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Structure and Histochemistry of the Extrafloral Nectary of *Acacia terminalis* (Salisb.) MacBride (*Leguminosae, Mimosoideae*)

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Summary

The extrafloral nectary of *Acacia terminalis* is of the flat type and is located on the adaxial surface of the petiole of the bipinnate leaf. The secretory area is restricted to the base of the trough and no gaps or pores were detected by staining with vital dyes. Between the vascular bundles beneath the nectary and the surface cuticle there were three cell types. The cells of the flanking zone adjacent to the vascular bundles did not appear to be producing secretion whereas the cells of the glandular and secretory zones were secreting. The cells of the glandular zone were elongated whereas those of the surface secretory zone were spherical. Both had endoplasmic reticulum and Golgi bodies with secretory vesicles which were observed in close association with the plasmalemma. Secretion accumulated in the intercellular spaces of the glandular zone cells and forced the cells of the secretory zone apart. Symplastic contact was maintained in all cell types by plasmodesmata which were often associated with endoplasmic reticulum. Secretion accumulated beneath the cuticle which was distended but remained intact on the surface of the secretion.

Keywords: Acacia terminalis; Extrafloral nectary; Histochemistry; Secretion; Ultrastructure.

1. Introduction

The extrafloral nectary of *Acacia terminalis* (Salisb.) MacBride (*Leguminosae, Mimosoideae*) is situated on the petiole of the bipinnate leaf and can be up to 12 mm in length. Boughton (1981), in an extensive survey of Australian phyllodineous acacias, described three types of nectaries. Porate nectaries were found in 30 of the 43 species studied and had a deep depression with secretory tissues at the base of the pore. Non-porate nectaries were less common and had no depression, whereas the flat nectary, intermediate in morphology between the porate and non-porate nectaries was found in one phyllodineous species only, *A. macradenia.* The extrafloral nectary of *A. terminalis* is of the flat type and appears to be unusually large among bipinnate species. It has an unusual role as an attractant and food source for some of the local native avian population (Kenrick *et al.* 1982, Knox *et al.* 1985). Studies have shown that there is a significant increase in nectar production during the period of maximum flowering, a phenomenon first observed in acacias by Zimmerman (1932). In physiological tests performed in the field the nectaries showed a specific diurnal pattern of secretion (Knox *et al.* 1985). Maximum glandular activity occurred between the predawn hours until midmorning irrespective of ambient temperature and humidity.

There is very little information on the breeding system of the genus *Acacia,* which is important for timber production, ornamental horticulture and essential oil production. This study of the extrafloral nectaries is part of a wider programme on the reproductive biology of Australian acacias which includes their modes of pollination and fertilization. *A. terminalis* is self-incompatible (Kenrick *et al.* 1984) and cross-pollination is essential for seed setting. The extrafloral nectaries appear to be an adaptation for bird pollination, and in this paper we present an account of their anatomy, histochemistry and ultrastructure in order to investigate the pathway and mechanism of nectar secretion.

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2. Materials and Methods

Fresh plant material of *A. terminalis* for all experiments was collected from small trees, 1–2 years old, growing in a glasshouse. The trees form part of the progeny from breeding experiments near Erica, Victoria in 1982 (KENRICK *et al.* 1984). The average length of the nectaries on our experimental specimens was 3 mm. Actively secreting nectaries were selected in the morning, during the period of maximum secretion. The inactive nectaries selected had not secreted for a few days.

2.1. *Vital Staining for Cuticle Permeability*

Solutions of 1% aqueous neutral red (JOEL and JUNIPER 1982), 1% methylene blue (JOEL *et al.* 1983), and 1% toluidine blue O, the latter two buffered to neutrality with 0.01 M Tris-HCl, were added as drops to both active and inactive nectaries whilst attached to the plant. The nectaries were rinsed briefly in water to remove excess stain then examined under a dissecting microscope. Observations were made after 2, 15, 60, and 120 minutes contact with the stain. The experiment was performed in the morning to coincide with maximum glandular activity (KNOX *et al.* 1985). The above procedure was repeated with the wetting agent "Tween 20" added to the dyes to give final concentrations of 0.1, 0.4, and 1%.
Petioles with active and inactive nectaries were excised 2 cm below the extrafloral nectary and immediately immersed in vials containing the vital dyes. The nectaries were observed hourly for 5 hours for signs of dye uptake and secretion.

2.2. *Light Microscopy*

Active nectaries were transversely cut and fixed in FAA (formalin-/acetic acid/alcohol) for 24 hours at 0–4 °C with two changes of fixative (O'BRIEN and MCCULLY 1981). The tissue was then dehydrated in alcohols and infiltrated, and embedded in glycol methacrylate (GMA). Serial sections 2.5 µm thick were collected onto glass slides from which sections were then selected to represent glandular and non-glandular tissue and stained with periodic acid-Schiff's reagent (PAS) and toluidine blue O (TBO), Coomassie brilliant blue (FISHER 1968), Sudan III & IV (O'BRIEN and MCCULLY 1981) and Sudan black B (BRONNER 1975), for bright field microscopy. Calcofluor and Auramine O (HESLOP-HARRISON 1977) were applied to sections and examined by fluorescence microscopy.

2.3. *Scanning Electron Microscopy (SEM)*

Active and inactive nectaries were excised from the leaf petiole and attached to aluminium stubs by silver dag adhesive. They were observed uncoated at 10 kV in a Siemens ETEC autoscan SEM.

2.4. *Transmission Electron Microscopy (TEM)*

Active nectaries were cut from the plant in a pool of ice cold freshly prepared fixative which consisted of 4% glutaraldehyde, 4% paraformaldehyde (modified from KARNOVSKY 1965), 10% sucrose, 1% caffeine (MUELLER and GREENWOOD 1978) in 0.025 M phosphate buffer pH 7.2. Caffeine was included in the fixative as problems were encountered in the preservation of the tissue due to the presence of tannins. The petiole was almost completely removed from the base of the gland which was then cut transversely with consecutive sections placed in separate vials. Fixation proceeded on ice for 24 hours with fixative changed after 30 minutes, 1.5, 4, and 7 hours. The tissue was post-fixed with 1% osmium tetroxide on ice for 3 hours then for a further 1 hour at room temperature. Tissue was routinely dehydrated through an ethanol series followed by infiltration of Araldite with continuous rotation. The nectaries were then flat embedded in moulds and polymerized at 60 °C for 2 days.
Silver sections were cut with glass knives using a Reichert-Jung OM 2 microtome and mounted on collodion-coated mesh grids. Standard staining procedures were performed using uranyl acetate and lead citrate (REYNOLDS 1963). Sections were observed at 80 kV in a Phillips EM 400.

3. Results

3.1. *Morphology*

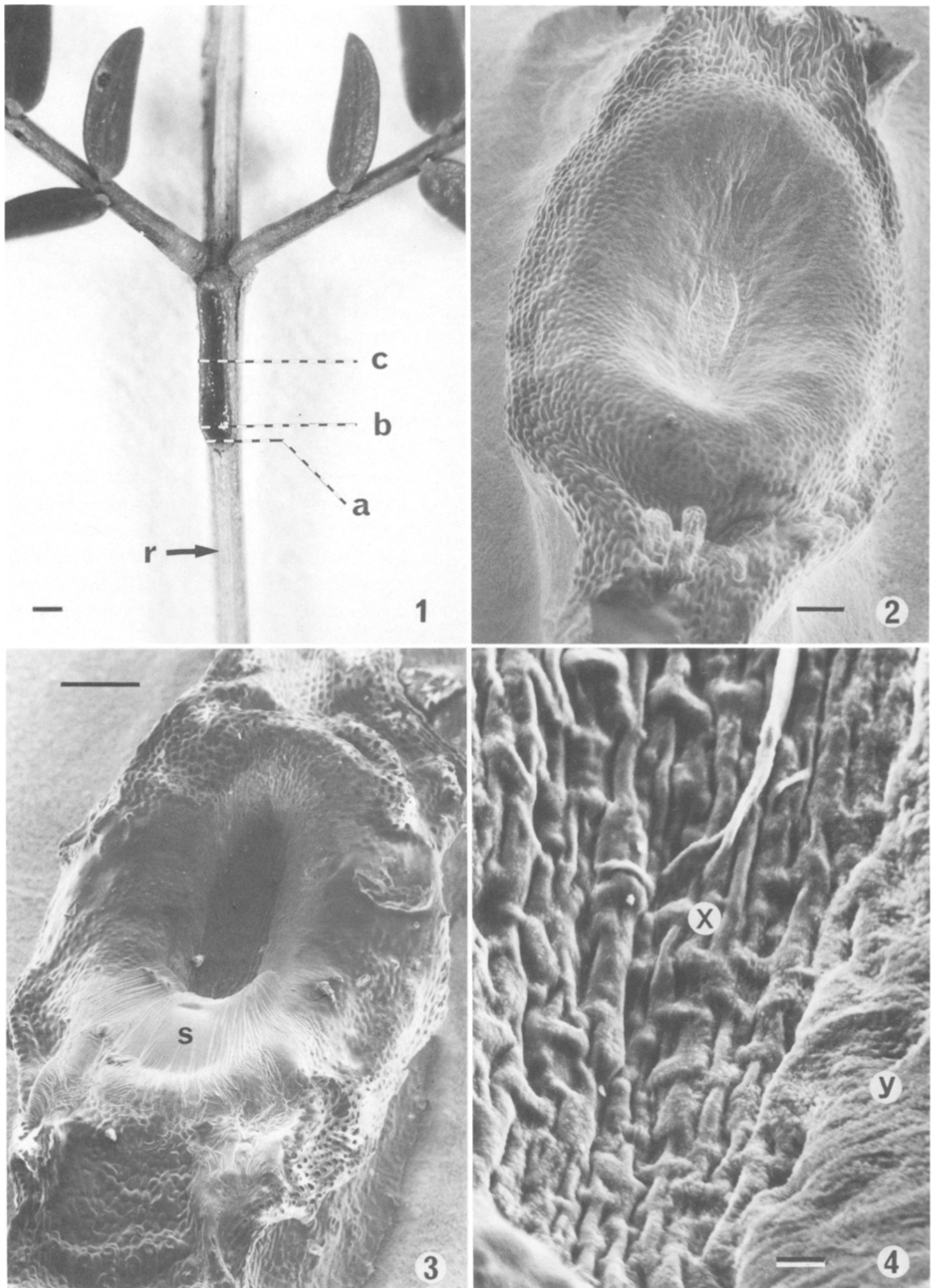
The extrafloral nectaries of *A. terminalis* occur singly on the adaxial surface of the petiole (Fig. 1). There are 4 ridges running longitudinally along the petioles and these are an important taxonomic feature in identifying this species. The nectary is an extension of the upper petiolar ridge. A buttress of cells rises from the ridge to form a trough shaped nectary (Figs. 2, 3, and 5). This shape enables the nectary to hold the copious quantities of secretion (Fig. 3), and the end of the trough distal to the leaflets is wider to facilitate this function. Secretion is diurnal, occurring during early morning hours. The sides of the nectary trough are highly pigmented and covered with a smooth cuticle (Fig. 2), whereas the base of the trough has a highly convoluted cuticle (Fig. 4). No stomata, ruptures or pores were seen in the trough area of the secreting or non-secreting nectaries examined.

Fig. 1. Part of a bipinnate leaf from a mature tree of *A. terminalis* showing the position of the extrafloral nectary on the petiolar ridge (*r*) relative to the pinnae. Transverse sections through areas *a, b, c* correspond to the cell arrangements illustrated in Figs. 6 and 7 (5 and 8) respectively. Bar represents 1 mm

Fig. 2. SEM of a nonsecreting extrafloral nectary. Bar represents 100 µm

Fig. 3. SEM of a secreting extrafloral nectary with a drop of nectar (*s*) which is somewhat collapsed under the electron beam. Bar represents 100 µm

Fig. 4. SEM of the internal surface of the nectary trough. The base of the trough (*x*) has a very convoluted cuticle while the cuticle on the sides of the trough (*y*) is relatively smooth. Bar represents 10 µm



Figs. 1–4

3.2. *Vital Staining Characteristics*

There was no vital staining of the nectary cells. Where stomates occurred on the petiole around the nectary the underlying cells were stained. With cut and immersed petioles, dye was taken up by the vascular tissue of the active but not of the inactive glands and transported to the nectary when experiments were carried out during the morning. When the faintly stained nectar was drawn off, the whole base of the gland but not the sides of the trough had taken up the stains, Toluidine blue O and methylene blue. The results with neutral red were difficult to interpret due to the red pigment of the nectary tissue. The staining results were similar with or without the detergent "Tween 20".

3.3. *Anatomy*

There are 3 distinct regions in the nectary (Fig. 1). A buttress is present at either end of the nectary (Fig. 1 *a*) which is an extension of the upper petiolar ridge that has differentiated into a plateau of cells (Fig. 6). In this area three major vascular bundles have branched from the adjoining petiolar vascular tissue (Fig. 5) each with an external cap of pericycle tissue. The vascular tissue also shows occasional branching into the region closer to the upper surface. The cortical cells surrounding the vascular bundle abut quadrate epidermal cells with occasional stomata (Fig. 6). At the edge of the trough (Fig. 1 *b*), the circle of vascular tissue has branched further, becoming crescent-shaped to accommodate a layer of cells which surround a cylinder of densely-staining glandular tissue (Fig. 7). This layer of polymorphic cells is termed the flanking zone as the cells are not thick-walled or suberized (BOUGHTON 1981). Loosening of the epidermal cells has occurred (apparently by dissolution of the middle lamella) accompanied by an increase in the number of these cells containing tannins, phenols and pigment. In the middle of the nectary the trough becomes much deeper and wider (Fig. 1 *c*). The surface secretory tissue, which occurs only at the base of the trough, consists of irregularly-shaped epidermal and hypodermal cells with large intercellular spaces (Fig. 8). The glandular tissue beneath consists of elongated cells with the long axis perpendicular to the nectary surface (Fig. 10). Transitional cells of the flanking zone separate the glandular cells from the large crescent of vascular tissue, which is continuous along the length of the groove of the gland. The cuticle is pushed up from the epidermal cells by the secretion but no ruptures in the cuticle are observed (Figs. 10 and 11).

3.4. *Histochemistry*

Histochemical analysis (Tab. 1) of the extrafloral nectary showed that the cuticle, stained by lipid stains Sudan III and IV, Sudan black B and Auramine O is continuous except at stomata which occur only on the petiole (Figs. 9 and 11). Phenolics, protein, carbohydrate and lipid were present in the extracellular secretion. A protein layer was present external to the cuticle and has been termed the epicuticular layer.

3.5. *Ultrastructure*

There are three cell types bounded by a cuticle in the area between the base of the trough and the crescent of vascular tissue (Fig. 10). These cell types are the flanking zone, the glandular zone and the secretory zone. Features common to all three cell types are numerous mitochondria with well developed cristae, a large number of ribosomes in the cytoplasm, large nuclei and abundant plasmodesmata. The cells of the flanking zone are round, parenchymatous with electron dense middle lamella and no intercellular spaces (Fig. 12). Other distinctive features of these cells are the small vacuoles, short cisternae of endoplasmic re-

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Figs. 5–9. Light micrographs of transverse sections of extrafloral nectaries of *A. terminalis*

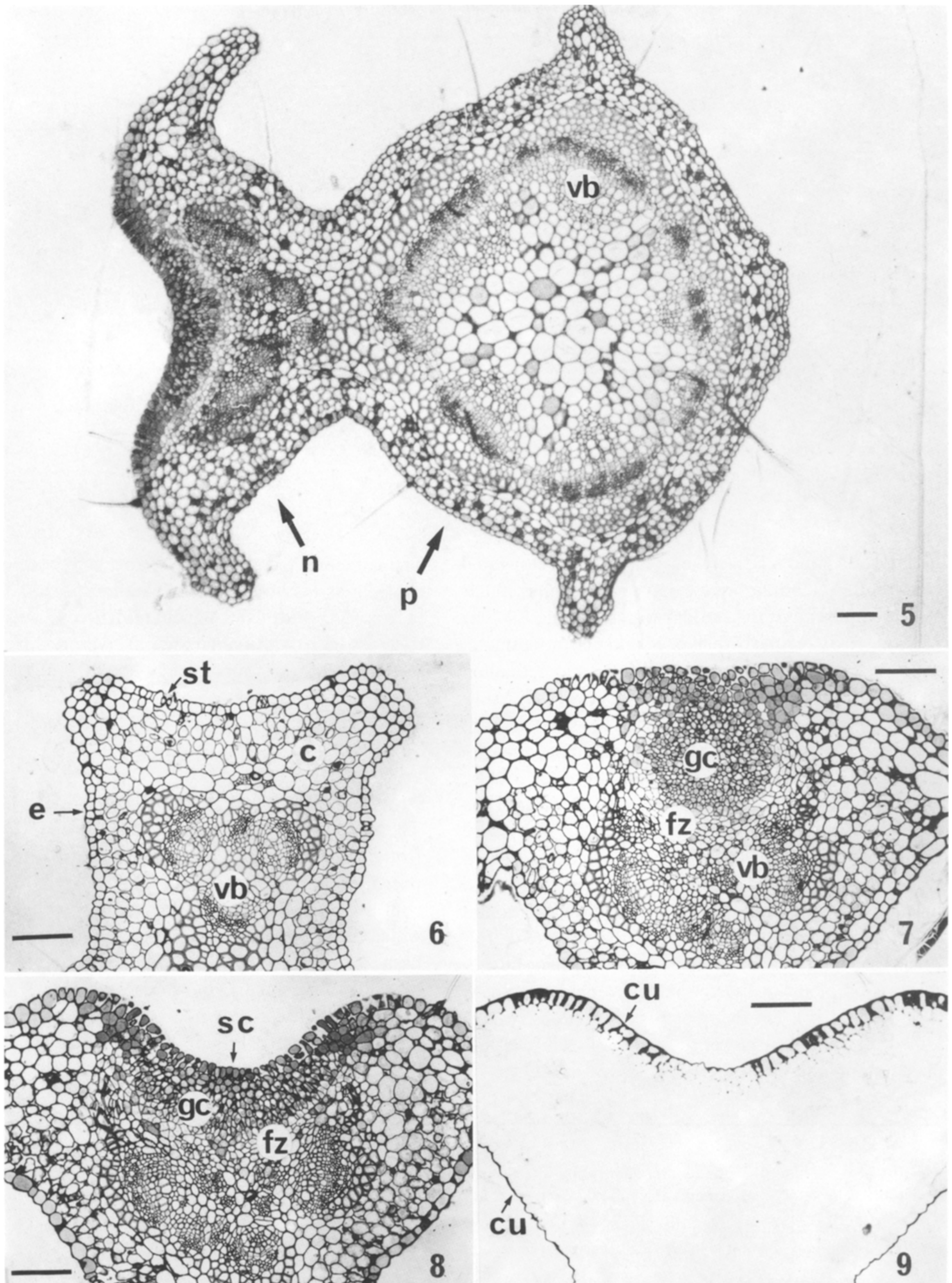
Fig. 5. Section through the extrafloral nectary (*n*) and petiole (*p*) at point *c* in Fig. 1 stained with PAS/TBO. *vb* vascular bundles. Bar represents 100 µm

Fig. 6. Section through the extrafloral nectary at point *a* in Fig. 1 stained with PAS/TBO and showing vascular bundles (*vb*), cortex (*c*), epidermis (*e*) and stomata (*st*). Bar represents 100 µm

Fig. 7. Section through the extrafloral nectary at point *b* in Fig. 1 stained with PAS/TBO showing glandular cells (*gc*), flanking zone (*fz*) and vascular bundles (*vb*). Bar represents 100 µm

Fig. 8. Section through the extrafloral nectary at point *c* in Fig. 1 stained with PAS/TBO showing flanking zone (*fz*), glandular cells (*gc*) and surface secretory cells (*sc*) which line the completely formed trough. Bar represents 100 µm

Fig. 9. Sudan black B staining lipid polymers and cuticle (*cu*). The stain shows that these compounds are not localized on the surface but penetrate between the cells with up to 3 layers deep at the base of the trough. Bar represents 100 µm



Figs. 5–9

Table 1. *Histochemistry of the secretory system of the extrafloral nectary of Acacia terminalis*

Stain	Specificity	Site			
		Epicuticular layer	Cuticle	Secretion	Vacuoles of secretory cells
Lipid					
Auramine O	cuticle, lipids	—	+ + +	+ + +	—
Sudan black B	cuticle, lipids	—	+ + +	+ + +	—
Sudan III & IV	cuticle, lipids	—	+ + +	+ + +	—
Protein					
Coomassie brilliant blue	protein	+ +	—	+	—
Polysaccharides and phenolics					
Toluidine blue O	acidic polyanions, phenolics and polysaccharides	—	—	+P	+G
Periodic acid-Schiff's reagent	carbohydrates with vicinal glycol groups	—	—	+	—

G, green denotes acidic polyanions and phenolics. P, purple denotes polysaccharides. — no staining. + weak staining. + + moderate staining. + + + strong staining.

ticulum (ER) and relatively few plastids. The elongated cells of the glandular tissue have small intercellular spaces which contain a granular secretion (Fig. 13). The cell walls are irregularly thickened and plasmodesmata, often associated with rough endoplasmic reticulum (RER), are present in thin areas of the wall (Fig. 14). The cytoplasm contains stacked RER (Fig. 13), tannin-containing vacuoles, and plastids containing ferritin. ER and Golgi with secretory vesicles are present in close association with the plasmalemma of cells of both the glandular and secretory zones (Fig. 14). The surface secretory cells have large vacuoles, some of which contain tannin (Fig. 17), and ferritin is present in the plastids (Fig. 16). Apparently active Golgi bodies and associated vesicles are present in the cells (Fig. 16). There are large intercellular spaces formed by accumulation of surface secretion between adjacent cells (Figs. 15 and 17). The cytoplasmic connection is maintained in the non-thickened areas of cell wall by plasmodesmata, ER and RER (Fig. 15).

The cuticle in the trough of the nectary is apparently continuous, without any detectable ruptures. The cuticle is forced up from the epidermal cell walls at the base of the trough under the positive pressure of the secretion to form a subcuticular space (Fig. 18). In the subcuticular space the secretion is floccular, fibrillar and/or granular in appearance (Figs. 17 and 18).

4. Discussion

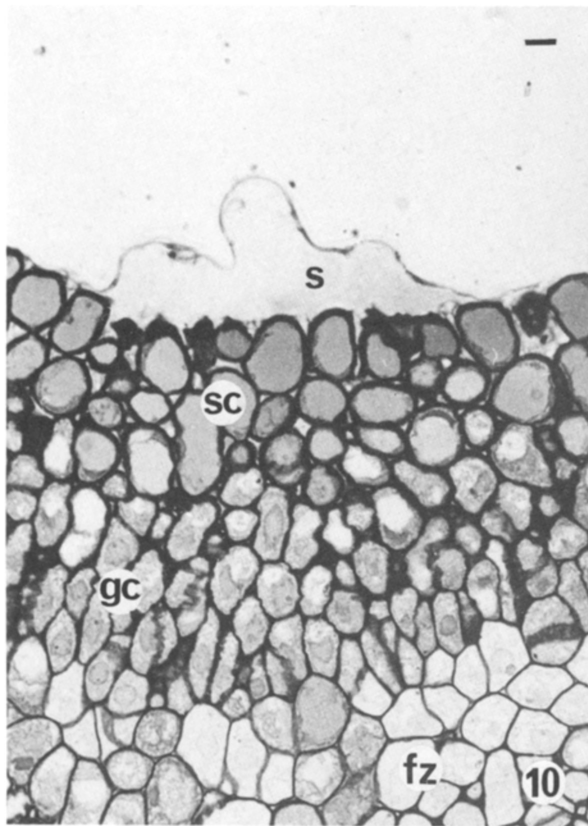
The extrafloral nectary of *A. terminalis* is an elongated trough-like structure in which the raised rim is composed of parenchyma cells. The secretory tissue is confined to and underlies the cavity of the trough in which the secretory material accumulates. The nectary tissue is subtended by vascular traces from which sugar probably enters the tissue of the flanking zone. The glandular and secretory tissues extend from the flanking zone to the overlying cuticle which coats, continuously, the sunken exterior surface of the trough.

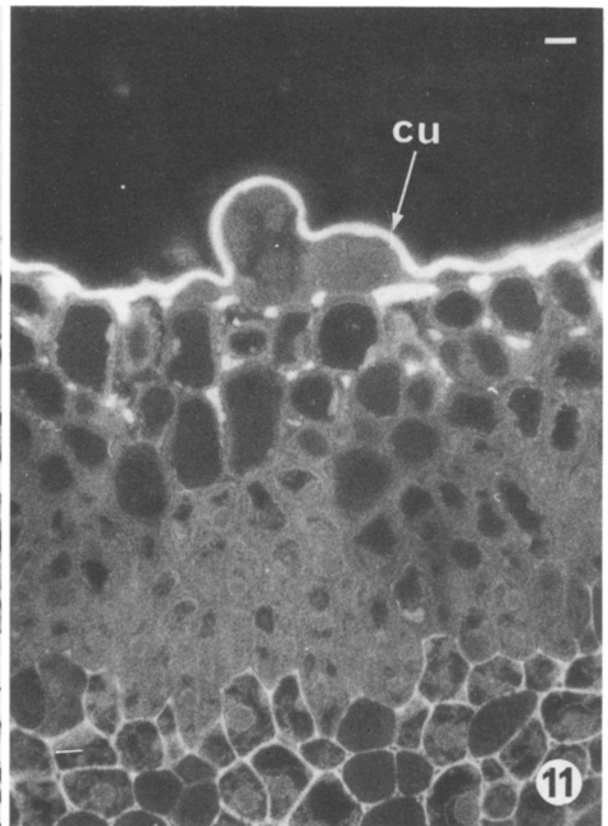
Fig. 10. PAS/TBO stained semithin section showing the lifted cuticle and subcuticular space filled with faintly staining secretion (*s*), and the cells of the surface secretory (*sc*), glandular (*gc*) and flanking zones (*fz*). Bar represents 10 μm

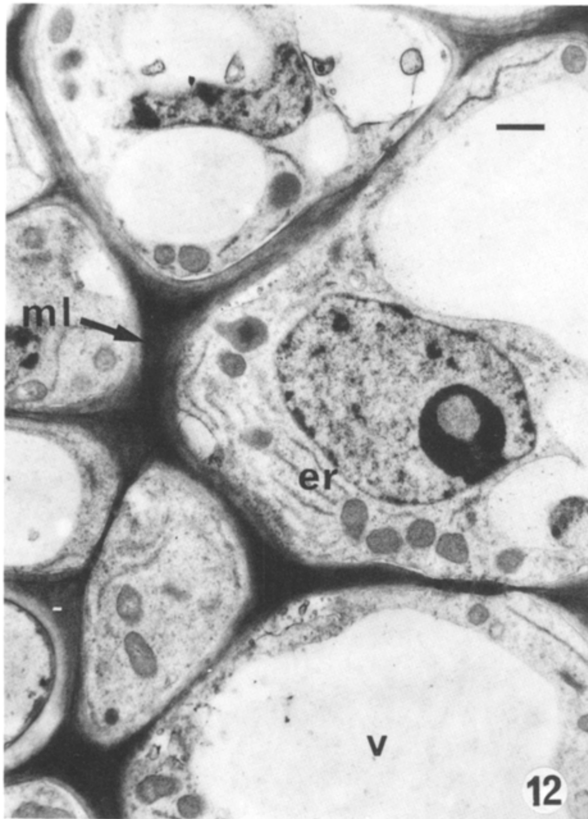
Fig. 11. Auramine O staining a neighbouring section to that shown in Fig. 10 showing the secretion is bound by an unbroken cuticle (*cu*) with cuticle and lipid polymers in the intercellular spaces. Bar represents 10 μm

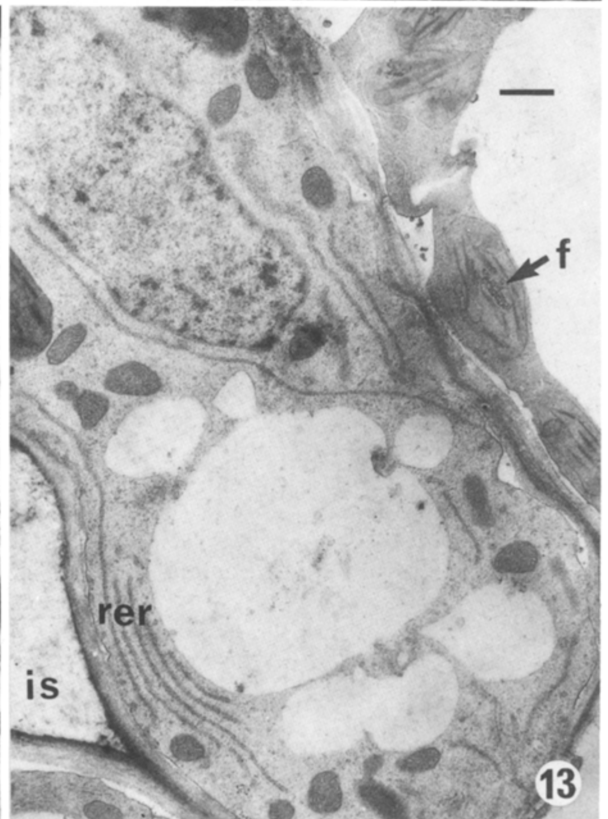
Fig. 12. Electron micrograph (EM) of cells of the flanking zone showing endoplasmic reticulum (*er*), vacuole (*v*) and middle lamellae (*ml*). Bar represents 1 μm

Fig. 13. EM of glandular cells showing secretion in the intercellular spaces (*is*), stacked rough endoplasmic reticulum (*rer*), and ferritin (*f*) in the plastids. Bar represents 1 μm









Figs. 10–13

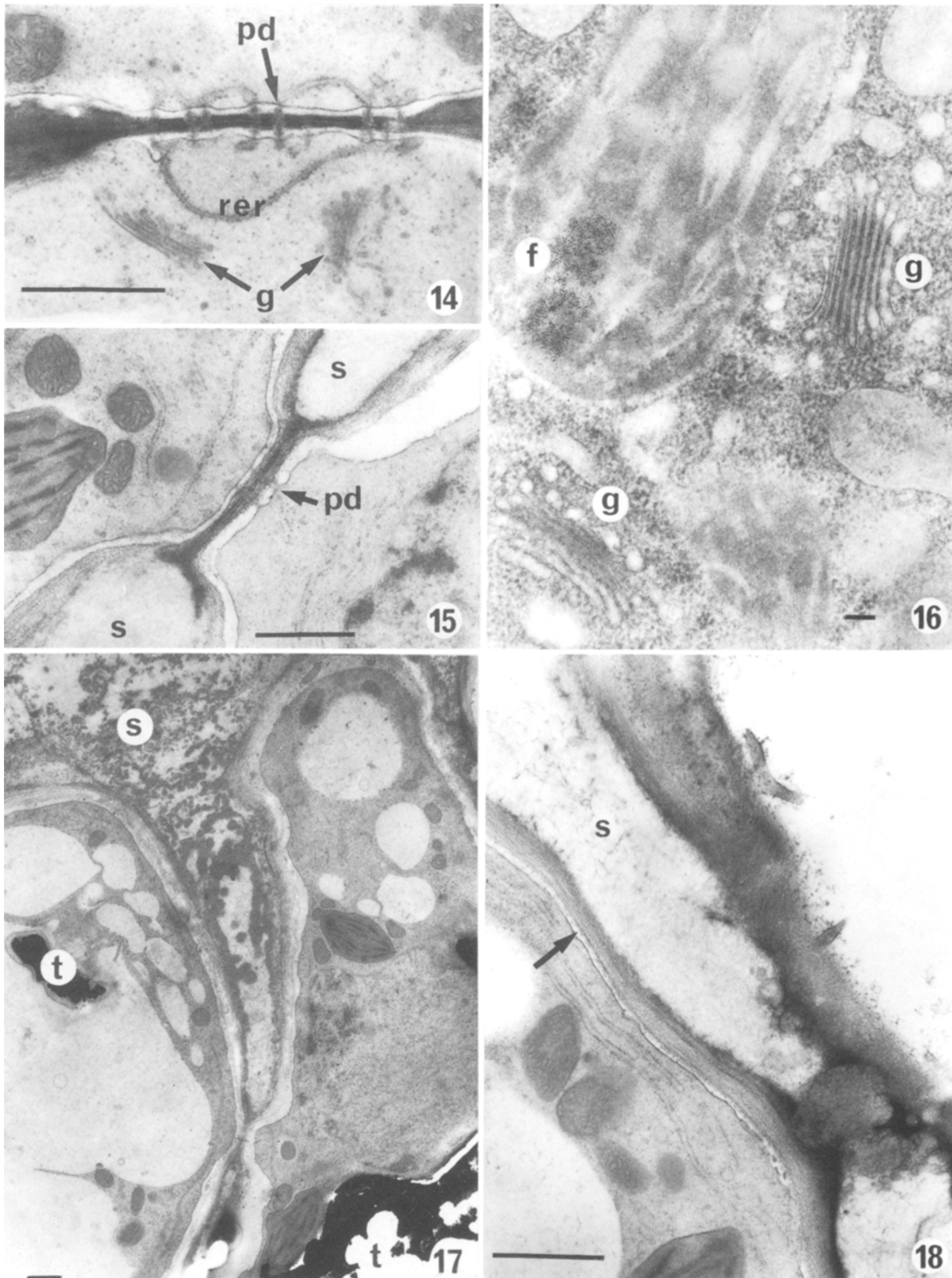


Fig. 14. EM of glandular cells showing rough endoplasmic reticulum (*rer*) and Golgi bodies (*g*) associated with plasmodesmata (*pd*). Bar represents 1 μm

Fig. 15. EM of secretory cell showing plasmodesmata (*pd*) and secretion (*s*). Bar represents 1 μm

Fig. 16. EM of secretory cell showing Golgi cisternae (*g*) and associated vesicles, and ferritin (*f*) granules in the plastid. Bar represents 0.1 μm

Fig. 17. EM of surface secretory cells with granular and floccular secretion (*s*) filling intercellular and subcuticular spaces. Tannin (*t*) vacuoles are a prominent feature of these cells. Bar represents 1 μm

Fig. 18. EM of secretory cell showing accumulation of fibrillar and granular secretion (*s*) in the subcuticular space which occurs in the base of the nectary trough. Granular substance accumulates in the periplasmic space (arrowed). Bar represents 1 μm

The inner tightly packed cells of the flanking zone lack intercellular spaces, which become more apparent toward the outer surface where an extensive intercellular network occurs. Accumulation of secretory product primarily in this outer intercellular network between the epidermal and subepidermal cells suggests that these cells are involved in secretion of some of the nectar components. However, all cells are richly interconnected by plasmodesmata which indicated that the primary passageway for the movement of assimilates to the surface secretory cells is via the symplast.

The suggestion that the epidermal and subepidermal cells are primarily involved in the secretion of the nectar is also supported by ultrastructural observations. It is in these cells that Golgi, secretory vesicles and ER associated with the plasmalemma are predominantly observed. This suggests that granulocrine secretion may be involved in the transfer of secretion to the intercellular location. Such a system has been proposed for nectaries of *Tropaeolum* (RACHMILEVITZ and FAHN 1973) and *Musa* (FAHN and BENOUAICHE 1979). The secretion has a granular appearance, but no intracellular accumulation of similar components was observed. This suggests that eccrine secretion (molecular transport across the plasmalemma) is also occurring. The histochemical evidence shows that the secretion contains protein and carbohydrate. The nature of the proteins and carbohydrates is not known as the appropriate analysis has not been undertaken. Nectar analysis performed on this species (KNOX *et al.* 1985) detected 18 amino acids, with phenylalanine and glutamine predominant. Samples contained a mean of 16% sugars comprising sucrose, fructose, and glucose. The source of the sugar is through the phloem of the branched vascular traces that cradle the glandular and secretory cells (FREY-WYSSLING 1955). The exact method of sugar transport has not been elucidated to date though many hypotheses including eccrine secretion have been proposed (DURKEE 1983).

The cells located at the surface of the secretory layer are covered by a cuticle. The contents of the intercellular spaces also stained positively for lipids. This material may be involved in turnover of the cuticle, particularly during active secretion when the cuticle is separated from the secretory cells to form the subcuticular space. This pattern has also been described in leaf cuticles of *Euphorbia* (BARTHLOTT and WOLLENWEBER 1981) and the stigma cuticle of *Amyema* (BERNHARDT and KNOX 1983). Such structures have not previously been described for other types of extrafloral nectaries. The distended appearance of the cuticle suggests that with an increasing accumulation of secretory material in the intercellular spaces, a pressure develops resulting in this deformation. We also suggest that backflow may be restricted by the dense and closely packed walls of the inner flanking layer. Evidence from scanning and transmission electron microscopy, light and fluorescence microscopy, and the application of vital dyes all confirm that the surface cuticle remains intact during active secretion. The cuticle does not rupture or possess channels or pores. This was also noted by BOUGHTON (1981) from light microscopy studies of a number of *Acacia* species. Lipid is present in the nectar as long chain wax esters (T. Douglas, personal communication, 1984). This suggests that the cuticle is in a soft fluid state which does not form a hard protective barrier like the cuticle on other parts of the plant. It is likely that the fluid cuticle is removed by foragers along with the rest of the nectar.

In conclusion, symplastic continuity appears to have a very important role in the extrafloral nectaries of this species. The continuous cell to cell contact may allow the passage and concentration of nectar constituents from the vascular traces through the 10 cell layers to the loosely arranged epidermal cells. The nectar components may then be transported outside the cell by both eccrine and granulocrine processes and accumulate in the subcuticular space. It appears likely that the whole nectar drop including fluid cuticle, is removed from the extrafloral nectary by the birds and insects which feed on the nectar.

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References

BARTHLOTT, W., WOLLENWEBER, E., 1981: Zur Feinstruktur, Chemie und taxonomischen Signifikanz epicuticularer Wachse und ähnlicher Sekrete, 67 p. (Tropische und subtropische Pflanzenwelt, 32.) Akademie der Wissenschaften und der Literatur: Mainz. Wiesbaden: Steiner.

BERNHARDT, P., KNOX, R. B., 1983: The stigmatic papillae of *Amyema* (*Loranthaceae*): Developmental responses to protandry and surface adaptions for bird pollination. Amer. J. Bot. **70**, 1313–1319.

BOUGHTON, V. H., 1981: Extrafloral nectaries of some Australian phyllodineous acacias. Aust. J. Bot. **29**, 653–664.

BRONNER, R., 1975: Simultaneous demonstration of lipids and starch in plant tissues. Stain Technol. **50**, 1–4.

DURKEE, L. T., 1983: Ultrastructure of nectaries. In: The biology of nectaries (BENTLEY, B., ELIAS, T., eds.), pp. 3–29. New York: Columbia University Press.

FAHN, A., BENOUAICHE, P., 1979: Ultrastructure, development and secretion in the nectary of banana flowers. Ann. Bot. **44**, 85–93.

FISHER, D. B., 1968: Protein staining of ribboned epon sections for light microscopy. Histochemie **16**, 92–96.

FREY-WYSSLING, A., 1955: The phloem supply to the nectaries. Acta Bot. Neerl. **4**, 358–369.

HESLOP-HARRISON, Y., 1977: The pollen-stigma interaction: pollen tube penetration in *Crocus*. Ann. Bot. **41**, 913–922.

JOEL, D. M., JUNIPER, B. E., 1982: Cuticular gaps in carnivorous plant glands. In: The plant cuticle (CUTLER, D. F., ALVIN, K. L., PRICES, C. E., eds.), pp. 121–130. London: Academic Press.

— REA, P. A., JUNIPER, B. E., 1983: The cuticle of *Dionaea muscipula* Ellis (Venus's Flytrap) in relation to stimulation, secretion and absorption. Protoplasma **114**, 44–51.

KARNOVSKY, M. J., 1965: A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. J. Cell Biol. **27**, 137 A.

KENRICK, J., KAUL, V., KNOX, R. B., 1984: Self incompatibility and the site of pollen tube arrest in Australian species of *Acacia*. Incompatibility Newsletter **16**, 3–4.

— MARGINSON, R., BERESFORD, G., KNOX, R. B., 1982: Birds and pollination in *Acacia terminalis*. In: Pollination '82 (WILLIAMS, E. G., KNOX, R. B., GILBERT, J. H., BERNHARDT, P., eds.), pp. 102–109. Proceedings of a Symposium, November 24, 1982, University of Melbourne, Victoria, Australia.

KNOX, R. B., KENRICK, J., BERNHARDT, P., MARGINSON, R., BERESFORD, G., BAKER, I., BAKER, H. G., 1985: Extrafloral nectaries as adaptations for bird pollination in *Acacia terminalis*. Amer. J. Bot. (in press).

MUELLER, W. C., GREENWOOD, A. D., 1978: The ultrastructure of phenolic-storing cells fixed with caffeine. J. exp. Bot. **29**, 757–764.

O'BRIEN, T. P., MCCULLY, M. E., 1981: The study of plant structure. Principles and selected methods. Melbourne, Australia: Termarcarphi Pty. Ltd.

RACHMILEVITZ, T., FAHN, A., 1973: Ultrastructure of nectaries of *Vinca rosea* L., *Vinca major* L. and *Citrus sinensis* Osbeck cv. Valencia and its relation to the mechanism of nectar secretion. Ann. Bot. **37**, 1–9.

REYNOLDS, R. S., 1963: The use of lead citrate at high pH as an electron-opaque stain in electronmicroscopy. J. Cell Biol. **17**, 208–213.

ZIMMERMAN, J. G., 1932: Über die extrafloralen Nektarien der Angiospermen. Botanisches Zentralblatt, Beihefte **49**, 99–196.