2007 Final Report

Provided to BARD Project No. US-3345-02R

Thomas L. Davenport

Abstract: The original objective was to determine the impact of temperature on floral behavior and pollen tube growth and humidity on the proportions of self-, close, and cross-pollinated avocado fruit on trees growing in humid, coastal and dry, inland CA climates. Because self-pollination was demonstrated to be the prevailing mode of pollination in Florida cultivars in warm humid conditions, it was appropriate to determine if similar rates of self-pollination occur in a dry Mediterranean climate present in California. The conclusions of the work were that despite limiting cool temperatures present in Ventura County, where the research was conducted, self-pollination within Stage 2 flowers is the dominant mode of pollination at both the humid and dry sites. Moreover, it was determined that pollen transfer is mediated by wind and bees have a negligible role in pollen transfer. Temperatures that are marginally warm enough to allow somewhat normal floral opening and closing behavior are still insufficient to provide pollen tube growth to the ovule before abscission of the flower. These results provide the basis for understanding why growers utilizing solid block avocado plantings achieve good yields without bees.

Achievements:

As more information became available during the study, the objective was expanded to better understand the mechanisms of pollen transfer during cross- and self-pollination events. The *primary objective* of the funded research was to determine if self-pollination during Stage 2 floral opening occurs in the Mexican x Guatemalan cultivar, Hass, growing in a Mediterranean climate of California, as was found to occur in West Indian and Guatemalan cultivars growing in a humid sub-tropical environment such as south Florida. A *second objective* was to determine if flowers can be pollinated during Stage 1 by pollen transferred by wind from flowers of complimentary, Type B cultivars dispersing pollen during the morning hours and during Stage 2 floral openings from anthers dispersing pollen within 'Hass' flowers.

To do this, replicate trees were protected from large, flying insects such as bees by enclosing branches in cages constructed of shade cloth with large opening sizes in the weave that would facilitate air passage while eliminating exposure to bees during the floral anthesis periods. That pollen, which is only available from nearby cross-pollinizing, complimentary cultivars could be deposited on Stage 1 'Hass' flowers inside the cages proved that pollen is blowing in the wind. This is the first study to recognize this important point. The lack of greater pollination rates in flowers outside the cages over that found on flowers inside the cages during each year of study at the two locations indicated that bees do not pollinate avocado flowers despite the large numbers of bee hives placed in the study orchards and their enhanced activity in the orchards. The implication of these findings is that orchards are best managed in such a way as to facilitate air movement within them. There appears to be no advantage to providing bees. Because self-pollination is prevalent and self-pollinated fruits are a major component of yield in California and Florida avocados (Previous research and current CAC-funded project) there may be no advantage to interplanting complimentary cultivars. Historical use of solid block plantings of 'Fuerte' and current large block

plantings of ‘Hass’ bear this out. There may be an advantage to interplanting in areas with marginally cool temperature conditions since cross-pollinated flowers have one extra day of pollen tube growth potential over self-pollinated flowers before they abscise.

Appendix

Technical Details of Research and Results

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Introduction

Avocado cultivars display one of two types of flowering behavior. Type A flowering cultivars, such as 'Hass' - the main commercial cultivar grown in California, first open their flowers to display their stigmas synchronously throughout the trees during morning hours (Stage 1). The Stage-1 flowers then close about midday. They reopen about midday the following day as Stage 2 flowers to present the anthers, followed by dehiscence of the anthers to reveal the pollen before permanently closing. Complimentary, type B cultivars display the reverse pattern by synchronously displaying Stage 1 flowers during the afternoon hours of the first day before closing in late afternoon. They then reopen as Stage 2 flowers during the following morning hours. Because of the synchronous opening and closing of hundreds of thousands of flowers by each cultivar type, interplanting of the two types, thus, offers the possibility of pollen transfer from Type B cultivars to Type A cultivars in the morning and transfer of pollen from Type A to Type B cultivars during afternoon hours (cross pollination). Because many stigmas are still receptive during Stage 2 openings, pollen can also be transferred from anthers to stigmas within flowers to provide self pollination to insure production of viable seed in case cross pollination did not occur.

The *primary objective* of the funded research was to determine if self-pollination during Stage 2 floral opening occurs in the Mexican x Guatemalan cultivar, Hass, growing in a Mediterranean climate of California, as was found to occur in West Indian and Guatemalan cultivars growing in a humid sub-tropical environment such as south Florida. A *second objective* was to determine if flowers can be pollinated during Stage 1 by pollen transferred by wind from flowers of complimentary, Type B cultivars dispersing pollen during the morning hours and during Stage 2 floral openings from anthers dispersing pollen within 'Hass' flowers.

Materials and Methods

Research in each year of the project was conducted as described in the proposal protocols and summarized here. Commercial 'Hass' orchards were chosen in locations to represent cool humid and warm dry "Mediterranean" conditions. An orchard owned by Mr. Paul Debusschere, located on the coastal plain near Oxnard, California, was used to represent the cool, humid environment. An inland orchard owned by Mr. Logan Hardison, characterized by low humidity in mountainous terrain, represented the warm day, dry environment. Beehives were provided nearby at both sites (See description of bees and hives in the project report by Mary Lu Arpaia). Complimentary cultivars were interplanted in each multiple of six 'Hass' rows across the humid, coastal site, thus providing eight potential cross-pollinizing cultivars; Zutano, Bacon, Fuerte, Ettinger, Harvest, Surprise, Marvel, and Nobel in various sections within the orchard (Fig. 1). The same cross-pollinizing cultivars were randomly interplanted at close spacing among

rows of ‘Hass’ trees such that branches substantially overlapped with their neighbors at the dry, inland site.

Four replicate cages were assembled and installed over tree branches at the humid coastal site and the inland dry site during February or early March of each year. Enclosure was accomplished during floral bud development but before the beginning of floral anthesis. Two caged trees and their in-row, open pollinated partners (Fig. 2) were each established in the second and third row, (rows 43 and 44 in Fig. 1) respectively, from a row interplanted with ‘Fuerte’ (row 41 in fig. 1)and three and four rows, respectively, from a row interplanted with ‘Zutano’ (row 47 in Fig. 1). ‘Hass’ trees located at the dry, inland site are arranged in rows with close interplanting of the same set of complimentary cultivars. The four caged trees and their open pollinated partners were located in one row traversing the middle of the orchard (Fig. 3).

Cages (~10 ft per side) were constructed of Baycor 40% LENO/LOCK shade cloth supported by 1 inch PVC pipe frames reinforced inside with ¾ inch galvanized steel electrical conduit in order to completely cover major sections of tree canopy without touching the observed inflorescences (Figs. 2 & 3). The size of the openings in the cloth matrix was 2x4 mm, which is sufficient to prevent honeybee penetration but large enough to allow passage of wind and pollen if present. No bees have ever been observed in the cages during the three years of study. Sticky traps were enclosed inside and outside each cage at both locations to determine the incidence of small insects that could possibly penetrate the shade cloth and pollinate flowers. The opening and closing times of Stage 1 and 2 ‘Hass’ flowers were recorded to determine the possible incidence of overlap of floral openings potentially resulting in close pollination, which could be mistaken for cross pollination. Stage 2 anther dehiscence times and pollen release times of flowers on the complimentary, type B, cultivars was also observed to determine the availability of pollen during ‘Hass’ Stage 1.

As many flowers as possible (up to 200 per replicate tree) were collected from each of the four caged and open pollinated trees at the closing of Stage 1 and 2 floral openings at the two study locations. Flowers were plucked using forceps and placed in 50-ml vials containing Carnoy’s solution composed of 25% acetic acid and 75% ethanol. Experience demonstrated that it was not informative to collect flowers on days in which the diurnal temperatures were too cool to allow normal flower opening and closing behavior. Such conditions never resulted in fruit set due to a similar sensitivity of pollen tube growth to cool night periods. If delays in opening and closing of the morning Stage 1 flowers occurred, then Stage 2 flowers were nearly always late in opening and closing as well. Therefore, the number of sampling days available each season was governed by the availability of periods with sufficiently warm diurnal temperatures so as to allow normal floral behavior. Although the floral opening and closing time data are not presented here, there was no overlap of ‘Hass’ floral stages at any time during the harvest days in any year except for one day in 2005. Stage 1 flowers typically closed before opening or anther dehiscence in Stage 2 flowers. There was, therefore, no possibility that pollen from ‘Hass’ panicles bearing Stage 2 flowers on nearby branches could be transferred to late closing Stage 1 flowers (close pollination) on any day (except the one previously noted) during the study.

Different technicians were hired each year in California to assist in setting up and breaking down the cages, collecting the flowers, and making the other necessary measurements and observations. As a result of limited and variable numbers of days with sufficiently warm temperatures and the limited ability of the technical worker to oversee the two study sites on a timely basis, few collections were successfully made in

the 2003 flowering season. No sampling was accomplished in the 2004 flowering season due to extended periods of cool temperatures until a sudden warming trend during the final week of floral anthesis, which was unfortunately missed by the technician. Two technicians were, therefore, hired in 2005 (one for each site), and I personally made the flower samplings in 2006. A sufficient number of warm temperature flowering days were, thus, obtained both years.

Alternate bearing of the inland orchard at Hardison's farm in 2006 prevented my ability to harvest due to lack of sufficient numbers of available flowering stems. Therefore, pollination data are limited to the humid coastal site at Debusschere's farm in 2006.

The vials containing the sampled flowers were each labeled and stored until sampling was completed for the season. They were then transferred to the lab in Florida and immediately analyzed. At that time, the stigmas and styles were excised from the preserved flowers, placed on microscope slides (1 slide per vial) coated with a gel containing aniline blue to enhance visibility of the pollen grains, and observed under a light microscope. The total number of observed stigmas, the number of stigmas with one or more pollen grains attached, and the total number of pollen grains on each pollinated stigma were recorded and averaged. The percent of pollinated stigmas was calculated for flowers collected in Stages 1 and 2 inside and outside the cages. ANOVA was conducted on the arcsine transformed data comparing pollinations in Stages 1 and 2 inside and outside the cages.

In order to keep the 2003 to 2006 flowering season results of the BARD-supported research in perspective, to retain a chronological presentation, and to provide additional data to support the conclusions of the research, I am first presenting results of experiments conducted during the 2001 flowering season, which was funded by the California Avocado Commission. Other than utilizing a different dry inland site (Rancho Simpatica) located near the Hardison site described above, the experiments were conducted using the same cages and protocols described herein for the BARD supported-experiments and will serve as an added replication of the experiments representing four years of data collection (substituting for the lost 2004 results) when published. The reader will note that the tables and figures reporting the various years' results have slightly different presentations due to the fact that different technicians were utilized each year to analyze the data and create the tables and figures. The information presented however, is consistent from year to year and serves the needs for this report.

Results and Discussion

Pollen deposition that occurred during Stage 1 and 2 flower openings inside and outside the cages at the Debusschere farm on the humid coastal plain in 2001 are shown in Table 1. Flowers were collected at the end of Stages 1 and 2 inside and outside the cages over a period of 8 observation days. Missing data indicates that collections were not made due to delayed opening or closing times of both stages or to rain. The average proportion of flowers receiving pollen during the morning, Stage 1 floral opening, inside the cages during the observation period was 3.40% and outside the cages was 3.72%. Because there was no 'Hass' pollen available during this period, the only pollen that could possibly be deposited on these flowers during this period was pollen from nearby Stage 2 flowers of complimentary cultivars. Because the caged tree flowers were protected from bees or other large insect pollinators that might transfer pollen from the complimentary cultivars, the pollen must have arrived on the wind. Similar pollination rates between the two treatments indicated that bees, although present in the orchard, contributed little, if any, to pollen transfer. Self-pollination in

Stage 2 flowers over the same period occurred in 18.59% of the caged tree flowers and in 18.81% of the open pollinated flowers after correcting for the already present average proportion of earlier deposited pollen in Stage 1. There was, therefore, approximately 5-fold more Stage 2 self-pollination within flowers than Stage 1 cross-pollination during the observation days. Stigmas were pollinated by only one pollen grain in about 75% to 85% of the pollinated flowers regardless of floral stage or location inside or outside the cages (Fig. 4). The balance of pollinated stigmas bore 2 or more pollen grains in decreasing frequency.

The proportions of cross and self pollinations were similar during the 10-day observation period conducted at the inland dry site, Rancho Simpatica, in 2001 (Table 2). The proportion of cross-pollinated flowers inside the cages was 4.88% and outside the cages was 7.39%; however, the difference between the two was not significant according to ANOVA. These results, along with the 18.76% and 17.59% self-pollination that occurred inside and outside the cages, respectively, were similar to the results found at the humid site in the same year (Table 1). Again, pollen from neighboring Type B complimentary cultivars was apparently transferred to Stage 1 'Hass' flowers by wind and self-pollination within Stage 2 flowers was the dominant mode of pollination. As at the humid site (Fig. 4), stigmas were pollinated by only one pollen grain in about 70% to 80% of the pollinated flowers regardless of floral stage or location inside or outside the cages, and the balance of pollinated stigmas bore 2 or more pollen grains in decreasing frequency (Fig. 5).

The BARD-supported research conducted in 2003 was not as successful due to few days available with sufficiently warm temperatures to allow proper floral behavior during the period when the hired technician was available. The results of data collection during 4 days at the humid coastal site (Table 3) and 2 days at the dry, inland site (Table 4) were less informative than those data obtained in the 2001 flowering season. Little if any cross-pollination occurred in Stage 1 at either site; however, substantial self-pollination, 40% inside and outside the cages, was revealed in Stage 2 flower samples at the inland dry site, and about 20% inside and 17% outside the cages resulted at the humid coastal site. Other than trends consistent with the previous year's study, little could be determined.

Despite the few warm days available during the period when flowers could be collected, there were sufficient numbers of warm days at the inland, Hardison site at other times in 2003 to produce a good crop in 2004 that stimulated an "off" year in the 2004 flowering season. There were substantial numbers of fruit borne on both caged and open-pollinated experimental trees. Fruit were, therefore collected from the 4 caged and 4 open pollinated trees at the Hardison site when fruit were marble sized and again when fruit were near mature. The embryos of marble sized fruit were subjected to SSR genetic analysis to determine the pollen parent of each fruit as part of a companion project to determine the pollen parents of fruit collected in each row of 'Hass' at the coastal site. The near mature fruit embryo samples were lost due to a lightning strike that destroyed our ultra low temperature freezer resulting in thawing of the embryo tissues. The results of analysis of the marble-sized fruits are displayed in Table 5. On average, 95% of the pollinated flowers that developed into fruit were cross-pollinated and 5% were self-pollinated regardless of whether the flowers were inside the cages or outside. Most of the retained cross-pollinated flowers were pollinated by 'Zutano', 'Fuerte', 'Ettinger', 'Bacon', or one of three other possible cultivars, Nobel, Marvel or Lamb Hass. All of the complimentary cultivars were closely interplanted with branches

overlapping the 'Hass' trees. It was, therefore, not surprising that a high proportion of cross pollination occurred due to the close proximity of pollen from the complimentary cultivars. The same rates of self- and cross-pollination within and outside the cages corroborate the conclusion that pollen was transferred by wind. Moreover, the lack of higher cross-pollination proportions outside the cages compared to inside again indicates that bees contributed little to none of the pollination events.

The 2004 flowering season was worse than the 2003 season due to the fact that temperatures at the humid coastal site remained cool with night temperatures ranging from about 8º to 12ºC and the day temperatures from about 15º to 20ºC until the last week of floral anthesis when the temperatures suddenly rose. There was no fruit set during the extended cool period, and the unanticipated change was so sudden that the technician was unable to respond before the flowering period was over. All of that year's fruit set to produce the crop at that location occurred during the short warm period lasting less than one week. Little flowering occurred at the inland site due to the influence of the high crop load so no data were taken at the site.

Overall, the results at both sites during the 2005 flowering season were similar to those obtained in the 2001 and 2003 flowering seasons. In six days of flower collections at the humid coastal site (Table 6) and five days of collections at the dry, inland site (Table 7), we observed the same levels of Stage 1 cross pollinations inside cages as those outside in the open pollinated trees despite bee activity on the latter. For example, pollen deposition on stigmas at the coastal site occurred in 0.31% of Stage 1 flowers inside the cages and in 0.2% of the flowers of trees outside the cages. As before, bees working the flowers did not transfer any pollen from complimentary cultivars. All transfers were mediated by wind. Self pollination (3.24% of Stage 1 flowers) was about 10-fold greater than cross pollinations in the cages and about 40-fold greater (8.56% of Stage 2 flowers) than cross-pollination events in flowers outside the cages. In comparison, the inland site Stage 1 flower collections resulted in 2.38% and 3.00% of the flowers available for cross pollination bearing pollen in flowers from inside and outside the cages, respectively. Stage 2 pollinations occurred in 10.6% and 23.6% of available flowers inside and outside cages, respectively.

The proportion of pollinated flowers with only one pollen grain was, as in 2001, about 75% regardless of whether deposition occurred in Stage 1 or 2 , inside or outside cages at either site (Figs. 6 and 7).

Assuming these observation days are typical of those in which the daily temperatures were sufficiently warm to provide adequate pollen tube to the egg, it again supports the results of our other observations, that self-pollination is dominant at both sites. This is based on the fact that pollen transfer within flowers is the only way the stigmas could have received the pollen in Stage 2. Despite high bee activity in both orchards, the amount and proportion of pollination (cross pollination) that occurred in Stage 1 inside the cages, which had no possibility of bee transfer, was the same as Stage 1 cross pollinations outside the cages. This was confirmed by ANOVA of the arcsine transformed data this year. This result, as in previous studies, indicates that bees provide little if any pollination over that of wind-carried pollen. There was slight floral overlap on one observation day in 2005 that could have contributed to some "close" pollination in Stage 1, i.e. pollen transfers from Stage 2 'Hass' flowers to late closing Stage 1 'Hass' flowers. If this occurred it would have contributed to a higher proportion

of apparent self-pollinations and would have required wind to move the pollen from flower to flower within the caged and open 'Hass' trees.

Observations in 2006 provided 8 days of flower samplings. During this observation period, winds were light and variable but mostly calm, as measured by a hand-held anemometer, in the coastal, Debusschere orchard. Average cross pollinations occurred in 1.14% of Stage 1 flowers inside the cages and in 3.43% of the Stage 1 flowers in the companion, open-pollinated trees (Table 8). Stage 2 self pollinations occurred in 9.81% of the flowers inside cages and 26.60% of flowers in the open pollinated trees. The significantly higher pollinations of both Stage 1 and 2 flowers in the open-pollinated trees could have been due to the fact that there was little breeze to move pollen into and within the cages. Despite the relatively large openings of the shade cloth used to construct the cages, the cloth presents substantial resistance to air flow through it, especially in light wind conditions. The slight or greater proportions of pollinated Stage 1 and 2 flowers outside the cages in any year could, therefore, easily be explained by light winds as was apparent in this year's observations. The higher proportions of pollinated stigmas with more than one pollen grain per stigma in Stage 2 flowers outside the cage support this possibility (Fig. 8)

Hand pollinations were done on Stage 1 and Stage 2 flowers during the same week as flower collections were being made. 'Fuerte' or 'Ettinger' pollen was used to pollinate Stage 1 flowers whereas 'Hass' flowers were utilized to self pollinate Stage 2 flowers. They were then collected 24, 48, and 72 hrs after pollination and stored in Carnoy's solution until analyzed in Florida. The flowers were rinsed in water. Pistils were excised and treated with 8M Na OH to soften them and stained with aniline blue. They were then individually squashed by pressing them between the cover slip and the slide. Squashed pistils collected at the various times were observed under a fluorescent microscope to observe the number of fluorescent pollen tubes per flower traveling specific distances towards the egg apparatus on each day following hand pollination. Approximately 10 flowers were collected at each time after pollination of each pollination stage. The distances traveled by the pollen tubes in each pollination stage (1 and 2) and time (24, 48, and 72 hrs) are presented in Figure 9. The greatest distance traveled by pollen tubes 72 hrs after pollination in stages 1 and 2 was halfway to the base of the style. This was at a time when floral abscission was beginning to occur. These results indicate that despite day and night temperatures that are sufficiently warm to allow normal flower opening and closing behavior, temperatures were still too cool to allow successful pollen tube growth to the egg apparatus before the flowers abscised from the tree.

Conclusions

The four years of data successfully collected at the two sites are consistent within themselves and with genetic studies currently being conducted at the humid coastal site, Debusschere orchard, in which the integrated result of fruit set is dominated by self-pollinations. Pollen is primarily dispersed in the wind and carried to receptive complimentary cultivars to facilitate cross-pollination. In addition, wind facilitates transfer of pollen within flowers to facilitate self-pollination. There was no indication of bee pollinations in either stage of 'Hass' flowers in avocado trees grown in either location despite the presence of numerous bees in the orchards during floral anthesis. Despite

the presence of sufficiently warm temperatures to allow normal flowering behavior, such temperatures are still too cool to support pollen tube growth to ovules.

Figure 1. Layout of Debusschere orchard plots B2 (north half) and A2 (south half). Orchard is bordered by tall windbreak rows of Poplar to the west and Eucalyptus to the east. 'Hass' (x) trees are interplanted with 'Ettinger' (ET), 'Nobel' (BL-567) (67), 'Fuerte' (F), and 'Zutano' (Z) in the indicated rows of the north half of the orchard. 'Hass' (x) trees are interplanted with 'Marvel' (BL-516) (16), 'Harvest' (HV), 'Bacon' (B), and 'SirPrize' (SP) in the indicated rows of the south half of the orchard. 'Lamb Hass' is interplanted with 'Hass' in rows 29, 35, 41, and 47 in the adjacent section immediately south of the displayed plotted section. Caged (xc) and open partner (xo) 'Hass' trees are located in rows 43 and 44.

												NORTH ROW														
26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	TREE	
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	1	
W			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	2	
I			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	xc	xc	x	x	x	x	x	x	3	
N			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	xo	xo	x	x	x	x	x	x	4	
D			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	5	B2
B			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	6	
R			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	xc	xc	x	x	x	x	x	x	7	
E			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	xo	xo	x	x	x	x	x	x	8	
A			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	9	
K			ET						67						F						Z					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	10	
			ET						67						F						Z				TREE	
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	11	
W			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	12	
I			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	13	
N			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	14	
D			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	15	A2
B			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	16	
R			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	17	
E			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	18	
A			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	19	
K			16						HV						B						SP					
	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	20	
			16						HV						B						SP					
26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50		

↓Lamb Hass↓

Figure 2. Caged and adjacent open-pollinated companion trees at the humid coastal site, Debusschere orchard. Another set of two cages and companion trees were located in the next row to the left of the one pictured here.

Figure 3. Caged and adjacent open-pollinated companion trees at the dry inland site, Hardison orchard. Four caged and alternating open-pollinated companion trees in 'Hass' row are closely interplanted with complimentary cultivars to left and right of row.

Table 1. Proportions of flowers receiving pollen during Stage 1 and 2 floral openings inside and outside cages on the indicated dates at the humid coastal plain site in 2001. Corrected average is the result of subtracting the proportion of flowers receiving pollen in Stage 1 from the total proportion of flowers bearing pollen in Stage 2.

POLLINATION SUMMARY AT DEBUSSCHERE (2001)				
DAILY AVERAGE PERCENTAGE (%)				
	INSIDE		OUTSIDE	
DATE	STAGE 1	STAGE 2	STAGE 1	STAGE 2
4/18	4.98		3.41	
4/26	2.10	30.28	3.91	33.61
4/27		21.40		18.36
4/30	3.00	37.01	2.92	36.28
5/1	2.14		3.95	
5/2		13.77	1.85	15.18
5/3		17.68		14.76
5/4	4.79	11.77	6.30	17.00
AVERAGE	3.40	21.98	3.72	22.53
CORRECTED	3.40	18.58	3.72	18.81

Table 2. Proportions of flowers receiving pollen during Stage 1 and 2 floral openings inside and outside cages on the indicated dates at the humid coastal plain site in 2001. Corrected average is the result of subtracting the proportion of flowers receiving pollen in Stage 1 from the total proportion of flowers bearing pollen in Stage 2.

POLLINATION SUMMARY AT RANCHO SIMPATICA (2001)				
	DAILY AVERAGE % POLLINATION			
	INSIDE		OUTSIDE	
DATE	STAGE 1	STAGE 2	STAGE 1	STAGE 2
5/7	1.00		6.01	20.00
5/8	8.42	25.61	12.48	29.28
5/9	10.07	28.79	9.38	26.73
5/10	4.59	20.90	5.21	26.80
5/11	3.56	24.54	10.69	43.64
5/14	3.16	20.58	5.53	29.53
5/22	4.35	23.58	5.73	18.79
5/24	1.24	29.49	7.29	21.33
5/25		16.11		15.10
6/1	3.92	19.61	4.17	18.61
Ave.	4.48	23.24	7.39	24.98
Corrected	4.48	18.76	7.39	17.59

Figure 4. Proportion of pollinated stigmas receiving one or more pollen grains during Stage 1 and 2 openings inside and outside the caged enclosures at the humid site, Debusschere orchard in 2001.

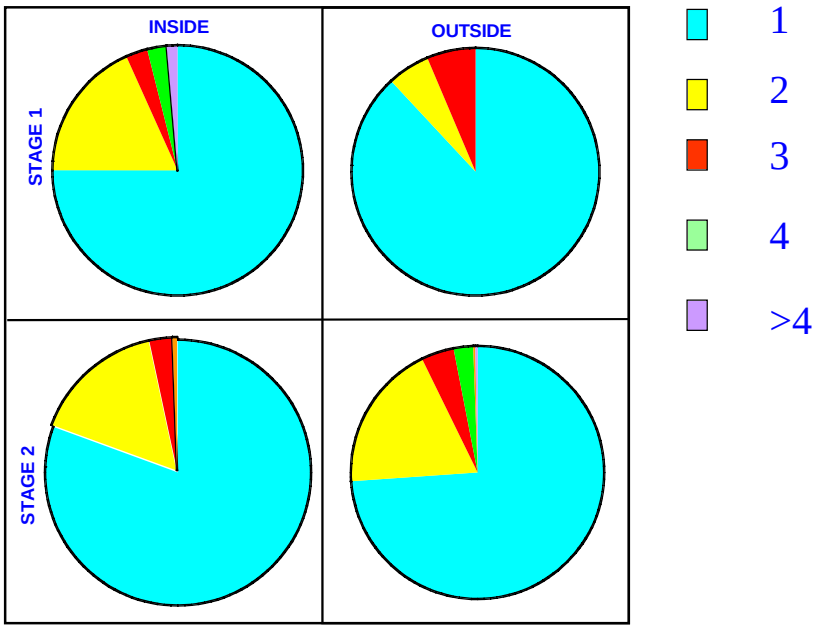


Figure 5. Proportion of pollinated stigmas receiving one or more pollen grains during Stage 1 and 2 openings inside and outside the caged enclosures at the dry inland site, Rancho Simpatica in 2001.

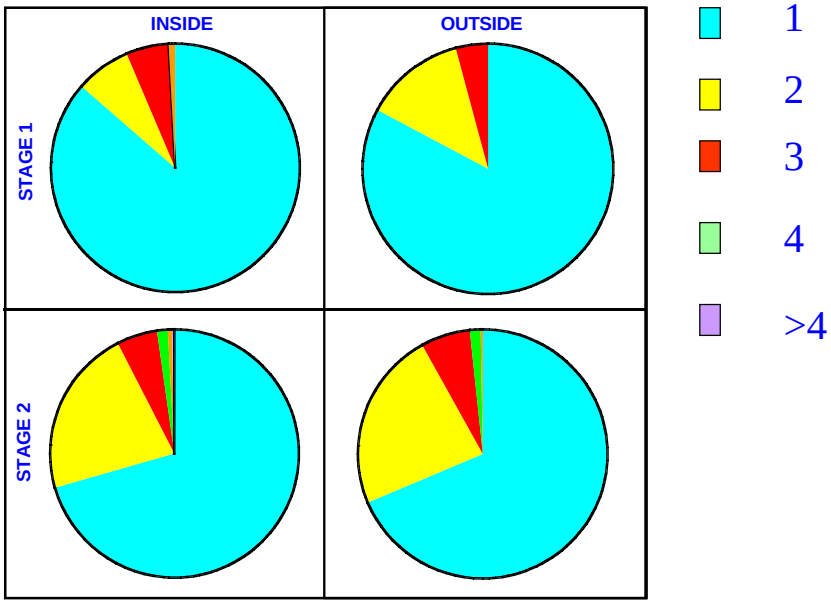


Table 3. Proportions of flowers receiving pollen during Stage 1 and 2 floral openings inside and outside cages on the indicated dates at the humid coastal plain site, in 2003.

POLLINATION SUMMARY AT DEBUSSCHERE (2003)				
DAILY AVERAGE PERCENTAGE (%)				
	INSIDE		OUTSIDE	
DATE	STAGE 1	STAGE 2	STAGE 1	STAGE 2
21-Apr		44.2		35.6
23-Apr	0.0	34.0	1.1	44.2
24-Apr		32.7		34.3
25-Apr	0.0	49.6	0.3	43.6
AVERAGE	0.0	40.1	0.7	39.4

Table 4. Proportions of flowers receiving pollen during Stage 1 and 2 floral openings inside and outside cages on the indicated dates at the dry inland site in 2003.

POLLINATION SUMMARY AT HARDISON (2003)				
DAILY AVERAGE PERCENTAGE (%)				
	INSIDE		OUTSIDE	
DATE	STAGE 1	STAGE 2	STAGE 1	STAGE 2
6-May	0.0		0.0	
7-May		20.1		16.8
AVERAGE	0.0	20.1	0.0	16.8

Table 5. Marble-sized fruit harvested on May 27, 2003 from caged and open-pollinated trees in the dry inland site, Hardison orchard. The frequency of pollinations of individual fruits by the pollen parents are listed in each column for each tree.

	Tree Locations																Summary			
	Tree #2		Tree #3		Tree #4		Tree #5		Tree #6		Tree #7		Tree #8		Tree #9		All Caged		All Open	
Paternity	Caged #5		Open #5		Caged #6		Open #6		Caged #7		Open #7		Caged #8		Open #8		Trees		Trees	
Fruits	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%	#	%
Zutano	5	35.7	11	52.4	10	62.5	9	40.9	9	39.1	10	62.5	9	45.0	13	65.0	33	45.2	43	54.4
Hass	0	0.0	0	0.0	2	12.5	0	0.0	0	0.0	2	12.5	1	5.0	2	10.0	3	4.1	4	5.1
Fuerte	0	0.0	0	0.0	0	0.0	3	13.6	3	13.0	1	6.3	1	5.0	1	5.0	4	5.5	5	6.3
Ettinger	5	35.7	4	19.0	3	18.8	2	9.1	2	8.7	1	6.3	5	25.0	1	5.0	15	20.5	8	10.1
Bacon	1	7.1	0	0.0	0	0.0	0	0.0	1	4.3	1	6.3	0	0.0	2	10.0	2	2.7	3	3.8
M/N/L	3	21.4	6	28.6	1	6.3	8	36.4	8	34.8	1	6.3	4	20.0	1	5.0	16	21.9	16	20.3
Total	14	100.0	21	100.0	16	100.0	22	100.0	23	100.0	16	100.0	20	100.0	20	100.0	73	100.0	79	100.0
Selfing %		0.0		0.0		12.5		0.0		0.0		12.5		5.0		10.0		4.1		5.1
Crossing%		100.0		100.0		87.5		100.0		100.0		87.5		95.0		90.0		95.9		94.9

Table 6. 2005 Pollination of Stage 1 and 2 'Hass' avocado flowers at the humid, coastal site, Debusschere orchard, in tree branches located inside and outside cages that prevent bee pollinations. TS represents the total number of stigmas observed. SP represents the number of pollinated stigmas among those observed, and % is the proportion of total stigmas that were pollinated in each stage on each date of observation.

	INSIDE						OUTSIDE					
	STAGE 1			STAGE 2			STAGE 1			STAGE 2		
DATE	TS	SP	%	TS	SP	%	TS	SP	%	TS	SP	%
4-Apr	132	1	0.8	178	3	1.6	221	2	0.9	208	8	3.8
6-Apr	366	0	0	303	21	6.5	383	0	0	452	53	11.7
11-Apr	183	2	1.09	49	3	6.25	195	0	0	133	22	16.5
12-Apr	425	0	0	184	3	1.6	186	0	0	175	9	5.14
14-Apr	193	0	0	198	4	2	193	0	0	194	16	8.2
15-Apr	192	0	0	200	3	1.5	195	0	0	197	12	6
TOTAL	1491	3	1.84	1112	37	19.5	1373	2	0.9	1359	120	51.3
AVE	248.5	0.5	0.31	185	6.17	3.24	228.8	0.33	0.2	226.5	20	8.56

Table 7. 2005 Pollination of stage 1 and 2 'Hass' avocado flowers at the dry, inland site, Hardison orchard, in tree branches located inside and outside cages that prevent bee pollinations. TS represents the total number of stigmas observed. SP represents the number of pollinated stigmas among those observed, and % is the proportion of total stigmas that were pollinated in each stage on each date of observation.

	INSIDE						OUTSIDE					
	STAGE 1			STAGE 2			STAGE 1			STAGE 2		
DATE	TS	SP	%	TS	SP	%	TS	SP	%	TS	SP	%
5-Apr	164	0	0	285	14	4.91	159	6	3.7	321	31	9.65
6-Apr	179	16	8.9	394	19	4.82	200	15	7.5	419	91	21.7
11-Apr	450	11	2.45	439	85	19.3	428	3	0.7	388	121	31.1
15-Apr	523	3	0.57	557	74	13.3	789	9	1.1	549	181	32
25-Apr	176	0	0	ND	ND	ND	250	5	2	ND	ND	ND
TOTALS	1492	30	11.9	1675	192	42.3	1826	38	15	1677	424	94.5
AVE	298	6	2.38	418.8	48	10.6	365.2	38	3	419.3	106	23.6

Figure 6. Proportion of pollinated stigmas receiving one or more pollen grains during Stage 1 and 2 openings inside and outside the caged enclosures at the humid site, Debusschere orchard, in 2005.

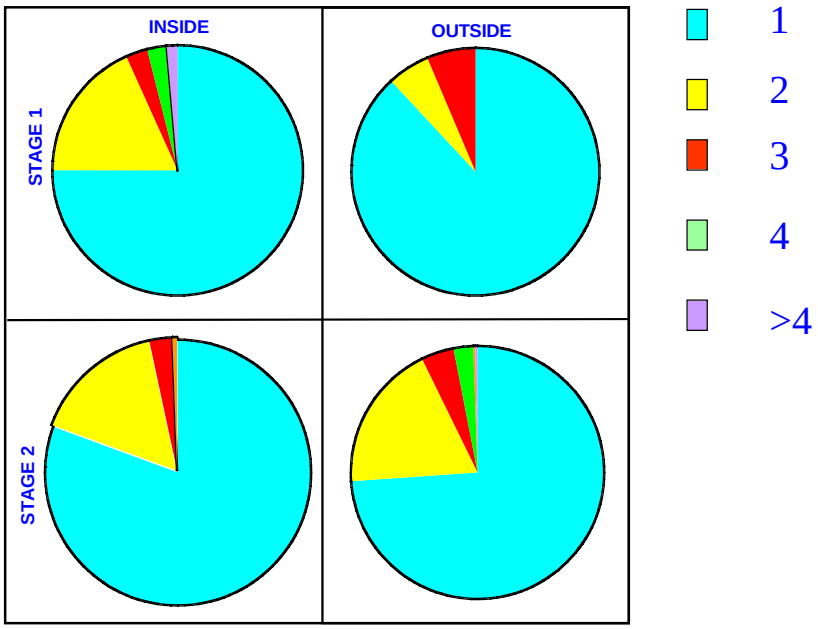


Figure 7. Proportion of pollinated stigmas receiving one or more pollen grains during Stage 1 and 2 openings inside and outside the caged enclosures at the dry inland site, Hardison orchard, in 2005.

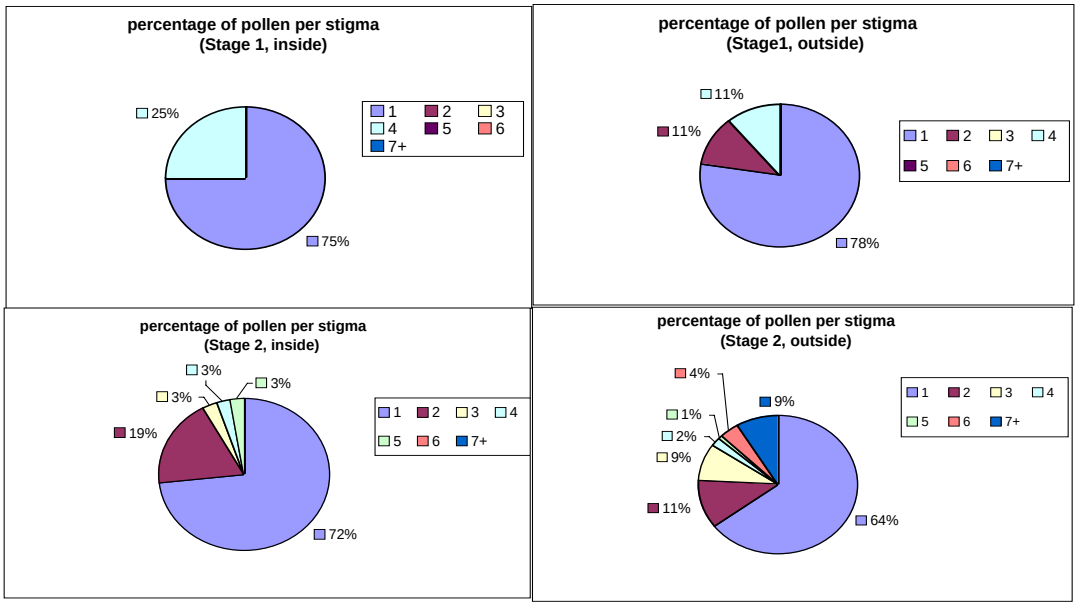


Table 8. Proportions of flowers receiving pollen during Stage 1 and 2 floral openings inside and outside cages on the indicated dates at the humid coastal plain site, in 2006.

	STAGE 1		STAGE 2	
DATE	INSIDE	OUTSIDE	INSIDE	INSIDE
16-MAY	1.15	2.62	14.75	40.63
17-MAY	0.74	2.37	16.14	34.57
18-MAY	0.84	2.68	11.90	20.67
19-MAY	3.09	4.08	9.14	20.12
20-MAY	0.63	3.49	7.54	20.41
21-MAY	0.89	3.81		
22-MAY	0.52	4.47	6.59	20.45
23-MAY	1.28	3.91	2.59	29.31
Average	1.14	3.43	9.81	26.60

Figure 8. Proportion of pollinated stigmas receiving one or more pollen grains during Stage 1 and 2 openings inside and outside the caged enclosures at the humid site, Debusschere orchard, in 2006.

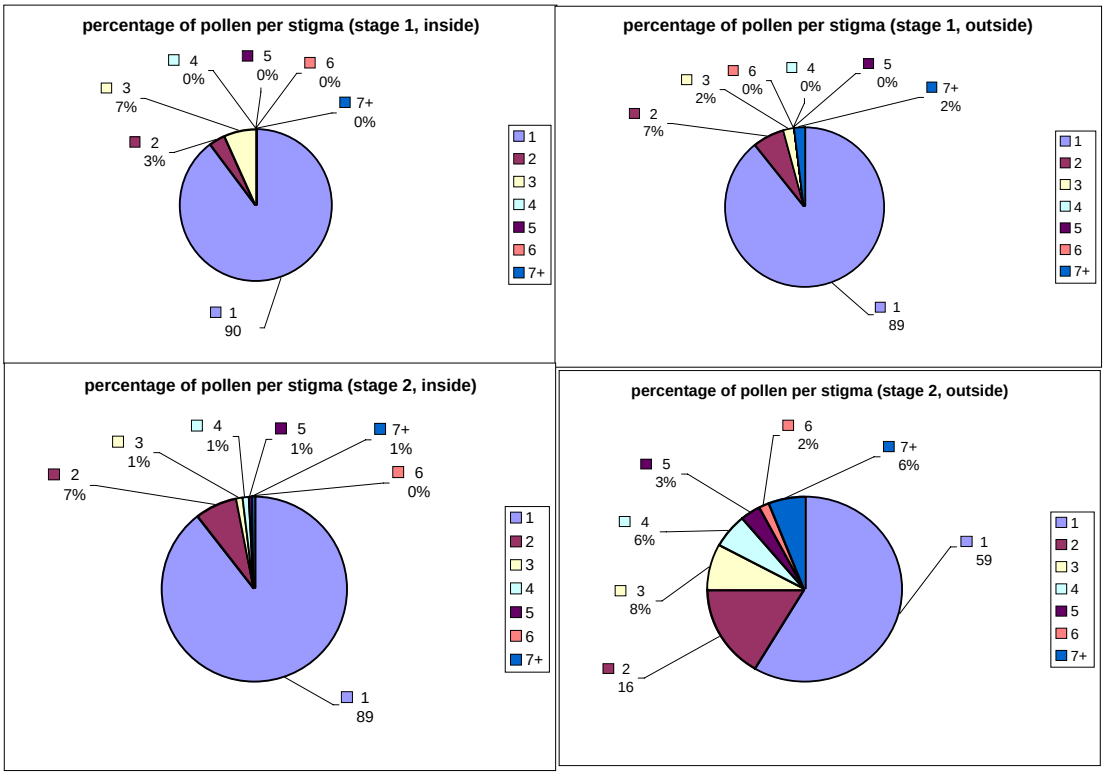
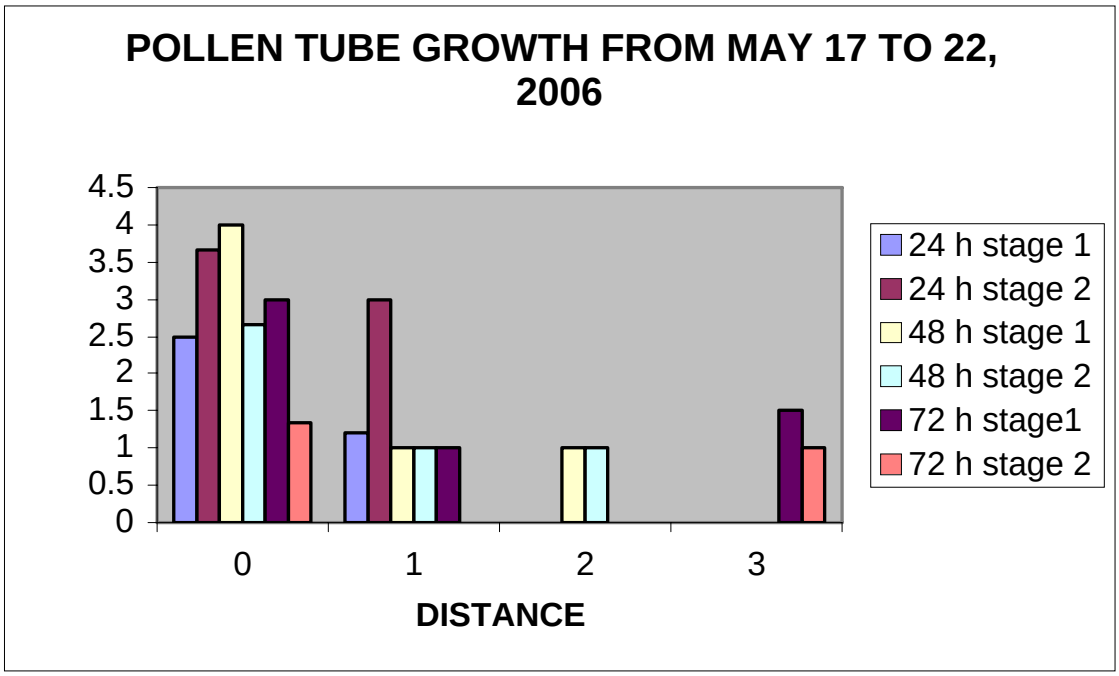
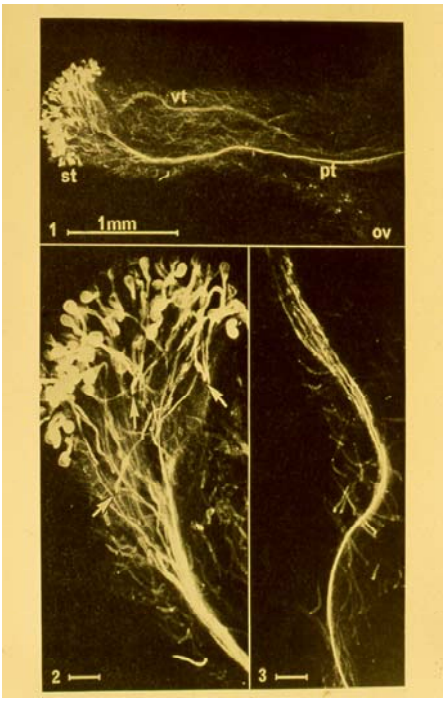


Figure 9. Average number of pollen tubes per flower growing the indicated distance in avocado floral pistils harvested 24, 48, and 72 hrs after hand pollination in Stage 1 or 2 flowers. Distance traveled numbers are defined below figure. Photos at bottom right depict pollen tubes in avocado as viewed under florescence microscopy.



- D0 = No germination
- D1= Just germinated but no travel
- D2 = Half way down style
- D3 = Between 1/2 and base of style
- D4 = In ovary
- D5 = Base of ovary
- D6 = In egg apparatus



Photos from Sedgley (1976)
New Phytol. 77:149-152.