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Pericarp ontogenesis with emphasis on the dispersal apparatus of three weed species of Faboideae (Fabaceae)

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ABSTRACT

It is known that the efficient dispersal is one among other features that amplify the invasion potential of a plant. Knowledge of the ontogeny, morphology and fruit dispersal of species is indispensable for weed control in crops. To identify the pericarp structures involved in the fragmentation and dehiscence processes and other features related to dispersal, we studied the pericarp development of *Aeschynomene evenia* Wright, *Desmodium incanum* (Sw.) DC. and *Vigna luteola* (Jacq.) Benth. (Fabaceae-Faboideae), all of which are considered weeds in certain situations. For light and scanning electron microscopy studies, we fixed and processed buds, flowers and fruits, according to usual methods, at different stages of development. We observed that the sclerenchymatous endocarp is essential for dehiscence in legumes, as well as for fragmentation in loments. We also found that the presence of hook-shaped trichomes, sclereid nests in the mesocarp, septum, hypodermis and the formation of false septa are essential to the diaspore dispersal of the species studied.

Key words: dehiscence, fragmentation, legume, Leguminosae, loment

Introduction

Fabaceae is the third largest family of flowering plants, in terms of the number of species; it is distinguished by the diversity of forms of its species and number of habitats in which they are found; and it is a family of great agricultural, economic and ecological importance (Wojciechowski 2003; Lewis *et al*. 2005). In general, Fabaceae is divided into three subfamilies (Judd *et al*. 2009): Caesalpinioideae, Faboideae and Mimosoideae. Faboideae is by far the largest of the three and is also important because of the number of genera comprising species that are considered weeds in crop fields (Kissmann & Groth 2000; Doyle & Luckon 2003).

Weeds are wild plants that arise and reproduce spontaneously (i.e., are not cultivated), growing on agricultural lands and in areas of human interest. These undesirable plants causing economic losses to farmers and livestock producers, as well as being a cause for concern regarding human life and health (Lorenzi 2000; Brighenti 2001). Knowledge of the ontogeny, morphology and fruit dispersal of weeds is indispensable for their control (Souza 2006).

Aeschynomene is one of the 20 largest genera of Fabaceae, with 180 species, among which are hydrophilic plants that grow in wetlands, making these plants a problem in lowland or flood-irrigated crops, especially rice (Kissmann & Groth 2000; Lewis *et al*. 2005). *Desmodium*, with a total of 275 species, is the twelfth largest genus of 29 genera of Fabaceae in number of species (Lewis *et al.* 2005). Several of those species are considered weeds on lawns and among perennial crops, as well as occurring in wastelands and along roadsides (Kissmann & Groth 2000). *Vigna* is comprised of 104 species (Lewis *et al*. 2005). One such species is *Vigna unguiculata* (L.) Walp., which is a common weed among soybean crops in southern Brazil (Kissmann & Groth 2000).

Fahn & Zohary (1955) stated that, historically, the fruits of Fabaceae preserved their basic pattern and at the same time evolved to quite diverse shapes and ways of dispersal. The Faboideae genera *Aeschynomene*, *Desmodium* and *Vigna* comprise species that are considered weeds. Those weeds have certain characteristics that provide them with competitive advantages over other species, principally the efficient dispersal of their diaspores (Brighenti 2001; Ziller 2001). To investigate this assumption, we attempted to determine which structural features of the pericarp would be responsible for successful diaspore dispersal in the Faboideae species *Aeschynomene evenia* Wright, *Desmodium incanum* (Sw.) DC. and *Vigna luteola* (Jacq.) Benth.

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Materials and methods

Collect and fixation

From specimens of *Aeschynomene evenia*, *Desmodium incanum* and *Vigna luteola*, we collected buds, flowers and fruits at different stages of development. Samples were collected at the following locations (coordinates) in Brazil: *A. evenia* in the municipality of São Francisco do Sul (26°11'46.1"S; 48°31'45.8" W), in the state of Santa Catarina; *D. incanum* in the municipality of Maringá (23°24'16.2"S; 51°56'53.7"W), in the state of Paraná; and *V. luteola* in the municipality of São Francisco do Sul (26°10'08.0"S; 48°32'06.5"W).

We fixed the collected material in a solution of formalin, acetic acid and 50% ethyl alcohol. We preserved the material in 70% alcohol (Johansen 1940). We deposited vouchers in the Herbarium of the (Paraná) State University at Maringá (code, HUEM), under the accession numbers *Aeschynomene evenia* 19927, *Desmodium incanum* 19933 and *Vigna luteola* 19932.

Slide preparation

For histological analysis, we embedded the botanical material in methacrylate resin (Historesin; Leica Microsystems, Wetzlar, Germany), as described by Guerrits (1991). The resin blocks were transversely and longitudinally sectioned in a rotation microtome, and the sections were stained with toluidine blue in 0.1 M phosphate buffer, pH 4.7 (O'Brien *et al.* 1964). Slides were prepared with Permount histological mounting medium (Johansen 1940). We obtained photomicrographs by image capture in a light microscope (BX50; Olympus, Tokyo, Japan) with a digital microscope camera (ICC50; Leica Microsystems).

Histochemical tests

To detect certain substances, we carried out histochemical tests in free-hand sectioned samples. We used Sudan IV for lipids; ferric chloride for phenolic compounds (Johansen 1940); Lugol's solution for starch; phloroglucinol in alcohol and acid solution for lignin (Berlyn & Miksche 1976); ruthenium red for pectic substances (Johansen 1940); and mercuric bromophenol blue for total proteins (Mazia *et al.* 1953).

Scanning electron microscopy

The fixed samples of ovaries and fruits at different stages of development were dehydrated in a graded series of ethyl alcohol, for 45 min in each solution. We then dried the samples in a critical point dryer (CPD 030; Bal-Tec AG, Balzers, Liechtenstein), using CO_2 (Horridge & Tamm 1969), after which they were mounted on stubs and sputter-coated with gold in an ion coater (IC-50; Shimadzu, Kyoto, Japan). Finally, we analyzed the samples under scanning electron microscopy (SEM) in an SS 550 microscope (Shimadzu), creating scanning electron micrographs with scales printed on them.

Morphological and anatomical description

The terminology used in the morphological and anatomical descriptions was based on studies in the botanical literature, including Roth (1977), Radford (1986) and Barroso *et al.* (1999). We used the strict sense criteria to delimitate the exocarp, mesocarp and endocarp (Roth 1977).

Results

Bud stage

At the bud stage in *Aeschynomene evenia*, *Desmodium incanum* and *Vigna luteola*, the ovary is stipitate, superior, monocarpellary and unilocular; in transversal section, it is ovate; and the ovules have marginal placentation (Fig. 1). In *A. evenia*, the outer epidermis of the ovary is strigose, whereas in *D. incanum* and *V. luteola*, it is ciliated, with non-glandular trichomes, especially in the dorsal and ventral regions of the ovary. At this stage, glandular trichomes are present in *D. incanum* and *V. luteola*. In all three species, the outer epidermis is uniseriate (Fig. 1).

In all three of the species evaluated, the ovarian mesophyll is composed of parenchymatous cells. All three species have one dorsal and two ventral vascular bundles. *Desmodium incanum* and *Vigna luteola* present large caliber phenolic idioblasts among the bundle cells (Fig. 1A, B). The inner epidermis of the ovary is uniseriate and glabrous, covered by a thin cuticle, with cells of dense cytoplasm and conspicuous nuclei in the three species (Fig. 1).

Anthesis stage

In *Aeschynomene evenia*, the outer epidermis of the ovary consists of papillary cells, a characteristic that persists in the early developing fruits; in *Desmodium incanum* and *Vigna luteola*, there are non-glandular and glandular trichomes distributed homogeneously over the entire surface of the ovary, in unripe and ripe fruit (Fig. 1).

In the mesophyll of all three of the species studied, the lateral procambial strands are pronounced. As in the ovary at the bud stage, phenolic idioblasts occur among the bundle cells in *Aeschynomene evenia* and *Vigna luteola*. In *V. luteola*, the two most internal mesophyll layers begin cell division on different planes, those layers giving rise to the middle and inner mesocarp in the advanced stages of development in this species (Fig. 1).

The inner epidermis of *Aeschynomene evenia* remains as in the previous stage. In *Desmodium incanum* and *Vigna luteola*, the cells undergo periclinal divisions making the in-

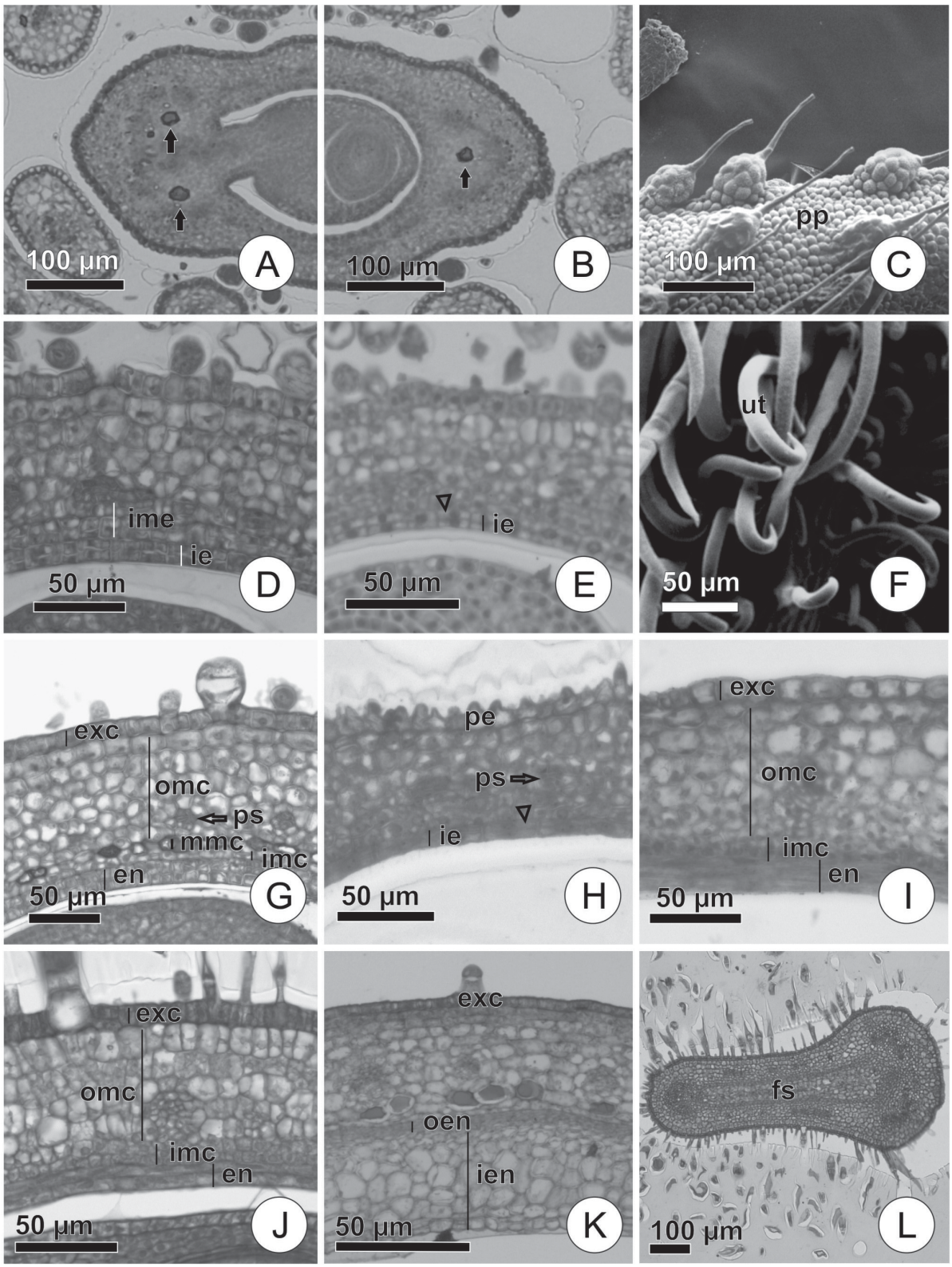

Figure 1. Transversal sections. A,B—Floral bud ovary of *Aeschynomene evenia* Wright; C, F—Ovary outer surface of *A. evenia* and *Desmodium incanum* (Sw.) DC; D,E,H—Anthesis flower ovaries of *Vigna luteola* (Jacq.) Benth., *D. incanum* and *A. evenia*; G. Pericarp of *V. luteola* in the early stage of development; I-K—Pericarps of *A. evenia*, *D. incanum* and *V. luteola* in development. L—False septum of *D. incanum* in early development. Note phenolic idioblasts among the vascular bundle cells (solid arrows) and parallel cell divisions (arrowheads).
en – endocarp; exc – exocarp; fs – false sept; ie – (biseriate) inner epidermis (or ventral meristem); ien – inner endocarp or seed cushion; imc – inner mesocarp; ime – inner mesophyll; mmc – middle mesocarp; oen – outer endocarp; omc – outer mesocarp; pe – papillary epidermis; pp – papillae; ps – procambial strand; ut – uncinate trichome.

ner epidermis biseriate, settling into the ventral meristem, from which the endocarp will arise in later stages (Fig. 1).

Post-anthesis stage

The fruits in early development present uniseriate exocarps, with anticlinal divisions. In *Aeschynomene evenia*, the exocarp cells are papillary with non-glandular trichomes. In *A. evenia*, the surface of the fruit remains strigose as in the ovary (Fig. 1). In *Desmodium incanum*, there are uncinate nonglandular trichomes of different sizes, making the fruit surface tomentose (Fig. 1F). In *Vigna luteola*, the presence of straight and thin non-glandular trichomes makes the fruit surface pubescent.

In *Aeschynomene evenia* and *Desmodium incanum*, the mesocarp retains the same characteristics described for the previous stage. In *Vigna luteola*, the mesocarp is divided into three regions (Fig. 1): the outer mesocarp, composed of seven to nine cell layers with division in different planes; the middle mesocarp, consisting of a phenolic layer of cells; and the inner mesocarp, the cells of which are smaller than are those of the outer mesocarp. In the ventral region of *V. luteola*, the formation of the septum can be observed.

The endocarp of *Aeschynomene evenia* is biseriate at this stage (Fig. 1), as seen in *Desmodium incanum* and *Vigna luteola* in the previous stage; in *D. incanum* the endocarp remains unchanged from the previous stage, and in *V. luteola*, it has three cell layers (Fig. 1).

Following the post-anthesis stage

There is an intense cell elongation throughout the pericarp, resulting in an increase in the size of the fruits.

In *Aeschynomene evenia*, the cells of the exocarp are no longer papillary and present external straight anticlinal walls and dense content. In *A. evenia* and *Desmodium incanum*, the mesocarp is divided into the outer mesocarp, composed of seven to nine parenchymatous cells, and the inner mesocarp, composed of one, smaller cell (Fig. 1). In both species, the inner mesocarp will differentiate into a crystal layer at the end of its development (Fig. 2). In *Vigna luteola*, the mesocarp retains the characteristics from the previous stage (Fig. 1).

The fibers which partially involve the ventral and dorsal vascular bundles are in differentiation in all three of the species evaluated, and the lateral bundles, which are collateral, are already differentiated. In *Aeschynomene evenia* and *Vigna luteola*, the phenolic idioblasts remain among the cells of the dorsal and ventral bundles.

In all three of the species studied, the endocarp is divided into an external region, composed of parallel, flattened, elongated cells, which in a later stage give rise to the sclerenchymatous endocarp, and an internal region, composed of parenchymatous cells (Fig. 1). In *Desmodium incanum* and *Vigna luteola*, the outer endocarp has two cell layers, whereas it has four layers in *Aeschynomene evenia*. The inner endocarp is composed of only one cell layer in *A. evenia* and *D. incanum*, whereas in *V. luteola* the cells are in markedly divided in several planes, resulting in a great number of layers that will compose the seed cushion (Fig. 1).

In the regions among the seeds of *Aeschynomene evenia* and *Desmodium incanum*, the inner endocarp of a valve becomes closer to the other due to the increase of cell layers in this region, and both coalesce, creating false transversal septa which subdivide the seed chamber (Fig. 1). In *Vigna luteola*, the septum becomes more prominent between the ventral and dorsal bundle cells, being continuous from the exocarp to the endocarp.

Ripe fruit

Aeschynomene evenia, *Desmodium incanum* and *Vigna luteola* all present elongated, stipitate, dry fruit with brown coloration, flattened in *A. evenia* and *D. incanum* and cylindrical in *V. luteola*. The ripe fruit of *A. evenia* and *D. incanum* are indehiscent loments that break apart transversely into one-seeded segments. In *A. evenia*, the one-seeded segments are more or less tetragons, with parallel borders, whereas in *D. incanum* the segments have a straight ventral region and a sinuous dorsal region. The segments separate easier in *A. evenia* than in *D. incanum*. In *V. luteola*, the ripe fruit is a dehiscent legume (Fig. 2).

In all three of the species evaluated, the exocarp presents stomata and non-glandular pluricellular trichomes (Fig. 2). In *Desmodium incanum* and *Vigna luteola*, the exocarp presents glandular trichomes. The surface of *V. luteola* is tomentose at this stage (Fig. 2).

Vigna luteola presents a hypodermic mesocarp, consisting of cells that are quite thick-walled, especially the anticlinal and external periclinal cells. In contrast with the cells of the sclerenchymatous endocarp, the cells of the *V. luteola* mesocarp have an oblique orientation (Fig. 2). Neither *Aeschynomene evenia* nor *Desmodium incanum* has an hypodermic mesocarp.

In *Aeschynomene evenia*, the outer mesocarp consists of voluminous parenchymatous cells, and groups of sclerenchymatous stratum, composed of sclereids (Fig. 2). The increase in the size of those cells results in a rugose appearance in superficial view (Fig. 2), and, in transversal section, the fruit wall is sinuous. In the fruit of *Aeschynomene evenia* and *Desmodium incanum*, the inner mesocarp consists of idioblasts contain prismatic crystals (Fig. 2).

Immersed in the mesocarp, the lateral collateral bundles, in all of the studied species, are of small caliber (Fig. 2). In the region of the ventral and dorsal bundles, there is a fiber calotte, which partially involves the vascular bundles, composed of differentiated cells, with thick, lignified walls, which follow the ventral and dorsal septa (Fig. 2).

In all three of the species studied, the outer endocarp becomes sclerenchymatous and the fibers present oblique

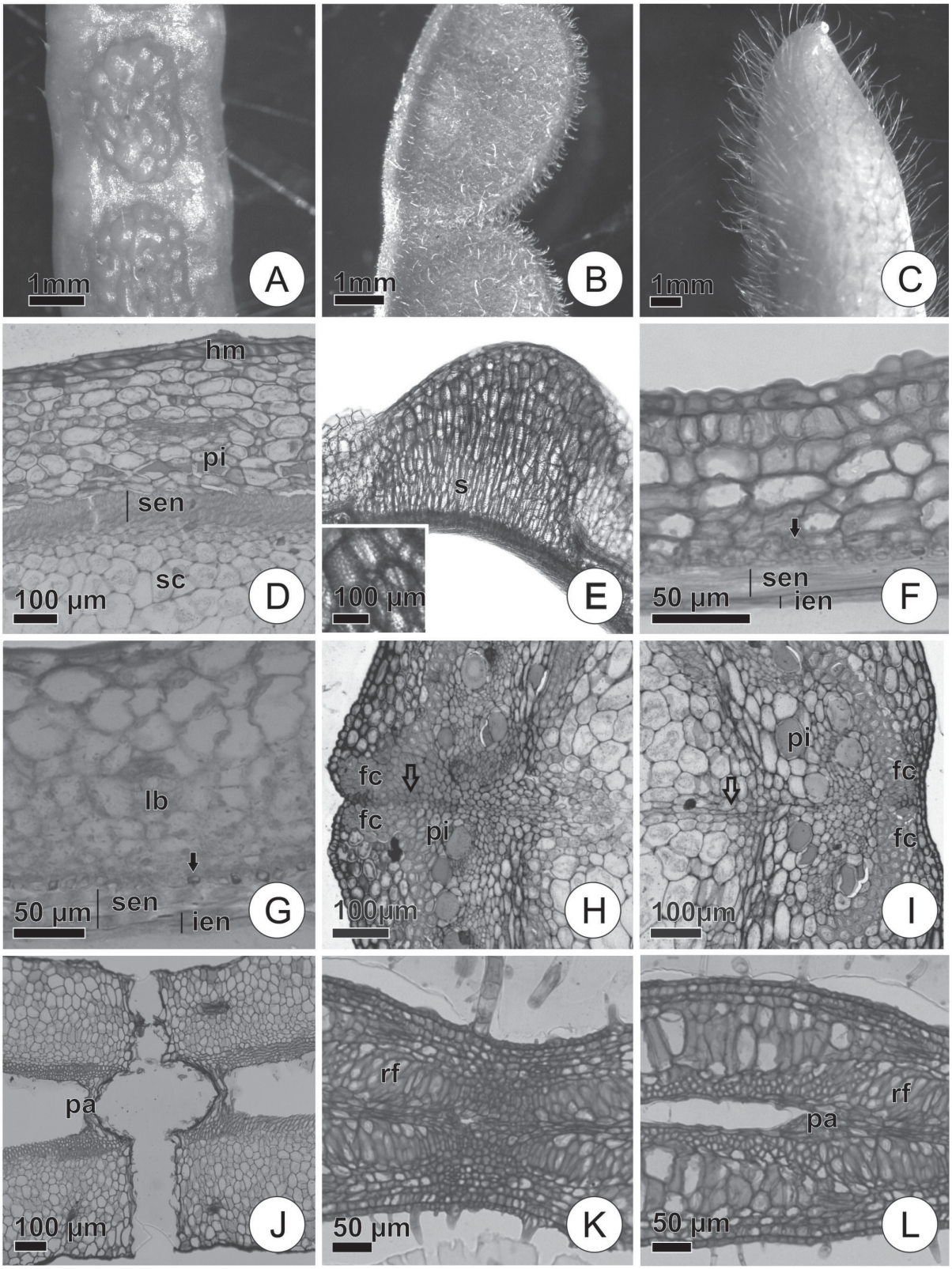

Figure 2. Ripe fruit. **A-C.** General aspects of part of the fruits, emphasizing the indumentum. **D-I.** Transversal sections. **J-L.** Longitudinal sections. A—*Aeschynomene evenia* Wright; B—*Desmodium incanum* (Sw.) DC. C—*Vigna luteola* (Jacq.) Benth.. D,F-G—Ripe pericarp of *V. luteola*, *D. incanum* and *A. evenia*. E—Sclereid islands on mesocarp of *A. evenia*. H—Dorsal region of the pericarp of *V. luteola* and septum. I—Ventral region of the pericarp of *V. luteola.* J—Joint of the loments in *A. evenia.* K-L—Joint of the loments in *D. incanum.* Note crystal layer (solid arrows) and septa (transparent arrows). fc – fibers, calotte; hm – hypodermic mesocarp; ien – inner endocarp; lb – lateral (collateral) bundle; pa – parenchyma; pi – phenolic idioblast; rf – radially oriented fibers; s – sclereids; sc – seed cushion; sen – sclerenchymatous outer endocarp.

orientation in transversal section (Fig. 2). In *Vigna luteola*, the inner parenchymatous endocarp occupies the entire seed chamber, constituting the seed cushion (Fig. 2). In the joint of the loments in *Aeschynomene evenia* and *Desmodium incanum*, the outer sclerenchymatous endocarp is interrupted by parenchymatous cells (Fig. 2). In the longitudinal plane of *D. incanum*, the fibers of the sclerenchymatous stratum near the joint become radially elongated (Fig. 2) whereas the cells present tangential elongation in the more distant regions. In *A. evenia*, a locule is formed in the joint of the loments (Fig. 2), whereas no such space is formed in *D. incanum*.

Discussion

The three species studied here present different dispersal syndromes. Barroso *et al.* (1999) claimed that there is an evolutionary tendency, associated with the dispersal and protection of the diaspores, which leads to adaptation of the pericarp. Fahn & Zohary (1955) reported that, despite the fact that the general structure of the Fabaceae pericarp is extremely uniform, there is considerable variation in the dimensions, location and cell orientation of each type of tissue, as well as in the nature of other pericarp elements. In the species studied, the variations in the individual structural characteristics of each pericarp makes dispersal more efficient and adapted to the environment in which they occur, expanding the invasion potential of these plants.

Aeschynomene evenia and *Desmodium incanum* present loments; this type of fruit is often indehiscent (Barroso *et al*. 1999) and constituted by only one carpel that disarticulates into single-seeded segments (Spjut 1994). According to Roth (1977), the legume, as produced by *Vigna luteola*, is the most frequent fruit type in Faboideae. A legume is derived from the follicle through the dorsiventral dehiscence, followed by the development of false septa in the valve walls; the legume gave rise to the loments and their derivatives (Roth 1977).

Vigna luteola presents autochorous dispersal, by elastic dehiscence. When the pericarp becomes ripe and undergoes desiccation, the internal pressure increases in the valves, which therefore become twisted, opening in two longitudinal slits, one in the ventral suture and the other in the dorsal region, thereby ejecting the seeds (Barroso *et al.* 1999; Souza 2006; Judd *et al*. 2009). In the ripe legume of *V. luteola*, the presence of dome of fibers in the sclerenchymatous endocarp and hypodermic mesocarp, associated with the difference in the cell orientation of these tissues and probably with the crossed micellar cellulose structure of these cell walls, allows the cells to shrink and stretch in different directions, while the fruit loses water, thereby contributing to the twisting of the valves. The septum that extends from the exocarp to the inner endocarp is the fragile region in which the pericarp opens when both valves become twisted, as described by Fahn & Zohary (1955).

The seed cushion observed in *Vigna luteola* is a parenchymatous tissue located internally to the sclerenchymatous stratum of the pericarp and originating from the same source, the adaxial meristem. During their development, the seeds are enveloped by the seed cushion, which provides them protection, as well as storing a great amount of water, thus maintaining a sufficient level of humidity in the seed chamber. The most internal cells of the seed cushion are smaller and present periclinal divisions, adding cells in tissue in a radial direction (Roth 1977). In *V. luteola*, these cells compose part of the septum.

The indehiscence and the presence of false transversal septa in the pericarp of *Aeschynomene evenia* and *Desmodium incanum* are characteristics that demonstrate a certain level of specialization of these fruits, as emphasized by Dudik (1981). In the region of the false septa, the modification of the sclerenchymatous tissue in *D. incanum* and the parenchyma intersection on the sclerenchymatous endocarp in *A. evenia* and *D. incanum* makes the separation region of the loments more fragile (Nemoto & Ohashi 2003). The presence of a locule in the region of the false septa facilitates the separation of the one-seeded segments in *A. evenia* only.

In *Aeschynomene evenia*, the presence of sclereid groupings on the outer mesocarp probably decreases the density of the one-seeded segments, thus making them more well adapted to hydrochorous dispersal, as reported by Barroso *et al*. (1999) for other species of *Aeschynomene*. This characteristic probably potentiates the *Aeschynomene* invasion of fields where lowland or flood-irrigated crops, such as rice, are grown.

Desmodium incanum presents one-seeded segments covered by uncinate trichomes of varying sizes, which makes the species well adapted to epizoochorous dispersal, because those segments are dispersed by attachment to the bodies of animals (Barroso *et al*. 1999; Souza 2006; Judd *et al*. 2009). This pericarp peculiarity facilitates the dispersal of these weed species, especially to abandoned lands, lawns, fields where perennial crops are cultivated and roadsides, where animals move freely.

The sclerenchymatous endocarp, essential for the legume dehiscence of *Vigna luteola* and for the one-seeded segment fragmentation of *Aeschynomene evenia* and *Desmodium incanum* loments, arises from the inner epidermis of the fertilized ovary, which, according to Souza (1984), is common in the subfamily Faboideae. In the loments, the false septa and the legume seed cushion both originate from the adaxial meristem (Roth 1977).

Another characteristic that seems to contribute to the success of these species in protecting the diaspores are the presence of the phenolic idioblasts. Phenolic compounds confer unpalatability and toxicity on plant organs, which are then avoided by phytophagous and herbivorous animals (Roshchina & Roshchina 1993). Therefore, the presence of phenolic idioblasts among the vascular bundle cells of *Aeschynomene evenia* and *Vigna luteola* and in the middle mesocarp of *V. luteola*, might confer unpalatability on these fruits, as well as ensuring immunity to bacterial and fungal infection.

We conclude that the structural variations in the pericarp of the three Faboideae weed species studied here make their dispersal efficient and adapted to the environments in which they grow. That increases the invasion potential of these plants.

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