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Outcrossing Rate and Fruit Yield of Hass Avocado Trees Decline at Increasing Distance from a Polliniser Cultivar

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Abstract: Optimal fruit production from many tree crops relies on the transfer of cross-pollen between trees of different cultivars rather than the transfer of self-pollen between trees of the same cultivar. However, many orchards are established with wide blocks of single cultivars, which can result in high percentages of self-fertilised fruit and sub-optimal yield and quality. We aimed to determine whether outcrossing rates and yield of Hass avocado fruit decline with increasing distance from polliniser trees of cultivar Shepard and whether selfed fruit are smaller than outcrossed fruit. Outcrossing rates declined from 49% at six trees (40 m) from a block of Shepard trees to 30% at thirty trees (160 m) from a block of Shepard trees. Tree yield across this distance declined by 44% as a result of a 69% decline in the number of outcrossed fruit per tree, without a significant decline in the number of selfed fruit per tree. Outcrossed Hass fruit were 12% heavier than selfed Hass fruit, with 3% greater diameter and 5% greater length. The study results demonstrate the importance of interplanting Type B avocado pollinisers closely with Type A Hass trees to increase fruit yield and size.

Keywords: cross-pollination; mating system; *Persea americana*; outcrossing; pollenizer; self-compatibility; single-nucleotide polymorphisms; xenia

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1. Introduction

Fruits and nuts contribute approximately 8.5% of the annual global food production [1]. Many fruit and nut crops rely heavily on pollination services to generate high yields [2–4], but shortfalls in the populations of both wild and managed pollinators are placing pressure on food production [4–8]. In addition, the flowers of many fruit and nut crops are partially or completely self-incompatible, requiring the deposition of cross-pollen from a different cultivar onto the stigma for optimal pollen tube growth, ovule fertilisation and fruitlet set [9–15]. Some fruit and nut trees selectively retain cross-fertilised fruitlets at the expense of self-fertilised fruitlets during fruit growth and development, resulting in much of the final crop being outcrossed [16–21]. The cross-fertilised fruit are often larger than the self-fertilised fruit [9,21–27]. Therefore, optimising food production from many fruit and nut crops relies on effective pollination services that maximise the transfer of cross-pollen between different cultivars rather than transferring self-pollen among trees of the same cultivar. However, many fruit and nut orchards are established with wide blocks of trees, with each block containing a single cultivar. The wide separation of trees of the different cultivars can reduce the opportunities for cross-pollen deposition, especially in the middle of single-cultivar blocks [13,18,19,28]. This can result in high proportions of self-fertilised fruit [18,19,28–30] and reduced yield or quality [26,31–35].

Avocado trees are regarded as bee-pollinated and self-compatible, with orchards often producing a mixture of self-fertilised and cross-fertilised fruit [17,29,36–40]. Self-fertilised and cross-fertilised fruit of the most widely-grown cultivar, Hass, sometimes differ little in size or nutritional quality [29], although cross-fertilised fruit were heavier with greater diameter than self-fertilised fruit in one recent study [39]. Despite the self-compatibility of Hass flowers, Hass orchards typically include polliniser trees of another cultivar to maximise the opportunities for effective pollination. The polliniser trees are used because there is limited overlap each day between the opening of the male and female phases of Hass flowers. Avocado displays heterodichogamy, with the flowers of Type A cultivars such as Hass typically opening in the female phase in the morning of one day, closing in the middle of the day, and opening in the male phase on the afternoon of the following day [39–43]. The flowers of Type B cultivars such as Shepard and Fuerte typically open in the female phase in the afternoon, close overnight, and open in the male phase for the following morning. Therefore, the opportunities for pollen deposition on female Hass flowers may be greatest when they have ready access to pollen from nearby Type B cultivars in the morning. However, the levels of cross-fertilisation (i.e., outcrossing) among mature avocado fruit often decline with increasing distance from polliniser trees [16,17,29,36,44,45]. This suggests that there is limited pollen flow between the different cultivars when the Type A and Type B cultivars are each planted in wide, single-cultivar blocks.

In this study, we hypothesised that (a) outcrossing levels among Hass avocado fruit would decline with increasing distance from polliniser trees; (b) tree yields would also decline with increasing distance from polliniser trees; and (c) cross-fertilised fruit would be larger than self-fertilised fruit. The results of this study have important implications for optimising avocado orchard designs and maximising food production in the face of global shortfalls in pollinator populations.

2. Materials and Methods

The experiment was conducted in Eastridge avocado orchard (25°13′17′′ S 152°18′45′′ E), near Childers, Queensland, Australia. This 95 ha orchard has a red clay-loam soil and contains the cultivars Hass, Shepard, Carmen Hass, and Maluma in large single-cultivar blocks [38]. We selected trees in the 57th to 76th rows of a 132-row block of 9-year-old Hass trees, i.e., 20 consecutive rows (Figure 1). We selected the 2nd, 6th and 30th trees from the northern end of each of the 75-tree-long rows. The first tree at the northern end of each row was adjacent to a block of Shepard trees but separated from the Shepard block by approximately 15 m. Tree spacing within the Hass block was 10 m between rows and 5 m within rows. Therefore, the 2nd, 6th and 30th trees in each row were 20 m, 40 m and 160 m, respectively, from the block of Shepard polliniser trees. Honeybee hives had been established prior to flowering at the northern ends of the 5th to 18th rows, the 80th to 81st rows, and the 116th to 130th rows in the 132-row block of Hass trees (Figure 1).

We harvested ten mature fruit from each tree (i.e., 10 fruit × 60 trees = 600 fruit in total) on 17–19 May 2022 using a stratified sampling design. We divided each tree into two quadrants on each side of the tree (i.e., four quadrants in total) and harvested two fruit per quadrant, one from the inside and one from the outside of the canopy. Two additional fruit were harvested near the trunk. These ten fruit were weighed collectively. We then counted all remaining fruit in the tree canopy. Tree yield was calculated by multiplying the average fruit mass in the ten-fruit subsample × the total number of fruit per tree. Each of the ten fruit in the subsample was then weighed individually, and its diameter and length were recorded.

We extracted DNA from the embryo of each of the ten fruit using methods described previously [29]. Each embryo was genotyped using the Agena MassARRAY platform (Agena Bioscience, San Diego, CA, USA) to assign paternity by amplifying DNA regions with unique single nucleotide polymorphisms (SNPs) that we identified previously from all cultivars in the orchard [46].

Cultivars Hass and Carmen Hass appear to be genetically identical [46] and were, therefore, considered to be the same cultivar when assigning seed paternity. Each potential cross-pollen parent in the orchard had DNA sequences with unique homozygous SNPs, and so the presence of one of these unique SNPs in DNA sequences from Hass seeds identified the pollen parent [46]. We calculated the proportion of harvested fruit that were outcrossed in each tree, and we estimated the total number of outcrossed fruit per tree by multiplying the proportion of harvested fruit that were outcrossed × the total number of fruit per tree.

Figure 1. (**A**) Schematic diagram of the layout of Eastridge (ER) avocado orchard with blocks of Carmen Hass, Hass, Maluma and Shepard trees. The experimental trees, shown as red dots, were located in Hass blocks ER5 and ER14, opposite Shepard blocks ER7 and ER8. The approximate locations of honeybee hives are shown in yellow boxes. (**B**) Honeybee hives located between blocks of Hass and Shepard trees.

We assessed the effect of distance to another cultivar on outcrossing rate, fruit number and tree yield via random block analysis of variance (ANOVA), regarding the 20 different orchard rows as blocks. Tukey's Honestly Significant Difference (HSD) tests were performed when differences among the three means were detected via ANOVA. We also assessed the effect of distance to the nearest honeybee hive on yield using regressions, with yield regarded as the dependent variable and distance to the nearest hive regarded as the independent variable. We assessed the effect of pollen parentage on fruit diameter, fruit length and fruit mass by three-way ANOVA, with pollen parentage, orchard row, and distance to another cultivar as the factors. Means were regarded as significantly different at $p < 0.05$. Means are reported with standard errors (SEs).

3. Results

The percentage of Hass fruit that were outcrossed declined from 52 ± 3% and 49 ± 3% at two trees and six trees away from cultivar Shepard, respectively, to 30 ± 4% at 30 trees away from cultivar Shepard (Figure 2A). Almost all outcrossed fruit were pollinated by Shepard, except that two fruit were pollinated by Maluma. The number of selfed fruit per tree did not differ significantly with increasing distance from cultivar Shepard (Figure 2B). However, the number of outcrossed fruit per tree declined by 69% at 30 trees, compared with either two trees or six trees, from cultivar Shepard (Figure 2B). As a result, the total number of fruit per tree declined by 43% or 45% at 30 trees, compared with 2 trees or 6 trees, respectively, from cultivar Shepard (Figure 2B). Tree yield declined by 43% or 44% at 30 trees, compared with at two trees or six trees, respectively, away from cultivar Shepard (Figure 2C). Outcrossing rate, fruit number and tree yield did not differ significantly between the second and sixth trees along the Hass row (Figure 2A–C).

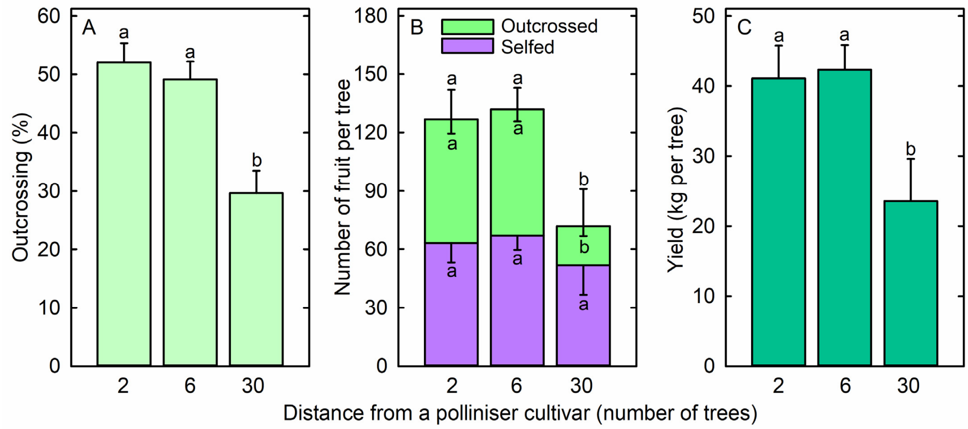

Figure 2. (**A**) Outcrossing rate among fruit, (**B**) number of outcrossed fruit, number of selfed fruit, and total number of fruit, and (**C**) fruit yield on Hass avocado trees located at 2 trees (20 m), 6 trees (40 m) or 30 trees (160 m) from a polliniser cultivar (Shepard). Means (+SE for outcrossing rate, total number of fruit, and fruit yield; −SE for number of selfed fruit, and number of outcrossed fruit) with different letters are significantly different (random block ANOVA and Tukey's HSD test; $p < 0.05$; $n = 20$ trees).

Tree yield was not related significantly ($r^2 < 0.001$; $p = 0.886$) to the distance to the nearest honeybee hives when all 60 trees were included in the analysis (Figure 3). Tree yield was also not related significantly ($r^2 < 0.001$; $p = 0.943$) to the distance to the nearest honeybee hives among the 20 trees that were located only two trees away from cultivar Shepard. However, tree yield was related positively to the distance to the nearest honeybee hives for the 20 trees that were located six trees away from cultivar Shepard ($r^2 = 0.227$; $p < 0.05$) as well as for the 20 trees that were located 30 trees away from cultivar Shepard ($r^2 = 0.120$; $p < 0.05$).

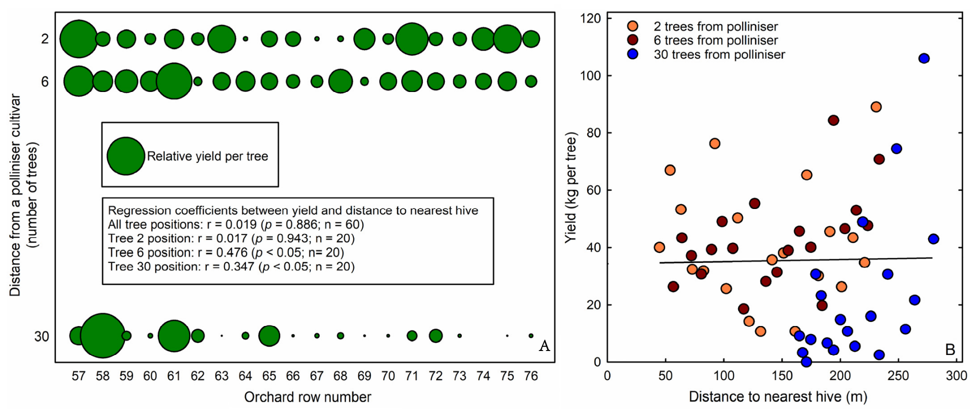

Figure 3. (**A**) Bubble plot showing relative yield of Hass avocado trees located at 2 trees (20 m), 6 trees (40 m) or 30 trees (160 m) from a polliniser cultivar (Shepard). The row numbers (57–76) in the Hass orchard block are shown. Coefficients are provided for the regressions between yield and distance to the nearest honeybee hives. (**B**) Regression line between yield and distance to the nearest honeybee hives for all experimental trees ($r^2 < 0.001$; $p = 0.886$; $n = 60$).

The outcrossed Hass fruit that were pollinated by cultivar Shepard had 2.6 mm greater diameter and 4.8 mm greater length than selfed Hass fruit (Table 1). These outcrossed fruit were 12% heavier than selfed fruit (Table 1).

Table 1. Diameter, length and fresh mass of selfed and outcrossed Hass avocado fruit.

Parameter	Mother Cultivar × Father Cultivar	
	Hass × Hass (Selfed)	**Hass × Shepard (Outcrossed)**
Diameter (mm)	77.8 ± 0.3 a	80.4 ± 0.3 b
Length (mm)	99.3 ± 0.5 a	104.1 ± 0.6 b
Fresh mass (g)	306 ± 3 a	344 ± 4 b

Means (±SE) with different letters within a row are significantly different (three-way ANOVA; $p < 0.05$; $n = 322$ selfed fruit and 232 outcrossed fruit).

4. Discussion

Our results demonstrated that tree yields in the orchard were limited by the amount of cross-pollen transferred from the Type-B Shepard polliniser trees to flowers in the middle of the Type-A Hass block. The predominant flower visitors at the study site in Australia are honeybees [38], as with avocado orchards in other countries [47–50]. These foragers appeared to transfer pollen from male-phase Hass flowers to female-phase Hass flowers at similar levels throughout the block. Hass flowers are unlikely to display significant levels of autogamous self-pollination due to the wide temporal separation of their female and male flower phases [39–43], although close pollination among flowers of the same tree or of trees of the same cultivar can occur depending on the length of the overlap between sexual stages. The results, therefore, suggest that the abundance of pollinators did not change significantly throughout the block. However, the pollinators did not transport sufficient cross-pollen into the middle of the block. Honeybees can forage over much longer distances than 160 m [51–53], but individual workers may forage over shorter distances or remain within the same row when large orchards of mass-flowering trees are at peak flowering during spring [54–56]. This short foraging range may explain why the outcrossing rates or yield of orchard trees often decline in the middle of single-cultivar blocks [26,30,31,57,58]. We considered whether the declining yield in the middle of the block might be due to the increasing distance to the nearest honeybee hives, but we found no significant relationship between yield and proximity to the nearest hives when all 60 experimental trees were included in the analysis. In fact, we found that yield in the middle of the Hass block was related positively, rather than negatively, with increasing distance from the honeybee hives. To further understand the potential interactions between honeybee hive proximity, honeybee foraging range, and polliniser location, we are currently assessing the abundance of honeybees and other insects at different locations within the orchard blocks and determining what percentages of each foraging species are carrying self-pollen and cross-pollen at each location.

Outcrossing rates among avocado trees have declined at increasing distances from polliniser trees in blocks of Ettinger and Fuerte in Israel [17,36] and in blocks of Hass in Israel, California and Spain [16,44,45]. The yield of Ettinger avocado trees declined between the second row (12 m) and the seventh row (42 m) in one orchard and between the first row (6 m) and the eighth row (48 m) away from the polliniser trees in another orchard [17]. The yields of individual Hass trees in three orchards in Israel and California were correlated positively with outcrossing rates [16,45,59], although they were not correlated significantly in three other Californian orchards [45,60]. Hass yields declined between the fourth row (30 m) and the eighth row (60 m) from polliniser trees in one orchard in Israel [16] and between the first row and the fifth row (unknown distance) from polliniser trees in four orchards in California [45]. In contrast, Hass yields did not decline significantly between the first row (20 m) and the third row (36 m) from polliniser trees in Spain [44], and Ardith yields did not decline significantly between the first row (6 m) and the fifth row (30 m) from polliniser trees in Israel [17]. These latter results are similar to the current findings that Hass yields did not decline between two trees (20 m) and six trees (40 m) from the polliniser trees in Australia. However, the Hass yields in Australia did decline between 6 trees (40 m) and 30 trees (160 m) from the polliniser trees. The current study adds to previous findings by demonstrating that the number of selfed fruit per Hass tree was similar at different distances from Type-B polliniser trees but that the differences in yield could be attributed directly to differences in the number of outcrossed fruit per tree.

Outcrossed Hass fruit were 12% heavier than selfed Hass fruit, having both greater diameter and greater length. Avocado produces a single-seeded fruit, and so the effects of pollen parentage on fruit characteristics represent true xenia effects that are not confounded by pollen–parentage effects on the number of seeds per fruit [22,26]. Similar effects of outcrossing have been observed on other single-seeded fruits, with cross-fertilisation increasing fruit or nut mass by 9–28% in almond [61,62], 3–23% in hazelnut [23], 6–7% in lychee [21,63], 10–32% in macadamia [26,35] and 23–26% in plum [25]. The production of mainly selfed fruit, with lower mass and diameter, in the middle of single-cultivar Hass blocks is not optimal for fruit quality or financial returns because avocado growers are often paid a premium for cartons that contain larger fruit [64–67]. Furthermore, selfed Hass fruit possess lower concentrations of calcium in the flesh [29], and low calcium concentrations in the fruit flesh are associated with reduced shelf life [68–70].

The results from several countries, therefore, demonstrate the importance for avocado yield and fruit quality of planting Type-B polliniser trees more closely alongside Type-A Hass trees. Wide blocks of individual cultivars allow convenient and cost-effective management of pests, diseases, irrigation, fertilisers, harvesting and post-harvest processing because orchard management can be targeted at each cultivar [29,57,71–73]. There appears, though, to be a balance between establishing blocks with many rows of a single cultivar for cost-effective management versus interplanting different cultivars more closely to maximise yield and fruit quality. Cross-pollen was transported effectively for at least 30–36 m in previous studies of Hass and Ardith orchards [16,17,44] and at least 40 m in the present study. Yield declines have been observed by 42–48 m from polliniser trees in Ettinger blocks [17]. They have also been observed by 60 m [16] and, in the current study, by 160 m from polliniser trees in Hass blocks. Blocks of Hass trees may need to be no more than 120 m wide if there are compatible, synchronously flowering, Type-B pollinisers on both sides of the block and no more than 60 m wide if the pollinisers are planted on only one side. However, further research is required to gain a finer-scale understanding of the optimal widths of Hass blocks that maximise yield and fruit quality while minimising management costs. This research will be guided by identifying how far bees forage from their hives in avocado orchards, what proportions of bees carry cross-pollen at different distances from polliniser trees, how this proportion can be improved with better hive placement, and whether technologies such as pollen-dusting of bees or pollen-spraying on flowers have the potential to increase outcrossing levels.

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Data Availability Statement: The data presented in this study are available upon request from the corresponding author and with the permission of Hort Innovation.

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