A MORPHOLOGICAL STUDY OF THE FRUIT AND SEED OF CERTAIN SOUTHERN AFRICAN MEMBERS OF THE ANACARDIACEAE

by

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Submitted in partial fulfilment of the requirements for the degree

DOCTOR SCIENTIAE

in the Faculty of Science (Department of Botany)

UNIVERSITY OF PRETORIA

PRETORIA

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FEBRUARY 1988

This thesis is dedicated to

MY ADORABLE LORD OF MERCY AND LOVE

source of all inspiration, strength, ...

and secondly to my dear parents

Die Kenntnis der Natur ist der Weg
zur Bewunderung der Grösse des Schöpfers.

Liebig.

"Es geht doch nichts über die Freude, die uns das Studium der Natur gewährt. Ihre Geheimnisse sind von einer unergründlichen Tiefe; aber es ist uns Menschen erlaubt und gegeben, immer weitere Blicke hineinzutun. Und gerade, dass sie am Ende doch unergründlich bleibt, hat für uns einen ewigen Reiz immer wieder zu ihr heranzugehen und immer wieder neue Einblicke und neue Entdeckungen zu versuchen."

From "Gespräche Goethes mit Eckermann"
Goethe 1831, one year before his death.

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Underlined page numbers signify title pages of chapters/papers dealing extensively with the respective genera.

CHAPTER 1

INTRODUCTION

1. BACKGROUND

The Anacardiaceae is a natural family consisting of some 60-80 genera (Cronquist, 1981). It is closely related to the Burseraceae and Julianaceae and broadly akin to the Simaroubaceae, Aceraceae, Coriariaceae, Sapindaceae and Hippocastanaceae (Cronquist, 1981; Dahlgren, 1983). Regarding seed characters it seems to be most similar to the Simaroubaceae (Corner, 1976). Engler (1892*) distinguished five tribes in the Anacardiaceae, namely Mangifereae, Spondieae, Rhoideae, Semecarpeae and Dobineeae. Except for the latter, the tribes and most of the genera conform to his earlier monographic treatment of the family. In this study Engler's (1892) spelling of tribal names is followed, which complies with the I.C.B.N.

A study of the taxonomy of the Anacardiaceae reveals some important questions. Whereas Cronquist (1981) regarded the Julianaceae as a separate family, Dahlgren (1983) included it in the Anacardiaceae. Dahlgren, however, distinguished the Dobineeae as a separate family, the Podoaceae, thereby confirming the suggestion of Airy Shaw (1973). In 1987 Wannan _et al._, however, recommended the retention of the Dobineeae within the Anacardiaceae. Airy Shaw (1973) in "Willis's Dictionary of the Flowering Plants and Ferns" also considers the Australian genus _Blepharocarya_ F. v. Muell. as the type of a separate family, which he established earlier. However, the Australian workers Wannan _et al._ (1985, 1987) and Jessup (1985) advocated the retention

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* Although the date 1897 is printed on the title page of this publication and was initially used in several publications of the present author, this fascicle 73 of Engler's "Natürliche Pflanzenfamilien" appeared in 1892 (Stafleu & Cowan, 1976).

of *Blepharocarya* in the Anacardiaceae, and demonstrated its close affinity with genera of the tribe Rhoideae. *Pistacia* L. is another genus that has been separated from the Anacardiaceae and was also regarded as the type of the family Pistaciaceae (Hou, 1978).

Because of its edible fruit and nuts, the Anacardiaceae is of substantial economic importance and has played a significant role in the affairs of man since the dawn of history. The cashew-nut (*Anacardium occidentale* L.), the mango (*Mangifera indica* L.), the hog-plum (*Spondias cytherea* Sonnerat) and the pistachio nut (*Pistacia vera* L.) are well-known and widely cultivated. Other species are grown more locally for their fruit, and the large trees of various genera supply valuable timbers. Species of *Rhus* L. and *Gluta* L. supply lacquers. Some species of *Schinopsis* Engl. and other species of *Rhus* are exploited for the extraction of tannins. Other members are notorious since resins in leaf, stem and fruit contain poisonous constituents causing dermatitis (Hou, 1978).

According to Hou (1978) and Cronquist (1981) the Anacardiaceae may be distinguished from related families by the combination of the following characters:

1. exstipulate leaves; 2. presence of secretory ducts; 3. presence of a mostly intrastaminal disc; 4. unilocular ovary with a single ovule; 5. anatropous ovule; 6. drupaceous fruits; and 7. exalbuminous seeds. Diagnostic chemical characters include the formation of phenols and anacard acid, or in their absence, the accumulation of resins and tannins in leaves and bark (Hegnauer, 1964).

2. SCOPE OF THIS PROJECT

The present project mainly deals with the development and structure of the fruit and seed of the southern African Anacardiaceae. Ten genera are represented in southern Africa. These belong to two of the five tribes, namely the Spondieae and the Rhoideae. Where more than one species occurs in a genus, the choice depended on the availability of material. Common names are from Von Breitenbach (1986) and author citations according to Gibbs Russel _et_ _al_. (1987). The following ten species were chosen for this investigation.

TRIBE SPONDIEAE

Harpephyllum Bernh. ex Krauss - (monotypic);

_H_. _caffrum_ Bernh. ex Krauss (Kaffir Plum, Wild plum, "Wildepruim").

Lannea A. Rich.;

_L_. _discolor_ (Sond.) Engl. (Tree Grape, Live-Long, "Dikbas").

Sclerocarya Hochst.;

_S_. _birrea_ (A. Rich.) Hochst. subsp. _caffra_ (Sond.) Kokwaro (Marula, "Maroela").

TRIBE RHOIDEAE

Heeria Meisn.; (1)

_H_. _argentea_ (Thunb.) Meisn. (Rockwood, "Kliphout").

Laurophyllus Thunb.- (monotypic);

_L_. _capensis_ Thunb. (Iron Martin, "Ystermartiens").

Loxostylis Spreng. f. ex Reichb. - (monotypic);

_L_. _alata_ Spreng. f. ex Reichb. (Tarwood, "Teerhout").

Ozoroa Del.;

_O_. _paniculosa_ (Sond.) R. & A. Fernandes (Common Resin Tree, "Gewone Harpuisboom").

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(1) The proposal by Fernandes & Fernandes (1965) to separate _Ozoroa_ from the Cape genus _Heeria_, has generally been accepted in South Africa. Dyer (1975) mentioned that possibly two species of _Heeria_, i.e. _H_. _argentea_ and _H_. _concolor_ (Presl) O. Kuntze, occur in southern Africa. However, De Winter (1974) proposed the new combination _Ozoroa_ _concolor_ (Presl ex Sond.) De Winter for the latter species. This leaves _H_. _argentea_ as the only southern African species of _Heeria_ (Gibbs Russell _et_ _al_., 1987). It remains to be seen whether the view of Fernandes & Fernandes (1965) can be substantiated by evidence from various sources. Besides numerous taxonomic problems on the generic and specific levels (some of these will be raised in different places in the thesis), it is clear that the taxonomy of this important family needs clarification.

Protorhus Engl.;

P. longifolia (Bernh.) Engl. (Red Beech, "Rooi Boekenhout").

Rhus; R. lancea L.f. (Karree, "Karee"); and

Smodingium E. Mey. ex Sond. -(monotypic);

S. argutum E. Mey. ex Sond. (Rainbow Leaf, "Pynbos").

The observations reported in this thesis focus mainly on the three species of the Spondieae and Rhus lancea.

Before the start of this project, the ontogeny and structure of the fruit of members of the Anacardiaceae had not been studied in detail. Roth (1977) mentioned the mature pericarp of the mango and described the mature structure of the "double fruit" of Anacardium occidentale. Recent studies, e.g. those of Boesewinkel (1987) and Bouman & Meijer (1987), re-emphasized the great taxonomic importance of seed characters. However, the information on the seed of this large family is tantalizingly inadequate (Corner, 1976).

The purpose of the present study is therefore not only to fill the gaps in the knowledge of the fruit and seed of the Anacardiaceae, but also to assess the taxonomic significance of the results, and to elucidate possible evolutionary relationships. An integration of the structural inquiries, including histochemical tests, with physiological and ecological aspects during and after germination of the seed is also attempted. The literature consulted during the study of the seed-coat of the indigenous species revealed that the ontogeny of the seed-coat of the exotic species, Mangifera indica (tribe Mangifereae) has not been investigated. The study of the mango ovule by Robbertse et al. (1986) disclosed new ovule characters in the Anacardiaceae. The reasons for including studies on Mangifera indica and a few other exotic taxa, such as Operculicarya decaryi H. Perr. and Tapirira guianensis Aubl. subsp. guianensis, in a thesis dealing with southern African taxa, are the following:

- to fill a definite gap in our knowledge of the Anacardiaceae;
- to supplement the mentioned ovule studies;
- to facilitate a comparison of the seed structure in the five tribes; and
- to assist in speculations on phylogenetic relationships.

3. STRUCTURING OF THESIS

This thesis consists of contributions in the form of published papers and manuscripts presented as photocopies. The manuscripts, have all been accepted for publication.

Chapters 2 to 7 deal with the tribe Spondieae, chapter 8 is concerned with the tribe Rhoideae, mainly the fruit of Rhus lancea, and chapter 9 deals with the seed of the tribe Mangifereae. The General Discussion in chapter 10 comprises a synthesis of the results with some additional unpublished data, as well as the available information from the literature regarding the fruit, seed and seed germination. It also includes phylogenetic considerations regarding the gynoecium, fruit, ovule and seed. Aspects that had received little attention previously are elaborated upon, e.g. ovule characters, especially the hypostase. The association of recalcitrance with particular ovule and seed characters, as well as habit and habitat is pointed out for the first time. The work on the Anacardiaceae served as the point of reference in these associations. Finally a speculation on the relative great age of the family is included in the General Discussion.

4. EXPLANATORY NOTES

The reader will notice some unfortunate irregularities in author citations: " A.Rich." and "Richard" both refer to A. Richard and "Sond." and "Sonder" to O. W. Sonder. Author citations in papers published in, or manuscripts accepted by, the Botanical Journal of the Linnean Society are given according to Meikle (1980). The manuscripts were submitted in the course of several years to two different journals and had to comply with different requirements. It will also be noticed that initially the term 'secretory canals' and later on 'secretory ducts' was used, the latter being more generally used in English papers.

The published papers contain a number of errors. All the corrections are listed separately in the Appendix. The Appendix also contains a paper of which I am co-author and two non-refereed papers published in a semi-scientific journal. Since papers and manuscripts corresponding to the various chapters, contain the relevant, 'Material and methods' a separate chapter on this topic was considered unnecessary.

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CHAPTER 2

PERICARP OF *Sclerocarya birrea* subsp. *caffra*

Botanical Journal of the Linnean Society (1986), *92:* 303–322. With 15 figures.

Development and structure of the drupe in *Sclerocarya birrea* (Richard) Hochst. subsp. *caffra* Kokwaro (Anacardiaceae), * with special reference to the pericarp and the operculum

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Received June 1985, accepted for publication September 1985

VON TEICHMAN, I. & ROBBERTSE, P. J., 1986. **Development and structure of the drupe in *Sclerocarya birrea* (Richard) Hochst. subsp. *caffra* Kokwaro (Anacardiaceae), with special reference to the pericarp and operculum.** * The development and structure of the exo-, meso- and endocarp of the drupe of *Sclerocarya birrea* subsp. *caffra* were examined. The mature exocarp comprises the outer epidermis with stomata and lenticels, subepidermal collenchyma and parenchymatous layers with secretory canals. This exocarp *sensu lato* develops from the outer epidermis and the outer layers of the ovary wall. The fleshy parenchymatous mesocarp or sarcocarp also contains secretory tissue. The mesocarp develops after endocarp differentiation and lignification. The developmental sequence within the pericarp corresponds to the general pattern in drupes. The endocarp or sclerocarp, which is not stratified, consisting mainly of brachysclereids, fibres and vascular elements, develops from the inner epidermis and adjacent tissue of the young ovary wall including the procambium strands. The operculum represents a well-defined part of the endocarp. Early in its development a parenchymatous zone already clearly demarcates the operculum. The literature on the pericarp of the Anacardiaceae drupe is discussed to establish the diagnostic value of these morphological characteristics for future taxonomic studies.

ADDITIONAL KEY WORD:—Anatomy.

CONTENTS

INTRODUCTION

Sclerocarya birrea (Richard) Hochst. subsp. *caffra* Kokwaro is one of the best * known and most highly valued indigenous fruit trees in southern Africa. Its common name, 'marula', will be used throughout this paper. *Sclerocarya* belongs * to the tribe Spondieae in the family Anacardiaceae (Scholz, 1964). It is one of

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*for corrections
see Appendix

the 10 genera of this family indigenous to southern Africa. Although not yet of such renowned economic importance as the closely related *Mangifera indica* L. (mango) and *Anacardium occidentale* L. (cashew nut), the marula has definite economic potential. A survey of the literature and other sources regarding the various uses of the marula and the possible pharmacological importance of the bark has previously been compiled (Von Teichman, 1983). Marula fruit juice and Amarula liqueur, prepared mainly from the mesocarp of the fruit, are already commercially available. Realizing this potential of the marula, selective breeding towards improvement of the wild stock is being undertaken by Prof. L. C. Holtzhausen (University of Pretoria). Propagation of the marula is by cuttings and seed.

The fruit of the marula is a specialized stone fruit. According to Roth (1977) a stone fruit or drupe represents a highly developed fruit type. The pericarp is differentiated into the exo-, meso- and endocarp; the latter is usually stony and protects the seed. Hill (1933, 1937) described several devices which have evolved to permit the escape of the embryo from the stony endocarp of stone fruits during germination. In *Sclerocarya* and some other genera of the Spondieae, as well as in members of other families, a specialized structure, the operculum, is found. The importance of the operculum during germination was confirmed during a recent study of the germination behaviour of the marula (Von Teichman, Small & Robbertse, in press). We know of no literature covering the development and structure of the operculum of the drupe in the Anacardiaceae.

Several authors have reported, amongst other aspects, on the pericarp structure of various drupes of the Anacardiaceae, particularly in species of economic or pharmacognostic importance. Herzog (1910) discussed the genera *Mauria* and *Euroschinus,* while Magen (1912) studied *Pistacia chinensis* Bunge, three species of *Schinus*, one *Lithraea* and one *Cotinus* species, as well as several *Rhus* species. Copeland & Doyel (1940) reported on *Toxicodendron diversiloba* (Torrey & Gray) Greene. Copeland (1955, 1959, 1961) also studied species of *Pistacia, Schinus* and *Anacardium.* Roth (1974, 1977) discussed an *Anacardium* species and published extensive data on the fruits of the Angiospermae. *Mangifera indica* was studied by several authors. There are no reports of pericarp anatomy of the genera in the tribes Spondieae, Semecarpeae and Dobineeae (Engler, 1897; Scholz, 1964). No literature is available on the southern African genera regarding either the development and structure of the fruit or the chemical and physiological changes occurring in the developing and ripening fruit.

MATERIAL AND METHODS

Flower buds, flowers and fruit in various developmental stages were collected from trees growing naturally on a northern slope of the Magaliesberg range near Pretoria.

Some material was preserved in FAA. For semithin sections, most of the material was immediately fixed in 6% glutaraldehyde in sodium cacodylate buffer (0.05 mol dm^{-3}). It was dehydrated, infiltrated and embedded in glycol methacrylate (GMA) according to Feder & O'Brien (1968). However, the monomer mixture consisted of 94% (v/v) hydroxyethyl methacrylate (i.e. the

94% HEMA was purified to remove most of the methacrylic acid), 5% (v/v) polyethylene glycol 200, and 0.1% (w/v) azobis-isobutyronitrile or later 0.6% (w/v) benzoyl peroxide. GMA sections, 2–3 µm thick, were cut on a Porter Blum MT-1 or Reichert OM U3 ultramicrotome. The 'Periodic Acid–Schiff Reaction' (PAS) was carried out according to Feder & O'Brien (1968) using 0.5% dinitrophenyl hydrazine (DNPH) in 15% acetic acid (for 30 min) as blocking agent. Sections were counterstained for 1–5 min in 0.05% toluidine blue O in benzoate buffer, pH 4.4 (Sidman, Mottla & Feder, 1961).

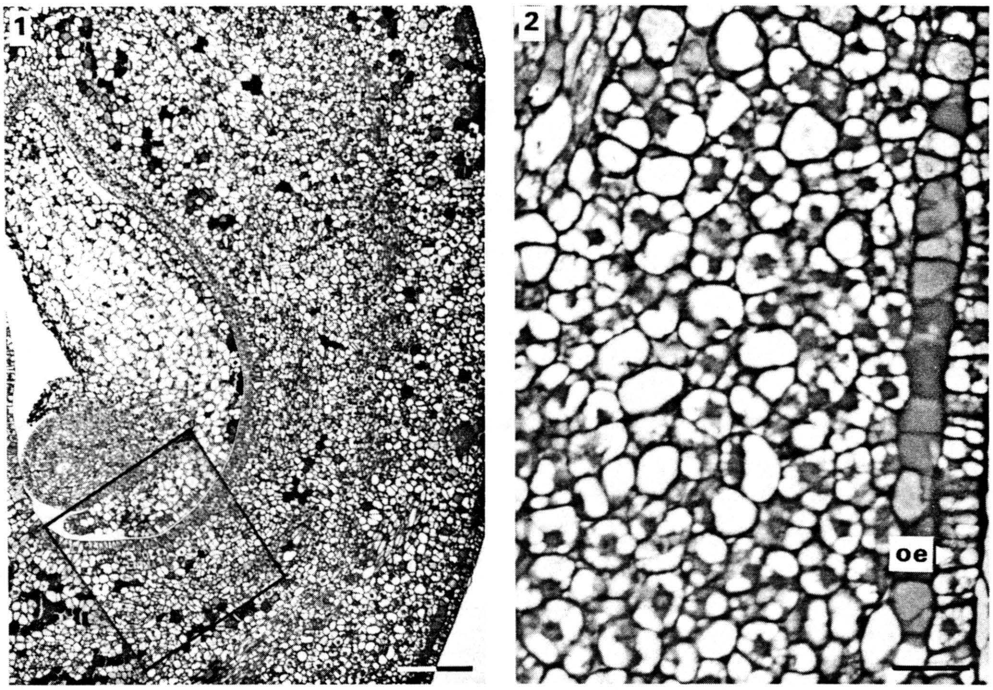
Other sections were stained for fatty substances, cutin and suberin, using sudan black B, and for proteins using acid fuchsin as well as amido black 10B as described by Von Teichman, Robbertse & Van Der Merwe (1982). The hydroxylamine–ferric chloride reaction (Jensen, 1962) was carried out to determine pectic substances in hand sections. To stain lignified cell walls, sections were mounted in a drop of saturated phloroglucinol in 20% HCl. Unstained GMA sections and hand sections were observed with phase-contrast optics or were examined under crossed polarizing filters.

For the study of the mature stony endocarp longitudinal and transverse sections were prepared as follows: thin slices of the stone were ground on a Standard Polisher Grinder (Buehler No. 41–1500). These were mounted on a glass slide using a Buehler epoxide resin mixed with a Buehler epoxide hardener. The resin was polymerized in an oven at about 50°C. The second grinding stage produced a second surface parallel to the first. The final thickness of the sections was about 30–45 µm. After a rinse in 50% ethanol, the sections were stained in safranine O made up according to Johansen (1940) for about 20 min. A water rinse was followed by a 2 min rinse in methyl cellosolve and a 1 min counterstaining in fast green (saturated in 90% ethanol). Finally, the sections were taken through three rinses in 100% *n*-butanol and two 5 min rinses in 100% xylol. Cover slips were applied using a drop of Canada Balsam.

Material preserved in FAA was used to prepare longitudinal sections of very young fruit and to dissect ovules from ovaries, the latter being cleared using a clearing fluid (Herr, 1971). Small pieces of the stony endocarp and pieces of the stony operculum were macerated by heating in a mixture of 50 cm^3 concentrated nitric acid and 1 g potassium chlorate. The macerated tissue was rinsed several times with distilled water before being mounted in the latter and viewed with phase-contrast optics. The photomicrographs of the semithin sections were taken either with a Leitz Orthoplan or a Reichert Univar microscope.

OBSERVATIONS

Most marula trees are dioecious. In an arbitrary sample of 119 trees, Baijnath (1983) found only 15 to be monoecious. These were predominantly male flowered, the female flowers being borne with male flowers on the basal one or two inflorescences of a particular twig. The female flowers generally have five crimson sepals and five pink to light crimson petals. A disc is present as well as about 21–26 staminodes. The ovary is usually bicarpellate; one or three carpels are frequent and rarely four locules are found. Each ovary locule can contain one pendulous ovule with a dorsal raphe at the tip of a massive funiculus. Shortly before anthesis the ovule with its two integument primordia (Fig. 1) is still in the process of bending towards the anatropus condition. *



Figures 1 & 2. Fig. 1. *Sclerocarya birrea*. LS of an ovary before anthesis illustrating the ovary wall, the pendulous ovule and massive funiculus. The framed part is magnified in Fig. 3; scale bar = 100 µm. Fig. 2. Magnification of the section illustrated in Fig. 1 showing the outer epidermis (oe), subepidermal cells, many of which were tanniniferous, and adjacent parenchyma. Scale bar = 20 µm.

In the young ovary wall (Figs 1–3) the following cell layers and zones could be distinguished:

the outer epidermis in which predominantly anticlinal cell divisions occurred (Fig. 2)

the subepidermal cells, many of which were tanniniferous (Fig. 2)

a parenchymatous zone, which in some sections already showed the occurrence of secretory cavities

another deeper-situated parenchymatous zone with many procambium strands (Fig. 3) which had partly differentiated into xylem vessels

a narrow layer of parenchyma cells which stretched up to the inner epidermis (Fig. 3), and often also contained tannin-like substances

the inner epidermis in which predominantly anticlinal cell divisions occurred (Fig. 3), although periclinal divisions were also observed.

In slightly older ovaries the operculum was already demarcated by the parenchymatous cells of the fifth zone mentioned above (Figs 4, 6, 7). Numerous cell divisions had taken place in the parenchyma constituting the bulk of the operculum at this stage (Fig. 4). These ovary walls contained numerous secretory cavities, some of which had become secretory canals (Fig. 5). These cavities developed schizogenously and were lined with epithelial cells. Vascular tissue never occurred in close association with the secretory canals and cavities. In the outer part of the zone with procambium strands anastomoses between

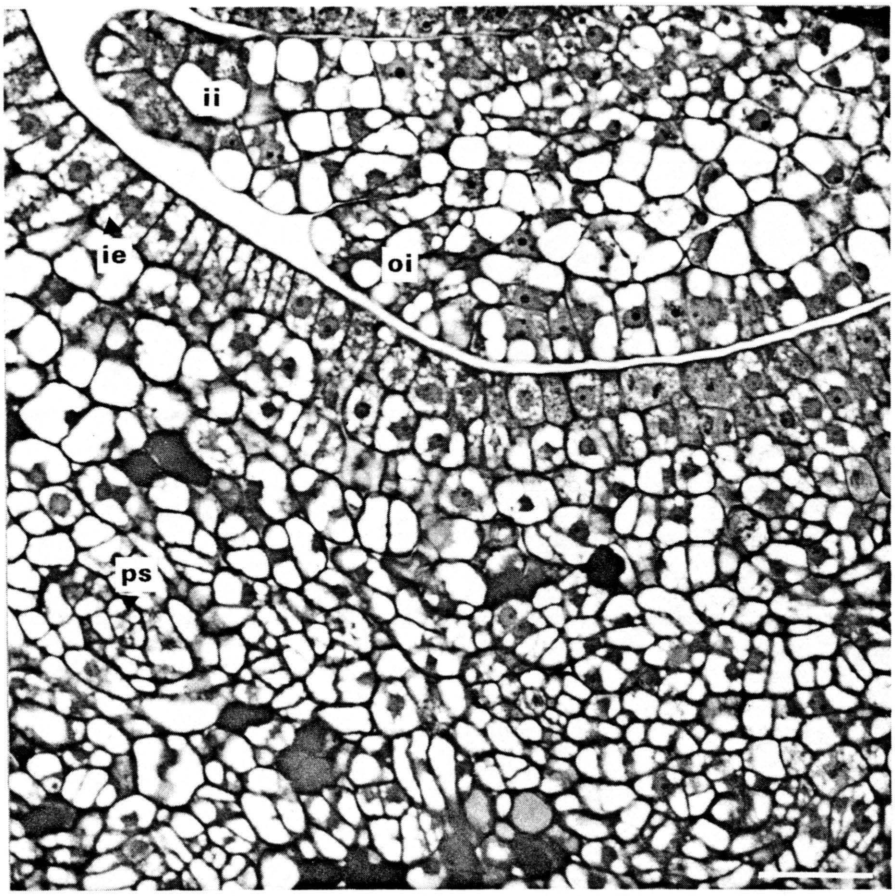


Figure 3. Magnification of the part framed in Fig. 1, illustrating detail of the outer (oi) and inner (ii) integument, the inner epidermis (ie) of the ovary wall and adjacent parenchyma with tanniniferous cells and procambium strands (ps). Scale bar = 30 µm.

these strands could be seen. The derivatives of the procambium cells formed bundles of narrow, elongated, thin-walled fibre initials of the future endocarp (Fig. 5).

In ovaries with fully developed ovules (Figs 6, 8) the following features were clearly discernible: the operculum had developed within the future endocarp opposite the prominent knee of the funiculus which may be called a ‘ponticular outgrowth’. This ‘ponticular outgrowth’ and other characteristic features of the ovule, especially the outer integument are discussed by Robbertse, Von Teichman & Van Rensburg (1986). A zone of parenchyma containing tanniniferous cells demarcated the operculum and also lined the ovary locule (Fig. 6). The part of the ovary wall adjacent to this zone contained numerous bundles of fibre initials interspersed with groups of more or less isodiametric parenchyma cells of the ground tissue. These cells which were often tanniniferous represented the sclereid initials of the future endocarp (Fig. 7). A number of the parenchyma cells in the operculum tissue itself had also elongated. These cells represented the fibre initials, while most of the

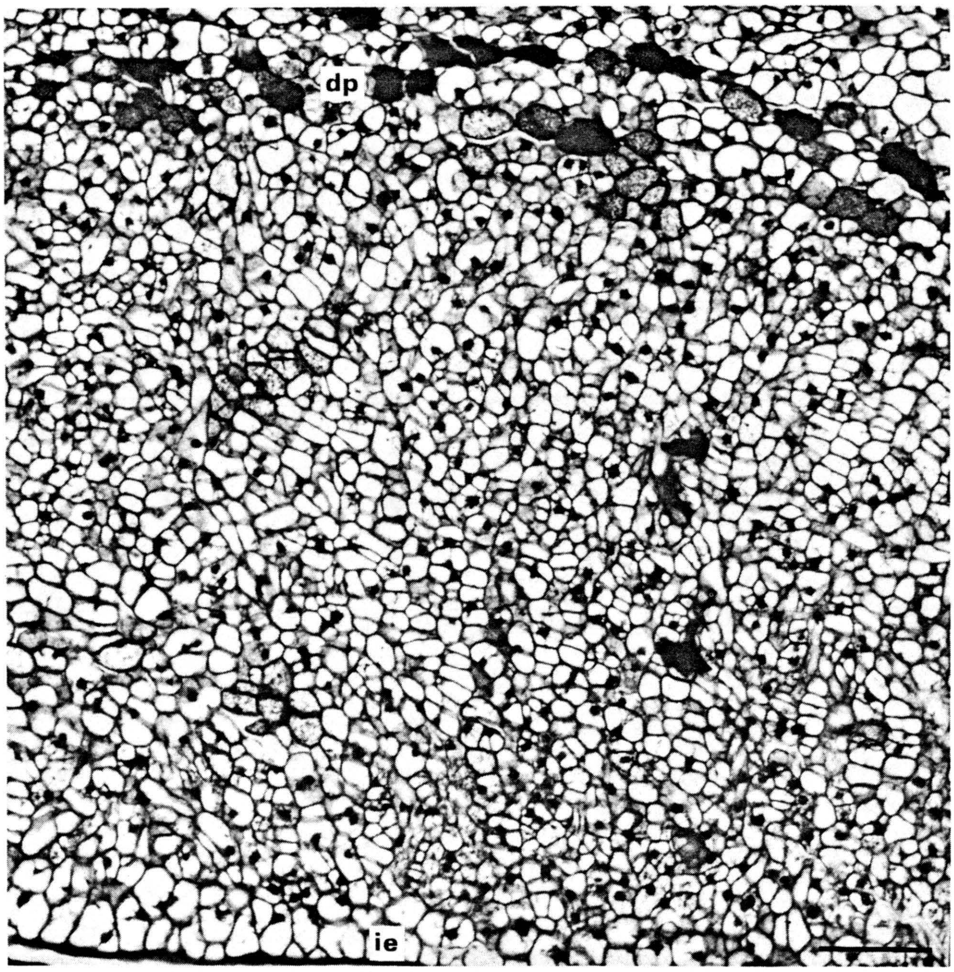


Figure 4. A part of the parenchymatous operculum with demarcating layers of parenchyma (dp) and inner epidermis (ie). Scale bar = 50 µm.

tanniniferous cells and large, isodiametric parenchyma represented the sclereid initials of the operculum (Fig. 7).

It was concluded that the operculum developed from the innermost epidermis of the ovary wall, as well as derivates of the subepidermal cells, but not the * parenchyma zone including the procambium strands as was the case for the rest of the endocarp. The operculum, which was made up of endocarp tissue, therefore had a slightly different origin from the rest of the endocarp. Figure 8 illustrates that the operculum does not include the placental tissue. This also explains why neither procambium strands nor differentiated vascular tissue could be distinguished in the operculum at any stage. In the very young fruit, which measured about 9.4 × 7.5 mm, the ovule and the funiculus occupied more than half of the ovary locule.

The next stage in the development of the pericarp is illustrated in Fig. 9. The outer epidermis, together with the subepidermal parenchyma, as well as the

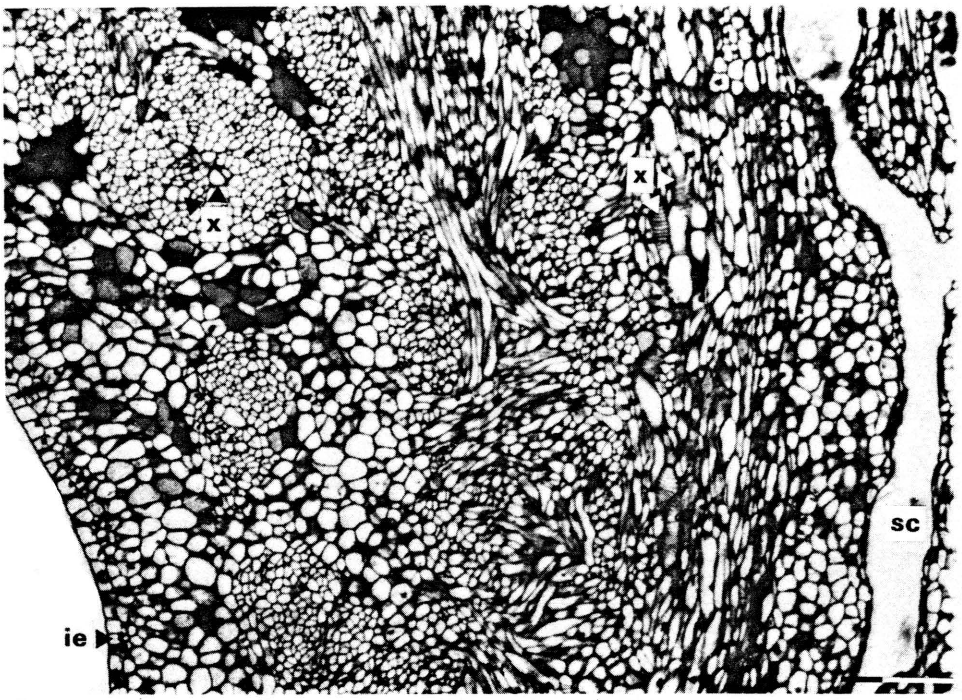


Figure 5. LS of an older ovary wall with a part of a secretory canal (sc). Differentiated xylem elements (x) and derivatives of the procambium cells occurring in bundles are shown. Nearer to the inner epidermis (ie) these bundles are cut in a transverse plane. Scale bar = 70 μm.

outer part of the zone with secretory cavities and canals, constituted the exocarp. The parenchymatous mesocarp initials could not be distinguished, but at this stage were represented by the inner part of the zone with secretory cavities and canals. Figure 9 also shows some characteristic features of the endocarp. The latter included the inner epidermis, the subepidermal parenchyma, groups of sclereid initials as well as numerous intertwining and anastomosing bundles of fibre initials. The width of the endocarp had increased markedly since the young fruit stage mentioned above. The fruit now measured about 20 × 15 mm. The very small seed with the massive funiculus occupied less than half of the fruit locule. The epidermis of the exocarp had just started to elongate radially and had accumulated tannin-like substances (Fig. 10). Stomata were more or less evenly distributed over the whole surface of the fruit. The operculum at this stage also consisted mainly of elongated intertwining bundles of fibre initials and groups of sclereid initials.

The mesocarp had begun to develop after the differentiation of the endocarp was more or less complete. The lignification of the endocarp had proceeded to such an extent that good semithin sections were unattainable. A radial elongation of the 8–10 cell layers of mesocarp initials was the beginning of the mesocarp development. At this stage the fruit measured about 30 × 20 mm and the very young seed, which was suspended from the end of the large funiculus, occupied only approximately one-third of the fruit locule. The fruit dimensions at this stage were about 75% of those of the mature fruit, while the seed

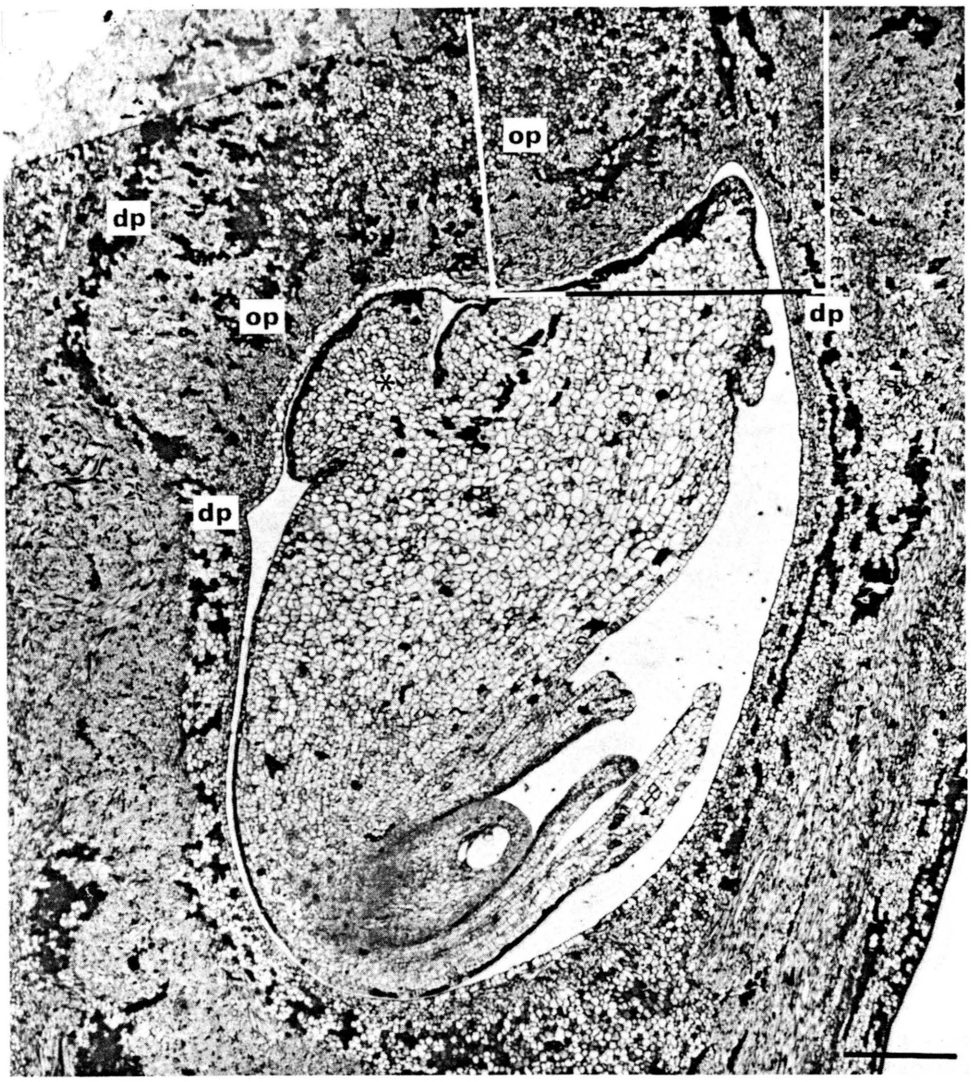


Figure 6. LS of an ovary with a fully developed ovule. The demarcating parenchyma layers (dp) surrounding the operculum (op), parts of the ovary wall and the 'ponticular outgrowth' (asterisk) are easily distinguished. Scale bar = 300 μm.

dimensions were only about 33% of those of the mature seed. The pericarp had therefore enlarged at a greater rate than the seed. The width of the exocarp at this stage was more than twice that of the mesocarp. The embryos in these seeds were either spherical or heart-shaped. In the larger embryos, 2.0 × 1.5 mm, cotyledons could be distinguished.

The fruit is abscissed when ripening commences so that final ripening of the mature fruit takes place on the ground. The fully ripened yellow fruit has a pleasant and characteristic odour. The shape and variation in dimensions of the fruit are illustrated in Fig. 11. The raised, brown remnants of the styles and stigmas as well as the minute brown lenticels could be distinguished. Figure 12 shows a transverse section of a ripe fruit. The exocarp and endocarp each

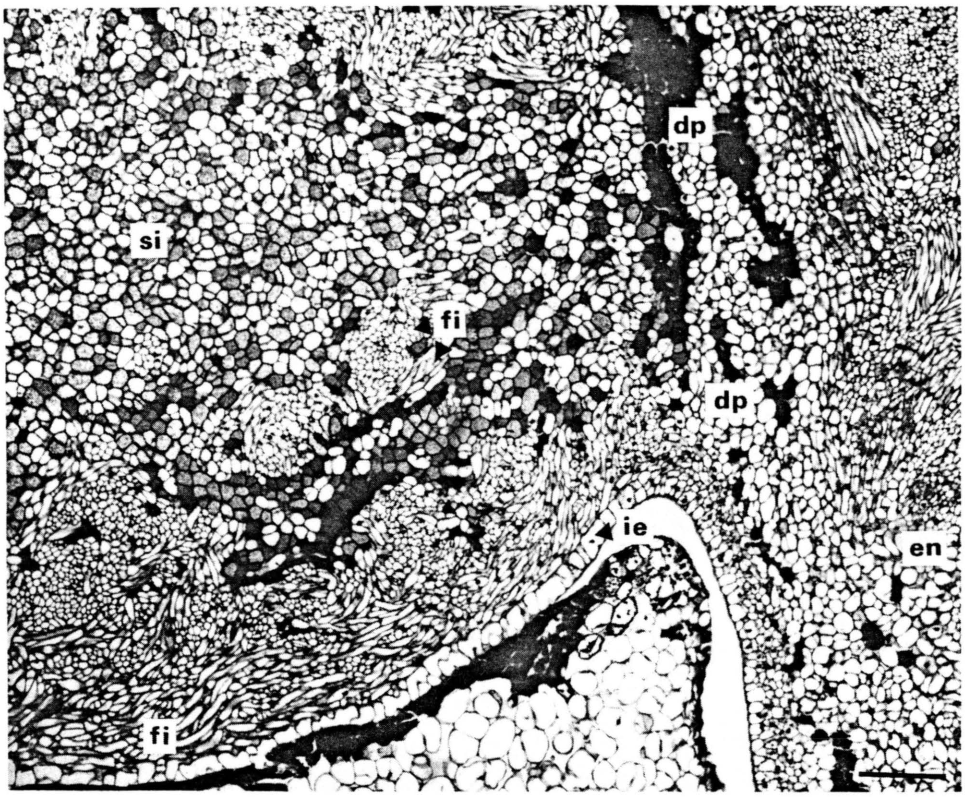


Figure 7. Higher magnification of framed part in Fig. 6. The parenchyma layers demarcating the operculum (dp), the fibre initials (fi), the sclereid initials (si) in the operculum as well as in the rest of the endocarp (en), and the inner epidermis (ie) are illustrated. Scale bar = 100 µm.

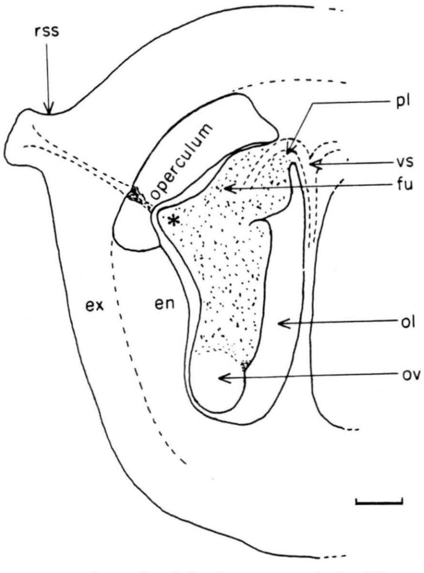


Figure 8. Diagrammatic representation of a LS of a young fruit. The ovule (ov) with 'ponticular outgrowth' (asterisk), and massive funiculus (fu), lying in the fruit locule (ol), the placental tissue (pl) and vascular tissue (vs) are shown. The thick exocarp (ex), endocarp (en), operculum and remains of stigma and style (rss) are also illustrated. Scale bar = 0.9 mm.

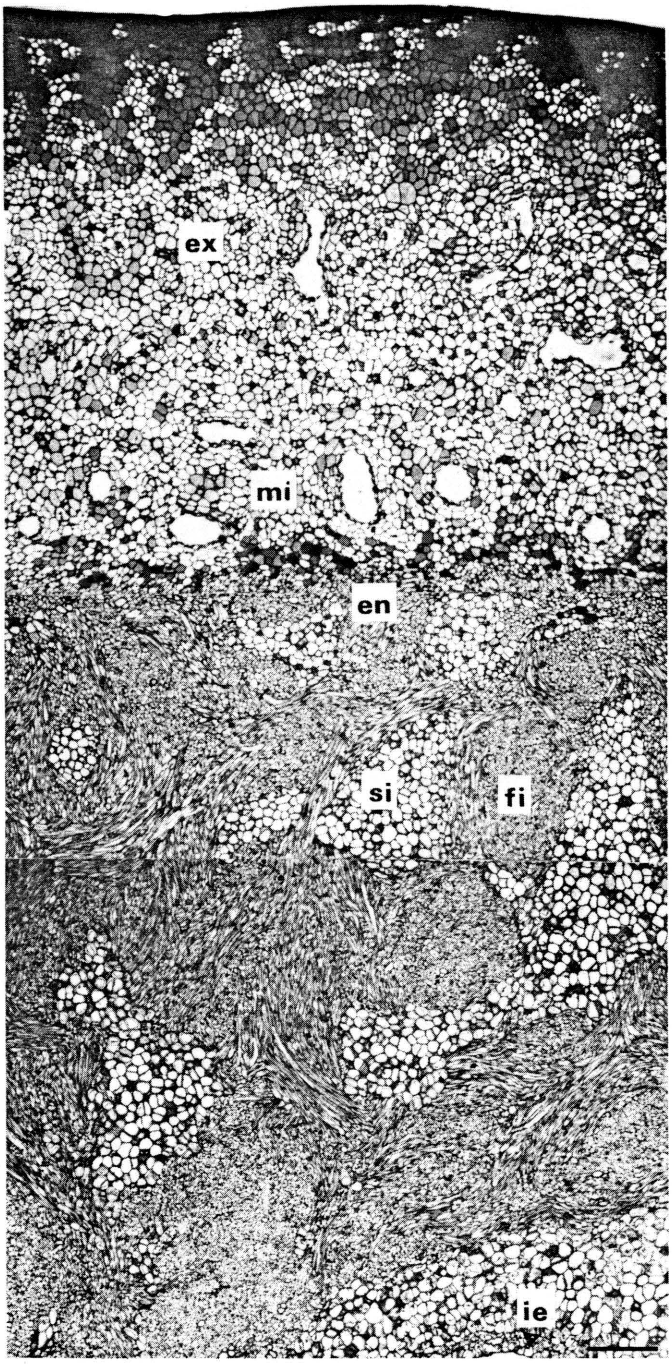


Figure 9. TS of the pericarp of a young fruit in which the endocarp (en) with fibre initials (fi), sclereid initials (si) and inner epidermis (ie) is well defined. The zone with mesocarp initials (mi) is represented by the innermost part of the exocarp (ex). Scale bar = 100 μm.

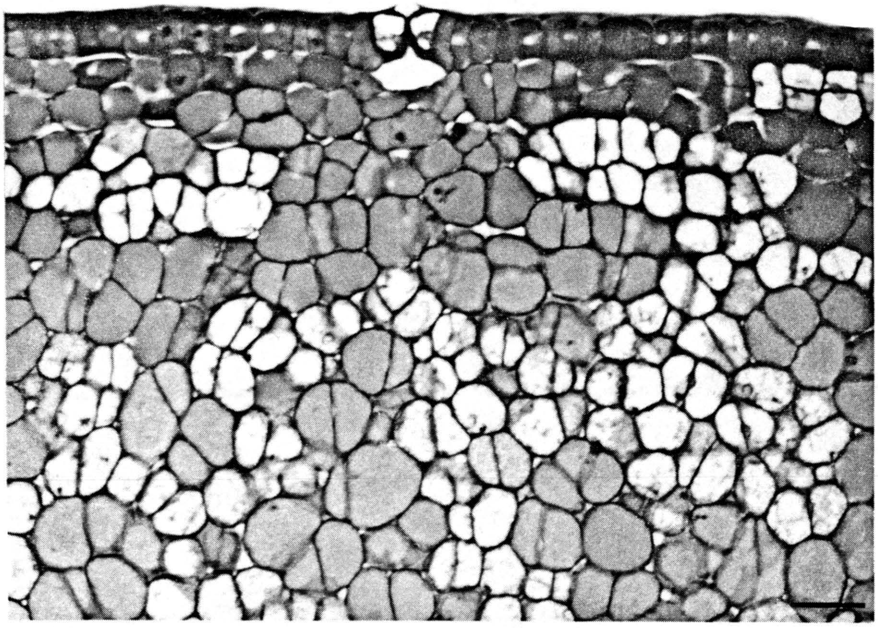


Figure 10. TS of a part of the exocarp of a young fruit to illustrate the stomata in the outer epidermis, and the presence of tannin-like substances in the latter and adjacent parenchyma. Scale bar = 20 µm.

comprised about 27% of the pericarp, while the mesocarp represented 46%. The epidermal cells of the exocarp had elongated and the outer walls as well as the outer part of the anticlinal walls were heavily cutinized (Figs 13, 14). A relatively thick cuticle which stretched far into the substomatal chamber (Fig. 14) was also present on these outer walls. The subepidermal parenchyma had become collenchymatous (Fig. 13). The cell walls of the latter and the parenchyma cells of the outer part of the exocarp gave a positive reaction for pectic substances. The exocarp cells are generally rich in tannin-like substances.

The parenchyma cells beneath some stomata gave rise to lenticels. As the cells of the phellem-like filling tissue lie in radial rows (Fig. 14) these phellem-like cells originated from a meristemoid phellogen by means of periclinal divisions. The suberized cell walls of the phellem-like cells showed the typical staining reaction of suberin with sudan black B. In the inner thin-walled parenchymatous exocarp secretory cavities and canals occurred. These were remarkably numerous in the transition zone between the exocarp and mesocarp, resulting in mechanically weak layers. The exocarp of the ripe fruit represents the relatively tough and somewhat leathery 'peel'. Ballschmieter & Torline (1973) reported that the characteristic flavour of the fruit is contained within this 'peel'. Recently the volatile flavour components of marula juice have been identified chromatographically (Pretorius *et al.*, in press).

In the mesocarp the secretory cavities appeared radially elongated to keep pace with the stretching of the adjoining parenchyma. The chemical nature of the secretions produced by the epithelial cells lining these cavities and canals was not elucidated. Generally, the secretions stained blue with toluidine blue O,

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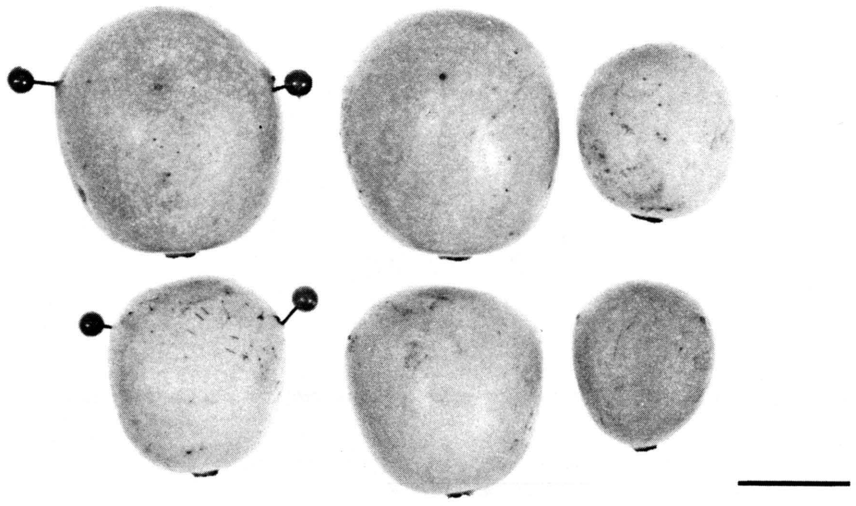

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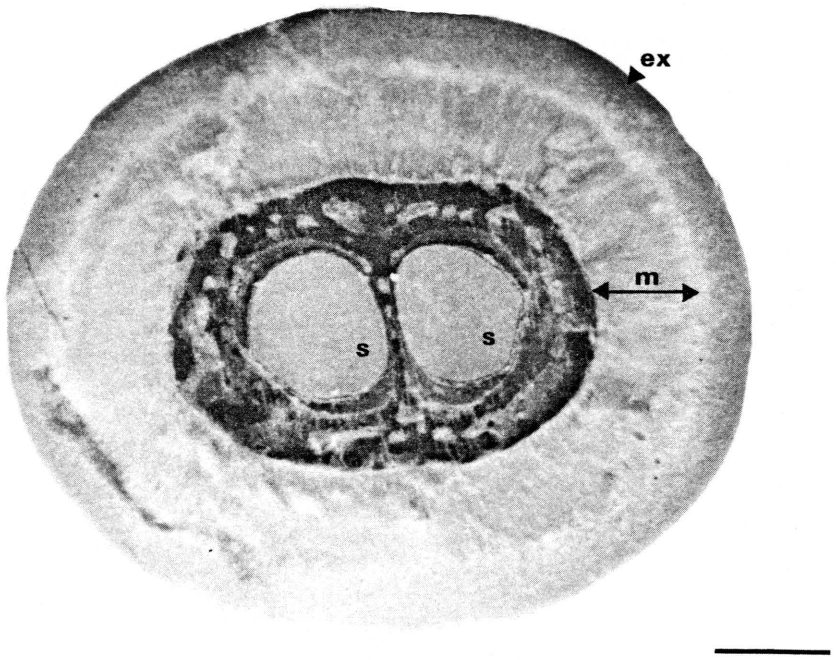

Figures 11 & 12. Fig. 11. Mature marula fruit collected in the field illustrating the remnants of styles and stigmas (near tips of needles), variation in size and shape. Scale bar = 20 mm. Fig. 12. TS of a mature marula fruit, illustrating two fruit locules with seeds (s), the well defined endocarp, the mesocarp (m) and exocarp (ex). Scale bar = 42 mm.

indicating the possible presence of polyphenolic compounds. Small rod-shaped inclusions were seen in these secretions. They showed neither birefringence with polarization microscopy nor exhibited phase contrast effects. However, these crystal-like structures gave positive results with acid fuchsin and amido black 10B indicating that they were probably proteinaceous.

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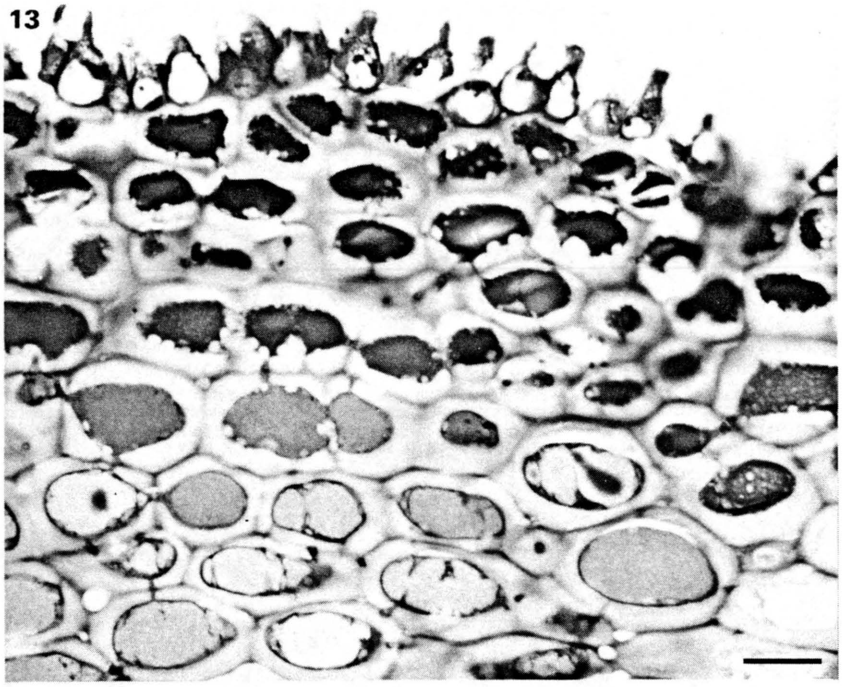

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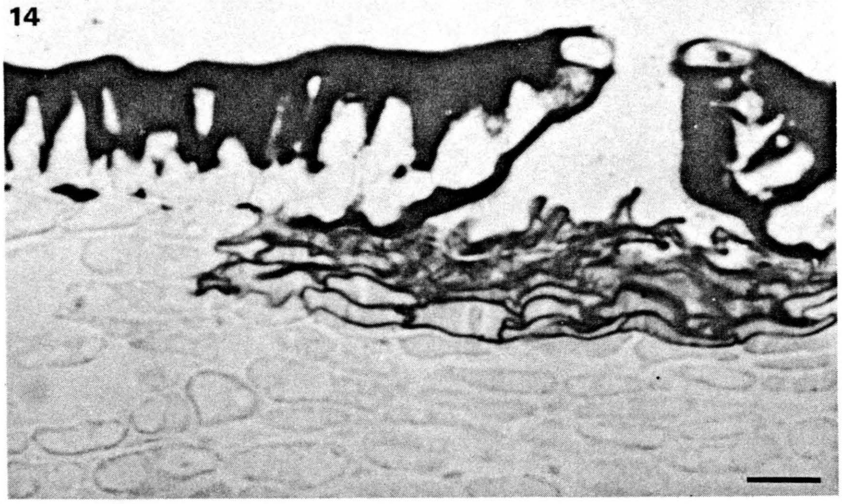

Figures 13 & 14. Fig. 13. TS of a part of the mature exocarp illustrating the epidermis and adjacent collenchyma cells. Scale bar = 20 μm. Fig. 14. TS of a part of the mature exocarp stained with sudan black B illustrating the cuticle, cutinized cell walls and the phellem-like filling tissue. Scale bar = 20 μm.

The mature mesocarp consisted mainly of thin-walled, severely stretched parenchyma. In contrast, the transition zone between the fleshy mesocarp and the stony endocarp consisted mainly of isodiametric sclereids. After fermentation of the ripe fruit the mesocarp could easily be removed, while these sclereids adhered tightly to the stone.

Figure 15 illustrates a longitudinal section of a ripe marula stone. The

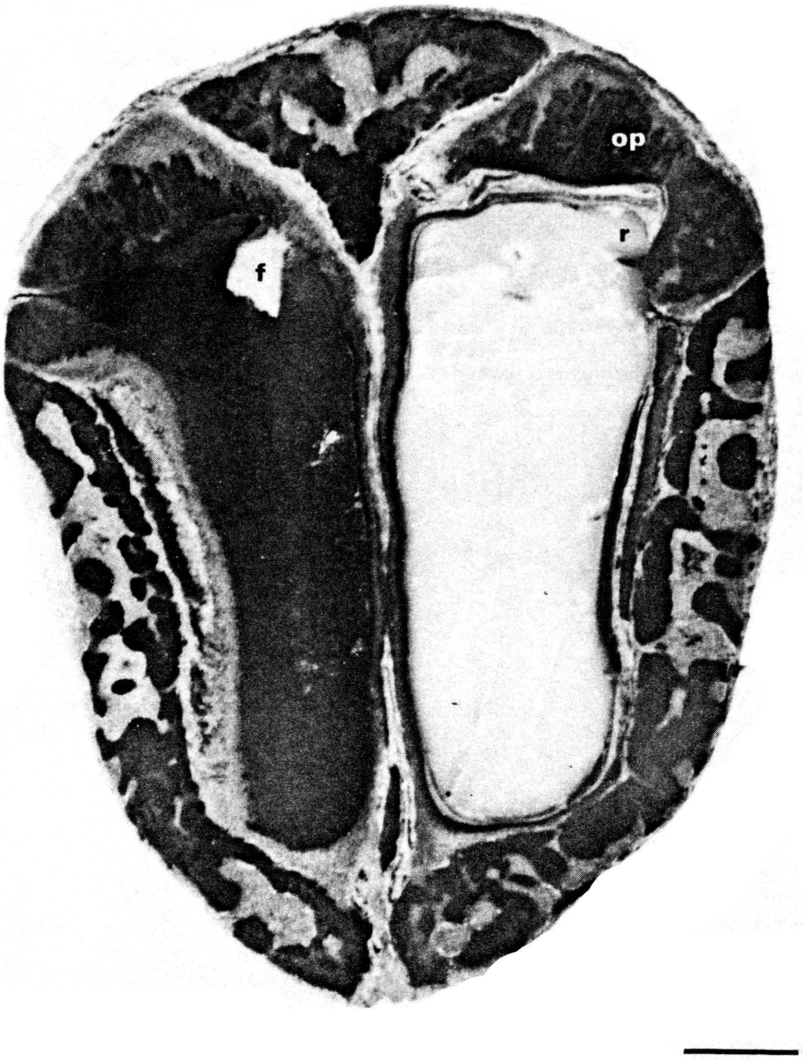

Figure 15. LS of a mature marula stone illustrating the operculum (op), a part of the funiculus (f) in an empty fruit locule, a seed with radicle (r) adjoining the operculum and the rest of the endocarp. Scale bar = 95 mm.

parenchymatous demarcating zone around the operculum can be distinguished clearly. In nature this zone, which apparently contained no lignified elements, is weathered and/or decayed, resulting in a loose operculum in old stones. During germination the operculum is then easily pushed out by the radicle. The dark, brown part of the operculum (Fig. 15) and remaining endocarp consisted mainly of brachysclereids which contained tannin-like substances. The lighter parts mainly represented intertwining, thick-walled, non-septate fibres in bundles and brachysclereids without tannin-like substances. The brachysclereids (Esau, 1977) were irregularly shaped and showed remarkable variation in form and size, sometimes with short lobes or arms diverging from the central body. In relation to the thickness of their cell walls, the cell lumens were generally large.

DISCUSSION

Exocarp: According to Esau (1977: 431) the term pericarp "in a strict sense, means the matured ovary wall", but may be extended to include extracarpellary tissue. Roth (1977: 9) stated that the exocarp (or epicarp), which has a protective function, "may consist only of the outer epidermis of the pericarp". This is the general definition for the exocarp used by Roth (1977). In the marula the exocarp consists of the outer epidermis and a large number of collenchymatous or parenchymatous cell layers. The latter represents an exocarp in the broader sense, as in this case "also sub-epidermal layers are included in exocarp formation" (Roth, 1977: 9). In *Mangifera* (Roth, 1977) and *Semecarpus anacardium* L. fil. (Weber, 1907) the exocarp consists of the outer epidermis as well as several layers of collenchyma and parenchyma respectively. In *S. anacardium* secretory cavities and vascular bundles occur in this hypodermal parenchyma. In some species of *Anacardium* (De Paula & Alves, 1973; De Paula & Heringer, 1978; Roth, 1974; Weber, 1907), *Buchanania lanzan* Spreng. (Mitra & Mehrotra, 1980), *Rhus typhina* L. (Bogaciński & Molski, 1969; Copeland & Doyel, 1940; Tabata, 1907) and *Schinus molle* L. (Copeland, 1959; Soos & Hausknost, 1951) the exocarp is represented by the outer epidermis and sometimes an additional hypodermal layer.

Therefore, the structure of the marula exocarp is similar to those of *Mangifera* and *Semecarpus anacardium.* In comparison with the exocarp of some species of the tribe Anacardieae and most species of the tribe Rhoideae the marula exocarp is exceptionally thick. The function of the latter is not only protection. It is, together with the mesocarp, the seat of the characteristic marula odour and flavour. These characteristics are possibly connected with the presence of the pectic and tannin-like substances, as well as the secretions produced by epithelial cells of the secretory cavities and canals.

In the exocarp of the mature marula fruit both stomata and lenticels are present. Sinha (1945) reported that in the mango fruit, lenticel development proceeded without involving a cambium. Unlike Sinha (1945) we found that in the marula fruit a meristemoid phellogen gives rise to the phellem-like filling tissue of the lenticel.

Mesocarp: Roth (1977) calls the mesocarp a sarcocarp when it is a fleshy parenchymatous tissue. In the Anacardieae the generally parenchymatous mesocarp constitutes the main part of the pericarp in the mature fruit. A very characteristic feature in species of *Anacardium* and *Buchanania* is the large secretory cavities, which lie close to one another, usually forming a circle as seen in a transverse section of the fruit. In *Anacardium* (Roth, 1974) and *Mangifera* (Joel, 1980) the vascular bundles are closely associated with the secretory cavities. In *Buchanania lanzan* fibro-vascular bundles are irregularly dispersed through the mesocarp (Mitra & Mehrotra, 1980).

In several genera of the Rhoideae, smaller and/or larger secretory canals, usually associated with the vascular bundles (Grundwag, 1976), also characterize the mesocarp. In contrast to some genera of the Anacardieae (e.g.

Anacardium and *Buchanania*) and several genera of the Rhoideae, the secretory canals in the marula and *Mangifera* are inconspicuous in the fleshy, parenchymatous mesocarp or sacrocarp. In the marula the vascular bundles are * not associated with the secretory canals.

Secretions occurring in the exo- and mesocarp: According to Esau (1977), secretory cells, cavities and canals are useful for diagnostic purposes in taxonomic work. The same applies to ergastic substances such as starch, tannins and crystals and the secretions occurring inside the cavities described above.

The presence of resin canals in the twigs and other organs is generally considered as one of the very characteristic features of the Anacardiaceae (Engler, 1897). This could imply that 'resins' are present in the secretory canals of fruit and other organs. However, Trease & Evans (1978: 410) stated that resins which constitute complex mixtures "are often associated with volatile oils (oleo-resins), with gums (gum resins) or with oil and gum (oleo-gum-resins)". The term 'secretion' is therefore preferred for the products of the secretory cavities and canals of the marula fruit, until these products have been analysed. Regarding the pericarp of the drupe of the Anacardiaceae, the secretory canals and/or cavities occur in the exocarp and/or the mesocarp. So far no detailed analysis of the secretions found in the pericarp of genera of the tribe Spondieae seems to be available. The secretions in the marula exo- and mesocarp probably contain polyphenolic compounds and proteinaceous crystal-like granules. Molisch (1931) found colourless, tombstone-like protein crystals in the milky secretions of the shoots of the mango and a species of *Odina, Schinus molle* and species of *Rhus.* Hegnauer (1964) and authors like Tischer, Illner & Seidl (1941) mention various types of secretions found in fruits of the Anacardiaceae, namely volatile oils, balsams, skin irritants and mucilages or gum-resins. In the parenchyma cells of the mesocarp of *Mangifera indica* starch grains occur (Roth, 1977). In *Anacardium occidentale* such cells contain druse crystals of calcium oxalate (Roth, 1974), while Mitra & Mehrotra (1980) noticed rosette crystals of calcium oxalate in *Buchanania lanzan.* Crystals occur very frequently in the 'transition layer' between the meso- and the endocarp.

Phenolic compounds, for example, tannins, occur in many fruits and have a definite influence on their flavour because of their astringency. In the fruit of the Anacardiaceae the occurrence of phenolic compounds or tanniniferous cells is a common phenomenon in the exocarp and/or mesocarp of the genera discussed. However, in the marula phenolic compounds occur especially in the tissues of the exocarp and the endocarp. The chemical nature of the secretions in the Anacardiaceae fruit is very complex and diverse.

Transition layer between the meso- and endocarp: Although not emphasized by previous authors, a very characteristic transition layer (i.e. between mesocarp and endocarp) seems to occur in many genera. This layer can consist of the following:

thickened, lignified cells, interrupted by thin-walled parenchyma as in *Anacardium* species (Weber, 1907)

parenchyma or lignified parenchyma cells with crystals, sometimes of calcium oxalate, occurring in *Pistacia* species (Grundwag, 1976), *Schinus molle* (Soos & Hausknost, 1951) and species of *Rhus, Lithraea* and *Cotinus* (Magen, 1912), as well as a *Toxicodendron* species (Copeland & Doyel, 1940) isodiametric sclereids in *Rhus typhina* (Bogaciński & Molski, 1969). In the marula pericarp a transition layer of sclereids tightly adhering to the endocarp occurs.

Endocarp: The endocarp protects the seed and together they form the stone (or pyrene) of the drupe, which is usually the dissemination unit. The endocarp, which is lignified and stony, may be referred to as the sclerocarp. If "only the inner epidermis of the pericarp corresponds to the endocarp" it is an endocarp "*sensu stricto*" (Roth, 1977: 9). Often subepidermal layers are "involved in the formation" of the endocarp, which then represents an endocarp "*sensu lato*" (Roth, 1977: 9). She also made the distinction between a homogeneous and heterogeneous endocarp. In the first case only fibres or sclereids or parenchyma constitute the endocarp and in the latter two or three of these cell types occur.

Two distinct kinds of endocarp have been distinguished in drupes of various genera of the Anacardiaceae. Most species studied have a stratified endocarp. It consists of several layers usually formed by the inner epidermis of the ovary wall by means of periclinal divisions. These layers may consist of the following:

a layer of lignified macrosclereids (bordering the fruit locule) and one or two layers of microsclereids, e.g. in *Anacardium*

about seven layers of irregularly shaped brachysclereids, e.g. in *Pistacia*

three palisade layers, consisting of thick-walled cells and one microsclereid layer between the outermost two palisade layers, as in species of *Schinus* and *Rhus*

three distinct layers, in *Toxicodendron* and *Euroschinus.*

Magen (1912), comparing the endocarp structure of representatives of the genera *Schinus, Rhus, Lithraea* and *Cotinus*, concluded that only slight differences exist in the size of the individual layers and the degree of lignification. The non-stratified kind of endocarp possibly occurs in fewer genera. In *Mangifera* a heterogeneous endocarp is described by Roth (1977: 421); that is, the inner epidermis forms "sclereids with strongly thickened, wavy walls" and the subepidermal layer forms an intricate network of fibres in which vascular bundles occur. Mitra & Mehrotra (1980) described the homogeneous endocarp of *Buchanania lanzan* as consisting of elongated astrosclereids orientated at right-angles or parallel to the fruit axis.

Therefore, most members of the Rhoideae possibly have a homogeneous, stratified endocarp consisting of sclereids of varying form and size. Only species of *Mauria* (tribe Rhoideae) have a completely different endocarp, since it consists of one layer of pavement epithelium ("Pflasterepithel", Herzog, 1910).

In the marula (tribe Spondieae) the endocarp originates from the inner epidermis as well as several subepidermal layers. This endocarp *sensu lato* represents the heterogeneous, non-stratified type because it ultimately consists mainly of fibres, brachysclereids and xylem vessels. It reveals some similarity to the endocarp of the mango.

The operculum and other opening mechanisms: Hill (1933) stated that the method of emergence of the embryo of seeds which are enclosed in a sealed lignified envelope is very interesting. The latter includes the stony endocarp found in several drupes of the Anacardiaceae. The opening devices described by Hill (1937) comprise the following:

irregular splitting
splitting into two halves
shutters or flanges
cork-like stoppers or plugs
caps or lids.

The last named, sometimes called opercula, represent the most sophisticated opening mechanisms. A number of genera, such as *Haematostaphis, Pseudospondias, Dracontomelon* and *Pleiogynium* (Hill, 1933, 1937), all of the tribe Spondieae, have endocarp opercula.

In other genera of the Anacardiaceae the operculum represents a mesocarp structure (Hill, 1937). These ingenious endocarp devices have also been found by Bonnefille & Letouzey (1976) in fossils closely related to *Antrocaryon* (Anacardiaceae). *Spondias* (Spondieae) can be added to the list of Hill (1937), since Hladik & Halle (1979) found inconspicuous opercula in several species of this genus. In addition to the marula, opercula were also found in the indigenous *Harpephyllum caffrum* Bernh. and species of *Lannea*, all belonging to the * tribe Spondieae.

Other opening mechanisms occur in the Anacardiaceae, as in *Buchanania lanzan* in which the endocarp splits vertically into two halves (Mitra & Mehrotra, 1980). In *Anacardium occidentale* (Weber, 1907: fig. 85) and *Pistacia vera* L. (Popov, 1971: fig. 2) the endocarp seems to split at the one side only. We have found the latter mechanism in *Rhus lancea* L. fil. Different kinds of opening devices occur in the drupes of the Anacardiaceae. It remains to be seen whether opercula occur only in the Spondieae or in the other tribes also.

Growth relations between fruit and seed, and within the pericarp: Our observations on the fruit and seed development in the marula correspond to those of Grundwag (1976) on four species of *Pistacia.* At anthesis the marula ovule more or less filled the ovary locule, whereafter the fruit developed quicker than the seed. The lignification of the endocarp preceded the growth of the embryo. However, embryo growth in *Mangifera* preceded the endocarp lignification, the seed usually filling the fruit locule at this stage (Kennard, 1955). Grundwag (1976) found that fruit development occurred prior to the growth of the ovule in some other genera of the Anacardiaceae.

Roth (1977) reported that in drupes the differentiation of the endocarp generally precedes that of the mesocarp, which only takes place when the stone is completely developed. In the marula the developmental sequence within the pericarp therefore corresponds to the general pattern.

CONCLUSIONS

According to the literature there seem to be morphological differences in the exocarp within a genus, for example *Anacardium.* It remains to be determined whether these differences may perhaps be ascribed to different interpretations by various authors. Secretory structures in the drupe of the Anacardiaceae occur mainly in the parenchymatous mesocarp and are very characteristic for a species. The secretions might even prove useful for diagnostic purposes in taxonomic work. The endocarp in the genera studied is generally well-defined. The structure of the endocarp shows relative uniformity within a genus and, considering the Rhoideae (excepting the genus *Mauria*), possibly even within a tribe. The kind of opening mechanism found in the sclerocarp of the genera studied up to date also seems to be uniform within a genus and even within the tribes. Information available suggests that drupes of the different species and even genera are morphologically easy to distinguish from one another. It remains to be determined at what level the pericarp features could be applied in future taxonomic revisions of the 79 genera of the Anacardiaceae.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria and the CSIR Foundation for Research Development.

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CHAPTER 3

SEED-COAT OF *Sclerocarya birrea* subsp. *caffra*[*]

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[*] Accepted (11.11.1987) for publication in *Bot. J. Linn. Soc.*

Notes on the ontogeny and structure of the seed-coat of *Sclerocarya* *birrea* (Richard) Hochst. subsp. *caffra* (Sonder) Kokwaro (Anacardiaceae)

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VON TEICHMAN, I., 198 . Notes on the ontogeny and structure of the seed-coat of *Sclerocarya birrea* (Richard) Hochst. subsp. *caffra* (Sonder) Kokwaro (Anacardiaceae). The pendulous, bitegmic, anatropous ovule with dorsal raphe is suspended at the tip of a massive funicle. A group of nucellar cells with intensively staining cell walls, the hypostase *sensu stricto*, is present. The initially plate-like tanniniferous chalazal-nucellar tissue, with suberin and lignin impregnated cell walls represents a hypostase *sensu lato*. The mature seed-coat is formed by the raphe, extensive chalaza, adjacent, well-developed, cup-like hypostase *sensu lato*, remnants of the two integuments and a cuticular layer. The exalbuminous seed of *S. birrea* subsp. *caffra* (the marula), is regarded to be a derived and phylogenetically advanced type. The undifferentiated seed-coat is very similar to that found in *Lannea discolor* which, like the marula, belongs to the tribe Spondieae. The similarities in the structure of the seed-coat and seed of the marula and *L. discolor* confirm their proposed close phylogenetic relationship.

ADDITIONAL KEY WORDS: chalaza - hypostase - raphe - seed.

CONTENTS

INTRODUCTION

Sclerocarya birrea (Richard) Hochst. subsp. caffra (Sonder) Kokwaro - henceforth referred to by its vernacular name "marula", is indigenous to southern Africa. It is a well-known fruit tree and is of potential economic importance. In South Africa it occurs mainly in open woodland and along rivers in the frost-free areas of the Transvaal and Natal. The distribution extends northwards to Ethiopia, westwards to South West Africa, Angola as well as Zaire and eastwards to the Malagasy Republic, occurring at altitudes up to 1200 m (Kokwaro & Gillett, 1980).

Sclerocarya Hochst. belongs to the tribe Spondieae with, amongst others, the indigenous genera Lannea A. Rich. and Harpephyllum Bernh. ex Krauss (Engler, 1897). The present work is part of a comparative study of mainly the fruit and seed of the genera of the Anacardiaceae indigenous to southern Africa. Von Teichman & Robbertse (1986,a) provide a survey of the literature on the pericarp of the Anacardiaceae drupe in general and on the marula in particular. Aspects of the pericarp and seed-coat of *Rhus lancea* L. fil., (Von Teichman & Robbertse, 1986,b) and *Lannea discolor* (Sonder) Engl. (Von Teichman, 1987 & in press) have previously been discussed. The relevant aspects in *Harpephyllum caffrum* Bernh. ex Krauss have been researched and are being prepared for publication. The present paper reports briefly on the seed-coat ontogeny and structure of the marula.

MATERIAL AND METHODS

A large number of flower buds, flowers and fruit in various developmental stages were collected from trees growing naturally on a northern slope of the Magaliesberg range near Pretoria. Voucher specimens (Von Teichman 542 & 543) are kept at PRU (H.G.W.J. Schweickerdt Herbarium, Department of Botany, University of Pretoria).

Pieces of mature seed-coat were fixed in 2.5% glutaraldehyde in 0.1 mol dm^{-3} buffer, consisting of Na_2HPO_4 and NaH_2PO_4 (Coetzee & Van der Merwe, 1985). Flowers at anthesis and various young fruit preserved in FAA, were thoroughly rinsed with and dissected in 50% ethanol. The details of the dehydration prior to the infiltration and embedding in glycolmethacrylate (GMA), as well as the preparation of the 2-3 μm thick sections, the PAS-reaction and toluidine blue O counterstain correspond with those described for the pericarp of the marula (Von Teichman & Robbertse, 1986,a).

Some sections were stained for lipidic substances, especially cutin and suberin, using a saturated solution of sudan black B in 70% ethanol. Staining for proteins was done with amido black 10 B (Bullock _et_ _al_., 1980). To test for the presence of lipids in the cotyledons, fresh tissue from ripe fruit was squashed on a slide and stained with, and mounted in, a saturated solution of sudan IV in 70% ethanol. To stain lignified cell walls, sections were mounted in a drop of saturated phloroglucinol in 20% HCl. Unstained sections were observed using crossed polarizing filters for polarizing microscopy.

OBSERVATIONS

The ovary of flowers at anthesis is usually bicarpellate and each locule contains one pendulous ovule with a dorsal raphe and a massive funicle with a knee-like protuberance (Von Teichman & Robbertse, 1986, a). The extremely well-developed outer integument (o.i.), the shorter inner integument (i.i.), extensive raphe, a hypostase *sensu stricto* and plate-like hypostase *sensu lato* (Von Teichman, in press) characterize the ovule. Proximally the o.i. is up to six cell layers thick, while the i.i. comprises about four cell layers. Fine tannin-like deposits occur only in the inner epidermis of the i.i. as well as in the cells of the hypostase *sensu lato* lying within the chalazal-nucellar region.

In very young fruit, the embryo shows limited growth. The very young seed (Figs 1 & 2) therefore still exhibits all the characteristics of the ovule. Subsequently an extensive development of the chalaza and adjacent hypostase *sensu lato* takes place in the young seed.

Mature, slightly compressed seeds are obclavate or variously shaped according to the space available in the fruit locule, and measure about 15 - 20 x 4 - 8 mm. The papyraceous seed-coat is cream-coloured externally, but on the inside it is shiny and light brown with a large, very characteristic dark brown saddle-like patch. The brown coloration is caused by the tanniniferous deposits in the inner epidermis of the i.i. and the hypostase *sensu lato*.

The mature seed-coat is formed by the raphe, the well-developed chalaza, extensive hypostase *sensu lato* (Fig. 3), remnants of the o.i. and i.i., as well as a cuticular layer between the squashed nucellar remnants and the intact, tanniniferous inner epidermis of the i.i.. The cuticular layer consists of the cuticles of the nucellar epidermis and inner epidermis of the i.i.. A very characteristic feature of the mature cells of the hypostase *sensu lato* is the distinct secondary impregnation of the cell walls with lipidic substances i.e. probably suberin and also with lignin. With polarization microscopy the walls showed birefringence. During maturation of the seed-coat lignification and a slight secondary thickening was also noted in the cell walls of the subepidermal cells of both the ovule wall (Fig. 3) and o.i.. This is taken as a slight mesotestal condition (*sensu* Corner, 1976).

In the mature seed the cotyledons contain reserve materials consisting of proteins and lipids, with no starch. The cellular endosperm remnants are insignificant and the seed is considered exalbuminous (Fig. 3).

DISCUSSION AND CONCLUSIONS

In the marula and *Lannea discolor* the anatropous ovule is pendulous, while in *Rhus lancea* it is basal. In all three species the ovule is characterized by a very well-developed o.i., and is suspended on a massive funicle which has a knee-like projection.

The mature seed-coat of *R. lancea* (Von Teichman & Robbertse, 1986, b) is probably of the endotestal type representing a differentiated seed-coat according to Corner (1976). In the ovule of *L. discolor* and marula a hypostase *sensu stricto* and hypostase *sensu lato* occur. In the paper on *L. discolor* (Von Teichman, in press) the occurrence of these hypostases in other taxa of the Anacardiaceae and the reason for their distinction was discussed. In *L. discolor* and the marula the contents of the hypostase *sensu lato* cells are tanniniferous, while in the marula an additional cell wall impregnation with lipids, probably suberin and lignin, occurs.

According to Corner (1976) and Tilton (1980) lignification of the cell walls of hypostase cells often occurs. Corner (1976) and Netolitzky (1926) also mention suberization of the chalaza and hypostase cell walls respectively, while Bouman (1984) reports on the impregnation of hypostase cell walls with cutin, suberin, lignin and callose, as well as the accumulation of tannin-like substances in the cytoplasm of the hypostase cells.

In the hypostase *sensu lato* of the marula this impregnation only takes place during maturation of the seed-coat. This implies a protective function rather than an impediment of the nutrition during megasporogenesis or embryo development. This impregnation could represent a vestige of the ancestral well-developed seed-coat which probably existed prior to the lignification of the endocarp. The participation of the chalaza, with adjacent hypostase *sensu lato*, in the formation of the seed-coat is greater in the marula than in *L. discolor*.

In *L. discolor* a slight exotestal condition is found, while in the marula traces of a mesotestal condition are present in the mature seed-coat. In both species the raphe, extended chalaza and adjacent hypostase *sensu lato*, together with remnants of the integuments and a cuticular layer form the mature seed-coat. This constitutes an undifferentiated type of seed-coat (*sensu* Corner, 1976).

The seed of these two species of the tribe Spondieae can be regarded as being of the "raphe-chalazal" type *sensu* Robbertse *et al*. (1986). They may be considered derived and phylogenetically advanced as discussed in the paper on *L. discolor* (Von Teichman, in press). Similarities in ovule and seed-coat structure, as well as in the type of reserve material in the cotyledons of the exalbuminous seed, also confirm the close phylogenetic relationship of the marula and *L. discolor*.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria, Margaretha Mes Institute for Seed Research, and the CSIR Foundation for Research Development. I wish to express my sincere thanks to Proff. P.J. Robbertse and A.E. van Wyk for valuable discussions and to Dr Elzabé Schoonraad for her constructive criticism of the manuscript. Rohm & Hass (Pty) Ltd. is sincerely thanked for the free samples of 94% HEMA supplied.

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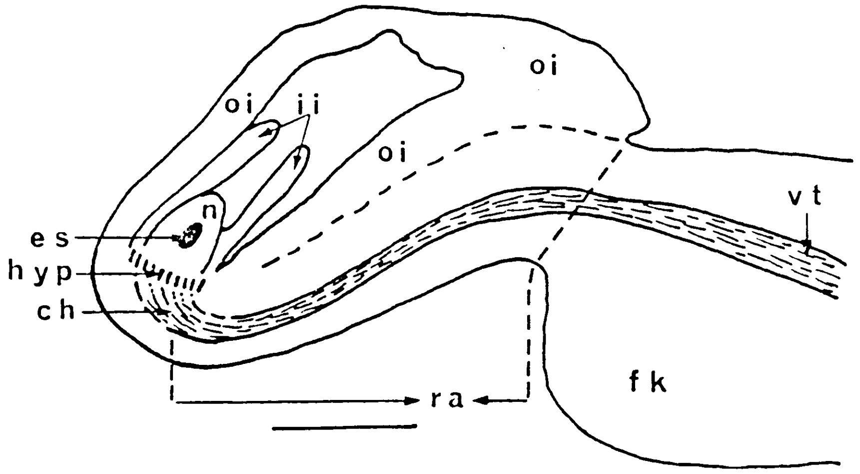

Figure 1. A reconstruction of a very young seed of the marula from serial longitudinal sections. It illustrates the massive funicle with funicular knee (fk), the vascular tissue (vt) in the funicle and raphe (ra), the chalaza (ch), plate-like hypostase <u>sensu</u> <u>lato</u> (hyp), the embryo sac (es) still surrounded by the nucellus (n), the shorter inner integument (ii) and well-developed, obliquely cut outer integument (oi). Scale bar = 0,5 mm.

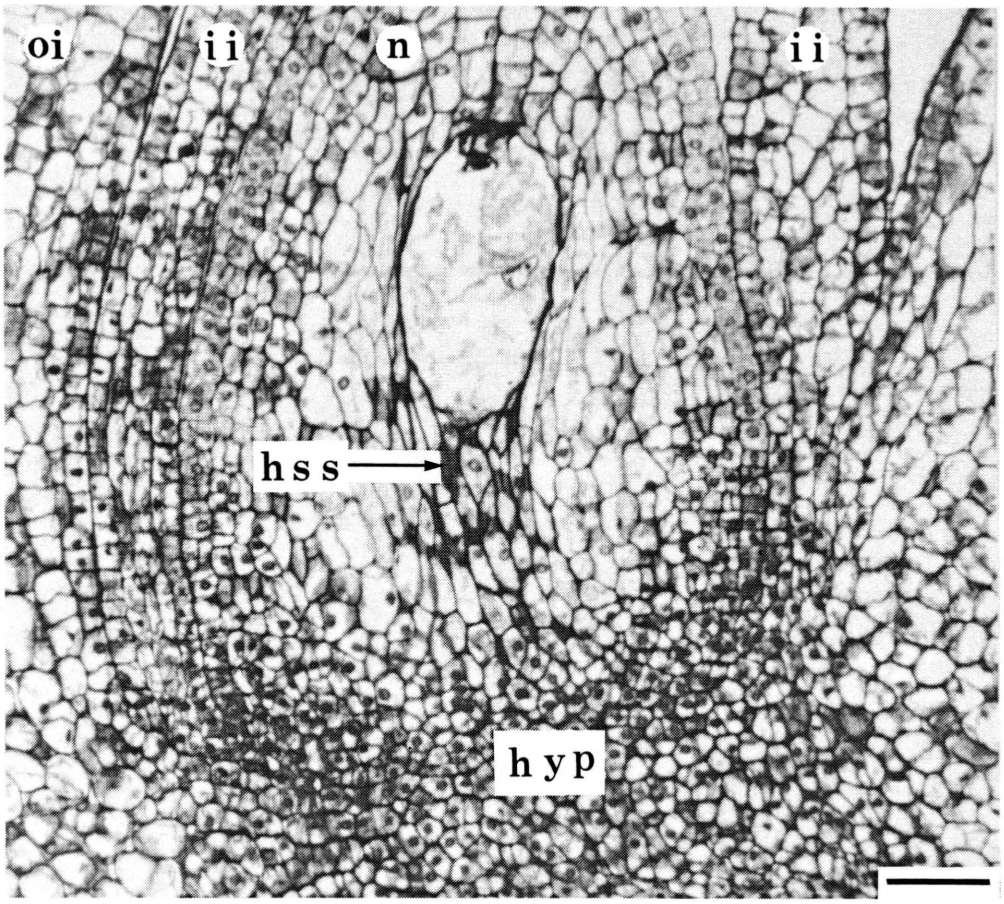

Figure 2. LS section of a very young seed i.e. an enlargement of a small part of Fig. 1, illustrating parts of the outer and inner integuments (oi) and (ii), the nucellus (n) surrounding the embryo sac, the hypostase <u>sensu</u> <u>stricto</u> (hss) and plate-like hypostase <u>sensu</u> <u>lato</u> (hyp) between the proximal ends of the ii. Scale bar = 40 μm.

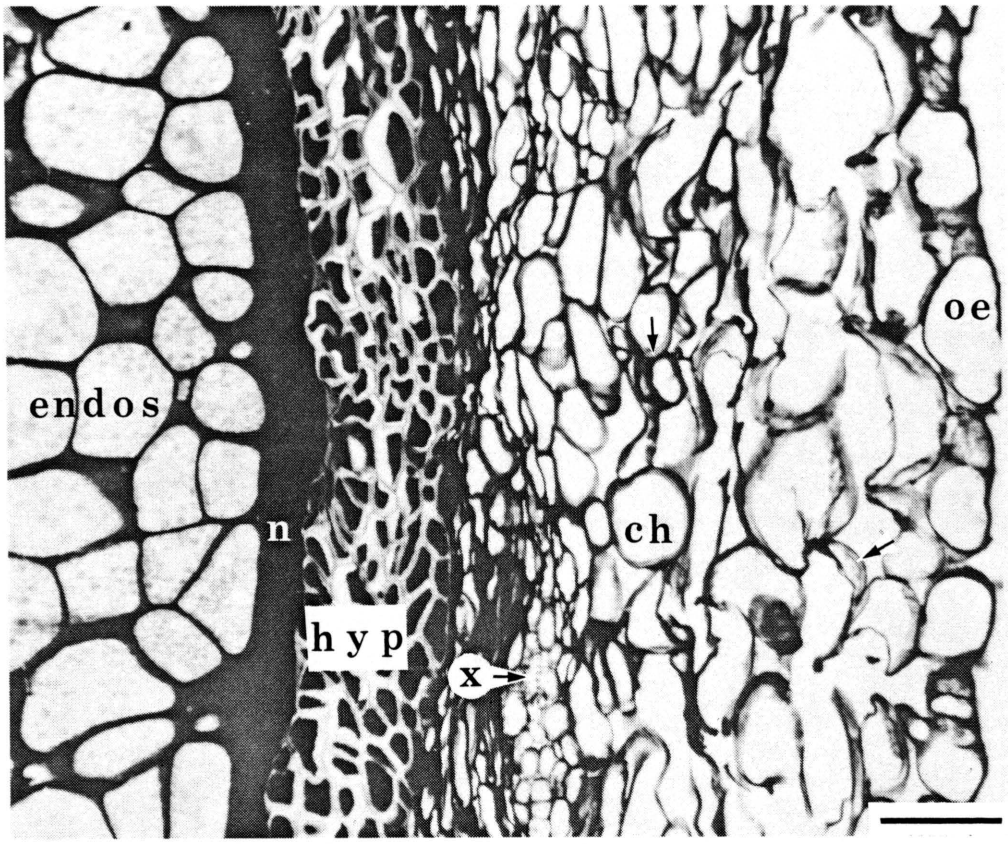

Figure 3. TS of the mature seed-coat at the brown saddle-like patch. The more or less intact outer epidermal cells (oe) of the chalaza (ch) and remnants of the subepidermal cells with secondarily thickened as well as lignified parts of cell walls (arrow heads) and tracheoids (x) are illustrated. The adjacent hypostase sensu lato (hyp) with tanniniferous cytoplasm and, in this PAS and toluidine blue stained section very light green-blue cell walls; the utilized and squashed nucellus (n) and cellular endosperm remnants (endos) are also shown. Scale bar = 30 μm.

CHAPTER 4

GERMINATION OF *Sclerocarya birrea* subsp. *caffra*

A preliminary study on the germination of *Sclerocarya birrea* subsp. *caffra*

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Seeds of *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, commonly known as marula, occur in locules within a hard lignified endocarp. The endocarp appears to restrict germination mainly by offering mechanical resistance. It may also restrict the leaching of germination inhibitors and possibly serve as a barrier to oxygen diffusion. The endocarp does not restrict water uptake. High germination counts were only obtained by removing opercula. Leaching slightly improved germination of seeds in intact endocarps. The optimum germination temperature of opercula-removed seeds was between 27° and 37°C. Storage improved rate of germination.

S. Afr. J. Bot. 1986, 52: 145 – 148

Sade van *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, algemeen bekend as maroela, kom voor in vrughokke binne 'n harde gelignifiseerde endokarp. Skynbaar verhoed die endokarp saadkieming hoofsaaklik weens die meganiese weerstand wat dit bied. Dit mag ook die uitloging van kiemingsremstowwe beperk en moontlik weerstand teen die diffusie van suurstof bied. Die endokarp beperk nie wateropname nie. Goeie saadkieming is alleenlik verkry na verwydering van operkulums. Loging het die kieming van sade in intakte endokarpe effens verhoog. Die optimum kiemingstemperatuur van sade, na verwydering van operkulums, was tussen 27° en 37°C. Opberging het die tempo van kieming verhoog.

S.-Afr. Tydskr. Plantk. 1986, 52: 145 – 148

Keywords: Endocarp, germination, operculum, *Sclerocarya birrea*

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Accepted 12 September 1985

Introduction

Some members of the family Anacardiaceae e.g. mango, cashew, pistachio and sumacs are of economic importance. The indigenous marula *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (Kokwaro & Gillett 1980), a member of the same family, serves as a source of food and shelter for many animal species. It also has ornamental value and plays a distinct role in the cultural rites of various black tribes. They utilize, amongst other things, the seed and especially the fruit extensively. The leaves and bark are used for medicinal purposes (Shone 1979).

Fruit juice and marula liqueur are already commercially produced. Research programmes on cultivation and improvement of marula have been initiated by Prof L.C. Holtzhausen, Department of Horticultural Science, University of Pretoria. As a prerequisite to such studies a thorough knowledge of the germination behaviour of this scientifically little known species is essential.

The fruit of the marula is a highly specialized drupe or stone fruit. The stone is embedded in an edible, fibrous, juicy flesh (mesocarp *sensu lato*) which is covered by the peel (exocarp *sensu lato*). One to four fruit locules occur in the hard lignified endocarp. Each locule contains a single seed, and appears to be a hermetically sealed unit with an orifice tightly closed by a relatively small lid, the operculum. The diameter of the operculum is always smaller than that of the seed and it is therefore completely impossible to remove intact seeds from the fruit. The operculum has to be ejected when germination commences. These opercula have a distinct cream to light brown 'cementing layer', which is more or less weathered in old fruit collected in the veld. The seeds are covered with a thin papery seed coat. The protective function of the seed coat is therefore taken over by the stony endocarp. This endocarp is the effective 'covering' or 'coat' of the marula seed.

In preliminary studies it was found that seeds do not germinate readily. The present study was aimed at identifying the reason for this. Emphasis was placed on the possible restrictive effect of the covering structures. Such structures are known to present chemical and physical barriers to germination (Ballard 1973). In addition the effect of temperature and fruit age on germination was studied.

Materials and Methods

Freshly ripened fallen fruits were picked up beneath trees in the wild at the Nylsvley Nature Reserve (grid reference 2428 DA Nylstroom) during March 1982 and February 1983. The fruit were brought to the laboratory where the peel and flesh were removed. The stones, henceforth termed fruit, were stored at room temperature in screw-capped glass jars. Before use the basal layer of mesocarp adhering to the stony endocarp was removed manually. In some studies opercula were removed by clamping the fruit in a vice and sawing along the 'cementing layer' before lifting them off with a scalpel.

Fruit were germinated in polystyrene dishes (176 × 152 × 35 mm) containing 450 cm^3 washed coarse river sand and covered with aluminium foil. Holes for drainage were pierced into the bottom of the dishes. The sand was moistened with 80 cm^3 of a 0,025% (m/v) Thiulin solution (seed dressing fungicide Bayer S.A.). In preliminary studies this was found to be the most suitable concentration.

Unless otherwise stated, germination tests were conducted in the dark at 27°C and 70% RH. Treatments were replicated four times with 10 fruit per dish, each fruit containing (1) 2 – 3 (4) perfect seeds. The initial germination percentage was calculated on the total number of seeds per dish. To count the total number of seeds per dish in the case of intact fruits, the opercula were removed after the experiment had been completed. The average germination percentage and standard deviation were calculated from the four dishes. Seeds were taken as germinated when the radicle protruded at least 5 mm. Details of other methods are given with the results.

Results

Effect of removal of opercula and leaching on germination

Removing opercula increased germination significantly (Figure 1). Germination of opercula-removed fruits was further increased by a preceding leaching in running tap water for 24 h. Germination of intact fruits was also slightly increased by leaching. However, for significant germination, removal of opercula was a prerequisite (Figure 1). An increase in germination after removing opercula was also obtained when fruits were previously soaked for 24 h in 1 mol dm^{-3} KOH. This treatment, however, did not increase germination of intact fruits (Figure 1). Acid scarification in concentrated sulphuric acid did not increase germination (data not shown).

Water uptake

Water uptake studies were conducted by placing fruits in a paper roll consisting of two sheets of Anchor seed germination paper outside, one layer of Salters absorbant cellulose wadding and one sheet of Anchor seed germination paper inside. Rolls were immersed in distilled water for 10 min whereafter excess water was allowed to drain off. To prevent desiccation, rolls were covered with polyethylene sheeting. Fruits were removed at various intervals, blotted and weighed. After 80 h moisture uptake had levelled off, judged by percentage moisture on an air dry basis (data not shown). Samples of cotyledon tissue were then rapidly removed from all seeds with a cork borer, weighed and then oven-dried at 85°C to constant mass. Removing opercula or damaging the endocarp by rasping did not increase water uptake over an 80 h period (Table 1). By this time some seeds from fruits of which opercula had been removed, showed signs of germination.

Effect of oxygen on germination

Polystyrene dishes containing fruits in Thiulin-moistened sand were placed in glass desiccators. The desiccator lids were modified and provided with gas in- and outlets. Fruits were incubated in air (21% oxygen) and in 100% oxygen. In the latter case 100% oxygen was flushed through the desiccator at a rate of about 2000 cm^3 min^{-1} for 10 min and then sealed. In both cases desiccators were kept sealed for the whole duration of the experiment.

The results in Figure 2 show that raising the initial oxygen concentration to 100% slightly increased germination of intact fruits. Germination of fruits in which the locule had been opened at the base increased significantly. Maximum germination, however, was only obtained with fruits from which opercula had been removed. Raised oxygen concentration did not affect germination of such fruits.

Effect of light on germination

In these experiments intact and opercula-removed fruits were incubated in the light and in the dark at 27°C. The distal parts of the fruits with the opercula were allowed to protrude above the surface of the sand to allow exposure to light. White light was provided by cool white fluorescent tubes (8,25 $\mu E\ m^{-2}\ s^{-1}$). Only opercula-removed fruits germinated. Germination was better in the dark (72,7% ± 8,7) than in white light (49% ± 14,4).

Effect of temperature on germination

Seed from opercula-removed fruits require high temperatures for germination (Figure 3) with an optimum between 27° and 37°.

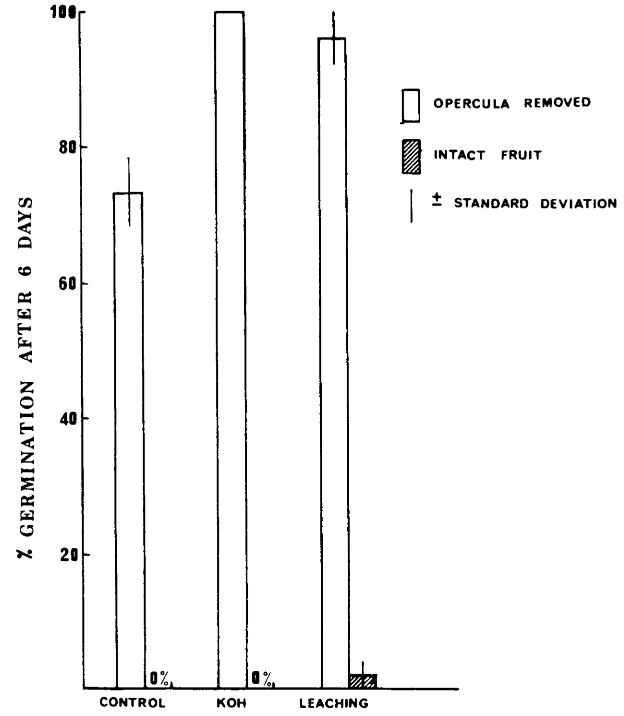

Figure 1 Effect of soaking for 24 h in 1 mol dm^{-3} KOH and tap water leaching (24 h) on germination of marula fruits in the dark at 27°C (fruit age 4,5 months).

Table 1 Moisture content of marula seeds incubated in the dark at 27°C for 80 h (fruit age 6 months)

Fruit treatment	% Moisture content
Intact endocarp	22,5 ± 1,9
Opercula removed	22,4 ± 1,4
Fruit-locule opened at base by rasping	22,8 ± 1,3

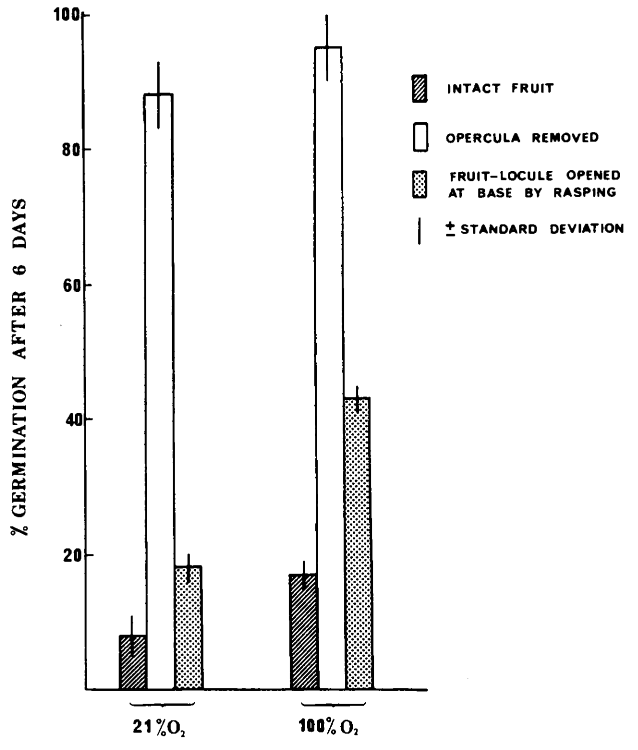

Figure 2 Effect of oxygen on the germination of marula in the dark at 27°C (fruit age 10,5 months).

Effect of fruit age on germination

Fruits were stored in closed glass containers in the laboratory at approximately 21°C. Germination was tested after various storage periods. The results in Table 2 show that with ageing, seeds require progressively shorter incubation times for maximum germination. The fruit lot of 1982 was used for the 14 and 23 months storage periods, while that of 1983 was used for the shorter storage periods.

Discussion

The results obtained in this study show that lack of germination of marula fruits cannot be ascribed to embryo dormancy. In other representatives of the Anacardiaceae e.g. *Rhus trilobata* embryo dormancy occurs and is overcome by cold stratification (Weber *et al.* 1982). The major barrier to germination in marula appears to reside in the mechanical resistance offered by the endocarp. Leaching the fruit with tap water consistently gave a significantly higher germination percentage in comparison with that of the control (Figure 1). It could therefore be reasoned that the endocarp also restricts the leaching of germination inhibiting substances. Another contributing factor of less importance is the barrier offered by the endocarp to oxygen diffusion.

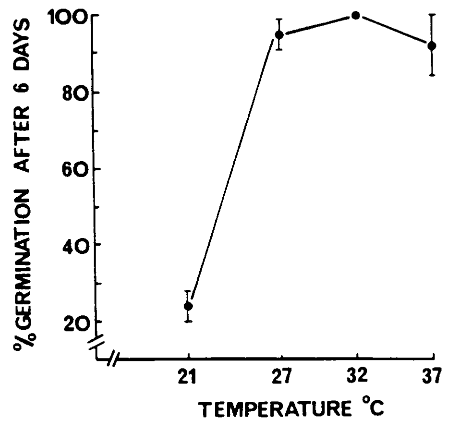

Figure 3 Effect of temperature on germination of opercula-removed fruits in the dark (fruit age 16,25 months).

A detailed morphological study of the endocarp of the marula showed that phenolic compounds i.e. tannin-like substances, occur abundantly in the operculum and the remaining part of the endocarp (Von Teichman & Robbertse, in press). Soaking the fruits in water increased the germination rate of *Schinus molle* and *Anacardium occidentale* (Nilsen & Muller 1980; Ibikunle & Komolafe 1973). In *Rhus trilobata* soaking speeded up seedling emergence and significantly increased total seedling emergence (Weber *et al.* 1982). The fact that acid scarification did not increase germination of marula, corresponds to some extent with the results of Nilsen & Muller (1980) on *Schinus terebinthifolius*. However, acid scarification produced significantly higher germination in several *Rhus* species (Morgeneyer 1956; Bear & Sievers 1957; Brinkman 1974; Farmer *et al.* 1982; Weber *et al.* 1982) and *Schinus molle* (Nilsen & Muller 1980). The latter authors stated that the scarification 'might have rendered the bound inhibitors leachable' and suggested an 'endogenous germination inhibition system of phenolic acids' for *Schinus molle* and *S. terebinthifolius*.

The endocarp of marula fruit does not restrict water uptake by seeds. This is in contrast to some other members of the Anacardiaceae (Morgeneyer 1956; Bear & Sievers 1957; Brinkman 1974; Farmer *et al.* 1982).

The slight inhibitory effect of white light on germination observed in this study is interesting and requires further study. In *Rhus glabra* (Anacardiaceae) better germination is obtained in light than in darkness (Brinkman 1974).

The high temperature requirement for germination is

Table 2 Effect of fruit age on germination of opercula-removed fruits in the dark at 27°C

Storage period (months)	% Germination after various incubation periods (days)				
	3	4	6	12	20
0,5 – 1,0	–	–	23,6 ± 6,4	56,7 ± 2,6	62,7 ± 1,7
2,0	0	5,7 ± 4,0	23,6 ± 8,0	79,7 ± 6,0	91,2 ± 3,0
14,0	17,4 ± 3,4	78,2 ± 12,8	80,3 ± 9,7	*	*
23,0	17,9 ± 11,7	65,4 ± 11,0	80,6 ± 7,0	*	*

– Results not available
* Experiment concluded

consistent with the fact that the marula is a subtropical plant. Unfortunately very little literature seems to be available concerning the optimum germination temperature of other subtropical species of the Anacardiaceae. Rocchetti & Panerai (1968) reported their results on the seed germination of the subtropical *Anacardium occidentale*, i.e. the cashew nut. Stones from Dahomey, Mozambique and Tanzania were germinated in well aerated soil at 95–98% RH and at constant temperatures of 10°, 15°, 20°, 25°, 30°, 35° and 40°C. Maximum 'germinability' (i.e. percentage germination) and maximum 'germinative energy' (i.e. expressed in average number of days before germination took place) were reached at 35°C. The moisture content of these *A. occidentale* seeds from Dahomey, Mozambique and Tanzania was 9,93%, 9,57% and 9,67% respectively. The shell, i.e. endocarp of the stones, presented a physical barrier to germination. All these results, therefore, correspond well with the optimum temperature of marula germination, the low moisture content of the marula seed and the mechanical resistance offered by the marula endocarp.

Like some other members of the Anacardiaceae (Brinkman 1974) seeds of marula apparently remain viable for a considerable period. During the present study seeds were found to germinate even after three years of storage in the laboratory.

Acknowledgements

This research was undertaken with grants from the University of Pretoria and the Foundation for Research and Development of the Council for Scientific and Industrial Research. Mrs Emsie du Plessis is thanked for her assistance with the manuscript.

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CHAPTER 5

PERICARP OF Lannea discolor

Botanical Journal of the Linnean Society (1987) *95:* 125–135. With 8 figures

Development and structure of the pericarp of *Lannea discolor* (Sonder) Engl. (Anacardiaceae)

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Received September 1986, accepted for publication October 1986

VON TEICHMAN, I., 1987. **Development and structure of the pericarp of *Lannea discolor* (Sonder) Engl. (Anacardiaceae).** The exocarp develops from the outer epidermis and subepidermal, parenchymatous cell layers of the ovary wall. A parenchymatous zone with secretory cavities more or less delimits the exocarp internally. The inner part of the parenchymatous mesocarp is tanniniferous. The parenchymatous transition zone between mesocarp and sclerenchymatous endocarp or sclerocarp, contains vascular tissue. The inner endocarp and operculum develop from the inner epidermis and subepidermal parenchyma of the ovary wall, while the outer endocarp develops from the parenchymatous zone with procambium strands. Comparing the pericarp of *L. discolor* with those of *Sclerocarya birrea* subsp. *caffra* and *Rhus lancea*, the close affinity with *Sclerocarya birrea* subsp. *caffra* is evident.

ADDITIONAL KEY WORDS:—Drupe – *Operculicarya* – operculum – sclerocarp.

CONTENTS

INTRODUCTION

The genus *Lannea* A. Rich. (Anacardiaceae) comprises approximately 40 species and occurs mainly in tropical and southern Africa, with one species in tropical Asia (Engler, 1897; Fernandes & Fernandes, 1966; Hou, 1978). It is placed in the tribe Spondieae with, amongst others, the genera *Spondias* L., *Dracontomelon* Blume and *Harpephyllum* Bernh. ex Krauss (Scholz, 1964). Other members of this tribe mentioned in the present paper are *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sonder) Kokwaro, henceforth referred to as the

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"marula", and *Operculicarya decaryi* H. Perr. from the Malagasy Republic (Perrier de la Bâthie, 1946). The striking similarity between the fruit of the latter species and those of *Lannea* will be pointed out in this paper.

Lannea discolor (Sonder) Engl. is a fairly common small deciduous tree indigenous to the savanna regions of southern Africa. Besides the many vernacular names used by the different African peoples, *L. discolor* is also known as live-long, tree grape and dikbas. *Lannea discolor* is mainly confined to rocky slopes and sandy flats in open woodlands. It is widespread in central and northern Transvaal, a small part of northern Natal, as well as in Swaziland and Botswana; and it also ranges to northeastern South West Africa and northwards as far as Zambia and Zaïre. The soft, light wood, bark and roots are utilized especially in the rural African household. Although the fresh fruit is eaten by humans and animals, it has no commercial value at present.

The chemistry of the gum exudate as well as the development and structure of the gum ducts have been investigated in *L. coromandelica* (Houtt.) Merr., a deciduous tree from India (Anderson & Hendrie, 1970; Venkaiah & Shah, 1984). Available literature does not cover any aspects of the development and structure of the pericarp in species of *Lannea*. Hence these aspects were studied, mainly in *L. discolor*, and form the subject of the present paper. The ontogeny of the seed-coat in this species will be discussed elsewhere (Von Teichman, in press). These papers form part of a comparative morphological study of mainly the fruit and seed in genera of the Anacardiaceae indigenous to southern Africa. The findings of the present study will be related to those of previous investigations on the marula (Von Teichman & Robbertse, 1986a) and *Rhus lancea* L. fil. (Von Teichman & Robbertse, 1986b).

MATERIAL AND METHODS

Flower buds, flowers and fruit in various stages of development were collected from trees growing naturally on a north-facing slope of the Magaliesberg range near Pretoria (grid reference: 2528 CB, Pretoria). A voucher specimen (*Von Teichman 550*) is kept at the H. G. W. J. Schweickerdt Herbarium, Department of Botany, University of Pretoria (PRU).

For the preparation of semithin sections the material was fixed in 2% glutaraldehyde (Coetzee & Van der Merwe, 1985) in 0.1 mol dm^{-3} buffer, consisting of Na_2HPO_4 and NaH_2PO_4. The material was dehydrated, infiltrated and embedded in purified glycol methacrylate (GMA) according to the method of Feder & O'Brien (1968). The monomer mixture consisted of 94% (v/v) hydroxyethyl methacrylate (i.e. the 94% HEMA was purified to remove most of the methacrylic acid), 5% (v/v) polyethylene glycol 200, and 0.6% (w/v) benzoyl peroxide. GMA sections, 2–3 µm thick, were cut on a Porter Blume MT-1 ultramicrotome. The 'Periodic Acid-Schiff Reaction' (PAS) was * carried out according to Feder & O'Brien (1968), using 0.5% dinitrophenyl hydrazine (DNPH) in 15% acetic acid for 30 min as blocking agent. Sections were counterstained for about 3 min in 0.05% toluidine blue O in benzoate buffer, pH 4.4 (Sidman *et al.*, 1961). Other sections were treated as follows:

(a) To test for fatty substances, especially cutin, a saturated solution of sudan black B in 70% ethanol, for 10 min was used. After rinsing in 70% ethanol, the sections were mounted in liquefied glycerine jelly.

* for corrections
see Appendix

(b) To stain lignified cell walls, sections were mounted in a drop of saturated phloroglucinol in 20% HCl.

(c) Unstained sections were observed under crossed polarizing filters.

Small pieces of the stony endocarp and a few stony opercula were macerated by heating in Schulze's solution (McLean & Cook, 1941). The macerated tissue was thoroughly rinsed with distilled water before being mounted in the latter and viewed with phase contrast optics.

OBSERVATIONS

Morphology of the female flower and fruit

Lannea discolor is dioecious. The small, creamish-yellow female flowers, about 5 mm long, are borne in racemes. The relatively short peduncle and the three * bracteoles at each node of the rhachis are covered with fine stellate hairs. The * calyx and corolla are usually tetramerous, although pentamerous flowers also occur. The sepal segments are glabrous while the base can be stellately tomentose. The oblong petals are imbricated in the bud and recurved at anthesis. A yellow, annular, intrastaminal, 8–10 crenate disc and 8–10 staminodes are present. Only about half of the flowers dissected had four styles, i.e. the syncarpous gynoecium consisted of four carpels, while the rest had five styles, i.e. five fused carpels. Therefore, some flowers have 10 staminodes, a pentacarpous ovary but a tetramerous perianth. The unilocular ovary is subglobose and laterally compressed. It contains a single pendulous anatropous ovule. The styles are relatively short and terminate in simple to subcapitate stigmas. In any one flower one of the four or five styles is always more strongly developed than the others and is centrally positioned on the gynoecium, the longitudinal axis of which is perpendicular to the long axis of the rhachis of the * inflorescence.

The ripening drupe (Fig. 1) is red, obliquely subglobose, laterally compressed and shows four or five style remnants. The ripe, purplish-black fruit has a grapelike flavour. Removing the exo- and mesocarp of ripe fruit, reveals the more or less alveolate surface of the stone (Fig. 2A, B). The stone is usually bi-operculate, rarely uni-operculate. In the former the two opercula mostly differ in size and shape (Fig. 2D) but two opercula of similar size were also observed (Fig. 2C). Approximately 4% of the fruit examined have a more or less triangular stone with two pairs of opercula (Fig. 2E) and two fruit locules.

Development and structure of the pericarp

At the time of anthesis the ovary wall of *L. discolor* shows the following structure (Fig. 3): the outer epidermis in which anticlinal cell divisions predominate; a hypodermal parenchymatous zone with schizogenous secretory cavities lined with epithelial cells; a second parenchymatous zone with the procambium strands partly differentiated into xylem vessels; and the inner epidermis, which, with the second parenchymatous zone, comprise the initials of the future endocarp.

Tanniniferous deposits are present in the outer epidermis and in some deeper lying parenchyma cells. Further development of the ovary wall involves an

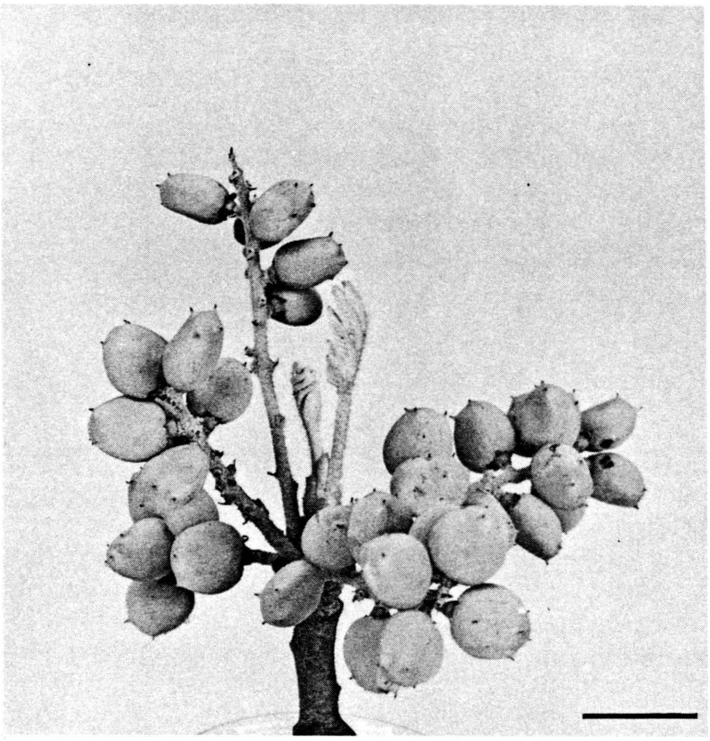


Figure 1. Mature fructification of *Lannea discolor* illustrating the size and morphology of the fruit, as well as the remnants of styles and stigmas. Scale bar = 15 mm.

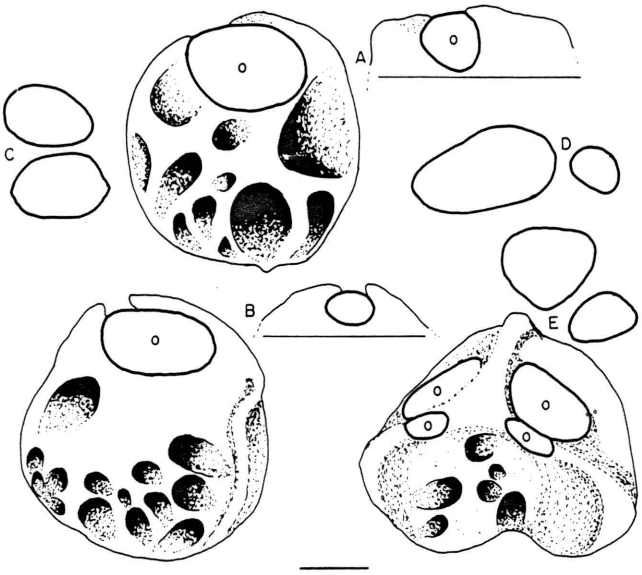
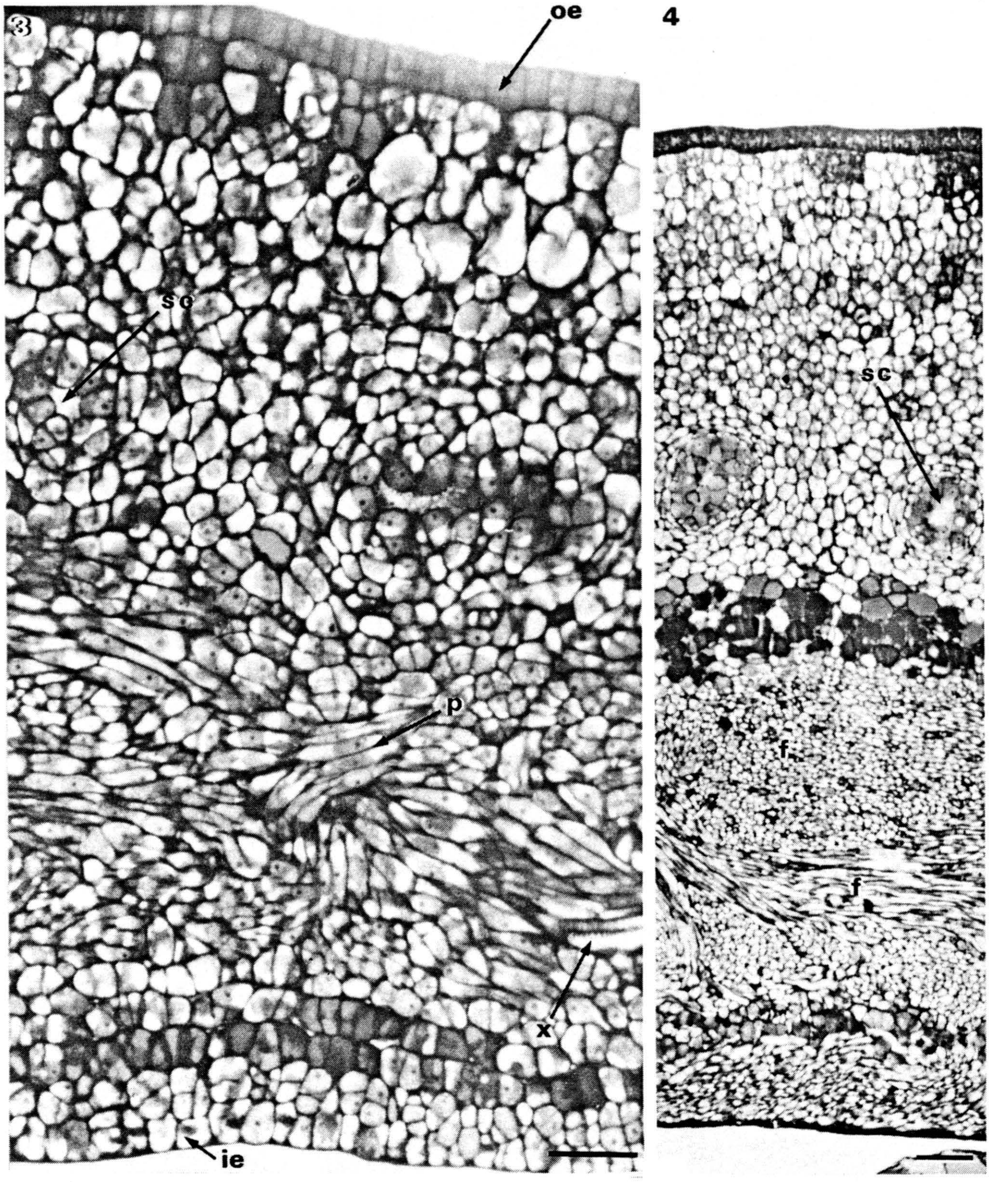


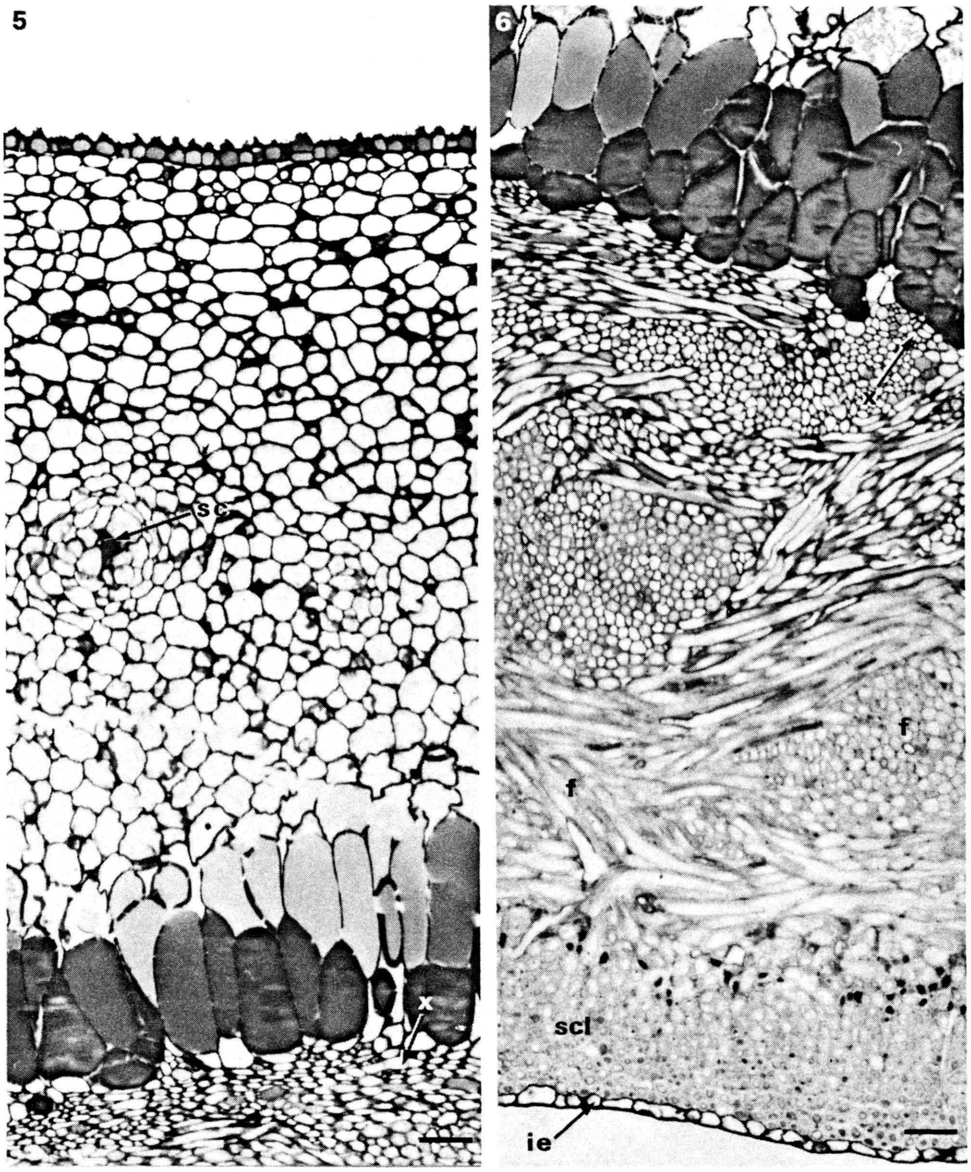
Figure 2. Mature, ripe stones with opercula (o). A & B. The more typical alveolate, laterally compressed stone in lateral view. The drawings on the right show the second, smaller operculum on the reverse side. C & D. Two pairs of opercula to illustrate the size and shape differences. E. One of the triangular stones more or less in apical view, with two pairs of opercula, and one pair of loose opercula. Scale bar = 2 mm.



Figures 3 & 4. Fig. 3. Transverse section of the ovary wall at anthesis, illustrating the outer epidermis (oe), parenchymatous zones, secretory cavities (sc), derivatives of procambium cells (p), xylem elements (x) and the inner epidermis (ie). Scale bar = 30 µm. Fig. 4. TS of the very young pericarp, showing the exo- and mesocarp, the inner part of the latter being tanniniferous. The bundles of fibres (f) of the endocarp (cut in ts and ls) are also distinct. Scale bar = 50 µm.

enormous proliferation of the procambium derivatives. Numerous well-defined bundles of thin-walled fibre initials develop. Tanniniferous substances are deposited in a narrow zone of parenchyma cells which form part of the future mesocarp (Fig. 4).

In young fruit collected one month after anthesis, the exocarp, which is the outer epidermis and about 17–20 layers of the outer parenchymatous zone, show only slight changes (Fig. 5). Since the mature exocarp becomes detached



Figures 5 & 6. TS sections of the young pericarp, illustrating the exocarp, approximately delimited on the inside by the secretory cavities (sc); the mesocarp, the inner part showing radially elongated tanniniferous parenchyma cells (Fig. 5); and the parenchymatous transition zone between meso- and endocarp with vascular tissue i.e. xylem elements (x) in Figs 5 & 6. The fibre bundles (f) in ls and ts, the sclereids (scl) and inner epidermis (ie) of the endocarp are shown in Fig. 6. Both scale bars = 50 μm.

from the meso- and endocarp more or less on the level of the secretory cavities, * this is regarded as the boundary between exo- and mesocarp. The mesocarp is now well-defined. The tanniniferous cells have elongated radially (Fig. 5) and about 8–10 cell layers of thin-walled outer mesocarp have undergone cell divisions and slight radial elongation.

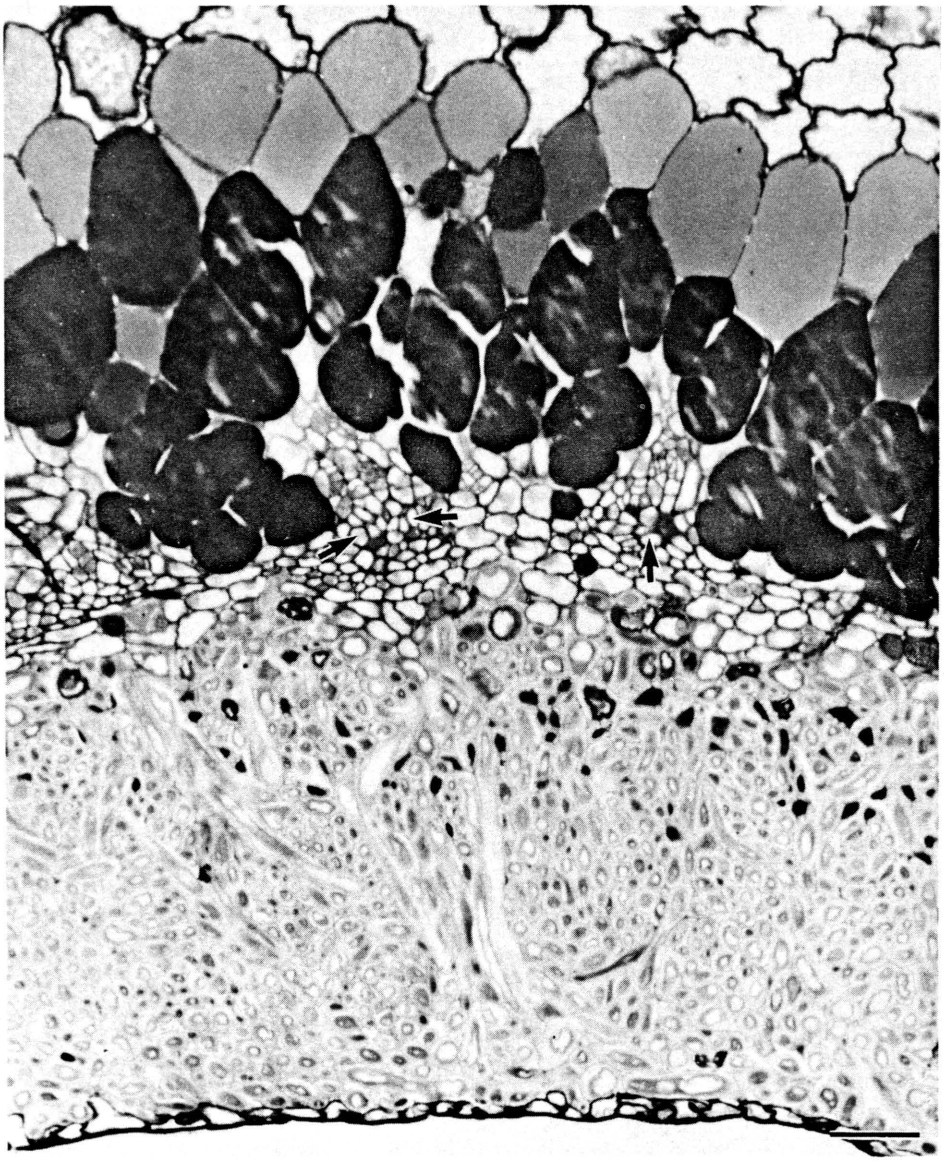


Figure 7. LS of young pericarp showing the tanniniferous mesocarp, the transition zone with xylem elements (arrow heads), and the narrow, sclerenchymatous (excepting the inner epidermis) endocarp which constitutes the operculum tissue. Scale bar = 50 μm.

The boundary between meso- and endocarp is a parenchymatous transition zone within which most of the vascular tissue occurs (Figs 6, 7 & 8A). In the endocarp, lignification has commenced and xylem elements have also differentiated within the bundles of fibres. The inner epidermis consists mainly of periclinally elongated parenchyma cells with intensely stained walls (Figs 6 & 7). Longitudinal sections of the fruit show that the endocarp is at its narrowest at the operculum (Fig. 8A & C) which is demarcated by a zone of parenchyma similar to the transition zone between meso- and endocarp. The operculum *

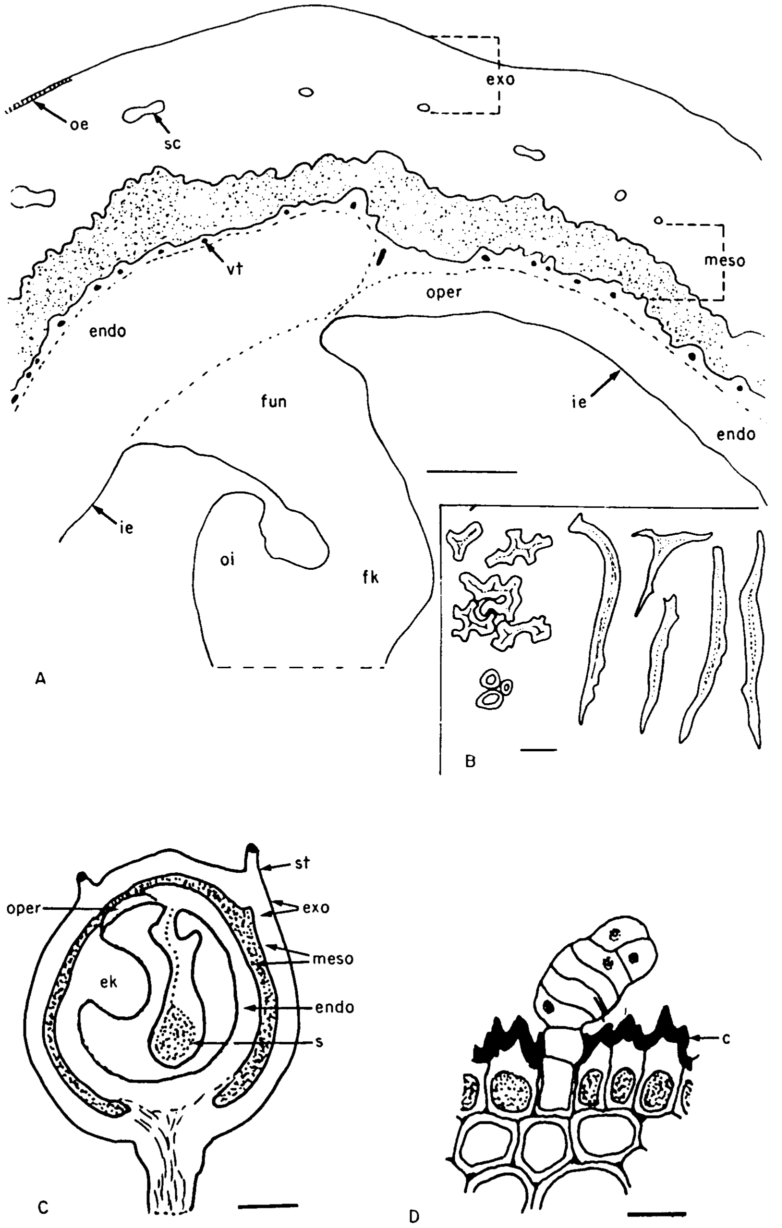


Figure 8. Drawings of a ls of a young pericarp in A; various sclereids of the operculum in B; ls of a whole young fruit in C and a high magnification of a small part of the outer epidermis with multicellular hair and adjacent hypodermis in D. The cuticle (c), endocarp knob (ek), endocarp ★ (endo), exocarp (exo), funicular knee (fk), funicle (fun), inner epidermis (ie), mesocarp (meso), outer epidermis (oe), outer integument (oi), operculum (oper), young seed (s), secretory cavities (sc), styl remnants (st) and vascular tissue (vt) are shown. Scale bars in A = 0.5 mm, in ★ B = 100 µm; in C = 2 mm and in D = µm.

probably develops from the inner epidermis and adjoining 10–15 parenchyma layers, containing no procambium strands. Opercula of ripe fruit consist mainly of sclereids (Fig. 8B) with no xylem elements or other vascular tissue. At the early stages of development, the distinct endocarp knob which projects into the fruit locule (Fig. 8C) does not differ anatomically from the rest of the endocarp, but in the ripe fruit it consists mostly of densely packed fibres.

In young and mature fruit the cells of the outer epidermis contain tanniniferous deposits. The outer tangential walls are sculptured (Fig. 8D) and covered with a thick cuticle. Stomata are on the same level as neighbouring epidermal cells or slightly raised above the surface and the guard cells show distinct outer and minute inner rims. Hairs with two-celled stalks embedded in * the epidermis and multicellular emergent bodies are present. In mature fruit lenticels occur directly under the squashed epidermis.

The slightly collenchymatous outer exocarp cells remain intact even in the old ripe fruit. The thin-walled parenchymatous layers of the inner exocarp, epithelial cells lining the secretory cavities and surrounding parenchyma, as well as the thin-walled outer mesocarp become squashed. At this stage the endocarp is stony. The inner epidermis now consists of thick-walled brachysclereids, which form the shining inner surface of the stone. Macerated tissue of the ripe, stony endocarp (excluding the operculum), shows mainly thick-walled fibres with round and slitlike pits. Small, often globose brachysclereids, with minute cell lumina and sclereids similar to those found in the operculum (Fig. 8B), are also present.

DISCUSSION AND CONCLUSIONS

The close alliance between the two members of the Spondieae indigenous to southern Africa, namely *L. discolor* and the marula is supported by the present investigation. However, the female flower of the marula (Von Teichman & Robbertse, 1986a) has many more staminodes (21–26), and the ovary is usually bicarpellate (or may be uni- to tetralocular) and thus seems to be phylogenetically more primitive than that of *L. discolor*. About 4% of the fruit of *L. discolor* presently investigated showed two fruit locules and therefore developed from a bicarpellate ovary. This type of fruit can be seen as a * phylogenetic 'link' between the usually unilocular *L. discolor* fruit and the usually bilocular ones of the marula.

However, regarding the development and structure of the pericarp, the close relationship between these two taxa is marked. The exocarp *sensu lato* (Roth, 1977) in both taxa consists of the outer epidermis and a number of more or less collenchymatous as well as some parenchymatous cell layers. The stomata are similar and lenticels occur in both species. The mesocarp of the marula is a typical broad, fleshy sarcocarp with extensively radially elongated parenchyma cells, while in *L. discolor* only the inner, tanniniferous layers are reminiscent of a sarcocarp. In *L. discolor* the parenchymatous zone between meso- and endocarp contains vascular tissue and some sclereids, while in the marula this transition zone consists mainly of isodiametric sclereids.

In both species the endocarp is a typical sclerocarp according to the definition of Roth (1977). Fibres develop from derivatives of procambium cells, while parenchyma cells differentiate mainly into brachysclereids. The sclereids in *L. discolor* resemble those of *Buchanania lanzan* Sprengel of the tribe Mangiferae (Mitra & Mehrotra, 1980). Significant is the presence of opercula. These are very sophisticated opening mechanisms of the stony endocarp enclosing the seed.

In both species under discussion the endocarp tissue of the operculum develops from the inner epidermis and adjacent parenchyma of the ovary wall excluding procambium strands.

Contrariwise, in *Rhus lancea* L. fil. of the tribe Rhoideae, the exocarp consists mainly of the outer epidermis and sclerenchymatous hypodermis (Von Teichman & Robbertse, 1986b) and is a brittle, papery, often loose skin. The mesocarp of *R. lancea* consists of an outer parenchymatous and inner sclerenchymatous part, the crystal layer being transitional between the meso- and endocarp. The secretory cavities in *R. lancea*, ramifying through the parenchymatous mesocarp adjacent to the sclerenchymatous part, are closely associated with the vascular bundles. The latter association does not occur in the marula and *L. discolor*. The endocarp of *R. lancea* consisting of 3 layers of sclereids, is also a sclerocarp. According to Roth (1977) this layered endocarp is stratified; since only sclereids occur, it is homogeneous. In the two species of the tribe Spondieae under discussion, the sclerocarp is non-stratified and heterogeneous, since fibres, sclereids, xylem vessels and parenchyma cells are present.

Concerning the growth relations between fruit and seed, the ovule of *L. discolor* more or less fills the ovary locule at anthesis as is the case in the marula and *R. lancea*. In all three species the lignification of the endocarp precedes the growth of the embryo. In *L. discolor* mesocarp differentiation and endocarp lignification occur more or less simultaneously, while in the marula differentiation and sclerification of the endocarp precedes mesocarp development. This latter developmental sequence corresponds to the general pattern in drupes (Roth, 1977).

During the present investigation fruit of the indigenous *Lannea edulis* (Sonder) Engl., L. *schweinfurthii* (Engl.) Engl. var. *stuhlmannii* (Engl.) Kokwaro and *Operculicarya decaryi* were also briefly investigated.

Most of the stones of *L. edulis* collected near Nelspruit resemble those of *L. discolor* in having alveolate sculpturing and two unequal opercula. However, stones of *L. edulis* from Cullinan were more elliptic–subglobose, compressed and showed fewer alveoles, and only one more or less ovate operculum per stone. However, it must be recognized that the taxonomy of this species has not been elucidated.

The stone of *L. schweinfurthii* var. *stuhlmannii* is only slightly sculptured and usually has only one more or less elliptical operculum. However, it is possible that a detailed investigation would reveal that two opercula per stone might also occur. Of the 60 fruits of *O. decaryi* examined, 54 had a single operculum, while five had two opercula more or less equal in size and one had two opercula differing in size. The stones of *O. decaryi* seem to have no alveoles but have prominent ridges. The pericarp structures of *L. discolor, L. edulis, L. schweinfurthii* var. *stuhlmannii* and *O. decaryi* seem to be similar, as is the seed morphology in general. The possible bearing of these findings on the generic status of *Operculicarya* will be discussed in a forthcoming paper.

Similarities in pericarp development and structure between *L. discolor* and the marula support the generally accepted view that these two species are particularly closely related. Once the pericarp ontogeny and structure of more Anacardiaceae fruit have been studied, the taxonomic significance of these characteristics can be assessed more fully.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria, Margaretha Mes Institute for Seed Research, and the CSIR Foundation for Research Development. I wish to express my sincere thanks to Dr A. E. van Wyk for valuable discussions, to Dr Elzabé Schoonraad for her constructive criticism of the manuscript and Mr C. F. van der Merwe for his help with the final printing of the photomicrographs. Rohm & Hass (Pty) Ltd are sincerely thanked for the free samples of 94% HEMA supplied.

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CHAPTER 6

SEED-COAT OF *Lannea discolor**

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*Accepted (December 1986) for publication in *Bot. J. Linn. Soc.*

Development and structure of the seed-coat of *Lannea discolor* (Sonder) Engl. (Anacardiaceae)

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VON TEICHMAN, I., 198 The development and structure of the seed-coat of *Lannea discolor* (Sonder) Engl. (Anacardiaceae). The bitegmic, anatropous ovule contains a group of nucellar cells with slightly thickened and intensively staining cell walls. Besides this hypostase *sensu stricto*, the nucellus cells in the chalaza become tanniniferous. This tanniniferous chalazal-nucellar tissue is initially plate-like. It is referred to as the hypostase *sensu lato*. The latter and the chalaza enlarge significantly. The raphe, extensive chalaza and well-developed cup-like hypostase *sensu lato* play an important role in the development of the seed-coat. The inner, tanniniferous epidermis of the inner integument persists in parts of the mature seed-coat. The outer, distinctly tanniniferous epidermis of the outer integument shows in the mature seed-coat a degree of secondary wall thickening. This undifferentiated type of seed-coat of *L. discolor* (tribe Spondieae) is remarkably similar to that of *Camnosperma minor* (tribe Rhoideae), both also showing tendency towards the exotestal type. In the Rhoideae the endotestal i.e. differentiated type of seed-coat is also present. The exalbuminous seed of *L. discolor* represents a derived and advanced type.

ADDITIONAL KEY WORDS: chalaza - embryo - hypostase - *Operculicarya* - ovule - raphe.

CONTENTS

INTRODUCTION

The development and structure of the pericarp (including a well-developed, stony endocarp) of Lannea discolor (Sonder) Engl. of the Anacardiaceae, tribe Spondieae, is described elsewhere (Von Teichman, 1987) to which the reader is also referred for a general introduction to the species. In this article the emphasis is on the ontogeny of the seed-coat. According to Netolitzky (1926) Lohde already during the previous century recognized the necessity of ontogenetic studies for a better understanding of the mature seed-coat structure. Once the seed-coat structure of more species of the Anacardiaceae is known, the taxonomic implications of this character can be effected. The seed of a few indigenous species of Lannea A. Rich. as well as those of Operculicarya decaryi H. Perr. from the Malagasy Republic (Perrier de la Bâthie, 1946) were investigated. The striking morphologic similarity of the seeds of all these Lannea species to that of O. decaryi will be elaborated upon.

Related studies include that of Srinivasachar (1940) on the development of the female gametophyte of Spondias mangifera Willd., that of Kelkar (1958, 1961) on the embryology and development of the endosperm of Lannea coromandelica (Houtt.) Merr. and the investigation by Corner (1976) of the seed of

Campnosperma minor Corner. Connections between the present study on *L. discolor* and the findings of these authors as well as those mentioned by Davis (1966) in her survey of older literature, will be pointed out.

MATERIAL AND METHODS

Material was collected fortnightly during a two month period, commencing middle September 1984. A large number of flower buds, flowers and fruit in various stages of development were obtained from trees growing naturally on a north-facing slope of the Magaliesberg range near Pretoria (grid reference: 2528CB, Pretoria). Details of voucher specimens and the preparation of the semithin glycolmethacrylate sections correspond with those described for the study on the pericarp of *Lannea discolor* (Von Teichman, 1987).

In addition to the staining techniques mentioned, some sections were treated as follows:

- staining for proteins *(sensu lato)* was done in a 1% aqueous acid fuchsin solution, for 1 to 5 min;
- staining for proteins in amido black 10B according to Bullock *et al*. (1980); as a control, proteins were removed by leaving sections from the same block in a solution of 1% pepsin in 0,01M HCl, pH

2,6 at 37°C for 20 h and rinsing in distilled water before staining with amido black 10B.

In order to test the reserve materials for lipids, fresh tissue of ripe cotyledons was squashed on a slide, stained with a saturated solution of sudan IV in 70% ethanol for 5 min and observed directly in the stain.

OBSERVATIONS

Old, dry fruit were rehydrated and the pericarp removed. The latter consists of a soft, but leathery exocarp, a relatively thin, somewhat fleshy mesocarp, with dark brown to black inner tanniniferous layers, and the stony endocarp (Von Teichman, 1987). The mature fruit mostly contains only one light brown, reniform seed with a characteristic dark brown, saddle-like patch and well-developed funicle (Fig. 1A). The bent, non-chlorophyllus embryo consists of the radicle and two flattened, reniform cotyledons (Fig. 1B).

The internal morphology of young seeds collected one month after anthesis, was reconstructed from serial longitudinal sections and is illustrated in Figure 2. This revealed:

- a knee-like protuberance of the funicle;

- two integuments, the nucellus, endosperm and small pear-shaped embryo;
- a well-developed raphe and chalaza; and
- a very characteristic cuplike zone of tanniniferous parenchyma cells partially surrounding the nucellus.

The origin of the tanniniferous parenchymatous zone was investigated in very young seeds collected 2 weeks after anthesis of the flowers. These tanniniferous cells occur in a plate immediately adjacent to the vascular bundle of the raphe where it fans out in the chalaza. This plate extends to the proximal ends of the inner integument (Fig. 3). As the chalazal-nucellar region cannot clearly be delimited from the rest of the nucellus this plate- and later cup-like zone of tanniniferous parenchyma cells will initially be referred to as the tanniniferous chalazal-nucellar tissue, henceforth the t.c.n. tissue. Some of the nucellar cells around the chalazal end of the embryo sac form a hypostase. The slightly thickened walls of these cells stain more intensively (Fig 3).

Transverse sections of young seeds of an age similar to the one shown in Figure 2 are illustrated in Figures 4 to 7. Of note are: (i) the importance of the cup-like t.c.n. tissue and chalaza in the formation of the seed-coat (Fig. 4); (ii) the nucellar epidermis and the inner integument (i.i.) with its inner epidermis easily distinguishable by the light blue colour in the PAS-toluidine blue stained sections (Fig. 5); and (iii) the outer integument (o.i.) which consists of an inner epidermis, about 2 central layers and an outer epidermis with intensely blue staining tanniniferous deposits (Fig. 5). A thin, discontinuous cuticular layer is present on the outer epidermis of the o.i. and ovule wall and a more or less continuous one occurs between the 2 integuments. The 3 cuticular layers covering the inner epidermis of the i.i., the nucellus (Fig. 5) and embryo are well-developed. The raphe with an amphicribral vascular bundle takes part in the formation of the seed-coat (Figs 6 & 7).

Six weeks after anthesis the fruit locule contains a young reniform seed with two cotyledons. The dark brown colour of the saddle-like patch on the young seed is caused by the tanniniferous deposits in the t.c.n. tissue (Figs 8 & 9). The integuments near or continuous with the t.c.n. tissue and chalaza are not significantly altered at this stage (Figs 8 & 9), but at the raphe the o.i., i.i. and the outer nucellar layers are reduced. All the cuticular layers are still present.

In the mature seed some endosperm layers persist (Figs 10 & 11), while the contents of the nucellar cells has been utilized and the cell walls squashed (Fig. 11). The epidermal cells of the ovule wall and o.i. with their tanniniferous contents and often secondarily thickened radial and inner tangential cell walls also contribute to the seed-coat. This constitutes a slight exotestal condition according to the definition of Corner (1976). In the mature seed-coat mainly the outer epidermis of the o.i. and the inner epidermis of the i.i. remain. The t.c.n. tissue, the vascular tissue, especially the xylem elements of the chalaza (Fig. 10) and raphe, and the remains of the two integuments constitute the mature seed-coat.

In the mature seed-coat the cuticles covering the nucellus and the inner epidermis of the i.i. are well-developed; however, no other cuticular layers could be distinguished (Fig. 11). The presence of well-developed cuticular layer(s) in the mature seed-coat of seeds enclosed in a stony endocarp was already reported for many such seeds by Netolitzky (1926).

The cotyledons of the young, as well as mature seed, contain lipids, but no starch, and the main reserve material is distinctly proteinaceous.

DISCUSSION

The present study indicates that the fully developed ovule of L. discolor is strikingly similar to that of Sclerocarya birrea (A. Rich.) Hochst. subsp. caffra (Sonder) Kokwaro, the 'marula' (Von Teichman & Robbertse, 1986,a) and Rhus lancea L.f. (Von Teichman & Robbertse, 1986,b). These similarities include: (a) an extensive o.i. that projects much further than the i.i., (b) a prominent funicular knee, previously referred to as a "ponticular outgrowth" and (c) the anatropous ovules. In L. discolor and R. lancea the raphal and chalazal tissues commence their extensive development only after fertilization. A re-assessment and ontogenetic study of the ovule and seed of L. discolor, therefore, indicate that the term 'raphe-chalazal ovule' suggested by Robbertse et al. (1986) is not applicable, as it would apply to a very young seed (Fig. 2), and the ovule is thus best described as being anatropous.

Engler (1897) described the anatropous ovule of the Anacardiaceae as having a dorsal raphe, the seeds as having little or no endosperm and the embryo as being relatively large, fleshy and either bent or straight. To Engler this latter character was taxonomically significant and he used it to subdivide the tribe Spondieae into the subdivisions Rectembryeae and Curvembryeae. The seed of all the indigenous species of *Lannea* investigated in the present study contains a bent embryo. Therefore the genus *Lannea* placed by him in the Rectembryeae belongs in the Curvembryeae.

In *L. discolor* the funicular and raphal vascular bundles are amphicribral. Bouman (1984) mentions several families where such bundles occur, although collateral bundles are much more common.

The reserve material in the young, as well as mature cotyledons consists of lipids and proteins. This corresponds to the condition found in the seed of *Rhus lancea* and other species of the Anacardiaceae (Von Teichman & Robbertse, 1986,b). The seed of *L. discolor* is regarded as exalbuminous since the cotyledons are fleshy and the endosperm remnants are not easily distinguished macroscopically as is the case in *L. coromandelica* (Kelkar, 1961). Cellular endosperm remnants also occur in the mature seed of species of *Dobinea* Buch.-Ham. ex D. Don (Netolitzky, 1926), *Pistacia chinensis* Bunge (Magen, 1912), several species of *Rhus* L. (Netolitzky, 1926; Von Teichman &

Robbertse, 1986,b) and *Schinus molle* L. (Copeland, 1959), whereas the embryo of *Semecarpus anacardium* L.f. (Srinivasachar, 1940) utilizes the endosperm totally. The seed of the Anacardiaceae therefore usually has little or no endosperm, as Engler reported as early as 1897. The embryo of *L. discolor*, the marula and *R. lancea* is non-chlorophyllous. This type of embryo would logically be found in seeds with a thick, lignified endocarp, and the presence or absence of chlorophyll in the embryo may, according to Dahlgren (1980) be taxonomically significant.

During the present investigation, seed of the indigenous *Lannea edulis* (Sonder) Engl. and *Lannea schweinfurthii* (Engl.) Engl. var. *stuhlmannii* (Engl.) Kokwaro, as well as *Operculicarya decaryi* from the Malagasy Republic was briefly compared with that of *L. discolor*. All examined seeds were reniform and have the dark brown saddle-like patch. In fact similarities are so striking that the species are obviously closely related. In view of these observations the generic status of *O. decaryi* needs reinvestigation, especially once female and male flowers are available, since these were not described by Perrier de la Bâthie (1946).

According to the suggested definition of Tilton (1980), the nucellar cells with slightly thickened walls at the chalazal end of the embryo sac may be called a hypostase. Corner (1976: 22) considers the typical hypostase to consist of cells "which become brown and suberized or lignified but lack differentiation in form". He thus uses the term in a general sense "for this plate or disc at the base of the nucellus". Bouman (1984: 148) defines the hypostase as "situated above the chalazal vasculature and, depending on the form of the nucellar base, it may be present as a cluster of cells, or a disc- or cup-like cell plate".

A hypostase occurs in a considerable number of taxa of the Anacardiaceae. In the tribe Mangiferae (Engler, 1897) a layer of tanniniferous cells with thickened cell walls is responsible for the reddish-brown colour of the seed-coat of Anacardium occidentale L. (Weber, 1907). In the tribe Rhoideae a hypostase with lignified cell walls was described for Rhus toxicodendron L. by Grimm (1912). In Toxicodendron diversiloba (Torrey & Gray) Green a cup of tanniniferous cells surrounds the base of the nucellus in young seeds (Copeland & Doyel, 1940). A hypostase with thick-walled cells and a "moribund appearance" occurs in *Schinus molle* according to Copeland (1959).

In *Pistacia chinensis* the darkly staining, probably tanniniferous cells of the hypostase later form a deep cup, which is externally recognizable by a darkening of the seed-coat (Copeland, 1955). A similar hypostase occurs in *P. vera* L. and inter alia also in *P. atlantica* Desf. and according to Grundwag & Fahn (1969) and Grundwag (1976) this tissue corresponds to a dark spot in the mature seed-coat. Netolitzky (1926), however, found a group of suberized cells ("Korkzellen") in the chalaza of species of *Pistacia* L. The seed of *Camnosperma minor* has an extensive chalaza and extensive hypostase, composed of "small contiguous cells with thin brown walls" (Corner, 1976: 68).

In the tribe Spondieeae a hypostase occurs at least in *Spondias mangifera* and *Lannea discolor*. In *S. mangifera* "a pad of tannin containing cells is formed at the chalazal region of the nucellus" (Srinivasachar, 1940: 86) and thus shows close similarity with the t.c.n. tissue of *L. discolor* as does the extensive hypostase and chalaza of *Camnosperma minor*.

The group of nucellar cells at the chalazal end of the embryo sac of *Lannea discolor* is not visible externally in the mature seed. It is proposed that these cells be interpreted as the hypostase *sensu stricto*, while the extensive t.c.n. tissue which persists as a conspicuous darkly coloured saddle-like patch in the mature seed-coat be called the hypostase *sensu lato*. The latter wider interpretation seems to fall within the concept of the more generalized application of the term hypostase by authors such as Corner (1976), Tilton (1980) and Bouman (1984).

A hypostase *sensu stricto* probably occurs in species of *Rhus* L., *Schinus* L. and *Lannea*, while the hypostase *sensu lato* seems to occur in *Anacardium occidentale*, *Toxicodendron diversiloba*, species of *Pistacia* L., *Camnosperma minor*, *Spondias mangifera* and *Lannea discolor*. The cells of the hypostase *sensu lato* probably serve initially as a storage place for tannin-like metabolic products and in the mature seed-coat it may have a strengthening/protective function.

In *L. discolor* the raphe, extensive chalaza, hypostase *sensu lato* and remnants of the integuments form the mature seed-coat. As reported for *Camnosperma minor* (Corner, 1976: 67) there are "traces of an exotestal state" in the seed-coat of *L. discolor* since the epidermis of the ovule wall and o.i. is not only tanniniferous but also often shows secondarily thickened radial and inner tangential cell walls. However, on the whole the seed-coat of *L. discolor* is undifferentiated. Such seed-coats are often found in drupaceous indehiscent fruit (Corner, 1976).

In *Rhus lancea* and other species of the Rhoideae (Von Teichman & Robbertse, 1986,b) it is probably the inner epidermis of the o.i. which persists and has lignified radial cell walls with conspicuous pits. This is an endotestal state *sensu* Corner (1976). Therefore, within the tribe Rhoideae, one finds undifferentiated to slightly exotestal seed-coats with extensive chalaza and hypostase (*Camnosperma*) as well as thin, differentiated, endotestal seed-coats (*Rhus*, *Pistacia* etc). The undifferentiated seed-coat and extensive hypostase described for *Lannea discolor* of the Spondieae is therefore not restricted to that tribe.

CONCLUSIONS

Boesewinkel & Bouman (1984: 572) state that the anatropous ovule, which is found in most families of the angiosperms, "gives rise to the so-called normal seed" and that due to local growth derived seed types develop.

Although the seed of L. discolor does not conform to any of the described types of Boesewinkel & Bouman (1984) it is nevertheless considered as derived and phylogenetically advanced. The reasons for this conclusion include: 1. the occurrence of the well-developed stony endocarp or sclerocarp which gives mechanical protection to the embryo. 2. the extensive chalaza and raphe which are secondarily developed, as well as 3. the exalbuminous condition of the seed.

Since a sclerocarp is present in the species of the Anacardiaceae discussed, it is not surprising to find undifferentiated and thin, endotestal (differentiated) seed-coats in this family.

The development of a sclerocarp in the pericarp of the Anacardiaceae probably preceded the reduction in mechanical strengthening layers in the seed-coat.

Despite the anatropous nature of the ovule, the mature seed has a campylotropous-like shape. The latter originates through secondary curvature of the developing seed, perhaps partly enhanced as a result of the space restrictions in the fruit locule caused by the projecting endocarp knob (Von Teichman, 1987, Fig. 8C). Since the characteristic saddle-like patch is derived from chalazal (i.e. hypostase sensu lato) tissues, the seed could also be regarded as an incipient or transitional stage towards the development of a pachychalazal seed.

The seed-coat of L. discolor is of the undifferentiated type and the raphe and extensive chalaza (including the hypostase sensu lato) play an important role in its development. The seed of L. discolor is regarded as a derived and phylogenetically advanced type and possibly could best be interpreted as a 'raphe-chalazal' type sensu Robbertse et al. (1986). The seed of Lannea discolor (tribe Spondieae) shows close resemblance to that of Camnosperma minor (tribe Rhoideae). However, more detailed ontogenetic studies of the seeds of the Anacardiaceae are required for a better understanding of the variation in the mature seed-coat structure. Thereafter the taxonomic significance of seed-coat structure in the Anacardiaceae can be assessed.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria, Margaretha Mes Institute for Seed Research, and the CSIR Foundation for Research Development. I wish to express my sincere thanks to Prof A E van Wyk for the valuable and indispensable discussions. Dr Elzabé Schoonraad is thanked sincerely for her constructive criticism of the manuscript, and Prof P J Robbertse for his advice. Rohm & Haas (Pty) Ltd is also thanked for the free samples of 94% HEMA delivered to us!

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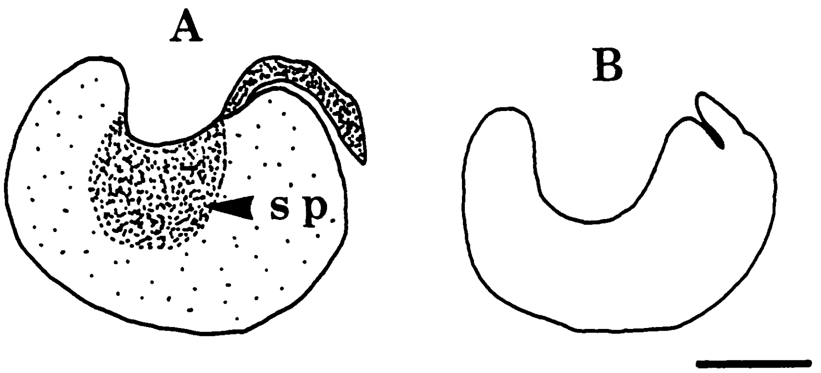


Figure 1. <u>Lannea</u> <u>discolor</u> seed (A) and embryo (B) in lateral view, showing their size and shape. In the seed the saddle-like patch (sp) in the seed-coat and the funicle are shown. The embryo consists of 2 cotyledons and the radicle. Scale bar = 2 mm.

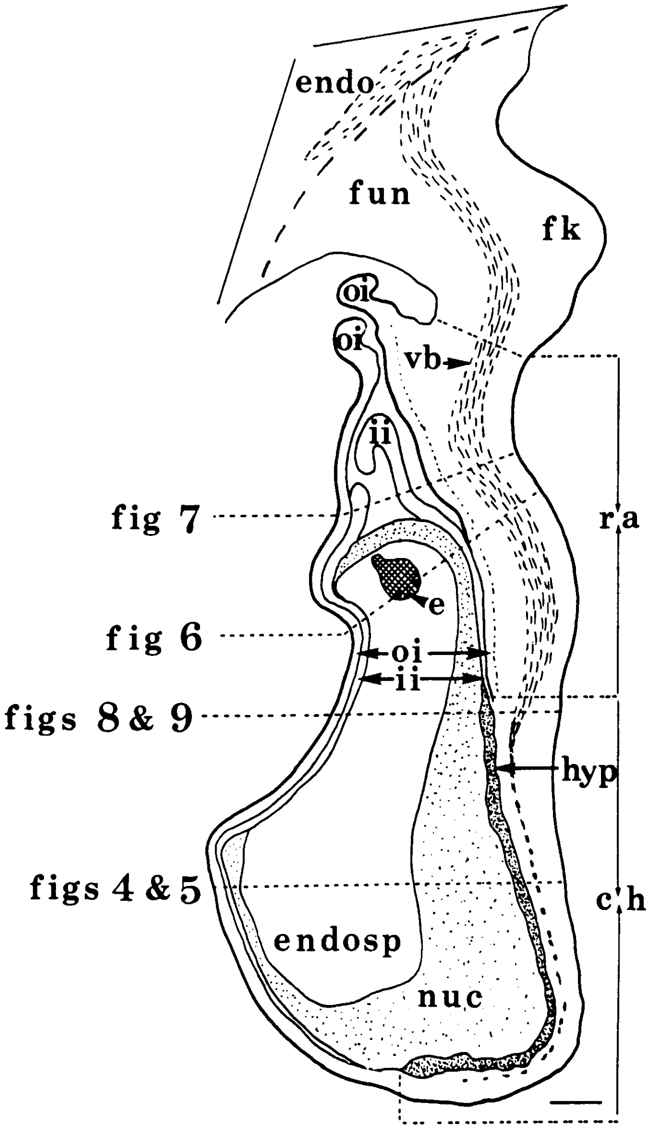


Figure 2. A reconstruction of a young seed (collected 1 month after anthesis) by means of several (serial) LS. It illustrates the funicle (fun) adjacent to the endocarp (endo), the funicular knee (fk), the vascular bundle (vb) inside the funicle and the well-developed raphe (ra), the extensive chalaza (ch) with vascular tissue and extensive cup-like zone of tanniniferous parenchyma (hyp). The outer (oi) and inner (ii) integuments, the nucellus (nuc), endosperm (endosp) and embryo (e) are also shown. The position of various TS of a seed of the same age (Figs 4-7) and those of older seeds (Figs 8 & 9) are indicated. Scale bar = 30 μm.

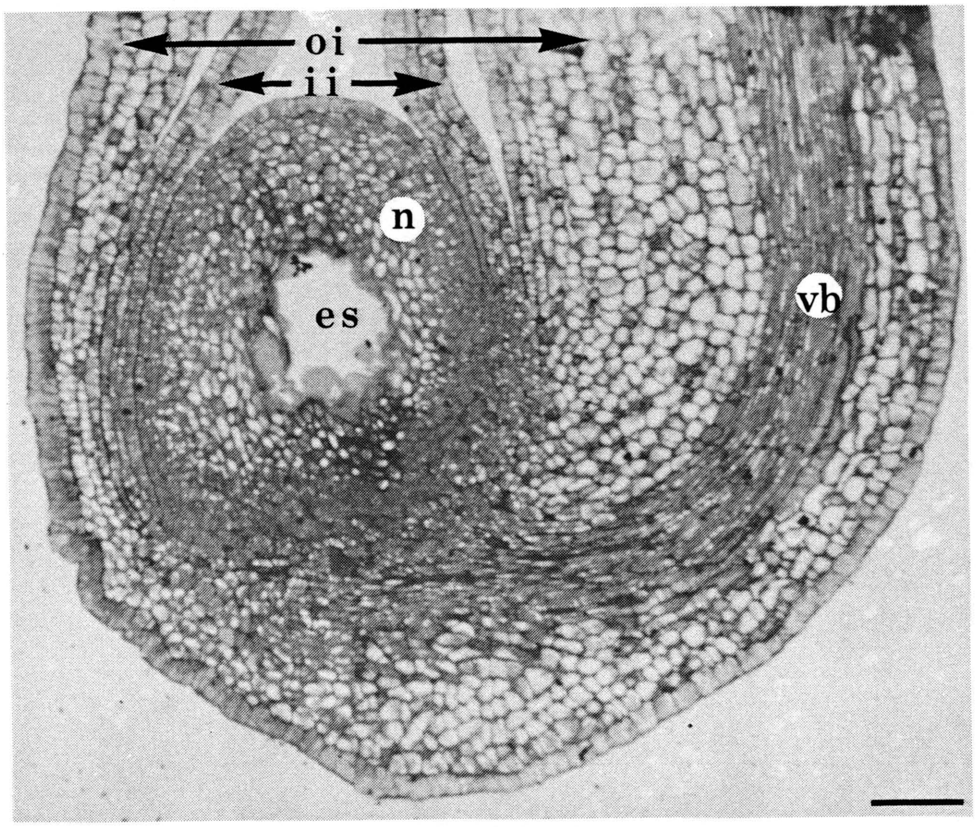


Figure 3. LS of a very young seed, collected 2 weeks after anthesis, showing the outer (oi) and inner (ii) integument, the nucellus (n) surrounding the embryo sac (es) which is lined with endosperm cells. The hypostase and plate of tanniniferous cells between the proximal ends of the i.i. are also shown. The vascular bundle (vb) of the funicle and raphe fans out in the chalaza. Scale bar = 90 μm.

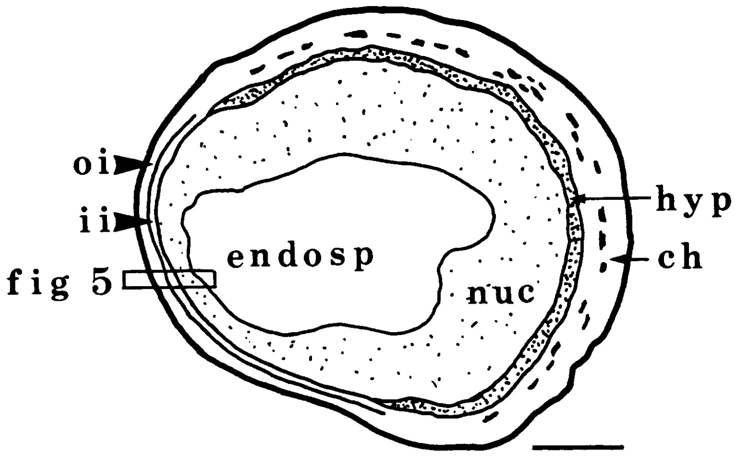


Figure 4. TS of a young seed, collected 1 month after anthesis, illustrating the extent of the well-developed chalaza (ch) and t.c.n. tissue (hyp), as well as the nucellus (nuc) surrounding the endosperm (endosp). The inner integument (ii) is continuous with the t.c.n. tissue. The structure of the small, framed part including the oi and ii is shown in Fig. 5. Scale bar = 300 μm.

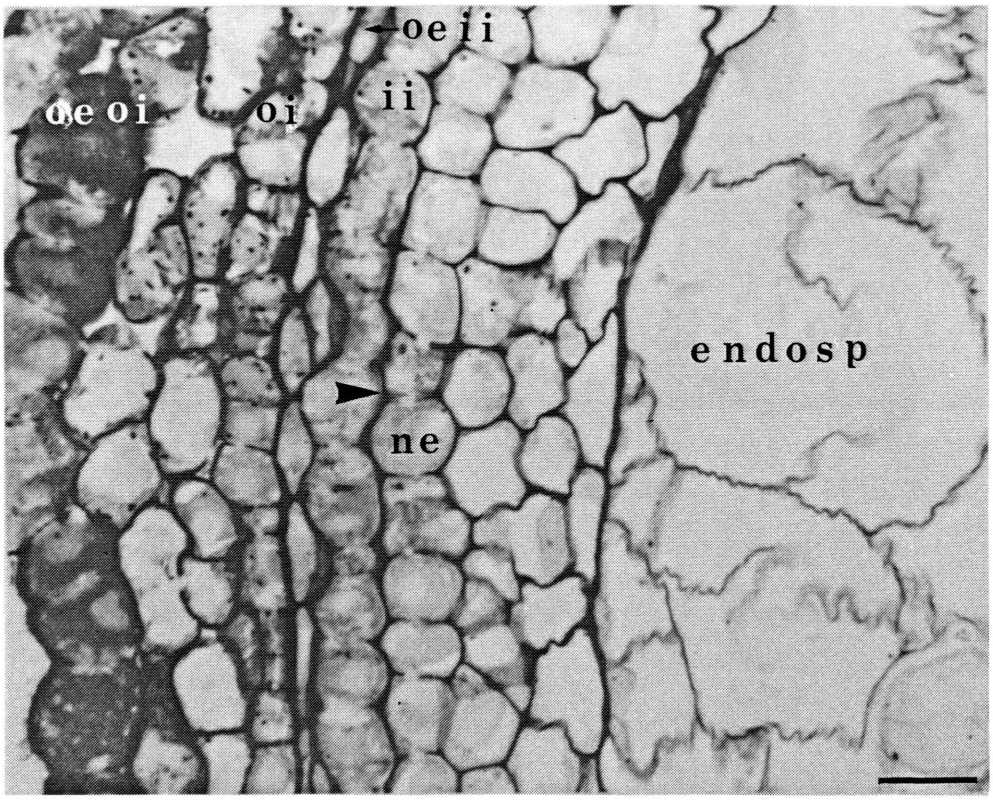


Figure 5. Higher magnification of framed part in Fig. 4, showing the outer epidermis (oe oi) which is tanniniferous and inner epidermis (oi) of the outer integument, as well as the outer epidermis (oe ii) and inner epidermis (ii) of the inner integument. The arrow head points to the well-developed cuticles. The nucellar epidermis (ne), remnants of nucellus and endosperm (endosp) are also shown. Scale bar = 20 μm.

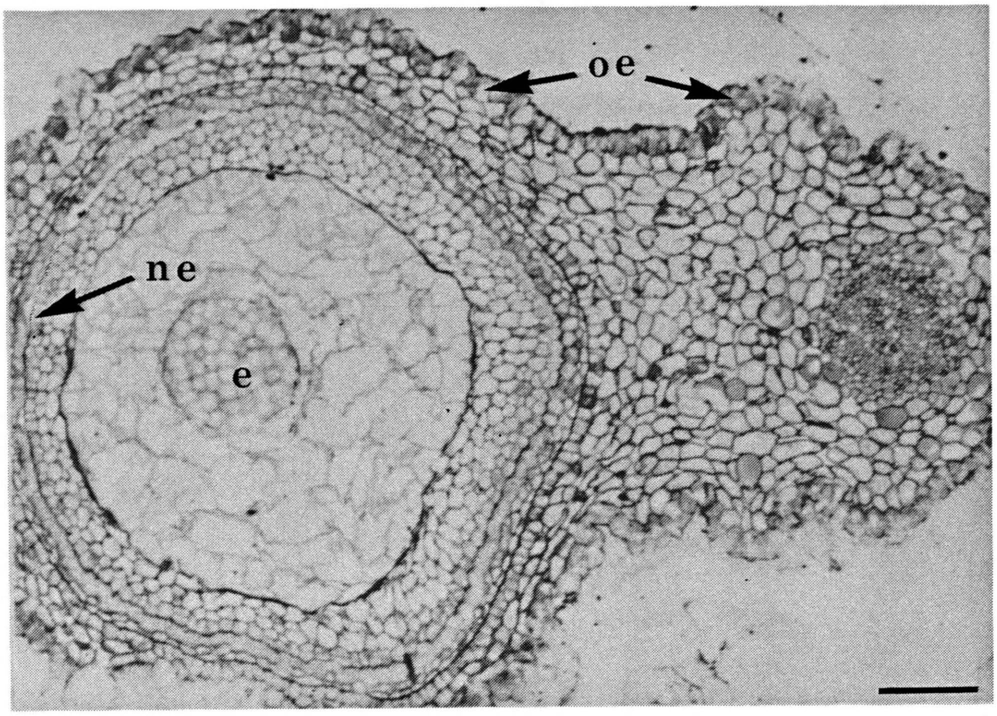


Figure 6. TS of the same young seed which is also illustrated in Figs 4, 5 & 7. The raphe with amphicribral vascular bundle, the outer epidermis (oe) of the oi and raphe, the remainder of the oi, the ii, the nucellar epidermis (ne) and endosperm surrounding the embryo (e) are distinguishable. Scale bar = 100 μm.

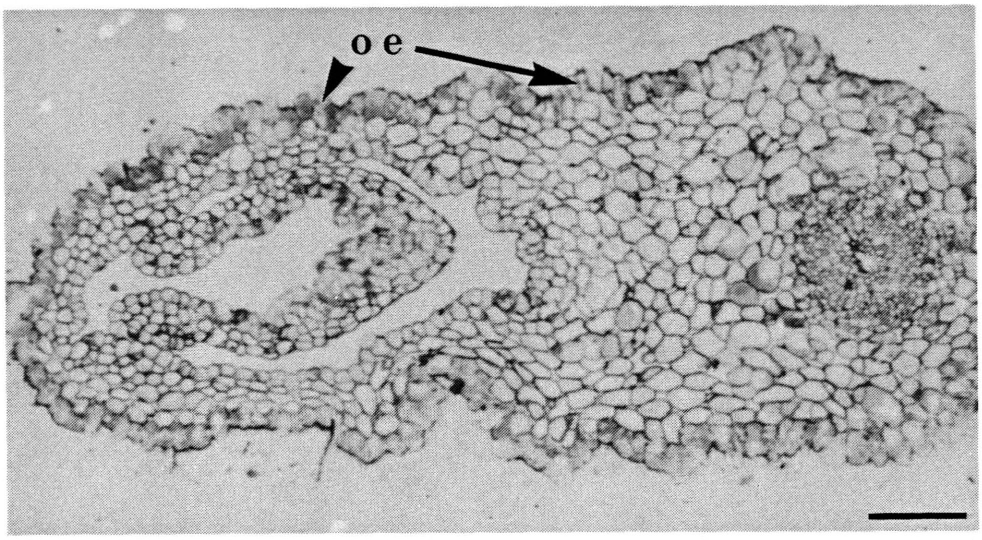
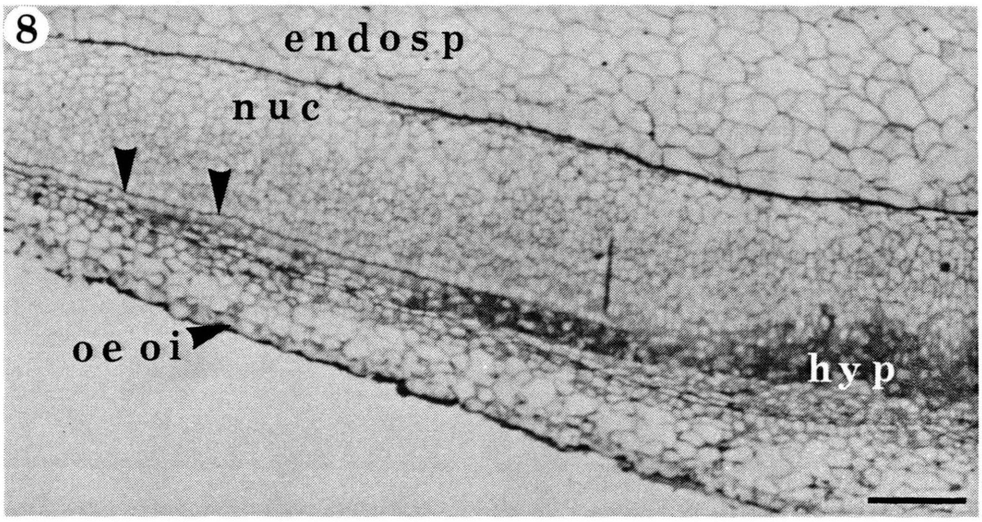
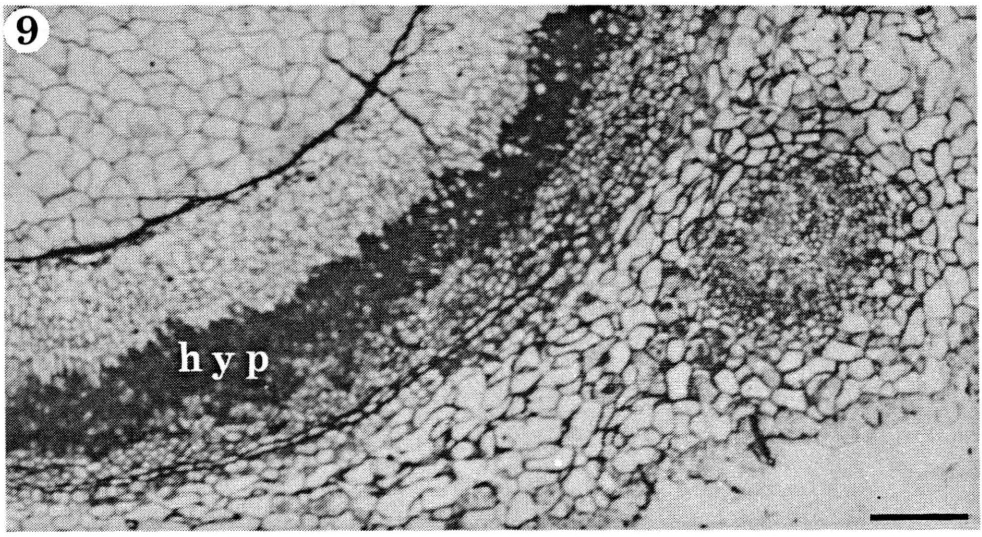


Figure 7. TS of the young seed, showing the amphicribral vascular bundle in the raphe, here closer to the funicle and the outer epidermis (oe) of the oi and raphe. The distal portions of the 2 integuments are also illustrated. Scale bar = 100 μm.





Figures 8 & 9. TS of the seed-coat of a seed collected 6 weeks after anthesis of the flower. Fig. 8 illustrates the part adjacent to the t.c.n. tissue (hyp), the endosperm (endosp) and the nucellus (nuc). The arrow heads indicate the cuticles of the nucellar epidermis and the inner epidermis of the ii. The cell contents of the latter layer and the outer epidermis of the oi (oe oi) is tannin-like. Fig. 9 illustrates the seed-coat continuous with that in Fig. 8 showing also the extent of the t.c.n. tissue (hyp) and the amphicribral vascular bundle of the raphe. Scale bars = 100 μm.

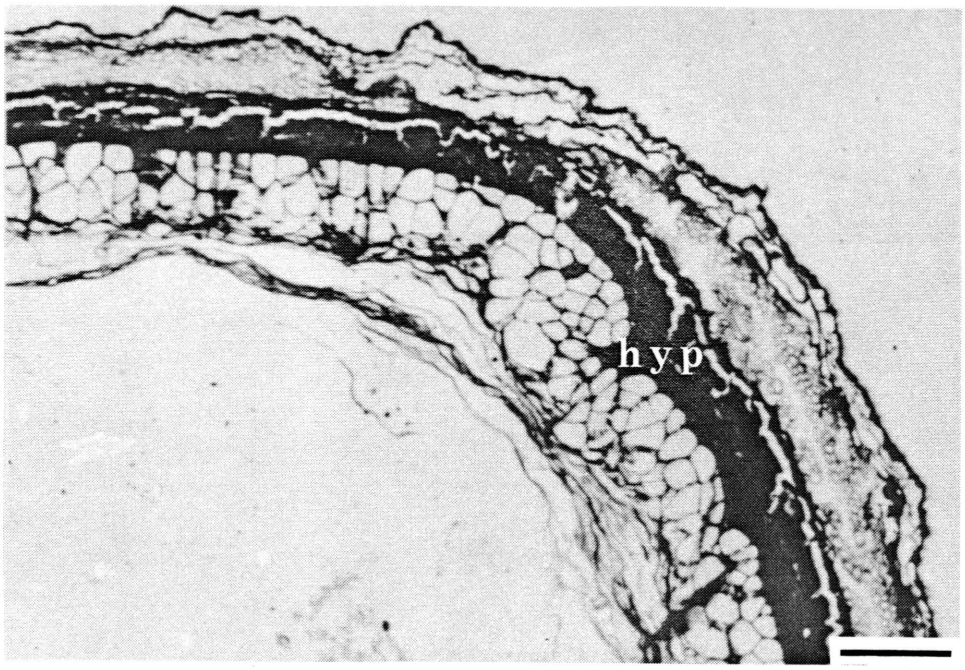


Figure 10. TS of the mature seed-coat at the brown, saddle-like patch. The tanniniferous chalazal-nucellar tissue (hyp) not distinguishable from the utilized and squashed nucellus is shown. To the outside the vascular bundles of the chalaza and to the inside endosperm remnants are present. Scale bar = 100 μm.

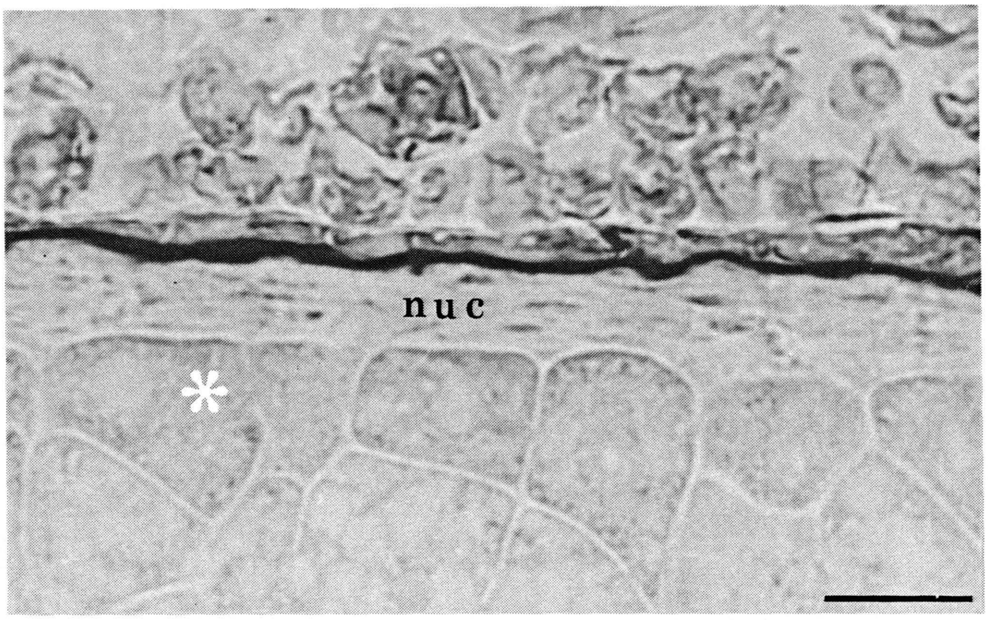


Figure 11. TS of the mature seed-coat near the saddle-like patch. This sudan black B stained section shows the persistent cuticles of the nucellar epidermis and of the remnants of the inner epidermis of the ii as one distinct layer. The remnants of the oi, the squashed nucellus (nuc) and the cellular endosperm remnants (white asterisk) are also illustrated. Scale bar = 100 μm.

CHAPTER 7

PERICARP AND SEED-COAT OF Harpephyllum caffrum*

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*Accepted (25.11.1987) for publication in Bot. J. Linn. Soc.

The ontogeny and structure of the pericarp and seed-coat of _Harpephyllum_ _caffrum_ Bernh. ex Krauss (Anacardiaceae)

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VON TEICHMAN, I. & VAN WYK, A.E., 198 . The ontogeny and structure of the pericarp and seed-coat of *Harpephyllum caffrum* Bernh. ex Krauss (Anacardiaceae). The exocarp *sensu lato* which develops from the outer epidermis and adjacent parenchyma of the ovary wall, consists of collenchyma cells with a stomatous epidermis. The fleshy, parenchymatous mesocarp or sarcocarp develops after endocarp differentiation. The endocarp is partly spongy and partly woody. The spongy endocarp contains most of the vascular tissue and fills the cavities and grooves of the intricately sculptured outer woody endocarp. The inner woody endocarp and adjacent woody, endocarpal operculum develop from the inner epidermis and subepidermal parenchyma of the ovary wall. The bitegmic, anatropous ovule develops into a derived, exalbuminous seed with an undifferentiated seed-coat. An extensive chalaza, extensive hypostase *sensu lato* and the raphe are important in the development of the seed-coat. The pericarp and seed-coat of *H. caffrum* is compared with those of *Sclerocarya birrea* subsp. *caffra* and *Lannea discolor*. The close phylogenetic relationship of these three species of the Spondieae is reaffirmed. The marked similarities in pericarp and seed structure between *H. caffrum* and species of the genus *Spondias* are noted.

ADDITIONAL KEY WORDS: chalaza - drupe - endocarp - hypostase - operculum - raphe - seed.

CONTENTS

INTRODUCTION

The monotypic genus Harpephyllum Bernh. ex Krauss is placed in the Anacardiaceae with 78 other genera occurring mainly in tropical and subtropical regions (Scholz, 1964). Harpephyllum belongs to the tribe Spondieae with, amongst others, the genera Lannea A. Rich., Sclerocarya Hochst. and Spondias L. (Engler, 1897).

Harpephyllum caffrum Bernh. ex Krauss is a dioecious evergreen tree, up to 30 m in height. The glabrous leaves are aggregated at the end of branches and persist for up to two years, after which they often turn bright red. The tree occurs in frost free coastal, lower montane and riverine forests of the eastern Cape Province, Natal and the Transvaal, the distribution extending northwards into Zimbabwe and Mozambique. It grows easily from seed and truncheons. Besides the vernacular names used by the various African peoples, it is also known as "kafferpruim", "suurpruim", wild plum and kaffir date. Although H. caffrum is currently not of particular economic importance, it is grown as an ornamental in gardens and is generally utilised in many other ways. The pale red wood makes a fine timber. The fruit with its pleasant sour taste and characteristic odour is eaten raw by humans and is relished by a variety of animals. An excellent table jelly and good soft drink can be prepared from the ripe fruit. Watt & Breyer-Brandwijk

(1962) mentions various medicinal uses of the bark by the Zulu. El Sherbeiny & El Ansari (1976) reported on the leaf polyphenolics and flavonoids. According to Williams (1930: 44) the leaves and twigs yield 11,4% tannins and the bark 18,2% which make it "worthy of further investigation as a possible commercial source of tanning material".

The present paper forms part of a comparative morphological study of mainly the fruit and seed of the genera of the Anacardiaceae indigenous to southern Africa. Aspects of the pericarp structure of *Sclerocarya birrea* (Richard) Hochst. subsp. *caffra* (Sonder) Kokwaro - henceforth referred to by its vernacular name "marula" - have previously been reported on (Von Teichman & Robbertse, 1986,a), as well as pericarp and seed-coat structure of *Rhus lancea* L. fil. (Von Teichman & Robbertse, 1986,b) and *Lannea discolor* (Sonder) Engl. (Von Teichman, 1987 and in press,a). The ontogeny of the seed-coat of the marula is described in a recent manuscript (Von Teichman, in press, b).

Harpephyllum caffrum is the third and last species of the Spondieae indigenous to southern Africa to be discussed in this series. In this paper the phylogenetic relationship, as reflected by fruit and seed structure, between *H. caffrum* and two native species of the Spondieae viz. the marula and *L. discolor*, as well as members of the exotic genus *Spondias*, will be discussed.

MATERIAL AND METHODS

Female flowers and fruit in various stages of development were collected from trees growing in a private garden in Pretoria. Voucher specimens (Von Teichman 537 & 551) are kept at PRU (H.G.W.J. Schweickerdt Herbarium, Department of Botany, University of Pretoria).

Semi-thin glycolmethacrylate sections were prepared according to the methods used for *Lannea discolor* (Von Teichman, 1987). The following additional histochemical tests were carried out:

- Staining for proteins, lipids and lignin was done as described for the seed-coat of the marula (Von Teichman, submitted). Pectic substances were tested for with ruthenium red according to Johansen (1940).

- Starch was tested for, in addition to the PAS reaction, with the potassium iodide-iodine (IKI) reaction (Jensen, 1962).

- In some sections the cellulose cell walls were treated with 0.1% optical brightener i.e. Calcofluor-White M2 R New (Hughes & McCully, 1975) and examined with a Reichert Univar microscope equipped with epifluorescence optics.

Small pieces of the stony endocarp and a few stony opercula were macerated by heating in Schulze's solution (McLean & Cook, 1941).

OBSERVATIONS

Structure of the female flower, fruit and seed.

The whitish to greenish-yellow female flowers are borne in 6-12 cm long terminal panicles. The flowers, which are about 6.0 mm in diameter with pedicels 2.5 to 5 mm long, appear in spring to early summer. They are bracteate and the median, deltate bracteole (about 0.5 mm long) lies between one or two minute (about 0.25 mm long) triangular ones. The rachis, pedicels and bracteoles are pubescent with the slender club-shaped, uniseriate hairs consisting of five to six cells.

The perianth is usually pentamerous. The oblong-ovate petals are imbricated in the bud (Fig. 1) and recurved at anthesis. Usually, ten conspicuous staminodes are inserted below the glabrous, 10-crenate, annular disc. The syncarpous, usually pentacarpellary, seldom tetracarpellary gynoecium consists of a subglobose, slightly laterally compressed ovary, separate short styles and slightly capitate to truncate papillate stigmas.

About 68% of the ovaries are unilocular, while 24% have one well-developed locule as well as either one very small, always sterile locule or one or two slits between two epidermal layers presumably representing one or two totally reduced locules. Only about 8% of the ovaries have two equally well-developed fertile locules. The latter may each contain one pendulous anatropous ovule with a dorsal raphe and a massive funicule. It is the anterior (sensu James & Clapham, 1935) carpel which is fertile and lies nearest to the subtending bracteole.

The ripe, subfleshy, oblong-obovate drupe (Fig. 2), 2.5 cm long and 1.2 to 1.5 cm in diameter, is red, smooth, glabrous, with minute style remnants. The slightly tough exocarp comprises about 8% of the pericarp thickness, the fleshy mesocarp about 25% and the endocarp about 67% (Fig. 2). The endocarp is partly woody and partly spongy (Fig. 3). The woody part is characteristically intricately sculptured.

About six irregular longitudinal ridges radiate from an inner woody core, forming heterogeneous and complexly shaped cavities. In fruit with one or two well-developed locules, one or two large cavities respectively occur at the apex (Fig. 3A). In these cavities one woody, dome-shaped bi-partite operculum (Fig. 3A & B) per locule is found, concealed by the ridges and covered by the spongy endocarp. The inner surface of the fruit locule is shiny as if polished. The woody endocarp resists weathering, while the softer spongy endocarp which fills the various cavities and grooves, is ephemeral.

The ripe, oblong, slightly reniform, flattened seed is about 11 to 13 mm long and 4 to 5 mm broad (Fig. 4). The light brown seed-coat features a characteristic dark brown saddle-like patch; the funicle is well-developed and the embryo is non-chlorophyllous, slightly bent and comprises the radicle and two flattened, usually white but occasionally light yellow cotyledons.

Ontogeny and structure of the pericarp

In flower buds (Fig. 1) the ovary wall features the following cell layers and zones:

- the outer epidermis in which various anticlinal cell divisions have taken place;

- an outer hypodermal zone of parenchyma ("zone A");
- a broad (middle) zone of partly tanniniferous parenchyma cells in which the procambium strands and vascular bundles mostly occur in close association with schizogenous secretory ducts, lined with large, cytoplasm-rich epithelial cells;
- an inner parenchymatous zone of about 6 to 8 cell layers ("zone B"), and
- an inner epidermis with predominantly anticlinal cell divisions.

Shortly after anthesis the middle zone containing the vascular tissue has developed most markedly (Figs 5 & 6). By now derivatives of the procambium cells have formed bundles of narrow, elongated, thin-walled fibre initials. Most of the secretory ducts are characteristically arranged in semi-circles as seen in TS, and are closely associated with, or even surrounded, by vascular tissue, to such an extent that it can be assumed that they were derived, as the vascular tissue, from the procambium strands. The parenchyma cells in zone B close to the inner epidermis divided anticlinally and periclinally.

In very young fruit, more and larger bundles of haphazardly orientated fibre initials (Fig. 7) differentiated in the middle zone. These fibre initials will eventually develop into the fibres constituting the bulk of the woody endocarp ridges. The spongy endocarp, which is also interspersed among the bundles of fibre initials, originates from the parenchyma cells (with or without tanniniferous contents), as well as vascular and secretory tissue of the middle zone (Fig. 7). Groups of tanniniferous parenchyma cells are also found in zone B (Fig. 7). The vestiges of reduced fruit locules could often be distinguished as a slit surrounded by epidermal cells (Fig. 7).

In slightly older fruit (Fig. 8), the future exocarp comprises about 20% of the thickness of the pericarp and consists of the outer stomatous epidermis and adjacent zone A of densely packed parenchyma cells, often containing tannin-like substances. At this stage the future endocarp comprises about 80% of the thickness of the pericarp, and can be subdivided into the following three main components:

- the spongy endocarp, which occurs on the outside of the future woody endocarp ridges, as well as interspersed between them;
- the future woody endocarp ridges consisting of large fibre bundles, spiral xylem elements and elongated tanniniferous parenchyma cells; and

- the parenchymatous operculum tissue, originating from a part of the inner epidermis of the ovary wall as well as the adjacent hypodermal parenchyma which later becomes partially tanniniferous. It is delimited by a few cell layers of much smaller parenchyma cells. No vascular elements occur in the operculum tissue.

About one month after anthesis the hypodermal layers of the exocarp are slightly collenchymatous (Fig. 9). The structure of the spongy and woody endocarp, at this stage of development, is illustrated in Figures 9 to 12. The spongy endocarp parenchyma exhibits numerous large intercellular spaces, which gave rise to the term "spongy" (Figs 9 & 10), while the outer woody endocarp (Figs 10 & 11) consists mainly of unlignified fibre bundles orientated in various directions. In the inner parenchyma zone B (Fig. 12) many of the cells now show intrusive growth and differentiation into fibre sclereids. The walls of the inner epidermal cells, however, have not yet become lignified or developed secondary cell walls (Fig. 12).

In fruits collected about 6 weeks after anthesis, the radial cell walls of the elongating mesocarp initials, viz. the innermost parenchyma cell layers of the exocarp, have now become twice as long as the tangential walls. All woody endocarp fibres are now lignified. Wall thickening and lignification to form brachysclereids have started in the inner epidermis, and brachysclereids have also developed from those parenchyma cells in zone B which had not differentiated into fibre sclereids. This sclerenchymatous zone forms the inner part of the woody endocarp.

The exocarp and mesocarp of the mature but not yet ripe fruit is partly illustrated in Figures 13 & 14. Especially the cell walls of the outer collenchymatous exocarp are impregnated with pectins, while starch grains occur in the inner exocarp cells. The mesocarp is now a typical sarcocarp since the parenchyma cells have elongated radially extensively. It comprises about 25% of the thickness of the pericarp.

The exocarp, or peel, of the ripe fruit consists of the outer, stomatous epidermis and approximately 25 distinctly collenchymatous hypodermal layers. The outer tangential walls, as well as the outer parts of the anticlinal walls of the epidermal cells, are heavily cutinized. The cuticle is about 3 μm thick and reaches extensively into the substomatal chambers. The stomata are slightly raised above or are even with the neighbouring epidermal cells. The guard cells show outer and minute inner rims (sometimes referred to as "ledges"). No lenticels were observed. The thin-walled mesocarp cells have more or less disintegrated at this stage. No distinct transition layer between mesocarp and endocarp can be distinguished but a large number of amphicribral vascular bundles with associated secretory ducts occur in this region (Fig. 14) and also alongside the ridges of the woody endocarp (Fig. 10). The fibrous nature of the spongy endocarp at this stage of development can probably be ascribed to the xylem elements of the vascular bundles. The parenchyma cells within the spongy endocarp are now mostly tanniniferous. The inner epidermis now consists of brachysclereids which constitute the shiny inner surface of the stone. The woody endocarp ridges consist mainly of fibres (Fig. 15) which are densely packed in bundles, as well as groups of brachysclereids. The operculum tissue and adjacent inner woody endocarp consist predominantly of brachysclereids, although fibre sclereids and stone cells also occur (Fig. 15).

Ontogeny and structure of the seed-coat

Very young seeds collected about one month after anthesis (Fig. 3C) reveal the following salient characteristics:

- a knee-like protuberance of the funicle;
- a well-developed raphe and ovule wall with tanniniferous epidermis;
- a plate-like zone of tanniniferous cells in the chalazal-nucellar tissue extending to the proximal ends of the inner integument (i.i.) (Fig. 16). These cells

and their derivatives are referred to as the hypostase *sensu lato* (Von Teichman, in press, a) and are continuous with the tanniniferous inner epidermis of the i.i. (Fig. 16)

- a weakly developed hypostase *sensu stricto* at the chalazal base of the embryo sac.
- nucellar remnants surrounding the endosperm and a minute embryo;
- a three cell layered i.i. (Fig. 16), which is much shorter than the outer integument (o.i.); and
- a proximally 3-4 cell layered, extensively developed o.i. (Fig. 16).

The hypostase *sensu lato*, as well as the chalaza and raphe develop extensively. The mature seed-coat (Fig. 17) consists of the following:

- an extensive raphe with amphicribral vascular bundle and chalaza;
- an extensive tanniniferous hypostase *sensu lato* which causes the dark brown colour of the saddle-like patch (Fig. 4);
- remnants of the o.i., especially the outer, tanniniferous epidermis and fragments of the subepidermal cell walls which show a slight secondary thickening and definite lignification. This constitutes a slight mesotestal (Corner, 1976) condition;

- remnants of the i.i., mainly the inner, very marked tanniniferous epidermis; and
- a cuticular layer formed by the cuticles of the nucellar epidermis and inner epidermis of the i.i. (Fig. 18).

In the ripe seed, the central cells of the o.i. are more disintegrated than those shown in Figure 18 and the nucellus is completely squashed and utilized. A few layers of endosperm persist. However, the seed is considered to be exalbuminous since the cotyledons are fleshy and the remnants of cellular endosperm are insigificant macroscopically. The reserve material in the cotyledons are proteins and lipids. No starch is found in the endosperm or cotyledons.

DISCUSSION AND CONCLUSIONS

The female flower, fruit and pericarp

The close relationship between Harpephyllum, Lannea and Sclerocarya suggested by Engler (1897) when he placed the three genera together in the tribe Spondieae, has been reaffirmed by the present study. The following floral characters are shared by H. caffrum, L. discolor (Von Teichman, 1987) and the marula (Von Teichman & Robbertse, 1986a): imbricate petals, disc, staminodes, relatively short and separate styles, a massive funicle, dorsal raphe and one pendulous, anatropous ovule per locule with the o.i. better developed than the i.i..

The fruit of the marula and *H. caffrum*, which are fleshy and sub-fleshy drupes respectively, are relished by humans and various animals, while the moderately palatable fruit of *L. discolor* is eaten by birds. The tanniniferous mesocarp in the fruit of *L. discolor* probably plays a part in the dispersal of the stones by birds.

The ovary in the marula is usually bilocular, whereas in *L. discolor* and *H. caffrum* it is mostly secondarily unilocular. The stone of *H. caffrum* has been described as being bony and bilocular by Sonder (1894) and according to Engler (1897) and Fernandes & Fernandes (1966) it has four locules of which only two are fertile. This implies that the ovary is usually at least bilocular with probably two additional rudimentary, sterile locules. We found only 8% of the ovaries to be bilocular and the rest unilocular. This could possibly be ascribed to the fact that the trees used in the present study were cultivated outside their natural range.

The exocarp of all three species is very similar and comprises the outer epidermis as well as hypodermal layers and therefore represents an exocarp *sensu lato* according to Roth (1977). The epidermises of the three species show significant similarities, especially regarding the stomata.

Multicellular hairs occur only in *L. discolor*. Lenticels were found in the marula and *L. discolor*, but not in *H. caffrum*. Pectic substances are abundant in the walls of the collenchyma cells in the marula and *H. caffrum*, but not in *L. discolor*. In the marula and *L. discolor* secretory canals occur in the inner exocarp and outer mesocarp, while in *H. caffrum* these mostly occur in the spongy and also woody endocarp.

In the marula and *H. caffrum* the mesocarp is a typical sarcocarp (*sensu* Roth, 1977) since it comprises fleshy parenchymatous tissue. In *L. discolor*, however, the mesocarp consists of 8 to 10 outer layers of parenchyma cells and 2 to 5 inner, slightly radially elongated tanniniferous parenchyma layers.

With regard to the interpretation of the spongy and woody endocarp tissues of *H. caffrum* the more general use of the term endocarp is followed. This was advocated by Airy Shaw & Forman (1967) in their study of *Spondias*. The entire endocarp of the marula and *L. discolor* is woody due to the extensive occurrence of sclerenchyma. It constitutes a typical sclerocarp which is heterogeneous (Roth, 1977) since fibres as well as sclereids occur. The woody part of the endocarp of *H. caffrum* falls into the same category. In all three species the operculum is part of the woody endocarp. The operculum originates from the inner epidermis of the ovary wall and adjacent parenchyma only, thus excluding the zone with procambium strands. Contrary to the major part of the woody endocarp, the operculum tissue contains no fibres. Most of the brachysclereids in the woody endocarp vary only slightly in the different species. In L. discolor and H. caffrum the long sclereids are best described as fibre sclereids as in H. caffrum (Figure 15 C). *

From the aforementioned it is obvious that although the structure of the pericarp is species-specific, the marked similarities indicate the close association of the three species under discussion.

The seed and seed-coat

As the structure of the ovule of Harpephyllum caffrum closely resembles that of the marula and Lannea discolor, it is not surprising that the seeds and seed-coats of these three species are also fairly similar, thereby reflecting a close association. The seeds of all three species are exalbuminous and the reserve material in the cotyledons consists mainly of proteins and lipids.

In H. caffrum and the marula secondary wall thickening and lignification of the subepidermal cell wall fragments of the o.i. is found, representing a slight mesotestal

*for corrections
see Appendix

condition (*sensu* Corner, 1976). However, in *L. discolor* traces of an exotestal condition are present.

Owing to the absence of tannin-like substances in the epidermal cells of the raphe, ovule wall and o.i. the seed-coat of the marula is cream-coloured. The outer cell layers conceal the presence of the dark brown saddle-like patch. However, in *L. discolor* and *H. caffrum* this patch is visible externally and the tanniniferous outer epidermal cells cause the rest of the seed-coat to be of a uniform light brown colour. In *H. caffrum* the hypostase *sensu lato* is up to 13 cell layers thick, while in the other two species it is not quite as well-developed, being four to five cell layers thick. A similar hypostase *sensu lato* is probably found in a number of taxa of the Anacardiaceae (Von Teichman, in press, a). Tannin-like substances, therefore, occur in the outer epidermis of two species, as well as in the inner epidermis of the i.i. and hypostase *sensu lato* of all three species discussed here. All three species also possess a cuticular layer between the nucellar epidermis and the inner epidermis of the i.i. providing the brittle seed-coat with additional support. The tannin-like substances, the cuticular layer and suberin and lignin wall impregnation of the hypostase *sensu lato* of the marula, all supply some protection to the embryo and possibly could be relicts of well-developed ancestral seed-coats existing prior to the development of a sclerocarp.

In these three species of the Spondieae, the raphe, extensive chalaza and hypostase sensu lato, as well as remnants of the integuments and the cuticular layer develop into an undifferentiated seed-coat (sensu Corner, 1976). A very similar seed-coat occurs in Campnosperma minor Corner of the tribe Rhoideae (Corner, 1976). However, in the genus Rhus L., also of the Rhoideae, probably an endotestal, i.e. differentiated seed-coat (sensu Corner, 1976) occurs (Von Teichman & Robbertse, 1986, b). More than one type of seed-coat therefore occurs in the large Rhoideae tribe and these seed-coat characters, amongst others, may be employed taxonomically for its subdivision. On the whole the seed-coat structure is species specific and certainly of diagnostic significance at the generic level.

The seed of the three species of the Spondieae discussed here could be interpreted as being of the 'raphe-chalazal' type (sensu Robbertse et al., 1986) and regarded as a derived and phylogenetically advanced type as was discussed previously (von Teichman, in press, a).

The measure of bending shown by the developing seed depends on the physical space available in the fruit locule. Due to the endocarpal knob projecting into the fruit locule, the seed of L. discolor becomes reniform.

According to Dahlgren (1980) the embryo of the Anacardiaceae may or may not contain chlorophyll. The embryo of the three species of the Spondieae under discussion is non-chlorophyllous.

Concerning the growth relations between fruit and seed, it is evident that in the three species of the Spondieae discussed here, lignification of the endocarp precedes the development of the seed. While in *L. discolor* mesocarp differentiation occurs more or less simultaneously with endocarp lignification, the latter precedes the mesocarp development in the other two species. The latter developmental sequence corresponds to the general pattern in drupes (Roth, 1977).

A study of the relevant literature left the distinct impression that *H. caffrum* is also very closely related to the species of the genus *Spondias*. This affinity is based on characters such as compound leaves, the complex structure of the woody endocarp, the presence of opercula, a spongy endocarp and a saddle-like patch on the seed-coat as well as similarities in the shape of the seed. *Spondias philippinensis* (Elmer) Airy Shaw & Forman, like *H. caffrum* has a slightly bent seed, a seed-coat with a dark patch and compound leaves (Hou, 1978: 480 Fig. 38). The four species of *Spondias* described by Hladik & Hallé (1979) have a complicated woody endocarp with ridges and cavities, and an apical operculum concealed in a deep cavity similar to that of H. caffrum. The endocarp of these species of Spondias as well as H. caffrum is well preserved which, according to the authors, renders it useful for field identification.

It is also noteworthy that, although the geographical range of the genus Spondias (i.e. Angola, Madagascar, tropical Africa and Indo-Malesia) and the genus Harpephyllum is presently mutually exclusive, both occur on former eastern Gondwana fragments. This may account for the marked similarities between these two taxa. The taxonomic delimitation of these possibly congeneric genera should therefore perhaps be reconsidered. This would require detailed comparison of a significant number of characters.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria, Margaretha Mes Institute for Seed Research, and the CSIR Foundation for Research Development. We wish to express our sincere thanks to Prof. P.J. Robbertse for discussions and to Dr Elzabé Schoonraad for her constructive criticism of the manuscript. Rohm & Hass (Pty) Ltd. is sincerely thanked for the free samples of 94% HEMA supplied.

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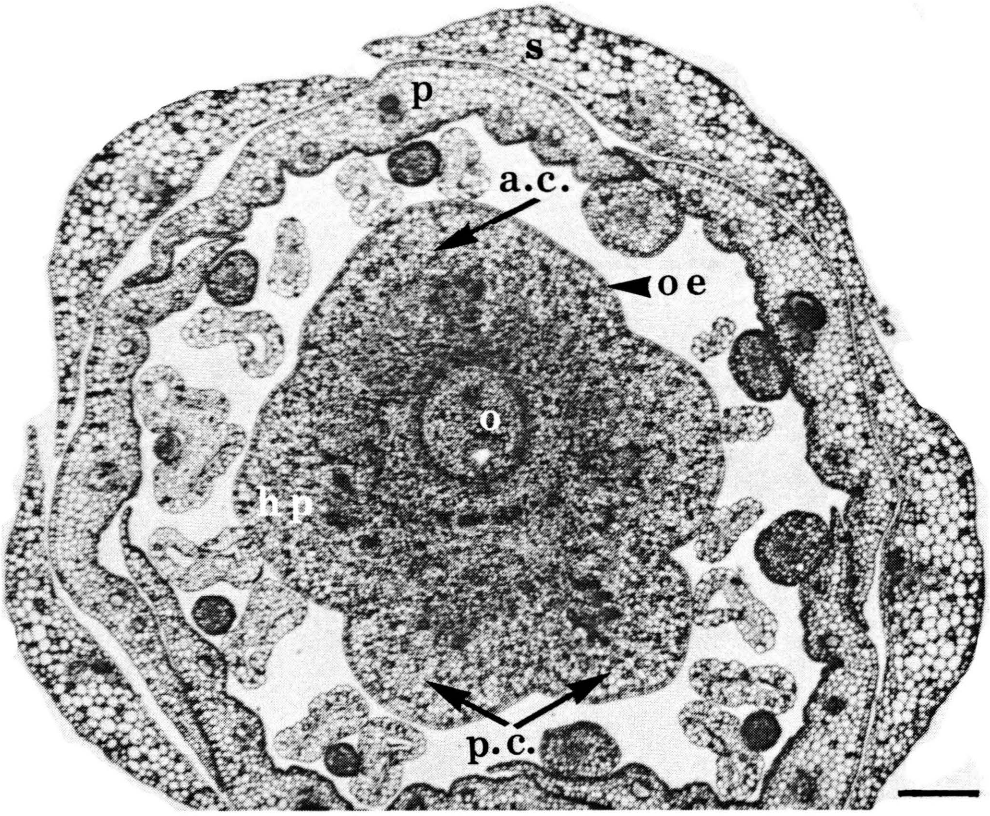


Figure 1. TS of the flower bud cut above the level of the disc. It illustrates the sepals (s), the imbricate petals (p), the whorls of staminodes, upper slightly lobed part of the pentacarpellary ovary with ovule (o). The two posterior carpels (p.c.) are small (i.e. always reduced), the anterior carpel (a.c.) is well developed. The outer epidermis of the ovary wall (oe), the hypodermal parenchyma zone (hp) as well as the adjoining zone with vascular and secretory tissue are also shown. Scale bar = 200 μm.

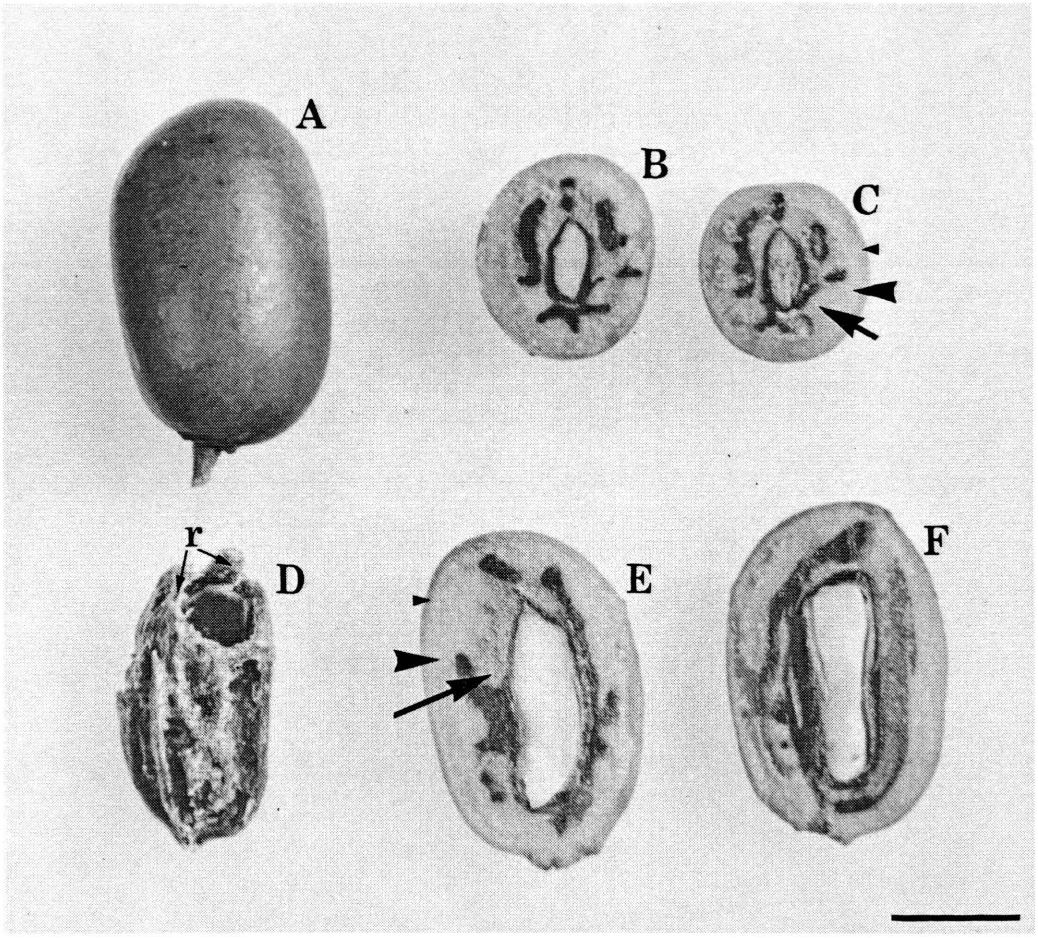


Figure 2. Mature, ripe fruit of <u>Harpephyllum</u> <u>caffrum</u> illustrating the size and form in A. Two median TS (B & C) and two median LS (E & F) show the exocarp (small arrow heads), mesocarp (large arrow head), spongy endocarp (large arrows), dark woody endocarp and white cotyledon. The woody endocarp with its ridges (r) and cavity is also illustrated in D. Scale bar = 10 mm.

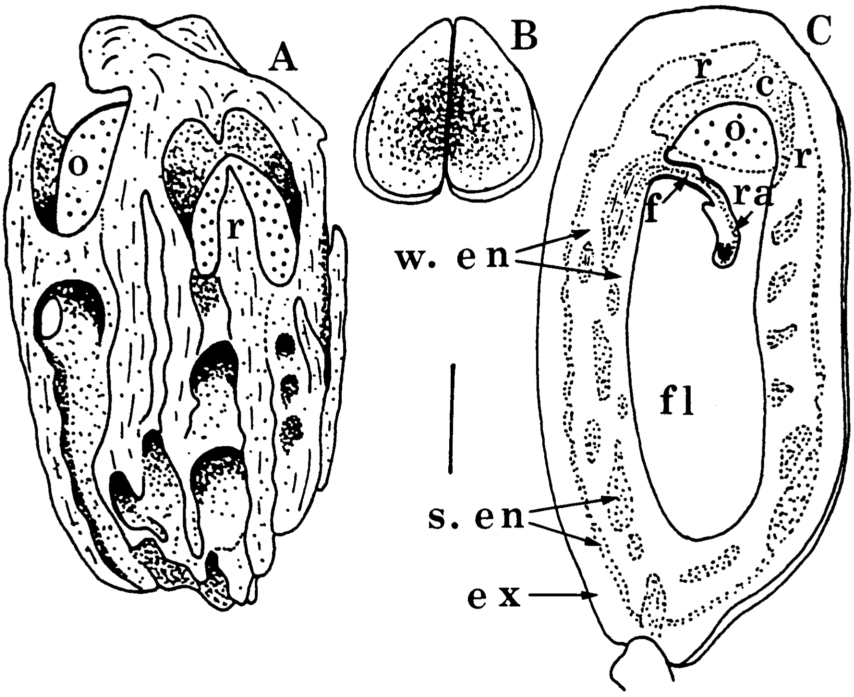


Figure 3. Drawings of the woody endocarp of a ripe fruit (A), a bipartite operculum (B) and LS of a young fruit in which the mesocarp had not yet differentiated (C). In A the operculum (o) of the better developed fruit locule is viewed from the side, while a ridge (r) partly conceals the other operculum. In the young fruit (C) the woody endocarp (w. en) with major ridges (r) and cavity (c), spongy endocarp (s. en), exocarp (ex), operculum (o) and adjacent inner surface of fruit locule (fl), as well as the funicle (f) and raphe (ra) of a very young seed are illustrated.
Scale bar = 4 mm.

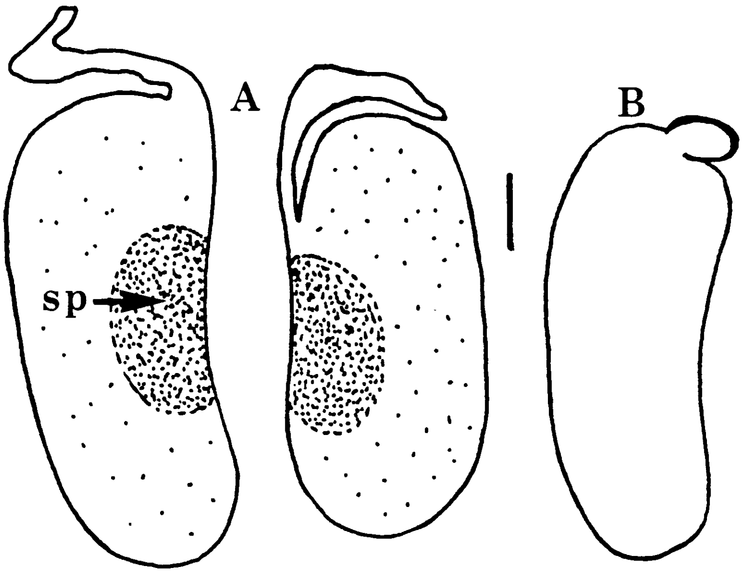


Figure 4. Drawings of two seeds with seed-coat and funicle in (A) and an embryo in (B) to show their size, form and the characteristic darker saddle-like patch (sp) on the seed-coat. Scale bar = 2 mm.

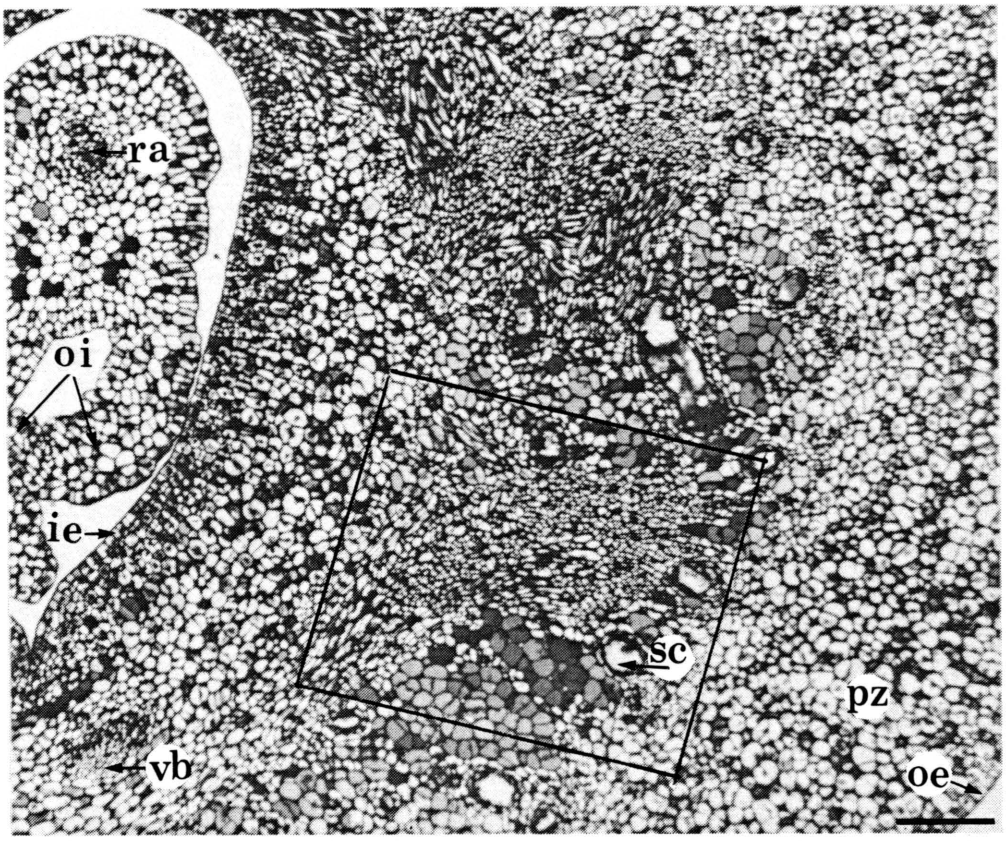


Figure 5. TS of ovary wall at anthesis, illustrating the outer epidermis (oe), adjoining parenchyma zone A (pz), secretory cavities (sc) in the middle zone, large vascular bundle (vb) of one of the posterior carpels, the inner epidermis (ie), as well as the vascular bundle in the raphe (ra) and the distal parts of outer integument (oi) of the ovule. Scale bar = 100 μm.

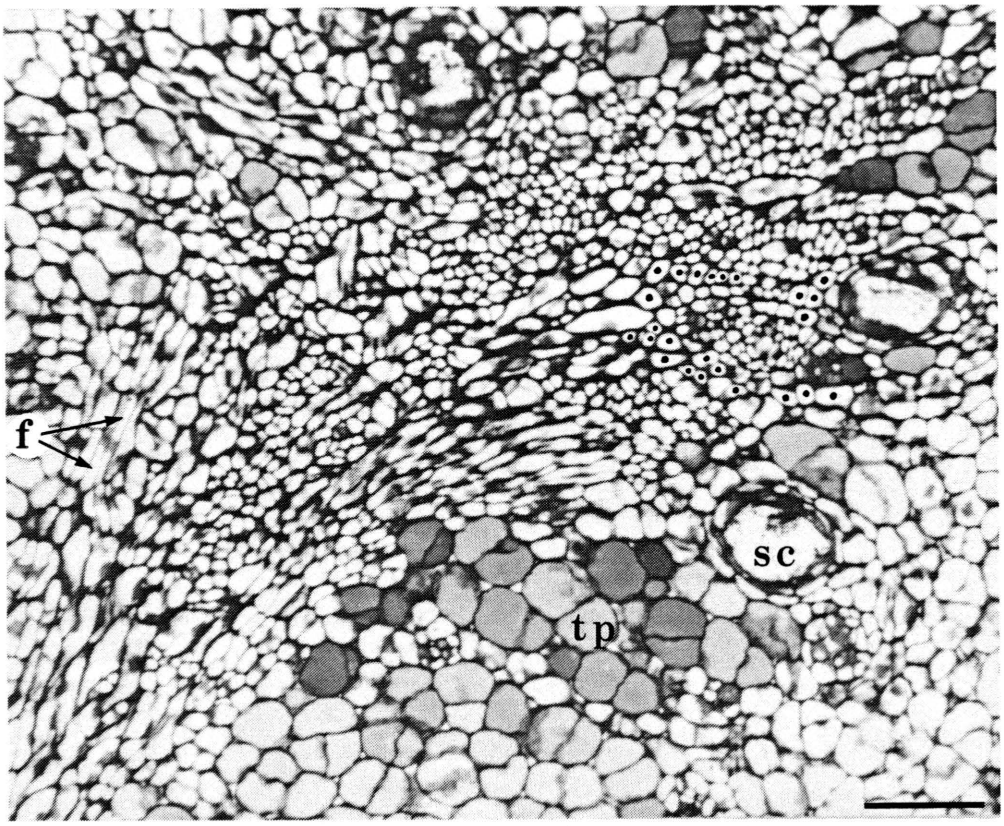


Figure 6. Magnification of the part framed in Fig. 5. The tanniniferous parenchyma (tp), secretory cavities (sc), with neighbouring xylem elements (marked with black dots) and fibre initials (f) in the middle zone are illustrated. Scale bar = 50 μm.

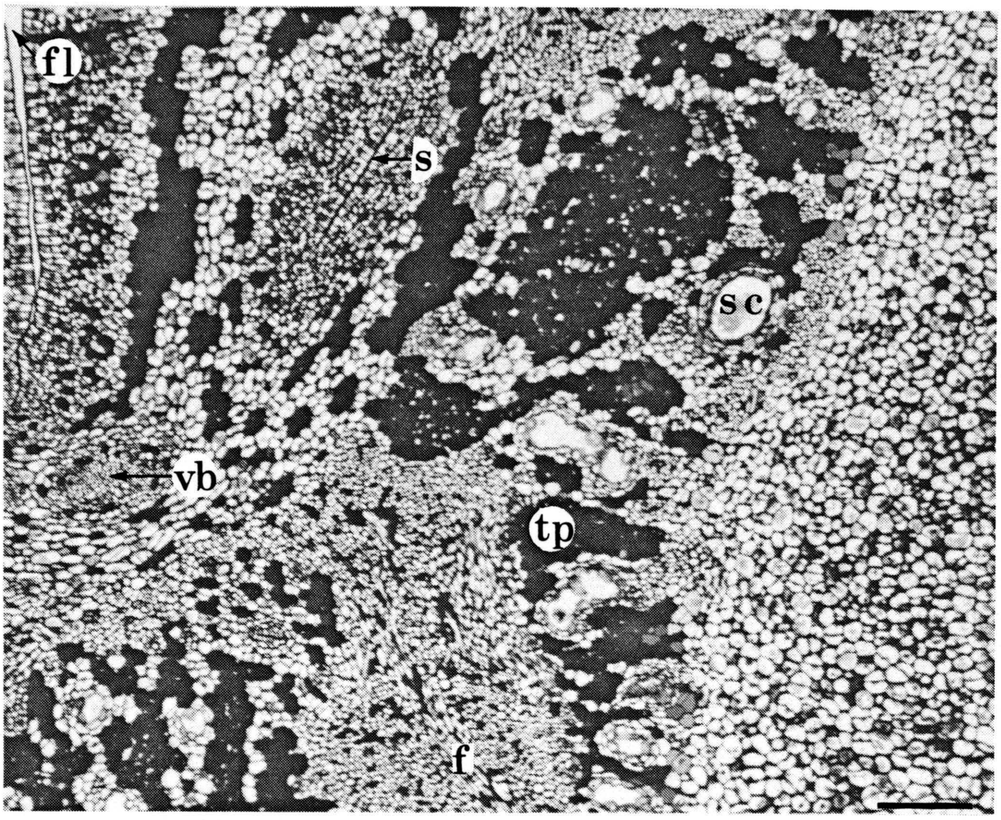


Figure 7. TS of a part of the pericarp of very young fruit. The tanniniferous parenchyma (tp), secretory cavities (sc) with vascular tissue, which will form the spongy endocarp, fibre initials (f) of the future woody endocarp, vascular bundle (vb) of one of the posterior carpels, the slit (s) of the reduced fruit locule and the fruit locule (fl) are shown. Scale bar = 100 μm.

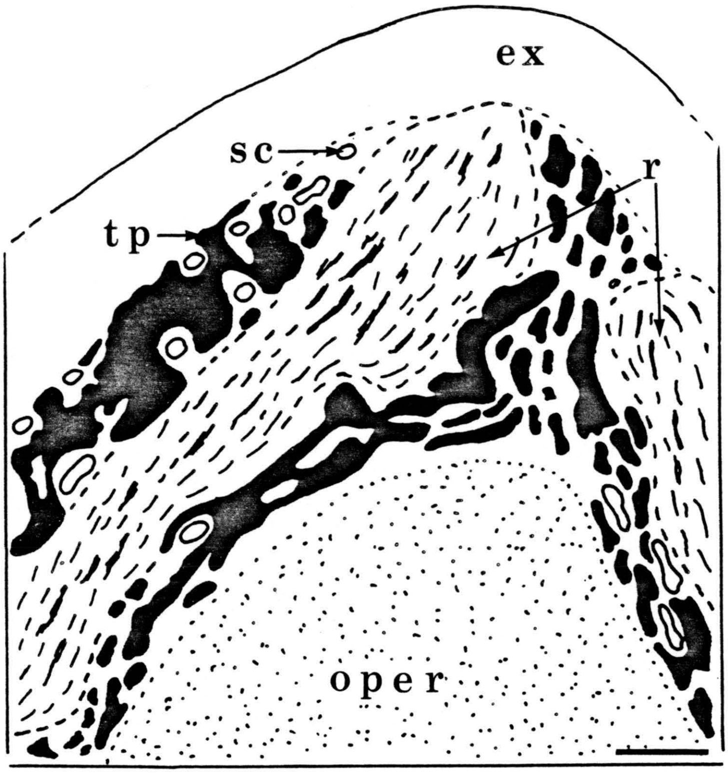


Figure 8. Drawing of a LS of a part of the pericarp of young fruit. The exocarp (ex), including mesocarp initial cells which cannot be distinguished yet, the woody ridges (r), the tanniniferous parenchyma (tp) and secretory cavities (sc) within the spongy endocarp and operculum tissue (oper) are illustrated.
Scale bar = 400 μm.

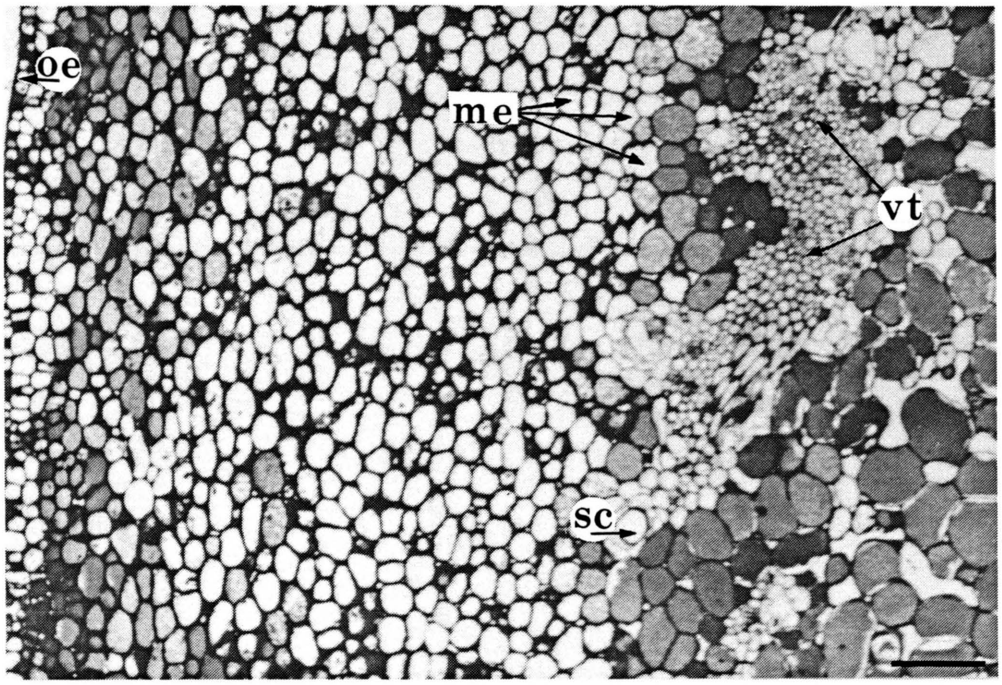


Figure 9. TS of young fruit illustrating a part of the exocarp, including the outer epidermis (oe); the future mesocarp zone (me) and the outer part of the spongy endocarp with, amongst others, secretory cavities (sc) and vascular tissue (vt).

Scale bar = 100 μm.

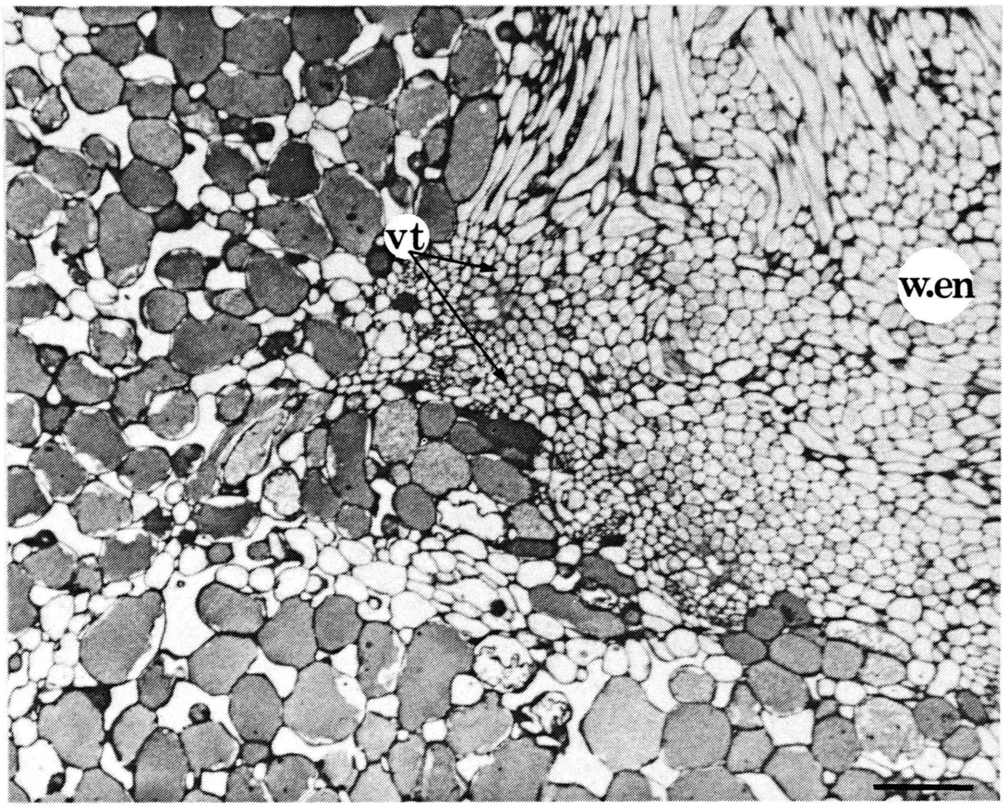
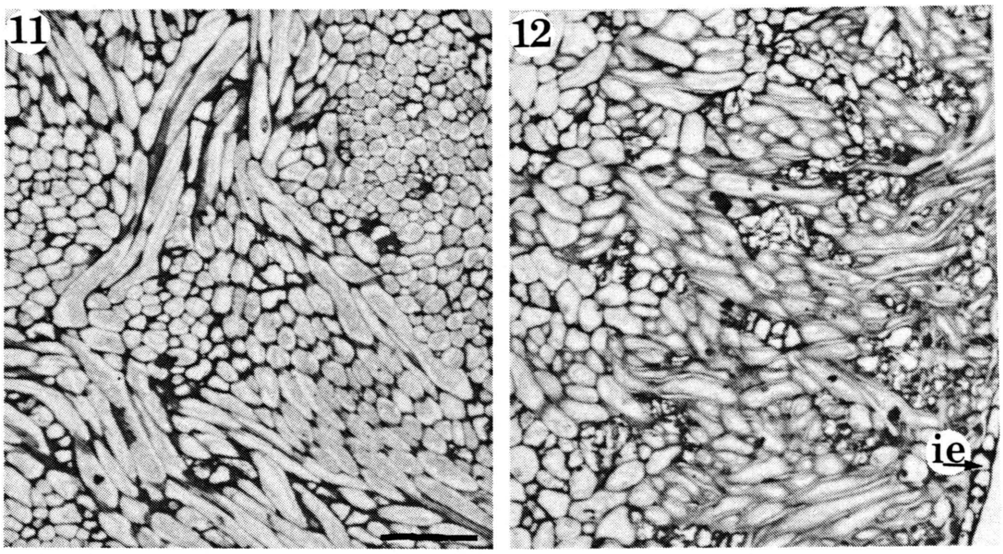
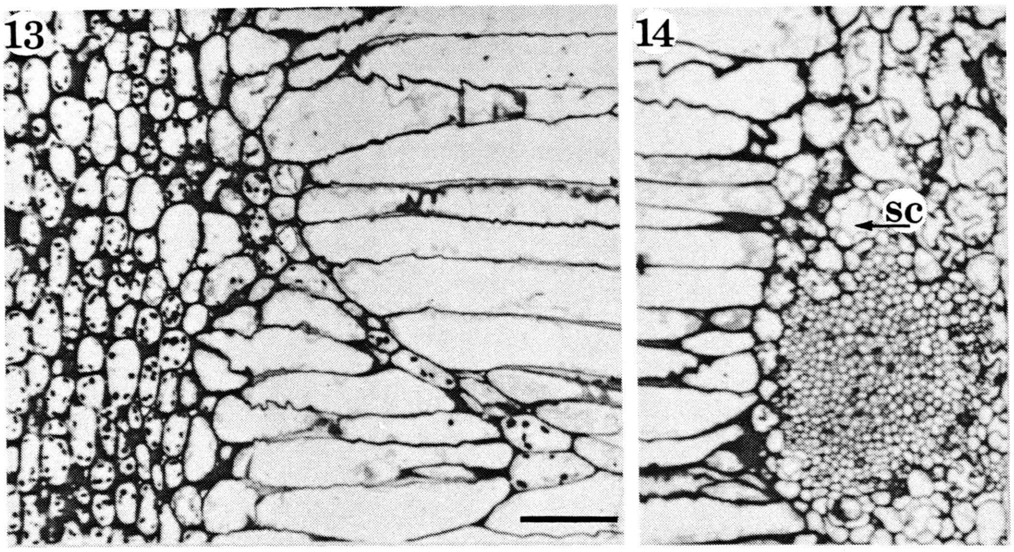


Figure 10. TS of young fruit. The micrograph was taken adjacent to that in Fig. 9, and shows the inner spongy endocarp, vascular tissue (vt) between the latter and the woody endocarp (w.en). These vascular bundles adhere tightly to the woody endocarp ridges in the ripe fruit. Scale bar = 100 μm.



Figures 11 & 12. TS of young fruit. Fig. 11 shows a part of the woody endocarp ridge, i.e. the fibre bundles. Fig. 12 shows the innermost part of the section which was also used for Figs 9 to 11. The inner epidermis (ie) and adjacent lignified fibre sclereids and interspersed parenchyma (darkly stained cell walls) are illustrated in Fig. 12. Scale bar of Fig. 11 and Fig. 12 = 100 μm.



Figures 13 & 14. TS of a part of the mature pericarp of a fruit which was preserved in F.A.A.. Figure 13 illustrates the inner part of the exocarp and adjacent mesocarp, while Fig. 14 shows a small part of the same section, i.e. the inner part of the mesocarp and adjoining spongy endocarp with one of the amphicribral vascular bundles and secretory cavity (sc). Scale bar of Fig. 13 and Fig. 14 = 100 μm.

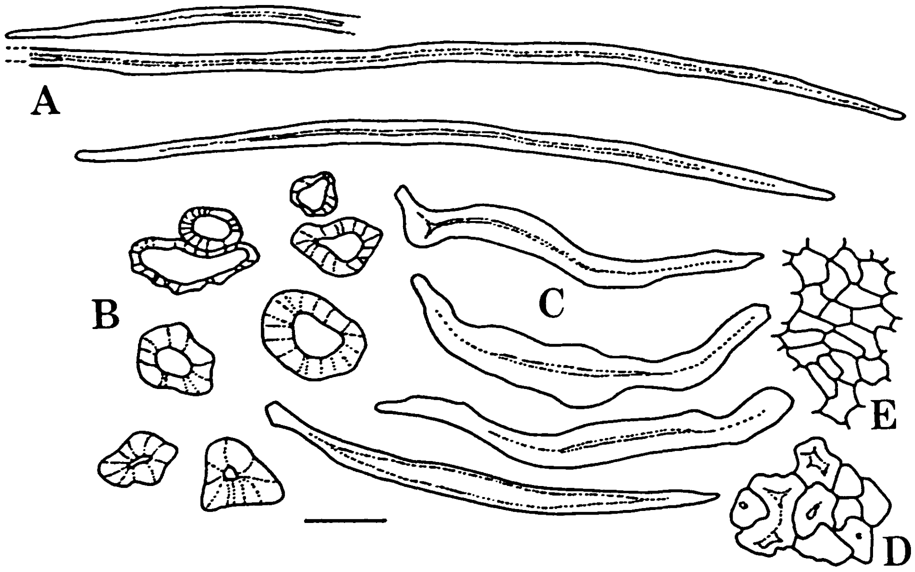


Figure 15. Drawings of sclerenchyma occurring in the woody endocarp, viz. partly in the operculum and partly in the adjoining woody endocarp, i.e. fibres (A), brachysclereids, varying in wall thickness (B), fibre sclereids (C) and groups of densely packed, smaller brachysclereids which probably originated from the inner epidermis (D) and (E). At this magnification the group E shows only the outlines of the cells fitting like a jigsaw puzzle. Scale bar = 500 μm.

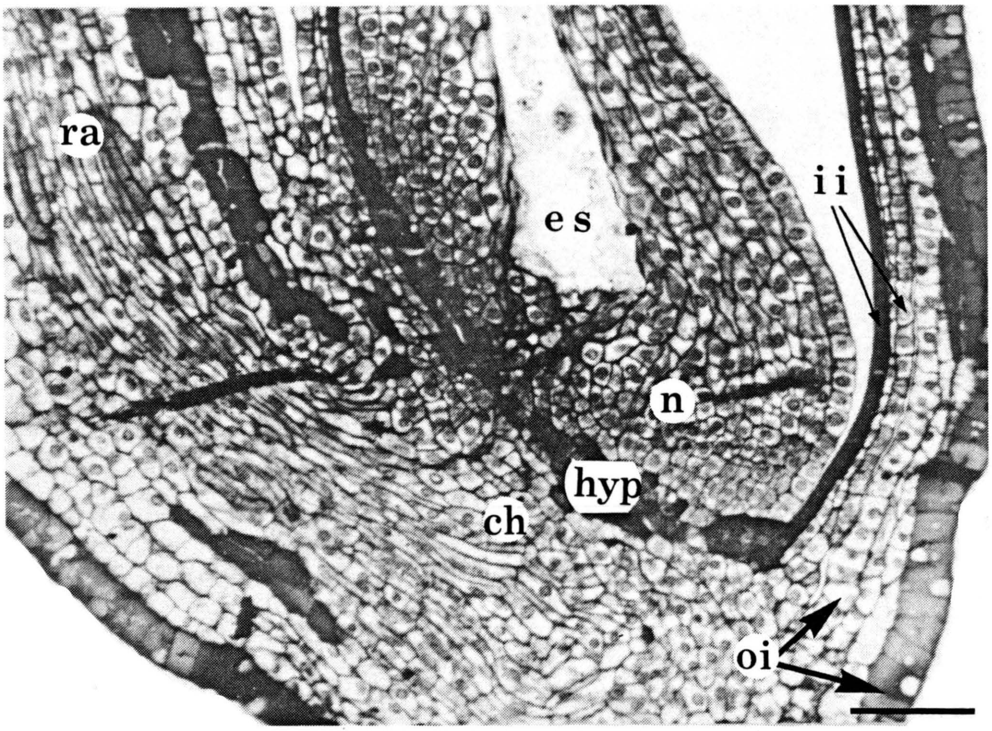


Figure 16. LS of the distal part of a very young seed. Parts of the raphe (ra), chalaza (ch), tanniniferous hypostase sensu lato (hyp), embryo sac (es), nucellus (n), inner integument (ii), and outer integument (oi) are shown. Scale bar = 50 μm.

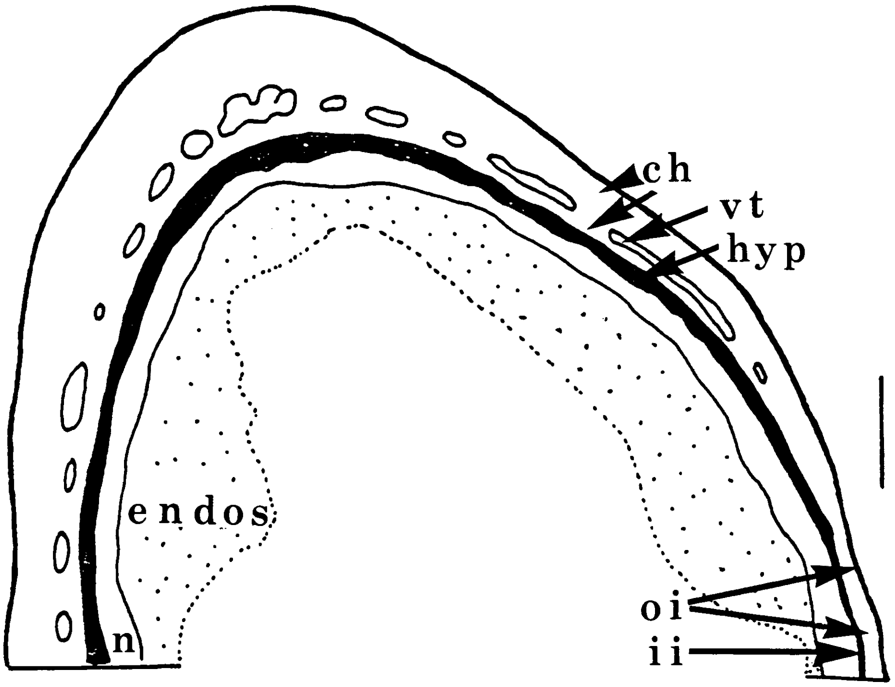


Figure 17. Drawing of a TS of the mature seed-coat, especially the chalazal region. It illustrates the role played by the chalaza (ch), with vascular tissue (vt), hypostase _sensu lato_ (hyp), the outer and inner integuments (oi) and (ii). The nucellus (n), adjacent to the endosperm (endos) is not completely utilized. Scale bar = 400 μm.

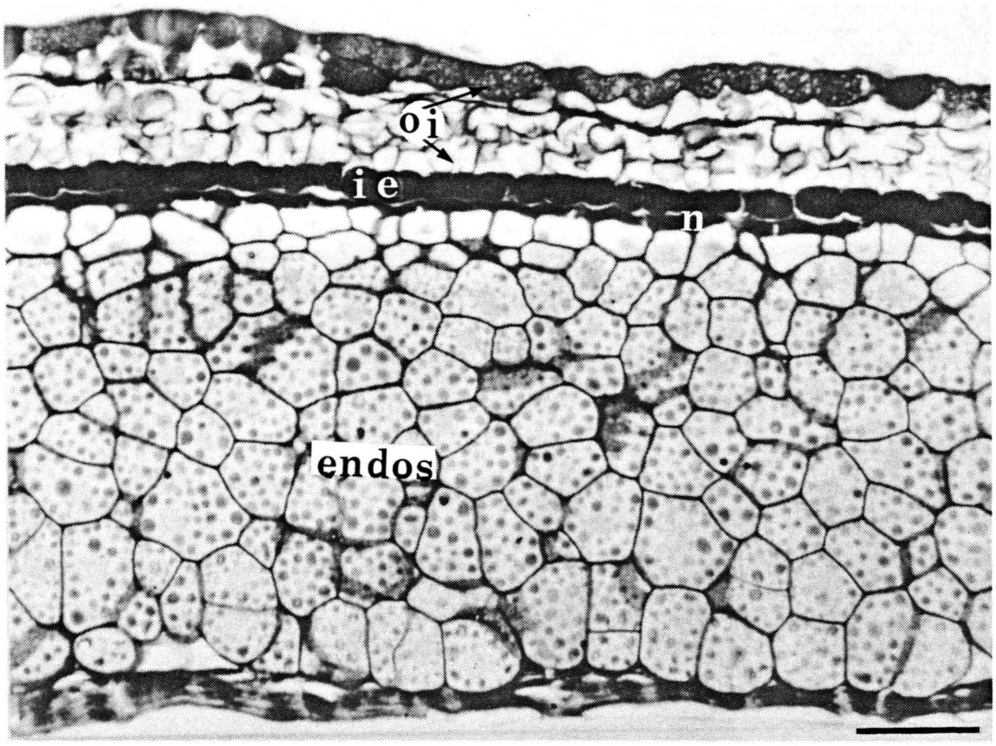


Figure 18. TS of a part of the seed-coat not including the chalazal region. This seed is older than that shown in Fig. 17 since the nucellus (n) is squashed at this stage. The outer integument (oi), inner integument remnant, namely the inner epidermis (ie), the cuticular layer (lying between the ie and n) and the cellular endosperm remnants (endos) are illustrated. Scale bar = 50 μm.

CHAPTER 8

PERICARP OF *Rhus lancea*

Botanical Journal of the Linnean Society (1986), *93:* 291–306. With 12 figures

Development and structure of the pericarp and seed of *Rhus lancea* L. fil. (Anacardiaceae), with taxonomic notes

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Received December 1985, accepted for publication January 1986

VON TEICHMAN, I. & ROBBERTSE, P. J., 1986. **Development and structure of the pericarp and seed of *Rhus lancea* L. fil. (Anacardiaceae), with taxonomic notes**. The pedicel of the female flower of *Rhus lancea* is distinctly articulated and usually carries three bracteoles. In the linear tetrad the micropylar megaspore forms the 8-nucleate embryo sac of the *Oenothera*-type. The single, bitegmic ovule is anatropous. The ripe, loose, papery exocarp consists mainly of the outer epidermis and a sclerified hypodermis. The mesocarp is not a typical sarcocarp, since the ridges and the inner layers are sclerenchymatous. The endocarp, originating from the inner epidermis, consists of four layers and its structure and microchemistry emphasize the close alliance of *Rhus* with other genera of the section Rhoideae. The endotestal seed indicates a * phylogenetic affinity between the Anacardiaceae and the Burseraceae.

ADDITIONAL KEY WORDS:—Anatomy – development – drupe – endocarp – seed coat.

CONTENTS

INTRODUCTION

Rhus lancea L. fil. is a well known tree indigenous to southern Africa and is locally known as karee or karree. It makes a hardy evergreen horticultural

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*for corrections
see Appendix

subject, although it is not a particularly useful timber tree. All parts of the tree have been or are used in some form or another (Esterhuyse, 1983; Moffett, 1984). Moffett (1984) gives an account of its propagation, nomenclature, geographical distribution and habitat. *Rhus* L. is the type genus of the tribe Rhoideae of the Anacardiaceae. The latest taxonomic revision of the species of *Rhus* in southern Africa was published by Schonland in 1930. The genus is currently being revised by R. O. Moffett for the *Flora of Southern Africa*.

The present study forms part of a comparative morphological study of the genera of the Anacardiaceae indigenous to South Africa. Very little detail is known about the seed coat of the species of the Anacardiaceae (Corner, 1976). A literature survey concerning the development and structure of the drupe in this family (Von Teichman & Robbertse, 1986) revealed that apparently no detailed study of these aspects regarding *Rhus* has yet been published. However, some authors touched on certain structural aspects, for example the reserve substances in the seed and seedling (Tabata, 1907), the seed coat and pericarp of several members of the tribe Rhoideae, including 14 species of *Rhus* (Magen, 1912), and the pericarp of *Rhus typhina* L. (Bogaciński & Molski, 1969).

The fruit of *R. lancea* is a drupe, the pericarp being differentiated into the thin exocarp, the partly fleshy and partly sclerenchymatous mesocarp and the hard endocarp. In contrast to the findings regarding *Sclerocarya* Hochst. (Von Teichman & Robbertse, 1986), the mesocarp in *R. lancea* is not a typical sarcocarp. The mature sclerenchymatous mesocarp and the endocarp together form the protective layers of the stone. Before a comparative taxonomic study of all the genera of the Anacardiaceae can be attempted, the definition of these protective layers needs re-evaluation as only homologous structures should be compared. Moreover, it is anticipated that a comparative study of seed and fruit structure could contribute considerably to the supra-specific taxonomy of the Anacardiaceae (cf. Brizicky, 1963). The present study is a contribution towards assembling the necessary comparative data. This paper reports on the morphological aspects of the female flower, fruit and seed, as well as on the development and structure of the pericarp and seed coat.

MATERIAL AND METHODS

Flower buds, flowers and fruit in various stages of development were collected from a tree grown on the campus of the University of Pretoria. Voucher specimens (*Von Teichman 512 & 544*) with flowers, young fruit, as well as mature fruit are kept at PRU (H. G. W. J. Schweickerdt Herbarium, Department of Botany, University of Pretoria). For reference purposes some material was preserved in FAA.

For the preparation of semi-thin sections most of the material was immediately fixed, initially in 6%, later in 3% glutaraldehyde in sodium cacodylate buffer (0.05 mol dm^{-3}). The material was dehydrated, infiltrated and embedded in glycol methacrylate (GMA) according to Feder & O'Brien (1968). The monomer mixture used is described in Von Teichman & Robbertse * (1986). GMA sections, 2–3 µm thick, were cut on a Porter Blum MT-1 or Reichert OM U3 ultramicrotome. The 'periodic acid–Schiff reaction' (PAS) was carried out according to Feder & O'Brien (1968), using 0.5% dinitrophenyl hydrazine (DNPH) in 15% acetic acid (for 30 min) as blocking agent. Sections were counterstained as described in Von Teichman & Robbertse (1986). Some GMA sections, on which the PAS reaction had been carried out, were subsequently either mounted in aniline blue or stained with the optical brightener Calcofluor-White M2R New (Smith & McCully, 1978). Sections of the same block were overstained with 0.1% brightener according to Hughes & McCully (1975). These sections were examined with a Reichert Univar microscope using epifluorescence optics.

Fresh tissue of the ripe cotyledons with endosperm was squashed on a slide. Staining in a saturated solution of Sudan IV in 70% ethanol for 1 min was followed by a brief rinse in 70% ethanol and mounting in glycerine jelly. Some GMA sections were stained for fatty substances, especially cutin, using Sudan black B, and for proteins using acid fuchsin as well as amido black 10B as described in Von Teichman & Robbertse (1986). To stain lignified cell walls, sections were mounted in a drop of saturated phloroglucinol in 20% HCl. Unstained sections were observed with phase-contrast optics or using crossed polarizing filters for polarizing microscopy. The photo-micrographs were taken with a Reichert Univar microscope.

Small pieces of the stone were macerated using the method described previously by Von Teichman & Robbertse (1986). In order to establish the nature of the cell walls of the osteosclereids FAA-preserved material was washed in 50% ethanol, dehydrated with ethanol and infiltrated with and embedded in Spurr's resin. The sections were contrasted with 4% (w/v) aqueous uranyl acetate and thereafter with lead citrate (Reynolds, 1963). A Philips TEM 301 was used for the examination.

Some blocks used for these thin sections were etched in a sodium ethoxide solution (100% ethanol saturated with sodium hydroxide) for 15 min and washed with tap and distilled water. After drying in a desiccator the mounted blocks were sputter-coated with gold and observed with a Philips SEM 501 at 12 kV. The crystals were examined with an EDAX.

OBSERVATIONS

Morphology of the female flower, fruit and seed

Rhus lancea is dioecious. The greenish-yellow female flowers are only about 3 mm in diameter. The pedicels are 2.5–4.0 mm long and distinctly articulated. The flowers are bracteate, two bracteoles usually occurring basally close to one another, and a third one about halfway up to the articulation on the pedicel.

The calyx and corolla are pentamerous and glabrous, the latter being imbricate. A yellow, annular, 5-crenate, intrastaminal disc is present. Five staminodes occur, alternating with the recurved, oblong petals. The tricarpellary, syncarpous gynoecium consists of an oblique, subglobose, slightly compressed, unilocular ovary, three filamentous, free styles and capitate stigmas. The female flowers produce obliquely subglobose, greyish-yellow, glabrous fruit measuring on average 6.0 × 4.2 mm (Fig. 1A–D). The ripe drupe usually has a loose papery exocarp. Minute brown remnants of styles and stigmas are visible on the exocarp. The outer mesocarp has a sticky consistency, is partly fleshy but is edible and has a sweet-sour taste.

In contrast to typical drupes the stone consists of the sclerified part of the

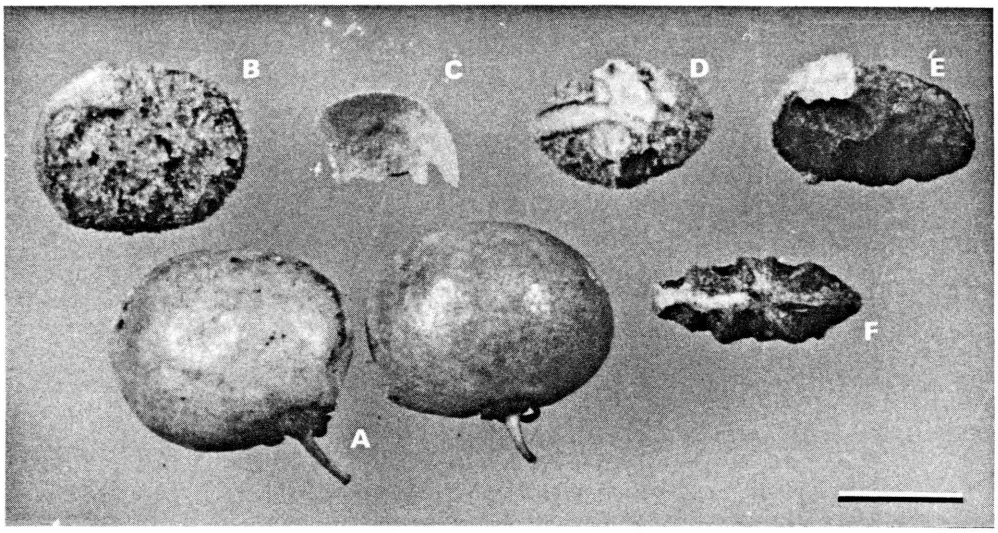


Figure 1. Mature, ripe fruit of *Rhus lancea* illustrating the size and morphology in A. B (lateral view) and D (distal view) show the mesocarp after exocarp (C) removal. The light coloured mesocarp ridge is illustrated especially well in D, as well as in E and F (lateral and proximal view of the stone). Scale bar = 3 mm.

mesocarp, the distinctly stratified endocarp and the single seed. The brown unilocular stone is distinctly compressed with prominent ridges (Fig. 1E & F) formed by the sclerenchymatous mesocarp. It also shows a light coloured softer mesocarp ridge which commences near the fruit stalk and ends more or less below the median style (Fig. 1D–F). During germination this ridge becomes swollen and, together with the endocarp, splits to allow the radicle to emerge.

A single, anatropous ovule develops from the base of the ovary. It is situated directly below the central style, that is if the gynoecium is viewed from the top this style lies on the longitudinal axis of the compressed ovary. The seed is of the 'raphe chalazal' type described for several South African representatives of the Anacardiaceae (Robbertse, Von Teichman & Van Rensburg, 1986).

A linear tetrad of megaspores was observed, the micropylar one forming the embryo sac. After lignification of the endocarp the ovule develops to form a compressed exalbuminous seed which is straight and measures on an average 4.0 × 1.8 mm. A pale yellow seed coat, which is not fused to the endocarp and which consists mainly of the inner epidermis of the outer integument, surrounds the yellow embryo.

Development and structure of the pericarp

The following structures are present in the ovary wall of small flower buds:

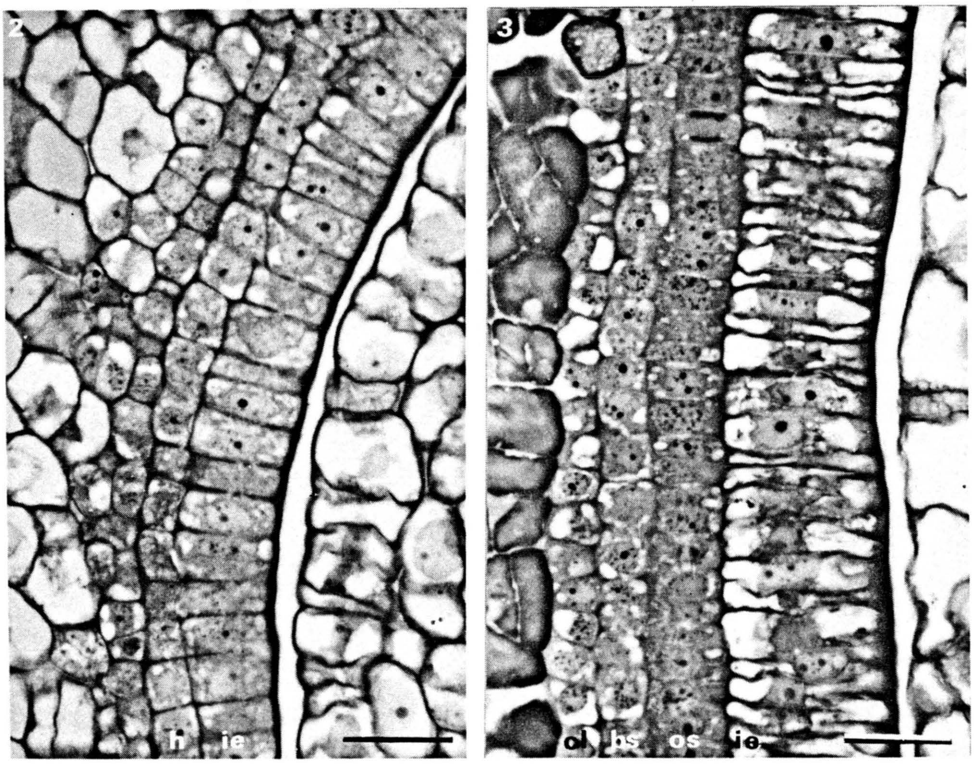
the outer epidermis of slightly radially-elongated cells, usually filled with tannin-like substances;

a parenchymatous zone with numerous tanniniferous cells, and collateral vascular bundles which are always closely associated with secretory cavities or canals. These schizogenous cavities, lined with epithelial cells, apparently originate with the vascular tissue from the procambium strands;

an inner epidermis and hypodermal layer, consisting of more or less radially arranged cells. In very young buds only the inner epidermis is embryonic and the hypodermal layer is therefore considered to be a derivative of this layer.

In ovaries of larger flower buds three embryonic cell layers could be distinguished. The cells of the two innermost layers are arranged radially and the newly formed hypodermis therefore also originates from the inner epidermis. At the time of anthesis the cells of the innermost layer are already radially elongated (Fig. 2, ie) and anticlinally divided. The cells of the third and fourth innermost layers often lie in radial rows (Fig. 2), indicating periclinal divisions of the third innermost layer which is also of dermal origin.

About 3 weeks after anthesis the initials of the four future endocarp layers are clearly distinguishable (Fig. 3). These, as far as could be ascertained, develop by periclinal divisions from the inner epidermis. At this stage the inner epidermal cells or proto-macrosclereids (Fig. 3, ie) are about four times their initial length and will elongate even further. The hypodermal cells which divide anticlinally now also elongate radially (Fig. 3, os) and represent the proto-osteosclereids. The third and fourth innermost layers represent the proto-brachysclereid and proto-crystal layers respectively (Fig. 3, bs, cl).



Figures 2 & 3. Fig. 2. Transverse section of a part of the ovary wall at anthesis, illustrating the inner epidermis (ie), the hypodermal layer (h) and two more layers of the future endocarp adjacent to the hypodermis. Scale bar = 20 μm. Fig. 3. Transverse section of the ovary wall slightly older than the one shown in Fig. 2. Initials of the four endocarp layers are shown, namely proto-macrosclereids (ie); proto-osteosclereids (os); proto-brachysclereids (bs) and proto-crystal layer (cl). Scale bar = 20 μm.

The next significant development in the pericarp is the drastic radial elongation of the young macrosclereids. Six weeks after anthesis these cells are 10 times the length of the neighbouring proto-osteosclereids. Transverse sections of these young fruits show the mesocarp with undulating ridges comprising parenchymatous tanniniferous cells (Fig. 4, scl), which later become sclerified. The vascular bundles with secretory canals usually occur closely associated with the mesocarp ridges. The phloem occurs in a tangential strip and is separated from the secretory canal by parenchyma and some tanniniferous cells (Fig. 4, tc). Parenchymatous sheath cells surround the epithelium cells of the secretory canals (Fig. 4, sh).

In somewhat older fruit (i.e. approximately 8 weeks after anthesis) the differentiation of the pericarp is well advanced, while the development within the ovule seems retarded (Fig. 5). The macrosclereids show irregular secondary wall thickenings. Observations of 2 µm thick transverse sections at the light microscope level indicated that an 'extra cell layer' may exist between the osteosclereids and the adjoining brachysclereids. However, using 0.5–1.0 µm thick Spurr's sections for light microscopy and TEM sections, it became clear that this 'extra cell layer' was actually the slightly constricted capitate tips of the osteosclereids (Figs 6 & 7). The nucleus usually lies in this capitate part and the radial cell walls are characteristically fluted (Figs 6–8). Secondary wall thickenings now occur in the osteosclereids as well as in the brachysclereids of

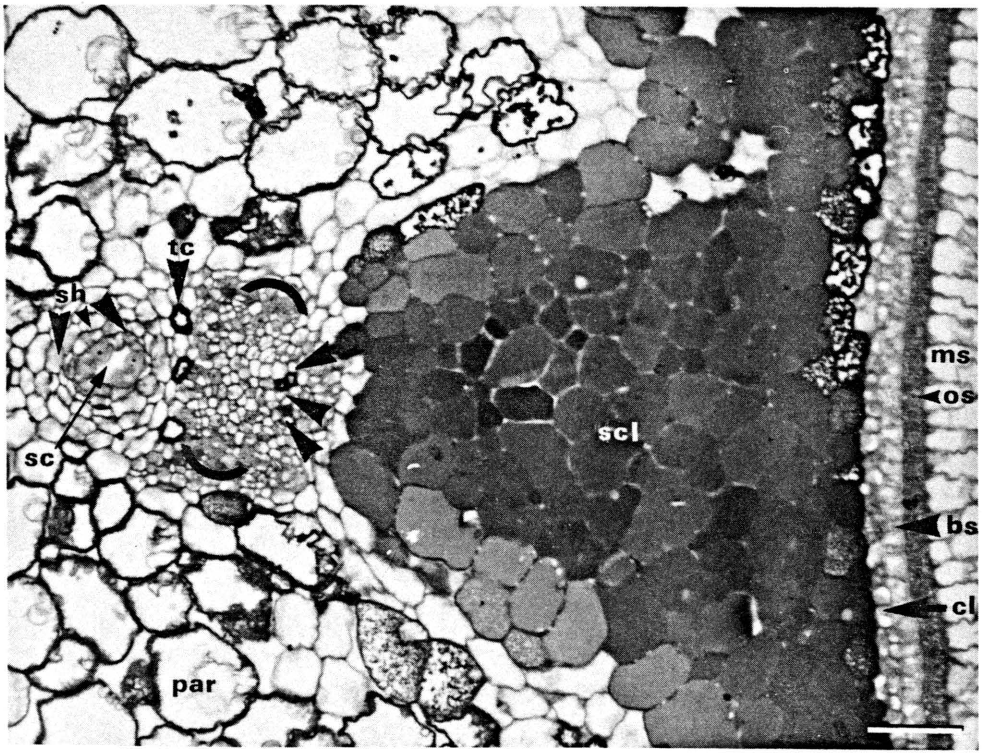


Figure 4. Transverse section of a very young pericarp, illustrating the macrosclereids (ms) in part, osteosclereids (os), brachysclereids (bs) and crystal layer (cl), tanniniferous mesocarp ridge (scl), which later sclerifies, parenchymatous mesocarp (par), xylem (arrowheads), phloem strip (inside brackets), secretory canal (sc), sheath cells (sh) and tanniniferous cell (tc). Scale bar = 50 µm.

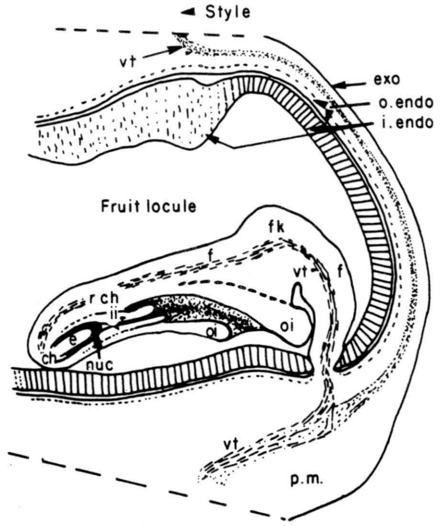


Figure 5. Diagrammatic representation of a longitudinal section of a young fruit, illustrating the vascular tissue (vt) within the funicle (f), the knee-like protuberance of the funicle (fk), and the raphe chalaza (rch) terminating in the chalaza (ch); the outer (oi) and inner integuments (ii), the nucleus (nuc) and zygote (e); and exocarp (exo), parenchymatous mesocarp (pm), outer endocarp (o. endo) of three layers and the inner endocarp or macrosclereid layer (i. endo).

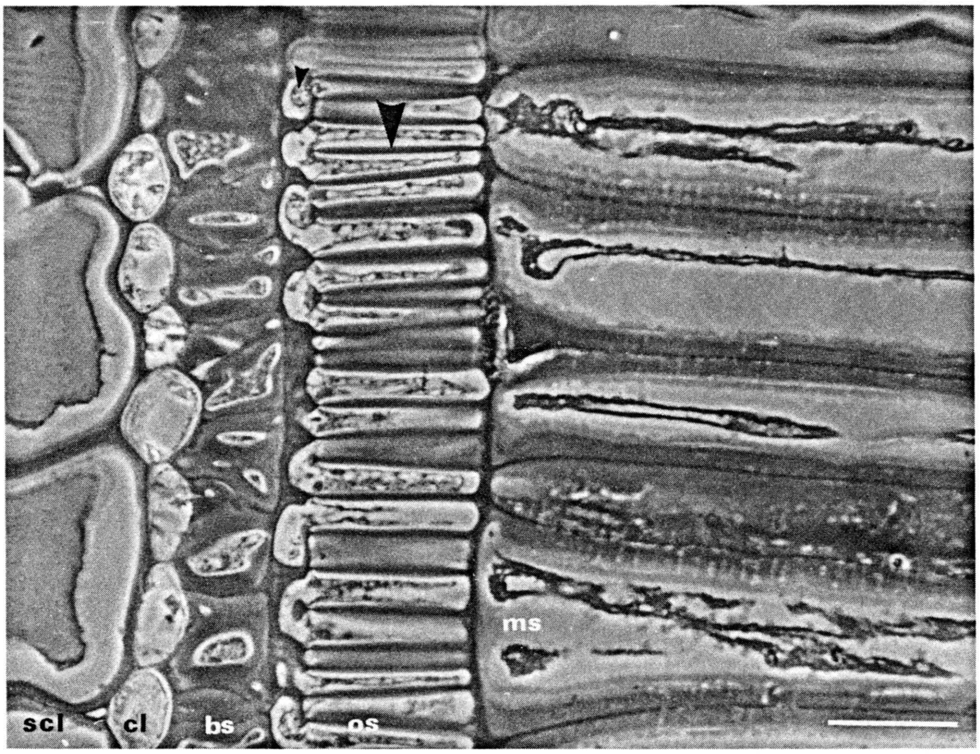


Figure 6. Transverse section of FAA preserved material (0.5 μm Spurr section) photographed with phase-contrast. Part of the endocarp is illustrated, namely macrosclereids (ms); osteosclereids (os) with cell wall flutes (large arrowhead) and capita (small arrowhead). Brachysclereids (bs); crystal layer (cl), brachysclereids of adjacent sclerenchymatous mesocarp (scl). Scale bar = 20 μm.

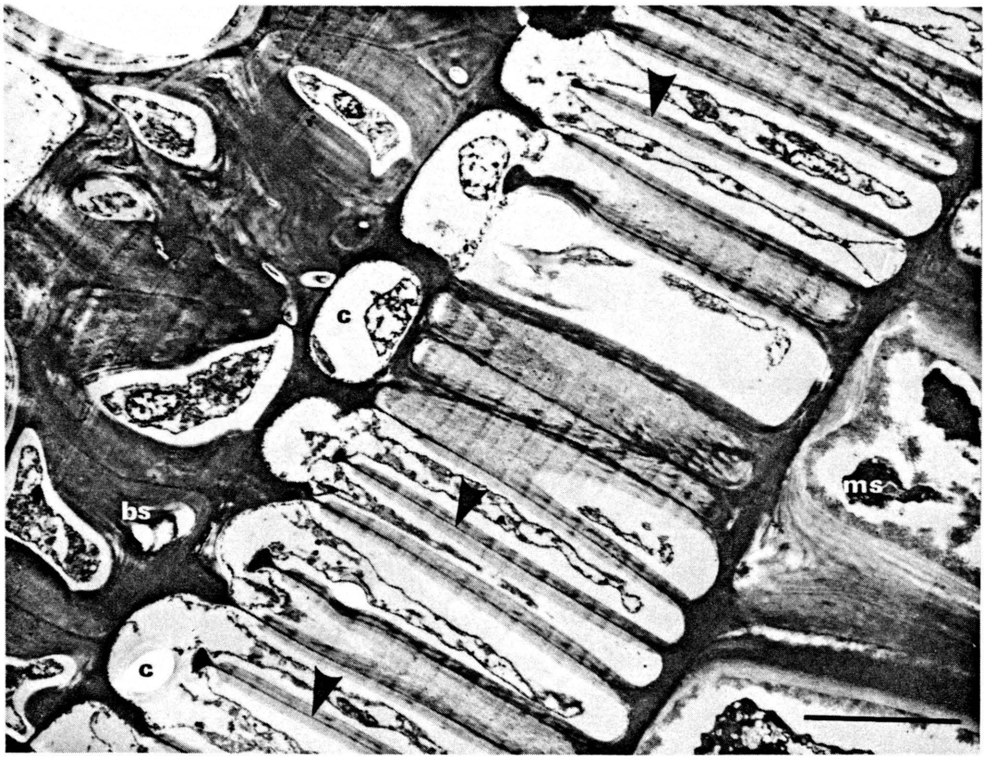


Figure 7. TEM through a part of the endocarp showing base of a macrosclereid (ms), the osteosclereids with capitate parts (c) and cell wall flutes (arrowheads) as well as brachysclereids (bs). The material was FAA preserved with resulting inadequate fixation and infiltration. Scale bar = 10 μm. *

the endocarp. Material prepared for TEM was subsequently etched and confirmed the structure of the osteosclereids and facilitated the investigation of the structure of the crystals. The crystals contain calcium, are rectangular prisms (Fig. 8) and are probably calcium oxalate. The tanniniferous mesocarp cells (compare Fig. 4) forming the inner sclerenchymatous mesocarp and the ridges adjacent to the endocarp differentiate to form brachysclereids (Fig. 6, scl) and some fibres.

The mature fruit is illustrated in Figures 9–11. The relative thicknesses of the * exocarp, the fleshy mesocarp, sclerenchymatous mesocarp with ridges, and endocarp were significant.

The loose papery exocarp consists of the outer epidermal cells, a layer of relatively larger, thin-walled hypodermal sclereids and remnants of mesocarp parenchyma. The epidermal cells contain tannin-like substances. The outer tangential cell walls are impregnated with cutin and the cuticle measures about 10 μm in thickness. Other epidermal cell walls are sometimes secondarily thickened. No lenticel-like structures were observed, but stomata occur abundantly. The guard cells, with characteristic upper ledges, are elevated above the surface of the epidermal cells.

The fleshy mesocarp consisting of thin-walled parenchyma contains some vascular strands. The sclerenchymatous mesocarp consists mainly of

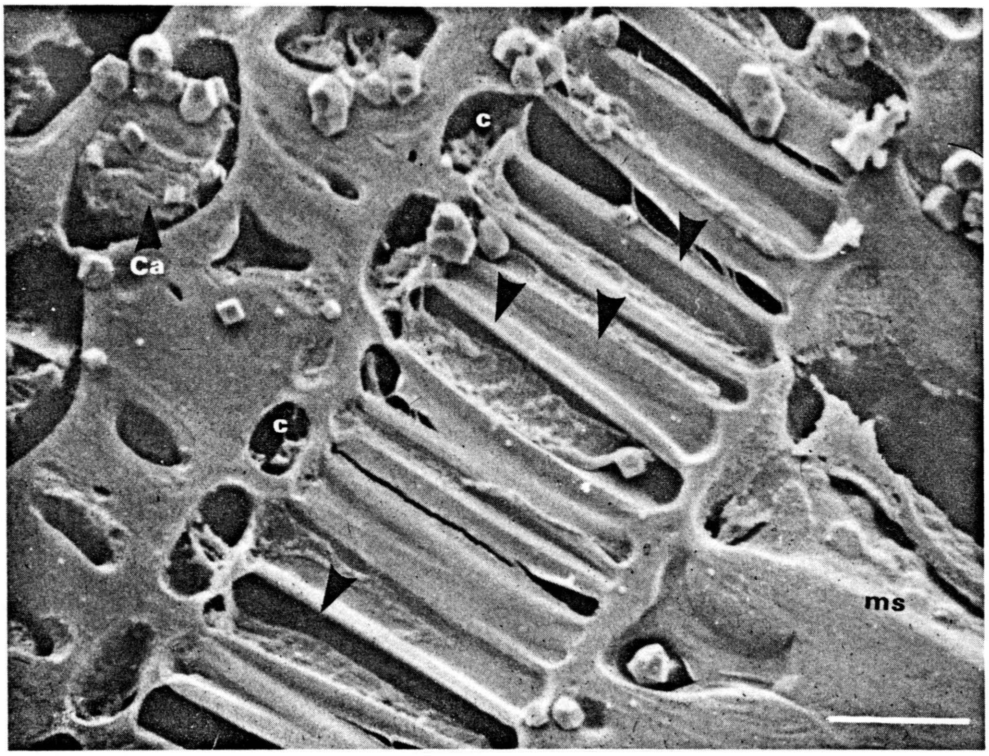


Figure 8. SEM of the same block used for Fig. 7 after etching in sodium ethoxide. Shown are the base of some macrosclereids (ms); the osteosclereids with capitate parts (c) and cell wall flutes (arrowheads); a few brachysclereids and one prismatic calcium oxalate crystal (Ca). Scale bar = 10 μm. *

brachysclereids and fibres. The more or less bilobed ridge (Fig. 9, ridge; Fig. 1, D–F), which becomes swollen and splits open when the radicle emerges, is lighter in colour than the other mesocarp ridges, probably because its sclereids contain fewer tannin-like substances. Parenchyma occurs centrally in the bilobed ridge, where it splits (Fig. 10). Most vascular strands are associated with these ridges, which can be very small and narrow (Fig. 11). At this stage one to three layers of sheath cells surround the layer of epithelium cells of the secretory * cavity (Fig. 11). Usually the cells of the innermost sheath layer have lignified cell walls. The heavily lignified brachysclereids and short fibres of the mesocarp form a strong additional protection for the seed.

The mature endocarp consists of the following:

an innermost layer of macrosclereids. These cells are about 180 μm long; secondary cell walls consist mainly of cellulose with some lignin;

a layer of osteosclereids. These measure about 30 μm and have strongly lignified secondary cellulose walls;

a layer of brachysclereids. Cells average 21 μm in length and have thick, secondary cellulose or even lignified walls, which proved to be wavy in macerated tissue; and

a more or less continuous layer of crystal cells, each about 12 μm long.

The pericarp and seed do not develop simultaneously. Lignification in the

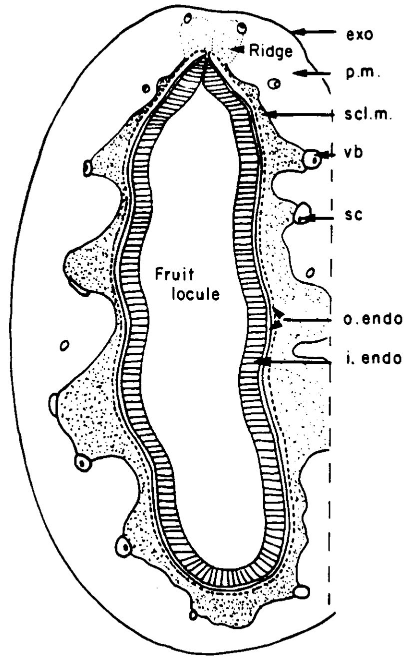


Figure 9. Diagrammatic representation of a transverse section of a mature fruit showing the relative thicknesses of the exocarp (exo); the parenchymatous mesocarp (p.m.) and sclerenchymatous mesocarp (scl.m.); the association of the secretory canals (sc) with the vascular bundle (vb); the outer and inner endocarp (o.endo and i.endo respectively, compare Fig. 5), as well as the 'light coloured' mesocarp ridge (Ridge).

endocarp starts about 2 months after anthesis when the seed is still small and occupies only a small part of the locule (Fig. 5).

Development and structure of the seed

The seed coat develops mainly from the outer integument (oi) (see Fig. 5) while the inner, much shorter integument (ii) degenerates. In the ripe seed (Fig. 12) the radial cellulose walls of the persistent inner epidermis of the outer integument show distinctly lignified thickenings with conspicuous pits (Fig. 12, inset). Sudan black B stained sections show that the cuticle of this epidermis is well developed. The outer epidermis of the outer integument and the central layers are crushed and scarcely discernible. The seed of *Rhus lancea* is therefore endotestal according to the definition of Corner (1976).

Following the interpretation of Vijayaraghavan & Prabhakar (1984), the seeds are exalbuminous. The endosperm remnants and cotyledons contain fats (seen as oil droplets in the squashed preparations) and abundant proteinaceous reserve materials. However, starch could not be detected in the young or ripe seeds, either with polarizing microscopy, or in the PAS sections.

DISCUSSION AND CONCLUSIONS

The division of the genus *Rhus* into four sections by Engler (1881, 1897) shows how important morphological characteristics of the fruit are in taxonomic considerations.

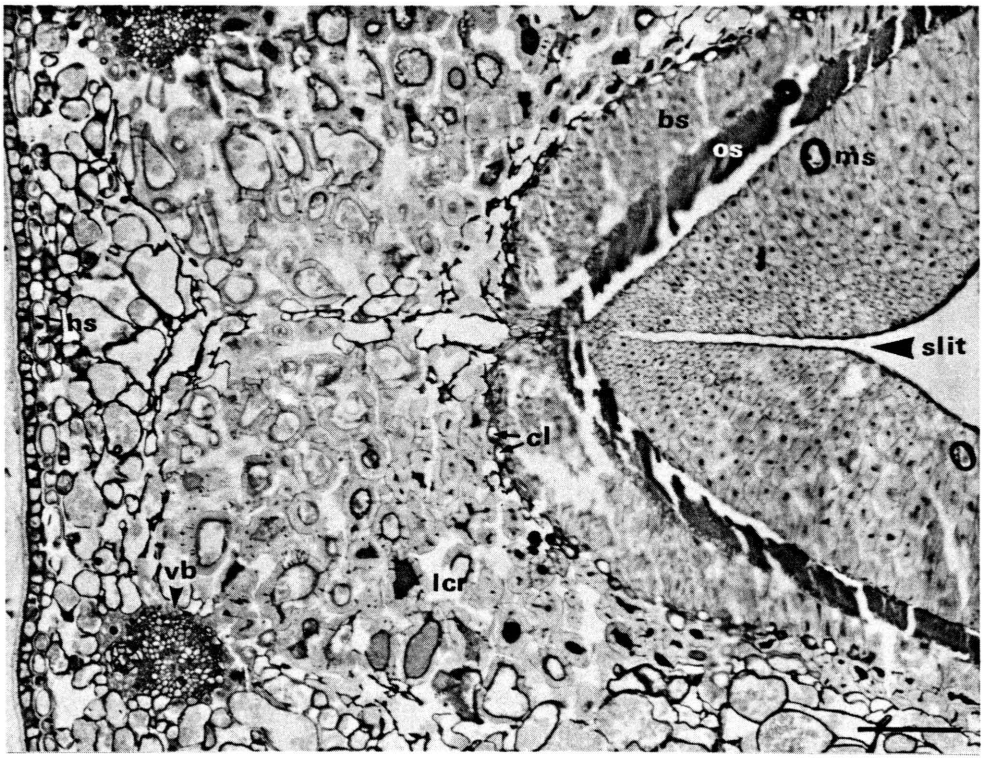


Figure 10. Transverse section of the mature pericarp, illustrating: the exocarp consisting mainly of the outer epidermis and the sclerified hypodermis (hs); the vascular bundles (vb) adjoining the 'light coloured' ridge (lcr) in the mesocarp; the endocarp layers of crystal cells (cl); brachysclereids (bs); osteosclereids (os) and macrosclereids (ms), the latter with slit. Scale bar = 100 μm.

The exocarp

According to our interpretation, the loose, ripe skin or exocarp of the fruit of *R. lancea* is formed by the outer epidermis, a sclerenchymatous hypodermis plus remnants of the outermost mesocarp. The term exocarp is therefore used *sensu lato* according to Roth (1977). However, Brizicky (1963) stated that in all the subdivisions of *Rhus* (for example *Rhus* subgen. *Toxicodendron*) the exocarp is always formed by a single layer. Bogacińsky & Molski (1969) also described the epicarp of *R. typhina* as consisting only of the outer epidermis with two types of trichomes. The difference between our use of the term and that of other authors is probably the result of different interpretations, ours being based on the ontogeny and anatomy of the ripe exocarp. According to Soos & Hausknost (1951) the exocarp in *Schinus molle* L. is formed by two cell layers, namely an epidermis and a hypodermis, both consisting of polygonal cells, those of the former being the smaller. The exocarp of *R. lancea* is therefore very similar to that of *S. molle*.

The mesocarp

According to Roth (1977) a fleshy parenchymatous mesocarp is referred to as a sarcocarp. The mesocarp of *Rhus* is usually described as being parenchymatous

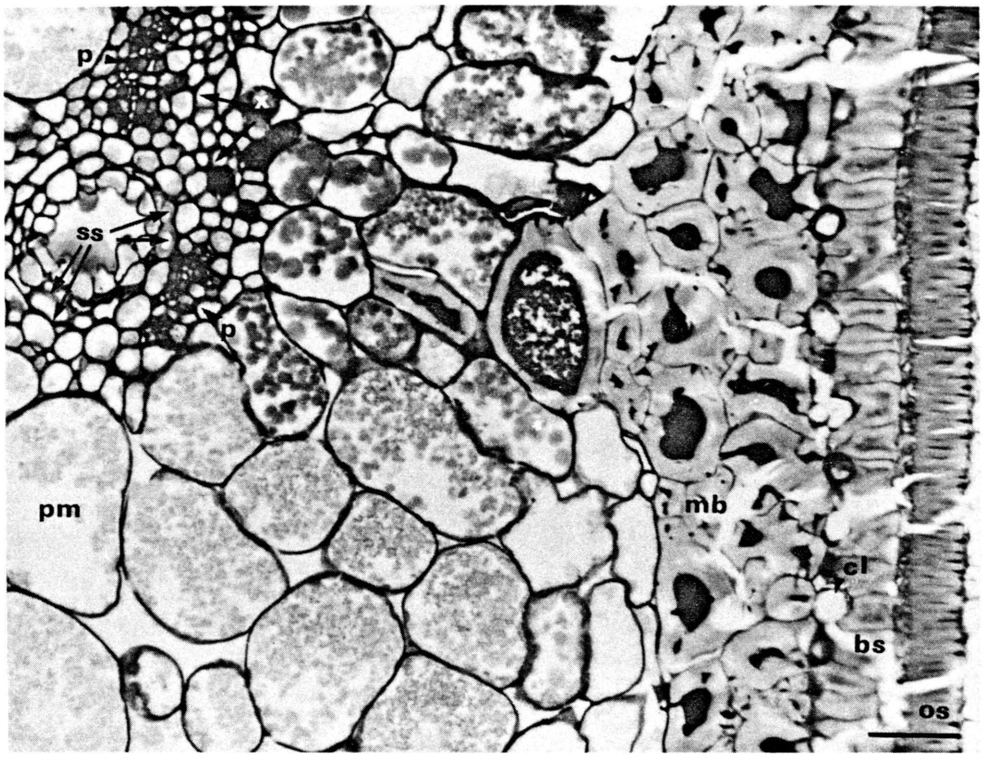


Figure 11. Transverse section of a part of the mature pericarp, namely osteosclereid (os), brachysclereid (bs) and crystal (cl) layers of the endocarp; the sclerified mesocarp with brachysclereids (mb) and fibres and parenchymatous mesocarp (pm) are illustrated. The secretory cavity with sclerenchymatous sheath cells (ss), the broken phloem strip (p) and the strip of xylem elements (x) are also shown. Scale bar = 100 µm.

throughout (Bogacińsky & Molski, 1969; Brizicky, 1963). However, two marked zones occur in the mesocarp of *R. glabra* L., *R. verniciflua* Stokes and other species of *Rhus* previously placed in the genus *Toxicodendron*, namely a larger, outer, parenchymatous part and an inner zone consisting of stone cells or small sclereids (Brizicky, 1963). The inner sclerenchymatous mesocarp of *R. lancea* which forms the outer layers of the stone and its prominent ridges therefore shows very close affinity to several other *Rhus* species.

The endocarp

In seeds and fruit, sclerenchyma attains its highest degree of differentiation and specialization (Roth, 1977). This certainly applies to the endocarp of *Rhus lancea* which is formed, apart from the outermost crystal layer, by three layers of sclereids. Considering the structure and possibly also the microchemistry of the endocarp, there are strong affinities between a number of genera of the tribe Rhoideae of the Anacardiaceae.

Magen (1912), whose work is relatively unknown, reported on the structure of the mature endocarp of *Pistacia, Schinus, Lithraea, Cotinus* and *Rhus*, all belonging to the Rhoideae. He showed a high degree of similarity in the

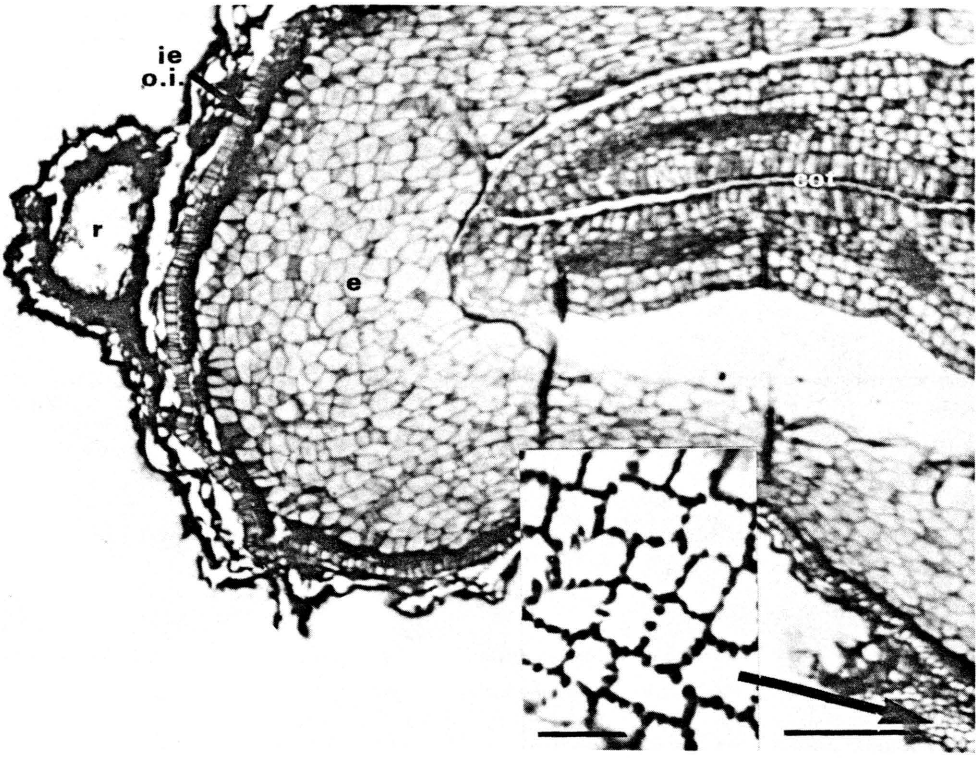


Figure 12. Transverse section of a part of a ripe seed illustrating the seed coat in which the inner epidermis of the outer integument (ie o.i.) is developed. The radial cell walls of the latter are shown in a paradermal section (inset). The vascular bundle of the raphe (r), the endosperm (e) and two cotyledons (cot) are also illustrated. Scale bar = 100 μm; of the inset = 10 μm.

endocarp of all these genera excepting *Pistacia*. The genus *Euroschinus*, studied by Herzog (1910) can probably be added to the list of genera showing these similarities, while *Mauria*, also of the tribe Rhoideae, has a completely different endocarp structure (Herzog, 1910). The endocarp of *Toxicodendron diversiloba* (Torrey & Gray) Greene studied by Copeland & Doyel (1940) is similar to that of *Rhus lancea*. Brizicky (1963) suggested that the genus *Toxicodendron* should be treated as a subgenus of *Rhus*. According to Copeland & Doyel (1940) and Copeland (1959) the endocarp of *T. diversiloba* and *Schinus molle* is derived entirely from the inner epidermis of the ovary and therefore represents an endocarp *sensu stricto*, according to Roth (1977). This most probably also applies to *R. lancea*. Soos & Hausknost (1951) found calcium oxalate crystals in the crystal layer of *Schinus molle*.

The description by Magen (1912) of the endocarp of *Rhus toxicodendron* subsp. *radicans* can be summarized as follows:

an outermost crystal layer, 9–15 μm thick, each cell containing one prismatic calcium oxalate crystal;
palisade layer no. 1, 22–57 μm thick;
microsclereid layer, 5 μm thick;
palisade layer no. 2, 30–43 μm thick; and
palisade layer no. 3 with wavy radial cell walls, 191–231 μm thick.

He initially expressed doubt whether the microsclereid layer, which he also observed *inter alia* in *Schinus molle*, was a real cell layer or not, and further showed that within the genus *Rhus*, this 'layer' tends towards reduction, so that in *R. lucida* L. it is absent. As only hand or wax sections were used for anatomical studies then, it seems likely that the microsclereid layer reported by Magen actually represented the capitate parts of the osteosclereids observed during the present study. Magen's drawing of *Rhus toxicodendron* subsp. *radicans* shows striking similarity to our observations made on 2 µm sections of *Rhus lancea*. Even the flutes on the radial walls of the "palisade no. 2 cells" are shown. Therefore, there is little doubt that in at least some of the *Rhus* species investigated by Magen, for example *R. typhina*, which was again reported on by Bogacińsky & Molski (1969), the same type of capitate osteosclereid of *R. lancea* occurs. In fact, not only the structure, but also the measurements of these layers, as well as the cell wall constitutents are similar.

Embryology

Several authors who have studied species of *Pistacia, Rhus* and *Schinus* (e.g. Copeland, 1955, 1959; Kelkar, 1958) stated that the ovule develops from the base of the ovary. This is in accordance with our findings. Kelkar (1958), Copeland (1959) and Grundwag (1976) stated that in some species of the tribe Rhoideae it is the chalazal megaspore of the linear tetrad that is functional. The latter gives rise to a *Polygonum*-type embryo sac. However, we found that it is the micropylar megaspore that forms the 8-nucleate embryo sac. According to Rutishauser (1969) this represents the *Oenothera*-type.

The seed coat

The extensive study on seed coats by Magen (1912) that has largely escaped attention included several genera of the Rhoideae mentioned previously. In *Pistacia chinensis* Bunge he found the radial walls of the inner epidermis of the outer integument to have pit-like secondary wall thickenings. Figures II and III of Magen (1912: 17) show these thickenings in transverse and paradermal sections. A very similar, somewhat tracheidal, seed coat is also illustrated for *Schinus* and he refers (Magen, 1912: 21) to this type of seed coat as the "Anacardiaceen-typus". *

Therefore, Magen (1912) described an endotestal seed in the genera of the Rhoideae, which was also found in *R. lancea*. In its seed coat characteristics, *R. lancea* shows a pronounced affinity with species of *Pistacia, Schinus, Lithraea, Cotinus* and other *Rhus* species. Magen (1912) mentioned further that the structure of the multilayered endocarp and the one-layered seed coat of the genus *Empetrum* of the Empetraceae points to a relationship with *Pistacia*, and on the basis of pericarp and seed coat structure he showed a possible relationship between the Anacardiaceae and *Juliana* of the Julianaceae. On the other hand he found no affinities between the Anacardiaceae and the Sapindaceae. Magen also studied the seed coat of members of the Aquifoliaceae, Celastraceae, Staphyleaceae and Aceraceae and mentioned previous work on the Balsaminaceae, Hippocastanaceae and Melianthaceae. *

In *Pistacia* species Copeland (1955) and Grundwag & Fahn (1969) found endotegmic seeds, with abundantly pitted radial cell walls of the inner epidermis of the inner integument. A reinvestigation of the integument(s) in *Pistacia* might resolve the problem of the origin of the seed coat. Corner (1976, Vol. I: 31) states that the "Anacardiaceae are most problematic. The unspecialized testa may degenerate to give unitegmic seeds. In some the inner epidermis of the tegmen becomes thick-walled. Neither feature accords with Rutales or Sapindales."

Corner (1976, Vol. II: 44) illustrates the endotestal seed coat of *Commiphora caudata* Engl. (Burseraceae) with irregular scalariform secondary thickenings on the outer and radial walls. These observations, the results of Magen (1912) concerning the seed coat in *Schinus* etc., and our findings regarding *R. lancea*, all combine to support the generally accepted idea that a strong phylogenetic affinity exists between the Anacardiaceae and the Burseraceae.

The reserve materials in the endosperm and cotyledons

Most authors who have investigated the endosperm in species of the Anacardiaceae found that it is nuclear and partially persisting. Tabata (1907) found starch, proteins and fats in the endosperm of the ripe seed of *Rhus succedanea* L., while the cotyledons contain proteins and fats only. When investigating the reserve materials in the seedling he found that the fats had been transformed into starch, which was then abundantly present in the cotyledons. Fats also occur in the seeds of *R. typhina* (Tischer, Illner & Seidl, 1941), while a non-drying oil occurs in the seeds of *R. glabra* (Frankforter & Martin, 1904). Brizicky (1963), referring to previous studies, reported the presence of fats and oils in fruit of various species of *Rhus*. The fats and proteins found in the endosperm and cotyledons of *R. lancea* therefore represent the reserve materials usually found in the genus *Rhus*.

Growth relations of pericarp and seed

In *Rhus lancea*, as in *Sclerocarya* (Von Teichman & Robbertse, 1986), endocarp differentiation precedes seed development. Grundwag (1976) reported the same developmental sequence of pericarp and seed in species of *Pistacia* and stated that similar growth relations between ovary wall and ovule were described for the stone fruits of various other members of the Anacardiaceae.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria and the CSIR Foundation for Research Development. We would also like to thank Dr Elzabé Schoonraad, Prof. J. Coetzee and Mr C. F. van der Merwe for their advice and help.

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CHAPTER 9

SEED OF *Mangifera indica**

Accepted for publication in *S. Afr. J. Bot.*

THE STRUCTURE OF THE SEED OF MANGIFERA INDICA L. AND NOTES ON SEED CHARACTERS OF THE TRIBE MANGIFEREAE (ANACARDIACEAE)

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ABSTRACT

The anatropous, unitegmic and pachychalazal mango ovule develops into the campylotropous, pachychalazal seed. The undifferentiated seed-coat is of dual origin , developing from the integument as well as from the pachychalaza. The term 'peritesta' is suggested for the peripheral, band-like integumentary part of the seed-coat. The major saddle-shaped chalazal part is associated with a tanniniferous hypostase. The discussion includes the anatomy and histochemistry of the seed. The pachychalazal seed with undifferentiated seed-coat probably characterizes the tribe Mangifereae. After consideration _inter alia_ of seed characters, it is concluded that, according to generally accepted criteria, the Anacardiaceae is phylogenetically one of the more advanced families.

OPSOMMING

Die anatrope, unitegmiese en pagichalasale saadknop van die mango ontwikkel tot 'n kampilotrope, pagichalasale saad. Die ongedifferensieerde saadhuid is van tweeledige oorsprong en ontwikkel uit die integument sowel as die pagichalasa. Die term "peritesta" word voorgestel vir die periferale bandagtige integumentêre deel van die saadhuid. Die grotere saaltjievormige chalasale deel is met 'n looisuurhoudende hipostase geassosieer. Die bespreking sluit in die anatomie en histochemie van die saad. Dit blyk dat die pagichalasale saad met ongedifferensieerde saadhuid kenmerkend mag wees vir die tribus Mangifereae. Na oorweging van o.a. saadeienskappe, kan volgens algemeen aanvaarde kriteria afgelei word dat die Anacardiaceae filogeneties een van die meer gevorderde families is.

KEYWORDS: _Anacardium_, _Buchanania_, _Gluta_, hypostase, mango, pachychalaza, peritesta.

INTRODUCTION

The family Anacardiaceae is chiefly tropical and subtropical in its distribution and includes about 70 genera with c. 600 species (Hou,1978). Although the general structure of the seed of the Anacardiaceae has been investigated by several authors, the seed-coat as such, according to the assessment of Corner in 1976, had received detailed attention in only a few species. The senior author of the present paper has since researched the ontogeny of the seed-coat in three species indigenous to South Africa, viz. *Lannea discolor* (Sonder) Engl., *Sclerocarya birrea* (Richard) Hochst. subsp.*caffra* (Sonder) Kokwaro, and *Harpephyllum caffrum* Bernh. ex Krauss (Von Teichman in press a & b and Von Teichman & Van Wyk in press).

Mangifera indica L., of the tribe Mangifereae, family Anacardiaceae, is a species with significant economic importance and one that ranks fifth in production among all fruit trees (Corbineau *et al*. 1986). However, low fruitset has been encountered in some South African cultivars. This has recently led to various studies aimed at possible solutions to the problem, e.g. pollination studies by De Wet *et al*. (1986) and an investigation of the mango ovule by Robbertse *et al*.(1986)

which also highlighted the broad taxonomic importance of ovule and seed structure. However, as no adequate description of the mango seed and seed-coat has been published before and also because of its relevance to the fruit industry, it was decided to provide this relevant information. This information is also needed to elucidate the phylogenetic position of the mango. In this article the structure of mainly the mature seed-coat of _Mangifera_ _indica_ is described as well as that of some other species of the tribe Mangifereae (_sensu_ Engler, 1892).

MATERIALS AND METHODS

Ovules and fruit in various stages of development of the mango cultivars Haden, Sensation and Zill were collected on the Lisbon Estate, Eastern Transvaal.

Free hand sections were prepared from fresh material and cleared with Herr's clearing fluid (Herr 1982). For the preparation of semi-thin sections of the mature seed-coat and cotyledons, the material was fixed in 2,5% glutaraldehyde in 0,1 mol dm^{-3} buffer consisting of Na_2HPO_4 and NaH_2PO_4 (Coetzee & Van der Merwe 1985). For better preservation of tanniniferous substances 0,5% caffeine was added to the fixative according to the recommendation of Mueller & Greenwood (1978).

The material was dehydrated, infiltrated and embedded in purified glycol methacrylate (GMA) according to the method of Feder & O'Brien (1968). The composition of the monomer mixture, procedures of the periodic acid-Schiff reaction (PAS) and counter staining with toluidine blue O was described in detail previously (Von Teichman 1987).

Lipids were stained for 10 minutes with a saturated solution of sudan black B in 70% ethanol. To stain lignified cell walls, sections were mounted in saturated phloroglucinol in 20% HCl. Protein staining was done with acid fuchsin and amido black 10B (Bullock et al. 1980). Starch was tested for with the PAS reaction and with potassium iodide-iodine (Jensen 1962). To localize callose, sections were mounted in a solution of 0,1% w/v water soluble aniline blue in 0,067 M phosphate buffer at pH 8,5. Pretreatment with toluidine blue O was done to eliminate autofluorescence of the lignin and of the aniline blue non-specifically bound to cell walls (Smith & McCully 1978). Sections were examined with a Reichert Univar microscope equipped with epifluorecence optics.

RESULTS AND DISCUSSION

Structure of the Ovule

In their discussion of the seed-coat in general, Boesewinkel & Bouman (1984) point out that structures that seem identical at maturity may sometimes arise along different ontogenetic pathways. The true nature of any seed-coat can therefore only be interpreted if the structure of the ovule is known and the ontogeny has been investigated. The relevant details concerning the mango are provided here.

According to Davis (1966),the ovule of the Anacardiaceae and therefore by implication also that of the mango, is anatropous, uni- or bitegmic and crassinucellar; endosperm is of the Nuclear type. Robbertse et al. (1986) confirmed the anatropous nature of the basal mango ovule and found that it was unitegmic due to the integumentary shifting, resulting in the inner integument remaining rudimentary. The chalazal tissue accommodates the greater part of the nucellus whereby a pachychalaza is formed. According to Corner (1976) such a pachychalazal ovule develops from basipetal, intercalary growth in the area where the chalaza adjoins the integument. Although Robbertse et al. (1986) did not mention whether or not they had observed a hypostase, it is evident from their figure illustrating a longitudinal section of the mango ovule (their Figure 3 B) that a tanniniferous hypostase is associated with the extensive chalaza. This pachychalazal nature of the ovule (Figure 1A) obviously has a significant effect on the eventual structure of the mature seed and seed-coat.

Development of the seed and seed-coat

During the development of the seed , the raphe becomes attached to the pericarp at the ponticulus (Figure 1A & B). Since the antiraphe develops much more extensively than the raphe, the seed becomes curved or campylotropous (*sensu* Corner 1976). The pachychalaza of the ovule including the hypostase, undergoes extensive radial expansion resulting in the formation of a large saddle-shaped pachychalaza (Figure 1B). The study of the young seed material revealed the presence of an active plate meristem, with mainly anticlinal divisions developing near the antiraphal base of the integument and causing the rapid growth of the integument at the antiraphal periphery (Figure 1B). At maturity the seed is therefore also pachychalazal according to the definition of Corner (1976).

Mature seed-coat

Two regions can be distinguished in the mature seed-coat: the first is a thin, fragile, narrow, light brown band or girdle (Figure 2) extending from the micropylar end around two thirds of the circumference of the seed and covering the convex antiraphal side. This region is at its broadest, i.e. 10 to 15 mm, near the micropyle. It consists of about 15 to 20 squashed cell layers containing single, scattered, less distorted tanniniferous parenchyma cells causing the light brown colour of this part. No endosperm remnants are found on the inner surface of this region. The second, and by far the largest, region of the seed-coat (Figures 2 & 3) is the dark brown, thick, chalazal part. This part originates from the pachychalaza of the ovule , while the narrow girdle covering the antiraphal part originates from the single short integument. The seed-coat is thus of dual origin.

The chalazal part is smooth on the outside and unevenly rugged on the inside due to the presence of the rigid vascular bundles. On the shallow, concave, raphal side it is about 1mm thick, relatively tough and consists of an outer thin-walled more or less squashed parenchymatous chalazal tissue with scattered groups or single tanniniferous cells (Figure 4). Numerous amphicribral, anastomosing vascular bundles on the inner side form a wickerwork , resulting in this region of the seed-coat being relatively tough. Longitudinal sections of vascular bundles reveal tracheoids (sensu Schmid 1986) with helical secondary wall thickenings. An extensive thin-walled, parenchymatous hypostase of about 10 cell layers is also present (Figures 3 & 4). The tanniniferous contents of the cells cause the dark brown colour of this region. According to Corner (1976) the hypostase typically consists of a few layers of small, angular, contiguous cells without intercellular cell spaces, which become brown and suberized or lignified but lack differentiation in form. The mango hypostase agrees with this description. In the maturing mango seed the cell walls are impregnated with lignin and lipids, probably suberin or cutin. If cutin is present this would be in accordance with the findings of Bouman (1984), who mentioned various kinds of wall impregnations in the hypostase, including cutin. We found that these walls also consist mainly of cellulose. According to Tiwari (1983) the hypostase wall impregnations of Torenia fournieri Lind. also contain cellulose, as well as callose and pectin. However, in the mango seed-coat no callose was found.

Other authors who have superficially investigated the mango seed-coat structure include Juliano & Cuevas (1932) who noted slight lignification; and Lindner (1971) who mentioned three types of stone cells , both authors describing the outer layers of the seed-coat. The latter author also mentioned an inner tannin containing layer which he referred to as 'braune Haut'. This is probably the first reference to the tanniniferous hypostase described in the present paper.

In the mango, the pachychalaza is therefore associated with an extensive tanniniferous hypostase, an association also noted by Corner for other species with pachychalazal seed or seeds with a more or less extensive chalaza.

However, the integumental part of the mature mango seed-coat seems to differ distinctly from the integumentary part of similar previously described pachychalazal seeds (*vide* Boesewinkel 1981). The seed-coat of e.g. *Trichilia grandifolia* Oliv. (Boesewinkel 1981), originating from a bitegmic pachychalazal ovule is also of dual origin but the integumentary part occupies approximately the lower one-third or micropylar area and is more or less restricted to the original position of the integuments. In the mango the integument expands peripherally after fertilization through intercalary meristematic activity. Anticlinal divisions occur and the integumentary part gradually expands and eventually virtually surrounds the chalazal part as a narrow band or "peritesta", a term that could be created in analogy with "perichalaza" *sensu* Corner (1976). This description of a "peritesta" is probably the first to be published, but this type of integumental seed-coat may be of much more general occurrence.

Boesewinkel & Bouman (1978) described the pachychalazal seed of *Glycosmis* cf. *arborea* (Roxb.) D.C., a member of the Rutaceae, and striking similarities can be noted with respect to the mango: the unitegmic condition of the ovule, derived through integumentary shifting; the pachychalazal proliferation and nature of the chalazal part of the seed-coat and the single, large exalbuminous seed that occurs in an animal-distributed fruit.

Embryo

The embryo of the mango has *inter alia* two cotyledons of unequal size, the edge of the smaller one causing a noticeable indentation on the inside of the seed-coat (Figure 2). The characteristic impression of the rough, chalazal part and narrow, smooth, integumentary part of the seed-coat on the cotyledon surface is illustrated in Figure 5. The cotyledons consist of a starchless parenchymatous epidermis, an inner and outer storage parenchyma and numerous amphicribral vascular bundles occurring in close association with schizogenous secretory ducts. Besides protein bodies and oil in elaiosomes, the cells of the storage parenchyma contain simple starch grains. The latter are described by Wuersch & Hood (1981) as being ellipsoidal, 8 to 20 µm in diameter, some with dimples in the outer surface, apparently due to the tight packing of the protein bodies and the grains.

The mature seed of other species of the tribe Mangifereae

As mentioned in the introduction, detailed seed-related ontogenetic studies have been undertaken by the senior author on species related to the Mangifereae. These, in conjunction with the present study on the mango, provide the background for the interpretation of research by other authors.

Communications on seed-related studies on other species of the Mangifereae include those of Weber (1907) and Ziesak (1972) on Anacardium occidentale L. (cashew-nut), that of Hou (1978) on Swintonia acuta Engl. and on species of Gluta L., and that of Hou (1978) and Mitra & Mehrotra (1980) on several species of Buchanania Spreng.

The account of Weber (1907) confirmed the close relationship of the cashew nut with the mango which was already pointed out by Engler (1892). In both species the seed-coat consists of an epidermis, a many layered parenchymatous tissue, the inner part of which contains numerous ramifying chalazal vascular bundles and an inner layer, 10 to 15 cells thick, of brown cells with thicker walls. According to Weber (1907) the latter layer is responsible for the brown colour of the seed-coat. This represents a hypostase. In the figures of the kidney-shaped cashew seed (Weber 1907, Tafel V1 Figs 79 & 80) the "Chalazastelle" is indicated. The seed of the cashew is doubtlessly also pachychalazal. The vascular bundles in the cotyledons are amphicribral (Weber 1907, Tafel V11 Fig. 96) and closely associated with the schizogenous secretory ducts, while starch grains, aleuron grains and oil also occur.

Ziesak (1972), possibly unaware of the work by Weber, also studied the cotyledons of A. occidentale. He states that the size of the simple starch grains, 1 - 8 µm in diameter, facilitate the identification of cashew seeds in confectionery. In the vascular bundles delicate spiral and reticulate tracheids (*sensu* Schmid 1986) are found. On studying the detailed illustrations of Hou (1978, Fig. 15g and Fig. 18g) of the embryos of *Gluta papuana* Ding Hou and *G. sabahana* Ding Hou respectively, one can conclude that these seeds are also pachychalazal. The same conclusion can be drawn regarding several species of *Buchanania* Spreng., viz. *B. sessilifolia* Bl., *B. macrocarpa* Laut. (Hou 1978); and *B. lanzan* Spreng. (Mitra & Mehrotra 1980) while the drawing of the embryo of the closely related *Swintonia acuta* Engl. (Hou 1978, Fig. 13j) indicates the occurrence of an extensive chalaza.

CONCLUSIONS

1. As far as the evidence at hand allows one to draw some conclusions, there are indications that certain seed characters occur constantly in numerous genera of the Mangifereae, viz.

- size relatively large;
- the absence of endosperm;
- an undifferentiated seed-coat (*sensu* Corner 1976) which is of dual origin;
- an extensive pachychalaza; and associated with it
- a hypostase .

Our study, therefore, confirms the observations of Corner (1976) that an extensive chalaza is often closely associated with a tanniniferous hypostase.

2. As far as the phylogenetic position of Mangifera indica is concerned, this species exhibits various characters that may be defined as either primitive or advanced. Primitive characters include the woody habit and moist tropical habitat, and the anatropous ovule with Nuclear endosperm. On the other hand, in the angiosperms unitegmy and a pachychalazal ovule are generally considered advanced characters (Corner 1976; Boesewinkel & Bouman 1978; Boesewinkel 1981). Therefore, the mango ovule is partly advanced. The mango seed also shows several advanced characters: it is exalbuminous and pachychalazal and the seed-coat is undifferentiated, representing advanced simplification. Characters that, on superficial examination, may be considered primitive are the large seed size and recalcitrant viability. Recalcitrance generally occurs in large seeded, woody species of the moist tropics (King & Roberts 1979) e.g. in Dipterocarpus Gaertn. (Tompsett 1987). However, Boesewinkel & Bouman (1978) reported that seed germination in Glycosmis cf. arborea, an advanced species of the Rutaceae, takes place immediately after fruit drop, indicating probable recalcitrance. Moreover,the large size of the mango seed is the result of extensive secondary development of the embryo, the integument and the strongly vascularised pachychalaza. The latter represents a type of derived specialization of the seed-coat which probably plays an important role in the transport of nutrients to the large embryo.

In the mango the protective function of the seed-coat is adequately taken over by the specialized sclerenchymatous endocarp and there is no necessity for a sclerenchymatous seed-coat. According to Stebbins (1974), this transference of the protective function of the seed-coat often occurs in groups with medium to large sized seeds and he, as well as Boesewinkel & Bouman (1978), consider this large, one-seeded, animal-dispersed drupe a more effective and advanced fruit type.

Therefore, according to generally accepted criteria, our investigation of the mango ovule and seed indicates a general advanced phylogenetic condition. This, together with the information gathered from other genera, confirms the opinion of Corner (1976) that the Anacardiaceae can be regarded as one of the more advanced families.

ACKNOWLEDGEMENTS

This research was undertaken with grants from the University of Pretoria, Margaretha Mes Institute for Seed Research and the CSIR Foundation for Research Development. We wish to express our sincere thanks to Professor A.E. van Wyk for his constructive discussions. Rohm & Haas (Pty) Ltd is sincerely thanked for the free samples of 94% HEMA.

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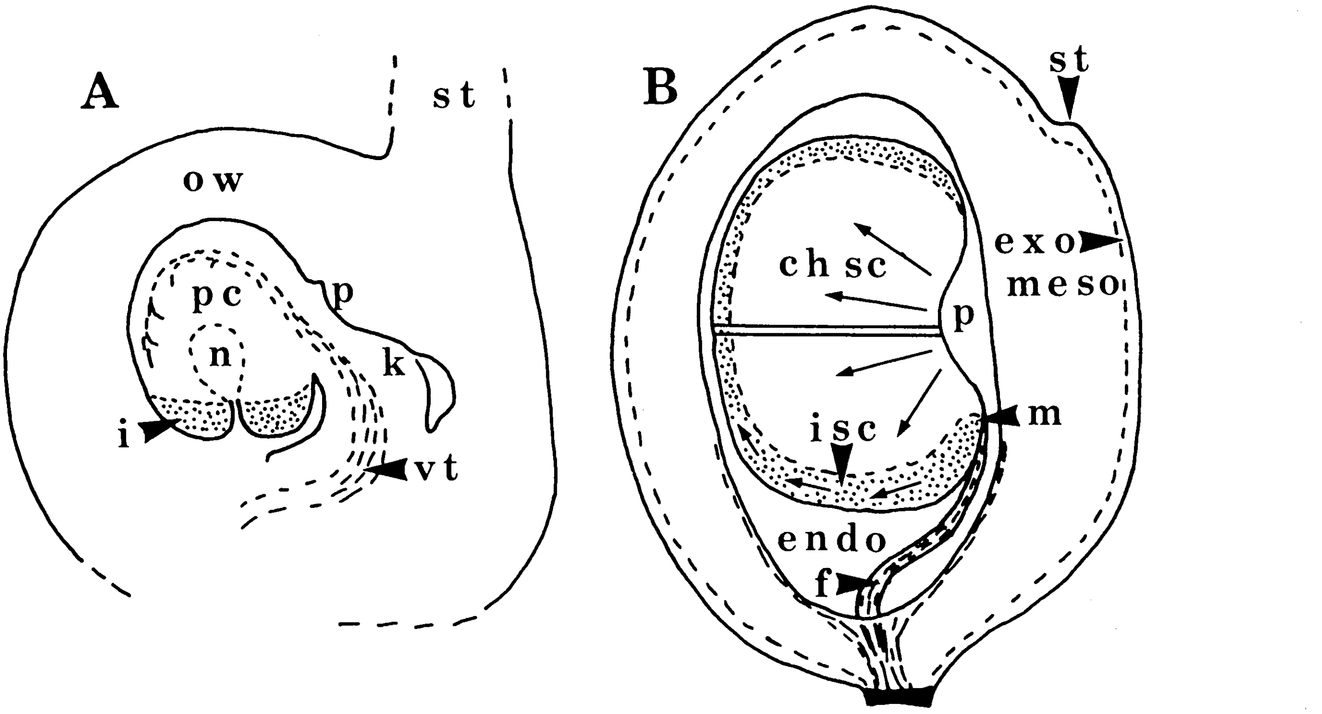

Figure 1. Diagrammatic illustrations of longitudinal sections of the pachychalazal ovule in the ovary (A) and fruit with an intact, mature seed (B). Parallel lines in B represent position of Figure 3. Ovary wall - ow, ponticulus - p, style base - st, short integument - i and dotted, funicular knee - k, nucellus - n, pachychalaza - pc, vascular tissue - vt, endocarp - endo, exocarp - exo, micropyle - m, mesocarp - meso. The seed, which is bending around the ponticulus is shown with chalazal seed-coat (chsc), funicle (f) and integumentary seed-coat (isc and dotted). Arrows indicate direction of meristematic growth.

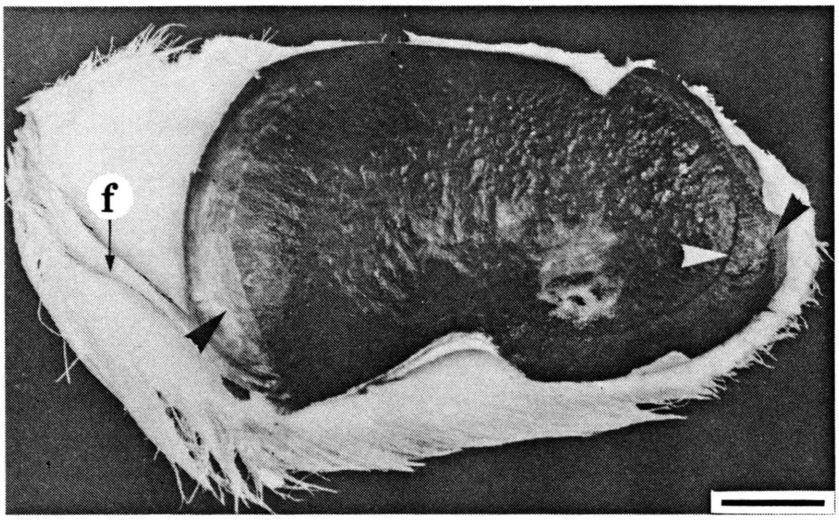

Figure 2. A part of the fibrous endocarp, nearly half of the seed-coat and the fibrous funicle (f) of the mango. The seed-coat is shown from the inner surface, the integumentary part (black arrow heads) surrounding the chalazal part. The mark left by the edge of the smaller cotyledon (white arrow head) is also illustrated. Scale bar = 10 mm.

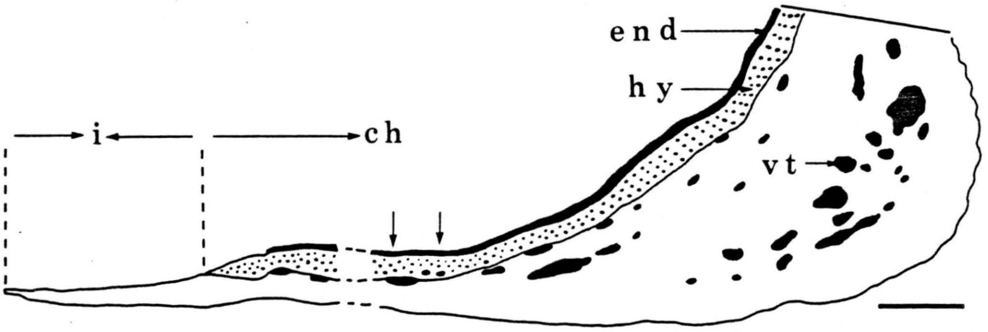

Figure 3. Drawing of a TS section of the seed-coat. The chalazal part (ch) represents only 40% of the section, while the integumentary part (i) is illustrated in full. The vascular bundles (vt) in the chalazal tissue, the hypostase (hy) as well as the nucellar and endosperm remnants (end) are shown. The part between the two small arrows is enlarged in Figure 4. Scale bar = 300 μm.

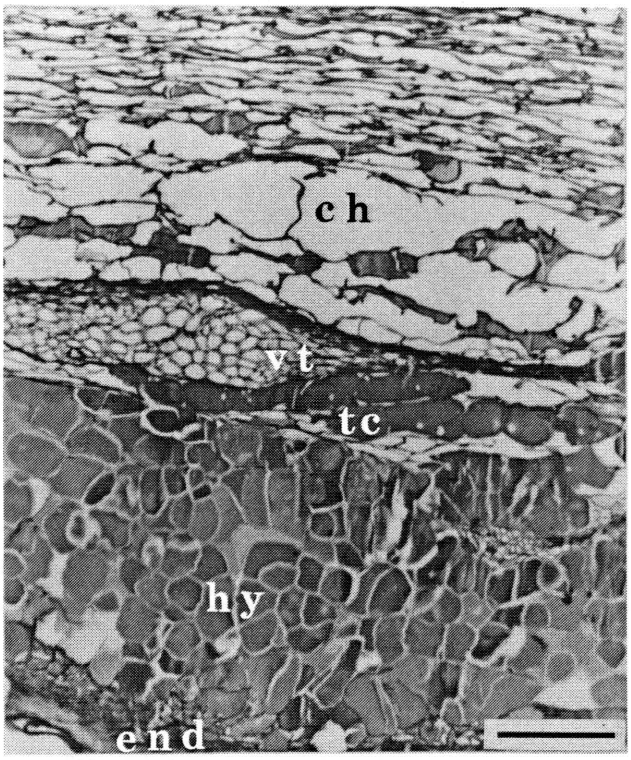

Figure 4. A transverse section of the chalazal part of the seed-coat. The chalazal parenchyma (ch), the vascular bundles (vt) and tanniniferous cells (tc) are illustrated. The hypostase (hy) with thin cell walls which appear nearly colourless in this PAS/TB section, nucellus and endosperm remnants (end) are also shown. Scale bar = 100 μm.

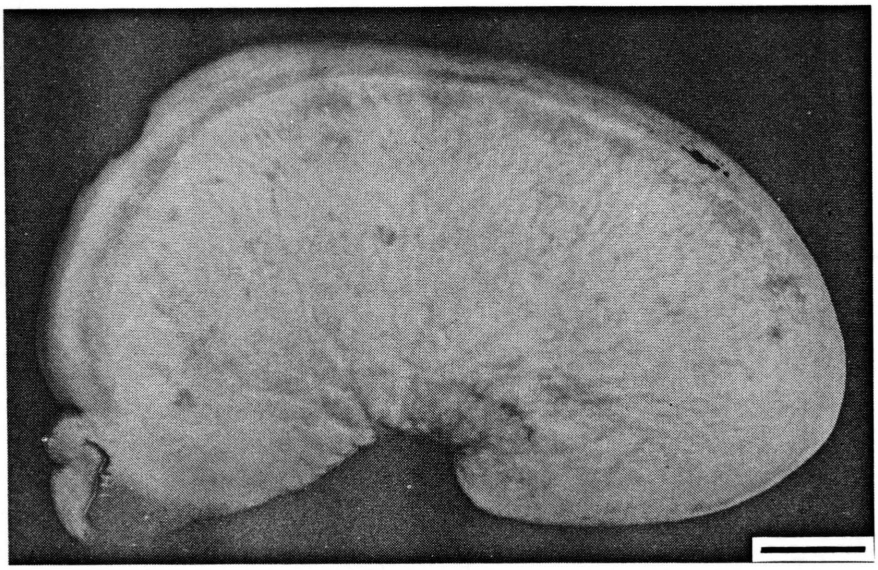

Figure 5.The embryo of the mango. The radicle, the impressions of the narrow, smooth, integumentary part of the seed-coat and rough chalazal part on the larger cotyledon are illustrated. Scale bar = 8 mm.

CHAPTER 10

GENERAL DISCUSSION

10.2 THE FRUIT

CHAPTER 10

GENERAL DISCUSSION

10.1 INTRODUCTION

This chapter briefly recaps the main results of the previous chapters. Simultaneously a synthesis of these results with existing information on the fruit, seed and seed germination will be attempted. Various own, unpublished data, some with accompanying figures, will be supplied. These results are essential for a better understanding of the variation in pericarp and seed-coat structure as well as seed germination. To distinguish the additional data from the conventional discussion, these parts will be supplied in a different typing. The tribe Spondieae is discussed in more detail and with a better insight, since the fruit and seed of about 25% of the genera comprising this tribe have been studied for this project.

In this discussion, reference will be made to taxonomic implications of the results. However, in the conclusions these will be summed up more pertinently. The phylogeny of the gynoecium, fruit, ovule and seed of the Anacardiaceae is mentioned in an attempt to elucidate the evolutionary status of this family.

Since the structure of the hypostase is mentioned in several of the previous chapters, without relating it to possible function, the hypostase as such is elaborated upon in this general discussion. The association of the hypostase with an extensive chalaza and pachychalazy is mentioned in detail, because these features often occur in large recalcitrant seeds. An association of recalcitrant seed viability with ovule and seed characters, as well as habit and habitat and the distribution of these features among dicotyledonous families sensu Dahlgren (1983) are outlined. Lastly a hypothesis based on this association is proposed and the features of a probably ancestral anacardiacious plant postulated. Speculations on the age of the Anacardiaceae and the list of literature references conclude this general discussion.

10.2. THE FRUIT

10.2.1 FRUIT TYPES AND FRUIT DISPERSAL IN THE ANACARDIACEAE IN GENERAL

To appreciate the large variety of fruit types found in the Anacardiaceae it is sensible to look initially at the variation occurring in the ovary. The superior ovary may consist of five free carpels, each with a single ovule, only one being fertile, e.g. Androtium Stapf (Hou, 1978). The carpels may also be incompletely connate as in the 5-carpellate ovary of Dracontomelon Blume and Koordersiodendron Engl. (tribe Spondieae), but usually also with only one fertile carpel (Hou, 1978). Jessup (1985) described Buchanania Spreng. with 4-6 carpels connate only at the base. Therefore, in the tribe Mangifereae only Androtium is apocarpous, whereas the ovary of Buchanania exhibits an extreme form of partial syncarpy, as also observed in the two genera of the Spondieae mentioned above. The ovary can be 1-carpellate and 1-locular, or syncarpous and 2—5(-12)-locular, but if 1-locular, there are usually three styles (Hou, 1978). Sometimes the ovary is partly or wholly immersed in the receptacle or disc and seemingly semi-inferior or inferior. The ovary is rarely truly inferior as in Drimycarpus Hook.f. It is usually sessile, sometimes stipitate as in Gluta L.

Possibly the first detailed descriptions of some fruit of the Anacardiaceae are those of Gaertner (1788) in his "Fructibus et Seminibus Plantarum". The informative illustrations show his sharp perceptivity. The fruit is mostly drupaceous (Hou, 1978). The drupe is a fleshy-stony indehiscent fruit or sarco-sclerocarpium (Roth, 1977). Roth considers the drupe a highly developed fruit type, with mostly two specialized parts. These are the fleshy mesocarp (i.e. sarcocarp) which, being attractive to animals, causes them to act as agents of diaspore dispersal. Secondly there is a hard, often sclerified endocarp (e.g. sclerocarp) which takes over the protective function of the seed-coat. Usually a reduced number of seeds, mostly a single seed, occurs in sclerocarpia (Roth, 1977). According to Engler (1892) and Hou (1978) some drupaceous fruits of the Anacardiaceae are subtended by enlarged sepals (e.g. *Parishia* Hook.f., *Astronium* Jacq. and *Loxostylis* Spreng.f. ex Reichb.) or enlarged persistent petals (e.g. *Swintonia* Griff., *Gluta* and *Melanorrhoea* Wall.). In the tribe Dobineeae the fruit develops on the subtending, enlarged bract. According to Engler (1892) these flying apparatuses are very similar to those of the Dipterocarpaceae. Some other fruits have increased their dispersal potential through wing development from the ovary wall itself. Samaras with a circular wing are found in *Smodingium* E. Mey. ex Sond., *Pseudosmodingium* Engl. and *Laurophyllus* Thunb.; large apical wings also occur, in e.g. *Schinopsis* Engl. and *Loxopterygium* Hook.f.

Stone (1972) mentions that in the Juglandaceae the samaroid, light, relatively small fruit are wind-distributed and that the seeds in these species germinate epigeally.

> It was found in the present study that the species with samaroid fruits, i.e. *Loxostylis alata* Spreng.f. ex Reichb., *Laurophyllus capensis* Thunb. and *Smodingium argutum* E. Mey. ex Sond., exhibit epigeous germination (Von Teichman, unpublished data).

According to Stone (1972) the wind-distributed fruits are primitive and animal-distributed fruits have evolved in two separate lines within the Juglandaceae. The latter fruit type is drupe-like, heavier and larger and germination in these species is hypogeous.

> In the Anacardiaceae similar animal-distributed fruits occur and in some of these larger drupes, as in *Heeria argentea* (Thunb.) Meisn. and *Protorhus longifolia* (Bernh.) Engl., germination is indeed hypogeous. However,in the indigenous species studied, the situation is more complex as will be briefly discussed later.

In *Anacardium* L. and *Semecarpus* L.f. the large, animal-distributed fruit has an enlarged, fleshy hypocarp, representing pedicel and receptacle. Roth

(1977) mentions that the double fruit of *A*. *occidentale* L. consists of the cashew apple, developing from the floral pedicel and the cashew-nut originating from the gynoecium. In the indigenous *Laurophyllus capensis* there is an extraordinary bracteate inflorescence with dense, flattened branches, which in the infructescence, confine the small narrowly winged seeds in small spaces, so that all the seeds do not get dispersed at the same time. According to Marloth (1925) the bracts close up in dry weather, thus preventing the seeds from being scattered under unfavourable conditions. In *Cotinus coggygria* Scop. the whole infructescence serves as a dissemination unit (Engler, 1892). The fruit pedicels elongate and are covered with long hairs. This infructescence is compared to a stiff wig which is rolled along by the wind. Hou (1978, p.400) reports that "some species of *Dracontomelon*, *Gluta* etc. growing in peat-swamp forests, on tidal riverbanks or occasionally in inundated areas, are dispersed by water".

10.2.2 FRUIT MORPHOLOGY AND DISPERSAL OF THE TEN SOUTHERN AFRICAN GENERA COMPARED WITH RELATED GENERA.

10.2.2(a) TRIBE MANGIFEREAE

Since no species of the tribe Mangifereae is indigenous in southern Africa, the fruits of this tribe will not be considered in detail here. Since Scholz (1964) preferred the tribal name Anacardieae to Mangifereae, the former has also been used in this study. In Von Teichman & Robbertse (1986, a) see chapter 2, the pericarp of some members of the Anacardieae is discussed in comparison with that of the marula. *Semecarpus anacardium* L.f. (Weber, 1907) is included in the tribe Mangifereae since Roth (1977, p.350) reported that the species studied by Weber concerned *Anacardium latifolium* Lamk., namely "*Semecarpus anacardium* (synon. *Anacardium latifolium*)". This synonymy should have been pointed out, since the genus *Semecarpus* naturally belongs to the tribe Semecarpeae. The fruit of the tribe Mangifereae are 1-locular drupes.

The exocarp usually consists of the outer epidermis which is often tanniniferous. In Buchanania lanzan Spreng. (Mitra & Mehrotra, 1980) the cell walls are thick and lignified. In most species, except B. lanzan, several hypodermal layers of collenchyma (in Mangifera L.) or parenchyma (in Anacardium) are considered also to be part of the exocarp. The mesocarp is a more or less typical sarcocarp, consisting of thin-walled, radially elongated parenchyma cells. In Mangifera indica L. (Roth, 1977) vascular bundles, secretory ducts and tanniniferous parenchyma are irregularly dispersed within the mesocarp. In the nut of Anacardium occidentale (Roth, 1977), B. lanzan (Mitra & Mehrotra, 1980) and Semecarpus anacardium (Weber, 1907) the large secretory ducts are a conspicuous feature of the mesocarp - these lie roughly in a circle and are often macroscopically visible. The vascular bundles are either associated with these cavities or distributed irregularly throughout the mesocarp.

The endocarp in M. indica, A. occidentale and S. anacardium consists of a characteristic palisade-like macrosclereid layer representing the inner epidermis. In M. indica fibres and vascular bundles constitute the remaining endocarp, whereas in A. occidentale and S. anacardium a layer of micro=sclereids follows the macrosclereid layer. In B. lanzan elongated astro=sclereids form the bony endocarp which splits into two pieces during seed germination. In A. occidentale (Weber, 1907 : Fig. 85) the endocarp seems to split at the one side only. In Gluta the leathery endo=carp is connate with the seed (Engler, 1982). In Swintonia the endocarp is described as coriaceous (Hou, 1978), while in Bouea Meisn. the mesocarp is fleshy and the endocarp fibro-crustaceous (Hou, 1978) Therefore, as far as the fruit of this tribe has been studied, the exocarp is sometimes relatively thick representing an exocarp *sensu lato*, the mesocarp often shows very large secretory ducts and the endocarp is distinctly sclerified in some genera. No special endocarpal opening mechanisms are present in this tribe.

10.2.2(b) TRIBE SPONDIEAE

Concerning the fruit of the tribe Spondieae, three indigenous species have been dealt with in great detail: *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro, henceforth referred to by its common name, the marula (chapter 2), *Lannea discolor* (Sond.) Engl. in chapter 5 and *Harpephyllum caffrum* Bernh. ex Krauss in chapter 7. Considering all the available literature, these are indeed the only species of which the ontogeny and structure of the pericarp have been studied. The close relationship of these three species was confirmed and their pericarp structure was compared in chapter 7. Therefore, only a brief survey will be given here to compare their structure especially with that of *Tapirira guianensis* Aubl. subsp. *guianensis* from Ecuador, henceforth referred to as *Tapirira*, and *Operculicarya decaryi* H. Perr. from the Malagasy Republic, two species of the Spondieae (Von Teichman, unpublished data).

The marula fruit is a relatively large, very fleshy drupe, cream-coloured and tasty when ripe and therefore distributed by various animals. The common names, distribution, habitat, morphology and propagation, as well as the important uses, i.e. nutritional, medicinal, cultural etc., of the marula are given in Von Teichman (1982 & 1983), see Appendix. The fleshy, dark red, plum-like and tasty drupe of *H. caffrum* is also animal-distributed, e.g. by birds, monkeys, baboons and bushbabies (Palmer & Pitman, 1972). The subfleshy, moderately palatable drupe of *L. discolor* is eaten by birds who disperse the stone; however, according to Palmer & Pitman (1972) monkeys, baboons, elephant and kudu also eat these red to purplish fruit.

Tapirira Aubl. is represented by 10 - 15 species in tropical America (Barfod & Holm-Nielsen, 1986) The fleshy, black-violet drupes which occur in a many-fruited infructescence are bird (i.e. dove)-dispersed (Engler, 1892).

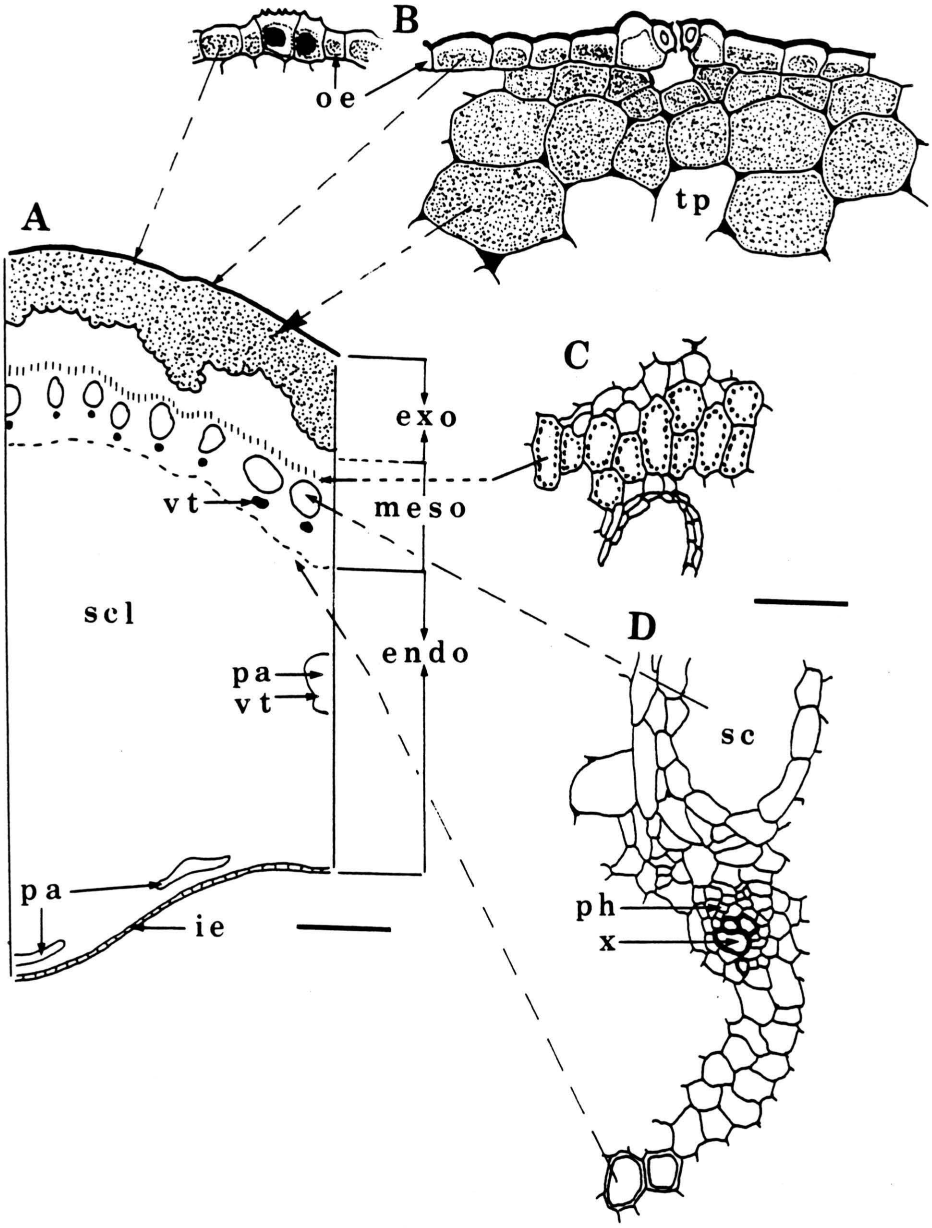

Figure 1. Transverse section of the pericarp of Operculicarya decaryi. A represents an entire TS of the exocarp (exo), mesocarp (meso) and endocarp (endo) of a young fruit. The vascular bundles (vt) near the sclerenchymatous (scl) endocarp, parenchyma islands (pa) within the latter and the inner epidermis (ie) are also shown. In B the detail of the outer epidermis (oe) and the hypodermal, tanniniferous parenchyma (tp) of the young pericarp are illustrated. C illustrates the parenchyma of the mature mesocarp. D shows a part of a secretory duct (sc), the adjacent vascular bundle with phloem (ph) and xylem (x), as well as parenchyma bordering on the sclereids of the young endocarp. Scale bars in A = 500 μm, in B, C + D = 50 μm.

The infructescence of Lannea discolor is also many-fruited. However, the subfleshy drupes of Operculicarya decaryi, which are strikingly similar to those of L. discolor, occur in small numbers (1-4) on the nodes. They are most probably also bird-dispersed. The fruit of Tapirira and O. decaryi are mostly 1-locular with a single pendulous seed, hanging from the apex of the locule, as in most L. discolor drupes.

10.2.2(b)(i) Exocarp

The exocarp of the three indigenous species is very similar, comprising the outer epidermis as well as hypodermal layers. This represents an exocarp sensu lato according to Roth (1977).

> The stomata in O. decaryi (Fig. 1B), which are slightly raised above the surface or are on the same level as the adjacent epidermal cells, and the guard cells having small outer and inner rims (also called ledges) are very similar to those of the indigenous species. In Tapirira the stomata seem always to be raised above the neighbouring epidermal cells and inner rims are possibly absent in the guard cells (Fig. 2B). In all five species the cuticle stretches into the substomatal cavity. The latter is filled with a tanniniferous substance in Tapirira. In O. decaryi the subepidermal parenchyma is densely tanniniferous (Fig. 1B) while in Tapirira the 17 - 20 subepidermal cell layers are tanniniferous collenchyma (Fig. 2B). Tannin-like substances also occur in the exocarp of the three indigenous species.
>
> Until more detailed studies can be done, it is proposed that the exocarp-mesocarp boundary in O. decaryi runs more or less along the tanniniferous parenchyma (Fig. 1A). The exocarp then constitutes about 20% of the pericarp thickness in the mature fruit in comparison with the about 22% in L. discolor. In Tapirira this boundary most probably runs within the zone of secretory ducts (Fig. 2A). This is being suggested since the peel or thick exocarp in L. discolor and the marula tears off in similar zones.

10.2.2(b)(ii) Mesocarp

In the very fleshy drupe of the marula the mesocarp (sarcocarp) constitutes about 46% of the pericarp thickness and consists mainly of 8 - 10 extensively radially elongated, thin-walled parenchyma cell layers. In comparison, the mesocarp in the fleshy drupe of Harpephyllum caffrum, and that of the subfleshy drupe of Lannea discolor, constitutes about 30% and 40%

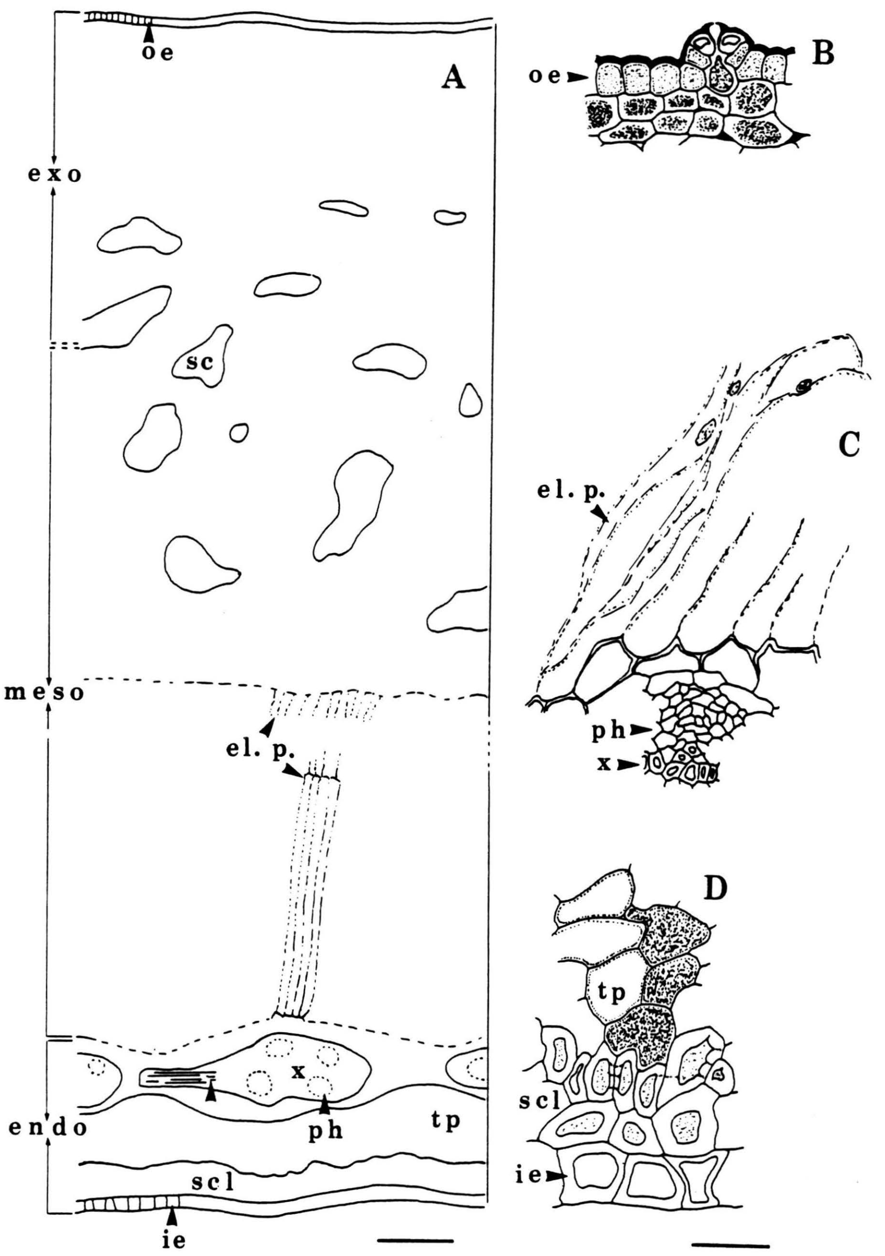

Figure 2. Transverse section of the mature pericarp of Tapirira guianensis subsp. guianensis. A represents an entire section of the exocarp (exo), mesocarp (meso) and endocarp (endo), showing the outer epidermis (oe), secretory ducts (sc), elongated parenchyma (el.p.), the xylem (x and arrow head), phloem (ph), the tanniniferous parenchyma (tp), subepidermal sclerenchyma (scl) and inner epidermis (ie). B illustrates a part of the outer epidermis and adjacent tanniniferous collenchyma, while C and D show detail of the mesocarp and endocarp. Scale bars in A = 150 µm, in B,C & D = 40µm.

respectively. In Lannea discolor the mesocarp consists of 8 — 10 parenchymatous cell layers and near to the endocarp of 3 — 4 tanniniferous, slightly radially elongated parenchyma cell layers.

> As indicated in Figure 1A, the parenchymatous mesocarp in Operculicarya decaryi contains schizogenous secretory ducts with relatively closely associated vascular bundles (Fig. 1D). In the mature fruit, about 2 cell layers on the outside of these ducts are granularly tanniniferous and slightly radially elongated (Fig. 1C). In Tapirira, the fruits obtained from herbarium specimens did not allow a precise reconstruction of the sarcocarp-like part of the mesocarp (Fig. 2A & C) since only remnants could be studied microscopically. Since, according to Engler (1892) and Barfod & Holm-Nielsen (1986), the mesocarp is fleshy, it probably includes the inner part of the parenchymatous zone with secretory ducts and several layers of extensively radially elongated thin-walled parenchyma cells. Provisionally the pericarp of Tapirira as seen in a transverse section, is suggested to consist of about 20% exocarp, and thin endocarp (approx. 15%)and about 65% mesocarp.

The mesocarp of the five species of the Spondieae studied in some detail therefore shows great variation. Its structure has been more liable to changes during the evolution of the species on the different continents with their differing climatic conditions and other evolutionary pressures.

10.2.2(b)(iii) Endocarp

In the Spondieae the endocarp is the most interesting part of the pericarp. In the three indigenous species it is wholly or partly a sclerocarp, since nearly all the cells are sclerified. In Harpephyllum caffrum the endocarp is very complex, since it consists of spongy and woody tissues. In all three species the sophisticated opening mechanism found in most of the drupes of the Spondieae, represented by the operculum, is part of the woody endocarp. The operculum originates from the inner epidermis of the ovary wall and adjacent hypodermal parenchyma. In the mature fruit the sclerenchymatous operculum is surrounded or delimited by parenchyma which permits the access of water before and during germination. This parenchyma is probably weathered and/or decayed to a larger or smaller extent in older stones in the veld, facilitating the "pushing out" of the operculum by the emerging radicle.

The operculum consists mostly of various brachysclereids, fibre-sclereids and stone cells. In the marula, *Lannea discolor* and *Harpephyllum caffrum* the endocarp, excluding the operculum, takes its origin from the inner epidermis of the ovary wall, adjacent parenchyma and the central zone of parenchyma with procambium strands. These zones are easily distinguishable in the ovary wall of flowers at anthesis (see Fig. 3, p.208). In the three indigenous species of the Spondieae discussed here, the exocarp differentiates from the outer epidermis of the ovary wall and the subepidermal parenchyma. The mesocarp differentiates more or less simultaneously with or usually after endocarp sclerification. The mesocarp seems to take its origin in the inner part of the young exocarp, i.e. the part that borders on the outer part of the young endocarp. The mature woody endocarp consists mostly of vascular tissue, fibres, fibre-sclereids and brachysclereids. The latter can be tanniniferous and show a remarkable variation in size and form. The inner epidermis of the ovary wall differentiates into the innermost layer of brachysclereids of the pericarp (Fig. 3, p.208) which lines the fruit locule, giving it its shiny appearance.

The endocarp *sensu lato* in *Harpephyllum caffrum* including the spongy tissue between the spinous, woody, processes radiating from the central woody part, represents about 62% of the pericarp thickness. In the marula, *Lannea discolor* and *Operculicarya decaryi* the endocarp thickness comprises about 30%, 38% and 40% respectively.

> The structure of the endocarp of *Operculicarya decaryi* is very similar to that of *L. discolor*. It is woody, operculate and the innermost epidermis consists of brachysclereids. Tightly packed bundles of fibres which, as in *Lannea discolor*, marula and *Harpephyllum caffrum*, differentiated from the procambium strands and their derivatives, are orientated in all directions. "Islands" of parenchyma cells and parenchyma associated with vascular bundles are present in this endocarp (Fig. 1A).

> However, the endocarp of Tapirira is relatively thin and crustaceous. It consists of an innermost layer of brachysclereids, most probably representing differentiated inner epidermal cells of the ovary wall. Adjacent to this is a continuous layer of tanniniferous brachysclereids, about 2-3 cell layers thick. This is followed by a zone of densely tanniniferous parenchyma (Fig. 2A & D) and a zone of parenchyma with large amphicribral vascular bundles. In O. decaryi and Tapirira the endocarp most probably also takes its origin from the three inner zones of the ovary wall mentioned above. However, in Tapirira cell divisions in the hypodermal parenchyma of the ovary wall take place only very rarely and the procambium strands differentiate into the vascular bundles.

A survey of the available literature yields only scanty information on the pericarp structure of the other species of the Spondieae*. The exocarp in Pleiogynium Engl. is membranous (Hou, 1978). In Cyrtocarpa H.B.K., Haematostaphis Hook.f., Koordersiodendron, Pegia Colebr. and Pseudospondias Engl. the mesocarp is reported to be resinous (Engler, 1892). In most of these species the endocarp is woody, but in Pegia it is thin and crustaceous. In their taxonomic treatment of the genus Spondias L. in tropical Asia, Airy Shaw & Forman (1967) reported on the remarkable and complex endocarp of several Spondias species. It can be more or less smooth or spinous processes diverging from the inner woody endocarp are partly or wholly connected with a peripheral layer of fibrous meshes. Hladik & Hallé (1979) pointed out that the endocarp characteristics of four Spondias species are well preserved and provide a good criterion for field identification. They also show how concealed the operculum in each fruit locule is, very similar to those described in Harpephyllum caffrum (chapter 7, p.109).

Tapirira and Pegia therefore have both a thin and crustaceous endocarp, instead of the usual stony endocarp found in the members of the Spondieae. From a functional point of view it was therefore unnecessary to provide such an endocarp with any special opening mechanism, such as an operculum.

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* Since the descriptive terms of different authors are given, data are not strictly comparable.

10.2.2(b)(iv) Opening mechanisms, i.e. the operculum, in general and specifically in the endocarp of the Spondieae

Hill (1933, p.881) remarked so appropriately "the family Anacardiaceae presents some of the most remarkable examples of seed protection by means of a hard woody endocarp, as well as some of the most ingenious devices to allow for the escape of the germinating embryos." Opercula, however, also occur in seeds as well as in various other lower and higher plant organs. A very brief survey of these opercula is given in Von Teichman (1984).

Concerning opercula in seeds, Kapil et al. (1980, p.568) defined these as follows: "Plug-like structures formed in the micropylar region of the seed and becoming disjoined during germination by circumscissile dehiscence." Several other names have been used for these opercula or seed-lids. Opercula are found mainly in monocotyledonous families such as Araceae, Zingiberaceae, Musaceae, Marantaceae, Commelinaceae, Lemnaceae and also Costaceae (Boesewinkel & Bouman, 1984). They are not so well known in dicotyledonous families (about 20, e.g. Nymphaeaceae, Melastomataceae, Droseraceae and Begoniaceae (Kapil et al., 1980). However, when the woody endocarp is responsible for seed protection some device has been evolved to permit the escape of the embryo from its stony enclosure. Hill (1933 & 1937, pp.255 & 256) describes these fascinating devices as follows:

- irregular splitting of the endocarp wall;
- simple splitting of the endocarp or endocarp loculi into two halves (at least at the apex);
- endocarp opening by throwing off shutters or flanges; and
- endocarp opening by throwing off lids or caps.

Besides the Anacardiaceae Hill mentioned species of the following families: Apocynaceae, Arecaceae, Burseraceae, Cornaceae, Elaeocarpaceae, Halorrhagidaceae, Humiriaceae, Juglandaceae, Meliaceae, Oleaceae, Pandaceae, Chrysobalanaceae, Sapotaceae, Verbenaceae and Zygophyllaceae. The stone in the

Spondieae seems to be characterized by lids, i.e. opercula, and the information available is the following.

In Pleiogynium the 12-locular drupes have 12 cap-like endocarp opercula. However, the mesocarp is also stony and shares the protective function with the woody endocarp (Hill, 1933). The black, ripe drupe of Dracontomelon can possess up to five fertile fruit locules. If only one is fertile, the orifice of the four reduced locules is also hermetically sealed by a smaller woody operculum (Hill, 1933; Wilkinson, 1968). The latter is very similar in shape to the unipartite ones found in the marula. In the marula up to four fertile locules are found, the operculum varying from bi-partite in a few stones, to mostly uni-partite. The "intermediary" forms have a small extra lobe on one side (Von Teichman, 1982, in Appendix). The sterile locules are also usually externally distinguishable by smaller opercula. Concerning the endocarp, which seems to vary little in the species of the respective genera of the Spondieae, the genera Dracontomelon and Sclerocarya Hochst. show the following remarkable similarities :

- the number of fertile locules, which can be reduced to one within the same species;
- the type of endocarp, probably including the anatomy;
- the structure of the opercula.

Hill (1937) described the stone of Antrocaryon micraster A. Chev. et Guillaumin and Choerospondias axillaris (Roxb.) B.L. Burtt et A.W. Hill as having five loculi and consisting of the inner stony part of the mesocarp, which also forms the opercula and the woody endocarp. The latter shows a median split underneath the mesocarp opercula. Engler (1892) already reported opercula in Dracontomelon, Lannea A. Rich. and in the 4-locular stone of Pseudospondias. These split into two halves just as those in the mostly 1-locular stones of Harpephyllum caffrum and the bi- or unilocular stones of Haematostaphis barteri Hook.f. Hill (1937) also described the stone of

Allospondias lakonensis (Pierre) Stapf. However, Airy Shaw & Forman (1967) reduced to *Spondias* both *Allospondias* Stapf and *Solenocarpus* Wight et Arn. In *Cyrtocarpa* the stony endocarp also possesses two or three opercula apically (Engler, 1892).

In *Lannea discolor* (chapter 5, p.56) the stone is described as bi-operculate, rarely uni-operculate, since the former opercula seemed to separate as two small structures which, in the case where the two parts were of unequal size, shared only a narrow common border. However, to avoid splitting hairs, one might also describe these opercula as dome-shaped bi-partite structures. From the discussion in Von Teichman (1987) (see chapter 5, p.63) of the other species of *Lannea* and the description of *L. coromandelica* (Hout.) Merr. (Hou, 1978) it is apparent that within this genus the operculum shows a distinct variation, often even within the species. A more extensive study might show that this variation is not more significant than that in the marula. Nearly all the stones of *Operculicarya decaryi* have a single, i.e. uni-partite, operculum but some stones were bi-operculate, i.e. bi-partite. From the foregoing it is clear that opercula are present in the stony endocarp or stone, i.e. partly including the mesocarp, of nearly all the genera of the Spondieae described by Engler (1892), as well as *Antrocaryon* Pierre and *Choerospondias* B.L. Burtt & A.W. Hill described later, *Pegia* and *Tapirira* being the only exceptions. Although the pericarp of *Tapirira* differs from that of *Lannea discolor*, these two genera (*Tapirira* and *Lannea*) have a number of characters in common: plants dioecious, perianth tetramerous or pentamerous, sepals imbricated in the bud, disc present, ovary mostly uni-locular, ovule single and pendulous, infructescence many-fruited, drupes purplish black and dispersed by birds. These similarities indicate a close relationship between these two genera, which is shown to a lesser extent by the seed-coat structure.

10.2.2(c) TRIBE RHOIDEAE

This is the largest and most diverse tribe of the Anacardiaceae and comprises 35 genera described by Engler (1892) and 12 additions according to Barkley (1957). In southern Africa the tribe is represented by seven genera.

10.2.2(c)(i) Rhus

Of these seven genera, only Rhus L. has been studied in detail. The pericarp of R. lancea L.f. was investigated by Von Teichman & Robbertse (1986, b), see chapter 8. The dryish drupe of R. lancea is animal-distributed, especially by birds. In the mature drupe, the pericarp comprises the following:

- a relatively thin, papery peel or exocarp consisting mainly of the outer epidermis and hypodermal sclereids;
- the mesocarp, consisting of a fleshy part of thin-walled parenchyma with secretory ducts in association with vascular strands, and the sclerenchymatous mesocarp; and
- the stony endocarp consisting of four layers, namely the innermost macrosclereids, followed by osteosclereids, brachysclereids and crystal cells.

Considering the origin of these pericarp parts, it was found that the exocarp takes its origin mainly from the outer epidermis of the ovary wall. The mesocarp develops from the central parenchyma zone with numerous tanniniferous cells, secretory ducts and associated vascular bundles. The endocarp originates from the inner epidermis of the ovary wall (chapter 8).

In a comparison of the endocarp of the indigenous species of the Spondieae with that of R. lancea, homologous structures have to be compared. However, the endocarp in these members of the Spondieae originates from the inner epidermis of the ovary wall, subepidermal parenchyma and the adjacent parenchyma zone with procambium strands (Fig. 3). Therefore, the interpretation of the endocarp of R. lancea should be altered, in the sense that homologous structures are considered in this comparison. Figure 3 shows this comparison diagrammatically. The interpretation given in chapter 8 was based mainly on the studies of Magen (1912) and especially the work of Brizicky (1963) who described some Rhus species with a parenchymatous mesocarp and other Rhus species with a partly parenchymatous and partly sclerenchymatous mesocarp. Magen (1912) described the endocarp of several Rhus species as being a layered or stratified sclerocarp, the structure being very similar to that of R. lancea as described in chapter 8. The inner sclerenchymatous "mesocarp" and adjacent vascular strands have therefore to be considered as part of the endocarp. The stone of R. lancea would then comprise the whole sclerenchymatous part of the pericarp, enclosing the single seed. The mesocarp of R. lancea would then consist of parenchyma and the circle of secretory ducts lying adjacent to the vascular bundles (Fig. 3).

10.1.1(c)(ii) Six indigenous genera of the Rhoideae

Before the scanty information on the pericarp structure of the various genera of the Rhoideae is briefly discussed, the fruit of the other indigenous genera will be considered. The sequence of the genera is determined more or less by that of Engler (1892).

The purplish black, one-seeded, sub-fleshy drupes of Protorhus longifolia are distributed by birds and monkeys (Palmer & Pitman, 1972).

> The pericarp differs from that of a typical drupe in the sense that the mesocarp is not a typical sarcocarp, consisting mainly of tanniniferous parenchyma and large secretory ducts. In a transverse section of the pericarp the latter lie roughly in two circles. The endocarp is not woody as described by Engler (1892), but the anatomical detail is not yet known. No details are available for Protorhus namaquensis Sprague, the only other species of Protorhus Engl. in southern Africa. P. namaquensis is endemic to a remote area in southern South West Africa.
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> The fruit of Loxostylis alata is described as an oblique, dry, somewhat flattened drupe with persistent calyx and remains of styles (Dyer, 1975). The enlarged sepals form a very effective flying apparatus for this light, most probably wind-distributed

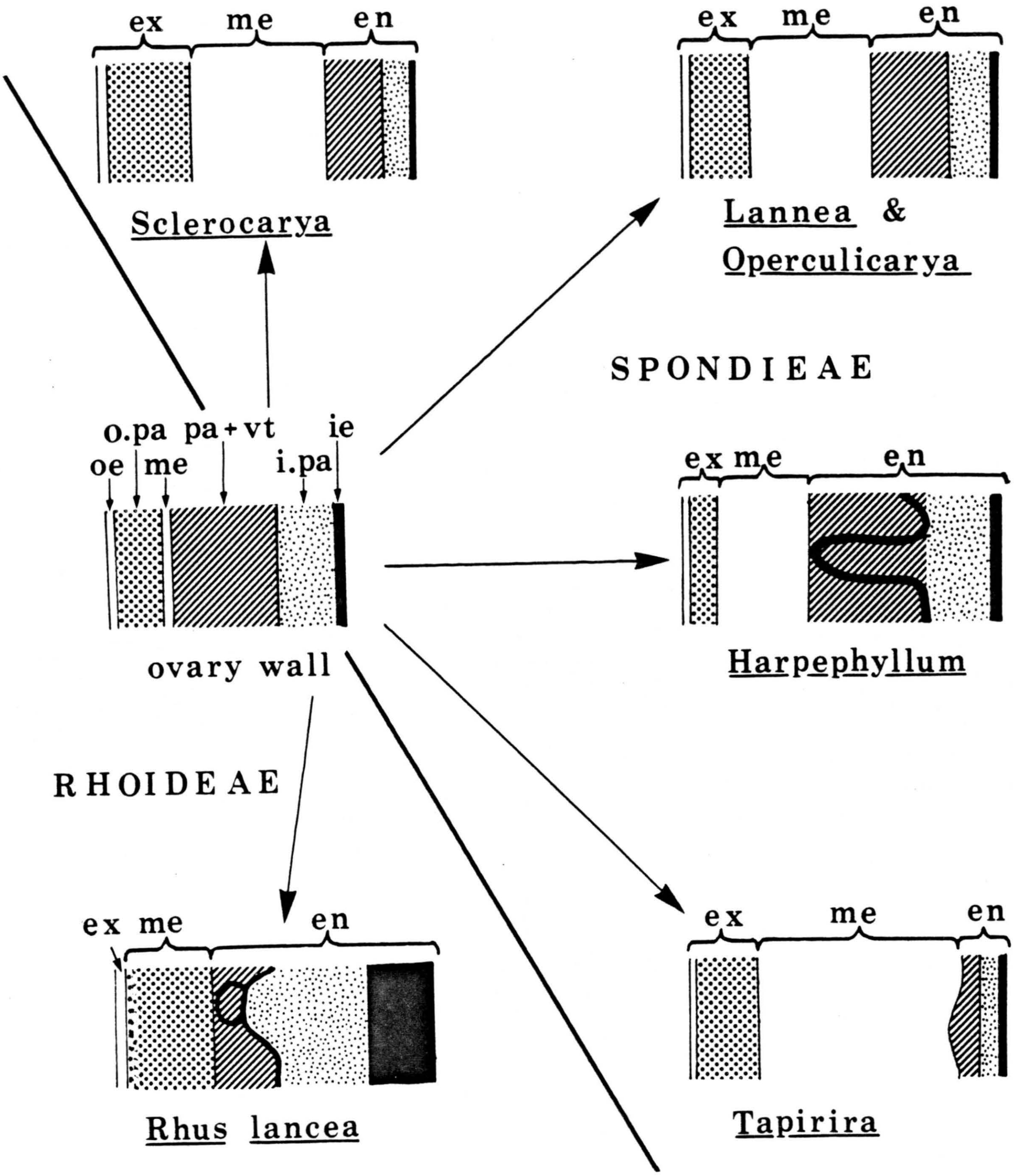

Figure 3. A diagrammatic representation of the parts of an ovary wall and the pericarp of different genera and Rhus lancea. In the ovary wall are shown: the outer epidermis (oe), outer parenchyma zone (o.pa), mesocarp initials (me), parenchyma zone with vascular tissue (pa + vt), inner parenchyma zone (i.pa), and inner epidermis (ie). In the pericarp the relative thickness and the origin of the exocarp (ex), mesocarp (me) and endocarp (en) are illustrated.

> fruit. After removal of the papery, thin exocarp the relatively thin mesocarp with the prominent secretory ducts which run close alongside each other, is exposed.

The endocarp which is not stony, but rather corneous as suggested by Engler (1892), constitutes the main part of the pericarp. Although it is not subtended by enlarged sepals, the fruit of *Micronychia macrophylla* H. Perr. from the Malagasy Republic, described by Perrier de la Bâthie (1946), shows close external similarities in the secretory ducts with *Loxostylis alata*. The mesocarp of this species is also described as very resinous.

The fruit of both *Laurophyllus capensis* and *Smodingium argutum* are described as small achenes by Dyer (1975). Roth (1977), in the extensive survey of the fruits of the angiosperms, considers the achene as a special form of dry, indehiscent fruit characteristic of the Asteraceae. This fruit originates from an inferior ovary of two carpels and the fruit wall may or may not be tightly attached to the seed-coat.

> The wind-distributed fruit of *L. capensis*, *S. argutum* and *Loxostylis alata* originate from superior, two- and three-carpellate ovaries and are best described as samaras. The fruit of *Laurophyllus capensis* and *Smodingium argutum* are compressed, narrowly winged along the margin, uni-locular and one-seeded.

A samara is "a dry indehiscent fruit of the type of a nut, producing only 1 or 2 seeds" and is furnished with one to several wings (Roth, 1977, p. 358). Samaras originate from ovaries consisting of one or several carpels (Roth, 1977).

> A transverse section of the small, brown, light but hard samara of *Laurophyllus capensis* shows that the wing is formed by the exocarp and mesocarp. The former is thin and the mesocarp is characterized by relatively large secretory ducts which are externally visible. The endocarp is relatively thick and hard but not stony. The pericarp of the not quite as small, reddish fruit of *Smodingium argutum* is very similar to that of *L. capensis*.

Considering the remarkable similarity in the perianth, the single pendulous ovule, the morphology of the samara, the anatomy of the exocarp and mesocarp and the toxins occurring in Smodingium argutum as well as the three species of Pseudosmodingium of the Mexican highlands, the close relationship between these two genera is very obvious.

Heeria argentea has dark green to black, one-seeded and almost dry drupes, the size of walnuts. According to Marloth (1925) these fruits are eaten by rock-rabbits. Hemm (personal communication*) observed that rats cherished these resinous, unpleasantly smelling fruit more than others in a nursery. Since this species grows amongst rocks and boulders on hillsides and mountains, it is possible that rodents serve to distribute the fruit.

> The pericarp differs from that of typical drupes, since no stony endocarp is differentiated. Remarkably large numbers of tanniniferous parenchyma cells and large secretory ducts characterize the mesocarp.
>
> Ozoroa paniculosa (Sond.) R. & A. Fernandes, previously placed in the genus Heeria Meisn., differs significantly in fruit characters. The relatively small, shiny, black, wrinkled, dryish, one-seeded drupes of O. paniculosa are bird-distributed. The pericarp consists of a relatively thin exocarp, a mesocarp characterized by conspicuous secretory ducts containing a blackish, oily secretion. The latter is similar in colour and consistency to that of Loxostylis alata. The well-developed endocarp is not stony but rather cartilaginous.

10.2.2(c)(iii) Exotic genera of the Rhoideae

As in Rhus, the exocarp of most of the remaining genera of the Rhoideae is also thin, membranous or papery. In Baronia Baker it is reported to be leathery (Engler, 1892) and in Rhodosphaera Engl. as chartaceous. The exocarp is pilose or hairy in Blepharocarya F. v. Muell. (Jessup, 1985) and Parishia (Engler, 1892).

The mesocarp of the drupes of these exotic genera of the Rhoideae is, as is

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also evident from the description of the southern African genera, mostly resinous, i.e. more or less parenchymatous and traversed by secretory ducts. However, the mesocarp of the light, wind-distributed samara of Schinopsis is thick and spongy (Engler, 1892). The genus Mauria Kunth of South America and the genus Sorindeia Thouars occurring mainly in tropical West Africa, share many characters of the leaves, perianth, stamens, three-carpellate ovary, ovule and drupaceous fruit. The mesocarp of the latter is more or less fleshy. The endocarp in Mauria is thin and consists of one layer of pavement epithelium ("Pflasterepithel" - Herzog, 1910). According to Barfod & Holm-Nielsen (1986) it is corneous and in Sorindeia it is crustaceous or woody* (Engler, 1892). Engler (1892) noted the close relationship between these two genera.

The close relationship between the monotypic genus Rhodosphaera of Australia and Schinus L. which occurs in South America (Engler, 1892; Jessup, 1985) is also noteworthy. The similarities on which this close alliance is based are mainly found in the unisexual flower, imparipinnate leaf, imbricate calyx, disc, the unilocular, tri-carpellate ovary and unilocular drupe with solitary seed. Although the mesocarp adheres to the endocarp in both genera, the pericarp structure shows distinct differences.

The similarity of the layered or stratified, stony endocarp of Rhus lancea with that of Toxicodendon diversiloba (Torrey & Gray) Greene and species of Schinus, Lithraea Miers and Cotinus Tourn. (Magen, 1912; Copeland, 1959; Copeland & Doyle, 1940) was pointed out in chapter 8. The stony endocarp in drupes of Pistacia L. is described by Magen (1912), Copeland (1955) and Grundwag (1976). It differentiates from about six cell layers and consists of stone cells. The latter are enlarged mainly in the radial

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* Since these descriptive terms of the different authors are given, these data are not strictly comparable.

direction and become interlocked by producing irregular outgrowths prior to lignification. The available literature yielded no further ontogenetic pericarp studies. Engler (1892), Hou (1978) and Jessup (1985), using various terms, supply only scanty information on the endocarp of genera such as *Camnosperma* Thwaites, *Euroschinus* Hook.f., *Pentaspadon* Hook.f. and *Trichoscypha* Hook.f.

Generally the endocarp in the Rhoideae is not at all as massive as in most genera of the Spondieae. The stony endocarp of *Rhus lancea* seems to be characteristic of the well-developed, stony type found in this tribe. No special opening mechanisms such as opercula have been evolved in this endocarp. However, in *R. lancea* the stony endocarp exhibits a pre-formed slit (chapter 8, p.156, Fig. 10) which most certainly facilitates the penetration of the radicle through the stony enclosure during germination. The stratified sclerocarp of *Anacardium occidentale* of the tribe Mangifereae also shows such a slit (Weber, 1907, plate VII, Fig. 85). This endocarp of *A. occidentale* is very similar to that of *Rhus* and its closely related genera of the Rhoideae.

10.2.2(d) TRIBE SEMECARPEAE

The scanty information on the fruit of the few genera of the tribe Semecarpeae can be summarized as follows:

- The drupes are unilocular and one-seeded.
- In *Melanochyla* Hook.f. and *Semecarpus* the fruit are basally more or less immersed in the fleshy hypocarp formed by the calyx and floral axis.
- In *Drimycarpus* and *Holigarna* Hamilt. the fruit is totally or to a great extent immersed in the fleshy hypocarp.
- In *Semecarpus* the exocarp is reported to be fleshy.
- In the four genera mentioned above the mesocarp is resinous.

- In Semecarpus curtisii King (Hou, 1978, p.501, Fig. 48f) the resin ducts which are very conspicuous are loaded with an acrid resin.
- The endocarp in Melanochyla is thick and bony, whereas in the other genera it is described by various other terms (Engler, 1892; Hou, 1978; Jessup, 1985).

10.2.2(e) TRIBE DOBINEEAE

Extremely interesting plants occur in this tribe. The female flower in both genera, namely Dobinea Buch.-Ham ex Don and Campylopetalum Forman, is naked. According to Engler (1892) the fruit in Dobinea, which is winged at the margin, is one- to three-seeded and strongly compressed. Since the ovule is solitary, in Campylopetalum, the fruit is one-seeded. The fruit in Campylopetalum is lens-shaped, not winged, but pubescent. The reticulate, indehiscent fruits in both genera develop on the subtending enlarged bract which is transparent and strongly nerved. This bract acts as a wing and forms a flying apparatus (Engler, 1892; Forman, 1953). It would be extremely interesting to study the pericarp of these fruit, since this would possibly help to resolve the "taxonomic dispute" of these genera and this tribe.

10.3 SEED AND SEED-COAT ONTOGENY AND STRUCTURE

10.3.1 INTRODUCTION

In the evolution of higher plants, the capacity to produce seeds was a significant evolutionary advancement. It enabled particularly the angiosperms to become dominant over the spore-bearing plants during recent geologic times. The seed provides continuity between successive generations (Esau, 1977).

According to Corner (1976) the morphology of the fruit and seed should play a major role in classification of flowering plants. Corner (1976, p. VIII) is furthermore of the opinion "that seed-structure is a prime inheritance. Its histological detail expresses genetic character. The many variations cannot be ascribed to environmental selection." He also gives his ideas on seed evolution as follows:

- The main trend is simplification by reducing the complexity and size. "In the long run seeds also become neotenic, small and simple."
- Alternatively, and as successfully, the number of seeds in fruit are reduced until the one-seeded fruit functions as a seed.

In sclerocarpia the integuments rarely differentiate a mechanical layer in the seed-coat and the endocarp performs the duty of protection, e.g. in the Anacardiaceae. When the cells of the integument of the ovule form more cell-layers by periclinal divisions, seeds with massive seed-coats develop. Corner (1976, p.8) refers to these seed-coats as multiplicative and explains that "seed-evolution has caused in the main loss of multiplication but also, as a diversion, an excess, and such seeds with the excessive multiplication" and without tissue-differentiation of the seed-coat, are called "overgrown seeds". The latter occur in various families, mainly those with drupes or nuts. "Fagaceae and most Anacardiaceae seem to have overgrown seeds. It may be the case with Ochnaceae and Simaroubaceae but in these families there is also the problem of the pachychalazal seed with its overgrowth." The genus Mangifera is listed with other genera as having overgrown seeds (Corner, 1976, p.8).

Corner (1976) has left us a unique classification of seed-coats of the dicotyledons. It is based mainly on the presence of mechanical layers. The type of seed-coat development represents an important characteristic of the species. However, "seemingly identical structures may arise along different ontogenetic pathways" (Boesewinkel & Bouman, 1984, p.575). Therefore, the knowledge of the ovule and the ontogeny of the seed-coat is a prerequisite for the precise interpretation of the mature seed-coat.

Netolitzky (1926) points out that in the mature seed-coat the parts originating from the raphe and chalaza have essentially the <u>same structure</u> as the part formed by the integuments. The integuments, the chalazal and raphal tissues, as well as the included nucellar tissue functionally partake in the development of the seed-coat (Boesewinkel & Bouman, 1984).

Schmid, (1986) reviewed the work of Corner (1976) and made some terminological recommendations. These include the following:

- the term "tracheoid" should replace "tracheidal" since the former refers to tracheary elements in general, whereas the latter refers to tracheids only;
- seeds may have more than one mechanical layer in the seed-coat and this should be referred to by the appropriate hybrid terminology, e.g. endotestal-exotegmic seeds; and
- the terminology can be shortened, e.g. exotestal-endotestal should be exo-endotestal.

The proposals of Schmid (1986), based on the system of Corner, make provision for unitegmic seeds, sarcotestal seeds, seeds lacking a mechanical layer and monocotyledonous seeds. Schmid (1986) notes that although the work of Corner (1976) has been extensively reviewed and cited, there has been little criticism on the classification of seed-coats. The second recommendation of Schmid mentioned above, rectifies an aspect that has been criticized. Schmid (1986) again emphasizes the importance of developmental studies in the interpretation of the mature seed-coat, an aspect somehow neglected by Corner.

10.3.2 <u>SEED AND SEED-COAT OF THE ANACARDIACEAE</u>

Corner (1976) remarks that seed studies have to begin with the ovule and end with germination. Therefore, the information on the ovule will be given initially for each tribe and the germination of the indigenous species will be briefly mentioned at the end. The study of the available literature on the Anacardiaceae reveals the shocking reality that very little is known of the seed of the about 70 genera. Even a relatively recent description of a new genus gives no more details of the seed than "Semina laevia testa membranacea, radicula erecta" (Forman, 1953, p. 556).

10.3.2(a) TRIBE MANGIFEREAE

The solitary ovule in this tribe is pendulous from a basal funicle. The ovule was described as anatropous, bitegmic and crassinucellar. Copeland (1961) considered the integument of Anacardium occidentale (the cashew) to be one massive structure, representing the two completely fused integuments. However, Robbertse et al. (1986) found the ovule of Mangifera indica (the mango) to be anatropous, unitegmic and pachychalazal (Appendix). During a recent investigation of the seed of the mango most of the available data of the seed and or seed-coat of the Mangifereae are given (chapter 9).

According to Hou (1978) the seed-coat is free from the endocarp in Buchanania, Anacardium and Mangifera. However, in the mango the raphe proved to be attached to the endocarp at the ponticulus (chapter 9). In Swintonia, Gluta and Bouea the seed-coat adheres to the endocarp (Hou, 1978). In all these genera the two cotyledons are completely free from one another (Hou, 1978). In chapter 9 the similarities in the seed-coat of the mango and the cashew are pointed out. The study of Weber (1907) of the seed of the latter seems to be the only detailed study of a member of the Mangifereae. Ziesak (1972) complemented it only with microscopic observations on the starch grains in the cotyledons. Considering the remarkable similarities in the seed of the cashew and the mango, a reinvestigation of the ovule of the former seems desirable, since it is probably also pachychalazal. In their pharmacognostical study of the fruit and seed of Buchanania lanzan, Mitra & Mehrotra (1980, p.69) describe the seed-coat as brown and "marked on both sides by the branching chalaza". Their illustration of this seed definitely suggests the presence of a saddle-shaped pachychalaza, since the seed is so similar to that of the mango. The illustrations of Buchanania sessifolia Bl. and B. macrocarpa Laut.(Hou, 1978, p.414, Fig 3f+g; p.419, Fig. 5e+f) of the fruit with half of the pericarp removed and of the seed viewed from the raphe side, certainly allow the conclusion that these seeds are pachychalazal. The detailed illustrations of the embryos of Gluta papuana Ding Hou and G. sabahana Ding Hou and the related Swintonia acuta Engl. (Hou, 1978) allow the deduction that the seeds of the species of Gluta are possibly pachychalazal and that those of Swintonia have an estensive chalaza or partial pachychalaza (chapter 9).

Therefore, there are certainly strong indications that the pachychalazal type of seed occurs in a number of the genera of the tribe Mangifereae. The seed-coat in the mango is undifferentiated (sensu Corner) and of dual origin (see chapter 9). It is chalazal and integumentary, which is also characteristic of this tribe. An extensive hypostase is closely associated with the pachychalazal tissue in the mango seed-coat. The cell walls of the hypostase cells are impregnated with lignin and lipids. Weber (1907) unknowingly also described an extensive hypostase in the seed-coat of the cashew. The latter is similar to that of the mango owing to the tanniniferous cell contents of the cells of the hypostase in both seed-coats.

10.3.2(b) TRIBE SPONDIEAE

In this tribe the anatropous ovule is pendulous from the apex of the ovary locule. Kelkar (1958) describes the development of the two integuments in Lannea coromandelica. This mainly concerns the splitting of a single primordium into two parts. Whatever the precise origin of the two integuments, the ovule of members of this tribe is bitegmic and also crassinucellar. The outer integument (o.i.) is better developed than the inner integument (i.i.). The former distally forms a long, flap-like structure, which is initially not easily distinguishable from the funicle. This structure has been called an obturator (Kelkar, 1958). However, as pointed out by Robbertse et al. (1986), see Appendix, the usage of the term obturator for this structure of the o.i. is incorrect. The funicle is well-developed in the species studied in detail and is characterized by a funicular knee. The ovule and/or very young seed of Lannea discolor, the marula and Harpephyllum caffrum are described in chapters 6, 3 & 7, i.e. Von Teichman (in press, a), Von Teichman (in press, b) & Von Teichman & Van Wyk (in press).

With regard to the hypostase in these ovules, the following distinction is made:

- the hypostase sensu stricto represents the nucellar cells adjacent to the chalazal end of the embryo sac; the slightly thickened walls of these cells stain more intensely with P.A.S./toluidine blue O; and
- the hypostase sensu lato is a plate of tanniniferous cells adjacent to the vascular bundle of the raphe, where the latter fans out in the chalaza. The reasons for this distinction are given in chapter 6 (p.80). For the present discussion it is important to repeat briefly that the hypostase sensu stricto is differentiated before embryo sac maturity; does not take part in the formation of the seed-coat and is not associated with an extensive chalaza. The hypostase sensu lato is usually differentiated more or less after embryo sac maturity; plays a role in the development of the seed-coat and is often associated with an extensive chalaza.

The occurrence of these two hypostases in all the tribes of the Anacardiaceae is discussed in the study of the seed-coat of L. discolor (chapter 6). A summary of this occurrence in the tribe Spondieae follows:

- a hypostase sensu stricto occurs in Lannea discolor, the marula and Harpephyllum caffrum; and
- a hypostase sensu lato occurs in the above mentioned species, as well as in Spondias mangifera Willd.

For the present discussion on the seed of the members of the Spondieae, a brief summary of the seed of the three indigenous species must be supplied. The thin, light brown seed-coat of the reniform seed of Lannea discolor is externally characterized by a dark brown saddle-like patch. The papyraceous, cream-coloured seed-coat of the more or less obclavate seed of the marula internally exhibits a light brown, saddle-like patch. The seed-coat of the oblong, slightly reniform seed of Harpephyllum caffrum is very similar to that of L. discolor.

Since the ovule structure of these three closely related, indigenous species is so similar, it is not surprising that the seeds, specifically the seed-coats, are also similar. The chalaza with the associated hypostase sensu lato and also the raphe to some extent, develop extensively. The mature seed-coat of these species consists of the following:

- a large raphe with usually distinctly amphicribral vascular bundle;
- an extensive chalaza with the associated tanniniferous hypostase sensu lato; the latter is responsible for the colour of the saddle-like patch and forms the smaller portion, i.e. the chalazal part of the seed-coat;
- remnants of the o.i., especially the intact outer epidermis which is tanniniferous in Lannea discolor and Harpephyllum caffrum; in L. discolor inner tangential and radial cell wall thickenings occur in this epidermis, constituting traces of an exotestal condition (sensu Corner, 1976); in H. caffrum and the marula a slight mesotestal condition (sensu Corner, 1976) occurs, since fragments of the cell walls of subepidermal cell layers show slight secondary thickening and lignification;
- remnants of the i.i., mainly the inner, tanniniferous epidermis; and
- a cuticular layer between the inner epidermis of the i.i. and the adjacent nucellar epidermis; the last three parts form the

larger, integumentary part of the seed-coat.

In the marula seed-coat, the cells of the hypostase sensu lato show cell wall impregnation with lignin and also lipids, namely Sudan black B-positive substances, probably suberin or cutin. This cell wall impregnation of the hypostase was also found in the mango seed-coat (Von Teichman et al., submitted, see chapter 9). Since the seed-coat described above, lacks differentiation of a definite mechanical layer or layers, it is called undifferentiated sensu Corner (1976).

The nearly mature seed-coat of Operculicarya decaryi and the mature seed-coat of Tapirira guianensis subsp. guianensis henceforth again referred to as Tapirira, have recently been examined (Von Teichman, unpublished data). Since detailed ontogenetic studies have been done on the indigenous, related genera, it is possible to interpret the mature structure of these exotic genera.

The reniform seed of O. decaryi consists of a white embryo and a light brown seed-coat characterized by a dark brown saddle-like patch. It is suspended from the apex of the fruit locule by a well-developed funicle. Transverse sections of the seed-coat at the point where the raphal vascular bundle has split to form one major and several minor bundles are illustrated in Figure 4. The greater, i.e. integumentary, part of the seed-coat consists of the following:

The outer epidermis of the o.i. is parenchymatous and tanniniferous, the three to four central layers are followed by the inner epidermis of the o.i. Only the inner epidermis of the i.i. is well-preserved and is followed by a cuticular layer. This most probably consists, as in the seed-coat of Lannea discolor, of the cuticles of the inner epidermis of the i.i. and of the nucellar epidermis.

The smaller, chalazal part of the seed-coat manifests externally as a dark brown saddle-like patch. It consists mainly of the tanniniferous epidermis of the ovule wall, the parenchymatous chalazal tissue with the vascular tissue more towards the inside and adjacent to the latter the tanniniferous, parenchymatous hypostase. In this still immature seed-coat the nucellus adjacent to the integumentary part is already totally squashed. However, adjacent to the hypostase it is still cellular. The endosperm remnants are mainly cellular at this stage (Fig. 4B). The cell walls of the hypostase cells show no impregnation with lipids. The form of the seed as well as the seed-coat shows a striking similarity with that of Lannea discolor. It remains to be seen whether the mature seed-coat of O. decaryi shows any signs of an exotestal condition.

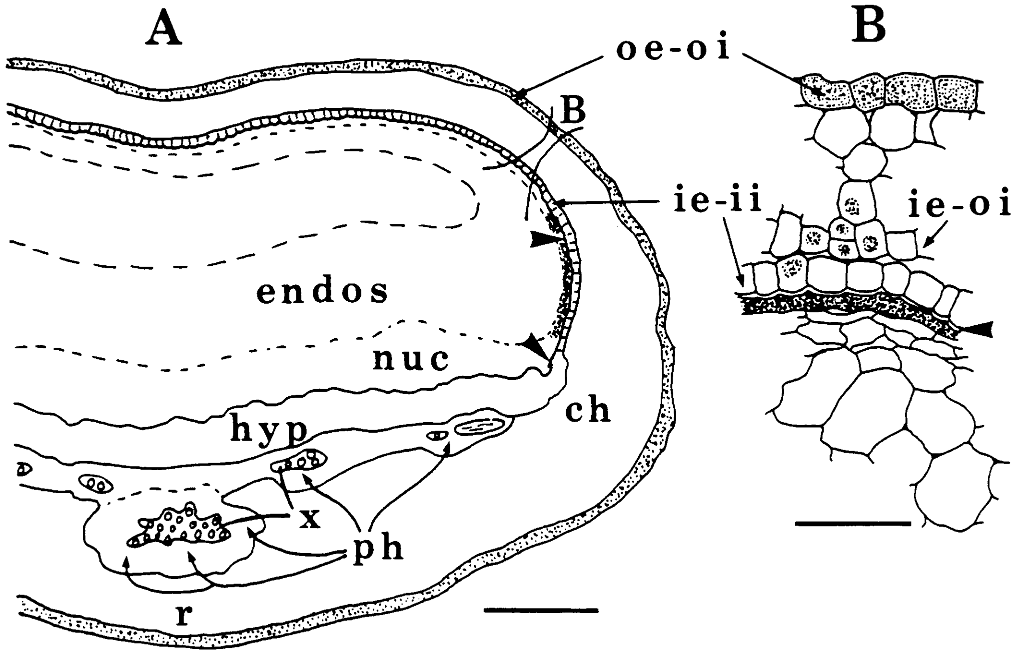

Figure 4. Transverse section of the seed-coat of Operculicarya decaryi at the transition of the raphe-chalazal and the integumentary part. The vascular bundle of the raphe(r), namely phloem (ph) surrounding the xylem (x) has split to form one major and several minor bundles, the hypostase (hyp) adjacent to the cellular nucellus remnants (nuc) and endosperm remnants (endos) are illustrated in A. In B details of the outer epidermis of the outer integument (oe-oi), the inner epidermis of the outer integument (ie-oi), and inner epidermis of the inner integument (ie-ii) are illustrated. The arrow heads in A & B indicate the cuticular layer, followed in B by squashed nucellar remnants and cellular endosperm remnants. Scale bars in A = 150μm, in B= 40μm.

The pendulous, more or less ovate to slightly bent mature seed of Tapirira consists of a thin, light brown seed-coat and a striate embryo. The plano-convex cotyledons with parallel purplish stripes and well-developed radicle characterize the embryo. The seed-coat shows the characteristic dark brown saddle-like patch internally more distinct than externally.

Transverse sections of the seed-coat at the dark brown patch, i.e. the chalazal part, with a part of the cotyledon, and sections of the integumentary part are illustrated in Figure 5A & 5B and 5C respectively. The outer part of the chalazal seed-coat, i.e. the tanniniferous epidermis with its distinct cuticle, the partly tanniniferous hypodermal chalazal tissue and the vascular tissue, do not significantly differ from that of the seed-coat of Lannea discolor. The exceptions are the outer cuticle and the greater number of hypodermal cells that are tanniniferous. The xylem consists mainly of tracheoids (sensu Schmid, 1986) with helical secondary thickenings, also noticed in the chalazal xylem of the other species. However, the hypostase tissue can be divided into an outer part and an inner part. In the former the cell walls are distinctly red to reddish brown. These pigments could be phlobaphenes which are red-brown and occur for example in the o.i. of Eriospermum Jacq., Eriospermaceae of the Lilliflorae (Huber, 1969, p.415). The cell walls of the inner part of the hypostase are stained light blue or are colourless in the P.A.S./toluidine blue O sections and stain positively with Sudan black B indicating lipids, namely suberin or cutin.

The outer hypostase is therefore different from any previously studied hypostase in the Anacardiaceae, whereas the inner hypostase is very similar to that found in the mango and the marula while it differs only slightly from that of L. discolor. Since an adjacent portion of the cotyledon was also sectioned (Fig. 5A & 5B), the purplish colour of the stripes can be connected directly with the outer, peripheral circle of secretory ducts and their secretions. Starch which was absent from the mature cotyledons of the indigenous species, seems to be an important part of the oily food reserve in the seeds of Tapirira. In this preliminary study the integumentary part of the seed-coat (Fig. 5C) reveals a tanniniferous outer epidermis of the o.i., similar to that of L. discolor. The central cells of the o.i. show fragmentary secondary wall thickenings, which possibly also occur in the inner epidermis of the o.i. This probably represents a slight meso-endotestal condition. A slight mesotestal condition was also found in the marula and Harpephyllum caffrum. Of the inner epidermis of the i.i. only remnants seem to be present. A well-developed cuticular layer, probably representing the cuticles of last mentioned epidermis and the nucellar epidermis, is present. The nucellus and endosperm remnants appeared mucilaginous.

Although many characters including some of the seed-coat are shared by Lannea and Tapirira, the outer hypostase of the chalazal part of the seed-coat, the purplish striations of the cotyledons, and the food reserves in the seed in Tapirira are different from those of any of the related genera of the Spondieae studied up to date.

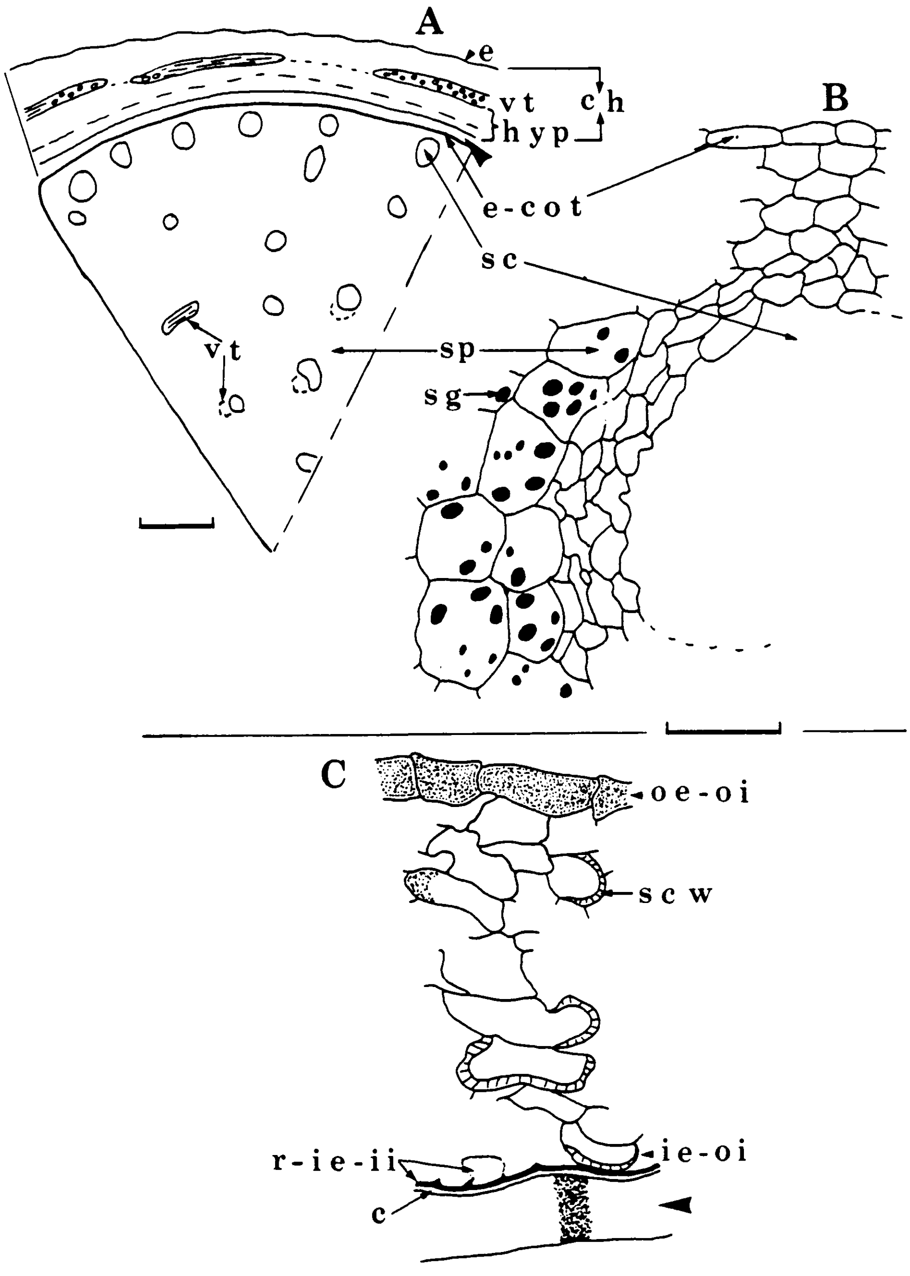

Figure 5. Transverse sections of the seed-coat and cotyledon of <u>Tapirira guianensis</u> subsp. <u>guianensis</u>. In A the chalazal part of the seed-coat (ch) with epidermis(e), vascular tissue (vt), hypostase (hyp) and adjacent mucilaginous nucellus and endosperm remnants (arrow head), as well as a portion of the cotyledon with vascular tissue (vt) and associated secretory duct (sc) are shown. In B details of the cotyledonary tissue, i.e. epidermis (e.cot), secretory duct (sc) and storage parenchyma (sp) with starch grains (sg) are illustrated. C illustrates the integumentary part of the seed-coat with outer epidermis of the outer integument (oe-oi), secondary cell walls (scw), inner epidermis of outer integument (ie-oi), the remnants of the inner epidermis of the inner integument (r-ie-ii), the cuticular layer (c) as well as nucellus and endosperm remnants (arrow head). Scale bars in A = 400μm, in B & C = 40μm.

It is mentioned in chapter 6, p.77 that the seeds of two other species of Lannea also show the reniform shape and the dark brown saddle-like patch. Since the latter occurs in several indigenous species of Lannea, the illustration of the seed of L. coromandelica by Hou (1978) is interpreted as having the same seed-coat structure. The illustration of the seed of Spondias pinnata (L.f.) Kurz clearly shows a dark saddle-like patch, (Hou, 1978, p.482, Fig. 39c). The same probably applies to S. philippinensis (Elmer) Airy Shaw & Forman (Hou, 1978, p.480, Fig. 38e & 38f). Srinivasachar (1940, p.86) describes a plate-like "pad of tannin containing cells" in S. mangifera so similar to the hypostase sensu lato. The presence of a saddle-like patch also applies to a species of Pegia (Hou, 1978, p.489, Fig. 43f).

Additional information from the literature concerns the nature of the seed-coat. It is said to be membranous in Spondias and thin in Dracontomelon, Antrocaryon and Pseudospondias (Engler, 1892). Hou (1978) reports that the seed-coat is free from the endocarp in the genera occurring in Malesia. This is also the case in the species studied presently and seems characteristic of this tribe.

While the seed-coat of the Mangifereae is described as probably pachychalazal, that of the Spondieae seems to be characterized by a partial pachychalaza. Externally it manifests as the saddle-like patch which occurs in the three indigenous genera and in Operculicarya, Tapirira, Spondias as well as Pegia. Seven genera represent about 50% of the tribe Spondieae. The seed-coat represents an undifferentiated type and has a dual origin — partly raphe-chalazal and largely integumentary.

Although the integuments play a more important part in the formation of the seed-coat than the chalaza, and the raphe plays only a minor role, these seeds might still be referred to as "raphe-chalazal". The latter term was suggested by Robbertse et al. (1986), see Appendix, who applied it to the ovules of the indigenous Spondieae, which proved to be very young seeds with a tiny embryo. The terms "pachychalazal" and "raphe-chalazal" both acknowledge the contributions of parts of the ovule other than the integument(s).

10.3.2(c) TRIBE RHOIDEAE

The anatropous ovule of members of this tribe is mostly attached basally on a short funicle. In other genera, it is suspended more or less laterally from the outer ovary wall, shortly below the apex. In most genera the ovule is bitegmic. Reviewing the older literature, Copeland (1959, p.23) suggests that the obturator, namely the "outgrowth at the base of the funiculus", which is present in some genera but not in *Schinus*, actually could be part of the o.i. However, Grimm (1912) who had made serial longitudinal sections of the ovule of a species of *Rhus* pointed out that this "outgrowth" is a part of the o.i. and not a funicular obturator. He also observed the characteristically well-developed funicle and a lignified hypostase *sensu stricto* in this *Rhus* ovule. The investigation of the very young seed of *Rhus lancea* (chapter 8, p. 152) showed without doubt that this structure is the distal part of the o.i. In *Pistacia chinensis* Bunge the ovule is said to be *unitegmic* (Copeland, 1955). Grundwag & Fahn (1969) found an incomplete o.i. in *P. vera* L. (the pistachio nut). According to these authors as well as Grundwag (1976), who studied four different species of *Pistacia*, the cup-shaped, tanniniferous hypostase in the chalaza corresponds to a darkening of the corresponding part in the mature seed-coat. In the Spondieae the ontogenetic studies of the seeds with a partial pachychalaza showed that the extended chalaza with tanniniferous hypostase manifested externally in the darker saddle-like patch of the seed-coat. Examination of these three articles on *Pistacia* therefore suggests the presence of a *small partial pachychalaza* with associated hypostase *sensu lato*. In his review of the earlier work Corner (1976) suggested by implication the presence of a pachychalaza in Pistacia and vaguely also in Schinus and Rhus.

Copeland (1955) and Grundwag & Fahn (1969) mention a thick-walled innermost epidermis of the integument in Pistacia. In P. vera this epidermis of the i.i. (the outer one being incomplete) becomes abundantly pitted. In P. chinensis this innermost epidermis extends as far as the margin of the hypostase. This extension is strikingly similar to that in the seed-coat of the indigenous species of the Spondieae. In P. vera a cuticle is present between this epidermis and the nucellar remnants, a fact which together with the extensive chalaza supports the above mentioned similarity even more.

In Toxicodendron diversiloba a cup-shaped, tanniniferous hypostase is present in the chalaza after fertilization (Copeland & Doyle, 1940). Unfortunately the mature seed and seed-coat of T. diversiloba are not described. Preliminary studies on the seed of Rhus lancea indicate the presence of a small, saddle-like, partial pachychalaza in the seed-coat of the light brown, slightly bent seed (unpublished data). Owing to the presence of the hypostase sensu lato in T. diversiloba and the close relationship of T. diversiloba with species of Rhus, the presence of a small, partial pachychalaza is expected in the mature seed-coat of T. diversiloba. In Schinus molle L. a hypostase appears in the chalaza when the embryo sac matures (Copeland, 1959). The illustration of the mature seed (Copeland, 1959, p.20, Fig. 37) clearly shows a small partial pachychalaza.

In Campnosperma minor Corner an elongated chalaza and brown, tanniniferous hypostase extends "along most of the raphe-side, the free integuments limited to the antiraphe and the sides of the seed" (Corner, 1976, p.67). It is pointed out in chapter 6 (p.79) that this extensive chalaza and hypostase of Campnosperma shows close similarity to these tissue in Lannea discolor. Therefore, a partial pachychalaza is suggested to be present also in Campnosperma. This undifferentiated type of seed-coat of C. minor of the

Rhoideae therefore shows striking resemblance with that of *Lannea discolor*, *Harpephyllum caffrum* and the marula of the tribe Spondieae.

The situation in some genera of the Rhoideae, however, is more complicated than in the Spondieae. Magen (1912) described the mature seed-coat of *Pistacia* as thin and membranous with pit-like secondary wall thickenings in the radial walls of the innermost epidermis of the *one* integument; this is an *endotestal* seed-coat *sensu* Corner (1976). He found the same type of mechanical layer in species of *Schinus*, *Lithraea*, *Cotinus* and *Rhus*. A re-examination of the German text and the fact that at least the ovules of *Schinus* and *Rhus* are doubtlessly bitegmic (with an endotegmic seed-coat), shows that a mistake came into the interpretation of Magen's work in chapter 8, p.159. The last paragraph, first line should read: "endotestal *as well as endotegmic* seeds", instead of "an endotestal seed". Therefore, in *Schinus* and *Rhus* Magen (1912) seemed to describe endotegmic seeds. In *Rhus lancea* (chapter 8) a similar pitting on the radial walls is said to be present in the inner epidermis of the o.i. Unfortunately this last interpretation was based on a brief study only and not as in *Lannea* etc. on a detailed ontogenetic study. The mechanical layer may originate from a different layer. This not only demonstrates the significance of ontogenetic studies in general, but gives priority to the ontogenetic study of the seed-coat of *Rhus lancea* in future research. The latter study will show to what extent the raphe, chalaza and the integuments contribute to the development of the mature seed-coat.

Concerning the other indigenous genera of the Rhoideae, brief preliminary studies of the seeds of *Heeria argentea* and *Ozoroa paniculosa* revealed an important difference between these genera. The seed of *H. argentea* is pachychalazal, whereas that of *O. paniculosa* shows a distinct, small, partial pachychalaza (unpublished data).

The available literature afforded very little additional information on the seed-coat structure of the Rhoideae. According to Hou (1978) the seed-coat is free from the endocarp in Pentaspadon, Pistacia, Campnosperma, Euroschinus and some Rhus species. In Parishia and other Rhus species it adheres to the endocarp. If any description is given of the seed-coat by Engler (1892) or Jessup (1985), it is said to be thin or thin and membranous. Therefore, in the large tribe Rhoideae with about 35 genera (Engler, 1892) the range of the structural seed-coat variation is large. It probably includes amongst others, the undifferentiated type described for Campnosperma by Corner (1976) and the differentiated type initially described by Magen (1912). The partial pachychalaza probably plays an important role in the development of the seed-coat.

10.3.2(d) TRIBES SEMECARPEAE AND DOBINEEAE

In the six genera of the tribe Semecarpeae (Barkley, 1957) the ovule is suspended on the funicle from the wall of the ovary above its middle or just below the apex (Hou, 1978). The latter author and Engler (1892) describe the seed-coat in most of the genera as thin or membranous. The seed-coat is either adherent to the endocarp or it is free. In the two fascinating genera of the tribe Dobineeae the solitary, anatropous ovule is basally attached. According to Engler (1892) and Forman (1953) the seed-coat is thin or membranous in this tribe.

10.3.3 ADDITIONAL NOTES ON THE SEED OF THE ANACARDIACEAE

This study on the seeds of the Anacardiaceae confirms the observation of Corner (1976) and Bouman (1984) that the extensive chalaza, namely the partial pachychalaza and pachychalaza, is often associated with a tanniniferous hypostase. In the Anacardiaceae the extensive chalaza plays a significant role in the development of the seed-coat. The chalaza and hypostase as well as their association with large, recalcitrant seeds in woody, tropical species will be discussed in more detail later.

Bouman (1984) mentions that the structure of the funicular and raphal vascular bundle has received little attention. In the ovule and seed-coat of Lannea and Harpephyllum Bernh. ex Krauss the raphal bundle is distinctly amphicribral (chapters 6 & 7). Although amphicribral bundles are not rare, collateral bundles are most common (Bouman, 1984).

The embryo of the Anacardiaceae may or may not contain chlorophyll (Dahlgren, 1980). The embryos of the three indigenous species of the Spondieae, as well as Operculicarya decaryi and most indigenous species of the Rhoideae are non-chlorophyllous. However, the embryos of Heeria argentea and Protorhus longifolia (unpublished data) as well as species of Mauria (Herzog, 1910) contain chlorophyll. According to Yakovlev & Zhukova (1980) chlorophyll is present in the embryos of Buchanania latifolia Roxb., Cotinus coggygria Parishia sp. and Schinus terebinthifolius Raddi, while those of Anacardium occidentale and Mangifera indica are non-chlorophyllous. Since the absence or presence of chlorophyll is of taxonomic significance (Dahlgren, 1980), this character supplies another important difference between Heeria Meisn. and Ozoroa Del.

The discussion on the seeds emphazises the close relationship of Harpephyllum with Spondias, since a partial pachychalaza seems to occur in both genera. Information on the seed and seed-coat is too scanty to confirm the alliance of Schinus and Rhodosphaera in this respect. Since the seed of Tapirira has some unique features the seed character only partially relate to those of Lannea. However, the similarities in the seed-coat of Lannea species with that of Operculicarya decaryi underline the suggested close alliance which was pointed out in chapter 5 & 6 and in this discussion. Kokwaro (1986) mentions a resemblance of Lannea alata (Engl.) Engl. to the genus Operculicarya H. Perr. This indicates that the investigation on O. decaryi urgently needs completion.

10.3.4 NUTRIENT RESERVES IN SEEDS OF ANACARDIACEAE

In the Anacardiaceae the formation of the endosperm is nuclear. It is persistent as a very thin, sometimes cellular layer in the seed or it is wholly absorbed. The nutrient reserves in the seed are oily or may also contain some starch (Corner, 1976). The analysis of the seed of the mango yielded amongst others, 72,6% total carbohydrate which was mostly starch, 13,7% oil and 7,08% crude protein (Moharram & Moustafa, 1982). In the mango seed starch therefore dominates the other food reserves. According to Ziesak (1972) the cotyledons of the cashew nut (Anacardium occidentale) also contain much starch besides the oil and protein reserves. In the seeds of the Spondieae protein and lipids, in the form of oil, occur. However, in Tapirira starch also occurs and forms an important food reserve.

Discussing the nutrient storage tissues in seeds in general, Boesewinkel & Bouman (1984) prefer the terms albuminous and exalbuminous to endospermous and exendospermous respectively, since the latter concerns the endosperm only. They also state that most seeds contain some protein reserves, stored in protein bodies or aleurone grains, to supply the seedling with nitrogenous compounds. According to Jacobsen (1984) cotyledons store protein, lipids and starch whereas living endosperm rarely stores starch, but commonly contains lipids, protein, phytin, calcium oxalate and carbohydrates in the form of thickened cell walls. The author points out that oily seeds may have almost any internal structure, whereas starchy seeds with the exception of cereals usually contain large embryos and little extra embryonic food reserves. The mango therefore has a typical starchy seed.

10.4 SEED GERMINATION IN ANACARDIACEAE

The germination of seeds is a very fascinating, but also an enormously large field on its own. The present discussion of the germination of mainly the indigenous species of the Anacardiaceae, is based on limited germination experiments. The exception is the study on germination in the marula described by Von Teichman *et al*. (1986), see chapter 4. The present superficial account does not take an extensive literature review into consideration. However, the unpublished data are interesting and will be presented.

> As mentioned at the beginning of this chapter, small, light, wind-dispersed fruit called samaras, are associated with an epigeal seed germination. This is indeed the case in *Loxostylis alata*, *Smodingium argutum* and *Laurophyllus capensis*. The larger, heavier, animal-distributed seeds were said to exhibit hypogeal seed germination. The latter is true for *Protorhus longifolia* and *Heeria argentea*. However, the seed germination of the relatively small, light, animal-distributed fruit of *Lannea discolor* is also hypogeal. The animal-dispersed fruit of the marula (*Sclerocarya birrea* subsp. *caffra*) and *Harpephyllum caffrum*, which are both relatively large, as well as the small fruit of *Ozoroa paniculosa* and *Rhus lancea* have an epigeal seed germination. To facilitate further discussion the species are tabulated:
>
> *epigeal*:
> - *Loxostylis alata* (wind)
> - *Laurophyllus capensis* (wind)
> - *Smodingium argutum* (wind)
> - *Sclerocarya birrea* subsp. *caffra**
> - *Harpephyllum caffrum*
> - *Ozoroa paniculosa* *
> - *Rhus lancea*
>
> *hypogeal*:
> - *Protorhus longifolia*
> - *Heeria argentea*
> - *Lannea discolor* *
>
> Three species are marked with an asterisk to indicate that the larger seedling has a thickened subterranean organ, possibly of hypocotylar origin. These three species occur in savanna regions subjected to periodic veld fires. It is feasible to interpret this subterranean organ as an ecological adaptation facilitating survival during fires and the long dry season.

Rizzini (1965) studied the seedling development of woody plants of the cerrado (Brazilian wooded savanna). The author points out that the critical stage is that of the young seedling. In the latter the roots and shoots usually have neither undergone intensive lignification and suberization nor has the differentiation of the swollen subterranean organ proceeded far in the very young seedling. Another efficient adaptation to survive drought and heat effects is the deposition of pectin in the walls of the root cap cells.

Pectin is important owing to its high water-holding capacity.

According to Rizzini (1965, p.424) "heavy lignification and suberization are quite well-known in the savannas, macchia, cerrado, campo and other areas." The author also mentions the role of fire in the destruction of seedlings and that seed-born young plants usually survive in only especially favourable spots such as moist, shaded depressions. Rizzini (1965) found that epigeal germination is generally associated with the trees of the area, whereas hypogeal germination is often related to undershrubby species and species with larger seeds. The germination of the species with a swollen subterranean organ is epigeal or hypogeal. Rizzini (1965) also reports that species with these underground water-storing structures do not make up the bulk of the flora. The observations and speculation on the indigenous species of Anacardiaceae confirm the results of Rizzini to a remarkable degree:

1) of the ten tree species investigated, seven show epigeal germination;
2) thickened subterranean organs are present in species with either type of germination; and
3) these organs are probably a fire survival adaptation.

It may also be mentioned that several cases of polyembryony were noted during the germination experiments on marula. In the mango, polyembryony is well-known. More extensive germination studies of species of the Anacardiaceae may show the significance of these observations.

<u>Heeria</u> <u>argentea</u> may be considered a relic of the generally extinct, moist, subtropical rainforests, which existed in southern Africa at the end of the Cretaceous and beginning of the Tertiary (Van Wyk, personal communication*; Deacon, 1983). During the climatic change from a moist subtropical to a drier, temperate climate in the distribution region of <u>H</u>. <u>argentea</u> in the Cape, specifically with a long, hot, dry summer, the hypogeal germination possibly represented the crucial or primary survival adaptation of <u>H</u>. <u>argentea</u>.

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The difference in seed germinations between Heeria argentea, occurring in the fynbos, and Ozoroa paniculosa is another seed character that justifies the separation of Ozoroa from the Cape genus. Here it is important to keep in mind that even among the species of one genus, both types of germination as well as transitions from epigeal to hypogeal may occur, (Eames, 1961).

Since the seeds of O. paniculosa germinate relatively easily, it would be interesting to investigate the mode of germination of the other indigenous species of Ozoroa. This could possibly confirm the suggested justification. Eames (1961) mentions that hypogeal germination is regarded as more advanced than epigeal germination. This is a fascinating aspect since animal dispersal, which is also related to hypogeal seed germination, is also considered more advanced than for example wind dispersal.

Lastly the germination of other species of the Anacardiaceae can be mentioned. Tabata (1907) reports amongst others on the epigeal germination of Rhus succedanea L. According to Forman (1953) the wind-distributed fruit of Campylopetalum is related to epigeal germination. The results of Hou (1978) which are tabulated below, indicate that the association of epigeal germination with trees and with animal dispersal of fruits usually holds to a certain extent in these genera of the Anacardiaceae.

"please turn over for Table 1"

Table 1. The germination type of various genera in the Anacardiaceae and association with habitus and type of fruit dispersal sensu Hou (1978).

Germination type	Genera and no of species in brackets	Habitus	Fruit dispersal
Macaranga type epigeal with thin cotyledons	Rhus (2)	shrub	animal
Sloana type epigeal with thick, food-storing cotyledons	Buchanania (2)	tree	animal/water
	Anacardium (2)	tree	animal
	Dracontomelon (1)	tree	animal
	Spondias (3)	tree	animal
	Rhus (1)	tree/shrub	animal
	Parishia (1)	tree	wind
Heliciopsis type/ subtype: cotyledons thin/thick, covered - pericarp & testa, secund at soil level (+- hypogeal)	Gluta (3)	tree	animal/water/ wind
	Mangifera (2)	tree	animal
	Melanochyla (1)	tree	animal
	Rhus (1)	shrub	animal
Heliciopsis type/ subtype Koordersiodendron: cotyledons thin/ thick, covered - pericarp & testa secund above the soil (+- epigeal)	Koordersiodendron (1)	tree	animal
	Swintonia (1)	tree	wind

10.5 PHYLOGENY OF THE GYNOECIUM, FRUIT, OVULE AND SEED OF THE ANACARDIACEAE

10.5.1 GYNOECIUM

The gynoecium in the Anacardiaceae demonstrates the principal evolutionary trends (Stebbins, 1974) from apocarpous to syncarpous, and from hypogyny to perigyny and epigyny. The gynoecium in *Androtium* is apocarpous; in genera such as *Buchanania* a partial syncarpy and in most other genera syncarpy occurs. The superior ovary in most genera represents hypogyny while epigyny is present in *Drimycarpus*. The third trend according to Stebbins (1974) is the shift from axile to parietal placentation. In the Anacardiaceae the placentation is basal or parietal —— the ovule is suspended from the ovary wall. The gynoecium in the Anacardiaceae is therefore relatively advanced.

10.5.2 FRUIT

An evolutionary trend in the fruit of the angiosperms was the shift from dehiscent to indehiscent fruit. Adaptations of the indehiscent fruit for animal dispersal are among the most widespread and significant mechanisms. For most plants animal dispersal is probably more effective than wind dispersal (Stebbins, 1974). The drupe in the Anacardiaceae which is usually animal-dispersed therefore represents an advanced fruit type. The exocarp and mesocarp are often attractive to animals, whereas the well-developed endocarp has taken over the protective function of the seed-coat. This transfer of the protective function of the seed-coat often occurs in groups with medium-sized to large seeds. The one-seeded drupe in most of the Anacardiaceae furthermore shows the derived condition of the reduction of seed number. The less effective wind dispersal of their fruit possibly is one of the reasons why three of the indigenous genera are monotypical. The one-seeded fruits of these monotypic genera, namely *Laurophyllus*, *Loxostylis* and *Smodingium*, have relatively small seeds.

10.5.3 OVULE

According to Stebbins (1974, p.311) the evolutionary "trends in the ovule have been the shift from anatropous to the campylotropous and orthotropous condition." According to Bocquet & Bersier (1960) nine different types of ovules can be distinguished. These authors suggest a possible origin of the anatropous ovule from the orthotropous one, and demonstrate that campylotropy and amphitropy can originate independently from orthotropous and anatropous ovules. Stebbins (1974) agrees with many authors that bitegmic ovules are less advanced than unitegmic ones and that the evolutionary shift has been from the crassinucellar to the tenuinucellar condition. In the Anacardiaceae the anatropous ovule is bitegmic in most genera and seems to be always crassinucellar — all of which points to a relatively primitive ovule. However, the ovule of the mango, and possibly also that of the closely related _Anacardium_ _occidentale_ (see chapter 9), are unitegmic and pachychalazal. The ovule of _Pistacia_ is probably unitegmic. Corner (1976) considers the pachychalazal ovule to be advanced since it develops by means of an intercalary basipetal growth which is the antithesis of the primitive acropetal growth of dicotyledonous organs.

According to Dahlgren (1975) unitegmic ovules are often also tenuinucellar or they are found in combination with a cellular endosperm development, suggesting a correlation between these characters. However, each of these three characters is distributed independently. The endosperm development in the Anacardiaceae is nuclear, which is considered by many authors (Takhtajan and Cronquist as cited by Stebbins 1974; Dahlgren, 1975) to be the primitive state. Dahlgren (1975) points out that primitiveness in one set of characters is not necessarily combined with primitiveness in others.

In most genera of the Anacardiaceae the reduction of the _number of ovules_ to one per gynoecium, which is regarded as advanced, has taken place with retention of the anatropous orientation. According to Stebbins (1974) this is common in the angiosperms.

10.5.4 SEED

The principal trend in seed evolution has been from copious to scanty endosperm (Stebbins, 1974), and a simplification by reduction in complexity and size (Corner, 1976). According to Stebbins (1974) the reduction of the endosperm and an increase in the size of the embryo are present in many trees in the tropics and in temperate regions. This represents one indication of the specialized nature of many tropical arboreal genera. Corner (1976) also mentions that large, overgrown seeds are a derived diversion, since they have excessive multiplication of tissue which usually shows little differentiation.

The seeds of the Anacardiaceae lack endosperm or have only remnants which can be cellular. Seeds of the tribe Mangifereae and Heeria argentea have a pachychalaza, whereas those of the Spondieae and some Rhoideae have a partial pachychalaza. The seed-coat is usually undifferentiated. However, some Rhoideae have a differentiated, probably an endotegmic, seed-coat.

Seed reproduction is so complex since it comprises seed development (dependent on successful pollination etc.), seed dispersal and the establishment of seedlings. These three processes can be widely separated from one another both in time and space (Stebbins, 1974). The establishment of the seedling is the weakest link in the colonization of new territory by plant species (Stebbins, 1974) Relatively large seeds with large embryos and therefore with sufficient nutrient reserves, have the best chance of establishing vigorous seedlings with optimal competitive ability. Heeria argentea possesses a relatively large seed and hypogeal germination occurs. This species could therefore adapt to the climatic change from moist subtropical to drier temperate conditions by means of, amongst others, vigorous seedlings.

The relatively advanced state of the seed of the Anacardiaceae is therefore indicated by their exalbuminous condition, the extensive chalaza and the simplified seed-coat. The size of the large, overgrown seeds in the mango, Heeria argentea and probably many other tree species supports this suggestion. The present study of the fruit and seed of especially the indigenous Anacardiaceae therefore seems to confirm the opinion of Corner (1976) that as far as fruit and seed characters are concerned this family which also occurs in forests, can be regarded as one of the more advanced families. However, as will be seen in the final discussion, my own opinion differs from that of Corner regarding some seed characters.

During the present study of the pachychalazal seed of the mango and Heeria argentea and the survey of the relevant literature, the following interesting association was noted. Recalcitrant seed viability occurs in plants with large seeds and woody plants of the tropics (King & Roberts, 1979). In those families in which species with recalcitrant seeds occur, there is a definite association with anatropous ovules, that are bitegmic and crassinucellar and ovules with a nuclear endosperm development. The significant role played by the extensive chalaza in the seed-coat development and to a lesser extent the occurrence of an unspecialized, i.e. undifferentiated, type of seed-coat are noteworthy in these families. However, before these associations are discussed, a review of the hypostase will be supplied, since it is so often associated with the extensive chalaza of recalcitrant and orthodox seeds.

10.6 ASSOCIATION OF RECALCITRANCE WITH OVULE AND SEED CHARACTERS, AS WELL AS HABITUS AND HABITAT, WITH SPECIAL REFERENCE TO THE HYPOSTASE AND PACHYCHALAZA

10.6.1 STRUCTURE, POSSIBLE FUNCTION AND DISTRIBUTION OF THE HYPOSTASE AS WELL AS ASSOCIATION WITH ADDITIONAL OVULE CHARACTERS AND PACHYCHALAZY

10.6.1(a) STRUCTURE

Reviewing the hypostase Tilton (1980) is of the opinion that the use of hypostase as an all-inclusive term, sensu Van Tieghem, is confusing and vague. Van Tieghem (1901, 1902) emphasizes that the hypostase is constant in structure and function, but variable in form and position. According to him the hypostase comprises a group of nucellar cells at the chalazal end of the embryo sac. It is mostly disc- or cup-shaped, consisting of isodiametric cells with lignified, but not very thick cell walls. In Loranthaceae and Nuytsiaceae the hypostase is tube-shaped surrounding a large part of the embryo sac. In Vitaceae it can be column-shaped. Furthermore, Van Tieghem (1901, 1902) mentions the presence or absence of the hypostase in various families.

The description of a "podium", i.e. a part of the nucellus at the antipodal base of the embryo sac, by Dahlgren (1940) does not significantly differ from Van Tieghem's hypostase. However, this term was used for the basal part of the nucellus in Euphorbiaceae (Bor & Bouman, 1974; Bor & Kapil, 1975). The "postament" is a column of nucellar tissue intruding basally into the embryo sac (Dahlgren, 1940) and is absorbed by the developing megagametophyte (Tilton, 1980). Narayana (1963) described a structure in Balsaminaceae similar to the "Zuleitungsbahn" or "Staerkestrasse" mentioned by Tilton (1980). This is a column-shaped group of deeply staining cells which connects the raphal vascular bundle with the antipodal end of the embryo sac (Narayana, 1963).

Tilton (1980, p.2065) who prefers to refer to these and other specific modifications of the nucellus by different terms, defines the hypostase as follows: "a group of modified cells with usually lignified walls, generally within the chalazal region of the ovule but which may surround a portion of the megagametophyte and extend partially into the micropylar half of the ovule". Corner (1976, p.22) considers the hypostase to consist "of a few layers of small, angular, contiguous cells without cell-spaces, which become brown and suberized or lignified but lack differentiation in form." He uses the term in the general sense for the plate or disc at the base of the nucellus. Bouman (1984, p.148) mentions that the hypostase has diverse features in the different taxa. These features, and the family or families in which they occur, are the following:

- dense cytoplasm: Trapaceae (Ghosh, 1956);
- thickening of walls; Loganiaceae (Bendre, 1975); Cyperaceae (Nijalingappa & Devaki, 1978); Onagraceae (Johansen, 1928; Khan, 1942); Trapaceae; Brassicaceae (Prasad, 1977);
- cell wall impregnation, which may or may not accompany the thickening of the cell walls, (a) with <u>cutin</u>: Onagraceae; (b) with <u>suberin</u> or <u>cutin</u>, i.e. lipidic substances: Euphorbiaceae; Anacardiaceae; (c) with <u>lignin</u>: Anacardiaceae; Scrophulariaceae (Tiwari, 1983); Fabaceae (Pillai & Raju, 1975); (d) with <u>callose</u>: Scrophulariaceae (Rodkiewicz, 1967; Tiwari, 1983); (e) with <u>pectins</u>: Scrophulariaceae (Tiwari, 1983); and
- accumulation of tanninlike substances: ?Trapaceae; ?Euphorbiaceae (Singh, 1970); ?Loganiaceae; ?Onagraceae; Anacardiaceae (Grundwag, 1976; the present study).

The question mark indicates "dense or granular contents" which can represent phenolic or tanniniferous substances. Depending on the form of the nucellar base, the hypostase may be a cluster of cells, a disclike or cuplike cell plate (Bouman, 1984).

10.6.1(b) FUNCTION

Since such diverse features characterize the hypostase, it is not surprising that various functions have been assigned to it. However, these were mostly speculations, since only a few authors undertook detailed light microscopic or electron microscopic studies of the hypostase cells. Van Tieghem (1901) postulated that the hypostase mainly restricts the growth of the embryo sac basally. Tilton (1980) found starch, protein and lipids in the hypostase cells of Agavaceae and starch as well as other carbohydrates in Liliaceae. Naturally she related the hypostase to the translocation of nutrients into the megagametophyte and embryo, and assigned the secondary function of storage to these cells.

According to Rolston (1978) wax, lignin, tannin, suberin and pectin constitute water proofing substances. Boesewinkel & Bouman (1984) agree that the hydrophobic lipids, namely suberin and cutin, form barriers to the diffusion of water and dissolved products. Therefore, the wall impregnations with lignin and lipids in the hypostase cells in the maturing seed-coat of the mango and marula, could mark the cessation of the translocation and the beginning of the protective function. Johansen (1928) noted that in the Onagraceae cutinization of hypostase cell walls takes place only after embryo development and that this impregnation served to stabilize the water balance of the resting seed. Prasad (1977) reports the presence of proteins in the hypostase cell during the globular stage of the embryo. When these reserves were consumed during the later part of seed development, the thickening of the hypostase cell walls took place.

Rodkiewicz (1967) found callose deposits in the hypostase cell walls in Scrophulariaceae during the diad stage of meiosis of the megasporocyte and the mature embryo sac stage. Tiwari (1983, p.124) detected callose at an even earlier stage in another species of the Scrophulariaceae and suggested that callose in the hypostase might serve "as the 'molecular filter' regulating the flow of nutrients to the embryo sac and filtering off undesirable large molecules." The walls of these hypostase cells consist mainly of cellulose and callose, with a considerable amount of pectin, but only little lignin. However, Kapil and Tiwari (1978a) point out that the electron microscopical studies of Diboll and Larson, as well as Woodcock and Bell showed the plasmodesmata to be absent between the embryo sac and the chalazal part of the nucellus. Therefore, if the symplastic transport of water and nutrients is ruled out, and the cell walls, namely the apoplastic thoroughfare, are additionally impregnated with waterproofing substances, the embryo sac or embryo is isolated from the surrounding tissues.

According to Boesewinkel & Bouman (1984) the functions of tannin in seed-coats could be the following: protection, increase in hardness and imparting colour. Since the seed-coat in the Anacardiaceae is protected by the sclerocarp, the main function of the tannin-like substances seems to be coloration. In the caryopses of grass species, Van Niekerk (1987) found the differentiation of the phenolic-rich closing layer, which closes off the chalazal region, to be associated with physiological maturity of the seed. Later this closing layer together with the cuticle in the remaining wall of the kernel effected resistance to desiccation.

Accumulation of tannin-like substances in the hypostase cells of the Anacardiaceae seed-coat has been observed by several workers. In <u>Lannea discolor</u> for example, this accumulation takes place in the young seed-coat. Possibly an apoplastic transport functions until in the maturing seed-coat the cell wall impregnations form the mentioned isolation. These remain speculations until T.E.M studies are done of the hypostase cells during the different ontogenetic stages. Lastly the possible association of seed-abortion, namely ovule senescence, with abnormal behaviour of the hypostase (Maheswari Devi & Chandrasekhara Naidu, 1979; Chandrasekhara Naidu 1985) and callose in the hypostase in Begoniaceae (Dumas & Knox, 1983) have to be mentioned.

The hypostase, therefore, differentiates during different stages, but generally it makes its appearance during the progress of meiosis in the macrospore mother cell. In Cyperaceae, Onagraceae and Euphorbiaceae (Venkateswarlu & Rao, 1975) it develops during the post-fertilization stage of the developing seed. As has been mentioned before, in the Anacardiaceae the hypostase <u>sensu stricto</u> (the group of nucellus cells adjacent to the embryo sac) is initiated early in the differentiation of the embryo sac and does not play a role in the seed-coat formation. However, the hypostase <u>sensu lato</u> (the plate of nucellus cells in the chalaza adjacent to the vascular bundle) develops after anthesis (Srinivasachar, 1940; Grundwag, 1976 and own observations) and does take part in seed-coat formation.

10.6.1(c) DISTRIBUTION OF THE HYPOSTASE AND ASSOCIATION WITH ADDITIONAL OVULE CHARACTERS

The hypostase occurs in the ovule and or seed of many Angiosperm families (Van Tieghem 1901, 1902; Netolitzky, 1926; Tilton, 1980; Bouman, 1984). According to these four authors, authors mentioned in the previous discussion on the structure and function of the hypostase and authors who will be mentioned along with the respective family, the hypostase occurs, amongst others, in the following 18 families:

1. Agavaceae
2. Anacardiaceae
3. Bixaceae
4. Cyperaceae
5. Euphorbiaceae
6. Fabaceae
7. Flacourtiaceae (Van Heel, 1973)
8. Lauraceae
9. Malvaceae (Chandra & Bhatnagar, 1976)
10. Moraceae
11. Myrtaceae
12. Onagraceae
13. Polygonaceae
14. Rosaceae
15. Sapindaceae
16. Theaceae
17. Trapaceae
18. Tropaeolaceae

It seems noteworthy that in all but two families the ovules are anatropous. This is to be expected, since according to Davis (1966) 204 out of 248 families have anatropous ovules. A survey of the number of integuments, nucellar condition and endosperm formation (Davis, 1966 or Corner, 1976) revealed that in 72% of these families the ovule is bitegmic, crassinucellar with nuclear endosperm formation.

An integumentary tapetum or endothelium, which is the inner epidermis of the i.i. that is differentiated as a specialized and cytoplasmic rich layer, occurs in the ovules of 65 families of dicotyledons (Kapil & Tiwari, 1978b). According to this review 53,8% of these families show unitegmic, tenuinucellar ovules and developing an "ab initio" cellular endosperm. Although the survey of the 18 families with a hypostase is meant only as a possible test sample, the association of the occurrence of the hypostase with bitegmic, crassinucellar ovules and nuclear endosperm development seems significant.

In four or possibly more families, a hypostase and an endothelium occur together in the usually anatropous or anacampylotropous ovule. As Table 2 shows, the ovule characters are partly as "typical" of ovules with hypostase and partly of ovules with endothelium.

TABLE 2. THE OVULAR CHARACTERS OF FAMILIES IN WHICH A HYPOSTASE AS WELL AS AN ENDOTHELIUM OCCUR

Family	ovular character		
Balsaminaceae	bitegmic	tenuinucellar	cellular
Brassicaceae	bitegmic	tenuinucellar	nuclear
Loganiaceae - subfamily Buddleioideae	unitegmic	tenuinucellar	cellular
Scrophulariaceae	unitegmic	tenuinucellar	cellular

If these four families are taken into account together with the 18 mentioned above, the hypostase occurs in association with bitegmic, crassinucellar ovules and nuclear endosperm in only 59,0% of these 22 families. A false endothelium, i.e. one of nucellar origin, is recorded in species of Begoniaceae, Droseraceae, Elatinaceae and Papilionaceae (Kapil & Tiwari, 1978b). In some families the endothelium persists in the mature seed as a pigment layer, e.g. in Polemoniaceae, Plantaginaceae, Linaceae and Erythroxylaceae (Boesewinkel & Geenen, 1980; Bouman, 1984). Considering the persistence of the tanniniferous hypostase of several families, these integumentary and nucellar layers appear to be analogous in the mature seed. The hypostase has originated independently in many families during the evolution of the angiosperms.

10.6.1(d) ASSOCIATION OF THE HYPOSTASE WITH AN EXTENSIVE CHALAZA

The present study on the Anacardiaceae confirms Corner's (1976) observation that in most seeds the hypostase occurs in association with a more or less extensive chalaza. The extensive chalaza occurs in different gradations, e.g. perichalazy, partial pachychalazy and pachychalazy, where the contribution of the integument to the seed-coat is respectively: absent or small and the seed-coat is mainly of chalazal origin. However, owing to the absence of ontogenetic studies, the pachychalaza is often overlooked (Boesewinkel & Bouman, 1984). Mathur & Gulati (1980) found an extended chalaza and associated hypostase in the Sapindaceae. In Swietenia Jacqu. (Meliaceae) a perichalaza, with adjacent hypostase in the form of a layer of small brown cells, is present (Corner, 1976). In the Spondieae and Rhoideae of the Anacardiaceae (chapters 3, 6 & 7) a partial pachychalaza is found as discussed here.

According to Corner (1976), pachychalazal seeds occur in the following families:
Balsaminaceae, Ebenaceae, Euphorbiaceae, Flacourtiaceae, Lauraceae, Meliaceae, Ochnaceae, Rosaceae, Sapindaceae and Simaroubaceae. A study of Corner's description of these pachychalazal seeds shows that the persistent hypostase is usually present as one of the following: a hypostasial layer, a hypostasial lining, expanded hypostase or apparently extended hypostase. The pachychalaza in Tropaeolaceae is associated with an ill-defined hypostase (Tiwari et al., 1977). Boesewinkel & Bouman (1978) described the pachychalazal seed in Glycosmis cf. arborea (Roxb.) DC. of the Rutaceae. This seed shows several striking similarities to that of the mango (chapter 9). The chalazal part of the seed-coat is layered, the inner tanniniferous zone possibly having the character of a hypostase (Boesewinkel & Bouman, 1978).

Van Wyk & Botha (1984) described a pachychalaza in Myrtaceae where it is probably also associated with a thin extended hypostase. These last three families can therefore be added to Corner's list of families in which pachychalazy definitely occurs. Pachychalazy may occur in a number of other families such as Anacardiaceae, Proteaceae and certainly the families in which Corner (1976) had already found pachychalazal ovules, namely Balsaminaceae, Tropaeolaceae and Ochnaceae.

The pachychalazal seed may therefore develop from a pachychalazal ovule or by means of an extensive post-fertilization development from an anatropous ovule. This latter, secondary development of the chalaza is already considered as derived by Netolitzky (1926). The intercalary growth of the chalaza below the proximal base of the one or two integuments forms the extended chalaza, which is considered as derived (Corner, 1976; Boesewinkel & Bouman, 1984 and other workers). In the Myristicaceae a "tegmic" pachychalaza is described (Van Heel, 1982; Corner, 1983) and here a basin-shaped chalazal meristem is involved.

As can be expected, this new container for the endosperm and embryo in the ovule, namely the pachychalaza, is associated with an extensive vascular supply. If the pachychalazal ovule develops into a large or overgrown seed, the network of vascular bundles can be traced to the expansion of the chalazal supply (Corner, 1976). In the mature seeds of several families such as Celastraceae, Clusiaceae, Flacourtiaceae, the chalazal cells form a sclerified plug to 'seal' the opening in the endotestal or exotegmic layer, the mechanical layer of the o.i. or i.i. (Corner, 1976).

Corner (1976) found that in pachychalazal seeds the mechanical layer(s) deteriorate or disappear from seeds of drupaceous fruit —— this is a transference of the protective function to the sclerocarp. Therefore an undifferentiated, unspecialized type of seed-coat is often associated with pachychalazy, e.g. in Anacardiaceae, Rutaceae and Meliaceae.

10.6.2 ASSOCIATION BETWEEN SOME SEED CHARACTERS AND OVULE CHARACTERS, HABITUS AND HABITAT

As mentioned above, an association between recalcitrant seed viability (recalcitrance), large seeds, woody plants of the tropics, ovule and other seed characters was noted during the present study. For Table 3 the article on "the storage of recalcitrant seeds, achievements and possible approaches" by King & Roberts (1979) was taken as the starting point. This article contains a list of the species with recalcitrant seeds, arranged alphabetically under the family with common name, best storage conditions reported and comments. Only those families in which the recalcitrant behaviours was confirmed, have been taken into account.

Since only the Gramineae (Poaceae) was left, this single monocotyledonous family was also not considered. The data for the ovule and seed characters, habitus and habitat were mostly taken from Corner (1976) and Cronquist (1981). For the taxonomical arrangement the classification of angiosperms outlined by Dahlgren (1983) was followed. Other sources used, are indicated by small numbers which are explained at the end of the table.

"please turn over for Table 3"

Table 3. Distribution of recalcitrant seed species among dicotyledonous families (<u>sensu</u> Dahlgren, 1983) as well as its association with some ovule and seed characters, habitus and habitat.

Superorder Order Family	Ovule			Seed			Habitus	Habitat
	no of integ.	nuc. devel.	endosperm	seed-coat	reserves	size		
1. Magnoliiflorae Laurales Lauraceae	bi	cra	nuc	undifffer. and plexus of vasc. bund.	oil & st	1	wo	trop
2. Malviflorae Malvales Sterculiaceae	bi	cra	nuc	massive testa ex teg(-) post chal. vasc.bund.	oil & st	1	wo	trop
ditto Dipterocarpac.	bi	cra	nuc	undiffer. or and chalazal lobes ex teg(-)	st & oil 1.	1	wo	trop
ditto Malvaceae	bi	cra	nuc	ex tes ex teg ch & hyp	oil & st(+/-)	s→ mod	wo 2.	trop 2.
ditto Bombacaceae	bi	cra	nuc	ex teg hyp? massive ch multipl.	oil & st 3.	1	wo	trop
7. ditto Urticales Moraceae	bi	cra	nuc	undiffer. → ex tes(-) ch & hyp	oil or -	s/l	wo 2.	trop
ditto Euphorbiales Euphorbiaceae	bi	cra	nuc	sarcotes tegmic pachy (+/o)	oil	s/l	wo	trop
9. Theiflorae Theales Ochnaceae	bi	ten	nuc	undiffer. pachy	oil & prot	ov	wo	trop
ditto Theaceae	bi	ten	nuc	me tes/ ex tes/ sarcotes	oil 4.	1 4.	wo	trop

Superorder Order Family	Ovule			Seed			Habitus	Habitat
	no of integ.	nuc. devel.	endo-sperm	seed-coat	reserves	size		
ditto Clusiaceae	bi	cra/ ten	nuc	undiffer. tes connate -endocarp pulp	oil	l	wo	trop
10.Primuliflorae Ebenales Ebenaceae	bi &endoth	ten	nuc	ch +-enlarg. ex tes ?pachy	oil	s/l	wo	trop
11.Rosiflorae Fagales Fagaceae	bi	cra	nuc	undiffer. ?pachy	st/oil	l 2.	wo	temp
ditto Betulaceae	uni	cra	nuc	undiffer. ?pachy	oil	l	wo	temp
ditto Juglandales Juglandaceae	uni	cra	nuc	?undiffer. ?pachy	oil	l	wo	temp → subtrop
ditto Rosales Rosaceae	bi/ uni	cra	nuc	undiffer. multipli. pachy+ hyp?	st & oil &prot	l 2.	wo	temp → subtrop
13.Proteiflorae Proteales Proteaceae	bi	cra	nuc	me tes wo pachy & hyp	oil	ov	wo	trop
14.Myrtiflorae Myrtales Myrtaceae	bi/ uni	cra	nuc	ex tes/ me tes; p.pachy/ pachy 5.	st 5.	ov 5.	wo	trop
16.Rutiflorae Sapindales Anacardiaceae	bi/ uni 6.	cra	nuc	undiffer. pachy/ p.pachy endo teg 7.	st,oil, prot	l ov s	wo	trop

Superorder Order Family	Ovule			Seed			Habitus	Habitat
	no of integ.	nuc devel.	endo-sperm	seed-coat	reserves	size		
ditto Sapindaceae	bi	cra	nuc	sarcotes pachy	oil & st	l	wo	trop
ditto Hippocastanac.	bi	cra	nuc	hilum & ?pachy	st	l	wo	temp
ditto Rutales Rutaceae	bi	cra	nuc	ex tes pachy, ext ch & hyp	oil & st (-) 8.	l	wo	trop
ditto Meliaceae	bi	cra	nuc	ex teg perich & hyp	oil & st 9.	l	wo	trop
ditto Geraniales Erythroxylac.	bi & endoth	cra	nuc	endoth ex teg	st	l 10.	wo	trop
24.Gentianiflorae Gentianales Rubiaceae	uni	cra → ten	nuc	ex teg p.pachy?	oil & st	l	wo	trop

A. Additional literature used:

1. Tompsett, 1987; 2. Bailey & Bailey, 1978; 3. Soepadmo & Eow, 1977;

4. Vaughan, 1970; 5. Van Wyk & Botha, 1984; 6. Robbertse et al., 1986;

7. Von Teichman et al. (submitted); 8. Boesewinkel, 1978;

9. Boesewinkel, 1981; 10. Boesewinkel & Geenen, 1980.

B. Abbreviations:

bi = bitegmic; ch = normal chalaza; cra = crassinucellar; endo teg = endotegmic;

endoth = endothelium; ext ch = extensive chalaza; ex teg = exotegmic;

ex tes = exotestal; hyp = hypostase present; l = large; me tes = mesotestal;

mod = moderate; multipl. = multiplicative; no of integ. = number of integuments; nuc - Nuclear type; nuc.devel. - nucellus development; ov = overgrown; pachy = pachychalaza; perich = perichalaza; post chal. = post chalazal; p.pachy = partial pachychalaza; prot = protein; s = small; sarcotes = sarcotesta; st = starch; subtrop = subtropical; ten = tenuinucellar; tes = testa; temp = temperate; trop = tropical; undiffer. = undifferentiated; uni= unitegmic; vasc. bund = vascular bundle(s); wo = woody; + = present; - = absent; (+/o) = present or absent; +- = more or less; (-) = not well-developed; ? = possibly; → = to.

Except for the Lauraceae (superorder Magnoliiflorae), families with recalcitrant seeds (recalcitrance) do not occur among the more primitive superorders, i.e. Nymphaeiflorae, Ranunculiflorae, Caryophylliflorae, Polygoniflorae and Plumbaginiflorae. Except for the Rubiaceae (superorder Gentianiflorae), recalcitrance is absent in the highly evolved groups such as Asteriflorae, Solaniflorae, Corniflorae, Loasiflorae and Lamiiflorae. The two superorders Malviflorae and Rutiflorae are best endowed with recalcitrance since six families occur in each of them. Its distribution in a number of orders suggests that this seed character evolved simultaneously in different groups of angiosperms.

As mentioned before, the anatropous ovule is the most common type of ovule in the angiosperms (Davis, 1966). Therefore it is not surprising that 66,6% of the families concerned have anatropous ovules, 8,4% have orthotropous and the remaining families have anacampylotropous or campylotropous ovules. Considering the number of integuments, 75% of the tabled families have bitegmic ovules, 12,5% bi- or unitegmic ovules and 12,5% have unitegmic ovules. The nucellus development is crassinucellar in 79,2%, tenuinucellar in 12,5% and crassinucellar or tenuinucellar in 8,3% of the families. Regarding the endosperm development, only the Nuclear type is present in the tabled families and could therefore have been omitted from the table. The presence of recalcitrance in these families is therefore definitely associated with bitegmic and crassinucellar ovules with a nuclear endosperm development — this ovule type occurs in 58,3% of the families. If the Anacardiaceae and Myrtaceae, which are mostly bitegmic, are added to the above, the association is 66,7%.

It is difficult to find a meaningful association of recalcitrance with certain seed-coat characters because the data are still fragmentary in most families. However, in 70,8% of the tabled families a modified or possibly modified chalaza in one of its gradations is present. This could be another feature which represents a strikingly high degree of incidence in these families. As pointed out previously, the extended chalaza is often associated with an undifferentiated type of seed-coat. The nutrient reserves in the seeds, tabled under seed reserves, do not yield a significant association since oil and starch, and oil alone, occur in about the same proportion of families, i.e. 41,7% and 33,3% respectively.

King & Roberts (1979) already observed that recalcitrance is found mainly in large-seeded, woody species and tropical fruit crops. Therefore it is not surprising that a relatively large seed size, woody habitus and tropical habitat show the remarkable association of respectively 95,8%, 100% and 79,2% with recalcitrance. It needs to be mentioned that overgrown was taken as large, and if small and large seeds are present in a family, this was taken as "large seeds present." Likewise, if the habitat of most genera in the family is tropical this was indicated as "tropical" in the table.

A remarkable association therefore exists between seed recalcitrance in a family, the bitegmic, crassinucellar ovule and a nuclear endosperm development, a relatively large seed, a woody habitus and tropical habitat.

10.6.3 A HYPOTHESIS

The results of the following studies reflect character associations very similar to those mentioned above. Among the 12 characters that are more abundant in rainforest families of dicotyledons than in the flora of the world, Sporne (1973) mentions the following: woody habitus, secretory cells, unisexual flowers, bitegmic and crassinucellar ovule and a nuclear endosperm development. Practically the same associativon as mentioned in the discussion above, is found in seeds of Glycosmis cf. arborea (Roxb.) DC. of the Rutaceae. This study of Boesewinkel & Bouman (1978) is referred to in more detail in chapter 9, the discussion of the mango seed-coat. It is not known whether the large, pachychalazal seed of Trichilia grandifolia Oliv., a woody species of the predominantly tropical Meliaceae, is recalcitrant. However, it is known that the ovule of T. grandifolia is bitegmic, anatropous and crassinucellar with a massive chalaza (Boesewinkel, 1981).

Studies on Eugenia L. of the Myrtaceae (Van Wyk & Botha, 1984), Cryptocarya R.Br. and Dahlgrenodendron J.J.M. van der Merwe & van Wyk of the Lauraceae (Van Wyk, personal communication*, and Van der Merwe et al., 1988) support the association of overgrown pachychalazal seeds with recalcitrance. In species of Dipterocarpus Gaertn. Tompsett (1987) found an association of a faster desiccation rate in seeds, with smaller seed size, an orthodox seed viability and a drier habitat. Other species of the same genus from a moister habitat, showed a slower desiccation rate of the large recalcitrant seed.

The recalcitrant seed viability is doubtlessly to be regarded as more primitive than the orthodox seed viability since the following are absent in the recalcitrant seeds: a "switching off" of some enzyme systems during the transition of cells from a hydrated, metabolically active state to a desiccated state with very low metabolic activity; degeneration of cytoplasmic organelles prior to dormancy and the reversal of these intricate

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processes during imbibition.

On the basis of the previous discussion and the studies mentioned above which support the evidence presented, the following hypothesis is proposed: The remarkable association of seed recalcitrance with primitive ovule characters, the woody habitus and tropical habitat, suggests that larger seed size and the frequently associated pachychalazy cannot be considered to be derived seed characters. Furthermore it is postulated that the ancestral, woody Anacardiaceae of the tropical forests had large, recalcitrant seeds with a pachychalaza. The hypothesis supports the view of Engler (1892) that the Mangifereae constitutes the most primitive tribe. *Mangifera indica* of this tribe retained the ancestral characters enumerated above. In view of this hypothesis, theories concerned with aspects such as seed size in the context of the evolution of the angiosperms, should be reconsidered.

10.7 SPECULATIONS ON THE AGE OF THE ANACARDIACEAE

The information gathered on the fossil record, the number of monotypic genera and the distribution of closely related species on different continents, allow a speculation on the age of this family. The fossil record may be summarized chronologically as follows:

- wood fossils from the Paleocene were recorded in Patagonia (Cronquist, 1981);
- fossil fruits, showing affinity to the tribe Spondieae, from the middle Eocene have been found in Kentucky (USA) by Grote & Dilcher (1987);
- in the same epoch the family was well represented in England (Cronquist, 1981);
- during the Miocene the Anacardiaceae played an important role in Europe (Engler, 1892); and
- this more northerly distribution of the family was recently confirmed by Biondi (1981) who described fossil wood from the lower Miocene found in Sardinia.

The study of the extensive treatise on the Anacardiaceae by Engler (1892) reveals the presence of a strikingly large number of monotypic genera. With the aid of the available literature a survey of the number of monotypic genera was compiled. The following 11 genera are monotypic: Androtium, Drimycarpus, Faguetia March., Haematostaphis, Harpephyllum, Koordersiodendron, Laurophyllus, Loxostylis, Pachycormus Coville, Rhodosphaera and Smodingium. Seven other genera are possibly also monotypic. The information of Engler (1892) on these genera could either not be verified or authors disagreed whether one or two species should be assigned to the genus. A further eight genera described by Engler (1892) as monotypic, proved either to contain more than one species, or the genus had been transferred to another genus.

Owing to the many characters shared by certain genera, the following close relationships between genera with widely differing geographical distribution were mentioned during this discussion:

- the genus Lannea occurring mainly in tropical southern Africa is closely related to the genus Tapirira represented in tropical South America;
- the monotypic genus Smodingium which is endemic in South Africa shows a close alliance with Pseudosmodingium of the Mexican highlands;
- the genus Sorindeia of tropical western Africa is closely allied to the southern American genus Mauria; and
- the monotypic genus Rhodosphaera, endemic in Australia, is closely related to the genus Schinus, a native of South America.

Lastly it was mentioned in this discussion, that Heeria argentea is considered a relic of the practically extinct, moist, subtropical rain=forests which existed in southern Africa at the end of the Cretaceous. Therefore, not only the fossil record and the large number of monotypic genera, but also the close relationships of these genera and H. argentea, a rainforest relic, call for an origin of the Anacardiaceae well before

Gondwanaland broke apart at the end of the Jurassic, or the beginning of the Cretaceous. The Anacardiaceae is therefore regarded as a very ancient family.

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CHAPTER 11

CONCLUSIONS

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11.1 CONCLUSIONS OF PREDOMINANTLY TAXONOMIC SIGNIFICANCE

The study of the ontogeny and structure of the fruit and seed, especially the pericarp and seed-coat, of indigenous members of the Anacardiaceae as well as the study of the relevant literature led to the following conclusions:

- the exocarp of the drupe of the Anacardiaceae may be thin and papery as in _Rhus lancea_ or it may be thick and represent an exocarp _sensu lato_, as in _Sclerocarya birrea_ subsp. _caffra_;
- the parenchymatous mesocarp, which is mostly characterized by conspicuous resin ducts, usually differentiates after endocarp lignification as is typical of drupes in general;
- the endocarp is very often differentiated as a sclerocarp. The latter is either a stratified, homogenous sclerocarp as in the tribes Mangifereae and Rhoideae, or the sclerocarp is non-stratified and heterogenous as in the tribe Spondieae;
- the endocarpal opening mechanism, namely the operculum, is restricted to those members of the tribe Spondieae with a well-developed sclerocarp;
- the pachychalazal seed and associated with it an undifferentiated type of seed-coat characterize most members of the tribe Mangifereae;
- the raphe-chalazal seed of many genera of the Spondieae has a partial pachychalaza and also an undifferentiated seed-coat;
- the seed of genera of the Rhoideae are either characterized by a pachychalaza, or a small partial pachychalaza or a differentiated (endotegmic) seed-coat;
- in the pachychalazal and raphe-chalazal seed the seed-coat is of dual origin, being derived mainly from the extensive chalaza as well as from the one or two integuments;

- the close relationship of Harpephyllum, Lannea and Sclerocarya is emphasized by the mutual occurrence of derived fruit and seed characters, namely animal dispersal of the fruit, tendency towards one-seededness of the drupe, a well-developed sclerocarp, the exalbuminous nature of the seed and a simplified seed-coat structure.
- the presence of various shared characters in Lannea and Operculicarya calls for a reinvestigation of the generic status of the genus Operculicarya;
- the monotypic indigenous genus Harpephyllum and the widespread tropical genus Spondias might be congeneric and therefore their taxonomic delimitation needs reconsideration;
- in the Anacardiaceae the structure of the pericarp and seed-coat is specific and therefore of diagnostic significance at least on the generic level;
- studies of the ontogeny and structure of the pericarp and seed-coat may play a significant role in resolving taxonomic disputes, e.g. whether the two genera of the tribe Dobineeae are to be included in the family Anacardiaceae or not;
- the fruit of the indigenous Laurophyllus capensis, Loxostylis alata and Smodingium argutum are considered to be samaras;
- the genus Heeria, represented in southern Africa by H. argentea, differs markedly from the genus Ozoroa, e.g. O. paniculosa. In addition to the ten characters that were used by Fernandes & Fernandes (1965) to separate the genus Ozoroa from Heeria in the Cape, the following distinguishing fruit and seed characters were found during this study:

character	Heeria	Ozoroa
fruit dispersal	rock-rabbits & rodents	birds
seed type	pachychalazal	raphe-chalazal
seed-size	overgrown	not overgrown
embryo colour	chlorophyllous	non-chlorophyllous
germination type	hypogeal	epigeal

These differences substantiate the separation of the two genera.

11.2 CONCLUSIONS BASED ON CHARACTER ASSOCIATIONS

Various results revealed the following associations:

- in the Anacardiaceae, as in other families, animal dispersal of large fruit may be associated with hypogeal seed germination;
- the woody habitus and fruit dispersal by wind are often associated with epigeal seed germination;
- the seedlings of species occurring in savannah or similar habitat with long, dry seasons, may have thickened subterranean organs;
- the extensive chalaza in its different gradations is frequently associated with a hypostase;
- the bitegmic, crassinucellar ovule with nuclear endosperm development is commonly regarded as being primitive, and during this study it was found that this type of ovule is distinctly associated with the presence of a hypostase;
- on the other hand a unitegmic, tenuinucellar ovule with cellular endosperm development is generally regarded as an advanced type of ovule. A literature study revealed that this type of ovule is significantly associated with an integumentary tapetum (endothelium);
- both the hypostase and integumentary tapetum originated independently in many families during the evolution of the angiosperms;
- the distinction made between a hypostase <u>sensu</u> <u>stricto</u> and a hypostase <u>sensu</u> <u>lato</u> is justified, since only the latter is associated with an extensive chalaza and participates in the seed-coat development;
- it is accepted that a woody habitus and tropical habitat are primitive, and these can be associated with recalcitrant seed viability (recalcitrance). During this study it was found that these characters and type of habitat are significantly associated with the primitive ovule as well as with a pachychalazal seed and large seed size (overgrown);
- although previous authors have concluded that a pachychalazal/overgrown seed can be regarded as being derived, it is evidenced here that these

seed characters should rather be regarded as primitive;

- therefore, it is also postulated that the ancestral woody Anacardiaceae of the rainforests probably had pachychalazal overgrown, recalcitrant seeds;
- the modern, woody Anacardiaceae of the forests have a relatively advanced gynoecium, a derived fruit, i.e. a often one-seeded, animal-dispersed drupe, and a seed which is partly derived, considering its exalbuminous condition, and partly primitive when it is overgrown, pachychalazal and recalcitrant; and
- lastly it is postulated that the crucial survival adaptation of *Heeria argentea* (a rainforest relic) was the overgrown seed which germinates hypogeally to establish a strong and competitive seedling.

SUMMARY

A MORPHOLOGICAL STUDY OF THE FRUIT AND SEED OF CERTAIN SOUTHERN AFRICAN MEMBERS OF THE ANACARDIACEAE

by

IRMGARD VON TEICHMAN UND LOGISCHEN

Promoter: Prof. Dr. P.J. Robbertse
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The Anacardiaceae is a natural family which is grouped into five tribes. Two of these occur in southern Africa. The Spondieae is represented by Harpephyllum Bernh. ex Krauss, Lannea A. Rich. and Sclerocarya Hochst. and the Rhoideae by Heeria Meisn., Laurophyllous Thunb., Loxostylis Spreng. f. ex Reichb., Ozoroa Del., Protorhus Engl., Rhus L. and Smodingium E. Mey. ex Sond. Although this study focusses mainly on the three species of the Spondieae and Rhus lancea L.f., observations on other species, e.g. of Heeria, Mangifera L., Operculicarya H. Perr. and Tapirira Aubl., are also described.

The thesis includes reprints and manuscripts of scientific papers, dealing mainly with the ontogeny and structure of the fruit and seed. The taxonomic significance of characters is stressed, supplemented with notes on evolutionary trends and seed germination. A comprehensive review of the literature is provided, including comments on the structure and function of the hypostase and occurrence of the pachychalaza.

The exocarp of the drupe in Anacardiaceae may be thin (R.lancea) or thick, representing an exocarp sensu lato (Sclerocarya). The mesocarp is characterized by conspicuous secretory ducts and usually differentiates after endocarp lignification. The endocarp is usually a sclerocarp. It is either stratified (R. lancea) or non-stratified (Spondieae). Opercula are restricted to the members of the Spondieae with well-developed sclerocarp.

Most members of the Mangifereae are characterized by a pachychalazal seed and undifferentiated seed-coat. A raphe-chalazal seed with partial pachychalaza and undifferentiated seed-coat occurs generally in the Spondieae as well as in Rhus and Ozoroa. The seed of Heeria is pachychalazal whereas other genera of the Rhoideae have a differentiated, i.e. endotegmic, seed-coat. In pachychalazal and raphe-chalazal seeds the seed-coat develops mainly from the chalaza and the integument(s), and is thus of dual origin.

Shared derived fruit and seed characters emphasize the close relationship of Sclerocarya, Lannea and Harpephyllum. Pericarp and seed-coat structure also proved to be of diagnostic significance on the generic level. The presence of numerous shared characters between Lannea and Operculicarya calls for a reinvestigation of the generic status of the latter genus. Since the monotypic genus Harpephyllum and tropical genus Spondias L. might be congeneric, the taxonomic delimitation of these genera should be reconsidered.

Differences in fruit dispersal, seed type, embryo colour and germination type provide additional characters to distinguish between Ozoroa and Heeria. Heeria argentea (Thunb.) Meisn., a Cape endemic, is considered a relict of a tropical floristic element now largely extinct in this part of Gondwana. It is suggested that the crucial adaptations ensuring its survival under the present more arid mediterranean climate, are the overgrown seed and hypogeal germination.

For the first time, the marked association between the presence of a hypostase in the ovule and other presumably primitive ovule characters (bitegmy, a crassinucellar state and nuclear endosperm development) is pointed out. In addition seed recalcitrance is found to be significantly associated with the latter ovule characters as well as with a woody habitus, tropical habitat, greater seed size (overgrown seed) and a pachychalaza. On the basis of these associations it is postulated that the latter two seed characters are most probably primitive and not derived as was hitherto suggested.

OPSOMMING

'N MORFOLOGIESE STUDIE VAN DIE VRUGTE EN SADE VAN SEKERE SUIDER-AFRIKAANSE LEDE VAN DIE ANACARDIACEAE

deur

IRMGARD VON TEICHMAN UND LOGISCHEN

Promotor: Prof. dr. P. J. Robbertse

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Die Anacardiaceae is 'n natuurlike familie wat in vyf tribusse gegroepeer is. Twee hiervan kom in suidelike Afrika voor. Die Spondieae word verteenwoordig deur *Harpephyllum* Bernh. ex Krauss, *Lannea* A. Rich. en *Sclerocarya* Hochst. en die Roideae deur *Heeria* Meisn., *Laurophyllus* Thunb., *Loxostylis* Spreng. f. ex Reichb., *Ozoroa* Del., *Protorhus* Engl., *Rhus* L. en *Smodingium* E. Mey. ex Sond. Alhoewel hierdie studie hoofsaaklik op die drie spesies van die Spondieae en *Rhus lancea* L.f. konsentreer, word waarnemings op ander spesies, bv. van *Heeria*, *Mangifera* L., *Operculicarya* H. Perr. en *Tapirira* Aubl. ook beskryf.

Die proefskrif sluit herdrukke en manuskripte van wetenskaplike publikasies in wat hoofsaaklik oor die ontogenie en struktuur van die vrug en saad handel. Die taksonomiese betekenis van kenmerke word beklemtoon, aangevul met aantekeninge oor die ewolusionêre tendense en saadkieming. 'n Omvangryke oorsig van die literatuur word voorsien, wat kommentaar op die struktuur en funksie van die hipostase en voorkoms van die pagichalasa insluit.

Die eksokarp van die steenvrug in die Anacardiaceae mag dun wees (*R. lancea*), of dik, in welke geval 'n eksokarp *sensu lato* verteenwoordig word (*Sclerocarya*). Die mesokarp word gekenmerk deur opvallende sekreetkanale en differensieer gewoonlik na endokarplignifisering. Die endokarp is gewoonlik 'n sklerokarp. Dit is óf gestratifiseer (*R. lancea*) óf nie gestratifiseer nie (Spondieae). Operkulums is beperk tot die lede van die Spondieae met goed ontwikkelde sklerokarp. Die meeste lede van die Mangifereae word gekenmerk deur 'n pagichalasale saad en ongedifferensieerde saadhuid. 'n Rafe-chalasale saad met gedeeltelike pagichalasa en ongedifferensieerde saadhuid kom algemeen by die Spondieae voor, asook by *Rhus* en *Ozoroa*. Die saad van *Heeria* is pagichalasaal, terwyl ander lede van die Rhoideae egter 'n gedifferensieerde, d.w.s. endotegmiese saadhuid het. By pagichalasale en rafe-chalasale sade ontwikkel die

saadhuid uit die chalasa en die integument(e) en is dus van tweeledige oorsprong.

Gemeenskaplike afgeleide vrug- en saadkenmerke beklemtoon die nou verwantskap van *Harpephyllum*, *Lannea* en *Sclerocarya*. Perikarp- en saadhuidstruktuur blyk ook van diagnostiese betekenis op die generiese vlak te wees. Die aanwesigheid van talryke gemeenskaplike kenmerke by *Lannea* en *Operculicarya* noodsaak 'n herondersoek van die generiese status van die laasgenoemde genus. Aangesien die monotipiese genus *Harpephyllum* en die tropiese genus *Spondias* L. kongeneries mag wees, behoort die taksonomiese afbakening van hierdie genera weer oorweeg te word.

Verskille in vrugverspreiding, saadtipe, embriokleur en saadkieming voorsien bykomstige kenmerke om tussen *Heeria* en *Ozoroa* te onderskei. *Heeria argentea* (Thunb.) Meisn., 'n Kaapse endemiese spesie, word beskou as 'n reliek van die tropiese floristiese element wat nou in hierdie deel van Gondwana grootliks uitgesterf het. Daar word voorgestel dat die oorgroeide saad en hipogeale kieming die beslissende aanpassing is wat sy oorlewing onder die huidige, droër mediterreense klimaat verseker het.

Vir die eerste keer word die aandag gevestig op die opvallende assosiasie tussen die aanwesigheid van 'n hipostase in die saadknop en ander vermoedelik primitiewe saadknopkenmerke (bitegmie, 'n krassinusellêre toestand en nukleêre endospermontwikkeling). Daarbenewens is gevind dat saad met 'n onortodokse lewenskragtigheid betekenisvol geassosieer is met laasgenoemde saadknopkenmerke, asook met 'n houtagtige groeiwyse, tropiese habitat, groter saad (oorgroeide saad) en 'n pagichalasa. Aan die hand van hierdie assosiasies word gepostuleer dat laasgenoemde twee saadkenmerke heelwaarskynlik primitief is en nie afgelei nie soos tot dusver beweer is.

ACKNOWLEDGEMENTS

It gives me great pleasure to express my sincerest gratitude to the many persons and institutions whose assistance, advice and encouragement made this thesis possible, namely:

Prof. P.J. Robbertse for suggesting this fascinating project, his confidence, advice, scholarly criticism and also administration in connection with this thesis.

Prof. A.E. van Wyk for sharing some of his knowledge, his constant encouragement and criticism.

Prof. A. Eicker, head of the Department of Botany, for his confidence bestowed on me and his interest.

Prof. H.A. van de Venter, Director of the Margaretha Mes Institute for Seed Research, for granting me study leave and the last minute secretarial assistance.

Prof. H.P. van der Schijff for encouraging my visits to foreign laboratories and the opportunity to introduce new techniques in our laboratory.

Prof. Margaret E. McCully, Carleton University, Ottawa, for teaching me the "GMA-techniques" and her kindness.

My dear parents for enabling me to enrol for the B.Sc. course, their never-failing encouragement and caring love. My little mother who is now on her own, for her intense interest in my research, the writing of this thesis and substantial financial help.

The following persons are sincerely thanked for their assistance with the collection of, or forwarding of plant material: Mr. A.T.D. Abbott, Dr. A. Barford (Denmark), Mrs. K.P. Clarke, Mrs. Emsie du Plessis, Mr. C.J. Geldenhuys, Prof. N. Grobbelaar, Mr. Dave Hardy, Mr. Willem Hattingh, Mr. Geoff Hemm, Mrs. Marissa von Wolff and Dr. Piet Vorster.

My friends Dr. Hester Kruger, Emsie du Plessis and Dr. Elzabè Schoonraad, for their inestimable help with the critical reading and improvement of the text.

Mrs. Kathy Harpur, Miss Thea Janse van Rensburg and Miss Ingrid Vis for the accurate typing of handwritten parts of the thesis and many manuscripts.

The staff of the Department of Botany and Electron Microscopy Unit, University of Pretoria for the interest and especially Dr. Isabella Claassen, Prof. J. Coetzee and Mr. Chris van der Merwe for their help.

Miss Helène Visser of Audiovisual Services, University of Pretoria, is thanked for the excellent photos of plants, fruits and seed.

The staff of the National Herbarium and Mary Gunn Library, particularly Miss E. Retief, Mrs. E. Potgieter and Mrs. B.F. Lategan for their valuable assistance.

The staff of the Merensky Library, University of Pretoria, particularly Mrs. Adri de Kock, Mr. Pieter de Kock, Miss Elna Stapelberg, Miss Martie van der Walt and friendly staff in the Inter-Library Loan Section for their kind help.

Prof. P.J. Robbertse, Dr. E. Schoonraad, Prof. J.G.C. Small and Prof. A. E. van Wyk for the incorporation of our joint papers or manuscripts.

The University of Pretoria, particularly also the Department of Botany and Margaretha Mes Institute, as well as the CSIR Foundation for Research Development for the financial assistance during this research.

Last but not least my brothers and sisters, other relatives as well as friends for their encouragement and interest. Very specially Mrs. S.C. Scheibert, Prof. Guillaume Theron and all my Yoga-friends.

CURRICULUM VITAE

Irmgard von Teichman und Logischen was born on 14 February 1940 in Grootfontein, South West Africa. She attended the Höhere Privatschule in Windhoek and matriculated from the Swakopmund High School.

1959 : Secretarial course, Technical College, Pretoria.

1960 - 1962 : Technical assistant, Veterinary Tuberculosis Research Unit, Onderstepoort. It was here that the love for research was realized and first experience gained in teaching new assistants.

1965 : B. Sc., (*cum laude*) University of Pretoria (henceforth referred to as U.P.) with Botany and Entomology as majors.

1966 : Margaretha Mes Memorial Prize for the best female Botany student at U.P. Technical assistant and later junior lecturer, Botany Department, U.P.

1967 : Studied under Prof. H. Merxmüller at the Institut für Systematische Botanik, Maximilian Universität, München, with the aid of a bursary from the Deutscher Akademischer Austauschdienst.

1968 : B.Sc. (Honns.) Botany (*cum laude*), U.P. Studied with the aid of a merit bursary from the C.S.I.R.

1969 : (January - June) junior lecturer, Botany, U.P.; (August 1969 - October 1970) technical assistant, Biology Department, Carleton University, Ottawa. Studied modern methods concerning the preparation of plant material for light and electron microscopy under Prof. Dr. Margaret E. McCully.

1971 : Junior lecturer, Botany, U.P.

1973 : M.Sc. (*cum laude*), U.P. (aided by a merit bursary from the C.S.I.R.) and later worked under Dr. T.P. O'Brien (Monash University) and Dr. Anne E. Ashford (Australian National University) both in Australia.

1974 : Technical assistant, Botany, U.P.

1975 - 1986 : Research Officer, Botany, U.P.

1981 : During sabbatical leave, research under Dr. J.D. Ross at the Department of Botany, University of Reading, England. Attended course in light microscopy at the Universität Tübingen and visited the Institut für Pflanzenbau und Pflanzenzüchtung, Technische Universität, München.
1987 : Senior Research Officer, Department of Botany, Margaretha Mes Institute for Seed Research, U.P.

A number of papers have been presented at local congresses and Prof. Dr. H.P. van der Schijff gave a joint paper on the Dioscorea research (M.Sc. project) in Genève (1974). Seminar lectures were given in the Botany Departments Würzburg (1967) and Reading (1981). Two scientific books have been translated from English to German. She is the author of nine popular scientific papers and author or co-author of 18 scientific papers.

In January 1988 she was elected a Fellow of the Linnean Society of London (F.L.S.).

APPENDIX

A re-evaluation of the structure of the mango ovule in comparison with those of a few other Anacardiaceae species

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The mango ovule has been described by some authors as unitegmic and by others as bitegmic, but in the latter case with integuments fusing quite early. The results of this investigation suggest that it is a pachychalazal ovule in that the greater part of the ovular envelope consists of chalazal tissue and that only the micropyle is formed by one massive integument. The pachychalazal ovule could possibly be a characteristic of the tribe Anacardieae. Bitegmic ovules in which the outer integument is continuous with two labellate outgrowths of the massive funicle ('raphe-chalazal ovules') are apparently characteristic of the tribes Spondieae and Rhoideae. All previously investigated species as well as *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro and *Lannea discolor* (Sond.) Engl. were found to be chalazogamous.

S. Afr. J. Bot. 1986, 52: 17 – 24

Die mango-saadknop is deur sommige outeurs as unitegmies en deur ander as bitegmies, maar dan met integumente wat vroeg versmelt, beskryf. Volgens die resultate van hierdie ondersoek is dit 'n pagichalasale saadknop deurdat die grootste gedeelte van die saadknop-omhulsel uit chalasale weefsel bestaan en dat slegs die mikropilum deur die een massiewe integument gevorm word. Die pagichalasale saadknop mag moontlik 'n kenmerk van die tribus Anacardieae wees. Bitegmiese saadknoppe waarvan die buitenste integument aaneenlopend is met twee lipvormige uitgroeisels van die massiewe funikulus ('rafe-chalasale saadknoppe') is blykbaar kenmerkend vir die tribusse Spondieae en Rhoideae. Chalasogamie kom by alle voorheen ondersoekte spesies asook *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro en *Lannea discolor* (Sond.) Engl. voor.

S.-Afr. Tydskr. Plantk. 1986, 52: 17 – 24

Keywords: Anacardiaceae, mango, ovule, pachychalazal

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Accepted 26 July 1985

Introduction

While working on a project on the pollination and fruit set of the mango (*Mangifera indica* L.) a lack of properly illustrated descriptions of the ovule as well as discrepancies in the literature on ovule morphology was noted. The interest * in the morphology and development of the seed and fruit of the South African Anacardiaceae (Von Teichman & Robbertse, in press) led to a comprehensive study of the mango ovule and a specific comparison of it with those of some other South African Anacardiaceae species. Furthermore all relevant literature available was taken into account. These investigations indicated that little effort has been made to compare the mango ovule with those of other Anacardiaceae species. Ovule characteristics have as yet not been used to delimit taxa in the Anacardiaceae. For example Scholz (1964) mentioned pendulous ovules for the Spondieae but ignored the diagnostic value of ovule characteristics in the other tribes.

Juliano & Cuevas (1932) described the mango ovule as being anatropous and pendulous with two integuments which fuse quite early. Singh (1961) stated that 'The gynoecium contains a single unitegmic, crassinucellate and anatropous basal ovule . . . '. The present findings do not quite agree with this rather simplistic description of the mango ovule and suggest a new interpretation of its various components.

Joel & Eisenstein (1980) described a ponticulus in the mango ovary, acting as a bridge for the pollen tube to enter the funicle on its way to the embryo sac. The presence of an obturator in the Anacardiaceae ovule was mentioned by Copeland & Doyel (1940) in *Toxicodendron*, Copeland (1955) and Grundwag (1976) in *Pistacia* and Kelkar (1958a) in *Lannea.* In view of the definition of an obturator (Schnarf 1929; Esau 1977), the reported existence of an obturator in the Anacardiaceae needs to be reconsidered.

Materials and Methods

Mango flowers and very young fruits were collected at the experimental farms of the Citrus and Subtropical Research Institute at Nelspruit and the University of Pretoria in Pretoria. Two mono-embryonic cultivars 'Haden' and 'Sensation' and two polyembryonic cultivars 'Peach' and 'Sabre' were used. Material of *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (the marula) and *Lannea discolor* (Sond.) Engl. was collected from different localities north of the Magaliesberg in Pretoria. Specimens of *Harpephyllum caffrum* Bernh. were collected from a tree * grown in a private garden in Pretoria and *Rhus lancea* L.f. from trees grown on the campus of the University of Pretoria.

The material prepared for the semi-thin sections was fixed

*see Corrections in Appendix

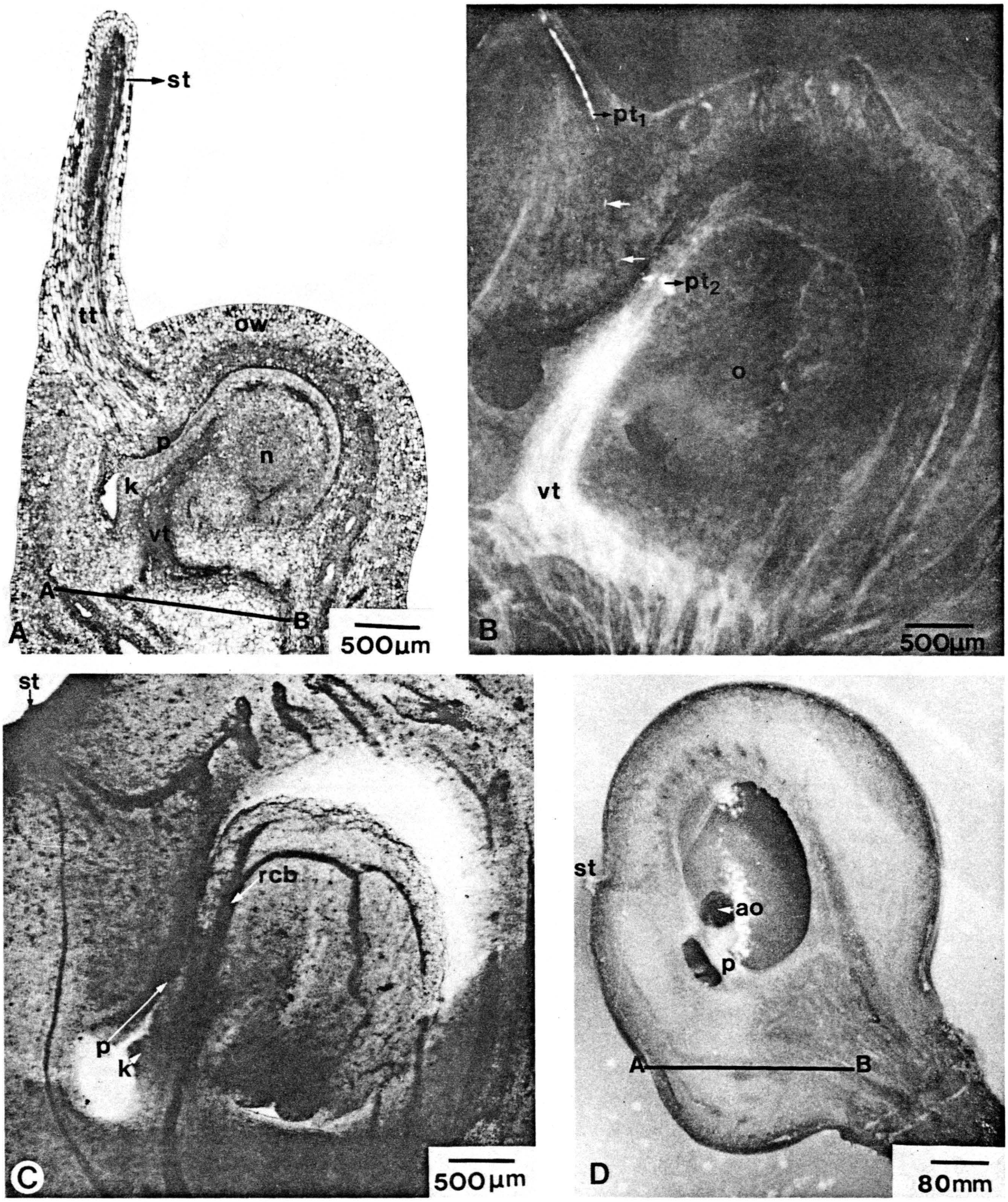

Figure 1 Sections of different developmental stages of mango ovaries. A. Longitudinal section of a 'Haden' ovary with ovule in the tetrad stage. B. Micrograph of LS of a 'Peach' ovary taken with epifluorescence optics; white arrows trace invisible part of pollen tube. C. Cleared section of 'Sabre' ovary. D. Cut surface of halved 'Haden' fruitlet. A – B — line of adnation between fertile and aborted carpels, ao — aborted ovule, k — knee-like protuberance of funicle, n — nucellus, o — ovule, ow — ovary wall, p — ponticulus, pt$_1$ — polen tube in style, pt$_2$ — pollen * tube crossing raphe-chalazal vascular tissue, rcb — raphe-chalazal bundle, st — style, tt — transmitting tissue, vt — vascular tissue.

in 6% glutaraldehyde in 0,05 mol dm^{-3} sodium cacodylate buffer. Thereafter it was dehydrated and embedded in glycol methacrylate (GMA) according to Feder & O'Brien (1968). The monomer mixture, however, consisted of 94% (v/v) hydroxyethyl methacrylate (that is, the 94% HEMA was purified in our laboratory to remove most of the methacrylic acid); 5% (v/v) polyethylene glycol 200, and 0,1% (w/v) azobis-iso-butyronitrile or later 0,6% (w/v) benzoyl peroxide. A Reichert OM U3 ultramicrotome and glass knives were used to cut the 2 – 3 µm thick GMA sections. The Periodic acid – Schiff Reaction (PAS) was carried out according to Feder & O'Brien (1968), using 0,5% dinitrophenyl hydrazine

(DNPH) in 15% acetic acid (for 30 min) as blocking agent. Toluidine blue O in benzoate buffer, pH 4,4 (Sidman *et al.* 1961), was used for counterstaining. Ovules dissected from fresh or FAA-preserved material were mounted directly in a clearing fluid (Herr 1971) and drawings were made with the aid of a drawing tube fitted to a Leitz microscope. For pollen tube studies either fresh or FAA-preserved material was cleared in a 0,8 mol dm^{-3} NaOH solution for 12 hours at 60°C after which it was stained in a 0,1% solution of water soluble aniline blue in a 0,3 mol dm^{-3} K_3PO_4 buffer, mounted in the same solution and photographed with a Reichert Univar microscope fitted with epifluorescence optics. Other micrographs were taken with the same microscope using standard illumination.

Results and Discussion

Number of carpels and position of the ovule

According to Scholz (1964) the tribe Anacardieae to which the mango belongs, can be five- or uni-carpellate. Although the mango is usually uni-carpellate, up to five apparently free carpels can occasionally occur in the cultivar 'Sensation' while rudimentary or aborted carpels are common around the base of the ovary in the cultivar 'Haden'. *Harpephyllum caffrum* has five adnate carpels, one of which is fertile. The marula has one to four fertile carpels, whilst *Lannea discolor* has three, only one of which is fertile. These three genera all belong to the tribe Spondieae. The gynoecium of *Rhus lancea* (tribe Rhoideae) consists of three carpels, one of which is fertile. All taxa in the Anacardiaceae have only one ovule per carpel. In marula (tribe Spondieae) the ovule is pendulous (Von Teichman & Robbertse, in press), but in the mango the ovules are reported to be basal (Mukherji 1949; Singh 1961) or becoming pendulous (Juliano & Cuevas 1932), while Sharma (1954) stated that the 'ovule is borne on a long ascending funicle attached sub-basally'.

The mango ovule is indeed basal (Figure 1). A vascular strand supplying the ovule, runs perpendicular to the axis of the flower. This vascular bundle is homologous with axial bundles in the fruit of marula (Figure 2A) along line C – D. If this vascular bundle in the mango ovule (Figure 1A) is 'lifted' into a vertical or axial position by elongation of cells along line A – B, the mango ovule would also be pendulous instead of ascending as shown in Figure 2B. Figures 2A, 2B and 1D clearly indicate that line C – D in the ovary of marula which is the line of adnation between the carpels, also exists in the mango ovary (A – B) between the fertile carpel and the undeveloped carpel primordium. With this in mind it could be argued that the so-called apocarpous carpels in the tribe Anacardieae (Scholz 1964; Heywood 1978) are not truly apocarpous, but that the carpels are adnate at the base. Owing to the abortion of carpels in the mango, growth along the line of adnation is retarded or stopped causing a lop-sided development of the ovary with a basal ovule. In contrast growth along the line of adnation is not retarded in *Harpephyllum caffrum, Lannea discolor* and marula (Figure 2A) with the result that pendulous ovules are formed. In *Rhus lancea* the ovule is basal.

Integuments

Integument initiation in the mango starts when the megaspore mother cell is fully developed (Figure 2C). Two integuments are initiated but at the tetrad stage only the outer integument remains (Figure 3C). Both integuments are initiated in the dermal layer. The initials of the inner integument are pushed aside by 'integumentary shifting' as described by Bouman & Calis (1977) in the ovule of *Eranthis*. The outer integument develops rapidly by means of periclinal and anticlinal cell divisions thus forming wedge-shaped files of cells. The epidermal and subepidermal nucellus cells also divide periclinally so that the micropylar extension of the nucellus keeps pace with the development of the integument for some time (Figure 3D). As a result of the nucellar extension the functional chalazal megaspore is deeply seated in the nucellus (Figure 3C).

Development of the integument does not continue beyond the stage shown in Figures 3C & D. It is succeeded by periclinal and anticlinal cell divisions in the chalaza to form a 'chalazal cup' which accommodates the greater part of the nucellus (Figure 3B). The mature ovule can therefore be described as pachychalazal in accordance with Corner's (1976) pachychalazal seed. The chalazal cup is highly vasculated while no vascular tissue was observed in the integument. Referring to Copeland (1955) and Grundwag & Fahn (1969), Corner (1976) cited a pachychalaza in *Pistacia*. Corner must have misinterpreted these papers since neither of these authors shows a true pachychalaza in their drawings of the ovules. Figure 3A of Grundwag & Fahn (1969) clearly shows that the nucellus is free from the integuments up to its proximal end. In the pachychalazal mango ovule, however, the free part of the integument is limited to the micropylar zone (Figure 3B). Although Copeland (1961) made no mention of it, a pachychalazal ovule seems to occur in the cashew nut, *Anacardium occidentale*. The cashew nut like the mango, belongs to the tribe Anacardieae. We have not seen material or literature dealing with ovules of other Anacardieae taxa but at least the ovules of the mango and cashew from this tribe can easily be distinguished from ovules of the other tribes on account of the pachychalaza.

The ovule of the marula differs from the mango ovule by already having two well differentiated integuments in the megaspore mother cell stage (Figure 4G). At a later stage the nucellus enveloped by the inner integument is situated at the end of a massive pendulous funicle. The outer integument is continuous with two plate-like outgrowths of the funicle (Figure 4H). This type of ovule, as was also stated by Grundwag (1976), seems to be typical of the majority of Anacardiaceae taxa. In the ovules of *Pistacia* species, *Lannea coromandelica* and *Rhus mysurensis* these unusual outer integuments have been misinterpreted as an obturator (Copeland 1955; Grundwag 1976; Kelkar 1958a; 1958b). During the present study this ovule type was observed in marula, *Harpephyllum caffrum, Lannea discolor* (Figure 4) and *Rhus lancea*. Esau (1977) defines an obturator as 'an outgrowth of the placenta or of the lining of stylar canal that brings the pollen transmitting tissue close to the micropyle'. The extended outer integument in the investigated species does not function as an obturator and cannot be interpreted as such. It is quite clear that the 'obturator' observed in *Pistacia* species by Copeland (1955) and Grundwag & Fahn (1969) is indeed part of the extended outer integument. In his observations on *Lannea coromandelica* and *Rhus mysurensis* Kelkar (1958a; 1958b) mentioned the flap-like structures of the outer integument.

Comparing Figure 2A & B and considering the reduction in integument differentiation in the mango ovule, it is quite possible that the mango ovule is a neotenic form of the marula ovule. In both the mango and marula ovules the proximal base of the outer integument is on the ventral side of the ovule intersecting line E – E in Figure 2A & B. The distal base of the outer integument intersects line F – F. Therefore in both

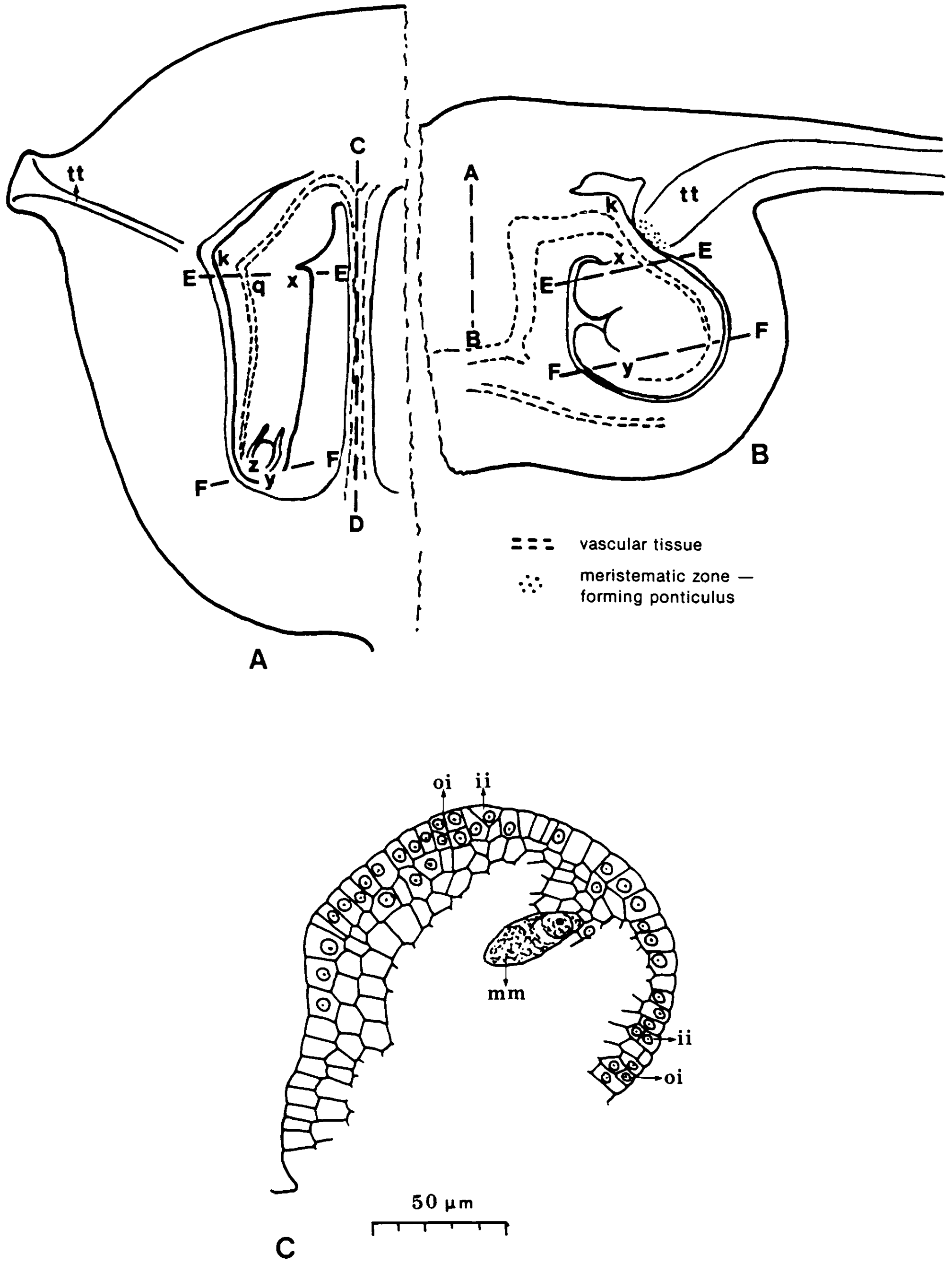

Figure 2 Drawings explaining ovule phylogeny and ontogeny. A. Longitudinal section of the ovary with an ovule of the marula. B. Drawing of Figure 1A turned 90° to match marula ovary. C. Mango ovule in megaspore mother cell stage. Lines A – B and C – D indicate the adnation zones of carpels; lines E – E — distal part of true funicle and proximal end of outer integument and raphe-chalaza; line F – F indicates distal end of raphe-chalaza; q, x, y & z indicate points as explained in text; ii — inner integument, k — knee-like protuberance of funicle, mm — megaspore mother cell, oi — outer integument, tt — transmitting tissue.

ovules the original chalaza as far as the base of the outer integument is concerned, stretches from point x on line E – E up to point y on line F – F. This implies an elongation between lines E – E and F – F in the marula ovule to form a partial perichalaza. The chalaza in the marula ovule now consisting only of a portion of the original chalaza, stretches between points y and z (Figure 2A). The vascular bundle between lines E – E and F – F in the latter ovule is therefore partly raphe and partly chalaza. This point is accentuated by the loop in the vascular strand at point q (Figure 2A) which can be regarded as the primary position of the nucellus and the chalaza. The vascular strand and adjacent tissue along line x – y must therefore be interpreted as secondary and of raphe-chalazal origin.

The present interpretation of the marula ovule also applies to the ovules of *Harpephyllum caffrum, Lannea discolor* (Figure 4) and *Rhus lancea*. It does not appear to conform with any type of ovule described in the literature and deserves a new name such as 'raphe-chalazal ovule', analogous to Corner's (1976) perichalazal and pachychalazal seeds. Therefore the basal ovules of the mango and probably also the cashew are unitegmic and pachychalazal. On the contrary the pendulous ovules of the presently investigated Anacardiaceae species are bitegmic and raphe-chalazal.

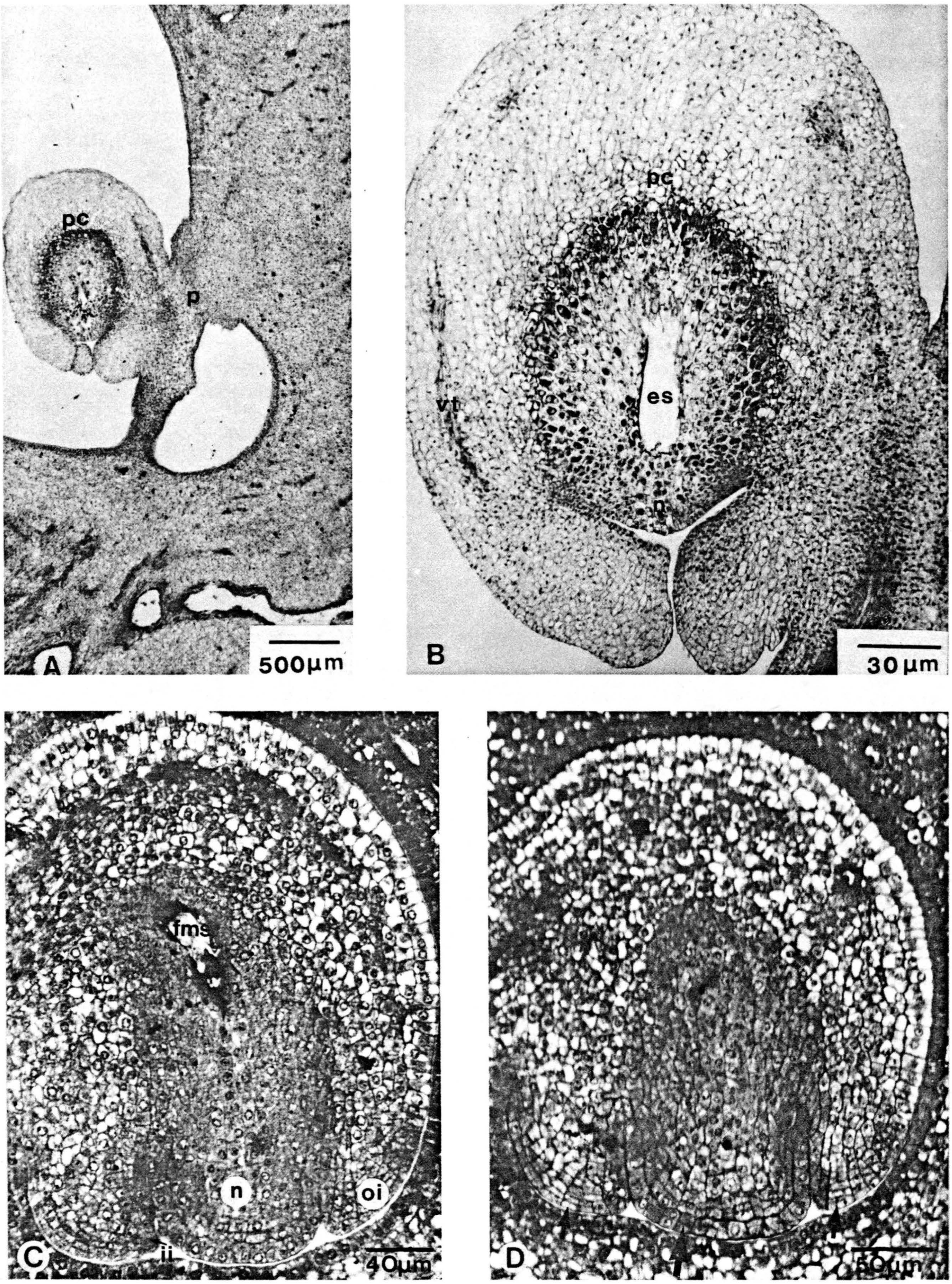

Figure 3 Mango ovules at different developmental stages. A. Section of very young fruit showing the orientation of young seed. B. Longitudinal section of ovule a few days after anthesis. C. Longitudinal section of ovule in the tetrad stage with degenerating megaspores partly cut away. D. Para-longitudinal section of same ovule as in C showing periclinal cell divisions in integument and nucellus indicated by arrow points. es — embryo sac, fms — functioning megaspore, ii — rudiment of inner integument, n — nucellus, oi — outer integument, p — ponticulus, pc — pachychalaza.

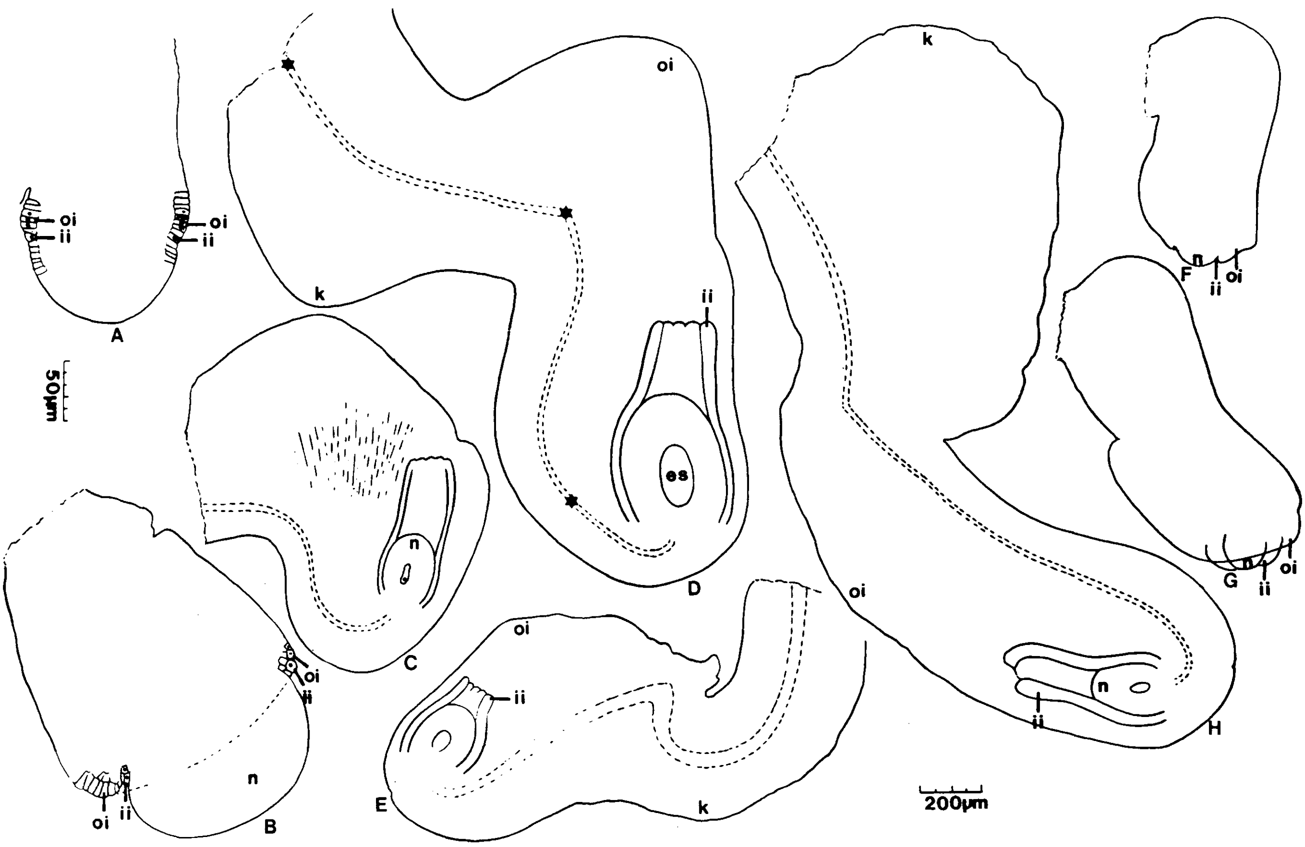

Figure 4 Anacardiaceae ovules in different developmental stages — drawings made from ovules cleared in Herr's fluid. A – D. *Lannea discolor* showing integument initiation in young flower bud stage (A&B), open flower stage (C) and few days after anthesis (D); broken lines indicate vascular tissue; stars indicate positions of micrographs A and B in Figure 5 taken from opposite side of a similar ovule. E. *Harpephyllum caffrum*. F – H. The marula in young bud stage (F), one day prior to anthesis (G) and a few days after anthesis (H). es — embryo sac, ii — inner integument, k — knee-like outgrowth of funicle, n — nucellus, oi — outer integument.

The ponticulus and related structures

Another common Anacardiaceae characteristic featuring in the mango and marula ovules is the knee-like outgrowth marked k in the two ovules (Figures 2A & B; 4D, E & H). The same structure was termed 'Höcker' by Grimm (1912) in ovules of *Rhus* and *Coriaria*. This knee or 'Höcker' usually points towards the base of the style and considering the fact that chalazogamy is a common feature in the Anacardiaceae (Grimm 1912; Copeland & Doyel 1940; Copeland 1955; Copeland 1961; Grundwag 1976; Joel & Eisenstein 1980), it was thought that this structure could function as a bridge for the pollen tube on its way to the embryo sac. The path of the pollen tube through the 'Höcker' was indeed described in *Rhus* and *Coriaria* by Grimm (1912) and also through the 'knob' in *Pistacia chinensis* by Copeland (1955). In the mango, however, the pollen tube does not enter the funicle through the knee (Figure 1B). As was also described by Joel & Eisenstein (1980), the pollen tube grows through an outgrowth of the ovary wall which fuses with the funicle to form a bridge or 'ponticulus'. In a depression of the funicle a bridge is therefore formed between the knee and the ovule (Figures 1 & 2B). After fertilization the ponticulus and funicle elongate as shown in Figure 3A. A similar bulge of tissue through which the pollen tube enters the raphe in *Anacardium occidentale* was described by Copeland (1961).

The path of the pollen tube in the marula and *Lannea discolor* was also investigated. In both species the pollen tube enters the funicle at the placenta and although a prominent knee occurs in both taxa (Figure 4) it probably has no particular function. In the species investigated the pollen tube then follows the vascular strand of the funicle through the chalaza to the nucellus. It then grows around the embryo sac and enters at the micropylar pole (Figure 5). These observations show that chalazogamy occurs in the marula, *L. discolor* and previously investigated species.

Taxonomy

According to Scholz (1964) the tribe Anacardieae is characterized by simple leaves and either apocarpous or unicarpellate gynoecia. Heywood (1978), however, recognized two groups in the Anacardieae, namely species with compound leaves and apocarpous gynoecia and species with simple leaves and unicarpellate gynoecia. No taxa with compound leaves were included in this study. In the light of the marked differences between the mango and cashew ovules on the one hand and those of the other taxa on the other, a re-evaluation of the delimitation of the tribes including ovule characteristics as one of the criteria, is suggested.

Concluding remarks

1. In the light of the morphology of the mango ovary and the adnation of the carpels in *Harpephyllum caffrum* it cannot be agreed that true apocarpous gynoecia occur in the Anacardiaceae. Therefore a new delimitation of the tribes based on various characteristics especially also those of the ovule, should be considered.
2. The mango ovule is pachychalazal with only one developed integument and may represent a neotenic form of other Anacardiaceae ovules.

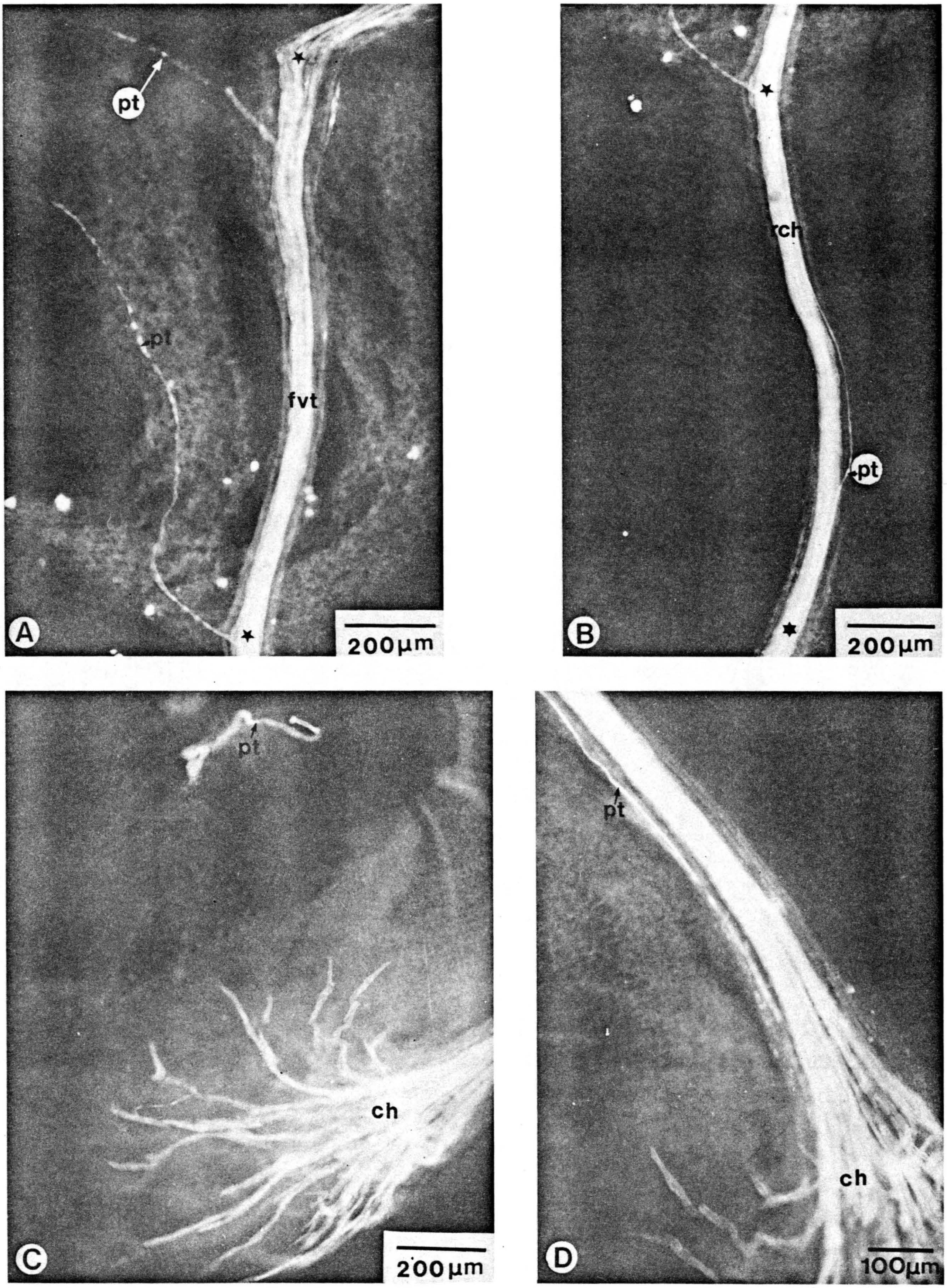

Figure 5 Epifluorescence micrographs showing the path of the pollen tube in the ovule of *Lannea discolor*. The position of the top and bottom stars in A corresponds to positions of proximal and middle stars in Figure 4(D) and those in B with middle and distal stars in Figure 4(D), but the mirror-image thereof. D shows the pollen tube in the chalazal area while C also shows pollen tube fragments in the micropylar end of the nucellus. ch — chalazal vascular tissue, fvt — funicular vascular tissue, rch — raphe-chalazal vascular tissue, pt — pollen tube.

3. Chalazogamy occurs in the mango, the marula and *Lannea discolor*, as well as several other species previously investigated.
4. A conspicuous knee-like outgrowth occurs in the ovules of all the species investigated. It probably has no particular function as in the mango, the marula and *Lannea discolor* the pollen tube does not enter the funicle through this knee.
5. The plate-like outgrowths of the 'funicle' in most Anacardiaceae ovules are extended lobes of the outer integument and cannot be regarded as an obturator. The area surrounding the vascular tissue extending from the nucellus to the proximal end of the outer integument is the raphe-chalaza.
6. The ovules of *Harpephyllum caffrum, Lannea discolor, Rhus lancea* and *Sclerocarya birrea* are interpreted as a previously undescribed type for which the term 'raphe-chalazal ovule' is suggested.

Acknowledgements

The authors wish to thank Dr E. Steyn for discussions and comments on the manuscript. Financial support from the F.R.D. of the Council for Scientific and Industrial Research, the University of Pretoria and the S.A. Mango Growers' Association is gratefully acknowledged.

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NOTES ON THE DISTRIBUTION, MORPHOLOGY, IMPORTANCE AND USES OF THE INDIGENOUS ANACARDIACEAE:

1. THE DISTRIBUTION AND MORPHOLOGY OF *SCLEROCARYA BIRREA* (THE MARULA)

By Irmgard von Teichman,
Margaretha Mes Institute for Seed Research, University of Pretoria.

Introduction

During germination experiments on marula a literature study was also undertaken. Literature on the marula is very extensive. This note therefore only allows a superficial survey, the aim being to emphasize again the beauty and importance of our indigenous plants. Personal observations of the author are included. The various uses of the marula are discussed in a later article.

Scientific and common name(s)

The correct name is now *Sclerocarya birrea* (A. Rich.) Hochst. subsp. *caffra* (Sond.) Kokwaro (Kokwaro and Gillett, 1980). *Sclerocarya* is derived from the Greek words sklēros, meaning hard and karyon, nut or nucleus (Holmes, 1979).

The Afrikaans and English common names were derived from tribal names.

Afrikaans:	maroela, olifantsappel (Shone, 1979).
English:	marula, maroola, meroola, elephant's apple (Shone, 1979); cider tree (Fox and Young, 1982).
Herero:	omuongo (Giess, 1965/1966).
Nama:	goaros
Ndebele:	iganu, ikanyi, umganu, umkano (Fox and Young, 1982).
Northern Sotho:	morula (Palmer and Pitman, 1972).
Central Sotho i.e. Pedi:	lehlabula la marula (fresh fruit), dikôkô (fresh seed), (Quin, 1959); morula, merula (plant), lerula, marula (fruit) (Fox and Young, 1982).
Swazi:	umganu (Codd, 1951).
Thonga:	nkanye (a tree), minkanye (trees), kanye (a fruit), makanye (fruits); mongo (seed) (Junod, 1912 & 1913).
Venda:	mufula (Palmer and Pitman, 1972); mukumbi (beer), thebvu (stone), thambo (seed) (Shone, 1979).
Zulu:	umganu (Codd, 1951) amaganu (fruits), umganu (seeds) (Fox and Young, 1982).

Shone (1979) and Fox and Young (1982) give more vernacular names used by more northerly tribes. The number 360 is allotted to the marula in The National List of Trees (de Winter *et al.*, 1978).

Distribution and habitat

According to Quin (1959), Hedrick (1972), van Wyk (1974), Shone (1979), Kokwaro and Gillett (1980), and Fox and Young (1982) *Sclerocarya birrea* subsp. *caffra* has a wide distribution in Africa. From the Sudan and Ethiopia in the north, through Kenya, Tanzania including the Island * Zanzibar also Zambia, Malawi, Mozambique, Malagasy Republic, Zimbabwe and Northern Botswana to northern Transvaal * and Natal in the South. In western Africa it is reported to occur in Senegal, Zaïre, Angola and northern parts of South West Africa.

In Transvaal marula occurs in the warm, frost-free areas, the most southern distribution being the northern slopes of the Magaliesberg range. It is found in open woodland, open bushveld at a low to medium altitude and along rivers. In the Kruger National Park it is the tree with the widest distribution as it can occupy all types of ecological niches. West of the Lebombo Mountains it is gaining dominance on the basalt plains where the closely associated *Acacia nigrescens* disappears (van Wyk, 1974). In Natal frost is also the limiting fac-

*see Corrections in Appendix

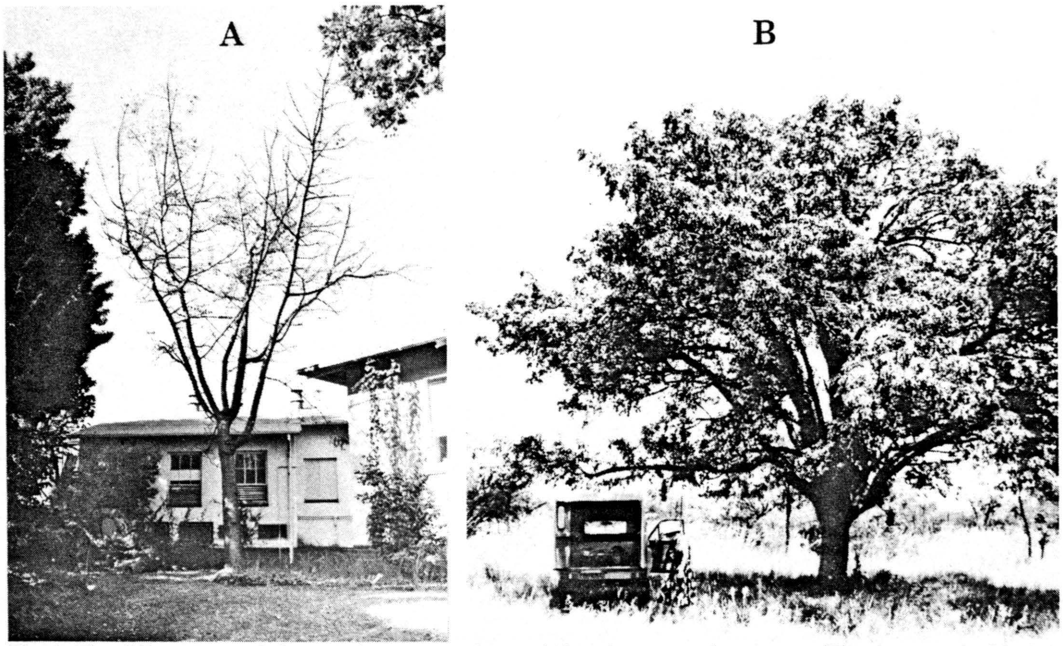

Fig. 1. The different growth forms, of a young male tree (A) and a mature female tree (B), photographed in July and February respectively.

tor in its natural distribution.

In Malawi, Kenya and Tanzania (in varied woodlands and savannas), marula occurs at altitudes of up to 1 200 m, (Kokwaro and Gillett, 1980). The soil preferred by marula seems to be red or stony sandy soil, sandy loams and sandy granite soil. It is often found associated with *Acacia* spp.

Habitus and morphology

Under ideal conditions the trees can reach a height of 15–18 m, and can be regarded as one of the fastest-growing indigenous trees, which usually start bearing fruit when about seven years old (Shone, 1979). Discussions of the author with farmers initially seemed to support the idea of "Blacks in northern Transvaal who maintain that male and female trees can be identified by their form i.e. the male tree being tall and slender, the female squat and spreading" (Shone, 1979). However, according to Robbertse* variations in growth form (Fig. 1) indicate differences in age. Shone (1979) includes figures of two young trees in their natural environment ("with branches inclined upwards"). It is possible that young female trees (which do not yet bear fruit) are sometimes interpreted as male trees.

Marula is deciduous but can be distinguished in winter by the sturdy twigs with their conspicuous leaf scars and blunt, thick tips and the grey, rough mottled bark flaking off in patches (Palmer and Pitman, 1972; Palgrave, 1981). The greyish, smooth bark of young trees differs markedly from that of the mature tree (Shone, 1979).

The marula has a well-developed root system which also serves to store water. A section, 1 m long and 7 cm in diameter, can yield a good cup of water (Shone, 1979). The large (about 15–30 cm long) compound, alternate and imparipinnate petiolate leaves are crowded near the ends of the twigs. The 7–13 (occasionally up to 17) leaflets are petiolate. Their form varies greatly * from ovate, oblong-elliptic to elliptic. The apices are usually acuminate, or mucronate, cuspidate or acuminate-caudate. The margin is entire or sometimes dentate-serrate on suckers, young trees and seedlings. The base is asymmetric, slightly cuneate or rounded. The adaxial surface is glabrous, pale-green to dark-green while the abaxial surface is dull and pale-green (e.g. Fernandes and Fernandes, 1966).

Contrary to some workers, who regard the flowers of marula to be bisexual, or oc-

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casionally bisexual, Baijnath (1983) and the author have only found unisexual flowers. Monoecious trees bear male and female unisexual flowers, while dioecious trees bear only male or only female unisexual flowers. In a random sample of 119 trees Baijnath (1983) found only 15 to be monoecious trees. These 15 trees were predominantly male-flowered, the female flowers being borne with male flowers on the lowermost 1–2 inflorescences of a particular twig. Most of the trees are therefore dioecious. Prior to Baijnath (1983), Palmer and Pitman (1972) were the only workers to observe the rare female flower in a male inflorescence. The flowers have 4–5 crimson sepals and 4–5 pink to light crimson petals. Herbarium specimens with flowers were generally collected at the end of September and during October. They appear more or less simultaneously with the young leaves of that season. About 20 male flowers are borne in a sparse, drooping raceme, about 50 to 220 mm long. In the male flower the disc and the 12 to 25 stamens are striking. The female flowers occur singly or in small groups. The ovary (waxy and white when young) is crimson and conspicuous when older. Usually there are 2 styles and stigmas, although occasionally one or three are present. The staminodes (sterile stamens) in the female flower are fewer and have somewhat smaller anthers than the stamens in the male flower, anthers often being absent. All the specimens in the National Herbarium in Pretoria (PRE) were examined with a good dissecting microscope. In no female flower could pollen (which is distinctly yellow in the male flowers) be distinguished i.e. no fertile stamens were present. Without an optical aid however, it is difficult to distinguish stamens from staminodes.

The fruit is shed during January to March before fully ripened. Ripening takes place while the fruit is lying on the ground, the ripening time depending on the temperature and rainfall. The marula fruit is a stone fruit or drupe. As was also noted by Palgrave (1981) the smooth, pale yellow fruit has "Three rather obscure points just below the apex". However, in the author's study usually only two of these "points" were present. Each "point" represents the remnants of a style. It became evident that fruit with two "points" were 2-locular while those with three "points" were 3-locular, with the respective fruit shapes differing too.

As "stone", "kernel", "pip", "nut", "seed", "embryo" have often been used indiscriminately, it seems worth considering the most appropriate terms. According to Holmes (1979) a stone fruit or drupe is "a more-or-less fleshy 1-celled fruit with one or more seeds having the pericarp differentiated into a thin epicarp, a fleshy mesocarp, and a hard stony endocarp". In the marula the exocarp (="epicarp") is the outermost part of the peel, the mesocarp (which is partly clinging to the exocarp and the endocarp) constitutes the edible, white, fibrous, juicy, flesh; and the endocarp containing the seeds is the stone. Subsequently I will refer to peel, flesh and stone. The stone can be 1, 2, 3 or 4-locular ("-celled"). These locules are chambers containing the seeds with one seed per locule. Each locule has an opening sealed by a hard, disc-like lid which varies in form (Fig. 2). Lids are not present in all types of stone fruit, but are characteristic for the fruits of some genera. Hill (1933), for example includes a figure of a somewhat flattened stone, with five locules, of *Dracontomelon mangiferum* (also a member of the family Anacardiaceae), with lids which are very similar to those of the marula. The hard stony endocarp thus takes over the protective function usually fulfilled by the seed coat, which is thin and papery in the seed of marula.

According to Quin (1959) an "Analysis of mature marula fruits yielded the following results:

Average weight per fruit	17.88 g
Peel per fruit	41.0 %
Stone per fruit	52.51 %
Edible flesh per fruit	6.47 %"

The average mass of 200 fresh fruit picked at random from about 3 000 fruit collected at Nylsvley Nature Reserve, was 21,82 g. The fruit mass is probably influenced by available moisture.

Of 50 stones, examined by Quin (1959) 38 % contained three seeds and 62 % two seeds. The average number of locules per fruit and percentage of locules without any seed varies, as shown in the table overleaf.

The stone fruit is therefore generally 2-locular, sometimes 1- or 3-locular, and seldom 4-locular. The solitary, pendulous ovule develops into a seed consisting of a thin seed coat, enveloping the embryo with fleshy cotyledons. The latter are usually white, with an oleaginous fine texture and pleasant, nutty, sweet flavour. No endo-

Locality and date of collection	Number of fruit	% "empty" locules	Average number of locules per fruit	Maximum number of locules per fruit
Nylsvley March 1982	600	45	2,12	4
Nylsvley April 1982	100	33,5	2,3	3
Pretoria Mountain View (garden)	128	53,2	1,88	3

Fig. 2. Stones of marula showing the different shapes and sizes as well as the lids of the seed-containing locules. One stone (upper left) without lids and seeds.

sperm is present.

Propagation
Marula can be propagated from seed. If seven to eight months old stones (Shone, 1979) are put out (untreated and undamaged, according to van Wyk, 1974) good germination is ensured. Shone (1979) mentions that the stones (best sown in September) should be covered with 30 mm of pure river sand. The nursery soil should be sandy loam which is particularly well-drained. In the Skukuza nursery* "Fresh" stones are put out in December and January. The medium in the black plastic bags (which are put in the sun) is also very well-drained. Considering personal observations (von Teichman, Small & Robbertse, in preparation) and the high average daily maximum temperature at Skukuza (i.e. 32.3 °C for December and January), relatively high temperatures are also required for marula seed germination. It is really fascinating to observe germination. The first compound leaves on the seedling look very unlike leaves on the mature tree, as the three leaflets of the seedling are deeply serrated (Fig. 3).

Viability of the seed is no problem. It is also not affected by passage through animals (Shone, 1979). Animals play a role in spreading the stones and it is known that the seeds germinate where for example the faeces of kudus is deposited. It is possible that the acids etc. in the digestive systems of animals, and the abundant water available during good seasons may "soften" the whitish layer which forms the cement of the locule lids. As part of my experiments "Peggy" an Indian female elephant in the National Zoo in Pretoria, was fed with marula fruit. The somewhat fermented whole stones, crushed stones and pieces of stone etc. were retrieved from her faeces (Fig. 4). Judging from the author's experiments, it is clear that stones with even very slightly cracked lids (Fig. 4) germinate quickly (i.e. in a few days), if fungal infections do not in-

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Fig. 3. Stages of marula germination. The narrow cotyledons (cot), which contain the organic reserve compounds of the seedling, are pulled out of the stone.

terfere. Walker (1982) provides a figure of three marula stones found in kudu faeces lacking lids. Exactly such stones were also found in Peggy's faeces (Fig. 4). Now the question arises why so few seedlings are found near trees in the veld, which produce thousands of fruit? I have found that about 44 % of all fruit-locules may contain no seed at all. Many human beings collect fruit and numerous animal species feed on the fruit.

The leaves of young trees and seedlings are relished by other animals. Fungal infections of the seed have been a very big problem in our experiments. The Skukuza nursery had similar problems when they placed the plastic bags in the shade. Furthermore the stones might need a covering layer of sand additional to the well-drained soil.

That these factors, especially human and animal utilization, play a decisive role was confirmed by Mrs Coetzee*. She noticed numerous seedlings under their tree in the enclosed farmyard near Mariepskop. Stones about six months old were collected under this tree. Most of these stones had been completely cleaned, others were still partly covered with shrivelled peel remnants. It was clearly noticeable that the cementing layer of the lids was partly eroded (by insects, bacteria etc.?). These lids could be removed relatively easily. It is possible that seeds from such an environment have a better chance to germinate than those in the open veld.

The marula can also be propagated vegetatively. According to Holtzhausen** branches 10–20 cm in diameter root fairly easily. Softwood cuttings from pruned trees root easily in spring. A well-aerated, well-drained medium with a good water-holding capacity is used, for example Perlite (⅓) + sand (⅓) + peat (⅓).

Conclusion

It is hoped that marula trees will be planted more extensively in our South African farmyards and large city-gardens instead of exotic trees. Marula trees always make a beautiful display. Under favourable conditions plants grow relatively fast and can bear fruit within seven years. Although they prefer frost-free areas, they will do well in colder regions if protected during the first few winters. The marula is reasonably drought resistant and could there-

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Fig. 4. Marula stones retrieved from the faeces of Peggy at the National Zoo. Whole stones (top left), stones without locule lids and separate lids (top right) and stones with "cracked" locule lids (in two bottom rows).

fore be planted along many of our rural roads. When the importance and potential of the marula will be fully realised by the white population as well, it might result in greater utilisation.

Acknowledgements

The constructive criticism of Professor N. Grobbelaar and the assistance of the veterinary personnel of the National Zoo are appreciated. Mrs Janet Botha is thanked for reading the manuscript. This research was financially supported by the Margaretha Mes Institute for Seed Research, the University of Pretoria and the C.S.I.R.

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NOTES ON THE DISTRIBUTION, MORPHOLOGY, IMPORTANCE AND USES OF THE INDIGENOUS ANACARDIACEAE.
2. THE IMPORTANCE AND USES OF *SCLEROCARYA BIRREA* (THE MARULA).

by Irmgard von Teichman,
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INTRODUCTION

The marula is one of the most highly valued indigenous wild fruit trees among the black tribes of Southern Africa. This is indicated by many descriptions of the role of the marula. Krige (1937), for example, discusses the importance of the marula to the Phalaborwa tribe. This tribe lives in a low-lying bushveld area with unpredictable rainfall, where they often experience food shortages. "Known and used wherever it grows, it is only in the drier areas that the marula assumes importance as a staple food". That the value of the marula is recognized is shown by the fact that it is protected. "In most tribes there is a fine attached to cutting the morula without special permission from headman or chief, and care is always taken to chop down only the male trees". "The morula as a food and a drink is used extensively all over the Northern Transvaal, but such is its importance in the economic life of the Phalaborwa that theirs may with justice be termed a morula culture".

The many diverse uses of the marula tree are discussed below under different headings, such as "medicinal uses", etc. The categories are artificial in the sense that a fruit, for example, can have medicinal properties because of its nutritional value.

NUTRITIONAL USES

The fruit

The fruit of the marula is delicious. According to Fox and Young (1982), Guy states that "the marula is one of the best known wild fruits of Africa and many a traveller has blessed it on a hot day in the bush in late summer ..." The Pedi (Central Sotho) classify marula trees according to the palatability of their fruit (Quin, 1959). Krige (1937) reports that the marula fruit ripens "just when the supply of corn is running low. Consequently especially in lean years (mokhope), the drink made from this fruit, comes as a welcome addition to the Native diet". Marula trees are prolific bearers, for example a total of 91 272 fruit with a mass of 1 647 kg was collected from one tree in 64 days (Quin, 1959). The results of a complete analysis of the fruit (Table 1) are available.

Table 1. Chemical analysis of fresh marula fruit (Wehmeyer, 1967)

mg/100 g edible portion	Marula flesh	Marula seed
Vitamin C	67,9	—
Calcium	6,2	106
Magnesium	10,5	467
Iron	0,1	0,42
Sodium	trace	338
Potassium	54,8	677
Copper	0,04	1,99
Phosphorus	8,7	836
Thiamin	0,03	0,04
Riboflavin	0,05	0,12
Nicotinic acid	0,25	0,71
g/100 g		
Moisture	91,7	4,0
Ash	0,2	4,2
Protein	0,5	30,9
Fat	0,1	57,0
Fibre	0,5	2,4
Carbohydrate (by difference)	7,0	1,5

According to Wehmeyer* (personal communication) the energy value of the marula fruit is 130 kJ per 100 g fruit flesh and 2 699 kJ per 100 g seed.

An outstanding feature of the composition of the fruit is its high vitamin C content. A wide range of analytical data is available. The highest vitamin C content was found in the fruit juice of marula by Fox and Stone (1938). Fox and Stone (1938) give the following interesting comparison (Table 2):

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Table 2. Milligram of vitamin C present in 100 ml of different beverages (Fox and Stone, 1938)

Orange juice	± 50,0
Milk	2,0
Home-brewed beer	0,8
Marula "beer"	100,0
Marula fruit juice	200,0

Fox and Stone (1938) state: "The vitamin C content of marula fruit is the highest of any we have yet tested, being approximately 2 mg. per cc. juice; this is no less than four times that of average orange juice". It is therefore not astonishing that many authors emphasize the high anti-scorbutic value of the fresh fruit. "Even jelly prepared from it by Europeans has been found to contain appreciable amounts of the vitamin" (Fox & Stone, 1938).

It is common knowledge that the marula fruit is highly prized by various African nations because of the alcoholic drink that can be prepared from it. For most of the drinks that are brewed from marula fruit, the designation (**marula cider**) (as suggested by Shone, 1979) is more appropriate than the commonly used term marula beer.

There is a common belief, popularized by the press and other media, that baboons and other animals get drunk after eating marulas. de Klerk and Watson (1974) question the validity of this belief. Their analysis of the fresh fruit shows only a minimal alcohol content. The fruit takes days to ferment, and in their experiments, baboons refused to eat rotten fruit. Baboons are slow eaters, and "food passes through the baboon's digestive system within hours of intake". They therefore could never eat enough fruit, nor would the fruit flesh and juice be in the stomach long enough to cause drunkenness. According to de Klerk and Watson (1974) "drunkenness can quite easily be induced in animals with the aid of drugs". This was confirmed by Gertenbach* (personal communication). He also agreed with the impression of the author that there is no proof (**to date**) that animals can get drunk on marula fruit.

Marulas are also used for flavouring. According to Palmer and Pitman (1972) Sim reports that on the west coast of Africa, marula juice is used for sweetening porridge after it has been boiled to a thick consistency. The Pedi of South Africa also use marula juice to flavour porridge.

Ballschmieter and Torline (1973), reporting on their work on marula flavour, state that the flavour is mainly concentrated in the peel.

Since the preparation of marula jelly and lemonade is such a simple and enjoyable task, recipes are given in an Appendix.

Lately the "New S.A. Liqueur" i.e. AMARULA, produced by Distillers Corporation in Stellenbosch from marula fruit has enjoyed great publicity.

The **seeds**, which are enclosed within a woody endocarp, are a recognized delicacy and are eaten raw like ground nuts, or cooked with porridge. Some Africans consider a gift of marula seeds as a sign of great friendship (Palmer & Pitman, 1972) and the Thonga appreciate the seeds to such an extent, that they regard them as "food for kings" (Junod, 1913).

Krige (1937) reports that the Phalaborwa subsist largely on the seeds in winter, mixing them with spinach or meat. In summer they mix them with green mealies or crush the seeds and make cakes which are eaten on their own. The Pedi flavour "kaffir-corn" stew and green leaf relishes with marula seeds (Quin, 1959).

The Phälaborwa women use a ‘modukulo’ and the Pedi women a ‘modikola’ to obtain the marula seeds after the hard endocarp (marula stone) has been cracked between two stones (Krige, 1937: Quin, 1959).

Marula fruits have probably been utilized for many centuries. Archaeologists found marula stones at Mapungubwe where Boskopoid people lived over 1 000 years ago. Specially fashioned pieces of bone typical of Iron Age sites in the Transvaal were possibly used to crack marula stones (Palmer and Pitman, 1972). Holtzhausen* (personal communication) mentioned that seed extraction is simple if the marula stones are immersed in boiling water for a couple of minutes and dried in the sun. The lids of the fruit locules then come off easily.

The extracted seeds are winnowed by the Phalaborwa and kept in closed calabashes where they keep for some days. The marula stones are stored in "hammock-like receptacles made of strings of bark, lined with grass and supported by four poles" (Krige, 1937). The Pedi store the stones on elevated platforms (Quin, 1959).

According to Ligthelm et al. (1951) the

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mass of the marula stones comprises 86,1 % "shell" (i.e. endocarp) and 13,9 % seed. The moisture content of the seed is given as 4,97 % and the oil as 53,5 %. An analysis of the oil is supplied by these authors. They conclude that the composition of the oil is typical of that of the oils found in the family Anacardiaceae.

The analysis of the marula seed supplied by Wehmeyer (1967) (see Table 1) gives a good idea of its food value. These results correspond well with those given by other authors, e.g. Quin (1959).

The seeds of marula were therefore successfully used instead of imported nuts in cakes and confectionery "during the war" (Fox and Young, 1982).

The root

Van Wyk (1974) reports that "among the Bantu in dry areas, the roots of the marula are a well known source of water". The method by which the water can be obtained is described as follows: "A section of the thick, superficial roots, about 1 to 2 metres in length, is exposed without damaging it by digging from the stem outwards. The root is then chopped free quickly and the bark around one end removed for about 10 cm. When the root is held upright with the debarked point downwards, clear water starts to drip from it within minutes".

MEDICINAL USES

The bark

According to Haerdi (1964) a bark extract (or decoction) is used by the people of the Ulanga district in Tanzania for washing wounds. A mixture of bark extracts from marula, *Dalbergia melanoxylon, Vitex doniana* and *Piliostigma thonningii* and a root extract of *Afrormosia angolensis* is used in a steam bath for poor circulation of the limbs.

Naude* (personal communication) reports that the bark is also an excellent remedy for haemorrhoids. Watt & Breyer-Brandwijk (1962) mention many uses of the bark of marula, namely: (a) In early days it was believed that a "brandy tincture of the bark" had prophylactic effects against malaria; while the powdered bark was used in treating malaria. (Spencer et al. (1974)), however, found no anti-malarial activity when testing the bark of marula). (b) "The Zulu use a decoction of the bark, externally and internally, as a prophylactic against gangrenous rectitis". (c) A bark decoction "is taken in half-pint doses in dysenteries and diarrhoeas" (Several older people in Pretoria have confirmed this use).

Van Wyk (1974) describes the experience of a person in the Kruger National Park who felt immediate relief when juicy, mashed inner bark was placed on painful blisters caused by hairy caterpillars. The blisters disappeared shortly thereafter.

Liengme (1981) reports that the roots and bark are used by the Tsonga of Gazankulu to make a cleansing medicine for the stomach.

According to Kokwaro (1976) *S. birrea* bark is used in East Africa as a medicine for dysentery and rheumatism. The bark of *S. caffra* is used as a medicine for toothache, constipation and stomach trouble (Kokwaro, 1976).

Ayensu (1978) reports that a bark decoction is used in Ghana for "skin eruptions".

The research of a South African pharmaceutical company, (Noristan Limited), "strongly suggests that the majority of the plants used in the folk-medicine of Southern Africa do indeed possess some pharmacological activity. This makes folk-medicine an excellent starting point for effective research". For the past 11 years, "Noristan Limited" has made a systematic evaluation of the Southern African flora. "During this period over 300 plant species have been investigated with a view to the identification of pharmacological activity and the isolation of novel therapeutically useful substances" (Fourie and Snyckers, 1982). "Noristan Limited" has preliminary results on crude extracts of the bark of marula, i.e., weak pharmacological activity was found in respect to: antihypertension, anti-inflammation and painkilling (analgesic) (Fourie*, personal communication).

The leaves

In the Ulanga district of Tanzania, a drink made from the juice of marula leaves is used for the treatment of gonorrhoea (Haerdi, 1964). An extract of the leaves is used by Black tribes for boils and abcesses (Shone, 1979) and the leaves are also "applied to burns, stings and wounds" (Grivetti, 1982). Similarly the people in the West African republic of Niger apply the leaves of the "eedi" tree (as they call the marula) as a poultice on scorpion bites (Beckwith, 1983).

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HOUSEHOLD USES

The fruit

A rather unique use of the fruit is reported by Watt and Breyer-Brandwijk (1962): the Zulu "regard the fruit as a potent insecticide" and use "the fruit for the destruction of ticks". The oil which is extracted from marula seeds is used by different tribes as a skin cosmetic (e.g. Quin, 1959; Watt and Breyer-Brandwijk, 1962). Palmer and Pitman (1972) describe how the Venda use the preservative qualities of this oil for preparing meat.

In Kavango (South West Africa/Namibia) seeds are pounded and placed in boiling water and the oil which can be collected is eaten together with the porridge (Fourie*, personal communication).

Krige (1937) reports that the peel is used for snuff, i.e. it is burnt to ashes and mixed with ground tobacco and Quin (1959) describes attractive mud-floor inlays made with marula stones.

The bark

According to Mrs Bode* (personal communication) the bark was used by the Voortrekker women to dye their sunbonnets. She mentioned that the red-brown colour faded with time but would have kept longer if salt had been added during the dyeing process.

The bark is also used by Africans for making a red dye, buckets for water and containers, while "the fibrous living bark is plaited into ropes" (Shone, 1979).

Williams (1930) analysed the bark of a comparatively young marula tree and found it to contain 10,7 % tanning matter. Further investigations will show whether marula bark can be used as tanning material. The same author reports that the gum of marula, which is also rich in tannin, is sometimes "used for making a substitute for ink by dissolving it and mixing with soot".

The wood

The marula is not very hard when fresh, and therefore pleasant to work with. The people of several Black tribes utilize it. Various kitchen utensils, stamping blocks, drums, and cattle yokes are made from marula wood (Krige, 1937; Quin, 1959; Liengme, 1981). Tribes in northern South West Africa/Namibia use the wood for sledges and wato's, i.e. boats made from tree trunks (Fourie,* personal communication). Shone (1979) gives full details of the marula's wood, e.g. working properties, texture and hardness.

CULTURAL USES

The marula plays a very important cultural role in the life of the Tsonga (Thonga), a tribe which historically occupied southern Mozambique and adjacent areas (Liengme, 1981). Junod (1912) describes how the "Feast of the First Fruit" is observed. The chief and elder brothers are the first to be allowed to eat certain fruit or drink bukanye, the drink made from marula fruit. The luma of the bukanye is a national feast starting in January. A sour liquor made from the first fruit "is poured out on the tomb of the deceased chiefs in the sacred wood; they are invoked to bless this new year and the feast which is about to be celebrated". "These feasts are the saturnalia, the bacchanalia, the carnival of the tribe!" Marula branches feature in funeral rites of the Tsonga. Palmer and Pitman (1972) reported that marula is called "the marriage tree" by the Zulu, while the Tsonga "give a brew of the bark in a cleansing ritual before marriage".

Other reports state that marula seeds are used by the Pedi for birth control (Wolhuter, 1973). "The Venda administer the powdered bark to an expectant mother to regulate the sex of the child, bark from a male tree for a boy and bark from a female tree for a girl" (Watt and Breyer-Brandwijk, 1962). The marula stone is an important part of the Shangana divining dice and "represents the vegetable kingdom or 'Medicine'" (Watt and Breyer-Brandwijk, 1962).

"Because of the marula's high fertility some of the trees have become fecundity trees". A clay pot for offerings is buried under particularly prolific bearers (Shone, 1979). Although doubted by some authors, even aphrodisiac properties have been ascribed to marula seed.

According to Grivetti (1982) the marula tree is used by the "Batlokwa of Botswana" as a calendrical marker (morule = December).

COMMERCIAL UTILIZATION OF THE MARULA

The wood

The wood has been subject to commercial

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*Dr T.G. Fourie, Noristan Limited, Private Bag X516, Silverton 0127 utilization for many years. Shone (1979) relates the "marula drama" which started during World War II, when, due to the critical timber shortage, a commercial exploitation began. The wood was primarily used for fruit boxes. Later paint brushes, handles, furniture and veneer were manufactured, while marula lavatory seats were even exported to Rhodesia and Australia. Eventually in 1962 complete protection of marula was legally enforced. Today applications to fell marulas have to be made through the local agricultural extension officer.

The fruit

Already in 1976 Redelinghuys et al. came to the conclusion that it is technically and economically possible to produce marula juice commercially. The fresh fruit juice has a pleasant flavour and high Vitamin C content. Up to a ton of juice can be retrieved from the fruit of a prolific bearer (in one season) when using the rack and cloth press method (Holtzhausen,* personal communication). Fully realizing the commercial potential and economic importance, Prof Holtzhausen started with the improvement of marula. This includes a selection programme in the field and then breeding in orchards with further selection to establish the desired characteristics genetically. For juice production big fruit with small stones would be the aim. Fruit which weigh 50 g or even 70 g have been found in the field (Holtzhausen,* personal communication). Marula juice forms part of a "New Range of Tropical Concentrates" sold by SIMO Products, PO Box 861, Tzaneen 0850. This is delicious and should sell very easily once it is also available on the supermarket shelves. AMARULA, a liqueur made from marula juice is also commercially available.

The research of du Toit et al. (1983) on the marula fruit-fly is worth noting, especially as it is reported that this pest also is the dominant fruit-fly species on mango. This naturally has great economic implications.

THE VALUE OF THE MARULA TO ANIMALS AND OTHER PLANTS

The fruit and leaves of marula are utilized extensively by many wild and domesticated animals. The trees are browsed as high as the animals can reach (van Wyk, 1974; Shone, 1979). Marula bark is eaten to such an extent by elephants in the Kruger National Park, that "trees which have been completely debarked are a common enough sight in the over-grazed areas along the rivers". For many rodents for example the bush squirrel, (*Paraxerus cepapi*) the seeds are a very valuable source of nutrition (van Wyk, 1974).

The marula tree hosts many insects, for example at least eight butterfly species, mosquitos, bees and a small beetle (*Polydada*), the larvae of which are very poisonous and used by the Bushmen in the preparation of their arrow poison (Palmer and Pitman, 1972; van Wyk, 1974).

A very valuable fodder grass occurs under marula trees (Shone, 1979). van Wyk (1974) mentions that the marula hosts several mistletoes, of which *Erianthemum dregei* (= *Loranthus dregei*) is the most important species.

CONCLUSION

The marula is one of the best known and most highly valued indigenous fruit trees. The seeds which are a recognized delicacy are regarded as food for kings. The fruit has economic potential as juice and liqueur are manufactured from it. The bark has distinct medicinal value. The tree as such has ornamental value and is an excellent shade and fodder tree. Therefore the marula which is legally protected is highly recommendable for improvement and cultivation.

ACKNOWLEDGEMENTS

Mrs Janet Botha is thanked for her help with the language and layout. The help of the personnel of the Botanical Research Institute and all the other people who shared their knowledge is appreciated. This study is part of a project conducted by the Margaretha Mes Institute for Seed Research supported by the University of Pretoria and the C.S.I.R.

APPENDIX

Marula jelly

It is best to select fruit that are not quite ripe. They are washed and the peels cut cross wise. Covering the fruit with water, they are boiled slowly (about one hour) until the peel is very soft. If the mixture is allowed to cool completely it is easier to get a clear solution when slowly strained through muslin.

A simple pectin test is made as follows: two tablespoonfuls methylated spirits and one tablespoonful marula-solution are mixed

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*Prof L.C. Holtzhausen, Department of Horticultural Science, University of Pretoria, 0002 cautiously by swirling in a glass before pouring into a saucer. If a "gelatin-cake" forms equal amounts of sugar and marula-solution are used. The author prefers to add a bit of lemon juice. The solution is brought to boiling point and **then** the sugar is added very slowly so as not to stop the boiling. When the ordinary "jelly-test" is positive the jelly is ready. Marula jelly is delicious, has a fine flavour and a beautiful clear amber colour.

Marula lemonade

"Wash fruit, peel by cutting a round 'lid' off the tops and squeezing out the fruit. Discard peels. To every kilogram of fruit, add about one litre of water and 250 g of sugar. These quantities are subject to individual taste and are merely a guideline. Let the mixture stand for about two days. Bottle and keep cool. Most refreshing on a scorching summer's day. Remember to seal the bottles with corks and allow some breathing space" (Onderstall, 1979).

Note by the author: As the 'peels' contain the pectin and characteristic (i.e. unique) flavour needed for jelly preparation, these peels can be used for preparing jelly instead of being discarded.

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CORRECTIONS

Chapter 2

Irmgard von Teichman & P.J. Robbertse: Development and structure of the drupe in Sclerocarya birrea subsp. caffra.

p.11: In the title, the abstract and first line of the introduction insert: (Sonder), i.e. Sclerocarya birrea (Richard) Hochst. subsp. caffra (Sonder) Kokwaro.

p.11: last para., 2nd last line, insert Hochst., i.e. Sclerocarya Hochst. belongs

p.13: penultimate line: "anatropous", not anatropus

p.16: 4th line: "derivatives", not derivates

p.26: 3rd line: "sarcocarp", not sacrocarp

p.28: para 3, 2nd last line: "Bernh. ex Krauss", not Bernh.

Chapter 5

Irmgard von Teichman: Development and structure of the pericarp of Lannea discolor.

p.55: para 5 5th last line: "Blum", not "Blume"

p.56: para 3 2nd line: "pedicel", not "peduncle"
para 3 3rd line and 2nd last line: "rachis", not "rhachis"

p.59: para 1 1st line: "at", not "on"

p.60: para 1 2nd line: "wherein", not "within which"

p.61: Figure legend, 3rd line: "glandular hair", not "hair" and
2nd last line: "style", not "styl"

p.62: para 2 5th line: "Glandular hairs", not "Hairs";
para 4 3rd last line: "bicarpellate ovaries", not "a bicarpellate ovary"

Chapter 7

Irmgard von Teichman & A.E. van Wyk: Pericarp and seed-coat of *Harpephyllum caffrum*.

p.119: Add to first para: "The other brachysclereids illustrated in Figure 15 approach the spheroidal (Fig. 15 B & E) and vesiculose (Fig. 15 D) types of monomorphic sclereids proposed by Rao & Bhupal (1973).

p.126: Insert before Robbertse *et al.*: "RAO, T.A. & BHUPAL, O.P., 1973. Typology of sclereids. *Proceedings of the Indian academy of Science, section B*, *77*: 41-55.

Chapter 8

Irmgard von Teichman & P.J. Robbertse: Pericarp and seed of *Rhus lancea*.

p.146: Abstract 2nd last line: "tribe", not section

p.147: Last para 5th line: "by", not in

p.153: Figure legend: "Transmission electron micrograph of", not TEM through

para 2 1st line: "pericarp", not fruit

p.154: Figure legend: "Scanning electron micrograph", not SEM

para 1 7th line: "epithelial", not epithelium

p.159: para 3 4th line: "one", not outer

para 4 1st line: "endotestal and endotegmic seeds", not an endotestal seed

p.161: 2nd last line: "*caffra* (Sond.) Kokwaro", not *caffra* Kokwaro

Robbertse, P.J., Irmgard von Teichman & Helene J. van Rensburg: Re-evaluation of the mango ovule.

p.282: Introduction 4th line: "were", not was

p.282: Material and Methods: "Bernh. ex Krauss", not Bernh.

p.283: Figure Legend 4th line: "pollen tube", not polen tube

p.289: Last reference: "_caffra_ (Sond.) Kokwaro, not _caffra_ Kokwaro

Irmgard von Teichman: Distribution and morphology of the marula.

p.290: Distribution and habitat 7th line: "island" not Island

10th line: "northern" not Northern

p.291: 2nd last para 9th line: "shape", not form