Almond Board of California

RECEIVED
AUG 1 2 2005
ALMOND BOARD

Final Report 2004- 05

Project Title: **Pollen flow in almond orchards: Do individual cross- compatibility factors (S-alleles) differ in their ability to set seed?**

Project Leader: Tom Gradziel

Cooperating Personnel: A. Dandekar M.A. Thorpe, S. Uratsu, A. Vezvaei and K. Barckley

Location: Department of Plant Sciences, University of California at Davis, CA

Objective:

1. Determine whether specific S-alleles/combinations differ in their ability for successful cross-pollination.

2. Compare other determinants of pollinizer quality, particularly levels of pollen production among pollinizer varieties.

Results.

Cross-pollination during the limited flowering season is one of the most important determinant of final crop yield. Because almond pollen is self sterile or self-incompatible on its own flowers, pollen needs to be transferred by honeybees from another cross-compatible variety. Self-incompatibility is controlled by the S-gene. Each almond variety, because it is a diploid species, has two forms or alleles of this gene; one inherited from each parent. The resultant S-genotype will determine which varieties it will successfully set seed on and which varieties it will fail to set seed on (i.e. cross-incompatibility group or CIG). Unlike the tree and flower, almond pollen is haploid, containing only one S-allele. Following pollination pollen grows through the flower style. Growing pollen tubes containing any S-alleles in common with the 2 alleles in a flower will be recognized as 'self' and growth arrested in the lower style before fertilization can take place. Most California almonds are derived from crosses between Nonpareil with the S-alleles (S7S8) and Mission (S1S5) and so possess any of the 4 resulting S-combinations (S1S7, S1S8, S5S7, or S5S8). Different varieties that have the same S-allele pairs such as Price (S1S7) and Rosetta (S1S7) are cross-incompatible and so not able to cross-pollinate each other. Most California varieties have at least one and, less frequently, two S-alleles different from the variety they're intended to pollinate, and so are cross-compatible. A critical decision facing growers is the appropriate source of the pollinizer pollen. Two common questions addressed in this research are:

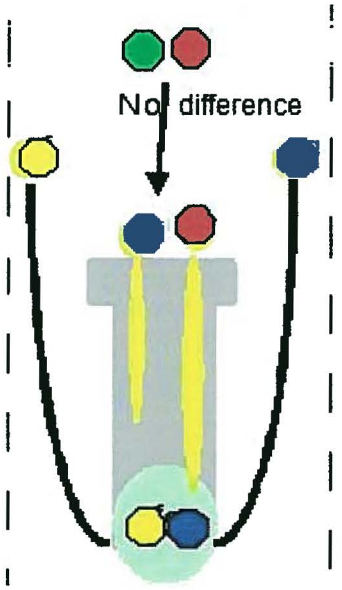
(1) Does a variety such as Winters (S1S14) which, because it has no pollen cross-incompatibility group (CIG) factors (S-alleles) in common with Nonpareil (S7S8) (and so will have all of its pollen capable to fertilize Nonpareil), provided a better pollen source than a variety such as Carmel (S5S8) which has one pollen CIG factor cross-compatible and one factor in common? and

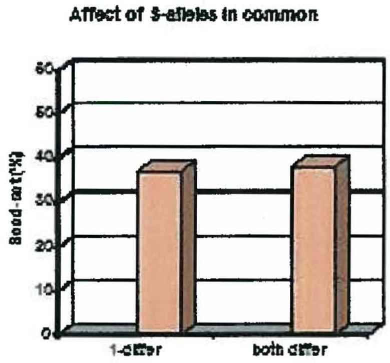
(2) Are some varieties such as Fritz better at achieving seed set than others under otherwise cross-compatible field conditions?

1). A comparison of pollination success when one vs. both pollen S-alleles differ from the seed parent.

The efficiency of specific pollen-parent/seed-parent CIG group combinations on final crop set was evaluated in 2004 in over 1000 additional crosses using a wide range of S-allele combinations representing the extent of current California almond germplasm. Almond crosses, in which known CIG groups were combined in multiple combinations using hand pollinations under under relatively controlled field conditions, were studied. Flowers were not emasculated prior to pollination, since all genotypes tested were self-incompatible. Results showed no advantage in final seed set success when both S-alleles contributed by the pollen donor variety differed from the seed parent as compared to pollen parents possessing only one S-allele differing from the seed parent . These results are in agreement with previous 2002-03 results, where a total of over 5000 crosses were evaluated. Combined multi year results evaluating a total of over 6000 crosses are presented in Figure 1. Seed set is virtually identical regardless of whether the donor or pollen had one or both S-allele's differing from the seed parent variety. As discussed in the 2003 report , environmental affects on pollen and flower fecundity, including temperatures and moisture levels during pollination, were the major determinant of final set, overshadowing any difference in pollen S-allele identity (as long as the parents shared at least some cross-compatible pollen). This trend was again observed even when very small amounts of pollen were applied to the recipient flowers, as described in 2003. With the larger, pooled data, the environmental affects, (which appear independent of S-allele identity), average out for different S-allele types, resulting in the near identical final sets regardless of whether one or both, pollen S-alleles

Do improved seed sets result from having both S - alleles differ in the pollen donor (vs. only 1 of 2 alleles different)?





Seed set following cross with 1-different vs. 2 dif. Cross - incompatibility factors.

Fig. 1. Comparison of over 6,000 controlled crosses using pollen parents with either one or both cross-incompatibility factor or S-alleles distinct from seed parent. No difference in seed set was observed.

differed from the seed variety (as would be expected if environmental differences rather than specific cross-compatible S-genotype group were the major factor determining fruit set).

This and previous studies of both pollen tube growth through the flower style (using tissue staining and fluorescence microscopy) as well successful seed set following the use of different cross-incompatibility groups (CIG) parents in controlled crosses, support an action of cross pollinations in almond were both self-and cross-pollen show good growth in the upper style, while only those pollen whose S-allele differs from the flower being pollinated, show consistent growth through the lower style to fertilization. Good continued growth of cross-compatible pollen tubes was found in the lower style and ovary, however, only when large numbers of pollen tubes (typically greater than 20) were found growing in the upper style. Both the growth of cross-compatible pollen and self-incompatible pollen (including from the flower being pollinated), appeared equally successful in stimulating the growth of the smaller number of cross-compatible pollen tubes through the lower style. Having large numbers of pollen tubes growing in the upper style thus is an important determinant of fertilization success. Results 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 a pollinizer as a variety such as Fritz which has both CIG groups different. Observations of honeybee cross pollinations of almond in the field also indicate that large amounts of self pollen are deposited onto the stigma during the transfer of cross pollen carried by the honeybee. In such honeybee 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.

(2) Pollination efficiency of the variety Fritz relative to Sonora under standard honeybee cross-pollinated field conditions.

To evaluate differences in pollenizer effectiveness under standard orchard conditions, we identified an isolated commercial orchard where Fritz and Sonora were the alternating pollinizers for Nonpareil. We have determined the S-allele genotype for Fritz to be (S6S1) with both alleles being cross-compatible with Nonpareil (S7S8). The S6 allele is unique to Fritz while the S1 allele is common in many other pollinizers of Nonpareil including Mission, Price, Monterey and Padre. The S-allele genotype for Sonora is (S13S8) where S13 is unique to Sonora while S8 is found in Nonpareil in many of its pollinizers including Carmel, Livingston, Sauret#1 Plateau, Avalon and Butte. Therefore, only the S13 containing pollen will be cross-compatible on Nonpareil, while S8 pollen will be recognized by Nonpareil flowers as 'self' pollen with S8 pollen tube growth arrested before fertilization can be achieved. Favorable pollination conditions existed during the 2004 season, with good winter chilling, moderate pollination temperatures, and freedom from rains. The Sonora variety began to bloom approximately 7 days before Nonpareil with relatively good bloom overlap with Nonpareil for the first four days of the Nonpareil bloom. The Fritz variety

Paternity testing shows slight advantage of Sonora over Fritz pollen in San Joaquin grower field tests in 2004 -05.

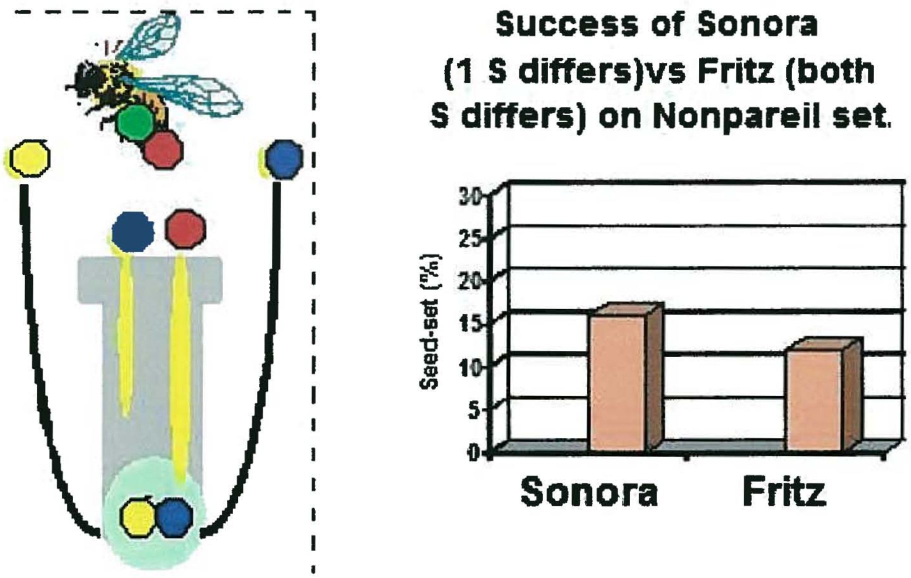
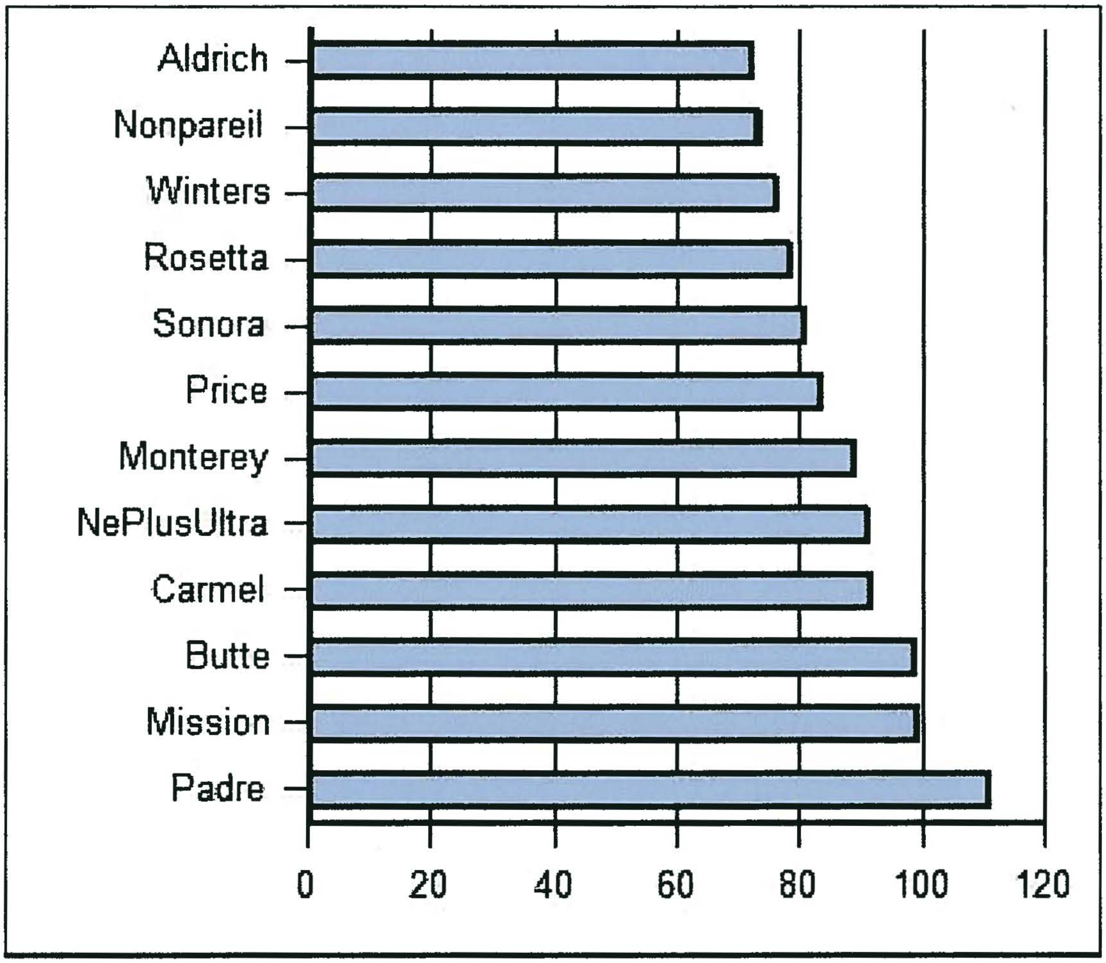


Fig. 2. Paternity determination of 40 Nonpareil almond seed from standard grower field where pollinizers were Sonora and Fritz showing slight advantage for Sonora (probably due to better bloom overlap in 2004 and not specific S-allele types.

bloomed approximately 2-3 days after Nonpareil and reached full bloom approximately 3 days after Nonpareil. Following Nonpareil harvest, seedlings from 40 mature Nonpareil kernels were germinated and molecularly analyzed for the relative proportion of Fritz (S1 or S6) to Sonora (S13) contribution with results plotted in Figure 2. Results show a small but significant increase in seed resulting from pollination by Sonora relative to Fritz. Based on our experience with such field crosses in almond, we believe the seed-set advantage of Sonora on Nonpareil was entirely the result of its earlier bloom and better boom overlap with the crucial early Nonpareil flowers. Any advantage of the full cross-compatibility of the Fritz pollen donor variety was thus masked by its later flowering time. [Fritz typically flowers closer to nonpareil so that 2004 paternity success will vary with year. Consequently, these results do not fairly address the question of S-allele importance to final seed set. Results do however, underscore the overriding importance of optimal boom overlap for optimal seed set on Nonpareil. Bloom overlap, particularly for the very important early Nonpareil bloom, presently appears to be the major determinant of pollinizer success (paternity) given at least some cross-compatible S-alleles in the pollen donor variety.

2). Differences in pollen quality among varieties.

To compare differences in pollen quality among varieties, pollen production at the popcorn stage of blossoming was determined for flowers of Nonpareil and the following pollinizers: Sonora, Carmel, Aldrich, Winters, Butte, Monterey, Rosetta, Price, Padre, and Mission. Test samples were from virus-free trees of the FPMS foundation orchards at Davis, CA. Consistent and statistically significant differences were observed among varieties with Padre, Mission and Butte among the top pollen producers (Fig. 3). In some cases, increased pollen production was associated with greater number of pollen bearing stamens, (Figure 4) though other varieties produced more pollen on fewer stamens. Flowering almond trees are one of the first sources of pollen for the honey bee hives coming off overwintering and abundant supplies of good quality pollen are required for honeybee colonies to come to strength quickly. Because the almond flowering season is over quickly, the stronger beehives will primarily benefit later



1Fig. 3. Differences in pollen production (mg/20 flowers) among common almond pollinizers.

crops. Many beekeepers and almond growers, however, believe that because pollen is such a crucial food source, that bees will preferentially harvest from varieties producing more abundant pollen. Greater pollen gathering activities would in turn make these varieties, better pollinizers for a major variety such as Nonpareil. While our work has shown that sizable differences in pollen production exist among varieties, we have not yet tested whether these differences in production, correlate with differences in honey bee foraging activity.

Fig. 4. Nonpareil flower at 2 days after bloom opening showing relatively large number of dehiscing anthers yet relatively low pollen per flower.

Summary and application to 2005 bloom season.

Combined 2002-2004 results support a model for pollination in almond which is distinctly different from other pomological crops. (In addition to our own findings, this model is supported by developmental studies of almond flowering by Dr. Vito Polito, Plant Sciences, UCD, and colleagues doing similar research in Spain). Perhaps as a consequence of the harsh environments typical for native wild almond populations in their Central Asian origins, almond has evolved an inherent resilience for overcoming inclement weather during bloom (including cold winter rains followed shortly thereafter by the intensive heat and drought common to spring and summer in the Central Asia). To optimize pollination

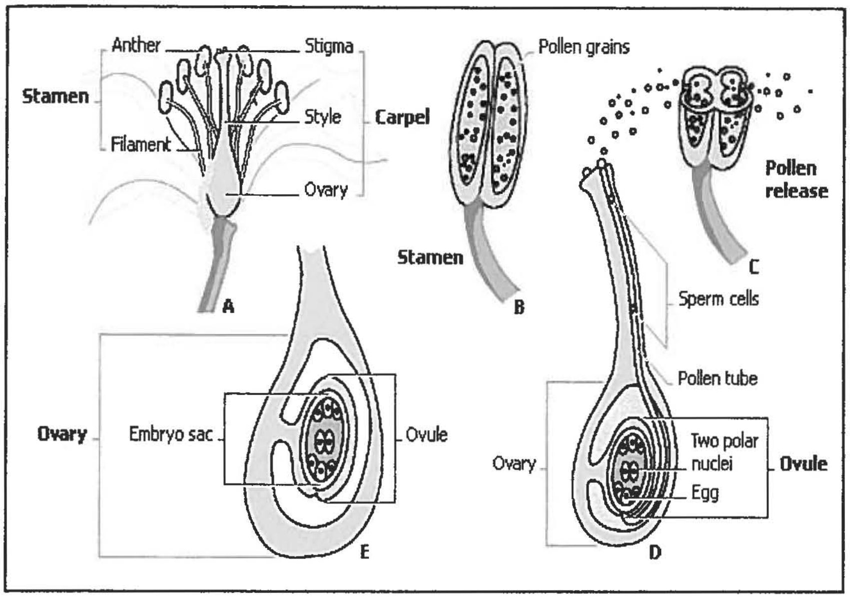


Fig. 5. Flowering and fertilization in almond.

success, almond trees have evolved to flower very early in the spring when temperatures are cooler and water more available. To further compensate for this very early bloom season, almond anthers (Figure 5 a,b) have adapted to dehisce (release pollen, Figure 5 c) under higher humidities than most other Prunus tree crops. Pollen is mature and dehiscing shortly after the flowers first open (Figure 5, a). Although the pollen is mature, the developing ovary in the base of the flower has been arrested at a state of development approximately 3 days before maturity (receptivity) (Figure 5, e,d). Ovule development resumes only when growing pollen is detected in the upper style of the flower (Figure 5 d). Depending upon temperature, it takes approximately 3 days for pollen to traverse the style. This initial asynchrony between ovary and pollen development ultimately acts to synchronize the pollination event, so that the egg will be mature and receptive when the pollen tubes finally traverse the style and enter the ovule for fertilization. This synchronization between ovule development and pollen tube growth to fertilization also allows almond a unique capacity to outlast the cold, rainy storms common during flowering. Because both pollen growth and ovule development are temperature dependent, both developments will be suppressed by the cold temperatures typical of February storms in California. This sort of semi-dormancy delays continued flower development until after the storm passes and warmer spring temperatures return. [In contrast, for both peach and apricot, the flower ovules are much closer to receptivity at the time of initial flowering (when pollen first starts to grow at the top of the style). Consequently, cold weather has a greater suppressing effect on the pollen tube growth than ovule maturation so that by the time pollen tubes reach the ovule, the egg may have aged to the point that it is no longer receptive and fruit set does not occur].

Higher day and night temperatures during the unusually warm rains seen in the 2005 bloom, however, were less effective in slowing down flower development and resulted in a large proportion of the early Nonpareil flowers opening before the storm had run its course. Due to the rainy weather, honeybee pollinators were less able to move pollen resulting in reduced transfers of compatible pollen and reduced total pollen load (both self and cross) deposited to the flower stigma. (The rains also acted to delay flowering of later pollinizers which typically flower one to two days after Nonpareil but instead occurred four to six days after initial bloom which further reduced cross-pollination). In addition to reduced cross-pollination, reduced honeybee visits led to a lower number of total (self- plus cross-) pollen grains on the stigma and so low numbers of pollen tubes growing in the upper style (where both cross- and self-pollen grow uninhibited). Since high numbers of pollen tubes growing through the upper style are required to stimulate successful growth of the relatively small number of cross-compatible pollen tubes through the lower style to fertilization, seed set will further suffer. Often, pollination in the lower style but without fertilization, will induce early fruit development and enlargement without seed development. With no seed development occurring, however, these fruit will abort after 3 to 6 weeks, as was commonly observed in Nonpareil and other mid-flowering period varieties in 2005.