- Nothing is urgent with the cultivation of *Lithops*.
- The only time, which is crucial, is flowering time when the hand pollination has to be done, that is to say if you want to produce your own seeds.

They are marvelous little plants!

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Propagation Strategies to Support a Wide Hybridization Breeding Program Within the *Chamelaucium* Alliance©

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INTRODUCTION

The activities of the Floriculture Group are focused on the selection and development of the West Australian flora with the object of improving industry capacity. Breeding within the *Chamelaucium* alliance is our most advanced activity, however we also work on a range of other species within genera such as *Grevillea*, *Geleznowia*, *Boronia*, *Banksia*, *Dampiera,* and a range of arid zone daisies.

Our breeding activities with the *Chamelaucium* alliance have focused on wide hybridization within a group of five related genera including *Chamelaucium* sp., *Verticordia* sp., *Actinodium* sp., *Pileanthus* sp., and *Darwinia* sp. The objective of the breeding program is to introduce novel cut flowers and amenity plants to industry. We assess hybrids on the basis of flower color, shape, and stem architecture as well as flowering time, productivity and post harvest performance. Some plants are tested for use in the flowering pot-plant section of the nursery industry and scheduling experiments are undertaken to assess nursery performance.

The Floriculture Group is in a unique position to undertake a breeding program of this type because Western Australia is the natural home of most of these plants. Plant propagation features prominently in the overall activities of the Floriculture Group. Our nursery is accredited to Nursery Industry Accreditation Scheme, Australia (NIASA) standards. The Group adopts and maintains a policy of industry best practice where possible including our plant tissue culture laboratory. Our greenhouse is a double-skinned plastic pneumatic inflated style with ridge venting and thermal screens.

In our plant tissue culture laboratory we have five laminar-flow stations and two sizable culture rooms. The lab is capable of a range of plant tissue culture activities including conventional micropropagation through to maintenance of cell culture lines for somatic hybridization experimental work. There is a strong focus on applied research and nursery integration.

The Floriculture Group also has a seed testing and grafting laboratory, which is used in conjunction with the breeding activities but also sustains stand alone research into these areas on a wide range of Western Australia plants. The Floriculture Group uses a research and development model, which includes plant selection,

propagation (using both sexual and asexual techniques), nursery management, and assessment and commercial release.

STOCK PLANT SELECTION AND PREPARATION

A breeding program is only as good as the capacity of the parents to contribute to the next generation. The breeding program selects parents on the basis of genotype, specifically its phenotypic attributes (e.g., flower color, flowering time, stem architecture, disease resistance, and availability). Populations of wild plants are identified through experience, professional connections, and within the bush-harvesting business, and Conservation and Land Management flora databases. Plants are collected under license. Selection of females and males normally takes place during the flowering season. At this stage of the life cycle it is possible to assess and select and capture the extent of variation within plant populations. Hidden characteristics, such as disease resistance or post-harvest life, are selected with random sampling techniques (Growns, 1998). As cutting material, it is poor at this stage of growth. It can be difficult to strike roots on cuttings taken at flowering time for many species and/or genotypes. Species in the *Chamelaucium* alliance are no different, and we may use grafting at this point. Because we have significant numbers of hybrids growing at our field station we are now in a position to select from our own F1 progeny as well. Producing superior parents through controlled crosses is now a significant part of our breeding strategy. Our objective is to have all females and males represented in the nursery so that logistically it is easier to manage the controlled breeding program. Some species, for example *C. megalopetalum,* require ongoing grafting for building up as stock plants as they otherwise don't thrive or survive. Rootstock selection for survival in pots is therefore critical. Such rootstocks may be different to those that would be used for in-ground cultivation. This process takes a maximum of 2 years and is part of the longer term planning within the breeding activity.

BREEDING PROGRAM AND CROSSING ACTIVITY

We aim to undertake all of our crosses under controlled conditions and therefore need to prepare and emasculate the females and undertake pollen assessment using similar strategies to those used in seed testing. There are three broad types of crosses we undertake. The first is intraspecific crosses between selected genotypes of the same species. Crosses between two different species, such as *C. uncinatum* and *C. megalopetalum* are interspecific crosses. We call these crosses our Pearl or Gem series. For these plants we have a white (Pearl) and pink or purple (Gem) range and we select successful hybrids to fill a timeline and further develop the seasonal length that these flowers can be produced. We also have a program of producing wide intergeneric crosses between *Chamelaucium* and *Verticordia* sp. Our hybrids between *C. uncinatum* and *V. plumosa* are part of our Star flower series.

Chamelaucium sp. are pollen presenters and are prone to self fertilization. During flowering, pollen is deposited onto the style and easily collected and stored at this point. Emasculation must be done prior to the opening of the flower otherwise pollen will have been deposited on the style increasing the risk of self-fertilization. Collecting and storing pollen allows us to access the male half of the breeding equation at any time during the flowering season. Plants within the *Chamelaucium* alliance are all pollen presenters and pollen is available to be collected off of the tip

of the style immediately post anthesis. Females are emasculated and in a properly emasculated flower the style will continue to elongate and mature. At this point a small amount of prepared pollen is placed directly on the stylar dome thus completing the controlled cross. After about 28 days, the females are revisited and the previously crossed fruits are collected and labeled and sent to the plant tissue culture laboratory for early embryo rescue.

We use early embryo rescue techniques in our breeding program to ensure that any putative hybrids we produce have the maximum chance of surviving through to field assessment. In any given crossing season we average about a 10% return on our breeding investment. The first step is fruit preparation and culture initiation. The objective of this activity is to introduce viable embryos into sterile culture conditions. Once the embryos are removed from the fruit the testa oxidizes and needs to be removed otherwise normal germination is impaired. The second stage is stock plant management in vitro. The objective of the next stage is to produce rooted micro-cuttings as quickly as possible. Our aim is to produce rooted micro-cuttings capable of acclimatization in the nursery for each embryo we rescue. These rooted micro-cuttings are then progressed through the normal nursery handling system to harden off tube-stock and then planted out at our field station for assessment. We identified medium hypoxia in vitro as the principle limiting cause of poor rooting in our putative hybrid culture lines and developed a rooting protocol called in vitro soilless medium culture (IVS) (Newell et al., 2003). The IVS forward integrates an aerobic propagation medium into Stage 3 culture and fits into the overall propagation activity with the least amount of effort.

ASEXUAL PROPAGATION

Clonally derived parental material is required in the breeding program so that suitable numbers of controlled crosses can be done for each selected cross.

Although *Chamelaucium* can be propagated by various types of cuttings throughout the year, the preferred cutting material is semihardwood tips. Nodal cuttings can be used but these are best if they contain at least one actively growing axillary shoot. Cuttings typically strike 1 to 2 roots out of leaf axils after 3–6 weeks in an open, free-draining propagation mix on hot beds. Rooted cuttings are transferred into 100-mm tree tubes and are ready for field planting after a further 3–6 weeks. Root binding is a problem, particularly after nursery stock is held back in smaller tubes. In the field the problem manifests in two ways. Early symptoms include shoot twisting and breakage under windy conditions and finally foliage symptoms similar to nutrient deficiency. Plant death is primarily due to a collapse of the transpiration system during summer demand.

We use grafting to propagate bush-selected material, which is unsuitable to use for cuttings or for those genotypes that do not grow for long periods in pots. Rootstock is prepared as cuttings from selected genotypes. The typical stem diameter of the graft is less than 2 mm. Best results are achieved when the rootstock is actively growing and still small in the pot. The typical graft is a wedge or cleft graft. The scion is hand prepared and the finished graft is wrapped with parafilm tape before going out to the cloche to take.

The Floriculture Group has a small but active plant tissue culture laboratory with five laminar flow stations. Plant tissue culture technology is integral to the breeding project. It is also used for the selection and propagation of elite genotypes

of Australian plants with horticultural potential either as cut flowers or for the amenity nursery industry.

We are conducting an investigation into the possibility of using somatic hybridization to double up the chromosome numbers in some of our F1 hybrids to restore fertility and to continue the wide hybridization of genotypes within the *Chamelaucium* alliance. This involves asexually fusing two protoplasts from two plants, which would normally not sexually hybridize.

CONCLUSION

The Floriculture Group relies on the application of modern plant propagation practices for the selection, development, and breeding activity. Good propagation is the cornerstone to achieving the objective of the group, which is the development of novel plant products for commercial application. The group uses both sexual and asexual propagation techniques as well as conventional and sophisticated propagation technologies.

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Shoot Dieback of Geraldton Wax©

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Geraldton wax has been observed exhibiting signs of shoot dieback. This disease causes the plant to die back from the tips, often leading to whole plant death. This disease is a problem in all stages of production, but is worst during propagation when the young plants are most susceptible to disease.

Isolation studies proved the fungus *Colletotrichum* sp. to be the cause of this disease. It was found that *Colletotrichum* sp. is not influenced by wounding; however a heat stress period and humidity are requirements for infection to occur. Fungicide trials indicated that a preventative treatment is needed for this disease to be managed. Control of this pathogen was found to be effective with the use of Amistar® when applied as a preventative treatment. This article describes a part of the experimental work carried out as an honours project: Shoot Dieback of Geraldton Wax (Diplock, 2004).

INTRODUCTION

Chamelaucium uncinatum Shauer (Geraldton wax) is one of Australia's most important native floriculture products. One of the limitations growers face when producing a high quality product is the limited amount of information available on diseases of this plant. There is a recognized need for pathogen identification and control of diseases of Geraldton wax (Fuss et al., 1992). Shoot dieback was observed