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## **Ecological study of a forest humus by observing a small volume. I.**

### **Penetration of pine litter by mycorrhizal fungi**

By J. F. PONGE

#### **Abstract**

Observed on a 5 x 5 cm small surface of litter in a 35-years-old Scots pine stand with bracken and the moss *Pseudoscleropodium purum*, the F<sub>1</sub> layer is extensively invaded by a mycelial mat made of several mycorrhizal fungi. Observations under a light microscope gave circumstantial evidence of the role of these fungi in advanced stages of decomposition: they seem to protect the partly decayed plant material and the faeces deposited by soil animals from subsequent attack by soil bacteria. Penetration of pine needles and cadavers of soil arthropods is prominent in so far as animals have previously made entries by tunnelling into the substrates. In addition the black mycorrhizal fungus *Cenococcum geophilum* was observed to penetrate bracken epidermal cells by its own means and to make lysis zones in dead arthropod cuticles. Consequences for forest soil ecology and tree nutrition are discussed in view of existing literature.

#### **1 Introduction**

A small volume of soil in a Scots pine (*Pinus sylvestris* L.) stand was observed and described from an ecological point of view (PONGE 1984, 1985a, 1985 b, 1988). This micromorphological study focussed on interrelationships between soil animals and microorganisms during litter decomposition and root development. Since in the top first centimeters the bulk of living and dead organisms was made of mycorrhizal fungi and fine roots of pine, it was judged necessary to publish a separate paper on this topic. Our aim was to ascertain the importance of the root system of trees and its associate mycelia in the decomposition processes.

Field studies on the occurrence of fungi during pine needle decomposition have been made both by direct observation (KENDRICK and BURGESS 1962; BERG 1977; MITCHELL and MILLAR 1978; MITCHELL et al. 1978) and isolation methods (GREMMEN 1957; KENDRICK 1958; KENDRICK and BURGESS 1962; KENDRICK 1963;

SARBHOY 1964; HAYES 1965a, 1965b; BRANDSBERG 1969; WATSON et al. 1974; GREMMEN 1977; BLACK and DIX 1977; SOMA and SAITO 1979). Other studies on coniferous species may be added for comparison such as the work of GOURBIERE (1981, 1982, 1983) and GOURBIERE and PEPIN (1983) on *Abies alba* Mill. No occurrence of mycorrhizal fungi inside or at the periphery of pine and fir needles was noticed, although SOMA and SAITO (1979) indicated the importance of mycelial spread of ectomycorrhizal fungi in the Ao (litter) layer and presumed that they could be involved in decomposition processes. Reasons for this lack of information may be found in the methods used (isolation on agar plates or observation of fructifying fungi in damp chambers) and in the stage of decomposition at which the studies were conducted.

The importance of decaying wood in the establishment of mycorrhizal root systems has nevertheless been stressed by BOULLARD and DOMINIK (1966), MEYER (1979), KONDAS (1980), KROPP (1982), MASER and TRAPPE (1984).

The presence of different materials such as pine needles, wood debris, bracken leaflets, faecal pellets and the pine root system in the same small volume of soil enabled us to identify relationships which generally escaped observers working on specific substrates, often in isolation from their natural environments.

The interest of such morphological studies for forest ecology and management emerges from the following points: assessment of the health of the absorbing root system of trees and its degree of mycotrophy, knowledge of the origin of organic matter that accumulates in the humus part of the soil (here mainly chitin from fungal walls and arthropod cuticles), knowledge of the organisms that take place in decomposition processes for each component of the litter. These last two points must be highlighted, because direct observation gives more information on what happens in the soil at a given time than current methods in microbiology and soil organic matter chemistry.

## **2 Material and methods**

A small surface (ca. 5 × 5 cm) of moder humus was sampled in a 35-years-old *Pinus sylvestris* plantation (Orleans forest, France) on 11/VIII/81 and microstratified in the field into L<sub>1</sub> (brown needles), L<sub>2</sub> (black needles), F<sub>1</sub> (broken needles + pine roots and widespread mycelial mat) and other layers not described here. Observation of the underlying layers showed that they accumulate faecal material from soil fauna, arthropod cuticles and fungal cell walls, mainly from dematiaceous fungi. Biological activity mainly takes place in the F<sub>1</sub>

layer where young feeding roots of pines are actively growing together with their associate fungi. Consequently the results presented here will concern, for the main part, the F<sub>1</sub> layer, although a lot of information was derived from previous investigation of the L layers. Fixation was made immediately, before any transport, in 95% ethyl-alcohol. A more complete description of the stand has been given in PONGE (1984) and ARPIN et al. (1986).

Observations were made under a light microscope at 400 × magnification after sorting the plant and animal material under a dissecting microscope. Pine needles, wood and bark and other dense fragments were cut in 7.5 μm sections and mounted in chloral-lactophenol (25 cc lactic acid + 50 g chloral hydrate + 25 cc phenol). Phase contrast allowed one to discern between dead and living cells at the time of fixation. Presence of cytoplasm is easily discernable by the opacity of cell contents, provided the fungal walls are not melanized (FRANKLAND 1974). When necessary (e.g. for dematiaceous fungi), methyl blue was used in lactophenol as a staining agent for cell contents. Animals were either dissected (oribatid mites) or observed after clearing (other groups) in order to analyse their gut contents. The faecal material was embedded and cut like the plant material. Recognition of the fragments by external characters before cutting them proved to give a more reliable interpretation than direct soil sectioning. The nature of the mycelial mat and its connection to the fungus mantle of the mycorrhizae and to the internal colonizers of plant debris and cadavers were also ascertained by direct observation at a low magnification in addition to the microscopic characters observed in sections.

### 3 Results

#### 3.1 Mycorrhizal types and their associate mycelia

The black mycorrhizal ascomycete *Cenococcum geophilum* (= *Cenococcum graniforme*) forms typical mycorrhizae on short roots which may be dichotomous (Fig. 1) or not (Fig. 2). Identification of the fungus was made by observing the structure of the mantle surface (FERDINANDSEN and WINGE 1925; HATCH 1934; TRAPPE 1971). Not only short roots may be colonized by this fungus, but also long roots. Beyond the elongation zone, where root hairs occur on nonmycorrhizal roots, this fungus often forms a collar (Figs. 1 and 2), with characteristic mantle and penetration of hyphae between the cortical cells (Hartig net), as on short roots. Some long root apices are also transformed into mycorrhiza-like structures. In this case, the mantle is present but the meristem tissues remain active, with numerous mitoses, and no penetration by hyphae is visible: only elongation of the root seems to be impeded. These observations agree with the findings of WILCOX (1968b), who

demonstrated that in growing roots, growth of the fungus was not at the same rate as the root, thus protecting the apex from being colonized, when the root grows well. It may be postulated that long roots with mycorrhizal apices stopped their growth for some obscure reason. These roots could not have been dormant because of the lack of metacuticized layer (WILCOX 1968a), which made possible colonization of the apex by the fungus. The extra-matrical mycelium of *Cenococcum geophilum* is clearly visible: It is made of brown (melanized walls) hyphae that sprout at a right angle from the mantle of the mycorrhizae (Fig. 1). This feature was also encountered at the periphery of its sclerotia (Fig. 3) or when the fungus had developed inside of faecal material, e.g. faeces of lumbricid worms. It must be noticed that the presence of hyphae attached to sclerotia is not a constant feature (MEYER, personal communication, see also TRAPPE 1969). Nevertheless, in the present study, all sclerotia were observed in the immediate vicinity of the roots and connected by hyphae to mycorrhizal mantles.

Another common mycorrhizal fungus is a basidiomycete tentatively identified as *Hyphodontia* (BOIDIN, personal communication), more precisely belonging to a group of three strongly related species [*H. pallidula* (Bres.) John Erikss., *H. alutaria* (Burt) John Erikss., *H. arguta* (Fr.) John Erikss.] that possess lagenocystidia (ERIKSSON and RYVARDEN 1976). These cystidia are present not only in the hymenium of the carpophores but also on the vegetative hyphae and this typical character was found on the specimen studied. Connection of this fungus to a type of mycorrhiza was characterized because the hyphae cannot be detached from the orange-brown mycorrhizae which are often present on the same long root as the former species (*C. geophilum*, Fig. 2) and from hyphal characters (hyphal width, clamp connections, mode of branching). The influence of this fungus on the root system of pine seems to be more pronounced than this of *C. geophilum*: repeated branching of the short roots (Fig. 2), thickness of the Hartig net (1 to 2 cell layers, compared with only 1 for the ascomycete), intensity of the defence reaction of the host (2 layers of tannin cells, against 1 only for *C. geophilum*), penetration of the endodermis in the long roots, prime invasion of the stele once long roots have died (PONGE 1988).

In contrast, *Hyphodontia* seems not to have a strong affinity towards growing parts of the roots, since there is an absence of mantle around apices of short roots and absence of development of this fungus just beyond the elongation zone, unlike *C. geophilum* (PONGE 1988). *Hyphodontia* seems to be responsible for the main part of the mycelial mat that spreads throughout the F layer in the small surface under study. Around the orange-brown mycorrhizae, there appears to be a profuse cottony white mass embedding the root (on Figure 2 the mycelium connected to the mycorrhiza was partly stripped off in order to show the root). We may wonder whether this fungus is a true mycorrhizal fungus and not a parasite of dying roots. Two points may prove it to be mycorrhizal: its constant association with the same type of root colour and branching, and the fact that it has

been found around and in the youngest roots, i.e. in the L<sub>2</sub> layer (PONGE 1985a). Penetration of the endodermis was observed only in long roots, never in short roots (feeder roots).

Less common in the small volume studied is a pale pinky type of mycorrhiza with a smooth mantle (Fig. 4). The colour of this mycorrhiza is perhaps not exactly the same as in the living state, since alcohol had probably dissolved some pigment. Nevertheless its smooth aspect makes it readily recognizable. Connection to a given mycelium is consequently difficult to determine. Analogy between mantle hyphae (PONGE 1988), other hyphae present in the mycelial mat and hyphae of a rhizomorph with characteristic asterocystidia makes it belongs possibly to *Resinicium bicolor* (Fr.) Parm. (= *Odontia bicolor*). But this identification is far from reliable, contrary to the two former cases.

### 3.2 Mycelial mat

The general aspect of the mycelial mat is that of a dense network of hyphae where *Hyphodontia* is largely dominant. It can be seen embedding animal faeces or any other material or be free of organic remains.

Detailed examination of the hyphae revealed the basidiomycetous (dikaryotic) nature of the second (orange-brown mycorrhizae) and third (pale pink mycorrhizae) type of mycorrhizal fungi. The production of calcium oxalate crystals is of very common occurrence in the mycelium of *Hyphodontia*, where they often entirely coat the hyphae, the size of the crystals varying strongly from one place to another (Fig. 5).

The hyphae of *Cenococcum geophilum*, in the mycelial mat, may vary greatly in appearance, with walls either being covered by wart-like protuberances (Fig. 6) or smooth (Fig. 7), the latter being of more common occurrence. The degree of melanization of the walls may also vary greatly (Fig. 8), but generally lack of melanization is associated with penetration of plant material (see later). The results concerning the mycelial characters of this fungus and its polymorphism are in fairly good agreement with the cultural observations of MIKOLA (1948). Differences in the stage of development may be invoked to explain the degree of melanization and of roughness of the fungal walls (MEYER, personal communication). Compared to the other two fungi, the hyphae of *C. geophilum* are also characterized by the thickness of their cell walls (at least in melanized parts), which protect them from grazing by some small animal groups, even when they are specialized fungal feeders (Collembola, some oribatid mites, PONGE 1988). For that reason, the mycelia of the two basidiomycetes seem to be more heavily and selectively consumed by members of the soil fauna. In addition, incrustation of the walls by

phenolic pigments (melanin, FLAIG 1972) make them difficult to digest. Oribatid mites of the camisiid species, however, proved to be able to digest these walls (PONGE 1988). The association of pigments to chitin and protein parts (MANGIN et al. 1986) confers to the walls of this fungus strong similarities to arthropod cuticles (PETER et al. 1986), with which it shares the same resistance to degradation. It must be noticed that other dematiaceous fungi develop similar mycelia, such as *Phialocephala* (WANG and WILCOX 1985), only connection to mycorrhizal roots enabling us to identify *C. geophilum*.

### 3.3 Penetration of the plant material by mycorrhizal fungi

#### 3.3.1 Pine needles

Penetration of pine needles by *Cenococcum geophilum* in the F layer was considered as a particular stage in their decomposition process (PONGE 1988). This stage follows invasion by other fungi, whose remains are visible in the mesophyll and the stele, but no development of *Verticicladium trifidum* Preuss (the commonest internal colonizer in the L layer) was recorded before the entry of the mycorrhizal fungus. This was interpreted as a difference in the seasonal development of these two fungi (PONGE 1988). The observed fungus develops a dense felt of intermingled hyphae, whose aspect and mode of branching strongly differs from that observed in the aerial mycelium of *C. geophilum*. Presumption that it is the same fungus comes from zones where the two forms are co-existing (Fig. 9). MIKOLA (1948) observed the same sudden changes in his cultures. Here also identification by mean of mycelial characters proves to be impossible. In the present case, no hyphal connection was observed with pine roots, thus keeping unsolved the question of what species this fungus belongs to, really. Growth of the fungus is restricted to the mesophyll part of the needles, just under the hypodermis. Since no penetration of cell walls was observed, we may postulate that the fungus grew freely, splitting the plant tissues, thus covering the inside of the needle by its own prosenchym-like tissue. Although these hyphae appear to be dead, we did not observe any bacterial development in the needles invaded by this fungus, contrary to our observations in the L layer where fungal colonies were dead or senescent. This suggests some antibiotic properties of the walls of this fungus (*Cenococcum geophilum* is known to produce a substance strongly active against Gram-positive bacteria, KRYWOLAP and CASIDA 1964). The need to use lipid dissolving solvents to extract this substance (op. cit.) probably indicates that it is produced in the walls, thus deterring bacteria even after the death of the fungus. The needles penetrated by this dematiaceous mycelium seem very palatable to soil animals, especially oribatid mites (phthiracarid and eu-phthiracarid species, PONGE 1988), although the fungal

tissue itself is not consumed. Consequently; a lot of needles colonized internally by this fungus are tunnelled by mites, which allows mycelia of mycorrhizal fungi (*Hyphodontia* and aerial form of *Cenococcum*) to freely enter the needles (only the cavities, never the cell structures).

### 3.3.2 Pine wood

Observations on a young fallen branch (5 years, included in the L layer and sampled with the other debris, PONGE 1985a) showed intense development of a basidiomycetous fungus with numerous lagenocystidia. This characteristic feature enabled us to identify this fungus as *Hyphodontia*, i.e. the same genus forming the orange-brown mycorrhizae (see lagenocystidia on aerial parts of the extra-matrical mycelium, Fig. 10). *Hyphodontia* spp. are only known as wood fungi, causing white rot (ERIKSSON and RYVARDEN 1976), and not as mycorrhizal ones (this genera is absent from a comprehensive review by TRAPPE 1962, although other Corticiaceae are noticed). The present observations suggest that this fungus could live both saprophytically and symbiotically, although existence of a hyphal connection between the wood substrates and roots has not been demonstrated here.

### 3.3.3 Pine bark

Pine bark was studied on various fragments from the fallen branch previously described, several pine twigs and free fragments probably detached from the tree stem. *Cenococcum geophilum* was widespread on the surface of all these fragments, mainly as a hyphal mat (Fig. 7), but in some cases it formed a pseudo-parenchymatous tissue (Fig. 11). Penetration of hyphae to the inside of bark pieces was never observed, although bark from pine twigs was, in the F layer, a site of intense activity of soil animals and bacteria (PONGE 1988).

### 3.3.4 Pine roots

In this paper we consider that dead roots are incorporated into the litter compartment of forest soil. Thus the fate of mycorrhizal fungi after death or senescence of the root is in the scope of this study.

*Cenococcum* mycorrhizae were not observed to decay in the studied layer. Resistance of this fungus to



summer or adverse sites dryness is well-known (WRIGHT 1963; SALEH-RASTIN 1976; MEYER 1987) and is used, among other characteristics such as tolerance to acidity (MIKOLA 1948), to explain its widespread occurrence in raw humus.

On the contrary, decay features were commonly observed in the orange-brown mycorrhizae. They became fairly brown in colour and their most conspicuous characteristics was the contrast between the mantle, which was rapidly invaded by bacteria and algae (compared to *C. geophilum*, see below) and the inside of the roots. The Hartig net (fungus) and the tannin cells (tree) remained intact probably a long time after the root began to senesce.

The most pronounced features of decomposition were observed on long roots. Of observed phenomena, the penetration of the stele by *Hyphodontia* was the main characteristic of this early stage of decomposition. Sometimes this fungus was accompanied by *Cenococcum geophilum* (typical form). No penetration of the cortical cells by mycorrhizal fungi was observed, unlike observations of MIKOLA (1948) on *Cenococcum* mycorrhizae of birch.

### 3.3.5 Other plant material

The most pronounced features of fungal penetration by mycorrhizal fungi were observed in the epidermis of bracken fern [*Pteridium aquilinum* (L.)] leaflets. Figures 12 and 13 show *Cenococcum* hyphae penetrating plant cell walls by means of haustoria. Some penetrating hyphae are melanized (Fig. 12), some others not (Fig. 13), perhaps according to their developmental stage. In the cases here described, where the material was directly mounted without being cut, the physical connection between these internal hyphae and the mycelium spread on the surface of the leaves may be easily followed by varying the focusing plane.

Penetration by mycorrhizal fungi (both *Cenococcum geophilum*, *Hyphodontia* and the fungus supposed to make pink mycorrhizae) was observed inside bracken petioles after their invasion by soil animals, and in some other substrates such as moss stems which also contained holes made by fauna.

### 3.3.6 Animal corpses

The inside of arthropod corpses (mainly oribatid mites whose cuticle is resistant to degradation) was invaded by

other animals such as enchytreid worms that deposit their faeces (PONGE 1988), but also by numerous fungi that thrive well on this N-rich substrate. Among them, the mycorrhizal *Hyphodontia* colonizes essentially the remains of phthiracarid or eu-phthiracarid mites after they have lost their prostomium (which allows the fungus to enter freely the bubble-shaped abdomen). The most interesting feature is the attack of the chitinous part of the cuticle of arthropod remains by *Cenococcum geophilum* (Fig. 14): a hollow zone is clearly visible around the dark hyphae that penetrate the chitinous part of the cuticle.

#### 4 Discussion

The present findings first give strength to the idea that mycorrhizal fungi are not purely dependent on the tree for their nutrition but may also benefit from nutrients produced during the decay of organic materials. For most species the problem has so far been unsolved as to whether these fungi rather feed on the tree or on the litter, and whether nutrition of the tree benefits from them or not. Our study cannot answer these disputed points, which requires experiments to discern between the causes and the effects. Nevertheless, we can try to analyse our results in the light of existing knowledge.

The fact that mycorrhizae and their associate mycelia develop well in pure organic substrates was ascertained by study of the vertical distribution of short roots of pine in soils with raw humus (MIKOLA and LAIHO 1962; BOWEN 1964; HARVEY et al. 1976). The same holds true for beech (MEYER 1964; MEYER and GÖTTSCHE 1971) and spruce (MIKOLA 1962). Wood and charcoal were also noticed as good media for mycorrhizal root development (BOULLARD and DOMINIK 1966; HARVEY et al. 1976), probably through their role as nitrogen acceptor (MEYER 1985).

From our results it may be thought that *Hyphodontia* and *Cenococcum* exploit the F layer in a non-random way: Each organic fragment, either from moss, bracken or pine origin, is embedded and, when passages have been made through it by the activity of soil fauna, these fungi may gain access. Faecal masses, mainly from lumbricid and enchytreid worms (with a high production of mucus), are undoubtedly preferred in view of the mycelial mat embedding and spreading throughout them, as is the case for cadavers. The question which arises is: does it matter for tree nutrition? In poor soils, where most mycorrhizal root tips are located in the Ao horizon, many nutrients are in an organic form and confined to the litter and humus layers. The poor surface/volume ratio of tree roots and the failure of root hairs to take water and nutrients when the pores are not of the capillary size

(or the channels often interrupted) make necessary the association with a fungus. We may hypothesize that when nutrients are confined to decaying organic fragments mycorrhizal fungi are an obligate partner in tree nutrition. From the point of view of forest strategies, MEYER'S idea (personal communication) is that trees with obligate ectomycorrhizae compete better in soils with a holorganic humus layer, unlike pioneer trees without obligate mycotrophy which are more successful in mineral soils.

Whether mycorrhizal fungi are direct agents of plant litter decomposition has been debated for a long time. Some species of mycorrhizal fungi are able to decompose litter, holocelluloses or lignocelluloses even in pure culture: *Laccaria laccata* (MIKOLA 1956), *Tricholoma* spp. (NORKRANS 1950; LUNDEBERG 1970; TROJANOWSKI et al. 1984), *Suillus* spp. (DAHM et al. 1987), *Xerocomus subtmentosus* (LINDEBERG 1948; LUNDEBERG 1970), *Lactarius* spp. (LINDEBERG 1948; LUNDEBERG 1970; GILTRAP 1982), *Clitopilus prunulus* (Lundeberg 1970), *Cenococcum geophilum*, *Amanita muscaria*, *Rhizopogon* spp. (TROJANOWSKI et al. 1984). In our observations, *Cenococcum geophilum* seems to be able to penetrate the epidermic cells of *Pteridium aquilinum*, but no lysis of the plant cell wall was observed on a wide scale, contrary to *Verticicladium trifidum* and *Marasmius androsaceus* in pine needles (PONGE 1985a). *Hyphodontia*, although very active on pine wood, was not observed to degrade pine needles, but rather to benefit from the activity and autolysis of other groups of soil fungi, permeating and embedding the dead plant and faecal material. That mycorrhizal fungi can absorb and metabolize complex organic substances produced during plant and animal decomposition has been proved: this is for instance the case for proteins with *Cenococcum geophilum* (MIKOLA 1948; BOTTON et al. 1986), *Suillus bovinus*, *Rhizopogon roseolus* and *Pisolithus tinctorius* (ABU ZINADAH and READ 1986), *Paxillus involutus* (READ 1987) and for fulvic acids with *Pisolithus tinctorius* (TAN and NOPAMORNBODI 1979). From that literature and what we observed to occur in a humus sample, we can hypothesize that with differing opportunities in their environment, mycorrhizal fungi may or may not express their lytic potentialities. Perhaps this is the main point that separates them from true decomposer fungi, although strain differentiation may also be invoked (LUNDEBERG 1970).

The last point we want to discuss is the antagonism between mycorrhizal fungi and other soil microorganisms. We observed an intense bacterial (and algal) development on the inside as well as on the periphery of pine needles in the L layer (PONGE 1985a), i.e. in the absence of a dense mycorrhizal mycelial mat. In the F layer, contrary to what was expected, bacteria were much less numerous and lysis of bacterial colonies was observed inside some pine needles (PONGE 1988). The only places where bacteria seemed to be in an active state were some micro-sites in faecal pellets, generally near the surface, but these colonies were made of a few

cells and probably in a quiescent stage. Antibiotic properties of mycorrhizal fungi against soil bacteria were stated (KRYWOLAP and CASIDA 1964; SASEK and MUSILEK 1967, 1968; MARX 1968a, b; GRAND and WARD 1969; MARX 1972), but these properties are shared by many other basidiomycetes (WILKINS 1948). It is therefore difficult to have a clear view of these antagonisms from the existing literature. Concerning the relationships between mycorrhizal and other fungi, we observed that plant fragments with an active saprophytic fungal flora were not colonized by *Cenococcum geophilum* or *Hyphodontia* sp. This suggests that there exists two groups of fungal colonizers which are not coexisting during the decomposition process of pine needles: the one is able to penetrate entire needles by its own means and digest lignocelluloses (*Verticicladium trifidum*, *Marasmius androsaceus*, PONGE 1985a), the other can penetrate needles only after tunnelling by soil animals and probably benefits from their previous digestion (mycorrhizal fungi, such as, here, *Cenococcum* and *Hyphodontia*). A depressive effect of mycorrhizal fungi against true decomposer fungi was ascertained by the experiments of GADGIL and GADGIL (1975), confirmed on the field by their trenching and clearfelling experiment (GADGIL and GADGIL 1971, 1978). Unfortunately their results were not supported by other studies: trenching of pine roots (BERG and LINDBERG 1980), observations in pure cultures on *Cenococcum geophilum* (MIKOLA 1948). The work of DIGHTON et al. (1987) even concludes the opposite, pine roots stimulating cellulose decomposition even in sterile conditions, this effect being enhanced in the presence of the mycorrhizal *Suillus luteus* or suppressed in the presence of the decomposer *Mycena galopus*, the latter being alone or associated with *Suillus*. Since the same experiments made with another mycorrhizal basidiomycete, *Hebeloma crustuliniforme*, gave rise to reduced decomposition by this fungus (as compared to roots alone), we may tentatively conclude that the nature of the fungus is quite decisive and that no generalization can be made concerning these antagonisms.

From the points discussed above we may wonder whether certain mycorrhizal fungi are useful in forests from the angle of tree nutrition (apart from auxin and antibiotic production, that might stimulate growth of the trees and protect them against pathogens). The fate of the melanized walls of *Cenococcum geophilum* is worthy to note in respect to this problem, since they seem to be the main products (with cuticles from oribatid mites) that strongly resist degradation in our humus sample. Faecal pellets of numerous animal groups contain the walls of this fungus, which are rarely digested to some extent (PONGE 1988). Then the question arises of an indirect role of *Cenococcum geophilum* in the non-accessibility of nitrogen (and probably also phosphorus) in moder humus types, although their organic layers are richer in this element (DELECOUR and PRINCE-AGBODJAN 1975). Moreover this fungus may be responsible by itself for the formation of a moder or a mor humus (MEYER 1964) if

it inhibits by its development some major animal species. This is purely conjectural but requires further study, given the importance of mycorrhizal fungi in the diet of the animals we studied (PONGE 1984, 1985a, b; PONGE 1988). Although some works indicate a benefit of this fungus on tree nutrition and growth (BOULLARD and DOMINIK 1966; LAMB and RICHARDS 1971; SIHANONTH and TODD 1977), *Cenococcum geophilum* seems to be less effective than other fungi in stimulating plant growth (MARX et al. 1978) or even deleterious as in the experiments of LUNDEBERG (1970) with raw humus as a substrate. In the latter work the author proved that the depressive effect on seedling development was caused by net immobilization of nitrogen by this fungus. In addition, our own observations show that the extent of penetration of the root is less with this fungus than with the basidiomycete *Hyphodontia*. We think then that environmental conditions which encourage the development of *Cenococcum geophilum*, as compared to other mycorrhizal fungi, must be ameliorated as far as possible, unless resistance to adverse conditions (summer drought, waterlogging) is required. Unfortunately, the effects of some major factors of importance to forestry practice, such as light, are contradictory (MIKOLA 1948; WRIGHT 1963), thus, at the present stage of our knowledge, it is difficult to select an appropriate method to influence the composition of the mycorrhizal fungal flora once the young tree has been planted in the field.

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#### **Summary**

This study was conducted in order to better understand the interrelationships between the fine root system of pine, the abundant mycelial mass spreading throughout the litter, and the decomposing plant and animal material. Fungi were determined to be mainly mycorrhizal, by observing their connection to roots. The two main species are the ascomycete *Cenococcum geophilum* (with dematiaceous walls) and the basidiomycete *Hyphodontia* sp. (with hyaline walls). They were observed to embed and penetrate plant and animal remains. Preferences were for fragments from an animal origin which were richer in nitrogen: faeces of oligochetes (lumbricid and enchytreid worms) and cadavers, mainly of oribatid mites. It seems that in most cases

mycorrhizal hyphae do not penetrate the decaying fragments by their own means, but rather benefit from tunnels and cavities previously made by soil fauna. Nevertheless in particular substrates the two species studied behave as true decomposers: *Cenococcum geophilum* can penetrate bracken epidermis cells by the means of haustoria and *Hyphodontia* sp. develops itself as a white-rot fungus in pine wood. The bacterial and algal development which was starting in the lower part of the L layer seems to be hindered in the F layer, probably under the influence of the antibiotic activity of mycorrhizal fungi. Consequences for forest soil ecology and tree nutrition are discussed in view of existing literature, and the role of *Cenococcum geophilum* which probably acts as a sink for some nutrients (N, P) in humus types where this fungus is too much abundant was pointed out.

### Résumé

*Etude écologique d'un humus forestier par l'observation d'un petit volume. I. Pénétration de la litière de pin par les champignons mycorrhiziens*

Cette étude a été entreprise dans le but de mieux connaître les relations existant entre le système racinaire fin des pins, la masse abondante de mycélium parcourant la litière et le matériel végétal et animal en décomposition. Les champignons ont été définis comme essentiellement mycorrhiziens, par l'observation de leur rattachement aux racines. Les deux espèces principales sont l'ascomycète *Cenococcum geophilum* (à parois dématées) et le basidiomycète *Hyphodontia* sp. (à parois hyalines). On peut les voir emballer et pénétrer les résidus végétaux et minéraux. Leurs préférences vont aux fragments d'origine animale et donc plus riches en azote: déjections d'oligochètes (lombrics et enchytréides) et cadavres, essentiellement d'acariens oribates. Il semble que dans la plupart des cas les hyphes ne pénètrent pas par leurs propres moyens les fragments en décomposition, mais profitent plutôt des tunnels et des cavités produites par la faune du sol. Cependant dans des substrats particuliers les deux espèces étudiées se comportent comme des décomposeurs vrais: *Cenococcum geophilum* est capable de pénétrer les cellules épidermiques de la fougère aigle au moyen d'haustoria et *Hyphodontia* sp. se développe comme une pourriture blanche au sein du bois de pin. Le développement bactérien et algal qui démarrait dans la partie inférieure de la couche L semble être entravé au niveau de la couche F, probablement sous l'influence de l'activité antibiotique des champignons mycorrhiziens. Les conséquences pour l'écologie des sols forestiers et la nutrition des arbres ont été discutées sur la base de la littérature existante, et le rôle de *Cenococcum geophilum*, qui immobilise en pure perte des éléments essentiels (N, P) dans les formes d'humus où ce champignon est trop abondant, a été mis en évidence.

### Zusammenfassung

*Ökologische Untersuchungen über einen Forsthumus durch Beobachtung eines geringen Volumens. I.*

*Durchdringung der Kiefernstreuschicht durch Mykorrhizapilze*

Diese Untersuchungen wurden durchgeführt, um die Beziehungen zwischen dem Feinwurzelsystem der Kiefern, den Pilzen der Streuschicht und den pflanzlichen und tierischen Resten besser zu verstehen. Die Pilze wurden auf Grund ihrer Hyphenverbindung zu den Wurzeln als Mykorrhizapilze definiert. Die zwei hauptsächlichsten Gattungen sind der Ascomycet *Cenococcum geophilum* (mit dunklen Zellwänden) und der Basidiomycet *Hyphodontia* sp. (mit hyalinen Zellwänden). Man kann sehen, daß sie die pflanzlichen und tierischen Reste umwachsen und in sie eindringen. Sie bevorzugen Teile tierischer Herkunft, die also stickstoffreich sind, wie Exkreme von Oligochäten (Lumbriciden und Enchytraeiden) und Leichen, insbesondere von Oribatiden. Es scheint, daß die Hyphen in der Mehrzahl die in Zersetzung befindlichen Reste nicht aus eigener Kraft durchdringen, sondern daß sie die Tunnel und Hohlungen, die durch die Bodenfauna entstanden sind, nutzen. In besonderen Substraten verhalten sich die zwei untersuchten Gattungen jedoch wie echte Zersetzer: *Cenococcum geophilum* kann die Zellen der Epidermis von *Pteridium aquilinum* mit Haustorien durchdringen und *Hyphodontia* sp. wächst als Weißfäulepilz im Kiefernholz. Es scheint, daß die Entwicklung von Bakterien und Algen, die in der unteren L-Schicht begann, in der F-Schicht erschwert ist, vermutlich durch antibiotische Aktivität der Mykorrhizapilze. Die Beobachtungen werden unter dem Aspekt der Waldbodenökologie und der Baumernährung diskutiert, und die Rolle der *Cenococcum geophilum* bei der Festlegung wichtiger Elemente (N, P) wird dargestellt.

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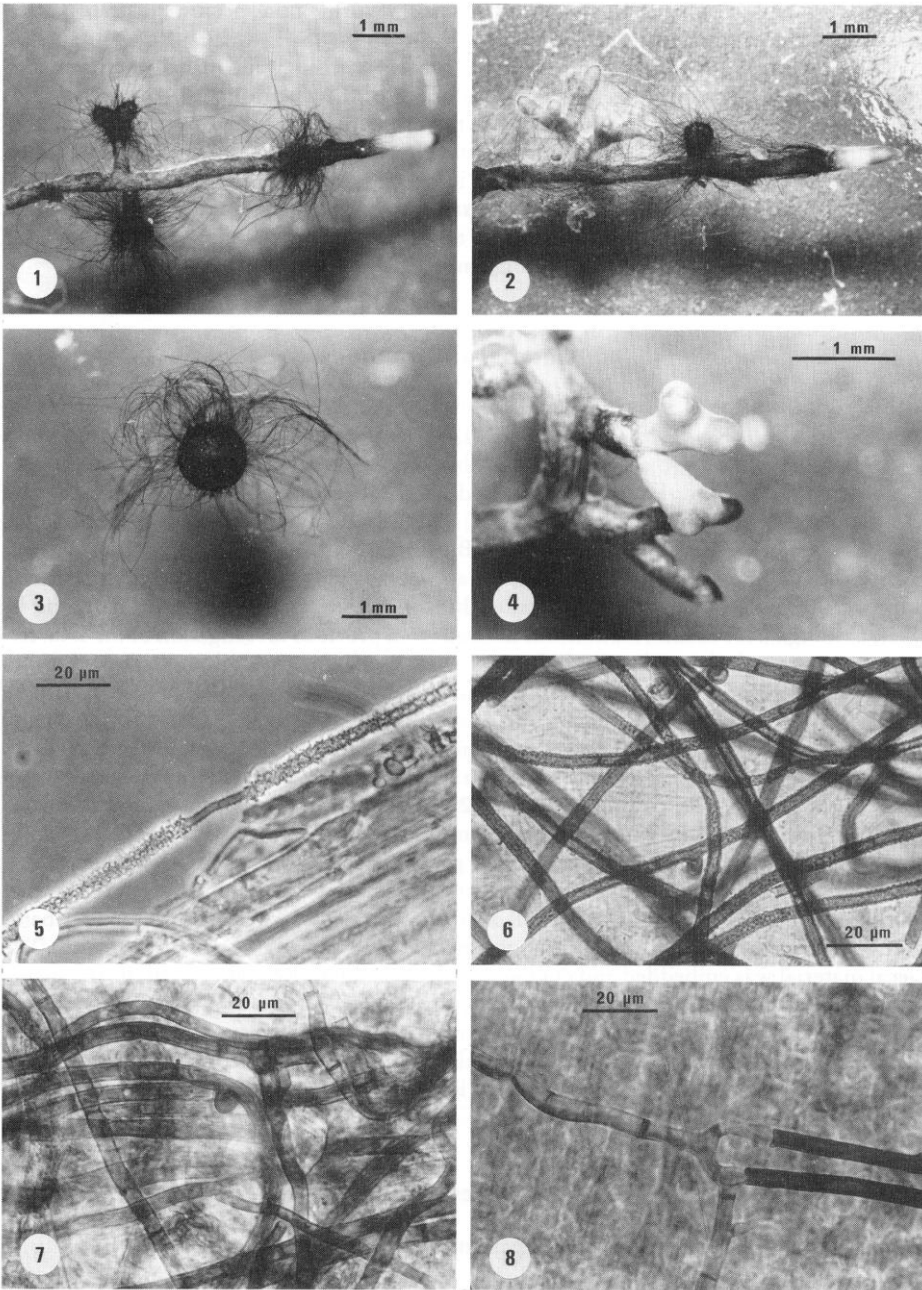
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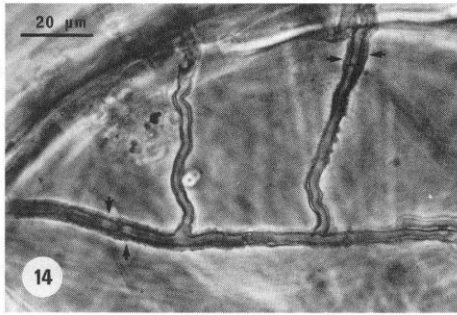
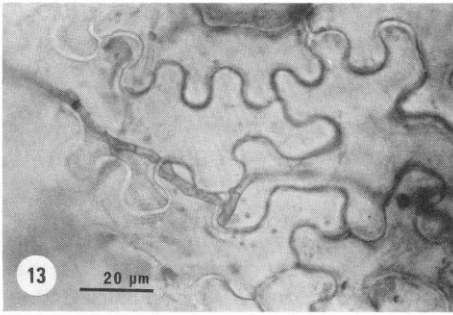
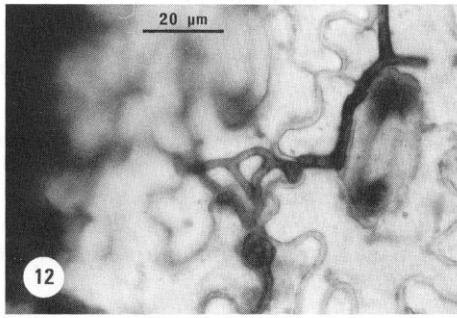
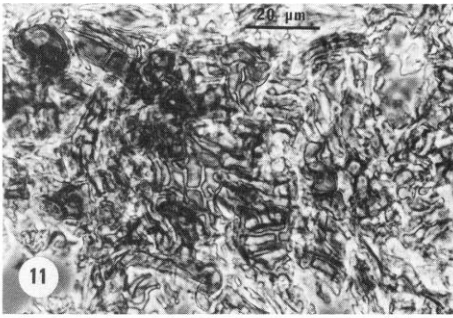
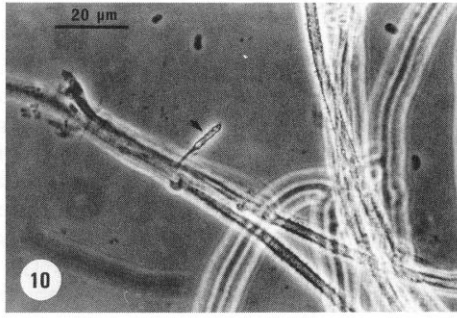
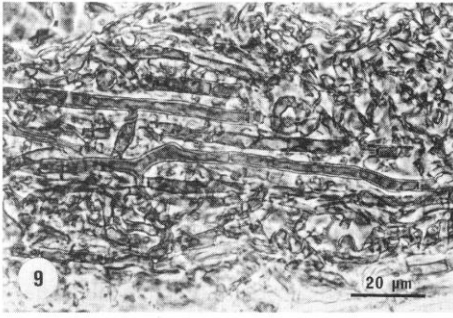
## Legends of figures

- Fig. 1.** Colonization of a long root and two short roots of pine by the mycorrhizal fungus *Cenococcum geophilum*. F<sub>1</sub> layer
- Fig. 2.** Two pine mycorrhizae, the one formed by the basidiomycete *Hyphodontia* sp., the other by the ascomycete *Cenococcum geophilum*, on the same long root. Note infection of the “root hair zone” by the latter fungus. F<sub>1</sub> layer
- Fig. 3.** Black sclerotium of *Cenococcum geophilum* with radiating hyphae. F<sub>1</sub> layer
- Fig. 4.** Pink mycorrhizae with a smooth surface and no attached mycelium. F<sub>1</sub> layer
- Fig. 5.** Hypha of *Hyphodontia* sp. covered with calcium oxalate crystals. F<sub>1</sub> layer
- Fig. 6.** Aerial hyphae of *Cenococcum geophilum* with a rough surface. F<sub>1</sub> layer
- Fig. 7.** Aerial hyphae of *Cenococcum geophilum* with a smooth surface. F<sub>1</sub> layer
- Fig. 8.** Aerial hyphae of *Cenococcum geophilum* with melanized and unmelanized cell walls. F<sub>1</sub> layer
- Fig. 9.** Presumed passage from the typical aerial form of *Cenococcum* hyphae to the aforementioned form at the inside of pine needles. F<sub>1</sub> layer
- Fig. 10.** Aerial hyphae of *Hyphodontia* with lagenocystidium (arrow). F<sub>1</sub> layer
- Fig. 11.** Pseudoparenchymatous tissue formed by *Cenococcum geophilum* at the surface of a piece of bark. F<sub>1</sub> layer
- Fig. 12.** Penetration of bracken epidermal cells by melanized hyphae of *Cenococcum geophilum*. F<sub>1</sub> layer
- Fig. 13.** Penetration of bracken epidermal cells by unmelanized hyphae of *Cenococcum geophilum*. F<sub>1</sub> layer
- Fig. 14.** Lysis zones (arrows) along *Cenococcum* hyphae growing on the chitinous (inside) part of the cuticle of the oribatid mite *Oribatula tibialis*. F<sub>1</sub> layer



Figs. 1-8





Figs. 9–14