

USE OF RAPD MARKERS TO INVESTIGATE THE EFFECTS OF POLLEN PROCESSING AND POLLEN STORAGE ON POLLEN VIGOR

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Abstract: Supplemental mass pollination (SMP) is a management technique often used in seed orchards when clonal phenology inhibits uniform male gamete contribution to the overall seed crop. To help ensure that each male in the orchard contributes equally to the crop, equal amounts of pollen from each clone in the orchard is used in a polymix. One of the major assumptions of the polymix approach is equal male gamete success. It has also been suggested that stored pollen, although viable based on *in vitro* germination experiments, may fail to produce filled seeds due to reduced vigor. In order to better understand the effects of processing and storage on male gamete success we utilized RAPD markers in combinations of fresh (F), processed (P), and stored (S) pollen from two males applied in equal amounts in a factorial array to a single female. Parent-specific RAPD markers were identified by screening 10-mer primers against eight megagametophytes from each of the parents [male clones 81028 (referred to as parent A) and 81069 (referred to as parent B), and one female clone 81077]. Due to their dominance, RAPD markers had to uniquely distinguish a single pollen parent from each of the other clones. Thirty-two primers were screened to identify pollen parent-specific markers which were not present in female clone 81077. Marker 1672000 was found to be specific to, and heterozygous in, pollen parent A, and two markers 111_1400 and 122_0875 were specific to, and heterozygous in, pollen parent B. Given equal treatment and assuming equal male gamete success, we expected to find the pollen parent-specific RAPD alleles in 25% of our controlled cross progenies. A total of 192 progenies from each treatment combination: FAFB, FAPB, FASB, PAFB, SAFF, PAPB, PASB, SAPB, SASB were assayed for the presence of our RAPD alleles. Treatment FAFB suggested that fresh pollen from parent B was more successful (excess of 10%) in producing viable offspring than was fresh pollen from parent A. Using stored or processed pollen from parent B gave similar results (excess of 9% in FAPB, and 9% in FASB), indicating that processing and storage did not effect viability of pollen from parent B. There did, however, appear to be a positive processing and storage effect on pollen from parent A, resulting in an excess of viable offspring from parent A (excess of 10% in PAFB, and 3% in SAFFB). Pollen from parent A was found to be more successful in producing viable offspring in all other treatment combinations, again suggesting some effect of processing or storage on pollen from parent A. Results of this experiment suggest that handling of pollen may influence its viability and that such effects may be different for different males in a pollen mix. Effects on pollen



Further research is necessary to examine which if any of these factors are at work.

Keywords: RAPD, pollen processing, pollen storage, SMP, polymix