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Compatibility Relationships in 'Manzanillo' Olive

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Abstract. **We investigated pollination and fruit set parameters in 'Manzanillo' olive (*Olea europaea* L.) following self-pollination and pollination with 'Sevillano', 'Ascolano', and 'Mission' pollen. Results of analyses and experiments conducted over 2 years in central California indicated that 'Manzanillo' behaves as a self-incompatible cultivar (index of self-incompatibility = 0.22 to 0.24). Pollination with 'Sevillano' resulted in a more than 4-fold increase in fruit set over self-pollination. When 'Mission' or 'Ascolano' pollen was used, there was no increase over self-pollinated samples. Analyses of pollen tube growth, fertilization, initial fruit set, and final fruit set were consistent with 'Manzanillo' being considered as a self-incompatible cultivar cross-incompatible with 'Mission' and 'Ascolano'. Our results indicate that 'Manzanillo' is likely to be more productive when interplanted with 'Sevillano' rather than when planted without a pollinizer or with 'Mission' or 'Ascolano'.**

U.S. production of table olives is located entirely in California. 'Manzanillo', the most widely planted cultivar, accounts for 61% of California's total olive acreage and two-thirds of the state's production (Connell, 1994). Other cultivars in the California industry are 'Sevillano', 'Mission', and 'Ascolano'. Taken together, these four cultivars account for more than 98% of table olive production in the United States (Connell, 1994). In an effort to clarify pollination requirements of the olive cultivars grown in California, Griggs et al. (1975) conducted an extensive study over several years. Although their results are somewhat contradictory and difficult to interpret, they do indicate that 1) 'Manzanillo' shows at least partial self-incompatibility; 2) the self-incompatibility response varies among years; and 3) commercial fruit set and productivity can be improved by cross-pollination with an appropriate pollinizer cultivar. Despite these results and those of other researchers who have demonstrated increases in fruit set under cross-pollination conditions (Lavee and Datt, 1978; Sibbett et al., 1992), many California growers continue to grow 'Manzanillo' in solid blocks. Indeed, in a manual on California olive production (Ferguson et al., 1994), no consideration is given to using pollinizers in olive production. In addition, even where a benefit is recognized for cross-pollination in 'Manzanillo', uncertainty regarding its pollination requirements has resulted in growers selecting a pollinizer cultivar on the basis of its fruit qualities without sufficient consideration being given to its suitability as a pollinizer. For example, 'Sevillano' has experienced waning popularity in recent years in the California olive industry (Connell, 1994), which has led to a reluctance among growers to plant the cultivar as a pollinizer.

With this background in mind, we initiated a study of the self-incompatible behavior of 'Manzanillo' and its responses to the application of 'Sevillano', 'Mission', and 'Ascolano' pollen. These three cultivars were selected because they are common in the California olive industry, and each is sometimes planted as a pollinizer in 'Manzanillo' orchards. Our objective was to determine the biological parameters (pollen–pistil interactions, fertilization, and fruit set) associated with self- and cross-pollination of 'Manzanillo' in order to elucidate compatibility relationships among the cultivars.

Materials and Methods

The study was conducted in 1994 and 1995 in an experimental orchard at the Univ. of California, Davis. The orchard received regular irrigation and fertilization, and management practices were generally comparable to those of a well-managed, commercial orchard. Four 'Manzanillo' olive trees of uniform size and with high flowering loads were selected each year. In 1994, a set of selected shoots on each tree received the following treatments: self-pollination, and cross-pollinations with either 'Sevillano' pollen, 'Mission' pollen, or with a mixture of 'Sevillano' and 'Mission' pollen. The last treatment was intended to explore a possible synergic effect of the application of pollen of the two genotypes, often suggested for open-pollination treatments (Griggs et al., 1975; Morettini, 1972; Tombesi et al., 1982). Because pollen viability did not differ between 'Sevillano' and 'Mission' pollen (see results), pollen from the two cultivars was combined 1 'Mission' : 1 'Sevillano' pollen (by weight) for the mixture treatment. In 1995, the mixture treatment was replaced with a treatment using 'Ascolano' pollen.

Unwanted pollinations were prevented by bagging the shoots before anthesis and keeping the bags on the shoots until loss of stigma receptivity. Handmade tissue paper bags, although fragile in the event of rain, were used because tissue paper allows proper gas exchange and does not affect greatly temperature and moisture conditions inside the bag (Cuevas, 1992). Flowers within the bags were not emasculated in an effort to replicate field conditions; thus, for all treatments self pollen was present in addition to the experimental pollen used, as it would be in an open-pollinated olive orchard. Pollen used in the experiments was collected from trees growing in southern California, where olive bloom occurs several days earlier than in our experimental trees, and stored at 4 °C until use. Pollen viability was evaluated by the fluorescein diacetate fluorescent reaction (Heslop-Harrison and Heslop-Harrison, 1970; Pinney and Polito, 1990).

Cross-pollination treatments were performed by opening the bags, applying the appropriate pollen to the receptive stigmas of the flowers with a fine paintbrush, and reclosing the bags. Hand pollinations were made every other day from anthesis to 6 d after anthesis, a total of four pollinations for each bagged sample, with each flower within a bag receiving at least two pollinations. Self-pollination treatments were performed by bagging the shoots and shaking the bags to ensure pollination. Effective pollination was confirmed by observing stigma pollen load.

Pollen tube growth and three parameters leading to fruit set were examined: fertilization, fruit set at 21 d (initial fruit set), and fruit set at 60 d (final fruit set). Fertilization was determined by microscopic examination of pistils from random samples of 20 flowers (five from each of four trees) collected 4, 8, 12, and 15 d after anthesis in 1994; and 5, 10, and 15 d after anthesis in 1995. Pistils were dissected and styles and ovules examined separately. Pollen tube growth in the style was analyzed using the method of Cuevas et al. (1994). Fertilization was evaluated using the aniline blue fluorescence technique to detect the presence of pollen tubes penetrating the micropyles of the ovules (Polito and Pimienta, 1982). On each of the four trees, eight shoots, each bearing 10 inflorescences with fertile pistillate flowers, were left intact for fruit set measurements. Initial fruit set was determined as the number of enlarged fruitlets per inflorescence 21 d after bloom. Final fruit set was measured on the same shoots at 60 d after bloom, by which time abscission of young fruitlets has ended and subsequent fruit drop through harvest is insignificant (Rallo and Fernández-Escobar, 1985). Shotberries, small, worthless, parthenocarpic fruits typical of several self-pollinated olive cultivars (Griggs et al., 1975; Sibbett et al., 1992), were not considered.

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In 1995, similar experiments were also conducted on four 'Mission' trees in the same orchard. These received the following treatments: self-pollination, cross-pollination with 'Manzanillo' pollen, and open pollination. ('Manzanillo', 'Ascolano', 'Sevillano', and 'Barouni' trees were present in the orchard.) Each pollination treatment was conducted on eight shoots per tree and initial and final fruit set were evaluated as described above.

Analysis of variance (randomized complete-block design with subsamples within blocks) for quantitative data and chi square tests for qualitative measurements (i.e., fertilized vs. nonfertilized ovules) were used to analyze the results.

Results and Discussion

Pollen viability was high for all cultivars in both years. In 1994, mean percent viability for 'Sevillano' and 'Mission' pollen was 78.2% ± 9.8% and 88.6% ± 3.6%, respectively. In 1995, 'Manzanillo', 'Sevillano', 'Mission', and 'Ascolano' pollen viability was 81.2% ± 0.5%, 60.9% ± 2.1%, 79.2% ± 2.0%, and 67.6% ± 2.5%, respectively.

During both years, final fruit set of 'Manzanillo' following cross-pollination with 'Sevillano' pollen was significantly higher than that obtained following self-pollination (Table 1). Pollination with 'Sevillano' produced more than a 4-fold increase in final fruit set over that of self-pollination (4.15 times in 1994 and 4.63 times in 1995). In addition, parthenocarpic shotberries occurred in relative abundance following self-pollination, a situation that also has been observed by others (Fernández-Escobar and Gómez-Valledor, 1985; Griggs et al., 1975; Sibbett et al., 1992). Index of self-incompatibility (ISI), a concept introduced by Lloyd (1965) and subsequently used by Zapata and Arroyo (1978) in analyses of tree breeding systems, is useful for assessing the degree of self-incompatibility in plant species. ISI is defined as the ratio of fruit (or seed) set following self-pollination to that following cross-pollination. For this work, ISI for 'Manzanillo' relative to 'Sevillano' cross-pollination, i.e., the ratio of set in 'Manzanillo' following self-pollination to set of 'Manzanillo' following cross-pollination with 'Sevillano', was 0.24 (1994) and 0.22 (1995).

The increase in fruit set following cross-pollination with 'Sevillano' was apparent at the time of initial fruit set measurements (Table 1) and was correlated with poor performance of self-pollen tubes to grow to the ovules and achieve fertilization (Tables 2 and 3). Fertilization levels from the first assessment at 4 (1994) or 5 (1995) d after anthesis through 15 d after anthesis did not differ within any pollination treatment (Tables 2 and 3); therefore, data were pooled within treatments to increase sample sizes. Although fertilization rates were reduced after self-pollination relative to that following cross-pollination with 'Sevillano', in 1995 the degree of reduction was less than that for fruit set. Indexes of self-fertilization (ISF = fertilization following self-pollination / fertilization following cross-pollination) for 'Manzanillo' selfed relative to 'Manzanillo' × 'Sevillano' was 0.27 (1994) and 0.53 (1995). The higher rate of fertilization following selfing in 1995 did not, however, produce a higher rate of fruit set. This suggests the possibility that the incompatibility response of olive may involve a postzygotic, as well as a prezygotic, mechanism. Because the phenomenon was observed in only 1 of the 2 years, it is not clear that this is the case. Verifying the possibility of a postzygotic basis for incompatibility in olive will require further investigation.

Application of 'Mission' or 'Ascolano' pollen to 'Manzanillo' flowers produced no increases over self-pollination in either initial or final fruit set (Table 1), and shotberries were also abundant. The reduced fruit set in these cases again was correlated with decreased pollen tube growth and fertilization (Tables 2 and 3), similar to the situation in the self-pollination treatment.

Observations of 'Manzanillo' flowers following the 'Mission' and 'Ascolano' treatments, as well as the self-pollination treatment, yielded results similar to the olive self-incompatibility response previously characterized by Cuevas (1992); pollen tube growth typically stopped shortly after tubes had penetrated the stigma surface. In a low percentage of flowers some pollen tubes, commonly only one, escaped the incompatibility reaction to achieve fertilization. As a result, levels of fertilization were reduced relative to pollination with 'Sevillano' pollen.

No significant differences between 'Sevillano' vs. the mixture treatment were detected, in either fruit set or fertilization ability (Tables 1 and 2). These results and the poor performance of 'Mission' pollen alone indicate that the incorporation of 'Mission' pollen into the mixture was not beneficial. Thus, there appears to be no potential advantage to interplanting the two cultivars as pollinizers in 'Manzanillo' orchards.

Incompatibility between 'Manzanillo' and 'Mission' was bidirectional, as fruit set of 'Mission' following pollination with 'Manzanillo' pollen did not differ from that following self-pollination (Table 4). Fruit set in open-pollinated 'Mission' flowers (where the potential pollen parents included 'Sevillano', 'Ascolano', and 'Barouni', in addition to 'Manzanillo') was two to three times that of the self-pollination and 'Manzanillo' pollination treatments.

These results indicate that 'Mission' and 'Ascolano' are incompatible with 'Manzanillo' and that these cultivars are unsuitable for interplanting as pollinizers in 'Manzanillo' orchards. This information is relevant, as the scarcity of data available for California olive growers (Ferguson et al., 1994) and the relative abundance of these cultivars in the California industry have often led to their being interplanted with 'Manzanillo' (Sibbett et al., 1992). Previous results reported by Griggs et al. (1975) also suggested cross-incompatibility between 'Manzanillo' and 'Mission', but their results were inconclusive regarding 'Ascolano'.

Fruits were harvested and weighed in Nov. 1994. Fruit fresh mass from the self-pollination treatment was 15% greater than that for the 'Sevillano' pollination treatment (Table 1). This increase is consistent with the reduction in fruit set, and the effect might have been

Table 1. Initial and final fruit set (fruits/inflorescence) and fruit mass (g) of 'Manzanillo' olive following self-pollination and pollination with either 'Mission', 'Ascolano', 'Sevillano', or a mixture of 1 'Mission' : 1 'Sevillano' pollen (by weight).

	Fruit set[z]				Fruit mass at harvest (g)
	Initial		Final		
Pollen source	1994	1995	1994	1995	1994
Selfed	0.160 a[y]	0.110 a	0.122 a	0.100 a	4.02 b
Mission	0.150 a	0.172 a	0.094 a	0.147 a	3.64 a
Ascolano	---	0.257 a	---	0.245 a	---
Sevillano	0.782 b	0.569 b	0.506 b	0.463 b	3.41 a
Mixture	0.915 b	---	0.575 b	---	3.54 a

[z] Initial = enlarged fruitlets at 21 d after bloom; final = fruits at 60 d after bloom.
[y] Mean separation within columns by Duncan's multiple range test at $P < 0.01$.

Table 2. Percentage of 'Manzanillo' flowers with fertilized ovules following self-pollination and cross-pollination in 1994 using either 'Mission', 'Sevillano', or a mixture of 1 'Mission' : 1 'Sevillano' pollen (by weight) sampled at 4, 8, 12, and 15 d after anthesis. Fertilization levels did not vary significantly during the sampling period for any treatment (P ranged from 0.52 to 0.86); therefore, data were pooled to increase sample sizes.

	Days after anthesis				
Pollen source	4	8	12	15	Total[z]
Selfed	6	5	5	14	8.9 a
Mission	5	20	12	19	14.5 a
Sevillano	37	30	27	39	33.3 b
Mixture	45	40	35	50	42.1 b

[z] Mean separation by chi square at $P < 0.01$.

Table 3. Percentage of 'Manzanillo' flowers with fertilized ovules following self-pollination and cross-pollination in 1995 using either 'Mission', 'Ascolano', or 'Sevillano' sampled at 5, 10, and 15 d after anthesis. Fertilization levels did not vary significantly during the sampling period for any treatment (P ranged from 0.32 to 0.86); therefore, data were pooled to increase sample sizes.

	Days after anthesis			
Pollen source	5	10	15	Total[z]
Selfed	10	10	25	15.0 a
Mission	5	10	10	8.3 A
Ascolano	5	5	15	8.3 A
Sevillano	20	30	35	28.3 b

[z] Mean separation by chi square at $P < 0.10$ (lowercase) or 0.01 (uppercase), respectively.

Table 4. Initial and final fruit set (fruits/inflorescence) in 'Mission' olive trees following self-pollination, cross-pollination with 'Manzanillo' pollen, and open pollination.

Pollen source	Fruit set[z] Initial	Fruit set[z] Final
Self-pollination	0.278 a[y]	0.243 a
Manzanillo	0.409 a	0.378 a
Open pollination[x]	0.955 b	0.753 b

[z] Initial = enlarged fruitlets at 21 d after bloom; final = fruits at 60 d after bloom.

[y] Mean separation within columns by Duncan's multiple range test at $P < 0.01$.

[x] Potential pollen parents include 'Sevillano', 'Ascolano', 'Barouni', and 'Manzanillo'.

greater if the pollination treatments had been extended to the whole-tree level. Because olive is an alternate-bearing species, a reduction in return bloom might also be expected as a result of increases in fruit set following effective cross-pollination.

The self- and cross-incompatible response of 'Manzanillo' agrees with previous observations elsewhere (Cuevas, 1992; Lavee and Datt, 1978). The results are also convergent with those obtained in field experiments conducted by Sibbett et al. (1992), who showed increases in fruit set and reduction in shotberry production in a 'Manzanillo'–'Ascolano' orchard after supplemental pollination using 'Sevillano' pollen.

In conclusion, our results show that 'Manzanillo' is self-incompatible and cross-incompatible with 'Mission' and 'Ascolano'. These results suggest that under California conditions, interplanting with 'Sevillano' trees or using 'Sevillano' pollen in a supplementary pollination program (Sibbett et al., 1992) may enhance 'Manzanillo' olive productivity.

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