Finding the best pollinizer for 'Hass' avocado and the effect of bees as pollinators

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ABSTRACT

The study was done over a period of three seasons. The results of the first two seasons were published in the two previous SAAGA yearbooks and this report will therefore mainly cover the results of the 2017-2018 season which entailed an *in-vitro* pollination study and a field trial. The *in vitro* tests were conducted in September 2017. 'Hass' female phase flowers were pollinated using pollen from eight 'new' B-cultivars as well as from 'Bacon', 'Hass' and 'Zutano'. In addition, 'Maluma Hass' female phase flowers were pollinated with 'Bacon', 'Ettinger' and 'Zutano' pollen. The effect of temperature and time on pollen tube growth was done by using 'Ettinger', 'Hass' and 'Zutano' pollen on 'Hass' female phase flowers.

The *in vitro* results were based on the number of pollen tubes entering the ovary and ovule. Data showed that 'new' cultivars 15 and 17 could qualify as pollinizers for 'Hass'. 'Bacon' and 'Ettinger' showed to be better pollinizers for 'Maluma Hass' compared to 'Zutano'. For all three pollen donors, 'Hass', 'Bacon' and 'Zutano', tempertures of 25-27 ºC were found to be optimal for pollen tube growth. At temperatures above 35 ºC, pollen tube growth was negatively affected. At optimal temperatures, pollen tubes can reach the ovule within 15 hours after pollination.

The field trial included fruit set determination on a) caged trees with bees ('Hass' x 'Hass' and 'Hass' x 'Zutano'), b) open pollinated trees and c) caged trees without bees ('Hass' x 'Hass' and 'Hass' x 'Zutano'). Fruit set counts were taken in October 2017 and again in January 2018. Further counts will be done in April/May 2018 during harvest time. 'Hass' x 'Zutano' in cages (nets) gave a slightly higher fruit set, but maybe not significantly enough to justify inter-planting 'Zutano' with 'Hass' according to the 2017-2018 season. Bees in the nets increased fruit set dramatically.

INTRODUCTION

Bender (2002) gave a short description of the origin of 'Hass' and the cultivar characteristics. He found that the 'Hass' cultivar was selected by Rudolph Hass in the 1920s and originated as a chance seedling variety. He also mentioned that 'Hass' has a very long harvest season and is known to be the top quality avocado available, but it is also known to have poor fruit set in some locations. Growers are continuously looking for higher yields and believe that it should be possible to increase the present yields by finding the best pollinizer. 'Hass' being an A-type cultivar, the pollinizer must be a B-type cultivar.

The benefits of cross-pollination against self-pollination for increasing fruit set and yield was already reported by Clark and Clark (1923, 1926). Since then many attempts have been made to 'prove' that avocados are out-breeders and that pollinizers are essential for good fruit set. Degani *et al*. (1997) and Degani and Goldring (1989) showed that abscission of avocado fruitlets and fruit characteristics were greatly influenced by the pollen parent. Garner *et al*. (2008) had contradictory results and found that outcrossing is not the primary factor affecting fruit persistence and ultimately yield. This report highlights the fact that the question about the effectiveness of pollinizers is not yet properly answered and requires more research. This study was therefore conducted to re-investigate the problem under South African conditions.

A good pollinizer without pollinators is of no use and Clark (1923) already reported the importance of bees as pollinators. Peterson (1955) concluded that large dipterous and hymenopterous insects are necessary for pollinating avocados. Ish-Am and Eisicowitch (1993 and 1988) wrote several articles that implied the importance of bees as pollinators for avocado. According to an article written by Afik (2006), bees have to collect pollen as well as nectar in order for them to visit the male and also the female flowers. If they collect only pollen, they will not visit the female flowering stages and pollination cannot occur.

According to Dixon (2004), eight equivalent hives should be used per hectare for trees six to ten meters high. He also mentioned that no less than four hives should be used per hectare. About 20-30 bees need to be working on one tree in order to attain good enough pollination. Weather conditions such as cold (<17 ºC), wet, windy and very cloudy days will reduce bee activity and cause a reduction in pollination.

Arpaia and Hofshi (2004) discussed different aspects regarding avocado pollination and about pollinizers and cross pollination. They mentioned three points to consider: 1. Synchrony of flowering cycle with 'Hass' flowering cycle, 2. Multiple pollinizers give a better overlap, and 3. Spatial placement of pollinizers. They also emphasized the importance of pollinating insects and the importance of bees.

In his report on avocado pollination, Dixon (2004) wrote that flower opening is highly dependent on temperature and this can affect the overlap of the male pollinizer flowers with the female 'Hass' flowers. Such temperatures will have a great influence on cross pollination success. Bender (2002) also pointed out that low temperatures can cause delayed and irregular flowering. Robbertse *et al*. (1998) also found that pollen tube development down the style can be greatly affected by temperature.

There are critical stages in the process that lead to fruit set and fruit development. Bender (2002) gave a short description of the processes that lead to fruit set. First pollen has to reach the pistil, it then has to germinate and form a pollen tube. The pollen tube has to move successfully down the style to reach the ovule. The pollen tube should then deliver the sperm cells to the egg cell and central cell inside the ovule to accomplish successful fertilization. The ovary will form the flesh of the avocado fruit and the ovule will form the seed with the embryo. The seed provide plant growth regulators that will aid the development of the fruit according to Bender (2002). If one of these stages are interrupted or disturbed, fruit formation and development will be halted.

Dixon (2004) stated that competition between pollen tubes in the style leads to stronger seed. This means that the higher the amount of pollen tubes moving down the style, the higher the competition between pollen tubes which should allow the strongest pollen tube to reach the ovary. The fruit that develop from that pollen tube that fertilized the ovary will be more vigorous and less prone to fall. Degani (1989) stated that more vigorous fruit can be obtained by improving the embryo and endosperm and also mentioned that this can be done by introducing an improved pollen parent with better a competitive ability.

Garner *et al.* (2008) explained in their report on fruit abscission and alternate bearing that even if fruit set were successful, critical abscission stages will cause a lot of the fruit to drop before maturity is reached. Garner *et al*. (2008) concluded that fruit drop can be more severe with adverse climatic conditions, poorly applied cultural practices and poor tree health. Most of the research mentioned in this introduction was done either in the United States or in European countries and it was regarded necessary to repeat some of the work under South African conditions. The aim of this study was therefore to study the effectiveness of different pollinizers for 'Hass' and the role of bees in pollination of avocados under South African conditions.

The project is running for the third season. The results of the 2015-2016 and 2016-2017 seasons were presented in the previous two SAAGA yearbooks. The results presented in this report are mainly from the 2017-2018 season.

MATERIALS AND METHODS

The study was done in orchards of ZZ2. It comprised of two parts, namely (A) *in vitro* pollination trials and (B) field trials. Data trees were subjected to the same standard cultural practices as the remaining orchard trees.

In vitro pollination

Four different tests were performed for the *in vitro* trials. a) The pollen of eight 'new' B-cultivars were used to pollinate 'Hass' female phase flowers; b) Pollinated 'Hass' flowers were exposed to different temperatures; c) Pollinated 'Hass' flowers were exposed to optimal temperature for different durations; d) 'Maluma Hass' flowers were pollinated with pollen from three different pollen donors.

In all four experiments, 'Hass' or 'Maluma Hass' flowers in early female anthesis were collected from four randomly selected trees in the respective orchards. The collected flowers were placed in petri-dishes containing a gel made up of 5% agar, 10% sucrose and 0.05% boric acid and allowed to open. B-cultivar flowers that were open in the female phase were collected the previous afternoon and placed in petri-dishes containing the same medium. The B-cultivar flowers were kept at 25 ºC during the night and they opened the next morning in the male phase, providing the pollen for *in vitro* pollination with the female phase A-cultivar flowers. The A-cultivar flowers were then pollinated with the respective B-cultivar pollen, and then they were subjected to the different treatments after which they were fixed in a Carnoy solution (ethanol, chloroform and acetic acid at a ratio of 60:30:10). Fixation of the flowers from each Petri-dish was done in separate glass test tubes and marked according to treatment and date of pollination.

The fixed flowers were taken to a laboratory at the University of Pretoria where the pistils were excised and the five pistils from each Petri-dish placed in a small container containing 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 Aniline Blue for staining the pollen tubes. Thereafter, the samples were kept in the dark until further treatment. 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 moving down the style, the number of pollen tubes reaching the ovary and the number of pollen tubes entering the ovules. The experiment took place on the 23-24th of August 2017 and was repeated on the 24-25th of August.

'New' B-cultivar pollen on 'Hass'

In the afternoon of day one, four Petri-dish sets of ten flowers from each of the eight 'new' B-cultivars were collected in the female phase and their pollen used the following day to pollinate the 'Hass' female flowers. On day two, 'Hass' (A-cultivar) flowers, in early female phase anthesis, were collected in order to have four petri-dishes, each with five 'Hass' flowers available for each of the eight B-cultivars. Further procedures as described above, ware then followed.

Effect of temperature on pollen tube growth

In the afternoon of day one, 4 petri-dishes x 10 flowers from 'Bacon' and 'Zutano' trees were collected in the female phase and their pollen were then used the following day to pollinate the 'Hass' female flowers. In the morning of day two, 4 petri-dishes x 5 flowers in early female phase were collected from 9 'Hass' trees in total, in order to have 3 trees x 4 petri-dishes with five flowers in each available for the two B-cultivars ('Bacon', 'Zutano') as well as for 'Hass' (A-cultivar). This means that one set of 'Hass' flowers needed to be placed in a fridge at about 4 ºC to prevent them from closing until 12:00 when they were returned to ambient temperature in order to warm up before pollination. 'Hass' flowers at early male phase anthesis were collected and the anthers allowed to open for providing pollen for self-pollinating the female 'Hass' flowers that were kept in the fridge.

The pollinated 'Hass' flowers were separated into three groups. One group (four petri-dishes per cultivar) was kept at 5-8 ºC for 24 hours before they were fixed. The second group was kept at 25-27 ºC and the third group at 35-40 ºC.

Effect of time on pollen tube time growth

On day one, 4 Petri-dish sets x 10 flowers from each of about 4 randomly selected 'Ettinger' and 'Zutano' trees were collected in the female phase and their pollen were then used the following day to pollinate the 'Hass' female flowers. On day two, 4 petri-dishes x 5 flowers of early female phase 'Hass' flowers were collected from 4 randomly selected 'Hass' trees for every pollen donor. Again, one set of 'Hass' flowers were placed in a fridge at about 4 ºC to prevent them from closing, until 12:00 when they were returned to ambient temperature in order to warm up before pollination. 'Hass' flowers at early male phase anthesis were collected and the anthers were allowed to open to provide pollen for self-pollinating the female 'Hass' flowers which were kept in the fridge.

The pollinated 'Hass' flowers were separated into four groups. One group (four petri-dishes per cultivar from four different trees) were fixed 5 hours after pollination, the second group after 15 hours, the third group after 20 hours and the last group after 25 hours.

Pollinizers for 'Maluma Hass'

On day one, 4 petri-dishes x 10 flowers from 'Ettinger', 'Bacon' and 'Zutano' were collected in the female phase and their pollen used the following day to pollinate the 'Hass' female flowers. On day two, 'Maluma Hass' flowers were collected in early female phase anthesis in order to have three petri-dishes with each five 'Maluma Hass' flowers for each of the 3 B-cultivars. Pollinated 'Maluma Hass' flowers were then incubated at 25 ºC for 24 hours before they were fixed.

Field trial

The trial was conducted in the Tzaneen area on an eight-year old 'Hass' orchard inter-planted with 'Zutano' trees, belonging to ZZ2. Four cages (covered with nets) containing both 'Hass' and 'Zutano' trees with bees and four cages containing only 'Hass' trees with bees were used. For open pollination, trees in rows containing both 'Hass' and 'Zutano' trees were used as well as rows containing only 'Hass' trees. Two cages were also set up with no bees inside, one net with 'Hass' trees only and the other with 'Hass' and 'Zutano'. All cages contained eight trees each. Two trees were randomly selected per treatment where fifteen shoots per tree on four sides of the tree (N, E, S and W) were tagged for counting fruit set.

Fruit set counts were made in October 2017 and again in January 2018. Final yield per treatment will be done during April/May 2018.

Pollen trap

A pollen trap was installed in two different orchards on the farm to determine whether bees do collect avocado pollen.

Statistical analysis

The data were analyzed using the statistical program GenStat® (Payne, 2014). A generalized linear model (GLM) analysis was applied to the *in vitro* pollination trial with a logarithmic link function, to test for differences between the treatment effects. Means were compared with Fisher's protected least significant test at the 5% level. REML, or linear mixed model, analysis was applied to the total number of fruit set. 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

***In vitro* pollination trial**

The results for the 2017-2018 season are presented in Figures 1 to 8.

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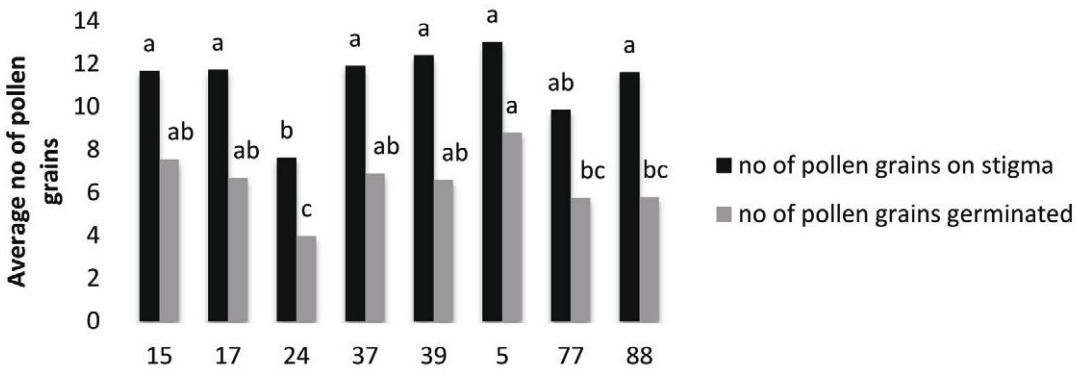

Figure 1: Comparison of pollen performance of eight 'new' avocado cultivars on 'Hass' pistils.

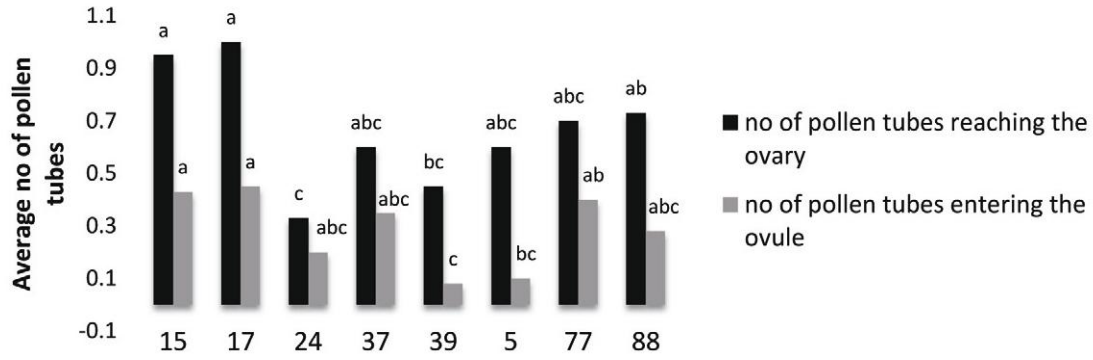

Figure 2: Comparison of pollen tube growth of eight 'new' avocado cultivars in 'Hass' pistils.

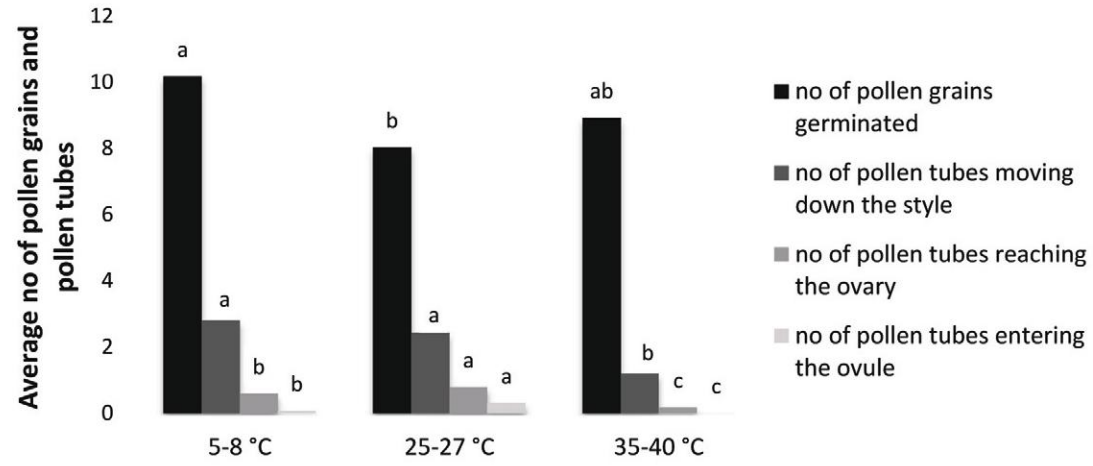

Figure 3: Combined average results of pollen performance of 'Bacon', 'Hass' and 'Zutano' pollen on 'Hass' pistils at 5-8 ºC, 25-27 ºC and 35-40 ºC.

'New' B-cultivars

Very little pollen was available in the anthers of cultivar 24 which could explain the low performance. The other cultivars had sufficient amounts of pollen and the germination was satisfactory.

According to Figure 2, pollen tubes of cultivars 15 and 17 were more successful in reaching the ovary and also in entering the ovule, meaning that there is a higher chance for fertilization compared to the other cultivars and they may therefore qualify as possible pollinizers for 'Hass'.

Effect of temperature on pollen tube growth

According to Figure 3, there was no big difference in pollen germination between low (5-8 ºC), average (25-27 ºC) and high (35-40 ºC) temperatures. For some unknown reason, pollen germination at the average temperatures (25-27 ºC) was slightly lower compared to more extreme temperatures. Low and high temperatures did have an effect on pollen tubes reaching and entering the ovule. The best results in this regard were obtained at 25-27 ºC which agrees with Sedgley and Annells (1981) who

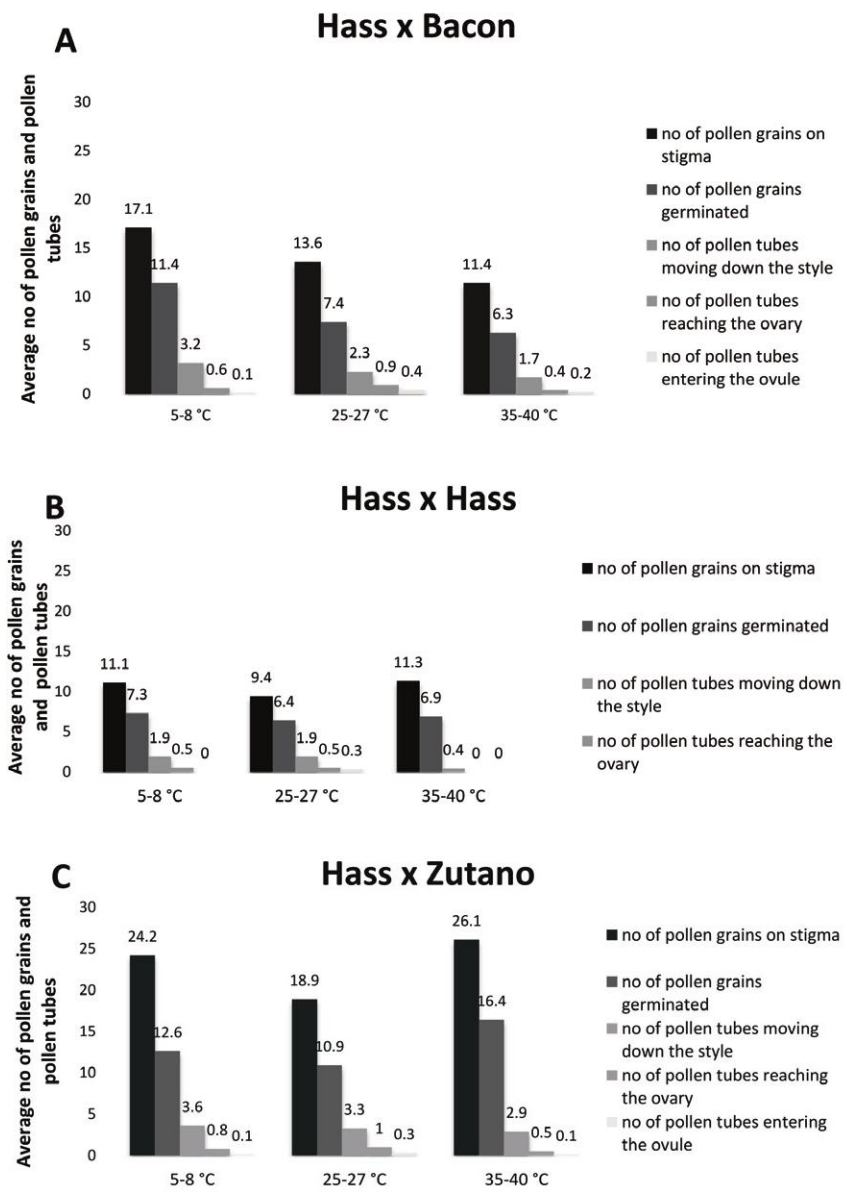

Figure 4: Comparison of pollen performance of three pollen donors ('Bacon', 'Hass' and 'Zutano') at three different temperature regimes on 'Hass' pistils. A = 'Bacon' on 'Hass'. B = 'Hass' on 'Hass' and C = 'Zutano' on 'Hass'.

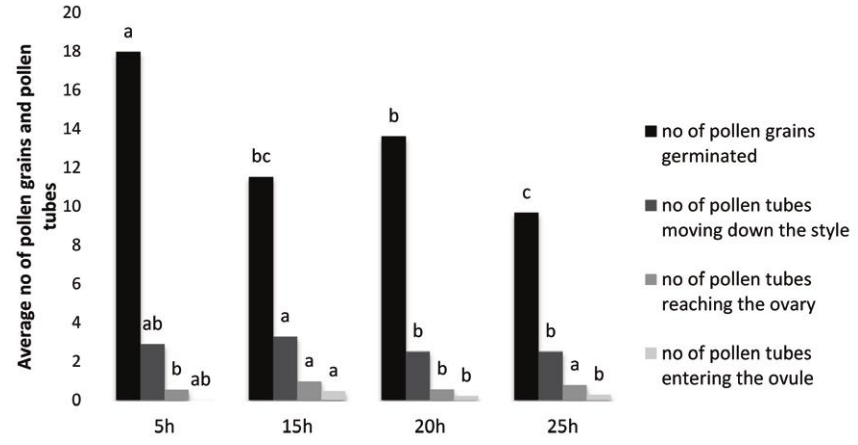

Figure 5: Combined average pollen performance results of three pollen donors ('Ettinger', 'Hass' and 'Zutano') at 5 hours, 15 hours, 20 hours and 25 hours after pollination.

found 25/20 ºC to be optimal for pollen tube growth and embryo development of avocado. Figure 4 gives a comparison of the pollen performance of the three pollen donors.

From the results in Figure 4 it is clear the avocado pollen germination and pollen tube growth is not very sensitive to low or high temperatures. 'Hass' pollen on 'Hass' stigmas (Fig. 4B) gave the poorest results.

The effect of time on pollen tube growth

As seen in Figure 5, most pollen grains had already germinated five hours after pollination, although ovule penetration only occurred 15 hours after pollination. The variation in pollen performance was probably due the variation in pollen quality.

Pollinizers for 'Maluma Hass'

No significant differences were found between the pollen performances of the three pollen donors apart from the penetration of pollen tubes into the ovules, where 'Zutano' had the lowest figure in spite of the highest number of pollen grains on the stigma.

Figures 7 and 8 again show that 'Zutano' had the highest amount of pollen available, a higher number of pollen grains that germinated and more pollen tubes that moved down the 'Hass' style, although entrance of pollen tubes into the ovules seems to be a problem. 'Ettinger' and 'Hass' have the same number of pollen grains on the stigma. The same number of pollen tubes was found in the 'Hass' style when pollinated with either 'Hass' or 'Ettinger' pollen. There were no significant differences in the number of pollen tubes reaching or entering the 'Hass' ovary when pollination of 'Hass' flowers was done with either 'Zutano', 'Ettinger' or 'Hass' pollen. The 2017 *in vitro* pollination results agrees with Sedgley (1979) that the female parent (pistil) could have had more control over pollen performance.

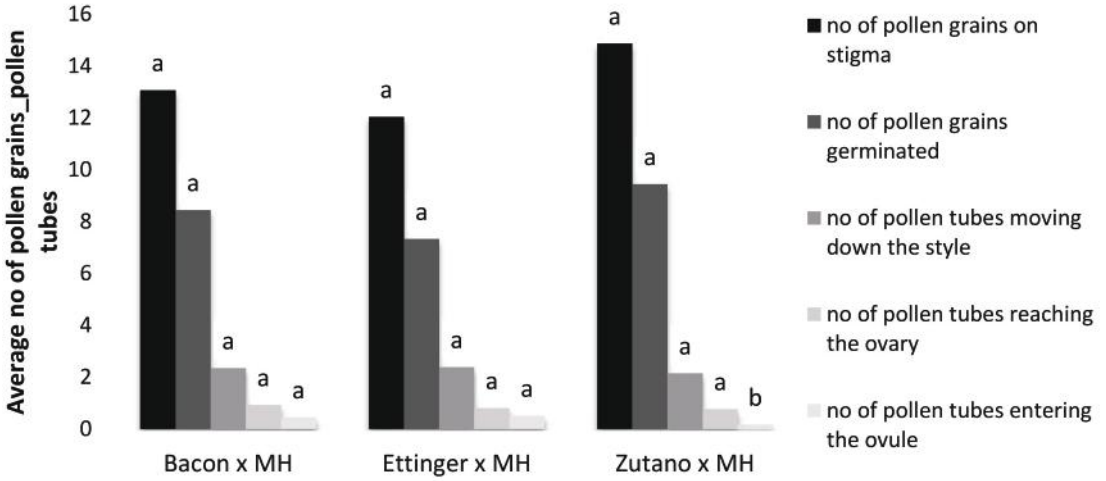

Figure 6: Performance of pollen of three pollen donors ('Bacon', 'Ettinger' and 'Zutano') on 'Maluma Hass' pistils.

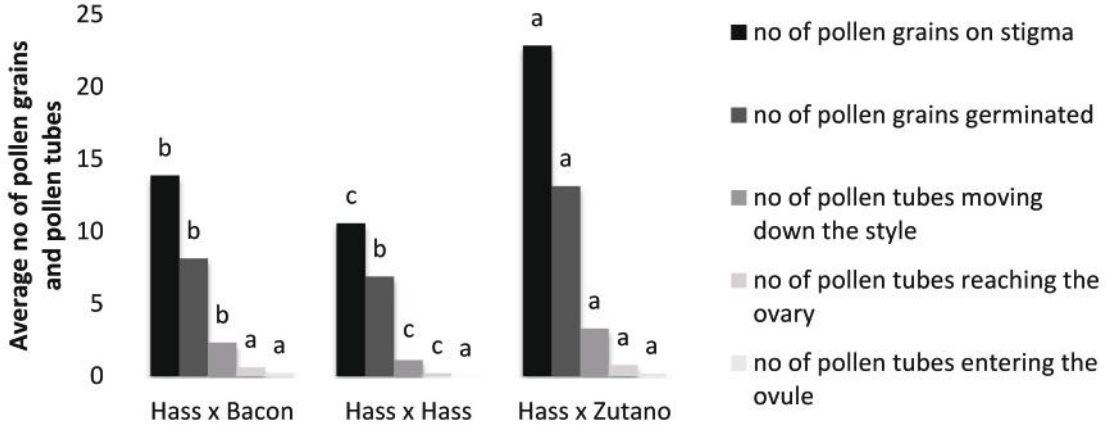

Figure 7: Combined data for *in vitro* pollen performance of three pollen donors at three different temperature regimes, 5-8 ºC, 25-27 ºC and 35-40 ºC.

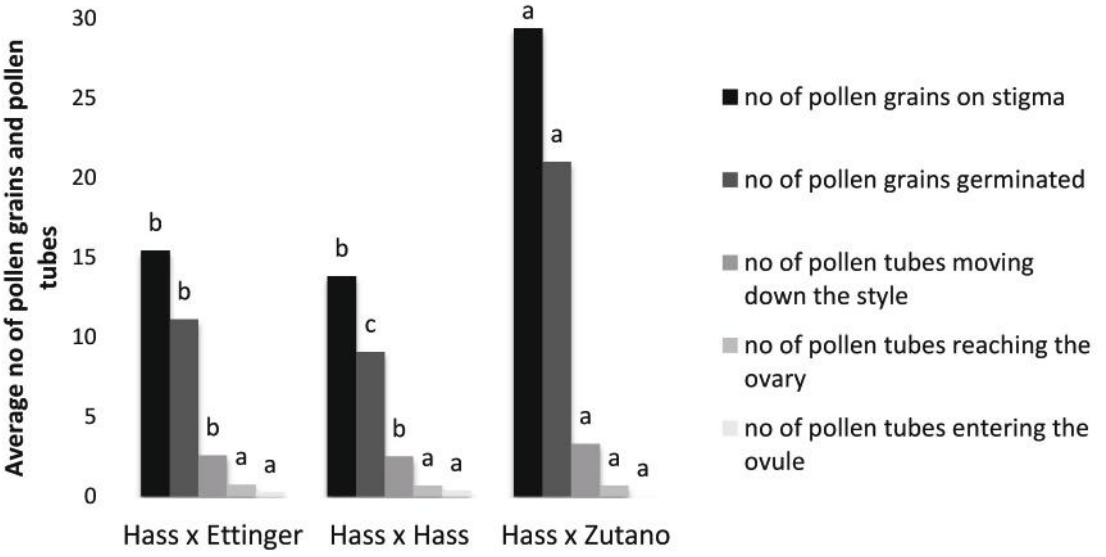

Figure 8: Combined data for pollen performance of three pollen donors at four different periods (5, 15, 20 and 25 hours) after pollination.

Field trial

The results for the 2017-2018 season are given in Figures 9 to 13.

The first fruit set count was done in October 2017 and the second fruit count in January 2018 (Fig. 9). During the first count, 'Hass' x 'Zutano' trees, both inside the cages as well as open pollinated trees, had a higher fruit set compared to pure 'Hass' x 'Hass' treatments. During the second count there was very little differences between treatments, except for the open pollinated 'Hass' x 'Hass' treatment that had the lowest fruit set. Fruit drop, indicated by the differences between the first and second fruit counts, was higher in the H x Z treatments than in the H x H treatments.

From Figure 10 it is clear that pollinizer 'Zutano' performed better regards to number of fruit counted on the branches. It also shows that pollinators are extremely essential for fruit set in avocado and that honeybees are effective pollinators. The fruit set in the 'Hass' x 'Zutano' cage without bees must have been caused by some pollinators the managed to get into the netted cage.

The importance of sunlight in avocado pollination is demonstrated in Figure 11.

Fruit set on the eastern side of the trees was significantly higher than on the other sides, followed by the southern and northern sides, while fruit set on the western side was the lowest. It can be argued that the reason of the higher fruit set on the east side, could have been the result of the amount of direct sunshine received by the eastern side of the tree when the 'Hass' flowers were in the female phase. The trees in the rows of the experimental orchard were orientated north-south. The field trial started in August 2017 when the sun was rising slightly north of east and when the 'Hass' flowers started to open in the female phase at about 09:00, the eastern side of the trees received the full amount of sunshine. The same applied to the 'Zutano' (pollinizer). The honeybees seemed to be very active and even more active on the open and well lit eastern sides of the trees, which could have resulted in better pollination and better fruit set compared to the 'shaded' western side. Also, on the southern and northern sides of the experimental trees, there was an overlap of branches of these adjacent trees which seemed to be less accessible to the bees.

Harvesting data

The harvesting data for the 2017-2018 season is not yet available, but since the data of the 2016-2017 season was not available for the previous year's report, (Bezuidenhout, Du Toit & Robbertse, 2017), it is now presented in Figures 12 and 13.

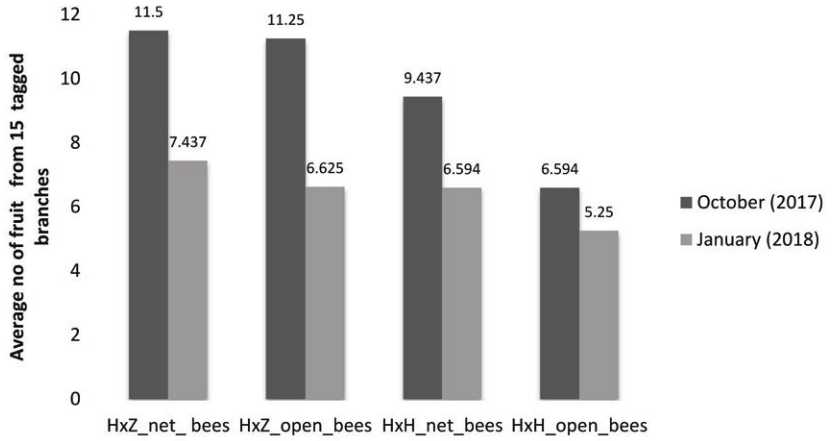

Figure 9: Average fruit set count taken in October 2017 and January 2018 for 15 shoots in four different treatments.

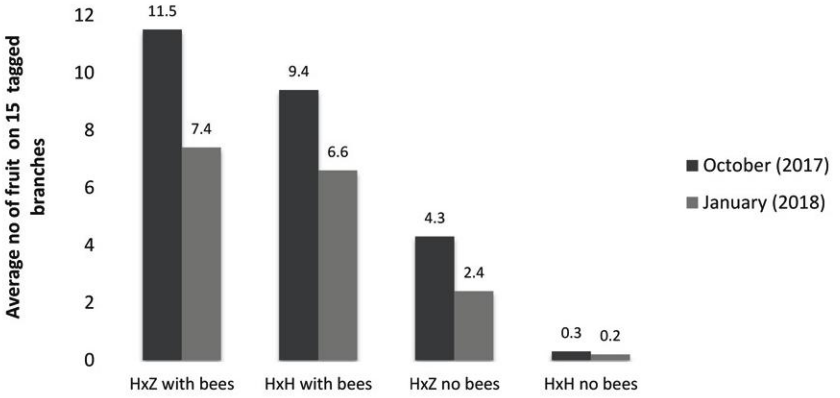

Figure 10: Average fruit counts in cages with bees and cages without bees taken in October 2017 and January 2018.

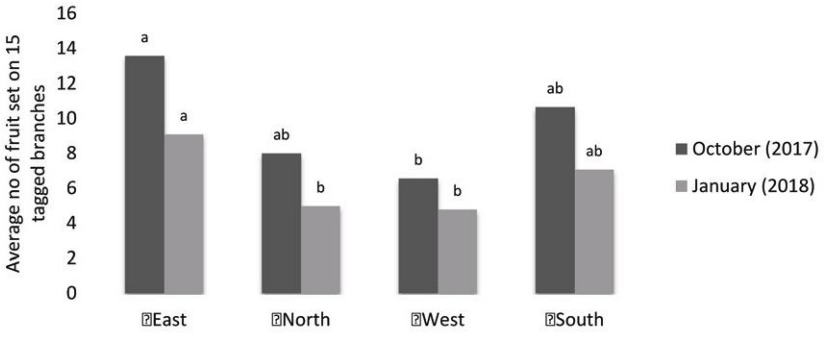

Figure 11: Average fruit set count taken in October 2017 and January 2018 from the Eastern, Northern, Western and Southern side of each tree.

The concentration of bees inside the nets clearly improved fruit set compared to the open pollinated trees which again emphasizes the importance of bees as pollinators for avocado. During the three seasons when this project was running, flowering synchronization between 'Hass' and 'Zutano' was poor. In spite of this, 'Zutano' trees inter-planted between the 'Hass' trees in the orchard, did improve fruit yield inside the cages (with bees) as well as on the open pollinated trees.

Pollen trap

During the 2016-2017 season, pollen kernels captured in the pollen trap showed that bees placed in the avocado orchard, travel long distances to collect macadamia pollen. About 60% of the kernels consisted purely of macadamia pollen with only 40% avocado pollen kernels. The procedure was repeated this season with an improvement of 56% avocado pollen and only 36% macadamia pollen, however, there were also kernels consisting of Compositae pollen (8%).

DISCUSSION

For interpreting the results of the *in vitro* pollination experiments, the success rate of pollen tubes reaching and ultimately entering the ovule was the most important criterion in deciding whether a B-cultivar could be a good pollinizer or not, when only considering fertilization success and will be applied in further discussions.

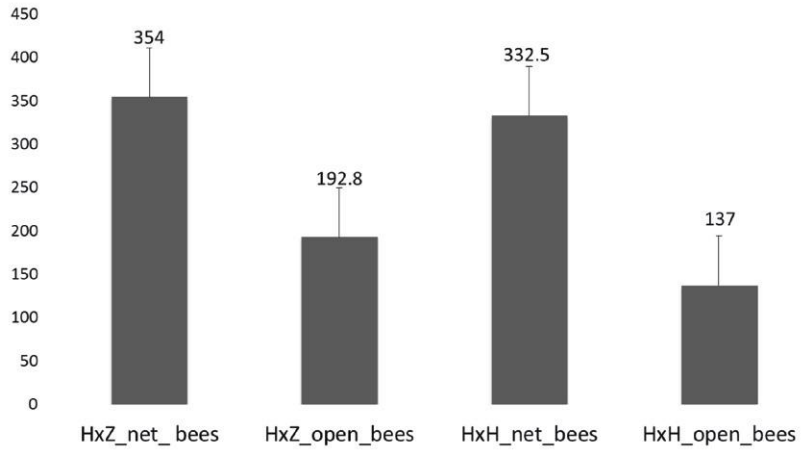

Figure 12: Final harvesting figures of the 2016-2017 season presented as number of fruit per tree.

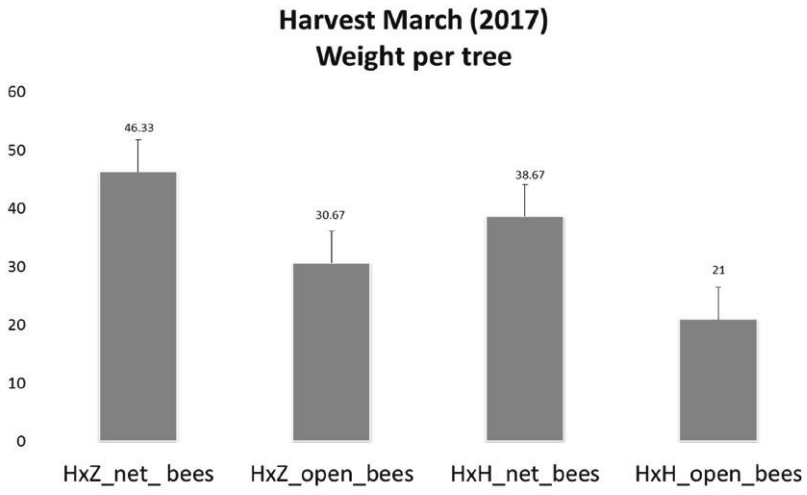

Figure 13: Final harvesting figures for the 2016-2017 season presented as kg per tree.

***In vitro* pollination trial**

Success rate of pollen tubes reaching and ultimately entering the ovary were the most important criteria in deciding whether a B-cultivar could be a good pollinizer or not. It is not possible to observe the fertilization process under a microscope and for the purpose of this project, it is assumed that fertilization is possible when the pollen tube enters the ovule.

According to *in vitro* data, cultivars 15 and 17 gave the best results regarding pollen tubes entering the ovule and may qualify as pollinizers for 'Hass' and cultivars 37, 88 and 77 could also be considered. Based on pollen tube penetration of ovules, 'Bacon' and 'Ettinger' may be suitable pollinizers for 'Maluma Hass', as they were slightly better than 'Zutano'.

Pollen of the three B-cultivars tested, performed almost equally at the three temperate regimes and most pollen tubes entered the ovules at 25-27 ºC (Fig. 3). Loupassaki *et al*. (1995) also found that 'Ettinger', 'Bacon', 'Zutano' and 'Hass' pollen germinate the best at 25 ºC. Similar results were also obtained by Loupassaki *et al.* (1997), Sedgley (1987), Sedgley and Annells (1981) and Robbertse *et al*. (1998). On the other hand, extreme high temperatures decreased pollen tube performance by decreasing the movement of pollen tubes towards the ovary and their ability to enter the ovary. Schaffer *et al*. (2013) found that pollen tube development towards the ovary diminish at 33 ºC which is in agreement with our results in Figure 3.

At more extreme temperatures, 'Hass' pollen and pollen tube performance were lower than those of 'Zutano' and 'Bacon'. 'Zutano' pollen seems to cope with temperatures above 30 ºC. Hofshi (1999) stated that stressful conditions like heat or cold can enhance the effect of cross-pollination on 'Hass'. Temperature can also affect ovule development (Schaffer *et al*., 2013). At different temperatures the ovule viability might be affected, which can have a great impact on fertilization success even though the pollen tube entered the ovule (Schaffer *et al*., 2013).

At 5 hours after pollination most of the pollen had germinated, but pollen tubes entering the ovules were only observed 15 hours after pollination. Schaffer *et al.* (2013) stated that pollen tubes were able to travel the length of the style within one to two hours, although penetration into the ovule occurred at about 18 to 24 hours after pollination, which is consistent with our findings. According to Schaffer *et al.* (2013), the number of pollen grains on the stigma does not limit fertilization, but Ish-Am (2005) on the other hand mentioned that more pollen on the stigma increases the chance of fertilization and that at least 20 pollen grains are needed to have successful fertilization.

Field trial

In the caged trees, a higher initial fruit set was found in the 'Hass' x 'Zutano' cages, but after abscission there was no significant difference in fruit set between 'Hass' x 'Hass' trees and 'Hass' x 'Zutano' trees, inside as well as outside the cages for the 2017-2018 year. These results are in contradiction with the results from the 2016-2017 year, where in cages fruit set between 'Hass' x 'Zutano' trees were significantly higher than that of 'Hass' x 'Zutano' and 'Hass' x 'Hass' trees outside the cages (Bezuidenhout, Du Toit & Robbertse, 2017). The cages did not significantly increase fruit set, which indicate that the hives placed in the orchard were healthy and the bees active in comparison to the hives in the cages. However, Robbertse *et al*. (1998) also found no difference between caged 'Hass' x 'Hass' and open 'Hass' x 'Hass' trees. The method applied for the fruit set counts this year, may not have been reliable, since the harvest figures in Figures 12 and 13 clearly shows that fruit counts inside the cages were significantly higher than on open pollinated trees.

Figures 12 and 13 clearly show that bees are absolutely essential for pollinating avocado flowers to get proper fruit set. Arpaia and Hofshi (2004) also emphasized the importance of bees as pollinators for avocado. Figure 12 also shows that in the nets with bees, there is a slight difference in fruit counts between the 'Hass' x 'Zutano' and the 'Hass' x 'Hass' treatments. Based on the present results it might be more important to introduce hives into the orchard, and in combination with the ideal pollinizer, to make sure that the bees in the hives are healthy and active. Garner *et al*. (2008) found that outcrossing is not the primary factor affecting fruit persistence and ultimate yield. Whiley and Winston (1987) concluded that, "… provided there is a compatible flowering/compatible relationship, single variety blocks can be planted without the consideration of cross-pollination". Our results, however, show that cross-pollination can cause some increase in fruit set and yield. The question to be solved is, does this increase in production compensate with the extra managerial aspects of harvesting two different cultivars in the same orchard?

The 'Hass' orchard in which our experiments were done was inter-planted with 'Zutano' of which the flowering did not quite synchronize with the flowering of 'Hass'. Although 'Zutano' has an extended flowering period, in all three seasons of the duration of the project 'Zutano' flowering was close to its end when the 'Hass' trees started flowering. It could well be that another pollinizer like the two 'new' cultivars 15 and 17, might have given different results.

The sun plays an important role in fruit set since the avocado flowers will open on the sides were the sun shines first in the mornings when 'Hass' is in female stage and 'Zutano' in the male stage. If flowering of the two cultivars are well synchronized, cross-pollination can occur. Schaffer *et al*. (2013) also mentioned that yield could be increased when pollinizer trees are planted close to the subject trees. On the other hand, self-pollination could occur from residual 'Hass' pollen on the bees from the previous 'Hass' male phase. Pollen can stay viable for up to 24 hours. As soon as the female 'Hass' flower open, pollination can occur and the sun directs this process into action. When sunlight is low and temperatures are lower, the flowers delay opening. Robbertse *et al*. (1998) found that the optimum temperature for flower opening is 25 ºC. In our findings, the sun played an important role in successful pollination. Increasing sunlight penetration into the tree would increase flower opening and consequent possible pollination by bees. Ish-am (2005) proclaimed that avocado pollination could be improved with an open canopy that allows direct sunlight to most areas of the tree.

CONCLUSION

This project again emphasized the importance of bees in avocado pollination, but it is important to keep the bees in the orchard. 'Zutano' did show promise, but does not seem to be the best pollinizer for 'Hass', due to its flowering period that is not perfectly synchronized with that of 'Hass'. *In vitro* pollination showed that there are other potential pollinizers for 'Hass', like 'new' cultivars 15 and 17, provided that their flowering period is synchronized with that of 'Hass'.

Based on the cultivars tested it seems that avocado pollen germination and pollen tube growth is fairly tolerant to low (below 10 ºC) and high (above 30 ºC) temperatures although the preferred temperature for pollen tube growth is around 25 ºC.

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