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Quantitative analysis of avocado outcrossing and yield in California using RAPD markers

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Abstract

The rate of outcrossing in orchards containing 'Hass' avocado (*Persea americana* Mill.) was determined using RAPD markers. The data represented 2393 fertilization events sampled from two climatic regions of southern California over a period of 4 years. In addition, three potential pollen sources (cultivars 'Bacon', 'Fuerte' and 'Zutano') were investigated using RAPD markers specific to each pollen source. Finally, yield data were collected from sampled trees to investigate the relationship between outcrossing and yield. Log-linear analyses of the resulting data showed that 'Fuerte' was the most effective pollinator of 'Hass' maternal trees independent of climatic region. The analyses also showed that outcrossing rate was strongly dependent on distance from a potential pollen source. There was a weak positive correlation between outcrossing and yield. Most of the variation in yield appeared to be accounted for by other causes. The total data suggested that self-fertilization was responsible for a substantial fraction of fruit set in California groves.

Keywords: *Persea americana*; Outcrossing rate; Molecular marker; RAPD

1. Introduction

The complex breeding system of cultivated avocado (*Persea americana* Mill.) is thought to be an adaptation to insure cross-pollination. Avocado cultivars are divided into two breeding categories, types A and B. During the first floral opening of type A flowers, which occurs during the morning hours, the stigmatic

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surface becomes receptive to pollen, but type A flowers do not produce pollen until approximately 30 h later. The anthers of type A flowers dehisce during the second floral opening which occurs in the afternoon of the following day (approximately 30 h later). Type B trees have a complementary flowering sequence with stigmatic receptivity occurring in the afternoon during the first floral opening. Then the flower closes and reopens the following morning when the anthers dehisce (Stout, 1923; Davenport, 1986). The complementarity is believed to insure that B type pollen is available to fertilize A type flowers and conversely A type pollen is available to fertilize B type flowers. This system of flowering is referred to as synchronous dichogamy.

Davenport (1989, 1992) and Davenport et al. (1994) have explored the consequences of the synchronously dichogamous breeding system in avocado in an extensive series of experiments in Florida. This work was based on direct measures of pollination including microscopic examination of stigmas exposed to insect pollination, observation of insect visitation behavior, and the enclosure of tree branches in cheesecloth bags to prevent cross-pollination. The results of these experiments indicated that (1) self-pollination was a major cause of fertilization; (2) all self-fertilization occurred during the second floral opening period; (3) variation in relative humidity was an important variable in determining the propensity for self-pollination in late stage flowers; and (4) insect vectors did not appear to play an important role in mediating cross-pollination. The general conclusion reached from these experiments was that self-pollination was the major cause of fruit set in commercial cultivars in Florida (Davenport et al., 1994).

In contrast, research in California has tended to support the idea that cross-pollination was important for fruit set. For example, Bergh (1967) concluded that bee pollination was very important in California in direct contrast to the Florida observations described above. Vrecenar-Gadus and Ellstrand (1985) used isozymes to establish a weak correlation between outcrossing and yield in California ‘Hass’ groves. Similarly, Markle and Bender (1992) reported that yield was substantially enhanced in an orchard, where cross-pollination was facilitated by the interplanting of type A and type B trees. However, the interplanting of type A and B trees has changed over the last 20 years to commercial plantings of large monocultures of a single genotype (‘Hass’) in California. The trend towards large ‘Hass’ monocultures is correlated with a long-term trend of decreasing fruit yields. This raises the question of the importance of cross-pollination with the possibility that the trend in decreasing fruit yield is a consequence of the absence of pollination sources (type B trees) available in large ‘Hass’ (type A) monocultures.

The California market is habituated to the ‘Hass’ fruit, which has many desirable qualities. Fruit of other (type B) cultivars, which are suitable as pollen sources, tend to bring a lower market price. Thus, a loss in yield may be more

than compensated for by market price, but there is clearly a point where profit may be maximized by mixed plantings. Determining this point is subject to many uncertainties. First, temperature and humidity during the flowering season, soil type and a host of other abiotic factors combine to influence yield and some of these factors vary in an unpredictable fashion from year-to-year (Bergh, 1967). It is, therefore, necessary to collect data over a number of consecutive years and in a number of different locations to arrive at some average measure of the importance of cross-pollination. A second source of variation is the effectiveness of various type B cultivars as potential pollen sources for 'Hass' maternal parents. The determination of effectiveness as a pollen source requires that genetic markers be used to identify the specific pollen source of successful pollination events. Moreover, the precise quantitative measurement of outcrossing rates requires the use of genetic markers to detect individual outcrossing events.

Isozymes lack the genetic resolution in avocado to detect most outcrossing events, owing to the fact that Hass is heterozygous for the small number of isozyme markers available (J. Clegg, personal communication). Using a DNA approach, it was necessary to dissect the embryonic tissue out of the seed and prepare DNA from this tissue to determine the pollen source of individual fruit. With the small DNA yields (30–150 ng) from avocado embryos, RFLP (restriction fragment length polymorphism) was ruled out as a possible technique due to the requirement of at least 5 μg of DNA to produce one southern blot. Small amounts of DNA available for analysis dictate that a polymerase chain reaction (PCR) based method be employed. One class of PCR-based markers that is particularly promising for investigations of avocado outcrossing are RAPD (random amplified polymorphic DNA) markers (Williams et al., 1990). These markers are generated by the amplification of random DNA segments of the avocado genome with single primers of arbitrary nucleotide sequence. No knowledge of target DNA sequence is required. Moreover, a large number of potential RAPD markers is available for use in the development of cultivar-specific markers. With cultivar-specific markers, the actual source of individual outcrossing events could be identified.

The objectives of the research described in this article are: (1) to measure outcrossing rates in commercial groves using RAPD markers; (2) to investigate the putative relationship between outcrossing and climatic region (coastal and inland regions of California); (3) to investigate the yearly variance in outcrossing rate over a period of four consecutive years; (4) to use specific markers for the cultivars 'Bacon', 'Fuerte' and 'Zutano' to determine the cultivar that is most effective as a pollen source in 'Hass' commercial groves; (5) to investigate the relationship between pollination success and distance from a potential pollen source; and (6) to estimate the correlation between fruit yield and outcrossing rate to test the hypothesis that the long term decline in fruit yield in 'Hass' commercial groves is accounted for by the absence of type B pollen sources.

2. Materials and methods

2.1. Plot design

The experimental materials consisted of 'Hass' fruit from trees in private groves at several locations in southern California. The first criterion for selection of the collection site was based on the presence of a tree (or trees) of 'Bacon', 'Fuerte' or 'Zutano' (all have type B flowering pattern) along the edge row of the orchard to serve as a potential pollen donor. The second criterion was to have sites from the inland and coastal climatic regions of southern California. In the inland region, three groves in Riverside county were chosen each of which included the pollen sources, 'Bacon', 'Fuerte' and 'Zutano'. For the coastal region, three groves were chosen in Ventura county that contained each of the three pollen sources. One grove in Santa Barbara county was selected that had both a 'Bacon' and a 'Fuerte' site.

Fruit collection for cross-pollination analysis was done at the beginning of 'Hass' avocado harvest, usually late November to early December. At each site, two trees were selected from each of the three rows, 1, 5 and 15 rows from the potential pollen source. From these six trees, 20 fruits were collected and the number of total fruits on the tree was counted. In any particular year, the maximum number of fruit collected from the eight sites was 960. This number could change due to a number of different factors, such as the alternate-bearing nature of 'Hass' and weather factors, such as high wind, which could cause early fruit drop.

2.2. DNA isolation

DNA was isolated from each embryo of the collected fruit, using a modified DNA extraction procedure of Rawson et al. (1982). Each embryo was removed from the cotyledons and placed in a mortar with liquid nitrogen. The embryo was ground to a fine powder and transferred to a 1.5 ml microcentrifuge tube. To the tube, 200 μl extraction buffer (0.35 M sucrose, 100 mM Tris, 50 mM KCl, 5% polyvinylpyrrolidone (FW 40 000), 25 mM EDTA and 10 mM diethyldithiocarbamic acid) was added. Additional grinding was done with a micropestle until the powder was completely moistened. Along with 500 μl of lysing buffer (100 mM EDTA, 50 mM Tris), 50 μl 25% Triton X-100, 50 μl 20% sarkosyl and 2 μl proteinase K (20 mg/ml) were added to the mixture and inverted several times. The tube was incubated at 37°C for a total of 40 min, with one inversion of the tube at 20 min. The tube was centrifuged at 13 000 rpm for 5 min at 4°C. The supernatant was transferred to a fresh microcentrifuge tube. With the addition of 750 μl isopropanol, the mixture was allowed to precipitate overnight at −20°C. The microcentrifuge tube was centrifuged at 13 000 rpm for 15 min at 4°C and the supernatant discarded. The pellet was washed with 75% ethanol and resuspended in 500 μl water. The enzyme, RNAse A (20 μg) was added to the

resuspended mixture to digest any contaminating RNA and the tube was incubated at 37°C for 30 min. To remove the enzyme and any other contaminating protein, phenol/chloroform extraction was performed. This involved the addition of an equal volume of phenol/chloroform (50:50 v/v) to the tube. The tube was inverted several times and centrifuged for 5 min. The top layer was transferred to a fresh tube and an equal volume of chloroform/isoamyl alcohol (24:1 v/v) was added. The tube was inverted several times, centrifuged at 13 000 rpm for 5 min and the top layer transferred to a fresh tube. The final precipitation of the DNA was done by adding an equal volume of isopropanol and $\frac{1}{10}$ volume of a 3 M sodium acetate. The resulting DNA pellet was washed with 75% ethanol, then resuspended in 500 μl water and stored at 4°C. Typically, 5 μl of embryo DNA was used in a PCR amplification reaction.

2.3. PCR amplification of RAPD markers

Each 25 μl PCR volume included 5 μl of the embryo DNA, 1X Taq buffer (without $MgCl_2$), 1 mM $MgCl_2$, 0.1 mM dNTP, 0.2 μM primer, 1 unit Taq DNA polymerase. The PCR was performed in a MJ Research PTC-100 Thermalcycler (Watertown, MA) with the cycling program of 45 cycles of 1 min at 94°C, 1 min at 37°C, 30 s at 54°C, and 2 min at 72°C and with a final extension of 15 min at 72°C. The PCR products were electrophoresed in 1.7% agarose gel in TBE at 50 V for 4 h. The gel was stained in ethidium bromide and photographed using UV fluorescence.

2.4. Verification of marker inheritance

Because RAPD markers are typically dominant, the verification of the inheritance of each cultivar-specific marker was essential. Progeny tests were used to establish the genotype of each cultivar of interest with respect to a RAPD marker. For example, self-pollinated progeny of the 'Hass' cultivar were assayed for RAPD markers to verify inheritance patterns when the marker was heterozygous in the 'Hass' parent. Fig. 1 displays a RAPD gel for segregating 'Hass' self-pollinated progeny which exhibits the expected 3:1 segregation pattern. Progeny tests were therefore employed to determine whether the cultivar was homozygous or heterozygous for the RAPD marker. When a cultivar-specific RAPD marker was homozygous, all outcrossing events could be directly scored; however, if the RAPD marker was heterozygous, only half of the outcrossing events could be detected and the actual outcrossing rate was estimated by doubling the observed frequency of outcrossing events.

The inheritance of a marker was further assessed using a panel of 30 'Hass' embryos determined to be cross-pollinated with 'Bacon', 'Fuerte' or 'Zutano' by RFLP analysis (Davis et al., 1998; unpublished data). Based on the criteria listed

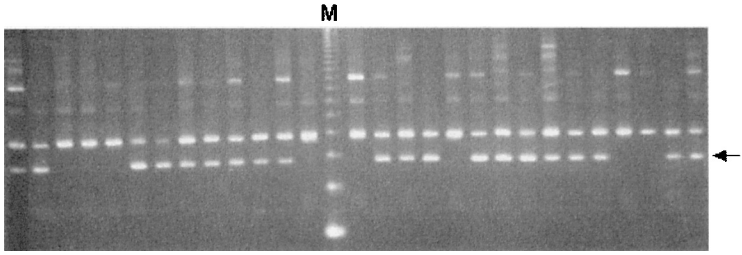

Fig. 1. RAPD banding pattern produced with primer OPA-15 (5′-TTCCGAACCC) in 28 self-fertilized progeny of a 'Hass' parent. Arrow indicates a marker segregating according to the expected 3:1 ratio of the presence versus absence of a band (χ^2=0.191, NS), indicating that the 'Hass' parent is heterozygous for this marker. Lane M is 1 kb ladder molecular weight standard.

above, the RAPD marker OPD-16 was homozygous for 'Bacon' and 'Zutano'; absent in 'Hass' and 'Fuerte'. The 'Fuerte' RAPD marker, OPE-14, was heterozygous, indicating that the observed 'Hass' outcrossing rate with 'Fuerte' must be doubled to account for all the outcrossing events. This RAPD band was absent in 'Hass', 'Bacon' and 'Zutano'.

3. Results

3.1. Characterization of climatic regions

When comparing the average maximum and minimum temperatures, and mean temperatures during avocado flower bloom (March–mid-May, Arpaia, 1997) of the two climatic regions, the coastal area was slightly cooler than the inland region (Table 1). Precipitation in the coastal regions was approximately 2–5 times

Table 1
Temperature and precipitation for the inland and coastal regions of southern California in March, April, and May[a]

	Inland region			Coastal region		
	March	April	May	March	April	May
Average maximum temperature (°C)	20.9	23.9	26.1	19.6	20.8	21.4
Average minimum temperature (°C)	7.1	8.7	11.2	7.7	9.2	10.8
Mean temperature (°C)	14.0	16.3	18.7	13.6	15.0	16.1
Average total precipitation (in.)	1.82	0.78	0.25	4.19	2.77	1.17

[a] Data source: Western Regional Climate Center (www.wrec.dri.edu). Recording period for inland region was from July, 1948 to December 1998, and that for coastal region was from December, 1927 to December, 1998.

the precipitation in the inland area. These climatic factors were taken into consideration when examining cross-pollination and fruit yield.

3.2. Selection of cultivar specific markers

A total of 332 decamer random primers (Operon Technologies, Alameda, CA) were screened to find cultivar-specific RAPD markers for 'Bacon', 'Fuerte' and 'Zutano'. The first criterion was the primer producing a polymorphic banding pattern among the cultivars. The second criterion was the presence of a band in 'Bacon', 'Fuerte' or 'Zutano' and its absence in 'Hass'. Five primers, OPC-2, OPC-7, OPE-12, OPE-13, OPE-18, produced a band in 'Bacon' that was absent in 'Fuerte', 'Zutano' and 'Hass'. One primer, OPD-11, produced a band in 'Zutano', not found in 'Bacon', 'Fuerte' and 'Hass'. For 'Fuerte', five primers, OPC-18, OPE-13, OPE-14, OPF-11, OPG-7, produced a band which was not present in 'Bacon', 'Zutano' and 'Hass'. There were a number of primers which produced a band in two or more varieties: 'Bacon' and 'Fuerte' shared a marker for one primer (OPB-5); 'Bacon' and 'Zutano' had shared a marker for 14 primers (OPA-4, OPB-11, OPC-5, OPC-9, OPC-15, OPD-5, OPD-6, OPD-16, OPD-20, OPE-11, OPF-2, OPG-8, OPG-13), 'Bacon', 'Fuerte' and 'Zutano' shared a marker for two 10-mers (OPA-1, OPA-9).

3.3. Statistical analysis

A total of 2393 fertilization events were assayed. Each assay was classified as to RAPD phenotype (and hence genotype based on knowledge of the parental genotypes), location of the maternal tree, year and maternal tree fruit yield. These data were arranged into a multidimensional contingency table, where each cell contained the number of fruit per tree on each row (averaged over two trees) in the various design categories. Initial analysis indicated that outcrossing rate did not vary by years. The outcrossing rate averaged over 4 years, all pollen sources, and all locations was 0.371. Table 2 presents outcrossing rates and yield data by location, averaged over years and replicate trees. The log-linear model for analysis of variance for categorical variables (Bishop et al., 1974) was fitted to the data combined over years, first with main effects and two-way interactions, and then by adding more terms to the model. The fit of the data to the model was tested by comparing the reduced model to the full model by a likelihood ratio test using PROC CATMOD in SAS (SAS Institute, 1989). The final model used was the following:

$$\begin{aligned}\log m_{ijkl} = {} & \mu + A_i + B_j + AB_{ij} + C_k + AC_{ik} + BC_{jk} + ABC_{ijk} + D_l \\ & + AD_{il} + BD_{jl} + ABD_{ijl} + CD_{kl} + ACD_{ikl} + BCD_{jkl} \end{aligned} \quad (1)$$

Table 2
Outcrossing rate and fruit yield averaged over 4 years (1993–1996) and over two trees on each row in three geographic regions

Region	Pollen source	Row	Outcrossing rate (%)	Fruit yield (fruit/tree)
Riverside	Bacon	One	32.6	178.7
		Five	27.8	81.7
		Fifteen	16.0	22.5
	Fuerte	One	104.5	162.0
		Five	44.8	108.1
		Fifteen	34.9	74.6
	Zutano	One	28.8	272.9
		Five	18.4	78.8
		Fifteen	6.6	63.9
Ventura	Bacon	One	18.2	217.2
		Five	27.7	146.2
		Fifteen	11.7	35.0
	Fuerte	One	65.8	67.0
		Five	46.4	139.2
		Fifteen	33.0	330.5
	Zutano	One	52.6	33.7
		Five	41.8	35.3
		Fifteen	36.7	39.2
Santa Barbara	Bacon	One	18.7	235.3
		Five	25.0	212.2
		Fifteen	26.0	148.3
	Zutano	One	58.8	177.8
		Five	48.0	160.4
		Fifteen	24.4	225.8

where $\log m_{ijkl}$ is the logarithm of cell frequency (fruit count) of lth type of mating on kth row with jth pollen source at ith location, μ the overall mean of the logarithm of cell frequency, A_i the effect of location (i=1, 2, 3), B_j the effect of pollen source (j=1, 2, 3), AB_{ij} the interactive effect of location and pollen source, C_k the effect of row number (distance to pollen source) (k=1, 2, 3), AC_{ik} the interactive effect of location and row number, BC_{jk} the interactive effect of pollen source and row number, ABC_{ijk} the interactive effect of location, pollen source, and row number, D_l the effect of mating (outcrossing or selfing) (l=1, 2), AD_{il} the interactive effect of location and mating, BD_{jl} the interactive effect of pollen source and mating, ABD_{ijl} the interactive effect of location, pollen source and mating, CD_{kl} the interactive effect of row number and mating, ACD_{ikl} the interactive effect of location, row number and mating, and BCD_{jkl} is the interactive effect of pollen source, row number and mating. Note that only the interactive effects of mating and other sources of variation are of interest in our

experiment. Significance of the various components of variance was determined by maximum likelihood approach using PROC CATMOD in SAS (SAS Institute, 1989).

The relationship between outcrossing rate and yield (number of fruits per tree) was examined for the three locations (Riverside, Ventura and Santa Barbara counties) separately, where data for each year were treated as independent data points to retain sufficient sample size for each population. A Pearson correlation coefficient was calculated using PROC CORR in SAS (SAS Institute, 1989).

The likelihood ratio test was non-significant (P=0.4997) indicating that the current model with three-way interactions fits the data and does not deviate from the full model (Table 3). Maximum likelihood analysis of variance showed that there was marginally significant effect of location and pollen sources on outcrossing rate (terms AD_{il} and BD_{jl}, respectively, Table 3), whereas there was highly significant effect of row number on outcrossing rate (term CD_{kl}). On average, outcrossing rate in the coastal regions (Ventura and Santa Barbara) was higher than in the inland region (Riverside) (Fig. 2A). Populations growing with the 'Fuerte' variety had the highest outcrossing rate, whereas those growing with the 'Bacon' variety had the lowest outcrossing rate (Fig. 2B). There was, however, a highly significant location×pollen source interactive effect on

Table 3
Maximum-likelihood analysis-of-variance of outcrossing frequency in avocado

Source[a]	DF	Chi-square	Probability
A_i	2	25.79	0.0000
B_j	2	26.04	0.0000
AB_{ij}	4	28.58	0.0000
C_k	2	1.33	0.5153
AC_{ik}	4	1.18	0.8806
BC_{jk}	4	2.25	0.6906
ABC_{ijk}	8	11.38	0.1809
D_l	1	41.92	0.0000
AD_{il}	2	6.14	0.0464
BD_{jl}	2	7.44	0.0243
ABD_{ijl}	3	36.64	0.0000
CD_{kl}	2	24.13	0.0000
ACD_{ikl}	4	7.83	0.0979
BCD_{jkl}	4	7.78	0.1001
Likelihood ratio	6	5.35	0.4997

[a] Source of variation: A_i, location; B_j, pollen source; AB_{ij}, location×pollen source; C_k, row number; AC_{ik}, location×row number; BC_{jk}, pollen source×row number; ABC_{ijk}, location×pollen source×row number; D_l, mating; AD_{il}, location×mating; BD_{jl}, pollen source×mating; ABD_{ijl}, location×pollen source×mating; CD_{il}, row number×mating; ACD_{ikl}, location×row number×mating; BCD_{jkl}, pollen source×row number×mating.

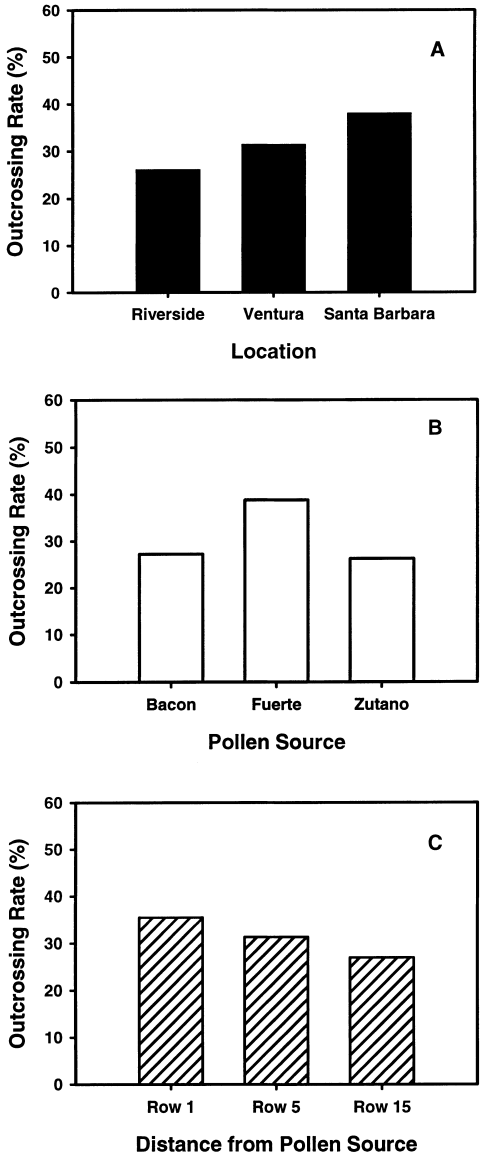

Fig. 2. Average outcrossing rate in avocado by: (A) locations; (B) pollen sources; and (C) distance from pollen source.

outcrossing rate as evidenced by the significant ABD_{ijl} term (Table 3). This was because there was a change in ranks of outcrossing rate with pollen sources 'Bacon' and 'Zutano' between locations (Fig. 3). In the inland location, Riverside, populations growing with 'Bacon' had higher outcrossing rate than those growing with 'Zutano', whereas in the coastal location, Ventura,

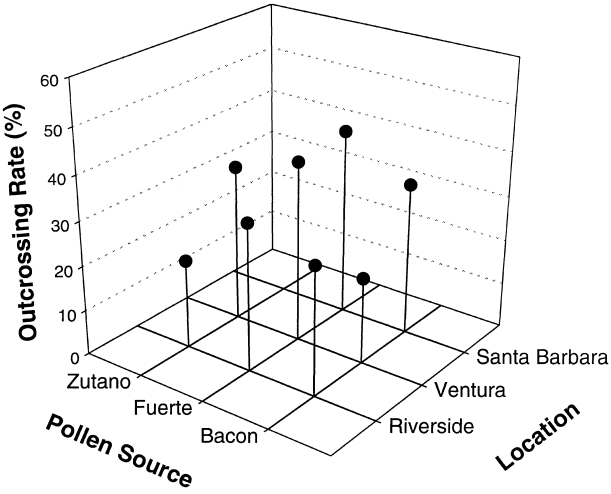

Fig. 3. Average outcrossing rate of avocado at three locations with three pollen sources. Location×pollen source interaction is evidenced by the rank change of 'Bacon' and 'Zutano' between Riverside and Ventura.

populations growing with 'Zutano' had higher outcrossing rate than those growing with 'Bacon'. The average outcrossing rate decreased as the distance from a pollen source increased from row 1 to row 15 (Fig. 2C).

There was a marginally significant positive correlation between outcrossing rate and yield at Ventura, while there was no significant correlation at Riverside and Santa Barbara (Fig. 4). The proportion of variation in yield attributable to outcrossing rate was rather small (R^2=0.01–0.25, or 1–25%).

4. Discussion

The data set analyzed in this experiment derives from the RAPD assay of 2393 individual fertilization events. Despite this relatively large scale, a number of factors comprised the experimental design, including different locations, years and pollen sources. Thus, the power to detect deviations from the null hypothesis is limited. It is therefore noteworthy that there are consistent differences in outcrossing between the inland (lower outcrossing) and the coastal (higher outcrossing) regions. Evidently higher outcrossing is favored in the cooler and more humid coastal regions. Higher outcrossing rates in 'Hass' orchards is also strongly influenced by proximity to a type B pollen source. The outcrossing rate falls off significantly when the pollen source is 15 rows away from the maternal tree. We conclude that both climatic region and proximity to a pollen source are important factors controlling the mating process.

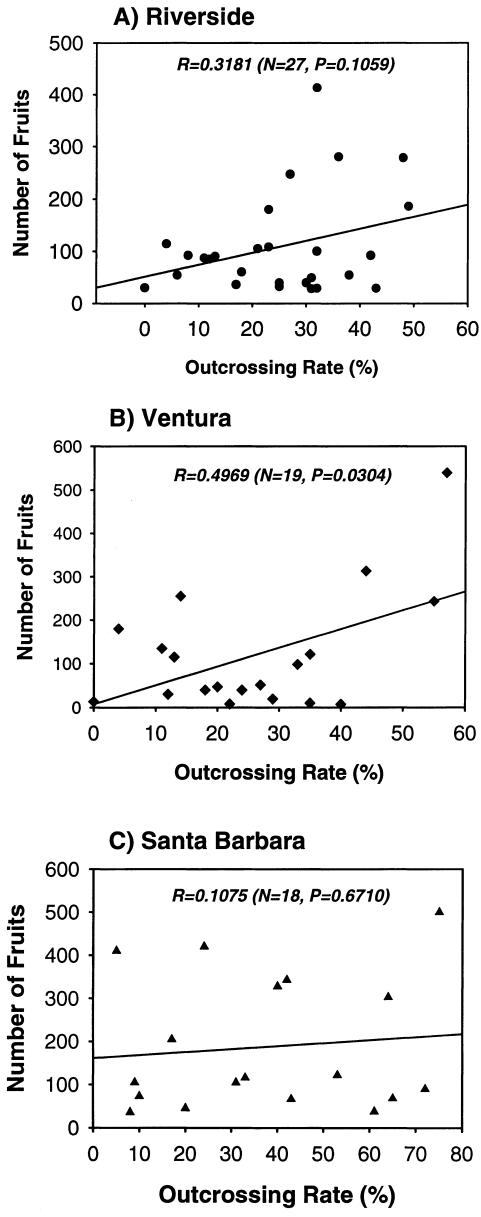

Fig. 4. Scatter plots of outcrossing rate vs. yield (number of fruits) of avocado at three locations (with simple regression line and correlation coefficient).

An important feature of the experimental design was the ability to classify outcrosses by pollen source. This allowed an assessment of the efficacy of three different cultivars as pollen sources (Fig. 2B). The results clearly establish that 'Fuerte' is the most effective pollen source for 'Hass' maternal trees independent

of climatic region. Accordingly, we recommend 'Fuerte' as a pollen source when it is deemed advantageous to have a mixed planting.

The data indicate a positive correlation between outcrossing rate and yield (data not shown), but when the data are partitioned by location the significant effect is accounted by the Ventura location. The Riverside location has two data points that may be outliers and if these points are removed, then the probability value is 0.0481, which is significant; however, the fraction of the total variance in yield accounted for by variation in outcrossing is small (R^2=16%). These results are consistent with work by Vrecenar-Gadus and Ellstrand (1985) who used isozyme markers and a somewhat different experimental design to measure the correlation between outcrossing and yield in a single year at a single south coast location (R^2=10.8%). The consistency between the present results, which span two major climatic regions and 4 years, and those reported earlier is impressive and further increases our confidence in the conclusion that outcrossing rate variation accounts for a small fraction of the total variation in yield in California.

Many factors cause variation in avocado yield including the alternate bearing tendency of 'Hass' avocados, climatic variations from year-to-year and the availability of pollination vectors. The results of this experiment suggest that variation in outcrossing rate is not the dominant cause of yield variation. Management techniques such as careful control of water and fertilizer regimes may be as effective as mixed plantings in increasing yields and these are much less expensive to implement. The fact that the data from this experiment span an interval of 4 years and cover two major climatic regions suggest that the average values reported here are useful guides for avocado management.

Work on avocado genetics has revealed relatively high levels of heterozygosity and a diverse gene pool (Furnier et al., 1990; Mhameed et al., 1997; Davis et al., 1998). In addition, the synchronously dichogamous mating system is evidently an adaptation to promote outcrossing which would suggest relatively low levels of inbreeding under natural conditions. Based on these observations, we expect genetic loads to cause early fruit abortion following self-fertilization. Research of Degani et al. (1997) in Israel using isozyme markers has shown a substantial increase in the apparent outcrossing rate between immature and mature fruit stages. Evidently abscission of selfed fruitlets occurs with a much higher probability than for fruit derived from out-pollination. Our sampling design involved the collection of mature fruit subsequent to early fruit drop, so the genetic census data may be biased in the direction of higher outcrossing, owing to the selective abortion of some fruitlets that originated from self-fertilization. This bias suggests that the rates of self-fertilization observed in all locations are an underestimate of the true frequency of self-fertilization at the time of union of gametes. In view of these considerations it is important to note that rates of self-fertilization are substantial in all regions and this is especially true when one recalls that the experimental design required selecting orchards with other pollen

sources adjacent to the grove, thereby biasing outcrossing estimates upwards. We must therefore conclude that a substantial proportion of the avocado fruit that set in California orchards are the result of self-fertilization.

It is possible that fruits that derive from outcrossing may be retained longer on trees during periods of extreme climatic stress. The four years included in this study did not include any episodes of extreme stress such as very high temperatures during the period of pollination and early fruit set. Nevertheless, the conclusions of this study would appear to be fairly robust and certainly applicable to normal growing years in California. The conclusions of particular importance to avocado growers are that the majority of fruit derive from self-pollination and there is a very weak correlation between fruit yield and outcrossing. Based on this conclusion it would not appear to be economically justified to grow mixed plantings of type A and B cultivars, especially if fruit of the type B cultivar brings a lower market price.

Acknowledgements

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