powder-marked infloresecences, the counts of bees during daylight decreased with increasing distance from the hives, indicating a scarcity or absence of feral bees in other parts of the orchard. The counts also show that under the conditions of this particular orchard with six hived and four feral bee colonies, an acceptable or average number of pollinating bees was not found beyond about 200m from the hives. This would imply that groups of 6-10 hives should be placed 400m apart in an avocado orchard. For conditions in California, Bergh (1967) recommended that groups of hives be placed no more than 440 yards apart, with about 200 yards being preferable.

In the Politsi orchard there was an almost total absence of bees on Hass trees except in very close proximity to the hives (figures 9 & 11). On the Ettinger trees, however, the bee numbers ranged from 2-5/20 inflorescences (figure 11). In the western and higher-lying southern part of the orchard, away from the apiary, some Hass trees were bee-visited. The scarcity of bees on Hass trees was ascribed to the following:

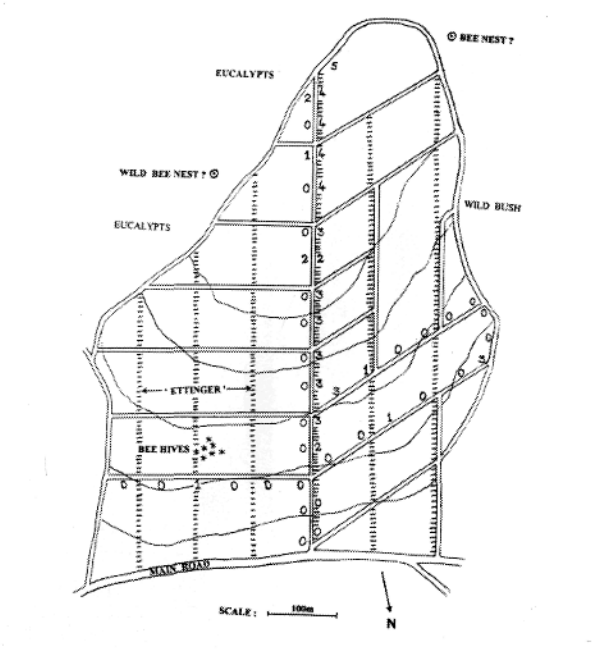

Figure 11: Number of foraging bees per 1m² of flowering tree canopy (or per 20 inflorescences) at 50m intervals in an avocado orchard at "Silverfontein", Politsi

1. Competitive beeplants attracted foragers away from avocados. Possibilities were late-flowering saligna gum *Eucalptus grandis* or the orchard weeds blackjack *Bidens* spp. and blue floss flower *Ageratum conyzoides.* Papademetriou (1976) and Eisikowitch & Melamud (1982) refer to the drastic reduction of bees in avocado orchards when beeplants with a stronger attraction, like *Citrus* crops, were flowering 3km or less from the hives.
2. Ettinger flowers have a higher nectar index than Hass flowers, i.e. they are more attractive to bees (Johannsmeier *et al.,* 1997). The high bee counts in the southern part of the orchard 550m from the hives (figure 11) could possibly have been the result of feral bee nests, which, we were told, were present, but did not see.

Honeybee colony development

No published information exists on honeybee colony development or honey production in flowering avocado orchards. This information would be useful for both beekeeper and avocado farmer when rentals of hives for pollination are negotiated. At Mooketsi during 1997 the trees flowered irregularly, and for a period of 6 weeks, which is unusual. During the first two weeks there was an increase in colony size, brood, honey and pollen (figure 12), whereafter brood and pollen production declined (the two are linked). At Politsi (figure 13) there was a slight increase in all the measured parameters except for a reduction in brood towards the end of the 4-week flowering period. Whether the increase in honey production towards the end of the flowering period resulted from

avocado flowers or competitive beeplants or both, is unknown. Two of the eight Politsi colonies had prepared for swarming towards the end of flowering, mainly because of a lack of space in the hives.

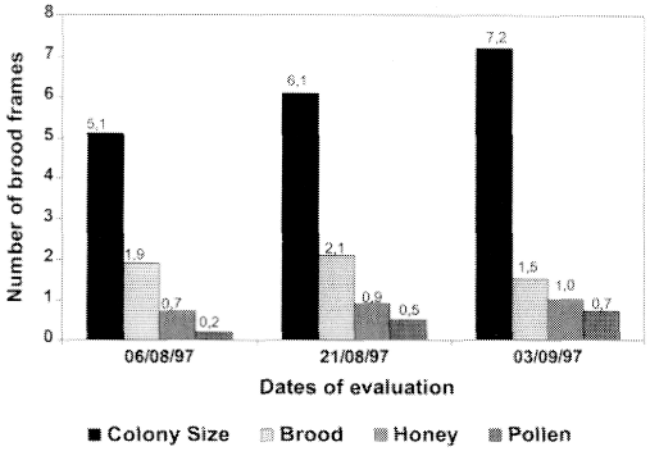

Figure 12: Development of honeybee colonies during six weeks in a flowering avocado orchard at Mooketsi

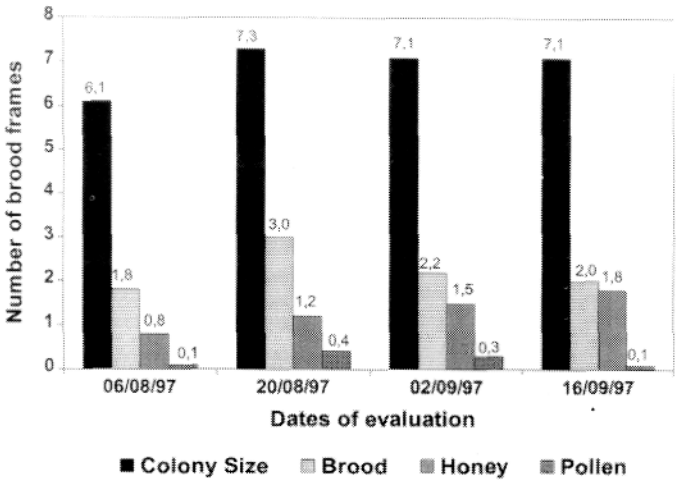

Figure 13: Development of honeybee colonies during one month in a flowering avocado orchard at Politsi

Recommended minimum standards for a pollination colony are 8 brood frames of bees and 2 brood frames containing brood of different stages in a queenright colony (Johannsmeier, 1997). The average size of the Mooketsi and Politsi colonies, which were provided by a beekeeper free of charge, was below the recommended norm, indicating that some weak colonies were involved.

ACKNOWLEDGEMENTS

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