EPIDERMAL STRUCTURE AND STOMATAL ONTOGENY IN SOME CELASTRACEAE

By DIVYA DARSHAN PANT and PARVEEN F. KIDWAI

Department of Botany, The University, Allahabad, India

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SUMMARY

Leaves of *Celastrus paniculatus*, *C. stylosus*, *Elaeodendron glaucum*, *Euonymus japonicus* and *E. pendulus* are essentially hypostomatic, although a few stomata were observed near veins in the upper epidermis of *Celastrus paniculatus*. Their irregularly orientated stomata show guard cells partly underarched and partly surrounded by a ring of three to eight subsidiary cells. Surface walls of epidermal cells in *Euonymus pendulus* show pits whose impressions are also seen in the cuticle.

Rehfous (1914) had suggested that the sunken subsidiary cells of *Celastrus europaeus*, *Catha edulis* and *Euonymus japonicus* and four other species of the genus are formed in an unusual manner from segments of the guard cells. Our studies on the contrary indicate that the subsidiary cells in all our plants, including *E. japonicus*, are typically perigene.

Mesophyll of all leaves is armed but the form of midrib bundles varies in different species and to some extent in different regions of the same leaf.

INTRODUCTION

According to Rehfous (1914), the guard cells of the stomata in *Celastrus*, *Catha* and *Euonymus* divide to give rise to daughter cells which are situated below them (cf. Metcalfe and Chalk, 1950). As far as we know, such a mode of stomatal development is not only peculiar to these species but entirely unknown in any other plant. It was thought, therefore, that the details of the ontogeny of stomata in some Celastraceae were well worth a re-investigation.

MATERIAL AND METHODS

Material of *Celastrus paniculatus* Willd., *C. stylosus* Wall., *Euonymus japonicus* Linn., *E. pendulus* Wall., and *Elaeodendron glaucum* Pers. was obtained from Dehra Dun. Epidermal peels of young leaves were prepared in 2% acetocarmine and those of mature leaves in glycerine jelly. Microtome sections were also cut.

RESULTS

Mature leaves

Margins of leaves in all the plants are toothed in the middle and apical regions. Each marginal tooth ends in a glandular tip. The epidermal cells of the glands as well as most of the mesophyll cells of the region show thick granular contents (Fig. 3m and n). At the base of the teeth are situated blind veins whose ends are thickened (Fig. 3m). Stomata occur on the lower side of the thickened vein end but they are of the usual type. In an old leaf the glandular tips of the teeth break off, usually in an acropetal succession.

Leaves of all investigated plants are hypostomatic but in *Celastrus paniculatus*, a few stomata occur on the upper surface adjacent to veins. Stomata are usually confined to areas between veins but a few of them occur over the vein areas in *C. stylosus*, *Euonymus japonicus* and *E. pendulus*. Anticlinal walls of epidermal cells on both sides of leaves are sinuous in *Celastrus stylosus* (Fig. 1b and g), but they are straight or slightly arched (Fig. 1a, c–f and h–j) in all other species. Each cell wall in the cuticle of *Euonymus pendulus* is clearly marked by three lines representing the two secondary walls on either side of the middle lamella (Plate 12, No. 5). The outer wall of the epidermal cells in this species is heavily cutinized and sectional views of leaves show peculiar canal-like pits in the surface wall of each cell (Plate 12, No. 3). The pits extend up to the inner face of the cuticle and in paradermal views of cuticular preparations these structures are marked by rounded spots in the central part of each cell (Plate 12, No. 5). Cuticular preparations of this species also show some peculiar shining bodies (Plate 12, No. 5, sb) in many cells of the upper epidermis. Their shapes vary and they are either attached by short stalks (extending into other cells) to the anticlinal walls or to the surface wall. Similar bodies are present only in the cells of the stomatal region in the lower cuticle. Here and there, among the usual sized polygonal cells of the epidermis of all species are found much

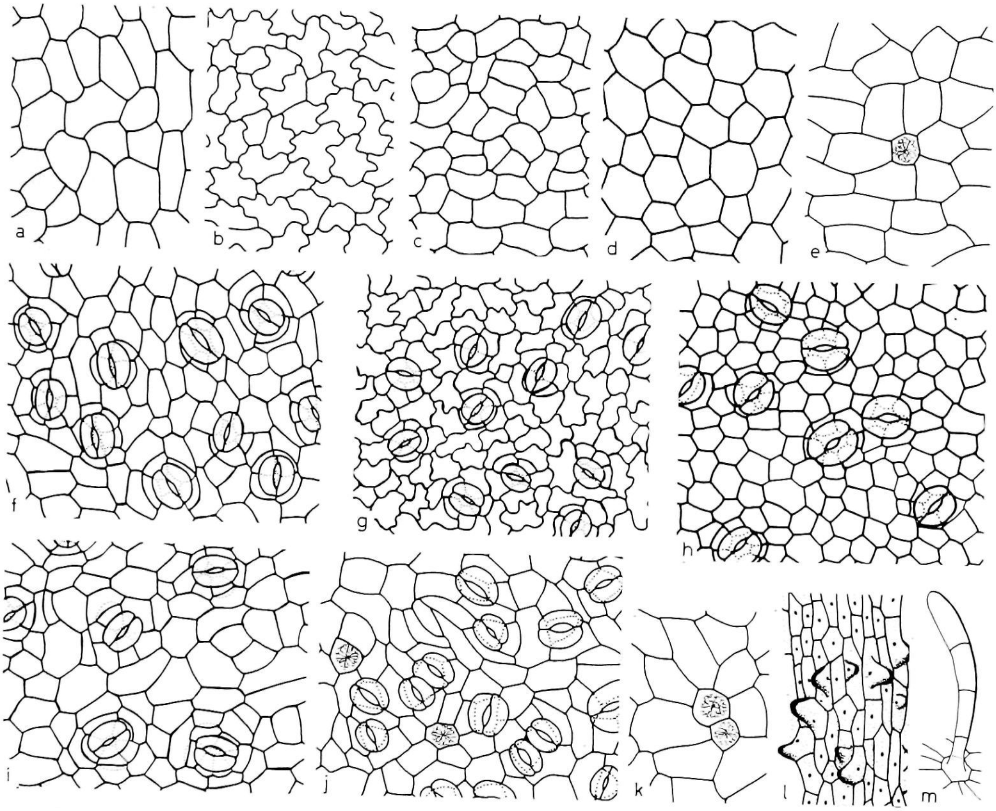

Fig. 1. (a–e) Upper epidermis of leaves. (a) *Celastrus paniculatus*; (b) *C. stylosus*; (c) *Euonymus japonicus*; (d) *E. pendulus*; (e) *Elaeodendron glaucum*. (f–j) Lower epidermis of leaves. (f) *Celastrus paniculatus*; (g) *C. stylosus*; (h) *Euonymus japonicus*; (i) *E. pendulus*; (j) *Elaeodendron glaucum*; (k) Portion of upper epidermis of *E. glaucum* showing contiguous sphaerraphide idioblasts; (l) *Celastrus stylosus*—portion of epidermis over midrib showing papillate cells; (m) *C. paniculatus*—a multicelled uniseriate hair. (a–k) ×200; (l–m) ×100.

smaller, often rectangular cells, particularly on the upper side of leaves. Elongated narrower cells mark the vein areas on both sides of leaves in *Celastrus paniculatus* and *C. stylosus*, but in all other species cells over the vein courses are differentiated only on the lower side.

Cutinized papillae or uniseriate, multicellular hairs with rounded ends occur in areas below veins in the lower epidermis of *C. paniculatus* (Fig. 1m). Cells over veins and midrib of leaves in *C. stylosus* often show short surface papillae (Fig. 1l). Here and there, in the epidermis of *Elaeodendron*, isolated shorter cells occur which contain sphaerraphides (Fig. 1e and j). Occasionally a pair of sphaerraphide idioblasts may be contiguous (Fig. 1k). Epidermal sphaerraphides, papillae and hairs are typically absent in both species of *Euonymus* investigated.

The stomatal apparatus consists of a median lenticular pore surrounded by a pair of kidney-shaped guard cells. The surface of the guard cells shows thickened cutin lamellae (Plate 12, No. 4) over the outer walls of the guard cells and thickly cuticularized outer ledges in all species. In *Celastrus paniculatus* and *Euonymus pendulus*, the common wall between the two guard cells is also thickly cutinized. Partly surrounding the guard cells and partly underarching them is a ring of subsidiary cells with slightly bulging centripetal walls (Figs. 2a–f, and 3j and k). The outlines of these cells become obscure in cuticular preparations (Plate 12, No. 4) indicating that their walls are poorly cutinized. Surrounding the sunken subsidiaries is an irregular ring of encircling cells which are only slightly differentiated from other epidermal cells. Each subsidiary cell generally has a corresponding encircling cell by its side. Sometimes the encircling cells partially overlap the subsidiaries (Fig. 2e). Arrested stomatal developments like those mentioned by Pant (1965*b*) occur in the lower leaf epidermis of all plants (see Figs. 1f–i and 3l). Contiguous stomata were seen in *Celastrus paniculatus* and *Elaeodendron*. Leaf cuticles are thinnest in *Celastrus stylosus* and *Elaeodendron* being about 0.5 μ thick on the lower side and 1.5 μ on the upper side. In *Celastrus paniculatus* they are 1 μ thick on the lower side and about 2 μ on the upper side. Thickest cuticles occur in *Euonymus pendulus* and *E. japonicus* where they are about 2.5 μ on the lower side and 3 μ on the upper side. The outer walls of epidermal cells are sometimes highly cutinized as in *E. pendulus*.

Simple dentate unbranched fibres accompany veins on the phloem side in *Celastrus paniculatus*, *Euonymus pendulus* and *Elaeodendron*. They are present only at vein ends in *Euonymus japonicus*. No fibres were observed along the veins of *Celastrus stylosus*. Blind ends of veins in *Euonymus japonicus* show a bunch of short and wide tracheoids spreading round the tip (Fig. 3o). The walls of the short tracheoids show simple or branched thickened bars which may appear scalariform or reticulate but are basically spiral (Fig. 3p) like those of the typical xylem elements of the veins. Leaves of *Elaeodendron* show a clearly differentiated layer of thick-walled hypodermal cells below the upper epidermis.

In *Euonymus pendulus*, the vascular tissue at the base of the midrib forms a closed planoconvex cylinder with a semicircular pith and a flat adaxial face (Plate 12, No. 1). In the middle region of the leaf the continuity of the vascular cylinder is broken on one or both flanks. In all others the vascular bundle is shaped like a gutter appearing as a C-shaped arc in transverse section (Plate 12, No. 2). The arc in the basal and middle regions of *Celastrus stylosus* comes closest to the cylinder in *Euonymus pendulus*, its flat side showing only a short gap. Next comes *Celastrus paniculatus* which has a much wider opening in the vascular arc in the middle region of the lamina on the flat side but the ends of the C are still incurved. The vascular arcs in basal and middle regions of midrib in *Elaeodendron*

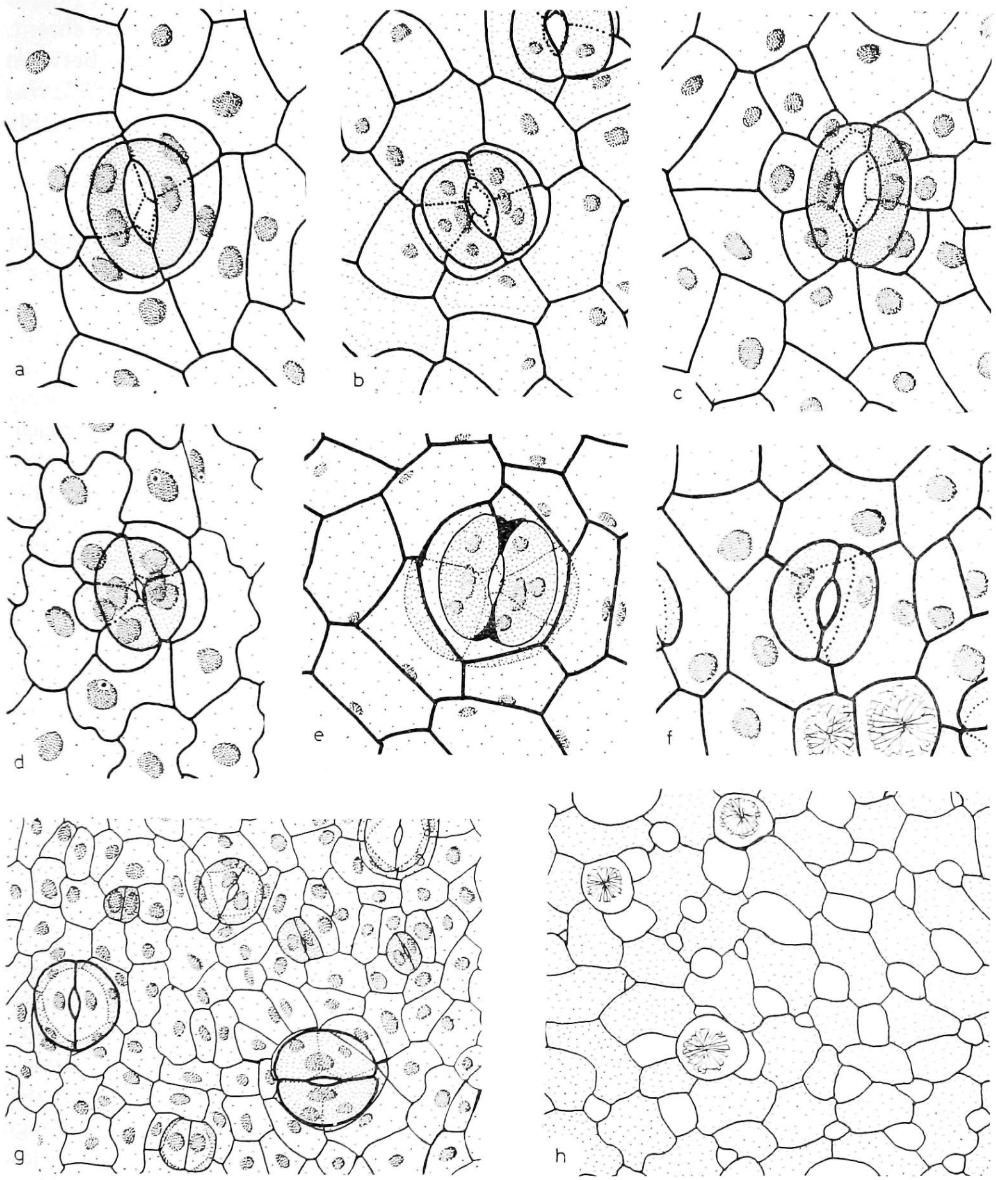

Fig. 2. (a–c) *Celastrus paniculatus*—stomata with four, five and eight subsidiary cells respectively, × 600; (d) *C. stylosus*—stoma with five subsidiary cells, × 600; (e) *Euonymus japonicus*—stoma with five subsidiary cells, some of which are overarched by the inner sides of encircling cells, × 600; (f) *Elaeodendron*—stoma with three subsidiary cells, × 600; (g) *Celastrus stylosus*—patch of protoderm showing stomata in different stages of development, × 450; (h) *Euonymus japonicus*—lobed mesophyll cells, air spaces and sphaerraphide idioblasts, × 300.

and basal region in *Celastrus paniculatus* are just like those of *C. stylosus* but their incurved ends are separated from the curved back of the arc and it is thus divided into three strands. The vascular bundle in *Euonymus japonicus* and in the apical region of leaves of all these plants has only the curved back of the arc—its incurved ends are absent.

Paradermal views of mesophyll show armed cells and large or small air spaces between their lobes (Fig. 2h). Tanniferous cells were observed in the mesophyll of *Celastrus paniculatus*, *Euonymus japonicus* and *E. pendulus*. Sphaerraphide idioblasts are invariably present in the mesophyll of all our plants (Fig. 2h).

Development of stomata

Development of stomata is of the mixed type (Fig. 2g) although younger stages of development are more frequent towards the bases of leaves. A cell of the protoderm (meristemoid) divides unequally, forming a smaller cell in one corner and a larger cell by its side (Fig. 3a and e). The smaller cell turns into the guard-cell mother cell. Its sister cell is recognizable for a short while only, by its conformity with the outline of the smaller cell. Subsequent growth makes it indistinguishable from other neighbouring cells (Fig. 3b). The guard cell initial, which has granular, darker-stained protoplasm and a prominent nucleus, divides and gives rise to a pair of guard cells (Fig. 3d, f and i). Meanwhile, its neighbouring cells start dividing tangentially (Fig. 3c, g and h). Generally these divisions take place first in the lateral neighbouring cells and then in the polars. As a result, an inner ring of perigene subsidiary cells and an outer ring of sister encircling cells is differentiated but often in *Elaeodendron* and occasionally in other plants also the neighbouring cells may turn directly into the subsidiaries without division (Figs. 1j, and 2c and f). In any case the inner sides of the subsidiary cells subsequently grow inwards (Fig. 3d) below the guard cells and become somewhat papillate. Thus the subsidiary cells become clearly differentiated. The encircling cells, however, become indistinguishable from the ordinary epidermal cells although they may sometimes overlap the outer sides of the subsidiaries.

DISCUSSION

Solereder (1908) mentions the 'lack of a uniform type of stoma' and the absence of any 'special subsidiary cells' in the Celastraceae. The mature stomata of the Celastraceae have been described by Metcalfe and Chalk (1950) as 'usually cruciferous to ranunculaceous; but rubiaceous in *Kurrimia*; guard cells surrounded by a rosette of smaller epidermal cells in *Mortomia*'. The stomatal structure of the present Celastraceae, except for a brief mention of *Elaeodendron glaucum*, is not described by any of the above authors. It would therefore appear that all our species of *Celastrus*, *Elaeodendron* and *Euonymus* are consistently similar and peculiar in having a ring of three to eight well-differentiated subsidiary cells which are almost completely covered by the two guard cells although their outer extremities are usually bare. The placement of the subsidiary cells below the guard cells is comparable with that of certain Magnoliaceae (see Bailey and Nast, 1945; Pant and Gupta, 1966).

The development of stomata in *Euonymus verrucosa*, *E. europaea*, *E. latifolia*, *E. macroptera*, *E. japonicus*, *Celastrus europaeus* and *Catha edulis* was first described by Rehfous (1914). According to him, the sunken subsidiary cells of the Celastraceae arise by the division of the guard cells. The present study brings out clearly that the subsidiary cells in the stomata of two species of *Celastrus*, one species of *Elaeodendron* and two species of *Euonymus* including *E. japonicus* are not formed by periclinal divisions

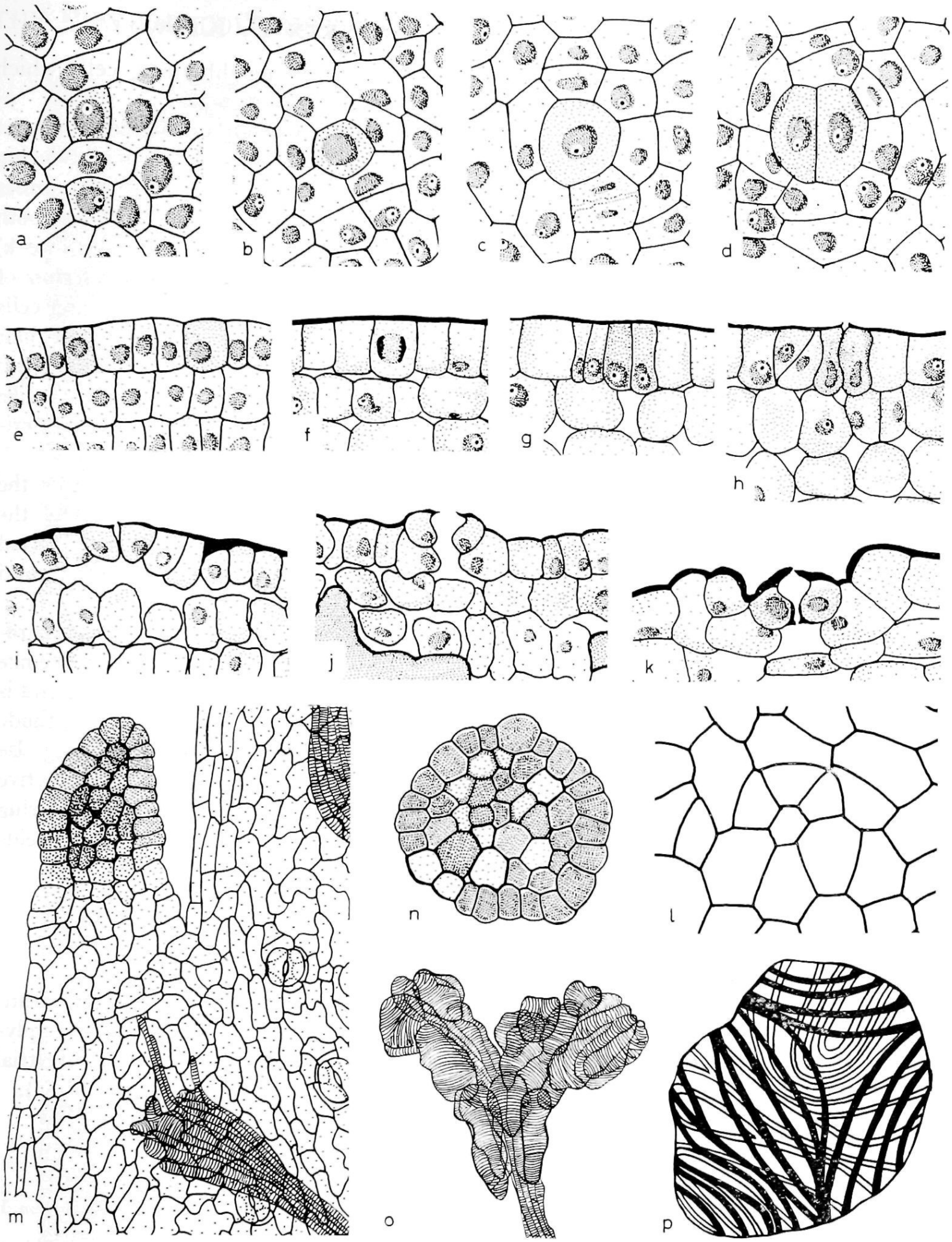

Fig. 3. (a–d) *Euonymus japonicus*—paradermal views of successive stages in development of stomata; (e–h) *E. japonicus*—sectional views of successive stages in development of stomata; (i) *Celastrus paniculatus*—young stomata whose subsidiaries are not yet overlapped by the guard cells; (j) *C. paniculatus*—mature stoma; (k) *Euonymus japonicus*—mature stoma; (l) *Celastrus paniculatus*—arrested stomatal development showing three neighbouring cells which have divided by tangential walls; (m) *C. stylosus*—portion of a leaf transparency showing a glandular tipped marginal tooth with a thickened vein end at its base; (n) *Euonymus japonicus*—transverse section of a glandular tooth showing glandular cells with dark contents; (o) *E. japonicus*—vein end from a leaf transparency showing a bunch of short and wide tracheoids; (p) *E. japonicus*—a short tracheoid from a vein end magnified to show simple and branched thickened bars. (a–k and p) × 600; (l and n) × 300; (m) × 225; (o) × 150.

of the guard cells but by anticlinal divisions of perigene neighbouring cells which initially surround the guard cell mother cells.

The conclusion that the subsidiary cells are formed from neighbouring cells and not from guard cells, was confirmed not only by observing stages where the neighbouring cells have just completed their divisions but also by the usual conformity in the number and outlines of the subsidiary cells and their sister encircling cells. The fact that both the epidermis and the hypodermis are regularly layered in these leaves (Fig. 3e–k) and that this pattern is never disturbed by any periclinal divisions in the epidermis of young leaves lends further support to our conclusions. Clearly, the neighbouring cells which initially stand level with the guard cells, divide at a later stage and thereafter their centripetal segments (subsidiaries) creep under the guard cells. The stomata of Celastraceae cannot therefore be regarded as multistratose bicelled pores (Pant, 1965*a*), unlike the stomata of *Equisetum* where the first division of the meristemoid is reportedly periclinal (Chatterjee, 1964).

A few arrested stomata observed in some of our preparations also confirm that the subsidiary cells are formed from neighbouring cells. In a few cases the tips of the neighbouring cells towards the side of the guard-cells mother cell had already started creeping below them before the guard cells were formed, proving thereby that the guard cells and subsidiary cells have an independent origin.

The presence of glandular teeth at the margins of leaves of the present plants is reported for the first time although similar structures have been reported by Reinke (1876, *vide* Solereder, 1908), in *Catha cassinoides*. At the base of each tooth a vein end is present which is thickened like the strand present at the base of an epithem hydathode (Haberlandt, 1914), but no differentiated epithem was observed and there is nothing else to indicate that the structures are hydathodes unless they are in the category of 'active hydathodes' of Haberlandt. Bunches of loosely packed, short and wide tracheoids occurring at the vein ends in leaves of *Euonymus japonicus* resemble the similarly attached sclereids of many other angiosperm leaves.

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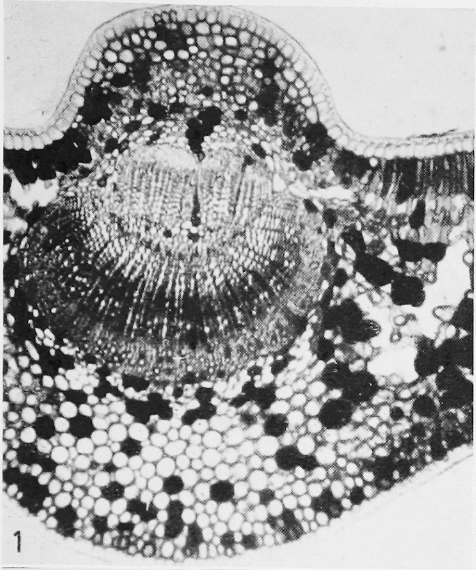
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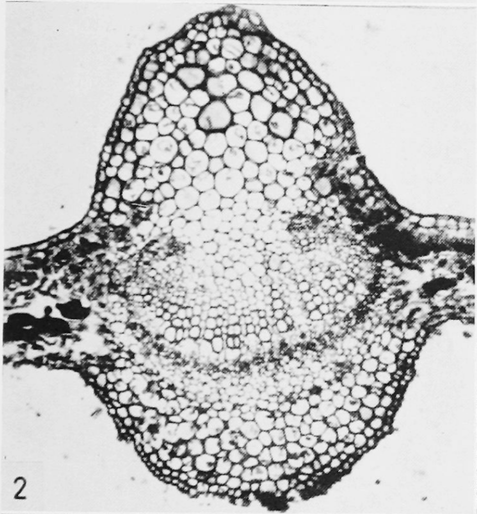
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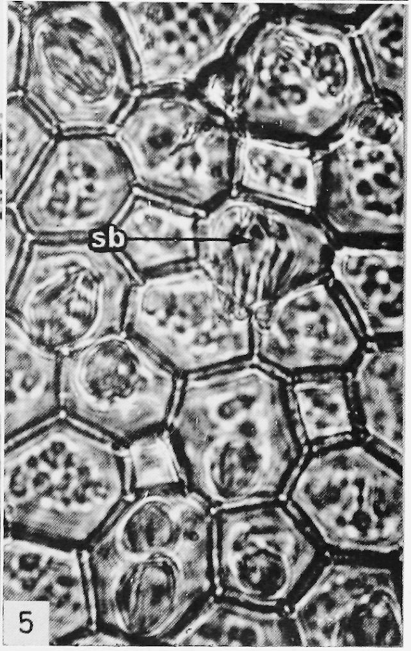

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DIVYA DARSHAN PANT AND PARVEEN F. KIDWAI—*EPIDERMAL FEATURES OF CELASTRACEAE*

(*facing page* 294)

EXPLANATION OF PLATE 12

No. 1. *Euonymus pendulus*—transverse section of leaf showing a closed plano-convex vascular cylinder in the midrib, × 60.

No. 2. *Celastrus paniculatus*—transverse section of leaf showing a C-shaped vascular bundle in the midrib, × 80.

No. 3. *Euonymus pendulus*—Portion of transverse section of upper epidermis of leaf showing canal like pits in the cutinized wall, × 1040.

No. 4. *Euonymus japonicus*—Cuticle showing cutin lamellae over the guard cells of a stoma, × 780.

No. 5. *Euonymus pendulus*—Upper cuticle of leaf showing small pits in all cells. A few cells show larger shining bodies (sb), × 550.