Almond Board of California

Project Report 2003-04

Project Title: **Effect of pollen S-allele combinations (one or both cross-compatible) on seed-set success**

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Objective:

1. Determine whether pollinizers that have one pollen cross-incompatibility factor (CIG = S-allele) in common with the seed parent lead to reduced seed-set compared to pollinizers where both pollen cross-incompatibility factors differ from the seed parent.

2. Determine whether otherwise fully cross-compatible pollen cross-incompatibility factors (CIGs = S-alleles) including self-compatible genetic variants, differ in their effectiveness for achieving honeybee transfer, fertilization and ultimately, seed set in field pollinations.

Summary.

Studies of both in-vivo pollen tube growth as well as the rate of successful transfer of pollen cross incompatibility groups (CIG) genotypes to progeny following controlled crosses, support a model of cross pollinations in almond were both self-and cross-pollen show good growth in the upper style while only cross pollen shows consistent growth through the lower style to fertilization. Good growth of cross-compatible pollen tubes was found in the lower style and ovary only when large numbers of pollen tubes (typically greater than 20) were found growing in the upper style. However, the growth of either cross-compatible pollen or self-incompatible pollen (that is, from the flower being pollinated), appeared equally successful in stimulating the growth of of cross-compatible pollen tubes through the lower style.

The success of specific pollen CIG groups on final crop set was also evaluated by assessing their successful transfer through fertilization to the seed and resulting seedlings plant. No difference in seed set was observed for pollinizers that shared a single incompatibility factor with the seed parent (for example, Carmel pollen on Nonpareil flowers) compared to pollinizers

where both incompatibility factors were different (for example, Mission pollen on Nonpareil). Similarly, early results from paternity testing indicate that while successful fertilization by self-pollen is rare no clear difference exists in fertilization success of different CIG groups.

Combined results support a model for pollinations in almond were high numbers of either self-incompatible and/or cross-compatible pollen tubes growing through the upper style strongly stimulate successful growth of a small number of self-compatible pollen tubes through the lower style fertilize the single ovule typically present in California cultivars. Having large numbers of pollen tubes growing in the upper style thus is an important determinant of fertilization success. Results from 2003/2004 support earlier conclusions that a cultivar such as Carmel which has only one cross incompatibility group (CIG) in common with Nonpareil will be just as effective of pollinizer as a variety such as Winters which has both CIG groups different. Observations of honeybee cross pollinations of almond in the field also indicate that large amount of self pollen is deposited onto the stigma during the transfer of cross pollen carried by the honeybee. In such honeybee cross-pollination, this self-pollen could provide much of the upper style pollen tube growth needed to invigorate the relatively few, lower-style pollen tube growth needed for consistent fertilization and seed set.

Introduction.

Because cross-pollination during the limited flowering season has been shown to be the most important determinant of final crop yield, the critical decision facing growers is the appropriate source of the pollinizer pollen. This is a crucial question both in the selection of pollinizer varieties as well as the use of pollen inserts to encourage cross-pollination. Two frequent grower and Farm Adviser questions concerning specific sources of pollen have been: (1) Does a variety such as Mission (SaSb) which has no pollen cross-incompatibility group (CIG) factors (S-alleles) in common with Nonpareil (ScSd) provided a better cross-pollination pollen source than a variety such as Carmel (SaSd) which has one pollen CIG factor different and one factor in common? (2) Are some pollen CIG factors (S-alleles) better at achieving seed set than others under otherwise cross-compatible field conditions? Although isozyme and PCR based pollen CIG (S-allele) markers have recently become available, these markers could not unequivocally determine pollen paternity following seed-set since these isozymes and PCR markers are shared by a great number of California almond varieties (due to the highly inbred nature of California almonds). We have now developed PCR and SSR based markers that can accurately identify the specific pollen donor contributing to seed-set.

1. Assessment of possible seed set disadvantage when donor pollen has one CIG factor in common with seed parent.

As in 2002, controlled hand pollinations were made using pollen from a variety [SaSg] having one cross incompatibility factor in common with seed parent [SaSb] (Fig. 1, a) and, separately, using pollen from a variety [SfSg] were both cross incompatibility factors differ from those of the seed parent (Fig. 1, b).

No difference in seed set was observed for pollinizers that shared a single incompatibility factor (for example, Carmel pollen on Nonpareil) vs. pollinizers where both incompatibility factors were different (for example, Mission pollen on Nonpareil). Results from 2003 agreed with those from 2002 with the combined data charted in Figure 2. Data from over 5000 controlled pollinations were examined in this study. Sizable differences in seed set were observed in the field for different crosses and even for the same cross when done in different locations or different years. These differences results from the different environments during and following pollinations and appear to be the most important factor for determining seed set when at least some cross-compatible pollen is readily available to honeybee pollinators.

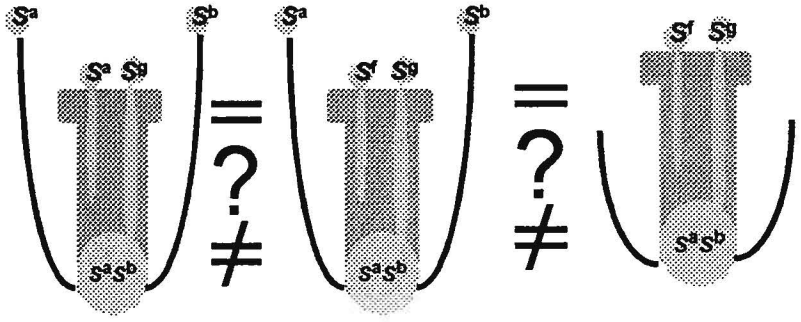

Fig.1. (a) (b) (c)

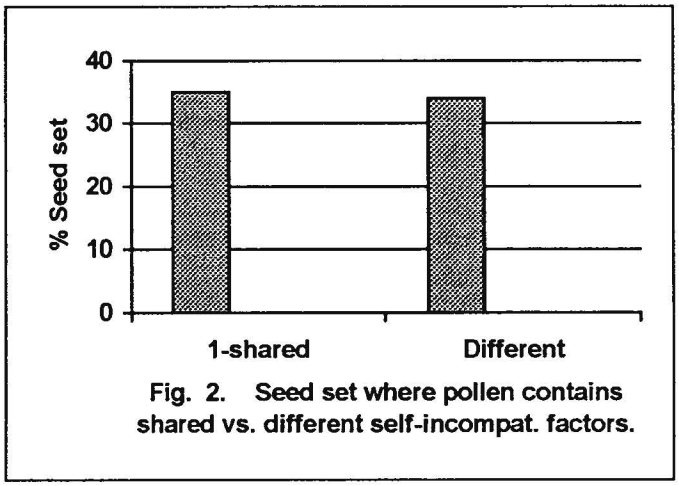

Fig. 2. Seed set where pollen contains shared vs. different self-incompat. factors.

The emasculation of flowers prior to controlled pollinations (Fig. 1,c) to avoid self-pollinations and was again evaluated in 2003 . Regardless of the cross-compatible pollen dosage, however, all crosses using escalated flowers (approx. 900, total) showed reduced final seed set relative to crosses onto un-emasculated flowers (Figure 3). The early flower drop associated with crosses to emasculated flowers indicates that flowers were often damaged during the emasculation process, with their subsequent abortion being a primary cause of the reduced seed-set. Average seed sets of just under 20% were achieved. Crossing success using emasculation followed by pollination with different CIG sources also indicated no difference among the sources evaluated.

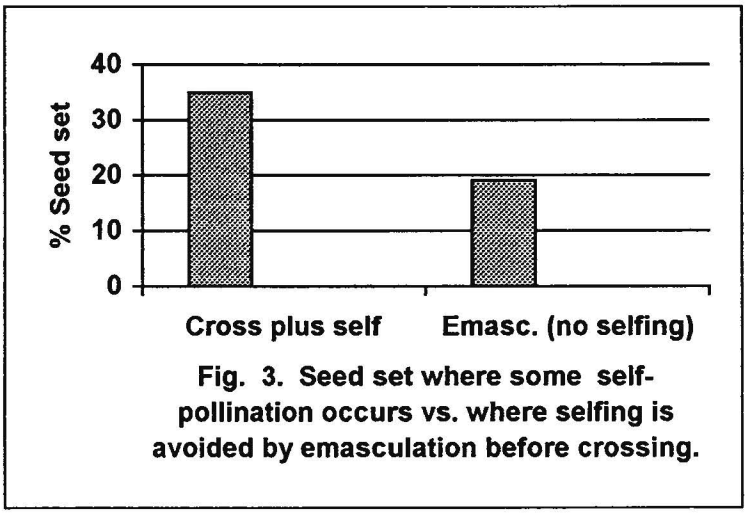

Fig. 3. Seed set where some self-pollination occurs vs. where selfing is avoided by emasculation before crossing.

Also studied was fertilization success when using very low doses of pollen onto emasculated flowers where both CIG groups were different and, separately, pollen were only one CIG group was different. As with previous studies, no difference in average through set was observed between the two groups (Figure 4). Low pollen doses were achieved by first mixing the almond pollen with inert Lycopodium spores in a ratio of 8 to 1. While low differences were observed between treatments, very low seed sets resulted from this treatment. Microscopic examination of pollen tube growth in the styles showed very low numbers of pollen tubes present in the upper styles and often the complete absence of pollen tubes in the lower styles and ovaries. Results support the need for sufficient pollen tube numbers in the upper style to stimulate growth in

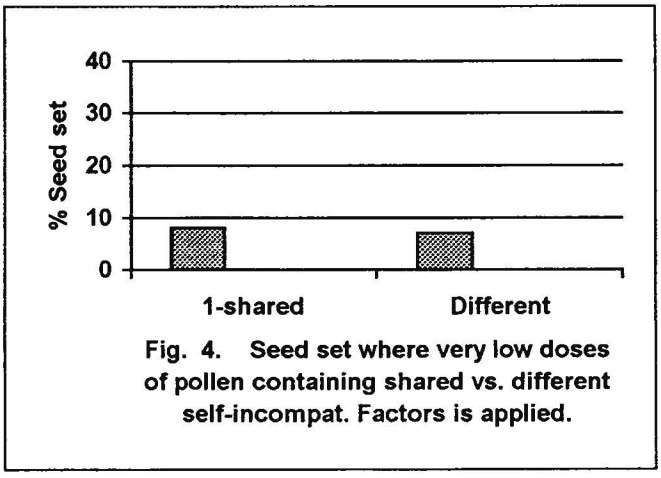

Fig. 4. Seed set where very low doses of pollen containing shared vs. different self-incompat. Factors is applied.

the lower style. Since tested flowers were first emasculated, no self pollen was applied during cross-pollination. If flowers were not emasculated, large numbers of self pollen would probably be applied to the stigma during honeybee visits leading to sufficient pollen tube growth in the upper style to encourage growth of fertilization of the few cross compatible pollen present. This specific scenario was not tested in this experiment, however.

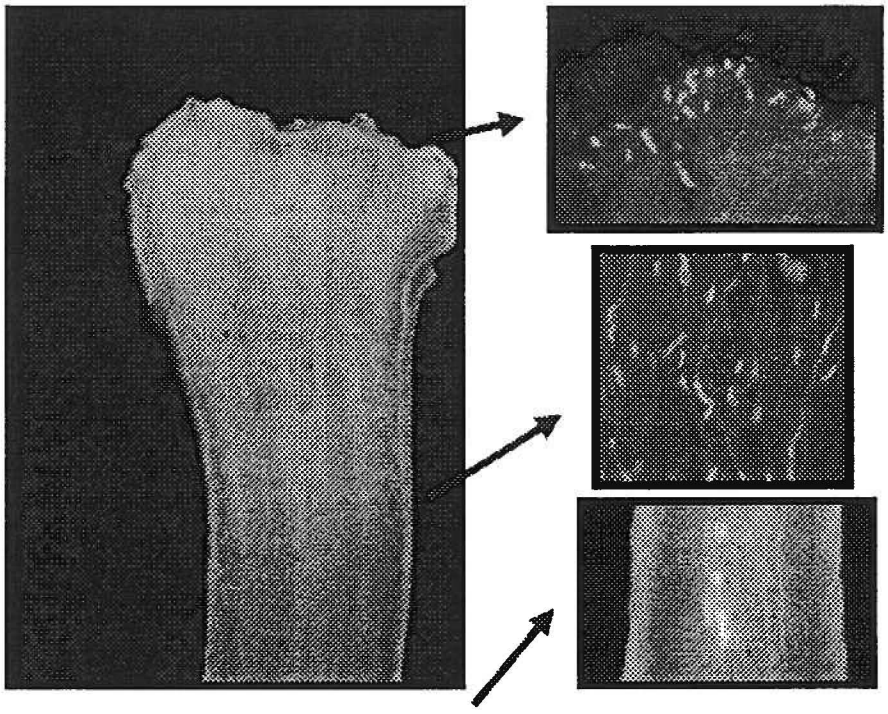

Fig. 5. Examples of pollen tube in the stigma (top) upper style (middle right) and style base near the ovule (bottom right).

The microscopic analysis of pollen tube growth through the styles of unemasculated (intact) flowers showed a large numbers of pollen grains germinating and growing through the upper and occasionally into the mid style sections (as illustrated in Figure 5). [Figure 5 shows cross-incompatible pollen tube growth response in a Solanaceae species which is very similar to that observed in almond. The almond style tissue, however, is almost identical in color to the autofluorescence observed in almond pollen tubes, making it much more difficult to get clear images of pollen tubes in vivo.] When a sufficient number of pollen tubes were present in the upper style (typically ~ 20 pollen tubes or more, often much more), good pollen tube growth was observed in the lower style (typically 4-9 pollen tubes observed, though additional pollen

tubes were probably present but not observed in the sections examined). Successful fertilization was rarely observed when no pollen tubes were observed in the lower style (though it is assumed that pollen tubes were present but not observed in the sections examined when the rare fertilization was achieved). The results demonstrate the need for a sizable number of pollen tubes growing through the upper style to stimulate growth of a lower number of cross-compatible pollen tubes through the complete style. Both self pollen and cross pollen appear to be capable of vigorous growth in the upper style and appear comparable in their ability to stimulate growth in the lower style. In crosses were both CIG groups differ from the flower, all pollen would be capable of growing through both the upper and lower style. Under these conditions, however, most pollen tube growth was observed in the upper style with typically 4 to 10 pollen tubes observed in the lower style. In crosses were only a single CIG. group differed from the pollinated flower, large numbers of presumably cross and self-compatible pollen tubes were observed in the upper style with, again, 4 to 10 pollen tubes observed in the

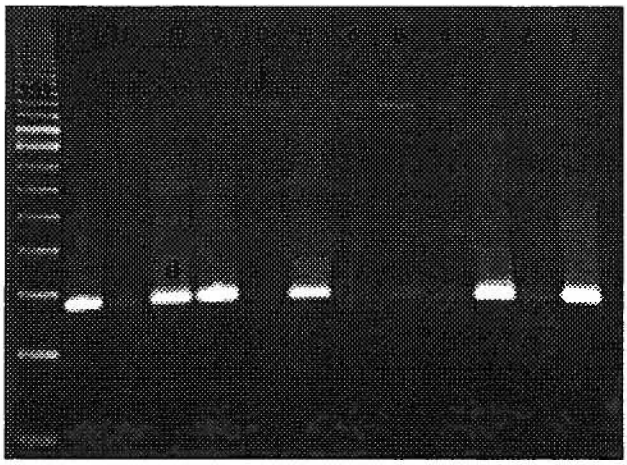

Fig. 6. Gel showing segregation of UCD developed markers for almond CIG groups d & j.

lower style. Observation of naturally pollinated almond flowers from the field, typically showed higher numbers of germinating pollen grains at the stigma and pollen tube growth in the upper style, presumably resulting from the high number of self pollen deposited to the stigma during the honeybee visit. The number of pollen tubes in the lower style was similar to that seen in earlier observations (though only seven naturally field pollinated styles were examined in this study).

2. Determine whether otherwise fully cross-compatible pollen CIG factors differ in their fertilization effectiveness following honeybee transfer of pollen.

Two field tests were undertaken; one involving caged trees and the other involving honeybee cross-pollination of an isolated single variety Mission block using a pollen insert on one of 2 hives.

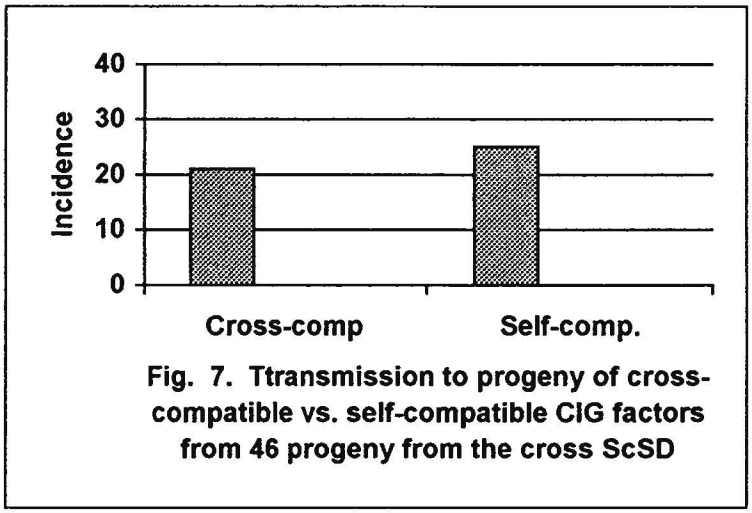

Fig. 7. Ttransmission to progeny of cross-compatible vs. self-compatible CIG factors from 46 progeny from the cross ScSD

Caged trees. A 14-year-old almond tree enclosed with mesh screening to exclude outside pollinators was honey-bee cross pollinated with pollen from potted trees of a breeding line having entirely different CIG factors (as in 2002, but a universally self-compatible CIG factor was compared to the cross-compatible CIG factor.). Forty-six of the resultant seed from the 2002 study have been germinated and analyzed using PCR markers (Fig. 6) developed at UCD to determine pollen source (paternity) and identify whether any CIG factor showed significantly higher rates of seed set as compared to the other. Initial results showed no difference between the otherwise fully cross compatible CIG factors (Fig. 7). Additional germinated and transplanted seedlings from 2002 and 2003 crosses are presently been evaluated using updated molecular probes and final results should be available for the 2004 Almond Board Conference.

Use of pollen inserts in an isolated Mission block. Two honeybee hives were placed at bloom in an isolated solid Mission planting (of approximately 100 trees) located at UCD. Fresh pollen having entirely different CIG factors (SxSy) than Mission (SaSb in Fig. 8) was prepared and placed in a hive-insert at one of the to hives following the recommendations of Tom Ferrarri (Pollen Bank, Bakersfield CA).

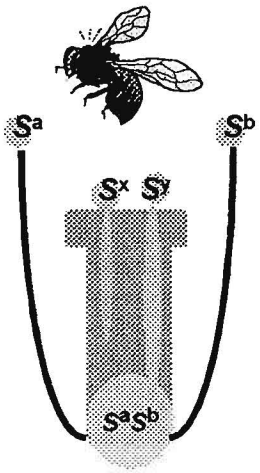

Fig. 8

As in 2002, mixing of the pollen within the beehive was observed but no mixing of pollen and between hives was detected. Low levels of between hives pollen mixing may have occurred which could not be detected by the pollen/lycopodium spore auto-fluorescence detection method used). Unfortunately, the test orchard was removed in the spring of 2004 because of budget cutbacks, and nuts could not be collected for paternity testing.

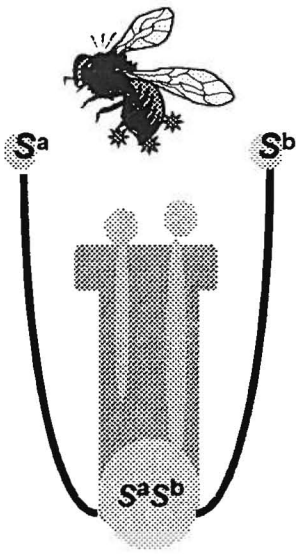

Fig. 9.

A second study was set up to evaluate the movement of almond pollen by honeybees following the removal of the yellow, sporopollenin coating. The yellow, sporopollenin coating of almond pollen is believed to be the compound which makes it attractive to honeybees, leading to the fairly fastidious grooming of pollen from foraging bees returning from the field. Almond pollen from which the yellow, sporopollenin coating has been removed may be less prone to grooming and thus allowing greater numbers of pollen grains to remain with the foraging bee on subsequent field trips leading perhaps, to greater efficiency of hive-insert applied pollen. At full bloom, a low dose sporopollenin-free pollen (< 1 g) was applied on two consecutive days to hives inserts containing standard amounts of normal pollen. The resultant seed has been harvested and is being prepared for planting. The paternity testing of germinated seed will be used to determine the level of pollination by sporopollenin-free pollen compared to standard hive insert pollen. The chemical treatment to remove the sporopollenin coating resulted in a loss in pollen germination rate compared to untreated pollen (Fig. 5) though the level of germination appeared adequate for normal field pollinations.

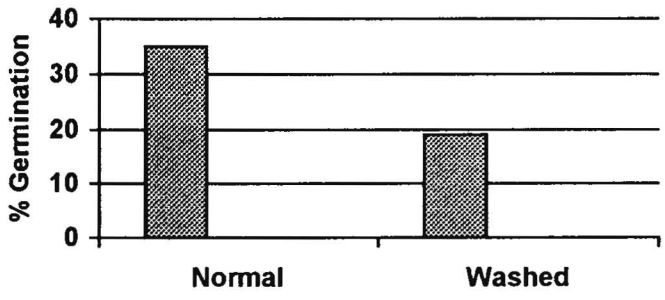

Fig. Germination of untreated and pollen from which the exterior sproropollenin has been washed.