FLORAL BIOLOGY AND PROPAGATION OF BLUE-FLOWERED *CONOSPERMUM* SPP.

by

Lynley M. Stone

B. Biotech (Hons) Flin.

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DECLARATION

I declare that the work in this thesis is of my own research, except where reference is made, and has not previously been submitted for a degree at any institution.

Lynley Stone

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ABSTRACT

Blue-flowered *Conospermum* are endemic to Western Australia, and show great potential as cut flowers. Propagation from cuttings or seed proved difficult, and root initiation *in vitro* is problematic. This thesis examines the floral biology of the species and the possibility of using somatic embryogenesis to overcome propagation problems.

A survey of explant tissue types for *C. eatoniae* and *C. caeruleum* was carried out to identify tissue that could be induced into embryogenic pathways. Vegetative, semi-floral and floral buds were initiated into culture from February to June, but were found unsuitable for embryogenesis, producing shoots, callus or dying in culture. Leaves from *in vitro* leaf cultures formed callus in the presence of 2,4-D and BAP, but were unable to differentiate into embryos in the presence of a variety of growth regulator combinations and concentrations. Immature zygotes died in culture. Direct embryogenesis and/or embryogenic callus was observed on mature zygotes of the species *C. caeruleum, C. spectabile, C. dorrienii* and *C. brownii*, and somatic embryos were maintained in culture for up to 18 months for *C. caeruleum*.

Maturation and germination of somatic embryos proved difficult; treatments of cold, ABA, desiccation or mannitol did not induce maturation. It appears that developmental pathways in *Conospermum* are well defined and are difficult to alter *in vitro*. It was concluded that somatic embryogenesis has limited commercial potential in these species.

Conospermum species have an active pollination mechanism where the style is held in a state of tension when the flower opens. When pressure is applied at the base of the style by an insect, the style flicks downwards, striking the insect pollinator and releasing pollen from the anther in a single dusty mass. However, the breeding systems of blue-flowered *Conospermum* have not previously been well explored.

Flowers on a *C. eatoniae* inflorescence opened from the basal end upwards acropetally, with the terminal two or three buds never opening. Fruit and seed set occurred only from the basal one to three buds. Isolation of *C. eatoniae* and *C. amoenum* flowers showed they were unable to self-pollinate in the absence of insect pollinators.

Experiments to determine the timing of the peak of stigmatic receptiveness were inconclusive. Pollen germinated and penetrated the stigma 0 – 6 days after anther dehiscence. Pollen loads on the stigma did not relate to the number of pollen tubes observed down the style. Controlled pollinations of cultivated *C. eatoniae* at a field station using self and cross pollen, revealed compatibility with a range of pollen genotypes, as pollen tubes were observed extending down the style. However, late-acting incompatibility could not be ruled out as controlled crosses failed to set any seed as flowers were shed from the bush.

DNA analysis of open pollinated *C. eatoniae* seed progeny from two plants from a field station and two plants in natural bushland revealed very different pollination habits. Plants from the field station showed no outcrossing, with progeny closely resembling the maternal parent, whereas plants from the wild population showed

outcrossing with several different paternal parents. These results suggest self-pollinated seed can be reliably obtained in a plantation situation using stands of ramets of the same clone. Alternatively, assuming that the required insect pollinators are present in a cultivated stand, it should be possible to obtain cross pollinated seed by surrounding the maternal plant with the desired paternal parent.

Unusual pollen behaviour was observed for many blue-flowered species, a white-flowered species of *Conospermum,* and close relative, *Synaphea petiolaris.* Up to three pollen tubes emerged from the triporate pollen *in vitro,* and at rates of up to 55 μms^{-1}. This rate was maintained for only 2 s but is greater than 20 times faster than reported in the literature for any species, *in vitro* or *in vivo.* Pollen with multiple tubes was also observed on the stigma *in vivo* in *C. amoenum* flowers.

Changing the osmotic pressure of the germination medium by altering sucrose concentration influenced the number of tubes to emerge from the pollen grain; generally the number of tubes decreased as sucrose increased. However, the rate of tube growth was unaffected. The addition of calcium channel blockers to the germination medium had no effect on *Conospermum* growth rate, nor did they eliminate pulses of tube growth.

Observation of *Conospermum* pollen ultrastructure revealed similarities to *Gramineae* pollen. The tube cytoplasm was packed with vesicles filled with material of similar electron density to the cell wall. Few golgi were identified, and the apical end of the tube contained these vesicles, smaller secretory vesicles and mitochondria. This is atypical of the tip, which is normally free of large vesicles. Distinct zones in the

cytoplasm were not identified, which is similar to *Gramineae.* Like the grasses, *Conospermum* appears to pre-manufacture cell wall material and store it in vesicles ready for rapid germination and extension.

A biological function of multiple pollen tube emergence with such rapid growth was not elucidated.

This research has shown *Conospermum* to be a complex and very interesting genus. Further investigation into the remarkable growth of multiple pollen tubes would enhance our knowledge of the biological processes involved in tube growth and the process of fast wall formation. The potential benefits to the cut flower industry of commercialising some of these species warrants further effort to find an efficient method of propagation. Introduction into horticulture may be the only means by which these threatened species will survive.

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TABLE OF CONTENTS

CHAPTER:

ABBREVIATIONS

WA: Western Australia

MS : Murashige and Skoog media (1962) including MS salts and vitamins

TDZ: thidiazuron

BAP: 6-benzylamino purine

2,4-D: 2,4-dichlorophenoxyacetic acid

IBA: indole-3-butyric acid

K: kinetin

GA_3: gibberellic acid

NAA: 1-napthaleneacetic acid

ABA: abscisic acid

FDA: fluoroscein diacetate

LIST OF PUBLICATIONS

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Stone, L., McComb, J.A., and Seaton, K.A. (2002) Propagation of blue-flowered *Conospermum* species. International Association for Plant Tissue Culture and Biotechnology – Australian Branch: The Importance of Plant Tissue Culture and Biotechnology in Plant Sciences. Armadale, NSW, Australia, January 20-23, 2002.

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