mechanisms in doubt. The best previously reported evidence for fossilized pollination droplets is found in the seed fern ovule *Pachytesta* (Medullosaceae), where a resinous-appearing substance has been reported in two species (*4*). Unfortunately, this substance does not extend from the micropyle of either ovule, but is confined to a small area at the tip of the pollen chamber. Also, no pollen or spores are trapped within the substance in either ovule. If the material in *Pachytesta* does represent a pollination droplet, then it must be the postpollination remnant of what was formerly a much larger, externally protruding exudate. There is, therefore, no conclusive evidence that pollination droplets similar to those of extant gymnosperms characterize Paleozoic seed plants.

Within the Callistophytaceae, a seed fern family of Pennsylvanian age, some remarkable reproductive aspects have recently been documented. These include microsporogenesis, microgametophyte development, ovule and megagametophyte development, and ultrastructural features of the pollen exine (*5*, *6*). The presence of pollen within the pollen chambers of immature ovules assignable to *Callospermarion* (*6*) indicates that pollination occurred relatively early in the Callistophytaceae, as it does in many extant gymnosperms. In addition, the occurrence of a pollen grain with a branched pollen tube within the pollen chamber of a Middle Pennsylvanian *Callospermarion*-type ovule (*7*) supports the proposal that these grains were potentially functional, rather than chance contaminants.

An additional immature *Callospermarion*-type ovule from Middle Pennsylvanian sediments (Illinois number 6 coal, Carbondale Formation) has recently been discovered with a noncellular substance extending from the micropyle (Fig. 1A). The substance is 0.51 mm long and expands in diameter at increasing distances from the outer orifice of the micropylar canal (Fig. 1B). It is convoluted and quite dark at the periphery, and several palynomorphs (isolated pollen and spores) either are embedded in or adhering to the surface of the substance (arrows in Fig. 1B).

Small size (1.7 mm long) and relatively undifferentiated integument are features consistent with immature ovules of extant gymnosperms at the time of pollination and with the previously proposed developmental sequence for *Callospermarion* (*6*). However, other features of the specimen are not what one would expect of a normally developing *Callospermarion* ovule at the time of pollination. The nucellus is only one cell layer thick and the sclerotestal cells are fully differentiated at the apex (Fig. 1A), features typically associated with more mature ovules. Also, none of the palynomorphs within the droplet conform to the genus *Vesicaspora*, pollen of the Middle Pennsylvanian Callistophytaceae (*8*) thought to be that associated with this ovule. Interpretation of the structure as a pollination droplet must therefore rely on the presumption that the specimen does not exhibit the features of a normal ovule. Since one would expect normally developing ovules to have matured into seeds that were subsequently destroyed by the developing embryo, only ovules that have had their developmental sequence disrupted would remain to be fossilized. With respect to the specimen at hand, the apparent discrepancy in developmental completeness of the various features is what one could expect of an abortive ovule.

By comparison with the physical and developmental features of extant gymnosperm ovules and those known to occur in *Callospermarion pusillum* (*6*), the following sequence of events can be interpreted as having led to the preservation of this specimen with a recognizable pollination droplet. The ovule presumably matured in the normal fashion up to the time when a pollination droplet was produced. At that point, because of either the absence of the appropriate pollen (*Vesicaspora*) or other unknown factors, ovule abortion occurred. Before metabolic activity ceased completely, the nucellus was reduced in thickness and the sclerotestal cells in the micropylar region differentiated and became sclerified. At the same time the pollination droplet shrank and partly collapsed due to desiccation. This was quickly followed by fossilization of the specimen.

The available evidence does not demonstrate whether pollen was drawn into the pollen chamber by shrinkage of the droplet, or the grains floated upward into the micropyle of the inverted ovule with the aid of the saccus (*2*). The discovery of a structurally preserved pollination droplet in a *Callospermarion*-type ovule does, however, document the occurrence of this type of reproductive mechanism among Paleozoic seed plants and supports the proposal that some early seed-fern ovules followed developmental and reproductive sequences similar to those of extant gymnosperms.

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References and Notes

1. A. Foster and E. Gifford, *Comparative Morphology of Vascular Plants* (Freeman, San Francisco, ed. 2, 1974), pp. 429, 455, 512–515, and 537–538.
2. J. Doyle, *Sci. Proc. R. Dublin Soc.* **24**, 43 (1945).
3. F. Oliver and E. Salisbury, *Ann. Bot.* (*London*) **25**, 1 (1911); R. Graham, *Bot. Gaz.* (*Chicago*) **95**, 453 (1934); G. Rothwell, *Palaeontographica* **131B**, 167 (1971).
4. T. Taylor, *Palaeontographica* **117B**, 1 (1965).
5. J. Hall and B. Stidd, *Palaeontology* **14**, 431 (1971); M. Millay and D. Eggert, *Am. J. Bot.* **61**, 1067 (1974); M. Millay and T. Taylor, *Palaeontographica* **147B**, 75 (1974).
6. G. Rothwell, *Am. J. Bot.* **58**, 706 (1971).
7. ______, *Science* **175**, 772 (1972).
8. M. Millay and D. Eggert, *Am. J. Bot.* **57**, 50 (1970).

8 August 1977; revised 11 October 1977

Calcium Oxalate: Occurrence in Soils and Effect on Nutrient and Geochemical Cycles

Abstract. *Whewellite and weddellite, calcium salts of oxalic acid, have been found in the litter layer of several different soils, indicating that oxalate is a major metabolic product of fungi in natural environments. The presence of oxalate in soil solution speeds weathering of soil minerals and increases the availability of nutrients to vegetation.*

The minerals whewellite and weddellite, the mono- and dihydrate, respectively, of calcium oxalate, have been considered extremely rare in geologic environments (*1*, *2*) although they are common in plant and animal tissue (*3*). We have found that crystals of weddellite and whewellite adhere to the outer surfaces of fungal hyphae in the litter layer of forest soils from each of five widely separated locations in the United States.

The apparently widespread occurrence of these minerals in contact with percolating solutions has a large effect on biological and geochemical processes in soils because (i) the crystals are a reactive reservoir of calcium for the ecosystem; (ii) even small amounts of oxalate in solution increase the effective solubility of iron and aluminum by several orders of magnitude (*4*, *5*); (iii) oxalate, since it is both the anion of a weak acid and a chelator of iron and aluminum, affects the pH of the soil solution; and (iv) the chelation of iron and aluminum keeps phosphorus available to plant roots, since phosphorus is scavenged from solution by the precipitation of iron and aluminum as hydroxides (*6*).

Samples of both litter layer and soil A horizon were collected from undisturbed forests of Douglas fir (*Pseudotsuga menziesii*), Benton County, Oregon; Engelmann spruce and subalpine fir (*Picea engelmanni* and *Abies lasiocarpa*) in the Tesuque Watershed Study Area (*7*), Santa Fe County, New Mexico; coast redwood (*Sequoia sempervirens*), Sonoma County, California; red pine (*Pinus resinosa*), New Haven County, Connecticut; and mixed oak and maple (*Quercus* and *Acer* spp.), also New Haven County, Connecticut. The New Mexico sample was collected and air-dried in September 1976; all other samples were collected in February and March 1977 and kept in sealed plastic bags.

Hysterangium crassum, a probable ectomycorrhizal basidiomycete, is a dominant fungal species at the Oregon location (*8*). Species were not determined at the other sites.

A portion of each sample was desiccated and examined with an ETEC scanning electron microscope. Euhedral crystals, 1 to 5 μm in length, adhered to all hyphae examined that were taken from the litter layer. The most common habit was a flattened, twinned tetragonal dipyramid (Fig. 1A), which is typical of weddellite (*1*). A variety of other morphologies having apparent monoclinic symmetry were also common (Fig. 1B). Whewellite, which is monoclinic, is known to form several of the observed morphologies (*9*); the reentrant groove along twin planes is distinctive.

Hyphae of *H. crassum* taken from interstices of the A horizon are also covered with well-developed crystals, but hyphae in contact with mineral grains are bare (Fig. 1C). Hyphae taken from the A horizon of soils at other locations are either bare or covered with much smaller crystals than those found in the litter layer.

We analyzed crystals from the New Mexico site with a Kevex energy-dispersive x-ray spectrometer, which detects all elements heavier than neon. Calcium was the only element detected.

Mycelia were separated from a moist portion of each sample, stuffed into a capillary tube, and exposed to copper x-radiation in a Debye-Scherrer camera. Diffraction patterns were obtained from all fungi from litter layers and from *H. crassum* taken from the A horizon. Weddellite is associated with the Oregon and California fungi; whewellite with those from New Mexico and Connecticut. Although a few tetragonal dipyramids typical of weddellite were observed in the electron micrographs of the New Mexico fungi, no single diffraction pattern indicated the presence of both minerals. Some quartz and illite were present in some samples; they were recognized by the spottiness of their diffraction lines. All of the measured lines matched published data for either whewellite or weddellite (Table 1) (*10*).

Table 1. Calculated *d*-spacings from x-ray diffraction pattern obtained from hyphae of the fungus *Hysterangium crassum* compared with standard *d*-spacings for weddellite (*10*). (A portion of an *H. crassum* hypha with attached crystals is shown in Fig. 1A.) The intensity of *H. crassum* lines was estimated by eye (S, strong; M, moderate; W, weak; and V, very). The intensity of the standard pattern was measured photometrically and expressed as a percentage of the strongest line. For standard weddellite all reflections of intensity greater than or equal to 10 percent are listed.

H. crassum		Standard weddellite	
d (Å)	Intensity	*d* (Å)	Intensity
6.23	VS	6.18	100
4.41	S	4.42	30
3.93	M-W	3.91	8
3.64	M-W	3.68	12
3.10	M-S	3.09	10
Missing*		2.82	14
2.778	VS	2.775	65
2.406	S-M	2.408	16
2.223	S	2.243	25
2.118	M	2.118	8
2.021	VW	2.024	6
1.959	M	1.957	10
1.897	M	1.899	16
1.838	M	1.836	10

*Obscured by 2.778?

It is possible that weddellite was present in the New Mexico sample at the time of collection but dehydrated to whewellite before analysis. No change was noted, however, in the diffraction pattern of natural weddellite after vacuum desiccation for 50 hours. Conversion to whewellite did occur after heating at 220°C for 15 hours. The resulting diffraction pattern had much broader peaks than that of natural whewellite. Samples from Connecticut were x-rayed within 24 hours of collection, so it is unlikely that these samples dehydrated.

The sampling sites are diverse climatically as well as geographically. In each environment, fungi exude oxalic acid or oxalate abundantly enough to cause the precipitation of its calcium salt. Calcium accumulations on fungal rhizomorphs are also reported in tropical soils (*11*) which are similar to accumulations described at the Oregon location (*12*). Because calcium oxalate is slightly soluble (solubility product $\approx 10^{-8.5}$) (*13*), we assume that the presence of calcium oxalate crystals implies the presence of oxalate in solution.

Many species of fungi produce oxalic acid in laboratory culture (*14*). Smith (*15*) found oxalate in the root exudate of several species of trees in a northern hardwood forest. Bruckert (*16*) and Bruckert and Jacquin (*17*) found oxalic to be the most abundant low-molecular-weight organic acid present in solution at the base of a mor humus layer. Furthermore, in preliminary work we have found a total oxalate concentration of 1.0×10^{-5} mole/liter in soil solution from the Oregon location. The sample was collected from a depth of 20 cm and analyzed by the gas chromatographic method of Mee

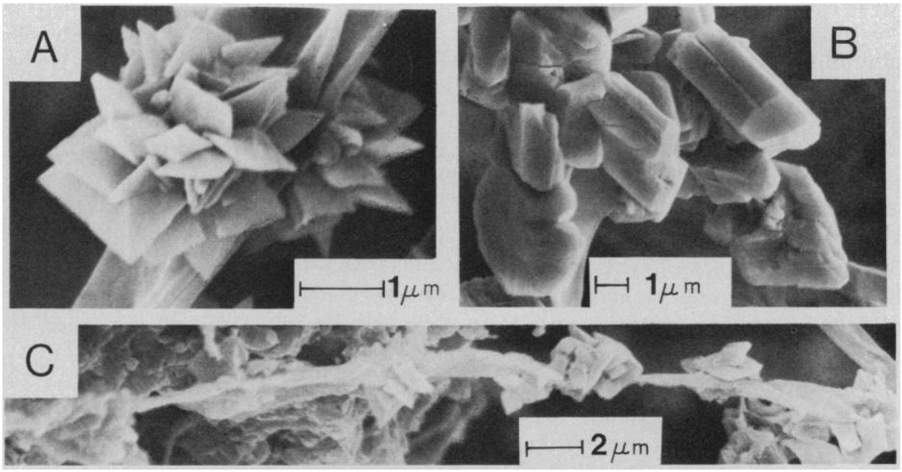

Fig. 1. (A) Crystals of weddellite, $CaC_2O_4 \cdot 2H_2O$, adhering to a hypha of the fungus *H. crassum* taken from the A horizon of a soil developed under Douglas fir in Benton County, Oregon. (B) Crystals of whewellite, $CaC_2O_4 \cdot H_2O$, adhering to fungal hyphae in the litter layer of a soil developed under Englemann spruce and subalpine fir in Santa Fe County, New Mexico. (C) Hyphae of *H. crassum* from the A horizon. Note that crystals are attached to hyphae only in the interstitial space: the hypha in contact with the solid phase of the soil is bare.

and Stanley (*18*). It therefore appears likely that oxalate is commonly a component of soil solutions.

Oxalate is destroyed metabolically by many soil organisms (*12, 19*). As a result, its abundance in soil is largely determined by the difference between rates of formation and destruction and the location where these processes take place.

The unusual chemical properties of oxalate in nonbiologic reactions suggest that it enters into the chemistry of the soil in several ways that affect both mass transport and plant nutrition. As well as forming a sparingly soluble precipitate with calcium, oxalate strongly complexes iron and aluminum. Because these are multiligand complexes, a complete solution-solid equilibrium model is complicated (*20*). The general nature of the interaction can be illustrated by the reaction

$$3H^+(aq) + Al(OH)_3(s) + 2CaC_2O_4(s) = Al(C_2O_4)_2^-(aq) + 2Ca^{2+}(aq) + 3H_2O$$

where aq means aqueous and s solid. The mass-action expression for this reaction has the form

$$[Al(C_2O_4)_2^-] = \frac{K[H^+]^3}{[Ca^{2+}]^2}$$

Thus, the amount of complexed aluminum in solution is sensitive to both pH and calcium activity. If aluminum hydroxide is in the form of microcrystalline gibbsite, the equilibrium constant K is calculated to be $10^{5.2}$, using published stability constants (*4, 13*). Figure 1C suggests the significance of this reaction; oxalate exuded by the hypha in the interstitial space has reacted with calcium in solution to precipitate weddellite, but oxalate exuded near the mineral surface has apparently reacted with aluminum or iron to form a soluble complex.

Production of oxalate thus serves to retain calcium, an essential nutrient required in large amounts, in the rooting zone. Because of their small size, these calcium oxalate crystals will presumably dissolve rapidly, releasing calcium, if vigorous root uptake lowers calcium activity in the soil solution enough for it to become undersaturated with respect to calcium oxalate.

Complexation of aluminum by oxalate tends to increase the supply of potassium, magnesium, and calcium to solution, since the sources of both these nutrients and aluminum are the common silicate minerals. Both kinetic and equilibrium effects are involved: oxalic acid has been shown to attack biotite more rapidly than other low-molecular-weight organic acids or HCl (*21*); if aluminum activity is lowered by chelation, the equilibrium activities of other components of the mineral in contact with the soil solution are raised.

Precipitation of iron or aluminum hydroxide efficiently removes phosphate from solution (*6*). Oxalate in the rooting zone chelates iron and aluminum, inhibiting both their precipitation and the removal of phosphate from solution. If oxalate is destroyed at greater depths, iron and aluminum are liberated to solution. As a result, they will tend to precipitate as hydroxides and trap phosphate from solution into the solid phase of the soil, thus reducing the loss of phosphorus from the ecosystem.

These predicted effects of oxalate are consistent with soil chemistry observed at the New Mexico location, where there is a net production of organic acids in the top 30 cm of the soil. Below that depth, their concentration in solution decreases rapidly. Iron and aluminum are present in solution at concentrations in agreement with those calculated from the model. There is an increase in the iron, aluminum, and phosphorus content of the fine-grained fraction of the soil at the depth where organic anions and trivalent metals are disappearing from solution (*22*).

The reaction of oxalate with an aluminous or ferric silicate or hydroxide to form a soluble trivalent metal complex consumes H^+. If the ligand is later destroyed, the liberated metal will remove OH^- from solution as it precipitates. The effect of transport is to reduce the acidity in the zone of ligand production and to increase it in the zone of ligand destruction.

The magnitude of these complexing effects depends strongly not only on the amount of oxalate in solution but also on the solubility of the soil minerals. The solubilities of both iron and aluminum hydroxides vary by more than two orders of magnitude depending on their crystal form (*4, 23*). At a particular oxalate concentration, we would expect higher solution concentrations of the trivalent metals in soils developed on igneous rocks than in soils developed on oxidized sedimentary rocks (*24, 25*).

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References and Notes

1. J. Marlowe, *J. Sediment. Petrol.* **40**, 499 (1970).
2. P. Leavens, *Am. Mineral.* **53**, 455 (1968); H. Lowenstam, *Science* **162**, 1129 (1968).
3. H. Arnott, in *Biological Mineralization*, I. Zipkin, Ed. (Wiley, New York, 1973), p. 609; C. Frondel and E. Prien, *Science* **95**, 431 (1942).
4. C. Lind and J. Hem, *U.S. Geol. Surv. Water Supply Pap. 1827-G* (1975).
5. W. Norvell, in *Micronutrients in Agriculture*, J. J. Mortvedt, Ed. (Soil Science Society of America, Madison, Wis., 1972), p. 115.
6. P. Struthers and D. Sieling, *Soil Sci.* **69**, 205 (1950); B. Bache, *J. Soil Sci.* **15**, 110 (1964); A. Wild, *ibid.* **1**, 221 (1950).
7. J. R. Gosz, *For. Sci.* **22**, 167 (1977).
8. R. Fogel, *Can. J. Bot.* **54**, 1152 (1976).
9. V. Goldschmidt, *Atlas der Krystallformen* (Winters, Heidelberg, 1923), vol. 9, pp. 42–47.
10. Powder Diffraction File, Joint Committee on Powder Diffraction Standards, Swarthmore, Pa., cards 20-231 and 17-541.
11. N. Stark, *BioScience* **22**, 355 (1972).
12. K. Cromack, Jr., P. Sollins, R. Todd, R. Fogel, A. Todd, W. Fender, M. Crossley, D. Crossley, Jr., in "Soil organisms as components of ecosystems," U. Lohm and T. Persson, Eds. (Proceedings of the 6th International Soil Zoology Colloquium, Stockholm), *Ecol. Bull.* (*Stockh.*), in press.
13. A. Ringbom, *Complexation in Analytical Chemistry* (Interscience, New York, 1963), pp. 324–347; L. G. Sillén and A. E. Martell, *Stability Constants of Metal-Ion Complexes* (Spec. Publ. 17, The Chemical Society, London, 1964); *ibid.*, *Supplement 1* (Spec. Publ. 25, The Chemical Society, London, 1971).
14. J. Foster, *Chemical Activities of Fungi* (Academic Press, New York, 1949).
15. W. H. Smith, *Ecology* **57**, 324 (1976).
16. S. Bruckert, *Ann. Agron.* **21**, 421 (1970).
17. _____ and F. Jacquin, *Soil Biol. Biochem.* **1**, 275 (1969).
18. J. Mee and R. Stanley, *J. Chromatogr.* **76**, 242 (1973).
19. T. Chandra and Y. Shethna, *Antonie van Leeuwenhoek J. Microbiol. Serol.* **41**, 101 (1975).
20. Lind and Hem (*4*) did not consider calcium or iron complexes with oxalate; hence the amount of complexed A1 they calculated is high. Norvell (*5*) assumed a high calcium concentration in solution and therefore calculated a low concentra- of complexed trivalent metals. The amount of complexation is proportional to the second or third power of oxalate activity, and as a result model predictions are very sensitive to assumptions about oxalate activity or concentration.
21. J. Boyle, G. Voigt, B. Sawhney, *Soil Sci.* **117**, 42 (1974).
22. W. C. Graustein, *Geol. Soc. Am. Abstr. Programs* **8**, 891 (1976).
23. D. Langmuir and D. Whittemore, *Adv. Chem. Ser.* **106**, 209 (1971).
24. We have found calcium oxalate associated with two more genera of fungi: weddellite with *Rhizopogon* sp. in mycorrhizal association with western white pine (*Pinus monticola*) in the H. J. Andrews experimental forest, Lane County, Oregon, and whewellite with *Gautieria* sp. under Engelmann spruce at the Benton County, Oregon, location.
25. D. Jones, W. J. McHardy, and M. J. Wilson [*Trans. Br. Mycol. Soc.* **66**, 153 (1976)] have shown that many species in the Mucorales bear external spines composed of weddellite. The absence of tetragonal crystals in these spines suggests that their growth is directly controlled by the fungi, rather than by the nonbiologic precipitation of excreted oxalate with calcium in soil solution that we presume to be the origin of crystals described in this report.
26. We thank the Tesuque Ranger District, Santa Fe National Forest, and the Pacific Northwest Experiment Station of the U.S. Forest Service for their cooperation. R. A. Berner, C. Eberhart, D. K. Fujita, G. R. Holdren, Jr., F. M. McCorison, W. A. Norvell, and J. M. Trappe assisted in sample collection or discussion of results, or both. R. B. Addison, C. Y. Li, J. E. McLane, and A. Pooley provided technical assistance. Portions of this work were supported by NSF grants DEB 74-20744-A02 and DEB-76-10188. Serendipity was funded by NSF grant BMS-75-20324 to the Elemental Cycling Conference. Contribution 294 from the Coniferous Forest Biome, United States–International Biological Program, and contribution 1187 from the School of Forestry, Oregon State University.

21 June 1977; revised 9 August 1977