

## **Pollen Parent Effect on Outcrossing Rate in 'Hass' and 'Fuerte' Avocado Plots during Fruit Development**

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**Abstract.** Outcrossing rates in 'Hass' and 'Fuerte' avocado (*Persea americana* Mill.) blocks were determined during fruit development using the isozyme systems of malate dehydrogenase (MDH, EC 1.1.1.37), leucine aminopeptidase (LAP, EC 3.4.11.1), and triosephosphate isomerase (TPI, EC 5.3.1.1) as genetic markers. Abscission of avocado fruitlets was selective and was greatly influenced by the pollen parent. Thus, in 'Hass' trees subjected to cross-pollination by 'Ettinger' and 'Fuerte', the population of 'Hass' fruitlets 1 month after fruit set consisted mainly of 'Hass' selfs; however, during fruit development, the rate of 'Hass' selfs decreased and the rate of 'Hass' hybrid fruitlets produced by 'Ettinger' and 'Fuerte' increased. By the end of fruit abscission, the surviving mature 'Hass' fruits were mostly 'Ettinger' hybrids. The 'Hass' fruit yield was found to correlate significantly with the rate of Outcrossing with 'Ettinger'. When 'Ettinger' served as a pollen parent for 'Fuerte', the Outcrossing rate in trees adjacent to 'Ettinger' was about 40%, which shows that cross-pollination among avocado cultivars of the same flowering group can be substantial at close proximity. The Outcrossing rate in 'Fuerte' diminished with increasing distance from 'Ettinger', but no effect on yield was observed.

Avocado is considered to be an outcrossing plant due to the synchronously dichogamous nature of its flowering behavior (10, 15, 23-25). Thus, to ensure adequate pollination and optimum yields, planting of two complementary cultivars in the same plot was recommended (3, 22, 26). On the other hand, it was noted that some cultivars were successfully self-pollinated, since their screened trees produced well (8, 9, 15), and large solid blocks of 'Hass' in California consistently set good crops (21).

The question of whether interplanting of cultivars is indeed necessary in commercial

orchards was addressed by Bergh et al. (4-7), who studied the effect of interplanting on avocado yield. They found that, when several avocado cultivars were planted adjacent to a complementary cultivar, significantly higher yields were obtained. The effect was limited, however, to the first row adjacent to the pollen donor. These authors concluded that cross-pollination was the primary cause of yield increase. Furthermore, Bergh (4) recommended that, in order to bring about cross-pollination, "the branches of the two varieties must be close together and preferably overlapping."

The development of isozymes as genetic markers in avocado (19, 20, 29, 30) made it possible to distinguish hybrid offspring from selfs (11) and to determine rates of outcrossing between cultivars in the orchard (19, 28, 32). In the present work, isozyme analysis was used to study the effect of pollen parent proximity on the percentage of hybrids during fruit development, with the objective of determining the difference, if any, between the degree of hybrid production at harvest time and the initial outcrossing rate.

### **Materials and Methods**

The research was carried out in two avocado plots planted in 1979 in commercial orchard of Kibbutz Givat Brenner, near Rehovot, Israel.

The 'Hass' plot (Fig. 1, plot 3) has 28 rows (7.5 m apart), with 17 trees per row (4.5 m apart). The plot has two rows of 'Ettinger' at its western end and is in proximity to one younger 'Ettinger' row (planted in 1981), across a road at the eastern end. On the north side, among 'Hass' trees in the adjacent plot 4, is a group of 'Fuerte' trees (Fig. 1), with another group of 'Fuerte' and 'Reed' trees at the east end of that plot. Two trees in plot 3 were identified as 'Fuerte' and 'Reed' and are marked in Fig. 1.

The 'Fuerte' plot has trees 7.5 x 6 m apart (Fig. 2, plot 7), with 'Ettinger' trees eight rows removed to the west from the sampled area. Plot 8 has much closer 'Ettinger' trees on the north side of the 'Fuerte'.

During May-Oct. 1984, fruitlets and fruits were sampled at various developmental stages. Fruitlet and fruit samples were kept at 4°C. Fruitlets (up to 10 g) were analyzed within 24 hr. Fruits were sometimes kept for 2 to 4 days before analysis. Their embryos were assayed for three isozyme systems: malate dehydrogenase (MDH), leucine aminopeptidase (LAP), and triosephosphate isomerase (TPI). In very young fruitlets (50-100 mg), the embryo with its endosperm were crushed in 100 µl of extraction buffer (11), and the extract was absorbed on a 4 x 6 mm Whatman 3MM paper wick. Extracts for electrophoresis were prepared according to Torres (27) from embryos of larger fruitlets.

The enzyme systems were separated by horizontal starch gel electrophoresis (starch obtained from Sigma) and stained as described previously for LAP (12), MDH (11), and TPI (19).

### **Results**

*Hybrid percentage from fruit-set to maturity in 'Hass'.* Fruitlets were sampled from the same trees on three dates, from the middle of May to the end of October. On each occasion, four pairs of adjoining trees were sampled; five average-sized fruit lets were picked from each tree. The fruitlets were assayed for TPI-1, LAP-2, and MDH-1. TPI-1 is a dimeric enzyme having fast (F) and slow (S) as the most common alleles (19, 31). LAP-2 is a monomeric enzyme having F and S alleles (30). MDH-1 is a dimeric enzyme

having F and S alleles (29). Table 1 presents the isozyme genotypes in these three systems for the cultivars present in the area (Fig. 1).

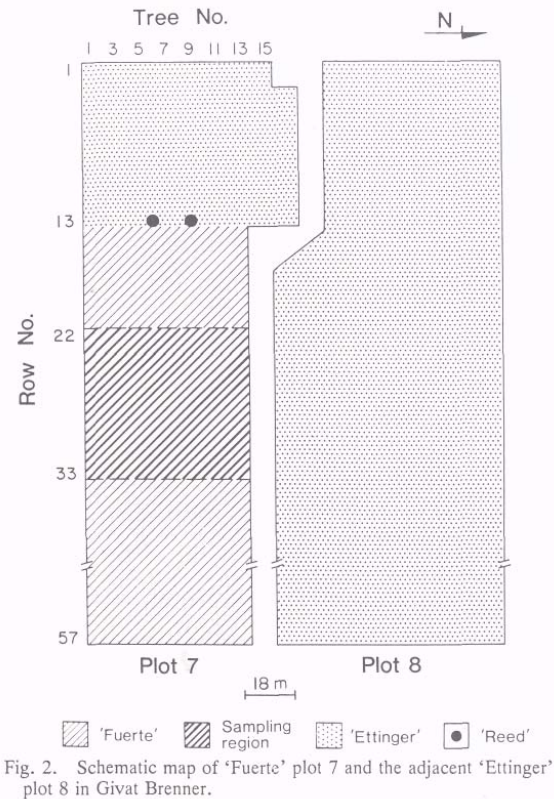
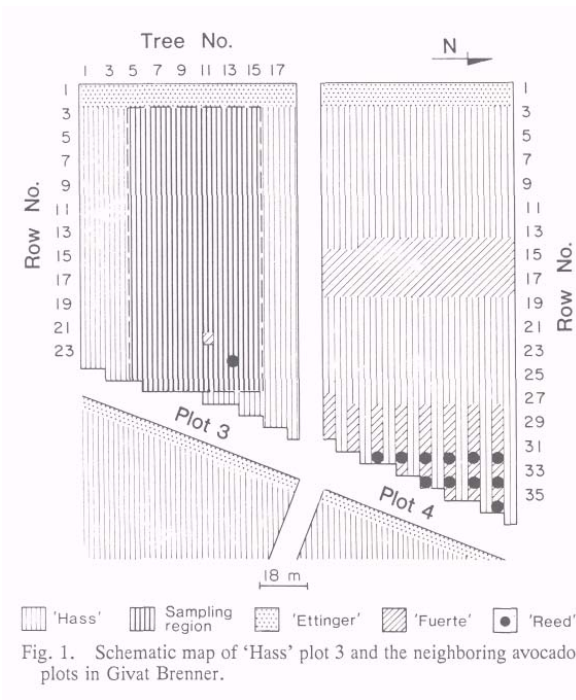


Table 1. Isozyme genotypes of four avocado cultivars present in the studied plot areas<sup>a</sup>.

Cultivar	<i>Tpi-1</i>	<i>Mdh-1</i>	<i>Lap-2</i>
Hass	SS	SS	FF
Ettinger	FS	SS	FS
Fuerte	FS	FS	FF
Reed	SS	FS	FF

<sup>a</sup>See Figs. 1 and 2.

'Hass' embryos heterozygous for *Tpi-1* have as a pollen parent either 'Ettinger' or 'Fuerte'. Those heterozygous for *Lap-2* are 'Ettinger' hybrids, while those heterozygous for *Mdh-1* could be the offspring of either 'Fuerte' or 'Reed'. Since only a few 'Reed' trees were present in the area (Fig. 1), and since 'Reed' started to flower only at the end of the 'Hass' bloom, we may assume that 'Hass' embryos heterozygous for *Mdh-1* were 'Fuerte' hybrids.

The hybrids described above are between a heterozygote ('Fuerte' for *Mdh-1*, 'Ettinger' for *Lap-2*, and both for *Tpi-1*) and a homozygote ('Hass' for all three). The hybrids may thus be expected to segregate in a homozygote to heterozygote ratio of 1:1 in each of the three isozyme systems. The actual rate of outcrossing should therefore be double the

observed rate of heterozygosity (however, see the discussion below on excess heterozygosity).

Table 2 gives the observed rates of heterozygosity in 'Hass' fruitlets for each of the three isozyme systems determined on the three sampling dates. The contribution of the two potential pollen donors, 'Ettinger' and 'Fuerte', can then be calculated. As indicated by the percentage of heterozygotes for *Tpi-1*, the hybrid survival proportion increased consistently during fruit development and abscission up to a value of about 84% (41.8 x 2) close to maturity. The percentage of 'Fuerte' offspring (calculated from the percentage of heterozygotes for *Mdh-1*) increased from a value of 0.8% at early fruit development up to 22.4% in October. The percentage of 'Ettinger' offspring (calculated from the percentage of heterozygotes for *Lap-2*) reached a value of 74% at the verge of fruit maturity.

Table 2. Heterozygosity in 'Hass' fruits assayed by three isozyme systems at different stages of fruit development.

Date	No. tree pairs studied	No. fruitlets assayed	Average fruit wt (g)	Percent heterozygotes <sup>z</sup>		
				<i>Tpi-1</i>	<i>Mdh-1</i>	<i>Lap-2</i>
5 May	46	596	0.07	11.4 b	0.4 b	---
10 Sept.	48	355	121	36.2 a	10.7 a	29.3 b
22 Oct.	52	398	184	41.8 a	11.2 a	36.8 a

<sup>z</sup>Results (in columns) followed by different letters are significantly different ( $P < 0.001$ ), according to Duncan's multiple range test.

<sup>y</sup>LAP-2 was not detected in very young fruitlets.

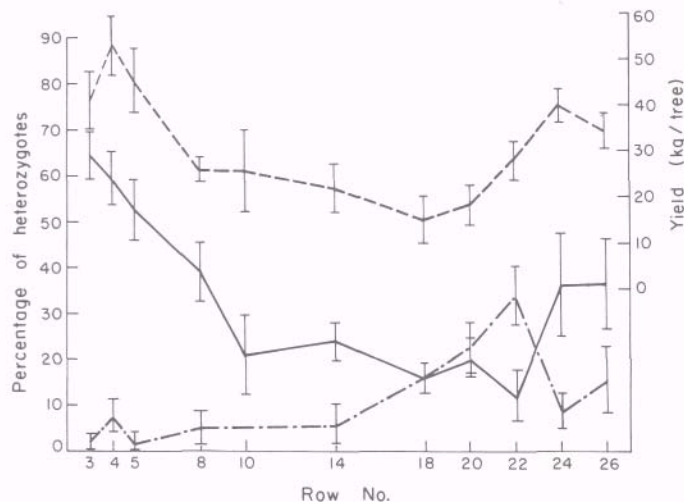


Fig. 3. Average yields (----) and mean percentage of 'Hass' embryos heterozygous for *Mdh-1* ('Fuerte' offspring, ---) and for *Lap-2* ('Ettinger' offspring, —) at the verge of fruit maturity (September–October), in various rows of the 'Hass' plot. The vertical lines represent the SE of the mean values.

The distance-dependence of the effect of each pollen parent on hybrid percentage was examined close to maturity (September–October). Figure 3 shows the average percentage of heterozygotes for *Lap-2* ('Ettinger' progeny) and of heterozygotes for *Mdh-1* ('Fuerte' progeny) for the sampled 'Hass' rows. With increasing distance from 'Ettinger',

i.e., towards the center of the plot, the rate of heterozygosity decreased, reaching its lowest value about rows 18 to 22 (135 and 165 m from 'Ettinger' at the western end of the plot). It rose again on approaching the 'Ettinger' row to the east end of the plot (see Fig. 1). The rate in rows 3 to 5 was higher than the assumed 50% maximum.

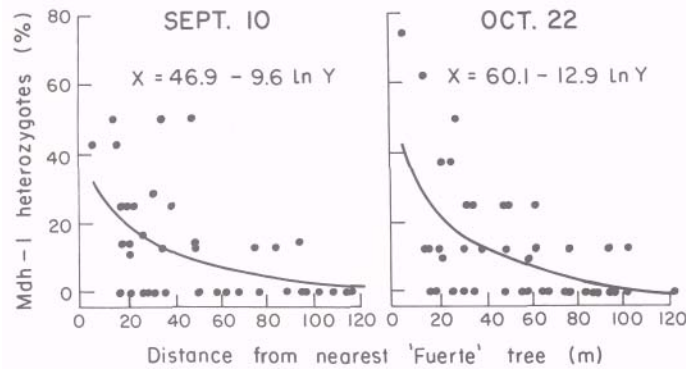


Fig. 4. Percentage of 'Hass' embryos heterozygous for *Mdh-1* ('Fuerte' offspring) as function of the distance of the 'Hass' tree from the nearest 'Fuerte' tree.

The percentage of heterozygotes for *Mdh-1* ('Fuerte' progeny) was quite low, with pronounced increase at about row 22. In Feb. 1985, all 'Hass' fruits were harvested. Figure 3 also presents average yield per tree (17). The data show a decrease in yield with increasing distance from 'Ettinger' ( $r = 0.85$ ).

Figure 4 presents the percentage of 'Fuerte' hybrids in the 'Hass' trees sampled in September and October as a function of the distance between the 'Hass' tree and its nearest 'Fuerte' pollen donor. As indicated by the two equations in Fig. 4, the percentage of 'Fuerte' offspring (i.e., the percentage of heterozygotes for *Mdh-1*) is a linear function of the natural logarithm of the distance from the nearest 'Fuerte' tree [ $\ln(\text{Dis } F)$ ].

Statistical analysis of the relationship between heterozygotes for *Mdh-1* ('Fuerte' offspring) and  $\ln(\text{Dis } F)$  and percentage of heterozygotes for *Lap-2* ('Ettinger' offspring) showed significant correlations for September (Eq. 1) and October (Eq. 2).

September:

$$\% \text{ Mdh-1} = 40.5 - 6.4 \ln(\text{Dis } F) \\ 0.20\% \text{ Lap-2}; R^2 = 0.27 \quad P < 0.001 \quad [1]$$

October:

$$\% \text{ Mdh-1} = 51.4 - 7.7 \ln(\text{Dis } F) \\ 0.27\% \text{ Lap-2}; R^2 = 0.38 \quad P < 0.001 \quad [2]$$

The conclusion to be drawn from these equations is that the survival rate of 'Fuerte' progeny depends on its distance from both 'Fuerte' and 'Ettinger' trees. Increasing the distance from 'Fuerte' and approaching 'Ettinger' (Fig. 1) resulted in a lower survival rate of 'Fuerte' offspring.

*Hybrid percentage from fruit-set to maturity in 'Fuerte'.* On nine dates, from the beginning of May to the end of October, fruitlets and fruits were sampled from eight 'Fuerte' rows at different distances from 'Ettinger' (Fig. 2). Because of the low fruit-set in the orchard, a

small number of fruitlets was sampled from a relatively large number of trees. Fruitlets of average size were selected and then embryos were assayed for LAP-2. The only possible pollen donor responsible for 'Fuerte' embryos heterozygous for *Lap-2* was 'Ettinger'.

In very young fruitlets, no LAP-2 activity was detected. Table 3 presents the average rates of heterozygosity from consecutive periods of fruit development. No significant differences were found between these rates. Figure 5 presents the average rate of *Lap-2* heterozygote in each row, and shows a decrease in the rate of 'Ettinger' offspring with increasing distance from 'Ettinger' trees. No correlation was found between rates of outcrossing and yields.

Table 3. Percentage of 'Fuerte' fruitlets heterozygous for *Lap-2*, which identifies 'Ettinger' pollination.

Sampling period <sup>z</sup>	No. trees sampled	No. fruitlets examined	Percent heterozygous for <i>Lap-2</i>
May-June	67	264	11.5 ± 2.6
July	87	234	11.7 ± 2.4
August	75	237	11.9 ± 2.2
October	61	246	11.8 ± 2.0

<sup>z</sup>10, 24 May; 6 June; 7, 17 July; 8, 22 Aug.; 7, 10 Oct.

<sup>y</sup>Mean ± SE.

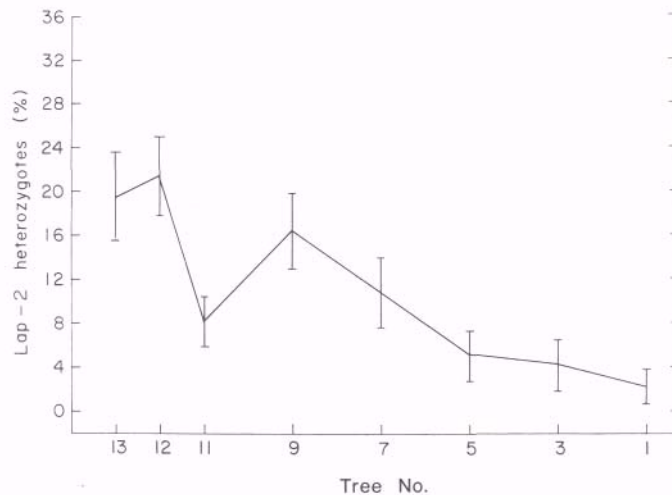


Fig. 5. Mean percentage of 'Fuerte' embryos heterozygous for *Lap-2* in relation to the distance from the 'Ettinger' plot. Trees are numbered as in Fig. 2, i.e., trees numbered 13 are closest to 'Ettinger'. The vertical lines represent the SE of the mean value for data obtained on four dates for equidistant trees in rows 22 through 33.

## Discussion

A cross between heterozygous and homozygous plants is expected to yield a maximum of 50% of heterozygous offspring. Our results (Fig. 3) show that the percentage of 'Hass' fruits heterozygous for *Lap-2* in the rows adjacent to west-end 'Ettinger' (i.e., rows 3-5) was higher than 50%, reaching a maximum value of 65%. These findings are consistent with the results of a preliminary study carried out 1 year earlier in the same plot that

showed that the rate of heterozygosity for *Lap-2* in the four rows adjacent to 'Ettinger' ranged between 60% and 74% (18). In addition, studies of outcrossing rates in another solid block of 'Hass' close to 'Ettinger' showed rates of heterozygosity for *Lap-2* of 56% and 58% (19). The consistency of these results suggests that the unexpectedly high ratio of heterozygotes to homozygotes is unlikely to be due to chance. This phenomenon probably arises from genetic selection, similar to that found in self-pollinated 'Ettinger' progeny, which results in a high preference of heterozygotes over homozygotes (12). Obviously, under conditions of genetic selection favoring heterozygotes, the overall rate of outcrossing is a product of multiplication by a factor lower than 2. The precise magnitude of this factor remains to be determined. This genetic selection, resulting in a better chance of survival of heterozygotes for *Lap-2*, is probably responsible for the fact that, in 'Hass' fruits (Table 2), the observed rate of 'Ettinger' progeny (heterozygous for *Lap-2*) is higher than the difference between the total outcrossed progeny (heterozygotes for *Tpi-1*) and 'Fuerte' progeny (heterozygous for *Mdh-1*) and not equal to this difference, as would be expected for an independent assortment of the loci.

The dependence of the outcrossing rate in 'Hass' on the distance from the 'Ettinger' pollen parent is shown in Fig. 3. 'Ettinger' (flower type B), previously shown to be a potent pollen parent (11, 19), was again found to excel as a pollen parent for 'Hass', a complementary cultivar (flower type A). On the verge of fruit maturity (September-October), the percentage of 'Hass' fruit identified as 'Ettinger' progeny (heterozygous for *Lap-2*) in the three rows adjacent to the adult west-end 'Ettinger' trees (rows 3 to 5, Fig. 3), was in the range of 55% to 65%, indicating that almost all fruits in these three rows were 'Ettinger' hybrids. A clear and consistent decline in the percentage of 'Ettinger' hybrids can be seen up to row 14, followed by a fairly constant value of  $\approx 20\%$  (corresponding to  $\approx 40\%$  'Ettinger' progeny) up to row 20. On approaching the younger 'Ettinger' trees at the east end, the rate of identified 'Ettinger' hybrids increased again to 40%. The relatively lower percentage of hybrids to the east end of the 'Hass' block is probably attributable to the small size of the young 'Ettinger' trees and their relatively greater distance from 'Hass' trees (Fig. 1). The percentage of 'Hass' fruits identified as 'Fuerte' progeny (heterozygous for *Mdh-1*) has a value of  $\approx 6\%$  (corresponding to  $\approx 12\%$  'Fuerte' progeny) up to row 14. From this row on, the rate of 'Fuerte' hybrids increased, apparently due to the effect of 'Fuerte' from plot 4. It reached a maximum value of 34% (corresponding to  $\approx 68\%$  'Fuerte' progeny) at row 22, probably because of the 'Fuerte' tree right in this row (Fig. 1).

The proportion of identified 'Ettinger' hybrids in the 'Fuerte' rows adjacent to the 'Ettinger' block was  $\approx 20\%$  (Fig. 5). As 'Fuerte' and 'Ettinger' belong to the same flowering group B, these results demonstrate that cross-pollination among cultivars of the same flowering group can be fairly efficient at close proximity. Except for an unexplained drop in trees numbered 11 (three trees away from the 'Ettinger' plot), there is a gradual and consistent decline in the rate of 'Ettinger' progeny down to a value of 2% in the trees numbered 1 (13 trees away from 'Ettinger' plot; i.e., a distance of  $\approx 80$  m).

Although the percentage of 'Hass' and 'Fuerte' heterozygotes show a consistent gradual decline with the distance from the pollen parent (Figs. 3 and 5), a significant percentage of cross-pollinated progeny occur even at a considerable distance. The fact that cross-pollination occurred at great distances from the pollen parent is probably the result of

indirect pollen transfer by pollen exchange in the beehive (13). This might be responsible for the constant levels of 'Ettinger' and 'Fuerte' hybrids, even at a considerable distance from the pollen parents (Fig. 3).

Our results (Figs. 3 and 5) reconfirm our previous findings that significant outcrossing does occur at a great distance from a potential pollen donor (19). Thus, the conclusion reached in California (2) that, for breeding purposes, "outcrossing rate should be practically nil within 100 m isolating distance", is not supported by our results.

It should be noted that rates of cross-pollination, or cross fertilization, were not determined in this study. Hybrid tissues (embryo and endosperm) could be separated from maternal tissue (seed coat) only after the fruitlets had reached a minimum weight of 50 mg. At that time, the fruitlets were already about 4 weeks old (14, 16), and a massive abscission of flowers and fruitlets had occurred. The extent of this abscission may be affected by the pollen parent (1). Fruitlets originating from different pollen parents exhibit different rates of abscission; therefore one can not assume that the percentage of hybrids in 1- month-old fruitlets reflects the rate of cross-pollination. In fact, we found significant changes in the percentage of 'Ettinger' and 'Fuerte' hybrids during 'Hass' fruit development (Table 2). One month after fruit set, the population of 'Hass' fruitlets consisted mainly of 'Hass' selfs, the rate of hybrid occurrence being 22% (11% identified). At the end of the abscission period, the rate of hybrids had increased to 84% (42% identified). This increase in percentage of hybrids resulted from selective abscission in which progeny of 'Ettinger' and 'Fuerte' displayed an advantage over 'Hass' selfed progeny. A similar trend was found by Gazit and Gafni during the first 3 weeks of fruit development following hand-pollination (16).

When 'Ettinger' served as a pollen parent for 'Fuerte', no selective abscission of fruitlets occurred (Table 3). It should be noted, however, that we had to start the isozyme analysis with fruitlets weighing 100 mg, since LAP-2 activity could not be detected until that stage. It is, therefore, possible that, in the early stages of fruit development, selective abscission of 'Fuerte' selfs vs. 'Ettinger' progeny took place. This may be expected from the finding that a higher survival of 'Fuerte' x 'Ettinger' hybrid fruitlets than of 'Fuerte' selfs occurred after hand-pollination (16).

The selective abscission of fruitlets may be affected by factors such as genetic selection, sensitivity to environmental conditions in the orchard, and the ability to compete with vegetative growth and with neighboring fruitlets. The enormous number of flowers found in avocado and the massive abscission of fruitlets point to the importance of tolerance and competitive ability in the survival of fruitlets. It is possible to increase the survival chances of the fruitlets of a given cultivar by genetic improvement of the embryo and endosperm, using a pollen parent that confers traits of improved tolerance and greater competitive ability. Although 'Ettinger' was found to be a potent pollen parent for 'Hass' (Fig. 3 and ref. 19), it cannot be assumed that 'Ettinger' will always excel as a pollen donor. The positive effect of 'Ettinger' on yield is probably due mainly to its capability to bestow properties such as greater tolerance to environmental stress and greater ability to compete with vegetative growth. However, these effects may not be manifested under conditions where such stress or competition do not occur.

A high percentage of hybrid fruits does not necessarily ensure a high yield (19). Usually,



however, a substantial increase in outcrossing rate may be assumed to be accompanied by a significant increase in fruit yield. A correlation between outcrossing rate and yield in 'Hass' groves was demonstrated in the present study (Fig. 3), as well as by Vrecener-Gadus and Ellstrand (32).

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