Almond Board of California
Project Report - 2005

Project Title: Effect of pollenkit -free pollen on enpollination success under controlled and commercial conditions.
[Pollenkitt is the more appropriate term for the washable pollen-coating while the previously used term, sporopollenin, is the tough, inert, and relatively indigestible pollen wall coating].

Project Number: **05-TG-02**

Project Leader: Tom Gradziel

Cooperators: A. Dandekar, M.A. Thorpe K. Barckley, and A. Vezvaei.

Location: Dept. of Plant Sciences, University of California at Davis

Objectives:

1. Determine if pollenkit -free pollen is retained on foraging honeybees longer than normal almond pollen (presumably because it is not perceived by the honeybees as pollen and so is not as fastidiously groomed from returning foragers).

2. Verify that pollenkit-free pollen applied to the hive entrance in enclosed tree/hive cages, is successfully transferred by honeybee foragers to receptive flowers resulting in successful nut set.

3. Attempt to document the successful honeybee transfer and fecundity of pollenkit-free pollen under natural orchard conditions.

Summary:

Pollenkit, the mucilaginous yellow coating typical of honeybee transferred pollen, can be removed without significant loss in pollen viability or fecundity. [Initially, this material was identified as sporopollenin, but as recently pointed out by Dr. Robbin Thorpe, pollenkitt is the more appropriate term for the washable pollen-coating while the previously used term, sporopollenin, refers to the tough, inert, and relatively indigestible pollen wall material remaining after washing (even with harsh acids)].

Initial results also support an improved efficiency of hexane-washed pollen for honeybee cross-pollination when applied using standard enpollination techniques. Because of the preliminary nature of this work, however, the cost efficiency of this approach relative to standard enpollination practices remain untested. The removal of the pollenkit was effective in removing the pollen clumping agents as well as much of the anther sac and flower parts usually found in bulk pollen. Consequently, hexane-washed pollen was easier to handle, transport and store.

Almond pollen, as with most honeybee collected pollen, is coated by pollenkit, a mucilaginous, yellow compound which causes the pollen to clump together and which appears to act as an attractant for bees. If the pollenkit is the principal attractant for honeybees, its removal may make the pollen 'invisible' to honeybee collectors resulting in less harvesting within the hive and so more enpollinated pollen retained by the foraging honeybees and so available for cross-pollination.

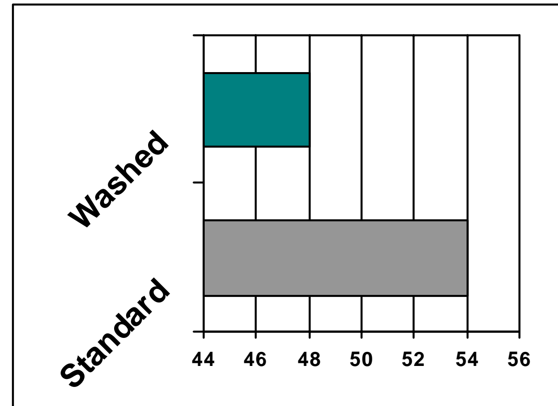

Figure 1. Comparison of pollen germination success on agar media for washed and unwashed pollen.

Pollenkit-washing.

Lab tests in 2005 confirmed that the pollenkit could be ely washed from almond pollen using various non-polar chemical solvents. Washing fresh pollen with enough hexane to remove all traces of the natural yellow pollenkit color, proved the most effective, provided that the hexane solution was first purged of any remnant moisture using various drying agents. When even small amounts of water were present in the hexane, the pollen was killed, presumably because the moisture triggered activation of the otherwise dormant pollen membranes allowing hexane to enter the pollen interior where it is toxic. Following the removal of remnant moisture, however, hexane effectively removed all visible pollenkit with only about an 8 % reduction in pollen viability once the pollen was thoroughly air-dried (Fig. 1).

Figure 2. Magnified image of unwashed Nonpareil pollen (left) compared to washed and filtered pollen (right).

The following wash procedure proved simple, effective and efficient, and so was adopted as standard procedure for subsequent evaluations. All moisture was first purged from the hexane by storing it with previously microwave heated micro-seive desiccant (1 min. per 5 g micro-seive). (The heating purges all moisture from the micro-seive making it an effective 'sponge' for remnant moisture in the liquid hexane). Moisture-free hexane was then used to wash pollen samples until all yellow pigment was removed. The subsequent anther sac/pollen/hexane slurry was then passed through a 50uL Nalgene filter to remove anther sacs and other flower debri greater than 50uL (allowing the 30 uL pollen and hexane liquid to pass. The, thus purified pollen slurry was then passed through a 10 uL, 115 ml Nalgene Filter Unit which allowed the hexane liquid to pass through the filter but retained the purified pollen. Because hexane

is very volatile, it readily evaporates after washing, particularly if an air flow is established across the washed pollen. [Both hexane as well as the washed pollen are very flammable and so must be handled with appropriate care. The flammability of washed pollen is a result of it's very small size and so relatively large surface area: analogous to the explosive grain dust in wheat storage.] Treated pollen was talc-like in its color and consistency and so much easier to manipulate and apply than natural pollen (Figure 2).

Pollen viability testing.

Following thorough air-drying, (using vacuum filtration with the Nalgene filter to force dry air across the pollen/filter) treated pollen could be stored for over eight months and still retain viability. Pollen viability was determined by testing in-vitro germination on a standard sucrose/agar artificial medium (Fig. 3). Pollen tubes lengths of at least 4 times the the pollen spore diameter (approximately 100uL) were counted as successful germinations. Germination was evaluated after 10 h at approximately 25C. Approximately 2000 treated & control pollen were scored in this test.

Figure 3. Pollen germination and growth of hexane-washed pollen on a sugar/agar artificial gernination media.

Effectiveness of hexane-washed pollen under field conditions.

To test pollen viability and fecundity under standard 2005 field conditions, hexane-washed pollen was applied using a camel's hair paintbrush to recently opened Nonpareil flowers located at the Wolfskill Experiment Station, Winters, CA. All flowers had previously been enclosed in mesh bags to prevent insect cross-pollination. Final seed set (Figure 4) was comparable to control pollinations using standard collected pollen, though all test showed relatively low seed sets owing to the poor weather conditions during Nonpareil flowering. A total of over 1000 pollinations were evaluated. The pollen parent used was UCD97,3-40 owing to it's unique S-genotype which could be used for later DNA paternity testing.

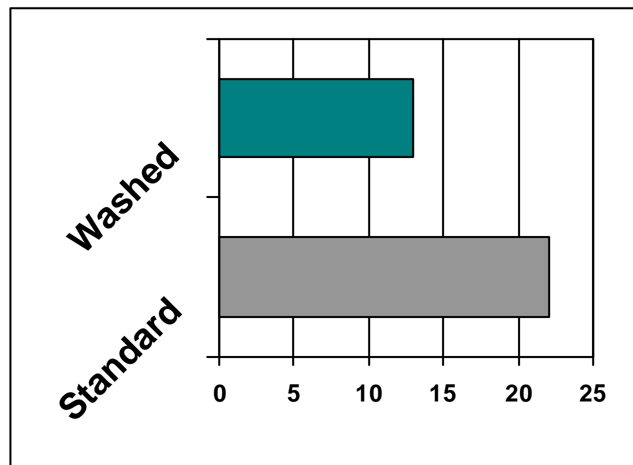

Figure 1. Nonpareil fruit set averags for standard and hexane-washed pollen.

Evaluating honeybee transfer and fecundity of pollenkit-free pollen under orchard conditions.

To assess the efficiency of honeybee transfer of hexane-washed pollen under field conditions, 1 g of hexane-washed pollen was applied to the hive entrance using standard enpollination practices, and, in a separate experiment, applied directly to foraging honeybees. In both experiments, the Nonpareil tree and the honeybee hive were enclosed within a large screened cage to prevent outside insect pollination. Natural pollen from a separate, genetically distinguishable source was also applied either as enpollination pollen (again 1 g) at the hive entrance and as bouquets of flowering branches placed in water filled buckets near the hive entrance and so available throughout the flowering season. Mature seed were harvested and germinated and DNA extracted from leaf samples. Molecular 'fingerprinting' techniques using unique S-genotypes (Sj from the UCD97,3-40 hexane washed parent), determined that up to 10% of the resultant seed were derived from the 1 g of hexane-washed pollen when applied to the hive entrance according to standard enpollination practice. [Approximately 50% of the resultant seedlings have been evaluated to date, though the ratio appears to be holding.]

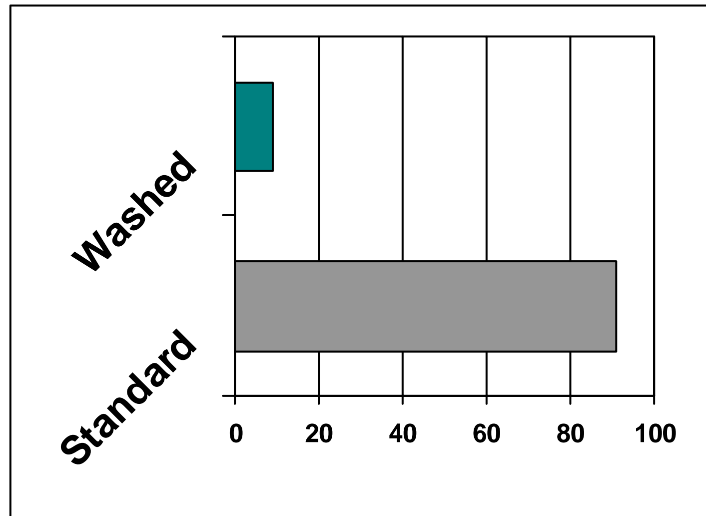

Figure 2. Paternity results from seed-set on a caged Nonpareil tree showing that a small amount of hexane-washed pollen placed at the hive enterance was effectivelt transferred by bees to Nonpareil flowers.

Evaluating honeybee transfer and fecundity of pollenkit-free pollen under natural orchard conditions.

In a final study, hexane-washed pollen was applied in an open-field situation to a beehive using standard enpollination techniques with open-pollinated seed later collected from a nearby very-late flowering almond at the Bee-biology field laboratories in Davis, CA.. The unusual winter/spring conditions of 2005 allowed an unusually extended bloom season with the almond genotype being targeted for enpollination being the last tree in the area to be in bloom. A strong hive was located within 100 m of the tree and bees from this hive were observed visiting this tree.

This study would thus test cross-pollination efficacy following standard enpollination using hexane-washed pollen. Resultant seed have now been collected and germinated and are being grown in Wolfskill Research fields so that leaves can be used for DNA extraction as part of the DNA fingerprinting study. Leaf samples should be ready for DNA analysis by late summer, 2006.