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THE EFFECT OF PLANTING DESIGN ON OUT-CROSSING RATE AND YIELD IN THE 'HASS' AVOCADO

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ABSTRACT

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Two enzyme loci were used to measure out-crossing rates in pure and inter-planted groves of 'Hass' avocados. Both out-crossing rate and yield per tree were found to be significantly higher in the inter-planted grove. Out-crossing rate and yield were significantly correlated overall, but the relationship within groves was too weak for significance. This information should prove useful as a basis for future studies on methods for maximizing avocado fruit production.

Keywords: avocado; electrophoresis; isozymes; *Persea*.

INTRODUCTION

The avocado (*Persea americana* Mill.) is a tropical fruit tree grown throughout coastal areas of Southern California. Over the years, the avocado has been the subject of several reproductive studies due to its unusual breeding system. Avocado flowers are known to exhibit a dual cycle of opening known as complementary synchronous dichogamy (Nirody, 1921; Stout, 1922, 1932; Bergh, 1969). When a flower first opens, the stigma is receptive and the anthers have not yet dehisced. Therefore, the flower is functionally female. After remaining open for several hours the flower closes, but re-opens the following day. This time, the stigma is dried and unreceptive, but the anthers are mature. The flower is now functionally male.

Furthermore, there are two avocado breeding types. All flowers on Type A trees are in their female stage one morning and re-open as males the following afternoon. Those flowers on Type B trees, on the other hand, first open as female in the afternoon and re-open as male the next morning. Under ideal conditions, all the flowers on a given tree or cultivar will go through their cycle in synchrony. This complementary floral opening between breeding types is thought to promote out-crossing (Bergh, 1969).

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In an agricultural situation, therefore, the inter-planting of two complementary cultivars would seem to be necessary to obtain optimum yields (Bergh, 1977). However, this dichogamous phenology is known to be affected by temperature. Low temperatures cause delayed flower opening which increases the opportunity for self-pollination (Lesley and Bringhurst, 1951; Bergh, 1969; Sedgley and Annells, 1981; Sedgley and Grant, 1983).

Because of the possibility of self-pollination, the question arises whether inter-planting of cultivars is actually necessary in a commercial orchard. Several attempts have been made to address this problem. Clark (1922, 1923) found no difference in yield between isolated trees and those exposed to other cultivars. Nets were used both to isolate trees and to confine several cultivars together. However, using field studies, Bergh and co-workers (Bergh and Gustafson, 1958; Bergh and Garber, 1964; Bergh et al., 1966) discovered that avocado trees planted adjacent to (or grafted with) a complementary cultivar had significantly higher yields. Cross-pollination between the two cultivars was presumed to be the cause. Although Bergh's studies clearly implicated out-crossing as the primary factor in producing higher yields, no method was available to them to directly measure out-crossing rates.

Progeny testing with genetic markers allows for the direct evaluation of whether a fruit is the product of self- or cross-pollination. In particular, the biochemical genetic markers detected by gel electrophoresis, isozymes, have proved to be especially useful for the measurement of out-crossing rates because they can be assayed both from parent and progeny (seed) tissue (Tanksley and Orton, 1983a, b). Torres et al. (1978) introduced isozymes to avocado to characterize known cultivars. Since that time, isozymes have also been used to distinguish hybrid offspring from selfs and to determine out-crossing rates between cultivars (Torres and Bergh, 1978a, b; Degani and Gazit, 1984).

The work presented in this paper used electrophoretic markers to test whether cross-pollination between complementary avocado cultivars correlates with yield and to determine the effect of planting design on these parameters.

MATERIALS AND METHODS

'Hass' orchards with two different planting designs were located on the Irvine Ranch in Orange County, California. One grove (the "mixed" grove) was inter-planted with 'Bacon' trees (1 row 'Bacon': 1 row 'Hass'). This planting design was continuous for approximately 4 ha. The study was located in the center of this orchard. The orchard was isolated from other cultivars by over 800 m. The second grove (the "pure" grove) consisted solely of 'Hass' trees, isolated from another cultivar ('Bacon') by over 80 m, a distance generally considered to preclude cross-pollination by bees (McGregor, 1976).

Care was taken to choose groves with trees of similar age growing under approximately the same conditions. Both orchards occur on east-facing slopes less than 2.5 km apart. The pure 'Hass' grove is planted on a 5 × 6 m spacing, while the mixed grove is a 5 × 7 m design; any effects of tree-spacing should be about the same for both areas. Furthermore, the orchards chosen differ in age by only 1 year. The trees in the pure 'Hass' grove were planted in 1975, and the mixed grove was established in 1976. Thus, if age influences yield or out-crossing rate, the measurements should be slightly biased in favour of the older orchard. Irvine Ranch Company records also show that the trees at both sites have a coordinated alternate bearing cycle. Therefore, alternate-bearing habits should not influence any yield comparison.

Two plots of 121 'Hass' trees were marked within each grove. Twenty randomly selected trees per plot were tagged and yields were obtained using a hand counter. Fruits approximately 6 months old were collected from November to December 1983. A sample of 10 fruits per tree was taken when possible, but very low yields on some trees made it necessary to take as few as 5. A total of 777 fruits were collected from all 4 plots. These were then stored in a cold room (4°C) until electrophoresis was to be performed.

Extracts from a cotyledon of each seed were prepared and run according to a previously described procedure (Vrecenar, 1984). The isozyme loci, malate dehydrogenase-1 (MDH-1) and triose phosphate isomerase-1 (TPI-1) were used to detect out-crossing, since 'Hass' is SS for both loci while 'Bacon' is FS in both cases. Slow (S) and fast (F) refer to the location of the bands on a stained gel, relative to the origin. Therefore, FS offspring for either locus must be the result of a 'Hass' × 'Bacon' cross. Some SS offspring may also result from an out-cross. Since one-fourth of the out-cross gametes cannot be detected, the number of definite out-crosses (FS offspring) was multiplied by 1.33 for each tree. The two loci have been shown to assort independently with Mendelian segregation (Vrecenar, 1984).

After out-crossing rates were calculated, an ANOVA was performed to test the effects of replications and planting design on out-crossing rate and yield. Finally, an ANOCOVA was done to determine of any relationship existed between out-crossing rate and yield for both sites.

RESULTS

Out-crossing rate in the 'Hass' avocados assayed was positively correlated with yield when analyzed over all replications and planting designs ($P < 0.004$, $r^2 = 10.8\%$, Fig. 1). However, within each grove, although yield and out-crossing rate were correlated, the relationship was not significant (pure: $P < 0.26$, $r^2 = 3.6\%$; mixed: $P < 0.21$, $r^2 = 4.3\%$).

Planting design had a significant effect on both yield and out-crossing rate. The mean number of fruits per tree was 138.8 for the mixed grove and 92.7 for the pure grove. This difference was significant ($P < 0.01$, Fig. 2).

An average of 89.6% of the fruits sampled in the mixed grove resulted from an out-cross. This out-crossing rate was significantly higher than the 42.2% out-crossing rate observed in the pure grove ($P < 0.001$, Fig. 3). Replication had a marginally significant effect on out-crossing rate at the pure site ($P < 0.056$). However, yield was not affected by this parameter ($P < 0.81$).

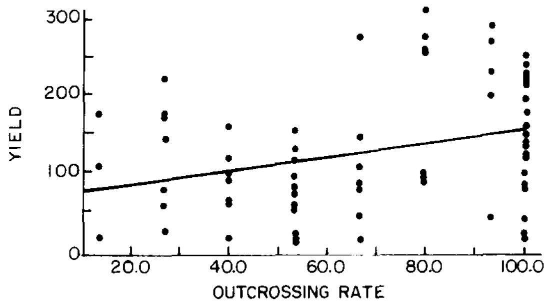
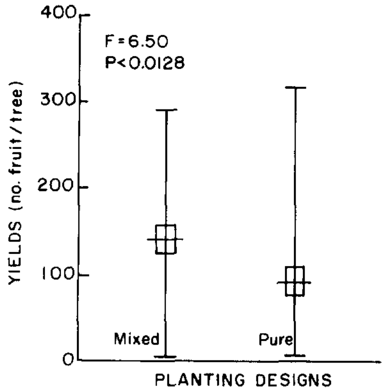


Fig. 1. Out-crossing rate vs. yield in 'Hass' from both pure (all 'Hass') and mixed (inter-planted with 'Bacon') groves (see text). Dots represent individual trees. Yield is measured in number of fruits per tree. Out-crossing rate is in percentage of sample.



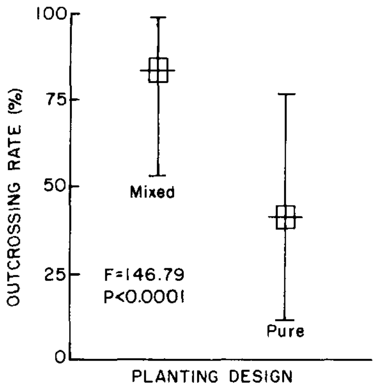


Fig. 2. The effect of planting design on yield from both pure (all 'Hass') and mixed (inter-planted with 'Bacon') groves (see text). The middle horizontal lines represent the means, the vertical lines represent the ranges, and the boxes indicate 1 standard error.

Fig. 3. The effect of planting design on out-crossing rate. The middle horizontal lines represent the means, the vertical lines represent the ranges, and the boxes represent 1 standard error.

DISCUSSION

Out-crossing between the two avocado cultivars 'Hass' and 'Bacon' correlated significantly with the number of 'Hass' fruits per tree. A mixed planting design of 'Hass' and 'Bacon' correlated with increased 'Hass' fruit production. The improved 'Hass' yield for the inter-planted 'Hass' and 'Bacon' avocados is best explained by the fact that the planting design promotes cross-pollination between the two cultivars. While it is possible that the minor differences of inter-tree spacing and the age of the groves could also have an impact on yield, the positive, but weak, relationship of out-crossing to yield *within* groves still implicates cross-pollination.

The out-crossing rates observed in the pure 'Hass' plots are high for an isolated grove (cf. Torres and Bergh, 1978a). However, the nearest beehives were approximately 600 m away in the center of a pure 'Bacon' orchard. Bees which foraged on the 'Bacon' trees in the morning could presumably then travel to the 'Hass' grove and accomplish pollination. The distance involved is not unusual for bees foraging on avocado trees (McGregor, 1976). This foraging pattern could also explain why replicates in the pure 'Hass' grove had a significantly different out-crossing rate. One plot is downhill and slightly closer to the 'Bacon' orchard. This replicate had relatively higher out-crossing and higher yields. Still, this semi-isolated grove did exhibit significantly lower yields than the mixed grove. Further studies using more isolated groves should be performed to address how out-crossing and yield decrease with isolation.

Several factors could explain the non-significant correlation observed between out-crossing rate and yield within each site. Replication of the pure site had a significant effect on out-crossing rate for this planting design. As stated above, Replicate 2 was less isolated than Replicate 1. Although the general trend between out-crossing rate and yield was still valid for Replicate 2, there was enough variation to affect the entire distribution of out-crossing rate for the pure planting design. The mixed site had such a high percentage of out-crossing that this out-crossing distribution was skewed (22 trees showed 100% out-crossing). As a result, no linear correlation was detected between this parameter and yield.

Out-crossing rates were very high in the mixed grove plots. This observed out-crossing rate could be due not only to the complementary flowering cycles of the two cultivars, but also to differential abortion of 'Hass' selfed fruits. Although there is apparently no cultivar difference in fertilization success rate (Sedgley, 1979), some studies suggest that certain cultivars make superior pollen parents and that selfed fruits are differentially aborted relative to fruits sired by these parents (Gazit, 1977; Degani and Gazit, 1984). Thus, the 4-month-old fruits sampled in this study may have exhibited inflated percentages of cross-pollination due to preferential abortion. To test this hypothesis, fruits should be analyzed to determine their parentage prior to abortion. However, such a study will necessitate the development of electrophoretic systems for extremely immature avocado seed tissue or embryo culture of very young seeds (S. Gazit, personal communication, 1984).

Although inter-planting was advantageous to 'Hass' fruit production, the optimum ratio of 'Bacon' or other pollinators to 'Hass' required per orchard is still unknown. 'Bacon' avocados are not currently as commercially desirable as certain other cultivars. Therefore, limiting the number of these trees while still deriving maximum pollination is advisable. Some other planting designs, based on those used in apple and almond orchards, have been suggested for interplanting avocados (Bergh, 1966; Lee, 1973). Electrophoretic markers for detecting out-crosses will allow testing of these designs and others for adequate pollen dispersal and optimal fruit set.

This study has shown that inter-planting 'Hass' and 'Bacon' avocados is beneficial to 'Hass' yield. The higher yields observed in inter-planted orchards is correlated with cross-pollination between the two cultivars. This information may prove useful as a basis for future studies on methods for maximizing avocado fruit production.

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