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A Comparative Study of the Ontogeny of Macrosclereids and Osteosclereids in the Integument of *Cassia Fasciculata* and *Desmodium Canadense*

PHILIP J. ARNHOLT¹

Abstract. Developmental patterns, developmental sequence, deposition of secondary wall material, and maturation of macrosclereids and osteosclereids were similar in *Desmodium canadense* and *Cassia fasciculata*. Both species apparently lack a well defined strophliolar region. Two distinct differences were observed in the mature seed coat. One is the presence of a single row of macrosclereids in the hilar region of *Cassia fasciculata* and a double row of macrosclereids in the hilar region of *Desmodium canadense*. The second is the appearance of "spicule-like" structures, apparently enveloped laterally by a cellulosic matrix in the cuticularized layer of *Cassia fasciculata*.

Taxonomists differ in their treatment of the legume group. Engler and Prantl (1891), Pool (1929), and Gleason and Cronquist (1963) divide the group into three families. Bentham and Hooker (1862-1867), Rendle (1925), Rehder (1940), and Gray (1950) treat the legumes as a single family composed of three sub-families. The family names Mimosaceae, Caesalpiniaceae, and Fabaceae applied to the legumes by Gleason and Cronquist will be used in this paper.

Corner (1951) and Isely (1955) have made studies of seed characters in the legumes. Both workers recognize two basic seed types within this group: (1) the Mimosaceae—Caesalpiniaceae type and (2) the Fabaceae type.

An anatomical study of the ontogeny of osteosclereids and macrosclereids of the seed coat in *Cassia fasciculata* Michx. and *Desmodium canadense* (L.) DC. members of the Caesalpiniaceae and Fabaceae groups respectively, may yield additional data which would help to show the relationship between these two groups.

In many leguminous species the epidermis of the seed is highly specialized at maturity and consists of elongated cells with thickened walls covered by a well-defined cuticle. These cells have been designated as malpighian cells (Tozzetti, 1855), macrosclereids (Coe and Martin, 1920), and palisade cells (Zimmerman, 1936). Underlying the epidermal layer there is often a hypodermal layer composed of elongate cells with a concave constriction along the radial walls. These cells generally exhibit a uniform secondary wall thickening of cellulose (Pammel, 1899). Terms used to

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describe these are osteosclereids (Pammel), sand glass cells (Pitot, 1935), and hour glass cells (Zimmerman, 1936). The terms macrosclereids and osteosclereids will be used in this paper, for their histological classification is considered more desirable than descriptive references to their appearance.

Macrosclereids are further characterized by fluted and twisted secondary wall thickenings, most pronounced in the outer tangential portions of the cell. In the inner tangential portion of the cell the wall thickenings are less pronounced and the secondary wall surrounding the lumen is rather uniform in thickness. Coe and Martin (1920) cited Rees (1911) as reporting that the macrosclereids of *Melilotus alba* were composed of pectose and hemicellulose compounds. The much discussed "light line" of macrosclereids can generally be observed as a tangential line near the point where the lumen terminates. According to Esau (1960), the "light line" is the result of a high degree of refraction in a restricted region in the epidermal wall. The lumen often extends into the thickened outer radial and tangential walls as minute "pore canals", a term originally applied by Pammel (1899) and subsequently used by other early investigators. These canals are believed to be the result of specialized secondary wall thickenings and are thought to have no relation to the pits of contemporary plant anatomy.

REVIEW OF LITERATURE

Pammel completed an anatomical study of the seed coats of legumes in 1899. He found both macrosclereids and osteosclereids in *Cassia chamaesrista* (now known as *C. fasciculata*), *Cassia nictitans*, and *Cassia marylandica*, although the osteosclereids were poorly differentiated in *C. nictitans*. He also recorded both macrosclereids and osteosclereids in *Desmodium canadense* and *Desmodium nudiflorum*. In *D. strictum* he indicated that macrosclereids were present but did not mention osteosclereids.

Pitot (1935) studied the ontogeny of macrosclereids and osteosclereids in *Dolichus lablab*, *Pisum sativum*, *Anagyris foetida*, *Cicer arietinum*, and *Phaseolus vulgaris*. He concluded that macrosclereids and osteosclereids arose from the external integument and that the internal integument was usually crushed or dissolved. When present, the osteosclereids usually appeared after the macrosclereids.

Zimmerman (1936) studied the strophiole and its relation to the hardness of the seed coat and its permeability to water. He indicated that there was probably some relation between the thickened cuticle and elongate macrosclereids in the strophioliar region but was unable to determine the significance of these variations. The remainder of his paper was devoted to the development of the seed coat in the region of the strophiole.

The ontogeny of the angiosperm ovule as a nucellar primordium, the development of the integuments, and its embryogeny have been dealt with by Hayward (1938) for *Pisum sativum*, the garden pea. The ontogeny of the sclereids in this same species has been investigated by Reeve (1946), and without a doubt his is the most exhaustive and detailed ontological study of the legume integument.

Reeve studied the comparative histogenesis of macrosclereids and osteosclereids of *Pisum sativum*. He found that macrosclereids are derived from a well-defined proteoderm in the young ovule in which early ovule growth is accompanied by rapid anticlinal division and later growth occurs through elongation and enlargement in tangential directions of these cells. He noted that osteosclereids do not appear until the macrosclereids are rather well-defined. His work indicated that both periclinal and anticlinal divisions precede the development of the hypodermal layer. Periclinal divisions cease and the osteosclereids differentiate in a regional pattern, as in the development of the macrosclereids.

Reeve (1946) also studied the structural composition of the sclereids in the integument of *Pisum sativum*. He detected the presence of pentosans in the secondary wall thickenings of macrosclereids and osteosclereids in the integuments of pea and lima bean. Arabinose and xylose were tentatively identified as sugars of the pentosan-cellulose complex of the secondary walls. He stated that galactose, galacturonic acid and possibly arabinose occur as constituents of the middle lamellar pectins. The "light line" of the macrosclereids was considered by him to be a phenomenon of light refraction and caused by a deposition of secondary wall material.

MATERIALS AND METHODS

Individual flowers of plants growing in their natural habitats were tagged with strings and observed daily from July 28, 1965, to August 12, 1965. *Cassia fasciculata* Michx. was found growing near the Charleston Water Pumping Station in Charleston, Illinois, while *Desmodium canadense* (L.) DC. was found growing along the New York Central Railroad tracks about two miles east of Mattoon, Illinois. Measurements of the length and width of the ovaries of twelve plants of each species were taken daily over this period from the time of anthesis. This data was used later in determining the approximate age of ovaries selected for sectioning and is summarized in Table 1. Pods in various stages of development were collected from surrounding plants, killed, fixed, and stored in either Craff I or F.A.A. fixatives. The pods were then measured to determine their approximate age, dehydrated in the tertiary butyl alcohol series as described by Sass (1958), infiltrated, and embed-

ded in tissue mat paraffin. More mature seeds were difficult to infiltrate with paraffin and were dissected out of the pods before infiltration. Sections 10 μ thick were made with a rotary microtome, the cut being perpendicular to both the long axis of the hilum and the long axis of the ovary. In addition, a few sections were cut in a plane parallel to the long axis of the hilum and parallel to the long axis of the ovary. The sections were then fixed to slides with Haupt's adhesive, stained in safranin, and counter-stained in fast green. Illustrations were prepared with the aid of a Zeiss drawing attachment for a microscope.

TABLE 1. Ovary Length in Relation to Days of Development after Anthesis

| Days | <i>Desmodium canadense</i> | <i>Cassia fasciculata</i> |
|------|----------------------------|---------------------------|
| 1 | 6 mm | 11 mm |
| 2 | 6-7 mm | 12-13 mm |
| 3 | 7-9 mm | 14-16 mm |
| 4 | 11-12 mm | 17-19 mm |
| 5 | 12-13 mm | 20-21 mm |
| 6 | 16-18 mm | 22-24 mm |
| 7 | 24-25 mm | 29-31 mm |
| 8 | 29-31 mm | 34-36 mm |
| 9 | 31-33 mm | 39-41 mm |
| 10 | 34-36 mm | 44-46 mm |
| 11 | 34-36 mm | 48-52 mm |
| 12 | 34-36 mm | 52-56 mm |
| 13 | 34-36 mm | 52-56 mm |
| 14 | 34-36 mm | 52-56 mm |
| 15 | 34-36 mm | 52-56 mm |

OBSERVATIONS

A. *Desmodium canadense* (L.) DC.

The first stage of development to be studied intensively was immediately following anthesis. At this stage the exposed ovary is approximately 5 mm long in *Desmodium canadense*. Fig. 1 shows a median longitudinal section of an ovule cut perpendicular to the long axis of the hilum and through the funiculus. The epidermal layer of the outer integument appears distinct from the underlying cells. These epidermal cells are clearly meristematic, based upon

the evidence of anticlinal divisions. Both anticlinal and periclinal cell divisions are present in the hypodermis and underlying layers. The cells of these regions are undifferentiated. Likewise, at this stage there is no evidence of cell differentiation in either the epidermis or hypodermis of the hilar region. For convenience, areas of the integument not related to the hilar or micropylar regions will be referred to as "lateral sides." With the possible exception of periclinal division in the hilar region, the epidermal layer in *D. canadense* seems to remain as a discreet layer of cells throughout the stages of development as a result of anticlinal cell division only.

Five days after anthesis in *D. canadense* the ovary is approximately 18 mm. long. There is distinct differentiation in the area of the hilum (Fig. 2) where a double row of radially elongate macrosclereids is evident, suggesting that perhaps periclinal division of the epidermal cells has taken place. Likewise, the epidermal cells in the area of the micropyle have become differentiated from the underlying layers because of their pronounced radial elongation. At this stage, however, very little secondary wall material has been deposited in the epidermal cells of either the hilar or micropylar regions and there is evidence of continued anticlinal cell division. Elongation of the epidermal cells of the lateral sides is considerably less pronounced than in the areas of the hilum and the micropyle (Fig. 3). At this time the cells of the hypodermal and underlying layers are characterized by a slight tangential elongation, but without noticeable change in thickness of the cell wall.

Increase in size of the ovary ceases ten days after anthesis, but enlargement of the ovule continues. A progressive development of the epidermal cells into macrosclereids continues from the regions of the hilum and micropyle towards the lateral sides and thin well-defined cuticle becomes apparent (Fig. 4). In the more mature macrosclereids deposition of secondary wall material is pronounced in the outer tangential wall and the outer portions of the radial walls, causing the lumen to become "flame-like" in appearance. These are apparently cellulose thickenings, for they have a strong affinity to fast green.

PLATE 1.

Desmodium canadense (L.) DC.

All illustrations of ovules are from median longitudinal sections cut perpendicular to the long axis of the hilum and through the funiculus.

- Fig. 1. Cross section of an ovule and ovary at anthesis. The epidermal layer of the ovule (e) is distinct from the underlying tissue. X159.
- Fig. 2. Ovule about 5 days after anthesis showing differentiation in the hilar region. X159.
- Fig. 3. Ovule about 5 days after anthesis showing development along the lateral side. X254.
- Fig. 4. Ovule about 9-10 days after anthesis. Radial elongation of the

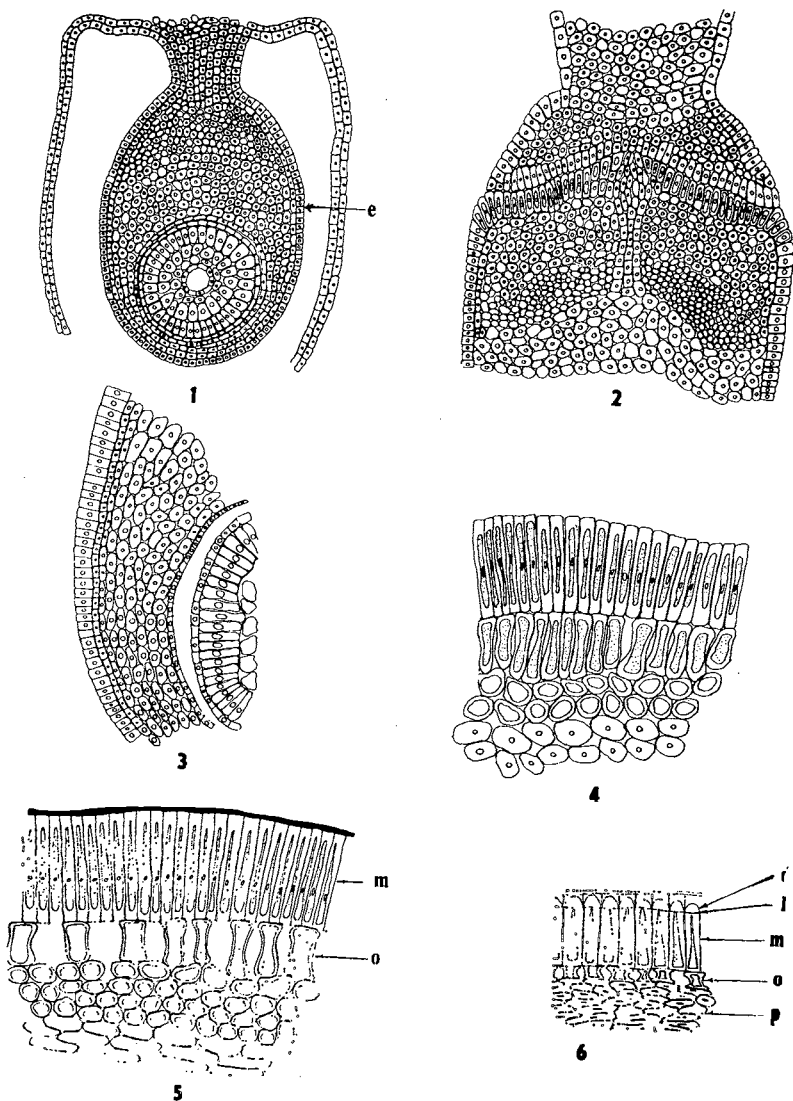


Fig. 5. Ovule about 11-12 days after anthesis showing further development of macroscleireids (m) and osteosclereids (o). At this time the intercellular spaces are well developed in the osteosclereid layer.

Fig. 6. Mature seed coat of the ovule collected about 3 weeks after anthesis showing macroscleireids (m), light line (l), domes (d), osteosclereids (o), and thick-walled parenchyma (p).

At this time the cells of the hypodermal layer begin to assume the characteristics of osteosclereids through the deposition of secondary wall material. These thickenings also show a strong affinity for fast green and are probably of cellulose. The deposition of secondary wall material is accompanied by an elongation and constriction of the radial walls, resulting in large intercellular spaces (Figs. 4 and 5). Secondary wall thickenings are also apparent in the layer of parenchyma underlying the osteosclereids, but these parenchyma cells retain their isodiametric form. Differentiation of osteosclereids occurs first around the micropylar and hilar regions and progresses towards the "lateral sides" in a pattern, as in macrosclereid development.

In the mature seed coat the osteosclereids and macrosclereids are characterized by a decrease in length without an appreciable change in diameter. This is caused by increased crystallinity or a change in cellulosic orientation within the cell walls, according to Reeve (1946). The outer tangential portion of the mature macrosclereid is capped by two non-cellular layers: (1) an outer thin layer, the cuticle, and (2) an underlying thick layer known as the cuticularized layer. Designations for these layers were first given by Pammel (1899) and were followed by subsequent investigators. A thin light line separates the constricted tip of the lumen from the dome shaped cap of the macrosclereid (Fig. 6). At this point, the osteosclereids have acquired their familiar hour-glass shape with thickened secondary walls. These walls are constricted along the radial portions, producing large intercellular spaces. The parenchyma cells of the underlying layer have elongated tangentially and have thickened secondary walls, which cause the cell lumen to appear as a thin line.

B. *Cassia fasciculata* Michx.

At the time of abscission of the corolla, the *Cassia fasciculata*, ovary is about 11 mm. in length. Figure 7 shows a median longitudinal section of the ovule cut perpendicular to the long axis of the hilum and through the funiculus. The cells of the epidermis form a layer distinct in appearance from the underlying cells. These epidermal cells seem to be meristematic on the evidence of anticlinal cell divisions. The cells of both the epidermis and hypodermis are nearly isodiametric in shape (Fig. 7). At this stage of development the hilar region is not clearly defined. As in *Desmodium canadense*, there is evidence of only anticlinal cell division in the epidermal layer, while both anticlinal and periclinal divisions occur in the underlying tissue.

In the next stage, eight to ten days after anthesis, the ovary is 34-45 mm in length (Figs. 8, 9, and 10) and the differentiating macrosclereids in the region of the hilum and micropyle show considerable radial elongation. In contrast, the cells of the lateral sides that will differentiate into macrosclereids show slight radial elongation and evidence of many anticlinal cell divisions. Deposition of cellulosic secondary wall material is evident in the areas of the hilum and micropyle only, as these areas show an increased affinity for fast green. At this same stage of development, the cells of the hypodermal layer show some tangential elongation and evidence of both anticlinal and periclinal divisions. The macrosclereids of the hilar region are interrupted by a bundle of vascular tissue, the ending of which is referred to by Tschirch and Oesterle (1893-1897) as a "tracheid island", a group of cells of unknown function (Fig. 8).

Although sections were made of many later stages of ovary maturation in *C. fasciculata*, intermediate steps in differentiation of the cells of the hypodermis into osteosclereids were not found. The cells of the hypodermal layer become distinct as a layer of tangentially elongate cells approximately six to ten days after anthesis, but no evidence of early stages in the radial elongation or the deposition of secondary wall thickening of these cells was observed. The cells of the hypodermal layer probably differentiate rapidly sometime after cessation of ovary growth.

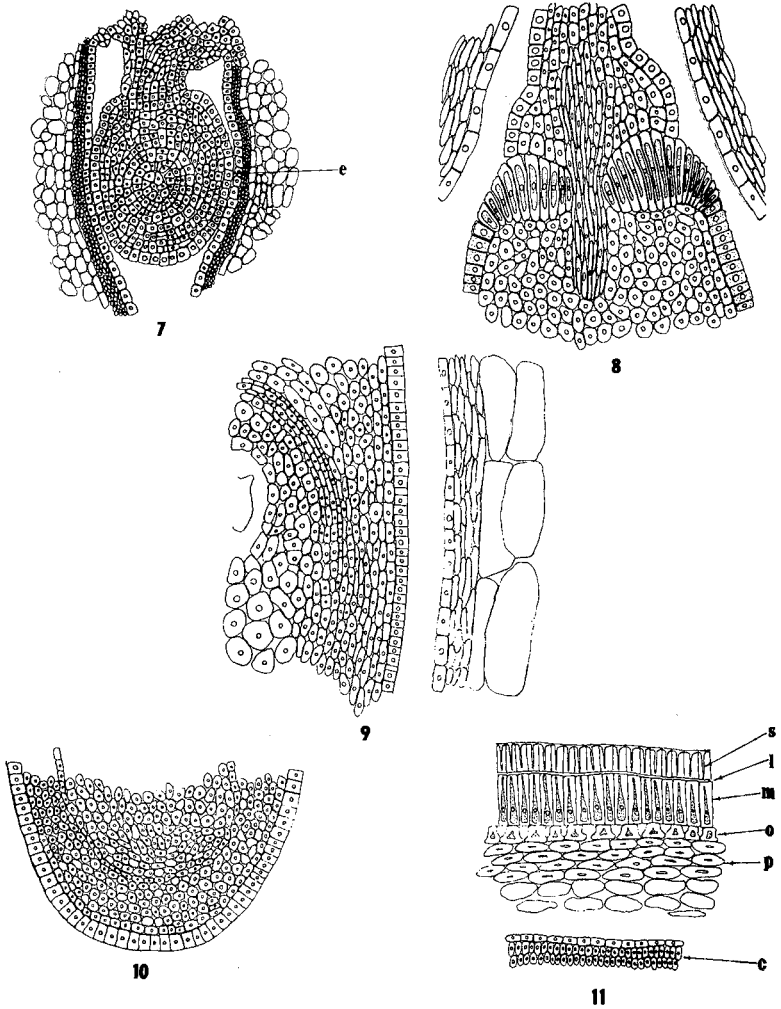
In the mature seed coat of *C. fasciculata*, both macrosclereids and osteosclereids form a well-defined uniform layer around the ovule. There is an underlying layer of thick-walled parenchyma and evidence of partially crushed endosperm. The macrosclereids are elongate with prominent secondary wall thickenings. The deposition of secondary wall material is more pronounced in the outer tangential wall and the outer portions of the radial walls, thus causing the "flame-like" appearance of the lumen. The light line is apparent just above the point where the lumen terminates. Above the light line is a complex layer which appears to be the result of differentiation of the distal portion of the macrosclereids. This layer is composed of "spicule-like" bodies thought to be composed of cutin because they retain safranin. The spicules are enveloped laterally by material of probable cellulosic composition, since it loses safranin readily in the destaining process and has a strong

PLATE 2.

Cassia fasciculata Michx.

All illustrations of ovules are from median longitudinal sections cut perpendicular to the long axis of the hilum and through the funiculus.

Fig. 7. Cross section of the ovary and ovule at anthesis. The epidermal layer of the ovule (e) is distinct from the underlying tissue. X159.



- Fig. 8. Ovule 9 days after anthesis showing development of macrosclereids in the hilar region and the interruption of the macrosclereid layer by vascular tissue. X254.
- Fig. 9. Ovule 9 days after anthesis showing differentiation of the epidermis along the lateral side. X254.
- Fig. 10. Ovule 9 days after anthesis showing evidence of anticlinal cell division along the lateral side in an area most distal to the hilum. X254.
- Fig. 11. Mature seed coat of an ovule collected about 3 weeks after anthesis showing macrosclereids (m), spicules (s), light line (l), osteosclereids (o), underlying thick-walled parenchyma (p), and part of Cotyledon (c). X254.

affinity for fast green. In sectioning, this layer separates very easily from the underlying macrosclereids. The osteosclereids form a well-defined hypodermal layer. The radial walls of the osteosclereids exhibit a slight constriction, while the inner tangential wall shows a prominent lateral expansion and the outer tangential wall remains narrow, thus resulting in a tapered osteosclereid very distinctive in form (Fig. 11).

DISCUSSION AND CONCLUSION

Comparison of early stages of seed coat development of *Cassia fasciculata* Michx. and *Desmodium canadense* (L.) DC. reveals very little structural difference. In both species the epidermal layer is distinguished by the presence of anticlinal divisions only. The hypodermal layer shows evidence of both anticlinal and periclinal cell division and is difficult to distinguish from the underlying layers of cells.

Development of osteosclereids and macrosclereids, in both species, is initiated in the areas of the hilum and micropyle, this differentiation progressing towards the lateral sides. Both macrosclereid and osteosclereid development is apparently initiated three to five days earlier in *D. canadense* than in *C. fasciculata*.

As maturation progresses, three structural differences become apparent. The first is the development of a single row of macrosclereids in the hilar region of *C. fasciculata*, and a double row of macrosclereids in the hilar region of *D. canadense*. The second structural difference is found in the composition of the cuticularized layer. This layer is very pronounced in the *Cassia* material and has a highly complex cellulosic matrix. Reeve (1946) referred to this layer as being composed of a combination of the old primary cell wall and cutin, while Pammel (1899) refers to this as a cuticularized layer of undetermined origin. The cuticle of *D. canadense* lacks the thickness and the apparent "spicule-like" complex found in the macrosclereid layer of *Cassia*. The third structural difference is the form of the osteosclereids of the species of *Cassia* and *Desmodium* studied. In *D. canadense* the mature osteosclereids exhibit the characteristic hour glass shape. Osteosclereids in *C. fasciculata* have inner tangential walls which are longer than the outer tangential walls, the radial walls showing a slight constriction and tapering towards the outer tangential wall.

With few exceptions, the development of macrosclereids and osteosclereids follows a pattern similar to that reported by earlier workers for other leguminous species. These similarities were found in the types of cell division which occur in the epidermal and hypodermal layers of the outer integument, regional development of

both macrosclereids and osteosclereids, presence of a "flame-like" lumen in macrosclereids, and an outer thickened cuticularized layer topped with a thin cuticle.

This study points to two striking features evident in *Cassia fasciculata*, one of which has not been observed in earlier studies on leguminous species. This feature is the presence of the "spicule-like" bodies, very probably made up of cutin, in the cuticularized layer. The second feature is a single row of macrosclereids in the hilar region which has been reported previously in species of Caesalpiniaceae by Corner (1951). Another feature revealed in this study is the strophliolar region as described by Zimmerman (1936) in *Melilotus albus*, *Lupinus cruikshanksii*, *Lathyrus latifolius*, and *Pisum sativum*. This area is apparently not well-defined in either *C. fasciculata* or *D. canadense* as indicated by longitudinal sections. Development of the sclereids in this area was comparable to, but not in excess of, the development of sclereids in the hilar and micropylar areas. The apparent absence of a strophliolar region in the material of *C. fasciculata* and *D. canadense* studied raises a question as to the importance of this structure in the delineation of the subfamilies of the legume group.

Most of the ontological studies of macrosclereids and osteosclereids have been done in the Fabaceae, because of many economic species. Ontological studies of macrosclereids and osteosclereids in the Caesalpiniaceae, as well as in the Mimosaceae, have not been previously made. This study has pointed out significant structural differences in the hilar region and cuticularized layer of *Cassia fasciculata* as compared to *Desmodium canadense* and other species of the Leguminosae that have been investigated. Although close phylogenetic relationship is implied by the similarity of the gross development of the seed coat, developmental studies of the testa of other genera of the Caesalpiniaceae, Mimosaceae, and Fabaceae are certainly needed in order to determine the potential taxonomic significance of: (1) the single row of macrosclereids in the hilar region (2) the strophiole, and (3) the "spicule-like" bodies in the cuticularized layer of *Cassia fasciculata*.

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