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Calcium Oxalate Bipyramidal Crystals on the Basidiocarps of Geastrum minus (Lycoperdales)¹

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Geastrum minus (Persoon) Fischer is a temperate-zone, litter-associated fungus that displays bipyramidal crystals of calcium oxalate dihydrate (weddelite) on the peridial surfaces of its basidiocarps. The chemical nature and habit of the crystals were determined by X-ray diffraction and elemental energy dispersive analyses, and scanning electron microscopy. This study represents the first known report of calcium oxalate crystals being associated with Geastrum and extends the list of soil, litter, and wood-rot fungi that produce crystals during some stage of their life cycles. The occurrence of crystal-forming fungi in these micro-environments is intriguing for a variety of reasons that include their involvement in soil formation, soil fertility, and retention and/or cycling of elements necessary for plant growth.

INDEX DESCRIPTORS: calcium oxalate, weddelite, Geastrum, fungus, basidiomycetes, crystals.

Many fungi (Hodgkinson, 1977), a few lichens (Jackson, 1981), and myxomycetes (Schoknecht and Keller, 1977) produce calcium (Ca) oxalate (CaC₂O₄·n H₂O) crystals during some phase of their life cycle. The occurrence of these crystals, especially among organisms associated with dead and decaying organic matter or the soil/litter environment, is receiving increasing attention (Table 1) because of the potential value of the oxalate in Ca retention and cycling in the microenvironment in which the fungus occurs.

The pathways of oxalate biosynthesis have been described for a number of fungi (Hodgkinson, 1977). However, it is uncertain as to whether the oxalate is excreted into the environment where it combines with Ca or whether the Ca oxalate crystals are entirely a product of intracellular processes. Graustein et al. (1977) and Crumack et al. (1979) suggested in their studies that the fungi excreted oxalate into the soil water where it combined with Ca to form aggregates of crystals external to the hyphae. In contrast, Arnott (1982), Horner et al. (1983), and Arnott and Webb (1983) working with leaf-litter and wood-rot fungi, showed evidence for intracellular formation of the Ca crystals. The results of these three studies are consistent with abundant evidence for intracellular origin of similar crystals in a variety of higher plants (Arnott and Pautard, 1970, Franceschi and Horner, 1980).

How microorganisms such as fungi and bacteria help to cycle Ca in the environment, is an intriguing question. Furthermore, it is important to understand the extent of oxalate-forming fungi in the soil and litter environments in order to assess their contribution to Ca cycling. The present study adds another fungal species to the growing list and provides new information about the habitat (outward form) of the crystals associated with Geastrum, a temperate-zone litter-associated fungus, probably with a worldwide range (Cunningham, 1942).

MATERIALS AND METHODS

Collections — Six collections (Table 2, A.-F.) of dry, mature basidiocarps of Geastrum (Geastrum; small earth star) observed in this study were all identified as G. minus (Persoon) Fischer (Cunningham, 1942). Two of the collections, representing the basis for this study, were collected by one of the authors (L.H.T.) in the Loess Hills area of western Iowa: A. Tyson Bend, Harrison Co., (1982); B. Waubonsie State Park, Fremont Co., (1983). Both collections of basidiocarps, formed the previous fall, were made from prairie sites in late May. The remaining four collections were obtained from the fungal herbarium at the Department of Botany, University of Iowa, and include two personal collections of T. H. McBride: one from Iowa (C.) and one from South Dakota (D.). The other two collections (E. and F.) were made in Europe. The location and previous taxonomic identifications of these four collections are included in Table 2.

Fifty-three partial or complete basidiocarps (Table 2) were observed with a dissecting microscope for the presence of crystals. Locations of crystals on the basidiocarps were identified, recorded (Table 2), and photographed via light microscopy (LM) and scanning electron microscopy (SEM). Regions of the basidiocarps are designated in Figure 1.

Microscopy. — The endoperidia and exoperidia were observed and photographed with a dissecting microscope (Wild, M7A) fitted with fiber optics and an automatic 35 mm camera. Kodak Techpan film was used to record the images. For SEM, both endoperidia and exoperidia were mounted on brass disks with double-stick tape and silver paint, coated with approximately 15 nm gold-palladium in a Polaron #5100 sputter coater, and observed and photographed with a JEOL JSM-35 SEM and Polaron Type 665 film. The interior of the endoperidium was exposed by handsectioning with a razor blade. The calcium portion of the crystals was identified and mapped with a Kevex X-ray energy dispersive system attached to the SEM.

X-ray diffraction analyses — Crystals were scraped from the peridia of a relatively unweathered specimen from Collection B., mounted in a 114 mm Debye-Sherrer X-ray powder diffraction camera, and exposed to 7 hours of nickel-filtered CuKα radiation generated at 20 mA and 40 kV. Standardized samples of weddelite (cod) from Begonia sanguinea (Horner and Zindler-Frank, 1982) and whewellite (com) isolated from Quilaja (Zindler-Frank, unpublished) were analyzed in the same way. Visual comparison was made among the lines on the three negatives and d-spacings of diffraction patterns were measured and compared with American Society for Testing Materials (ASTM) X-ray standard file cards 17-541 for cod and 20-231 for com.

RESULTS

All six basidiocarp collections (A. through E.) from diverse geographical locations, displayed crystals (Table 2). The most common location was on the outer surface of the endoperidium, followed by peristome, the inner exoperidium, and the stalk (see Fig. 1). There were no crystals on the interior of the endoperidium, and the gelatin region contained only the capillitium and masses of cinnamon to brown, warted spores with an average diameter of 4.5 μm. The spore characters as well as the other morphological details of the basidiocarp, including the presence of the crystals, supports our

¹ This study is dedicated to the memory of Dr. Jessie Augusta Parish, on the twentieth anniversary (1960-1985) of the gift of her farm to the Iowa Academy of Science (see Iowa Acad. Sci. Proc. 82:33-46, 1975). Her hands, mind, and heart captured the Iowa Geastrum in her original drawings as a young student under the tutelage of Prof. T.H. Macbride. Department of Botany, Iowa City (Macbride, T.H. 1912. Mycologia 4:84-86; plate LXII).
Table 1. Scanning Electron Microscopic Studies Demonstrating Presence of Calcium Oxalate Crystals Associated with Soil, Leaf-Litter, and Wood-Rot Fungi.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Microenvironment</th>
<th>Location on Fungus</th>
<th>Ca Oxalate cod/com*</th>
<th>Author(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basidiomycetes</td>
<td>Gasteromycetes</td>
<td>soil</td>
<td>hyphae</td>
<td>cod and/or com</td>
</tr>
<tr>
<td></td>
<td>Hymenogastrales</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lycoperdales</td>
<td>Geastrum minus</td>
<td>litter</td>
<td>basidiocarp peridia</td>
<td>cod</td>
</tr>
<tr>
<td>Unnamed</td>
<td>soil hyphae</td>
<td>cod</td>
<td></td>
<td>Arnott and Webb (1983)</td>
</tr>
<tr>
<td>Ascocormes</td>
<td>Discomycetes</td>
<td>litter</td>
<td>sterile hairs of apothecia</td>
<td>cod</td>
</tr>
<tr>
<td>Helatales</td>
<td>Cunninghamamella echinulata</td>
<td>culture</td>
<td>asexual spores</td>
<td>cod</td>
</tr>
<tr>
<td></td>
<td>Mucor hiemalis</td>
<td>soil/leaf litter, culture</td>
<td>sporangia</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Mucor mucedo</td>
<td>culture</td>
<td>zygospores and sporangiophores</td>
<td>cod</td>
</tr>
<tr>
<td>Mucor plumbeus</td>
<td>culture</td>
<td>sporangiophores</td>
<td>cod</td>
<td>Jones et al. (1976)</td>
</tr>
<tr>
<td>Rhizopus oryzae</td>
<td>soil/leaf litter, culture</td>
<td>sporangia</td>
<td>—</td>
<td>Powell and Arnott (1984)</td>
</tr>
<tr>
<td>Unnamed</td>
<td>leaf litter</td>
<td>hyphae</td>
<td>cod</td>
<td>Arnott (1982)</td>
</tr>
</tbody>
</table>

* cod = weddellite or dihydrate form; com = whewellite or monohydrate form.

Table 2. Presence and Location of Crystals on Basidiocarps of Geastrum minus (Persoon) Fischer

<table>
<thead>
<tr>
<th>Collection</th>
<th>No. Basidiocarps observed</th>
<th>Percent of Structures Bearing Crystals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Outer endo-periderm</td>
<td>Inner exo-periderm</td>
</tr>
<tr>
<td>A Tyson Bend, Harrison Co., Iowa 1982</td>
<td>31</td>
<td>(30) 97%</td>
</tr>
<tr>
<td>B Waubonsie State Park, Fremont Co., Iowa 1983</td>
<td>2</td>
<td>(2) 100%</td>
</tr>
<tr>
<td>C (G. juniperinus Mach.) Scott Co., Iowa 1909 under juniper trees</td>
<td>4</td>
<td>(4) 100%</td>
</tr>
<tr>
<td>D (G. minus Schw.) Black Hills, S. Dak. 1897</td>
<td>11</td>
<td>(10) 91%</td>
</tr>
<tr>
<td>E (G. quadrifidum Nees) Europe, &lt;1900, J.B. Ellis</td>
<td>4</td>
<td>(3) 75%</td>
</tr>
<tr>
<td>F (G. fornicatus (Huds.) Fr.) Europe, 1891, J.B. Ellis</td>
<td>1</td>
<td>(1) 100%</td>
</tr>
<tr>
<td>TOTAL = 6</td>
<td>53</td>
<td>(50) 94%</td>
</tr>
</tbody>
</table>

1 All of these collections, as well as the Fungal Herbarium, Department of Botany, University of Iowa mentioned in Materials and Methods, are now permanently housed in the herbarium in the Department of Botany, Iowa State University.
identifications of all specimens of the collections as Geastrum minus (Persoon) Fischer (Cunningham, 1942).

A comparison of X-ray diffraction patterns (Fig. 2) of the crystals from Geastrum minus (collection B; G in Fig. 2), Begonia sanguinea (Begoniaceae) (B in Fig. 2), and Quillaja sp. (Rosaceae) (Q in Fig. 2) confirms that the G. minus crystals are Ca oxalate dihydrate (cod, weddelite). All of the crystals from basidiocarps of the six collections have the distinct bipyramidal habit characteristic of weddelite. Therefore, specimens from all six collections (A - F) possess Ca oxalate dihydrate crystals.

When observations of all of the basidiocarps are summarized, the crystals occur over the outer surface of the endoperidium, the outer peristome surface, the stalk, and the inner surface of the exoperidium. Crystals therefore occur at the interface between the exo- and endoperidia which were in contact with each other and possibly joined in early development.

Figure 3 shows the outer surface of the endoperidium and the peristome. The white, ivory, or brown surface is studded with single crystals and aggregates of crystals which are distinct bipyramids (Fig. 4). Some crystals penetrate each other (Fig. 5). All crystals display surface cracks or linear pitting which we interpret as partial decomposition due to weathering. Sometimes crystals are in an advanced state of decomposition and their interior is exposed (Figs. 6 and 7).

The crystals on the outer endoperidium either are external to all hyphae (Fig. 4) or are at most covered by a few strands of hyphae (Fig. 5). The small spherical bodies in Fig. 4 are spores that have been released through the peristome and occur everywhere on the basidiocarp (see later). Infrequently, other larger spherical bodies of unknown origin and chemical composition appear among the crystals and dispersed spores (Fig. 8).

The mature peristome consists of radially-oriented hyphae (Fig. 9) and is bordered by a distinct collar. Both regions usually exhibit crystals and spores (Figs. 10 and 11) which are on the surface of the hyphae. Crystals are less frequent there, and sometimes absent (Table 2); when present they display the same bipyramidal habit and pitted surface texture (Fig. 12). Radial aggregates of thin, platelike crystals are occasionally seen (Fig. 13) and are much smaller than the bipyramidal crystals.

Most of the basidiocarps had short stalks (≤ 1 to 2 mm in length) which were relatively free of crystals (Table 2) but, in two collections (D and E.), the stalks were covered with crystals.

The inner surface of the exoperidium did not have distinct hyphae as did the endoperidium, but appeared as a featureless substrate studded with microspores. Bipyramidal crystals with a more weathered appearance typically covered the inner exoperidium (Fig. 14).

Fig. 2. X-ray diffraction patterns for two hydration forms of Ca oxalate; cod = dihydrate (weddelite); com = monohydrate (whewellite). Three patterns are from crystals of Begonia sanguinea = B, Geastrum minus = G (Collection B); Quillaja sp. = Q.

The crystals appeared to be partially embedded (Fig. 15) and gave positive elemental X-ray peaks for Ca (Fig. 16). Crystals were not present on the inner surface of the exoperidium of some specimens (Table 2; i.e., collections A and B.).

The compactness of the hyphae (Fig. 17) of the endoperidium is evident in a longitudinal razor blade section. They are very closely associated in the outer surface of the endoperidium along with the crystals and less compact on the inner surface of the endoperidium in connection with the capillitium. Masses of spores occurred throughout the capillitial matrix (Fig. 18) where no crystals or other recogniz-

Fig. 3. Entire endoperidium and peristome of basidiocarp. Crystals are visible over entire surface. Collection B. 10X.

Fig. 4. Bipyramidal crystals on middle portion of outer endoperidium. Small spheres (arrowheads) are dispersed spores. Outer endoperidium is composed of distinct hyphae. Collection B. 443X.

Fig. 5. Bipyramidal crystals on upper portion of outer endoperidium. Two crystals have penetrated each other during growth. Fits and cracks are due to weathering. Collection B. 900X.

Fig. 6. Edges of severely weathered bipyramidal crystals on peristome. Collection A. 2600X.

Fig. 7. Enlargement of Fig. 6 showing pattern of weathering of larger crystal. Weathered internal structure is different from that observed on surfaces of crystals. Collection A. 8600X.

Fig. 8. Portion of middle endoperidium with infrequently-observed spherical object near lower right corner. Bipyramidal crystals and spores are also visible. Collection B. 1200X.
CALCIUM OXALATE BIPYRAMIDAL CRYSTALS
The soil organic materials may be associated with the separation of Ca oxalate dihydrate. Even though Cunningham (1942) described "minute glistening particles" covering the endoperidium of *G. minus*, neither he nor any previous investigator mentioned that they were crystals or that they were Ca oxalate. Quite often the crystals found associated with fungi are very small and they usually develop only during one stage of the life cycle (see Table 1). White crystalline substances often have been misidentified as Ca carbonate (Scholte and Kellner, 1977). Pobequin (1954), however, states that Ca oxalate is commonly produced by all organisms and occurs in and on cells of fungi belonging to Ascomycetes, Basidiomycetes, and Zygomycetes.

In addition to fungi from soil/litter/wood-rot environments, a pathogenic fungus, *Sclerotium rolfsii*, has been reported to produce Ca oxalate and has been studied both in culture and in infected tissue (Punja and Jenkins, 1984). Crystals formed along the infecting hyphae and were attributed to the ability of the fungus to produce oxalic acid which, in turn, sequestered calcium from the host to form the crystals. The authors hypothesized a role for oxalic acid in promoting pathogenesis.

*Geastrum minus* is a litter inhabitant, presumably associated with soil organic materials. Observations of 53 samples from six collections show that the crystals formed by this fungus are specifically located at the interface of the exo- and endoperidium of the basidiocarp. The absence of crystals in certain samples is possibly a result of extreme weathering before they were collected, since crystals were pitted or deeply eroded in all other collections we examined.

Unfortunately, young, developing basidiocarps were not available and, as a result, the origin and development of the crystals have not been studied. The question of whether the crystals were an intracellular product, as in most other plants (Arnott, 1982; Arnott and Webb, 1983; Hornet et al., 1983; Franceschi and Hornet, 1980) remains unanswered.

We believe that the location of the crystals between the two peridia may be associated with the separation of the peridium during basidiocarp development. This is an extension of the suggested role of crystals and crystal idioblasts in the formation of air spaces in *Epiba* leaves (Kausch, 1980; Kausch and Hornet, 1981) and in other aquatic plants (Hornet and Franceschi, 1978; Franceschi and Hornet, 1980).

Scholte and Kellner (1977) found Ca oxalate crystals associated with the peridia of certain members of the slime mold genera *Perichaeta* and *Diasmena*. All of the species they examined exhibited Ca deposits on the peridia. The researchers related variations in total Ca and other elements to environmental conditions, primarily weathering. The substrates in most of these collections were bark and decaying materials such as tobacco stalks and rabbit dung.

The identification of Ca oxalate crystals on *Geastrum minus*, a fungus producing basidiocarps at the soil surface, adds this species to a rapidly growing list of fungi that are important biological components of soil and organic litter. The Ca oxalate-producing fungi, along with other microorganisms involved in oxalic acid and Ca oxalate production and/or degradation, may be very important contributors to soil formation, soil fertility, and retention and/or cycling of elements necessary for plant growth. Graustein et al. (1977) and Cromack et al. (1979) emphasized this for *Hysterangium crassum*, the soil fungus they studied. Plant and fungal generated oxalic acid which has the ability to degrade primary minerals and bind with various cations in forest soils deserves further investigation.

All of the Ca oxalate produced by the soil/litter/wood rot fungi has been identified as the dihydrate form (weddelite, Table 1), with two exceptions (Graustein et al., 1977). The diversity of crystal shapes produced by these fungi suggests the influence of additional factors beyond environmental (physical and chemical) ones. Crystals occur as bipyramidal in *Geastrum* (this study) and *Sclerotium* (Punja and Jenkins, 1984); in aggregates of crystals called druses in *Hysterangium* (Graustein et al., 1977, Cromack et al., 1979), *Dasycypha* (Hornet et al., 1983), and an unnamed fungus (Arnott and Webb, 1983); as rod-shaped crystals in an unnamed fungus (Arnott, 1982), and as small spine-like crystals in *Mucor* (Jones et al., 1976; Urbanus et al., 1978; Powell and Hornet, 1984), *Cunninghamella* (Jones et al., 1976), and *Rhizopus* (Powell and Hornet, 1984). This diversity of crystal shapes is repeated and enlarged upon in the higher plants where crystal formation is an intracellular process that appears to be under genetic control (Kausch and Hornet, 1984) which, in turn, may influence the presence of certain ions and substances that could alter crystal shape (Cody and Hornet, 1984). The significant point, however, is that each organism produces characteristic crystals in a manner that is not disruptive to itself. How these factors (i.e., genetic, physical, chemical) relate the organism to its environment, especially with regard to the fungi discussed here, is intriguing and suggests that this entire microcosm warrants further study of the roles of oxalate and Ca oxalate crystals.

ACKNOWLEDGEMENTS

The authors wish to thank the Graduate College for partial support of this study. Aspects of this study were carried out in the Bessy Microscopy Facility in the Department of Botany and the X-ray Diffraction Laboratory in the Department of Earth Sciences. We thank Drs. George Knaphus and Nels Lersten for reviewing the manuscript and Mrs. Lillian Johnson for typing it.

**Fig. 9.** Peristome of basidiocarp. Hyphae are arranged in radial fashion. Collar surrounds peristome. Collection B. 24X.

**Fig. 10.** Middle portion of peristome. Bipyramidal crystals and spores are evident. Collection A. 200X.

**Fig. 11.** Portion of collar around peristome. Variously-sized bipyramidal crystals occur on peristome, collar, and endoperidium. Collection A. 180X.

**Fig. 12.** Weathered bipyramidal crystals on peristome. Collection A. 1600X.

**Fig. 13.** Aggregate of thin, platelet crystals found among bipyramids on peristome. These aggregates were infrequent. Collection A. 4400X.

**Fig. 14.** Bipyramidal crystals on inner exoperidium. Crystals vary in size and typically appear more corroded than those on endoperidium. Collection A. 120X.
REFERENCES


Fig. 15. Enlarged region from Fig. 14 showing severely weathered bipyramidal crystals. These crystals appear partially embedded in inner surface of exoperidium. This surface does not consist of visible hyphae as does endoperidium (see Fig. 4). Collection A. 440X.

Fig. 16. Same region and orientation as Fig. 15. X-ray elemental map for Ca. Bipyramidal crystals show positive Ca identification. Collection A. 440X.

Fig. 17. Razor blade section through endoperidium exposing its internal structure. Endoperidial wall consists of compact hyphae. Bipyramidal crystals are visible on outer surface and threads of capillitium extend from inner surface. Collection B. 495X.

Fig. 18. Portion of capillitium containing many spores. Collection B. 500X.

Fig. 19. Many released spores occur over peristome and outer surface of endoperidium. Spores average 4.5 µm in diameter. Collection B. 1200X.

Fig. 20. Spherical spores with warty surfaces. Collection B. 3600X.