Effects of Washed Pollen on Enpollination

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Interpretive Summary:

Enpollination is the practice of supplying previously collected cross-compatible pollen to a special dispenser at the hive entrance to augment honeybee pollen transfer and so field pollination in almond orchards. 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. In this study we have removed the pollenkit to make the pollen more 'invisible' to honeybee collectors resulting in less harvesting within the hive mounted dispenser and so more enpollinated pollen retained by the foraging honeybees and so available for cross-pollination. We have found that pollenkit can be removed without significant loss in pollen viability or fecundity by careful washing with either mineral oil or the chemical solvent hexane. Field results support good efficiency of washed pollen for honeybee cross-pollination when applied using standard enpollination techniques. 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. Hexane-washing was more demanding owing to its potential toxicity to hydrating/germinating pollen but resulting pollen was easier to handle, transport and store. Pollenkit removal is easier with mineral oil. Mineral oil washed pollen gave better germination on artificial media but resulted in stickier pollen which clumped too much for easy field application. Adding commercially available Lycopodium spores allowed a more typical pollen consistency but lowered pollen germination rates on artificial media. Seed set following controlled field pollinations was successful for both wash treatments. Both hexane and mineral oil washed pollen placed in honeybee hive enpollination dispensers was successful in allowing seed set in caged tree studies. Resulting seed has been germinated and molecular markers used to document pollination success (paternity) of each treatment.

Objectives:

1. Test light mineral oil as an alternative pollen-wash solvent to the more toxic and so difficult to use Hexane.

2. Compare field seed sets (using previously established caged tree/beehive set-ups) to compare washed-pollen efficacy with normal honeybee cross-pollination.

Materials and Methods:

1. Untreated pollen and pollen in which the pollenkit coating has been removed by either hexane or light mineral oil was tested for germination on both artificial media and on flower stigmas in the field. Both germination rates and field seed set were compared in determining treatment effect.

2. Washed-pollen was applied to hive entrance enpollination dispensers and directly to pollen foraging honeybees in a caged Nonpareil tree. Molecular markers were used to determine paternity of resultant seed and so efficiency of the pollen wash treatments.

Fig. 1. Hexane washed pollen at right compared to Nonpareil pollen contained in dehiscing anther sacs as typically harvested for enpollination.

Results and Discussion:

Germination tests

The pollenkit was effectively washed from fresh, field collected almond pollen using hexane, a non-polar chemical solvent. Washing pollen with sufficient hexane to remove all traces of the natural yellow pollenkit color, proved most effective, provided that the hexane solution was first purged of any remnant moisture using drying agents. If even small amounts of water remained 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

Fig. 2. Pollen washed and filtered using light mineral oil showing sticky consistency before diluting to a powder using commercially available

of remnant moisture, however, hexane effectively removed all visible pollenkit with less than 10 % reduction (combined 2005-06 results) in pollen viability once the pollen was thoroughly air-dried. Treated pollen was talc-like in its color and consistency and so much easier to manipulate and apply than natural pollen (Fig. 1). Treated pollen could be stored for over 12 months at 0°C and remain viable.

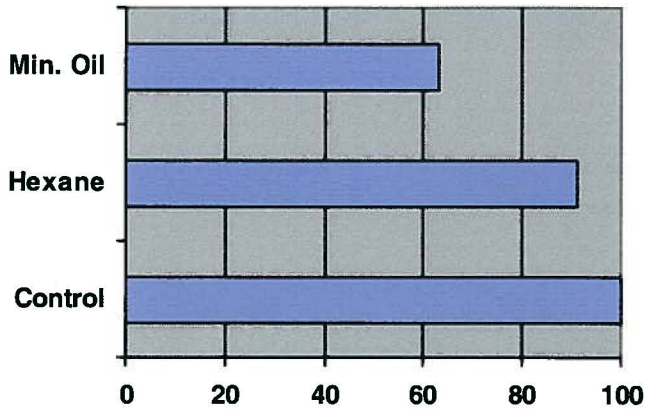

Fig. 3. Germination of treated pollen on agar media set (as a proportion of control).

Because light mineral oil is not toxic to pollen, it was used without special precautions to wash pollen until all yellow pollenkit color was removed. As with hexane-wash, the pollen was then vacuum filtered and dried. Filtered pollen remained coated with small amounts of mineral oil which served as a protectant but also resulted in a sticky consistency (Fig. 2). To obtain a more talc-like powder, this material was then mixed with Lycopodium spores. [Lycopodium spores are commercially available killed and cleaned spores of the Lycopodium moss, which due to its very small size (approximately 30u) and consistency, is very similar to almond pollen]. Germination of mineral oil washed pollen on artificial media was, on average, only 6% lower than untreated (32% vs. 38% in 2006). When Lycopodium spores were added to the mineral oil washed pollen to absorb the excess oil and make the oil-washed pollen more powdery and so manageable in the field, the germination rate fell to 24%, presumably as a result of a detrimental effect of the commercial Lycopodium product used (Fig. 3).

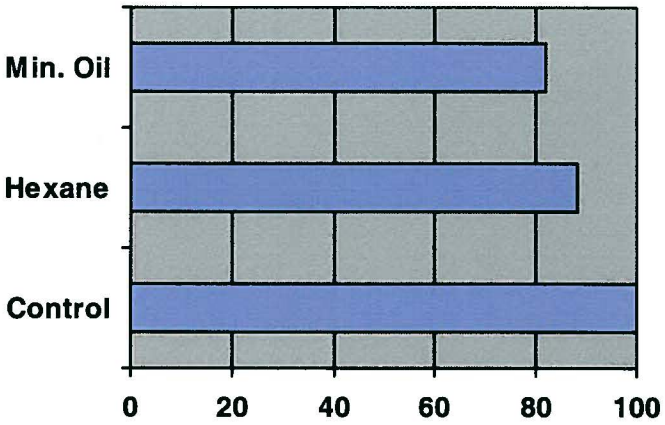

Fig. 4. Final seed set (as a proportion of control) for treated pollen on mesh bag enclosed Nonpareil branches.

Field tests

To test pollen viability and fecundity under standard field conditions, washed pollen was applied to recently opened Nonpareil flowers. All flowers had previously been enclosed in mesh bags to prevent insect cross-pollination. Final seed set for both hexane- and oil-washed pollen were slightly reduced from control pollinations using natural pollen, though all test showed very low seed sets (less than 10%) owing to the cold and rainy conditions during Nonpareil flowering in 2006 (Fig. 4).

Control pollinations used in this study involved similar controlled crosses on adjacent enclosed Nonpareil branches using untreated pollen (as shown on the left in Fig. 1). Final seed size and germination rate were indistinguishable from control generated seed.

Honeybee transfer

To assess the efficiency of honeybee transfer of washed pollen under field conditions, 1.0 ml of hexane-washed pollen was applied to the hive entrance using standard enpollination practices. The tree and the honeybee hive were enclosed within a large 20' x20' x20' screen-covered cage to prevent outside pollination. Cut flowering branches from cross-compatible breeding lines were also placed in the enclosure to serve as a typical pollen source for honeybee collection and transfer. All seed were harvested at hull split. Resulting seed were cleaned, germinated and grown to the seedling stage using standard nursery practices. Leaf samples from resultant seedlings were evaluated for identity of the form of the S-gene, since the different pollen sources (cut flowers and washed pollen) differed for this gene allowing its use as a marker for paternity (pollen origin). Molecular 'fingerprinting' analysis of over 100 seed showed that approximately 6% of the seed originated from hexane-washed pollen (Fig. 5). This is a surprisingly high level of successful honeybee mediated pollination given the small amounts of hexane-washed pollen used and the fact that it was only applied during a 2 day period at full bloom while the cut flowers were available from 5% bloom to petal drop. (New flowering branches were brought in aprox. every 5 days of the 15 day Nonpareil bloom period in 2006). There was no observable difference in seedling quality (size, form, growth rate, etc.) between the 2 treatments.

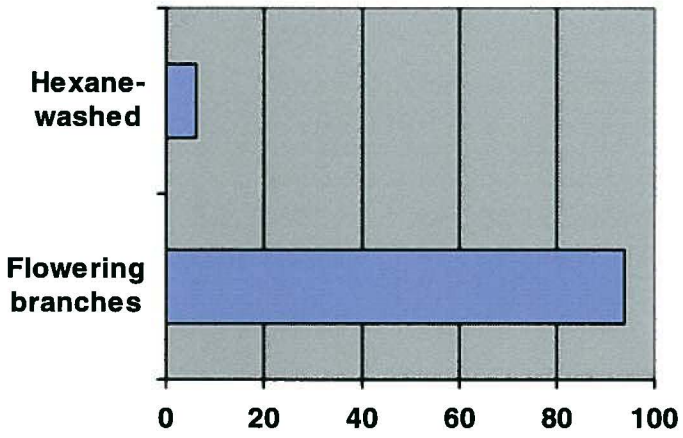

Fig. 5. Seed paternity (%) following honeybee cross-pollination with flowering branches or 1 ml of 'hexane-washed pollen applied to hive evtrance dispensers.

In a related experiment, mineral oil-washed pollen was also successfully transferred to flowers (with subsequent fertilization & seed set) when applied to caged bumblebees, though no alternative pollen source was tested in these studies.

Recent Publications:

Martínez-Gómez, P., Sánchez-Pérez, R., Rubio, M., Gradziel, T. M.,Sozi, G.O. 2005. Application of Recent Biotechnologies to PrunusTree Crop Genetic Improvement. Ciencia Investigacion Agraria. 32 (2).

Martinez-Gomez,-P; Sanchez-Perez,-R; Vaknin,-Y; Dicenta,-F; Gradziel,-TM. Improved technique for counting chromosomes in almond. Scientia-horticulturae. 2005 May 30; 105(1): 139-143.

Peace,-CP; Ahmad,-R; Gradziel,-TM; Dandekar,-AM; Crisosto,-CH. The use of molecular genetics to improve peach and nectarine post-storage quality. Acta-horticulturae. 2005 June, no 682(1); 403-409.

Martínez-Gómez, P., Sánchez-Pérez, F., Dicenta, W. Howard, P. arus, T.M., Gradziel.. (2006). Almond. In Chittra Kole: Genome Series Vol. 9. Science Publishers Ltd. Helsinki.

Barckley, K.K.,S.L. Uratsu, T.M. Gradziel and A.M. Dandekar (2006) Multidimensional analysis of S-alleles from cross-incompatible groups of California almond cultivars. J Amer Soc Hort Sci 131:632-636.

Gradziel, T., B. Lampinen J. Connell and M. Viveros. (In Press). 'Winters' Almond: an Early-Blooming, Productive and High Quality Pollenizer for 'Nonpareil'. HortScience.