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9. ABSTRACT

Phloem tissues of Microcorium scolopendria (Polypodiaceae) and Psilotum nudum (Psilotaceae) were examined with light and electron microscopes. The characteristic refractive spherules in the sieve elements of M. scolopendria apparently develop from endoplasmic reticulum-derived cytoplasmic vesicles. In both taxa they have not been observed to be related spatially to plastids or mitochondria. Refractive spherules contain protein and often occur in the peripheral cytoplasm of mature sieve elements. In M. scolopendria they also occur in pericycle cells. Significant differences in refractive spherule substructure occur between the two taxa studied.

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Refractive Spherules in Phloem of *Microsorium scolopendria* and *Psilotum nudum*

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With 10 Figures

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Summary

Phloem tissues of *Microsorium scolopendria* (*Polypodiaceae*) and *Psilotum nudum* (*Psilotaceae*) were examined with light and electron microscopes. The characteristic refractive spherules in the sieve elements of *M. scolopendria* apparently develop from endoplasmic reticulum-derived cytoplasmic vesicles. In both taxa they have not been observed to be spatially related to plastids or mitochondria. Refractive spherules contain protein and often occur in the peripheral cytoplasm of mature sieve elements. In *M. scolopendria* they also occur in pericycle cells. Significant differences in refractive spherule substructure occur between the two taxa studied.

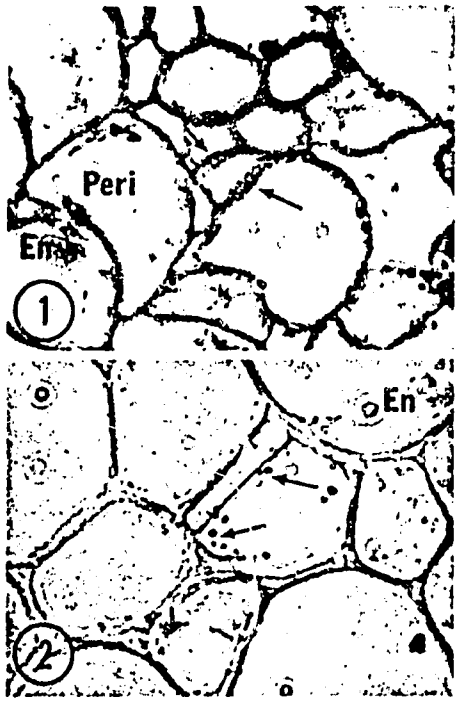
1. Introduction

Sieve elements of vascular cryptogams characteristically contain refringent granules termed refractive spherules. These spherules were reported to be pectic in nature (STILES 1910), mucilagenous (HUME 1912), or proteinaceous (JANCZEWSKI 1882, TERLETZKI 1884, THOMAE 1886, BOODLE 1901, LAMOUREUX 1961, ESAU 1969). JANCZEWSKI (1882) reported that they did not stain with osmium and were not lipoidal. MAXE (1964, 1966) described their appearance in the electron microscope as electron opaque spheres enclosed by a single membrane.

It has been suggested that refractive spherules are plastids, similar to the protein-containing monocotyledon sieve element plastids (STRASBURGER 1891, PERROT 1899, BEHNKE 1965, 1969). However, these suggestions are based on the similarity of staining reaction and appearance under the light microscope. Recently, refractive spherules have been related to the endoplasmic reticulum in origin (LIBERMAN-MAXE 1971). This study was initiated to describe the fine structure of refractive spherules and to trace their development.

2. Material and Methods

Samples 2.5 mm in length were taken from rhizomes of *Microsorium scolopendria* (Burm.) Copel. and aerial stems of *Psilotum nudum* (L.) Griseb. by simultaneous cuts at both ends. Immediately after cutting, the samples were placed in formaldehyde-glutaraldehyde fixative (Karnovsky 1965) and aspirated for 10 minutes. Center portions of the samples were then cut into 1 mm cubes and fixed for an additional 4 hours in fresh fixative at about 0 °C.



Figs. 1 and 2. Photomicrographs of cells showing refractive spherules in *Microsorium* and *Psilotum*. Fig. 1. Refractive spherules in *Microsorium*, stained with mercuric bromphenol blue, occur as dark spots near walls of sieve elements (arrows). Endodermis (*En*) and pericycle (*Peri*) occur to lower left. ×1,200. Fig. 2. Refractive spherules in *Psilotum* stained with mercuric bromphenol blue (arrows). Endodermis (*En*) is to upper right. A pericycle is lacking. ×1,200

Samples were washed with three changes of 1/15 M phosphate buffer (pH 7.2), post-fixed 2 hours in 2% osmium tetroxide in phosphate buffer at about 0 °C, washed with three more changes of buffer, dehydrated with EtOH, and embedded in epon. Thin sections were obtained with a diamond knife in a Sorvall—Porter Blum MT-2B Ultramicrotome and were placed on bare copper grids. Sections were stained with lead citrate (Reynolds 1963) and 2% aqueous uranyl acetate. They were viewed and photographed with a Philips EM-75 (Fig. 5) and a Hitachi HS-8-1 electron microscope of the St. John Plant Science Laboratory Electron Microscope Facility. Thick sections of 6% glutaraldehyde fixed material and free hand sections of fresh material were also subjected to a variety of histochemical tests as described below.

Figs. 3 and 4. Electron micrographs showing organelles in sieve cells of *Microsorium*. Fig. 3. Refractive spherules (*r*), endoplasmic reticulum (*er*), and dictyosomes (*d*) in developing protophloem sieve cell. ×14,000. Fig. 4. Dictyosome vesicles (*dv*), plasma membrane (*pm*), refractive spherules (*r*), plastids (*p*), and mitochondria (*m*) in developing metaphloem sieve cell. ×36,000

Figs. 3 and 4

3. Observations

The refractive spherules of *M. scolopendria* vary in diameter from 0.1 to 0.6 μm. The average size from 50 measurements was 0.35 μm. In mature sieve elements they occur predominantely in peripheral regions of the cell (Fig. 1). Histochemical tests of these spheres with Sudan IV for lipids, ruthenium red stain for pectic substances, and the periodic acid-Schiff's base reaction for polysaccharides (JENSEN 1962) were negative. A weak osmium reaction is probably with the proteins which are easily detected with the mercuric bromphenol blue (MAZIA, BREWER, and ALFERT 1953) reaction (Fig. 1).

In the electron microscope the refractive spherules appear as electron dense regions surrounded by a single membrane. During development the membrane may appear irregularly shaped (Figs. 4–6). The electron dense core appears granular and in most observations is spherical in shape (Figs. 3–6). Irregularly shaped cores were observed, but not shown, in young metaphloem sieve elements and they occurred only infrequently.

Plastids, mitochondria, and dictyosomes have not been observed spatially associated with the developing spherules (Figs. 3–6). In most cells refractive spherules appear in association with the endoplasmic reticulum. In some sections the membrane surrounding the refractive spherules appears continuous with the endoplasmic reticulum (Figs. 5 and 6). During development refractive spherules may occur throughout the cell (Figs. 3–5). However, in mature sieve elements they are almost entirely restricted to the peripheral cytoplasm and occur in greater numbers in regions adjacent to the pores (Fig. 9).

Mature refractive spherules of protophloem and metaphloem appear similar in structure. They are composed of an electron dense spherical core with a single surrounding membrane. The core may be crystalline; however, no distinct crystalline arrangement has been observed. Refractive spherules which are present in pericycle cells differ from above: they appear as electron dense regions bounded by a single membrane (Figs. 7 and 8), but the core is often not entirely spherical and is less electron dense than those of sieve elements.

The refractive spherules of *P. nudum* are larger than those in *M. scolopendria*. They range in diameter from 0.5 to 2.0 μm, with the average size, for

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Figs. 5–8. Electron micrographs of refractive spherules in *Microsorium*. Fig. 5. Immature metaphloem sieve element. Refractive spherules (*r*) are associated with the endoplasmic reticulum (*er*), but not with plastids (*p*) or mitochondria (*m*). Dictyosomes (*d*) and lipid droplets (*L*) are also present. ×16,500. Fig. 6. Section of maturing metaphloem sieve element showing association between refractive spherules and endoplasmic reticulum (arrows). ×30,000. Fig. 7. Refractive spherule in pericycle cell. Protophloem is mature at this stage. ×27,000. Fig. 8. Refractive spherule in pericycle cell. Metaphloem is mature at this stage. ×45,000

Figs. 5–8

50 measurements, 0.8 µm. There also appear to be fewer refractive spherules per sieve element in *P. nudum* and sections as in Figs. 2 and 10 with few refractive spherules are frequently observed. Results of histochemical tests of these spherules were identical with those for *M. scolopendria*, with a strong positive reaction for the mercuric bromphenol blue test for proteins (Fig. 2).
The refractive spherules in *P. nudum* appear to have a halo of less electron opaque material surrounding the electron dense core (Fig. 12). This type of structural arrangement of the core has not been observed in *M. scolopendria*. The developmental relationships of refractive spherules to the endoplasmic reticulum was not established for *Psilotum*. However, they have never been observed spatially related to plastids, mitochondria, or dictyosomes.

4. Discussion

The refractive spherules in *M. scolopendria* and *P. nudum* do not appear to be spatially related to plastids, mitochondria, or dictyosomes. In *M. scolopendria* they appear to develop from specialized regions of the endoplasmic reticulum. This finding is in agreement with Liberman-Maxe (1971) who described the development of refractive spherules from dilated tips of endoplasmic reticulum in *Polypodium vulgare*. The refractive spherules in *M. scolopendria* are also similar in structure to those of *P. vulgare* which is in the same family, *Polypodiaceae*. Refractive spherules and monocotyledon sieve element plastids do not appear to be related. However, structures observed in leptoides of *Polytrichum* (Hebant 1970, Esau 1969) may be similar.
It appears that refractive spherules may differ in substructure among the taxa. Indeed, the refractive spherules in *Psilotum* are different from those of *Microsorium*. Further study of refractive spherules of different taxa may reveal further differences among the vascular cryptogams and mosses. However, the basic structure of an electron dense core enclosed in a single unit membrane will probably be constant.
Refractive spherules are probably not homologous to phloem protein. However, it appears significant that all phloem sieve elements contain a protein in either the fibrillar form (angiosperms, gymnosperms) or a spherical form (vascular cryptogams). In angiosperms, spiny vesicles appear to be related to phloem protein development (Newcomb 1967, Cronshaw and Esau 1968,

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Figs. 9 and 10. Electron micrographs of refractive spherules in *Microsorium* and *Psilotum*. Fig. 9. Refractive spherules in pore region between two mature sieve cells of *Microsorium*. ×50,000. Fig. 10. Sieve cells of *Psilotum nudum* with open pores. Note refractive spherules (*r*), mitochondrion (*m*), plastid (*p*), and cell wall (*w*). The plastid contains a granum. ×16,000. Fig. 10*a*. An enlarged area of Fig. 10 showing a refractive spherule under higher magnification. Note the difference in structure of this refractive spherule from those shown in Fig. 9. ×60,000

Figs. 9 and 10

STEER and NEWCOMB 1969, ESAU and GILL 1970 a). However, this relationship may not always be consistent (ESAU and GILL 1970 b). The spiny vesicles may arise from dictyosome vesicles (NEWCOMB 1967, CRONSHAW and ESAU 1968) or possibly from the endoplasmic reticulum (NEWCOMB 1967, STEER and NEWCOMB 1969). WOODING (1969) has also observed P-protein within the endoplasmic reticulum. The relationship between refractive spherules and the endoplasmic reticulum in *Microsorium* and *Polypodium* (LIBERMAN-MAXE 1971), in all probability, merely reflects a similar process of protein synthesis related to the endoplasmic reticulum. If the refractive spherules in *Microsorium*, *Psilotum*, and *Polypodium* correspond to the phloem proteins of higher plants, they obviously assume a different form.

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