Hybridisation Biology Within the *Chamelaucium* Alliance—Preliminary Studies

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Intraspecific, interspecific, and intergeneric crosses involving nine species from the genera *Chamelaucium*, *Verticordia*, and *Darwinia* were conducted. Pollen-pistil interactions and the formation of seed were studied in order to locate any hybridisation barriers that may exist. Whilst seed set was recorded from 16 crosses indicating no hybridisation barriers, the presence of barriers was observed in the remaining 55 crosses. The long styles of *D. squarrosa* and *Darwinia* spp (novo) may have been responsible for the incompatibilities when used as female parents, as the pollen tubes of the shorter styled species were unable to transcend the longer styles. According to the above results, methods to overcome the hybridisation barriers were suggested to facilitate the union of desirable characteristics in new hybrids.

INTRODUCTION

Myrtaceae is one of the largest and most important families in Australian flora, containing 144 generic groups (Briggs and Johnson, 1979). The genera *Chamelaucium*, *Verticordia*, and *Darwinia* form part of the tribe Chamelaucieae (Bentham and Mueller, 1967) belong to the *Chamelaucium* alliance, and are largely endemic to Western Australia. The *Chamelaucium* alliance contains genotypes which have attractive flowers, with *Chamelaucium uncinatum* being one of the major floricultural crops of the export flower market. There is a constant demand for new genotypes with novel flower types and colours, which flower at different times of the year.

Hybridisation is an effective method for plant improvement by creating new genetic combinations (Maluszynski et al., 1995). Although intraspecific, interspecific, and intergeneric hybridisation all have the potential to improve the germplasm, interspecific and intergeneric hybridisation are more likely to produce spectacular results. The vibrant flower colouration of *Verticordia* spp. and *Darwinia* spp. combined with the vigour of *Chamelaucium* spp. would produce a desirable hybrid.

Difficulties have been reported when attempting to create interspecific and intergeneric hybrids within the *Chamelaucium* alliance. The failure of seed set in interspecific crosses within *Chamelaucium* (Lamont, 1989) and *Verticordia* (Tyagi et al., 1991) suggests the presence of incompatibilities. It is recognised that interspecific and intergeneric crosses are difficult to accomplish and the complex cytogenetic background of *Verticordia* indicates that wide crosses with this genus may be challenging. However, a number of interspecific and intergeneric hybrids within the *Chamelaucium* alliance have been identified confirming that wide crosses are possible.

Much of the work conducted within Myrtaceae has been on *Eucalyptus*, with interspecific breeding barriers being discovered in this genus (Ellis et al., 1991). In order to devise methods to overcome the breeding barriers in the *Chamelaucium* alliance, the site of the incompatibility must first be identified. Pollen-pistil interactions via aniline blue staining and fluorescence optics are used to locate the site of hybridisation failure. Once the hybridisation barriers are located, techniques can be employed in order to overcome them to facilitate wide crosses.

MATERIALS AND METHODS

Plant Material. Nine genotypes from the *Chamelaucium* alliance were selected: *C. uncinatum* (514*), *C. uncinatum* (772*), *C. uncinatum* (773*), *C. floriferum*, *V. helmsii*, *V. multiflora*, *V. plumosa*, *D. squarrosa*, *D.* spp. (novo). * These numbers represent accessions held at the University of Western Australia.

Pollen Viability. A pollen viability assessment was conducted for each plant within the breeding program to ensure that pollen was viable for use in the pollinations. The pollen was germinated in vitro in a 20% sucrose solution for 24 h at 25C, stained with aniline blue, and observed under fluorescence optics.

Pollination. The presence of secondary pollination mechanisms in all of the genera used necessitated the need for emasculation in order to prevent self pollination. *Darwinia squarrosa* and *Darwinia* spp. (novo) were not emasculated. Branches containing emasculated flowers were covered with a perforated plastic bag to prevent insect pollination. Reciprocal crosses were conducted using the nine genotypes. Flowers were pollinated approximately 10 days after emasculation and five flowers from each cross were collected 48 h after pollination for pollen tube investigations. The styles were stained in aniline blue and observed under fluorescence optics. Where pollen tubes were seen at the ovary end of the style, the ovaries were also examined for pollen tube presence.

Plants Collected for Seed Set. At least 10 flowers from each cross were left intact for seed set assessment. The fruits were harvested when mature and raised via embryo-rescue techniques. Once cuttings had developed roots, they were placed in Growool™ and moved into a glasshouse environment. The well established plants were then transferred to potting mix for evaluation.

Floral Measurements. Due to the large variation in style length between members of the *Chamelaucium* alliance and the expected implications on pollination success, the style length of each genotype used in this program was measured.

RESULTS

Pollen Viability. All of the pollen used for pollinations was of sufficient viability. *Verticordia multiflora* had between 20% to 50% germination; *D. squarrosa* and *D.* spp (novo) had 50% to 70% germination; and *C. uncinatum* (514, 772, and 773), *C. floriferum*, *V. helmsii*, and *V. plumosa* all had excellent viability with greater than 70% germination.

Pollen-Pistil Interaction. Table 1 summarises the results of the pollen-pistil interactions.
Seed was recovered from *C. uncinatum* (514, 772, and 773) suggesting that this species is a superior female parent. Conversely, *V. helmsii* and *D.* spp (novo) showed poor female parentage with little or no pollen tube growth and subsequent lack of seed production.

Style Length. Whilst the stigmas of *Chamelaucium* spp. and *Verticordia* spp. used in this program were relatively similar in length, ranging between 2.9 to 5.4 mm, the *Darwinia* spp. were much longer with *D. squarrosa* being 16.6 mm and *D.* spp. (novo) 14.6 mm in length.

Table 1. Pollen tubes scores for intraspecific, interspecific, and intergeneric crosses within the *Chamelaucium* alliance.

	Male								
Female	*C. uncinatum* 514	*C. uncinatum* 772	*C. uncinatum* 773	*C. floriferum*	*V. helmsii*	*V. multiflora*	*V. plumosa*	*D. squarrosa*	*D.* spp. (novo)
C. uncinatum 514	5	6	6	6	0	4	4	4	3
C. uncinatum 772	6	6	6	6	0	6	6	4	4
C. uncinatum 773	6	6	6	5	6	6	5	6	6
C. floriferum	0	1	5	4	0	1	4	0	0
V. helmsii	0	0	0	0	0	0	1	3	0
V. plumosa	4		4	4	4	4	4	4	4
D. squarrosa	5	4	0	2	0	0	5	5	5
D. spp. (novo)	1	0	0	0	0	0	0	0	0

[1]*C.* = *Chamelancium*, *V.* = *Verticordia*, *D.* = *Darwinia*.

Scores are classified as follows: 0, no pollen germination; 1, pollen germination; 2, pollen tube penetration into the stigma; 3, pollen tube penetration into the style; 4 pollen tube presence at the ovary end of the style; 5, pollen tube presence in the ovary; 6, seed set.

DISCUSSION

Of the 71 crosses conducted (involving nine species within the *Chamelaucium* alliance) epifluorescence microscopy indicated that 28 combinations showed immediate pollen-pistil incompatibility with pollen failing to germinate, or pollen tubes not penetrating the stigmatic surface. Such prefertilisation incompatibilities may be overcome by the use of mentor pollen (Pryzywara et al., 1989) or the addition of growth hormones to aid the germination of pollen and the growth of pollen tubes (Shrivastava and Chawla, 1993).

Three crosses, including *D. squarrosa* 5 *C. floriferum*, displayed pollen tube arrest in the stigmatic or stylar tissue. The hybridisation barrier in some crosses were caused by differences in pistil length where the pollen tubes of the species with shorter pistils were unable to transcend the styles of species with longer pistils. The *D. squarrosa* and *D.* spp. (novo) styles were much longer than the other genotypes and were responsible for the incompatibility. This may explain why the reciprocal crosses involving the two species were different.

Epifluorescence microscopy indicated that some crosses were apparently viable with pollen tubes reaching the ovary ends of the styles. The lack of seed from such crosses indicates that postzygotic barriers may have been present preventing hybrid zygote formation. In vitro pollination (Zenkteler et al., 1987) or earlier embryo rescue are effective techniques for overcoming postzygotic barriers.

Although breeding barriers have been detected in the *Chamelaucium* alliance in interspecific and intergeneric crosses, there remains great potential for the exploitation of breeding on all levels for the creation of new cultivars once work has been invested into overcoming these barriers.

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