

Methods used for conducting controlled cross-pollination in Citrus for the conventional hybridisation program at CSIRO Plant Industry, Merbein.



The aim of the following method is to prevent accidental cross-pollination, self-pollination or other form of pollen contamination when making controlled pollinations between different parents in the citrus breeding program.

Contamination can occur through insect-pollinator activity (see left) or via operator error due to contaminated tools or fingers.



To avoid pollen contamination all tools are sterilised with ethanol between each operation.

Tools need to be dried in the air after dipping in ethanol to prevent death of target pollen.

Fingers should also be cleaned with ethanol between crosses.



To avoid self-pollination, and unless they are pollen sterile, flowers of the female parent must be emasculated before the anthers dehisce their pollen. Before this, however, the flower to be pollinated has to be selected. The target flower should be just at the stage before opening and this can be gauged by carefully rolling the flower between thumb and forefinger, which will cause the petals to separate. Inspection within the flower will show that the anthers have not released their pollen but the stigmatic surface is shiny due to a secretion. If the anthers have released their pollen or the stigma is not shiny, the flower should be removed and another found. All flowers and flower buds not to be cross-pollinated should be removed as in the pictures on the left.



The pictures on the left illustrate the emasculating process. The petals of the selected flower from the female parent are peeled away and the stamens carefully removed without damaging the pistil.

The petals can also be removed or left in place.

If the petals are left, the emasculated flower needs to be contained within a bag to prevent pollinator visits.

We have found that by removing the petals as well, the flower can remain un-bagged, especially if it is cross-pollinated immediately, which is the normal case at Merbein.



The pictures on the left demonstrate emasculated flowers ready for controlled pollination.

Note where the petals have been left, the flower has been bagged to prevent pollinator visits.



Controlled cross-pollination.

Pollen from the male parent can be applied directly using anthers taken from the male parent. In this case the flower of the male should have been bagged prior to anthesis to prevent contamination by visiting pollinators.

Alternatively, pollen can be collected by drying non-dehisced anthers from unopened flowers over silica gel at room temperature. The dried pollen can then be stored in vials at 4°C until needed. Stored pollen can be applied to the stigma of the female parent via a soft paintbrush.



The cross-pollinated flower of the female parent should be labeled with the details of the cross. If the petals have been retained on the flower, it needs to be protected from contamination from insect-pollinators. If the petals have been removed, the flower is no longer attractive to insect-pollinators and can be left un-bagged.



As the fruit from the cross-pollination develops and grows, it is a good idea to place it within a bag. This prevents accidental loss of the fruit when or if it drops from the tree before it can be harvested.

The developing fruit is left on the tree until at least colour break occurs and preferably longer to ensure the seeds reach full development.

When harvested, the seeds are carefully extracted, surface dried so that the outer seed coat can be removed, and then sown in the seedbed.

In some cases with polyembryonic seeds, germination is conducted aseptically *in-vitro* to ensure maximum embryo growth, which increases the chances of recovering the zygotic or hybrid seedling.

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