

# The role of ectomycorrhizal fungi in calcareous soil tolerance by "symbiocalcicole" woody plants

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**Summary** — There are now a few examples in the literature of trees or dwarf shrub which can tolerate calcareous soils only in association with mycorrhizal fungi; these plants could be termed symbiocalcicole. An integrative flow-diagram which summarizes probable interactions between calcareous soil, mycorrhizal fungi and roots of symbiocalcicole plants is presented and discussed. Solubilisation, mobilisation and/or assimilation of phosphorus, calcium, nitrogen, iron and carbonate from calcareous soil are considered successively.

**mycorrhizas / calcareous soil / calcium / calcifuge / symbiocalcicole**

**Résumé** — Les champignons ectomycorhiziens et la tolérance des sols calcaires par les plantes ligneuses "symbiocalcicoles". Quelques cas d'arbres ou d'arbustes nains tolérant les sols calcaires uniquement lorsqu'ils sont associés à des champignons ectomycorhiziens ont fait l'objet d'une publication. Ces plantes pourraient être dénommées "symbiocalcicoles". Un diagramme résumant les interactions probables existant entre sol calcaire, champignon mycorhizien et racine d'une plante symbiocalcicole est présenté et discuté. Sont envisagées successivement, la solubilisation, la mobilisation et/ou l'assimilation du phosphore, du calcium, de l'azote, du fer et des carbonates d'un sol calcaire.

**mycorrhizes / sol calcaire / calcicole / calcifuge / symbiocalcicole**

## INTRODUCTION

It has long been known that some plants, including tree species, can be categorized according to their ability to grow in calcareous soils or acidic soils, *ie* the calcicole plants growing in calcareous soil, and the calcifuge plants unable to tolerate calcareous soils. From a practical point of view, both foresters and agronomists have taken

this into consideration in the selection of plant species for the different soil types to achieve maximum results. The physiological basis for this classification is still the subject of active investigation since no complete explanation as to the mechanism for the differential tolerance of the two types of soil is currently available. Many hypotheses have been proposed, and these have been the subject of a number of reviews (Burstrom, 1968; Kinzel, 1983).

Invariably, ion balances have been implicated but in most cases the experimental models have included growing plants in aseptically conditions or in soils where the mycorrhizal status was not determined. However, during the last 10 years, 4 studies comparing sterile and non sterile conditions for plant growth in calcareous substrate have indicated that some plants can tolerate calcareous soils only in association with mycorrhizal fungi. This suggests that the ecological and physiological status of the plants have been altered in the presence of a symbiotic partner. These four published studies will be reviewed here. To understand the possible role of mycorrhizal fungi in plant tolerance to calcareous soil, hypotheses based on current knowledge about calcareous soil toxicity and plant/fungus relationship will be proposed and discussed.

### CASE REVIEWS

There have been 4 reported examples to-date of plants showing tolerance to calcar-

eous soil due to their association with mycorrhizal fungi. A summary of these results and experimental conditions is presented in table I.

It is interesting to note that, although these experiments were not carried out under the same conditions, the general conclusions are remarkably similar. In the 4 specific examples published, plant growth and development was compared in the presence and absence of mycorrhizas either in calcareous soil only (Kianmehr, 1978; Piou, 1979), or in calcareous and acidic substrates (Le Tacon, 1978; Lapeyrie and Chilvers, 1985). In the first situation the calcareous soil toxicity was indicated in leaf chlorosis and plant death, and this was relieved by mycorrhizal infection. In the second situation, the calcareous soil toxicity was even more obvious when comparing plant growth and mortality between sterile acidic and sterile calcareous substrates. While growth was strongly inhibited in calcareous sterile substrate, following inoculation there was no difference between plant growth in both types of substrate, acidic or calcareous.

**Table I.** Case reviews of plant tolerance to calcareous soil due to mycorrhizal fungus.

<i>Host plant</i>	<i>Culture substrate</i>	<i>Inoculum type</i>	<i>Endo/ecto</i>	<i>Reference</i>
<i>Pinus nigra</i> <i>nigrians</i> Host	Sand $\pm$ CaCO <sub>3</sub>	Seedlings pre-infected or not, into unsterile soil	Ecto	Le Tacon (1978)
<i>Helianthemum chamaecistus</i> Mill	Sterile calcareous soil	Cenococcum graniforme sclerotia	Ecto	Kianmehr (1978)
<i>Pinus halepensis</i> Hill	Calcareous soil	Sterile <i>versus</i> unsterile soil	Ecto	Piou (1979)
<i>Eucalyptus dumosa</i> A Cunn ex Schau	Calcareous or acidic soil & potting mix $\pm$ CaCO <sub>3</sub>	Unsterile soil reintroduced into sterile substrate	Ecto & Endo	Lapeyrie and Chilvers (1985)

Different techniques were used to introduce the mycorrhizal fungi, ranging from monospecific inoculum (Kianmehr, 1978), 10% of unsterile soil (Lapeyrie and Chilvers, 1985), 100% of unsterile soil (Piou, 1979), or plantation of seedlings previously raised in a non sterile soil (Le Tacon, 1978). In three out of four cases, ectomycorrhizas were found conferring tolerance to calcareous soils (Kianmehr, 1978; Le Tacon, 1978; Piou, 1979), in one case the host plant was infected simultaneously with endomycorrhizal and ectomycorrhizal fungi (Lapeyrie and Chilvers, 1985). In this example, the endomycorrhizas were the dominant mycorrhizal form during the first two months conferring resistance to calcareous soil, being progressively replaced by ectomycorrhizas after this period (Chilvers *et al*, 1987). This suggests that both endomycorrhizas-VA and ectomycorrhizas have similar protecting effects on plants growing in calcareous soils.

While the four species cited in table I, *Eucalyptus dumosa*, *Pinus halepensis*, *Helianthemum chamaecistus* and *Pinus nigra nigricans* can tolerate calcareous soils following mycorrhizal infection, others, including *Cupressus sempervirens* or *Cupressus arizonica*, are indifferent to the presence of calcium carbonate even in sterile conditions (Piou, 1979). Yet, another group of plants, including *Eucalyptus dalrympleana*, *E populnea*, *E grandis*, *E largiflorens*, *E dives*, *E gunii*, *E maidenii*, *E globulus* sp *bicostata* (Lapeyrie, 1987) or *Picea excelsa* (Le Tacon, 1978) do not tolerate calcium carbonate even after infection by the same mycorrhizal strain which were protecting other species.

Since these studies did not aim to investigate the physiological aspects of resistance to calcareous soil, questions remains as to the mechanisms involved. However, where *Pinus nigra nigricans* was used as a test plant and grown in calcare-

ous soil, normal nitrogen metabolism, *ie* amino acid and protein synthesis, was restored following plant inoculation (Clément *et al*, 1977). Improvement of plant phosphorus nutrition was observed with *Eucalyptus dumosa* (Lapeyrie and Chilvers, 1985). Reduction of the calcium concentration in the leaves was noticed with *Eucalyptus dumosa* (Lapeyrie and Chilvers, 1985) and *Pinus nigra nigricans* (Le Tacon, 1978).

## DISCUSSION

### *Calcicole and symbio-calcicole plants*

It appears that the ecological classification between calcicole and calcifuge plants could be enriched by taking into account their mycorrhizal dependency on calcareous soils, some plants being able to tolerate calcareous soils only in association with mycorrhizal fungi while other do so even under sterile conditions. The new group of plants, could be termed "symbiocalcicole plants", implying that their ability to tolerate calcareous soil is strictly dependent on their symbiotic status. The definition of calcicole and calcifuge plants would therefore be altered slightly: the calcicole plants would refer to plants which tolerate calcareous soils even in the absence of mycorrhizal fungi, the calcifuge plants would become plants which do not tolerate calcareous soils even in the presence of mycorrhizal fungi.

Obviously, the existence of strictly calcicole trees could be questioned because, while in their ecosystem, trees are always associated with mycorrhizal fungi and because pot experiments in sterile substrate are always carried out for a limited period of time; always very short compared with the tree life span. It could therefore be

argued that survival in sterile calcareous soils (Piou, 1979), is a temporary phenomenon. However, if we refer to annual plants, carnations produced commercially either in soil or under hydroponic conditions are, in both cases, behaving as a calcicole species. The optimum nutrient solution for hydroponic culture is characterized by high pH and calcium concentration (Brun and Montarone, 1987). Endomycorrhizal fungi are absent in such conditions, without any symptoms of toxicity for the plant, while the same medium would be toxic for a calcifuge crop species.

Such distinction into three groups could be important to consider, before undertaking any comparative physiological work aimed at understanding why some plants tolerate calcareous soils and others do not. To explain the physiological differences between a calcifuge plant and a symbiocalcicole plant, *ie* why the latter can be rendered tolerant to calcareous soil by the fungus while the former cannot, two hypotheses can be considered. The first where both plants do not suffer the same metabolic disorders when planted in sterile calcareous soil; the metabolic disorders encountered by the symbiocalcicole plant would be such that the associated mycorrhizal fungus could counteract them, whereas in the case of the calcifuge plant, the fungus could not rectify these metabolic disorders. The second hypothesis supposes that the calcifuge and symbiocalcicole plants suffer the same metabolic disorders when planted in sterile calcareous soil; however, the plant-fungus relationship would involve different metabolic pathways in both cases; the symbiotic metabolism involving the symbiocalcicole plant would be able to counteract the host plant stress while in the calcifuge plant it could not. This implies that different plant fungus combinations have specific metabolic pathways involved. Evidence sup-

porting this statement has been demonstrated by Dell *et al* (1988) who showed that, for at least the fungal NADP glutamate dehydrogenase, its activity can be expressed or repressed in ectomycorrhizas depending on the host plant.

### ***Fungus-calcareous soil interface***

Irrespective of the direct action of the fungus on the plant metabolism (Al Abras *et al*, 1988) including hormonal metabolism (Gay, 1987) or on the plant gene expression (Hilbert and Martin, 1988), the role of mycorrhizal fungi in calcareous soil could also be considered through their action at the soil-plant interface. It is clearly established that some fluxes of ions are dependent on the presence of the symbiotic fungus (Rygiewicz and Bledsoe, 1984). In the specific case of calcareous soils, some pathways for the movement of ions, which could be very important for the host plant status, are presented in figure 1.

### **Nitrogen nutrition**

Nitrate is the prominent form of nitrogen in calcareous soils. Chlorosis in trees can be partly related to their nitrogen nutrition as found with Nordmann fir where different types of chlorosis can be induced either by nitrate or calcium carbonate (Khalil *et al*, 1989). Perturbation of nitrogen metabolism observed on calcareous soil in the absence of mycorrhizas (Le Tacon, 1978) appears to be overcome through the symbiosis.

It is well established that the mycorrhizal fungus actively participates in plant nitrogen nutrition. Mycorrhizal infection improves the nitrogen absorption, and simultaneously modifies the ratio of influx and efflux of ions (Rygiewicz *et al*, 1984a;

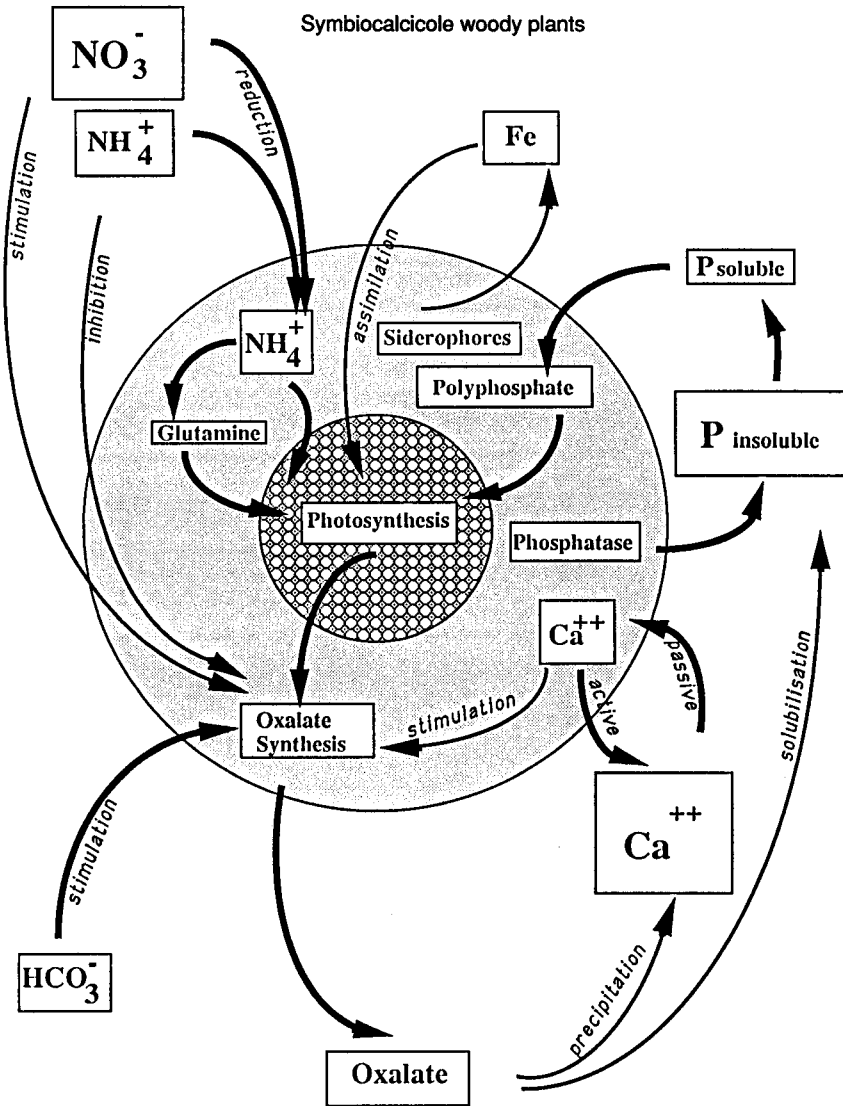


Fig 1. Schematic representation of pathways for the movement of ions at the soil-fungus interface in calcareous soil. : Host-plant root; : Ectomycorrhiza fungal sheath.

1984b). These experiments have been performed at acidic and neutral pH, and therefore the conclusions cannot be easily extrapolated to calcareous soils. However, it has been demonstrated on many occa-

sions that ectomycorrhizal fungi exhibit a nitrate reductase activity (France and Reid, 1979; Salsac *et al*, 1982). Free amino acids can be incorporated by mycorrhizal fungi (Carrodus, 1966) and mycorrhizal fungi

possess proteases (Botton *et al*, 1986; Plassard *et al*, 1986) giving them access to soil proteins. Then, the transfer of nitrogen to the plant occurs either as ammonium or as glutamine and this process is still under investigation (France and Reid, 1983; Martin *et al*, 1986), but it has been shown that composition of the free amino acid pool in the plant is dependent on its symbiotic status (Krupa *et al*, 1973; Krupa and Branstrom, 1974; Vésina *et al*, 1989).

### Calcium fluxes

According to another hypothesis, calcium ions may be responsible for calcareous soil toxicity (Jefferies and Willis, 1964; Hall, 1977). *In vivo* as well as *in vitro*, calcium ions are absorbed in excess by roots of calcifuge plants from calcareous soil or calcium ion solutions (Anderson and Ladiges, 1978; Salsac, 1973, 1980). As a consequence, chloroplast thylakoid structure would be affected (Cournier *et al*, 1982), as well as C3 or C4 photosynthesis (Portis *et al*, 1977; Chevalier and Paris, 1981; Gavalas and Manetas, 1980a, b; Portis and Heldt, 1976). These differences in calcium absorption and accumulation have been related to different composition of the plasma membrane of calcicole and calcifuge plants (Rossignol, 1977; Rossignol *et al*, 1977; Lamant and Heller, 1975; Lamant *et al*, 1977). Calcium ions enter the cell passively, the flow only being dependent on the nature of the membrane. At present, we do not have any information about the composition of the plasma membrane of symbiocalcicole plants compared to calcicole or calcifuge plants. The internal cation concentration of cells is also dependent on an active calcium efflux (Hager and Hermsdorf, 1981).

While mycorrhizal fungi are more or less tolerant to calcareous soils, depending on their ecological origin, they tolerate

extremely high concentrations of calcium ions (Lapeyrie *et al*, 1982). At ecological concentrations, the mycorrhizal fungus would mediate most of the nutrient fluxes from the soil to the plant, and could therefore prevent the plant from an over-accumulation. Primarily, mycorrhizal fungi possess an active efflux regulating the calcium accumulation (Lapeyrie and Bruchet, 1986), secondarily, calcium ions precipitate outside the fungal cell as calcium oxalate. Such crystals have been observed on many occasions *in situ* (Malajczuk and Cromack, 1982) as well as *in vitro* (Lapeyrie *et al*, 1984a). These calcium ions precipitated in the close rhizosphere are no longer free for absorption.

Using transmission electron microscopy, fungal intracellular vesicles, concentrating calcium associated with carbon hydrogen and oxygen, thought to be amorphous calcium oxalate vesicles have been observed (Lapeyrie *et al*, 1990). They have been described in fungal cell in pure culture as well as in association with a host plant. They occur in the sheath and as far as the Hartig net when calcium carbonate is provided in the external medium. Their role, internal storage or excretion, is still to be determined; presently no excretion figure have been found, suggesting that amorphous calcium oxalate content can be easily solubilized if some excretion occurs.

### Phosphorus nutrition

While in calcareous soils phosphorus evolves toward more and more crystalline, and less and less soluble forms (Duchaufour, 1970), fungal oxalic acid could be another important factor. The role of oxalic acid in mineral weathering has been well recognized and studied *in vitro* (Cornell and Schindler, 1987), as well as *in vivo* with lichens where the oxalic acid is secreted by the mycobiont (Jones *et al*, 1980;

Jones and Wilson, 1985). Oxalic acid is an acid as well as chelating agent and after excretion in the soil it is particularly efficient in minerals alteration (Robert *et al*, 1979). In calcareous soil, by triggering the formation of complexes with metal ions (Ca, Al, Fe), oxalic acid would release phosphorus from insoluble phosphates (Graustein *et al*, 1977; Coleman *et al*, 1983).

Abundant oxalic acid synthesis by mycorrhizal fungi is characteristic of calcareous soils: the synthesis is stimulated by nitrate but inhibited by ammonium ions, it is slightly stimulated by calcium ions and highly stimulated by carbonate ions (Lapeyrie *et al*, 1987). Carbonate ions from the soil, which can be toxic for the fungus as well as for the plant, are used by the fungus as a carbon substrate, including for oxalate synthesis either directly from oxaloacetate or *via* citrate, isocitrate and glyoxylate (Lapeyrie, 1988). Furthermore, the release of fungal phosphatases will allow the solubilization of organic phosphate (Bousquet *et al*, 1986).

After absorption by the fungus, phosphorus is stored in vacuoles as polyphosphate granules, eventually containing calcium, before being translocated to the host plant when required (Ling Lee *et al*, 1975; Strullu *et al*, 1982; Lapeyrie *et al*, 1984b; Martin *et al*, 1985; Orlovich *et al*, 1989). The plant phosphorus nutrition in calcareous soil is even more dependent on its mycorrhizal status than in acidic soils.

### Iron assimilation

Iron deficiency has been seen as the key point of calcareous soil toxicity. Indeed, calcareous soil chlorosis symptoms can be relieved by iron-chelate fertilization, suggesting that iron could not be absorbed in calcareous soil by the roots of the calci-

fuge plant. However, in most of the cases investigated, no consistent iron deficiency has been found in the leaves (Marschner, 1986). Today, rather than the iron concentration, its status in the plant is considered with reference to metabolically "active" or "inactive" iron (Oserkowsky, 1933; Katyal and Sharma, 1980; Mengel *et al*, 1984). It has been suggested that the calcifuge plants on calcareous soil synthesize in the root system some sort of "iron inactivator" (Rhoads and Wallace, 1960; Falade, 1973; Brown and Jones, 1975). As we know that some mycorrhizal fungi excrete siderophores (Szaniszlo *et al*, 1981; Watteau, 1990), as do most soil microorganisms; these iron-complexing molecules could interact with iron in the soil as well as in the plant organs, counteracting any inactivation.

### CONCLUSION

A characteristic difficulty in understanding the behaviour of calcifuge and calcicole plants is the multiplicity of factors affecting their response (Kinzel, 1983). It is now obvious that all these factors interact together with the plant, but we do not understand yet all the complexities of these interactions. However, it seems that an extra factor, the mycorrhizal fungus, has been neglected in most of the physiological studies aimed at understanding the calcicole calcifuge phenomenon. The presence of a fungus associated with the root system defines new soil-plant interactions, the fungus-soil interface becomes the dominant one. However, as previously mentioned, direct interactions between plant and fungus should not be neglected either, in an attempt to understand the way in which plants operate in calcareous soil.

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