**AEROBIOLOGIA**
9 (1993), 157 - 167

# Fungal physiology and the formation of calcium oxalate films on stone monuments

DANIELA PINNA

*SUMMARY. Extensive, uniform, yellow-brown films are observed on many monuments. The origin of these films, composed predominantly of calcium oxalate, has been investigated by several authors. Oxalate film formation may be related, in some cases, to the activity of such microorganisms as fungi, which presumably form oxalic acid via the metabolic transformation of organic substances already present on the stone. The present work provides an overview of the physiological factors affecting oxalate synthesis by fungi and of oxalic acid in fungi metabolism.*

*Key words: stone monuments, Ca-oxalate films, oxalic acid, fungi.*

Daniela Pinna, Soprintendenza Beni Artistici e Storici, Via Belle Arti 56, I-40126 Bologna, Italy.

## INTRODUCTION

This investigation deals with the origin of the yellow-brown films observed on many monuments (Fig. 1, 2, 3). The films are extensive, homogeneous and mainly composed of calcium oxalate, both monohydrate (whewellite) and dihydrate (weddellite). According to some authors (Matteini and Moles, 1986; Alessandrini *et al.*, 1989) these films are the result of the transformation of organic substances applied to the stone for protective and/or aesthetic purposes.

Lazzarini and Salvadori (1989) suggest that, in some cases, bacteria and fungi may be responsible for the metabolic transformation of the organic substances on the stone with the final production of oxalic acid (hereinafter referred as OA).

Rossi-Manaresi *et al.* (1989) have demonstrated calcium oxalate formation in exposure experiments; bacteria and fungi found in the samples were probably responsible for the OA production (Ciccarone and Pinna, in press). Calcium oxalate films were produced on marble samples from cultures of *Penicillium oxalicum* (Chiari *et al.*, 1989). These films, formed of fibrous crystals orthogonal to the surface, are on the whole resistant to drastic acid attack (HCl 35%).

Another hypothesis ascribed film formation to a natural cause, i.e. to OA production from lichens which could have colonized the monument surfaces; such colonization would have been favoured by the unpolluted atmosphere of the past (Del Monte and Ferrari, 1989; Del Monte, 1991).

*Figure 1.* Certosa of Pavia. Fragments of Carrara marble sculpture with Ca oxalate films (from G. Alessandrini, Centro CNR «Gino Bozza», Milano).

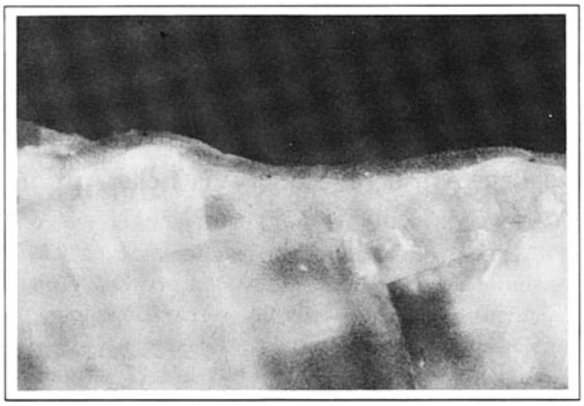

*Figure 2.* Certosa of Pavia. Polished cross section (90x). The colour film is a perfect replica of the marble surface (from G. Alessandrini, Centro CNR «Gino Bozza», Milano).

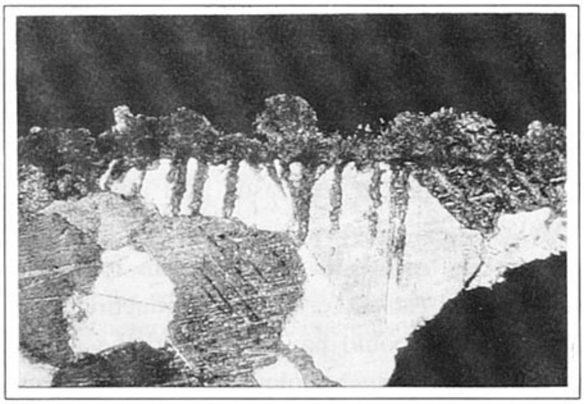

*Figure 3.* Roma. Ludovisi collection, sarcophagus with Paride judgement: thin section, Nicols x (68x). Calcium oxalates in the marble fractures (from M. Tabasso, ICCROM, Roma).

This review is focused on the hypothesis involving fungi metabolism. To obtain a better knowledge of the physiological factors involved in oxalate synthesis in fungi and the function of OA, papers concerning OA and oxalate production in fungi published from 1980 to 1992 have been examined.

## OXALIC ACID SYNTHESIS IN FUNGI

Two pathways of oxalic acid synthesis have been reported (Fig. 4).

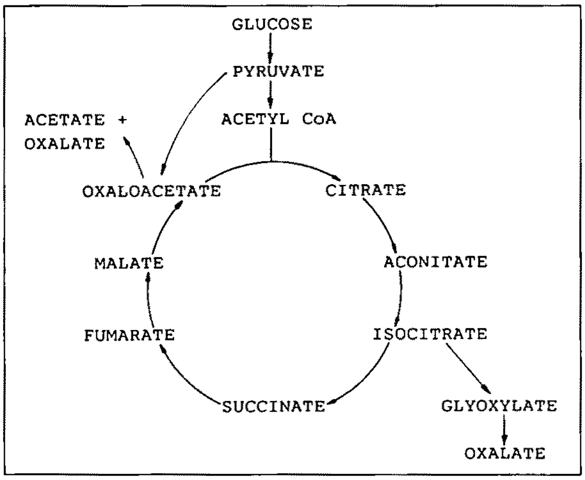

*Figure 4.* Summary of metabolic pathways of oxalic acid synthesis in fungi.

1) The hydrolitic cleavage of oxaloacetate to oxalate and acetate. This mechanism involves the tricarboxylic acid cycle (TCA), since oxaloacetate is one of the products of this cycle. This pathway occurs in several Ascomycetes: *Aspergillus niger* (Mueller, 1986), *Cryphonectria (Endothia) parasitica* (Havir and Anagnostakis, 1985) and *Sclerotinia sclerotiorum* (Maxwell and Bateman, 1968). Kubicek *et al.* (1988) suggest a pathway of oxalate accumulation by *Aspergillus niger* which occurs completely in the cytoplasm and does not involve the TCA. The enzyme responsible for this is oxaloacetate hydrolase which appears to be a cytoplasmic enzyme in

*Aspergillus niger* and is induced by shifting the pH of the culture from 6 to 8. *Aspergillus niger* is able to form oxaloacetate without the reactions of the TCA because it contains a cytoplasmic pyruvate carboxylase. In contrast, Tomoyeda *et al.* (1988) suggest that in *Aspergillus niger* oxalate seems to be yielded via both oxaloacetate and glyoxylate (see below). When pyruvate, citrate, glyoxylate or oxaloacetate were added separately to the growing fungus, oxalate was produced in all media tested. They found enzyme activity indicative of both pathways in the cell-free extracts of the mycelia in the growth culture.

2) Oxalate is produced via citrate (from TCA), isocitrate and glyoxylate. Maxwell and Bateman (1968) studied this pathway in *Sclerotium rolfsii* and showed that the final stage is catalysed by glyoxylate dehydrogenase, the activity of which depends to a considerable extent on the pH of the culture medium. The same pathway has been attributed to *Paxillus involutus* by Lapeyrie *et al.* (1987). This mycorrhizal fungus, growing on a medium containing ammonium, produced consistently low oxalate levels. The authors suggested that in this fungus most oxalate is synthesized via glyoxylate, because ammonium ions inhibited glyoxylate dehydrogenase activity.

The two mechanisms are similar to those described for the plants (review in Franceschi and Horner, 1980). While in the plants there is evidence for another pathway, i.e. the production of OA from L-ascorbic acid, none of the papers examined were concerned with this mechanism.

## SHAPE OF CALCIUM OXALATE CRYSTALS

Studies in literature indicate that calcium oxalate dihydrate (weddellite) is the most common form produced by fungi; only five studies report the production of whewellite (Tab. I).

The factors that determine the crystalline form of the oxalate are not yet clear. According to Punja and Jenkins (1984a) the availability of Ca may influence the morphology and hydratation state of the crystals. Keller (1985) has proposed that crystallization is genetically controlled while Horner *et al.* (1985) have suggested that high pH and a high Ca/oxalic acid ratio are generally necessary conditions for weddellite formation. Under conditions of low pH, weddellite becomes unstable, dissolves and is reprecipitated as whewellite. Rao and Tewari (1987) have suggested that the two forms are related to the amount of water present.

In regard to the mechanism of crystal formation, Horner *et al.*, (1983), Powell and Arnott (1985) and Whitney and Arnott (1986a, 1986b, 1987) have suggested that the crystals are formed within the wall of the hyphae and, as they increase in length, their distal ends protrude through the hyphal wall. This conclusion is in agreement with evidence for intracellular origin of similar crystals in several higher plants (review in Franceschi and Horner, 1980). Whitney and Arnott (1986a) have pointed out that, once the crystal protrudes, it is not clear whether its subsequent growth is the result of calcium and oxalic acid provided from sources within or outside the parent hypha.

## ANALYTICAL METHODS FOR OXALIC ACID AND CALCIUM OXALATE

There are several tests to determine the presence and quantity of OA and Ca oxalate produced by fungi. Polarizing and fluorescence light microscopy, scanning and transmission electron microscopy are used to observe specimens and study the crystal morphology as well as the relationship between fungus and host plant in the case of mycorrhizal fungi.

*Table I.*

| *Fungi* | *Author(s)* | *WED* | *WEW* | *Ca oxalate* |
|---|---|---|---|---|
| *Agaricus bisporus* | Whitney and Arnott (’87) | + | | |
| | Garibova *et al.* (’87) | | | |
| *Aphilloporales* | Keller (’85) | | | + |
| *Aspergillus niger* | Mueller (’86), Tomoyeda *et al.* (’88) | | | + |
| Basidiomycetes (litter fungi) | Horner *et al.* (’91) | + | | |
| *Cristulariella pyramidalis* | Kurian and Stelzig (’79a) | + | | |
| *Cryphonectria parasitica* | Bennet and Hindal (’89, ’90), Havir and Anagnostakis (’83, ’85), Hilman *et al.* (’90) | | | + |
| *Dasyscypha capitata* | Horner *et al.* (’85) | + | | |
| *Geastrum* species | Krisai and Mrazek (’86) | + | | |
| *Gaestrum minus* | Horner *et al.* (’85) | + | + | |
| *Gaestrum saccatum* | Whitney and Arnott (’86b) | + | | |
| *Gilbertella persicaria* | Whitney and Arnott (’86a, ’88) | | | + |
| *Hysterangium crassum* | Cromack *et al.* (’79) | | | + |
| *Inocybe* species | Ceruti *et al.* (’83) | | | + |
| *Irpex lacteus* | Keller (’85) | | + | |
| *Leucostoma cincta* and *L. personii* | Traquair (’87) | + | | |
| *Mucor hiemalis* and *Rhizopus oryzae* | Powell and Arnott (’85) | | | + |
| *Mycena citricolor* | Rao and Tewari (’87) | + | | |
| *Paxillus involutus* | Lapeyrie *et al.* (’87) | | | + |
| | Lapeyrie (’88), Duchesne (’89) | | | + |
| *Penicillium oxalicum* | Ikotun (’84) | | | + |
| | Chiari *et al.* (’88) | | + | |
| *Pleurotus osteatus* | Garibova *et al.* (’82) | | | + |
| Radiomycetaceae (genera *Hesseltinella, Radiomyces)* | Benny and Khan (’88) | | | + |
| *Sclerotinia sclerotiorum* | Marciano *et al.* (’83), Godoy *et al.* (90) | | | + |
| *Sclerotinia trifoliorum* | Callahan and Rowe (’91) | | | + |
| *Sclerotium ceviporum* | Stone and Armentrout (’85) | | | + |
| *Sclerotium rolfsii* | Punja and Jenkins (’84a, ’85) | + | + | |
| | Smith *et al.* (’86) | | | + |
| *Scopuloides hydnoides* | Keller (’85) | + | | |
| Brown rot fungi | Espejo and Agosin (’91) | | | + |
| Forest litter fungi | Arnott (’82) | + | | |
| Fungi from compost litter | Arnott and Fryar (’84) | + | | |
| Fungi in semiarid environments | Verrecchia (’90a, b) Verrecchia *et al.* (’90) | | + | + |
| Mycorrhizal fungus | Snetselaar and Whitney (’90) | + | | |
| Wood rot fungus | Arnott and Webb (’83) | + | | |

X-ray elemental analysis using an EDS microprobe connected with SEM is useful to localize individual elements more specifically. X-ray diffraction and infrared spectrometry are carried out to determine the presence of Ca oxalate crystals and to identify the monohydrate and the dihydrate form. Some authors use nuclear magnetic resonance and mass spectrometry to determine the presence of OA.

The identification of Ca oxalate can also be based on solubility characteristics: KMnO$_4$ titration, positive staining with silver-nitrate dithiooxamide and precipitation by the addition of a calculated quantity of Ca acetate. KMnO$_4$ can also be used to estimate the OA content.

Other useful techniques to detect OA are thin layer and gas chromatography and UV absorption. High performance liquid chromatography can be used to detect the concentration of OA present in the mycelium or in the culture medium. The ability of the species to utilize a certain compound from solution for oxalate synthesis has been demonstrated by labeling the compound (for example NaH$^{14}$CO$_3$) and detecting the labeled element in the OA molecule.

## FACTORS AFFECTING THE SYNTHESIS OF OXALIC ACID AND ITS FUNCTIONS

### Effect of calcium, bicarbonate, nitrate, pH, light

Several papers dealt with the relationship between OA and calcium. OA formation would be a process of biomineralization evolved by fungi and other eukaryotic organisms as a means to deal with high levels of calcium (Kazmierczak *et al.*, 1985).

The hypogeous fungus *Hysterangium crassum* exudes large amounts of OA in the soil (Cromack *et al.*, 1979) which causes more intense weathering of the clay within the fungal mats than in adjacent uncolonized soil. According to Arnott and Fryar (1984), the Ca oxalate crystals produced by forest litter fungi may be important in the long term maintenance of Ca concentration in the soil. Mineral uptake for the surrounding vegetation may be enhanced through the gradual breakdown of the crystals. Whitney and Arnott, examining oxalate crystals in *Agaricus bisporus* (1987) and *in vitro* growth of *Gilbertella persicaria* (1988), suggested that OA production in these two fungi could act to reduce Ca ion concentration in the environment.

Bennet and Hindal (1989) showed that the presence of Ca ions in a substrate stimulates OA production in vitro by some strains of *Cryphonectria parasitica.* At high calcium concentration, growth was reduced but OA synthesis was enhanced. Ca oxalate reduces the Ca cytoplasmic concentration to less than toxic levels and growth continues.

Lapeyrie *et al.* (1987) studied the influence of three ions characteristic of calcareous soils, Ca$^{2+}$, HCO$_2^-$, NO$_3^-$, on oxalate production by the ectomycorrhizal fungus *Paxillus involutus.* Since extremely high concentrations of exogenous Ca$^{2+}$ caused a reduction in fungal growth and a lowering of oxalate concentration, the authors suggested that OA synthesis is not primarily an adaptation to calcium *per se.* Small additions of bicarbonate ions substantially increased OA production when nitrate was present. When Ca was present, most of the OA was associated with the mycelium, probably as Ca oxalate; in the absence of Ca, most of the OA occurred free in the culture medium. Calcium ions could, however, directly stimulate oxalate synthesis to mantain intracellular ion balance. In this case, OA would be involved in osmotic pressure regulation. This study showed that bicarbonate provides a strong stimulus to OA synthesis and, based on this result, it is likely that bicarbonate in calcareous soil is a major cause of oxalate synthesis. The source of nitrogen also was found to be very important in oxalate production. Colonies grown on nitrate produced large quantities of oxalate in comparison to those grown on ammonium. Nitrate assimilation produces $OH^-$ and the resulting proton imbalance could stimulate OA synthesis to control cell pH. In such a case, oxalate production in the form of soluble Ca salt would be a mode of osmoregulation, as already reported by Raven and Smith (1976).

On the basis of previously mentioned results, Lapeyrie (1988), investigating the role of bicarbonate ions, showed that they can be a substrate for OA synthesis. He suggested that $HCO_3^-$ ions are incorporated into oxaloacetate by pyruvate carboxylase and concluded that abundant OA synthesis seems to be specific to fungi growing in calcareous soils, since they require carbonate ions as a substrate where nitrate ions are the main nitrogen source. In the symbiotic situation oxalate synthesis by fungi could be an efficient way of improving the host mineral nutrition.

Another hypothesis related to the symbiotic situation has been suggested by Snetsselaar and Whitney (1990). The amount of Ca released when phosphate is liberated from polyphosphate granules may be substantial in *Monotropa uniflora* mycorrhizal mantles. Ca oxalate crystals can provide a solution to the problem of disposing of Ca released when the granules dissolve to free the phosphate. Jurinak *et al.* (1986) have also related mycorrhizal fungi to phosphorous uptake by plants in semiarid soils. These authors examined mycorrhizal *Pascopirus smithii* and proposed that the solubility of Ca apatite increased when oxalate was present. The result was a marked increase in soluble phosphorus.

Other factors can influence OA synthesis. Kurian and Stelzig (1979a) demonstrated that the optimal pH for OA production by *Crystulariella pyramidalis* was within the range of 3.4-4.5. Bennett and Hindal (1989) and Punja and Jenkins (1984b), respectively, showed that *Cryphonectria parasitica* and *Sclerotium rolfsii* accumulated the greatest amount of oxalate when the pH of the media was 5.0.

Another study (Hillman *et al.*, 1990) concerned OA production by *Cryphonectria parasitica* under different light regimens (10,000 lx; 2,500 lx; dark). Oxalate accumulation was significantly stimulated at high light intensity.

### Oxalic acid as a factor in mineral weathering

OA may act as a mineral weathering factor because of its complexing as well as its acid properties. Kanatchinova and Kasymbekov (1986) isolated *Penicillium roquefortii* and *P. lividum* from a Russian sandstone. In vitro growth of *P. roquefortii* (Czapek medium + sandstone) yielded calcium oxalate. The authors suggested a relation between decomposition of the sandstone and production of OA by fungal activity.

Kanatchinova and Zemlyakov (1987) isolated fungi producers of OA from Russian ores, phosphorites and bauxites. The biosynthetic activity of the fungi depends on the pH of the medium and on the quantitative content of calcium in the natural substrate.

Verrecchia (1990a, 1990b) and Verrecchia *et al.* (1990) examined calcrete soils in semiarid environments in Israel, soils which have a particular vescicular microfabric. The calcrete horizon is hardened but highly porous and all the pores are coated with a grey layer of secondary calcium carbonate. Two kinds of needles are formed inside the pores: thin and regular needles (calcite) and filaments with very sharp spikes (Ca oxalate) that are of fungal origin. In desert sediments and soils, microflora are very abundant, especially cyanobacteria and fungi. They observed fungi developing in the micropores of the limestone. Under biological control, Ca oxalate coexists with Ca carbonate; both contribute to rock diagenesis. In the pores, biological activity promotes a complex cycling of Ca leading to recementation of the matrix and further lithification. Thus, the authors concluded that this kind of calcrete is due to geological evolution as much as to biochemical control.

## Oxalic acid as a pathogenic and/or fungitoxic factor

OA is moreover involved, as a pathogenic factor, in the action of plant pathogenic fungi. OA, a strong chelator of divalent cations, disrupts the integrity of the plant cell wall and facilitates lytic enzyme activity. Godoy *et al.* (1990) demonstrated that mutants deficient in production of OA from populations of UV-radiated *Sclerotinia sclerotiorum* are non pathogenic on beans, even though the mutants produce degradative enzymes at levels equal to the wild type. Evidence from various studies (Kurian and Stelzig, 1979a; Havir and Anagnostakis, 1983; Ikotun, 1984; Punja *et al.*, 1985; Stone and Armentrout, 1985; Smith *et al.*, 1986) indicate that OA:

1) increases the permeability of the host plasmamebrane,

2) decreases the pH to a value favorable for fungi growth and the activity of the fungal endopolygalacturonase,

3) chelates the calcium of the cell wall, calcium pectate, thus changing this polysaccharide to a form that can be hydrolized by the fungal enzyme.

This role and mechanism, however, is not completely established. Callahan and Rowe (1991) in a study on *Sclerotinia sclerotiorum*, suggested that OA is not the sole pathogenic factor responsible for the disease, but other macromolecular components play a codeterminat role.

Marciano *et al.*, (1983) showed that OA does not directly effect cell-wall-degrading enzyme activity. According to these authors, OA may inhibit polyphenoloxidase and thus limit the production of the phenolic oxidation compounds that protect the pectic substances in the cell walls.

Three studies supported the conclusion that oxalate could have a role of protection against plant diseases. Doubrava *et al.* (1988) showed that oxalate is a substance which induces systemic resistance to anthracnose, a disease caused by *Colletotrichum lagenarium*. According to the authors, the observations of enhanced susceptibility and resistance caused by oxalate are not in conflict because cell damage of the host may be necessary for the induction of systemic resistance. Duchesne *et al.*, (1989) suggested that OA is a fungitoxic and/or fungistatic substance against *Fusarium oxisporum* produced by mycorrhizal *Paxillus involutus*. Mucharromah and Kuc (1991) showed that oxalate induce systemic resistance against plant diseased by some fungi.

## Other functions of oxalic acid

OA can have other functions. Whitney and Arnott (1986a, 1987) examinig oxalate production in *Gilbertella percaria* and *Agaricus bisporus* suggested that the crystals:

1) can provide a physical or chemical barrier against attacks by grazing arthropods,

2) may serve as an «exoskeletal system» (only in *G. persicaria)* ensuring the rigidity of the sporangiophore wall.

Two papers dealt with other mechanisms of degradation in which OA seems to be involved. Orlowska *et al.* (1986) proposed that the mechanism of leaching metals from their oxides is probably linked with production of OA fungi capable of carrying out this process. Espejo and Agosin (1991) demonstrated that OA produced by three brown-rot fungi has a role in cellulose depolymerization.

## CONCLUSIONS

A relationship between Ca and OA synthesis has been demonstrated in some fungi. Excess Ca, toxic for the cell, could be removed by precipitation of calcium oxalate. The primary factor giving rise to OA formation is not yet clear. Some authors suggested that Ca may be the primary inductive factor, while according to others soil bicarbonate ions, acting as a substrate for fungal growth, and nitrate have the main responsibility.

There is agreement on the conclusion that Ca oxalate producing fungi may be very important contributors to soil formation and that oxalate crystals may have a role in the long term maintenance of Ca concentration in the soil and in retention and/or cycling of elements necessary for plant growth.

On the basis of the above mentioned considerations, it is possible that the material of a stone work of art, including the eventual organic substances applied, can act as a suitable substrate for oxalate production by fungi which, therefore, would have taken part, under certain conditions, in oxalate films production. Nevertheless, it is not yet possible to establish a relationship between fungi isolated from stone monuments and oxalate films because only some of them are known as OA producers; many other species need further examination to establish if they are OA producers or not. Moreover, further study is required regarding the kind of fungi which can colonize on a stone surface and the environmental conditions which favour such growth.

According to the data in the literature, oxalic acid may have various functions which are not, however, mutually exclusive, while calcium oxalate, too, can have several functions, although not necessarily concomitantly.

The shape of Ca oxalate crystals is a property of the hydratation form of the Ca oxalates. The factors that determine the crystalline form of the oxalates need further study.

Another topic which requires further study is that of the oxalic acid pathway from L-ascorbic acid. It would be interesting to know if this mechanism, which is one of the major precursors of OA in plants, occurs also in fungi.

## ACKNOWLEDGEMENTS

The author is grateful to Dr. G. Alessandrini and Dr. M. Tabasso for having kindly provided the photographs, and wishes to thank Dr. R. Rossi-Manaresi for stimulating discussions.

## REFERENCES

ALESSANDRINI G., BONECCHI R., PERUZZI R., TONIOLO L., (1989) — *Caratteristiche composizionali e morfologiche di pellicole ad ossalato: studio comparato su substrati lapidei di diversa natura.* Proceedings of the Symposium «Le pellicole ad ossalati: origine e significato nella conservazione delle opere d'arte», Milano, 137-150.

ALESSANDRINI G., BUGINI R., PERUZZI R., (1986) — *I trattamenti superficiali effettuati nel passato.* La Certosa di Pavia: passato e presente nella facciata della chiesa, CNR, Roma, 291-319.

ARNOTT H.J., WEBB M.A., (1983) — *The structure and formation of calcium oxalate crystal deposits on the hypae of a wood rot fungus.* Scanning Electron Microsc., **4**:1747-1758.

ARNOTT H.J., FRYAR A., (1984) — *Raphide-like fungal crystals from Arlington, Texas (U.S.A.) compost.* Scanning Electron Microsc., **4**:1745-1750.

ARNOTT J.J., (1982) — *Calcium oxalate (weddellite) crystals in forest litter.* Scanning Electron Microsc., **3**:1141-1150.

BENNETT A.R., HINDAL D.F., (1989) — *Mycelial growth and oxalate production by five strains of* Cryphonectria parasitica *in selected liquid culture media.* Mycologia, **81 (4)**:554-560.

BENNETT A.R., HINDAL D.F., (1990) — *Mycelium formation and calcium oxalate production by dsRNA-free virulent and dsRNA-containing hypovirulent strains of* Cryphonectria parasitica. Mycologia, **82 (3)**:358-363.

BENNY G.L., KHAN S.R., (1988) — *The Radiomycetaceae (Mucorales; Zygomycetes). Calcium oxalate crystals on the sporangiolar wall and aerial hyphae.* Scanning Microscopy, **2**:1199-1206.

CALLAHAN F.E., ROWE D.E., (1991) — *Use of a host-pathogen interaction system to test whether oxalic acid is the sole pathogenic determinant in the exudate of* Sclerotinia trifoliorum. Phytopathology, **81**:1546-1550.

Ceruti A., Marra D., Fusconi A., Berta G., (1983-84) — *Sulla struttura microscopica e submicroscopica di specie di «Inocybe».* Allionia, **26**:39-48.

Chiari G., Sampò S., Torraca G., (1989) — *Formazione di ossalati di calcio su superfici marmoree da parte di funghi.* Proceedings of the Symposium «Le pellicole ad ossalati: origine e significato nella conservazione delle opere d'arte», Milano, 85-90.

Ciccarone C, Pinna D., (1993) — *Calcium oxalate films on stone monuments-Microbiological investigations.* Aerobiologia, **9**:33-37.

Cromack K., Sollins P., Graustein W.C., Speidel K., Todd A.W., Spycher G., Li C.Y., Todd R.L., (1979) — *Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus,* Hysterangium crassum. Soil Biol. Biochem., **11 (5)**:463-468.

Del Monte M., (1991) — *Trajan's Column: Lichens don't live here anymore.* Endeavour, New Series, vol. 15, n. 2, 86-93.

Del Monte M., Ferrari A., (1989) — *Patine da biointerazione alla luce delle superfici marmoree.* Proceedings of the Symposium «Le pellicole ad ossalati: origine e significato nella conservazione delle opere d'arte», Milano, 171-182.

Doubrava N.S., Dean R.A., Kuc J., (1988) — *Induction of systemic resistance to anthracnose caused by* Colletotrichum lagenarium *in cucumber by oxalate and extracts from spinach and rhubarb leaves.* Physiological and Molecular Plant Pathology, **33**:69-79.

Duchesne L.C., Ellis B.E., Peterson R.L., (1989) — *Diseases suppression by the ectomycorrhizal fungus* Paxillus involutus*: contribution of oxalic acid.* Can. J. Bot., **67**:2726-2730.

Espejo E, Agosin E., (1991) — *Production and degradation of oxalic acid by brown rot fungi.* Applied and Environmental Microbiology, **57 (7)**:1980-1986.

Franceschi V.R., Horner H.T., (1980) — *Calcium oxalate crystals in plants.* Botanica Review, **46**:361-427.

Garibova L.V., Kozlava R.G., Losyakova L.S., Safonova N.V., (1982) — *Oxalic acid formation during growing of seed mycelium of the edible mushrooms* Agaricus bisporus *and* Pleurotus ostreatus *on loose nutritive substrates.* Biol. Nauki. (Mosc.), **0 (3)**:79-84.

Godoy G., Steadman J.R., Dickman M.B., Dam R., (1990) — *Use of mutants to demonstrate the role of oxalic acid in pathogenicity of* Sclerotinia sclerotiorum *on* Phaseolus vulgaris. Physiological and Molecular Plant Pathology, **37**:179-191.

Guidobaldi F., Laurenzi Tabasso M., Meucci C., (1984) — *Monumenti in marmo di epoca imperiale a Roma: indagine sui residui di trattamenti superficiali.* Bollettino d'Arte, **24**:121-134.

Havir E.A., (1983) — *Oxalate production by virulent but not by hypovirulent strains of* Endothia parasitica. Physiological Plant Pathology, **23**:369-376.

Havir E.A., Anagnostakis S.L., (1985) — *Oxaloacetate acetylhydrolase activity in virulent and hypovirulent strains of* Endothia (Cryphonectria) parasitica. Physiological Plant Pathology, **26**:1-9.

Hillman B.I., Shapira R., Nuss D.L., (1990) — *Hypovirulence-associated suppression of host functions in* Cryphonectria parasitica *can be partially relieved by high light intensity.* Phytopathology, **80**:950-956.

Horner H.T., Tiffany L.H., Cody A.M., (1983) — *Formation of calcium oxalate crystals associated with apothecia of the discomycete* Dayscypha capitata. Mycologia, **75 (3)**:423-435.

Horner H.T., Tiffany L.H., Cody A.M., (1985) — *Calcium oxalate bipyramidal crystals on the Basidiocarps of* Geastrum minus *(Lycoperdales).* Proc. Iowa Acad. Sci., **92 (2)**:70-77.

Horner H.T., Tiffany L.H., Knaphus G., (1991) — *Crystal forms associated with rhizomorphs on oakleaf litter.* Am. J. Bot., **78** (6 suppl.):26.

Ikotun T., (1984) — *Production of oxalic acid by* Penicillium oxalicum *in culture and in infected yam tissue and interaction with macerating enzyme.* Mycopathologia, **88**:9-14.

Jurinak J.J., Dudley L.M., Allen M.F., Knight W.G., (1986) — *The role of calcium oxalate in the availability of phosphorus in soils of semiarid regions: A thermodynamic study.* Soil. Sci., **142 (5)**:255-261.

Kanatchinova M.K., Kasymbekov B.K., (1986) — *Decomposing activity of microscopic fungi and its causative factors.* Mikol. Fitopatol., **20 (1)**:47-52.

Kanatchinova M.K., Zemlyakov S.V., (1987) — *Production of oxalic acid by microscopic fungi, desctructors of rocks.* Mikrobiol. Zh. (Kiev), **49**:55-61.

Kazmierczak J., Ittekkot V., Degens E.T., (1985) — *Biocalcification through time: environmental challenge and cellular response.* Paleont. Z., **52**:15-23.

Keller J., (1985) — *Les cystides cristalliferes des Aphyllophorales.* Mycologia Helvetica, **5**:277-341.

Krisai I., Mrazek E., (1986) — *Calcium oxalate crystals in* Geastrum. Pl. Syst. Evol., **154**:325-341.

Kubicek C.P., Schreferl-Kunar G., Wohrer W., Rohr M., (1988) — *Evidence for a cytoplasmic pathway of oxalate biosynthesis in* Aspergillus niger. Applied and Environmental Microbiology, **54 (3)**:633-637.

Kurian P., Stelzig D.A., (1979a) — *Growth of and oxalic acid production by* Cristulariella pyramidalis *on selected culture media.* Phytopathology, **69 (7)**:712-714.

Kurian P., Stelzig D.A., (1979b) — *The synergistic role of oxalic acid and endopolygalacturonase in bean leaves infected by* Cristulariella pyramidalis. Phytopathology, **69 (12)**:1301-1304.

Lazzarini L., Salvadori O., (1989) — *A reassessment of the patina called «scialbatura».* Studies in Conservation, **34**:20-26.

Lapeyrie F., (1988) — *Oxalate synthesis from soil bicarbonate by the mycorrhizal fungus* Paxillus involutus. Plant and Soil, **110**:3-8.

Lapeyrie F., Chilvers G.A., Bhem C.A., (1987) — *Oxalic acid synthesis by the mycorrhizal fungus* Paxillus involutus *(Batsch. ex Fr.) Fr.* New Phytol., **106**:139-146.

Marciano P., Di Lenna P., Magro P., (1983) — *Oxalic acid, cell wall-degrading enzymes and pH in pathogenesis and their significance in the virulence of two* Sclerotinia sclerotiorum *isolates on sunflower.* Physiological Plant Pathology, **22**:339-345.

Matteini M., Moles A., (1986) — *Le patine di ossalato sui manufatti in marmo.* O.P.D. Restauro (Num. spec.), Restauro del marmo/Opere e problemi:65-73.

Maxwell D.P., Bateman D.F., (1986) — *Oxalic acid biosynthesis by* Sclerotium rolfsii. Phytopathology, **58**:1635-1642.

Mucharromah E., Kuc J., (1991) — *Oxalate and phosphates induce systemic resistance against diseases caused by fungi, bacteria and viruses in cucumber.* Crop Protection, **10**:265-270.

Mueller H.M., (1986) — *Utilization of gluconate by* Aspergillus niger: *II. Enzymes of degradation pathways and main end products.* Zentralbl. Microbiol., **141 (6)**:461-469.

Orlowska B., Golab Z., Starosta J., (1986) — *The participation of fungi in leaching metals from their oxides.* Acta Mycol., **18**:281-288.

Powell M.D., Arnott H.J., (1985) — *Calcium oxalate crystal production in 2 members of the Mucorales.* Scanning Electron Microsc., **1**:183-190.

Punja Z.K., Huang J.S., Jenkins S.F., (1985) — *Relationship of mycelial growth and production of oxalic acid and cell wall degrading enzymes to virulence in* Sclerotium rolfsii. Can. J. Plant Pathol., **7 (2)**:109-117.

Punja Z.K., Jenkins S.F., (1984a) — *Light and scanning electron microscopic observations of calcium oxalate crystals produced during growth of* Sclerotium rolfsii *in culture and in infected tissue.* Can. J. Bot., **62**:2028-2032.

Punja Z.K., Jenkins S.F., (1984b) — *Influence of medium composition on mycelial growth and oxalic acid production in* Sclerotium rolfsii. Mycologia, **76 (5)**:947-950.

Rao D.V., Tewari J.P., (1987) — *Production of oxalic acid by* Mycena citricolor, *causal agent of the American leaf spot of coffee.* Phytopatology, **77**:780-785.

Rao D.V., Tewari J.P., (1989) — *Occurrence of magnesium oxalate crystals on lesions incited by* Mycena citricolor *on coffee.* Phytopatology, **79**:783-787.

Raven J.A., Smith F.A., (1976) — *Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation.* New Phytol., **76**:415-431.

Rossi-Manaresi R., Grillini G.C., Pinna D., Tucci A., (1986) — *La formazione di ossalati di calcio su superfici monumentali: genesi biologica o da trattamenti?* Proceedings of the Symposium «Le pellicole ad ossalati: origine e significato nella conservazione delle opere d'arte», Milano: 113-125.

Smith V.L., Punja Z.K., Jenkins S.F., (1986) — *A histological study of infection of host tissue by* Sclerotium rolfsii. Phytopathology, **76**:755-759.

Snetsselaar K.M., Whitney K.D., (1990) — *Fungal calcium oxalate in mycorrhizae of* Monotropa uniflora. Can J. Bot., **68**:533-543.

Stone H.E., Armentrout V.N., (1985) — *Production of oxalic acid by* Sclerotium ceviporum *during infection of onion.* Mycologia, **77**:526-530.

Tanaka K., Nonaka F., (1981) — *Synergistic action of oxalic acid pectolytic enzyme on the rot of onion bulb caused by* Aspergillus niger. Ann. Phytopathol. Soc. Jpn., **47 (2)**:166-174.

Tomoyeda M., Inari T., Koshino Y., (1988) — *Mechanism of oxalic acid fermentation by* Aspergillus niger. Nippon Nogeikagaku Kaishi, **62 (6)**:965-970.

Traquair J.A., (1987) — *Oxalic acid and calcium oxalate produced by* Leucostoma cincta *and* Leucostoma persoonii *in culture and in paeak bark tissues.* Can. J. Bot., **65 (9)**:1952-1956.

Tu J.C., (1985) — *Tolerance of white bean* (Phaseolus vulgaris) *to white mold* (Sclerotinia sclerotiorum) *associated with tolerance to oxalic acid.* Physiological Plant Pathology, **26**:111-117.

Verrecchia E.P., (1990a) — *Litho-diagenetic implications of the calcium oxalate-carbonate biogeochemical cycle in semiarid calcretes, Nazareth, Israel.* Geomicrobiology Journal, **8**:87-99.

Verrecchia E.P., (1990b) — *Incidence de l'activité fongique sur l'induration des profils carbonatés de type calcrete pédologique. L'exemple du cycle oxa-*

*late-carbonate de calcium dans les encroutements calcaires de Galilée (Israele).* C.R. Acad. Sci. Paris, t. 311, série II:1367-1374.

Verrecchia E.P., Dumont J.L., Rolko K.E., (1990) — *Do fungi building limestones exist in semi-arid regions?* Naturwissenschaften, **77**:584-586.

Whitney K.D., Arnott H.J., (1986a) — *Morphology and development of calcium oxalate deposits in* Gilbertella persicaria *(Mucorales).* Mycologia, **78 (1)**:42-51.

Whitney K.D., Arnott H.J., (1986b) — *Calcium oxalate crystals and basidiocarp dehiscence in* Geastrum saccatum *(Gasteromycetes).* Mycologia, **78 (4)**:649-656.

Whitney K.D., Arnott H.J., (1987) — *Calcium oxalate crystal morphology and development in* Agaricus bisporus. Mycologia, **79 (2)**:180-187.

Whitney K.D., Arnott, H.J., (1988) — *The effect of calcium on mycelial growth and calcium oxalate crystal formation in* Gilbertella persicaria *(Mucolares).* Mycologia, **80 (5)**:707-715.