CYSTOLITHS IN THE SECONDARY XYLEM OF SPARATTANTHELIUM (HERNANDIACEAE)

by

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Abstract

Cystoliths were observed in the secondary xylem of *Sparattanthelium* (Hernandiaceae). Their shape, size, distribution and chemical composition is described. The systematic value of cystoliths in the Hernandiaceae as well as in general is discussed.

Introduction

Cystoliths are internal, stalked outgrowths of the cell wall that project into the cell lumen. They consist of cellulose and are impregnated with calcium carbonate, they are irregular in shape and sometimes fill up the cell completely. Cystoliths may occur in parenchymatous cells in various parts of the plant, including even xylem and phloem rays. They are most frequently found in the epidermis, in hairs or in special large cells which are termed lithocysts (Fahn, 1967). In spite of the presence of calcium carbonate, this type of cell inclusions was not included in the comprehensive study on the occurrence and shape of crystals made by Chattaway (1955, 1956). Others, like Metcalfe & Chalk (1950), Esau (1963) and Fahn (1967) also described cystoliths as a separate category, apart from crystals.

The occurrence of cystoliths in various parts of the plant, but especially in the leaves, is restricted to a few families (Metcalfe & Chalk, 1950; Pireyre, 1961), particularly in Cannabinaceae, Moraceae and Urticaceae.

Cystoliths in the secondary xylem were up till now only known in a single family, Opiliaceae (Record, 1925, 1927; Kanehira, 1921; Metcalfe & Chalk, 1950). Record described cystoliths as 'deposits of calcium carbonate in special structures in the rays of 7 genera, viz. *Agonandra, Cansjera, Champereia, Lepionurus, Meliantha, Opilia* and *Rhopalopilia*'. According to Metcalfe & Chalk (1950), *Agonandra* is the only genus which lacks the cystoliths.

By chance I observed in a section of *Sparattanthelium* deposits which looked like a bunch of grapes. At first sight, these deposits appeared to be similar to the well-known cystoliths in the leaves of *Ficus*. As cystoliths have also been described for the leaves of *Sparattanthelium* (Solereder, 1899) and have been accepted as a feature of systematic value (e.g. Kubitzki, 1969), a supplementary examination of the wood of other species was undertaken.

Methods

Both stained and unstained sections were studied. A double staining of Astrablue and Safranin was applied to differentiate between unlignified and lignified cell components. The usual light-microscope, polarized-light and SEM-techniques were used. Finally, the mineral composition of the cystoliths was determined with an Energy Dispersive X-ray Analyzer.

Materials

According to Kubitzki (1969), the genus *Sparattanthelium* comprises 13 species, lianas or shrubs, restricted to the neotropics. For this study wood samples of 5 species were available. In addition samples of other Hernandiaceae and of a few taxa of the Opiliaceae and of the Acanthaceae were investigated.

Material seen:

Hernandiaceae – *Gyrocarpus americanus* Jacq. ssp. *americanus*, FHOw 701, Sri Lanka; Pulle 3217, New Guinea; *Hernandia didymantha* Donn., Uw 10383, P.H. Allen, Costa Rica, ex USw 30169; *H. guianensis* Aubl., Uw 380, Stahel 380, Suriname; Uw 1509, Lanjouw & Lindeman 1547, Suriname; *H. peltata* Meissn., B.W. 7670, New Guinea; *Illigera pentaphylla* Welw., Uw 22123, Versteegh & den Outer 588, Ivory Coast; *Sparattanthelium aruakorum* Tutin, Uw 12148, van Donselaar 3754, Suriname; *S. borororum* Mart., Uw 24192, R.C. Gill 43, Equador, ex SJRw 36125; *S. glabrum* Rusby, Uw 24073, Schunke 2539, Peru, ex USw 41876; *S. guianense* Sandw. Uw 24087, Fanshawe, F.D. 3938, Guyana, ex FHOw 13266; *S. wonotoboense* Kosterm., Uw 24191, A.C. Smith 3390, Guyana, ex SJRw 35917.

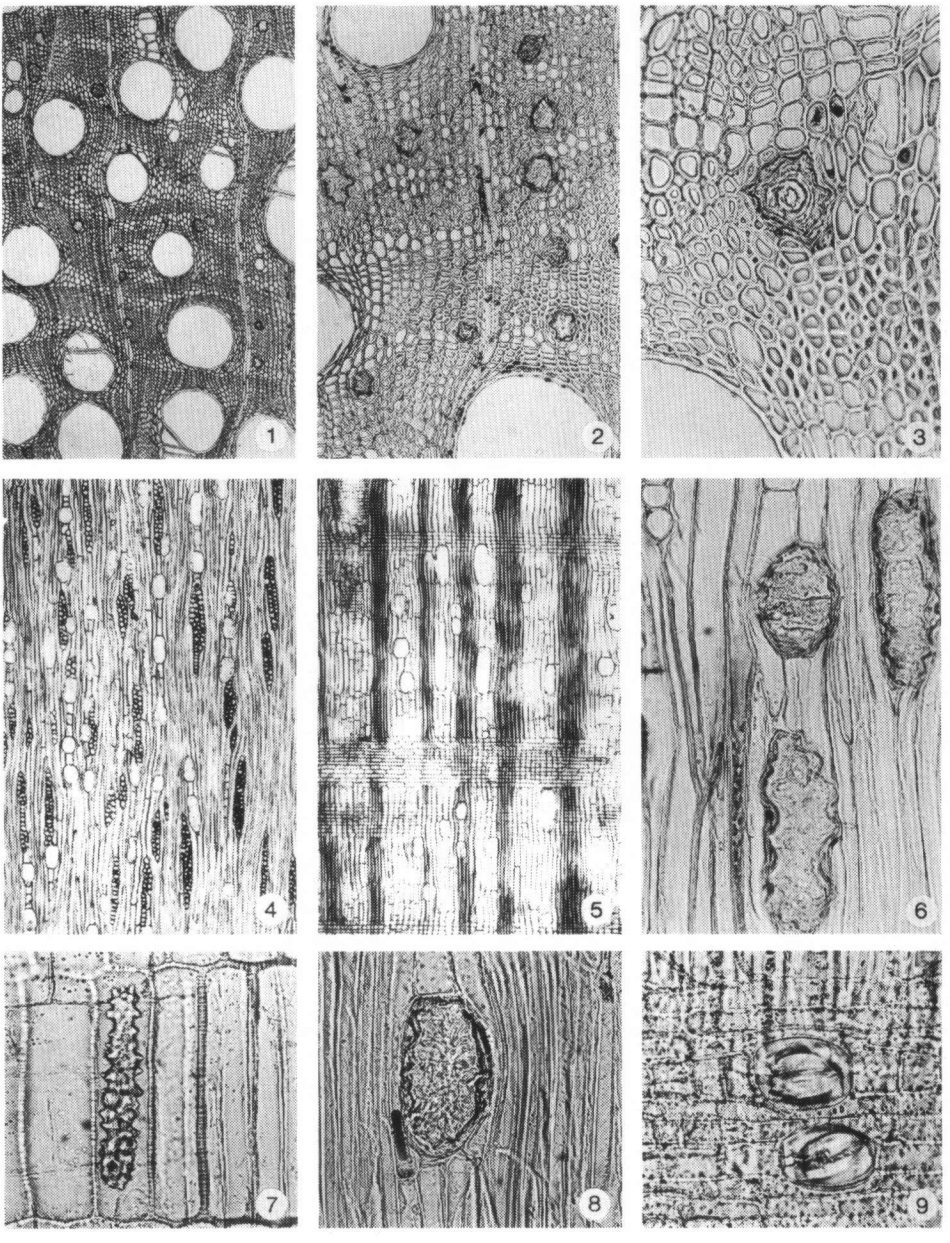


Fig. 1–9. Light-microscopical appearance of cystoliths in the secondary xylem. –– 1, 2 & 3: *Sparattanthelium aruakorum* Tutin. (Uw 12148). Cross section, x 35, x 90 & x 220 resp. –– 4: *Sparattanthelium guianense* Sandw. (Uw 24087). Tangential section, x 35. –– 5: *Sparattanthelium wonotoboense* Kosterm. (Uw 24191). Radial section, x 35. –– 6 & 8: *Sparattanthelium aruakorum* Tutin. (Uw 12148). Tangential and radial section resp., x 220. –– 7: *Trichanthera gigantea* (Humb. & Bonpl.) Steud. (Uw 5476). Radial section, x 220. –– 9: *Champereia manillana* Merrill. (Jacobs 7611). Radial section, x 220.

Opiliaceae – *Agonandra silvatica* Ducke, Uw 3402, Lindeman 4995, Suriname; Uw 6806, Schulz 8329, Suriname; *Champereia manillana* Merrill, Jacobs 7611, Philippines; *Opilia cf. amentacea* Roxb., Jacobs 9600, Papua New Guinea; *O. celtidifolia* (Guill. & Perr.) Endl. ex Walp., Breteler 2261, Cameroun.

Acanthaceae – *Aphelandra tetragona* (Vahl) C.G. Nees ab Esenb., Uw 10932, Florschütz & Maas 2431, Suriname; *Trichanthera gigantea* (Humb. & Bonpl.) Steud., Uw 2001, Lanjouw & Lindeman 3433, Suriname; Uw 5476, Geyskes s.n., Suriname.

Results

Sparattanthelium

Cystoliths were found in all species of *Sparattanthelium* examined. Their shape, size and distribution was variable, both within a sample and between different samples.

Shape – Slightly oval and oblong in *S. aruakorum, S. glabrum* and *S. guianense*. In *S. borororum* and *S. wonotoboense* only oblong cystoliths occurred.

Size – The oval cystoliths measure from c. 45 µm up to 60 µm and the oblong ones from 100 x 40 to 160 x 60 µm. In *S. wonotoboense*, a cystolith of 240 x 64 µm was observed. They usually fill up the cell completely.

Structure – The surface is always more or less granular, but without the sharp points characteristic for crystals like druses. Only occasionally the cystoliths show faint radiating lines (Fig. 10). On cross sections concentric lines around the centre of the cystoliths can often be noticed (Fig. 2 & 3).

Distribution – Average values for the number of cystoliths per square mm (cross section), based on 10 measurements, were computed. The averages vary from 2 to 29, but in general there are less than 10 per square mm (Fig. 1).

Lithocysts – The shape of these cells commonly resembles that of the cystoliths, since the latter fill up the cell completely. The outline of the lithocysts in longitudinal sections is rounded (oval or oblong, Fig. 4 & 5), and rather irregular in cross sections (Fig. 3).
Cystolith-containing cells in *Sparattanthelium* are always part of a parenchyma strand, but they are much larger than the other parenchyma cells. In general the cells show about the same size and shape as oil or mucilage cells, well known from the wood of e.g. Lauraceae.

Pedicel – The presence of a pedicel is of essential importance for the development of cystoliths (Fahn, 1967). However, in the material examined pedicels were rarely observed and short.

Chemical composition – A thorough examination of the chemical composition of the cystoliths is beyond the scope of this study. However, in order to establish the cystolith-nature of the observed inclusions, some information on this subject is necessary. Therefore, sections were double-stained with Safranin and Astrablue according to the method recommended by Von Aufsess (1973) to differentiate between lignin and cellulose. In both transverse and longitudinal sections the cystoliths are stained bright blue. Nevertheless the intensity of the blue colour shows various grades. There is no trace of red stained material in the cystoliths which indicates the absence of lignin. If pedicels do occur, they remain uncoloured.
Subsequently SEM/EDXA techniques were used to determine the mineral components of the cystoliths. The only element identified is calcium (Fig. 14, 15 & 16). In polarized light the cystoliths show little or no birefringence.

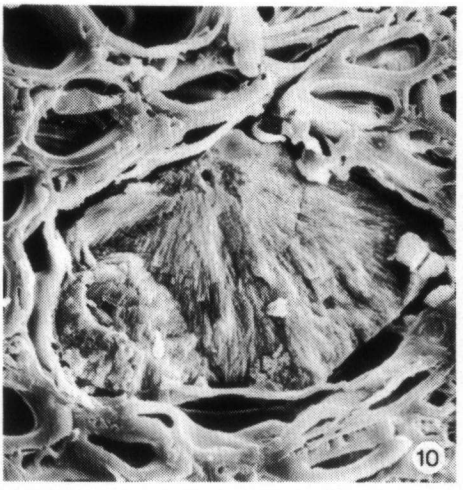
The SEM investigations gave some indication for the presence of a thin layer of unidentified nature between the wall of the lithocyst and the cystoliths (Fig. 13). This layer stained blue with the Astrablue-Safranin staining. Although cystoliths usually are not classified as crystals, this layer is comparable with integuments described for crystals in the secondary xylem of e.g. Flacourtiaceae (Miller, 1975).

The occurrence of the cystoliths in *Sparattanthelium* is restricted to a certain part of the stem. Shutts (1960) already noticed in this genus the presence of two distinct types of vessels in one sample, indicated by diameter and element length. On a transverse section these two different types are very clear. It is remarkable that the cystoliths often occur at the place where the diameter of the vessels changes abruptly.

According to Kubitzki (1969), cystoliths occur in the leaves of all species of *Sparattanthelium* and *Gyrocarpus*, together constituting the tribe Gyrocarpoideae. However, contrary to the results obtained with the secondary xylem of *Sparattanthelium*, no cystoliths were observed in the wood of *Gyrocarpus*. In view of the fact that occurrence of cystoliths is restricted to a small number of families (Solereder, 1899, 1908; Pireyre, 1961), it seemed worthwhile to examine the wood of some families from the leaves of which cystoliths have been reported.

Acanthaceae

Cystoliths are abundant in the ray cells of two samples examined of *Trichanthera gigantea*. The ray cells are not enlarged and similar to the other ray cells. Cystoliths occur in both square and upright cells. As a result their shape varies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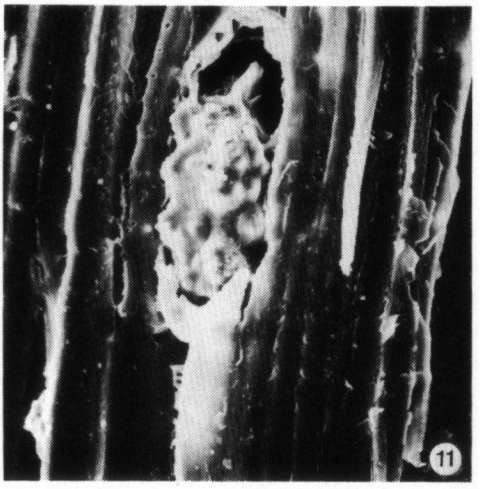
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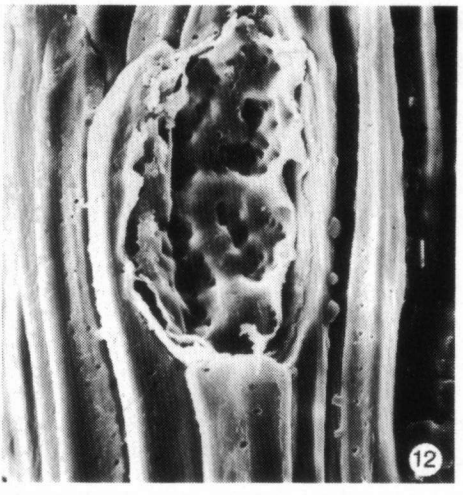
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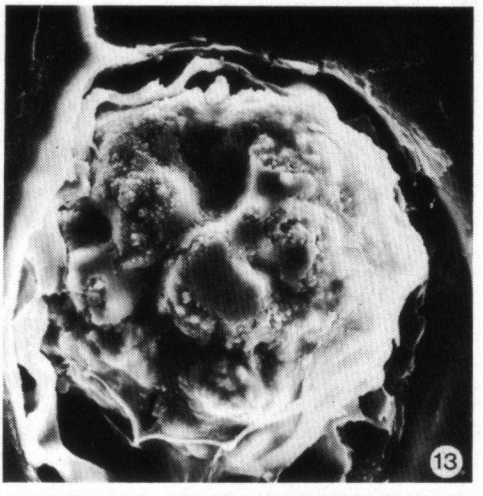
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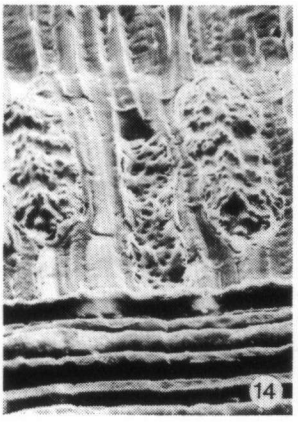
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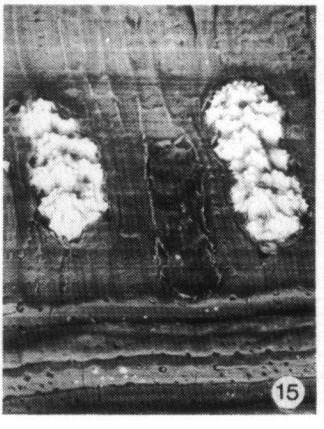
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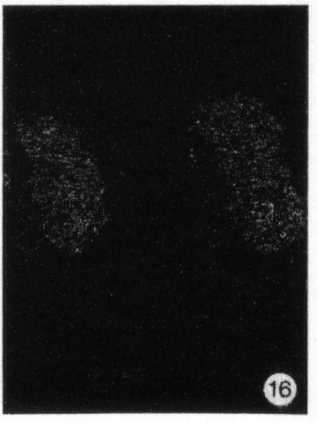
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from more or less square or round to very often oblong, respectively c. 40 μm and from 120 x 30 to 180 x 30 μm (Fig. 7). They never completely fill the cell lumen. In polarized light the cystoliths are birefringent or not. Pedicels are rare or absent, but if present they are very faint. The cystoliths are very fragile. A pretreatment of boiling in water completely destroyed most of them. In the particular case of the Acanthaceae, the following method turned out to be useful: use cold water when sectioning, remove air by boiling in water for less than 20 seconds and mount in glycerin. Finally, the best results were obtained using sections of 30–40 μm thick.

Boraginaceae

Seven species of *Tournefortia* and *Cordia* were examined. Cystoliths were not observed in the secondary xylem.

Discussion

The shape of the cystoliths has been described as very variable (e.g. Solereder, 1899, 1908), but as a result of her investigations, Pireyre (1961) limited the number of types to three:

a. round cystoliths;
b. oblong cystoliths the pedicel of which is attached at one end of the long axis, or oblong cystoliths the pedicel of which is attached perpendicular to the long axis;
c. cystolith-like structures.

There is no doubt that the cystoliths observed in *Sparattanthelium* belong to type b. This conclusion is strenghtened by the occurrence of a thin-walled layer between cystoliths and the parenchyma cell wall. According to Pireyre (1961) such an integument is usually connected with cystoliths of type b. Pedicels are rare and usually weakly developed in both stained and unstained sections of *Sparattanthelium*. However, after a pretreatment with hydrochloric acid the calcium carbonate dissolves and in some cystoliths the pedicels become clearly visible. Cystoliths of type b often show short or invisible pedicels (Pireyre, 1961).

In general, the pedicel and the cystolith body are composed of callose, cellulose and pectin (Fahn, 1967; Pireyre, 1961). After the first stage of its formation, the body is incrusted or impregnated with amorphous calcium carbonate (Frey-Wyssling, 1935; Esau, 1963; Pireyre, 1961). The few tests used in my investigation to obtain information on the chemical composition of the cystoliths in *Sparattanthelium* are in agreement with these data from the literature. All evidence clearly demonstrates that the structures observed in the secondary xylem of various *Sparattanthelium*-species are real cystoliths.

The occurrence of cystoliths in various parts of the plants has always been considered as a useful character in identification and it has often proved to be of systematic value (Solereder, 1899 & 1908; Metcalfe & Chalk, 1950; Fahn, 1967; Pireyre, 1961). Their presence in the leaves of *Gyrocarpus* and *Sparattanthelium* is of systematic value (Solereder, 1908; Kubitzki, 1969), as it is one of the characters used to divide the family of the Hernandiaceae into two tribes. As already mentioned before, cystoliths occur in the wood of all examined samples of *Sparattanthelium*. Although size, shape and distribution are variable, their occurrence is constant and therefore they constitute a character of systematic value. The cystoliths were not observed by Shutts (1960) who studied the secondary xylem of the Hernandiaceae. In *Sparattanthelium* he noticed the occurrence of 'secretory cells, swollen, common in vertical parenchyma'. Before sectioning he softened the wood samples in a 1 : 1 solution of hydrofluoric acid and 50 % ethyl alcohol for three to six weeks. As boiling in water sometimes destroys the cystoliths, it seems plausible that his pretreatment completely dissolved the cystoliths. As Shutts (1960) described these 'swollen secretory cells' not only for *Sparattanthelium* but also for *Hernandia*, I studied the secondary xylem of some samples of *Hernandia* myself. Neither cystoliths nor swollen parenchyma cells were observed. This result is rather striking since these cells were not only recorded by Shutts but also by Garratt (1933) and Record (1944) but not, however, by Brazier & Franklin (1961).

Fig. 10–16. SEM and Energy Dispersive X-ray Analyzer appearance of cystoliths in the secondary xylem of *Sparattanthelium aruakorum* Tutin. (Uw 12148). — 10: Cross section through a cystolith showing radiating lines, x 630. — 11: Radial section. At the top of the cystolith a short pedicel can be seen; x 320. — 12: Radial section showing the parenchymatous cell wall of the idioblast, x 320. — 13: Radial section. A thin layer between the cystolith and the cell wall is indicated by the arrow. — 14, 15 & 16: Radial section. Two cystoliths and an empty idioblast. In Fig. 16 the X-ray element map shows the location of calcium in these cystoliths.

The positive results of my own observations on the occurrence of cystoliths in *Trichanthera* (Acanthaceae) might be an indication of a more widespread distribution of these deposits. As most of the cystoliths in this genus are destroyed by boiling in water, a careful preparation is essential. Finally, a more detailed examination of the crystals described by Chattaway (1955) as 'druses, attached to the cell wall by a peg' should be considered. These crystals occur in *Celtis*-species (Ulmaceae). This family together with the Urticaceae, Moraceae and Cannabinaceae constitutes the order Urticales. Especially in this order cystoliths are a common character for the leaves.

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