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# T.E.M. Study of Intracellular and Extracellular Calcium Oxalate Accumulation by Ectomycorrhizal Fungi in Pure Culture or in Association with *Eucalyptus* Seedlings*

F. LAPEYRIE,[1] C. PICATTO,[1] J. GERARD[2] and J. DEXHEIMER[2]

[1]*I.N.R.A. Centre de Recherches Forestières de Nancy, 54280 Seichamps – France.*
[2]*Université Nancy I, Biologie des Ligneux, 54500 Vandoeuvre – France.*

**Abstract**

Calcium oxalate deposition, mainly amorphous, could be observed by TEM, outside hyphae in pure culture as well as in vacuolar or protoplasmic vesicles. In ectomycorrhizae, extracellular deposition were found in the fungal sheath, while intracellular ones were present as deeply as in the Hartig net.

## Introduction

Calcium oxalate crystals are frequently observed *in vivo* covering mycorrhizae and external hyphae. Fungal oxalic acid synthesis and excretion could play an important role in the ectomycorrhizal symbiosis. Indeed, in calcareous soil oxalic acid will precipitate large amounts of calcium which could be otherwise toxic for plants. In acidic soil, calcium oxalate accumulation especially in litter could be a reserve of calcium. Being a strong complexant of calcium aluminium and iron, fungal oxalic acid will be an efficient mobilization agent for phosphorus, by acidolyse as well as complexolyse (Cromack et al., 1978).

Presently no intracellular oxalate crystals have been described in ectomycorrhizal fungi suggesting that most of the fungal oxalic acid is excreted to crystalize outside the cell with external calcium. The aim of the present study was to determine, using TEM, if the mycorrhizal fungus *Paxillus involutus*, which can synthesize large amounts of oxalic acid (Lapeyrie et al., 1987; Lapeyrie, 1988), accumulates calcium (Lapeyrie and Bruchet, 1986) and accumulates oxalate as external depositions (Lapeyrie et al.,

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1984), accumulates as well calcium oxalate in the cell. The same study was conducted on ectomycorrhizae synthesized *in vitro* between *Pisolithus tinctorius* and *Eucalyptus globulus ssp bicostata.*

## Material and Methods

The fungus was grown in pure culture on basal medium with nitrate as nitrogen source (Lapeyrie et al., 1987) and 0.2% $CaCO_3$. The ectomycorrhizal synthesis was made according to the "paper sandwich" technique (Chilvers et al., 1986), using the same medium as previously without glucose and with 0.5% $CaCO_3$ for plant, and using PDA medium (Patato Dextrose Agar) for the fungal inoculum.

## Result and Discussion

The hyphae (H) of *Paxillus involutus* (Fig. 1) (60 days old) contrasted by uranyl acetate and lead citrate are covered with calcium oxalate crystals (C). As the crystals have been dissolve by uranyl acetate, only their geometrical shape can be observed. Few crystals appear to be covered by some biological material (⇒) in continuity with the external layer of the fungal cell wall (Fig. 3) and reacting positively to the Patag test for polysaccharides characterization. As it is found only on old hyphae it must be mucilage excreted by the ageing hyphae, covering the crystals after their complete external growth. This can not be considered as an evidence of the excretion throughout the wall, of an internally grown crystal as previously suggested (Whitney and Arnott, 1987).

Using non-contrasted sections, it was possible to avoid crystal solubilization and to observe their structure (Fig. 2) At high magnification, they appear heterogeneous, made of spherical vesicles (C). These vesicles varied in size and density. Some crystals have geometrical shapes. As well as external deposition, internal inclusions are observed (in), they have as well an heterogeneous structure. These inclusions are protoplasmic (Fig. 4) or vacuolar (Fig. 2).

Refereeing to previous study (Lapeyrie et al., 1984), the external depositions have been firstly considered as mainly calcium oxalate crystals, however, while some depositions gave a electron microdiffraction spectrum characteristic of crystalline compounds, most of them are amorphous. This is more in agreement with the "vesicular structure" of these external depositions observed in T.E.M.

As the calcium concentration of the internal vesicles (125 000 cps/$\mu m^2$), determined by X microanalysis, is in the same range than the calcium concentration of the external deposition (120 000 cps/$\mu m^2$), and as they have the same structure they are considered of closely related composition. Only the more crystalline external deposition were stable enough for a complete X microanalysis: Three elements are present, Ca, O and C. This is in agreement with calcium oxalate, $Ca(COOH)_2$.

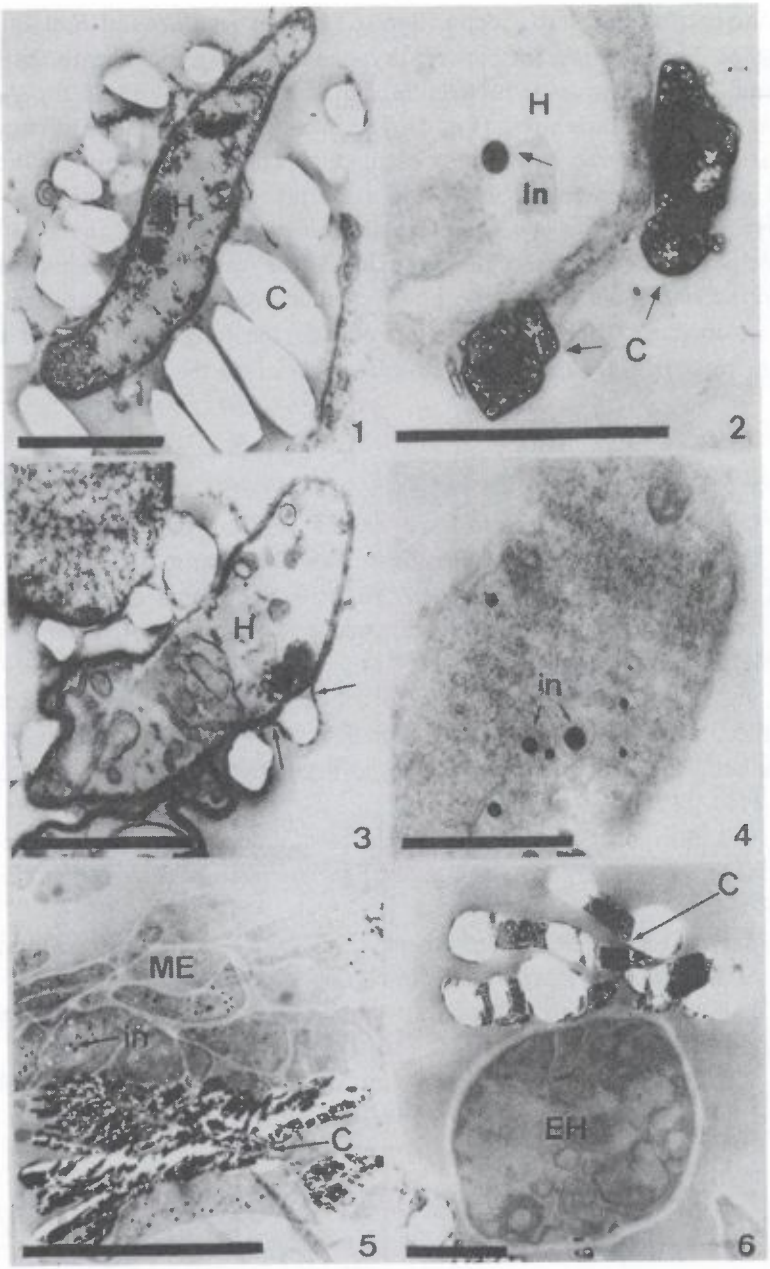


Figure 1 to 4. Mycorrhizal fungus in pure culture (*P. involutus*).
Figure 5 to 6. Mycorrhiza (*E. globulus*/*P. tinctorius*)

Abbreviations: C, crystal; EH, external hyphae; H, hyphae; in, intracellular inclusion; ME, external mantle. Bar = 1 $\mu$m.

In ectomycorrhizae, external deposition (C) can be seen around radiating hyphae (HE) (Fig. 6). They are present between hyphae in the external sheath (ME) (Fig. 5) and internal sheath, but never between fungal and host plant cell. In the sheath they seem to aggregate forming sticks (Fig. 5). The intracellular inclusions (in) are found in the fungal cells, but never in the host plant cells. They are common in the external sheath (Fig. 5), in the internal sheath, and as deeply as in the Hartig net.

Ectomycorrhizal fungi seems therefore able to immobilize calcium ions as amorphous calcium oxalate in intracellular vesicles. These vesicles could move freely along hyphae in the Hartig net. Their amorphous content could be resolubized (much more easily than crystals), either for excretion outside the fungal cell (in the sheath or outside the mycorrhizae), or for calcium translocation to the host-plant.

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