Succession of fungi and fauna during decomposition of needles in a small area of Scots pine litter

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Abstract

During micromorphological investigations on Scots pine litter, several decomposition stages have been recognized on fallen pine needles, each being associated with the activity of animal and microbial organisms, both. To well-known fungal successions that have been so far described by mycologists we must add succession of animal groups such as nematodes, amoebae, enchytraeids, sciarid larvae, oribatid mites and earthworms. A bacterial development was observed in the L_2 layer, following penetration by microfauna (nematodes, amoebae). After that stage pine needles were actively tunnelled by enchytraeids, sciarid larvae and oribatid mites and at the same time were nibbled on by epigeic earthworms (L_2 and F_1 layers). When the fine root system of pine developed through accumulated old needles (F_1 layer), mycorrhizal fungi penetrated the needles and seemed to impede any further bacterial development. Pine foliar tissues were progressively incorporated into the fecal material of earthworms and other members of the soil fauna. A more realistic scheme was suggested for plant litter decomposition in moder humus.

Introduction

Fungal successions during foliage senescence and decomposition have been extensively studied by plant and soil

mycologists, especially on pine needles (Black and Dix, 1977; Hayes, 1965; Kendrick and Burges, 1962; Mitchell and Millar, 1978; Mitchell et al., 1978; Rack and Scheidemann, 1987; Soma and Saito, 1979; Watson et al., 1974), but similar observations on animals were very scarce (Burges, 1967; Styles, 1967). In addition to pines, other coniferous trees have been investigated and strong resemblances may be observed with silver fir (Gourbière, 1981; 1982; 1983; 1988; Gourbière and Pépin, 1983). The role of soil fauna has been largely underestimated except when micromorphology was used to follow decomposition processes, as was the case with Douglas fir (Bal, 1970). When faunal results are discarded, which are in fact difficult to interpret in terms of successions, the commonly admitted sequential pattern for pine needles fungi may be summarized as shown by Table 1. The group of mycorrhizal fungi was only mentioned by Soma and Saito (1979), but their role in the late colonization of pine needles has been fully recognized by Ponge (1988; 1990).

The observations presented here are part of a morphological investigation which was carried out on a small area of pine litter in a temperate forest. Several papers have been already published (Ponge, 1984; 1985a, b; 1988; 1990; 1991). In the present paper, animals and micro-organisms that colonize decaying pine needles are described and the way they succeed each other was studied through comparison of superposed litter layers.

Material and methods

A small area (ca. 5 x 5 cm) of moder humus was sampled on 11.8.81 in a 35-year-old *Pinus sylvestris* L. plantation (Orleans forest, France) and microstratified in the field into L_1 (brown needles), L_2 (black needles), F_1 (broken needles + pine roots and widespread mycelial mat) and other layers not investigated here. Fixation of each layer was made immediately, before any transport, in 95% ethanol. A more complete description of the stand has been previously given (Ponge, 1984).

Observations were made under a light microscope at 400 x magnification after sorting the plant and animal material under a dissecting microscope. Pine needles and other plant fragments were sectioned to 7.5-μm thickness (microtome Stiassnie of Minot type with stainless steel knife, after embedding in paraffin wax) and mounted in chloral-lactophenol (25 cm^3 lactic acid + 50 g chloral hydrate + 25 cm^3 phenol). Phase contrast allowed one to discern between dead and living cells at the time of fixation (Frankland, 1974): presence of cytoplasm was easily discernible by the opacity of cell contents, provided the fungal walls were not melanized. When necessary (e.g. for dematiaceous fungi), methyl blue was used in lactophenol as a staining agent for cell

contents. It helped to observe living fungi and bacteria. Animals were either dissected (oribatid mites) or observed after clearing (other groups) in order to analyse their gut contents. Faecal pellets were embedded and cut like plant material. Recognition of the fragments by external characteristics before cutting them provided a more reliable interpretation of morphological features than direct soil sectioning. The nature of the mycelial mat and its connection to the fungus mantle of mycorrhizae and to the internal colonizers of plant debris and cadavers were also ascertained by direct observation at a low magnification.

Fungal colonizers were identified by morphological characteristics directly observed on the fixed material, without any cultivation. See Ponge (1984; 1985a; 1988; 1990) for further details on the anatomical characteristics that were used for due identification of fungi. Nomenclature for plant anatomy was taken from Esau (1965).

Results

Stage I: primary fungal colonizers

Fungal colonization may be easily revealed by the fruiting bodies of *Lophodermium pinastri*, *Ceuthospora pinastri* (Fig. 1) and *Lophodermella* sp. Of these three fungi, *L. pinastri* was the most commonly encountered. Several colonies of this ascomycete may occur on the same needle, each one being delimited by two black diaphragms. Figure 1 shows that *L. pinastri* and C. *pinastri* may coexist on the same needle, but in distinct colonies separated by the black stromatic lines of the first species which always excludes any other fungus. Examination of needles collected by a panel several decimeters above the ground surface did not reveal the presence of C. *pinastri*. Thus we may think that fruiting of this fungus took place after the needles had fallen on the forest floor, which was not the case for the two other fungi. It nevertheless belongs to the same decay stage, since we never observed it succeeding to the two other fungi. The internal development of these three fungi was similar, with hyphae travelling through free spaces in the mesophyll tissues, without any penetration of plant cells. Mesophyll cells appeared to be collapsed, with brown walls. In the stele, phloem tissue was brown and collapsed, but xylem tracheids and vascular fibers kept intact their structure and clarity (Fig. 2). Thus these fungi did not attack lignified tissues. Cellulose walls did not disappear, but seemed to be slightly transformed (distinct browning).

Many needles, light brown in colour and seemingly free of any fungal contamination, when sectioned, proved to be invaded by a hyaline fungus. No fruiting bodies were visible. The most intense development was observed in the substomatal chambers, probably at the expense of the obturating cell, which had disappeared. Nevertheless, hyphae of this fungus were present in all parts of the sections. Living cells were penetrated and their cytoplasm had disappeared, being replaced by fungal mycelium. No penetration was observed in tracheids, fibres, nor in the epidermal cells, but mesophyll, endodermis, phloem and stele parenchym cells were penetrated. A distinct browning was visible in all cellulosic walls, but lignified walls remained transparent (Fig. 3). Thus, contrary to the aforementioned three other species, this fungus had an internal development that affected cytoplasm (except lignified epidermal cells) and to a slight extent all cellulose walls. Since this fungus did not sporulate in the observed needles, no attempt was made to identify it, but it shares similar features with the white "stomata" category designated by Hayes (1965) for some freshly fallen pine needles.

Stage II: secondary fungal colonizers

Verticicladium trifidum was the most important fungus in the decomposition of pi ne needles. It has been encountered in a living state from the most superficial layer (L_1) to the F_1 layer, suggesting that its persistence in each colonized needle probably exceeded one year (see discussion). This fungus may be easily identified through its typical conidiophores protruding from stomatal apertures. *V. trifidum* commonly acted as a secondary colonizer (after the aforementioned fungi), but several needles in the L_1 layer proved to have been colonized for the first time by this fungus. This was ascertained by the absence of any other fungal species and for some of them by the presence of starch grains in the mesophyll and transfusion tissue parenchyma cells. By comparing many needles invaded by *V. trifidum* in the three layers investigated, several stages may be recognized in its internal development. Melanized hyphae penetrated through stomatal apertures and developed a stroma that filled the front cavity above the guard cells. This stage was observed on needles colonized by *L. pinastri* and may be considered as a quiescent period for *V. trifidum*, whose internal development may take place once the former species has senesced. Coincident development of these two fungi was never observed in sectioned needles. The next step was the penetration of all needle tissues by hyaline hyphae. These were about 5 μm thick and had relatively thick walls that were easily visible in cross sections. They seemed to invade preferentially elongated cells like xylem tracheids, and bundles of hyphae were observed filling resin ducts (Fig. 4). Hyaline hyphae were also present inside stomatal guard cells. Thus, at this stage, melanized stromata were restricted to

the front cavity of stomatal apertures. A tangential section through the lower part of stomata showed that the outer walls of guard cells began to be incrusted by a melanin-like substance. This observation deserves attention, because it is a starting point for needle blackening. No action on cell walls (whether they were made of cellulose or lingo-cellulose) was observed at this stage. Blackening of the needles occurred during the following step. On sections it may be seen that this was due to impregnation of the hypodermal basis by melanin-like substances (Fig. 5). Melanization may reach part of the mesophyll columns (Fig. 5). Ligno-cellulose cell walls were disrupted. This was especially true for transfusion tissue, where in some parts pit borders were the only tracheid remnants that were still recognizable. Hyaline hyphae may give rise to stromatic structures in the zones where pine tracheids had completely disappeared. Xylem tracheids seemed to be more resistant, or penetration may have been delayed as compared to transfusion tracheids: their walls seemed to have been reduced in thickness, but they were still perceptible. In the xylem tissue, bordered pits were the commonest route for the fungus passing from one tracheid to another.

Marasmius androsaceus is a basidiomycete known for its typical brown, flattened rhizomorphs (Fig. 6) that commonly enter decaying needles of several conifer species. Emergence of fruit bodies from the needles was not observed in our sample, but the common appearance of this fungus on decaying coniferous litter was taken as presumptive evidence of its identification (Gourbière, personal communication). Mesophyll cells were replaced by a pseudo-parenchymatous fungal tissue, but no lysis was observed in the ligno-cellulosic tracheids. The development of this fungus was conspicuous only in the L_2 layer and it seemed to be active only during win ter months.

The mycorrhizal ascomycete *Cenococcum geophilum* Fr. [= C. *graniforme* (Sow.) Ferd. et Winge] (ascribed to this group by Trappe (1971) for its resemblance with *Elaphomyces* spp.) was observed to have a well developed mycelium inside some needles in the F_1 layer (Fig. 7). These were not colonized by *V. trifidum.* Thus we may hypothesize that *V. trifidum* was inhibitory to C. *geophilum* and that the latter could not act as a tertiary colonizer. C. *geophilum* showed a preference for growing between the hypodermis and the mesophyll. The fact that hyphae of this fungus did not possess any cell content and showed some signs of autolysis indicated that colonization had probably occurred in the previous autumn.

Stage III: ingestion and penetration by fauna

After the stages of fungal decomposition, all needles appeared to have been attacked by soil animals. Fauna may penetrate inside pine needles (microfauna, mesofauna) or may ingest entire pieces that were compacted into faeces (macrofauna). Action of fauna was associated with a bacterial and algal development which was never observed in the primary and secondary fungal stages.

Presence of bacteria (visible through staining with methyl blue) was observed only after needles had been broken up or nibbled on by animals. In several cases, bacterial development was recorded without any penetration by an animal species, but these needles always showed some signs of a faunal attack. In a first stage, bacteria were observed to develop at the expense of fungi, whose hyphae were still recognizable, as was the case with *V. trifidum.* At a more advanced decay stage, bacterial development was recorded throughout the whole needle and in most cases the only visible plant remains were bordered pits. Bacteria were accompanied by algae, whose chloroplasts were of a deep purple when stained with methyl blue, or by cyanobacteria.

Protozoa were traced by the presence of cysts, but nematode worms and rotifers were also commonly encountered. Bacteria were present too. It seems that these animals were able to penetrate the needles by their own means, since some needles with microfauna did not present signs of attack by larger-sized animals. Thus they could be considered as a specific stage, half-way between the two first fungal stages and the other faunal stages. Nevertheless, penetration by microfauna was commonly associated with nibbling or fragmentation by other animal species. The same situation was encountered with *V. trifidum* (see above).

Colonization by mesofaunal groups was known to be active at the sampling time, since in addition to recognizable fecal pellets each group was represented by living animals with filled intestines.

Enchytreid worms were the most commonly encountered mesofaunal group in the three layers investigated. Although pine needles were not their only food, at the time of sampling (summer) they were found actively tunnelling pine needles in the L_2 and F_1 layers (Fig. 8). They were not identified at the species level, but probably belonged to the species *Cognettia sphagnetorum* (Vejdovsky, 1877) because of the observed fragmentative reproduction and high acidity of their environment (Healy, 1980; Standen, 1973). Deposition of fecal pellets in the interior of pine needles was observed, but in most cases the faeces were deposited outside the needles. Bacteria proliferated in the fecal material, both inside and outside the needles, except in the F_1 layer where they were replaced by mycorrhizal fungi, in some cases together with filamentous cyanobacteria. Parenchymatous pine cells were crushed and their shape was no longer recognizable, but lignified cells were

preserved. Action of these animals on the cellulosic material was not fully ascertained (in the most obvious cases digestion seemed to be only partial), but they certainly digested hyaline fungal material (Fig. 9). Dematiaceous hyphal walls were preserved (Fig. 9). Galleries of enchytraeid worms at the inside of pine needles were lined with mucus, which was the centre of an intense bacterial development. Much debris, which the animals had been carrying on their tegument, was found lining the galleries, such as pollen grains or Testacean cysts.

Oribatid mites that were tunnelling through pine needles belonged to the two closely related families Phthiracaridae [*Rhysotritia duplicata* (Michael, 1880)] and Euphthiracaridae (*Phthiracarus* spp.). No differences were found between these species concerning the ingested tissues, the fate of the ingested material and the way they deposited their fecal pellets, except that pine needles were an obligate growth medium for Phthiracarus larvae, which was not necessarily the case for Rhysotritia. Thus they will be considered together for describing their role in decomposition processes. Oribatid mites deposited their fecal pellets inside the pine needles (Fig. 10). Due to the high degree of comminution of pine cells after they had been broken up by the buccal apparatus, the ingested tissues were not identifiable in the faeces. There was a fairly good relationship between the size of the animals and the degree of comminution of the plant material. Fecal pellets contained cell wall remains, but there was probably a loss of the crystalline structure of cellulose in the inner part of each pellet: cell walls were observed to become dull brown and amorphous. This phenomenon occurred during passage through the mite intestine, since this transformation could be followed on the same individual between two successive food boli. Microbial development wasnot observed in or on the pellets found inside pine needles. Undoubtedly a choice was made among the different types of fungal colonized needles and it seemed that needles colonized by the mycorrhizal *C. geophilum* (see above) were preferred to needles colonized by *V. trifidum,* but no attempt was made to quantify this preference.

Pine needles were also ingested by larvae belonging to the Sciaridae family. They were observed tunnelling the needles in a fashion similar to enchytreid worms, i.e. most fecal material was deposited on the outside. Examination of the faeces gave only evidence of resistant pine structures, such as guard cells. Nevertheless, intestinal guts were filled with pine cells in the process of being dissolved, bordered pits remaining slightly visible, which proved that digestion of ligno-cellulose occurred, at least with the thin-walled transfusion tissue. No microbial development was recorded in the studied faeces, except cyanobacteria at the periphery.

Macrofaunal activity was restricted to the F_1 layer. This feature was not shared by mesofaunal species, which start their activity in the L_1 layer, as was shown above.

Lumbricid worms were the most active macrofaunal group in the studied layers, judged by accumulation of their faeces in the F_1 layer (Fig. 11). They belonged probably to the epigeic species *Dendrobaena octaedra* (Savigny, 1926) since it was the only one recorded on this site (Bouché, unpublished data). Pine needle tissues were ingested, but they were always mixed with other material (fungi, faeces, pollen grains, etc.). The degree of comminution of pine material was high, although not comparable to that by oribatid mites, due to transit in the gizzard. No transformations seemed to occur in the cell walls. Microbial development was feeble and restricted to some micro-sites or to the periphery. Earthworm faeces were in most cases penetrated by the brown hyphae of the mycorrhizal fungus C. *geophilum* (Fig. 12).

Two other types of macrofauna faeces were recorded, but they were far fewer in number than earthworm faeces. Their connection to identified species or group of species was highly hypothetical, thus they will be referred to as type A and type B (Ponge, 1988). Type A faeces were tentatively ascribed to slugs (Fig. 13). They were made of needle pieces that had been rejected without any change in their appearance when observed under a dissecting microscope. Examination at a higher magnification under a light microscope proved that pine tissues were intact, even the most delicate ones. Fungal material was present in great abundance inside of ingested needles, attesting that they had been consumed in the litter, not in the tree foliage. No cytoplasm remains were recorded, thus fungal cytoplasm was probably the only food source for these animals. Microbial colonization of type A faeces was restricted to some micro-sites. Similar features were observed in type B faeces, although the ingested needles had been broken into smaller pieces (Fig. 14). They were tentatively ascribed to woodlice (Ponge, 1988). They seemed not to be a good medium for microbial development, algae or bacteria being restricted to micro-sites as in the previous case. Nevertheless they had been penetrated by mesofaunal groups such as oribatid mites, which deposited their fecal pellets in small cavities. The age of some of the type B faeces was ascertained by autolysis of bacterial colonies, the pine material remaining intact. Penetration by mycorrhizal fungi was not recorded in type A and B faeces, contrary to earthworm faeces.

Stage IV: penetration by mycorrhizal fungi

Faunal activity led to the presence of holes in all pine needles in the F_1 layer, thus allowing the dominant fungi to enter the pine material. Penetration by C. *geophilum* was recorded above, but with mycelial growth quite distinct from that of fungi growing freely in open space. In the present case, C. *geophilum* acted as a saprobic species.

After the needles had been tunnelled by mesofaunal species, the aerial form of the two main mycorrhizal fungi present, namely *C. geophilum* and the hyaline basidiomycete *Hyphodontia sp.*, developed inside the hollow needles without any change in their anatomical features.

Discussion

The proposed scheme for decomposition of pine needles has no universal value, since it was derived from a microscale study strongly limited both in time and space. Nevertheless, comparison with information in the literature on the same subject leads to the conclusion that the observations on the first stages of fungal decomposition were in accordance with those of more comprehensive studies, thus providing credibility to the present results on later stages which were not studied earlier.

L. pinastri, *C. pinastri* and *Lophodermella* spp. (also called Hypoderma and Hypodermella in older literature) are widespread pine-specific fungi which have been recorded frequently on living or senescent pine needles (Gremmen, 1957; 1977; Hayes, 1965; Kendrick and Burges, 1962; Minter et al., 1978; Minter, 1981; Mitchell and Millar, 1978; Mitchell, Millar and Minter, 1978; Mitchell, Millar and Williamson, 1978; Rack and Scheidemann, 1987; Soma and Saito, 1979). These fungi were assigned to one decomposition stage (stage 1), due to uniformity in the mode of colonization (extra-cellular hyphae in the mesophyll) and to coexistence of these species in the same superficial layer (needles shed a few months before sampling date).

V. trifidum was also abundantly recorded as a secondary colonizer specific to fallen pine needles (Black and Dix, 1977; Burges, 1967; Gremmen, 1957; 1960; 1977; Hayes, 1965; Hughes 1951; Kendrick and Burges, 1962; Mitchell, Millar and Minter, 1978). Observations on the internal development of *V. trifidum* were scarce, apart from formation of black stromata in the sub-stomatic chambers, from which the conidiophores emerged. Nevertheless, Kendrick and Burges (1962) indicated that internal tissues were infected by hyaline hyphae, dark hyphae being located in the peripheral zone, and that a distinct browning of the host epidermis and hypodermis cell walls occurred under the influence of this fungus. No reference was made in the literature to the action of *V. trifidum* upon ligno-cellulose (transfusion tracheids) and the formation of scattered microstromata in later stages of development.

M. androsaceus was known as a non-specific internal colonizer of coniferous needles (Burges, 1967;

Gourbière, Pépin and Bernillon, 1987; Gourbière and Corman, 1987; Mitchell, Millar and Minter, 1978; Newell, 1984; Soma and Saito, 1979). Deterioration of internal tissues was known to be the action of a white-rot fungus, i.e. ligno-cellulolytic. Accordingly, a nearly complete disappearance of the mesophyll tissues was observed in the zone where the fungus had penetrated, but no lytic action towards tracheids was observed. Colonization was restricted to a small zone near the point of penetration. Thus, the impact of *M. androsaceus* was less drastic than that of *V. trifidum*, which is not in accordance with the weight losses commonly attributed to *M. androsaceus* (Gourbière and Corman, 1987). This discrepancy might arise from the fact that in the present sample *M. androsaceus* seemed to have been active just during a short cold period (winter months) inside pine needles.

The aforementioned fungal successions were already well described in pine species and they were analogous to what was observed on other coniferous genera (Gourbière, 1981; and personal observations).

In contrast, some of the present observations are not supported by the literature. *C. geophilum* was found at the same decay stage as *V. trifidum* and *M. androsaceus. C. geophilum* was surprisingly absent from reports of Kendrick and Burges (1962) and others on fungal decomposition of pine needles, probably due to lack of appropriate cultural methods. These workers used a combination of classical isolation techniques and direct observations on the needles in damp chambers. Using this approach, the recovery of mycorrhizal fungi was certainly impossible.

Colonization of pine needles by fauna has been studied by a few authors. Styles (1967) placed pine needles in nylon-mesh bags in forest litter and followed meso- and macrofaunal populations during four years. Unfortunately, no mention was made of enchytreid worms or microfauna, and the mesh size he used (1 mm) prevented earthworms and other large animals from penetrating the bags. The main similarity with the present results was that phthiracarid mites were present only in well decayed needles. This was also supported by the cultural observations of Hayes (1963) on sever al phthiracarid species. Other studies on the role of soil animals in pine needle decomposition (Berg et al., 1980; Elliott, 1970; Hartenstein, 1962; Kowal and Crossley, 1971; Lundkvist, 1978; Metz and Farrier, 1969; Soma and Saito, 1983) are difficult to discuss in this respect, since they were not conducted for an assessment of the impact of soil fauna upon decomposition processes. Direct relevance to decomposition processes could be found in laboratory experiments with enchytreid worms and spruce litter or humus (Abrahamsen, 1990; Williams and Griffiths, 1989). Both these studies and many others on different substrates and animal groups gave evidence of an increased net mineralization rate for nitrogen, but they differed in their answers to the question: is mineral nitrogen (or near-mineral substances such as urea)

produced directly by the animals or indirectly, through their action upon microorganisms? The question seems unsolved at the present time, and the matter probably needs more refined methods to be assessed. Another study made with the same material (Ponge, 1991) establishes a direct influence of some soil animals upon plant material through digestion processes.

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References

Abrahamsen G 1990 Influence of *Cognettia sphagnetorum* (Oligochaeta: Enchytraeidae) on nitrogen mineralization in homogenized mor humus. Biol. Fertil. Soils 9, 159−162.

Bal L 1970 Morphological investigation in two moder-humus profiles and the role of the soil fauna in their genesis. Geoderma 4, 5−36.

Berg B, Lohm U, Lundkvist H and Wiren A 1980 Influence of soil animals on decomposition of Scots pine needle litter. *In* Structure and Function of Northern Coniferous Forests: An Ecosystem Study. Ed. T Persson. Ecol. Bull. (Stockholm) 32, 401−409.

Black R L B and Dix N J 1977 Colonization of Scots pine litter by soil fungi . Trans. Br. Mycol. Soc. 68, 284−287.

Burges A 1967 The decomposition of organic matter in the soil. *In* Soil Biology. Eds. A Burges and F. Raw. Pp 479−492. Academic Press, London.

Elliott H J 1970 The role of millipedes in the decomposition of *Pinus radiata* litter in the Australian Capital Territory. Aust. For. Res. 4, 3−10.

Esau K 1965 Plant Anatomy. 2nd edition. Wiley, New York. 767 p.

Frankland J C 1974 Importance of phase-contrast microscopy for estimation of total fungal biomass by the agar-film technique. Soil Biol. Biochem. 6, 409−410.

Gourbiere F 1981 Vie et Décomposition des Aiguilles de Sapin *(Abies alba* Mill.): Etude des Microflores Fongiques Associées. Unpublished Doctorate Thesis. 140 p.

Gourbière F 1982 Champignons des aiguilles de sapin *(Abies alba* Mill.). VIII. Observation directe des microflores. Bull. Soc. Mycol. France 98, 129−138.

Gourbière F 1983 Champignons des aiguilles de sapin *(Abies alba* Mill.). IX. Microflores internes. Bull. Soc. Mycol. France 99, 203−215.

Gourbière F 1988 Structure spatio-temporelle de la mycoflore des premiers stades de décomposition des aiguilles *d'Abies alba*. Soil Biol. Biochem. 20, 453−458.

Gourbière F and Corman A 1987 Décomposition des aiguilles d'*Abies alba*: hétérogénéité du substrat et de la mycoflore, rôle de *Marasmius androsaceus.* Soil Biol. Biochem. 19,69−75.

Gourbière F and Pepin R 1983: Champignons des aiguilles de sapin *(Abies alba* Mill.). X. Observation en chambre humide. Bull. Soc. Mycol. France 99, 325−335.

Gourbière F, Pépin R and Bernillon D 1987 Microscopie de la mycoflore des aiguilles de sapin (*Abies alba*). III. *Marasmius androsaceus* . Can. J. Bot. 65, 131−136.

Gremmen J 1957 Microfungi decomposing organic remains of pines. Fungus 27, 34−42.

Gremmen J 1960 A contribution to the mycoflora of pine forests in the Netherlands. Nova Hedwigia 1, 251−288 + 8 inlet plates.

Gremmen J 1977 Fungi colonizing living and dead tissue of *Pinus sylvestris* and *P. nigra.* Kew Bull. 31, 455−460.

Hartenstein R 1962 Soil Oribatei. VII. Decomposition of conifer needles and deciduous leaf petioles by *Steganacarus diaphanum* (Acarina: Phthiracaridae). Ann. Entomol. Soc. Am. 55, 713−716.

Hayes A J 1963 Studies on the feeding preferences of some phthiracarid mites (Acari: Oribatidae). Entomol. Exp. Appl. 6, 241−256.

Hayes A J 1965 Studies on the decomposition of coniferous leaf litter. II. Changes in external features and succession of microfungi. J. Soil Sci. 16, 242−257.

Healy B 1980 Distribution of terrestrial Enchytraeidae in Ireland. Pedobiologia 20, 159−175.

Hughes S J 1951 Studies on micro-fungi. IX. *Calcarisporium*, *Verticicladium*, and *Hansfordia* (gen. nov.) Mycol. Papers No 43, 25 pp.

Kendrick W B and Burges A 1962 Biological aspects of the decay of *Pinus silvestris* leaf litter. Nova Hedwigia 4, 313−344 + 14 inlet plates.

Kowal N E 1969 Ingestion rate of a pine-mor oribatid mite. Am. Midl. Nat. 81, 595−598.

Kowal N E and Crossley D A Jr 1971 The ingestion rates of micro-arthropods in pine mor, estimated with radioactive calcium. Ecology 52, 444−452.

Lundkvist H 1978 The influence of soil fauna on decomposition of pine needle litter: A field experiment. Swed. Conif. For. Proj. Techn. Rep. No 18, 15 pp.

Metz L J and Farrier M H 1969 Acarina associated with decomposing forest litter in the North Carolina Piedmont. *In* Proceedings of the 2nd International Congress of Acarology, Sutton Bonington, 1967. Ed. G O Evans. pp 43−52. Akadémiai Kiadó, Budapest.

Minter D W 1981 Lophodermium on pines. Mycological Papers No 147, 54 pp. + 16 inlet plates.

Minter D W, Staley J M and Millar C S 1978 Four species of Lophodermium on *Pinus sylvestris.* Trans. Br. Mycol. Soc. 71, 295−301.

Mitchell C P and Millar C S 1978 Mycofloral successions on Corsican pine needles colonized on the tree by three different fungi. Trans. Br. Mycol. Soc. 71, 303−317.

Mitchell C P, Millar C S and Minter D W 1978 Studies on decomposition of Scots pine needles. Trans. Br. Mycol. Soc. 71, 343−348.

Mitchell C P, Millar C S and Williamson B 1978 The biology of *Lophodermella conjuncta* Darker on Corsican pine needles. Eur. J. For. Pathol. 8, 108−118.

Newell K 1984 Interaction between two decomposer basidiomycetes and a Collembolan under Sitka spruce: Distribution, abundance and selective grazing. Soil Biol. Biochem. 16, 227−233.

Ponge J F 1984 Etude écologique d'un humus forestier par l'observation d'un petit volume, premiers résultats. I. La couche L_1 d'un moder sous pin sylvestre. Rev. Ecol. Biol. Sol 21, 161−187.

Ponge J F 1985a Etude écologique d'un humus forestier par l'observation d'un petit volume. II. La couche L_2 d'un moder sous *Pinus sylvestris,* Pedobiologia 28, 73−114.

Ponge J F 1985b Utilisation de la micromorphologie pour l'étude des relations trophiques dans le sol: La couche L d'un moder hydromorphe sous *Pinus sylvestris* (Forêt d'Orléans, France). Bull. Ecol., 16, 117−132.

Ponge J F 1988 Etude écologique d'un humus forestier par l'observation d'un petit volume. III. La couche F_1 d'un moder sous *Pinus sylvestris.* Pedobiologia 31, 1−64.

Ponge J F 1990 Ecological study of a forest humus by observing a small volume. I. Penetration of pine litter by mycorrhizal fungi. Eur. J. For. Pathol. 20, 290−303.

Ponge J F 1991 Food resources and diets of soil animals in a small area of Scots pine litter. Geoderma 49, 33−62.

Rack K and Scheidemann U 1987 Über Sukzession und pathogene Eigenschaften Kiefernnadeln bewohnender Pilze. Eur. J. For. Pathol. 17, 102−109.

Soma K and Saito T 1979 Ecological studies of soil organisms with references to the decomposition of pine needles. I. Soil macrofaunal and mycofloral surveys in coastal pine plantations. Rev. Ecol. Biol. Sol 16, 337−354.

Soma K and Saito T 1983 Ecological studies of soil organisms with references to the decomposition of pine needles. II. Litter feeding and breakdown by the woodlouse, *Porcellio scaber.* Plant and Soil 75, 139−151.

Standen V 1973 The production and respiration of an enchytraeid population in blanket bog. J. Anim. Ecol. 42, 219−245.

Styles J H 1967 Decomposition of *Pinus radiata* litter on the forest floor. II. Changes in microfauna population.

N. Z. J. Sci. 10, 1045−1060.

Trappe J M 1971 Mycorrhiza- forming ascomycetes. *In* Mycorrhiza. Ed. E Hacskaylo, pp 19−37. USDA Forest Service Miscellaneous Publications No. 1189.

Watson E S, McClurkin D C and Huneycutt M B 1974 Fungal succession on loblolly pine and upland harwood foliage and litter in North Mississipi. Ecology 55, 1128−1134.

Williams B Land Griffiths B S 1989 Enhanced nutrient mineralization and leaching from decomposing Sitka spruce litter by enchytraied worms. Soil Biol. Biochem. 21, 183−188.

Legends of figures

Fig. 1. Infection by *Lophodermium pinastri* (tip of the needle) and *Ceuthospora pinastri* (minute black spots). L_1 layer. *Bar* = 3 mm.

Fig. 2. Infection by *Lophodermium pinastri.* From right to left: vascular fibres, phloem (note the browning), xylem, transfusion tissue. L_1 layer. Staining with methyl-blue. *Bar* = 50 μm.

Fig. 3. 'White stomata' fungus. Phloem (brown) and xylem (clear). L_1 layer. Staining with methyl-blue. *Bar* = 50 μm.

Fig. 4. Cross-sectioned hyphae of *Verticicladium trifidum* filling a resin duct. L_1 layer. Staining with methyl-blue. *Bar* = 50 μm.

Fig. 5. Blackening of hypodermis and outer part of mesophyll rays following invasion by *Verticicladium trifidum.* From right to left: central cylinder, transfusion tissue, endodermis, mesophyll rays, hypodermis, epidermis. Staining with methyl-blue. L_2 layer. *Bar* = 50 μm.

Fig. 6. Rhizomorph of *Marasmius androsaceus.* Phase contrast. L_2 layer. *Bar* = 50 μm.

Fig. 7. Internal development of *Cenococcum geophilum*. Two forms: poorly ramified (aerial form) and strongly ramified (internal form). Phase contrast. F_1 layer. *Bar* = 50 μm.

Fig. 8. Two enchytraeid worms *(arrows)* penetrating a needle at the time of fixation. F_1 layer. *Bar* = 2 mm.

Fig. 9. Dematiaceous and hyaline hyphae in the intestine of *Cognettia sphagnetorum.* Phase contrast. L2 layer. *Bar* = 50 μm.

Fig. 10. Partially dissected pine needle showing accumulation of phthiracarid mite fecal pellets. F_1 layer. *Bar* = 3 mm.

Fig. 11. Earthworm fecal pellet. F_1 layer. *Bar* = 1 mm.

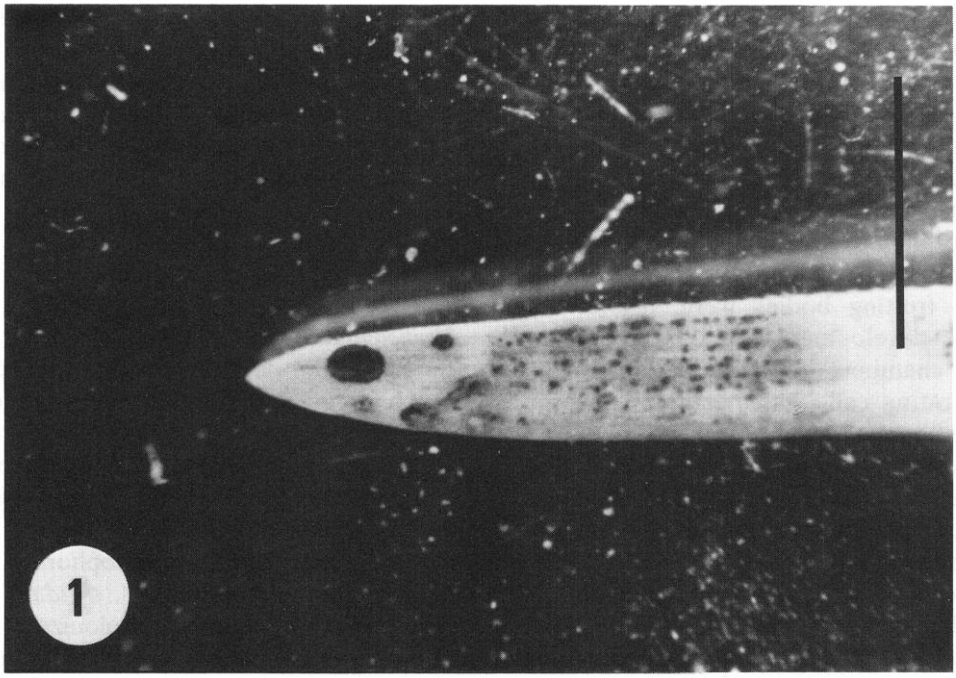
Fig. 12. Cenococcum geophilum hyphae protruding from an earthworm fecal pellet. F_1 layer. *Bar* = 2 mm.

Fig. 13. Type A faeces. F_1 layer. *Bar* = 2 mm.

Fig. 14. Type B faeces. Broken pine needles. F_1 layer. *Bar* 1 mm.

Table 1. Succession of fungi during decomposition of pine needles

Saprophytic senescence fungi	*Lophodermium pinastri* (Schrad.) Chev.
	Ceuthospora pinastri (Fr.) Hohnel (imperfect stage of *Phacidium lacerum* Fr.)
	Lophodermella spp.
Litter-decomposing micro-fungi	*Verticicladium trifidum* Preuss (imperfect stage of *Desmazierella acicola* Lib.)
Litter-decomposing basidiomycetes	*Marasmius androsaceus* Fries
	Collybia spp.
Mycorrhial fungi	unidentified



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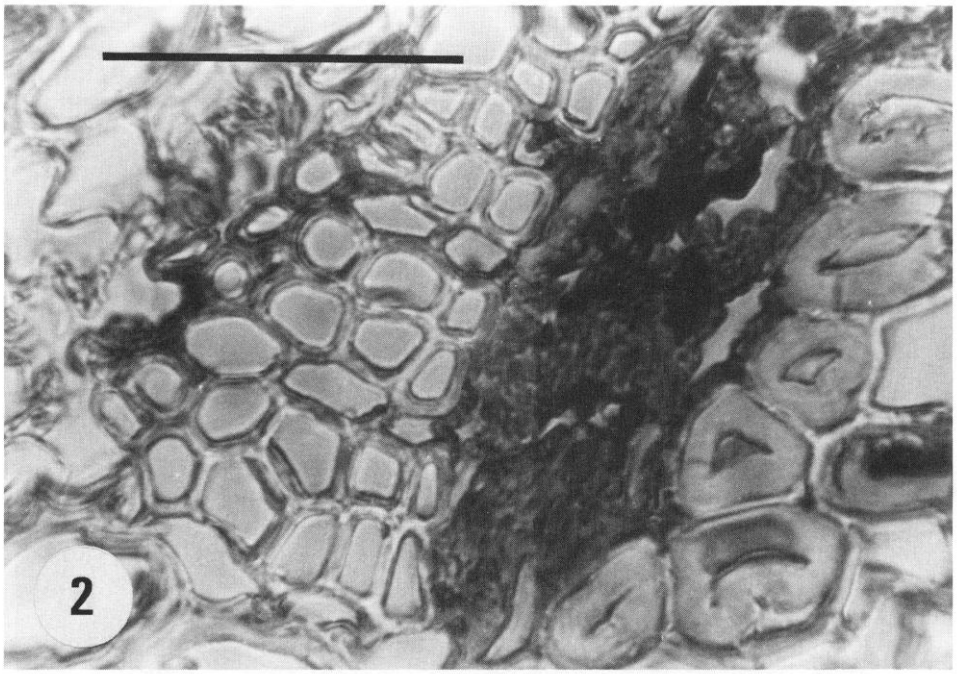

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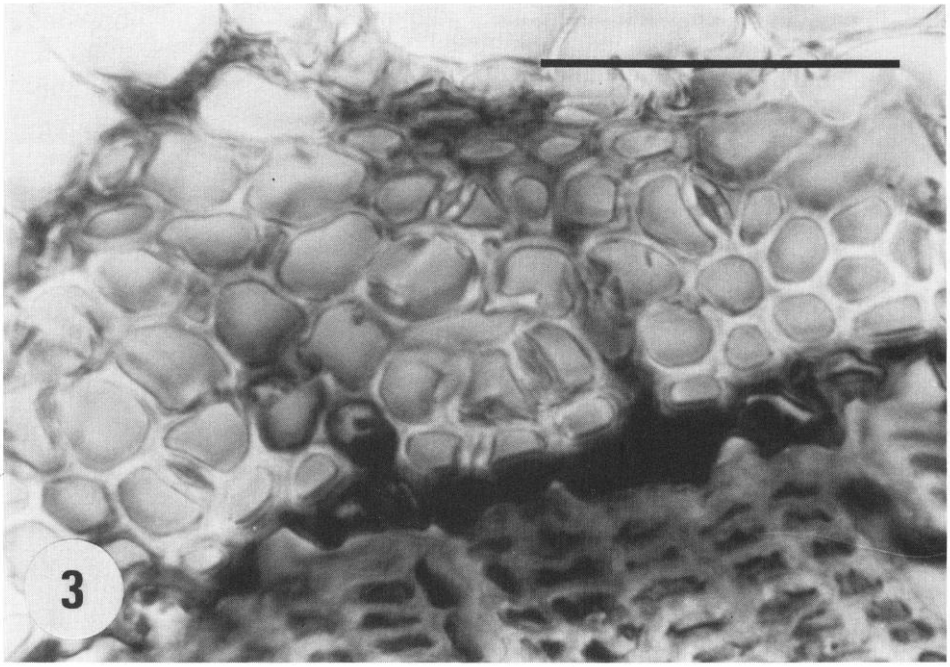

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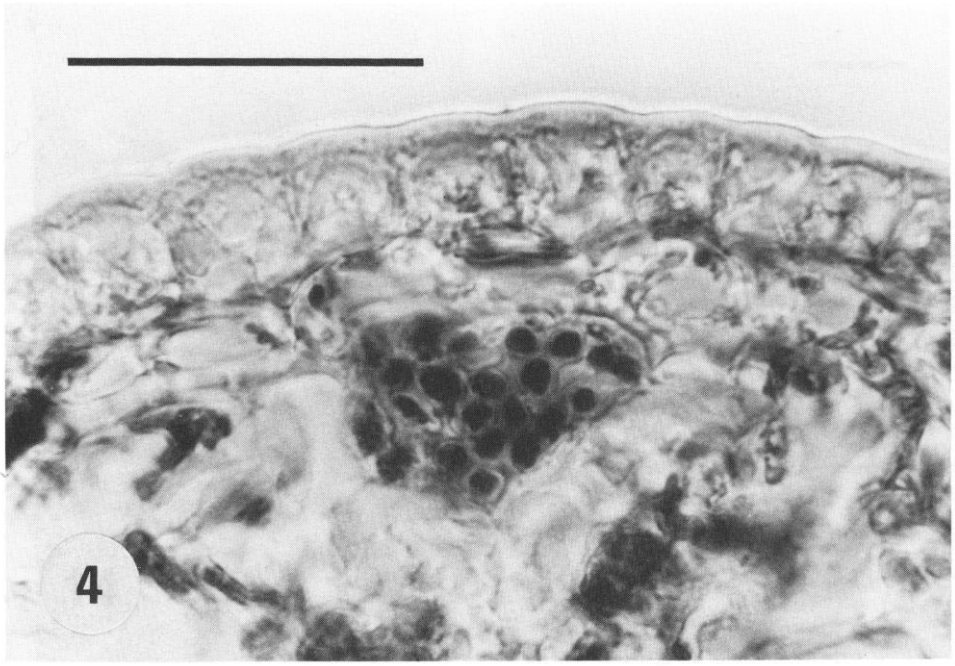

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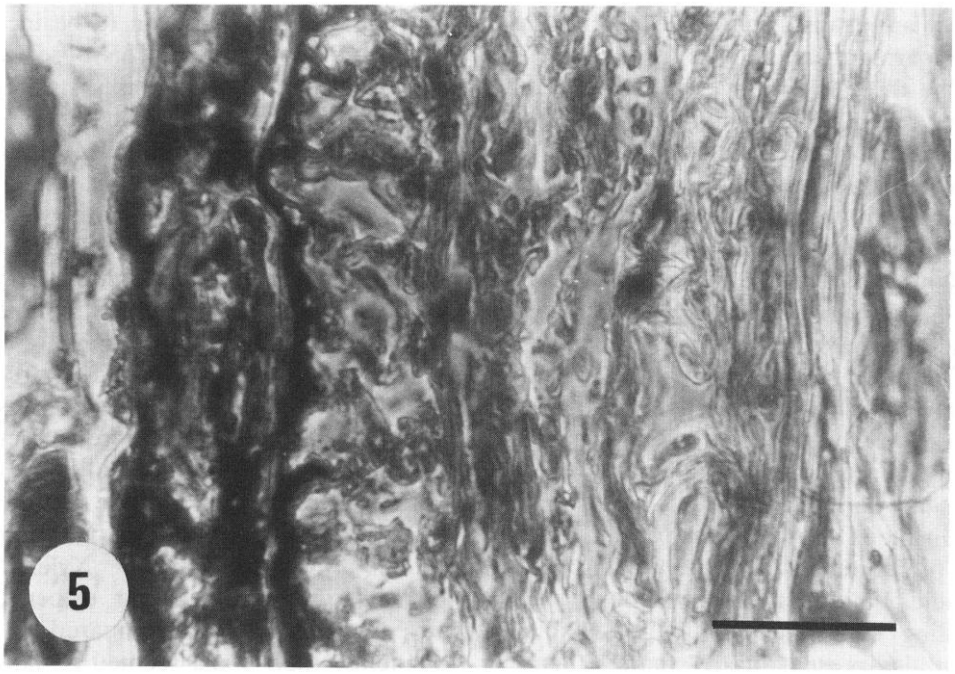

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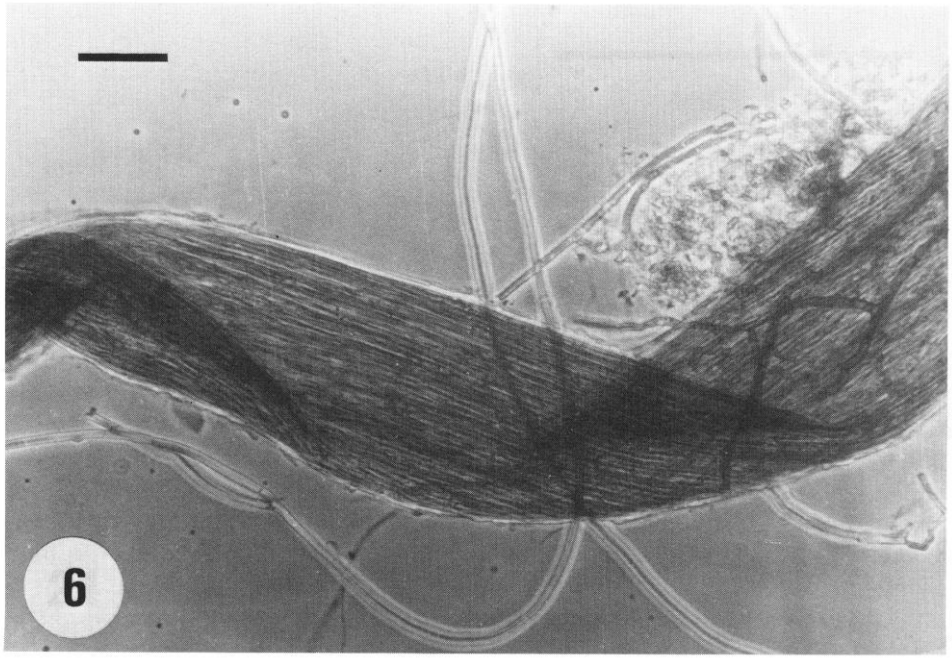

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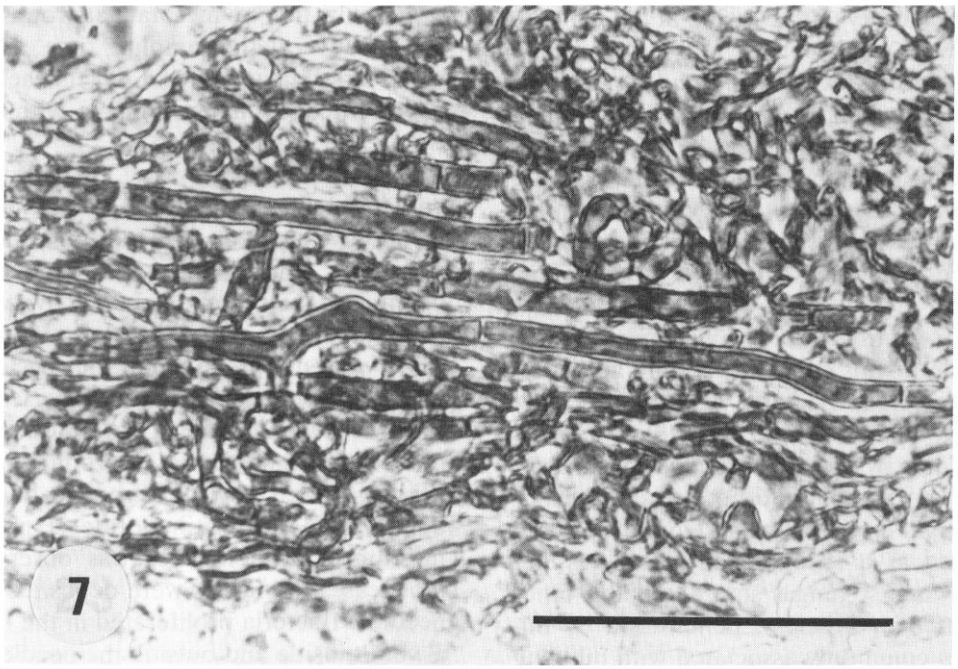

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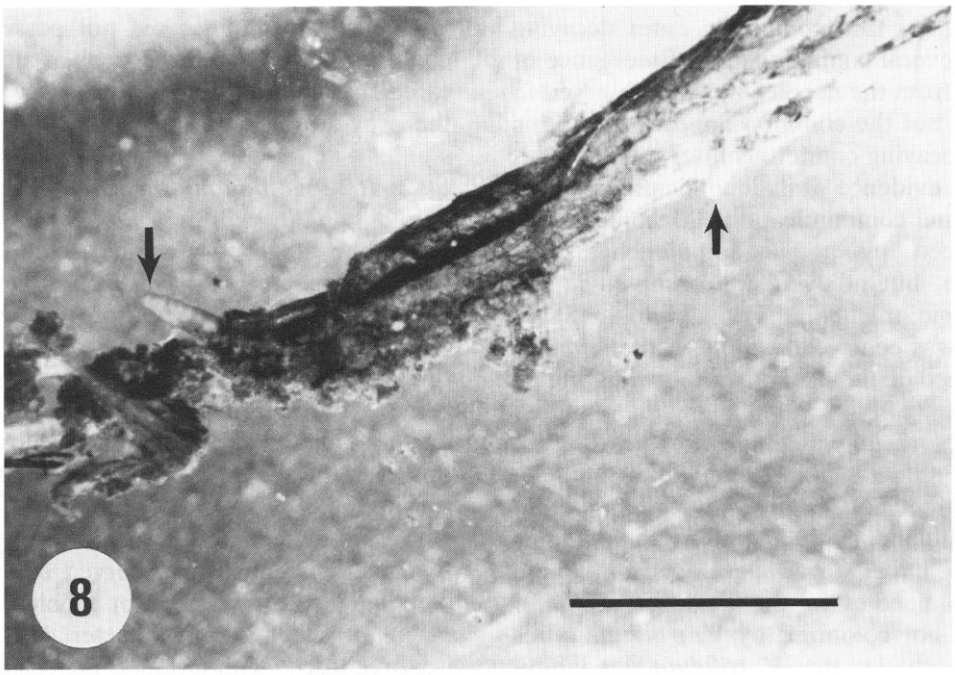

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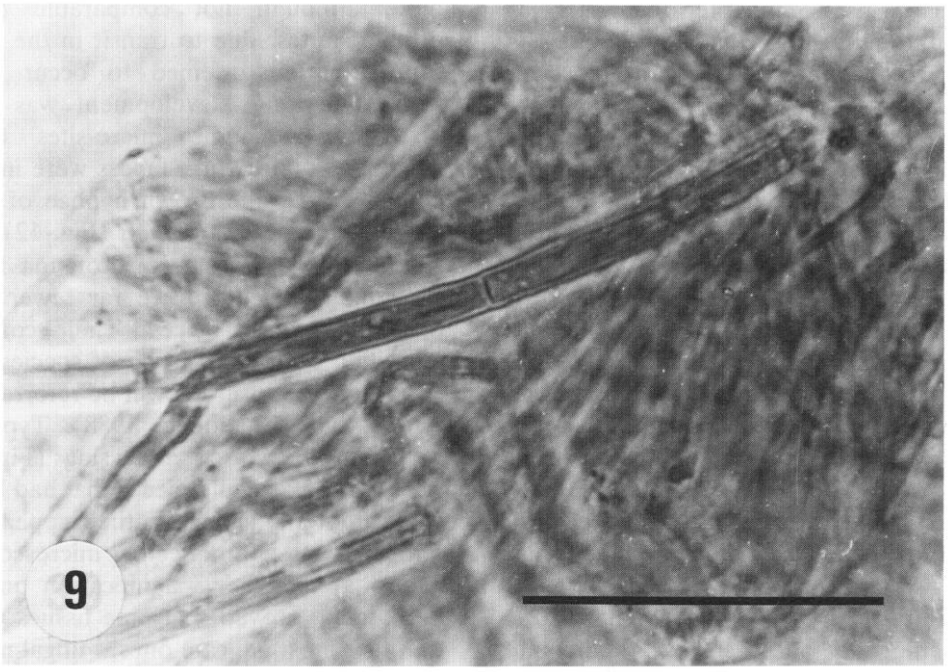


Fig. 9

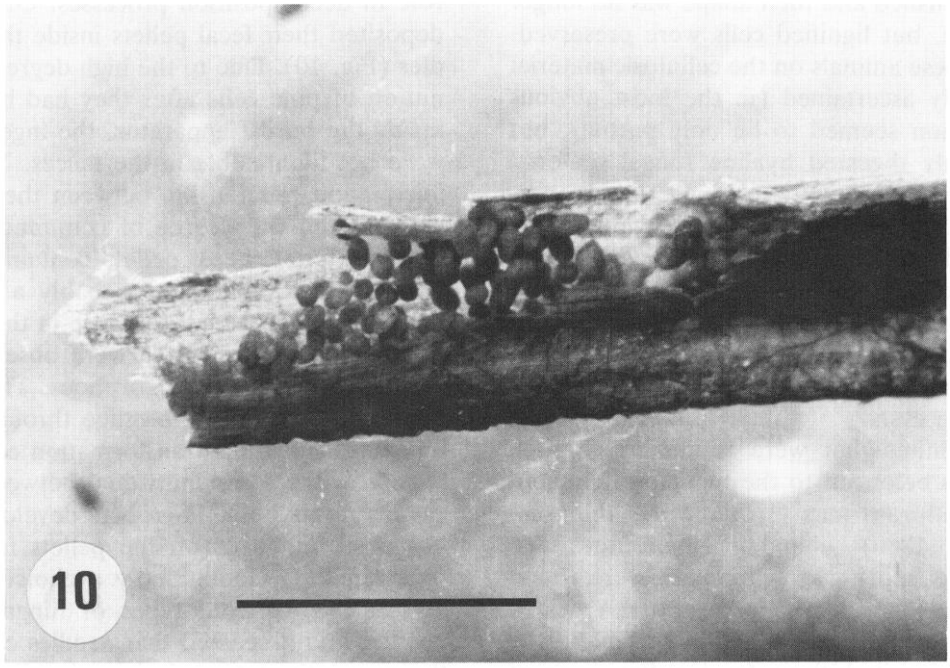
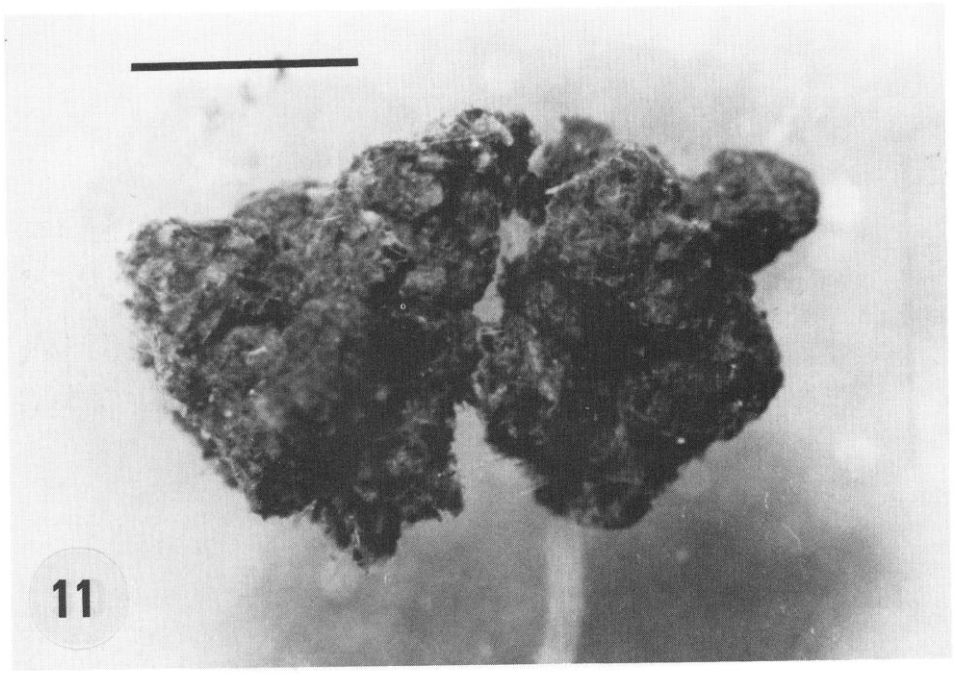


Fig. 10



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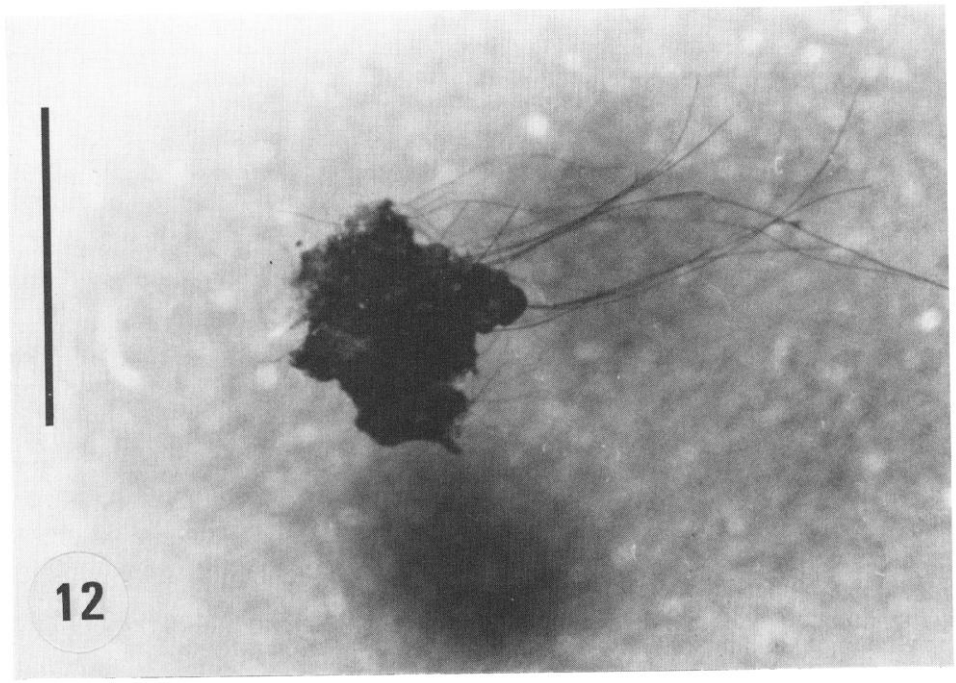
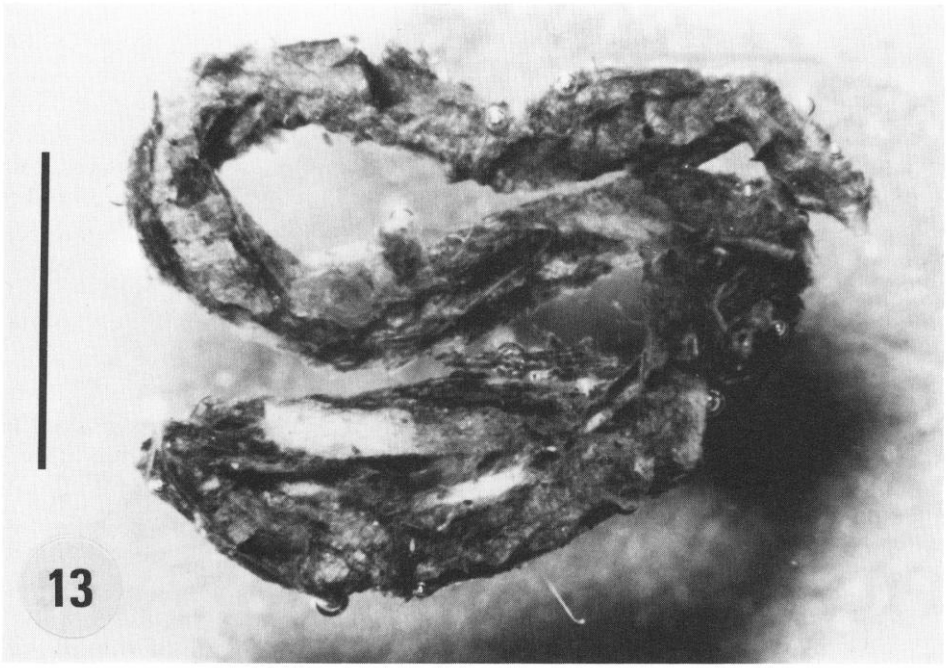


Fig. 12



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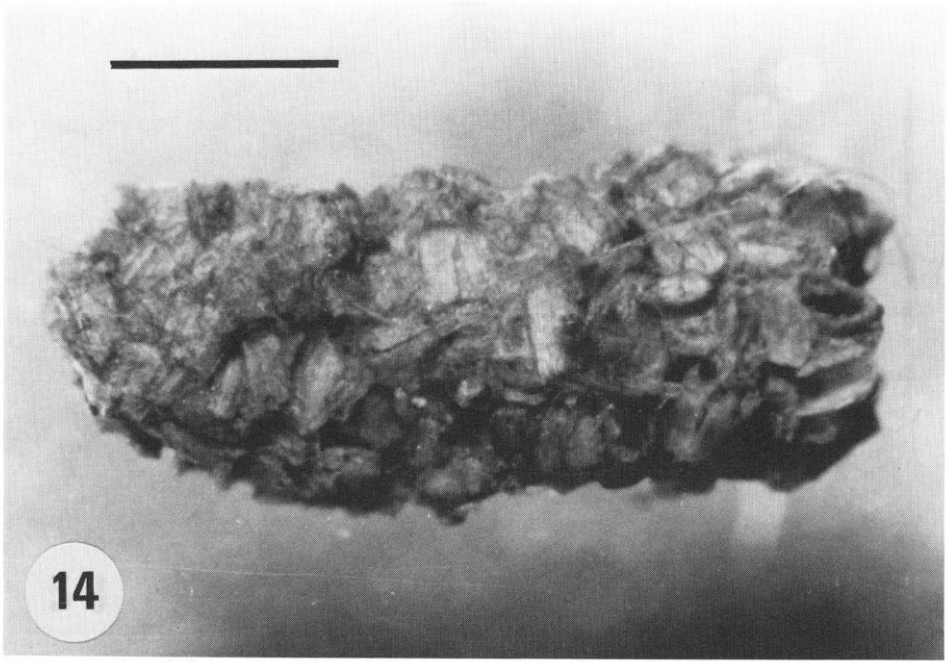

Fig. 14