

Observations on the distinctive cream-coloured leaves of *Combretum nelsonii* Dümmer

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The ultrastructure of the cream-coloured leaves subtending the flowers and of the leaves of *Combretum nelsonii*, is described. The cream leaves, which are shed as the fruits mature, lack functional plastids within the irregularly-shaped palisade, although the spongy mesophyll cells are photosynthetically active. Abundant air-spaces below the papillate upper epidermis and between the palisade cells cause light to be refracted within the cream leaves, contributing to their pale colour. Cream leaves do not regreen but become obscured by younger green leaves.

Die ultrastruktuur van die roomkleurige blare net onderkant die blomme en die groen blare van *Combretum nelsonii* word beskryf. Die roomkleurige blare, wat na bevrugting afval, is sonder funksionele plastiede in die onreëlmatig-gevormde palissadeselle, hoewel die sponsagtige mesofilselle fotosinteties aktief is. Lugruimtes onder die getepelde adaksiale epidermis en tussen die palissadeselle kom volop voor, en veroorsaak ligrefraksie binne die roomkleurige blare wat tot die bleek voorkoms bydra. Roomkleurige blare word nie weer groen nie, maar word later tussen jong groen blare verberg.

Keywords: *Combretum nelsonii*, cream leaves, ultrastructure.

Introduction

Amongst the shrubs and grasses growing on the stony, open hillside below the chalets at F.H. Odendal resort in the Blyde River Canyon Reserve, Pilgrims Rest district, Eastern Transvaal, South Africa (grid ref. 2430 DB), are several small shrubby trees, *Combretum nelsonii* Dümmer. In September (spring), these trees are particularly conspicuous because they are covered with a mass of cream-coloured leaves associated with the flowering shoots. This distinctive creamy colouration is first seen as the trees burst into leaf in September, and persists during the flowering period and the development of the young fruits in November. However, the trees become less conspicuous as the winged fruits develop, and by December there are fewer cream leaves to be seen, and the overall colour of the tree is green. Cream-coloured leaves associated with flowering shoots are also seen in other *Combretum* Loefl. spp., for example, *C. erythrophyllum* (Burch.) Sond. and *C. kraussii* Hochst., but to a lesser extent than that displayed in *C. nelsonii*.

There are two explanations which could account for the change in overall colouration of these *Combretum* spp. The first, which is widely supposed (Carr 1988; van Wyk 1984; Palgrave 1977), is that the cream leaves which subtend the peduncle of the inflorescence, turn green as the fruits begin to develop. The second is that new green leaves develop above the cream leaves, changing the predominant colour of the tree to green, and then the cream leaves drop off as the fruits mature. If the latter is the case, then two separate sets of leaves are lost every year, the cream leaves in summer, from January onwards, and the green leaves later in the autumn, from May onwards.

In order to establish which mechanism is involved in the colour change in *C. nelsonii*, observations were made on

trees growing at the Blyde River Canyon Reserve, Eastern Transvaal, during 1983, 1984 and 1985. Herbarium material was collected, and the morphological and ultrastructural differences between cream and green leaves were examined by light and electron microscopy.

Materials and methods

Voucher specimens of flowering and fruiting material identified as *Combretum nelsonii* Dümmer by Mrs L.E. Davidson, have been deposited in the Moss Herbarium, University of the Witwatersrand, Johannesburg, South Africa (Davidson 3773, 3792, 3797). Exell (1968, 1978) put *C. nelsonii* into synonymy under *C. kraussii* Hochst., but there are sufficient differences in habit and leaf epidermal features for it still to be considered a distinct species (Carr 1988).

Leaf material for light and transmission electron microscopy was chopped and fixed in 3% glutaraldehyde in 0.05M cacodylate buffer (pH 7.2) for 2 h at room temperature. The tissue pieces were washed in three changes of cacodylate buffer and post-fixed overnight in 1% osmium tetroxide in the same buffer. The tissue was dehydrated through a graded ethanol series and embedded in Epon resin. Sections showing silver-grey interference colours were collected on copper grids and stained sequentially in uranyl acetate and lead citrate, and examined and photographed in an AEI 801 electron microscope. For light microscopy, sections 1 μm thick were stained in 1% toluidine blue in 1% borax (pH 10 – 11). For scanning electron microscopy, pieces of leaf, 5-mm-square, were fixed in glutaraldehyde and osmium tetroxide as described above, then dehydrated in an acetone series, critical-point-dried and sputter-coated with a layer of gold approx. 20 nm thick. Both epidermal surfaces were examined at 20 kV in a Cambridge Stereoscan S4.

Results

Field observations

The following description is a compilation of observations made over the three-year period 1983–85. In the month of September, the shrubby trees of *C. nelsonii* were con-

spicuous amongst other shrubs and grasses because of their distinctive cream colour (Figure 1). Closer examination of the flowering shoots at the tips of the branches (Figures 2, 3a) revealed that the oval leaves which subtend the flowers were cream-coloured with a matt surface. Those further



Figures 1–3 *Combretum nelsonii*. 1. Shrubby tree of *C. nelsonii* at Blyde River Canyon Reserve, Eastern Transvaal, showing the distinctive cream-coloured leaves (Sept. 1983). 2. Leaves subtending the flowers are cream-coloured, those lower down may have patches of green between leaf veins. 3. Herbarium specimens of *C. nelsonii*, all $\times 0.5$. a. Flowering shoots subtended by cream leaves. Green leaves are emerging at the tip (Oct. 1984, L.E.D. 3797). b. Clusters of young fruit in the axils of cream leaves (late Nov. 1983, L.E.D. 3773). c. Branch showing winged fruit. All cream leaves have been shed from the axils of the fruit (arrowed), leaving only green leaves (June 1984, L.E.D. 3792).

down the stem were also predominantly cream, but with pale green patches between the lateral veins of each leaf. At the tip of some shoots, above the flowers, was a pair of partially-expanded lanceolate leaves approx. 25 mm long (Figure 3a), greenish brown in colour with a glossy 'varnished' appearance. By the end of November, the cream leaves were still present subtending the developing fruit (Figure 3b), but the greenish-brown leaves at the tips of many shoots were fully expanded, shiny and up to 60 mm long. By the end of December, these green leaves were responsible for the predominantly green colour of the branches because they overtopped the cream leaves. The cream leaves were still present at the end of January, but by June they had been shed and two or three additional pairs of green leaves had formed above the fruits (Figure 3c). The arrows in Figure 3c indicate the positions from which the cream leaves have been lost.

Structure of the green leaves

Transverse sections of the green leaves (Figure 4) show the general distribution of the main cell types. The leaves are approximately 200 μm thick with a single layer of elongated palisade cells beneath the upper epidermis. The spongy mesophyll cells occupy the lower part of the leaf in the conventional arrangement.

Both the upper and lower surfaces have a characteristic 'varnished' appearance and the younger leaves in particular may be sticky. Detailed examination, by scanning electron microscopy, of the leaf surface of critical-point-dried material revealed that much of the epidermis is covered with a broken layer of amorphous material (Figure 5, arrows) in which glandular peltate scales (Figure 5, s) are embedded. Beneath this resinous layer, which is soluble in chloroform, can be seen the outline of the epidermal cells (Figure 5, e). The cells of the upper epidermis (Figure 6) are more or less isodiametric and the subcuticular wall (outer tangential cell wall) is heavily thickened. The underlying palisade cells of the green leaves are densely packed, *i.e.* firmly attached to the epidermis and to each other (Figures 6 and 7). Within these cells are well-developed chloroplasts containing starch and plastoglobuli, and there are numerous mitochondria within the dense cytoplasm (Figure 7). The cells of the spongy mesophyll also contain well-developed chloroplasts with starch and plastoglobuli (Figure 8). Both palisade and spongy mesophyll cells contain large globules of lipid-like material usually associated with the vacuoles (Figures 7 and 8).

Structure of the cream leaves

Transverse sections of cream leaves (Figure 9) show that the general layout of the leaf structure differs markedly from that of the green leaves (Figure 4), primarily because of the disarray of the cells of the palisade.

As in the green leaves, both the upper and lower epidermal layers are covered with a resinous layer (Figure 10, arrow) produced by peltate glandular scales (Figures 9 and 10, s). However, in cream leaves, the subcuticular wall of each of the upper epidermal cells is elongated (Figure 11), giving the surface of the upper epidermis a slightly papillate appearance (Figures 9 and 10). The cells of the lower epidermis are not elongated in this way (Figure 9).

The palisade cells of the cream leaves are strikingly different from those of green leaves, not only in their shape and arrangement, but also in their contents. In shape they are irregular and branched, and separated by large air spaces more typical of spongy mesophyll cells (Figures 9 and 12). Although the tips of the palisade cells are attached to the upper epidermis, the epidermis can easily be stripped off in sheets. The palisade cells are highly vacuolate with a centrally suspended nucleus and a thin peripheral layer of cytoplasm. The most conspicuous feature of the cell are the spheres of electron-dense material within the vacuoles (Figures 9, 11 and 12). Only vestigial plastids are present and there are comparatively few mitochondria (Figure 12).

In contrast, the spongy mesophyll cells are very similar to those in green leaves but with more intercellular spaces (Figure 13). The chloroplasts contain starch grains and there are large globules of lipid present in the cytoplasm and vacuoles (Figure 13). The undersides of cream leaves are light green in colour. However, when cream leaves are immersed in water under vacuum, the upper surfaces gradually become light green as the intercellular spaces become infiltrated with water.

Discussion

Field observations made over several seasons' growth show quite clearly that the cream leaves which subtend the flowers and the developing fruit of *C. nelsonii* do not regreen. The gradual changing of the predominant colour of the tips of the branches from cream to green is due to the development of new green leaves above the developing fruit. Hence the trees appear green, even though the cream leaves are still present below the green leaves. Later in summer (from January onwards) the cream leaves do fall off, but the green leaves persist until autumn (from May onwards).

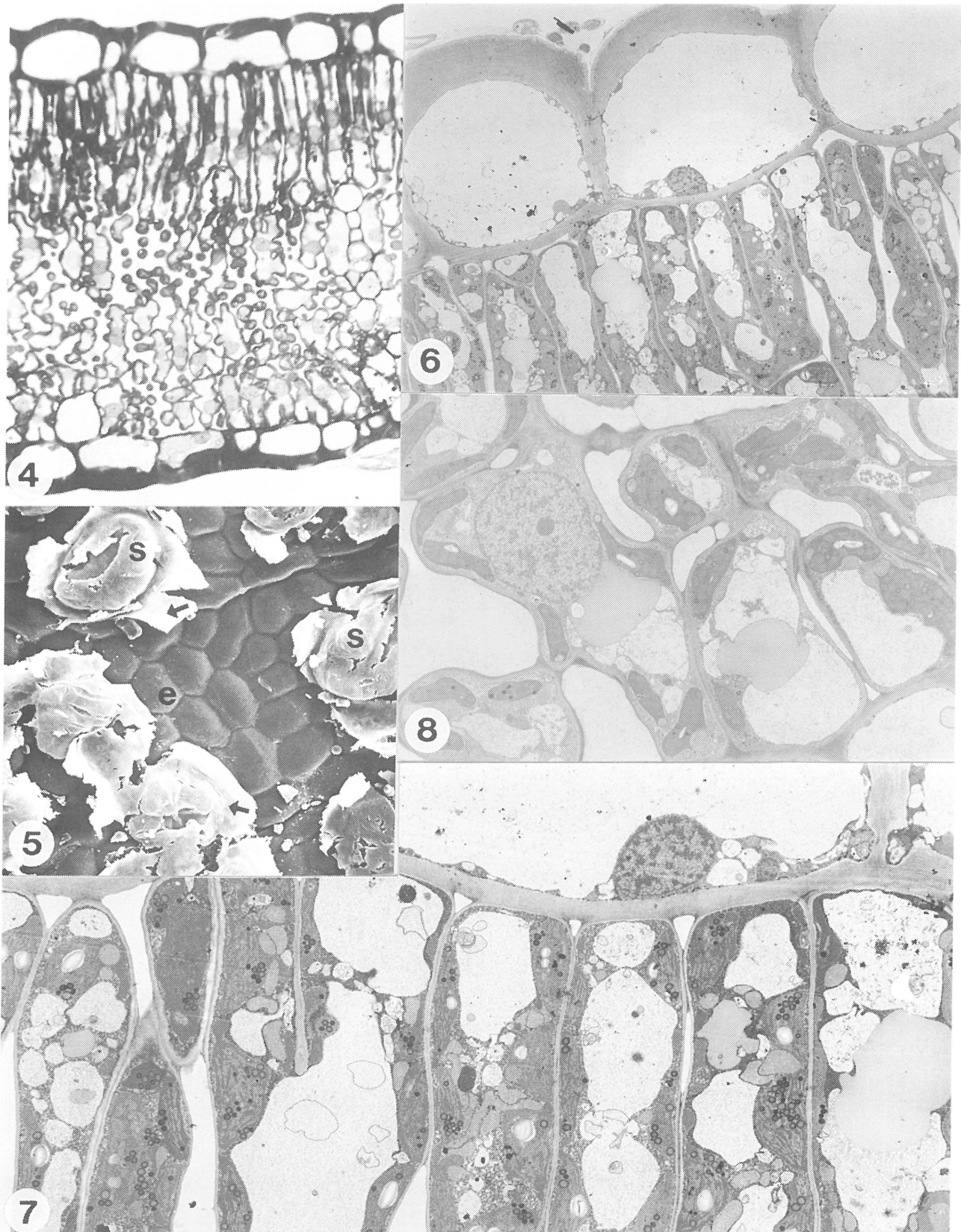
The main anatomical differences between green and cream leaves of *C. nelsonii* are found in the cells of the upper epidermis and the palisade layer. Differences include the lack of functional chloroplasts within the palisade cells, and increased intercellular air spaces around the irregularly shaped palisade cells, and below the adaxial epidermis. The subcuticular wall of each epidermal cell is also extended into papillae. It is most unlikely that the extreme development of these morphological features could be reversed.

Variation in leaves may be caused or augmented by features other than reduced levels or lack of chlorophyll. Variegation resulting from anatomical anomalies, termed 'false variegation' (Kirk & Tilney-Bassett 1967), has been investigated in a range of plants showing variable patterns of leaf variegation. White or silvery marks on leaves were found to fall into three main categories, namely lack of chlorophyll, the presence of extensive air spaces within the palisade and below the epidermis, and enlarged epidermal cells (Hara 1957).

All three of these features are evident in cream leaves of *C. nelsonii*. The palisade cells lack functional chloroplasts, and the photosynthetic function of the leaves is apparently carried out by the underlying spongy mesophyll which contains numerous active plastids. The upper epidermis is weakly attached to the palisade layer and can be detached in

sheets from the abnormally shaped palisade, though less easily than in the silver-grey leaves of the *Argenteum* (*Arg*) mutant of peas where the air spaces are even more extensive (Hoch *et al.* 1980). In addition, the epidermal cells are enlarged into papillae.

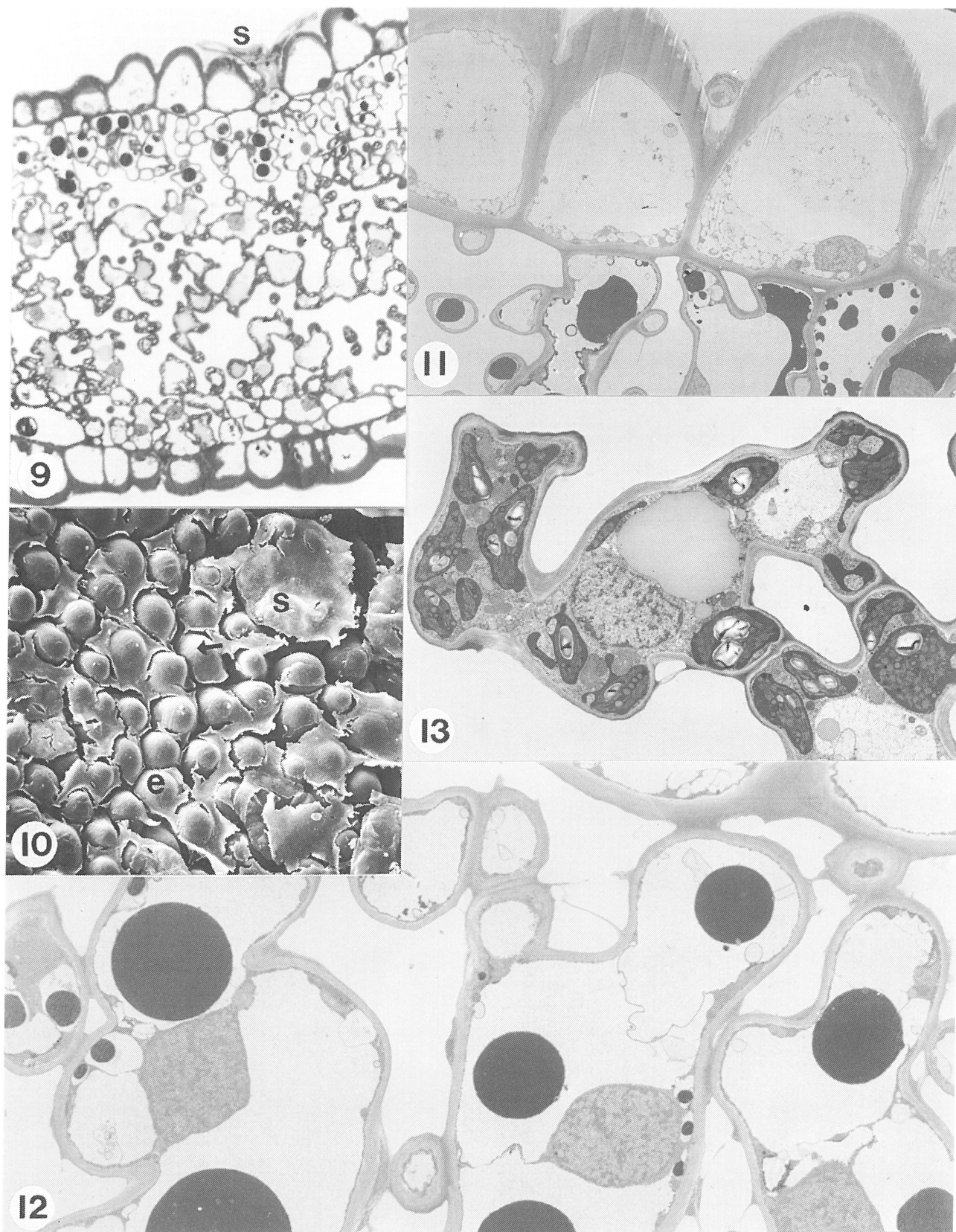
Increased air space beneath the epidermis and within the palisade layer causes light to be refracted internally within the leaf, so that the leaf appears white in that area, as reported for the flecks on the leaves of *Medicago truncatula* Gaertn. (McComb 1974) and white markings on clover (*Tri-*



Figures 4 – 8 Green leaves of *C. nelsonii*. 4. Section through leaf showing ordered palisade and mesophyll, $\times 400$. 5. SEM of upper surface of leaf showing resin layer (arrow) secreted by glandular scales (s) with underlying epidermis (e), $\times 320$. 6. Upper epidermis and palisade layer, $\times 1480$. 7. Palisade layer, $\times 3400$. 8. Spongy mesophyll cells, $\times 3600$.

folium repens L.) leaves (Carnahan *et al.* 1955). If this internal refraction in *C. nelsonii* is prevented by filling the intercellular spaces of the leaf with water under vacuum, then the green colouration of the underlying spongy meso-

phyll shows through. It is also possible that the papillate surface of the epidermis may contribute to the scattering of light on the leaf surface. Interestingly, illustrations of the epidermal cells of the white flecks on clover show them also



Figures 9 – 13 Cream-coloured leaves of *C. nelsonii*. **9.** Section through leaf showing disorganized palisade with extensive air spaces and glandular scale (s), $\times 400$. **10.** SEM of upper surface of leaf with resin layer (arrow), glandular scale (s) and papillate epidermis (e), $\times 320$. **11.** Papillate adaxial epidermis, $\times 1480$. **12.** Irregularly-shaped palisade cells with large intercellular spaces, $\times 3400$. **13.** Spongy mesophyll cells, $\times 3600$.

to be papillate (Carnahan *et al.* 1955), although this point is not specifically mentioned in the text.

Why this particular species of *Combretum* produces such a conspicuous flush of cream-coloured leaves subtending the flowers is not certain. However, it seems clear from the timing of this phenomenon that the blanched leaves provide a visual guide for pollinators. Further work is needed to follow the internal development of these leaves from their initiation at the apex to the fully expanded state described here, and to compare their ontogeny with that of the more persistent green leaves.

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