1967

A Comparative Study of the Ontogeny of Macrosclereids and Osteosclereids in the Integument of Cassia fasciculata and Desmodium canadense

Philip John Arnholt
Eastern Illinois University

PAPER CERTIFICATE #3

To: Graduate Degree Candidates who have written formal theses.

Subject: Permission to reproduce theses.

The University Library is receiving a number of requests from other institutions asking permission to reproduce dissertations for inclusion in their library holdings. Although no copyright laws are involved, we feel that professional courtesy demands that permission be obtained from the author before we allow theses to be copied.

Please sign one of the following statements.

Booth Library of Eastern Illinois University has my permission to lend my thesis to a reputable college or university for the purpose of copying it for inclusion in that institution's library or research holdings.

August 4, 1967
Date

I respectfully request Booth Library of Eastern Illinois University not allow my thesis be reproduced because _____

_____

_____

_____ _____
Date Author

A COMPARATIVE STUDY OF THE ONTOGENY OF MACROSCLEREIDS AND OSTEOSCLEREIDS IN THE INTEGUMENT OF CASSIA FASCICULATA AND DESMODIUM CANADENSE

(TITLE)

BY

PHILIP JOHN ARNHOLT

B.S. in Ed., Eastern Illinois University, 1963

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

MASTER OF SCIENCE IN EDUCATION

IN THE GRADUATE SCHOOL, EASTERN ILLINOIS UNIVERSITY
CHARLESTON, ILLINOIS

1967
YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

august 1, 1967
DATE

August 1, 1967
DATE

TABLE OF CONTENTS

ACKNOWLEDGMENT

The author wishes to express sincere thanks to Dr. Wesley Whiteside, under whose supervision this investigation was performed, for guidance in selection of the topic, and for guidance and criticisms in the preparation of this paper.

The writer also wishes to express thanks to his wife for assistance in typing and preparation of materials, and to Pastor C. Knauft of Milford, Illinois, for assistance in translation of articles written in German.

I. INTRODUCTION

Taxonomists disagree as to the status of the legume group. Engler and Prantl (1891), in their Naturlichen Pflanzen Familien, divide the legume group into three families, Caesalpineaceae, Mimosaceae, and Papilionaceae. Pool (1929), also divided the legume group into three families. However, Bentham and Hooker (1862-1867), in their Genera Plantarum, and Rendle (1925), in his Classification of the Flowering Plants, place the legumes in the family Leguminosae and divide them into the three subfamilies Papilionaceae, Caesalpinieae, and Mimoseae. This discrepancy exists in more recent literature, as Rehder (1940) and Gray (1950) treat the group as one family with three subfamilies, while Gleason and Cronquist (1963) place the legumes into the three separate families Mimosaceae, Caesalpiniaceae, and Fabaceae. The names applied to these groups by Gleason and Cronquist will be used in this paper.

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

Sclereids are cells with thick secondary walls, simple pits, and a protoplast that is generally short-lived. They are widely distributed in the plant body and are found in leaves, stems, roots, and fruits. Sclereids vary much in shape and may occur in clusters, layers, or as isolated cells. The cells of the integuments of an ovule generally differentiate as sclereids, resulting in a mature seed coat of one or more layers of thick-walled sclereids.

In many leguminous species the epidermis of the seed is highly specialized at maturity into elongated cells with thickened walls and 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). Osteosclereids (Pammel), sand glass cells (Pitot, 1935), and hour glass cells (Zimmerman, 1936) are terms which have been used to describe these cells. 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 which are 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. According to Coe and Martin (1920), Rees (1911) reports that the macrosclereids

of Melilotus alba are 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.

Macrosclereids form a tough outer seed coat and are thought to protect the seed from variations in moisture. The presence of these highly specialized cells is thought to be a factor in the ability of many legume seeds to retain their viability for long periods of time. The seeds of some legumes having testa of macrosclereids are disseminated primarily by either ocean or fresh water currents. Other species are disseminated largely through the digestive tracts of herbivorous animals. The fact that such seeds retain their viability is attributed to the impermeability to both water and digestive enzymes of the macrosclereids of the testa. According to Mattirolo (1899), water content of the seed is partly controlled by the presence of macrosclereids. Water may enter the seed via the micropyle and cause the testa to expand. This in turn causes an enlargement of the pore canals of the macrosclereids and water passes through the region of the light line and into the underlying layers by

capillary action. When transpiration occurs, there is little water to repair the loss and the pore canals close. This prevents any further water loss from the seed.

Later investigators (Cavasas, 1959; Coe and Martin, 1920), maintain that the light line region of the macrosclereids is highly impermeable. Experiments with dyes by Coe and Martin (1920) indicated that the light line region presents an effective barrier to their passage.

The hilar region seems to act as a hygroscopic valve, according to Hyde (1954). A fissure occurs along the groove of the hilum which opens when the seed is surrounded by dry air and closes when the seed is surrounded by moist air. Thus the moisture content of the seed is controlled and the seed retains its viability over long periods.

II. 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 that both macrosclereids and osteosclereids occurred in Desmodium canadense and Desmodium nudiflorum. In D. strictum he indicated that macrosclereids were present but did not mention the presence of osteosclereids.

Coe and Martin (1920) studied the relationship between the structure and chemical nature of the seed coat and seed coat permeability to water. They reported the presence of both macrosclereids and osteosclereids in Melilotus alba and M. officinalis. They concluded that permeability to water depends upon the secondary wall thickening of the macrosclereids rather than the chemical nature of the cells of the seed coat.

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) identified three types of macrosclereids, one with a flattened outer tangential wall characterized by (Phaseolus vulgaris), one with a rounded outer tangential wall characterized by (Lupinus luteus), and one with a pointed outer tangential wall characterized by (Trigonella foenum graecum). He concluded that macrosclereids with a pointed outer tangential wall usually result in hard seed coats while the other varieties produce soft seed coats. All three types had similar secondary wall thickenings and possessed a light line. He described the hypodermal layer of osteosclereids and referred to them as supporting cells having the shape of a sand glass. His work indicated the presence of large intercellular spaces in the osteosolereid layer.

Zimmerman also studied the strophicle 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 strophiolar 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 Reeves (1946), and without a doubt this 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 protoderm in the young ovule. He reported that early ovule growth is accompanied by rapid anticlinal division in the protoderm, and that 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 similar to 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.

III. 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, and this data was used later in determining the approximate age of ovaries selected for sectioning and is summarized in table I. Pods in various stages of development were collected from surrounding plants, killed, fixed, and stored in either Craf 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, infiltrated, and embedded in tissue mat paraffin. More mature specimens were difficult to infiltrate with paraffin and were dissected out of the pods before infiltration. Sections ten microns in thickness 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 section 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. Cover glasses of #1 thickness were then fixed to the stained slides with permount. Illustrations were prepared with the aid of a Zeiss drawing attachment for a microscope.

IV. OBSERVATIONS

A. Desmodium canadense (L.) DC.

The first stage of development that was studied intensively is immediately following anthesis. At this stage the exposed ovary is approximately 5 mm. long in Desmodium canadense. Figure 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 is distinct in appearance from the underlying cells. These epidermal cells are clearly in the meristematic condition 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 Desmodium 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 Desmodium 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 as compared to 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 there is no noticable 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 a 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 thickenings are apparently of cellulose because of their strong affinity to fast green stain.

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 similar to that of macrosclereid development.

In the mature seed coat the osteosclereids and macrosclereids are characterized by a decrease in length without any appreciable change in diameter. This is believed to be 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. The designations for these regions 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 (see 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 abcission of the corolla of Cassia fasciculata, the 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 in the meristematic condition based upon the presence 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 anticlinal cell division only 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 35-45 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 stain. 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," this being a group of cells of unknown function (fig. 8).

Although sections were made of many later stages of ovary maturation in Cassia 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. It is probable that the cells of the hypodermal layer differentiate rapidly sometime after cessation of ovary growth.

In the mature seed coat of Cassia 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 highly complex non-cellular layer. This layer is composed of "spicule-like" bodies thought to be composed of cutin because of their retention of 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 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).

TABLE I. OVARY LENGTH IN RELATION TO DAYS OF DEVELOPMENT

Days	Desmodium canadense	Cassia fasciculata
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

V. DISCUSSION AND CONCLUSION

Comparison of early stages of 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 Desmodium canadense than in Cassia 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 Cassia fasciculata, and a double row of macrosclereids in the hilar region of Desmodium canadense. The second structural difference is found in the composition of the cuticularized layer. This layer is very pronounced in the species of Cassia studied 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 Desmodium canadense lacks the thickness and the apparent "spicule-like" complex found in the testa of Cassia. The third structural difference is the form of the osteosclereids of the species of Cassia and Desmodium studied. In Desmodium canadense the mature osteosclereids exhibit the characteristic "hour glass" shape. Osteosclereids in Cassia 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 found by earlier workers for other leguminous species. These similarities were found in the types of cell division which occurs 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 differences evident in Cassia fasciculata that have not been observed in earlier studies on leguminous species. One of these is a single row of macrosclereids in the hilar region. Apparently all of the earlier workers found a double row of macrosclereids at this point. The second is the presence of the "spicule-like" bodies, very probably made up of cutin, in the cuticularized layer. These "spicule-like" bodies have not been described by earlier workers. Another inconsistency exists in the strophiolar 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 Cassia fasciculata or Desmodium canadense. Longitudinal sections cut through the seed perpendicular to the long axis of the hilum revealed little specialized activity in this region. 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.

Most of the ontological studies of macrosclereids and osteosclereids have been done in the Fabaceae because of the presence of many species of economic importance. 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 significent structural inconsistencies 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 in the gross development of the seed coat, developmental studies of the testa of other genera of the Caesalpiniaceae are certainly needed in order to determine the significance of the single row of macrosclereids in the hilar region and the "spicule-like" bodies in the cuticularized layer of Cassia fasciculata and their role in the delineation of taxonomic groups within the legumes.

LITERATURE CITED

Bentham, G., and J. D. Hooker. 1862. Genera Plantarum. Vol. I. London, Reeve and Co. 1040p.

Cavazza, L. 1950. Recherches sur l'imperméabilite des graines dures les Légumineuses. Schweiz. Bot. Gesell. Ber., 60:596-610.

Coe, H. S., and J. N. Martin. 1920. Sweet-clover seed. II. Structures and chemical nature of the seed coat and its relation to impermeable seeds of sweet-clover. U.S. Dept. Agric. Bull. 844. 39p.

Esau, Katherine. 1962. Anatomy of seed plants. New York, John Wiley and Sons, Inc. 331p.

Gleason, H. A., and A. Cronquist. 1963. Manual of vascular plants of Northeastern United States and adjacent Canada. New York, D. Van Nostrand Co., Inc., 810p.

Fernald, M. L. 1950. Gray's manual of botany, 8th ed., New York, American Book Co.

Hyde, E. O. C. 1954. The function of the hilum in some Papilonaceae in relation to ripening of the seed and the permeability of the testa. Ann. Bot. 18:241-256.

Mattirolo, O. Il tegumento seminale delle Papilionaceae nel mecoanismo della respirazione. Nuovo Giornale Bot. Ital. 23:161-165.

Pammel, L. H. 1899. Anatomical characteristics of the seeds of Leguminosae, chiefly of Gray's manual. Trans. Acad. Sci. St. Louis 9:91-275.

Pitot, A. 1935. Le développement du tégument des graines de Légumineuses. Bull. Soc. Bot. France 82:311-314.

Pool, R. J. 1929. Flowers and flowering plants. New York, McGraw-Hill Book Co., Inc. 428p.

Rees, Bertha. 1911. Longevity of seeds and structures and nature of seed coat. Proc. Roy. Soc. Victoria N.S. 23:392-414.

Reeve, R. M. 1946a. Structural composition of the sclereids in the integument of Pisum sativum L. Amer. Jour. Bot. 33:191-204.

_____, 1946b. Ontogeny of the sclereids in the integuments of Pisum sativum L. Amer. Jour. Bot. 33:806-26

Rehder, A. 1940. Manual of cultivated trees and shrubs. New York, The Macmillan Co. 496p.

Rendle, A. B. 1925. The classifications of flowering plants, Vol. II. Cambridge, University Press.

Sass, J. E. 1958. Botanical microtechnique. Ames, Iowa, The Iowa State College Press. 228p.

Taubert, P. 1891. Leguminosae. In Engler and Prantl. Die Natürlichen Pfanzenfamilien. Leipzig, Wilhelm Engelmann III, 2:70-384.

Tozzetti, T. A. 1855. Saggioni di studi intorno al guscio dei semi. Mem. R. Accad. Sci. Torino 15:359-445.

Tschirch, A., and Oesterle, O. 1893-1897. Anatomischer Atlas d. Parmakognosie U. Nahrungsmittel kunde. 12:260. 60 pl.

Zimmermann, K. 1936. Zur physiologischen Anatomie der Leguminosentesta. Landw. Versuchs.-Sta. 127:1-56.

VII. ILLUSTRATIONS

PLATE I

Desmodium canadense (L.) D C

All illust ations of ovules a e 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. X250.

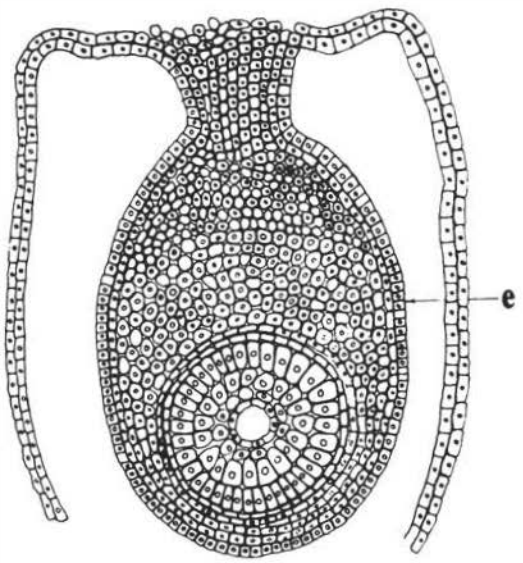
Fig. 2. Ovule about 5 days after anthesis showing differentiation in the hilar region. X250.

Fig. 3. Ovule about 5 days after anthesis showing development along the lateral side. X400.

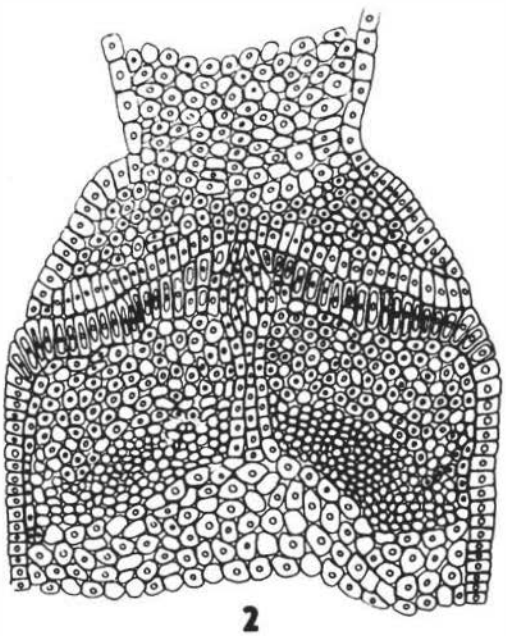
Fig. 4. Ovule about 9-10 days after anthesis. Radial elongation of the macrosclereids is evident and differentiation of the osteosclereids has begun.

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

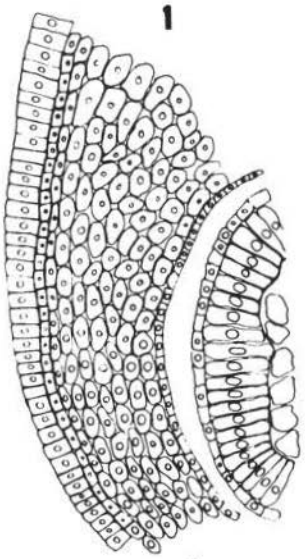
Fig. 6. Mature seed coat of the ovule collected about 3 weeks after anthesis showing macrosclereids (m), light line (l), domes (d), osteosclereids (o), and thick-walled parenchyma (p).



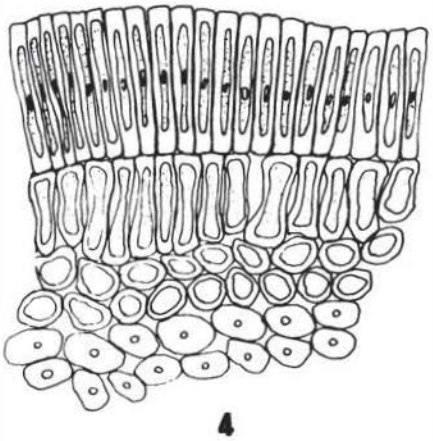
1



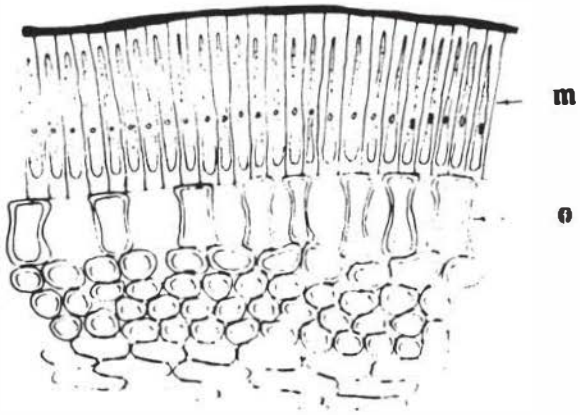
2



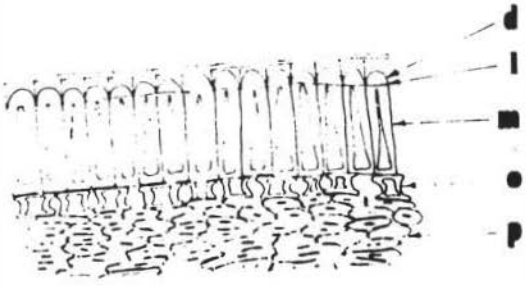
3



4



5



6

PLATE II.

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.

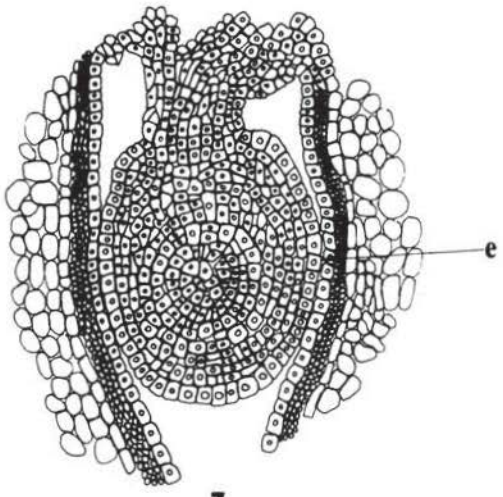
Fig. 7. Cross section of the ovary and ovule at anthesis. The epidermal layer of the ovule (e) is distinct from the underlying tissue. X250.

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. X400.

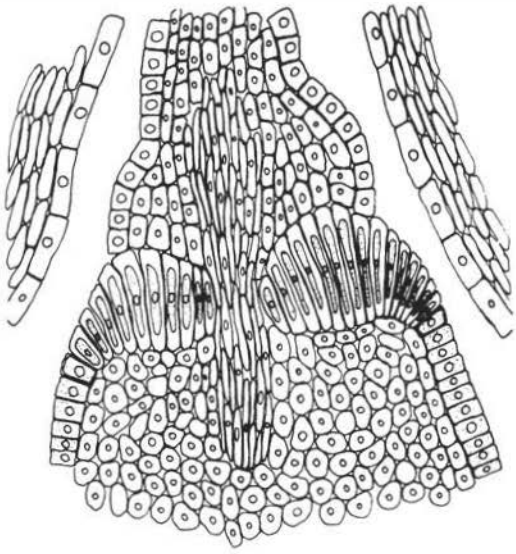
Fig. 9. Ovule 9 days after anthesis showing differentiation of the epidermis along the lateral side. X400.

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. X400.

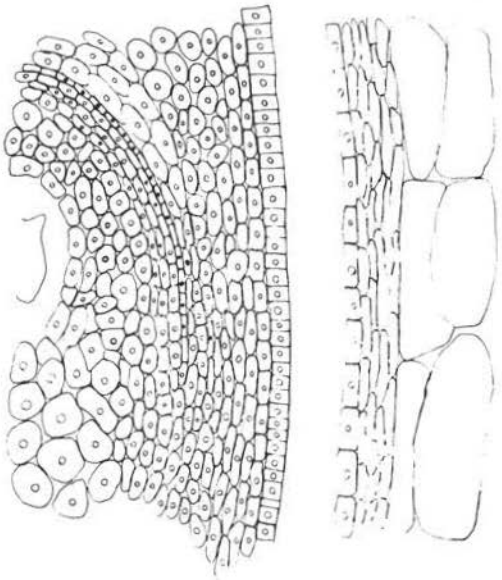
Fig. 11. Mature seed coat of an ovule collected about 3 weeks after anthesis showing macrosclereids (m), spicules (s), light line (l), osteosclereids (o), underlying thick-walled parenchyma (p), and part of cotyledon (c). X400.



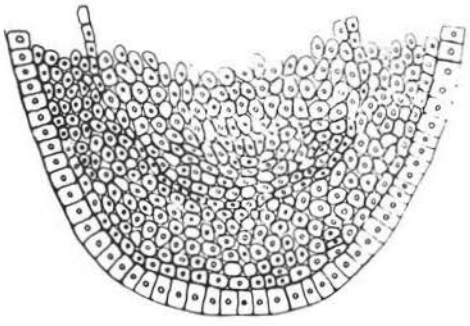
7



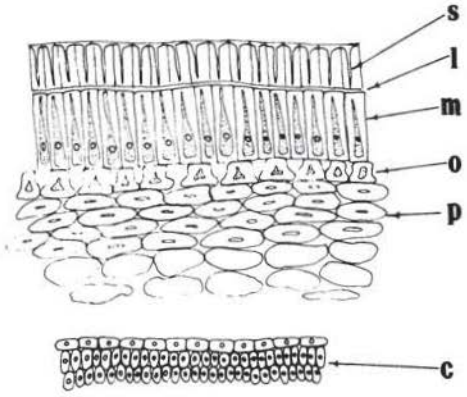
8



9



10



11