Pollination Studies in Hass Avocado in Relation to the Small Fruit Problem

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ABSTRACT
This season’s results of semi-in vivo pollination experiments confirm findings of last year that Ettinger is an excellent pollen donor for Hass and Ettinger pollen outperformed Hass pollen on Hass pistils. Fresh Ettinger pollen still germinated on Hass stigmas aged for 24 hours, but pollen tubes did not reach the ovary. Aged Ettinger pollen performed poorly compared to fresh pollen. Caged Hass + Ettinger trees with bees gave higher fruit retention than caged Hass + Hass trees with bees. The above-mentioned results confirm findings by different authors that avocado is a natural out breeder and pollen donors should be provided for assuring better fruit set and fruit retention.

INTRODUCTION
An extensive introduction for this project was given in the 1996 report (Robbertse et al., 1996). The present report basically deals with a repetition of the experiments that were done last year with the objective to compare the results of two different seasons. More details about Entomological aspects appear in the article by Johannsmeier et al, elsewhere in this volume.

MATERIAL AND METHODS
As was reported last year, the trial was carried out in two Hass avocado orchards planted in 1992 at Goedgelegen Farm, near Mooketsi. Details of the orchard lay-out and the position of the beehives in the orchards can be found in the SAAGA Yearbook, Volume 19 (Robbertse et al., 1996).

Pollen performance
The performance of Hass versus Ettinger pollen was done using semi-in vivo techniques. Sixteen x 25 Hass flowers were collected at the onset of the female phase. The flowers were placed in petri dishes (25 flowers per dish) containing agar with their pedicels submerged in the agar. As a pollen source, Ettinger flowers were collected at the onset of the male phase in the same way as the Hass flowers and incubated for about an hour at 28°C to allow the anthers to open. Eight x 25 Hass flowers were then
pollinated with Ettinger pollen after which they were incubated at 28°C for 24 hours, allowing pollen germination and pollen tube growth. The remainders of the Hass female phase flowers were kept at room temperature until the afternoon. Hass flowers at the onset of the male phase were collected and incubated at 28°C to allow pollen shed. This pollen was used to pollinate the remaining Hass female flowers. The latter flowers were then also incubated for 24 hours to allow pollen germination and pollen tube growth. After incubation the flowers were fixed in Carney's solution. The pistils were excised, cleared and stained with aniline blue for fluorescence microscopy. For each stigma, the number of pollen grains germinated on the stigma, number of pollen tubes in the style, number of pollen tubes reaching the ovary and number of pollen tubes reaching the ovule, were recorded.

**Pollen longevity and stigma receptivity**

Two experiments were conducted. To obtain aged stigmas, the perianth and stamens of female phase Hass flowers were removed and kept at room temperature for 24 hours. Fresh as well as aged stigmas were pollinated with fresh Ettinger pollen. The rest of the treatment was the same as for pollen performance.

**Fruit set experiments**

Fruit set on open-pollinated Hass trees was estimated 5m and 50m away from the row of Ettinger trees.

To test the effect of self and cross-pollination on fruit set, pairs of Hass + Hass and Hass + Ettinger trees were caged in bee-proof, 80% shade cloth tents, each pair with and without a hive of bees.

**RESULTS**

The results of the semi-in vitro experiments are shown in table 1. Due to pollen tube competition in the style, usually only one pollen tube will reach the ovule. For a better evaluation of pollen and pollen tube performance, we determined the percentage of pistils in which pollen had germinated as well as the percentage of pistils in which pollen tubes had reached a specific distance from the stigma, rather than looking at pollen or pollen tube numbers.
Pollen performance

In table 1 it can be seen that Ettinger pollen out-performed Hass pollen. Where Ettinger pollen was used, 94% of the stigmas contained germinated pollen grains, 59% of the styles were penetrated by pollen tubes and 10% of the ovaries were reached by pollen tubes compared to the figures 83%, 47% and 5% respectively, where Hass pollen was used.

Pollen longevity and stigma receptivity

The germination and pollen tube performance of the aged Ettinger pollen were much poorer compared to the fresh pollen. No stigmas were found where the pollen tubes, derived from aged pollen, could reach the ovary compared to the 10% and 28% where fresh Ettinger pollen was used. Pollen did germinate on aged Hass stigmas, but the pollen tubes lost their vigour with the result that only 4% of the styles were penetrated by pollen tubes compared to 59% where fresh stigmas were used.

Fruit set and retention

Where no bees were supplied in the cages, fruit set was extremely low (table 2) irrespective of whether two Hass trees were caged together or whether one Hass tree was caged with one Ettinger tree. Where bees were supplied in the tents, fruit retention on the Hass + Ettinger trees was significantly better than on the Hass + Hass trees.
Where Ettinger trees were situated between the beehives and the Hass trees, fruit retention on Hass trees 5m away from the Ettinger trees was 2.1 (estimated on a 0 = no fruit to 5 = good fruit set scale, compared to only 0.5, 50m away from the Ettinger trees.

**DISCUSSION**

The better performance of Ettinger as a pollen parent for Hass was not only demonstrated with the semi-*in vivo* experiments but was also manifested in the higher fruit retention on Hass trees closer to Ettinger along the line of bee activity (table 2). This is in accordance with similar results obtained by Guill and Gazit (1992) and Ish-Am (1994) in Israel. In the 1996 report we could not find a clear relationship between fruit retention on Hass trees and the distance away from Ettinger. The reason for this could be that the trees were much smaller in 1996 so that bees moved more freely in different directions in the orchard. The dry season that preceded flowering in 1995 caused a lack of indigenous plants flowering in the vicinity of the orchard. Local bees were therefore attracted to the avocado orchards, causing a high pollen pressure. Good rains preceded the 1996 avocado flowering with the result that sufficient indigenous flowers were available to keep the local bees away from the avocado orchards. The Hass trees were bigger so that the introduced bees were forced to move past the Ettinger trees to forage along the rows of Hass trees, carrying Ettinger pollen with them.

Degani *et al.* (1989) found that where Ettinger served as the pollen parent for Hass, fruit yield correlated significantly with the rate of out crossing. Although the rate of out crossing in our experiment with the caged trees has not yet been tested, the higher yield on the Hass trees that were caged in with Ettinger suggests similar results.

Aged stigmas of Hass flowers opening in the male phase could still sustain pollen germination, but the pollen tubes were arrested in the styles, thus preventing fertilisation. These results contradict Davenport's (1992) claim that self-pollination in the male phase of most commercial cultivars is a common phenomenon and are indirectly highlighting the importance of pollinators in avocado.

Similar to 1996, the aged pollen did germinate and pollen tubes did penetrate the style, but the general performance was poorer than that of 1996. We also did pollen viability

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Pollinator</th>
<th>Fruit set</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hass-Open, 5m from Ettinger</td>
<td>Natural bees</td>
<td>2,1</td>
</tr>
<tr>
<td>Hass-Open, 50m from Ettinger</td>
<td>Natural bees</td>
<td>0,5</td>
</tr>
<tr>
<td>Hass + Ettinger in cage</td>
<td>No bees</td>
<td>0,85</td>
</tr>
<tr>
<td>Hass + Ettinger in cage</td>
<td>With bees</td>
<td>3,05</td>
</tr>
<tr>
<td>Hass + Hass in cage</td>
<td>No bees</td>
<td>0,8</td>
</tr>
<tr>
<td>Hass + Hass in cage</td>
<td>With bees</td>
<td>1,5</td>
</tr>
</tbody>
</table>

*5 = very good to 0 = no fruit set*
tests by staining fresh pollen in Alexander’s stain (results not shown) which showed that only about 50% of the 1996 Hass pollen was viable. Although the score for Ettinger pollen was 99%, germination and pollen tube growth were poorer compared to the previous year.

REFERENCES


