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Hass Yield and Fruit Size as Influenced by Pollination and Pollen Donor — a Joint Progress Report

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ABSTRACT

Botanical, entomological and horticultural aspects were combined to study the influence of the pollen donor Ettinger and bees on Hass yield and fruit size. The experiment was carried out in two four-year-old Hass orchards bordered by a row of Ettinger. Honeybee colonies were introduced. Caged trees with and without bees were also used. Fruit set in caged trees with bees was significantly higher than in those without bees. *In semi-vivo* experiments Ettinger pollen outperformed Hass pollen in terms of germination and pollen tube growth. Ettinger pollen also had a positive influence on Hass seed size and large-seeded Hass fruit is likely to have an increased mass. However, a decline in yield in Hass trees with increasing distance from Ettinger pollen donors was not observed in this study.

INTRODUCTION

Fruit size and yield can be affected by various factors such as nutrition, climate, chemicals and also by the genetic expression of the cultivar. As far as the latter is concerned, only the seed coat, pulp and skin of the fruit originates from somatic tissue of the tree on which the fruit are borne, while the embryo is the product of fertilization and has a different genetic composition. Fruit development is controlled by phytohormones produced by the developing seed, and it is therefore possible that in the case of inter-cultivar cross-pollination, the genetic material derived from the pollen parent could have an influence on fruit and seed size. This phenomenon is referred to as xenia where only the morphology of the seed is affected and metaxenia where the morphology of the whole fruit is affected (Sedgley & Griffin, 1989). According to the latter authors, xenia and metaxenia were observed in some one-seeded fruits like the pecan and pistachio nuts, and nut size was influenced by the pollen donor. These phenomena have not yet been demonstrated to occur in avocado and we were interested in whether fruit size and yield in Hass avocado would be affected if the Hass flowers were cross-pollinated with Ettinger pollen.

Due to its dichogamous flowers, the avocado is a natural out breeder (Peterson, 1955; Davenport, 1986). Practical experience, however, showed that some cultivars are

successfully self-pollinated and satisfactory yields are obtained in commercial orchards consisting of one cultivar only. In Israel, trials conducted for four years in three different pure Hass orchards adjacent to an Ettinger orchard, showed significant yield increase in Hass trees close to an Ettinger orchard (Guill & Gazit, 1992; Ish Am, 1994). Degani *et al.* (1989) found that when Ettinger served as the pollen parent for Hass, fruit yield correlated significantly with the rate of out crossing.

In this study we applied a multidisciplinary approach to determine the effect of Ettinger as a pollen donor on fruit production and fruit size in Hass and the role of honeybees as pollinating agents.

MATERIALS AND METHODS

The trial was carried out in two Hass avocado orchards (orchards 1 and 2) planted in 1992 at Goedgelegen Farm, near Mooketsi. Each orchard has 20 rows of Hass bordered by Ettinger on two sides (figure 1.). The trees are planted 5 m x 5 m apart.

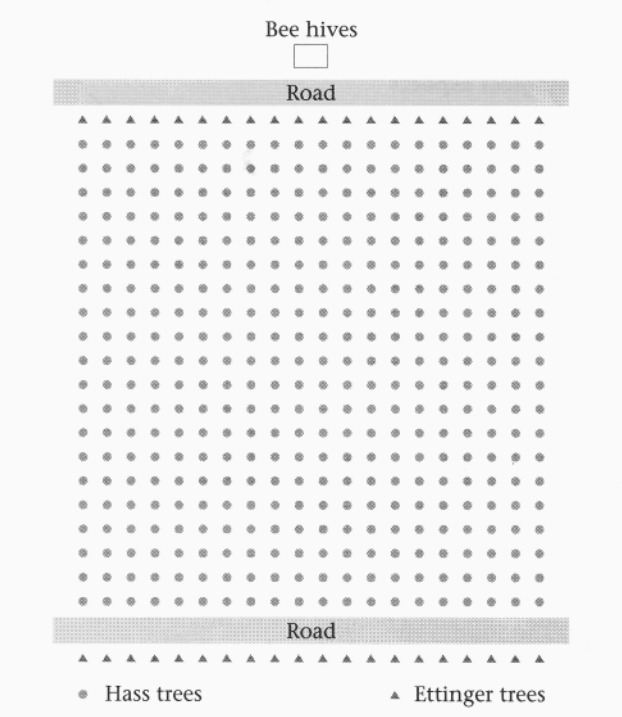

Figure 1
Schematic representation of cultivar layout at Goedgelegen Farm

Botanical aspects

Air-borne pollen

The possibility of wind-pollination in avocado was mentioned by Davenport (1992), and for this reason pollen traps, consisting of microscope slides covered with a thin layer of

petroleum jelly, were put out at various positions on wooden poles distributed through the orchard. On each pole, one slide was placed in a horizontal and another in a vertical position. The slides were collected after 12 h and the number of avocado pollen grains on each slide was recorded.

Pollen longevity and duration of stigma receptivity

No information is available on the longevity of avocado pollen, and the duration of stigma receptivity came under the spotlight when Davenport (1992) claimed that in Florida self-pollination during the male phase is the main means of pollination for most commercial cultivars. Pollen longevity was studied by pollinating freshly opened female phase Hass flowers with one-, two and three-day-old Ettinger pollen. Stigma receptivity was studied by pollinating emasculated Hass flowers, opening in the male phase, with fresh Ettinger pollen. Hass flowers collected during the onset of the female stage and kept on an agar medium under a controlled temperature regime, were allowed to open and close in both the female and male stages to allow spontaneous pollination.

Pollen performance

For studying performance of Ettinger versus Hass pollen, 8 x 25 Hass flowers were collected at the onset of the female phase. The flowers were arranged in petri dishes containing an agar medium consisting of agar, boric acid and sucrose (25 flowers per petri dish) and incubated at 30 °C until all flowers were fully open. Flowers were then pollinated with either fresh Ettinger or Hass pollen and incubated for 24 h at the same temperature to allow pollen germination and pollen tube growth. After incubation the flowers were fixed, pistils were excised, cleared and stained with aniline blue for fluorescence microscopy. For each pistil, the number of pollen grains on the stigma, number of pollen grains germinated and pollen tube growth up to one third of the style length, halfway down the length of the style, up to the ovary, up to the ovule and entering the ovule, were recorded.

This procedure was also followed for the pollen longevity and stigma receptivity tests.

Entomological aspects

Distribution of marked bees

Because feral honeybees were already present in high numbers on the earlier flowering Ettinger trees (average of nine bees/tree) throughout the orchard before the trial started, only five hived honeybee colonies were placed in orchards from the northwestern corner opposite to where the feral honeybees were presumed to come from.

Two hives were fitted with pollen dispensers filled with daylight-fluorescent 'astral pink' powder to mark outgoing foragers. Colour-marked and unmarked bees were counted five times on Hass trees at distances of 5, 10, 20, 40, 70 and 100 m from the hives on various days during the forenoon and afternoon. Every count was made in three different directions from the hives into the orchard, namely N, N-E and E.

Fruit set in cages

In a preliminary test an entire Hass tree was covered in bee-proof 80 % shade cloth and a beehive placed inside the tent. A second tree was similarly covered, but no honeybees were provided. A third tree was also covered, but the lower 1 m of the tent was open to allow orchard bees to enter. The tents were removed after flowering. There were two replications only for these treatments in two orchards.

Honeybee colony development

The condition of the five bee 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 twice at fortnightly intervals.

Horticultural aspects

At harvest (May 1995), Hass yield data were recorded at an increasing distance from Ettinger trees. Yield records for 160 trees in each of the two orchards were collected. From each tree, fruits were graded into two size categories, i.e. export-size fruit (fruit exceeding 61 mm in diameter, corresponding to count 24 fruit and larger) and small fruit (fruit less than 61 mm in diameter, corresponding to count 26 and smaller).

At harvest in May 1995 fruit were sampled for isozyme tests, and within 24 h after picking the embryos were assayed for TPI (triose phosphate isomerase) at the ITSC in Nelspruit to determine the pollen donor.

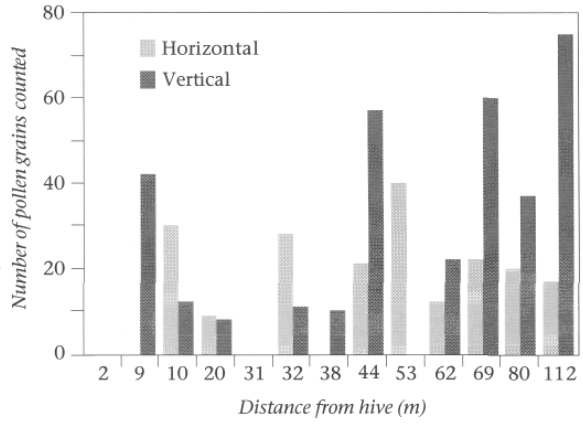

Figure 2
Number of pollen grains per pollen trap in relation to distance from bee hives

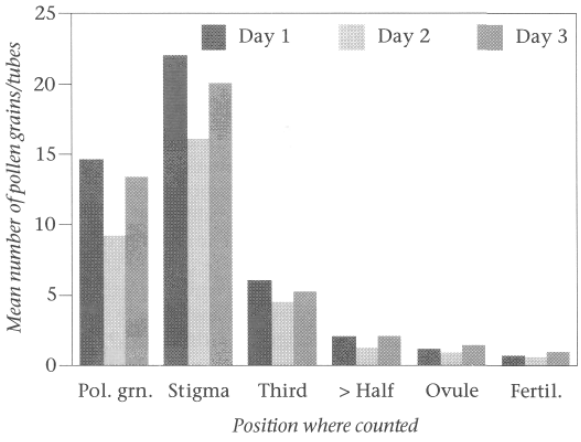

Figure 3
Performance of 1-, 2- and 3-day-old Ettinger pollen on Hass pistils

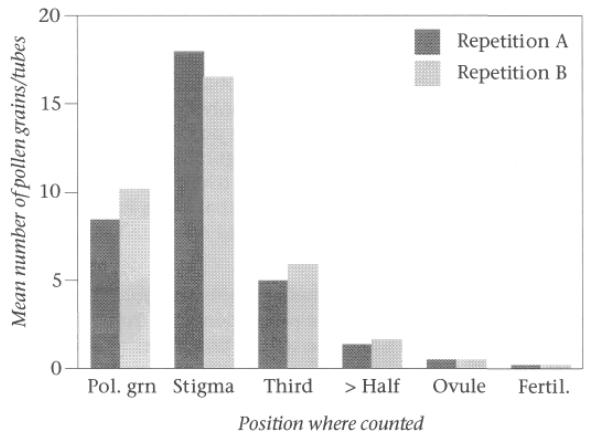

Figure 4
Performance of fresh Ettinger pollen on aged (30 h) Hass pistils. Results of two consecutive days.

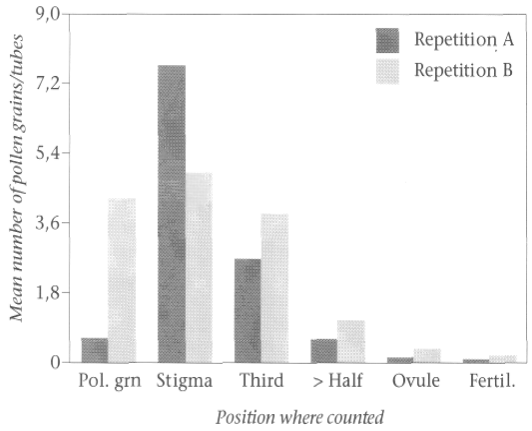

Figure 5
Performance of Hass pollen of spontaneous self-pollination during male phase. Results of two consecutive days.

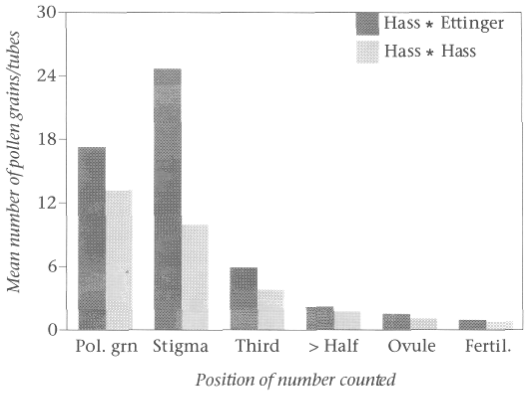

Figure 6
Performance of Hass vs. Ettinger pollen on Hass pistils

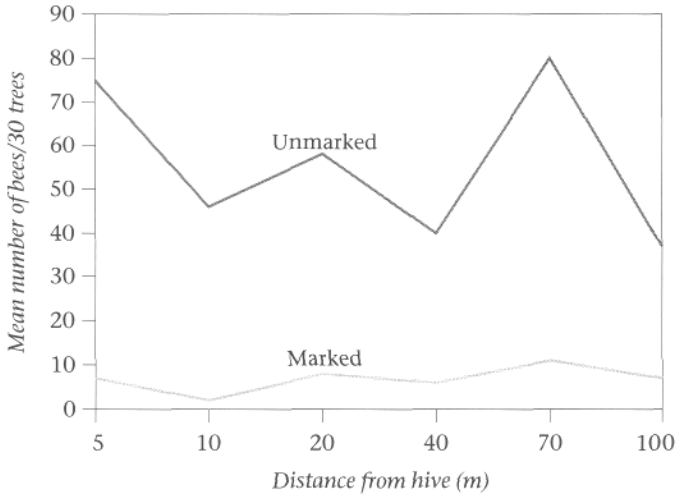

Figure 7
The number of colour-marked and unmarked bees on Hass trees at various distances from hives

RESULTS

Botanical aspects

Pollen traps

Figure 2 shows the distribution of air-borne pollen in the orchard. During the experiment the bees were marked with either a pink fluorescent powder or pine pollen (see section on Entomology). Stained avocado pollen and pine pollen were only found on the horizontal slides and it is, therefore, quite possible that the figures obtained from the horizontal slides could represent pollen that dropped from the bees in flight. More pollen was trapped on the vertical than on the horizontal slides. Pollen on the vertical slides

could be an indication of air-blown pollen, since a mild wind was blowing during the observation period.

Pollen longevity

There was no obvious difference in the performance of the fresh (day 1) and aged (days 2 and 3) pollen (figure 3). Day 2 was a cool day and Hass flowers did not open until about 10:00 and this might explain the slightly lower figures of day 2.

Stigma receptivity

Results of two consecutive days are presented separately in figure 4. The aged stigmas were still receptive for pollen germination, and pollen tubes managed to grow down the styles, but only a few tubes managed to reach the ovules. Where spontaneous pollination was allowed (figure 5), the stigmas were of the same age as in figure 3, and the results were nearly the same.

Ettinger vs. Hass pollen

The results are presented in figure 6. In Ettinger, pollen germination and pollen tube growth were significantly higher than in Hass.

Entomological aspects

Distribution of marked bees

No differences were found in the number of bees counted in the three different directions from the hives. The total of all the counts on various days and for afternoons and forenoons are shown in figure 7. There was no decrease in the number of marked bees at increasing distances from the hives up to the maximum distance of 100 m. The peak at 70 m distances for both marked and unmarked bees in all three directions cannot be explained, but could possibly be due to foragers from a wild ground nest in the orchard.

One pollen dispenser was filled once with pine pollen to determine onto which flower parts bees deposited pollen. This attempt failed, firstly because the pollen was so light that it was blown away by the bees and little stuck onto their bodies, and secondly, detecting the pine pollen grains, despite being relatively large, proved more difficult than anticipated with a stereo microscope. Flour as a carrier was subsequently mixed with the pollen, but not further tested.

Fruit set in cages

In this limited test there was a highly significant difference in fruit set with and without bees (table 1). The low fruit set of the tree caged with beehive in orchard 2 is probably the result of removal by hand of flowering panicles immediately prior to enclosure of the

tree by net.

The proportionately higher fruit drop of the tree caged with beehive treatments compared to the half-caged with feral bees treatments, could possibly be the result of selfing, i.e. pollination with own pollen. The flowers of caged trees were observed to be frequented by honeybees on various occasions.

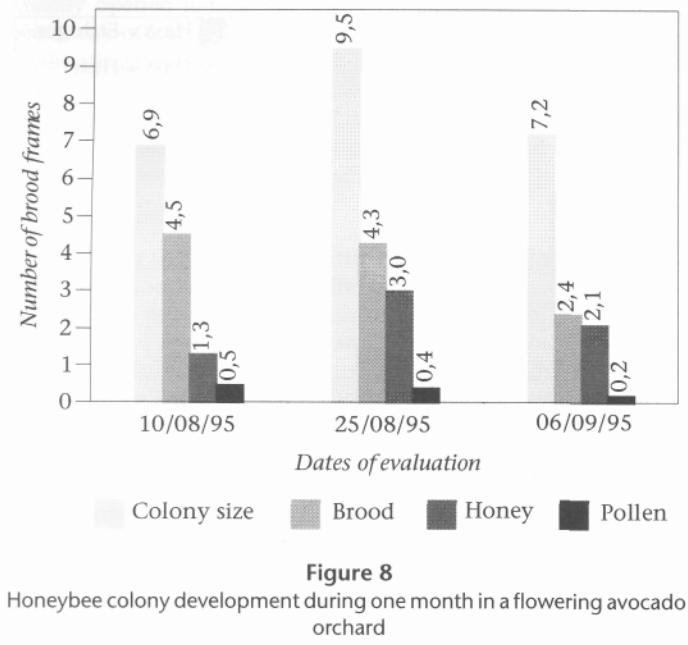

Figure 8
Honeybee colony development during one month in a flowering avocado orchard

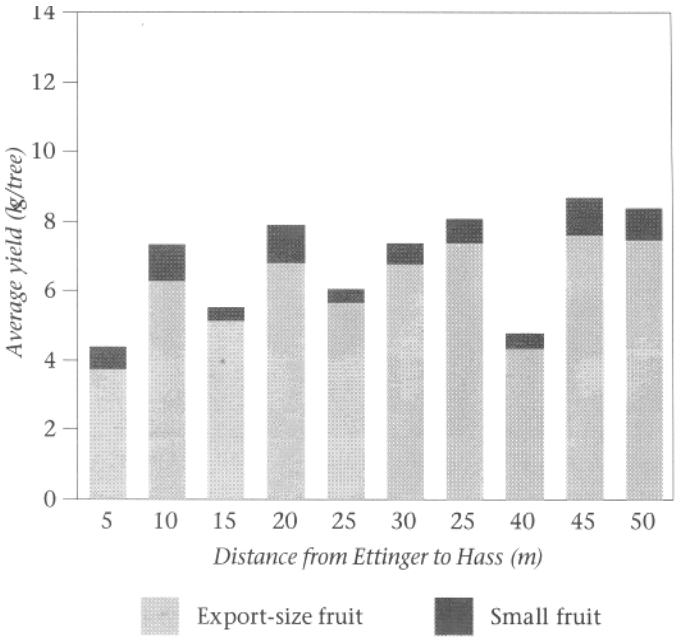

Figure 9
Hass yield in orchard 1, as influenced by distance from Ettinger

Honeybee colony development

The increase in colony size after the first two weeks is the result of emerged bees from the initial large amount of broods, namely 4,5 frames on average. Brood production was sustained at first due to a ready source of nectar and little pollen from avocado flowers (figure 8).

Horticultural aspects

In 1995, both Hass orchards showed no difference in yield with respect to an increasing distance from Ettinger. Hass fruit size was also not influenced by distance from Ettinger pollen donors (figures 9 & 10).

The isozyme test showed that there was nearly 100 % crossing in the two orchards regardless of the distance from Ettinger. Hass fruit derived from cross-pollination (as determined by the isozyme test) had a significantly larger seed mass than those obtained by selfing (data not shown).

Table 1
Fruit set of Hass avocado trees caged with and without bees

	Treatment	Number of fruits			
		Oct 95	Nov 95	Dec 95	Jan 96
Orchard 1	Caged (+ beehive)	97	96	88	17
	Caged (– beehive)	3	3	3	3
	Half-caged (+ feral bees)	88	88	80	50
Orchard 2	Caged (+ beehive)	14	14	13	8
	Caged (– beehive)	6	6	5	5
	Half-caged (+ feral bees)	120	88	46	40

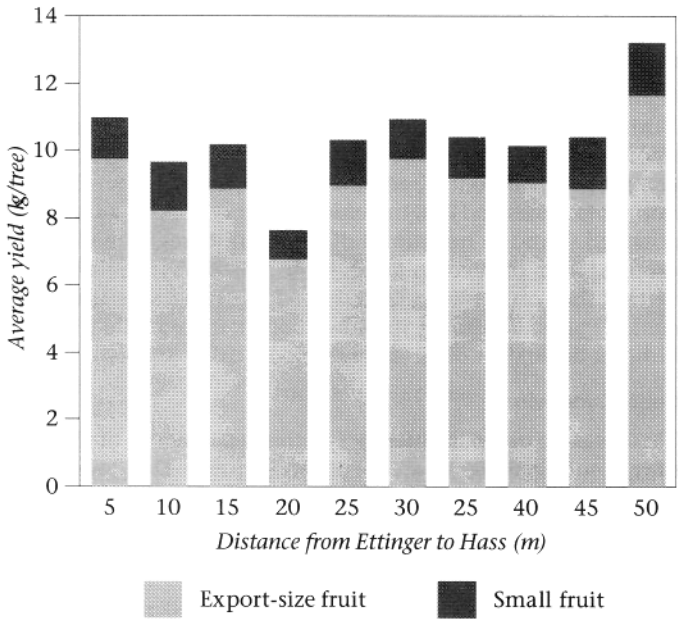

Figure 10
Hass yield in orchard 2, as influenced by distance from Ettinger

DISCUSSION

Botanical aspects

Pollen collected on the pollen traps confirmed the presence of air-borne pollen in an avocado orchard (figure 2). The quantities available are too small to have a significant effect on pollination, but do explain the odd fruit that were set on the tree that was enclosed in shade net (see Entomology section).

Ettinger pollen remains viable for at least three days (figure 3). It is, therefore, also possible that pollen collected on the bees will remain viable for some time which could explain fruit set in pure Hass orchards where no other pollen is available when flowers open in the female phase. However, we still have to supply evidence that sufficient amounts of pollen remain on the bees' bodies during the night.

Although Hass stigmas are still receptive when flowers reopen during the male phase (figures 4 & 5), the ovules are probably no longer 'receptive' as hardly any pollen tubes entered the ovules. These observations are in line with those of Gazit *et al.* (1995) where no fruit set was found when flowers were pollinated during the male phase. Further studies are needed to look at the structure of the ovule during the male phase.

In the experiment comparing the performance of Hass pollen with the performance of Ettinger pollen, Ettinger out-performed Hass by far (figure 6). Goldring *et al.* (1987) also found that amongst the potential pollen donors Hass, Ettinger and Reed, Ettinger excelled by producing almost all of the hybrid fruits.

Entomological aspects

Because the dry surrounding veld provided no additional forage for the bees, we suggest that avocado flowers can maintain colony size during the flowering period, but that brood production declines.

Horticultural aspects

The fact that we did not find declining yield in Hass at an increasing distance from Ettinger may be attributed to the small tree size in this study. Presently, there are still wide open spaces between the Hass trees and bees are easily able to travel through the orchard unhindered. As the Hass canopies close in years to come, this will change and may lead to similar results as reported by Guil & Gazit (1992).

CONCLUSIONS

Taking all our results into consideration, it is clear that pollination in Hass takes place during the morning when flowers are in the female stage if sufficient viable pollen, preferably from a B-type cultivar, is available. Ettinger is a B-type cultivar producing high quality pollen and proved to be an effective pollen donor for Hass. Hass orchards should, therefore be interplanted with Ettinger, but we still need to establish the ratio of Ettinger: Hass trees required for optimal production.

In 1995, we did not find any relationship between Hass yield and distance from Ettinger. However, Ettinger pollen had a positive influence on Hass seed size and large-seeded Hass fruit are likely to have an increased mass.

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