# Finding the best polliniser for 'Hass' avocado and the effect of honeybees as pollinators

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**ABSTRACT**

This study aims to find the best polliniser for 'Hass' avocado. A trial run was done with two cages during 2014 and a full experiment during 2015. The study entailed an in vitro pollination study, a field trial with encaged bees, open pollinated trees as well as time-lapse photography. For all treatments, two trees were used per treatment and ten flowering shoots per tree. Four sides of each tree were labelled for counting fruit set. Fruit set counts were made on 13 October and 8 December 2015. Flower behaviour and bee activity were monitored during the course of the study with the aid of time-lapse photography.

Results from 2014 and 2015 indicate that, in the in vitro pollination, 'Hass' x 'Zutano' gave higher figures than 'Hass' x 'Hass'. In the nets, 'Hass' x 'Zutano' also showed better fruit set during the first and second count in 2014, but not during all counts in 2015 due to the fact that 'Zutano' trees started flowering before the 'Hass' trees in the open pollinated trees. The fruit set did not differ significantly for both years.

## INTRODUCTION

The age-old question about the need for a suitable polliniser for 'Hass' avocado and the need for introducing honey bees as pollinators have not yet been adequately resolved. According to Alcaraz and Hormaza (2009), 'Hass' is the most important avocado cultivar worldwide, and one of the most popular cultivars for South Africa to export to European countries. Growers are continuously looking for higher yields and believe that it should be possible to increase the present yields by finding the best polliniser. Being an A-type cultivar, the polliniser must be a B-type cultivar.

In his report on avocado flowering, Davenport (1986) gives a detailed description of the avocado flower and also flower behaviour which is referred to as 'synchronous dichogamy'. He refers to Nirody (1922) who was the first person to recognise that avocado flowers were dichogamous and to Stout (1923) who described the unique dianthesis and synchronous dichogamous nature of avocado flowering behaviour. Stout (1923) classified avocado cultivars in two types, A and B types.

Gustafson (1966) gave a detailed overview of studies done on cross-pollination of avocados and mentioned that a single avocado flower can contain between 5000 and 10000 pollen grains, meaning that pollen supply is no problem in avocado pollination. Sedgley did several studies on pollination and pollen tube growth in avocado (Sedgley, 1979a; Sedgley, 1979b; Sedgley, 1979c; Sedgley & Buttrose, 1978; Sedgley & Grant, 1983) and also studied storage of avocado pollen (Sedgley, 1981). The viability of the pollen is also important. Papademetriou (1974) described avocado pollen as spherical with a thick wall and mentioned that under natural conditions (between about 7°C min. and 12°C max.) the pollen remained viable for at least 151 hours. Pollen stored under low temperatures can last for several days.

Tomer and Gottreigh (1975) described abnormalities in ovule development and defective ovules. Tomer *et al*. (1976) indicated that not every avocado flower has the potential to produce a fruit. Ish-Am and Eisicowitch (1988) showed that avocado flowers are not very attractive to honeybees. Hofshi (1995) supplied a summary of Dr Gad Ish-Am's seminar in which valuable information about avocado pollination can be found. He refers to the fact that avocado nectar consists of about 100% of sucrose, compared to nectar of other bee flowers that contain mostly fructose and glucose and also mentioned that avocado nectar contains another type of sugar called perseitol which is unique to avocado. Hofshi (1995) supplied a table showing how the avocado flower differs from 'honey bee flowers' and further mentioned the presence of sucrose as one of the reasons why bees do not prefer avocado nectar.

The benefits of cross-pollination against self-pollination for increasing fruit set and yield was already reported by Clark and Clark (1926, 1923). A good polliniser without pollinators is of no use and Clark (1923) already reported the importance of bees as pollinators. Peterson (1955) and Lemmerts (1942) concluded that large dipterous and hymenopterous insects are necessary for pollinating avocados, at least for type A cultivars. Since then many attempts have been made to 'prove' that avocados are outbreeders and that pollinisers are essential for good fruit set.

Degani and Goldring (1989) and Degani *et al*. (1997) showed that abscission of avocado fruitlets and fruit characteristics were greatly influenced by the pollen parent. Guil and Gazit (1992) found that over a period of four years, three 'Hass' orchards planted next to 'Ettinger' orchards showed significant yield increases. 'Hass' rows bordering 'Ettinger' produced 17 to 20 tonnes/ha annually, while at a distance of 50 m the yield was only 8 to 10 tonnes/ha and further away the yield decreased to 5 tonnes/ha. Gardiazabal and Gandolfo (1995) rated 'Hass' to be a weak self-pollinator and found that the pollinators which provided the highest cross-pollination results, in their combinations, were 'Zutano', 'Edranol' and 'Bacon'. Robbertse *et al*. (1994, 1995, 1996 & 1997) found that 'Ettinger' was a good polliniser for 'Hass'. Degani *et al*. (1997) also came to the conclusion that 'Ettinger' is a highly potent polliniser for 'Hass'. Alcaraz and Hormaza (2009) found that in southern Spain, 'Marvel' and 'Nobel' could be used in combination with 'Fuerte' as pollinisers for 'Hass'.

Arpaia and Hofshi (2004) discussed different aspects regarding avocado pollination and about pollinisers and cross pollination. They mentioned three points to consider: 1. Synchrony of flowering cycle with 'Hass' flowering cycle; 2. multiple pollinisers give a better overlap; and 3. spatial placement of pollinisers. They also emphasised the importance of pollinating insects and the importance of bees. In their presentation they list 259 references of which most refer to pollination and fruit set. Very recently, Hormaza (2014) stated that the necessity of pollinisers is not that clear. Hormaza (2014) reports further that in a solid 'Hass' block planted next to a solid 'Fuerte' block, the first row of trees adjacent to the 'Fuerte' block had 40 to 50% of the fruit that resulted from cross pollination; in the second row, about 30 to 40%, and in the third row, 20 to 30% of the fruit resulted from cross pollination. Therefore proximity of the polliniser trees from the commercially important trees should also be under consideration.

All the above positive reports on pollinisers have been questioned again with the publication of a report by Garner *et al*. (2008) who found that outcrossing rates were not related to yield or alternate bearing and that outcrossing is not the primary factor affecting flower and fruit persistence and ultimately yield. The latter contradictory report again highlights the fact that the question about the effectiveness of pollinisers is not yet properly answered and requires more research. This study was therefore conducted to reinvestigate the problem under South African conditions, since most of the research was done in northern hemisphere countries like North America, Israel and Spain.

## MATERIALS AND METHODS

This study was done in Tzaneen on a five-year old 'Hass' orchard belonging to ZZ2, inter-planted with 'Zutano'. The study entails an in vitro pollination study as well as a field trial with encaged and open pollinated trees. Data trees were subjected to the same standard cultural practices as the remaining orchard trees. For the in vitro pollination study, three sets of six 'Hass' flowers, in early anthesis (opening in the female phase), were collected from 11 different trees. The flowers were placed in petri-dishes containing a gel made up of 15% agar, 10% sucrose and 0.5% boric acid and allowed to open. 'Zutano' and Ettinger' flowers that were open in the female phase were collected the previous afternoon and placed in petri-dishes containing the same medium. The flowers were kept at 25ºC during the night and they opened the next morning in the male phase, providing the pollen for in vitro pollination with the female 'Hass' flowers. One set of 'Hass' flowers were then pollinated with the 'Zutano' pollen and another set where pollinated with 'Ettinger' pollen and kept at 25ºC until the next morning, allowing the pollen to geminate and pollen tubes to grow down the style. The flowers were then fixed in a Carnoy solution (ethanol, chloroform and acetic acid in the ratio of 60:30:10). The six flowers from each Petri-dish were fixed in a separate container.

The third set of opened 'Hass' flowers were placed in a fridge at about 4ºC to prevent them from closing until 12:00 when they were returned to an ambient temperature to warm up before pollination. 'Hass' flowers at early anthesis in the male phase were collected to allow the anthers to open and provide pollen for the self-pollinating female 'Hass' flowers. The self-pollinated 'Hass' flowers were also kept at 25ºC, until they were also fixed in Carnoy solution the next afternoon.

The fixed, pollinated flowers were then taken to a laboratory at the University of Pretoria where the pistil of each flower was excised and placed in a small container with 20% alcohol. The excised pistils were then placed in 5M NaOH to soften, followed by rinsing in tap water, cleared in 30% Jik, rinsed again before being placed in Anelin Blue to stain the pollen tubes. Squash preparations were made of each pistil and viewed under a fluorescent microscope. For each pistil the number of pollen grains on the stigma were counted as well as the number of pollen tubes germinated, the number of pollen tubes growing down the style, the number of pollen tubes reaching the ovary and the number of pollen tubes entering the ovary, as can be seen in Figures 1, 2, 3 and 4.

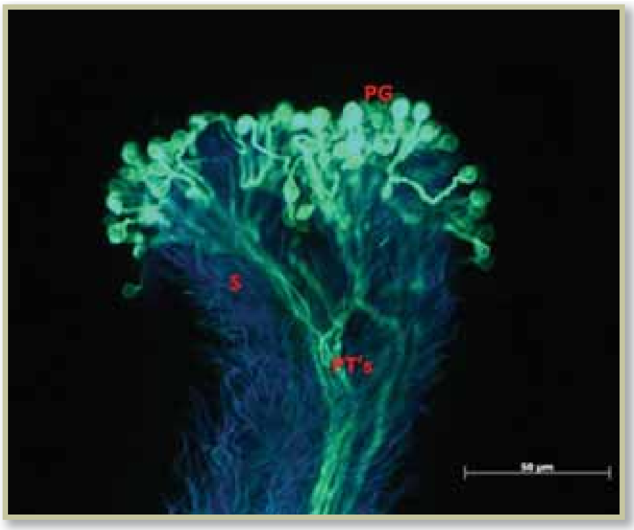


**Figure 1.** Pollen germinating on a stigma (PG: pollen grains; PT's: pollen tubes; S: Stigma).

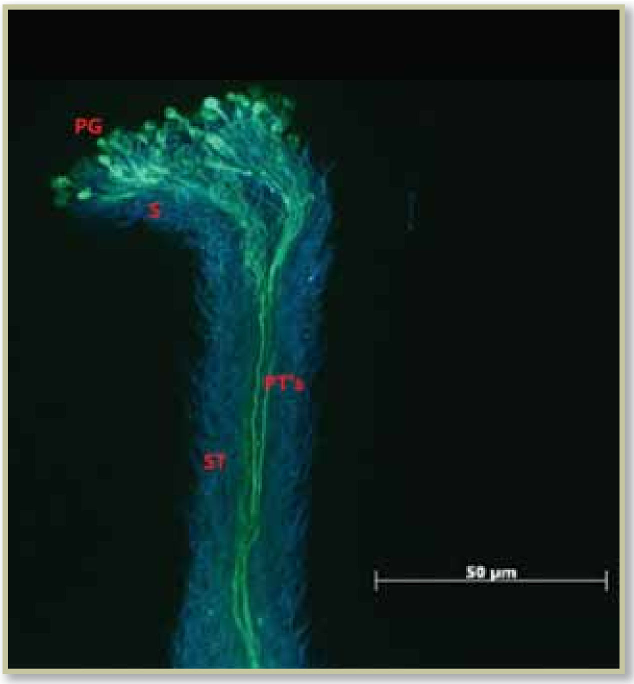


**Figure 2.** Pollen tubes in the style (PG: pollen grains; S: stigma; PT's: pollen tubes; ST: style).

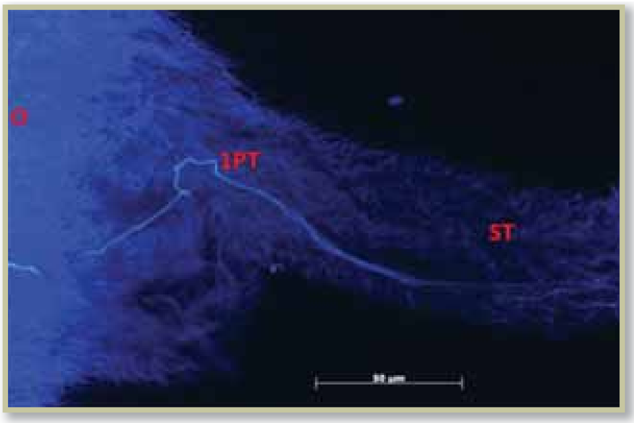


**Figure 3.** Pollen tube reaching the ovary (O: ovary; 1PT: one pollen tube; ST: style).

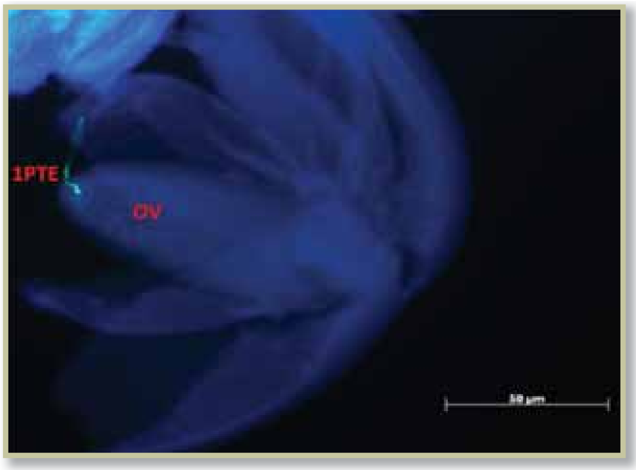


**Figure 4.** Pollen tube entering the ovule (1PTE: one pollen tube entering; OV: ovary).

For the field trial, four cages containing 'Hass' + 'Zutano' trees with bees, and four cages containing only 'Hass' trees with bees were used. For the open pollination part, trees in rows containing 'Hass' and 'Zutano' were used as well as rows with 'Hass' trees only. Two trees were used per treatment and ten flowering shoots per tree on four sides of the tree were marked for counting the fruit set. A trial run was done in 2014 with two nets and fruit counts were made during October 2014 and February 2015. During 2015 a full field experiment was done and the first fruit set count took place on the 13th of October, again on the 8th of December 2015 and lastly on the 16th of February 2016.

### Statistical analysis

The data was analysed using the statistical program GenStat® (Payne, 2014).

A generalised linear model (GLM) analysis was applied to the pollen data for 2015, with a logarithmic link function used to test for differences among the three treatment effects. These treatment effects include HxH, HxZ and HxE. Means were compared with Fisher's protected least significant test at the 5% level.

For 2015, REML (or linear mixed model) analysis was applied to the total number of fruit set on twenty shoots (count data, two trees), to test for differences between the effects of four treatments and fruit set counted on the four sides of the trees, as well as the treatment by side interaction. A pseudo split-plot analysis was used with treatments as whole plots and sides of a tree as split-plots. Means were compared with Fisher's protected least significant test at the 1% level as residuals after analysis were normal, but with heterogeneous treatment variances.

## RESULTS AND DISCUSSION

### In vitro pollination trial

The results of the 2014 experiment are presented in Figure 5 and the results of the 2015 experiment, in Figure 6.

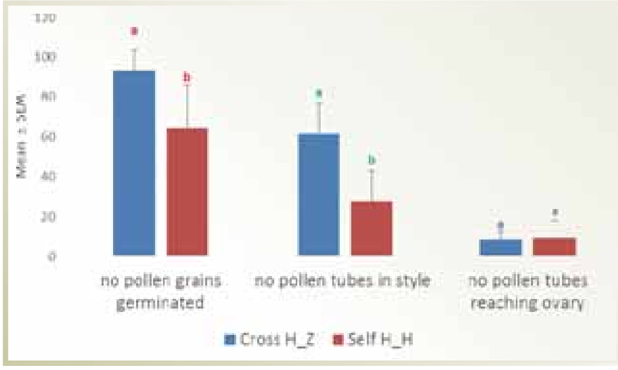


**Figure 5.** Pollen performance for in vitro pollinated 'Hass' x 'Zutano' and 'Hass' x 'Hass' flowers during 2014.

As depicted in Figure 5, pollen germination and pollen tube growth did better in the cross than in the self-pollinated treatment. However, there was no significant difference between 'Hass' and 'Zutano' pollen tubes that actually reached the ovary.

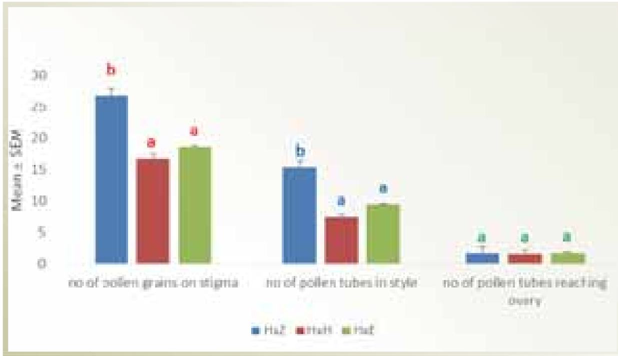


**Figure 6.** Pollen performance after in vitro pollinated 'Hass' x 'Zutano', 'Hass' x 'Hass' and 'Hass' x 'Ettinger' flowers during July 2015.

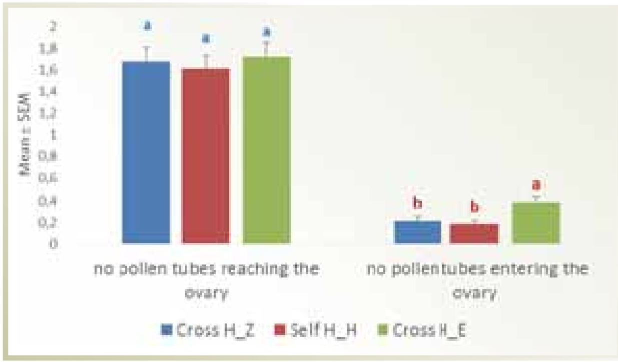


**Figure 7.** Pollen tubes reaching the ovary and ovule after in vitro pollinated 'Hass' x 'Zutano', 'Hass' x 'Hass' and 'Hass' x 'Ettinger' flowers during July 2015.

According to Figure 6, the number of 'Zutano' pollen on the stigma and the number of pollen tubes in the style were significantly more compared to 'Ettinger' and 'Hass.' However, similar to 2014, there was no difference in the number of pollen tubes that reached the ovary (Fig. 7). Due to the fact that the avocado ovary contains only a single ovule and competition of pollen tubes in the style, only one pollen tube usually reaches the ovary and enters the ovule. The entrance of the pollen tube into the ovule is an important criterion and was also used by Sedgely (1997a) as a measure for effective pollination. It is therefore clear that effective pollination was only achieved by 'Ettinger' pollen. Guil and Gaziet (1992) also found that better yields were obtained in 'Hass' orchards planted next to 'Ettinger', while Degani *et al*. (2004) regarded 'Ettinger' as a potent polliniser for 'Hass'.

**Field trial**

The results of the 2014 trial run are presented in Figure 8 and the results of the 2015 full experiment in Figure 9.


**Figure 8.** Fruit set counts for open field trial run 2014, based on total figures.

Only one replication per treatment was done and therefore no statistical analysis was possible. Figure 8 shows that during both counts, fruit set on 'Hass' trees inside the nets, and on trees in open rows containing 'Hass' and 'Zutano', were higher compared to nets and open trees with 'Hass' only.


**Figure 9.** Average fruit per 40 shoots per tree in four different treatments. (October 2015 and December 2015).

Contrary to Figure 8, in Figure 9 fruit set on 'Hass' trees together with 'Zutano' trees in nets was not higher than only on 'Hass' trees in nets. On open trees, fruit set on 'Hass' trees in rows containing both 'Hass' and 'Zutano' was higher. The proximity of the polliniser therefore seems important which agrees with Hormoza (2014) who also emphasised the importance of the proximity of the polliniser. Bender (2014) mentioned that commercial farmers noticed 'Hass' trees planted near 'Zutano' had a higher yield and that the effect is greater when the 'Hass' tree is only one tree away from the 'Zutano'. He also stated that results could vary from year to year and that it is not to say that there will be a higher fruit set every year from the 'Hass' x 'Zutano' combination. It could probably depend on synchronization of their flowering cycles, which can be affected by many external factors, like temperature or time of harvest. It could also be that some trees might be in alternate bearing cycles and could have a poor yield irrespective of whether the flowers are self or cross pollinated.

**Time-lapse photography**

Pictures of flowers on overlapping 'Hass' and 'Zutano' shoots were taken at 15 min. intervals. The date, time of the day and the temperature are recorded on each photo. Figure 10, taken 17 August 2015, shows that the 'Zutano' trees in the nets had finished flowering and only flowers closed after the male phase remain. The result is that there was no more 'Zutano' pollen left for cross pollinating the 'Hass' flowers. Therefore self-pollination took place in all nets resulting in similar results (Fig. 9). Open 'Hass' trees were exposed to more 'Zutano' trees which could have supplied cross pollen. Synchronisation is therefore a major problem which should be addressed in future. Arpaia and Hofshi (2004) also mentioned the importance of 'Hass' and the pollinator's synchronised flowering.

Figure 11 A and B (along with pictures of the same flowers taken the previous day and earlier in the morning – not shown) show the influence of temperature on the opening and closing of the flowers. At 07:00 in the morning, male phase flowers of 'Zutano' (B cultivar), were supposed to still be in the second bud stage, but as depicted in the picture (Fig. 11 A) and previous pictures, they opened the previous day (10 August), remained open during the night and only closed late afternoon on 11 August. They had no more pollen to pollinate the 'Hass' flowers that opened after 12:00 (Fig. 11 B) when they were supposed to be closing. Pollination under these conditions only allows self-pollination of 'Hass' flowers due to possible overlapping male and female phases.


**Figure 10.** Time-lapse picture of 'Zutano' (closed and finished flowering) and the open 'Hass' flowers on overlapping shoots indicating that 'Zutano' pollen would not be available for 'Hass' pollination.


**Figure 11.** Time-lapse pictures.
A – taken 11 August 2015 at 07:00, temperature 17ºC; B – taken same day at 13:00, temperature 25ºC ( Z: 'Zutano' flowers; H: 'Hass' flowers).

The above results are supported by Ish-Am (1993) who found that the flower opening times are strongly correlated with temperatures and that for every 1ºC drop in temperature, the flowering time is delayed by 30 to 60 minutes. By 17 August the temperatures were more favourable for the opening and closing of the flowers, but at that time there was no more 'Zutano' pollen available.

**CONCLUSION**

The in vitro pollination study showed that, although there was more 'Zutano' pollen grains on the stigmas and pollen tubes in the styles of 'Hass' pistils, there was no difference between the number of 'Zutano' and 'Hass' pollen tubes entering the ovules. Significantly more 'Ettinger' pollen tubes entered the ovules, indicating the possibility of using 'Ettinger' as a polliniser for 'Hass'. In the field trial-run, during 2014 the caged 'Hass' trees in combination with 'Zutano' provided higher fruit set than caged 'Hass' trees. During the 2015 field trial, however, there was no difference in fruit set on 'Hass' trees in cages inter-planted with 'Zutano' and 'Hass' trees only in cages. The latter results are somewhat skewed due to the early flowering of 'Zutano', causing a lack of 'Zutano' pollen to pollinate the 'Hass' trees. In the open field, fruit set on 'Hass' trees inter-planted with 'Zutano' was indeed higher than on 'Hass' trees, which were not in direct contact with 'Zutano'. Manipulating 'Zutano' trees to flower together with 'Hass' might still qualify 'Zutano' as a polliniser for 'Hass'. Time-lapse photography contributed to visual evidence for demonstrating the serious effect of low temperatures on the unsynchronised opening and closing of A and B type flowers during the female and male phases.

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