

**The Alleviation of
Dormancy in the
Horticulturally-
Important
*Chamelaucium
uncinatum* (*Myrtaceae*)**



Simone Dudley

CSIRO Centre for Mediterranean Agricultural
Research, Floreat, Western Australia, 6014 Australia

Anne Cochrane*

Threatened Flora Seed Centre, Department of
Conservation and Land Management, Locked Bag
104, Bentley Delivery Centre, Western Australia,
6983 Australia

* Corresponding author

Summary

Chamelaucium uncinatum Schau. is one of Australia's major floricultural crops for the export market. There is constant demand for new genotypes with novel flower types, colours and flowering times. Cultivation of plants for the nursery trade is by vegetative propagation, with seeds held within a receptacle dormant on dispersal. Embryo rescue techniques are used to raise hybrids, with hybridisation an effective method for plant improvement. Cheaper, less labour-intensive methods for propagation of *C. uncinatum* are presented. Results show variation in seed set between a self-pollinated selection from the wild (71%) and a bred hybrid (31%), as well as variation in response to temperature and treatment conditions, with percentage germination between 51–80% and 43–100% respectively. To stimulate germination, seed was surgically removed from the receptacle and treated with or without growth hormones and/or aqueous smoke solution and incubated at 15°C and 25/10°C. Maximum germination at 15°C occurred after smoke and GA₃ (25 mg l⁻¹) treatment for both genotypes. The hybrid demonstrated a two-fold increase in germination at 25/10°C [control and GA₃ (10 mg l⁻¹) treatments] compared to 15°C, with full germination attained using GA₃ (10 mg l⁻¹), with or without smoke. There was an increase in rate of germination at 15°C, compared to 25/10°C. These new techniques will enable large quantities of seed to be germinated quickly, leading to a high turnover of potential hybrids for the horticultural industry.

Introduction

The genus *Chamelaucium* belongs to the *Chamelaucium* alliance of the family Myrtaceae, and is largely endemic to Western Australia. Many species have attractive flowers and foliage, with *Chamelaucium uncinatum* being a major Australian floricultural crop for the cut-flower export market. There is constant demand for new genotypes of this species with novel flower types and colours, which flower at different times of the year. Hybridisation is an effective method for plant improvement.

The floriculture group within Agriculture Western Australia (AgWA) currently has a breeding program using genera within the *Chamelaucium* alliance, that includes both *Darwinia* and *Verticordia*. The aim of this breeding program is to produce new hybrids for the cut-flower and potted colour market. In this program, embryo rescue techniques are used to raise hybrids to maximise the likelihood of individual embryos germinating and eventually being established in the field for commercial assessment. Often only small numbers of seed are retrieved from each cross and if the embryos do not germinate normally, shoot growth can be induced adventitiously. Embryo rescue allows each putative hybrid embryo to be multiplied so that replications can be planted out for early assessment. Each hybrid remains in culture until at least 4 clones have been established in pots or in the field, then the culture line is terminated.

Collections from the wild and hybrid plants produced from the breeding program are planted at the AgWA's Medina Research Station in Western Australia. Large numbers of seed are produced from these plants via natural self pollination and cross pollination. Some of these seed may provide the germplasm for interesting and possibly successful new hybrids. It is too costly to raise large numbers of embryos of uncertain origin via tissue culture, thus an alternative cheap and low labour method is required.

Staff of the Western Australian Department of Conservation's *ex situ* seed conservation facility have collected and successfully germinated a range of *Chamelaucium*, *Verticordia* and *Darwinia* species listed as rare, threatened or poorly known. Removal of seed from the receptacle and treatment with aqueous smoke solution and the growth hormone, gibberellic acid, as GA₃, has proven to be the most successful protocol for germination used to date. This methodology for the germination of species from the *Chamelaucium* alliance presents a cheaper and less labour-intensive alternative to tissue culture.

The aims of these trials were to establish whether seed from two genotypes of *C. uncinatum* could be successfully germinated using this existing germination protocol, and to demonstrate the applicability of tested methods across a range of species within a genus or family.

Materials and Methods

1. Plant Material

Fruits were collected from plants of a self-pollinated selection (self) and a bred hybrid (hybrid) of *Chamelaucium uncinatum* from Agriculture Western Australia's Medina Research Station in late November 1998.

2. Seed Set

The percentage seed set was determined by sectioning fruits to establish the presence or absence of a healthy seed. The seed of *C. uncinatum* consists largely of an embryo-axis with a swollen hypocotyl as storage, located within a tough leathery receptacle. One hundred fruits from each genotype were examined and the number of embryos recorded.

3. Germination Test

Fruit of both *C. uncinatum* genotypes was soaked in distilled H₂O for 3 h to soften the receptacle prior to excising the seed. Seed was treated with either undiluted aqueous smoke solution (smoke) or distilled H₂O (nil smoke), for 24 h. Seed was rinsed with distilled H₂O before plating onto glass Petri dishes on agar (7.5 g l⁻¹) containing GA₃ at a concentration of 0 mg l⁻¹, 10 mg l⁻¹ or 25 mg l⁻¹. Experiments conducted on the self-pollinated selection and the bred hybrid ran over a period of 84 d and 68 d respectively. No treatment of seed of the self-pollinated genotype occurred at alternating temperatures due to the low availability of seed.

Petri dishes containing each combination of smoke/H₂O and GA₃ (0 mg l⁻¹, 10 mg l⁻¹ or 25 mg l⁻¹) were placed in growth cabinets set at a constant 15°C or in growth cabinets of alternating temperatures (10/25°C, 12 h/12 h). All growth cabinets had a photoperiod of 12 h. The agar medium was changed once during the experiment to overcome denaturing of GA₃ and dehydration of the agar. Germinated seeds were scored twice weekly and identified by emergence of the radicle. Experiments conducted on the wild species had two replicates of 23 seeds each, whilst those on the hybrid had one replicate of 25 seeds.

Results and Discussion: Seed Set and Germination

Sectioning of fruit indicated that seed set for the self-pollinated selection and the bred hybrid was 71% and 31% respectively. Germination ranged from 51% (control) to 80% (smoke + GA₃ 25 mg l⁻¹) for the self-pollinated seed. For the hybrid, germination ranged from 43% (control) to 100% (smoke/nil smoke + GA₃ 10 mg l⁻¹ + alternating temperatures) (Figure 29.1).

In both genotypes, the highest germination at 15°C occurred after treatment with smoke and GA₃ at a concentration of 25 mg l⁻¹ (96%). At 15°C, smoke out performed nil smoke at all levels of GA₃. Incubation with GA₃ at 25 mg l⁻¹ induced higher germination than at 10 mg l⁻¹, which in turn was superior to incubation without GA₃ for both the smoke and nil smoke treatments. At alternating temperatures, the highest germination for seed of the bred hybrid occurred after treatment with GA₃ at a concentration of 10 mg l⁻¹ with or without the application of smoke solution (100%). There was little difference between the treatments, with germination being very high in all cases.

The first treatment to reach 100% germination took 65 d, whereas 50% germination occurred within 19 d for some treatments (Table 29.1).

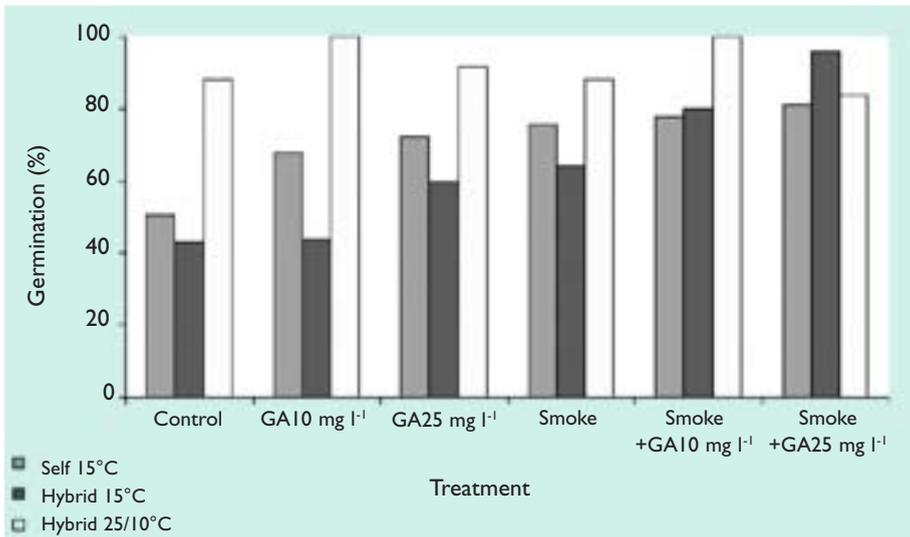


Figure 29.1 Percentage germination for self and hybrid seed of *C. uncinatum* under varying temperature and treatment conditions after removal of seed from receptacle.

Table 29.1 Time (days) to 50% germination (T_{50}) for *C. uncinatum* hybrid and self (in brackets) at 15°C and 25/10°C. No data for self-pollinated selection at alternating temperature

GA3 Conc.	15°C			Alternating Temperatures		
	0 mg l ⁻¹	10 mg l ⁻¹	25 mg l ⁻¹	0 mg l ⁻¹	10 mg l ⁻¹	25 mg l ⁻¹
Nil	- ¹ (77)	- (26)	55 (22)	41	24	31
Smoke	39 (34)	19 (20)	19 (23)	39	32	26

¹ Denotes treatments did not achieve 50% germination during the period of the experiment.

The self-pollinated selection of *C. uncinatum* showed a two-fold increase in seed set over the bred hybrid (71% as compared to 31%), indicating the possibility of barriers to pollen flow between genotypes. No further investigations were made, pollination mechanisms and genetic considerations of the species being outside the scope of the existing study.

After removal of the seed from the receptacle, germination was stimulated by the application of an aqueous smoke solution in combination with the growth hormone, gibberellic acid, as GA₃, when embryos were incubated at 15°C. The application of aqueous smoke has been shown to enhance the germination of other Western Australian natives (Dixon *et al.*, 1995; Roche *et al.*, 1997; Roche *et al.*, 1998). Growth hormones, such as GA₃, have also had a positive effect on the germination of Australian natives (Bell *et al.*, 1993; Bell, 1999), including a number of everlasting daisies (Plummer and Bell 1995). The synergistic effects of both smoke and GA₃ have been demonstrated in a range of Western Australian species (Cochrane *et al.*, 2002).

Incubation at alternating temperatures appears to overcome the need for smoke and GA₃, being sufficient alone to induce good germination. Changes in temperature and light regime create a similar environment to that which the seed is exposed to during natural germination.

Time to 50% germination was achieved very quickly using smoke and GA₃ at 15°C. Under these conditions, large quantities of seed can be germinated readily within a short period of time leading to a high turnover of potential hybrids. Uniformity in germination and subsequent seedling growth is of vital importance for cost-effective nursery management, with speed and synchrony of germination possibly of greater importance than final percent germination. At alternating temperatures, time to 50% germination was slower but more complete final germination was achieved.

Although increased germination was produced under protocols employing whole seed (100% germination as compared to 47% for embryo rescue techniques used at AgWA), these methods may not provide a substitute for embryo rescue in all species/hybrids. Instead, this new germination methodology may compliment the existing breeding program by providing a cheap, quick and low labour alternative to embryo rescue for producing large quantities of plants of *C. uncinatum* prior to screening for the breeding program. Further investigation is suggested to determine if after-ripening of fruits may overcome the requirement for GA₃ at lower temperature incubation.

This study has demonstrated that methods employed for the stimulation of germination of rare species can be applied across other, more common, species within a genus. It has also shown that seed-based research arising from *ex situ* conservation measures can make practical contributions to a wider understanding of seed biology in native species, thereby providing benefits to the horticultural industry.

References

- Bell, D.T., Plummer, J.A. and Taylor, S.K. (1993). Seed germination ecology in Southwestern Western Australia. *The Botanical Review* **59**: 1, 25–73.
- Bell, D.T. (1999). The process of germination in Australian species. *Australian Journal of Botany* **47**: 475–517.
- Cochrane, A., Kelly, A., Brown, K. and Cunneen, S. (2002). Relationships between seed germination requirements and ecophysiological characteristics aid the recovery of threatened native plant species in Western Australia. *Ecological Management and Restoration* **3**: 45–58.
- Dixon, K.W., Roche, S. and Pate, J.S. (1995). The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **101**: 185–192.
- Plummer, J.A. and Bell, D.T. (1995). The effect of temperature, light and gibberellic acid (GA₃) on the germination of Australian everlasting daisies (*Asteraceae*, Tribe *Inuleae*). *Australian Journal of Botany* **43**: 93–102.
- Roche, S., Dixon, K.W. and Pate, J.S. (1998). For everything a season: smoke-induced seed germination and seedling recruitment in a Western Australian Banksia woodland. *Australian Journal of Ecology* **23**: 111–120.
- Roche, S., Koch, J.M., and Dixon, K.W. (1997). Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restoration Ecology* **5**: 191–203.