

Original article

## Influence of floral visitation on nectar–sugar composition and nectary surface changes in *Eucalyptus*

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**Summary** — Floral nectaries and their production of major nectar carbohydrates were studied in three species of *Eucalyptus* in Australia. In *E. cosmophylla*, *E. grandis* and *E. pulverulenta*, the nectary is located on the inner surface of the hypanthium, below the stamen filaments. Nectary surfaces possessed hundreds of modified stomata that were solitary, distributed uniformly, asynchronous in development, and served as exits for nectar flow. Nectar yields per bagged flower were greatest in *E. cosmophylla* and least in *E. grandis*, correlating with flower size but not nectary stomatal density. The nectar of *E. pulverulenta* was sucrose-rich, but hexose-rich for the others. Few changes in nectar carbohydrate composition were detected between flowers whether protected or continually exposed to visitors (eg, honeybees), and whether young or old, indicating an overall constancy in composition for the long period of nectar availability.

*Eucalyptus* / honeybee / modified stomata / nectar carbohydrate / nectary surface

### INTRODUCTION

Floral nectar from *Eucalyptus* trees provides Australia's most important source of honey production (Brimblecombe, 1946; Clemson, 1985), allowing the country's average honey yields per bee colony to rank among the highest in the world (Anonymous, 1985, 1987). Today, their potential for honey and timber production, as well as their ornamental value, has fostered cultivation of eucalypts in many other countries (Pellett, 1923; Lovell, 1926; Vansell, 1941; Barbier, 1951; Lupu and Eisikowitch, 1990). Chem-

ical analyses of Australian honeys derived from individual eucalypt species are available (Chandler et al, 1974). However, little research has been devoted to *Eucalyptus* nectar, particularly within Australia (Bond and Brown, 1979; Moncur and Boland, 1989).

Although the overwhelming majority of species secrete floral nectar of relatively constant composition (Percival, 1961), some changes may occur. The carbohydrate composition may change with floral age, whether collected from species having short-lived

( $\leq 24$  h) flowers (Freeman, 1986; Petanidou et al, 1996), flowers of intermediate (2–4 days) duration (Loper et al, 1976), or long-lived (5–8 days) flowers (Christ and Schnepf, 1988; Kronstedt-Robards et al, 1989). An increased amino acid concentration is common in nectar of aging flowers (Gottsberger et al, 1990; Petanidou et al, 1996). Additionally, the composition of floral nectar may be altered by animal visits (Corbet, 1978; Willmer, 1980; Freeman and Wilken, 1987). Flowers of eucalypt species are generally long-lived, lasting 4–18 days post-anthesis (Ashton, 1975; Hodgson, 1976; Núñez, 1977; Griffin and Hand, 1979; Moncur and Boland, 1989; Lupo and Eisikowitch, 1990; Ellis and Sedgley, 1992). However, the constancy of *Eucalyptus* nectar composition either during flower phenology, or following animal visits, has not been examined in Australia.

Nectar escape in most Myrtacean flowers occurs from the inner surface of the hypanthium, in a region extending from the base of the staminophore to the upper surface of the ovary (Carr and Carr, 1987, 1990; Beardsell et al, 1989; Moncur and Boland, 1989; O'Brien et al, 1996). Structures referred to as stomates (Davis, 1968, 1969), stomata (Beardsell et al, 1989), stomatal-like (Moncur and Boland, 1989), modified stomata (O'Brien et al, 1996) and pore cells (Carr and Carr, 1987, 1990) now have been detected on the surfaces of floral nectaries from over 40 Myrtacean (mostly *Eucalyptus*) species. On the nectary surface of *E. stellulata*, Davis (1969) reported that these structures remain permanently open, whereas Carr and Carr (1987) postulated that they are able to open and close, and may regulate nectar flow through them.

The objectives of this study were to determine, in three *Eucalyptus* spp in Australia, various nectar characteristics including the constancy of nectar-carbohydrate composition following flower visits and as flowers age; and to examine the nectary surfaces,

to investigate further the mechanism of nectar escape.

## MATERIALS AND METHODS

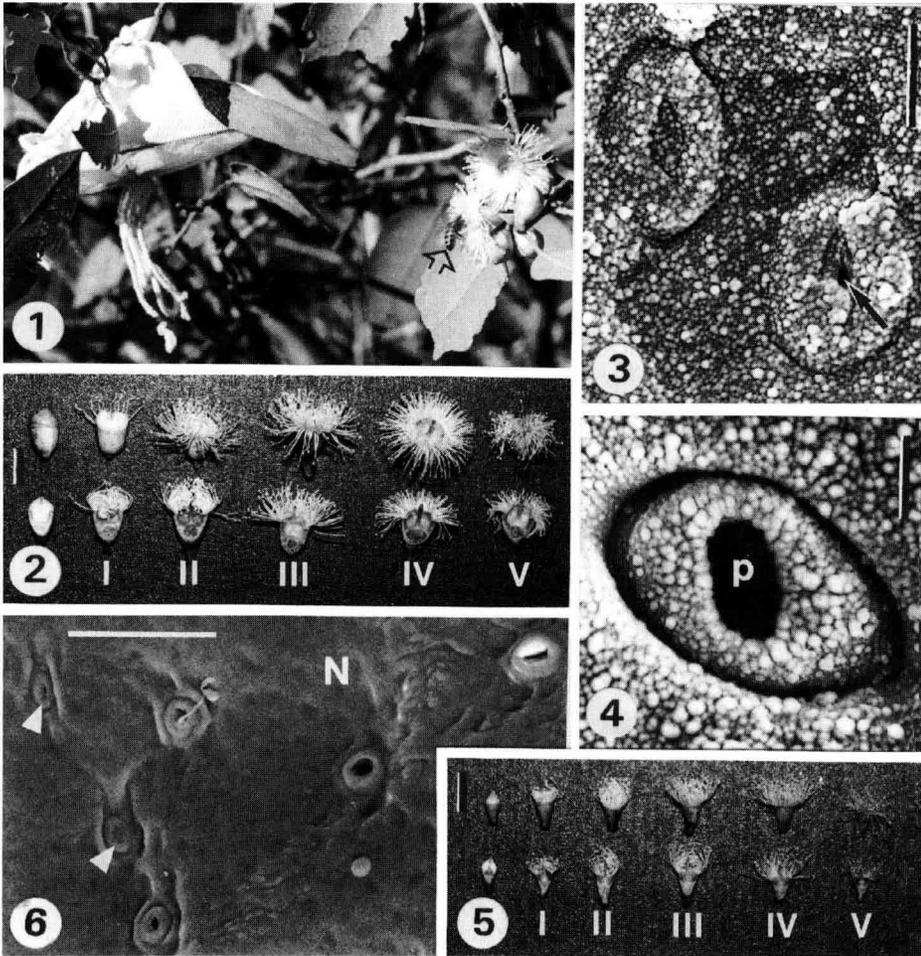
### Plant material

The trees investigated, one per species, were growing as ornamentals at The Australian National University in Canberra. *E. cosmophylla* F Muell (native to South Australia; Penfold and Willis, 1961) grew between buildings of the Department of Forestry and the Research School of Biological Sciences. Both *E. grandis* Hill (Maiden) (coastline of Queensland and New South Wales; Penfold and Willis, 1961) and *E. pulverulenta* Sims (southeastern NSW; Peters et al, 1990) were studied near the western border in the Culture Area of the Department of Botany. Of these species, *E. grandis* especially is recognized as an important honey plant (Penfold and Willis, 1961; Clemson, 1985).

### Floral stages

*E. cosmophylla* and *E. pulverulenta* typically possessed three buds per umbel (fig 1 and 7); *E. grandis* had seven. Five floral stages (I–V; see figs 2, 5 and 8) were designated. Flowers entered stage I following dehiscence of the bud cover (operculum; fig 2 top left), when the stamens commenced unfolding from their natal position occupying the hypanthial region. The protandrous flowers at stage II had most stamens still with their filaments bent inward; only a minority of innermost stamens still occupied the hypanthium. Continual unfolding of innermost stamens coupled with outstretching of the outermost stamens characterized stage III. A strong fragrance was evident by this stage. At stage IV, stamens were extended fully to form an obvious ring encircling the central style. Stage-V flowers still possessed all stamens, but in a collapsed ring owing to the shrivelled filaments.

To prevent visits by birds (honeyeaters) and large insects (primarily *Apis mellifera*; fig 1) to some flowers, many inflorescences per tree were protected (fig 1) when anthesis was imminent, by enclosure within bags (mesh size approx 2.5 mm; see fig 11 of Davis, 1992) fitted over wire frames. In *E. grandis*, dehiscence of the



**Fig 1-4. *E. cosmophylla*.**

Fig 1. Flowers protected inside bag (left) and exposed (right). *Apis mellifera* (arrow) collecting nectar. Fig 2. Floral stages I-V, preceded by operculum dehiscence (left). Top: intact flowers; bottom: flowers sectioned longitudinally. Bar = 1 cm. Fig 3-4. Nectary surface of mature bud. Fig 3. Immature (left) and maturing (right) modified stomata with ruptured cuticle (arrow) over pore. Bar = 10  $\mu$ m. Fig 4. Open pore (p). Bar = 5  $\mu$ m.

**Fig 5-6. *E. grandis*.**

Fig 5. Floral stages I-V, preceded by mature bud (left). Bar = 1 cm. Fig 6. Asynchrony of stomatal development on post-secretory nectary (N). Immature stages (arrowheads). Bar = 50  $\mu$ m.

operculum was preceded by its change from green to yellow (fig 5 top left; Hodgson, 1976); predictors of anthesis in the other species were not obvious.

### Nectar collection and analysis

Small numbers of flowers were harvested periodically from May–August 1990, and immediately carried into the laboratory nearby. In total, five (occasionally four or six) flowers per stage (both exposed and bagged) per tree were taken for nectar collection. Nectar was removed using a technique (Davis and Gunning, 1991) combining Drummond Microcaps® (1–10 µL) and then filter-paper wicks (McKenna and Thomson, 1988) to soak up residual nectar. Nectar-solute concentrations were assayed immediately by expelling nectar from a capillary onto Bellingham and Stanley refractometers (Tunbridge Wells, UK), then corrected to 20 °C. Concentrations were expressed as g per 100 mL using the formula of Búrquez and Corbet (1991). Wicks were allowed to air-dry before storage in clean labeled vials at room temperature.

Total nectar volume per flower was calculated by summation of filled capillaries, and that of the wicked nectar (< 2 µL) estimated from concentration data (above) and sugar yields (below).

Contents of sucrose, glucose and fructose in nectar were assayed enzymatically (Kronstedt–Robards et al, 1989). All enzymes were of analytical grade (Boehringer-Mannheim; Sigma). Briefly, sample wicks were placed in known volumes (0.5–1.0 mL) of distilled water in disposable Eppendorf centrifuge tubes and agitated on a Vortex Genie®. After 30 min elution time on ice, tubes were centrifuged before 5.0–200 µL aliquots were introduced into separate cuvettes containing 100 mM Tris-HCl buffer (pH 4.5, for sucrose determination; pH 7.6, for hexoses), 30 mM ATP, 5 mM NADP and 0.175 U glucose-6-phosphate dehydrogenase (EC 1.1.1.49). Depending on the assay, 5–µL aliquots of invertase (β-fructosidase, EC 3.2.1.26), hexokinase (EC 2.7.1.1) or phosphoglucose isomerase (EC 5.3.1.9) were added to initiate the reaction. Final volumes were 800–900 µL per cuvette. Reduction of NADP<sup>+</sup> to NADPH was measured spectrophotometrically (Eppendorf) at 340 nm using a chart recorder and compared to standards of freshly-prepared carbohydrate solutions.

Contents of sucrose, glucose and fructose per nectar sample were expressed as percentages. Means were calculated for each floral stage and compared by pooled, two-tailed *t* tests (*P* = 0.05). Ratios of glucose to fructose (G/F), and sucrose to hexose [S/(G+ F)], were also compared statistically.

### Examination of nectary surfaces

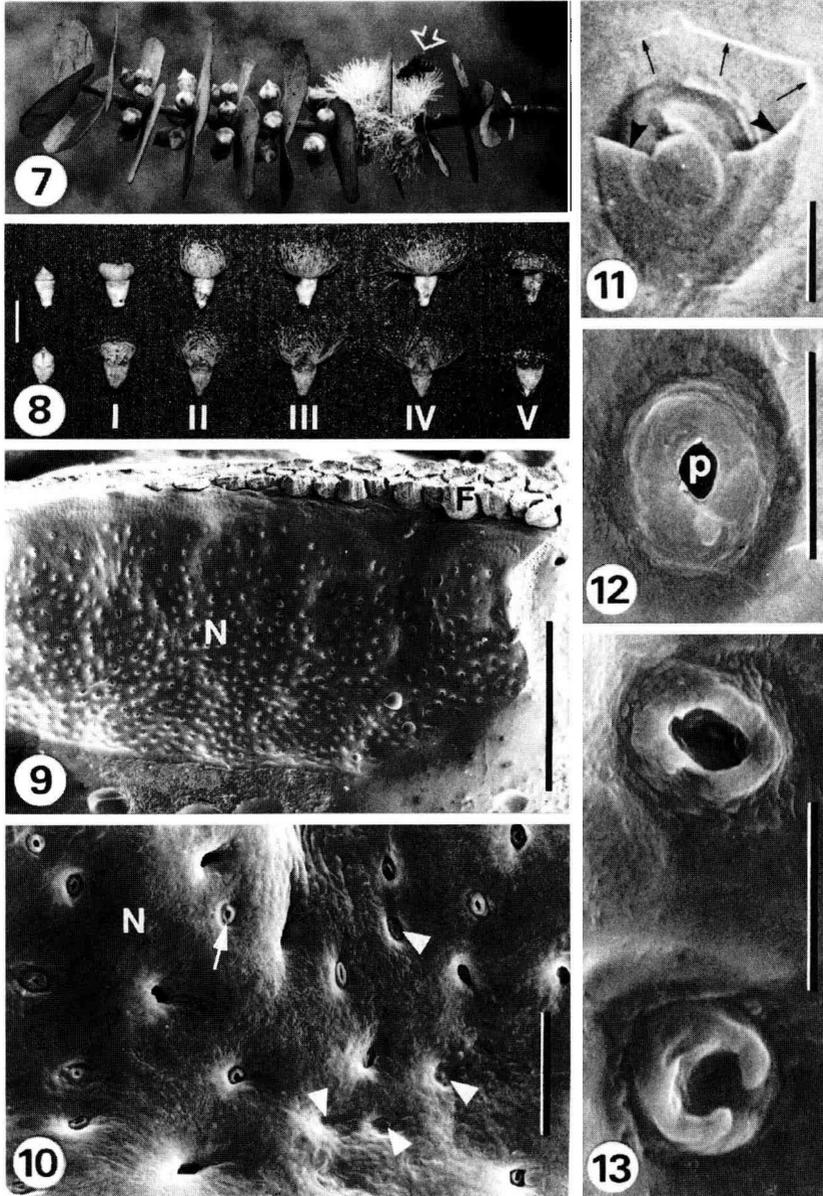
Mature, pre-secretory buds and post-secretory flowers of each species were harvested and immediately prepared for low-temperature scanning electron microscopy (SEM). Sliced buds and flowers were mounted nectary-side up with Tissue Tek®/colloidal graphite (1:1) on stubs and plunged into liquid N<sub>2</sub> slush (–230 °C). The flash-frozen specimens were then transferred under vacuum into a Hexland 1000A CryoTrans cold stage attached to a Cambridge Stereoscan 360 SEM. Nectaries were coated with gold, viewed directly at 15 kV and –180 °C, and photographed with Ilford SEM film.

## RESULTS

### Nectar volumes, concentrations and sugar quantities as flowers aged

Flower duration was shortest in *E grandis*, taking 4–6 days from anthesis to stage IV and about 6 days more to stage V. In *E cosmophylla*, stage III was reached 4–6 days after operculum dehiscence and stage V 7–9 days later. In *E pulverulenta*, stage III was reached 6–10-days, stage IV 12-days, and stage V 17-days post-anthesis, respectively.

In all species, nectar was absent in flowers with opercula just dehiscing, but was collectable by stage I (figs 14–16, upper plots) when flowers of *E cosmophylla* possessed 20–50 times more than the others. In that species, nectar secretion preceded anther dehiscence. At stage I, all *E cosmophylla* flowers secreted nectar, compared to *E pulverulenta* (70%) and *E grandis* (17%). Thereafter, nectar was available from stages II–V in each species.



**Fig 7-13. *E. pulverulenta*.**

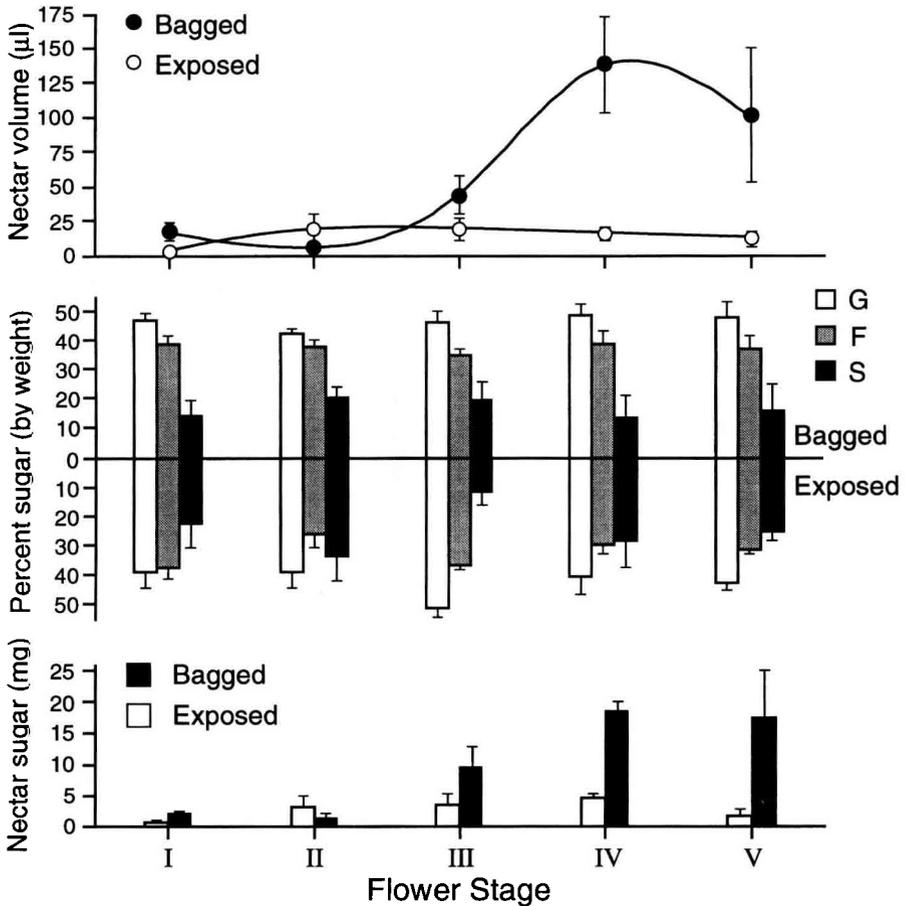
Fig 7. *A. mellifera* (arrow) visiting open flower. Fig 8. Floral stages I-V. Bar = 1 cm. Fig 9-13. Flower at stage I. Fig 9. Hypanthial surface (N) pitted with modified stomata. Filament bases (F). Bar = 1 mm. Fig 10. Various stages of modified stomata; immature (arrowheads), occluded (arrow). Bar = 100  $\mu$ m. Fig 11. Rupture through surface cuticle (arrows) to create the ostiole, revealing crater evident below cuticle (arrowheads) still spanning pore. Bar = 10  $\mu$ m. Fig 12. Open pore (p). Bar = 20  $\mu$ m. Fig 13. Pores occluded. Bar = 20  $\mu$ m.

Patterns of nectar-standing crop were similar for all species; flowers exposed continually to animal visits contained volumes that were not significantly different across stages II–IV (figs 14–16). By stage V, nectar yields declined.

In flowers that opened within bags and were continually inaccessible to large visitors, copious volumes of nectar accumulated (figs 14–16). Nectar yields were greatest at stage III in *E pulverulenta*, and at IV

in the others. At these peak levels, flowers of *E cosmophylla* averaged 4.4 and 8.9 times more nectar than *E pulverulenta* and *E grandis*, respectively. Bagged flowers of stage V contained less nectar than stage IV, but this difference was statistically significant ( $P < 0.05$ ) only for *E grandis*.

Nectar-sugar quantities per floral stage (figs 14–16, bottom plots) corresponded closely to the patterns for nectar volumes. This relationship occurred because, at each

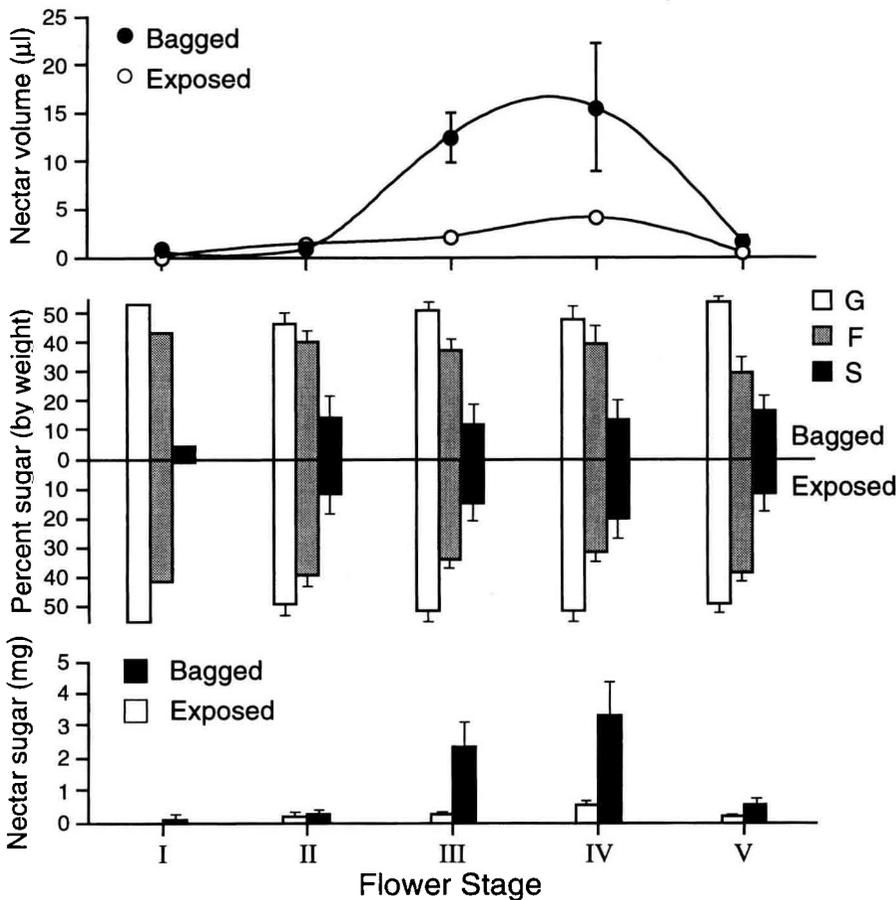


**Fig 14.** Nectar volume and nectar-sugar quantities and composition (glucose, fructose and sucrose) produced by five floral stages of *Eucalyptus cosmophylla* (30.v-9.viii 1990). Values are means  $\pm$  SE.

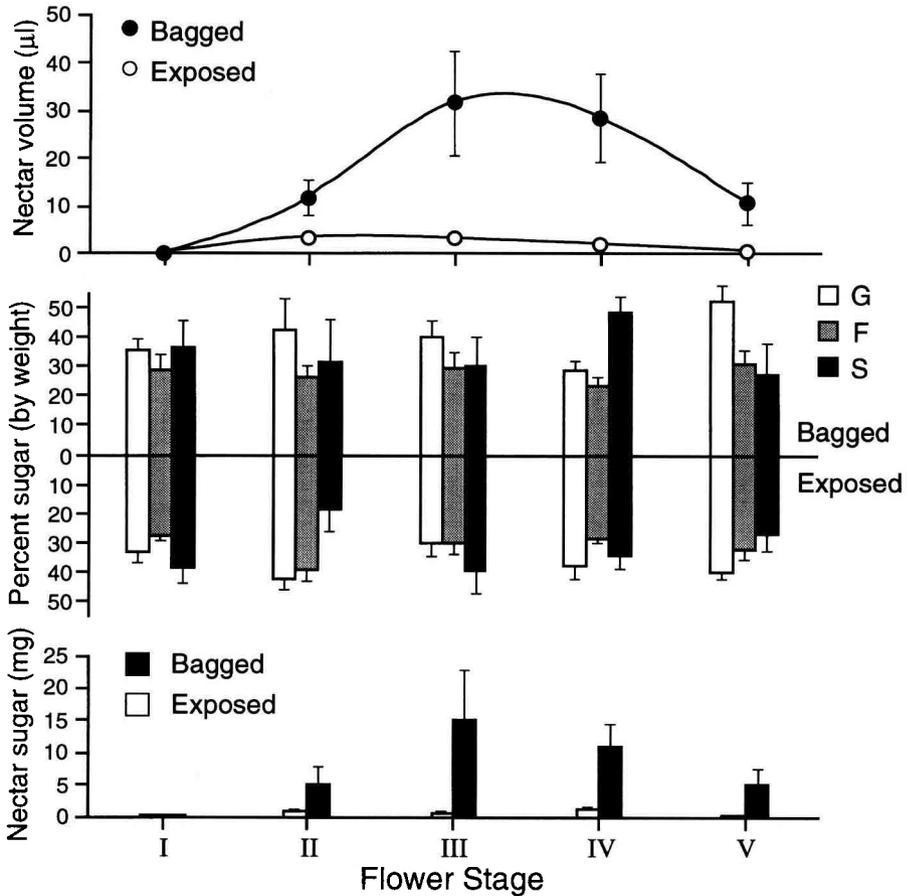
floral stage within a species, nectar-solute concentrations were not significantly different between bagged and exposed flowers. In bagged flowers, average quantities of nectar sugar were maximal at stage III (*E pulverulenta*) or IV (others), with *E cosmo-phylla* secreting 1.2 and 5.6 times more sugar per flower than *E pulverulenta* and *E grandis*, respectively.

Average nectar-solute concentrations at the five floral stages ranged from

16.0–37.3 g/100 mL (exposed) and 14.1–29.6 g/100 mL (bagged) in *E cosmo-phylla*. In *E grandis*, concentrations varied from 14.8–68.2 g/100 mL (exposed) and 19.1–41.9 g/100 mL (bagged). In *E pul- verulenta*, concentrations ranged from 17.8–30.6 g/100 mL (exposed) and 23.3–49.6 g/100 mL (bagged). Overall, nectar-solute concentrations were usually lowest at stage I and highest at stages IV and V.



**Fig 15.** Nectar volume and nectar-sugar quantities and composition (glucose, fructose and sucrose) produced by five floral stages of *Eucalyptus grandis* (15.vi-15.viii 1990). Values are means ± SE. At stage I, n = 1 flower.



**Fig 16.** Nectar volume and nectar-sugar quantities and composition (glucose, fructose and sucrose) produced by five floral stages of *Eucalyptus pulverulenta* (28.vi-18.viii 1990). Values are means  $\pm$  SE.

### Nectar-sugar composition in exposed and protected flowers, as flowers aged

In *E cosmophylla*, flowers that were protected from visits by netting showed no statistically-significant changes in quantity of each nectar carbohydrate (glucose, fructose, sucrose) as flowers aged (fig 14, middle

plot). The same held true for exposed flowers. In *E grandis*, the levels of the different sugars remained highly consistent for bagged and exposed flowers (fig 15). Similarly, no statistically significant differences occurred in *E pulverulenta*, whether bagged or exposed, as flowers progressed through stages I-V (fig 16). For both *E cosmophylla*

and *E grandis*, the sucrose content of nectar was usually lower than each hexose (figs 14 and 15; table I), whereas nectar sucrose in *E pulverulenta* (fig 16) averaged 33% over all flowering stages (table I). When the  $S/(G+F)$  ratios were compared between bagged and exposed flowers for each floral stage in all three species, and then overall (table I), no statistically significant differences were detected.

For all species the average content of glucose in nectar exceeded fructose (figs 14–16). Significant differences in the ratio of G/F between exposed and bagged flowers occurred at stage II in *E cosmophylla* (fig 14;  $P < 0.05$ ) and stage III in *E pulverulenta* (fig 16;  $P < 0.01$ ). When mean composition data were combined for all floral stages (table I), nectar of bagged flowers of *E pulverulenta* had a significantly greater G/F ratio than that of exposed flowers ( $P < 0.02$ ).

### Nectary features as flowers aged

The nectary surface spanned the inner hypanthium from the style base to near the staminophore and bore modified stomata, except for a zone of variable width below

the stamens: *E cosmophylla* (250–450  $\mu\text{m}$ ), *E grandis* (250  $\mu\text{m}$ ), *E pulverulenta* (fig 9; 200–250  $\mu\text{m}$ ). In all species, the modified stomata were usually solitary and distributed regularly (figs 6, 9 and 10). Stomatal frequency remained constant in pre- and post-secretory flowers and averaged  $348 \pm 15$  (SE,  $n = 7$ ; *E cosmophylla*),  $299 \pm 10$  ( $n = 3$ ; *E grandis*) and  $163 \pm 7$  ( $n = 3$ ; *E pulverulenta*) per  $\text{mm}^2$  of nectary surface.

In all species, modified stomata of various developmental stages could be detected in a single nectary epidermis. In pre-secretory buds could be found immature (figs 3 left and 10) and maturing (figs 3 right and 11) stomata, and those with open (figs 4, 10 and 12) pores, as well as modified stomata with occluded pores (figs 10 and 13). Similarly, this asynchrony in development was detected in post-secretory nectaries (fig 6). The occluding material of light electron density on the surface of the guard cells of *E pulverulenta* (fig 13) may correspond to the small red dots apparent with the naked eye, especially in fresh flowers of stages IV and V.

In *E cosmophylla* the nectary surface had a dull sheen and possessed numerous wax globules (figs 3 and 4) that were absent post-

**Table I.** Sugar composition of nectar collected from bagged or exposed flowers of three species of *Eucalyptus*, averaged ( $\bar{x} \pm \text{SE}$ ) over all floral stages. Within species, means in columns with the same superscript are not significantly different ( $P = 0.05$ ).

Species	Treatment	Glucose (G)	Fructose (F)	Sucrose (S)	G/F	S/(G+F)
<i>E cosmophylla</i>	Bagged	46.34 <sup>a</sup> ± 1.15	37.11 <sup>a</sup> ± 0.66	16.57 <sup>a</sup> ± 1.41	1.28 <sup>a</sup> ± 0.041	0.236 <sup>a</sup> ± 0.018
	Exposed	42.79 <sup>a</sup> ± 2.21	32.50 <sup>a</sup> ± 2.08	24.70 <sup>a</sup> ± 3.65	1.35 <sup>a</sup> ± 0.084	0.399 <sup>a</sup> ± 0.080
<i>E grandis</i>	Bagged	49.52 <sup>a</sup> ± 1.79	36.52 <sup>a</sup> ± 2.07	13.97 <sup>a</sup> ± 0.91	1.43 <sup>a</sup> ± 0.169	0.163 <sup>a</sup> ± 0.031
	Exposed	50.26 <sup>a</sup> ± 0.60	35.81 <sup>a</sup> ± 1.84	13.93 <sup>a</sup> ± 1.25	1.42 <sup>a</sup> ± 0.065	0.160 <sup>a</sup> ± 0.039
<i>E pulverulenta</i>	Bagged	37.81 <sup>a</sup> ± 2.57	27.71 <sup>a</sup> ± 1.38	34.61 <sup>a</sup> ± 3.68	1.36 <sup>a</sup> ± 0.015	0.689 <sup>a</sup> ± 0.097
	Exposed	36.77 <sup>a</sup> ± 2.16	31.66 <sup>a</sup> ± 2.02	31.56 <sup>a</sup> ± 3.88	1.18 <sup>b</sup> ± 0.058	0.535 <sup>a</sup> ± 0.089

secretion. In *E grandis* and *E pulverulenta* the nectary appeared shiny and, except for many small flecks of wax in mature buds of the former, lacked wax throughout (figs 6, 10–13).

Nectary color changed from mature bud to stage III to stage V, as follows: *E cosmophylla* — pale green to bright yellow to yellow-orange; *E grandis* — bright lemon-yellow to butterscotch (see Clemson, 1985) to light butterscotch with mottled black (fig 5, bottom right); *E pulverulenta* — pale green to bright orange to butterscotch with red or brown mottling (fig 8, bottom right).

## DISCUSSION

### Floral nectar characteristics

Nectar traits of these species differed in several respects. Both maximal nectar volume and sugar production per flower were related to flower size, being highest in *E cosmophylla* and lowest in *E grandis*. The tendency for nectar-solute concentration to increase during stages I–IV may relate to exposure of standing nectar to the atmosphere, during the progressive unfolding of the stamens during flower phenology. In stage V, further evaporation of water from nectar and a diminishment in rate of nectar volume secreted, probably contributed to the higher concentrations observed.

Nectar-sugar composition remained constant per species, in contrast to other species with long-lived flowers (see *Introduction*). Individual sugar levels remained similar, as did the ratios G/F and S/(G+F). Carbohydrate composition appeared most consistent for nectar of *E grandis*, which contained the lowest sucrose content (14%), and most variable for *E pulverulenta*, in which sucrose levels were highest (33%). In California, Vansell (1941) reported that for *E globulus*, the ratio between invert and total sugars varied over 5 days from 1:1.566 to 1:2.192,

but it was not disclosed which floral stages were involved nor whether this change was statistically significant. It would be interesting to determine whether pre-nectar of *Eucalyptus* flowers travels two separate routes (which may impact final nectar composition) through the nectary (Nichol and Hall, 1988), and if so, what proportion of the nectar travels each route, particularly during periods of differential rates of secretion (eg, stage I or V, versus III or IV). That G/F ratios were above unity (1.2–1.4) suggests that the secretory process in these eucalypts is more complicated than simple inversion of sucrose to equal quantities of the hexoses.

For reasons unknown, G/F ratios occasionally were significantly different, though not in a consistent pattern. In *E cosmophylla* (stage II) exposed flowers had a higher G/F ratio than bagged ones, but in *E pulverulenta* (III) the reverse was true. In *E cosmophylla*, variability in nectar-sugar composition was always greater from exposed flowers than bagged (see SE values in table I), but was irregular for the others. Willmer (1980) found changes in amino acid composition of nectar following visits; however, amino acids were not detected in eucalypt nectar (Núñez, 1977).

Microorganisms can occur in nectar and may affect its composition (Lüttge, 1961; Gilliam et al, 1983), and algae have been detected on nectaries of eucalypts (Carr and Carr, 1987). Although not sought, unidentified green-pigmented algae were found macroscopically on nectaries of two bagged flowers of *E grandis*, during nectar collection. However, the carbohydrate composition of these algal-contaminated nectars did not differ from others of stage III or IV. Evidently the algal spores entered the nectar of these two flowers by passing through the mesh airborne, or on the bodies of thrips, common inhabitants of all flowers studied.

Overall, the S/(G+F) ratios for nectar of *E cosmophylla* and *E grandis* averaged

between 0.1–0.499 ('hexose-rich'; Baker and Baker, 1983), but were in the range 0.5–0.999 ('sucrose-rich') for *E. pulverulenta*. These designations generally agree with observations of floral visitors to these species. For instance, short-tongued bees like *A. mellifera* are regular visitors of taxa that produce nectar having a wide range of sucrose/hexose ratios, but particularly those which are hexose-dominant or -rich (Baker and Baker, 1983). Similarly, species pollinated principally by honeyeaters (birds) have hexose-dominant or -rich nectar, only (Baker and Baker, 1983). These nectar-carbohydrate compositions are similar to previous analyses of other eucalypts in California (Vansell, 1941, 1944; Baker and Baker, 1990), Argentina (Núñez, 1977) and Australia (Moncur and Boland, 1989) which ranged from hexose-dominant to sucrose-rich. However, here the G/F ratios exceeded unity. Non-eucalypt nectars of the Myrtaceae are hexose-rich or -dominant (Percival, 1961; Beardsell et al, 1989; O'Brien et al, 1996).

The phenomenon of net reabsorption of nectar carbohydrates is well established in various taxa (Pedersen et al, 1958; Shuel, 1961; Corbet and Delfosse, 1984; Búrquez and Corbet, 1991) and now for *Eucalyptus*. Interestingly, no changes in sugar composition occurred here during the period of nectar reabsorption. Therefore, net reclamation of uncollected nectar did not occur selectively, for any of the three carbohydrates in particular, but instead as a mixture (Shuel, 1961; Freeman, 1986). Flowers of stage V appeared to resorb nectar at a rate inversely proportional to the volumes of stage IV (eg, compare *E. cosmophylla* and *E. grandis*, figs 14 and 15) that accumulated by protection from visitation. Contactable nectary-surface area available for reabsorption limits the process (Búrquez and Corbet, 1991). How pore occlusion influences reabsorption remains to be determined.

## Nectary surfaces

The floral nectaries differed in color and amounts of surface wax. Changes in color, such as the corolla, affect foraging behaviour (Weiss, 1995). In the absence of prominent petals, changes in nectary appearance during flower phenology may provide visual foraging cues to eucalypt visitors (O'Brien et al, 1996). However, the frequency of modified stomata on the nectary did not change during phenology, nor was it correlated with nectar volume or carbohydrate production.

The terms 'modified stomata' (Fahn, 1979; Davis and Gunning, 1992) are used here to represent the pore structures of *Eucalyptus* nectaries. They share specific developmental features (eg, breakthrough; Carr and Carr, 1987) and likeness with foliar stomata, but evidently are unable to close their pores by guard-cell movements (Davis, 1969). Instead, modified stomata (even on buds) can be found with pores occluded (Davis and Gunning, 1992). Occlusion apparently involves release of hydrophobic material(s) from the guard cells to seal the pore. Unknown is whether an occluded pore completely restricts the passage of exudate from the gland, or disallows any re-entry during nectar reabsorption. It would prove interesting to determine whether modified stomata without overlying nectar droplets (see Beardsell et al, 1989) are possibly immature or occluded. Unlike the stamens and style, the nectary does not abscind (Carr and Carr, 1990). Hence, it is possible that occlusion serves to deter pathogen entry through nectary pores to the developing seeds below.

Low-temperature SEM minimizes artefacts (Robards, 1984) and here provided no evidence that nectar flow in eucalypts is regulated by pore aperture movements (Carr and Carr, 1987). Rarely were modified stomata of the nectary observed to have guard cells almost meeting, or touching, across the pore. Instead, complete pore clo-

sure could occur by total occlusion. Interestingly, partial stomatal occlusion (as polar flaps and pseudo-outer stomatal ledges) also has been detected in leaves of certain eucalypts, where it involves deposition of new cuticle and wall (Carr and Carr, 1980). Even after nectar secretion had ceased, modified stomata with open pores were still found. Furthermore, pre-secretory buds possessed open pores on their nectaries. Therefore, the modified stomata did not control floral nectar secretion of these species, because commencement of nectar release did not coincide with synchronous, initial opening of pores of the modified stomata, nor did secretion cease as a result of stomatal closure. At any floral stage, modified stomata could be found on the nectary surface in all of four ontogenetic stages: immature, breaking through, open and occluded. This asynchronous pattern in development of modified stomata, itself sides against stomatal regulation of nectar flow. Indeed, like the nectary (fig 6), the leaf of *E grandis* shows asynchrony in development between nearby stomata (fig 20 of Carr and Carr, 1991). In an evolutionary sense, early land plants bore stomata before leaves, and stomata in the plant kingdom possess different degrees of functionality (Ziegler, 1987). These close similarities between leaf and nectary structures in *Eucalyptus* favor utilization of the existing terminology, 'modified stomata' (Fahn, 1979; Davis and Gunning, 1992) and 'guard cells', instead of 'pore cells' (Carr and Carr, 1987, 1990).

Further studies are required to investigate the anatomy and ultrastructure of *Eucalyptus* nectaries. A key question (Carr and Carr, 1987) still to be addressed in eucalypts is whether the nectary guard cells have plasmodesmata and are involved in symplastic transport of nectar. There is insufficient evidence from *Chamelaucium uncinatum*, the only Myrtacean species whose nectary modified stomata have been studied by transmission electron microscopy (O'Brien et al, 1996). However, in floral

nectaries of *Vicia*, the plasmodesmata detectable in primary pit-fields of guard-cell walls of immature modified stomata, were no longer complete at stomatal maturity (Davis and Gunning, 1992). Further evidence against the modified stomata making a direct contribution as part of a symplastic secretory pathway for nectar in that species, is considerable (Davis and Gunning, 1993).

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**Résumé — Influence de la visite florale sur la composition glucidique du nectar et sur les modifications de la surface nectarifère de l'*Eucalyptus*.** Les eucalyptus (*Eucalyptus* spp) constituent la source de nectar floral la plus importante pour la production de miel en Australie. Leur nectar a fait pourtant l'objet de peu de recherches. Les nectaires floraux et la production des principaux sucres du nectar ont été étudiés chez trois espèces d'eucalyptus poussant à Canberra, Australie : *E cosmophylla*, *E grandis* et *E pulverulenta*. Chez ces trois espèces, le nectaire est situé à la surface interne de l'hypanthium, sous le stamino-phore (fig 9). La microscopie électronique à balayage à basse température a montré que la surface des nectaires possédait des centaines de stomates modifiés et distribués relativement uniformément (figs 6, 9, 10), à raison de 163 (*E pulverulenta*) à 348 (*E cos-*

*mophylla*) stomates/mm<sup>2</sup>. Le développement des stomates modifiés est asynchrone (figs 6, 10), chaque nectaire examiné au stade présécréteur et postsécréteur possédant à la fois des stomates modifiés immatures (figs 3 gauche, 6, 10), en cours de maturation (figs 3 droite, 11), avec un pore ouvert (figs 4, 10, 12) ou fermé (figs 10, 13). On n'a pas trouvé de preuve que le stomate modifié s'ouvre et se ferme pour réguler la sécrétion nectarifère. Il est en revanche évident que la fermeture du pore se fait par occlusion et non par des mouvements de cellules stomatiques. Grâce à une technique combinée capillaire-mèche, le nectar a été prélevé à cinq stades floraux (I–V) sur chaque espèce (figs 2, 5, 8) : depuis les fleurs fraîchement écloses qui venaient de perdre leur operculum jusqu'aux fleurs de 12 à 17 jours dont les étamines étaient fortement flétries. À chaque stage, le nectar a été prélevé sur des fleurs ensachées (fig 1) quand l'operculum était déhiscent (fig 2 en haut à gauche) et sur des fleurs laissées libres d'accès aux visiteurs, principalement l'abeille domestique *Apis mellifera* (figs 1, 7) et le guêpier (oiseaux). Les rendements en nectar et les quantités de sucre par fleur ensachée avaient un niveau maximum chez *E cosmophylla* (fig 14), intermédiaire chez *E pulverulenta* (fig 16) et minimum chez *E grandis* (fig 15), conformément à la taille de la fleur mais pas au nombre de stomates des nectaires. L'analyse enzymatique du glucose, du fructose et du saccharose dans les échantillons de nectar a montré qu'*E cosmophylla* et *E grandis* sont riches en hexose ( $0,1 < S/(G + F) < 0,499$  ; tableau I), tandis que *E pulverulenta* est riche en saccharose ( $0,5 < S/(G + F) < 0,999$  ; tableau I). Le rapport glucose/fructose se situait autour de 1,2–1,4 pour les trois (tableau I). On a trouvé peu de changements dans la composition glucidique du nectar selon que les fleurs étaient jeunes ou vieilles, ensachées ou à l'air libre (figs 14–16), ce qui indique une constance dans la composition tout au long du processus de sécrétion des stades I–IV.

Au stade V, une réabsorption nette du nectar s'est produite (figs 14–16) et la constance dans la composition glucidique du nectar implique que cette réabsorption n'ait lieu de façon sélective pour aucun des trois sucres, mais plutôt sous forme d'un mélange complexe. Il est nécessaire de poursuivre les recherches pour étudier la fermeture des stomates modifiés et le rôle joué par les pores obturés dans l'écoulement et la réabsorption du nectar.

#### *Apis mellifera* / *Eucalyptus* / nectar/ glucide / nectaire / stomate

**Zusammenfassung — Einfluß des Blütenbeflugs auf die Zusammensetzung der Zucker im Nektar und Änderung der Oberflächenbeschaffenheit der Nektarien bei *Eucalyptus* ssp (Myrtaceae).** Obwohl Eukalyptusbäume in Australien die wichtigste Quelle von Blütennektar zur Honigproduktion darstellen, ist Eukalyptusnektar bislang nur wenig untersucht worden. Die floralen Nektarien sowie die Sekretion der wichtigsten Nektarkohlenhydrate wurde bei drei Eukalyptusarten in Canberra, Australien untersucht. Bei *E cosmophylla*, *E grandis* und *E pulverulenta* ist das Nektarium auf der inneren Oberfläche des Blütenbeckers unterhalb der Staminophore gelegen (Abb 9). Rasterelektronenaufnahmen bei niedrigen Temperaturen zeigten, daß die Oberflächen der Nektarien hunderte von modifizierten Stomata besitzen. Diese liegen zumeist einzeln und sind relativ gleichmäßig verteilt (Abb 6, 9, 10). Ihre Dichte beträgt 163 (*E pulverulenta*) bis 348 (*E cosmophylla*) pro mm<sup>2</sup> der Oberfläche. Die Entwicklung dieser modifizierten Stomata erfolgte nicht gleichzeitig (Abb 6, 10). Bei allen untersuchten Nektarien kamen diese sowohl vor als auch nach der Sekretion unreif (Abb 3 links, 6, 10) durchbrechend (Abb 3 rechts, 11), mit offenem (Abb 4, 10, 12) oder verstopftem Porus vor (Abb 10, 13). Es gab keine Anzeichen, daß sich die

Stomata zur Regulierung des Nektarflusses öffnen oder schließen. Stattdessen wurden die Poren durch Verstopfung verschlossen, offensichtlich nicht durch Bewegung der Begleitzellen. Mit einer Kapillar-Docht-Kombinationstechnik wurde Nektar von jeder Art aus fünf Blühstadien (I-V) gesammelt (Abb 2, 5, 8). Diese Stadien umfassten frischgeöffnete Blüten, die gerade ihren Knospendeckel verloren hatten, bis zu mehrere Tage älteren Blüten mit stark verwelkten Staubgefäßen. Bei jedem Stadium wurde Nektar sowohl von Blüten, die beim Aufplatzen des Knospendeckels (Abb 2, oben links) eingewickelt (Abb 1) wurden, als auch von Blüten, die ganzzeitig für Blütenbesucher (zumeist *Apis mellifera*) (Abb 1, 7), und Honigfresser (Vögel) zugänglich waren. Der Nektarertrag und die Zuckermengen pro eingewickelter Blüte waren bei *E cosmophylla* am größten (Abb 14) und bei *E grandis* am geringsten (Abb 15); bei *E pulverulenta* (Abb 16) lagen sie dazwischen. Diese Reihenfolge entsprach der Blütengröße, nicht aber der Anzahl der Stomata auf den Nektarien. Die enzymatische Analyse der Glucose, der Fructose und der Saccharose in den Nektarproben zeigte, daß *E cosmophylla* und *E grandis* reich an einfachen Zuckern (Hexosen) sind [ $0.1 < S/(G+F) < 0.499$ ; Tabelle I], während *E pulverulenta* vor allem das Disaccharid Saccharose enthält [ $0.5 < S/(G+F) < 0.999$ ; Tabelle I]. Das Verhältnis von Glukose zu Fructose betrug bei allen Arten im Mittel 1.2 bis 1.4 (Tabelle I). Es konnten nur geringe Unterschiede der Nektarzuckerzusammensetzung zwischen jungen und alten oder eingewickelten und zugänglichen Blüten (Abb 14-16) festgestellt werden, was auf eine gleichmäßige Zusammensetzung während des Sekretionsverlaufes der Stadien I-IV hindeutet. Im Stadium V trat eine Netto-Reabsorption des Nektars auf (Abb 14-16). Die auch in diesem Stadium festgestellte Gleichartigkeit der Nektarzusammensetzung lässt darauf schließen, daß diese Reabsorption nicht selektiv für einzelne der

drei Zucker ist, sondern die gesamte Mischung betrifft. Zur Klärung der Verstopfung der modifizierten Stomata und zu der Rolle der verstopften Poren während des Nektaraustritts und der Reabsorption werden weitere Untersuchungen benötigt.

### ***Apis mellifera* / *Eucalyptus* / Nektar / Zucker / Stomata / Nektarien**

### **REFERENCES**

- Anonymous (1985) World honey crop report no 22. *Bee World* 66, 131-133
- Anonymous (1987) World honey crop report no 23. *Bee World* 68, 186-187
- Ashton DH (1975) Studies of flowering behaviour in *Eucalyptus regnans* F Muell. *Aust J Bot* 23, 399-411
- Baker HG, Baker I (1983) A brief historical review of the chemistry of floral nectar. In: *The Biology of Nectaries* (B Bentley, T Elias, eds), Columbia University Press, New York, USA
- Baker HG, Baker I (1990) The predictive value of nectar chemistry to the recognition of pollinator types. *Isr J Bot* 39, 157-166
- Barbier EC (1951) La sécrétion de nectar chez les *Eucalyptus*. *Rev Fr Apic* 76, 529-532, 553-559
- Beardsell DV, Williams EG, Knox RB (1989) The structure and histochemistry of the nectary and anther secretory tissue of the flowers of *Thryptomene calycina* (Lindl) Stapf (Myrtaceae). *Aust J Bot* 37, 65-80
- Brimblecombe AR (1946) The relationship between starch content and flowering of trees as a possible means of predicting honey flows. *Australas Beek* 48, 93-97
- Bond HW, Brown WL (1979) The exploitation of floral nectar in *Eucalyptus incrassata* by honeyeaters and honeybees. *Oecologia* 44, 105-111
- Búrquez A, Corbet SA (1991) Do flowers reabsorb nectar? *Funct Ecol* 5, 369-379
- Carr DJ, Carr SGM (1980) *Eucalyptus* stomata with occluded anterior chambers. *Protoplasma* 104, 239-251
- Carr DJ, Carr SGM (1987) *Eucalyptus* II — The rubber cuticle, and other studies of the *Corymbosae*. Phytoglyph Press, Canberra, Australia
- Carr DJ, Carr SGM (1991) Development of the stomatal complexes during ontogeny in *Eucalyptus* and *Angophora* (Myrtaceae). *Aust J Bot* 39, 43-58
- Carr SGM, Carr DJ (1990) Cuticular features of the Central Australian bloodwoods *Eucalyptus*, sec-

- tion Corymbosae (Myrtaceae). *Bot J Linn Soc* 102, 123-156
- Chandler BV, Fenwick D, Orlova T, Reynolds T (1974) Composition of Australian honeys. CSIRO Div Food Res Tech Paper 38
- Christ P, Schnepf E (1988) Zur Struktur und Funktion von Asclepiadaceen-Nektarien. *Beitr Biol Pflanzen* 63, 55-79
- Clemson A (1985) *Honey and Pollen Flora*. Department of Agriculture, New South Wales. Inkata Press, Melbourne, Australia
- Corbet SA (1978) Bee visits and the nectar of *Echium vulgare* L and *Sinapis alba* L. *Ecol Entomol* 3, 25-37
- Corbet SA, Delfosse ES (1984) Honeybees and the nectar of *Echium plantagineum* L in southeastern Australia. *Aust J Ecol* 9, 125-139
- Davis AR (1992) Evaluating honey bees as pollinators of virgin flowers of *Echium plantagineum* L (Boraginaceae) by pollen tube fluorescence. *J Apic Res* 31, 83-95
- Davis AR, Gunning BES (1991) The modified stomata of the floral nectary of *Vicia faba* L. 2. Stomatal number and distribution as selection criteria for breeding for high nectar-sugar production. *Acta Hortic* 288, 329-334
- Davis AR, Gunning BES (1992) The modified stomata of the floral nectary of *Vicia faba* L. 1. Development, anatomy and ultrastructure. *Protoplasma* 164, 134-152
- Davis AR, Gunning BES (1993) The modified stomata of the floral nectary of *Vicia faba* L. 3. Physiological aspects, including comparisons with foliar stomata. *Bot Acta* 106, 241-253
- Davis GL (1968) Floral morphology and the development of gametophytes in *Eucalyptus melliodora* A Cunn. *Aust J Bot* 16, 19-35
- Davis GL (1969) Floral morphology and the development of gametophytes in *Eucalyptus stellulata* Sieb. *Aust J Bot* 17, 177-190
- Ellis MF, Sedgley M (1992) Floral morphology and breeding system of three species of *Eucalyptus*, section *Bisectaria* (Myrtaceae). *Aust J Bot* 40, 249-262
- Fahn A (1979) *Secretory Tissues in Plants*. Academic Press, London, UK
- Freeman CE (1986) Nectar-sugar composition in an individual of *Ruellia peninsularis* (Acanthaceae). *Madroño* 33, 300-302
- Freeman CE, Wilken DH (1987) Variation in nectar sugar composition at the intraplant level in *Ipomopsis longiflora* (Polemoniaceae). *Am J Bot* 74, 1681-1689
- Gilliam M, Moffett JO, Kauffeld NM (1983) Examination of floral nectar of citrus, cotton, and Arizona desert plants for microbes. *Apidologie* 14, 299-302
- Gottsberger G, Arnold T, Linskens HF (1990) Variation in floral nectar amino acids with ageing of flowers, pollen contamination, and flower damage. *Isr J Bot* 39, 167-176
- Griffin AR, Hand FC (1979) Post-anthesis development of flowers of *Eucalyptus regnans* F Muell and the timing of artificial pollination. *Aust For Res* 9, 9-15
- Hodgson LM (1976) Some aspects of flowering and reproductive behaviour in *Eucalyptus grandis* (Hill) Maiden at JDM Keet Forest Research Station (formerly Zomerkomst Forest Research Station). 1. Flowering, controlled pollination methods, pollination and receptivity. *S Afr Forest J* 97, 18-28
- Kronstedt-Robards EC, Greger M, Robards AW (1989) The nectar of the *Strelitzia reginae* flower. *Physiol Plant* 77, 341-346
- Loper GM, Waller GD, Berdel RL (1976) Effect of flower age on sucrose content in nectar of citrus. *HortScience* 11, 416-417
- Lovell JH (1926) *Honey plants of North America*. AI Root Company, Medina, Ohio, USA
- Lupo A, Eisikowitch D (1990) *Eucalyptus erythrorhiza*: a source of nectar and pollen for honey bees in Israel. *Apidologie* 21, 25-33
- Lüttge U (1961) Über die Zusammensetzung des Nektars und den Mechanismus seiner Sekretion. I. *Planta* 56, 189-212
- McKenna M, Thomson JD (1988) A technique for sampling and measuring small amounts of floral nectar. *Ecology* 69, 1036-1037
- Moncur MW, Boland DJ (1989) Floral morphology of *Eucalyptus melliodora* A Cunn ex Schau and comparisons with other eucalypt species. *Aust J Bot* 37, 125-135
- Nichol P, Hall JL (1988) Characteristics of nectar secretion by the extrafloral nectaries of *Ricinus communis*. *J Exp Bot* 39, 573-586
- Núñez J (1977) Nectar flow by melliferous flora and gathering flow by *Apis mellifera ligustica*. *J Insect Physiol* 23, 265-275
- O'Brien SP, Loveys BR, Grant WJR (1996) Ultrastructure and function of floral nectaries of *Chamaelucium uncinatum* (Myrtaceae). *Ann Bot* 78, 189-196
- Pedersen MW, LeFevre CW, Wiebe HH (1958) Absorption of C<sup>14</sup>-labelled sucrose by alfalfa nectaries. *Science* 127, 758-759
- Pellett FC (1923) *American Honey Plants*, 2nd edition. Am Bee J, Hamilton, IL, USA
- Penfold AR, Willis JL (1961) *The Eucalypts — Botany, Cultivation, Chemistry, Utilization*. Leonard Hill, London, UK
- Percival MS (1961) Types of nectar in angiosperms. *New Phytol* 60, 235-281
- Petanidou T, Van Laere AJ, Smets E (1996) Change in floral nectar components from fresh to senescent flowers of *Capparis spinosa* (Capparidaceae), a

- nocturnally flowering Mediterranean shrub. *Plant Syst Evol* 199, 79-92
- Peters GB, Lonie JS, Moran GF (1990) The breeding system, genetic diversity and pollen sterility in *Eucalyptus pulverulenta*, a rare species with small disjunct populations. *Aust J Bot* 38, 559-570
- Robards AW (1984) Fact or artefact — a cool look at biological electron microscopy. *Proc R Microscop Soc* 19, 195-208
- Shuel RW (1961) Influence of reproductive organs on secretion of sugars in flowers of *Streptosolen jamesonii* Miens. *Plant Physiol* 36, 265-271
- Vansell GH (1941) Nectar and pollen plants of California. Univ California Berkeley Agr Exp Stn, Bulletin 517
- Vansell GH (1944) Some western nectars and their corresponding honeys. *J Econ Entomol* 37, 530-533
- Weiss MR (1995) Floral color change: a widespread functional convergence. *Am J Bot* 82, 167-185
- Willmer PG (1980) The effects of insect visitors on nectar constituents in temperate plants. *Oecologia* 47, 270-277
- Ziegler H (1987) The evolution of stomata. In: *Stomatal Function* (E Zeiger, GD Farquhar and IR Cowan, eds) Stanford University Press, Stanford, USA