Anatomy of the Laminar Organs of *Commelina erecta* (Commelinaceae)

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Abstract - A study was undertaken to compare the anatomy of the laminar floral parts with that of the spathes and leaves of *Commelina erecta* L. Each flower has two types of petals and two types of sepals. In contrast to the other organs, the petals have a completely open venation system whose vein endings consist solely of modified bundle-sheath cells. Bundle sheaths of leaves and spathes, but not the floral organs, contain sclerified cells for support. The high density of hook-shaped trichomes on the outer surface of the spathe and of glandular microhairs on the inner surface might indicate protective and secretory functions, respectively. Anomalous stomatal apparatuses are more common on floral organs than on spathes or leaves. Leaves and spathes appear to have a more detailed developmental program than sepals and petals.

Introduction

Commelin (erect dayflower), with 170 species worldwide, is the largest genus within the Commelinaceae. Nine species are found in the US and only three of them are native (Faden 1993, 2000). *Commelina erecta L.* is the most widespread of the native species (Faden 1993). Its range includes much of the East Coast, Southeast, and southern Midwest (Faden 2000). The flowers have been thoroughly described for taxonomic purposes as having two different types of petals (two that are large, blue, and clawed; one that is small and colorless) and two different sizes of sepals (Brashier 1966, Pennell 1916, Radford et al. 1968). Thus, along with the leaves and spathes, which enclose the inflorescences, there are six different laminar organs in *C. erecta*. Since floral organs represent modified leaves (Weberling 1992), we decided to undertake a comparative anatomical study of the various laminar organs of *C. erecta*, with emphasis on vasculature and arrangement of stomatal apparatuses.

Materials and Methods

Specimens of vegetative and floral organs were collected from the property of the senior author in Lee County, AL. Some specimens were viewed in the living condition using bright-field microscopy. This technique was especially effective for studying the floral organs that were only a few cell layers in thickness. Other material was fixed in FAA (90 ml 50% ethanol, 5 ml glacial acetic acid, 5 ml formalin; Johansen 1940), then cleared in 50% ethanol and stored in the same fluid for an extended period of time. This material was viewed directly either using Nomarski optics (McCrone et al. 1978) or a bright

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field microscope following staining. Stain recipes included: 1) 1% safranin in 50% ethanol, 2) 0.5% aqueous toluidine blue O (TBO) in 0.02 M sodium benzoate buffer, 3) 2% ferrous sulphate in acidified formalin for tannin identification (Ruzin 1999), 4) 0.1% aniline blue in 0.1 N K_3PO_4 for callose identification (Martin 1959), and 5) 1% I_2KI for starch identification. Callose distribution was detected using a fluorescence microscope.

Material was preserved and embedded in plastic resin in two different ways. The first method involved fixation in 3% glutaraldehyde in 0.05 M potassium phosphate buffer (pH 6.8). Dehydration to 95% ethanol was followed by infiltration and embedment in JB-4 resin (Polysciences, Inc., Warrington, PA). Alternatively, some material was preserved in 3% buffered glutaraldehyde and, after washing in buffer, postfixed in 1% buffered OsO_4. The tissue was next dehydrated in an alcohol/acetone series and embedded in Spurr's resin (Spurr 1969).

Resin-embedded material was sectioned (6-μm thick sections for JB-4 material; 2-μm for Spurr material) using a Porter-Blum MT-2B ultramicrotome. The resulting sections were heat-fixed to glass microscope slides and stained with TBO. The stained sections were then covered with Permount mounting medium and a glass coverslip.

Photographs of slide material were made using either a 35-mm camera attached to a compound microscope with Ektachrome 64T color slide film, or a Nikon D70 Digital Camera attached to the microscope.

A dissecting microscope was used to observe both living and fixed and cleared material via both bright-field and dark-field options. Material was photographed with a Nikon D70 Digital Camera.

Air-dried leaves and spathes were ashed and the material viewed with both bright-field and scanning electron microscopy (SEM) in order to investigate mineral inclusions. Elemental analysis was accomplished with an energy dispersive spectrometer (EDS) attachment to the same microscope (Postek et al. 1980.)

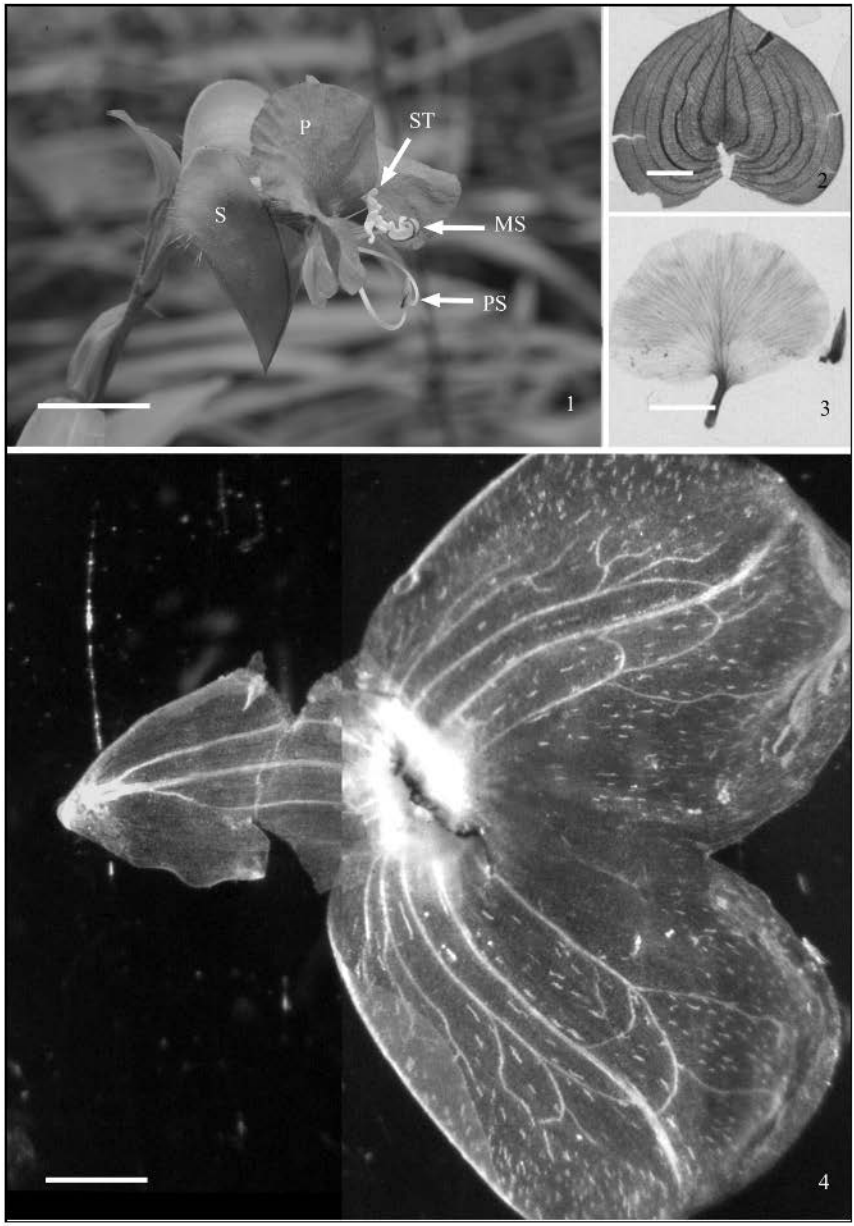
Supplementary material was fixed in buffered glutaraldehyde, dehydrated in ethanol followed by acetone, and dried by the critical-point method using liquid CO_2 (Postek et al. 1980). Dried material was attached to aluminum stubs with double-stick carbon tape. Specimens were coated with a gold-palladium vapor and viewed at 15 kV accelerating voltage using a Zeiss 940 Digital Scanning Microscope.

A voucher specimen was deposited in the Auburn University Herbarium (AUA #63659).

Results and Discussion

Floral morphology

Flowers of *C. erecta* arise from within a folded leaflike spathe that contains mucilage (Fig. 1). The leaflike nature of the spathe is evident once the organ is removed from the plant and unfolded (Fig. 2), although its shape differs from the lanceolate leaves. Unfolding the spathe requires some tear-



Figures 1–4. 1) Habit view of *C. erecta* flower and its spathe. MS = medial fertile stamen, P = posterior petal, PS = proximal fertile stamens, S = spathe, and ST = staminode. Scale bar = 10 mm. 2) Unfolded spathe showing its foliar outline. Scale bar = 5 mm. 3) Size comparison of large and small petal. Both organs have been cleared and then stained with safranin. Scale = 5 mm. 4) A composite photograph of three sepals—two large, one small—viewed by dark-field microscopy. Note the three main veins in each organ. Scale bar = 1 mm.

ing as the proximal margins are fused. The calyx consists of three sepals (Fig. 4); the two anterior ones are fused into a cup, the posterior one is free, smaller, and of a different shape. The corolla has two large, posterior, blue petals with claws (Figs. 1, 3). The anterior petal is much smaller, colorless to nearly so, and wedge-shaped. The difference between the two petal types is apparent in Figure 3. The androecium consists of three proximal fertile stamens and three distal staminodes (Fig. 1). The anther of the shorter, median fertile stamen is of a different shape from the others. It is referred to as the feeding anther because it is a primary attractant for insects (McCollum et al. 1984). The gynoecium consists of three fused carpels.

The population of *C. erecta* produced flowers from mid-June through early September. Three to five flowers extend (one at a time) from each enfolding spathe. Flower extension and anthesis from a given spathe varies from every other day to every fourth day. The period of anthesis for a given flower is brief; flowers are about one-third open an hour before sunrise, but are completely closed and wilted by about an hour after noon (R.R. Dute, pers. observ.).

General anatomy

Comparison of sectional views of the various organs shows consistent differences. Figure 5 shows a transection of a vegetative leaf. Anatomically, this organ is dorsiventral with a single, well-developed layer of palisade cells above a considerable volume of irregular, loosely packed, spongy parenchyma cells. Both cell types are chlorophyllous. In contrast, a sectional view of the spathe, the other photosynthetic organ, shows only a homogeneous, chlorophyllous mesophyll (Fig. 6). The mesophyll region of the floral organs also is homogeneous, but largely achlorophyllous (Fig. 7). Although a faint green color can sometimes be seen in these other organs, for example in the small sepal, the number of chloroplasts involved is miniscule in comparison to the leaves and spathes and cannot be observed by microscopy other than in the guard cells of the epidermis.

All organs have a considerable amount of intercellular space within the mesophyll; however, the limb of the large petal seems to be the most aerenchymatous of the organs (Fig. 8). The contrast in tissue density to that of the other organs, including that of the claw of the large petal (Fig. 9), is considerable. Added to the lack of density is the poor staining contrast of the limb tissues due to very thin cell walls. Adequate preservation of the limb tissues of the large petal proved difficult and could only be approached through glutaraldehyde fixation and plastic embedment. Even so, considerable cell shrinkage occurred.

Epidermal cells of the leaf are large and occupy a significant portion of the cross-sectional area of the leaf (41%, range 31–50%, N = 10; Fig. 5). These large epidermal cells are a typical feature of the Commelinaceae in general (Tomlinson 1966) and of *Commelina* in particular (Brückner 1926, Preston 1898). It has been hypothesized that these enlarged cells function in water storage (q.v. discussion in Tomlinson 1966). The ratio of epidermal area to the

entire cross-sectional area of the organ is significantly greater (P = 0.0027) in the spathe (62%, range 44–75%, N = 16; Fig. 6). Enlarged size of the epidermal cells does not extend to the guard or subsidiary cells (Figs. 5, 6).

Vascular tissue

Both leaves and spathes have the same arrangement of veins consisting of parallel longitudinal veins connected by narrower diameter transverse veins (Tomlinson 1969) or commissural bundles (Esau 1977) to give a closed venation system (Fig. 10). The venation patterns of large and small

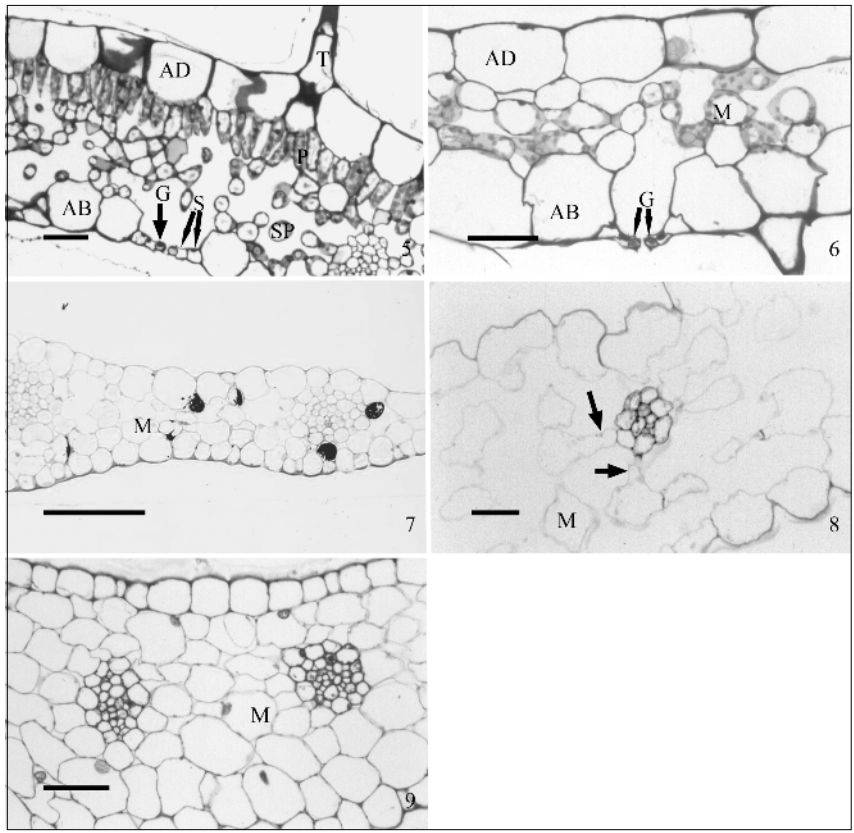


Figure 5–9. 5) Transection of vegetative leaf. AB = abaxial (lower) epidermis, AD = adaxial (upper) epidermis, G = guard cell, P = palisade parenchyma, S = subsidiary cells, SP = spongy parenchyma, and T = trichome. Scale bar = 50 μm. 6) Transection of spathe. The chloroplasts are visible as spots in the mesophyll (M). Note that the mesophyll is undifferentiated unlike that of the leaf. AD = adaxial epidermis, AB = abaxial epidermis, and G = guard cells. Scale bar = 50 μm. 7) Transection of small sepal. The mesophyll (M) is undifferentiated. Scale bar = 100 μm. 8) Transection of the limb of the large petal. The mesophyll (M) cells are separated by large intercellular spaces. The arrows indicate attachment of the mesophyll cells to the bundle sheath of a vascular bundle. Scale bar = 25 μm. 9) A transection of the claw of the large petal. The cells of the mesophyll are more tightly packed than those of the limb. Scale bar = 50 μm.

sepals are similar and consist of three main veins which not only are interconnected by small veins but which fuse distally (Fig. 4). In addition, there are branch veins in the large sepals that end blindly (Fig. 4). In contrast to the organs mentioned thus far, venation of the limb of the large petal is of a dichotomous, open type (Fig. 11). Venation of the small petal is also open, but of a simpler architecture and with fewer veins than its larger counterpart. Although both sepals and petals have three major veins, the origin of these

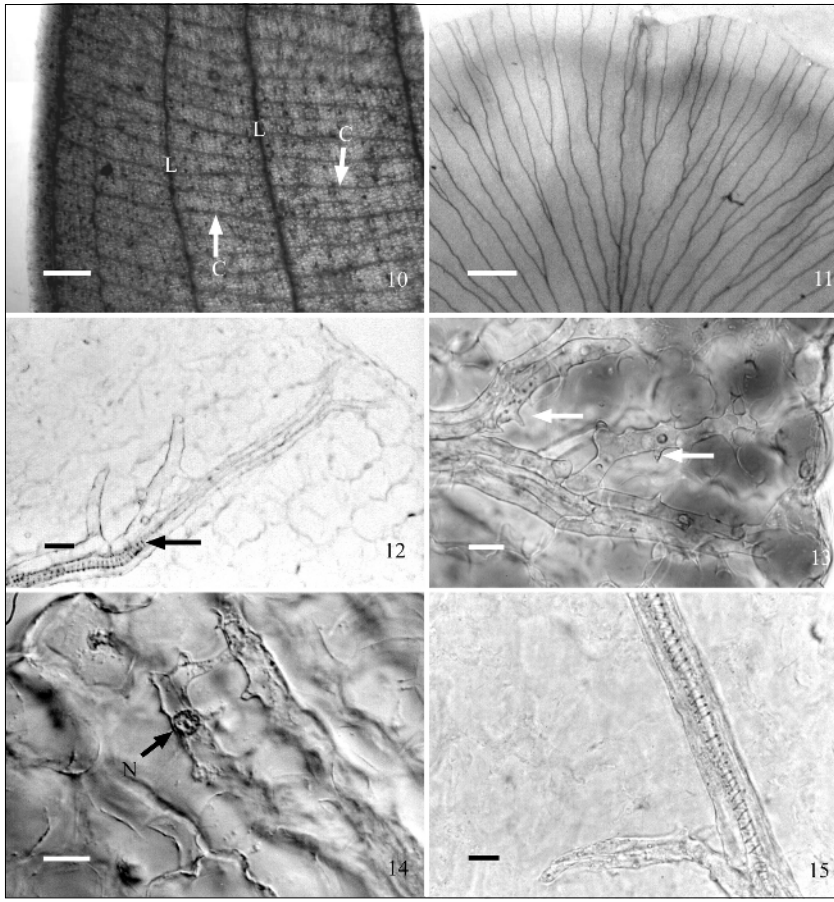


Figure 10–15. 10) Closed system of veins in spathe. C = commissural vein, and L = longitudinal bundles. Scale bar = 0.5 mm. 11) Dichotomous open venation of large petal (limb). Scale bar =1 mm. 12) Vein end in large petal. The arrow indicates termination of the xylem tissue. Bundle-sheath cells extend beyond this point as a branched vein prolongation. Scale bar = 50 μm. 13) Bright-field microscopic image of the edge of a petal showing a detail of a branched vein prolongation in a living petal. The arrows denote projections extending from the modified bundle-sheath cells. Scale bar = 25 μm. 14) Portion of vein prolongation as seen with Nomarski optics. The arrow indicates a nucleus. Scale bar = 25 μm. 15) A vein prolongation branching from a vein. This vein is located in the center of the limb away from the petal's edge. Scale bar = 25 μm.

veins from the receptacular stele differs. Namely, the primary laterals of the sepals share a vascular origin with the main veins of the petals, whereas the median veins of the sepals have a separate vascular origin (Hardy and Stevenson 2000a, b).

Detailed study of vein endings from cleared specimens of large petals indicates that "vein prolongations," consisting of modified bundle-sheath cells, extend beyond the xylem and phloem for a considerable distance (Fig. 12). The branching or "digitation" of these vein prolongations is not uncommon (Figs. 12, 13). The parenchymatous nature of these cells is indicated by their possession of nuclei and cytoplasm (Fig. 14). Similar vein prolongations can occasionally be found as minor branches from a vein at other locations in the petal (Fig. 15). Vein prolongations occur in small petals as well, but they lack the complexity (branching) of those found in large petals.

Confirmation of the structure of vein endings in large petals comes from serial cross sections beginning at the petal's edge. Only bundle-sheath cells of the vein prolongation are initially present (Fig. 16), followed by the appearance of a sieve element (Fig. 17), in turn followed by a tracheary element (Fig. 18). One can see the densely cytoplasmic nature of the bundle-sheath cells relative to other cells in the petal.

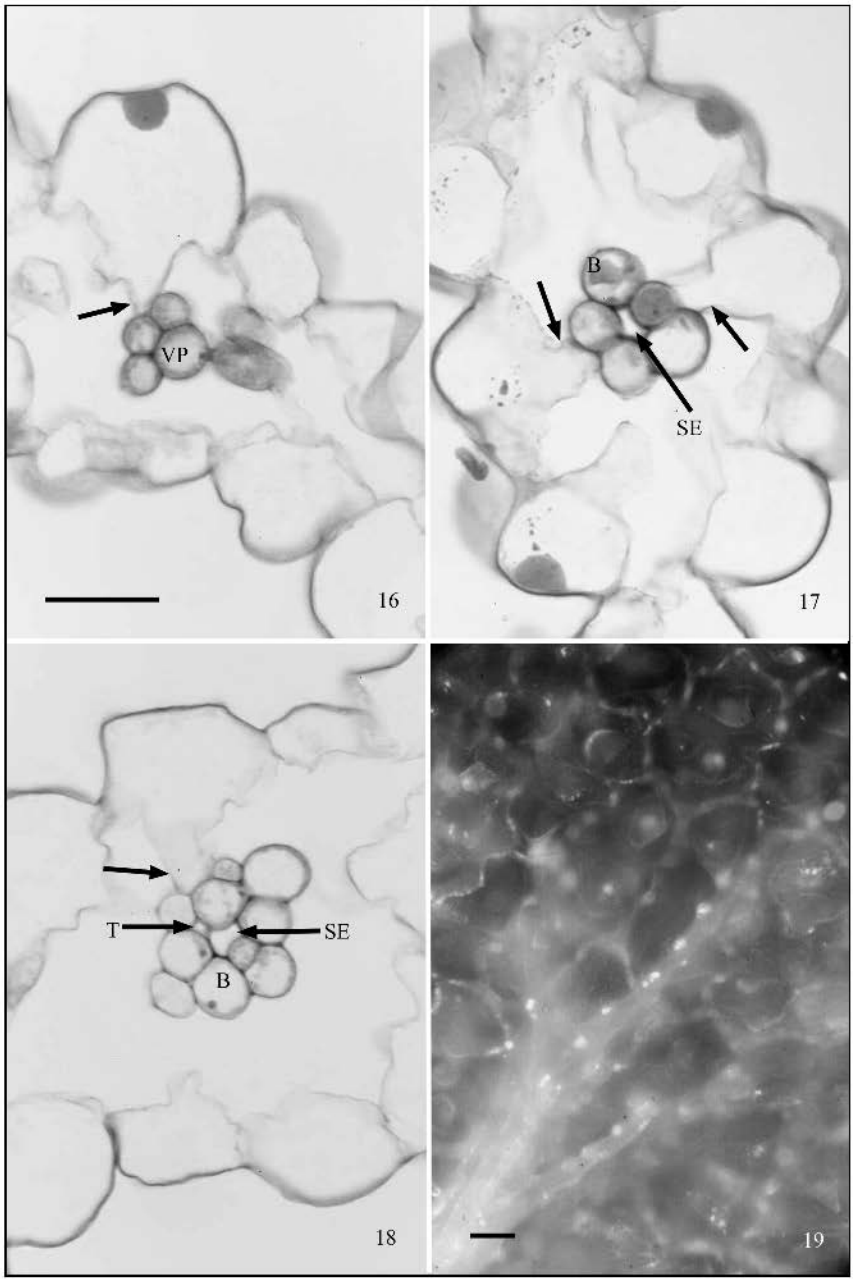
As the petal's margin is approached, the number of cell layers decreases, and near a vein's terminus, the vascular bundle is often surrounded by only cells of the two epidermal surfaces (Figs. 16–18). These epidermal cells are attached to the bundle-sheath cells by lobes (Figs. 16–18). These lobes of the epidermal cells probably result from restricted areas of cell wall growth as the petal expands. Localization of callose in the walls of vein prolongations is depicted using aniline blue fluorescence in Figure 19. Sites of fluorescence probably represent clusters of plasmodesmata connecting bundle-sheath cells with epidermal lobes (Currier 1957). In addition to epidermal lobes, cells of the vein prolongations themselves possess tiny projections from their surfaces (Fig. 13). The function of these projections is unknown.

In more proximal parts of the petal, the organ is thicker and there are more cell layers (referred to as the mesophyll, Fig. 8). In such instances, the mesophyll cells are attached to the bundle sheaths by lobes (Fig. 8, unlabeled arrows).

Bundle-sheath cells of the large petals contain large multigrained amyloplasts (Fig. 20) that give a distinct positive reaction to iodine, and that, in living cells, can be seen to move in response to cytoplasmic streaming. Size and number of starch grains show a gradient in the bundle-sheath cells from most numerous and largest in the proximal parts of the petal to absent near the margin.

There are two ways to view the vasculature of the large petals—ontogenetically and functionally. According to Weberling (1992), the open venation of perianth parts is the result of the reduced number of cell layers formed at the margin of the organ as it continues to divide. In other words, a certain amount

of petal thickness is necessary for the veins to join into a closed system. We hypothesize that the vein prolongations occur because there are not enough layers of cells near the margin to support the formation of xylem and phloem tissues. Thus, the vein prolongations represent a relictual vascular tissue that distributes water and food to the margin.



The importance of vascular bundle sheaths in transport of substances to and from vascular tissues is well-known (Ding et al. 1988; Evert et al. 1977, 1996a). The large petals and the other floral parts clearly function as sinks and import food and water. We hypothesize that movement of nutrients from bundle sheath cells to epidermal and/or mesophyll cells is symplastic via intercellular connections involving the cell lobes.

Cellular structure of the bundle sheath varies within and among organs. In general, sheath cells are elongate and appear circular when viewed in cross section. Bundle-sheath cells are highly cytoplasmic (in contrast to the surrounding mesophyll cells) and often contain large starch grains, especially in the proximal region of a given organ. In the parallel, elongate vascular bundles of leaves (except for the mid-vein), the bundle sheath has fibrous cells with more or less thickened (sclerified), lignified secondary walls where the sheath adjoins the phloem of the bundle (Fig. 21). Lignification of the walls of these cells is indicated by the blue-green hue imparted by TBO staining (O'Brien et al. 1964). In some instances, it appears as if the sheath is double at the fiber site, with the inner row consisting of fibers and the outer row consisting of either parenchyma cells or fibers (Figs. 21, 22). The remainder of the sheath's circumference consists of parenchyma cells. In vascular bundles from smaller diameter veins, the sclerified cells are still present but with thinner secondary walls. Commissural bundles at right angles to and connecting with the elongate vascular bundles possess a single-layered bundle sheath consisting only of parenchyma cells (Fig. 23). The architecture of the bundle sheath is similar in spathes, where the elongate vascular bundles have a sheath that has sclerified cells only opposite the phloem. The bundle sheath of the spathe is uniseriate. Here again, the sheath of commissural bundles is strictly parenchymatous. Tomlinson (1969) notes that for leaves of the Commelinaceae "the sheath (is) often completed either both above and below or only below by thick-walled, collenchymatous or even fibrous cells" and that transverse (commissural) veins are "sheathed by thin-walled elongated cells."

Commelina erecta is a C_3 monocot (Illinois Plant Information Network 2002), and interesting similarities can be found by comparing the anatomy of the bundle sheath of leaf and spathe with the bundle sheath in leaf veins of those C_3 species (grasses) that have been intensively studied. Sheaths of longitudinal veins of C_3 grasses consist of two concentric layers; an outer parenchyma sheath and an inner so-called "mestome" sheath whose walls are

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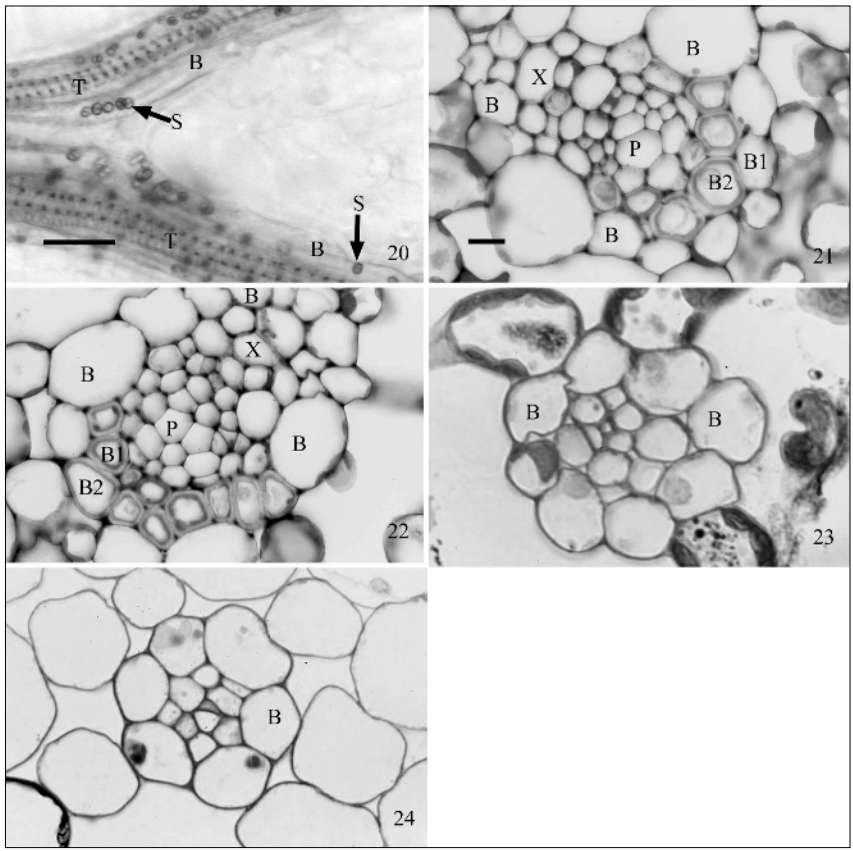
Figures 16–19 (opposite page). Sequential cross sections through the petal beginning near the margin and moving proximally. Initially, only cells of the vein prolongation (VP) are present (16), followed by an organized bundle sheath (B) containing a single sieve element (SE) (17). Further back from the margin, the first tracheary element (T) of the xylem appears within the vascular bundle (18). Extensions from the epidermal cells to the bundle sheath/vein prolongations are indicated by unlabeled arrows in Figs. 16–18. Scale bar for Figures 16–18 = 20 μm. 19) Fluorescence microscopy of branched vein prolongation showing sites of callose deposition. Scale bar = 25 μm.

asymmetrically thickened (O'Brien and Carr 1970). In barley, the thickness of mestome sheath walls is correlated with vein diameter, with increasing thickness from small to medium to large (Evert et al. 1996b). The walls of the mestome sheath cells contain suberin layers (Evert et al. 1996b; O'Brien and Carr 1970), a feature thought to restrict movement of photoassimilates from mesophyll to bundle-sheath cells to a symplastic pathway (Evert et al. 1996b). Experimental evidence in wheat shows that photoassimilate first enters the smaller longitudinal veins where it is passed by transverse (commissural) veins to the large diameter longitudinal veins for export from the leaf (Altus and Canny 1982).



Figures 20–24. 20) Multigrained amyloplasts (S) in bundle sheath (B) of branching vein in large petal. T = tracheary elements. Scale bar = 25 μm. 21) Cross section of longitudinal vein from leaf. The bundle sheath is a double layer (B_1, B_2) only adjacent to the phloem. The inner of these two layers (B_2) is sclerified. B = bundle sheath, P = phloem tissue, X = xylem tissue. Scale bar = 10 μm. 22) Another cross section of a longitudinal leaf vein where both bundle-sheath layers opposite the phloem are sclerified. Scale bar = 10 μm. 23) Commissural bundle of leaf; leaf bundle sheath (B) is entirely parenchymatous. Scale bar = 10 μm. 24) Parenchymatous bundle sheath (B) in vein of small petal. Scale bar = 10 μm.

The thickened bundle-sheath cells of *C. erecta* do not qualify as part of a mestome sheath because they have evenly thickened walls. Also, with few exceptions, thickened cells are exclusively located opposite the phloem. We hypothesize that the thick-walled cells of the vascular bundles function in support. Brückner (1926) also refers to sickle-shaped fiber masses associated with sieve elements in leaves of the Commelinaceae. According to him, these fibers serve for support. Thus, in *C. erecta*, commissural veins would provide little in the way of support because they consist exclusively of parenchyma cells. The support hypothesis is strengthened by the absence of thick-walled sheath cells in the temporary floral organs, which we hypothesize are supported only by turgor pressure. In these cases, the sheath is a more-or-less distinct layer of parenchyma cells (Fig. 24).

Trichomes

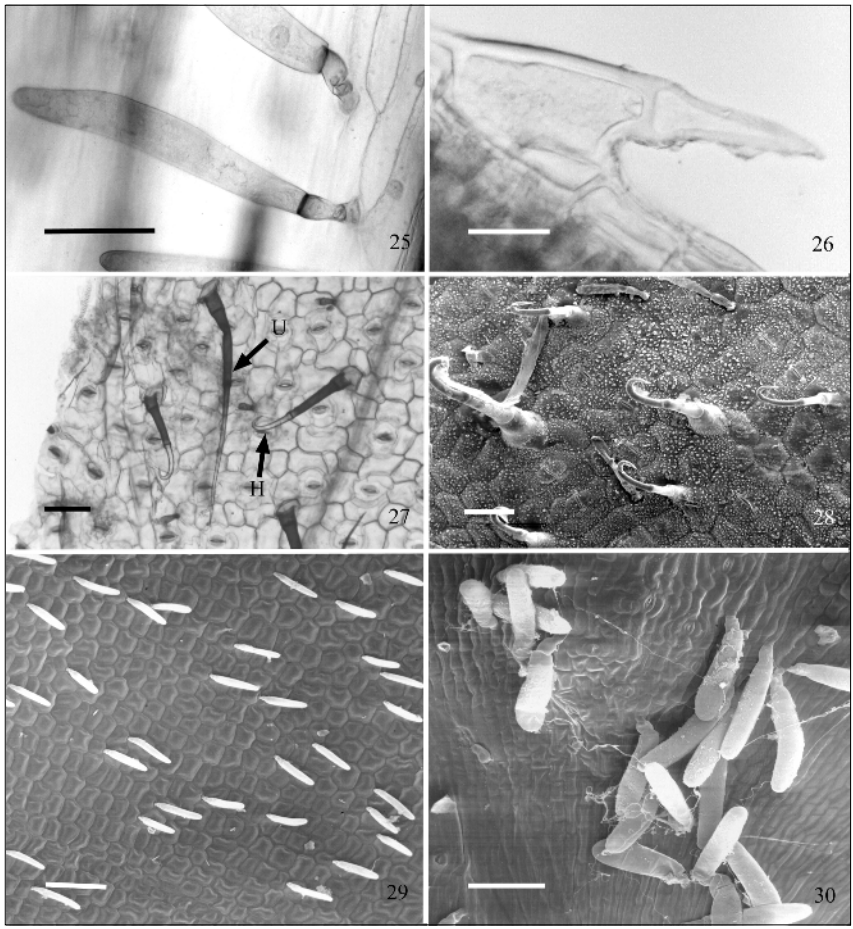
According to Tomlinson (1966, 1969), hairs on vegetative organs of the Commelinaceae can be divided into the following: glandular microhairs (clavate trichomes) (Fig. 25) and macrohairs. In the latter group, the types found on *Commelina* are two-celled prickle hairs (Fig. 26), hook hairs (Fig. 27), and uniseriate hairs of differing numbers of cells (Fig. 27—essentially elongate prickle hairs). EDS analysis of ashed leaves and spathes shows distal cells of hook and uniseriate hairs to be silicified. The response of prickle hairs to polarized light indicates possible silicification of their walls as well.

Two features of trichome distribution in *C. erecta* are worthy of comment (Table 1). The first feature is the large number of hook hairs on the abaxial surface of the spathe and their absence from the adaxial surface (Fig. 28). In contrast, the latter surface consists almost entirely of glandular microhairs (Fig. 29). The physical barrier provided by hook-shaped trichomes to insects is often lethal (q.v. Gilbert 1971 for a classic example), and the protection provided to the enclosed inflorescence makes perfect sense. Glandular microhairs of the adaxial (enclosed) surface of the spathe, in contrast, might be responsible for the mucilaginous substance that occupies the cavity formed by the folded spathe. Three-celled glandular hairs are implicated in mucilage secretion in *Tradescantia zebrina* Heynh. (inchplant) (Commelinaceae) (Thaler et al. 2001, as *Zebrina pendula* Schnizl.).

Table 1. Distribution of trichomes on the surface of various organs. ab = abaxial surface, ad = adaxial surface, and r = rare.

	Microhairs	Prickle hairs	Hook hairs	Uniseriate hairs
Large petal (ad and ab)				
Small petal (ad)				
Small petal (ab)	Y		Y (r)	
Large sepal (ad)	Y cluster		Y (r)	
Large sepal (ab)	Y		Y (r)	
Small sepal (ad)	Y cluster			
Small sepal (ab)	Y cluster			
Spathe (ad)	Y			Y
Spathe (ab)	Y		Y	Y
Leaf blade (ad)	Y	Y		Y
Leaf blade (ab)	Y		Y	Y
Leaf margin		Y		

The second feature of note is the clustering of glandular microhairs at the termination of the main veins of large sepals (adaxial surface) and small sepal (both surfaces) (Fig. 30). In such instances, the nature of the secretory material, if any exists, is unknown, and according to numerous studies (summarized in Fahn 2000), the substance could be one of many different chemical compounds. We would hypothesize, however, that the secreted material is water soluble and is transported in the xylem to the vein ending (in either its final form or as a precursor substance) and transferred to the cells of the trichomes for secretion.



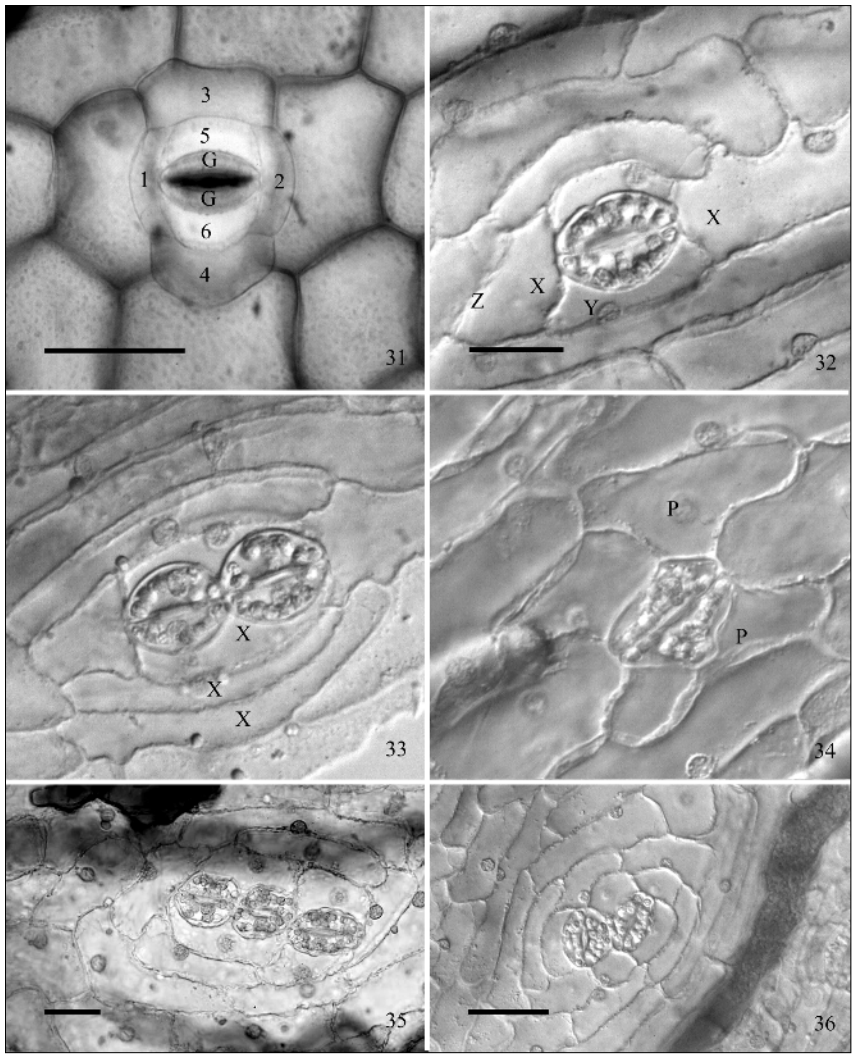
Figures 25–30. Trichomes. 25) Glandular microhairs on abaxial surface of small petal. Scale bar = 50 μm. 26) Two-celled prickle hair on leaf margin. Scale bar = 25 μm. 27) Hook hairs (H) and uniseriate hairs (U) on the abaxial leaf surface. Scale bar =100 μm . 28) Hairs on abaxial (outer) surface of spathe (SEM). Scale bar = 100 μm. 29) Glandular microhairs on adaxial (inner) side of spathe (SEM). Scale bar = 200 μm. 30) Cluster of glandular microhairs on abaxial surface of small sepal. Scale bar = 100 μm.

Stomata

Stebbins and Jain (1960) have given a detailed description of the structure and development of the stomatal apparatus in leaves of *Commelina communis* L. (Asiatic dayflower), and Kaushik (1971) has provided information on the structure of the stomatal apparatus in five other species of *Commelina*. According to Stebbins and Jain (1960), the mature apparatus consists of the two guard cells whose aperture is "oriented parallel to the long axis of the leaf." The guard cells, in turn, are surrounded by six subsidiary cells—a terminal subsidiary cell proximal (basal) to the guard cell pair, one distal (above) to the pair, and a pair of subsidiary cells (laterals) on either flank of the guard cells. The situation is the same for leaves of *C. erecta* (Fig. 31). In contrast, Preston (1898) considers the number of subsidiary cells in *C. nudiflora* (in old literature = *C. diffusa* Burm. f.) or *C. communis* (identity uncertain) to be four, but this observation was based only on cross-sectioned leaves and spathes.

In the present study, the structure of the stomatal apparatus was investigated using both regular bright-field microscopy (Fig. 31) and Nomarski optics (Fig. 32). The latter technique, in particular, enables one to clearly distinguish cell walls and nuclei as well as the numerous, distinct plastids that are a feature of the guard cells.

All laminar organs investigated in this study have stomatal apparatuses, although the frequency, arrangement, and structure of these features vary among organ types, and indeed, vary between adaxial and abaxial epidermal surfaces of a given organ. Stomata are densely distributed over the abaxial (lower) epidermis of the leaf, and while still common, are less densely distributed on the adaxial (upper) epidermal surface, typically in the costal regions. Stomata are densely distributed over the abaxial (outer) surface of the spathe, but, with the exception of a ring of stomata just inside the rim of the spathe's opening (over the marginal vein), are absent from the adaxial (inner surface). Few to no stomata exist on the adaxial surface of large sepals, but they are more common on the abaxial surface. In the latter case, most stomata are clustered over and around the vascular nexus formed by the anastomosis of the three main veins at the distal portion of the organ. A few stomata are also present on the costal region of the three main veins proximal to the nexus. The situation regarding the small sepal is similar to that of the large sepals, with more stomata on abaxial than adaxial surface. For example, counts of two adaxial surfaces gave numbers of only three and seven, whereas numbers of stomata on the corresponding abaxial surfaces were 34 and 30. On the abaxial surface, stomata are clustered over and around the vein nexus, with decreasing numbers located over the three main veins proximal to said nexus. The small petal, in contrast to the other organs, possesses a higher frequency of stomata on the adaxial surface (in a band from base to tip), whereas the abaxial epidermis has very few. No stomata have been found on the abaxial surface of the large petal, but a few scattered ones are present on the adaxial surface. Perhaps the near absence of stomata



Figures 31–36. Stomata. Figs. 31, 35 = bright-field; Figs. 32–34, 36 = Nomarski. 31) Typical stomatal apparatus on the upper leaf surface. Numbers 1 and 2 indicate terminal subsidiary cells and 3 thru 6 represent pairs of flanking subsidiary cells. G = guard cells. Scale bar = 50 μm. 32) Stomatal apparatus (on the abaxial surface of a small sepal) missing both terminal subsidiary cells (at X) and one lateral subsidiary cell (at Y). The partition at Z is just a fold in the tissue and not a cell wall. Scale bar = 25 μm. 33) A doublet stomatal apparatus on the abaxial surface of a small sepal. The Xs indicate three lateral subsidiary cells on one flank of the doublet. Scale bar = 25 μm. 34) The adaxial surface of a small sepal with a guard cell pair, but no subsidiary cells. The pavement cells flanking the guard cells are labeled with Ps. Scale bar = 25 μm. 35) A triplet stomatal apparatus on the abaxial side of a large sepal. Scale bar = 25 μm. 36) A doublet on the abaxial surface of a large sepal where the guard cell pairs point in different directions. Scale bar = 25 μm.

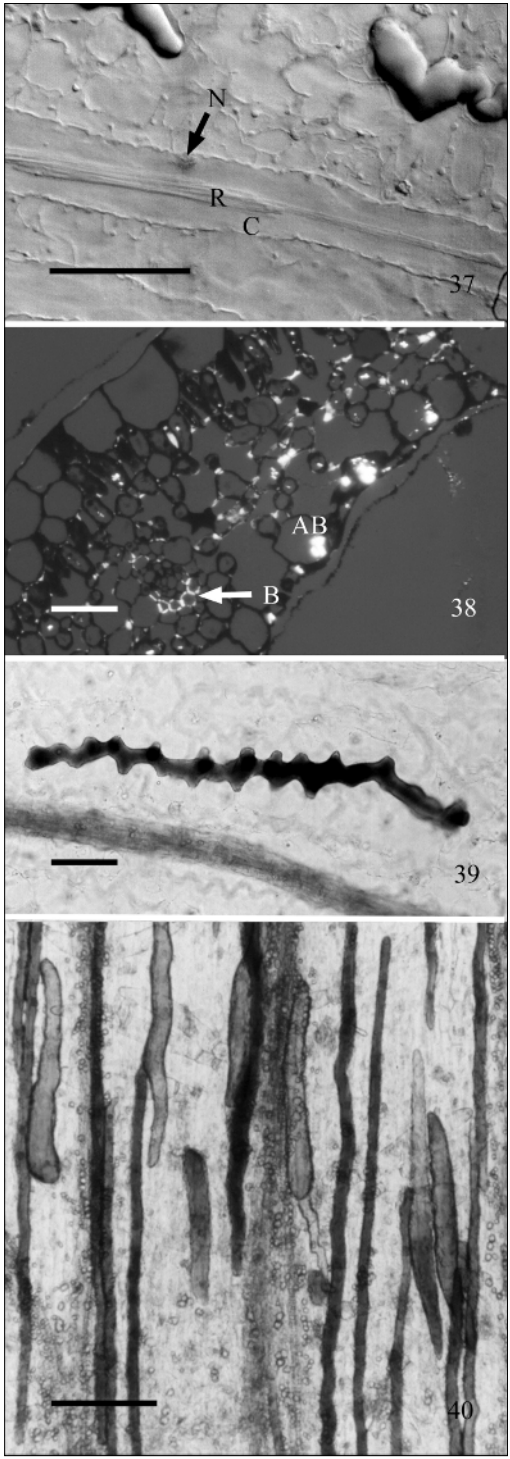
on the large petals is associated with the absence of a photosynthetic mesophyll as well as the extreme sensitivity of these organs to water loss.

Stebbins and Jain (1960) show for foliar stomata of *C. communis* that the first set of lateral subsidiaries form by asymmetric divisions of neighboring cells that border the guard cell mother cell. These divisions are followed by formation of the terminal subsidiary cells, and by asymmetric divisions of neighboring epidermal cells. Finally, the second set of lateral subsidiary cells arises from divisions of the first-formed lateral subsidiary cells at about the time the guard mother cell divides to form two guard cells. The end result is a stomatal apparatus consisting of a guard cell pair surrounded by six subsidiary cells.

The basic architecture (Fig. 31) of the stomatal apparatus is consistent on both leaf and spathe surfaces, with few exceptions (notably on the adaxial leaf surface). In the floral organs, however, stomatal abnormalities are frequent in number and diverse in nature. On the abaxial surfaces of large and small sepals, there are many cases where one or both terminal subsidiary cells are absent (Fig. 32), where a lateral subsidiary cell does not divide (Fig. 32), or where there are extra lateral subsidiaries (Fig. 33). The stomatal apparatuses of both petal types and of the adaxial surfaces of large and small sepals lack subsidiary cells entirely. In such instances, the regular epidermal cells (pavement cells) bordering the lateral sides of the guard cells assume the shape of butterfly wings (Fig. 34) or have no particular shape.

Another modification of the basic stomatal architecture is the manufacture of doublets (Fig. 33) or even triplets (Fig. 35) where more than one pair of guard cells are in direct contact with one another. The guard cell pairs involved can all have their long axes parallel to the long axis of the organ (Figs. 33, 35) or one guard cell pair can form its longitudinal axis at a slight angle to the organ's longitudinal axis (Fig. 36). Where subsidiary cells are involved with doublets, all guard cell pairs can share the same lateral subsidiary cells (Fig. 33) or have separate ones (Fig. 36). Doublets and triplets are not uncommon on the surfaces of floral organs. For example, on the abaxial surface of one large sepal, the guard cell pairs were grouped in the following arrangements: 25 singlets, five doublets, and one triplet.

Modifications of the architecture of the stomatal apparatus are not restricted to *C. erecta* in the Commelinaceae. Kaushik (1971) frequently observed abnormalities of the stomatal apparatus in leaves of five *Commelina* species, including missing or extra subsidiary cells and contiguous stomata. Stebbins and Jain (1960) found complexes in the leaf epidermis of *C. communis* where a terminal subsidiary cell was absent. Drawert (1941) found many aberrant examples in leaf sheaths of *Tradescantia virginiana* L. (as *T. virginica* (sic), Virginia spiderwort). Normally, the stomatal apparatuses in this species possess four subsidiary cells, but examples with only two lateral or two lateral plus one terminal were observed. Often the number of subsidiary cells was determined by the number of pavement epidermal cells adjacent to the guard cell initial



Figures 37–40. 37) Raphide canal (C) found in a large sepal using Nomarski optics. N = nucleus, and R = raphides. Scale bar = 25 μm. 38) Location of crystals (light spots) in a leaf cross section as visualized using polarizing microscopy. Note the occurrence of large crystals in the lower epidermis (AB). The sclerified walls of the bundle sheath cells are evident (B). Scale bar = 100 μm. 39) A tannin idioblast in a large petal. Scale bar = 25 μm. 40) Tannin idioblasts in a small petal. Scale bar = 100 μm.

(guard cell mother cell). Other aberrations are mentioned as well. Both Drawert (1941) and Stebbins and Jain (1960) comment on the migration of nuclei of the adjacent epidermal cells toward the common wall with the guard cell initial. Drawert speaks of this process in reference to the diffusion of division hormones emanating from the guard cell initial. Stebbins and Jain show nuclear migration to be a step in the polarization of the cytoplasm, ultimately leading to the asymmetric division of an epidermal cell into a small subsidiary cell and a larger (pavement) epidermal cell.

In a recent review article on stomatal development in *Arabidopsis thaliana* (L.) Heynhold (mouse-ear cress, Brassicaceae), Nadeau and Sack (2003) stress the importance of "intercellular signaling rather than mitosis allocated factors" in controlling division orientation in guard mother cells. They also discuss a mutant (too many mouths [tmm]) in which many guard cell pairs develop in contact with one another. Unfortunately, since *Arabidopsis* produces no subsidiary cells and is a dicotyledon, we do not know how applicable it would be to the study of *C. erecta*. Nevertheless, some form of communication involving signals as well as membrane receptors would be expected in *C. erecta*.

Why the architecture of the stomatal apparatus in *C. erecta* exists in such diversity in the laminar floral organs is unknown. Neither is the functional status of the guard cells known, although examples of open stomata have been observed in living specimens of small petals. Perhaps the transient nature of these organs releases them from the normal constraints on stomatal structure and function. Developmental and molecular studies of these structures can shed further light on these questions.

Other anatomical features

Clusters of calcium oxalate crystals are known to occur in elongate cells arranged in series in leaves and stems of the Commelinaceae (Brückner 1926, Tomlinson 1969). Such raphide-canals occur not only in the vegetative leaves of *C. erecta*, but also in all organs examined in this study (Table 2, Fig. 37).

Both Brückner (1926) and Tomlinson (1969) refer to the presence of crystals elsewhere in leaf cross sections. Brückner noted in *C. communis* massive crystals of varying shape in the lower epidermis of leaves.

Table 2. The distribution of some of the common anatomical features in the laminar organs of *C. erecta*. The pigment of the large petal is blue and is confined to the vacuoles of the epidermal cells, whereas that of the other organs is chlorophyll present in the chloroplasts of the mesophyll. Y = present, S = slight pigment, and N = not present.

	Pigment	Stomata	Raphides	Other crystals	Tannin tubes
Large petal	Y	Y	Y		Y
Small petal	N	Y	Y		Y
Large sepal	S	Y	Y	Y	Y
Small sepal	S	Y	Y	Y	Y
Spathe	Y	Y	Y	Y	Y
Leaf	Y	Y	Y	Y	Y

Tomlinson noted that "large rhombohedral crystals are otherwise common, especially in the hypodermis and epidermis of the lamina." This information is true also for *C. erecta* leaves, where polarizing microscopy indicates crystal size and distribution (Fig. 38). The relatively large size of crystals in the lower epidermis is quite obvious. However, crystals are absent from the guard cells and inner lateral subsidiary cells. The large crystals tend to be elongate, sometimes lozenge-shaped. X-ray analysis of ashed material indicates all crystals to consist of calcium salts. Very small crystals (crystal sand and small prisms) can be located in spathes and sepals with some difficulty. Identification is made easier by using polarizing microscopy on ashed spathes, where the mineral content is concentrated. No crystals (other than raphides) have been observed with certainty in the petals, but none of this material was ashed.

Tomlinson (1969) mentions the presence of tannin in palisade tissue of leaves in the Commelinaceae. Indeed, tannin idioblasts are common in the mesophyll of all organs investigated in the present study. When stained with a mixture of glacial acetic acid, formalin, and $FeSO_4$, these cells developed a blue precipitate indicative of tannins (Ruzin 1999). However, these same cells also take up other stains such as safranin, and thus might contain a mixture of ergastic materials. The shape of the idioblasts varies from that of a mesophyll cell (typical of the large petal; Fig. 39), to elongate tubes (e.g., the small petal in Fig. 40) to even small spheres (as found in vegetative leaves). Some organs can contain tannin idioblasts of more than one shape.

In conclusion, although similarities exist, the floral organs are of a simpler structure than the leaves and, to a lesser extent, the spathes. Lax developmental constraints or abbreviated ontogenetic sequences in petals and sepals appear responsible for the numerous stomatal aberrations as well as for vein prolongations (in the large petals) and absence of sclerified sheath cells. These features in turn are correlated with the transient nature of the reproductive organs. It would be interesting to search for similar aberrations in other genera of the Commelinaceae as well as to investigate the ontogeny of said structures during petal and sepal development.

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Literature Cited

Altus, D.P., and M.J. Canny. 1982. Loading of assimilates in wheat leaves. I. The specialization of vein types for separate activities. Australian Journal of Plant Physiology 9:571–581.

Brashier, C.K. 1966. A revision of *Commelina* (Plum.) L. in the USA. Bulletin of the Torrey Botanical Club 93:1–19.

Brückner, G. 1926. Beiträge zur anatomie, morphologie, und systematik der Commelinaceae. Beiblattzu den Botanischen Jahrbüchern 61:1–70.

Currier, H.B. 1957. Callose substance in plant cells. American Journal of Botany 44:478–488.

Ding, B., M.V. Parthasarathy, K. Niklas, and R. Turgeon. 1988. A morphometric analysis of the phloem-unloading pathway in developing tobacco leaves. Planta 176:307–318.

Drawert, H. 1941. Beobacktungen an den spaltöffnungen und den blatthaaren von *Tradescantia virginica* L. Flora 135:302–318.

Esau, K. 1977. Anatomy of Seed Plants. John Wiley and Sons, Inc., New York, NY. 550 pp.

Evert, R.F., W. Eschrich, and W. Heyser. 1977. Distribution and structure of the plasmodesmata in mesophyll and bundle-sheath cells of *Zea mays* L. Planta 136:77–89.

Evert, R.F., W.A. Russin, and A.M. Bosabalidis. 1996a. Anatomical and ultrastructural changes associated with sink-to-source transition in developing maize leaves. International Journal of Plant Sciences 157:247–261.

Evert, R.F., W.A. Russin, C.E.J. Botha. 1996b. Distribution and frequency of plasmodesmata in relation to photoassimilate pathways and phloem loading in the barley leaf. Planta 198:572–579.

Faden, R.B. 1993. The misconstrued and rare species of *Commelina* (Commelinaceae) in the Eastern United States. Annals of the Missouri Botanical Garden 80:208–218.

Faden, R.B. 2000. Commelinaceae R. Brown, Spiderwort Family. Pp. 170–197, *In* Flora of North America Editorial Committee. Flora of North America. Vol. 22. Oxford University Press, New York, NY. 352 pp.

Fahn, A. 2000. Structure and function of secretory cells. Advances in Botanical Research 31:37–75.

Gilbert, L.E. 1971. Butterfly-plant coevolution: Has *Passiflora adenopoda* won the selectional race with Heliconiine butterflies? Science 172:585–586.

Hardy, C.R., and D.W. Stevenson. 2000a. Development of the gametophytes, flower, and floral vasculature in *Cocliostema odoratissimum* (Commelinaceae). Botanical Journal of the Linnean Society 134:131–157.

Hardy, C.R., and D.W. Stevenson. 2000b. Development of the gametophytes, flower, and floral vasculature in *Dichorisandra thyrsiflora* (Commelinaceae). American Journal of Botany 87:1228–1239.

Illinois Plant Information Network. 2002. Illinois Plant Information Network. Available online at http://www.fs.fed.us/ne/delaware/ilpin/834.co. Accessed 2002.

Johansen, D.A. 1940. Plant Microtechnique. McGraw-Hill Book Company, Inc., New York, NY. 523 pp.

Kaushik, J.P. 1971. Studies on the foliar epidermis of Commelinaceae. Proceedings of the Indian Academy of Science, B 73:311–318.

Martin, F.W. 1959. Staining and observing pollen tubes in the style by means of fluorescence. Stain Technology 34:125–128.

McCollum, T.M., J.R. Estes, and J.R. Sullivan. 1984. Reproductive biology of *Commelina erecta* (Commelinaceae). Pp. 57–66, *In* N.V. Horner (Ed.). Festschrift for Walter W. Dalquest in Honor of his Sixty-sixth Birthday. Department of Biology, Midwestern State University, Wichita Falls, TX.

McCrone, W.C., L.B. McCrone, and J.G. Delly. 1978. Polarized Light Microscopy. Ann Arbor Science, Ann Arbor, MI. 251 pp.

Nadeau, J.A., and F.D. Sack. 2003. Stomatal development: Cross talk puts mouths in place. Trends in Plant Science 8:294–299.

O'Brien, T.P., and D.J. Carr. 1970. A suberized layer in the cell walls of the bundle sheath of grasses. Australian Journal of Biological Sciences 23:275–287.

O'Brien, T.P., N. Feder, and M.E. McCully. 1964. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 59:368–373.

Pennell, F.W. 1916. Notes on plants of the southern United States: I. Bulletin of the Torrey Botanical Club 43:93–111.

Postek, M.T., K.S. Howard, A. Johnson, and K.L. McMichael. 1980. Scanning Electron Microscopy. Ladd Industries. Williston, VT. 305 pp.

Preston, G.K. 1898. A species of *Commelina*. American Journal of Pharmacy 70:321–335.

Radford, A.E., J.E. Ahles, and C.R. Bell. 1968. Manual of the Vascular Flora of the Carolinas. The University of North Carolina Press, Chapel Hill, NC. 1183 pp.

Ruzin, S.E. 1999. Plant Microtechnique and Microscopy. Oxford University Press, New York, NY. 322 pp.

Spurr, A.R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructure Research 26:31–45.

Stebbins, G.L., and S.K. Jain. 1960. Developmental studies of cell differentiation in the epidermis of monocotyledons. I. *Allium*, *Rhoeo*, and *Commelina*. Developmental Biology 2:409–426.

Thaler, I., M. Gailhofer, and G. Zellnig. 2001. The ultrastructure of mucilage secreting trichomes of *Zebrina pendula* Schnizl. Phyton 41:227–245.

Tomlinson, P.B. 1966. Anatomical data in the classification of Commelinaceae. Journal of the Linnean Society (Botany) 59:371–395.

Tomlinson, P.B. 1969. Anatomy of the Monocotyledons, III. Commelinales–Zingiberales. Oxford at the Claredon Press, UK. 446 pp.

Weberling, F. 1992. Morphology of Flowers and Inflorescences. Cambridge University Press, Cambride, UK. 405 pp.