Ultrastructure and Function of Floral Nectaries of *Chamelaucium uncinatum* (Myrtaceae)

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Nectar is secreted for up to 11 d after anthesis in *Chamelaucium uncinatum*. The volume and sucrose concentration secreted varies between flowers, plants and days. The period of nectar secretion coincides with the period of pollen presentation and stigmatic receptivity suggesting nectar is part of an efficient reproductive strategy in *C. uncinatum*. The nectary of *C. uncinatum* consists of the entire upper surface of the ovary and hypanthium. The epidermis of the nectary is covered by a thickened cuticle which is only broken at the sites of the numerous modified stomata which are scattered across its surface. It is suggested that nectar is secreted onto the surface of the ovary via these modified stomata. The presence of extensive and well developed endoplasmic reticulum, mitochondria and Golgi bodies in the nectar secreting cells indicates that a granulocrine mechanism of secretion is occurring in *C. uncinatum*.

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**Key words:** *Chamelaucium uncinatum*, Geraldton Waxflower, floral nectaries, nectar production, modified stomata.

INTRODUCTION

Nectar is the most common reward offered by animal pollinated flowers (Simpson and Neff, 1983). The outcome of animal vectors foraging for nectar is the receipt and transfer of pollen resulting in pollination and fertilization of the flowers. The availability of nectar rewards whilst viable pollen and receptive stigmas are presented plays an important role in the reproductive success of a flower. Thus floral nectar secretion has a crucial role in the reproductive biology of many plant species with both the structure and function of nectaries receiving considerable attention from researchers during this century. The pathways of nectar secretion have been shown to vary between species. Both granulocrine transport in which vesicle membranes fuse with the plasmalemma and eccrine transport involving active molecular transport across membranes have been proposed (Durkee, 1983; Fahn, 1988). The release of nectar onto the nectary surface also occurs via a variety of mechanisms. Nectar may diffuse through thin secretory cell walls (Durkee, 1983; Fahn 1988). It may be secreted by trichomes or by modified stomata (Durkee, 1983; Fahn, 1988; Davis and Gunning, 1992; Zer and Fahn, 1992). Nectar may also accumulate beneath the cuticle of the nectary until the cuticle ruptures or is broken by the foraging vector releasing the nectar (Durkee, 1983; Marginson et al., 1985).

*Chamelaucium uncinatum* Schau. (Myrtaceae), commonly known as Geraldton Waxflower, is endemic to Western Australia and is widely grown in Australia, Israel and the USA as a commercial cut flower crop (Joyce, 1988). *Chamelaucium uncinatum* is an unusual species in that it possesses three separate secretory tissues. These are the floral nectary, the extrfloral nectary (O’Brien, 1995) and the anther glands (Slater and Beardsell, 1991). Little is known of the structural detail and function of floral nectaries in the commercially and ecologically important Australia family Myrtaceae although stomata have been implicated in floral nectar secretion in some myrtaceous species (Davis, 1968, 1969; Carr and Carr, 1987, 1990; Beardsell, Williams and Knox, 1989; Moncur and Boland, 1989; O’Brien, 1992). Flowers of *C. uncinatum* secrete considerable volumes of nectar. A study of the ultrastructure of *C. uncinatum* floral nectaries was made to investigate the pathway by which nectar is secreted in this species and to examine changes in the nectariferous tissue during and after the secretory phase. Given the structural similarity between stomata and the openings found penetrating the surface of the hypanthium and the known effects of abscisic acid on stomatal closure (Walton, 1980) we have investigated the effects of abscisic acid on nectar production, supplied through the cut ends of flower-bearing branches. To provide understanding of the function of the nectaries in relation to the reproductive biology of *C. uncinatum* as will be described in a future paper (O’Brien, 1996) field studies concerning nectar volume and concentration and insect behaviour and nectar removal rates were also conducted.

**MATERIALS AND METHODS**

**Plant material**

Floral nectary tissue was sampled from clonally propagated plants (cv. Alba and Purple Pride) grown in 15 cm pots in a shade house at the CSIRO Cunningham Laboratory, Brisbane (27°28’ S, 153°01’ N) Australia. Field nectar
Effects of abscisic acid on nectar production

Plants grown at a commercial flower farm near Gatton (27°34′ S, 152°17′ N), 90 km west of Brisbane.

Microscopy

Nectary tissue was fixed in 3% glutaraldehyde and 1% caffeine in phosphate buffer (0.1 M, pH 7.2) for 4 h at room temperature, rinsed in two changes of buffer, post-fixed in 1% OsO₄, rinsed again in buffer and dehydrated in a graded acetone series prior to embedding in Spurr's resin. Replicate material was dehydrated through a graded methanol series prior to embedding in LR White (medium grade). Sections 1 µm thick for light microscopy and ultrathin sections for transmission electron microscopy (TEM) were cut on a Reichert-Jung Ultracut microtome. Sections for light microscopy were stained with toluidine blue O (0.5% in 1% borax) for 1-2 min, rinsed with distilled water and viewed by bright-field or stained and mounted in auramine O and observed under epifluorescence (Carl Zeiss PH III). TEM sections were stained for 2 min in a saturated solution of uranyl acetate in 50% methanol then for 1 min in lead citrate (Reynolds, 1963) and viewed at 80 kV on a Philips 400T or at 100 kV on a Hitachi H-800 transmission electron microscope. Nectary tissue required for SEM was fixed and dehydrated as described for TEM. The dehydrated tissue was critical point dried, gold coated and viewed with a Philips 400 scanning electron microscope at 12 kV.

Results

Floral Nectaries of *Chamaeleucium uncinatum*

Field nectar secretion patterns

Patterns of floral nectar secretion and removal were observed on ten plants in Jul. and nine plants in Aug. 1993. Two comparable branches were selected on each plant and five flowers commencing anthesis marked on each branch. One branch was encased in an insect proof sleeve made from fly wire (2 × 1 mm) to prevent nectar removal by insects. The volume of nectar present in each of the marked flowers was measured between 0800 and 0900 h each day using a 5 µl micropipette until flowers no longer secreted nectar. The percent sucrose equivalent of the nectar (g solute per 100 g solution) was determined using a refractometer in conjunction with the July nectar volumes (Bolten et al., 1979). Variation in the volume and sucrose concentration of nectar produced between plants, treatments and days was compared using analysis of variance after the data underwent log transformation to improve normality. Changes to the structure and colour of flowers were observed during the period of nectar secretion.

Qualitative assessment of sugars in the floral nectar

The presence of sugars and their approximate relative amounts in the floral nectar was qualitatively assessed by gas chromatography/mass spectrometry by reference to known standards after formation of the trimethyl silyl derivatives following reaction with trimethyl silyl imidazole.

Ultrastructure of the nectaries

The epidermal cells of the nectary have few contents, staining weakly with toluidine blue O, and are covered with a thick and continuous cuticle (Fig. 1B). The cuticle is broken only at the sites of stomatal pores and stains intensely with auramine O (Fig. 1B and C). Scanning electron microscopy reveals the cuticle to be heavily sculptured and shows stomatal pores to be positioned at frequent, regular intervals (Fig. 1D). The secretory zone is five to seven cells deep and the dense cytoplasm of these cells stain blue with toluidine blue O (Fig. 1B). The secretory cells are clustered around the stomata with one cluster abutting the next due to the regular positioning of the stomata. The subglandular tissue beneath the nectariferous layer is composed of large parenchyma cells of which only the cell walls stain with toluidine blue O. The vascular tissue below the subglandular zone consists of both xylem and phloem.

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channels leading from the epidermal cells to the outer surface of the cuticle. The epidermal cells contain a single large electron translucent vacuole filling the entire cell. They contain no organelles and the vacuole is lined by a layer of electron opaque material and a small amount of granular cytoplasm (Fig. 1E). As previously stated, the cuticle is unbroken except at the position of the stomata. The cuticle lines the stomatal pores, tapering near the base of the pore and leaving only a narrow channel leading from the outer surface of the nectary into the substomatal space (Fig. 1F). A floccular substance, possibly nectar, is present in the pore. At the base of the pore abutting the substomatal space lie a pair of guard cells. The channel leading into the substomatal space is lined by a sheet of osmiophilic material and the substomatal space is lined with small globular electron opaque bodies. The substomatal space is bounded by the

Fig. 1. A, Half flower of *C. uncinatum* showing the location of the nectary (n), the style (s), the anthers (a), the staminodes (st), the ovules (o) and the oil glands (og). Bar = 2 mm. B, Light micrograph through the stomatal pore (arrowhead) in the nectariferous tissue stained with toluidine blue O showing the secretory cells (s), the unspecialised parenchyma cells (p), the vascular tissue (v) and the cuticle (c). Bar = 20 μm. C, Fluorescence micrograph through the stomatal pore (arrowhead) in the nectariferous tissue stained with auramine O showing the thick cuticle (c). Bar = 10 μm. D, Scanning electron micrograph of the sculptured cuticle of the epidermis of the nectary showing the numerous stomatal pores (arrowhead). Bar = 20 μm. E, Transmission electron micrograph (TEM) through the cuticle (c) and the epidermal cells (e) of the nectary. × 3500. F, TEM through the stomatal pore showing the secretory channel (arrowhead) and the presence of nectar in the pore (n), the cuticle (c), epidermal cells (e), the guard cells (g), the substomatal space (st) and secretory (s) cells. × 5600.
The nectariferous cells of secreting nectaries 2 d after anthesis contain numerous organelles within a granular cytoplasm. Many vacuoles are present with some surrounded by thin membranes only whilst others have a thick layer of electron opaque material lining the inner surface of the membrane. Some vacuoles may be empty and electron translucent, while some have granular contents, some contain membranous structures and others contain electron opaque bodies. Many cells within the nectariferous layer are connected by plasmodesmata although these were not observed in all cells (Fig. 2B). Golgi bodies are common and consist of stacks of three to four cisternae. Many have two guard cells and a number of secretory cells. The basal and upper wall of the guard cells is markedly striated and thickened. These cells contain vacuoles, starch grains and electron opaque bodies within a granular cytoplasm.
large vesicles at the ends of the cisternae indicating secretory activity (Fig. 2C). Mitochondria with well developed cristae are present within many of the nectariferous cells (Fig. 2C). Endoplasmic reticulum (ER) is well developed and present in several forms, occurring in convoluted and closely packed strands (Fig. 2D), as stacks of four to six cisternae (Fig. 2E) and as long strands of parallel cisternae (Fig. 2F). Starch grains, although uncommon, are also present in cells other than guard cells (Fig. 2F). Plastids and other electron opaque bodies are also found within the cytoplasm of nectariferous cells. The subglandular cells which abut the nectariferous cell layer contain a single large electron translucent vacuole filling most of the cell.

The ultrastructure of nectariferous cells 5 d after anthesis is similar to those 2 d after anthesis containing many organelles including well developed ER, mitochondria and vacuoles containing osmiophilic bodies. The cytoplasm is uniformly granular (Fig. 3A). However, by 10 d after anthesis the vacuoles of the nectariferous cells have expanded in size and take up most of the space within the cell. The cytoplasm around the vacuoles is granular in appearance and a few organelles remain. In some cells the vacuoles have merged to form one large vacuole taking up the entire cell space. The vacuoles are empty with the exception of a few containing osmiophilic bodies (Fig. 3B). By 16 d after anthesis cells of the nectariferous layer have few contents (Fig. 3C). All that remains is a granular cytoplasm lining the inner edge of the cell wall. Cell walls remain intact. The cells are now almost entirely filled by one large vacuole.

### Table 1. Mean total nectar production per flower of C. uncinatum on cut stems and intact plants under different conditions over 11 d. Different letters show means differ significantly (P < 0.05)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Test conditions</th>
<th>Nectar per flower (mg)</th>
<th>Significance</th>
</tr>
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<tbody>
<tr>
<td>Light</td>
<td>Stem + water</td>
<td>5.3 ± 3a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Stem + sucrose</td>
<td>8.3 ± 3a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Stem + ABA</td>
<td>6 ± a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Intact plant</td>
<td>37.1 ± 1b</td>
<td>b</td>
</tr>
<tr>
<td>Dark</td>
<td>Stem + water</td>
<td>3.8 ± a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Stem + sucrose</td>
<td>4.9 ± a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Stem + ABA</td>
<td>4.7 ± a</td>
<td>a</td>
</tr>
<tr>
<td></td>
<td>Intact plant</td>
<td>11.4 ± 4a</td>
<td>a</td>
</tr>
</tbody>
</table>

Fig. 3. A, TEM showing detail of a 5 d old nectariferous cell abutting the substomatal space (st). Many organelles are present within the granular cytoplasm as well as vacuoles containing electron dense deposits. Small intercellular spaces (arrowheads) are also present between cells. ×1500. B, TEM through a nectariferous cell of a 10 d old nectary showing a series of large vacuoles (v) filling the space within the cell. Granular cytoplasm (arrowhead) may still be seen outside these but few recognisable organelles are present. ×1500. C, TEM through a nectariferous cell of a 16 d old nectary showing the presence of a large vacuole (v) filling the cell leaving only a narrow band of cytoplasm (arrowhead) lining the cell wall. ×4600.
Effects of abscisic acid on nectar production

Abscisic acid had no significant effect on the amount of nectar production in the light or in the dark. Although nectar production was slightly higher when the stems were supplied with sucrose, the effect was not significant. Nectar production was significantly greater in flowers from intact plants, especially in the light (Table 1).

Field nectar secretion patterns

Nectar secretion occurs for up to 11 d after anthesis forming pools upon the surface of the nectary. The nectary surface of C. uncinatum is green at anthesis and remains so until approximately 7 d after anthesis when the colour begins to alter from green to red. By 10 d after anthesis the nectary surface is completely red. The volumes of nectar secreted by flowers and the number of days over which secretion occurred in C. uncinatum differed between Jul. and Aug. (Fig. 4A and B). In both months the standing crop of nectar was significantly less in unbagged compared with bagged flowers indicating bees and flies successfully removed at least half of the available nectar from flowers (F significant at P < 0.05). Nectar was produced continuously from the time of anthesis until the nectary tissue ceased to function. The volume of nectar produced varied significantly between plants, flowers and days (F significant at P < 0.05). Concentration of sucrose (Fig. 4C) in the bagged flowers was approximately double that of the unbagged flowers to which insects had access (F significant at P < 0.05). As with nectar volume, the concentration of sucrose varied significantly between plants, between flowers and between days (F significant at P < 0.05). Qualitative assessment of the extrafloral nectar by gas chromatography/mass spectrometry showed it to be proportionately composed of 30% fructose, 47% glucose and 23% sucrose.

DISCUSSION

Chamelaucium uncinatum is protandrous (Slater and Beardsell, 1991; O’Brien, 1996). Pollen is presented upon the stigma at anthesis with stigmatic receptivity developing 3 d after anthesis and reaching a maximum around 7–10 d after anthesis (O’Brien, 1996). The secretion of the nectar reward in C. uncinatum coincides with the presentation of pollen and the main period of stigmatic receptivity. The end of nectar secretion in C. uncinatum flowers coincides with the alteration of the colour of the nectary surface from green to red. Insects do not land upon flowers in which the nectaries have changed to red (O’Brien, 1996). Thus in C. uncinatum insects are encouraged to visit flowers during the period of pollen presentation and stigmatic receptivity through the provision of a nectar reward. The alteration in nectary colour provides a signal to the insects that no reward is offered by a particular flower thus forcing the insect to seek reward elsewhere. This enhances optimal foraging for the insect visitors and presumably also the reproductive success of each flower as insects are encouraged to visit only flowers with pollen or receptive stigmas. Further studies are required in natural populations of C. uncinatum to confirm this hypothesis.

Stomata as possible sites for the secretion of nectar in myrtaceous flowers have been reported previously in the genera Thryptomene, Leptospermum and Eucalyptus (Davis, 1968, 1969; Carr and Carr, 1987, 1990; Beardsell et al., 1989; Moncur and Boland, 1989; O’Brien, 1992; A. Davis, pers. comm.). As in C. uncinatum, it has been shown that a thickened cuticle covering the floral nectary of Thryptomene calycina (Myrtaceae) is broken only at the location of stomata and that it is through these breaks that nectar is secreted (Beardsell et al., 1989). Similarly, stomata located in the thickened cuticle of the nectaries of Leptospermum myrsinoides and L. continentale have been shown to be possible nectar secretory sites (O’Brien, 1992). The secretory pathway for the floral nectaries of C. uncinatum contrasts with that of the extrafloral nectary in which the nectar is secreted either through channels in the thin cuticle overlying the nectary or through breaks in the cuticle (O’Brien, 1995).

Stomata as avenues for nectar secretion have been reported in other plant families (Teuber et al., 1980; Durkee, 1983; Sammataro, Erickson and Garment, 1985; Davis and Gunning, 1992; Zer and Fahn, 1992). Stomata involved in nectar secretion have been described as ‘modified’ because they do not finely regulate flow rates of nectar as they do regulate gas exchange within a plant. All free surfaces of the guard cells of C. uncinatum are surrounded by thick cuticle which is continuous with that covering the other epidermal cells and implies that the guard cells of C. uncinatum are incapable of free movement. Similarly, Davis and Gunning (1992, 1993) concluded that the structure of the nectary of Vicia faba does not allow modified stomata to regulate nectar flow. Further, the addition of the plant hormone abscisic acid, involved in guard cell control (Walton, 1980), to cut flowering stems of C. uncinatum had no effect upon nectar flow rates. Inhibitors of stomatal mechanisms caused closure of leaf stomata in Medicago sativa while nectary stomata remained open (Teuber et al., 1980). These observations show that true stomatal function is not possessed by guard cells in the nectary of C. uncinatum and we suggest that these too should be referred to as modified stomata. Carr and Carr (1987, 1990) describing nectaries of Eucalyptus (Myrtaceae) have suggested the term ‘pore’ be used rather than stomata arguing that there is no evidence that the pore complexes originate in the same way as stomatal complexes. However, the structure of the guard cells in C. uncinatum nectaries reveals a strong similarity to leaf guard cells reported for other species (Pearson and Milthorpe, 1974; Sanchez, 1977; Wille and Lucas, 1984; Davis and Gunning, 1993) with similar thickenings of the cell walls and the presence of starch grains within the cytoplasm. We believe this similarity confirms that the nectary pores in C. uncinatum are formed by guard cells and are best described as modified stomata.

Two pathways of nectar transport from inside to the outside of the protoplasts of nectariferous cells have been postulated (Durkee, 1983; Fahn, 1988). Granulocrine secretion is characterised by an abundance of active ER, mitochondria and Golgi (Rachmilevitz and Fahn, 1973). Eccrine secretion is characterised by relatively few ER and Golgi but numerous plastids containing starch grains (Durkee, 1983; Zer and Fahn, 1992). Nectaries containing...
extensive and well developed ER and Golgi often contain few starch grains (Fahn and Benouaiche, 1979) and this is true in *C. uncinatum*. The activity of the extensive ER, the number of mitochondria and the presence of vesicles on the Golgi cisternae in *C. uncinatum* suggests that granulocrine secretion is the mode of transport of nectar in this species. Few starch grains are present in the nectariferous cells of *C. uncinatum* unlike the nectariferous cells of species with eccrine secretion such as *Passiflora* (Durkee, Gaal and Reisner, 1981) and *Rosmarinus* (Zer and Fahn, 1992) implying that starch reserves held within these flowers contribute little to the nectar secreted. Our observation that flowering stems and intact plants held in the dark showed significant decline in nectar secretion supports this conclusion. Nectar production in *C. uncinatum* appears to be heavily dependent upon current assimilation of photosynthate. Even supplying sucrose to cut stems was no substitute for photosynthetic activity.

Nectar volume and sucrose concentration in *C. uncinatum* varied between flowers, plants and days. The volume and length of the nectar secretion period was much less in Aug. compared with Jul. August was much warmer than July suggesting that the increased temperatures hastened the onset of senescence in the nectariferous tissue. The decrease in nectar production may also have resulted from water stress caused by increased temperature. The addition of water to plants of *Asclepias syriaca* increased nectar volumes approximately twofold (Wyatt, Broyles and Derda, 1992).

**Fig. 4.** A, Volume of nectar secretion in July in bagged and unbagged flowers from anthesis until secretion finishes. B, Percentage sucrose (g solute per 100 g solution) of nectar secretion in July in bagged and unbagged flowers from anthesis until secretion finishes. C, Volume of nectar secretion in August in bagged and unbagged flowers from anthesis until secretion finishes. ◆, Bagged; ■, open.
It is therefore likely that reduced water availability in *C. uncinatum* will decrease the volume of nectar secreted. The sucrose concentration of bagged flowers was greater than that of unbagged flowers. Insects removed nectar from the unbagged flowers very rapidly. Possibly during the 24 hours between the sampling of bagged flowers some evaporation occurred resulting in increased nectar concentrations. There is also evidence in some species that pools of nectar may be partially reabsorbed and reincorporated into a more concentrated nectar (Corbett, 1978). Variation in nectar production has been observed in other species (Zimmerman, 1988; Real and Rathcke, 1991; Shmida and Kadmon, 1991). Within plant variation or patchiness in nectar production will impact upon the way pollinators forage and therefore will also impact upon the reproductive success of individual plants. Consideration of the ecological implications of this observation would be best left to a study in the native habitat of *C. uncinatum*.

We have shown that nectar secretion in *C. uncinatum* occurs via the numerous modified stomata present in the epidermis of the nectaries of this species. We also suggest that the mode of secretion of nectar from the nectariferous cells is granulocrine although further research is required to confirm this hypothesis. The observations that the period of nectar secretion coincides with the period of pollen presentation and stigmatic receptivity suggests the evolution of an efficient reproductive strategy for *C. uncinatum*.

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**LITERATURE CITED**


