

Endocarp development in *Schinus terebinthifolius* Raddi (*Anacardiaceae*)¹

João Marcelo Santos de Oliveira² & Jorge Ernesto de Araujo Mariath³

¹Part of the Doctoral Thesis of the first author. Programa de Pós-Graduação em Botânica, Universidade Federal do Rio Grande do Sul.

²Universidade Federal de Santa Maria, Departamento de Biologia, Av. Roraima, 1000, CEP 97105-900, Santa Maria, Rio Grande do Sul, Brazil. linneau@yahoo.com.br.

³Universidade Federal do Rio Grande do Sul, Departamento de Botânica, Av. Bento Gonçalves 9500, CEP 91501-970, Porto Alegre, Rio Grande do Sul, Brazil.

Received 23.V.2014. Accepted 20.V. 2015.

ABSTRACT – The endocarp has taxonomic importance in *Anacardiaceae* and the study of its development is essential in order to identify its structure and whether it belongs to the *Anacardium*-type or *Spondias*-type. The placentation region is the ideal location for endocarp analysis. The fruits of *Schinus terebinthifolius* Raddi are drupes and the endocarp originates exclusively from the epidermis of the locular cavity. The periclinal divisions, responsible for endocarp formation, begin at anthesis, and finish within 72 hours. During this phase, the innermost cells of the ovarian mesophyll and the cells of the locular epidermis have similar structures. The endocarp develops in three organized layers of sclereids, making it the *Anacardium*-type. This endocarp differs from those previously described with four cell layers that include the crystalliferous layer, revealing a different pattern of development.

Key words: *Anacardiaceae*, crystal layer, development, endocarp, *Schinus*

RESUMO – **Desenvolvimento do endocarpo em *Schinus terebinthifolius* Raddi (*Anacardiaceae*).** O endocarpo possui importância taxonômica em *Anacardiaceae*, e o estudo do seu desenvolvimento é essencial para o reconhecimento de sua estrutura e se o mesmo pertence ao tipo *Anacardium* ou ao tipo *Spondias*. A região de placentação correspondeu ao local favorável para a análise do desenvolvimento do endocarpo. Os frutos de *Schinus terebinthifolius* Raddi são drupas e o endocarpo se origina exclusivamente da epiderme da cavidade locular. As divisões periclinais, responsáveis pela formação de endocarpo, iniciam na antese, e se encerram em até 72 horas. Durante essa fase, as células mais internas do mesofilo ovariano e as células da epiderme locular apresentam estrutura semelhante. O endocarpo se desenvolve em camadas organizadas de escleréides, com apenas três camadas, inserindo-o no tipo *Anacardium*. Este endocarpo difere daqueles previamente descritos com quatro camadas de células que incluem a camada de cristalífera, revelando um padrão diferente de desenvolvimento.

Palavras-chave: *Anacardiaceae*, camada cristalífera, desenvolvimento, endocarpo, *Schinus*

INTRODUCTION

The *Anacardiaceae* presents predominantly drupe-type fruits (Engler 1892, Pell *et al.* 2011), whose endocarp may be bony, fibrous-woody or chartaceous (Pell *et al.* 2011, Fleig 1987). The endocarp may originate from locular epidermis differentiation, as in

Schinus L., *Rhus* L. and *Lithraea* Miers, or from the locular epidermis and part of the ovarian mesophyll as in *Spondias* (Wannan & Quinn 1990, Carmello-Guerreiro & Paoli 2002, 2005). Wannan & Quinn (1990) consider the pericarp structure, including its number of layers, to be of considerable importance for tribal circumscription. A number of genera that

occur in *Anacardioideae*, including *Schinus* and other genera of the *Rhöeae* tribe (*sensu* Engler 1892), present an endocarp organized in a reduced number of sclereid layers and a crystal layer, classified as the *Anacardium*-type of endocarp (Wannan & Quinn 1990, Wannan 2006).

The study of the pericarp in this family has received much attention in the last few decades, especially since the endocarp structure has a high taxonomic and systematic value (von Teichman 1989, Wannan & Quinn 1990) due to its high degree of structural conservation among different genera (von Teichman 1989). Among the developmental studies of fruits in *Anacardiaceae*, those for *Rhus lancea* L. f. (von Teichman & Robbertse 1986), *R. problematodes* (von Teichman & van Wyk 1991), *Lannea discolor* Engl. (von Teichman 1987), *S. terebinthifolius* (Carmello-Guerreiro & Paoli 2002), and *Lithraea molleoides* (Vell.) Engl. (Carmello-Guerreiro & Paoli 2005) should be highlighted due to their detailed account of the pericarp anatomy.

However, due to the high degree of structural diversity in the pericarp of the *Anacardiaceae* taxa, insufficient studies have been performed. Currently, in a few genera, such as *Rhus* and *Schinus*, there is no consensus regarding the number of layers derived from the locular epidermis, especially with regard to the origin of the crystal layer (Copeland 1959, von Teichman & Robbertse 1986, von Teichman & van Wyk 1991, Carmello-Guerreiro & Paoli 2002).

Thus, the present study aims to determine the origin of the endocarp, the number of cell divisions involved in the process, and whether the ovarian mesophyll participates in the construction of the endocarp in *Schinus terebinthifolius* (Brazilian peppertree).

MATERIAL AND METHODS

Schinus terebinthifolius Raddi has a wide distribution in Brazil and in the state of Rio Grande do Sul (Fleig 1987). Floral buds, flowers, and young fruits of *Schinus terebinthifolius* Raddi were collected in Porto Alegre, Camará do Sul and Santa Maria, in the state of Rio Grande do Sul. The voucher was deposited in the ICN Herbarium (Universidade Federal do Rio Grande do Sul), labeled as specimen numbers 128956, 128959, 128960.

The samples were fixed in 1% glutaraldehyde and 4% formaldehyde, in phosphate buffer 0.1M, with pH 7.4 (McDowell & Trump 1976). Later the material was dehydrated in an ethylic series, embedded in

(2-hydroxyethyl)-methacrylate (Gerrits & Smid 1983), and sectioned using a sliding microtome (Leitz 1400) and an ultramicrotome (Leica Ultracut UCT) with a glass knife. Sections were stained with 0.05% toluidine blue O (Feder & O'Brien 1968). The observations and photomicrographies were performed using an Olympus BX41 microscope.

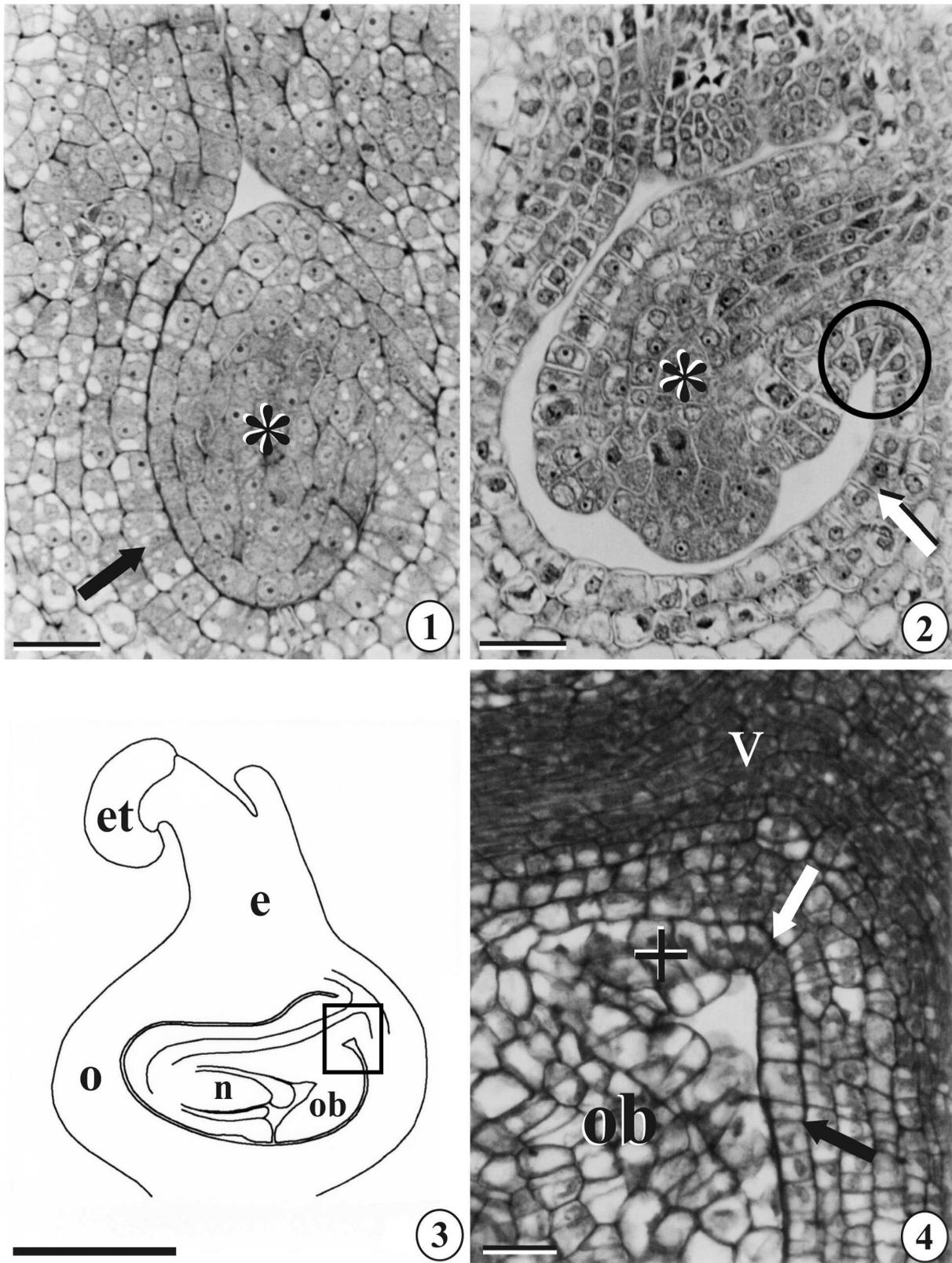
RESULTS

Morphological features of the fruits - The *Schinus terebinthifolius* fruits are of the drupe type. These drupes develop from pistillate, hypogynous, and unisepal flowers, with a single locular cavity. Only an anatropous ovule develops in the locular cavity. The mature fruits are dorsoventrally asymmetrical and slightly compressed laterally.

Ontogenetic considerations - During the initial phase of locular cavity differentiation, its epidermis is observed as a regular layer of cells with a meristematic structure. Especially in these initial ontogenetic phases, there is a continuity between the locular epidermis of the cavity and ovular epidermis (Figs. 1, 2).

Before anthesis, the locular epidermal cells undergo slight radial growth and an increase in the anticlinal divisions in this tissue. The first periclinal divisions of the locular epidermis, which start the layering process of the endocarp, occur during anthesis. In the placentation region (Fig. 3), there are differences between the epidermal cells from the locular cavity and the ovule, in terms of their volume and regular shape (Fig. 4). The periclinal cell divisions give rise to three layers of endocarp within a period of up to 72 hours following anthesis. This process usually begins at the base of the locular cavity and irradiates toward the cavity apex. The epidermis that covers the ovule remains unistratified (Figs. 5 - 7).

Immediately after the beginning of the first cycle of periclinal divisions, the innermost derived cells, i.e., those adjacent to the locular cavity begin growing in the radial direction and no longer divide periclinally. These cells give rise to the sclereids, usually called macrosclereids. During the initial phases, these sclereids are defined as proto-macrosclereids (Figs. 5 - 8). The second cycle of periclinal divisions, which occur in the external sister-group cells of macrosclereids, gives rise to proto-osteosclereids and proto-brachysclereids. The proto-osteosclereids form the intermediary cell layer in the three-layered structure (Figs. 6 - 8). Thus, part of the endocarp formation occurs through only two



Figs.1-4. Longitudinal sections of carpels of *Schinus terebinthifolius* at different phases of development. **1.** Detail of the ovule (asterisk), filling the locular cavity. Arrow indicates the locular protoderm. **2.** Detail of the ovule (asterisk), more developed when compared to the Fig. 1 and locular epidermis (arrow). Black circle indicates the boundary between the locular cavity epidermis and the ovule protoderm. **3.** General aspect of the carpel during anthesis. Square indicates detail of the placentation region under analysis. **4.** Detail of the black square indicated in **Fig. 3**. White arrow indicates boundary between the epidermis of the locular cavity (black arrow) and the epidermis (+) of the funicular obturator (ob); e = style; et = stigma; n = nucellus of the ovule; o = ovary wall; ob = funicular obturator; v = vascular tissue. Bars: **Figs. 1, 2, 4** = 25 μ m ; **Fig. 3** = 300 μ m.

cycles of periclinal divisions in the locular epidermal cells and their derivatives.

The latter differentiation reveals a trend toward radial growth, which is less intense in the proto-osteosclereids. Besides the typical periclinal divisions, there are anticlinal divisions in the cells of the three layers during a period of up to four weeks. In cross sections of the developing pericarp, the three to four innermost layers of the mesocarp present a cellular structure that is very similar to those derived from the locular epidermis (Fig. 8). Furthermore, the innermost cells of the mesocarp, particularly the crystal layer, occur more or less in alignment with the proto-brachysclereids during the initial phases of development.

During the third or fourth week of fruit maturation, when the endocarp already presents mature cells, there is no development of the proto-brachysclereid layer in regions of the secretory cavities (Figs. 9, 10), which results in a two-layered endocarp in such restricted areas. Thus the ensemble of cells differentiates into proto-osteosclereids. However, the innermost crystal layer of the mesocarp is usually differentiated in these regions.

DISCUSSION

The initial development process of the endocarp presented here for *Schinus terebinthifolius* shows similarities and differences in relation to other species of *Anacardiaceae*. The fruit development presents an initial period of cell division that occurs during anthesis and which is followed by a cellular growth phase. The meristematic cellular structure predominates both in derivatives of the locular epidermis and of the mesophyll cells. These general characteristics were demonstrated in the present study and were also documented for other *Anacardiaceae* species, such as *Rhus lancea* (von Teichman & Robbertse 1986), *Ozoroa paniculosa* (Sond.) R. Fern. & A. Fern. (von Teichman & van Wyk 1993), *Heeria argentea* Meisn. (von Teichman & van Wyk 1996), as well as for other angiosperms (Roth 1977).

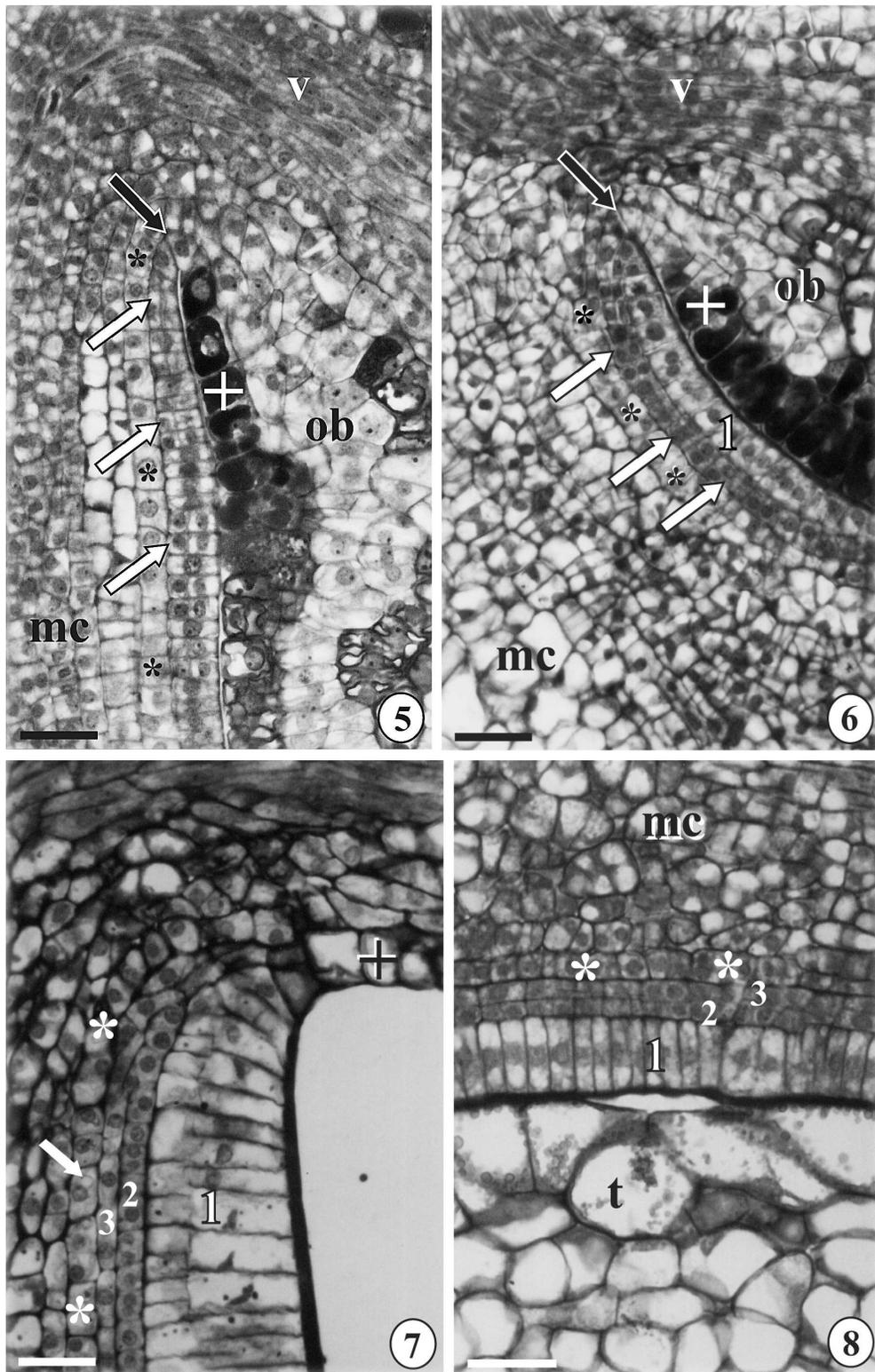
The first divisions for endocarp development in *Pistacia chinensis* Bunge (Copeland 1955) and *Anacardium occidentale* L. (Copeland 1962) begin during anthesis, similar to *S. terebinthifolius*. However, in *Rhus lancea* (von Teichman & Robbertse 1986) and in *Ozoroa paniculosa* (von Teichman & van Wyk 1993) the first divisions begin before anthesis.

Identifying general developmental patterns for angiosperm fruits is essential to establish homologies among different taxa. With regard to studies performed on *Anacardiaceae*, in general, the similarity between the inner portion of the ovarian mesophyll and the locular epidermis gives rise to difficulties in understanding the ontogenetic process and, consequently, to difficulties in demarcating the different regions of the pericarp, as observed in *Rhus lancea* (von Teichman & Robbertse 1986), *Ozoroa paniculosa* (von Teichman & van Wyk 1993), *Protorhus namaquensis* Sprague (von Teichman & van Wyk 1994) and in the present study for *S. terebinthifolius*.

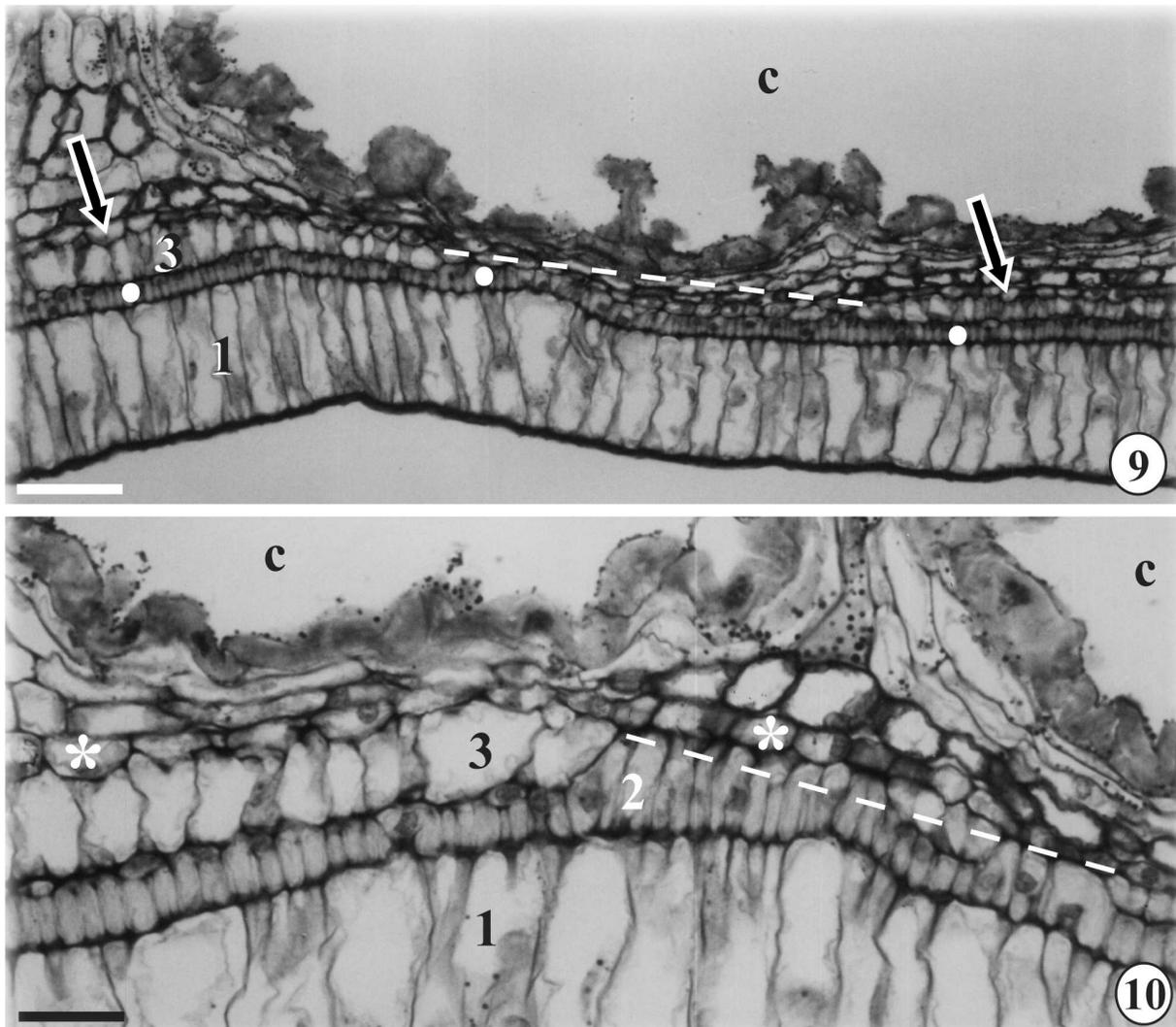
Previous studies of the ontogeny of the pericarp in *Anacardiaceae*, especially the endocarp, were performed in sections of the fruit that generally do not allow a clear distinction between what is derived from the locular epidermis and from the mesocarp (Teichman & Robbertse 1986, von Teichman & van Wyk 1991, Carmello-Guerreiro & Paoli 2002, 2005). The homology between the locular epidermis and the ovular epidermis shows that, besides each having distinct morphogenetic fates, the locular epidermis in *S. terebinthifolius* undergoes only two cycles of periclinal divisions.

In the species studied here it was also shown that the crystal layer cannot be considered a layer of the endocarp, but rather as the innermost layer of the mesocarp. Information about the origin of the crystal layer in other *Schinus* species is still not clear. In *Schinus molle* L., the developmental data presented by Copeland (1959) show an endocarp with only three strata. For *Schinus areira* L., although Wannan & Quinn (1990) described the crystal layer as part of the endocarp, no ontogenetic information was presented. In *Toxicodendron diversiloba* Torr. & A. Gray (Copeland & Doyel 1940) and *Rhus prolematodes* Merxm. & Roessl. (von Teichman & van Wyk 1994), species of genera very close to *Schinus*, the endocarp has only three layers. Wannan and Quinn (1990) described two types of endocarp in *Anacardiaceae*, where the *Spondias*-type constitutes irregularly-oriented sclerenchymatous cells, and the *Anacardium*-type presents few layers of sclereids in a palisade-like organization. Considering the results of this study, it is possible to classify the endocarp in *S. terebinthifolius* as the *Anacardium*-type.

The crystal layer is used by Wannan & Quinn (1990) as a morphological marker for transformations in the endocarp, when comparing different genera of *Anacardiaceae*, since it is present in several genera



Figs.5-8. Sections of the fruits of *Schinus terebinthifolius* at different phases of development. **5.** White arrows indicate the first periclinal divisions of the locular cavity epidermis (proto-endocarp). Sign + indicates uniseriate epidermis of the ovule. The continuity between both tissues indicated should be noted. The black arrow indicates a boundary between the proto-endocarp and the epidermis of the young seed. White arrows indicate the first cycle of periclinal divisions for endocarp formation. **6.** White arrows indicate the second cycle of periclinal divisions for endocarp formation. Black arrow indicates boundary between proto-endocarp and the epidermis of the young seed. **7.** Detail of the endocarp three weeks after anthesis. Arrow = crystal. **8.** Cross-section of the young fruit. Observe the similarity between the crystal layer and layers 2 and 3 of the endocarp. Asterisks = young crystal layer; mc = mesocarp; ob = funicular obturator; t = seed coat, v= vascular tissue; 1 = proto-macrosclereid layer; 2 = proto-osteosclereids, 3 = proto-brachysclereids. Bars = 25µm.



Figs. 9, 10. Cross-sections of young fruit of *Schinus terebinthifolius*. **9.** General aspect of endocarp. Arrows = crystal layer. **10.** Endocarp detail. Asterisks indicate crystal layer. Traced line in figures 9 and 10 indicates site where the brachysclereid layer (3) does not differentiate. c = secretory cavity; 1= macrosclereids; 2 = osteosclereids; 3 = brachysclereids; white circle = layer of osteosclereids. Bars: **Fig. 9** = 50 μ m ; **Fig.10** = 25 μ m .

of the *Rhœae* tribe. Thus, evolutionary tendencies, such as the reduction in the number of layers of the endocarp were mentioned. The uncertainty regarding whether the crystal layer origination begins at the locular epidermis does not invalidate its use as a morphological and topographical reference in comparative studies. Nevertheless, this issue should receive attention in further ontogenetic studies in *Anacardiaceae* fruits.

The results of this study highlight the importance of observing initial endocarp development in sections containing the placentation region. Following this methodological criterion, it was possible to describe the formation of an endocarp *sensu stricto* (*sensu* Roth 1977) with only three sclerenchyma layers for *S. terebinthifolius*. Thus, regarding the establishment of homologies for the endocarp in future studies

on *Schinus*, the crystal layer should be ignored, unless it is demonstrated that it has its origin in the locular epidermis. *S. terebinthifolius* presents the *Anacardium*-type of endocarp. Furthermore, considering that the endocarp structure is generally preserved in *Anacardiaceae* genera (von Teichman 1989, Wannan & Quinn 1990, Wannan 2006), we believe that all species of *Schinus*, and other phylogenetically related genera, present an endocarp formation similar to that described in the present study.

ACKNOWLEDGMENTS

The authors thank Professor Alfredo Elio Cocucci, Professor Carlos Frederico Widholzer and Rivete Silva de Lima for the critical review and

suggestions. The authors thank the undergraduate student Daniele Munareto Rodrigues for helping with the collection and processing of plant material.

REFERENCES

- Carmello-Guerreiro, S.M. & Paoli, A.A.S. 2002. Ontogeny and structure of the pericarp of *Schinus terebinthifolius* Raddi (Anacardiaceae). *Brazilian Archives of Biology and Technology* 45:73-79.
- Carmello-Guerreiro, S.M. & Paoli, A.A.S. 2005. Ontogeny and structure of the pericarp and seed coat of *Lithraea molleoides* Vell. Engl. (Anacardiaceae) with taxonomic notes. *Brazilian Archives of Biology and Technology* 48:599-610.
- Copeland, H.F. 1955. The reproductive structures of *Pistacia chinensis* (Anacardiaceae). *Phytomorphology* 5:440-449.
- Copeland, H.F. 1959. The reproductive structures of *Schinus molle* (Anacardiaceae). *Madroño* 15:14-25.
- Copeland, H.F. 1962. Observations on the reproductive structures of *Anacardium occidentale*. *Phytomorphology* 11:315-325.
- Copeland, H.F. & Doyel, B.E. 1940. Some features of the structure of *Toxicodendron diversiloba*. *American Journal of Botany* 27:932-939.
- Engler, A. 1892. Anacardiaceae. In *Die Natürlichen Pflanzenfamilien* (A. Engler, & K. Prantl, eds.). Engelmann, Leipzig, p. 138-178.
- Feder, N. & O'Brien, T.P. 1968. Plant microtechnique: some principles and new methods. *American Journal of Botany* 55:123-142.
- Fleig, M. 1987. Anacardiaceae: Floral ilustrada do Rio Grande do Sul. *Boletim do Instituto de Biociências*. 42:1-72.
- Gerrits, P.O. & Smid, L. 1983. A new, less toxic polymerisation system for the embedding of soft tissues in glycol methacrylate and subsequent preparing of serial sections. *Journal of Microscopy* 132:81-85.
- McDowell, E.M. & Trump, B. 1976. Histological fixatives for diagnostic light and electron microscopy. *Archives of Pathology & Laboratory Medicine* 100:405-414.
- Pell, S.K., Mitchell, J.D., Miller, A.J., & Lobova, T.A. 2011. Anacardiaceae. In *The Families and Genera of Vascular Plants. Eudicots: Sapindales, Cucurbitales, Myrtaceae* (K. Kubitzki, ed.). Springer, Heidelberg. p. 7-50.
- Roth, I. 1977. Fruits of Angiosperms. In *Encyclopedia of Plant Anatomy* (W. Zimmermann, S. Carlquist, P. Ozenda & H.D. Wulf, eds.). Gebrüder Borntraeger, Berlin. 675 p.
- von Teichman, I. 1987. Development and structure of the pericarp of *Lannea discolor* (Sonder) Engl. (Anacardiaceae). *Botanical Journal of the Linnean Society* 95:125-155.
- von Teichman, I. 1989. Reinterpretation of the pericarp of *Rhus lancea* (Anacardiaceae). *South African Journal of Botany* 55:383-384.
- von Teichman, I. & Robbertse, P.J. 1986. Development and structure of the pericarp and seed of *Rhus lancea* L. fil. (Anacardiaceae), with taxonomic notes. *Botanical Journal of Linnean Society* 93:291-306.
- von Teichman, I. & van Wyk, A.E. 1991. Taxonomic position of *Rhus problematodes* (Anacardiaceae): evidence from fruit and seed structure. *South African Journal of Botany* 57:29-33.
- von Teichman, I. & van Wyk, A.E. 1993. Ontogeny and structure of the drupe of *Ozoroa paniculosa* (Anacardiaceae). *Botanical Journal of the Linnean Society* 111:253-263.
- von Teichman, I. & van Wyk, A.E. 1994. The generic position of *Protorhus namaquensis* Sprague (Anacardiaceae): evidence from fruit structure. *Annals of Botany* 73:175-184.
- von Teichman, I. & van Wyk, A.E. 1996. Taxonomic significance of pericarp and seed structure in *Heeria argentea* (Thunb.) Meisn. (Anacardiaceae), including reference to pachychalazy and recalcitrance. *Botanical Journal of the Linnean Society* 122:335-352.
- Wannan, B.S. 2006. Analysis of generic relationships in Anacardiaceae. *Blumea* 5: 165-195.
- Wannan, B.S. & Quinn, C.J. 1990. Pericarp structure and generic affinities in the Anacardiaceae. *Botanical Journal of Linnean Society* 102:225-252.

