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Jean-François Ponge

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**The soil under the microscope: the optical
examination of a small area of Scots pine litter
(*Pinus sylvestris* L.)***

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* English translation of three papers published in French by Ponge (1984, 1985 and 1988). Some parts were rearranged for clarity but no attempt was made to update species and horizon names according to modern literature

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INTRODUCTION

The purpose of the present document is to show the interest of morphological studies for a better understanding of the biological interactions which take place in the soil ecosystem. Soil micromorphology was mainly developed following studies by Jongerius (1957) and Babel (1964), but Hesselmann's (1926) microstratification and observations and methodologies by Kubiëna (1938) set the stage for a science which associates the knowledge of the mineral world to that of the living world. On this base Bal (1970) and thereafter Brun (1978) were able to perform integrated studies which confirmed the interest of observing the soil for understanding its functioning as a living system.

On their part, zoologists made observations which allowed them to demonstrate that earthworms were not the only group playing a driving role in the building of soil structure (Zachariae, 1964; Van der Drift, 1964). Accordingly, the role of bacterial colonies in the formation of soil structure, in particular of soil aggregates, was demonstrated by microbiologists (Proth, 1978). This decisive entrance of biology in the field of soil science allowed the scientific community to realize how it was difficult to achieve an integrated study at a fine level of resolution.

Heterogeneity is great in the soil ecosystem and results obtained in a given micro-site cannot be transposed to another. In order to reach representativeness over a given site or a given soil type, the specialist must multiply laboratory experiments, observations and measurements, being forced to become specialized on particular taxonomic groups. In every case, the specialist is able to demonstrate the paramount importance of the group he/she is studying, to the detriment of a comprehensive understanding of soil biological functioning. However, several studies showed that biological interactions play a prominent role in the relationships between microbes, animals and the mineral substrate (Kilbertus & Vannier, 1979; Parkinson et al., 1979; Touchot et al., 1983).

In opposition to the multiplication of observations, measurements and experiments, it can be scheduled to take into account the widest possible array of interactions taking place in a restricted volume of soil. If we consider that most typical processes taking place in the soil ecosystem, such as plant litter decomposition, involve more vertical than horizontal movements of matter, then studying a profile, even on a restricted area, should provide a coherent view of soil biological functioning at this right place. For that purpose each layer or horizon will be considered as a source of information on the past history of the just overlying layer or horizon, using traces of biological activity in it as clues. This postulate is unavoidable in such an integrated study. It should be noted that the local examination of litter and soil must be replaced in the context of forest soil heterogeneity. My purpose is not to ascribe my results to a site, to a soil or to a humus type, but just to study a microcosm where interactions between soil organisms (microbes and animals), plant organic matter (litter and root system) and mineral particles (sand, silt and clay) could be studied in the most comprehensive manner within the limits of sampling and observational techniques.

In the following text, the term 'humus' will designate every part of the soil with a high amount of organic matter, i.e. for the soil under consideration the organic layers L, F, H, and the A₁ horizon, and not any type of organic matter which could be isolated by physical or chemical agencies. Bal (1970) underwent a sound discussion of the meaning of 'humus'.

Study site, sampling procedure and field observations

The humus profile under study is a dysmoder (sensu Manil, 1959) under Scots pine (*Pinus sylvestris* L.). The forest stand is a 35-yr-old plantation located in the Forêt d'Orléans (Loiret, France). The study site has been studied by Poursin & Ponge (1984) and Arpin et al. (1986a, b), which gave more details about it. The soil is leached acidic sandy with temporary waterlogging and is covered by a continuous carpet of the moss *Pseudoscleropodium purum* (L.), known for its common association with coniferous stands (Bournérias, 1979). Fronds of bracken, *Pteridium aquilinum* (L.), are sparsely distributed in the understory. The present paper deals only with the topmost three layers where most biological activity was shown to take place.

Sampling took place in August 1981. The different layers and soil horizons were separated directly in the field over a 5 x 5 cm area, using fingers and fine scissors, and were immediately fixed and preserved in ethyl alcohol (95% v/v) in separate plastic jars until study. The method of identification of litter layers and soil horizons by Babel (1971) was used, but we changed the nomenclature (Table 1). Babel's sub-horizons were called layers, considering that (i) organic deposits should not be confused with soil horizons, according to the opinion by Bal (1970), and (ii) faunistic and microbiological differences are such between sub-layers that they should be erected to the layer rank.

Table 1 summarizes most observations which were done on the field with the naked eye and were used for separating organic layers and first organo-mineral horizons of the topsoil. The top two layers are made of a continuous carpet of *Pseudoscleropodium purum*, living in L₁ (Fig. 1), dead in L₂ (Fig. 2). Thus the thickness of L₁ and L₂ does not correspond to freely accumulating pine litter: in these two layers most plant material was made of leafy shoots of mosses.

Table 1. Microstratification: qualitative features of the different layers and horizons (+++ = dominant in the layer/horizon considered; ++ = abundant but not dominant; + = present but not abundant)

Layers Horizons	Thickness (cm)	Transition with the underlying	Moss	Needles	Mycelia	Mycorrhizae	Faeces	Amorphous organic matter	Sand	Iron spots
L ₁	3	Fuzzy	+++ living	+ brown entire						
L ₂	2	Sharp	++ dead	++ Black entire						
F ₁	1	Fuzzy		+++ fragments	++		+			
F ₂	1	Fuzzy		++ fragments		+	++			
H ₁	0.5	Sharp		+ fragments		+	+++			
H ₂	0.5	Fuzzy					+	+++	++	
A ₁₁	1 to 4	Sharp						++	++	
A ₁₂									+++	+

Winter-spring waterlogging was visible through the presence of rusty spots in A₁₂, due to seasonal anoxia, and the transformation of animal faeces into an amorphous mass in H₂ (partly incorporated in A₁₁, with a sharp transition to A₁₂), due to groundwater movements. These features are

typical of hydromorphic forms of moder or hydromoder (Duchaufour, 1977). Measurements of pH_{water} (Poursin & Ponge, 1984) indicated a value of 3.3, which is very acid. This, together with the total volume of accumulated organic matter in so a young forest stand and the sharp transition between A_{11} and A_{12} , prompted me to interpret this humus profile as transitional to mor at the time of the study. In the A_{12} horizon, discoloured and spotted with rusty spots, there were rhizomes of *Pteridium aquilinum* and woody roots of *P. sylvestris*, some of which were decayed and transformed in dark masses of animal faeces, with a sharp transition to the surrounding bleached sandy horizon.

Preparation of the material for laboratory observation

Early observations were made on litter/humus material after spreading it in petri dishes filled with alcohol. These preliminary observations (accompanied or not by counts when necessary) were done under a dissecting microscope at varying magnification (x 10 to x 40). After a thorough examination of the whole material, specimens of the variety of decay stages (or other cause of variation) in the various plant organs were observed with a light microscope at higher magnification (x 100 to x 400), with or without phase contrast. Plant organs of varying nature (needles, twigs, bark fragments, roots, moss and bracken leaves and stems) were sectioned with a microtome (7.5 μm thickness) after dehydration in absolute ethyl alcohol and embedding in paraffin then sections were mounted either in chloral-lactophenol (to be observed under phase contrast) or in methylblue-Sudan III-lactophenol (to be observed in natural light). Methylblue stains indiscriminately cell organelles, allowing us to distinguish living bacterial and fungal cells within plant tissues, while Sudan III stains in red waxes, cutin and suber, and at last phase contrast was useful to study dead cell parts at varying stages of transformation (Locquin & Langeron, 1978). Following passage from ethyl alcohol to toluene, resins were badly conserved in sectioned plant material. Animal faeces were observed from the outside under a binocular microscope then mounted directly in lactophenol after squashing them or part of them under coverslides.

All animals, whatever their size (nematodes were collected as well as bigger fauna), were collected and identified at the species level whenever possible. Poorly sclerotized animals such as enchytraeids (potworms), springtails, some mites, insect larvae and nematodes were observed by transparency under phase contrast after mounting them in chloral-lactophenol, for the sake of identification and in order to examine their gut contents (when solid particles had been ingested). Heavily sclerotized animals (oribatid mites) were dissected then their gut parts were mounted in the same manner as above. The biovolume of each mounted animal was calculated using an approximate method taking into account the fact the actual volume of the body was lesser than that of a parallelepiped of same length, width and thickness, and higher than that of an octahedron of same dimensions, the ratio between both values being 4:1. It can be easily demonstrated that this approximation is true whatever the size and the shape of the animal, provided length, width and thickness could be calculated, and the animal was in a good state of turgor (which was insured by mounting it in chloral-lactophenol). The more an animal was squashed on the microscopic slide, the more a parallelepiped approximated its true biovolume. Corpses (empty cuticles of arthropods) were not measured because their volume could not be approximated with the above method.

THE L₁ LAYER: RECENTLY FALLEN NEEDLES AND LIVING MOSS



Figure 1. The L₁ layer before fixation and transport to the laboratory

A volume of ca. 50 mL of L₁ layer was sorted under the binocular microscope, which allowed me to separate different components, the volume of which was measured by meniscus displacement in graduated columns (Table 2). Exception was for animals, the biovolume of which was measured individually at higher magnification, as explained above. Animal faeces and fungal mycelia (mostly basidiomycetes), which were poorly represented and firmly appressed to their plant support (moss shoots, pine needles), were included in plant components.

Table 2. Volume of the different components of the L₁ layer sorted under the binocular microscope

Litter components	Volume (μL)
Moss	7500 ± 100
Pine needles	1000 ± 100
Pine bark	900 ± 100
Pine twigs	300 ± 100
Male flowers of pine	18 ± 2
Living fauna	0.4 – 2
Total	9700 ± 100

Table 2 shows that the total volume of litter components (~ 10 mL) was much less than the volume of layer sampled (50 mL), thereby indicating the importance of voids in this well-aerated environment.

The biovolume of fauna seems very weak compared to that of the plant material. However, it must be noticed that this superficial layer was the seat of the movement of big invertebrates which lived and moved on the ground surface, but could be temporary present or not. At the time and at the right place of sampling no woodlouse, millipede, centipede or lumbricid worm was observed, which would have been magnified the biovolume of fauna to a great extent, without increasing its role. This random factor will be neglected in the course of my study by taking into account mainly traces of animal activity, onto which my estimates of the respective impact of different animal groups will be based. However, living animals which were sorted under the binocular microscope allowed me (i) to study the transformation of plant and microbial substrates during intestinal transit time, and (ii) to assign faeces to animal groups. In the L₁ layer, I observed only a weak number of faeces, all of them were attributed to oribatid mites.

Sorting litter components under the binocular microscope allowed me to show the dominance of living shoots of the moss *Pseudoscleropodium purum* and the visible development of the ascomycete *Lophodermium pinastri* (Schrad.) Chev., which was reported in nearly all recently fallen pine needles (Fig. 1). This fungus formed black buttonhole-like subcuticular ascocarps (Ø 1 mm) and diaphragms which were visible from the outside and allowed identifying it with certitude (Minter et al., 1978; Minter, 1981). Figures 2 and 3 show parts of pine needles which were colonised by this fungus, known to infest foliage falling in spring or summer (Jones, 1935; Lanier, 1968).

Dating of the plant material present in the L₁ layer was possible thanks to the presence of male flowers of pine (known to fall at the end of June or beginning of July) and to the absence of euphylls (bracts, present in inflorescences and shoots of the year, known to fall at the beginning of June): I could conclude that the material present in L₁ had fallen during July and beginning of August.

Some needle parts appeared to be colonised by two other ascomycetes. *Ceuthospora pinastri* (Fr.) Höhn.* is the imperfect stage of *Phacidium lacerum* Fr. (Gremmen, 1959). I did not observe its pycnidia in litterfall baskets which were installed in the same site, but Hayes (1965) indicated that it fructifies only once the needle has fallen on the ground. Most needles infested by *Ceuthospora pinastri* showed signs of previous attack by *Lophodermium pinastri*, the former colonizing zones which had not been colonised by the latter (Fig. 3). *Ceuthospora pinastri* appeared in the form of numerous black subhypodermic pycnidia (Fig. 4), often grouped by two or three. *Lophodermella* von Höhn., which was not identified to species level by lack of mature fructifications, formed subhypodermic ascocarps (Fig. 5), contrary to *Lophodermium*, ascocarps of which are subepidermal or subcuticular according to species. *Lophodermella* is known to cause precocious fall of pine needles (Mitchell et al., 1978b) and this was verified through the small size of pine needles which were infected by this fungus.

Needles which were in contact with the underlying L₂ layer (the passage from L₁ to L₂ was progressive, see Table 1) were colonised by *Verticicladium trifidum* Preuss, the imperfect stage of the ascomycete *Desmazierella acicola* Lib. This fungus was easily recognized by its black erected conidiophores (Fig. 6), protruding through stomatal apertures from melanised stomata located in substomatic chambers. At an early stage, only substomatic chambers and ostioles appear in black, corresponding to the 'black stomata' stage of Hayes (1965). Thereafter, as far as the hypodermis becomes melanised, needles turn to black on their whole surface and become covered with a carpet of conidiophores of this fungus. *Verticicladium trifidum* was recognized as a major secondary coloniser

* The identification of this fungus was confirmed by Dr B.C. Sutton (Commonwealth Mycological Institute, Kew, Surrey, UK) who is warmly acknowledged

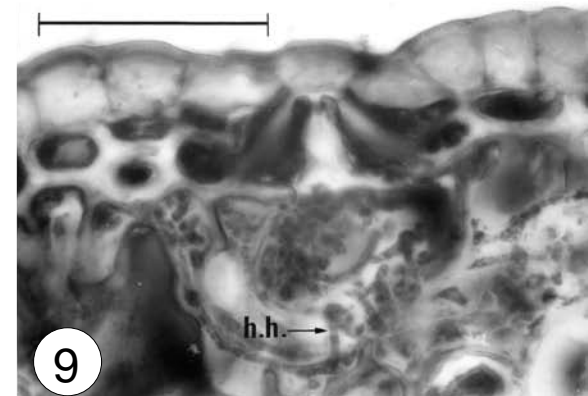
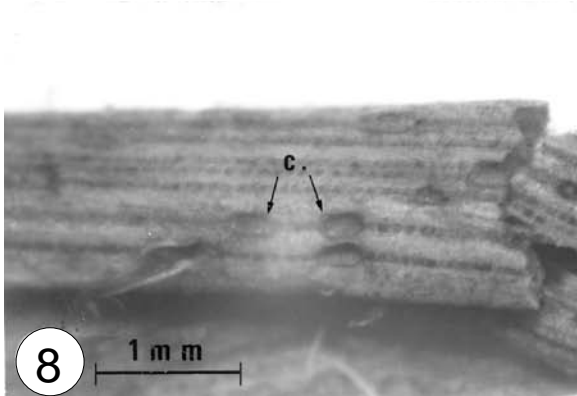
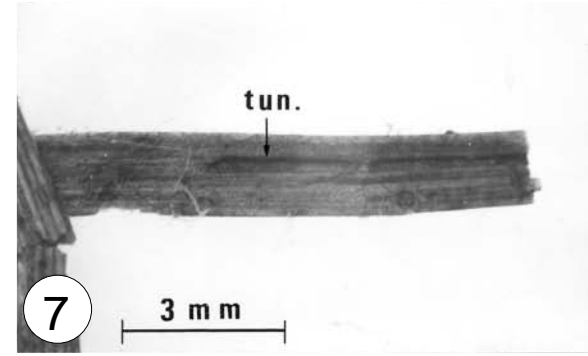
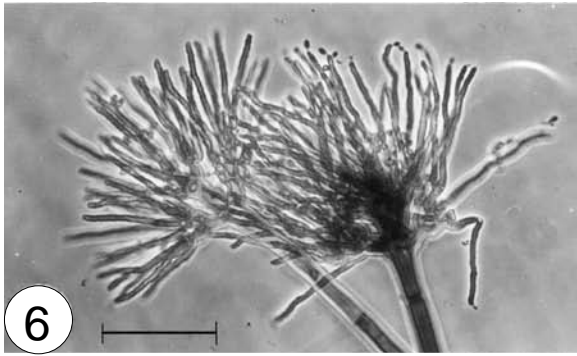
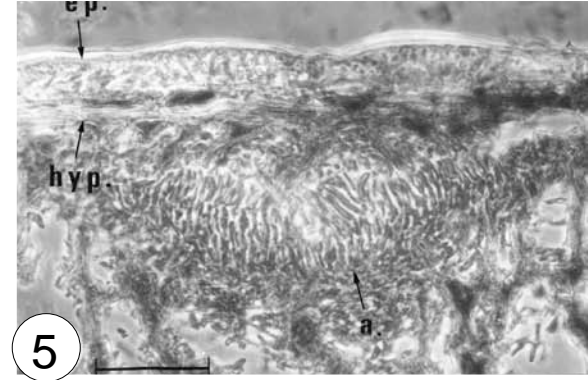
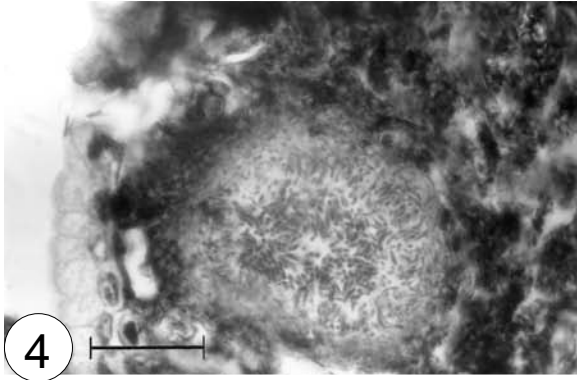
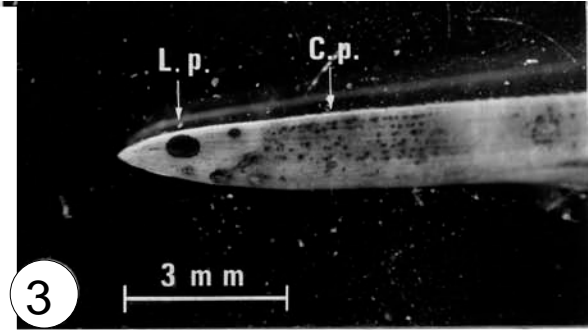
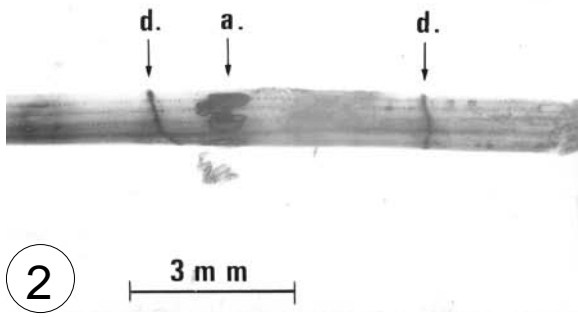


Fig. 2: Needle infested by *Lophodermium pinastri*; Fig. 3: Needle infested by *Lophodermium pinastri* and *Ceuthospora pinastri*; Fig. 4: Subhypodermic pycnidium of *Ceuthospora pinastri* (BMSL); Fig. 5: Subhypodermic immature ascocarp of *Lophodermella* (CP); Fig. 6: Conidiophores of *Verticicladium trifidum* (CP); Fig. 7: Pine needle attacked by fauna; Fig. 8: Idem, other mode of attack; Fig. 9: Light-brown pine needle without any fungal fructification, section through epidermis, hypodermis and mesophyll at the level of a stomatal aperture (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; d. = diaphragms; a. = ascocarps; c. = cavities caused by animal biting; tun. = faunal tunnels; ep. = epidermis; hyp. = hypodermis; L.p. = *Lophodermium pinastri*; C.p. = *Ceuthospora pinastri*; length of segment = 50 μm, unless otherwise indicated

of Scots pine needles by Kendrick & Burges (1962). However, in my sample, I also observed it as a primary coloniser.

At the level of the L₁ layer, the impact of soil animals was but feeble. Three kinds of traces of faunal attack were observed on pine needles, (i) small disjoint cavities, aligned along stomatal lines (Fig. 8), maybe indicating that substomatic stomata of *Verticicladium trifidum* were consumed (?), (ii) superficial grooves (Fig. 7), or (iii) tunnels at the inside of needles (not visible from the outside, see sections shown in Figs. 24 & 25). All these attacks were attributed to enchytraeids, for reasons which will be enlightened during the study of the underlying layer L₂ where these animals were abundant at the time of sampling. However, it must be noticed that such features of biting or tunnelling were exceptional in L₁, faunal feeding activity being mainly devoted to browsing of conidiophores and superficial mycelia, as was shown by the examination of gut contents.

Decaying needles

All needle types were sectioned (15 different morphological types), but only four of them will be presented here, as being particularly demonstrative.

Needles without any external sign of fungal attack

Some green needles were found in the L₁ layer. Sections revealed that they were identical to living foliage: chloroplasts (stained violet) were intact and no sign of fungal infestation was visible. These needles, which fell precociously probably because of branch fall or strong winds, are only mentioned for the record.

Some needles, although light-brown-coloured, did not show any visible trace of fungal development, to the exception of some hyaline hyphae crawling at their surface. When sectioned, these needles did not appear to be intact. They were invaded at the inside by a network of hyaline hyphae, without any fructification. Only living cells of pine seemed to be attacked, i.e. mesophyll and endoderm cells, living cells of transfusion tissue, phloem and vascular rays. In these tissues, cell walls were brown-coloured (Figs. 9 & 10), indicating a biochemical transformation of cellulose. Lignified dead cells (sclerenchyma, metaxylem tracheids with bordered pits, transfusion tracheids) were not invaded and lignin remained transparent (Fig. 11). Living cells with lignified walls (epidermis) were not invaded (Fig. 9). In all cases cytoplasm was replaced by hyphae of this fungus and cellulose seemed to be modified. Lignin seemed to arrest the development of the fungus, which was not able to penetrate tracheids through the areolae of bordered pits. However, suberin did not impede thin cell walls of the endodermis to be penetrated.

In the mesophyll, hyphae were seen growing in every part of the cells (Figs. 9 & 10). I hypothesize that this fungus was able to destroy cellulosic walls once cytoplasmic contents have been digested.

Penetration of the needles took place through stomatal apertures, given the dense development

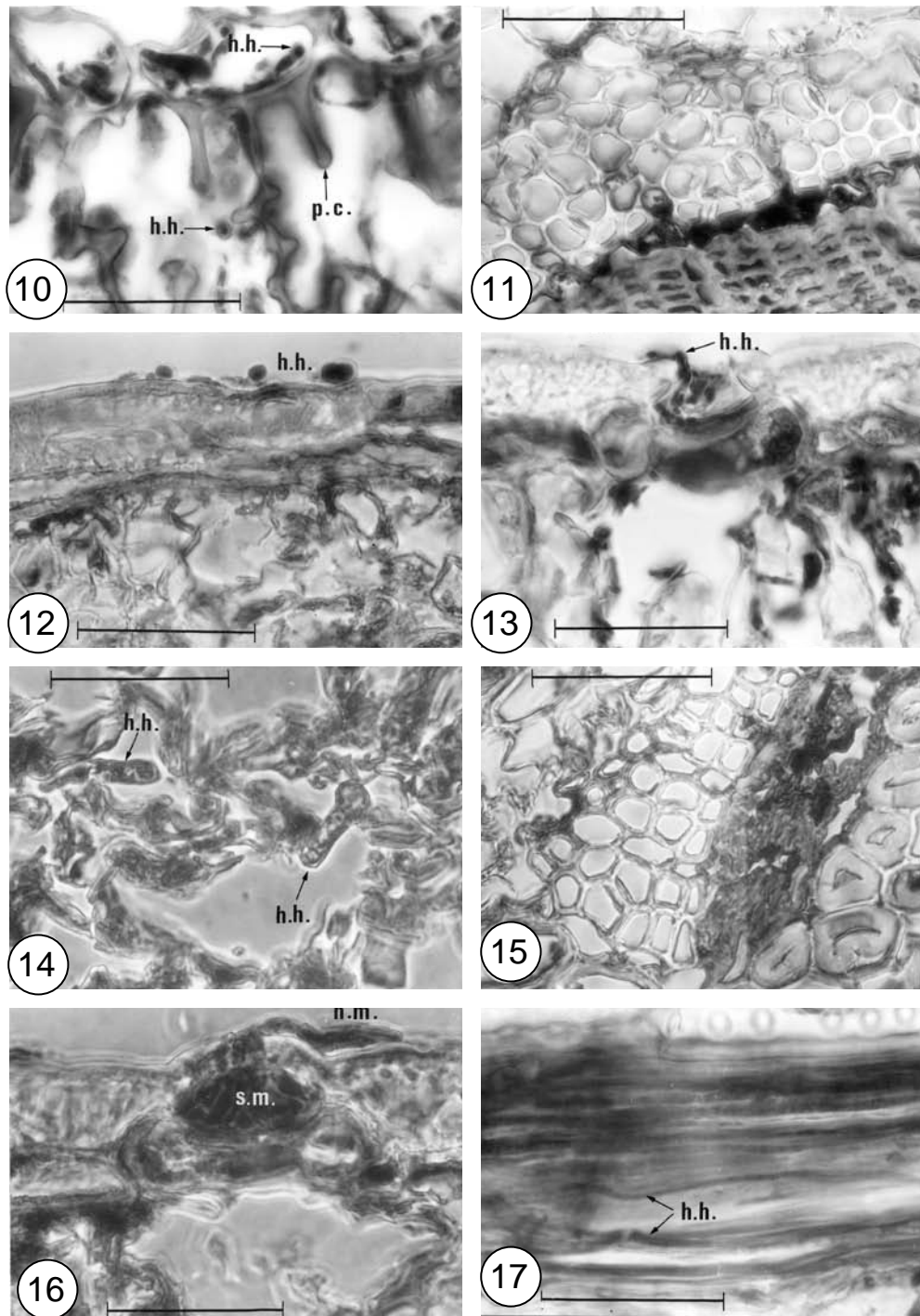


Fig. 10: Light-brown pine needle without any fungal fructification, transversal section through the mesophyll (BMSL); Fig. 11: Idem, transversal section through phloem, vascular cambium, metaxylem and protoxylem (BMSL); Fig. 12: Idem, transversal section showing hyphae appressed to the cuticle (BMSL); Fig. 13: Idem, hyphae penetrating through a stomatal aperture (BMSL); Fig. 14: Needle attacked by *Lophodermium pinastri*, longitudinal section showing hyphae in the mesophyll (CP); Fig. 15: Idem, transversal section showing transfusion tissue, xylem, phloem, perivascular sclerenchyma (BMSL); Fig. 16: Idem, longitudinal section showing melanised hyphae penetrating through a stomatal aperture and forming a melanised stroma (CP); Fig. 17: Needle attacked by *Verticicladium trifidum*, longitudinal section through phloem (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; h.h. = hyaline hyphae; h.m. = melanised hyphae; p.c. = cell walls; s.m. = melanised stromata; length of segment = 50 μ m

of hyphae which was observed in sub-stomatal chambers (Fig. 9) and the network of hyphae of similar size and shape which was observed externally (Fig. 12). Penetration through stomata was also occasionally observed (Fig. 13).

Light-brown needles remained turgescient. Not any identity could be given to this fungus, since sporulation did not occur in my sample, and the parasitic or saprophytic nature of the infestation could not be revealed to the observer. However, it must be noticed that early colonisation of pine needles by fungi seemed to be the rule in the studied sample, even when no external sign of fungal attack was visible.

Needles attacked by parasitic fungi

- *Lophodermium pinastri*

A network of invading hyphae was clearly visible in the mesophyll (Fig. 14). Hyphae were thin-walled and segmented, with frequent bulb-like expansions (haustoria?). However, they were never seen penetrating mesophyll cells, which were collapsed and brown-coloured (more than with the former, unidentified fungus). Phloem appeared also brown and collapsed, but xylem tracheids and sclerenchyma cells remained transparent (Fig. 15). Thus lignin, contrary to cellulose, did not seem to be attacked, at least at this stage.

Melanised stomata were often observed, stemming from hyphae crawling at the surface of needles which further penetrated the needles through stomatal apertures (Fig. 16). These stomata were interpreted as resting stages of *Verticicladium trifidum*, awaiting senescence of internal colonies of *Lophodermium pinastri*. This might explain the observed succession between *Lophodermium pinastri* and *Verticicladium trifidum* observed on ageing needles.

- *Ceuthospora pinastri* and *Lophodermella* sp.

Needles attacked by these two fungi exhibited the same signs than when attacked by *Lophodermium pinastri*, i.e. colonisation of the mesophyll by a network of extra-cellular hyphae, with concomitant collapse and browning of cell walls, without any degradation of lignin.

Needles attacked by the saprophytic Verticicladium trifidum

The internal development of this ascomycete (conidial stage of *Desmazierella acicola*) was characterised by long thick-walled hyaline hyphae stretched along cell files of phloem (Fig. 17), protoxylem (Fig. 18) and resin ducts (Fig. 19). Mesophyll cells were also invaded as well as those of

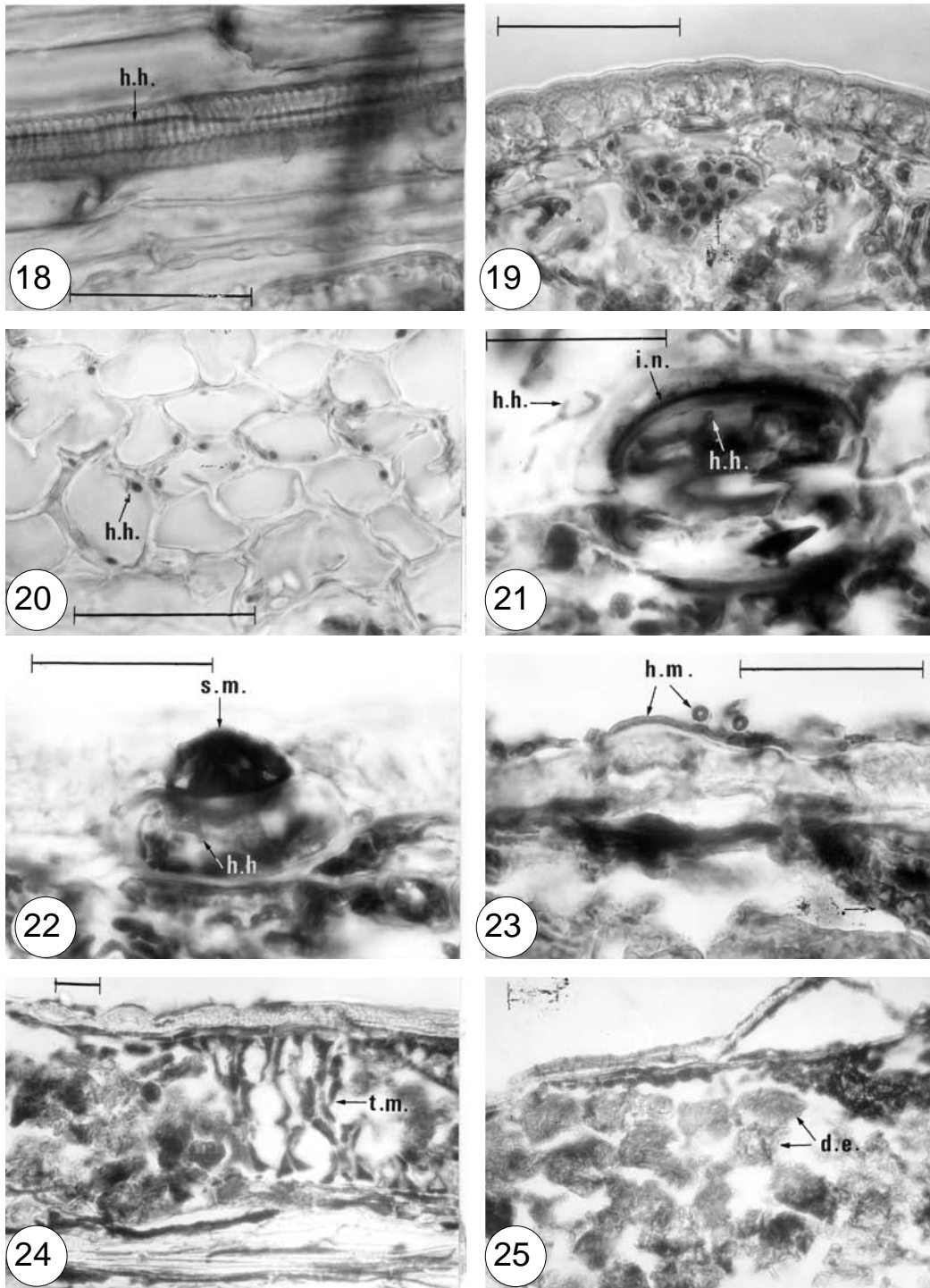


Fig. 18: Needle attacked by *Verticicladium trifidum*, longitudinal section through protoxylem and metaxylem (BMSL); Fig. 19: Idem, transversal section of a needle showing epidermis, hypodermis, resin duct and thick-walled hyaline hyphae (BMSL); Fig. 20: Idem, transversal section through transfusion tissue showing thick-walled hyaline hyphae appressed to intact pine cell walls (BMSL); Fig. 21: Idem, tangential section through hypodermis showing guard cells invaded by hyaline hyphae and their walls impregnated by dark deposits (BMSL); Fig. 22: Idem, longitudinal section through a stomatal guard cell showing hyaline hyphae at the inside (BMSL); Fig. 23: Needle attacked by *Lophodermium pinastri* and *Ceuthospora pinastri* then *Verticicladium trifidum* and penetrated by an enchytraeid, longitudinal section showing cuticle covered with melanised hyphae, epidermis, hypodermis with black deposits, mesophyll with bacteria (BMSL); Fig. 24: Idem, longitudinal section showing the passage from a zone attacked by fungi only (mesophyll cell files still present) to a zone tunnelled by an enchytraeid, with faeces deposition (BMSL); Fig. 25: Idem, longitudinal section showing enchytraeid faeces accumulated under the hypodermis, incrustated by black deposits (BMSL)

BMSL = methyl blue-Sudan III-lactophenol coloration; h.h. = hyaline hyphae; h.m. = melanised hyphae; s.m. = melanised stomata; i.n. = black deposits; t.m. = mesophyll cell files; d.e. = enchytraeid faeces; length of segment = 50 μ m

transfusion tissue (Fig. 20), into which starch grains could still be observed in abundance. The latter observation was indirect evidence that the needle was functional when still on the tree, contrary to needles previously parasitized by *Lophodermium pinastri*, *Ceuthospora pinastri* or *Lophodermella sp. Verticicladium trifidum*, known as a soil saprophyte (Gremmen, 1977), can be considered in the present case as a primary coloniser of fallen pine needles. At this stage, mesophyll, phloem, protoxylem and transfusion tissue were invaded, and only metaxylem was still untouched. Cell walls did not appear transformed, only cytoplasm being probably digested.

Melanised stomata of *Verticicladium trifidum* were observed in sub-stomatal chambers and stomatal apertures (Fig. 22). Melanin or melanin-like black substances seemed to diffuse and impregnate the walls of stomatal guard cells (Fig. 21) and hypodermis at the contact of melanised stomata, while hyaline hyphae invaded the inside of guard cells (Fig. 22).

The pine needles invaded by this fungus were brown-coloured, with lines of black stomata. At this stage conidiophores of *Verticicladium trifidum* were still not observed protruding singly or grouped by two or three from melanised stomata filling stomatal apertures.

Needles attacked by Verticicladium trifidum after Lophodermium pinastri and Ceuthospora pinastri

Like when *Verticicladium trifidum* was a primary coloniser, the presence of this fungus could be attested by its thick long hyphae colonizing longitudinally the needles and by its melanised stomata filling stomatal apertures. However, in the present case, blackening was generalized through the hypodermis, forming a thin opaque layer which gave a black aspect to the whole needle (Fig. 23). Previous colonisation by *Lophodermium pinastri* and *Ceuthospora pinastri* was attested by the presence of melanised diaphragms of the former (Fig. 23) and subhypodermic pycnidia of the latter.

The studied needle showed traces of tunnelling by an animal which deposited its faeces at the inside of the cavity it created. Figure 24 shows in the mesophyll the passage from a zone attacked by fungi only (mesophyll cell files still recognizable) to a zone which had been ingested then transformed into faeces by an animal. These faeces were round masses ca. 50-70 μm diameter closely packed under the blackened hypodermis, where they replaced the mesophyll (Fig. 25). Stromatic diaphragms of *Lophodermium pinastri* were not consumed, probably because of their toughness, and formed barriers to the tunnelling activity of the animal: the upper right side of Figure 25 also shows such a black diaphragm, together with an ascocarp which had been emptied after spore discharge (cuticle was peeled off). In other places vascular and transfusion tissues could be attacked as well.

Through observations which were made in L_1 , L_2 and F_1 layers, the tunnelling activity scarcely observed in L_1 was attributed to enchytraeids. At the inside of enchytraeid faeces plant cells were no longer recognizable, to the exception of some particular anatomical features. Cellulosic cell walls were crushed and lacked transparency (Fig. 26), but lignified walls of tracheids were still intact and recognizable (Fig. 27), indicating that vascular tissues were consumed. Hyaline hyphae (of unknown origin) were observed but they were dead (not stainable). Bacterial development was noticed everywhere, in some parts in the form of actively growing colonies (Fig. 28).

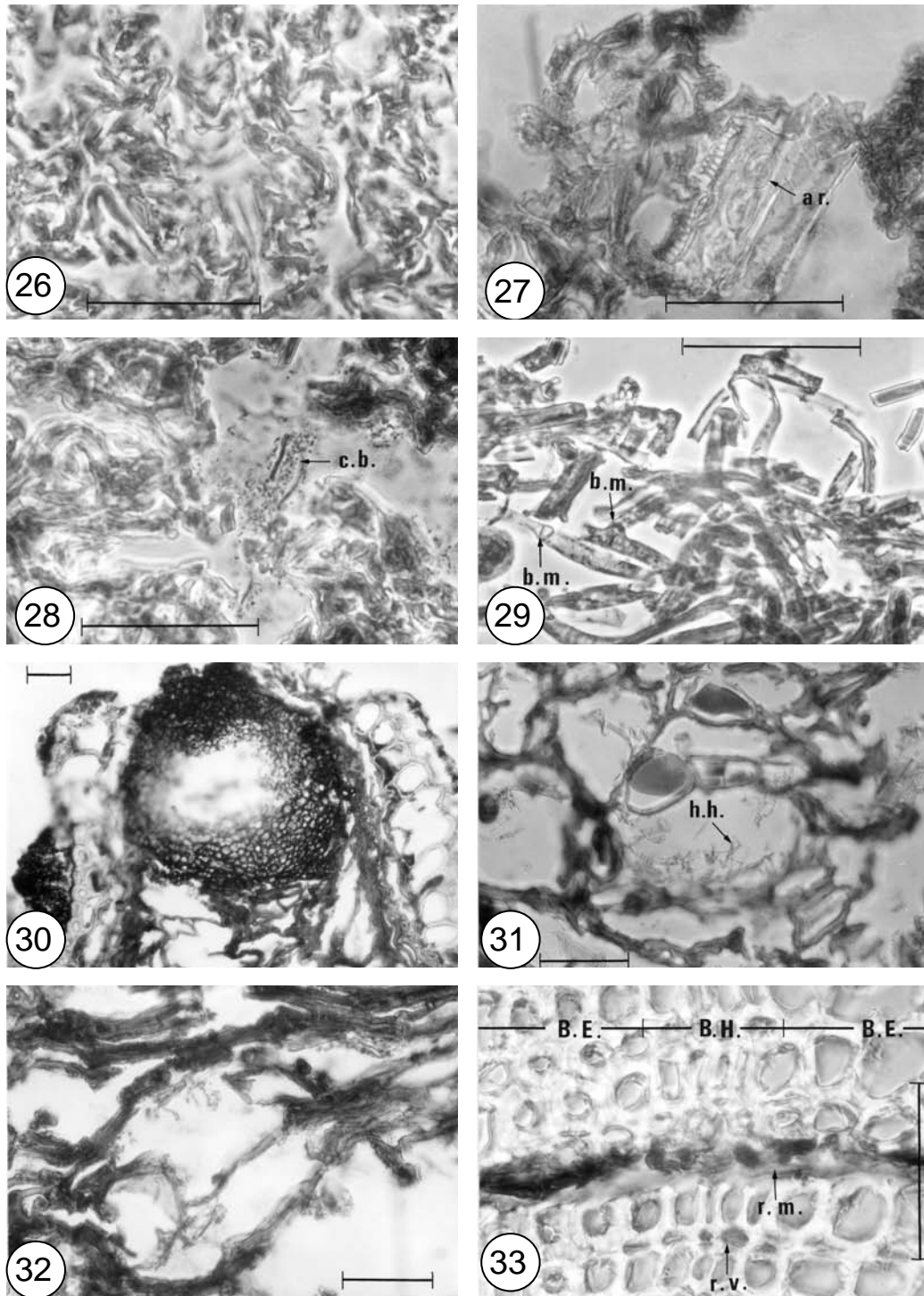


Fig. 26: Needle attacked by *Lophodermium pinastri* and *Ceuthospora pinastri* then *Verticicladium trifidum* and penetrated by an enchytraeid, detail of enchytraeid faeces showing plant cell wall remains and bacteria (CP); Fig. 27: Idem, detail of enchytraeid faeces showing remains of tracheids with bordered pits (metaxylem) (CP); Fig. 28: Idem, detail of enchytraeid faeces showing bacterial colonies (CP); Fig. 29: Idem, detail of basidiomycetous mycelium embedding the needle (CP); Fig. 30: Two-yr-old pine twig, transversal section showing suber, hypodermis and epidermis lifted off by a fungal pycnidium (BMSL); Fig. 31: Idem, transversal section showing medullar cells with intracellular hyphae (BMSL); Fig. 32: Idem, transversal section showing cortical cells, collapsed and with brown walls (BMSL); Fig. 33: Idem, transversal section showing metaxylem, medullary ray and vascular ray (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; a.r. = bordered pits; c.b. = bacterial colonies; b.m. = clamp connections; h.h. = hyaline hyphae; r.v. = vascular rays; r.m. = medullary rays; B.E. = earlywood; B.H. = latewood; length of segment = 50 μ m

Conclusions about decaying needles

Decay processes observed in L₁ pine needles were mainly due to the activity of pine-specific ascomycetes. Fungal development was mainly internal (to the exception of conidiophores of *Verticicladium trifidum*) but fungal hyphae were seen at the surface of needles and, in some cases, mycelia embedded needles. The bacterial development observed in one of the studied needles was interpreted as a contamination by an enchytraeid, the tegument of which probably carried propagules and deposited them at the inside of the needle. A relationship between bacterial development and fungal death at the inside of the needle was hypothesized but not ascertained.

The observed fungal successions were in full agreement with the observations by Kendrick & Burges (1962), to the exception of the time lapse they indicated for the fructification of *Lophodermium pinastri* and *Verticicladium pinastri*. In the study site, located much more southerly compared to England, these two fungi fructified within at most two months, i.e. much more rapidly than the 6-8 months these authors indicated. However, Soma & Saito (1979), in a study on the decomposition of needles of *Pinus thunbergii* in climate conditions comparable to the present study, found 6-9 months for the maturation of ascocarps of *Lophodermium pinastri*. For the sake of comparison, it should be also noted that *Ceuthospora pinastri* was erroneously called *Fusicoccum bacillare* by Kendrick & Burges (1962), as this could be ascertained from their figures, and that my L₁ layer corresponded to their L layer, while my L₂ layer corresponded to their F₁ layer.

Decaying twigs

A 2-yr-old twig was sectioned, its age being estimated from the succession of early- and latewood in the observed transversal sections.

Black pycnidia were present around the twig, protruding through peeled off bark and epidermis (Fig. 30). At least in some zones which were kept untouched, parenchymatous tissues were attacked by networks of hyaline and melanised hyphae, into which several species were seen but could not be identified. This was the case for the medulla (Fig. 31), the cortex (Fig. 32), medullary rays (Fig. 33) and vascular rays (Fig. 34). Figure 35 shows the passage from the intact protoxylem (compare with Figure 36) to the intact metaxylem, passing through a parenchymatous zone (medullary ray) which was the seat of an intense development of melanised hyphae.

When cells were attacked, cellulose became brown and lignin remained transparent. At this early stage of branchwood decay, the penetration of tracheids by hyphae from vascular rays was incipient (Fig. 37). Parenchymatous cells of vascular rays communicate freely with metaxylem tracheids through enlarged pits without torus (Fig. 34). Thereby the fungal colonisation of branchwood was both centripetal (through the cortex) and centrifugal (through the medulla) and was mainly mediated by vascular rays. At this stage, no internal development of ligninolytic basidiomycetes was registered.

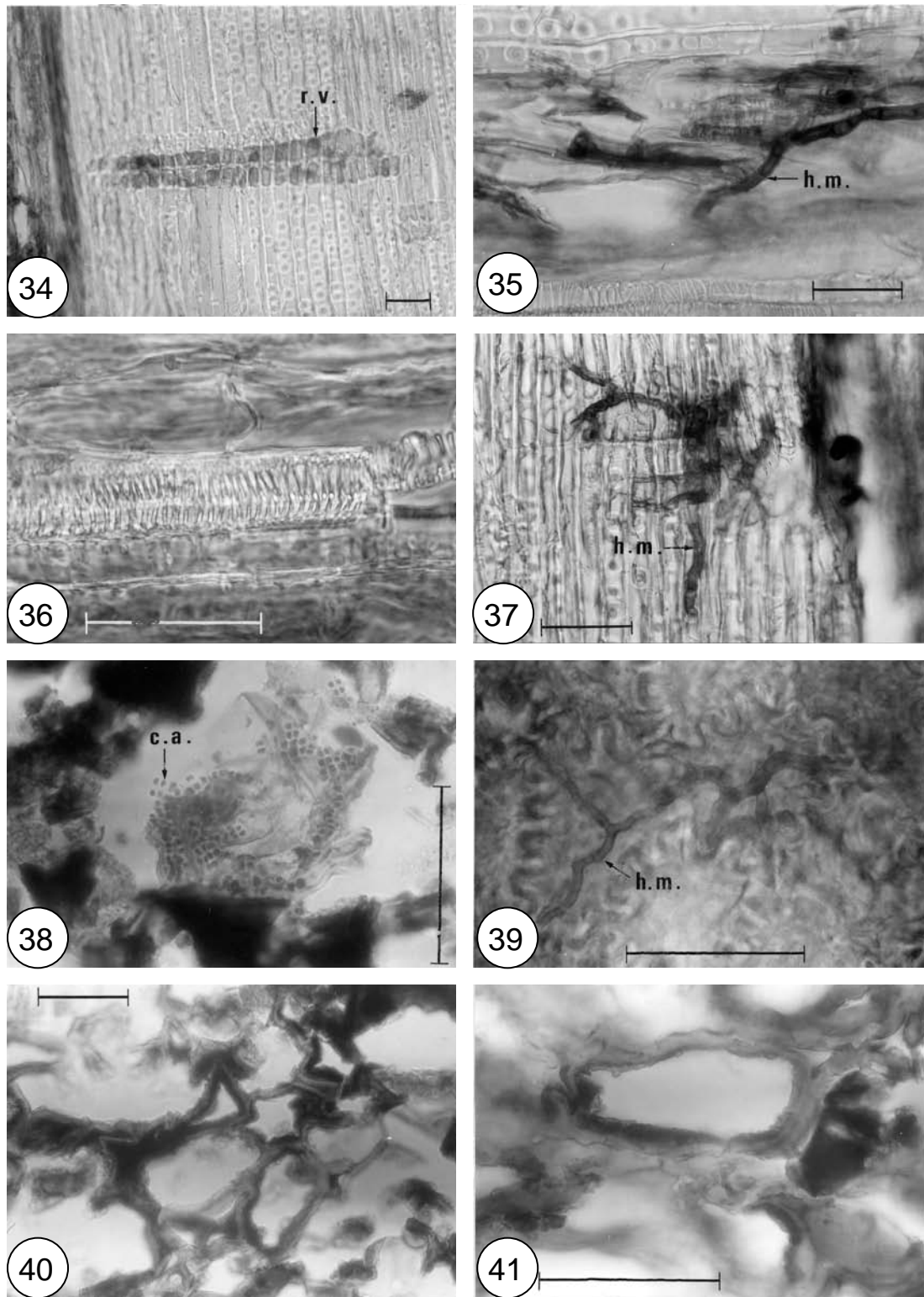


Fig. 34: Two-yr-old pine twig, longitudinal section showing a vascular ray (BMSL); Fig. 35: Idem, longitudinal section showing, from top to bottom, metaxylem, medullar parenchyma, protoxylem (BMSL); Fig. 36: Idem, longitudinal section showing a reticulated tracheid of the protoxylem (CP); Fig. 37: Idem, longitudinal section showing the passage of melanised hyphae from a vascular ray to adjoining tracheids (BMSL); Fig. 38: Piece of bark, section showing micro-algae in tetrads (BMSL); Fig. 39: Idem, melanised hyphae at the surface of the epidermis (CP); Fig. 40: Idem, brown deposits on walls of bark cells (BMSL); Fig. 41: Idem, detail (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; r.v. = vascular rays; h.m. = melanised hyphae; c.a. = micro-algal colonies; length of segment = 50 µm

Bark fragments

These bark fragments were detached from the tree. Their surface was covered with unidentified pleurococoid micro-algae, cells of which were $\sim 2 \mu\text{m}$ (Fig. 38). When epidermis was still present, it was covered with a network of melanised hyphae (Fig. 39). The walls of dead cells of bark (suber) were lightly brown and covered with dark brown deposits (Figs. 40 & 41). The lumen of some bark cells was near entirely filled with light brown masses (Fig. 42). No fungal development was visible.

Living and decaying moss (*Pseusceropodium purum*)

Intact leafy shoots of *Pseudoscleropodium purum* were the dominant component of the L₁ layer, showing typical anatomical features of living leaves (Fig. 43). Like other Bryales *Pseusceropodium purum* foliar limbs are made of a single layer of photosynthetic cells, without any lamella (de Ferré, 1963). I observed on some moss leaves that the photosynthetic tissue was directly attacked by dematiaceous fungi crawling at the surface of foliar limbs (Fig. 44).

Fauna

Inventory

Table 3 shows that oribatid mites were the most important invertebrate group, both in density (46 specimens) and in biovolume ($0.24 \mu\text{L} < V < 0.98 \mu\text{L}$). It should be noticed that a unique specimen of the springtail *Pogonognathellus flavescens* (Tullberg) occupied a biovolume comprised between 0.07 and 0.28 μL . This is exemplary of huge variations which could occur in soil animal biovolume (or biomass) when a big animal appears or disappears from a prospected area.

Concerning nematodes, as well as for the rest of microfauna, it must be noticed that my method does not allow collecting adequately tiny transparent animals such as nematodes, rotifers and protozoa. Only one nematode (of a big size for this group) was observed and studied, which is not apportioned to their actual abundance. However, I can assure with a reasonable degree of confidence that they were not numerous in the L₁ layer, given that, with the same method, many more specimens were found in underlying layers, as well as many cyst amoeba.

Enchytraeid worms

Although they could not be identified at the species level, given the methods used for fixing and preserving the studied material, all specimens present in the studied samples probably pertained to the genus *Cognettia* (A. Albrecht, personal communication).

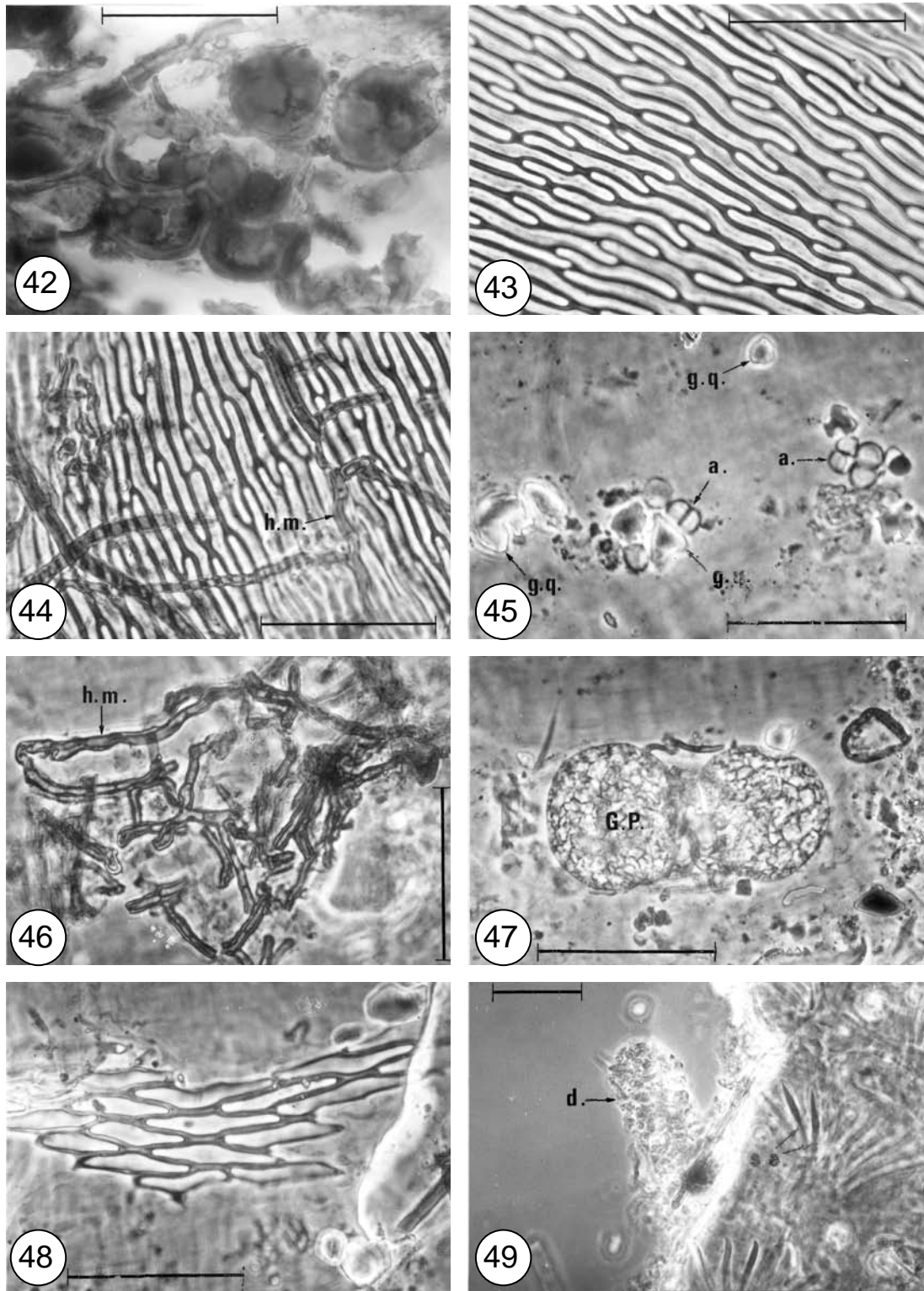


Fig. 42: Piece of bark, section showing light brown spherical masses filling the lumen of some bark cells (BMSL); Fig. 43: Leaving leaf of the moss *Pseudoscleropodium purum* (CP); Fig. 44: Melanised hyphae crawling at the surface and invading moss leaf cells; Fig. 45: Items carried on the tegument of enchytraeids, micro-algae in tetrads and silt-size quartz grains (CP); Fig. 46: Idem, melanised fungal hyphae with smooth walls (CP); Fig. 47: Idem, pollen grain of pine, intact (CP); Fig. 48: Idem, moss leaf fragment of *Pseudoscleropodium purum* (CP); Fig. 49: Idem, springtail faeces (CP)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; h.m. = melanised hyphae; g.q. = quartz grains; a. = micro-algae; d. = faeces; G.P. = pollen grains; length of segment = 50 μ m

Table 3. Taxonomic name, abundance and estimated biovolume of animals found in the L₁ layer

Taxonomic groups and species	Abundance	Upper estimate of biovolume (μL)	Lower estimate of biovolume (μL)
Nematodes	1	0.69 10 ⁻³	0.17 10 ⁻³
<i>Prionchulus punctatus</i>	1	0.69 10 ⁻³	0.17 10 ⁻³
Enchytraeids	7	0.22	0.06
Oribatid mites	46	0.98	0.24
<i>Platynothrus peltifer</i>	22	0.49	0.12
<i>Chamobates sp.</i>	3	0.61 10 ⁻¹	0.15 10 ⁻¹
<i>Parachipteria punctata</i>	3	0.55 10 ⁻¹	0.14 10 ⁻¹
<i>Oppia sp.</i>	3	0.13 10 ⁻¹	0.03 10 ⁻¹
<i>Oribatula tibialis</i>	3	0.26 10 ⁻¹	0.06 10 ⁻¹
<i>Camisia spinifer</i>	2	0.97 10 ⁻¹	0.24 10 ⁻¹
<i>Ceratoppia bipilis</i>	2	0.12	0.03
<i>Oppiella tricarinata</i>	2	0.60 10 ⁻²	0.15 10 ⁻²
<i>Oppiella subpectinata</i>	2	0.17 10 ⁻¹	0.04 10 ⁻¹
<i>Phthiracarus sp.</i>	1	0.42 10 ⁻¹	0.10 10 ⁻¹
<i>Carabodes femoralis</i>	1	0.19 10 ⁻¹	0.05 10 ⁻¹
<i>Adoristes ovatus</i>	1	0.26 10 ⁻²	0.10 10 ⁻²
<i>Minhuntozetes semirufus</i>	1	0.40 10 ⁻²	0.10 10 ⁻²
Miscellaneous mites	4	0.49 10 ⁻¹	0.12 10 ⁻¹
Uropodidae	3	0.44 10 ⁻¹	0.11 10 ⁻¹
Bdellidae	1	0.45 10 ⁻²	0.11 10 ⁻²
Spiders	2	0.49 10 ⁻¹	0.08 10 ⁻¹
Springtails	9	0.30	0.07
<i>Parisotoma notabilis</i>	8	0.16 10 ⁻¹	0.04 10 ⁻¹
<i>Pogonognathellus flavescens</i>	1	0.28	0.07
Fly larvae	7	0.38	0.10
Cecidomyiidae	5	0.25	0.06
Sciaridae	1	0.11	0.03
Chironomidae	1	0.17 10 ⁻¹	0.04 10 ⁻¹
Corpses	4		
Unidentified arthropod	1		
Unidentified oribatid mite	1		
<i>Platynothrus peltifer</i>	1		
<i>Phthiracarus sp.</i>	1		

- Transport on the tegument

The direct examination of enchytraeids showed that their tegument was covered with sticky mucus onto which a variety of items adhered and could be identified under the light microscope: pleurococoid green micro-algae (Fig. 45), melanised fungal hyphae with smooth (Fig. 46) or echinulate cell walls, and fungal spores. Fungal hyphae seemed to be intact and of a length indicating that they were more probably viable and thus prone to disperse the fungus. Pollen grains were also found (Fig. 47), as well as leaf fragments of *Pseudoscleropodium purum* (Fig. 48), springtail faeces (Fig. 49), nematodes (Fig. 50) and numerous quartz grains of silt size (Fig. 45). The presence of silt particles on the tegument of enchytraeids was the only trace of mineral uplift I saw in the L₁ layer. These observations testified for the important carrying activity exerted by enchytraeids: they disseminate micro-algae, fungi and bacteria and effect mineral uplift. Given the thickness of organic layers (8 cm) the presence of quartz grains of silt size in the L₁ layer indicated the important vertical movements effected by these animals.

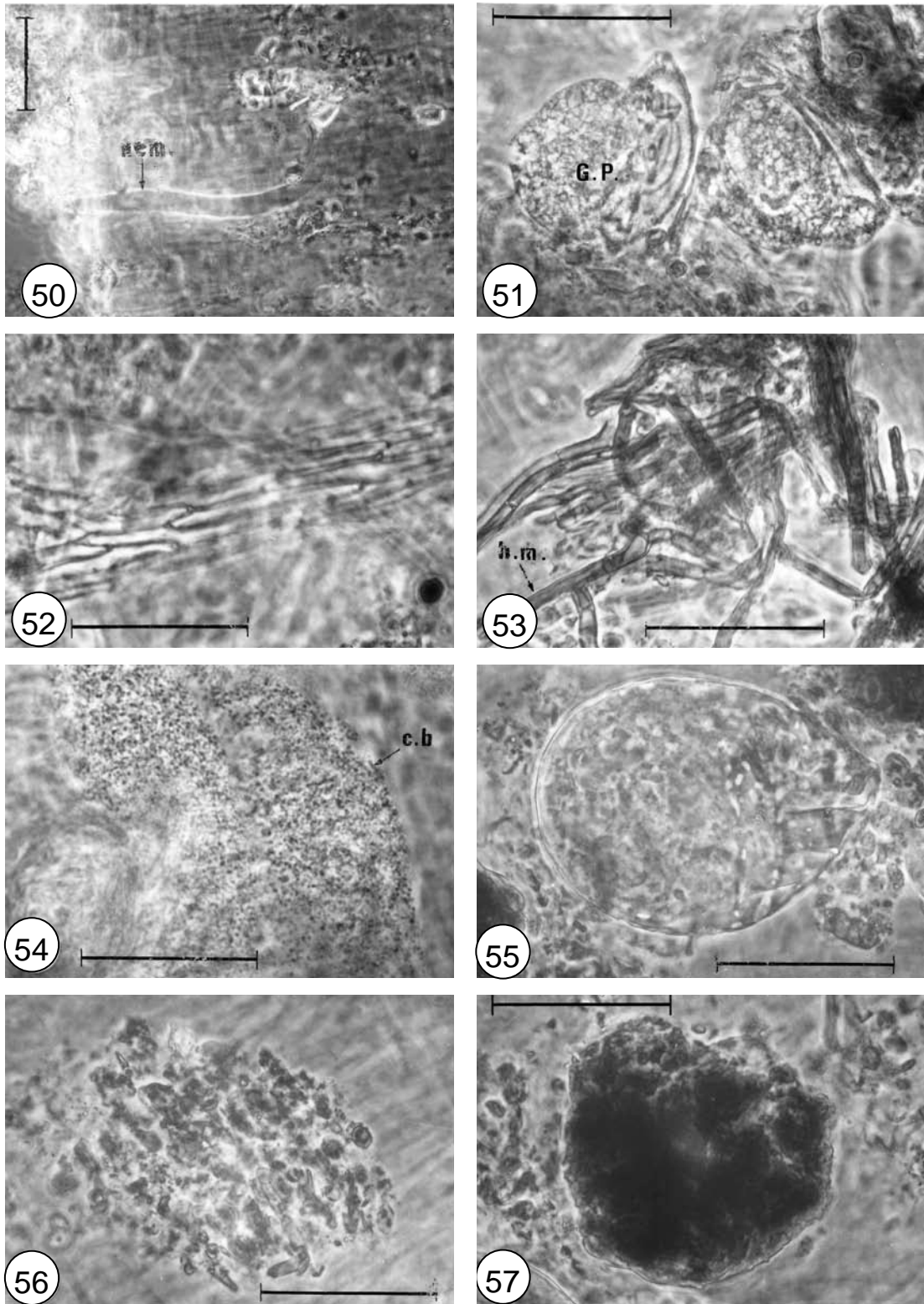


Fig. 50: Items carried on the tegument of enchytraeids, nematode (CP); Fig. 51: Gut contents of enchytraeids, observed by transparency, pollen grain of pine, collapsed (CP); Fig. 52: Idem, moss leaf fragment of *Pseudoscleropodium purum* (CP); Fig. 53: Idem, long fragments of fungal melanised hyphae (CP); Fig. 54: Idem, intestinal microflora (CP); Fig. 55: Idem, cyst amoeba (*Nebela collaris*) (CP); Fig. 56: Idem, faecal material of oribatid mites, with fungi (degraded) and bacteria (CP); Fig. 57: Idem, faecal material of oribatid mites, with melanised, highly compacted fungal hyphae (CP)

CP = phase contrast; nem. = nematode; G.P. = pollen grains; h.m. = melanised hyphae; c.b. = bacterial colonies; length of segment = 50 μ m

- Feeding activity

Gut contents of enchytraeids were observed by transparency: pollen grains (Fig. 51), fragments of leaves of *Pseudoscleropodium purum* (Fig. 52), non-fragmented melanised hyphae (Fig. 53). Bacterial colonies, which seemed living according to their neat and refringent contour under phase contrast, were attributed to intestinal microflora (Fig. 54). Cyst amoebae were also sometimes found, such as *Nebela collaris* (Ehrenberg) (Fig. 55). Mite faeces were also ingested. In this case fungal material seemed much more degraded, mixed with bacteria (Fig. 56) or highly compacted (Fig. 57). The necessity of a double digestion for the degradation of melanised fungal walls must be highlighted, which is reminiscent of observations made by Reisinger (1972) on the mite *Tyrophagus putrescentiae* (Schrank).

Nematodes

A single specimen, belonging to *Prionchulus punctatus* (Cobb) (Mononchidae), was found, but we expect nematodes were much more abundant. Their small size and transparency allowed them, together with other microfauna, to escape my observations with a dissecting microscope of the material fixed into ethyl alcohol. The observed specimen was considered as a predator (Arpin, 1980). By transparency, the degradation of the ingested material could be followed. In the anterior part of the intestine, I found mastax of rotifers (Fig. 58), thereby indicating that these animals served as food, only hard chitinous parts remaining undigested. Bacterial cells had slightly fuzzy contours, were poorly refringent under phase contrast, but they were still individualised. The medium part of the intestine showed more degraded bacteria, with a fuzzier contour, together with debris of melanised fungal spores (Fig. 59). In the posterior part, just before anus, numerous bacteria, highly compacted and with a hardly discernible contour, could be observed (Fig. 60). It must be noticed that from the examination of its gut contents the food diet of this 'predatory' nematode was mainly made of bacteria, which were progressively degraded during intestinal transit. Similar observations, using transmission electron microscopy, were done by Arpin & Kilbertus (1981) on the nearby species *Prionchulus muscorum*.

Oribatid mites

The species dominant in number and biomass (as ascertained by biovolume, see Table 3) was *Platynothrus peltifer* (Koch), which was represented by tritonymphs and adults. When isolated by dissection, the intestine showed by transparency several food boluses, similar in shape (oval) and size (50-100 µm) to oribatid faecal pellets, and bacterial colonies in special pouches (caecums) freely communicating with the intestinal lumen (Fig. 61). One or several colonies of intestinal microflora were found in all specimens, whether juvenile or adult, starving or feeding, which were dissected. All bacterial colonies living in the intestine of *Platynothrus peltifer* seemed identical morphologically. The ingested material was made of fungal hyphae, cut into pieces (contrary to what was observed in

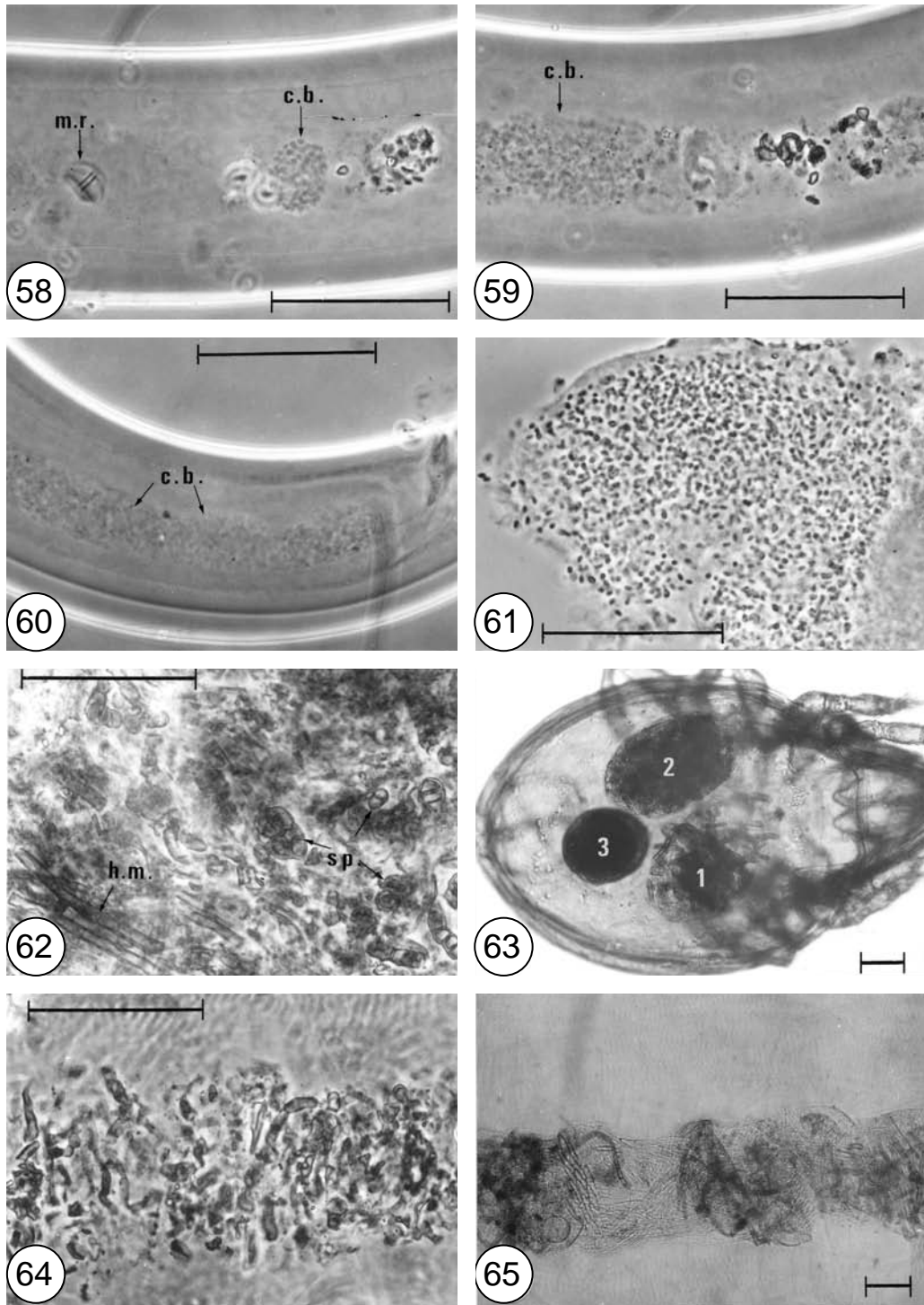


Fig. 58: Gut contents of the nematode *Prionchulus punctatus* (Mononchidae) observed by transparency, anterior intestine showing rotifer mastax, bacteria, unidentifiable organic matter (walls of melanised hyphae?) (CP); Fig. 59: Idem, medium intestine showing degraded bacteria and broken fungal spores (CP); Fig. 60 (x 400) Idem, posterior intestine showing strongly degraded bacteria (CP); Fig. 61: Gut contents of the oribatid mite *Platynothrus peltifer* observed after dissection, intestinal microflora (CP); Fig. 62: Idem, fungal spores and melanised hyphae (CP); Fig. 63: Gut contents of the oribatid mite *Adoristes ovatus* observed by transparency, showing three stages in the formation of faecal pellets (CP); Fig. 64: Gut contents of the springtail *Parisotoma notabilis*, degraded melanised hyphae and bacteria (CP); Fig. 65: Gut contents of fly larvae (Sciariidae) observed by transparency, leaves of the moss *Pesudoscleropodium purum* (CP)

CP = phase contrast; m.r. = mastax of rotifers; c.b. = bacterial colonies; h.m. = melanised hyphae; sp. = fungal spores; length of segment = 50 μ m

enchytraeids), mostly melanised (dematiaceous), and spores (Fig. 62). Fungal cell walls seemed not too degraded.

A similar food diet was found in *Ceratoppia bipilis* (Herman), *Camisia spinifer* and *Adoristes ovatus*. A photograph was taken by transparency through a specimen of the latter species, showing three stages of compaction of fungal hyphae which occurred during the formation of faeces (Fig. 63).

Springtails

Gut contents of *Parisotoma notabilis* (Schäffer) were, here too, formed of fungal material (Fig. 64) but, contrary to *Platynothrus peltifer*, bacteria were present in the food bolus and fungal hyphae were degraded, suggesting that a decaying and not a living fungal colony was ingested, probably coming from underlying layers (faecal material?).

Fly larvae

Only results about Sciaridae and Chironomidae will be presented, since not any solid particles were found in intestines of Cecidomyidae.

Sciarid larvae ingested mainly fragments of the moss *Pseudoscleropodium purum* (Figs. 65 & 66) but I found also melanised fungal hyphae, which had been ingested without being cut into pieces (Fig. 67), and pollen grains of *Pinus sylvestris* (Fig. 68). It must be noticed that fungal hyphae seemed to be ingested in the same manner as enchytraeids, without any previous fragmentation.

Fungal melanised hyphae were found in the intestines of chironomid larvae, together with hyaline hyphae. In the latter case, hyphae appeared compacted, with fuzzy contours, in way to be degraded (Fig. 69). Brown masses of organic matter (humus), of unknown origin, were also found (Fig. 70). It must be highlighted that the degradation of hyaline and melanised fungal hyphae was hardly comparable, melanin making hyphal walls of dematiaceous fungi resistant to gut enzymes (Reisinger, 1972).

Animal faeces

Only faeces of oribatid mites were observed in the L₁ layer. They were few abundant and deposited at the surface of needles and bark pieces. They appeared green in freshly collected material. Examination of faecal material under the light microscope showed that oribatid faeces contained decaying fungal material with numerous micro-algae in vegetative growth, as ascertained by preferential staining of chromosomes by methyl blue (Fig. 71).

Similar micro-algae were found in gut contents of *Platynothrus peltifer*, although in few

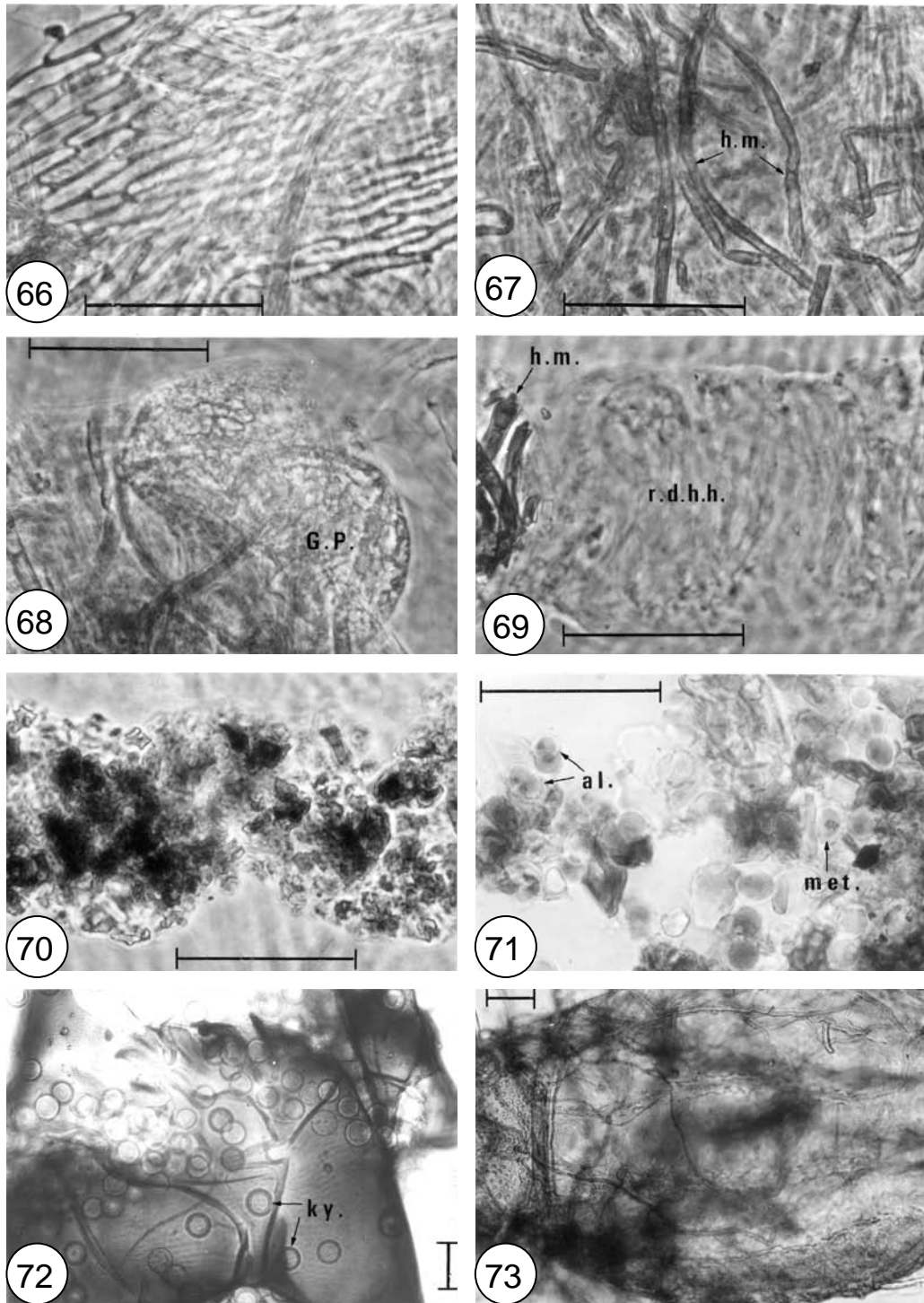


Fig. 66: Gut contents of fly larvae (Sciariidae), observed by transparency, leaf fragment of the moss *Pseudoscleropodium purum* (CP); Fig. 67: Idem, long fragments of melanised fungal hyphae (CP); Fig. 68: Idem, pollen grain of pine (CP); Fig. 69: Gut contents of fly larvae (Chironomidae) observed by transparency, hyaline hyphae, compacted and in way to be degraded (CP); Fig. 70: Idem, unidentifiable brown organic matter (melanised fungal walls?) (CP); Fig. 71: Micro-algal development (metaphases) in oribatid faeces (BMCL); Fig. 72: Unidentified cysts in an arthropod corpse (CP); Fig. 73: Development of melanised fungal hyphae in a corpse of *Platynocheilus peltifer* (observed by transparency) (CP)

CP = phase contrast; BMCL = methyl blue-chloral-lactophenol; h.m. = melanised fungal hyphae; G.P. = pollen grains; r.d.h.h. = dense network of hyaline hyphae; al. = micro-algae; met. = metaphases; ky. = cysts; length of segment = 50 µm

abundance. It is possible that micro-algal development started by some algal cells which were ingested then excreted in living stage in the faeces, thereafter developing in this favourable environment. Contrary to animal guts, into which fungal walls of melanised hyphae were still intact, the same material was in way to be degraded in faeces invaded by micro-algae.

Animal corpses

All corpses found in the L₁ layer were arthropod cadavers. A corpse of *Platynothrus peltifer* was shown to be invaded by a dense network of melanised hyphae (Fig. 72), while a corpse of an unidentified arthropod was filled with cysts of unknown origin (Fig. 73).

Conclusions about the L₁ layer

The decomposition of pine needles in the L₁ layer was mainly ensured by fungi. Even though the external development of fungi was but feeble, all needles were invaded by fungal hyphae which penetrated internal tissues (mesophyll, transfusion tissue) and replaced progressively the cytoplasm of plant cells. When needles were colonised on the tree (by *Lophodermium pinastri*, *Lophodermella* or *Ceuthospora pinastri*) part of this process had already started before needles fell on the ground.

Needles in the most advanced stage of decomposition (probably in contact with the underlying level L₂) were characterised by the internal development of *Verticicladium trifidum*. Blackening of hypodermic call walls under the influence of this fungus is worth to notice. Needle collapse was associated with the disappearance of cell cytoplasm, in particular in the mesophyll.

Similarly, parenchymatous tissues of pine twigs were colonised by fungi. The attack of wood was only incipient, some hyphae penetrating tracheids through vascular rays.

The impact of fauna seemed limited to grazing of fungi which grew externally on the decaying plant material. However, it must be noticed that leaves of the moss *Pseudoscleropodium purum* were a major food resource for sciarid larvae and to a lesser extent for enchytraeids. The transport of bacteria, fungal propagules (hyphae, spores) and mineral grains by the latter animal group was also noticeable in this superficial layer, exemplifying the importance of invertebrates in the early microbial colonisation of pine litter. Bacterial development at the inside of a pine needle tunnelled by enchytraeids was also indicative of the role of soil animals as carriers of bacteria.

THE L₂ LAYER: DECAYING NEEDLES AND DEAD MOSS



Figure 74. The L₂ layer before fixation and transport to the laboratory

Table 4 shows the respective volume of the gross components of the L₂ layer. Compared to the overlying L₁ layer, the L₂ layer was characterised by a greater diversity of plant material and a measurable amount of animal faeces.

Table 4. Volume of the different components of the L₂ layer sorted under the binocular microscope

Litter components	Volume (μL)
Pine wood	5100 ± 100
Moss	2600 ± 100
Pine bark	1700 ± 100
Pine needles	1700 ± 100
Animal faeces	220 ± 10
Pine twigs	200 ± 10
Bracken fronds	80 ± 10
Pine mycorrhizae	70 ± 10
Male flowers of pine	22 ± 2
Pine euphylls	12 ± 2
Living fauna	0.8 – 3.4
Total	11700 ± 100

Litter fall corresponding to the L₂ layer could be dated from previous December-January to previous June. The earlier date was deduced from the presence of bracken litter, *Pteridium aquilinum* (L.), in the form of leaflets and secondary and tertiary rachises detached from the fronds which currently fall in winter. The later date was deduced from the presence of pine euphylls, the fall of which currently occurs in June. Thus the L₂ layer embraced a period of time four times longer than the overlying L₁ layer.

The amount of moss material (22% of the total volume) was much less than in the L₁ layer (77%). However, this estimate must be amended because of the presence of a 5-yr-old branch of pine, the volume of which was estimated to 44% of the total volume of the sample. After discarding branchwood, the volume of dead moss was 39%, which still indicated a pronounced decrease compared to L₁. This should not be interpreted as resulting from a high decay rate of moss compared to pine needles, but rather to a low primary production of moss in winter and spring. At this stage, leafy stems of *Pseudoscleropodium purum* were deprived of pigment and invaded by an abundant mycelium but seemed still intact, at least to the naked eye (Fig. 74).

As in the previous layer, dating of the moss fraction was difficult. Kilbertus (1968a, b) showed that *Pseudoscleropodium purum* remained green for a year and a half then senesced and became brown for an additional three years, before decaying. At first sight this contradicts my observations on pine and bracken litter in the studied sample. Such a marked disagreement could be explained by the fact that (i) pine needles, when falling on the ground, penetrated to some extent through the moss carpet, (ii) moss continued to grow upwards after needles had fallen, and tended to cover them up during spring and summer months i.e. just previous to sampling which took place in August. However, this does not explain why dead foliage of *Pteridium aquilinum* (not likely to penetrate through moss) was found in the same layer than dead parts of *Pseudoscleropodium purum*. The present study cannot answer this question, but it should be noticed that similar discrepancies were found between my field observations and experiments on decomposition rates of pine needles performed by Kendrick & Burges (1962), as mentioned above. Guittet (1967) showed that the 'duration' of the L₂ layer was thrice that of the L₁ layer in Scots pine stand, which compares well with my observations. Unfortunately this author could not adequately date its litter samples.

Data on animal faeces presented in Table 4 did not embrace the whole faecal material. Although in much higher abundance in L₂ than in the overlying L₁ layer, faecal pellets of enchytraeids and microarthropods were too isolated and could not be taken with forceps, and thus their biovolume could not be measured adequately with my method. Rather, I estimated the volume of faeces of macroinvertebrates such as earthworms, woodlice and millipedes.

Voids were important (77%), although slightly less than in the overlying layer (80%), showing the absence of packing of pine and bracken litter through the moss carpet. The biovolume of fauna, mostly mesofauna, was feeble (0.8-3.4 μ L) although slightly higher than in L₁ (0.4-2 μ L).

Externally L₂ pine needles differed to a great extent from those found in L₁. They were black-coloured, often covered, as the whole material present in L₂, by a loose weft of mycelia, and most of them were collapsed (Fig. 74). Traces of previous colonisation by *Lophodermium pinastri* and *Ceuthospora pinastri* were still visible, but *Verticicladium trifidum* was dominant: many needles were covered by a net of its conidiophores. The folds of collapsed needles, where conidiophores of *Verticicladium trifidum* formed a dense network, were the main niche of nematodes, rotifers, ciliates and cyst amoebae, which could not be adequately collected extensively because of their small size and

transparency (a limit of my method, which was not convenient for microfauna). Brown rhizomorphs of *Marasmius androsaceus* Fries were seen protruding from some needles, which were in way to be discoloured. Similar observations of needle discoloration by *Marasmius androsaceus* were made by Soma & Saitô (1979) on *Pinus thunbergii* Parl., by Gourbière (1981) on *Abies alba* Mill., and by Latter (1977) on *Calluna vulgaris* Salisb. and several heathland species. This basidiomycete is a white rot (Hintikka, 1970). Its rhizomorphs have been considered by Gourbière (1981) to penetrate then to colonize coniferous needles through internal mycelia. In the present sample, rhizomorphs were short (a few cm), their width decreased then they ramified at distance from colonised needles, without linking them. They were interpreted as issuing from needles and not entering them. Mitchell & Millar (1978) showed that the cuticle of pine needles was penetrated by thin hyphae ramifying from rhizomorphs appressed on the cuticle and that rhizomorphs protruded from the needles by lifting out hypodermis, epidermis and cuticle. Although I did not observe any fungal rhizomorph penetrating the needles, I cannot discard that direct penetration by rhizomorphs of *Marasmius androsaceus* could have occurred previously. The fact that this fungus was absent from L₁ suggests that infection took place during previous winter or spring but was not extended to summer.

Some needles exhibited fungal sclerotia of particular toughness and shape, which could not be identified by lack of fructifications.

The action of fauna was prominent in the L₂ layer. In addition to macroinvertebrate activity, traced from the deposition of faeces (2% of the total volume of solid matter), most animal activity taking place at the time of sampling was attributed to enchytraeids. All pine needles showed signs of biting or penetration by these animals, and a needle had even been fixed with an animal penetrating it. Some other needles showed breaks, which could not be attributed to enchytraeids but rather to macroinvertebrates which deposited their faeces at this level. Most enchytraeids were retrieved in decaying leafy shoots of the moss *Pseudoscleropodium purum*, at the inside of fungal sheaths embedding stems and leaves. I noticed the behavioural tendency of these worms to penetrate any kind of decaying plant material. Several specimens were retrieved between bark and wood, among their faecal deposits, in the decomposing pine branch which was studied in the L₂ sample. Other deposits of enchytraeid faeces were observed at the surface of bark pieces, between needles when still assembled by pairs and appressed to each other, between stems and leaves of moss, or in fluffy masses invaded by mycelial wefts. Faeces of mites and springtails were more abundant than in L₁. They were of a dark brown colour and most of them were found isolated at the surface of needles.

Mycorrhizal fine roots of pine were not very abundant at this superficial level (0.6% of total volume of solid matter), being preferentially observed in F₂ and H₁ (Table 1). They were embedded in white-grey mycelial wefts of woolly aspect, quite identical to those observed around well-decayed pine needles, leafy shoots of mosses and enchytraeid faecal deposits.

The abundance of bark was also noticeable, with a biovolume comparable to that of pine needles (14.5%), as was already true in L₁. I want to notice that bark, a prominent component of pine litter, has been unexpectedly neglected in decomposition studies, if we except Kuhlman (1969, 1970).

Decaying pine needles

As for the overlying L₁ layer, only a small number of representative types will be described, although a wide variety of needles were sectioned (15 different morphological types).

Needles attacked by Verticicladium trifidum

Compared to L₁, the attack was much more advanced. Needles turned to full black over their whole surface. Sectioned needles showed that the contact zone between hypodermis and mesophyll was the main seat of blackening (Fig. 75): a thin black opaque layer, not made of fungal hyphae, was seen blanketing the hypodermis. Blackening diffused through cell files of the mesophyll, still recognizable at this stage of decay in the absence of living hyphae.

Cell walls of internal tissues were destroyed to a higher extent than in L₁. Endodermis was degraded, as well as the external part of transfusion tissue (Fig. 76). The degradation of lignin was effective, as ascertained by the presence of more or less identifiable bordered pits (areolae) within a mass of tracheid wall remains. The attack was more recent (less pronounced) at the level of vascular tissues: lignified walls were still intact, but I observed some living hyphae penetrating tracheids of the metaxylem through bordered pits, probably after disappearance of the torus (Fig. 77). Long hyaline hyphae of *Verticicladium trifidum*, stained blue by methyl-lactophenol, were observed running along vessels of secondary wood.

Although *Verticicladium trifidum* was often reported as a primary coloniser of fallen pine needles, mycological studies did not register its paramount importance in the decay process. Practically nothing was said of hyaline hyphae invading first living tissues, the cytoplasm of which was destroyed, then other tissues where cellulose and lignin were destroyed in turn. According to my observations, *Verticicladium trifidum* was always associated with the presence of black stromata, which seemed typical of this fungus. Mitchell & Millar (1978) observed that the bleaching (?) of pine needles was associated with the development of black stromata, to which they attributed the degradation of cellulose. Conversely, and more in conformity with my own observations, Lehmann & Hudson (1977) attributed to *Verticicladium trifidum* both blackening and softening of pine needles, the latter process being probably due to the degradation of lignin. Kendrick & Burges (1962) considered this fungus to be responsible for needle blackening and they demonstrated on a quantitative basis that sporulation (see conidiophore on Fig. 6) occurred in summer. They showed that it produced two kinds of hyphae, hyaline hyphae penetrating internal tissues, and melanised hyphae penetrating epidermis and hypodermis and thought to be responsible for needle blackening. The present study showed that at the inside of pine needles the mycelium of *Verticicladium trifidum* was only made of hyaline hyphae, melanised hyphae being entirely transformed in melanised stromata. Only hyaline hyphae were seen invading host cells and digesting their cytoplasm in the vicinity of stromata, such as for instance in stomatal guard cells (Figs. 21 & 22). Melanised stromatic nodules were seen from place to place in internal tissues, stemming from hyaline hyphae (Fig. 78). Such results conform to observations made in culture (Hughes, 1951; Gremmen, 1960) or by directly observing decaying needles (Hughes, 1951).

Needles attacked by Marasmius androsaceus

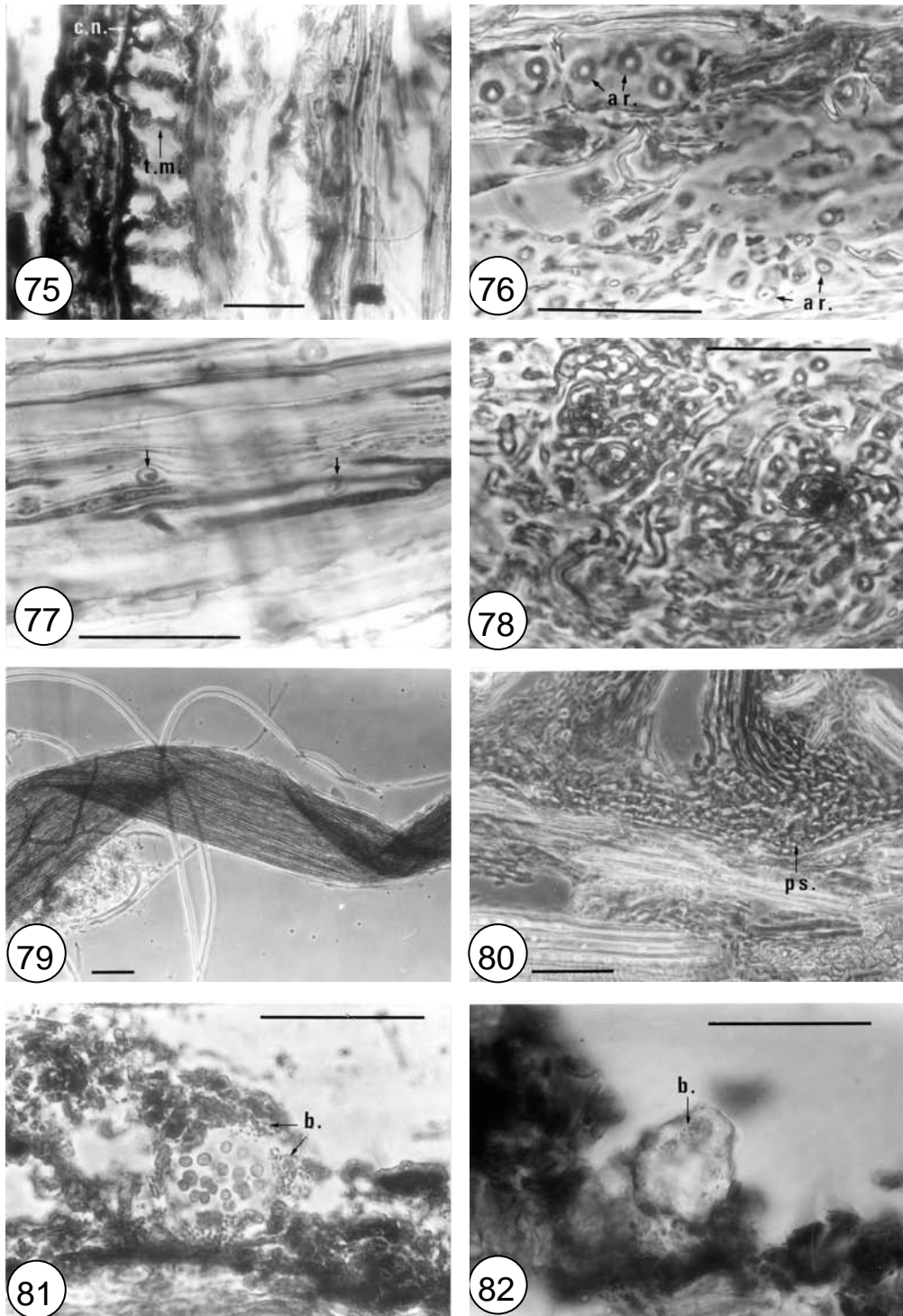


Fig. 75: Needle attacked by *Verticicladium trifidum*, longitudinal section showing epidermis, hypodermis, mesophyll, endodermis, transfusion tissue and perivascular fibres (BMSL); Fig. 76: Idem, detail of transfusion tissue showing partial degradation of cell walls (CP); Fig. 77: Idem, detail of metaxylem showing penetration of tracheids through bordered pits (arrows) (BMSL); Fig. 78: Idem, detail showing melanised stomata within transfusion tissue (CP); Fig. 79: Needle attacked by *Marasmius androsaceus*, brown rhizomorph (CP); Fig. 80: Idem, section through a zone from which started a rhizomorph, showing mesophyll, perivascular fibres, protoxylem and metaxylem (CP); Fig. 81: Idem, section through a fold of a collapsed needle showing composite bacterial colonies (CP); Fig. 82: Idem, showing a quartz grain of silt size covered with minute bacteria (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; c.n. = black opaque layer; t.m. = cell files of the mesophyll; ar. = areolae; ps. = pseudoparenchyma; b. = bacteria; length of segment = 50 μ m

This basidiomycete formed brown, leathery, flat rhizomorphs (Fig. 79), the anatomy of which was conform to descriptions given by Macdonald (1949) and Townsend (1954). According to criteria of maturity, such as the development of the internal lumen (responsible for flatness), rhizomorphs found in the L₂ layer were ageing. This is an additional argument for dating fungus infestation by *Marasmius androsaceus* to last autumn/winter.

In the vicinity of rhizomorph points of output the mesophyll appeared totally degraded, being replaced by fungal pseudoparenchyma (Fig. 80). Lignified walls of tracheids remained intact, but bacteria could be observed at the inside of tracheids with bordered pits of the metaxylem. This is another indication of ancientness of the colonisation process.

Needles colonised by *Marasmius androsaceus* were collapsed, due to disappearance of the mesophyll. Numerous folds were observed, with bacterial and micro-algal colonies, sometimes formed by several species in tight assembly (Fig. 81). Quartz grains, probably transported by enchytraeids or by earthworms, were also found in these microcosms: they were covered with minute bacteria lodging in irregularities (hollows) of the crystal surface (Fig. 82).

Marasmius androsaceus was suspected for a long time to be a lignin degrader (Lindeberg, 1944), which was confirmed by histological studies on infested needles (Mitchell & Millar, 1978; Mitchell et al., 1978a), but these authors did not show, as in the present study, any penetration of tracheids by this fungus. Bacteria, which were scarcely represented within tracheids, probably came from further contamination and did not show any sign of degradation of tracheid walls. However, the impact of the fungus on cellulose was prominent in the zone of rhizomorph rooting. I cannot discard the possibility of an erroneous identification of the fungus, which was done only through rhizomorphs and not through carpophores (which were absent from my sample).

Needles attacked by unidentified fungi

Some needles showed one or more black sclerotia at their surface. These needles were at a more advanced stage of decomposition (they were internally colonised by bacteria) and the action of the fungus previous to bacterial colonisation could not be ascertained.

Another species of fungus formed white excrescences at the surface of some needles. This fungus developed at the inside of needles, in mesophyll and transfusion parenchymatous tissues in which it formed long strands made of fibrous hyphae with thick wall and narrow lumen (Fig. 83). Hyphae of this fungus did not penetrate host cells, but rather its fibrous strands pulled aside host tissues, cells of which were squashed but kept intact their walls (Fig. 84). Externally, the fungus developed a network of fibrous hyphae forming the so-called white excrescences (Fig. 85). At this stage, needles were often embedded in a thick sheath made of dead melanised hyphae, which was colonised by bacteria (Fig. 86) and micro-algae (Fig. 87). This fungal-bacterial-micro-algal sheath was lifted from place to place by the development of white excrescences, which were able to pierce it and develop externally in the form of white, tough, smooth white masses. The external network of melanised hyphae was reminiscent of the 'network stage' which has been described by many authors (Kendrick & Burges, 1962; Hayes, 1965; Lehmann & Hudson, 1977; Mitchell et al., 1978a; Mitchell & Millar, 1978; Soma & Saitô, 1979; Gourbière, 1981). It was relict of an earlier stage of fungal

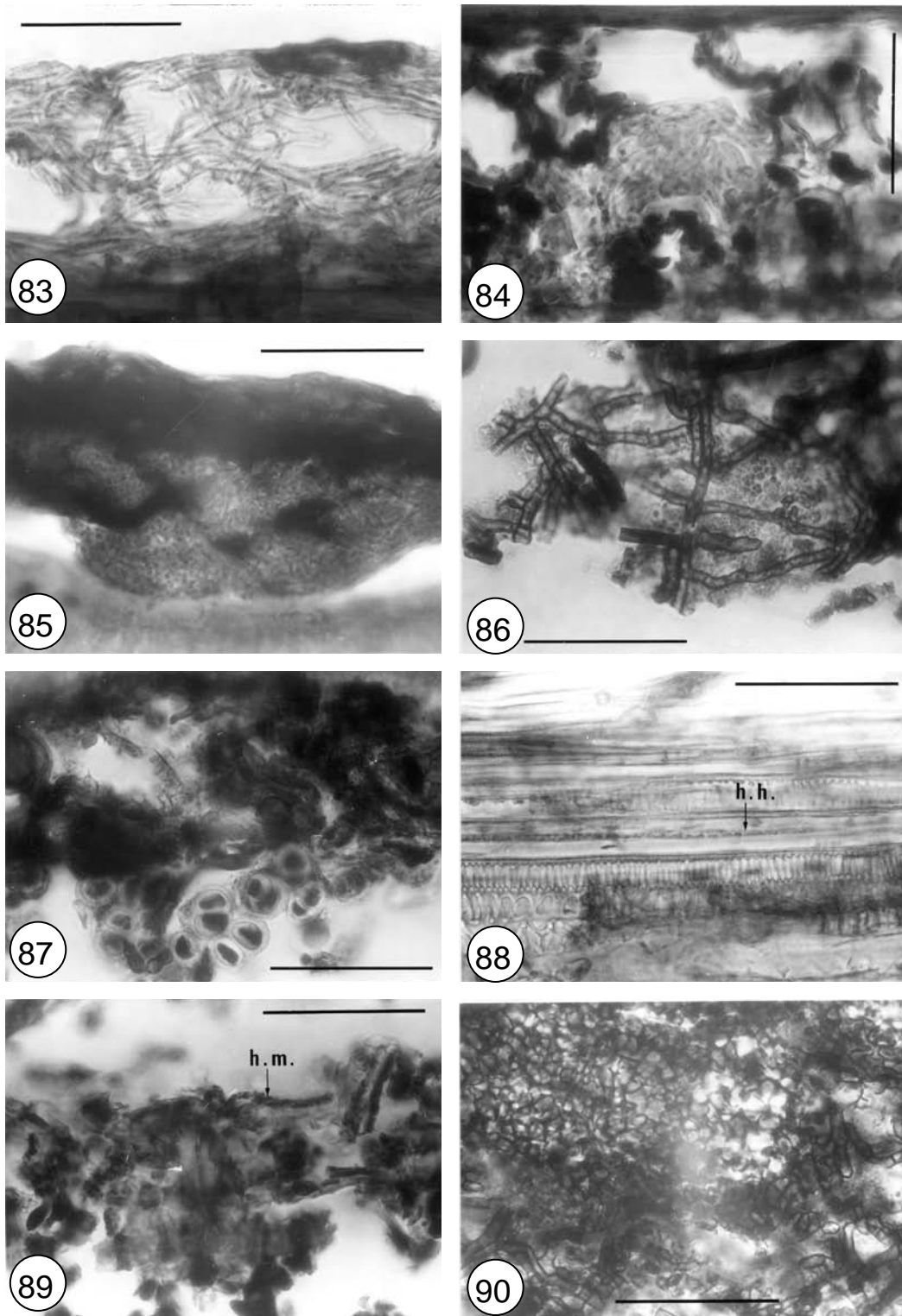


Fig. 83: Needle attacked by an unidentified fungus with white excrecences, longitudinal section showing transfusion tissue (BMSL); Fig. 84: Idem, mesophyll (BMSL); Fig. 85: Idem, dense fibrous mass at the surface of a needle (BMSL); Fig. 86: Idem, network of dead melanised hyphae with bacteria (BMSL); Fig. 87: Idem, with micro-algae (BMSL); Fig. 88: Needle with a development of bacteria, longitudinal section showing protoxylem (BMSL); Fig. 89: Idem, mesophyll (BMSL); Fig. 90: Needle with a development of bacteria and micro-algae, tangential section through epidermis (BMSL)

BMSL = methyl blue-Sudan III-lactophenol coloration; h.h. = hyaline hyphae; h.m. = melanised hyphae; length of segment = 50 μ m

colonisation, probably under moister atmosphere during previous winter or spring. The dry atmosphere of summer months might have limited the external development of fungi in L₁ (the past of L₂) while the internal development of *Verticicladium trifidum* did not seem to be affected by dry air, higher temperature allowing it to grow and fructify rapidly.

Needles with bacterial development

These needles were always broken or nibbled, indicating a previous attack by fauna. Bacteria seemed to attack preferentially fungal hyphae. In needle tissues where fungal attack by *Verticicladium trifidum* was still incipient and did not degrade host cells, such as xylem, hyphae of this fungus could be morphologically identified, with bacteria at their inside (Fig. 88). In the mesophyll, in which previous attack by fungi was more pronounced, melanised fungal walls were degraded. They were probably relicts of another (unidentified) dematiaceous fungus. Melanised walls appeared fragmented and agglomerated in coarse masses (Fig. 89). Studying at the right scale this process of transformation (humification?) of melanised fungal cell walls and establishing the role of bacteria would need the use of electron microscopy, which was out of the scope of my study.

Needles with bacterial and micro-algal development

These needles have been also previously broken by soil invertebrates. I interpreted this stage as a more advanced stage of decomposition compared to bacteria only. Needles were markedly collapsed, with numerous folds. Epidermis and hypodermis were replaced by a melanised fungal stroma, dead at this stage (Fig. 90), which was covered by a dense network of dead melanised hyphae, morphologically identical to the abovementioned 'network stage', with numerous bacteria.

The inside of needles was in an advanced stage of decomposition, under the influence of an intense bacterial development. Areolae from bordered pits were often the only trace of pre-existing tracheids (Fig. 91). The development of micro-algal colonies was visible at the inside of needles. I observed Chlorophyceae (Fig. 92), chloroplasts of which were coloured in violet by methyl blue-Sudan III (same colouration as in green needles and moss leaves), and Cyanophyceae of *Chroococcus* type (Fig. 93), the cytoplasm of which was uniformly coloured in blue without any nuclear or plastidial inclusion.

Some needles at this stage of decomposition showed a development of protozoa, as ascertained by the presence of numerous cysts (Fig. 94). Protozoan trophozoites were not observed and I was unable to identify them as ciliates or naked amoebae.

Needles penetrated by enchytraeids

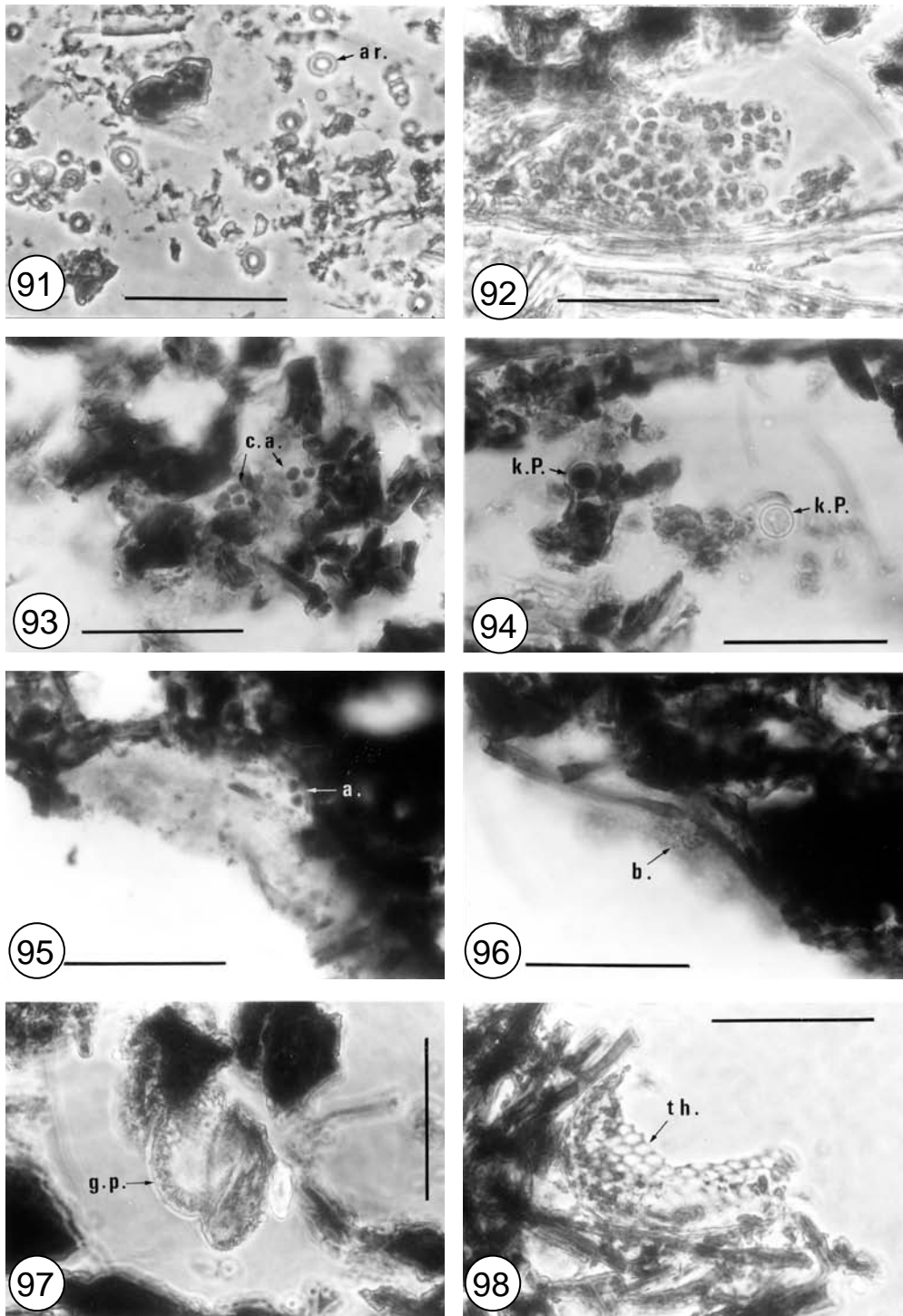


Fig. 91: Needle with a development of bacteria and micro-algae, section showing remains of transfusion tissue (CP); Fig. 92: Idem, section showing a micro-algal colony (Chlorophyceae) at the inside of a needle (CP); Fig. 93: Idem, section showing micro-algal colonies of Cyanophyceae (BMSL); Fig. 94: Idem, section showing protozoan cysts at the inside of a needle (BMSL); Fig. 95: Needle penetrated by enchytraeids, section showing mucus deposits, with micro-algae, along a gallery (BMSL); Fig. 96: Idem, section showing mucus deposits with bacteria only (BMSL); Fig. 97: Idem, section showing a pollen grain of pine at the inside of a gallery (CP); Fig. 98: Idem, section showing a thecamoeban cyst at the inside of a gallery (CP)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; ar. = areolae; c.a. = micro-algal colonies; k.P. = protozoan cysts; a. = micro-algae; b. = bacteria; g.p. = pollen grains; th. = thecamoeban cysts; length of segment = 50 μm

In addition to numerous bites and breaks made by these animals, some needles were penetrated by these oligochetes, more often than in L₁ where such penetration was exceptional. Mucus was deposited along galleries bored at the inside of pine needles by enchytraeids, with a development of micro-algae (Fig. 95) or bacteria (Fig. 96). Other items transported by these animals (see observations on enchytraeid worms in L₁) were deposited within their galleries, such as pollen pine (Fig. 97) or cysts of small Thecamoeba such as *Euglypha* spp. (Fig. 98).

When enchytraeids deposited their faeces at the inside of decaying pine needles (which was not always the case) then the excreted plant material (strongly degraded by fungi then by enchytraeids) was no longer recognizable (Fig. 99). Hyphal fragments can be observed, without any cell content and thus neither stained by methyl blue nor showing any opacity under phase contrast (Frankland 1974, 1975). Faeces deposited by enchytraeids at the inside of pine needles were the seat of an intense bacterial development (Figs. 100 & 101).

Remaining tissues were in an advanced stage of decomposition, with bacteria, algae and protozoa (Figs. 102 & 103). Numerous brown spherules of varying size were observed in the mesophyll (Fig. 104). Their origin could be deduced from observations made in less degraded tissues such as metaxylem (Fig. 105): they were issued from the condensation of a brown substance which was deposited on cell walls of pine tissues. Similar brown deposits were described by authors who studied in microscopy the decomposition of beech and oak leaves. Olah et al. (1978) were speaking of brown substances originating from condensation and oxidation of cell cytoplasm. Toutain (1981) interpreted them as brown pigments originating from polyphenol-protein complexes formed from the reaction of tannins (contained in vacuoles in living cells) and proteins, which occurs at the death of cells. This author considered this process as being responsible for the brown colour of dead leaves. If the formation of such condensed products by chemical reaction between proteins and tanning substances was experimentally demonstrated by Handley (1954) and Kononova (1961), no proof can be given that the same process occurred in the pine material here described. In the present material, brown spherules were observed in metaxylem tracheids, i.e. in cells without any cytoplasm but which could be filled with sap at the time of needle fall. Anyway, brown spherules were not observed in all needles, even those in an advanced stage of decomposition, and they could not be taken responsible for the observed darkening of needles.

Hyphal networks embedding decaying pine needles

Loose mycelial wefts were observed surrounding pine needles, sometimes agglutinating them (Fig. 106). They were comprised of two different fungi: the secondary mycelium with clamp connections (3 µm hyphal diameter) of a hyaline basidiomycete which was identified as *Hyphodontia* sp. (Fig. 107), and melanised hyphae (diameter 4 µm) of an ascomycete which was identified as *Cenococcum geophilum* Fr. (Fig. 108). As will be seen below these two fungi could be identified through a panel of morphological characters during the study of mycorrhizae and decaying wood. Cell walls of basidiomycetous hyphae were often seen encrusted with oxalate crystals (Fig. 108).

Hyphal networks were inhabited by minute animals such as nematodes. Faeces of mesofauna were also found, the content of which revealed that the secondary mycelium of *Hyphodontia* was actively consumed (Fig. 109). In the faecal material, hyaline fungal hyphae were deprived of oxalate

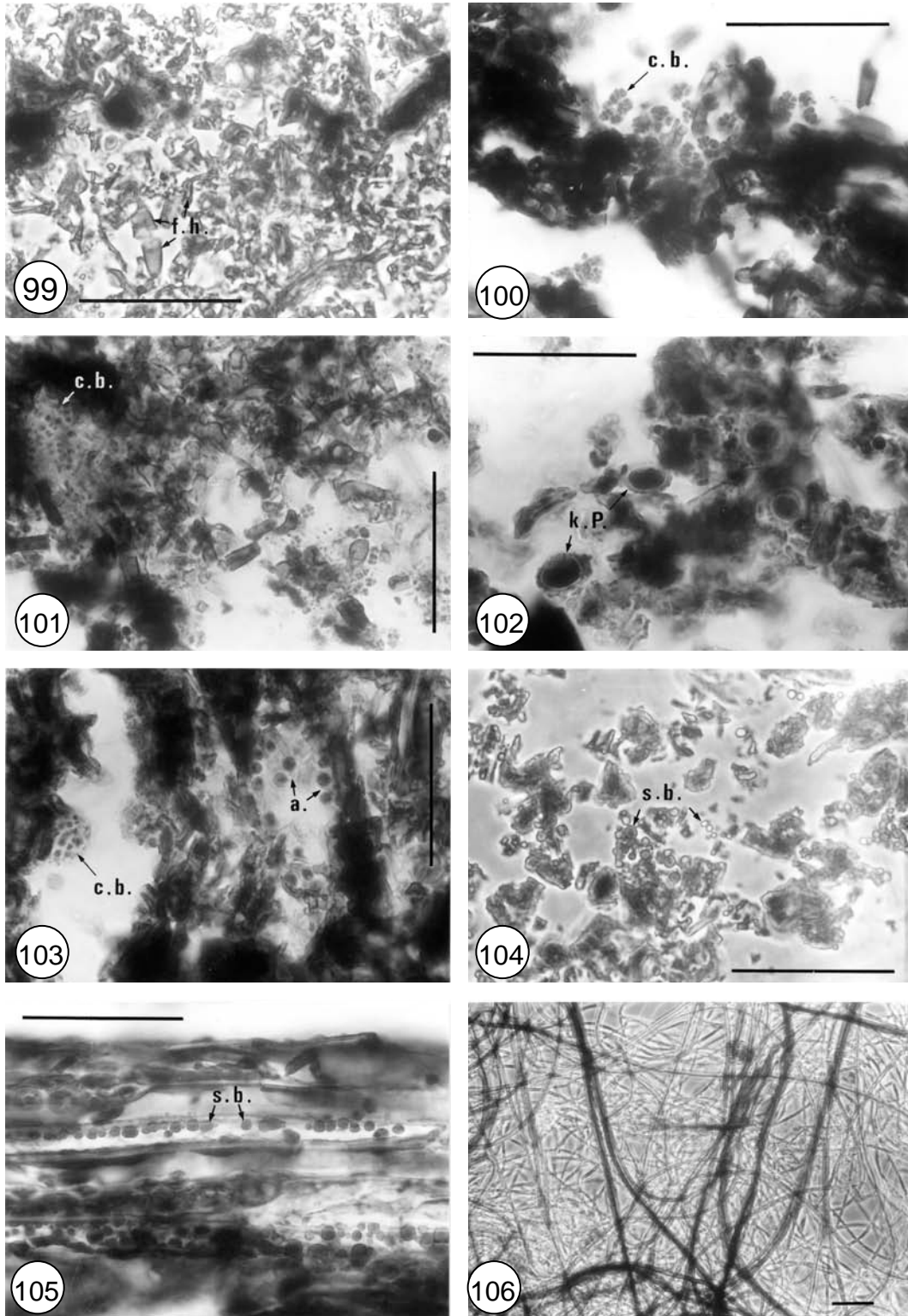


Fig. 99: Needle penetrated by enchytraeids, section through enchytraeid faeces showing their content (CP); Fig. 100: Idem, with bacterial colonies (BMSL); Fig. 101: Idem, showing another type of bacterial colonies (BMSL); Fig. 102: Idem, section showing protozoan cysts at the inside of a needle (BMSL); Fig. 103: Idem, section through mesophyll showing micro-algae and bacteria (BMSL); Fig. 104: Idem, showing brown spherules (CP); Fig. 105: Idem, brown spherules in metaxylem (BMSL); Fig. 106: Hyphal network embedding decaying pine needles, overall view (CP)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; f.h. = fungal hyphae; c.b. = bacterial colonies; k.P. = protozoan cysts; a. = micro-algae; s.b. = brown spherules; length of segment = 50 μ m

crystals and had a fuzzy contour, indicating a degraded state. The absence of any bacteria indicated that their degradation could have occurred in animal guts.

Conclusions about decaying pine needles

The degradation of internal tissues of pine needles through the development of *Verticicladium trifidum*, which was initiated in the L₁ layer, further developed in the L₂ layer. However, another saprophagous fungal species was observed (the basidiomycete *Marasmius androsaceus*), a secondary (or tertiary) fungal infection which probably took place during winter or spring. The lignin-free mesophyll completely disappeared under the cellulolytic action of these fungi, and the lignified transfusion tissue was degraded by *Verticicladium trifidum*. The latter species was considered as the main agent of needle blackening, a process taking place on the internal side of hypodermis.

Fauna, in particular enchytraeid worms, which were abundant at the time of sampling, opened needles by nibbling or biting the coarse layers (cuticle, epidermis, hypodermis) which isolated internal tissues from the external environment. Viable propagules of micro-algae and bacteria were carried on the tegument of these animals, and were propagated through their tunnelling activity at the inside of pine needles, thereby initiating a bacterial stage of needle degradation, which was accompanied by protozoan development. In some needles at an advanced stage of decomposition, the disappearance of internal tissues was probably completed within 8-9 months. Bacterial and micro-algal colonies developed both in internal tissues and in the numerous folds of collapsed needles. The shape of these colonies was reminiscent of what had been observed at greater depth in soil micro-aggregates (Arpin et al., 1980).

Decaying needles were covered by a network of melanised hyphae, mostly dead (probably relict from previous winter and spring), and were embedded in the oxalate-rich mycelium of the basidiomycete *Hyphodontia sp.*

Decaying pine twigs

Compared with the L₁ layer, where only tissues with cellulosic cell walls were invaded and degraded by fungi imperfecti, bacterial development was visible in the L₂ layer, even at the inside of metaxylem tracheids (Fig. 110). Previous fungal development was attested by the presence of dead hyaline hyphae, which could not be stained by methyl blue and were perfectly transparent under phase contrast. Tracheid walls exhibited incipient degradation (Fig. 111). Since no basidiomycetous development was observed, the succession of organisms taking place in the decomposition of pine twigs was ascomycetes (imperfect stages) → bacteria.

Dead hyphae of dematiaceous fungi were retrieved in vascular rays (see Figure 34 for living stages found in L₁), and numerous micro-algal colonies were observed in bark, quite similar to those found in L₁ (Fig. 38). Mesofaunal faeces were deposited at the surface of decaying pine twigs: their content was made of bacteria and dead fragmented melanised hyphae (Fig. 112).

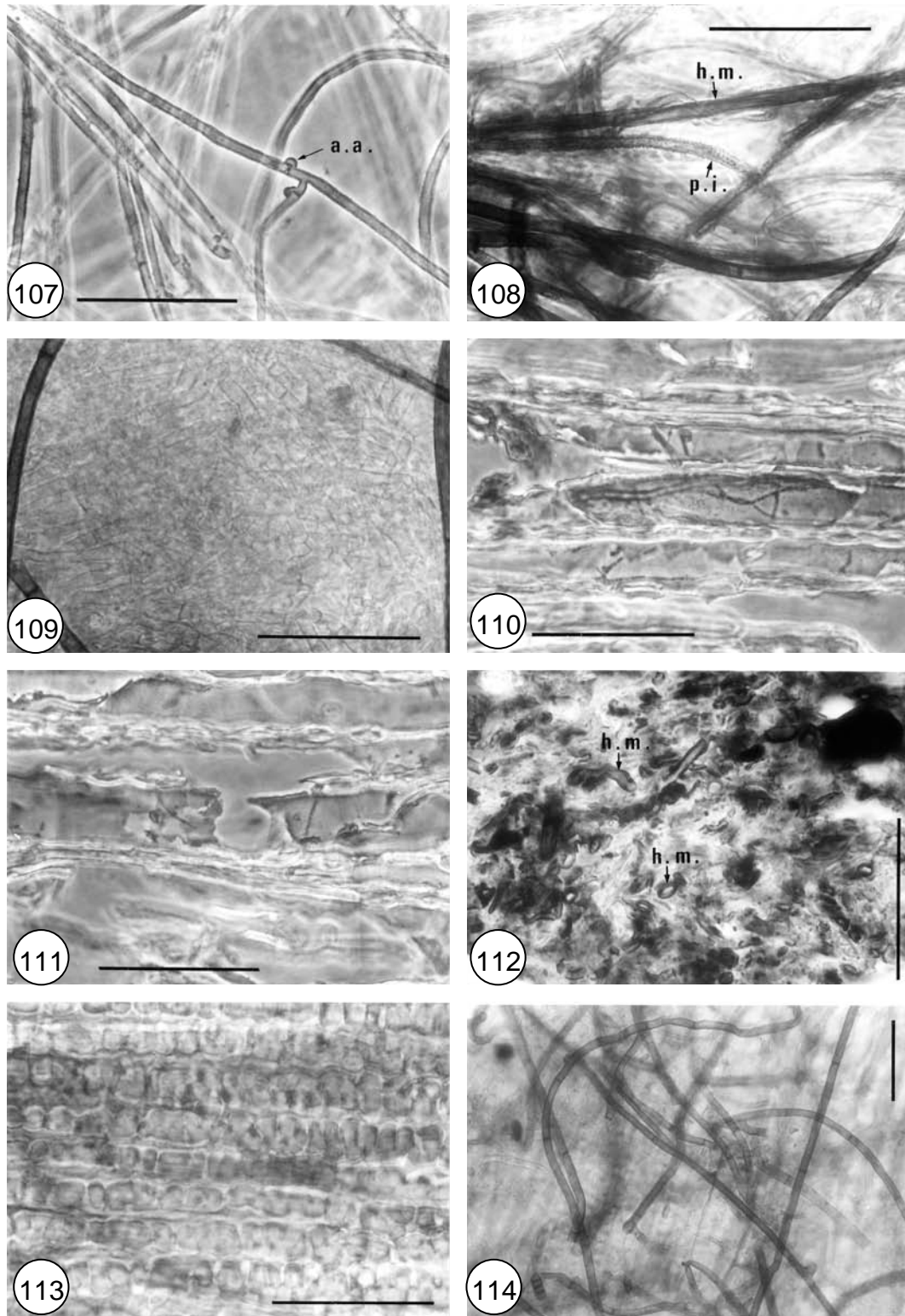


Fig. 107: Hyphal network embedding decaying pine needles, detail showing the secondary mycelium of the basidiomycete *Hyphodontia sp.* (CP); Fig. 108: Idem, detail showing melanic hyphae of the dematiaceous ascomycete *Cenococcum geophilum* mixed with hyaline hyphae of *Hyphodontia sp.* (CP); Fig. 109: Idem, detail showing the content of embedded mesofaunal faeces (CP); Fig. 110: Pine twig, longitudinal section showing metaxylem tracheids, with empty hyphae and bacteria (CP); Fig. 111: Idem, showing degraded tracheid walls (CP); Fig. 112: Idem, showing the content of deposited mesofaunal faeces (BMSL); Fig. 113: Pine euphyll, tangential optical section through epidermis (LN); Fig. 114: Idem, tangential optical section showing a superficial network of melanic hyphae (LN)

CP = phase contrast; LN = natural light without coloration; a.a. = clamp connections; h.m. = melanic hyphae; p.i. = fungal cell walls encrusted with oxalate crystals; length of segment = 50 μ m

Decaying euphylls of pine

Euphylls (flower and needle bracts) of pine were intact, which could be explained by their membranous texture and their recent fall (June). Euphyll epidermis exhibited a typical aspect (Fig. 113), quite different from that of pseudophylls (needles). They were covered by a network of poorly ramified melanised hyphae of 4 µm diameter (Fig. 114), quite similar to those attributed to *Cenococcum geophilum* in the mycelial network embedding needles (Fig. 108). Similarly to needles, pine euphylls were embedded in the mycelium weft formed by *Hyphodontia sp.* and *Cenococcum geophilum*.

Oribatid faeces were deposited at the surface of pine euphylls: their content was made of melanised fungal material and they were embedded in the mycelial network formed by *Hyphodontia sp.*

Decaying male flowers of pine

Bacteria, micro-algae and numerous quartz grains of silt size were observed at the inside of decaying male inflorescences of pine (Fig. 115). This association of silt-size mineral particles, bacteria and micro-algae was reminiscent of the material transported on the tegument of enchytraeids in the L₁ layer (Fig. 45), indicating that these worms penetrated male inflorescences, in which they fed on pollen (Fig. 51). The surface of quartz grains was punctuated with brown dots, which I interpreted as bacterial corpses.

Decaying branchwood of pine

The age of the young branch of pine which was collected in my sample was estimated to five years, taking into account the successive development of early- and latewood. From place to place bark was peeled off and sometimes was lacking. Below bark, external wood was softened to a great extent, being translucent and spotted black. Between bark and wood there was a fine layer of fine organic matter, with numerous enchytraeids, some of which were penetrating bark. This layer was made of enchytraeid faeces, which will be described below with the faunal material.

At the surface of wood, in those places where it was not covered by bark, there was a carpet of conidiophores of *Haplographium delicatum* Berck. & Br. (Fig. 117), which is the conidial (imperfect) stage of the ascomycete *Hyaloscypha dematiicola* (Berck. & Br.). Deeper in the wood, no prominent degradation was visible under the dissecting microscope, except that vessels were easily separated when crashed.

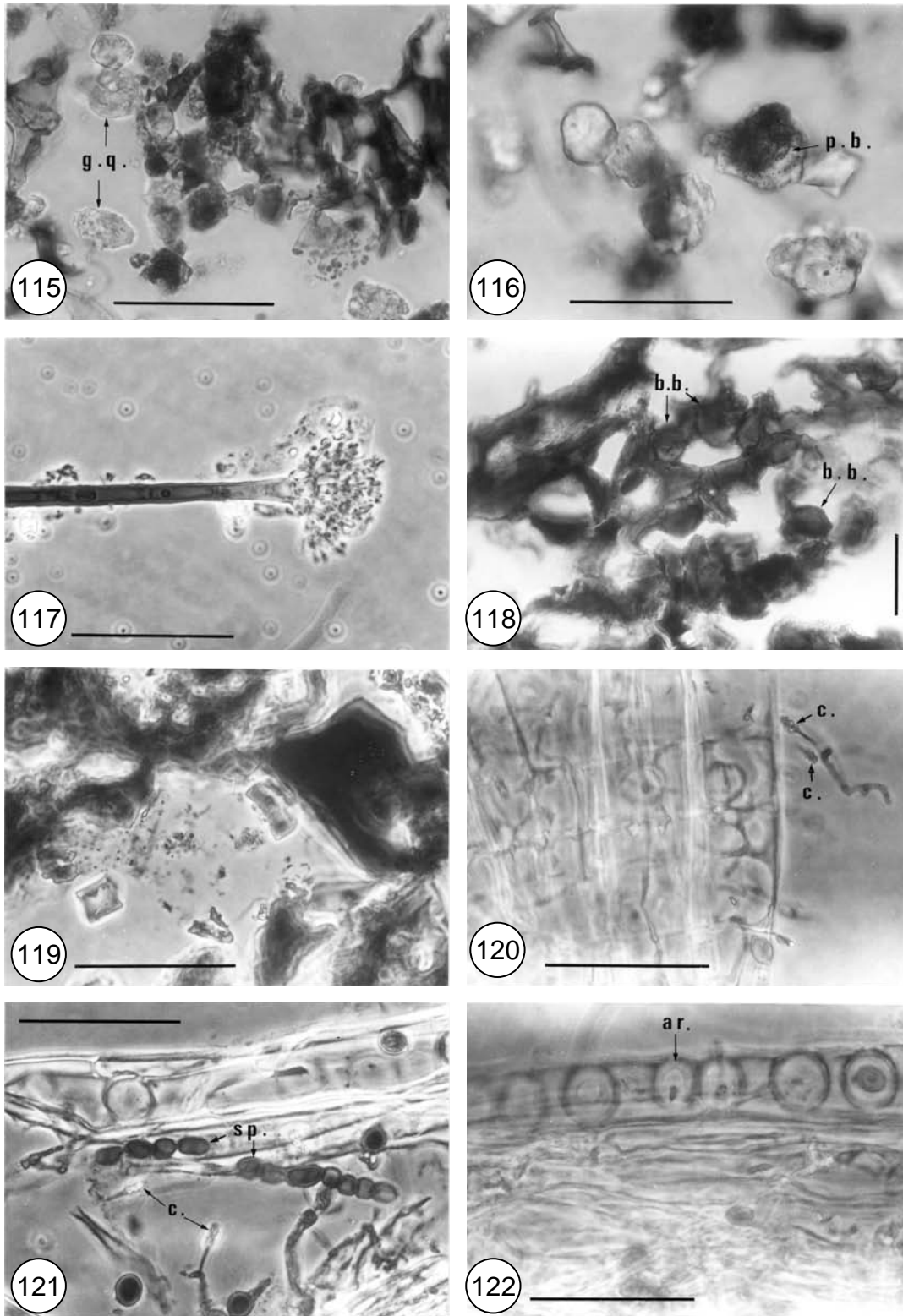


Fig. 115: Male flower of pine, detail showing quartz grains of silt size and bacteria (BMSL); Fig. 116: Idem, detail showing quartz grains with brown dots (BMSL); Fig. 117: Young pine branch, conidiophore of *Haplographium delicatum* (CP); Fig. 118: Idem, section through bark showing decaying cell walls and brown deposits (BMSL); Fig. 119: Idem, section showing bacterial colonies within bark (CP); Fig. 120: Idem, longitudinal section showing the secondary mycelium of *Hyphodontia sp.*, with cystidia, in metaxylem tracheids (CP); Fig. 121: Idem, showing chains of fungal spores and cystidia (CP); Fig. 122: Idem, showing degraded bordered pits (areolae) (CP)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; g.q. = quartz grains; p.b. = brown dots; b.b. = brown deposits; c. = cystidia; sp. = spores; ar. = areolae; length of segment = 50 μ m

Bark

Bark was in a stage of degradation, contrary to what was observed in L₁. Brown deposits, which were described at the inside of peridermic cells in L₁ (Figs. 40, 41 & 42), remained intact and were covered with bacteria (Fig. 118). Numerous micro-sites showed bacterial colonies (Fig. 119).

Wood

The internal part of wood was invaded by the secondary mycelium with clamp connections of *Hyphodontia* sp. (Fig. 120). Its hyphae were thinner (diameter 2 µm) and grew differently from what was observed in the mycelium embedding needles (Fig. 107). Numerous cystidia (short hyphae excreting oxalic acid) of particular size and shape were observed (Figs. 120 & 121), which were used for the identification of this fungus thanks to their taxonomic value at the species-group level. Cystidial types of the same kind have been described in cultures of wood-inhabiting basidiomycetes of the *Odontia* group (Corticaceae) such as *Echinodontium tinctorium* (Ell. & Ev.) according to Nobles (1965), and *Grandinia farinacea* (Pers.) according to Käärik & Rennerfelt (1957). Based on the particular morphology of its cystidia (lagenocystidia), this fungus was considered as belonging to one of the three species *Hyphodontia arguta* (Fr.) Erikss., *Hyphodontia pallidula* (Bres.) Erikss. or *Hyphodontia alutaria* (Burt) Erikss. (Boidin, personal communication).

Tracheids with bordered pits appeared to be degraded in peripheral branchwood, areolae having enlarged and lost their torus (Fig. 122). In the wood located just under bark, numerous spores, isolated or in chains (Fig. 121), were found, belonging probably to the genus *Scytalidium* Pesante (fungi imperfecti). In the zones deprived of bark cover, hyaline hyphae, wide (diameter 7-10 µm) and slightly stained by methyl blue (Fig. 123), were followed and were seen to be connected to conidiophores of *Haplographium delicatum*, thereby allowing identification of the mycelium. In enchytraeid faeces deposited between bark and wood, I found hyaline hyphae still living at sampling time, growing apices of which being strongly stained by methyl blue (Fig. 124). The pattern of fungal colonisation observed in the peripheral zone of branchwood was interpreted as a succession from ascomycetes (fungi imperfecti) to basidiomycetes, the latter (*Hyphodontia*) being able to penetrate more deeply the wood, and one of the ascomycetes continuing its growth in the mass of enchytraeid faeces accumulated between bark and wood. Such sharing of space and time by several fungal species colonizing the same pine branch was reminiscent of observations done by Butcher (1968) on *Pinus radiata* D. Don., i.e. superficial colonisation by several ascomycetes, followed a long time after by basidiomycetes penetrating deeper at the inside. More complicated patterns of succession were observed by Mangelot (1952) and Meredith (1959, 1960) when tree trunks were previously attacked by fungal parasites when still living. In their scheme first colonisers of fallen trunks were parasites, which continued their growth in dead wood, as I observed in some pine needles infected on the tree before decomposing in litter, then secondary and tertiary colonisers. If primary colonisation by parasites is skipped then a similar pattern emerges, with fungi imperfecti fructifying in places from which bark has been peeled off (Mangelot, 1952), followed by a wave of saprophagous basidiomycetes eliminating previous heartwood dwellers through antagonisms (Meredith, 1960).

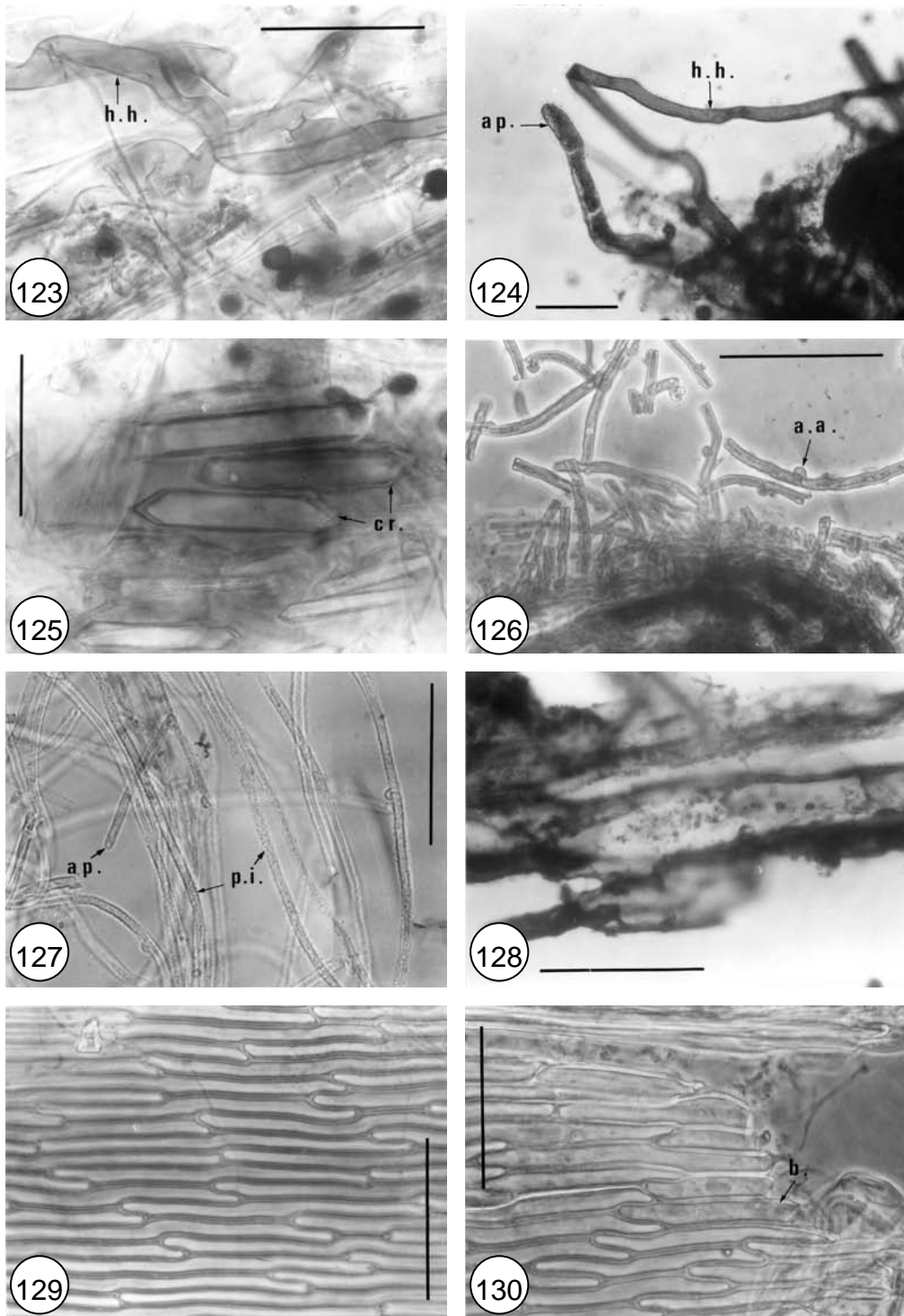


Fig. 123: Young pine branch, detail showing dead hyaline hyphae of *Haplographium delicatum* (BMSL); Fig. 124: Idem, detail showing intact hyaline hyphae with growing apices in enchytraeid faeces deposited between bark and wood (BMSL); Fig. 125: Idem, detail showing resin with crystals (LN); Fig. 126: Mycorrhiza, transversal section through fungal sheath and extra-matrical mycelium (CP); Fig. 127: Idem, detail of the extra-matrical mycelium, showing hyphae encrusted with oxalate crystals and smooth growing apices (BMSL); Fig. 128: Stem of the moss *Pseudoscleropodium purum*, longitudinal section through parenchyma, showing dead cells filled with micro-algae and bacteria (BMSL); Fig. 129: Leaf of the moss *Pseudoscleropodium purum*, showing intact cell walls (CP); Fig. 130: Idem, showing cells with bacterial development

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; LN = natural light without coloration; h.h. = hyaline hyphae; ap. = apices; cr. = crystals; a.a. = clamp connections; p.i. = fungal cell walls encrusted with oxalate crystals; length of segment = 50 μ m

It must be noticed, too, that resin was abundant in the peripheral zone of the studied branch, with rhombohedral crystals of unknown origin clearly visible in it (Fig. 125). Resin was accumulated between bark and wood, where it seemed to have been actively consumed by enchytraeids then retrieved in their faeces, as it will be described below.

Pine ectomycorrhizae

The fine root system of pine seemed near entirely mycorrhizal in the studied sample. Fine roots were not very abundant in the L₂ layer, since observation of the humus profile to the naked eye revealed fine roots mostly in F₂ and H₁ (Table 1). However, they were far from negligible in this very superficial layer. According to Dominik (1969), most mycorrhizae found in the L₂ layer could be classified in the A_e type, i.e. ectotrophic mycorrhizae (ectomycorrhizae) with a diffuse woolly extra-matrical mycelium, a prosenchymatous fungal sheath and a Hartig net.

The extra-matrical mycelium was constituted by a network of hyaline hyphae with clamp connections, morphologically identical to those observed embedding needles and euphylls, except that their cell walls were smooth (Fig. 126). In more remote parts of the extra-matrical mycelium, hyphae appeared encrusted with oxalate crystals, only growing apices being smooth (Fig. 127).

At this stage of the present study, a question arose about the nature of the fungi which embedded most plant remains: were they mycorrhizal or not? The answer was given while studying the underlying F₁ layer, as will be explained below.

Decaying moss (*Pseudoscleropodium purum*)

Stems

Cells of moss stems were invaded by micro-algae and bacteria (Fig. 128), and cell walls seemed degraded. These observations did not cope with the study done by Kilbertus et al. (1970) on the same species and in quite similar environmental conditions. These authors described cells with intact walls, however according to their photographs it seems that they studied thick-walled cells belonging to a supporting tissue similar to collenchyma rather than parenchymatous cells with thin walls, like I did it.

Leaves

When moss leaves were not torn internal attacks by microorganisms were never observed: cells were devoid of cytoplasm but walls were intact, middle lamella comprised (Fig. 129). When leaves were wounded, most probably by enchytraeids which abounded in moss material, then an intense bacterial

development (cocci) was observed to occur at the inside of broken cells, filling them near entirely (Fig. 130).

Most often moss leaves were embedded in the secondary mycelium of *Hyphodontia sp.*, which was frequently associated with that of another fungus, with clamp connections and smooth hyphae of a bigger (10 µm) diameter with thicker walls (Fig. 131).

Compared with the L₁ layer, it is worth to notice the disappearance of the sterile dematiaceous mycelia crawling at the surface of moss leaves, and its replacement by a hyaline woolly basidiomycetous mycelium, without any direct contact with the surface of leaves. These observations are in agreement with those of Kilbertus (1968a, b) and Kilbertus et al. (1970) on decaying *Pseudoscleropodium purum*. The white basidiomycete they described, hyphae of which were encrusted with oxalate crystals, is strongly reminiscent of the mycelium here described embedding mycorrhizae and permeating decaying litter. As in the present study, they thought it to be mycorrhizal. In addition, Kilbertus (1972) showed that there was a correlation between the abundance of *Pseudoscleropodium purum* and that of pine mycorrhizae in the top few centimetres, pointing on a possible influence of the moss on the process of mycorrhization, with the fungus as mediator.

Another question concerns the disappearance in L₂ of the dematiaceous mycelium which was observed at the surface of moss leaves in L₁ (Fig. 44). Given the amount of melanised hyphae which were found in faeces and guts of animals (oribatid mites, enchytraeids, fly larvae), it could be hypothesized that this fungus material was highly palatable to and was heavily grazed by small litter invertebrates. However, melanised hyphae were still visible at the surface of pine needles in L₂, although in a denser network where the visible impact of grazing was more limited than in L₁.

Decaying bracken foliage (*Pteridium aquilinum*)

Leaflets

Leaflets at several stages of decomposition were found, of which only two contrasted stages will be presented in the following.

- Leaflets with limb still present

To the exclusion of veins, only the upper epidermis was still present. Typical articulated epidermal hairs were observed (Fig. 132). Epidermal cells were invaded by hyaline hyphae (Fig. 133), as well as stomatal guard cells (Fig. 134). Similar to pine needles and moss shoots, bracken leaflets were embedded in the mixed mycelium formed by hyaline hyphae with clamp connections and oxalate crystals of the basidiomycete *Hyphodontia sp.* and by melanised hyphae of the ascomycete *Cenococcum geophilum* (Fig. 135). Previous fungal development (maybe before bracken fronds fell on the ground) was attested by the presence of empty fructifications (thyriothecia) of ascomycetous

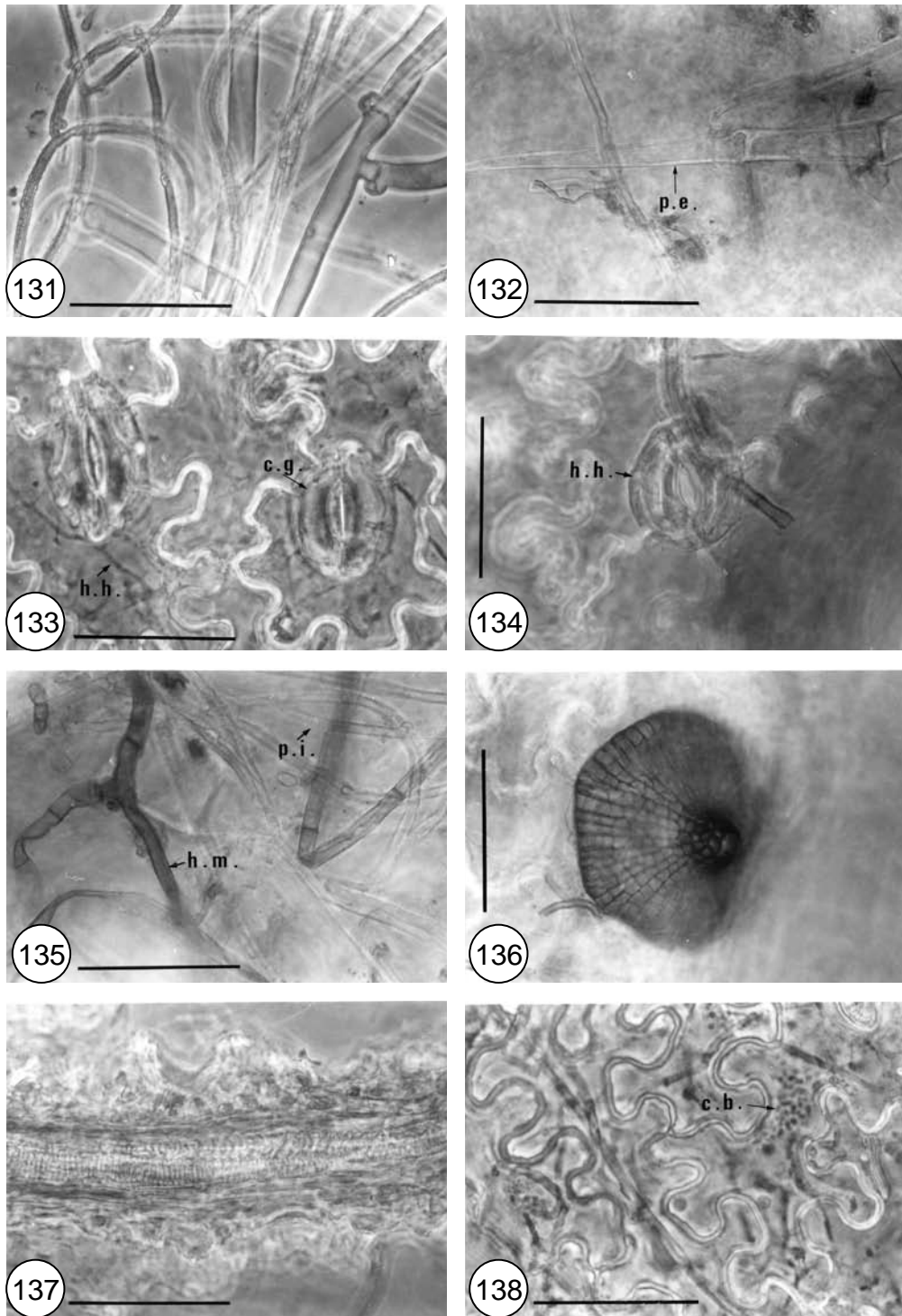


Fig. 131: Mycelium embedding leafy shoots of the moss *Pseudoscleropodium purum*, showing two kinds of basidiomycetous hyphae with clamp connections (CP); Fig. 132: Decaying leaflets of bracken, *Pteridium aquilinum*, detail showing an articulated epidermal hair (CP); Fig. 133: Idem, detail showing hyaline hyphae in epidermal cells (CP); Fig. 134: Idem, detail showing hyaline hyphae in stomatal guard cells (CP); Fig. 135: Idem, detail showing hyaline and melanised hyphae embedding foliar material (CP); Fig. 136: Idem, detail showing a thyriothecium of Microthyriaceae on the limb surface (CP); Fig. 137: Idem, detail showing a limb part nibbled by fauna, leaving intact a vein (CP); Fig. 138: Idem, detail showing a bacterial colony and fungal hyphae in epidermal cells (CP)

CP = phase contrast; p.e. = epidermal hairs; c.g. = guard cells; h.h. = hyaline hyphae; h.m. = melanised hyphae; p.i. = fungal cell walls with oxalate crystals; c.b. = bacterial colonies; length of segment = 50 µm

Microthyriaceae (Fig. 136). Faeces of oribatid mites were also observed, containing only fungal material.

- Vein networks

At this stage of bracken leaflet degradation, only networks of veins were still visible. Traces of nibbling by small invertebrates were present on both sides of remaining veins (Fig. 137). Enchytraeids were thought responsible for the disappearance of bracken limbs, given that their round-shaped, fuzzy-contoured faeces were found agglomerating at the surface of leaflets, their content asserting they had consumed bracken litter (see below).

Some small areas of upper epidermis, which were still present at this advanced stage of degradation, showed a bacterial development at the inside of epidermal cells (Fig. 138). Fungal loculae (pycnidia, ascocarps?) were found still adhering at the surface of intact veins or included in the dense fungal mycelium which replaced degraded bracken material (Fig. 139).

Rachises

The material present in the L₂ sample corresponded to secondary or tertiary rachises of *Pteridium aquilinum*, and not to primary rachises (bracken petioles). On the same fragment, several stages of decomposition could be found in the parenchymatous tissue. In some places, cell walls were still intact but the cytoplasm was invaded by the secondary mycelium with smooth walls of an unidentified basidiomycete (Fig. 140). In other places parenchymatous cells were degraded and invaded by rod-shaped bacteria (Fig. 141) or cocci (Fig. 142). Scalariform tracheids remained intact, even though they were invaded by hyphae of the same unidentified basidiomycete (Fig. 143). The examination of enchytraeid faeces which had been deposited at the surface of a bark fragment revealed the same bracken tracheids (Fig. 144) thereby indicating that enchytraeids consumed internal tissues of bracken rachises.

Comparison with results of other authors was difficult, given the scarcity of studies dealing with the decomposition of bracken foliage. Frankland (1966, 1969) studied fungal successions over two years of decomposition of bracken petioles and she showed that fauna intervened occasionally but not before the second year. In the present study I observed a much more rapid development of the decay process, the passage to a bacterial stage and concomitant ingestion by fauna (enchytraeids) occurring around six months after the fall of dead foliage. However, it must be noticed that the bracken frond ramifications I studied were thinner than petioles.

Fauna

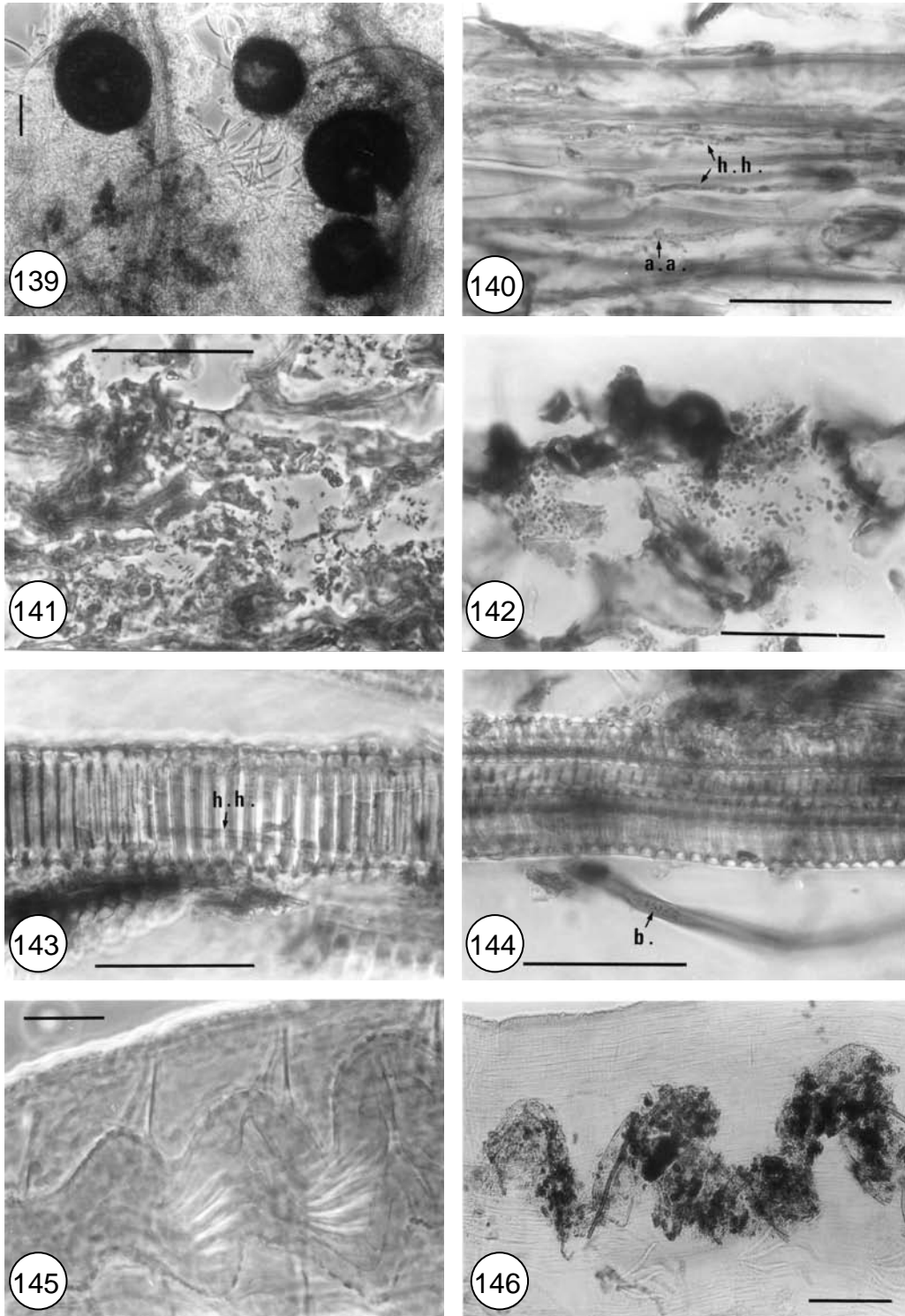


Fig. 139: Decaying leaflets of bracken, *Pteridium aquilinum*, detail showing thyriothecia of Microthyriaceae embedded in the secondary mycelium of *Hyphodontia* sp. (CP); Fig. 140: Decaying rachis of bracken, *Pteridium aquilinum*, longitudinal section showing parenchymatous cells with hyaline hyphae (BMSL); Fig. 141: Idem, detail showing rod-shaped bacteria (CP); Fig. 142: Idem, detail showing coccoid bacteria (BMSL); Fig. 143: Idem, detail showing a scalariform tracheid with hyaline hyphae at the inside (BMSL); Fig. 144: Similar scalariform tracheid in an enchytraeid excrement, with a melانىsed hypha invaded by bacteria (BMSL); Fig. 145: Enchytraeid worm observed by transparency, showing the zigzag shape of its empty intestine (CP); Fig. 146: Idem, overall view of gut contents observed by transparency (LN)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; LN = natural light without coloration; h.h. = hyaline hyphae; a.a. = clamp connections; b. = bacteria; length of segment = 50 μ m

Inventory

Table 5 shows biovolumes and densities of all animal groups which were found during sorting of L₂ litter. Compared to L₁, the diversity of animal groups was slightly greater, and oribatid mites decreased while enchytraeids increased in abundance. While the total biovolume of oribatids decreased by a factor of 10, that of enchytraeids increased by the same factor. Some care is requested when comparing data for springtails (Collembola), because a single specimen of *Pogonognathellus flavescens* occupied the totality of the biovolume of springtails in L₁, while several small species were present in L₂. Among them, two isotomids displayed contrasted distributional patterns between L₁ and L₂: *Parisotoma notabilis*, which was dominant in numbers in L₁, was replaced by *Folsomia manolachei* Bagnall. Fly larvae remained approximately at the same level of biovolume, but Cecidomyiidae, dominant in L₁, were replaced by Sciaridae, and nymphs of these insects appeared in L₂. Several groups of tiny invertebrates were represented by single specimens: tardigrades, pauropods and copepods.

Table 5. Taxonomic name, abundance and estimated biovolume of living animals found in the L₂ layer

Taxonomic groups and species	Abundance	Upper estimate of biovolume (μL)	Lower estimate of biovolume (μL)
Nematodes	21	0.10 10 ⁻¹	0.03 10 ⁻³
Dorylaimidae	8	0.60 10 ⁻²	0.15 10 ⁻²
<i>Anaplectus sp.</i>	6	0.16 10 ⁻²	0.04 10 ⁻²
<i>Tylolaimophorus sp.</i>	4	0.63 10 ⁻³	0.16 10 ⁻³
<i>Prionchulus sp.</i>	2	0.18 10 ⁻²	0.05 10 ⁻²
<i>Clarkus papillatus</i>	1	0.25 10 ⁻³	0.06 10 ⁻³
Enchytraeids	39	2.6	0.7
Tardigrades	1	0.95 10 ⁻³	0.24 10 ⁻³
Pauropods	1	0.27 10 ⁻³	0.07 10 ⁻³
Oribatid mites	12	0.92 10 ⁻¹	0.23 10 ⁻¹
<i>Oppia sp.</i>	4	0.81 10 ⁻²	0.20 10 ⁻²
<i>Platynothrus peltifer</i>	3	0.60 10 ⁻¹	0.15 10 ⁻¹
<i>Nanhermannia nana</i>	2	0.29 10 ⁻²	0.07 10 ⁻²
<i>Adoristes ovatus</i>	1	0.16 10 ⁻¹	0.04 10 ⁻¹
<i>Suctobelba sp.</i>	1	0.97 10 ⁻³	0.24 10 ⁻³
<i>Belboidea sp.</i>	1	0.48 10 ⁻²	0.12 10 ⁻²
Miscellaneous mites	5	0.42 10 ⁻¹	0.11 10 ⁻¹
Gamasina	2	0.34 10 ⁻¹	0.09 10 ⁻¹
Uropodina	1	0.59 10 ⁻²	0.15 10 ⁻²
Tydeidae	1	0.47 10 ⁻³	0.12 10 ⁻³
Acaridida	1	0.17 10 ⁻²	0.04 10 ⁻²
Copepods	1	0.95 10 ⁻³	0.24 10 ⁻³
Springtails	12	0.18 10 ⁻¹	0.04 10 ⁻¹
<i>Folsomia manolachei</i>	7	0.10 10 ⁻¹	0.03 10 ⁻¹
<i>Isotomiella minor</i>	1	0.17 10 ⁻²	0.04 10 ⁻²
<i>Allacma fusca</i>	1	0.20 10 ⁻²	0.05 10 ⁻²
<i>Lepidocyrtus lanuginosus</i>	1	0.10 10 ⁻²	0.03 10 ⁻²
<i>Parisotoma notabilis</i>	1	0.11 10 ⁻²	0.03 10 ⁻²
<i>Proisotoma minima</i>	1	0.20 10 ⁻²	0.05 10 ⁻²
Fly larvae	12	0.33	0.08
Sciaridae	8	0.33	0.08
Cecidomyiidae	3	0.22 10 ⁻²	0.06 10 ⁻²
Chironomidae	1	0.61 10 ⁻²	0.15 10 ⁻²
Fly nymphs	2	0.25	0.06
Sciaridae	2	0.25	0.06

Concerning microfauna, as this has been highlighted above, it should be clear that the present study can give only a truncated view of their importance in the studied litter profile. Despite of these shortcomings, numerous observations were made on cyst amoebae, other protozoa and nematodes. However, given the number of specimens of nematodes collected in L₂ (21) and the easiness with which they could be collected when seen under the dissecting microscope, this group was taken into account in Table 5. The fact that only one specimen of this group was observed in L₁, while 21 were observed with the same method and care in L₂, was indicative of their clear preference for the latter layer.

Enchytraeid worms

Generally speaking the intestines of enchytraeids showed a great variety of contents when observed by transparency in chloral-lactophenol. Their zigzag shape (Figs. 145 & 146) was reminiscent of the regular subdivision of the body into numerous metameres and explained why their faeces were loosely assembled in feeding places where they were deposited. Sometimes, but rarely, only one type of gut content was observed filling the intestine, such as for instance resin (Fig. 147). More generally, gut contents were composite, without any clear dominance of a category. When fungal material was particularly abundant; it was often accompanied by plant fragments (tracheids, for instance) which indicated that the animal had ingested internal tissues of needles or wood, together with their fungal colonisers. The most prominent character of enchytraeid gut contents, compared to other invertebrates, was the apparent absence of selective feeding.

Most hyaline hyphae ingested by enchytraeids corresponded to the secondary mycelium of the basidiomycete *Hyphodontia* sp. which embedded litter debris. These hyphae were often in way to be degraded in enchytraeid intestines (Figs. 148 & 149). Some hyaline hyphae of another type were also observed: their thicker walls did not appear degraded (Fig. 150).

Melanised hyphae of *Cenococcum geophilum* showed intact walls, the contrast with hyaline hyphae being better visible when both items were present in close vicinity (Fig. 151). Some melanised fungal walls were perforated (Fig. 152), resembling figures described for the attack of fungal walls by bacteria (Olah et al., 1978). In this case, it is highly probable that hyphae were in this state of degradation before being ingested. In some other cases melanised hyphae appeared to be more seriously degraded, their walls being made thinner and softer (Fig. 153). This could be due to the action of the animal, however, it may be questioned whether enchytraeids were capable to degrade fungal walls impregnated with melanin at first ingestion. Reisinger (1972, 1978) showed that at least two successive ingestions by the mite *Tyrophagus putrescentiae*, with microbial development in the meantime, were necessary to degrade melanised fungal walls. Other observations showed that some kind of melanised fungal material could be directly degraded by enchytraeids. Several individuals were shown to transport big dematiaceous (internally echinulate) spores of *Sphaeropsis sapinea* (Fr.) (Fig. 154). These spores were retrieved in the intestine, with their walls at several stages of degradation (Figs. 155 & 156). Thus enchytraeids, at least the species here studied, which I presumed to be *Cognettia sphagnetorum* (Vejdovský), had the potential to degrade melanised fungal walls as soon as first ingestion. However, the bulk of observations done on both guts and faeces showed that

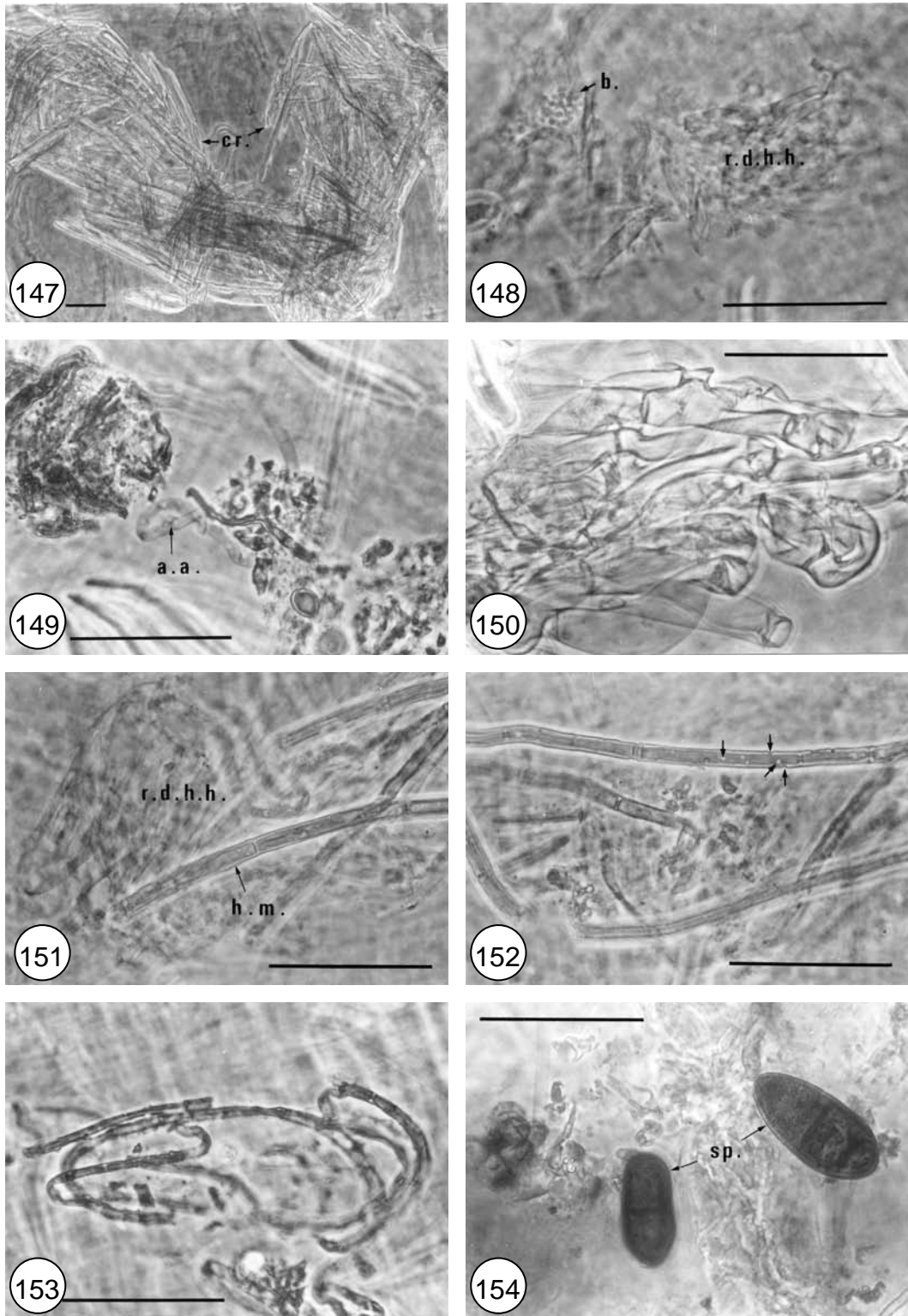


Fig. 147: Enchytraeid worm observed by transparency, gut filled with resin with crystals (CP); Fig. 148: Idem, detail of gut contents showing hyaline and bacteria in way to be degraded (CP); Fig. 149: Idem, basidiomycetous hyaline hyphae in way to be degraded (CP); Fig. 150: Idem, hyaline hyphae with intact walls (CP); Fig. 151: Idem, hyaline hyphae in way to be degraded, melanised hypha with near intact walls (CP); Fig. 152: Idem, melanised hypha with numerous perforations indicated by arrows (CP); Fig. 153: Idem, melanised hyphae in way to be degraded (CP); Fig. 154: Spores of *Sphaeropsis sapinea* on the tegument of an enchytraeid worm (CP)

CP = phase contrast; b. = bacteria; r.d.h.h. = dense networks of hyaline hyphae; a.a. = clamp connections; h.m. = melanised hyphae; sp. = spores; length of segment = 50 μ m

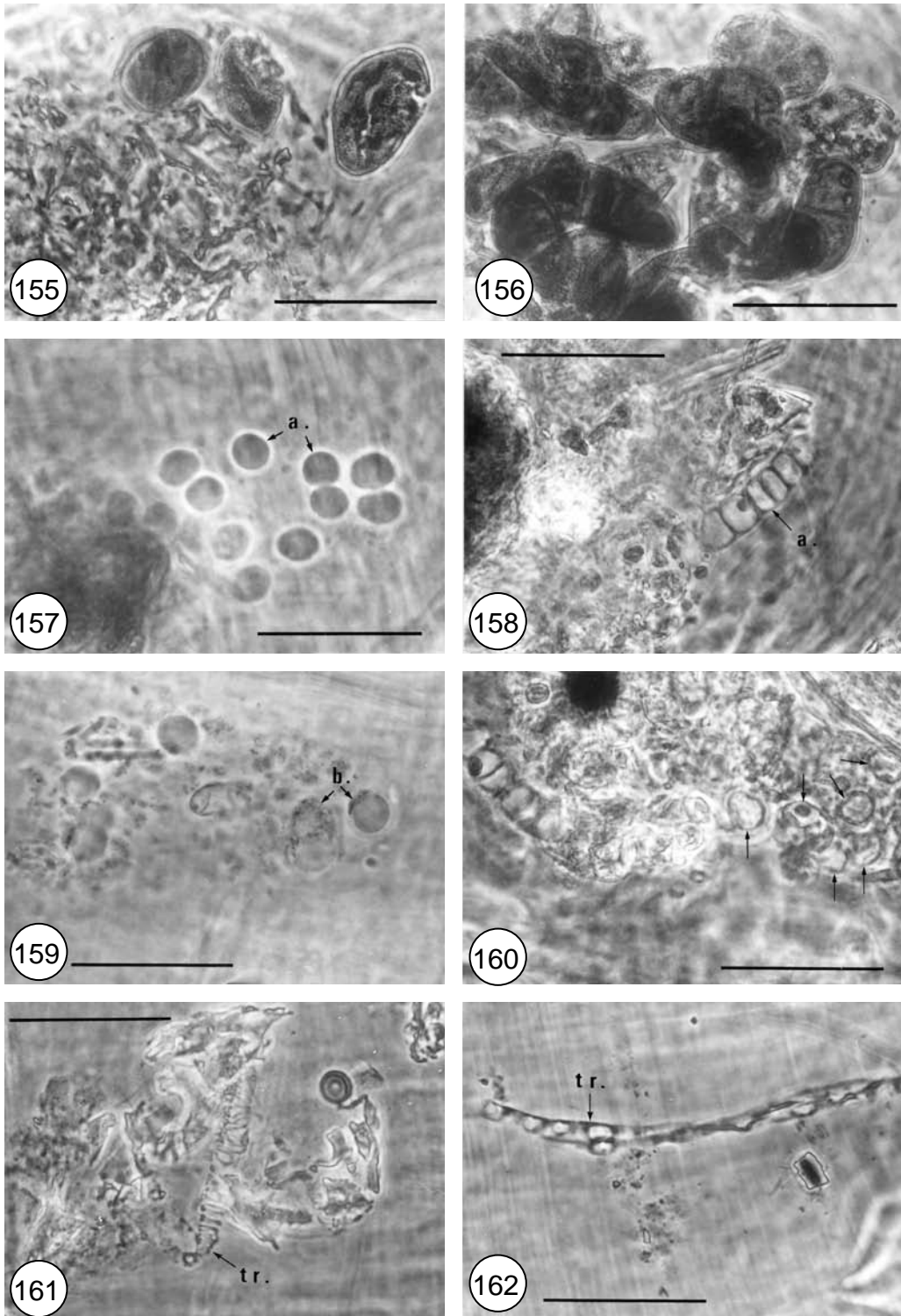


Fig. 155: Spores of *Sphaeropsis sapinea* at the inside of an enchytraeid intestine observed by transparency (CP); Fig. 156: Idem, showing several stages of degradation of spore walls (CP); Fig. 157: Enchytraeid gut contents observed by transparency, detail showing intact unicellular micro-algae (CP); Fig. 158: Idem, filamentous blue-green alga (cyanobacteria) with intact walls (CP); Fig. 159: Idem, unicellular micro-algae at different stages of degradation under the action of intestinal microflora (CP); Fig. 160: Idem, filamentous blue-green alga with dissociated cells indicated by arrows (CP); Fig. 161: Idem, reticulated tracheid and plant cells in way to be degraded (CP); Fig. 162: Idem, metaxylem tracheid in way to be degraded (CP)

CP = phase contrast; a. = algae; b. = bacteria; tr. = tracheid; length of segment = 50 μ m

this was far from being realized being realized during the time of transit through the intestine, in particular concerning hyphae of *Cenococcum geophilum*.

Algae were commonly observed in enchytraeid intestines, for instance unicellular Chlorophyceae (Fig. 157), quite similar to those found proliferating in oribatid faeces in L₁ (Fig. 71), or filamentous blue-green algae (Cyanophyceae), now called cyanobacteria (Fig. 158). In the first case, algal cells were often seen to be viable: they were turgescient, with their cytoplasm exhibiting opacity in phase contrast. Such features were never observed with fungal material, only walls of which appeared intact, at best. Algal cells were observed to be attacked by some bacteria from intestinal microflora: bacteria were seen agglutinated around algal cells, which became collapsed and devoid of cytoplasm. Several stages of algal degradation are visible on Figure 159. Filamentous blue-green algae (cyanobacteria) exhibited also some signs of degradation: cells were emptied and cells of the same file were separated, keeping intact their rigid cellulosic wall (Fig. 160). All these figures indicated that algae were somewhat hard to become digested, compared to fungal material which was more easily degraded. Studies in transmission electron microscopy by Kilbertus & Vannier (1979) showed that algae may pass intact through collembolan intestines.

Enchytraeid worms ingested needle plant tissues. Cells were always comminuted (Fig. 161). Recognizable cells were only those of protoxylem (Fig. 161) and metaxylem (Fig. 162), although they exhibited severe deformations. It is highly probable that the degradation of plant cell walls, which had been performed in a first stage by fungi and bacteria, reached a further stage in enchytraeid intestines. Resin, which abounds between bark and wood in fallen branches, was ingested, too. Crystals were found included in the ingested resin, which was found either as an isolated piece (Fig. 163) or completely filling the intestine (Fig. 147). Doubts may be raised about the nutritional value of pine resin. Maybe it provided the worms with some oligo-elements? The abundance of this food item in enchytraeid guts, often seen filling the whole intestine, may indicate it to be a preferred food resource. Another argument is that, compared to dead moss material which was dominant in the environment but was neglected by these animals in L₂ (but see above for living moss in L₁), resin was scarcely represented in the environment while voraciously consumed.

Another item which was frequently consumed by enchytraeids in L₂ was invertebrate faeces, most probably of microarthropods according to the small size of ingested masses (Fig. 164). These faeces exhibited numerous bacteria which were in way to be degraded (fuzzy contours in phase contrast) in enchytraeid intestines. Fragments of melanised hyphae, in an advanced stage of degradation, were also observed in ingested faeces.

Four other components of the enchytraeid food regime must be noted, too, because their abundance was far from negligible although they were not so much frequently encountered as the abovementioned items. Those were cysts of *Thecamoeba*, such as for instance *Trinema* spp., always in way to be degraded (Fig. 165), corpses or exuviae of microarthropods of which only setae were still intact (Fig. 166), quartz grains (Fig. 167) and pollen grains, most often of pine (Fig. 168) but also of other plant species growing in the study site such as the grass *Molinia caerulea* (L.) Moench (Fig. 169).

Intestinal microflora was richer and more diverse than in any other enchytraeid worm found in L₁. It was observed in all individuals I scrutinized, and it was distributed in all intestinal metameres. In addition to the minute bacteria already photographed surrounding algae (Fig. 159), swarms of slightly curved rod-shaped bacteria (Fig. 170) and cocci (Fig. 171) were observed. Several bacterial types

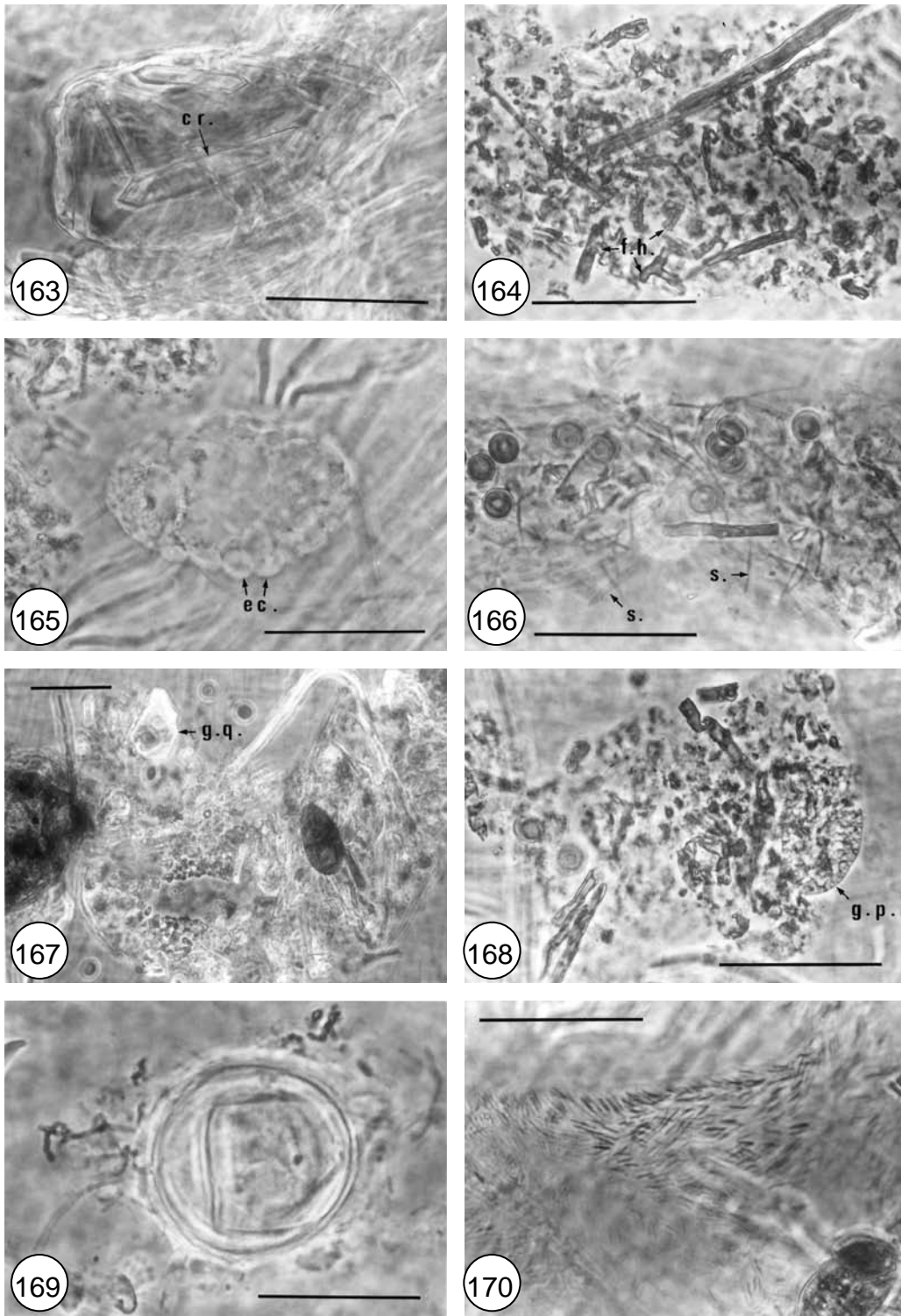


Fig. 163: Enchytraeid gut contents observed by transparency, detail showing a piece of resin with crystals (CP); Fig. 164: Idem, microarthropod faeces (CP); Fig. 165: Idem, cyst of *Trinema* sp. (cyst amoeba) in way to be degraded (CP); Fig. 166: Idem, microarthropod corpse or exuvia, in way to be degraded, and fungal spores (CP); Fig. 167: Idem, composite gut contents with quartz grains (CP); Fig. 168: Idem, fungal material, bacteria, fragment of pine pollen (CP); Fig. 169: Idem, pollen grain of *Molinia caerulea* with torn exine, fungal hyphae and bacteria in way to be degraded (CP); Fig. 170: Idem, intestinal microflora in the absence of food bolus (CP)

CP = phase contrast; cr. = crystal; f.h. = fragments of hyphae; ec. = scales; s. = setae; g.q. = quartz grains; g.p. = pollen grains; length of segment = 50 μ m

could be present in the same intestine, but never in the same metamere. Direct contact of intestinal microflora with the food bolus was exceptional (Fig. 159). Customarily, bacteria of intestinal microflora were seen as swarms surrounding the food bolus, or dispersed in the intestinal lumen when devoid of food, as photographed here.

At first sight the observed enchytraeids did not seem to select food resources. However, the absence of moss material and the abundance of resin let us think that they exhibited preferences. Such a phenomenon has been evidenced in Collembola by McMillan (1975).

Oribatid mites

Oribatid mites, which were the dominant invertebrate group in L₁, were replaced by enchytraeids in L₂. In this layer, dead oribatids (10 specimens) were near as abundant as living individuals (12 specimens), indicating that L₂ had been abandoned to the benefit of more superficial litter which was made of living moss and freshly fallen needles. At the inside of the oribatid group, it must be noted that big-sized species regressed in number, to the exception of *Nanhermannia nana* (Nicolet) which was absent from L₁ (Table 5), but small-sized species (mainly *Oppia* spp.) remained at the same level of abundance. Reasons for this apparent shift of oribatid species to more superficial litter were unclear, given that sampling took place during a dry and warm period (late August). Three non-conflicting hypotheses were raised to explain it, (i) intolerance of oribatid mites to the intense activity of enchytraeids in L₂, forcing them to live in the upper horizon L₁ where more drought-sensitive enchytraeids were at their disadvantage, (ii) intolerance to growing basidiomycetous mycelia (maybe causing a physical obstacle to foraging) or (iii) food preferences. Observations on the food bolus of oribatid mites seemed to sustain the third hypothesis, although the low number of non-starving individuals did not allow me to conclude neatly on that point.

Platynothis peltifer showed the same intestinal bacteria as in L₁ (Fig. 61), in swarms in close proximity to food boluses which were made of strongly transformed fungal material (Fig. 172). Other views showed even more strongly decayed fungal material where only some hyphal fragments were still recognizable (Fig. 173). In all cases, bacterial development was noticeable. By comparison with L₁, it can be hypothesized that *Platynothis peltifer* ingested faeces in L₂, most probably of oribatids given the small size of hyphal fragments found in them. Coprophagy had not been suspected in L₁.

All individuals of the genus *Oppia* which were not starving showed the same gut contents (Fig. 174): fragments of hyphae of *Hyphodontia* sp., with similar hyphal diameter and oxalate crystals as in the mycelium commonly embedding litter debris in L₂. Several stages of degradation of fungal walls were observed to occur at the inside of the intestine: first oxalate crystals were lost, and then fungal walls took fuzzy contours. The same basidiomycetous mycelium was never seen to be consumed by *Platynothis peltifer*. My hypothesis was that *Platynothis peltifer*, contrary to *Oppia* spp., did not find its preferred food in L₂ and rather ingested its own faeces which had been deposited some time before when L₂ was still a L₁ layer. Although numerous studies were performed on food habits of *Platynothis peltifer* (Hartenstein 1962a, b; Littlewood, 1969; Pande & Berthet, 1973; Anderson, 1975; Behan-Pelletier & Hill, 1983), these authors did not determine the stage of degradation of the ingested material, thus no definite conclusion can be reached about food preferences of this species.

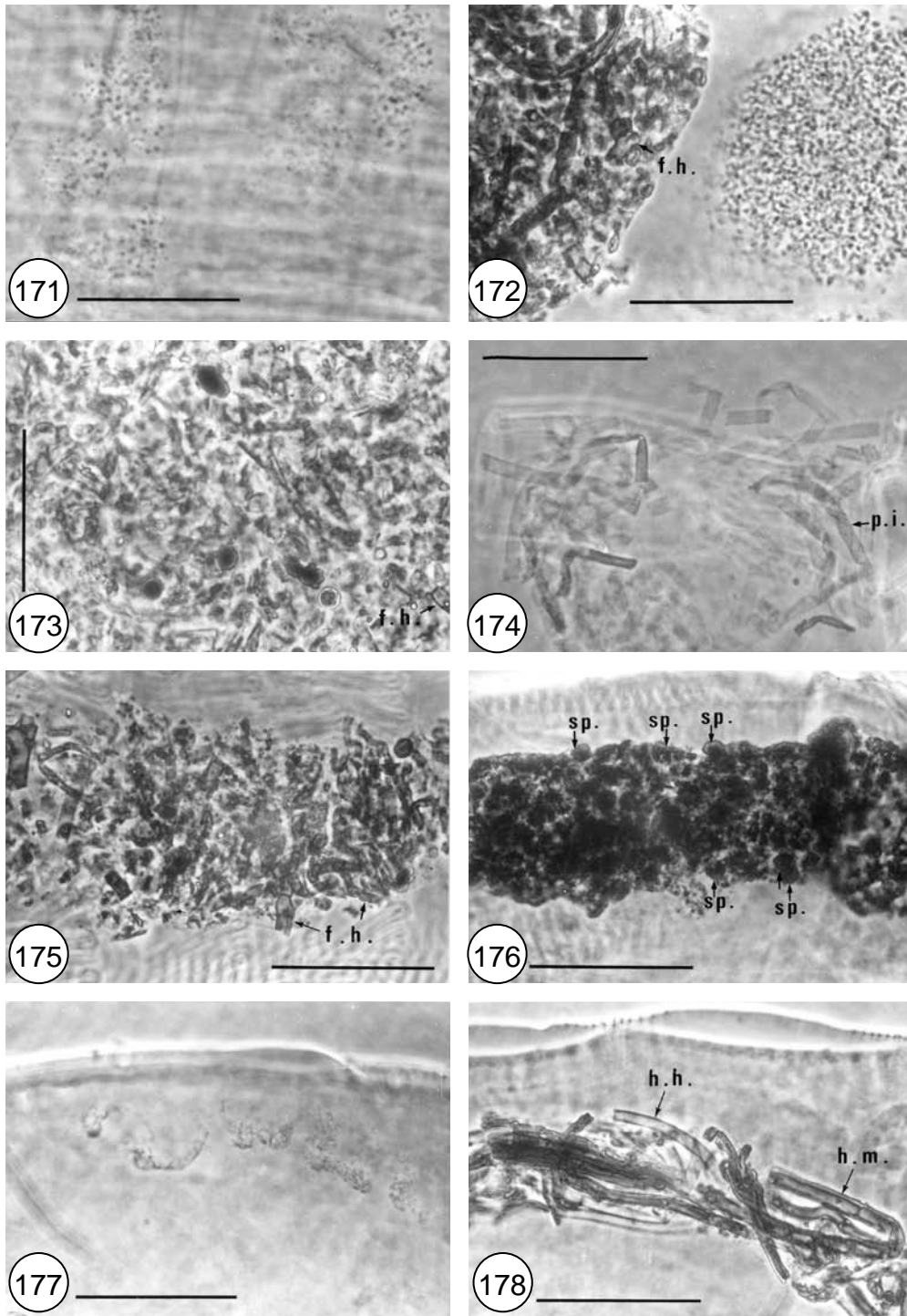


Fig. 171: Enchytraeid gut contents observed by transparency, intestinal microflora in the absence of food bolus (CP); Fig. 172: Gut contents of the oribatid mite *Platynocheilus peltifer* observed after dissection, detail showing intestinal microflora (right side) and food bolus (left side) made of decaying fungal material (CP); Fig. 173: Idem, detail showing strongly transformed fungal material and bacteria (CP); Fig. 174: Gut contents of the oribatid mite *Oppia* sp. observed after dissection, detail showing hyphae of the basidiomycete *Hyphodontia* sp. at different stages of degradation of cell walls and disappearance of oxalate crystals (CP); Fig. 175: Gut contents of the isotomid collembolan *Folsomia manolachei* observed by transparency, detail showing strongly decayed melanised fungal material and bacteria (CP); Fig. 176: Idem, detail showing strongly decayed and compacted melanised fungal spores (CP); Fig. 177: Gut contents of sciarid fly larvae observed by transparency, detail showing intestinal microflora (CP); Fig. 178: Idem, detail showing hyaline and melanised fungal hyphae, with intact walls (CP)

CP = phase contrast; f.h. = fragmented fungal hyphae; p.i. = fungal cell walls encrusted with oxalate crystals; sp. = spores; h.h. = hyaline hyphae; h.m. = melanised hyphae; length of segment = 50 µm

Springtails

Contrary to oribatid mites, Collembola were more numerous and species-rich in L₂ (12 specimens, 6 species) than in L₁ (9 specimens, 2 species). As in L₁, Isotomidae were dominant, *Parisotoma notabilis* being nevertheless replaced by *Folsomia manolachei*. As was observed in L₁, gut contents of isotomid springtails were mostly made of strongly degraded fungal material and bacteria, several stages of degradation being observed (Figs. 175 & 176). The latter photograph, displaying dematiaceous fungal spores compacted and in an advanced stage of transformation, is strongly reminiscent of what has been described as 'humus' or 'amorphous organic matter' by several authors (Knight & Angel, 1967; Bødvarsson, 1970; Gilmore & Raffensperger, 1970; McMillan & Healey, 1971; Hågvar & Kjøndal, 1981). My hypothesis, based on the great abundance of bacteria included in the food bolus, is that animal faeces were ingested, more particularly those of oribatid mites given the fungal nature of the food bolus and the small size of hyphal fragments. This was confirmed, as will be seen below, by the study of animal faeces found in L₂. Intestinal microflora was never observed in all collembolan specimens studied.

Fly larvae

The abundance of fly larvae was higher in L₂ (12 specimens) than in L₁ (7 specimens), but Cecidomyiidae were replaced by Sciaridae, nymphs of which were also seen, in addition to larvae. Chironomidae remained at a very low level of abundance, as in L₁.

Sciarid larvae possessed an intestinal microflora (Fig. 177). Contrary to what had been observed in L₁ (Figs. 65 & 66), only fungal material was ingested in L₂. As for enchytraeids, dead moss material, cells of which were devoid of cytoplasm, was totally neglected. Fungal hyphae kept their walls intact, whether melanised (Fig. 178) or hyaline such as those of *Hyphodontia* sp. (Fig. 179). The absence of any degradation of hyaline basidiomycetous hyphae in sciarid intestines was exceptional and was not registered in any other invertebrate group I studied in this sample. Plant material was never observed in sciarid guts.

Cecidomyiidae larvae, as was observed in L₁, did not possess any solid matter in their digestive tract. Liquid food can be suspected, as was hypothesized by Healey & Russel-Smith (1971) on the base of the anatomy of mouth pieces. However these animals transported numerous propagules on their tegument, probably viable according to phase contrast, for instance bacteria (Fig. 180) and microalgae (Fig. 181). The transport of microbial propagules adhering on a sticky (probably mucous) tegument is a character cecidomyiid larvae had in common with enchytraeids.

Gut contents of Chironomidae were made of strongly degraded fungal material, mixed with numerous bacteria (Fig. 182), as this had been observed in isotomid springtails (Figs. 175 & 176). Comparing these results with the observations made in L₁ on larval chironomid guts (Fig. 70) led me to conclude that they ingested animal faeces. However, the direct ingestion of fungal hyphae had been

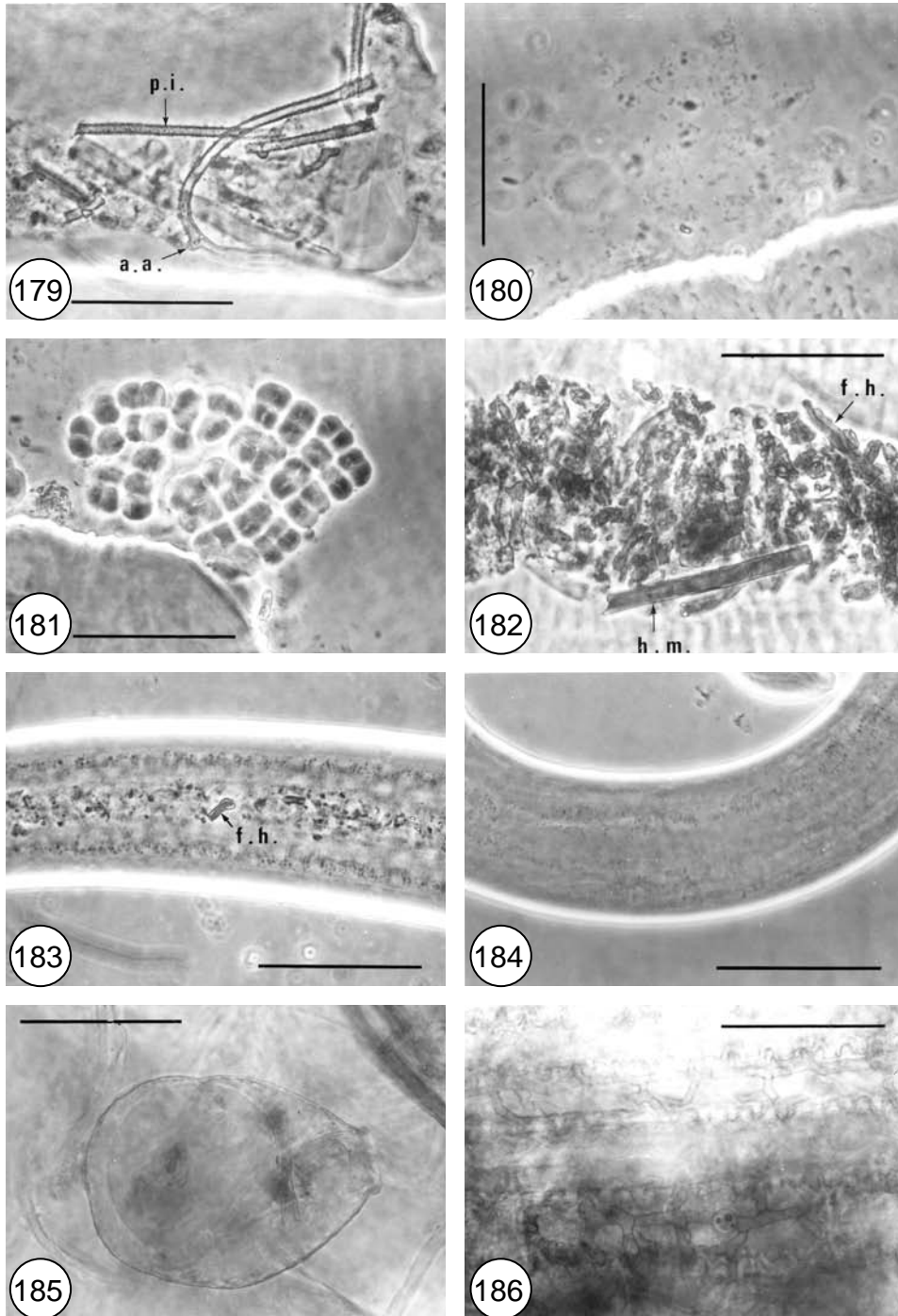


Fig. 179: Gut contents of sciariid fly larvae observed by transparency, detail showing hyphae of *Hyphodontia* sp., with intact walls (CP); Fig. 180: Bacteria transported on the tegument of cecidomyid fly larvae (CP); Fig. 181: Archethallus of Chlorophyceae transported on the tegument of cecidomyid fly larvae (CP); Fig. 182: Gut contents of chironomid fly larvae observed by transparency, detail showing strongly decayed fungal material and a melanised hypha with intact walls (CP); Fig. 183: Gut contents of the nematode *Anaplectus* sp. observed by transparency, showing intestinal microflora and small melanised fungal debris (CP); Fig. 184: Gut contents of the nematode *Tylolaimophorus* sp., showing intestinal microflora (CP); Fig. 185: The cyst amoeba *Nebela collaris* on a bracken leaflet of *Pteridium aquilinum* (CP); Fig. 186: Section through a macroinvertebrate excrement, detail showing a fragment of pine needle epidermis with a network of melanised hyphae (LN)

CP = phase contrast; LN = natural light without coloration; p.i. = fungal cell walls encrusted with oxalate crystals; a.a. = clamp connections; h.m. = melanised hyphae; f.h. = fragmented fungal hyphae; length of segment = 50 µm

also observed in L₁ (Fig. 69). Strong differences between sciarid and chironomid larvae both in food regime and impact on fungal walls have been also registered by Healey & Russel-Smith (1971).

Nematodes

Nematodes seem to abound in L₂, at least when compared with L₁ where only one specimen of Monochidae had been collected. A few specimens showed gut contents, if we except very small debris of melanised fungal walls in a female *Anaplectus sp.* (Fig. 183), two mastax of rotifers (not shown, but see Fig. 58) in a larva of *Clarkus papillatus* (Bastian) and a bacterial mass in way to be degraded (not shown, but see Figs. 58-60) in a larva of *Dorylaimus sp.* However, intestinal microflora was always visible, applied to the intestinal epithelium, in *Anaplectus sp.* (Fig. 183) and *Tylolaimophorus sp.* (Fig. 184).

Cyst amoebae

Cyst amoebae (Thecamoeba) were observed at the surface of plant material, mostly fern leaflets, when mounted for direct observation in chloral-lactophenol. Needles, which were sectioned for observing internal tissues, did not display cyst amoebae at the inside, but this failure can be ascribed to a methodological bias, as for most microfauna. Given that some sections showed cyst amoebae at the surface of collapsed pine needles (Fig. 94) it can be suspected that these protozoa abounded at the inside of decaying pine material, too. I observed *Nebela collaris* (Fig. 185), numerous *Euglypha sp.*, *Assulina semilunum* (Ehrenberg), *Phryganella acropodia* (Hertwig & Lesser) and *Corythion dubium* Taranek. It should be noticed that not any cyst amoebae had been found in the L₁ layer, to the exception of a specimen of *Nebela collaris* which had been ingested by an enchytraeid (Fig. 55), which indicates, if necessary, that L₂ was preferred to L₁.

Animal faeces

Macroinvertebrate faeces

Some macroinvertebrate faeces had a convoluted contour which was reminiscent of earthworm faeces. However, most of them were tightly appressed against moss leafy shoots or pine needles and their shape was hardly recognizable. In this case they could be allocated as well to millipedes or woodlice. In the absence of clear faunal connection, and waiting for more material found underneath (but see F₁ layer), macroinvertebrate faeces collected in L₂ were not attributed to any animal group. However, it should be noticed that microscopic characters did not differ between them: all of them were comprised of coarse fragments of moss, pine and bracken plant tissues, which were poorly transformed, pointing

to a common zoological origin. Plant cells were in the same state as described in the study of non-ingested decaying plant material and did not seem to be further degraded, for instance epidermis (Figs. 186 & 187), metaxylem tracheids (Fig. 188) or pine pollen grains (Fig. 189). However, some stomatal guard cells were found isolated from the rest of the epidermis (Fig. 190). Other plant tissues have disappeared, probably under the influence of the above described fungal development (as traced from the presence of empty hyphae) and of an intense development of bacterial and cyanobacterial colonies (Figs. 191 & 192). These microbial colonies did not differ from what has been observed in decaying plant material, but they seemed to proliferate in macroinvertebrate faeces. Moss material was mainly present in the form of entire leaves (Fig. 193) but sometimes moss cell walls were in way to be degraded (Figs. 194 & 195), a pattern which had not been observed in non-ingested moss material. Food selection did not seem evident, since not any plant or microbial material observable in L₁ and L₂ was absent from studied macroinvertebrate faeces. However, the particular abundance of corpses and/or exuviae of arthropods should be noticed (Fig. 189), which cannot be explained by random foraging. Living nematodes were observed in these faeces, as well as mycelia of the basidiomycete *Hyphodontia sp.*

Near all macroinvertebrate faeces were of a dark brown colour, due to their pure organic content comprising empty hyphae of dematiaceous fungi. However, a unique specimen of faeces, of convoluted contour and light colour, was found. Its content was purely mineral, made of quartz particles of silt size without any bacterial development (Fig. 196). This was the first clue to mineral uplift encountered in the studied profile, to the exception of mineral particles of similar nature which were transported on the tegument of enchytraeid worms.

Enchytraeid faeces

Enchytraeid faeces were always observed as fluffy clusters (Fig. 197). Their composition differed according to the place where they were deposited. Only plant tissues issued from needle consumption were found in enchytraeid faeces at the inside of pine needles, as well as in faecal masses accumulated between twin needles, for instance fragments (still intact) of hypodermis (Fig. 198) or stomatal guard cells isolated among strongly degraded remains of needle tissues (Fig. 199). Plant material was always mixed with numerous fragments of empty fungal hyphae. Fragments of pine tracheids and resin were found in enchytraeid faeces accumulated between bark and wood, together with brown spores of *Scytalidium sp.* (see Fig. 121). The mycelium of *Haplographium delicatum*, seen sporulating at the surface of naked wood (Fig. 117) developed itself within fluffy masses of enchytraeid faeces accumulated between bark and wood. Free clusters (not embedded in plant tissues) displayed fragments of varied origin such as pine needles, wood, bark or bracken fern foliage (Fig. 144), but never moss tissues. Faecal clusters were permeated by the mycelium of *Hyphodontia sp.*, in direct contact with decaying plant tissues (Fig. 200). In every case I noted an intense development of bacteria (Fig. 201) and cyanobacteria (Figs. 202 & 203).

Oribatid faeces

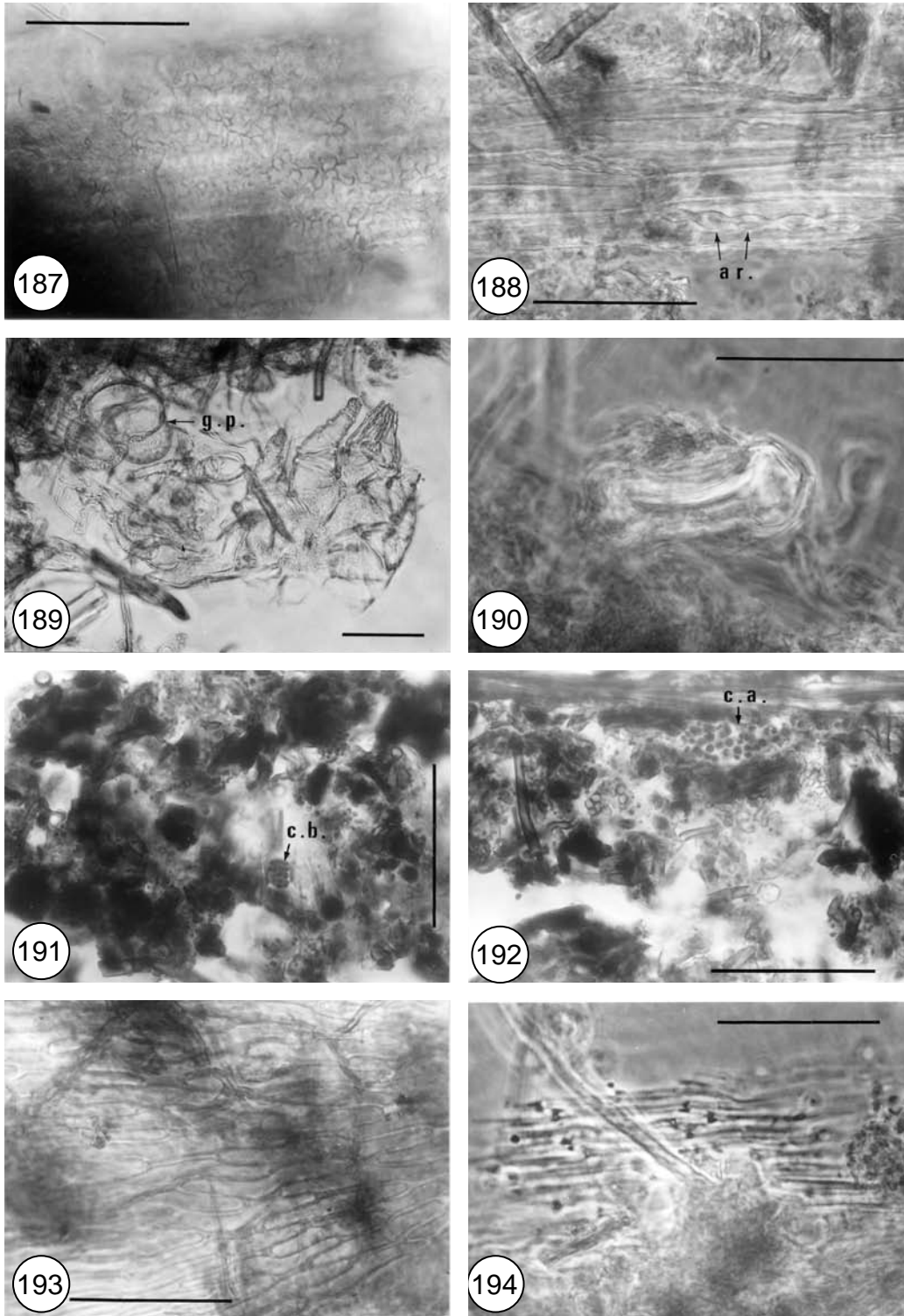


Fig. 187: Section through a macroinvertebrate excrement, detail showing a fragment of pine needle hypodermis, observed on its internal face (LN); Fig. 188: Idem, detail showing metaxylem tracheids of pine (CP); Fig. 189: Idem, detail showing a mite exuvia or corpse and a pine pollen grain (LN); Fig. 190: Idem, detail showing an isolated stomatal guard cell of pine (CP); Fig. 191: Idem, detail showing bacterial colonies (BMSL); Fig. 192: Idem, detail showing cyanobacterial colonies (BMSL); Fig. 193: Idem, detail showing a leaf of the moss *Pseudoscleropodium purum*, with intact walls (CP); Fig. 194: Idem, detail showing a fragment of moss leaf, with cell walls in way to be degraded (CP)

CP = phase contrast; LN = natural light without coloration; ar. = areolae; g.p. = pollen grains; c.b. = bacterial colonies; c.a. = cyanobacterial colonies; length of segment = 50 μ m

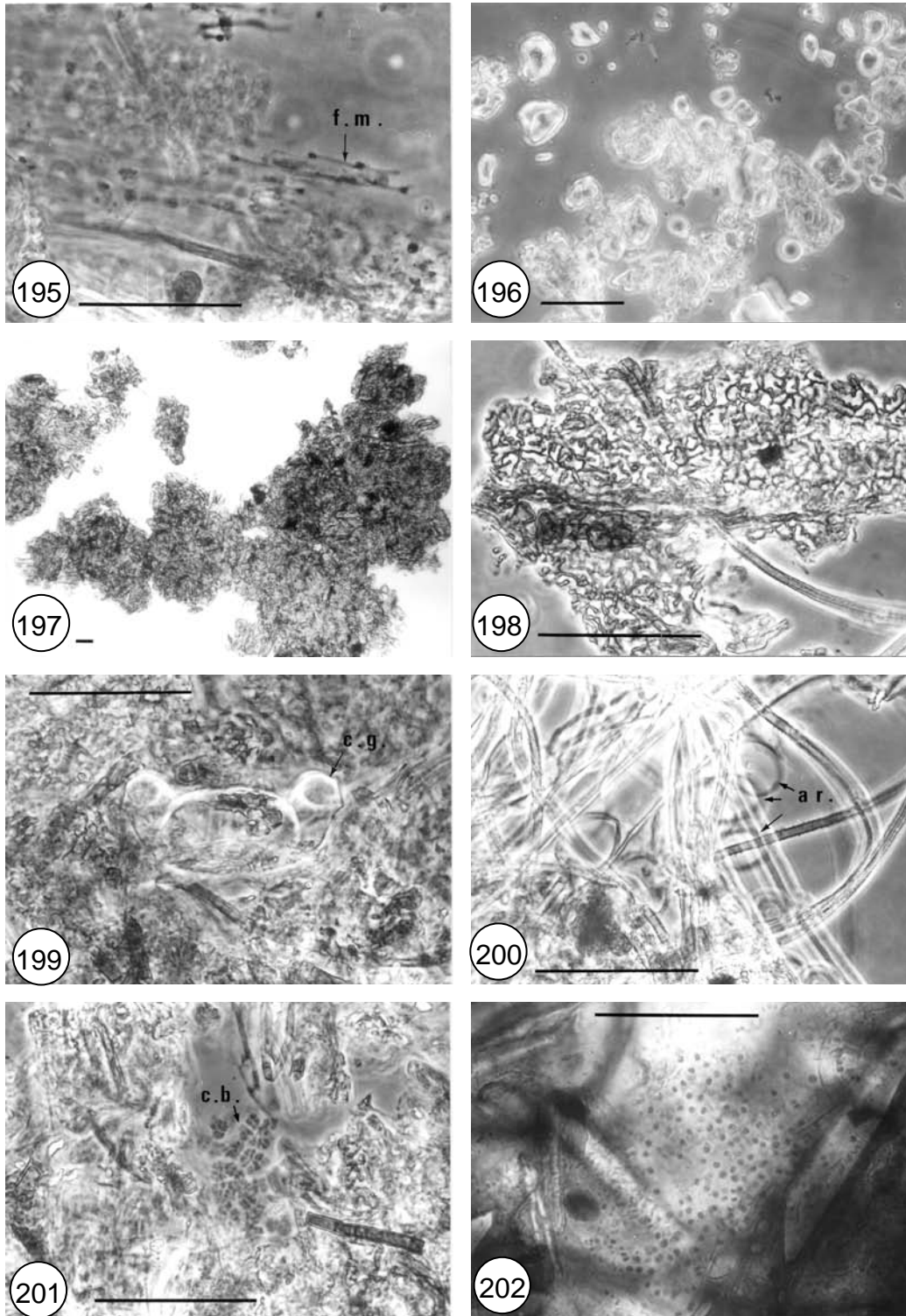


Fig. 195: Section through a macroinvertebrate excrement, detail showing a fragment of moss leaf, with cell walls in an advanced stage of degradation (CP); Fig. 196: Detail of macroinvertebrate excrement entirely comprised of silt-size quartz particles (CP); Fig. 197: Enchytraeid faeces clustered in a pine needle folding, overview (LN); Fig. 198: Idem, detail showing a fragment of pine needle hypodermis (CP); Fig. 199: Idem, detail showing a stomatal guard cell of pine needle (CP); Fig. 200: Enchytraeid faeces, detail showing pine tracheids with bordered pits and mycelium of *Hyphodontia* sp. (CP); Fig. 201: Idem, detail showing bacterial colonies (CP); Fig. 202: Idem, detail showing colonies of cyanobacteria (LN)

CP = phase contrast; LN = natural light without coloration; f.m. = moss leaves; ar. = areolae; c.b. = bacterial colonies; length of segment = 50 μ m

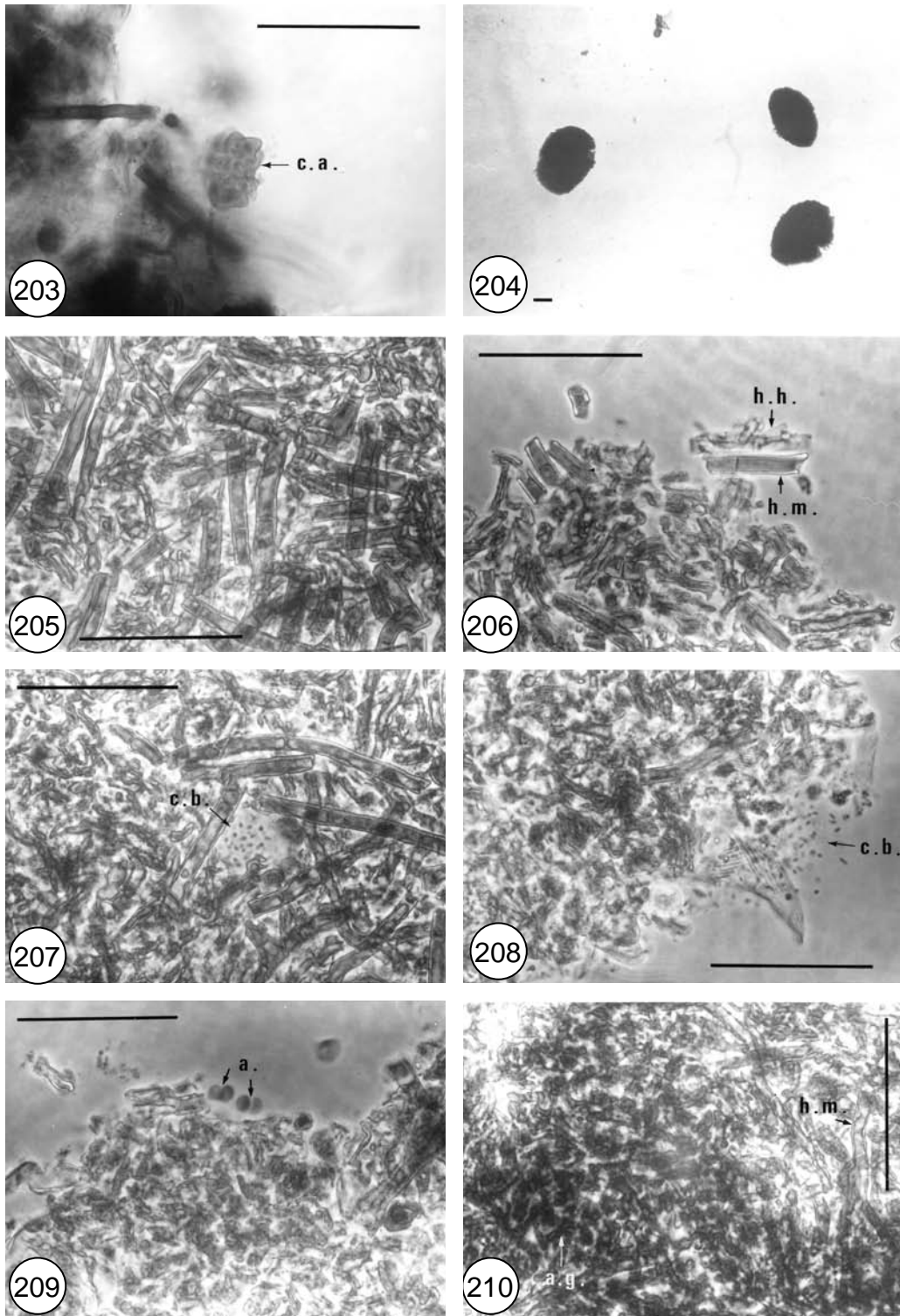


Fig. 203: Enchytraeid faeces, detail showing a cyanobacterial colony (LN); Fig. 204: Oribatid faeces, overview (LN); Fig. 205: Oribatid faeces, detail showing melanised hyphae of *Cenococcum geophilum*, with intact walls, cut into short pieces (CP); Fig. 206: Idem, detail showing melanised hyphae (intact) and hyaline hyphae (degraded) (CP); Fig. 207: Idem, detail showing a bacterial colony embedded in dematiaceous fungal material (CP); Fig. 208: Idem, detail showing dematiaceous fungal material, Collembolan scales and bacterial colonies at the periphery (CP); Fig. 209: Idem, detail showing algal development at the periphery (CP); Fig. 210: Idem, detail showing the passage from intact melanised fungal hyphae to a coarse assemblage of strongly degraded walls (CP)

CP = phase contrast; LN = natural light without coloration; c.a. = cyanobacterial colonies; h.h. = hyaline hyphae; h.m. = melanised hyphae; c.b. = bacterial colonies; a. = algae; length of segment = 50 μ m

Oribatid faeces were much more abundant than in the overlying L₁ layer. They were isolated, deposited at the surface of decaying plant material or embedded in the basidiomycetous mycelium of *Hyphodontia sp.* Their shape was characteristic (Fig. 204). To the exception of some fragments of pine pollen or scales of Collembola, oribatid faeces were comprised of fungal material, mostly melanised hyphae of *Cenococcum geophilum*, which were cut into short fragments (Fig. 205). When hyaline and melanised hyphae were found side by side, differences in their degradation stage were striking (Fig. 206). Bacterial colonies were observed, either in micro-cavities among compacted fungal hyphae (Fig. 207) or at the periphery of oribatid faeces (Fig. 208). Unicellular algae (Chlorophyceae) were seen proliferating at the surface of some faeces. These algae were of the same type as those observed in L₁ (Fig. 71), which were responsible for the green hue of oribatid faeces, but they were far less abundant, probably due to light interception by the overlying green moss layer.

I observed in some faeces a change in the aspect of melanised hyphae of *Cenococcum geophilum*. Cell walls had lost their neat contour, being transformed in a compact amorphous mass. Transitional stages could be observed at the inside of some ageing faeces (Fig. 210) until in some faeces the totality of the faecal material became amorphous (Fig. 211). I never observed such a phenomenon in the other invertebrate groups I scrutinized. Given that no microorganism was found associated with amorphous stages of *Cenococcum* degradation, I hypothesized that this degradation resulted from non-biotic processes, maybe involving free enzymes.

Exceptionally at this level, some oribatid faeces showed that pine needles could be consumed, too (Fig. 212). The presence of needle epidermis and the absence of tracheids suggested that internal tissues were not consumed, biting by oribatid mites being not followed by tunnelling activity (but see F₁ layer).

Springtail faeces

Faeces of Collembola were found at the surface of decaying pine needles, isolated as for oribatid faeces, but at a much lower level of abundance. Their dark colour was due to the abundance of melanised fungal material. Contrary to what has been observed in living specimens, the content of faeces found in L₂ (Figs. 213 & 214) indicated mycophagy rather than coprophagy. Hyaline hyphae were degraded while walls of melanised hyphae remained intact. Bacterial development was always intense (Fig. 215).

Animal corpses and corpse fragments

At first it should be noted that a lot of corpses and exuviae were included in invertebrate faeces (see Fig. 189). Thus most arthropod remains were not included in the list presented in Table 6, which takes into account only cuticles and fragments of cuticles which were found free in the environment. Oribatid mites were largely dominant. Two specimens of box mites (oribatid mites with complete closure of anterior part and legs in the body) were found (Table 3), indicating that this group, although not found alive in L₂ at the time of sampling, had previously colonised it.

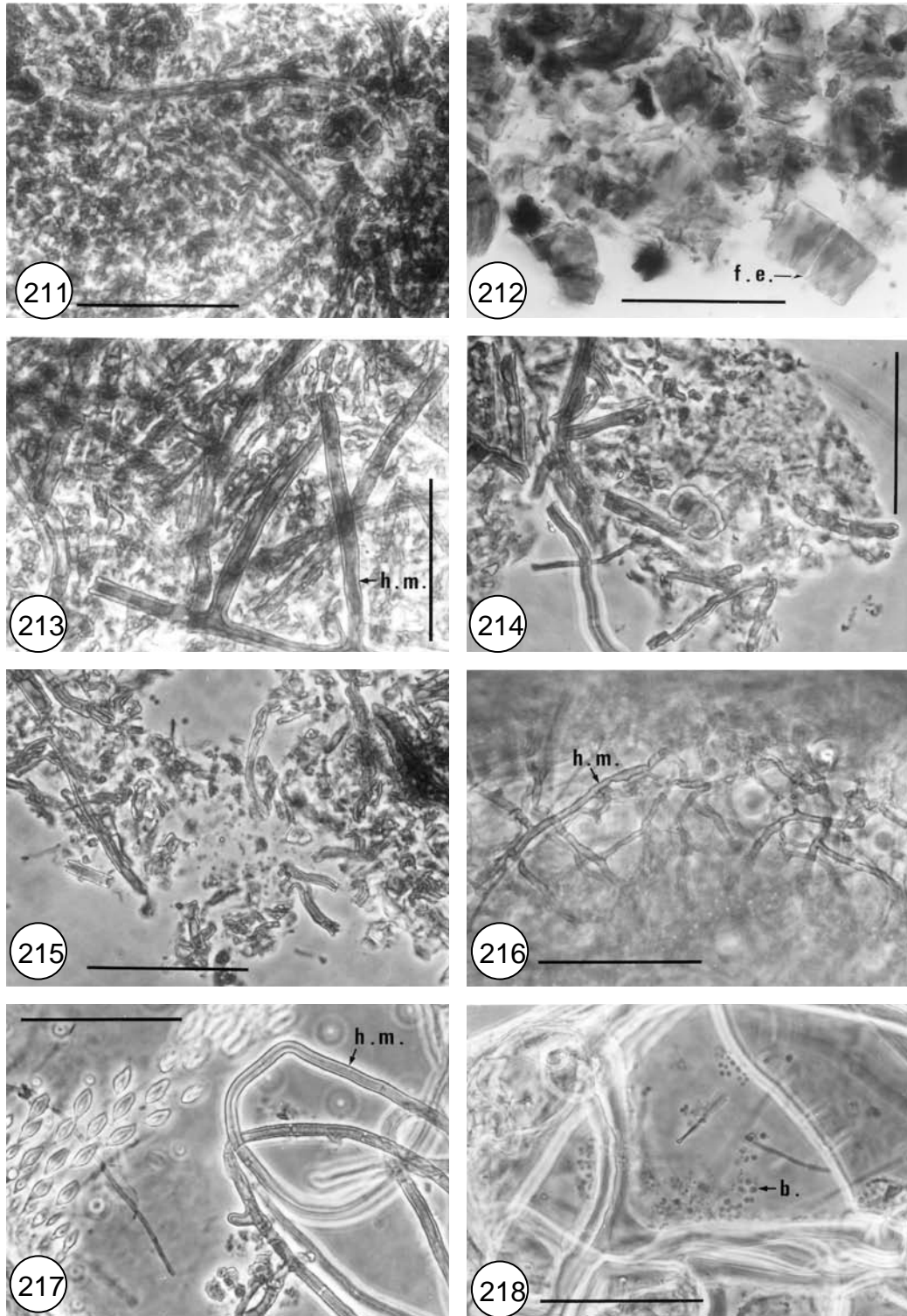


Fig. 211: Oribatid faeces, detail showing strongly decayed melanised hyphae of *Cenococcum geophilum* (CP); Fig. 212: Idem, detail showing fragments of pine epidermis and bacteria (LN); Fig. 213: Springtail faeces, detail showing fragmented melanised hyphae of *Cenococcum geophilum* (CP); Fig. 214: Idem, detail showing fungal material and bacteria (CP); Fig. 215: Idem, bacterial development and fungal material (CP); Fig. 216: Corpse of *Adoristes ovatus*, detail showing a network of melanised hyphae at the surface of the cuticle (CP); Fig. 217: Fly wing, detail showing melanised hyphae of *Cenococcum geophilum* (CP); Fig. 218: Corpse of *Oppia sp.*, detail showing bacterial development at the inside (CP)

CP = phase contrast; LN = natural light without coloration; f.e. = fragments of epidermis cell; h.m. = melanised hyphae; b. = bacteria; length of segment = 50 μm

Table 6. Number of animal corpses and corpse fragments found under the binocular microscope in the L₂ layer

Fragments of arthropod legs	2
Unidentified mesostigmatid mites	2
Unidentified oribatid mites	2
Unidentified box mites	2
<i>Adoristes ovatus</i>	2
<i>Oppia sp.</i>	1
<i>Oribatula tibialis</i>	1
<i>Platynocheilus peltifer</i>	1
<i>Nanhermannia nana</i>	1
Fly wing	1

As in the L₁ layer (Fig. 73) the surface of oribatid corpses was covered with a network of melanised hyphae (Fig. 216). A similar fungal development was observed at the surface of a fly wing (Fig. 217). However, chitinolysis was never registered. The inside of bodies was invaded by bacteria (Fig. 218), and protozoa could be observed, too, such as for instance the small cyst amoeba *Corythion dubium* (Fig. 219).

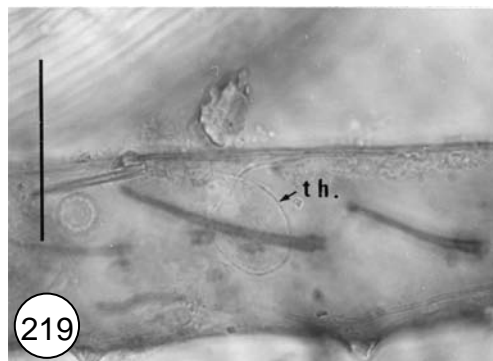


Fig. 219 (x 400) *Corythion dubium* (cyst amoeba) at the inside of an arthropod leg (natural light without coloration)
th. = thecamoeban cyst; length of segment = 50 µm

Conclusions about the L₂ layer

The process of fungal decomposition of litter initiated in L₁ was pursued in L₂, mainly under the influence of *Verticicladium trifidum*, a specific decomposer of pine needles. A novel stage could be defined, characterised by a bacterial and algal (cyanobacterial) development at the inside of pine needles, as well as outside them, in the numerous foldings issued from the collapse of parenchyma. This bacterial/algal development occurred mainly to the detriment of previous fungal colonisers, which achieved the destruction of internal tissues: lignified parts of pine needles were destroyed by *Verticicladium trifidum* while pine wood was destroyed by the white rot *Hyphodontia sp.*

The successional development of bacterial communities in such a superficial layer occurred mainly through the agency of enchytraeids and several macroinvertebrates, lumbricid activity being suggested by the deposition of typical earthworm faeces. Biting and nibbling of pine needles by fauna and the tunnelling activity of enchytraeid worms caused a disruption of the hard envelope (epidermis + hypodermis) which protected pine needles from the external environment, and microbial propagules were dispersed directly at the inside of decaying needles.

What was the fate of plant organic matter once incorporated into invertebrate faeces? The present study showed that an intense microbial (bacterial, algal) development was observed at the inside of faeces. The strong compaction of the fungal material within oribatid faeces, probably stemming in local anaerobiosis, was at the origin of a particular degradation of melanised hyphae, not accompanied with bacterial development. This formation of stable amorphous organic matter (humus sensu stricto) should be better described and followed in TEM microscopy. Microbial development seemed less intense in faeces of macroinvertebrates than in those of enchytraeids. In particular the development of algae and bacteria and the stage of degradation of the plant material in macroinvertebrate faeces did not seem different from what was observed in unconsumed pine needles. This was probably due to the fact that only coarse fragments were ingested by bigger animals, which did not result in disruption of plant cell walls. Such physical limit to the impact of soil animals on the degradation of plant litter was overlooked by most authors. Enchytraeid worms, by finely comminuting plant tissues and incorporating them in loosely packed faecal material (contrary to oribatid mites which compact it), favoured further bacterial development. However, at the studied depth level, the amount of plant litter which had transited by enchytraeid guts was still very feeble.

The algal (cyanobacterial) development which had been registered at the inside of pine needles was probably favoured by the shallow water table (pseudo-gley), which maintained a high level of available moisture even in summer months, as was indicated by the presence of *Molinia caerulea*. This might also explain the abundance of enchytraeids in full summer, as well as the presence of copepods, as will be seen below. The existence of algae at the inside of closed plant organs such as pine needles should deserve a special study on their metabolism when light is absent or strongly filtered out. When examined in water, these algal colonies appeared to be perfectly transparent, without any visible pigment. I suspect them to become heterotrophs when deprived of light, and thus to play the same role as bacteria and fungi in decomposition processes.

The optical method used in the present study allowed visualizing most interactions between microbes, animals, plants and minerals taking place in the L₂ layer, although the latter component was still negligible so near the surface. Within the abovementioned limits of the method (poor recovery of macroinvertebrates, imperfect assessment of microfaunal processes, absence of replication in time and space, poor representativeness) this method, cheap and easy to perform in every laboratory equipped with a good light microscope, was able to describe poorly known processes such as those which happens at the inside of a pine needle. In addition, the choice of a restricted volume to be studied allowed an overview of the soil ecosystem at the right place and at the right scale at which most interactions between plants, microbes and animals take place, thereby escaping the terrible problem of soil heterogeneity.

THE F₁ LAYER: FRAGMENTED NEEDLES, INVERTEBRATE FAECES AND MYCORRHIZAE



Figure 220. The F₁ layer before fixation and transport to the laboratory

Table 7 shows the respective volume of the gross components of the F₁ layer. Compared to the overlying L₂ layer, it should be noticed that the diversity of the observed material did not change longer.

Table 7. Volume of the different components of the F₁ layer sorted under the binocular microscope

Litter components	Volume (μL)
Pine wood	4200 ± 100
Moss	2700 ± 100
Macroinvertebrate faeces	1500 ± 20
Bracken fronds	150 ± 100
Pine bark	1200 ± 100
Pine twigs	1200 ± 100
Pine roots	800 ± 100
Pine euphylls	180 ± 20
Miscellaneous	100 ± 20
Male flowers of pine	55 ± 5
Complex assemblages	45 ± 5
Living fauna	4.6 – 18
Total	13500 ± 100

The abundant bracken litter, the presence of some strongly decayed fragments of birch leaves allowed to date approximately the fall of the plant material present in F₁ to a period extending from summer 1980 (a year before sampling date) to December 1980 or January 1981. This lapse of time embraced a peak of needle fall in September and October 1980 (Arpin et al., 1986a). The presence of euphylls and male flowers of pine (fallen in June) in F₁ was probably due to the penetration of these very small and light plant organs through loosely packed L₁ and L₂ layers, following intense rains which occurred in the course of summer 1981, previous to sampling.

The proportion of voids (73%) was only slightly less than in L₂ (77%): compaction was thus still poor at 5-6 cm depth (Table 1). Tables 2, 4 & 7 showed that F₁ was the first layer where pine needles were dominant and overwhelmed moss material. However, this should not be translated in terms of litter decomposition (which would be slower for pine than for moss), because maximum needle fall occurred during the period corresponding to the deposition of F₁ material. In addition, non-linear trends in litter decomposition were evidenced by the dramatic increase of macroinvertebrate faeces, which became the third litter component in volume in F₁ (11% of the total volume of solid matter against 2% in L₂). Root development was ten times more important in F₁ compared to L₂ (6% in place of 0.6%). The biovolume of living fauna, within the abovementioned limits of its calculation, was also higher than in L₂.

Pine needles were fragmented under the action of macrofauna (slugs, earthworms, woodlice, which were identified by their faeces) and mesofauna (enchytraeids, oribatid mites, which were directly observed). Oribatid mites deposited their typical egg-shaped faeces at the inside of needles (Fig. 221), external tissues remaining intact (their faeces could be observed only by breaking needles with scissors). Enchytraeids penetrated also pine needles but most of time only the anterior part of the body plunged into them (Fig. 222) and as a consequence they defecated mainly in the outside. Some sciarid larvae were also observed penetrating pine needles, in the same manner as enchytraeids. Thus, several widely different animal groups shared the same decaying plant material as food resource and cohabited in a small volume of litter, without any segregation in space or time. Such observations were in disagreement with commonly held views on competitive exclusion and niche separation (Barbault, 1981) but they were supported by the direct observation of multi-species cultures (Christiansen, 1967). When not ingested by fauna fragmented needles were embedded in the same woolly mycelium described in the L₂ layer. Observations on pine ectomycorrhizae showed that this mycelium, which was comprised of the ascomycete *Cenococcum geophilum* and the basidiomycete *Hyphodontia* sp., as mentioned above, were linked to the two ectomycorrhizal types which were dominant in F₁. Most often, external signs of previous fungal colonisation (pycnidia, ascocarps, diaphragms, conidiophores) had disappeared from the surface of pine needles, due to biting and nibbling by fauna. However, diaphragms of *Lophodermium pinastri* were still sometimes observed, together with conidiophore remains of *Verticicladium trifidum*. Unidentified black sclerotia which had been observed at the surface of some pine needles in L₂ were also observed in F₁, but rhizomorphs of the basidiomycete *Marasmius androsaceus* were absent. It should be also noticed that the past activity of the latter fungus was not registered on sectioned pine needles, pointing to a strongly seasonal development of this fungus (winter to spring, as mentioned above), which was probably not able at that time to colonize needles at a too advanced stage of decomposition. Decaying needles were strongly intermingled with other components of the F₁ layer. Pine ectomycorrhizae were often seen tightly appressed against fragments of pine needles, leafy shoots of moss and animal faeces, these assemblages being embedded in turn in fungus mycelium. Growing roots seemed to preferentially penetrate organic assemblages of decaying plant material, making their isolation particularly tedious.

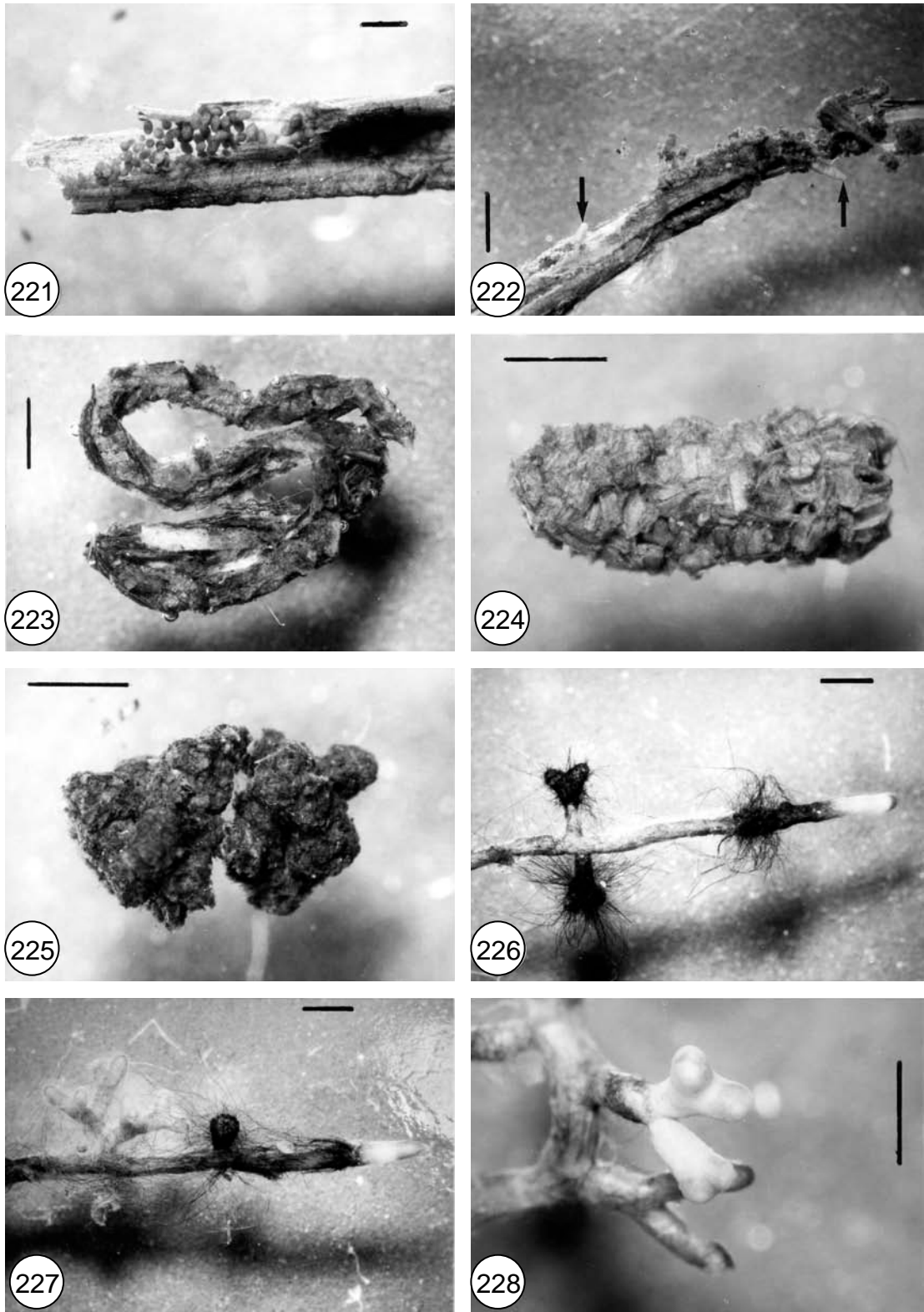


Fig. 221: Oribatid faeces at the inside of a pine needle; Fig. 222: Two enchytraeid worms (arrows) penetrating a pine needle, with deposited their faeces; Fig. 223: Type A excrement (slug?); Fig. 224: Type B excrement (woodlouse?); Fig. 225: Type C excrement (earthworm); Fig. 226: Long fine root of pine colonised by *Cenococcum geophilum* in the 'root hair' zone and with two ectomycorrhizae of the same fungus; Fig. 227: Long fine root of pine with growing apex and two different types of ectomycorrhizae (black ectomycorrhiza of *Cenococcum geophilum* and orange-brown ectomycorrhiza of *Hyphodontia* sp.); Fig. 228: Pink basidiomycetous ectomycorrhiza

length of segment = 1 mm

At last, all pine needles appeared to be strongly collapsed, with sometimes only the external envelope (hypodermis + epidermis + cuticle) remaining.

Moss leafy shoots seemed to be more microbially attacked than in the above L₂ layer. They were firmly appressed to each other, sometimes mixed with bracken leaflets, and abundantly covered (or intermingled) with animal faeces. As well as other plant material they were embedded in the at once ubiquitous fungal mycelium. Moss stems exhibited often a dark red colour, which had not been observed in L₂, and some of them were empty. In the latter case they were translucent and light coloured. White rhizomorphs were seen protruding from some decaying moss stems. Ramified moss stems were often seen totally deprived of leaves, probably following peeling by fauna.

Three types of macroinvertebrate faeces were observed in F₁. The A type (Fig. 223) was represented by more or less folded centimetric folded strands which were made of long fragments of pine needles which had been seemingly swallowed entire. The ingested plant material did not seem to have been transformed during gut transit. By their size and shape I interpreted these excrements as slug faeces, even though these animals were present in the study site but not in the studied sample. The B type (Fig. 224) was represented by truncated, flattened millimetric cylinders. Under the dissecting microscope, they appeared to be comprised of very small, compressed fragments of pine needles. Their colour was more or less dark brown, but never blackish. Fungal hyphae were observed under the light microscope, but they could come from secondary colonisation. By their size and shape these faeces were attributed to woodlice, despite of the fact that the two *Trichoniscus* larvae which were found in the sample did not exhibit a similar composition in their gut contents. The C type (Fig. 225) was by far the most abundant type of macroinvertebrate faeces found in the F₁ layer. Their colour was blackish and they were strongly convoluted without any definite external shape. Their content was finely ground, contrary to types A and B. Type C faeces were often found appressed against plant material, probably because of their pasty nature when excreted and of the movements of animals which pushed their way through litter. They were attributed to epigeic earthworms, in particular to *Dendrobaena octaedra* (Savigny) which was dominant in the site (Bouché, unpublished data). Other earthworm faeces were found in the sample, but they were not accounted for, given their poor representativeness. They were mineral, friable, white or light gray coloured. These excrements were produced either by another earthworm species or by *Dendrobaena octaedra* when dipping in underneath horizons: some authors like Martinucci & Sala (1979) classified this species as a 'weak burrower'.

Faeces of mesofauna (microarthropods, enchytraeids) were either dispersed at the surface of decaying material (oribatids, springtails) or agglomerated at the inside of needles or bark pieces (box mites, sometimes enchytraeids) or outside of them (enchytraeids, sciarid fly larvae). Despite of the intense activity of these animals (in particular enchytraeids and box mites) the total volume of faeces they produced was largely less than that of macroinvertebrates, in particular type C (earthworms).

The root network in F₁ was only comprised of fine roots. Long roots exhibited a growing apex, with an elongation zone (sometimes with absorbing hairs below it). They bore short ectomycorrhizal roots (Fig. 226). While the ectomycorrhizae present in L₂ were always of the coralloid orange-brown type, a wider variety of ectomycorrhizal types was depicted in F₁, probably because fine roots were older in F₁ compared to L₂ (Figs. 226 to 228). The dominant type was the black ectomycorrhiza of *Cenococcum geophilum* [= *Cenococcum graniforme* (Sow.) Ferd. & Winge]. This ascomycete is only known as a sterile mycelium (sometimes called 'jet-black mycelium') which exhibits many analogies with an *Elaphomyces* according to Trappe (1971). It produces spherical

sclerotia of varying size, which were found in abundance in the studied sample (Fig. 229). Long straight black hyphae protruded from ectomycorrhizae (Figs. 226 & 227) and sclerotia (Fig. 229) of *Cenococcum geophilum*, permeating the decaying plant material. Earthworm faeces (type C) were also a favourable seat for the development of this fungus (Fig. 230). Ectomycorrhizae of *Cenococcum geophilum* were short, dichotomous (Fig. 226) or monopodial (Fig. 227), and the fungus might also colonize the long root, in the zone normally carrying root hairs (Figs. 226 & 227). Cohabitation with the coralloid orange-brown type was observed, either on the same long root but on distinct short roots (Fig. 227) or even on the same short root. Most often, the growth of *Cenococcum geophilum* was slower than that of long roots, letting uninfected root apices but, as has been already shown by Wilcox (1968), it happened infrequently that the apex was colonised by the fungus, and root growth was checked (Fig. 231). My observations did not allow discerning which event (growth arrest of the root or fungus infection) occurred first. The coralloid type was always associated with a white woolly mycelium (Fig. 227) of the same type as the one embedding decaying moss shoots, pine needles, etc... The third ectomycorrhizal type, much more infrequent, was represented by pink, thick, velvety dichotomous ectomycorrhizae (Fig. 228), without any mycelial association visible under the dissecting microscope.

Decaying pine needles

Fungal mantle around pine needles

Most pine needles were embedded in a fungal mantle which was comprised of four distinct types, three of which proved to be associated with pine ectomycorrhizae. The most abundant type was the mycorrhizal ascomycete *Cenococcum geophilum*, in its typical aerial form (Fig. 232). This fungus was present in L₁ and L₂, but its mycorrhizal nature was verified in F₁, where it was associated with black ectomycorrhizae (Figs. 226, 227 & 231) and sclerotia (Fig. 229). The mycelium of *Cenococcum geophilum* was accompanied by the secondary mycelium of the white-rot basidiomycete *Hyphodontia sp.*, hyphae of which were encrusted with oxalate crystals (Fig. 233) or not (Fig. 232). Observations done in F₁ (see below) showed that this fungus, although definitely a white rot as already shown in L₂, (Figs. 120 & 121), was associated with the coralloid orange-brown ectomycorrhizae of pine. The third type was intermingled with the secondary mycelium of *Hyphodontia sp.* It was made of smooth hyaline hyphae of 10 µm diameter, at the inside of which intra-hyphal regrowth (hyphae of 3 µm diameter) was frequently observed (Fig. 233). Despite of the fact that clamp connections were not found on its hyphae in the F₁ layer, I thought that the same fungus had been observed embedding moss leaves in L₂, similarly in the company of *Hyphodontia sp.* (Fig. 131). This unidentified fungus was not found in association with pine ectomycorrhizae, but it cannot be excluded that such big smooth hyphae could differentiate from ordinary hyphae in the mycelium of *Hyphodontia sp.* Two arguments were in favour of this hypothesis, (i) hyphae issued from intra-hyphal regrowth of the third type were morphologically similar to hyphae of *Hyphodontia sp.* (when not encrusted with oxalate crystals), and (ii) both fungal types were always found tightly intermingled. The fourth type was rarer. It was made of smooth hyphae, somewhat larger (4 µm) than those of *Hyphodontia sp.* The same mycelium was found in association with pink velvety ectomycorrhizae of pine (Fig. 228).

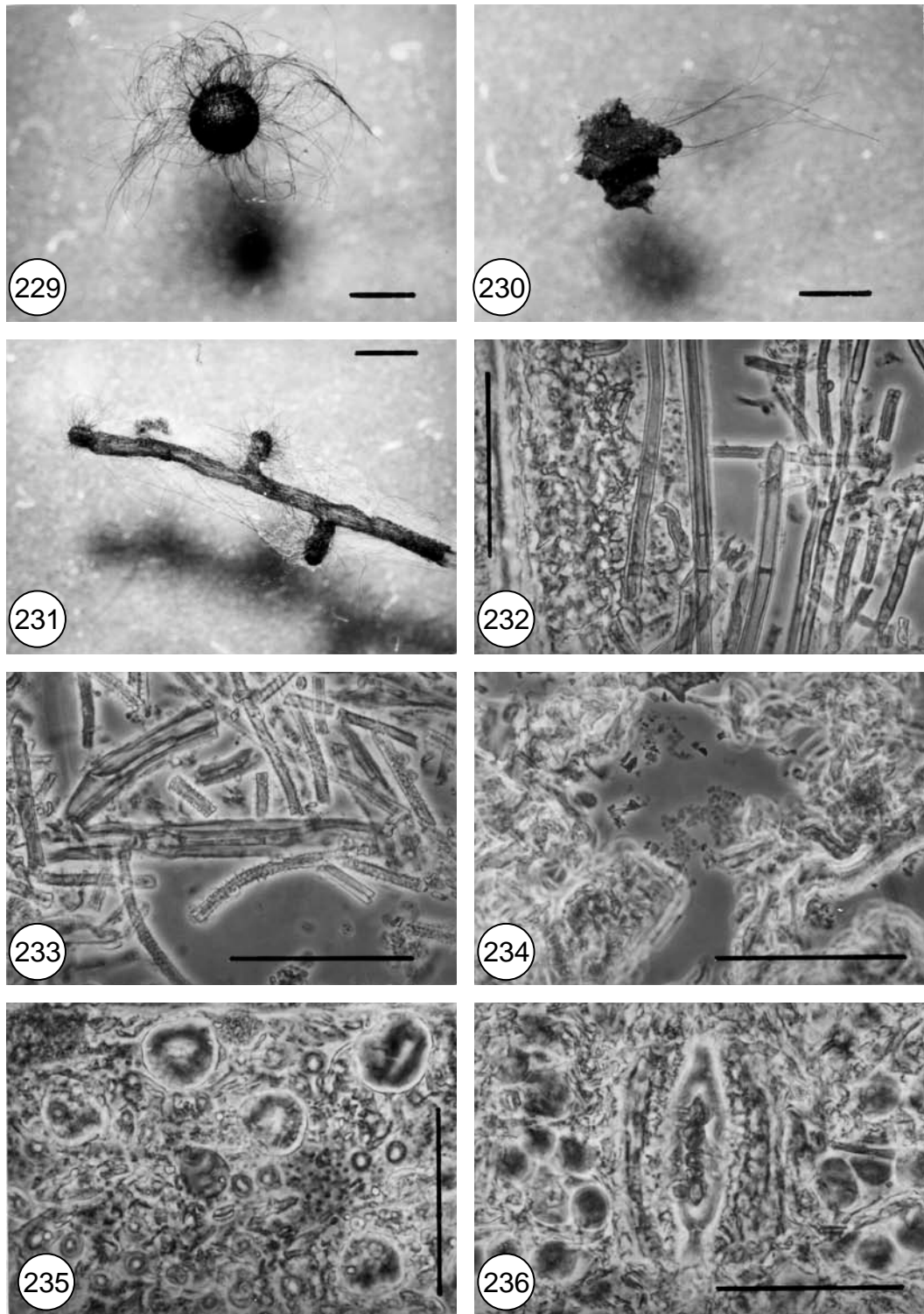


Fig. 229: Sclerotium of *Cenococcum geophilum*; Fig. 230: Type C (earthworm) excrement from which *Cenococcum geophilum* hyphae were protruding; Fig. 231: Long fine root of pine the growth of which was arrested and the apex colonised by *Cenococcum geophilum*; Fig. 232: Pine needle, section showing from right to left fine hyphae of *Hyphodontia sp.*, coarse hyphae of *Cenococcum geophilum*, bacterial development between hyphae, epidermis and hypodermis transformed in a melanised fungal stroma (CP); Fig. 233: Idem, showing fine hyphae of *Hyphodontia sp.* encrusted with oxalate crystals and smooth coarse hyphae with intra-hyphal regrowth (CP); Fig. 234: Idem, showing bacteria in way to be degraded (CP); Fig. 235: Idem, showing transfusion tissue transformed into a pulp, with bacterial development and brown spherules (CP); Fig. 236: Idem, showing a layer of unicellular algae just under the hypodermis, on both sides of a stomata (CP)

CP = phase contrast; length of segment = 1 mm (Figs. 229-231) or 50 µm (Figs. 232-236)

A bacterial development was observed in the fungal mantle embedding decaying pine needles (Fig. 232). Sometimes hyphae of *Cenococcum geophilum* were seen penetrating decaying fungal material, maybe corresponding to a successional process within the fungal mantle. The presence of another fungus, growing as a parasite on this fungus mantle, should be noted, too. It fructified in the form of tiny white funnels, the wall of which was made of firmly appressed hyphae, replaced at the inside by short free hyphae bearing conidia. Unfortunately they could not be sectioned correctly in order to study conidiogenesis. It could be possibly a *Myrothecium* sensu lato (Morelet, personal communication).

Inside of pine needles

The constant presence of fauna at the inside of pine needles found in F₁ must be highlighted, even when needles did not exhibit any sign of nibbling or penetration by invertebrates. Microfauna were always observed in sectioned needles, in the form of protozoa (naked amoebae, flagellates), rotifers or nematodes. Cyst amoebae, although frequently observed at the surface of bracken leaflets, moss leaves and pine needles, were never observed at the inside of pine needles except when these had been previously tunnelled by mesofauna. The decay stage which pine needles reached in F₁ was thus characterised by the ubiquitous action of a variegated fauna. Less generally, mycorrhizal fungi were seen penetrating pine needles, more especially *Cenococcum geophilum*. Non-mycorrhizal basidiomycetes were also observed at the inside of pine needles, but only in the form of dead hyphae, testifying for previous fungal colonisation. Most often, and this was true for *Cenococcum geophilum*, too, fungal hyphae were dead or senescent (no staining by methyl blue, no opacity under phase contrast), while bacteria or more exceptionally micro-algae (Chlorophyceae) were found alive. A replacement of fungi by bacteria had been already observed in L₂, but now the degradation of bacteria was observed, too (Fig. 234), which might indicate a check of bacterial colonisation in F₁. The same for chroococcal algae (cyanobacteria), which were abundant at the inside of pine needles in L₂, and in way to disappear in F₁.

- Needles attacked by *Verticicladium trifidum*, but not by *Cenococcum geophilum*

The presence of *Verticicladium trifidum* was attested by its hyaline, thick-walled hyphae, which were observed in a senescent state in xylem tracheids, or by its typical stromata developed at the inside of host tissues. Some needle fragments were observed, at the inside of which mesophyll and endodermis had been entirely transformed into melanised fungal tissues. These were devoid of cytoplasm at the time of sampling but their cell walls were still intact. Epidermis and hypodermis could be also transformed into a melanised fungal stroma (Fig. 232). In all cases of previous attested colonisation by *Verticicladium trifidum*, the transfusion tissue was transformed into a pulp, where the only more or less recognizable plant elements were bordered pit areolae, with fuzzy contours (Fig. 235). Bacterial development was intense (Fig. 235) except in the unique case observed where the fungus was still active (although senescent). Some traces of other types of fungal colonisation could subsist, too, such as empty hyphae and diaphragms of *Lophodermium pinastri*. Needles where the

development of *Verticicladium trifidum* was not arrested by the faunal transformation of pine needles were characterised by further cellulolysis in the central cylinder (to the exception of xylem) and the development of fungal stromata which replaced progressively epidermis, hypodermis, mesophyll and endodermis. Even when internal tissues were further invaded by bacteria, it should be noticed that the degradation of host cell walls was not caused by bacteria but rather by *Verticicladium trifidum*, since similar figures were observed in L₂ in the absence of bacteria (Fig. 76).

Other phenomena should be noticed, too, the importance of which and the assignment to *Verticicladium trifidum* activity were more disputable since they not repeatedly observed: the presence of big-sized brown spherules in the transfusion tissue of a pine needle (Fig. 235), and the development of a unicellular algal (Chlorophyceae) layer just under the hypodermis, which had been transformed into a melanised stroma (Fig. 236). The latter pattern, observed on a wide area of a unique needle fragment, had not been registered in the overlying L₂ layer.

- Needles attacked by *Cenococcum geophilum*

This ascomycetous ectomycorrhizal fungus was observed embedding needle fragments in F₁, where it was accompanied by basidiomycetous ectomycorrhizal fungi. However, it was also often seen penetrating pine needles, where the morphology of its mycelium was quite different from that of the aerial form. Contrary to *Verticicladium trifidum*, which invaded in a progressive manner the totality of internal tissues of pine needles, *Cenococcum geophilum* formed a dense network of entangled hyphae in the part of the mesophyll which was located just under the hypodermis (Fig. 237). Given that remains of pine tissues were not included within the mycelial network of *Cenococcum geophilum* but were pushed aside, it was thought highly probable that this fungus did not contribute to the degradation of pine needles. Entangled hyphae were convoluted, of smaller section (2 µm) than the aerial form (4 µm) and were less impregnated with melanin (grey vs jet black for the aerial form). Chlamydospores (Ø 4-7 µm) were observed from place to place. Although strongly different from needle-embedding hyphae, entangled hyphae found at the inside of decaying pine needles were attributed unambiguously to *Cenococcum geophilum*. Some zones were found, where both forms cohabited in the same mycelium and were connected to each other (Fig. 238). Both types of hyphae fall within the wide morphological variability of *Cenococcum geophilum*, which has been described in agar and liquid culture by Hatch (1934) and Mikola (1948), respectively. The morphological variety observed within the same fungal strain (see also strain varieties described by Mikola) is also at the origin of the numerous names given to this fungus in ancient studies (Hatch, 1934). The entangled hyphae of *Cenococcum geophilum* observed between the mesophyll and the hypodermis appeared always devoid of any cytoplasm, some were even in way to be degraded (Fig. 239). However, no bacterial development was registered. The mesophyll and the central cylinder showed signs of previous fungal colonisation, but typical melanised hyphae of *Cenococcum geophilum* were observed in some xylem tracheids. In this case, I suspected that needles were first fragmented by fauna then the sectioned central cylinder was colonised directly at the level of a break, since no hyphal connection was found with the mycelium which developed under the hypodermis. Needles colonised by *Cenococcum geophilum* seemed highly palatable to box mites, since tunnelling by these animals always occurred in needles previously colonised by the ascomycete. However, I observed that box mites ingested only internal tissues of decaying pine needles, not the melanised dense hyphal network

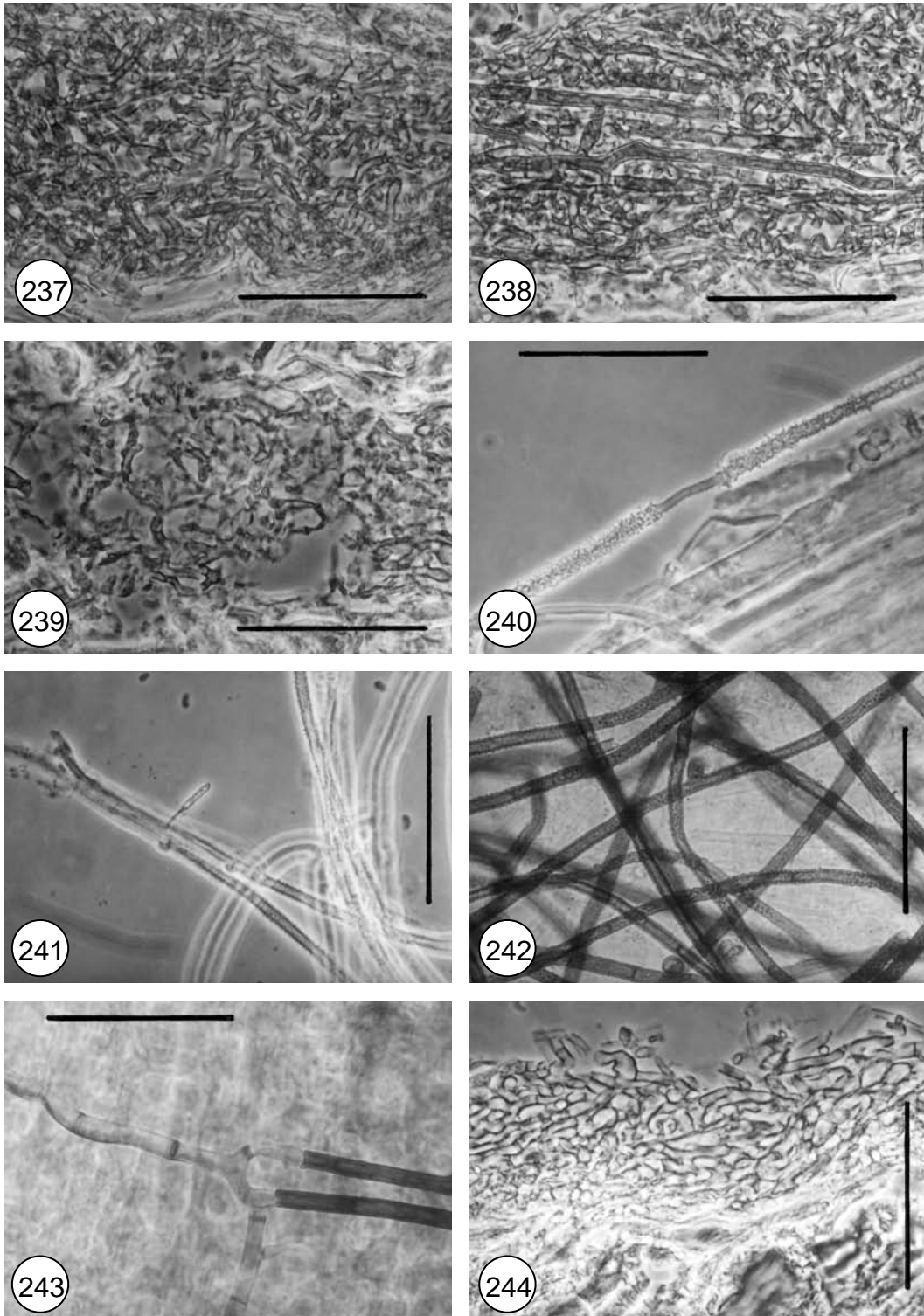


Fig. 237: Pine needle attacked by *Cenococcum geophilum*, section showing a network of entangled hyphae in the portion of the mesophyll located just under the hypodermis (CP); Fig. 238: Idem, showing a mixed network of large straight and small convoluted hyphae (CP); Fig. 239: Idem, showing entangled hyphae in way to be degraded (CP); Fig. 240: Coralloid orange-brown pine ectomycorrhiza with *Hyphodontia sp.*, detail of a hypha of from the extramatrical mycelium showing the passage from smooth wall to wall encrusted with oxalate crystals (CP); Fig. 241: Idem, showing a cystidium born by a hypha (CP); Fig. 242: Black pine ectomycorrhiza with *Cenococcum geophilum*, detail of the extramatrical mycelium showing melanised hyphae with encrusted walls (LN); Fig. 243: Idem, detail showing the passage from hyaline to melanised hypha on the same mycelium (CP); Fig. 244: Pink pine ectomycorrhiza, detail of the prosenchymatous fungal mantle (CP)

CP = phase contrast; LN = natural light without coloration; length of segment = 50 µm

produced by *Cenococcum geophilum*. The intense faunal activity which took place within needles colonised by *Cenococcum geophilum* made them rapidly emptied, and further colonised by other mycorrhizal fungi. However, mycorrhizal basidiomycetes were never observed at the inside of needle tracheids, they just colonised galleries created by tunnelling animals.

Brown spherules similar to what has been observed in other decaying needles in F₁ (Fig. 235) and L₂ (Fig. 104) were found only once. Their interpretation as polyphenol-protein complexes precipitating at the death of cells, a common phenomenon described by Handley (1954), is rather problematic, since they were observed only on a few needles. Thereby the question of their origin remains open.

The most prominent character of needles colonised by *Cenococcum geophilum* was the incompatibility of this fungus with bacterial and algal development. It must be highlighted that the degradation of bacterial cells (Fig. 234) was observed in a needle colonised by *Cenococcum geophilum*, a mycorrhizal ascomycete known for its production of antibiotics (Grand & Ward, 1969). However, it did not seem to be involved in the degradation of pine tissues, cells of which (in particular tracheids) were penetrated without any alteration of their wall. *Cenococcum geophilum* seemed also incompatible with *Verticicladium trifidum*, since needle fragments colonised by the former did not seem to have been previously colonised by the latter. The fact that hyphal walls of *Cenococcum geophilum* seemed devoid of cytoplasm might suggest that colonisation by this fungus took place during previous autumn and/or winter, while that by *Verticicladium trifidum* was more recent: viable hyphae of this fungus were visible in F₁ and were very abundant, with concomitant fructification, in L₂ and L₁ (seemingly deposited in spring and summer). Curiously, data on the seasonal development of *Cenococcum geophilum* mycelium were lacking in literature, only its mycorrhizae being studied in detail.

Pine roots and mycorrhizae, mycorrhizal fungi

Many authors described anatomical characters of pine ectomycorrhizae, either in light (Hatch & Doak, 1933; Hatch, 1934; McDougal & Dufrenoy, 1944; Robertson, 1954; Fassi & De Vecchi, 1962; Marks, 1965; Fassi & Fontana, 1966; Scannerini & Palenzona, 1967; Wilcox, 1968; Fortin et al., 1980) or electron microscopy (Foster & Marks, 1966; Marks & Foster, 1967; Scannerini, 1968; Hofsten, 1969). Extramatrical mycelia, although neglected by most authors, were described, in particular in the rhizosphere (Foster & Marks, 1967) and when formed of rhizomorphs (Duddridge et al., 1980; Foster, 1981; Brownlee et al., 1983). This is the reason why only poorly described features of mycorrhizae as well as root decomposition patterns will be illustrated here.

Living roots

- Coralloid orange-brown ectomycorrhizae

These mycorrhizae, already observed in L₂, were characterised by repeated dichotomous growth, stemming (not always, but often) in coralloid masses. The observation of the fungal mantle in tangential section allowed me to discern its prosenchymatous nature: it was formed of cemented hyphae, not separable with forceps. The thickness of the mantle was highly variable, sometimes of only one cell layer. It disappeared towards the apex of mycorrhizal branches. Contrary to external colonisation, internal colonisation was pronounced, even towards apices, with a 1-2 cell thick fungal Hartig net separating hypertrophied cortical cells of pine. Starch grains abounded at the inside of hypertrophied cortical cells. The peripheral cortical zone, just under the fungal mantle, was comprised of two layers of 'tannin' cells. In the peripheral zone of the fungal mantle the prosenchyma passed progressively (hyphae were more and more discernible, not cemented) to a woolly extramatrical mycelium made of smooth hyphae (3 µm diameter) with clamp connections, i.e. to the dicaryotic secondary mycelium of a typical basidiomycete. Within the limits of this purely morphological classification, coralloid orange-brown ectomycorrhizae can be classified in the type A_c of Dominik (1969).

The link between the smooth extramatrical mycelium of coralloid orange-brown ectomycorrhizae, the encrusted hyphae embedding pine needles and moss shoots and the white-rot fungus identified as *Hyphodontia* sp. (*arguta-pallidula-alutaria* group) was indirectly established through the observation of several anatomical characters. First, hyphal diameter, size and shape of clamp connections, ramification angle and wall thickness were identical between both mycelial forms. Second, the passage from smooth to encrusted zones was observed along the same hyphae (Fig. 240), pointing to versatility of oxalic acid excretion across hyphal walls. Third, a lagenocystidium was observed in the extramatrical mycelium of coralloid orange-brown ectomycorrhizae (Fig. 241), quite identical to cystidia observed in decaying branchwood (Figs. 120 & 121). This fungus was thus suspected to be both mycorrhizal and saproxylic, a behaviour which is not uncommon in Corticiaceae (Käärik & Rennerfelt, 1957; Vozzo & Hacskeylo, 1971).

- Black ectomycorrhizae

These jet-black ectomycorrhizae, unambiguously produced by the sterile ascomycete *Cenococcum geophilum*, corresponded to the G_a type of Dominik (1969). They were described under various names by many authors such as Hatch (1934) under *Mycelium radices nigro-strigosum*, Mikola (1948), Trappe (1971), Kiffer (1974) and Voiry (1981) under the name *Cenococcum graniforme*. Marks & Foster (1967) described quite identical black mycorrhizae but without naming the fungus.

The external morphology of black ectomycorrhizae was variable. They were either club-like (Fig. 227) or slightly dichotomous (Fig. 226) but never ramified. Straight hyphae merging at right angle from the mycorrhiza were typical of the 'jet-black mycelium' of Hatch (1934). In light microscopy, the thick fungal mantle appeared pseudo-parenchymatous, being formed of four layers of septate melanised hyphae, cemented in palisade arrangement. Contrary to the previous coralloid type, the thickness of the fungal mantle increased towards the apex. Melanisation of mantle cells decreased towards the interior of the mycorrhiza. The hyaline Hartig net was thinner than in the coralloid type, being formed of only one cell layer. Only one layer of host 'tannin' cells was formed under the mantle. Below it there was an external zone formed of dead parenchymatous cells, followed at the inside by alternating living and dead cortical cells. Thereby internal colonisation by the fungus was less

pronounced than in the coralloid type, but the host seemed more affected by *Cenococcum geophilum* than by *Hyphodontia sp.*

I often observed, in the proximal part of black ectomycorrhizae where the fungal mantle of *Cenococcum geophilum* was absent, an imperfect fungal mantle formed by the basidiomycete *Hyphodontia sp.*, hyphae of which were not cemented and bore clamp connections. When sectioned at this level (identified by a constriction of the cortex), black mycorrhizae showed the 2-cell Hartig net and the double layer of 'tannin' cells typical of orange-brown coralloid mycorrhizae, testifying for internal colonisation of the root by *Hyphodontia sp.* The coexistence of two fungi on the same mycorrhizal root has been already described by Marks & Foster (1967), who explained it by a successional process. The coexistence of *Cenococcum geophilum* and *Hyphodontia sp.* on the same short root was probably made possible by their different ratio of external and internal colonisation.

The mycelium of *Cenococcum geophilum* was comprised of melanised hyphae with thick walls (generally diameter 4-5 μm) most often smooth (Figs. 232 & 238) but sometimes encrusted with minute bumps (Fig. 242). Although not the rule (contrary to the inside of pine needles), the abrupt passage to melanin-free and thinner hyphae has been observed in the aerial mycelium, only intra-hyphal walls remaining impregnated with melanin (Fig. 243).

- Pink ectomycorrhizae

These mycorrhizae, of velvet aspect and always dichotomous (Fig. 228), bore a thick mantle which was strongly variable in its anatomical features, making their classification difficult according to Dominik (1969). When the mantle was pseudo-parenchymatous (true plectenchyma) they belonged to the F_e type but when the mantle was prosenchymatous, with recognizable hyphae (Fig. 244), they belonged to the B_e type. This pinpoints the awkward generalization of Dominik's classification. Melanised hyphae of *Cenococcum geophilum* were sometimes observed penetrating the mantle of pink ectomycorrhizae. The Hartig net was strongly developed, but only one layer of 'tannin' cells was observed in the cortex. Just below the 'tannin' layer brown spherules were often observed at the inside of cortical cells (Fig. 245), quite similar to those observed at the inside of pine needles (Figs. 104, 105 & 235). The mantle became thicker towards the apex, but Hartig net and 'tannin' cells disappeared, pointing to external colonisation only in the apical part of pink mycorrhizae.

Tangential sections revealed the prosenchymatous nature of the mantle, with numerous clamp connections (Fig. 246). Pink mycorrhizae were smooth, thus not in direct association with an extramatrical mycelium. However some aerial hyphae exhibited the same anatomical characters as those hyphae found in the mantle: presence of clamp connections, smooth thin hyphal walls of diameter 4 μm , i.e. more than those of *Hyphodontia sp.* The same mycelium was found embedding dead moss in the L_2 layer (Fig. 131), and decaying plant debris and animal faeces in the F_1 layer (Fig. 255), in association with thin encrusted hyaline hyphae of *Hyphodontia sp.* and thick melanised hyphae of *Cenococcum geophilum*.

- Long roots

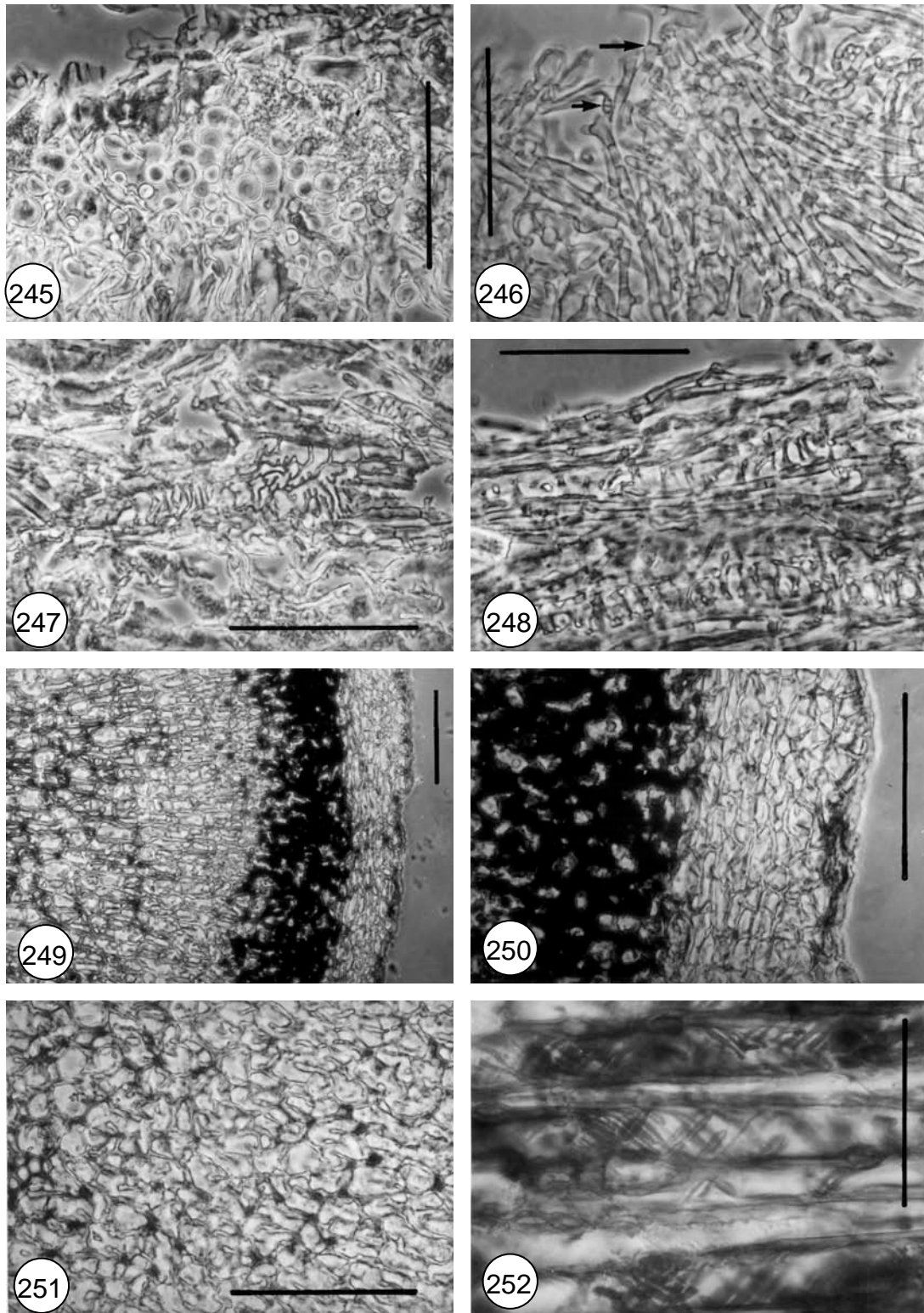


Fig. 245: Pink pine ectomycorrhiza, detail of the cortex, showing brown spherules in cortical cells (CP); Fig. 246: Idem, tangential section through the prosenchymatous fungal mantle with clamp connections (arrows) (CP); Fig. 247: Living long root of pine, section through the cortex showing a Hartig net (CP); Fig. 248: Decaying long root of pine, longitudinal section through the central cylinder showing the intracellular development of basidiomycetous hyphae (with clamp connections) of *Hyphodontia* sp. (CP); Fig. 249: Sclerotium of *Cenococcum geophilum*, overview of the section (CP); Fig. 250: Idem, detail of the wall (CP); Fig. 251: Idem, detail of the heart (CP); Fig. 252: Decaying pine twig, longitudinal section through the wood showing a soft-rot type of fungal attack of tracheids (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; length of segment = 50 μ m

Hair roots were rarely observed: they were located just behind the growth (elongation) zone. In this case no sign of fungal colonisation was visible, in particular there was neither Hartig net nor 'tannin' cells.

Even in the absence of externally visible sign, most long roots were colonised by mycorrhizal fungi. The internal development of *Hyphodontia sp.* was not accompanied by the formation of a mantle (except when a very thin fungal mantle was formed) but the Hartig net was present, like in true ectomycorrhizae (Fig. 247). The longitudinal penetration of this fungus was observed in cortical cells and in the endodermis, but the central cylinder was intact, with nuclei well visible within parenchymatous cells. No 'tannin' cell layer was observed in the cortex of long roots colonised by *Hyphodontia sp.*

The colonisation of long roots by *Cenococcum geophilum* was attested by the presence of a black mantle, with its typical palisade assemblage of fungal hyphae (like in black mycorrhizae), or by the presence of free hyphae running longitudinally along the root, giving it a brown colour (which could be erroneously interpreted as a sign of decay). At the inside of the root, the Hartig net was well developed, but cortical cells were not hypertrophied and 'tannin' cells were absent. A root, the apex of which had been colonised by *Cenococcum geophilum* (Fig. 231), showed under the fungal mantle an intact meristem, with numerous nuclei and the total absence of any internal colonisation by the fungus. I interpreted this pattern as the result of an arrest of growth of the pine root, maybe temporary, which was followed by superficial overgrowth of its apex by the mycorrhizal fungus. Check of the growth of the root was attested by the lack of the typical conical shape of the apex which was observed when roots were actively growing (Fig. 227).

It must be highlighted that mycorrhizal colonisation did not affect only true mycorrhizae (more or less transformed short roots) but also the long roots which bear them (Marks & Foster, 1973). In the case of *Hyphodontia sp.*, intracellular hyphae were observed in the cortex, which could be hypothesized to affect the uptake of water and nutrients by long roots. Robertson (1954) showed that the internal colonisation of short roots by mycorrhizal fungi did not spread from the mantle but rather from previously infected long roots. The colonisation of long roots by ectomycorrhizal fungi (in our case by *Hyphodontia sp.*) strongly resembles what has been described under the term 'ectendomycorrhizae' by Wilcox (1968, 1971).

Decaying roots

- Short roots

Decay patterns were observed only on coralloid orange-brown ectomycorrhizae. They turned neat brown. In all stages of decomposition I observed, no bacterial development was visible in internal (host) tissues. In particular, the cortex remained intact with its typical Hartig net and 'tannin' cells. Only the fungal mantle seemed affected by decay. Several stages of decomposition of the fungal mantle were observed, (i) mantle hyphae were devoid of cytoplasm, the contour of external hyphae

becoming fuzzy, indicating decay of their walls, (ii) the fungal mantle was the seat of an intense bacterial and algal development, hyphae becoming hardly recognizable, (iii) concomitant or succeeding to it, a stage where the mantle was invaded by other fungi, among which the basidiomycetous mycelium associated with pink ectomycorrhizae.

- Long roots

The incipient decay of long roots was characterised by the development of the secondary mycelium of *Hyphodontia sp.* in the central cylinder (Fig. 248). Some decaying long roots showed a joint development of *Hyphodontia sp.* and *Cenococcum geophilum*. The latter fungus was also observed at the surface of the decaying root, with its hyphae embedding it. The first stage of long root decomposition seemed to be the development in the stele of 'mycorrhizal' fungi, the only stage observed in this layer.

It must be noticed that, to the light of present observations, long roots and short roots seemed to behave quite differently concerning mycorrhization and processes associated with death of the root.

- Sclerotia of *Cenococcum geophilum*

The presence of sclerotia of *Cenococcum geophilum* has been widely attested in dysmoder humus, whether called 'mor' or 'raw humus' by authors (Ferdinandson & Winge, 1925; Meyer, 1964). In the present sample, they were in the form of near perfect spheres, of varying size, from which many hyphae radiated (Fig. 229). When sectioned they showed a three-layered wall and a more or less homogeneous heart (Fig. 249). The wall (Fig. 250) was comprised of (i) a 1-cell external layer, with thin melanised cell walls [analogous in tangential view to the mycorrhizal mantle according to Trappe (1971)], (ii) a 7-10-cell medium layer, arranged in circular files of cells, the walls of which were thin but less melanised than those of the external layer, and (iii) an internal 10-cell layer made of thick-walled, deep black cells with strongly reduced lumina and poorly observable cell limits. The internal layer was responsible for darkness and toughness of sclerotia. The sclerotial heart (Fig. 251) was made of radial files of cells, with thin slightly melanised walls. Meatuses between heart cells were filled with melanised wall material. I did not find hollow sclerotia of *Cenococcum geophilum*, but Ferdinandson & Winge (1925) noted that this phenomenon was strongly seasonal, preceding the 'germination' of sclerotia in spring.

Pine twigs

Preliminary remarks

The pine twigs which were present in the F₁ sample were about 2^{1/2}-yr-old and probably fell during previous winter. Two features must be highlighted. First, decay was in an advanced stage, under the joint activity of animals and fungi. Second, basidiomycetes, which had not been observed in pine twigs in the two overlying layers, now invaded all twigs found in F₁. For some of them, this was also the case for the ascomycete *Cenococcum geophilum*.

Decay processes observed in bark and wood seemed quite independent of each other, thus they will be studied apart although these superposed tissues pertained to the same twig fragments.

Wood

- Penetration by basidiomycetes without any attack of lignified walls

I observed several fungi at the inside of tracheids: a basidiomycete with thin smooth hyphae, which could be *Hyphodontia* sp. (associated with orange-brown mycorrhizae), a basidiomycete with smooth larger hyphae, which could be the one associated with pink mycorrhizae, and *Cenococcum geophilum*. Previous infestations were attested by the presence of empty melanised hyphae in vascular rays, a stage of fungal succession which was described in L₁ (Fig. 37), and pycnidia and acervuli in the peripheral bark.

- Penetration by fungi and bacteria with soft-rot attack

About half of the studied twigs showed signs of soft-rot attack, as attested by typical features (Fig. 252) which have been described by many authors (Bailey & Vestal, 1937; Courtois, 1963; Corbett & Levy, 1963; Corbett, 1965; Casagrande & Ouellette, 1971; Nilsson, 1973, 1974). Cavities were formed in cell walls of metaxylem tracheids, following the orientation of fibres, optically crossing at acute angle between adjacent tracheids (Fig. 252). Constricted micro-hyphae were sometimes observed out of abovementioned cavities. Some wood microbiologists asked why cavities were preferentially formed in some places, not in others. The answer lies in structural irregularities of tracheid walls, as exemplified in Figure 253, showing a portion of pine twig wood still not attacked by soft-rot fungi: micro-fibres (not visible at the used magnification) are assembled in fascicles, which are separated from each other at some places, and are joined at others. I hypothesize that soft-rot fungi preferentially used these open spaces for initiating the formation of cavities through external enzymic activity.

Pine twigs which exhibited features of soft-rot attack showed also an abundant development of other microorganisms, such as the three 'mycorrhizal' fungi already noted in the absence of soft-rot, and many bacteria which coexisted with fungi in the lumen of tracheids (Fig. 253). No faunal activity was registered at the inside of pine twigs, thereby indicating some delay in their decomposition, when compared with needles.

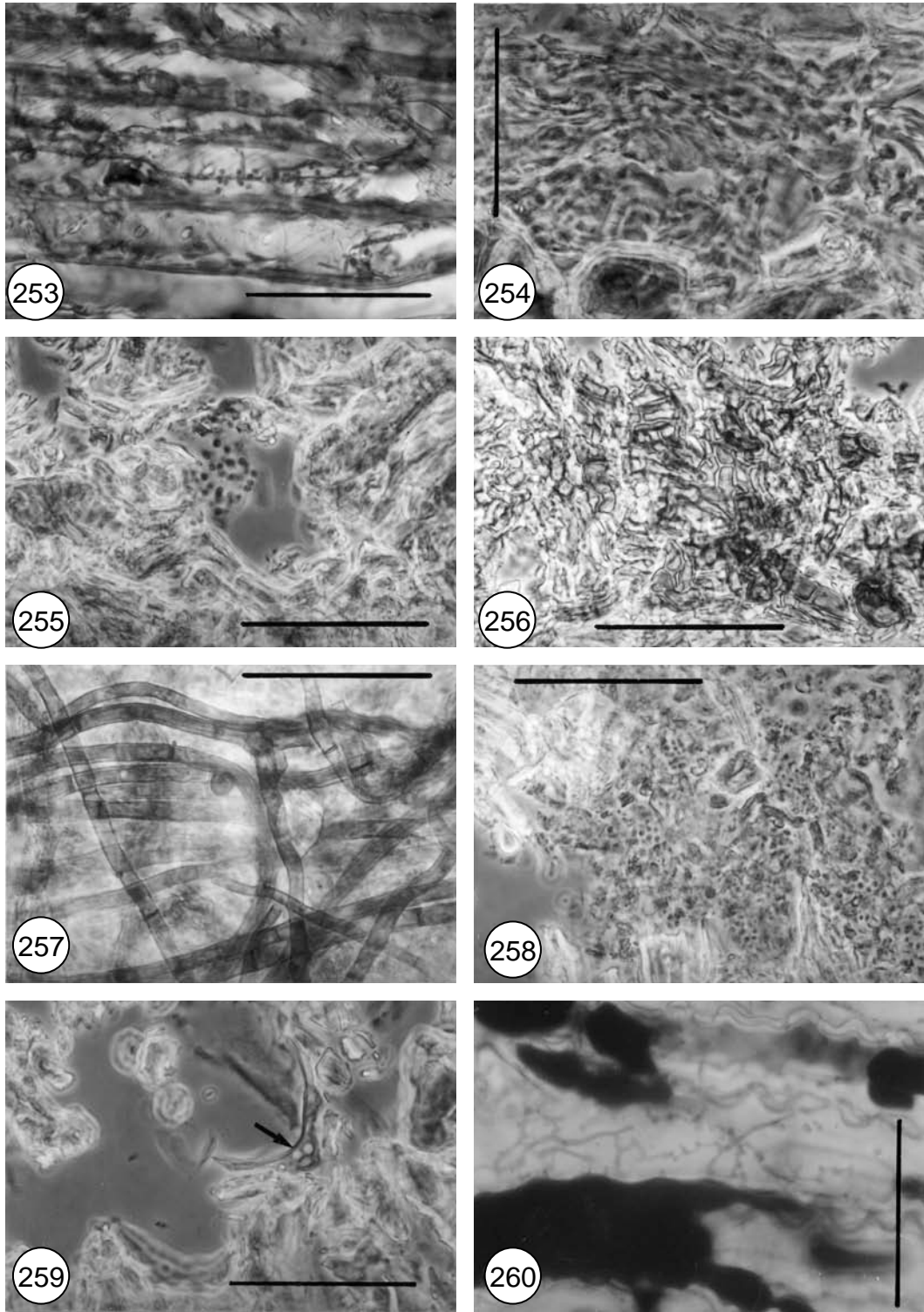


Fig. 253: Decaying pine twig, longitudinal section showing fungi and bacteria at the inside of wood tracheids (BMSL); Fig. 254: Idem, showing cortical tissues invaded by a fungus forming white rhizomorphs (CP); Fig. 255: Idem, showing a micro-site with bacteria in bark (CP); Fig. 256: Idem, showing the pseudoparenchyma of *Cenococcum geophilum* at the surface of bark (CP); Fig. 257: Idem, optical section of a thin bark fragment showing the mycelium of *Cenococcum geophilum* embedding it (CP); Fig. 258: Idem, section in a thick bark fragment showing bacterial development in a bark crevice (CP); Fig. 259: Idem, section showing holes of fungal origin in a cell wall piece (arrow) (CP); Fig. 260: Pine seed wing, optical section showing intracellular fungal hyphae (stained blue) and pigment deposits (opaque to natural light) (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; length of segment = 50 μ m

Bark

White rhizomorphs were often observed protruding from the bark of pine twigs. When sectioned (Fig. 254), cortical tissues appeared invaded by an unidentified fungus and host cells were transformed into a pulp, quite similar to pine needles penetrated by *Marasmius androsaceus* (Fig. 80). Only resin subsisted within this cellular pulp, in which limits of fungal cells were hardly visible. Despite of the fact that clamp connections were not observed, the basidiomycete nature of the fungus was highly probable.

Numerous micro-sites harboured bacterial colonies (Fig. 255). Protozoa, in particular cyst amoebae such as *Nebela collaris*, were frequently observed in micro-sites colonised by bacteria.

Pine twig bark was the seat of an intense activity of box mites [*Rhysotritia duplicata* (Michael), *Phthiracarus* sp., see below the chapter devoted to fauna], which deposited their faeces either in surface cavities (directly visible from the outside) or at the inside of bark tissues (visible only in sections). Enchytraeid faeces accumulated between bark and wood: their microscopic observation revealed the presence of pieces of resin, as this had been already observed in L₂ (Figs. 125, 147 & 163). Enchytraeids did not seem to be able to tunnel through bark, which was probably tough, too much for their soft mouth parts, limiting their penetration to liber and spaces between bark layers. Rotifers were found in the midst of enchytraeid faeces accumulated between bark and wood, but the observation of their gut contents did not reveal any other items than their intestinal microflora (Fig. 291).

The surface of bark was often covered by a dense network of melanised hyphae of *Cenococcum geophilum*, which in some places may form a pseudoparenchyma (Fig. 256), quite similar to the mantle of jet-black ectomycorrhizae.

Contrary to wood, the bark of pine twigs was the seat of an intense bacterial and faunal activity.

Pine bark

Notice

Pine bark means fragments of bark which fell detached from trunk of branches of pine, not bark still present around pine twigs, which had been studied above.

Thin bark fragments

These fragments correspond to the detachment of stem epidermis, which may fall on the ground together with some bark sheets still attached to it. They were characterised by the development of the mycelium of *Cenococcum geophilum* (Fig. 257), or that of *Hyphodontia sp.*, which embedded them in a fungal 'mantle', but the internal development of these fungi was never observed. Traces of previous fungal development were still visible (pycnidia, acervuli) but these fructifications were empty and could not be used to identify previous colonisers. However, I want to notice the presence of a melanised imperfect fungus with septate conidiophores and numerous dictyospores (septate conidia), which could be *Spadicoides xylogenum* (A.L. Smith) (Roquebert, personal communication). Below this fungus 'mantle' and in crevices of bark layers there was an intense bacterial development (Fig. 258).

Some bark fragments were covered with mucus, left after the passage of slugs or earthworms. Mucus was strongly transformed, showing numerous senesced or dead fungal hyphae (Fig. 281) and bacteria (Fig. 282). Naked amoebae were also often observed. Mucus was embedded in the abovementioned fungal network, a feature indicating its ancientness. Earthworm faeces (type C) were also observed tightly appressed against bark pieces. White rhizomorphs were present in contact with bark, but no penetration was observed, contrary to pine twigs.

No sign of biological activity (whether faunal or microbial) was found at the inside of thin bark pieces (when sectioned), contrary to surface (in particular mucus deposits) and crevices.

Thick bark fragments

An intense bacterial development was observed at the surface, quite similar to that observed on thin bark pieces. Bacteria were never found at the inside but traces of past fungal activity were still present. Cell walls were degraded, only debris being visible, with ancient holes due to the passage of fungal hyphae (Fig. 259). This was reminiscent of soft-rot activity, although this term was used only for wood. Dead and living hyaline hyphae were present, as ascertained by phase contrast (Frankland, 1974). More often, pieces of resin were still present among remnants of pulverised debris of ancient suberised cell walls.

It could be questioned why bacteria were so abundant at the surface of bark pieces and in bark crevices and were near absent at the inside of the same fragments. It is possible that resin and other chemical compounds which impregnate bark exert an antibiotic activity against bacteria but are tolerated by some fungal strains. Updegraff & Grant (1975) showed that, among 200 microbial strains isolated from the bark of *Pinus radiata* after previous enrichment, only 36 fungi imperfecti were able to grow on culture medium added with bark extract. Most of these fungi were able to degrade phenolic compounds which comprised a prominent part of bark (39% dry weight for phenolic acids, 60% when all ethanol-extractable compounds are taken into account). Not any bacteria or yeast was able to grow in such conditions.

Pine euphylls

Most euphylls showed zones browsed by fauna, with oribatid mite faeces deposited at their surface, thereby indicating the animal group responsible for faunal attacks. The microscopic examination of these faeces indicated that euphylls had been ingested. The epidermis was often the only remnant of euphyll tissues observed in browsed areas. The three 'ectomycorrhizal' fungi which had been repeatedly observed embedding decaying plant material were present as hyphae running at the surface of pine euphylls. Apart from their local consumption by oribatid mites, pine euphylls found in F₁ did not differ to a great extent from those found in L₂ (Figs. 113 & 114), which was probably due to their penetration through L₁ and L₂ till F₁. This might indicate that the fact that this substrate was ingested by animals in F₁ but not in L₂ was not due to insufficient conditioning by microflora in L₂ but rather to ecophysiological requirements of soil invertebrates, which were only fulfilled in the deeper F₁ layer where most of them were living (see Table 8 and compare with Tables 3 & 5).

Male flowers of pine

Most flowers and pollen grains found in F₁ were seemingly intact or only poorly decayed. In particular, pollen grains, which were often aggregated, were devoid of any bacteria or fungi. This was probably due to their drop through L₁ and L₂, as for pine euphylls. Most flower bracts showed cells with strong granulation but some cells were penetrated by tiny fungal hyphae, indicating incipient fungal colonisation. Such bracts starting to decay were generally embedded in an abundant fungal mycelium, at the inside of which bacteria were seen, but no bacterial development was seen at the inside of bracts.

It should be noticed that many pollen grains were observed at the inside of type C (earthworm) faeces, indicating that the activity of these animals in the F₁ layer mainly took place in last spring-early summer (June-July). On the contrary, pollen grains were not observed in faeces of type A (slugs?) and B (woodlice?), the deposition of which occurred probably in winter or early spring.

Miscellaneous pine material

Other pine remains were found in F₁, although only in weak amounts. Among them, bud scales most probably dropped through overlying moss and pine needles after they fell in April-May. Such tender material was nibbled by oribatids, which deposited their faeces below the external epidermis which subsisted after consumption of more tender tissues.

Pine seeds were also found. They were probably dispersed by wind, since no female cone of fragment of cone was found in their vicinity. Only seed tegument was still present, the albumen being entirely ingested by fauna. The microscopic observation of the tegument was made difficult by the presence of opaque brown-red pigments in some cells. The internal surface of the tegument was covered by numerous bacteria and actinomycetes. The very fine seed wing (made of a monocellular layer) could be observed by transparency and showed a network of intracellular fungal hyphae, some of them were senescent (Fig. 260).

Leafy shoots of moss (*Pseudoscleropodium purum*)

Preliminary remarks

Moss stems and leaves were always abundantly covered by a mixed mycelium made of hyaline encrusted hyphae of *Hyphodontia sp.* and melanised hyphae of *Cenococcum geophilum*, the latter fungus being sometimes observed alone.

Leaves

- Attack of soft-rot type

Thin elongated moss cells were invaded by fungal hyphae (without any clamp connection) which often filled them near entirely (Fig. 261). They were connected from a cell to another by microhyphae. This stage of fungal colonisation evolved either towards (i) a bacterial attack of fungal hyphae, the cytoplasm of which disappeared (lack of methyl blue staining), most cells being invaded by numerous bacterial rods (Fig. 262), or (ii) fragmentation and condensation of fungal cytoplasm (Fig. 263). The attack of moss cell walls was particularly visible when they were thicker (Fig. 264).

- Attack of white-rot type

Moss cells were invaded by basidiomycetous hyphae with clamp connections. Moss cell walls were thinned and became highly transparent (Fig. 265). No bacterial development was visible. Under the dissecting microscope, such leaves seemed strongly transformed, having lost rigidity and planar form.

From the beginning of the study, this was the first time moss cell walls appeared to be microbially attacked. However, it must be noticed that at this stage most leafy shoots of moss had lost their leaves, which had been ingested by fauna, leaf insertions still remaining well visible along stems. In this case, the decay of moss leaves followed another processing chain (see below, studies on fauna).

Stems

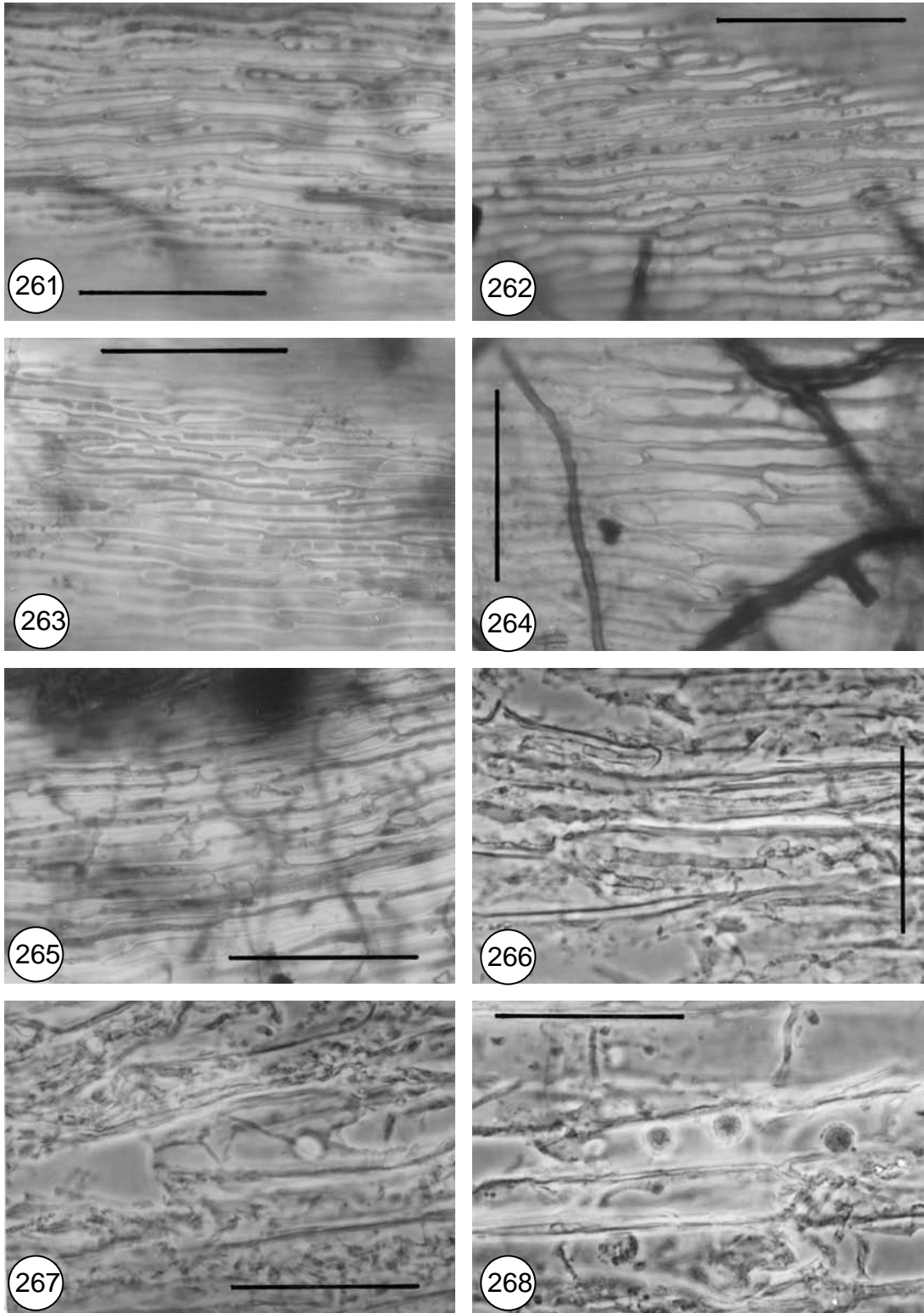


Fig. 261: Decaying moss leaf, detail showing fungal hyphae filling the inside of cells (BMSL); Fig. 262: Idem, showing cells invaded by rod bacteria (BMSL); Fig. 263: Idem, showing fragmentation and condensation of fungal cytoplasm (BMSL); Fig. 264: Idem, showing the irregular attack of moss cell walls (BMSL); Fig. 265: Idem, showing continuously thinned transparent cell walls and basidiomycetous hyphae at the inside (BMSL); Fig. 266: Decaying moss stem, detail showing dead (empty) basidiomycetous hyphae at the inside of moss cells (CP); Fig. 267: Idem, showing a living basidiomycetous hypha penetrating a moss cell through a pit (CP); Fig. 268: Idem, showing cysts of naked amoeba at the inside of moss cells (CP)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; length of segment = 50 μ m

- Notice

The internal anatomy of moss stems, which I studied through the observation of sections, showed an external layer of thick-walled cells, of collenchyma type, ensuring rigidity of the stem. The internal parenchyma was made of cells with much thinner walls. Pits corresponding to local thinning of the wall were observed (Figs. 267 & 269). A variety of decay stages of stem parenchyma could be observed in the F₁ layer.

- Cells with cell contents still present

The content of parenchymatous cells was granular, sometimes displaying remains of chloroplasts. This resembled what had been observed at the inside of male flower bracts. This was probably due to an autolytic process without any microbial involvement, at least at the beginning. However, it must be noticed that at this incipient stage cell walls became brown in the central part of the stem.

- Penetration of fungal hyphae

Moss stems were penetrated by basidiomycetes, the presence of which was attested, most often by empty fungal hyphae (Fig. 266), but sometimes hyphae were still living at the time of sampling, passing from a cell to another through pits, i.e. circular zones where the wall of moss cells was much thinner (Fig. 267). Moss cell contents (granulation) disappeared following fungal colonisation but at this stage walls did not seem to be affected at all. This decay stage might coexist with the previous stage, either in another part of the same stem or in the same part but in different cells. Living fungal hyphae were only found in the latter case, pointing to a succession between both stages even though all cells or stem parts did not evolve to the same rate. From their diameter and ramification type, basidiomycetous hyphae resembled those of the pink mycorrhizal fungus, but without any due certitude, morphological convergence being possible in the absence of more specific characters.

- Penetration of microfauna

Several types of microfauna were seen at the inside of internal parenchymatous tissues of moss stems. Their penetration was made possible through the numerous breaks caused by the activity of macrofauna. The presence of numerous type C (earthworm) faeces appressed against stem fragments and the abundance of moss material within them showed that the ingestion of moss by earthworms was prominent at this stage. Nematodes (bacteriophagous Plectidae were identified) were

observed in the central lumen and cells were penetrated by naked amoebae, the presence of which was attested by cysts (Fig. 268) and trophozoites (Fig. 269). It is possible that pits could be used by these tiny animals to pass from a cell to another, like fungi did (Fig. 267), but this was not supported by any definite observation. The colonisation of moss stems by microfauna was accompanied by bacterial (Fig. 270) and algal (cyanobacterial) development (Fig. 271) at the inside of parenchymatous cells. These stems, when observed under a dissecting microscope, exhibited a definite change of colour, passing from yellow to red, which could not be perceived on mounted sections. It should also be noticed that the penetration of hyphae of *Cenococcum geophilum* was observed in the central lumen of some decaying stems.

- Hollow moss stems

Some stems exhibited the total disappearance of their parenchymatous tissues, probably because they had been ingested by microfauna or mesofauna, but no deposition of faeces was observed, letting the question unsolved. Only the external layer of thick-walled cells remained intact, taking a brown colour which was well visible on the slides. Some remnants of parenchymatous cells were still attached to the internal side of this tough tissue. A rhizomorph-like fungal tissue, made of cemented empty hyphae, was often observed. Stems belonging to this decay stage were typically light-brown and transparent.

- Concluding remarks

There was an analogy between successional stages observed during the decay of moss stems and those of pine needles, although the former process seemed to be delayed and condensed: the whole variety of decay stages observed on pine needles from L₁ to F₁ were observed on moss stems in the only F₁ layer. Rather than a succession from canopy fungi colonising senescent needles (*Lophodermium pinastri*) to litter-dwelling saprophytes colonising dead needles (*Verticicladium trifidum*), 'ectomycorrhizal' basidiomycetes arrived directly at the inside of dead moss stems. The external collenchyma-like supporting tissue played a role similar to the epidermis-hypodermis external envelope of pine needles, allowing these 'tubes' to be voided by soil animals before complete disappearance. In both cases, bacterial development followed penetration by fauna and played only a secondary role in decomposition, at least concerning the physical disappearance of plant material.

Bracken fronds (*Pteridium aquilinum*)

Bracken leaflets

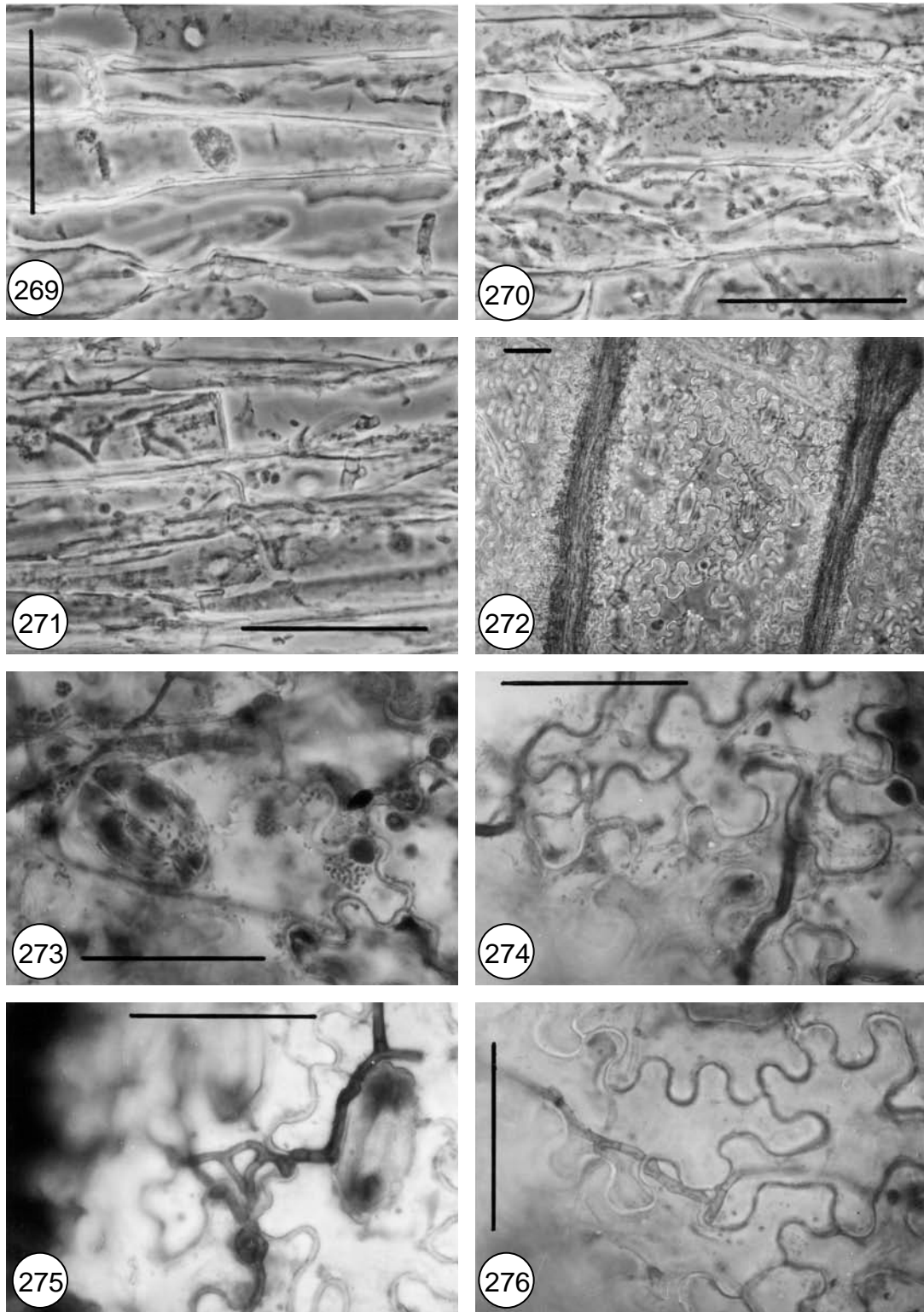


Fig. 269: Decaying moss stem, detail showing a trophozoite of naked amoeba (CP); Fig. 270: Idem, showing bacterial development (CP); Fig. 271: Idem, showing development of Cyanophyceae (CP); Fig. 272: Decaying bracken leaflet, detail showing upper epidermis still subsisting between veins (CP); Fig. 273: Idem, showing bacterial development in epidermal and stomatal guard cells (BMSL); Fig. 274: Idem, showing empty fungal hyphae (CP); Fig. 275: Idem, showing melanised hyphae of *Cenococcum geophilum* penetrating epidermal cells (LN); Fig. 276: Idem, showing lack of melanisation (LN)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; LN = natural light without coloration; length of segment = 50 μm

Most leaflets were reduced to their network of veins, with the upper epidermis often subsisting (Fig. 272). This resulted from the browsing activity of fauna, as attested by the deposition of faeces of oribatid mites, as will be seen below. In some particular cases, leaflet limbs were still preserved: this was the case when several leaflets were tightly packed in bundles into which they were firmly appressed against each other. It can be hypothesized that in this case some leaflets were out of reach of soil animals, in particular of mesofauna. More generally, decaying leaflets or bundles of leaflets were embedded in a mycelial weft formed by 'ectomycorrhizal' fungi, in particular encrusted hyphae of *Hyphodontia sp.* Abundant and varied microfauna were observed at the surface of bracken leaflets: nematodes, naked amoebae, cyst amoebae. Observations on the internal colonisation were made by transparency, without any need to section bracken leaflets. Two decay stages were observed, independently of the consumption of leaflet limbs by soil invertebrates.

- Bacterial stage

Epidermal and stomatal guard cells were invaded by bacterial colonies (Fig. 273). Previous colonisation by fungi was attested by the presence of empty hyphae (Fig. 274).

- *Cenococcum* stage

This fungus, which normally pertained to the mycelium embedding leaflets, in the company of *Hyphodontia sp.*, was seen penetrating epidermal cells (Figs. 275 & 276). The penetration of plant cells was accompanied by partial (Fig. 275) or total (Fig. 276) lack of melanisation. This morphological change of melanised hyphae was even more pronounced than during the colonisation of pine needles (Figs. 237 & 238) where host cells were not penetrated at all by this fungus. Haustoria (enlarged hyphae) were always formed before crossing a plant cell wall (Figs. 275 & 276). Other fungi were also observed in the company of *Cenococcum geophilum*, in particular an unidentified basidiomycete with clamp connections.

Rachises

Only fragments of secondary and tertiary rachises were found in the studied sample, without any true petiole (primary rachis). However, given that decay patterns did not differ in all fragments studied, whatever their size, it is highly probable that results of the present study may apply to bracken petioles, too. I always observed the partial or total disappearance of parenchyma, due to the intense tunnelling activity of mesofauna, as attested by their gut contents (see below). Microfauna were observed on plant sections, in particular the cyst amoebae *Corythion dubium*, and to a lesser extent *Nebela collaris*. Numerous cysts of protozoa were also observed. Invertebrate activity was characterized by the formation of cavities, in which animals were still present, together with plant and

fungal cell remains invaded by bacteria. Outside cavities, the parenchyma was invaded by bacteria (Fig. 277) and other microorganisms (algae, fungi?) which could not be identified (Fig. 278). Plant cell walls showed signs of ancient fungal attacks of soft-rot type (Fig. 279). Scalariform tracheids showed empty hyphae of the same basidiomycete described in L₂ (Fig. 143). The inner part of bracken rachises, often totally excavated by fauna, was colonised by the three 'ectomycorrhizal' fungi, *Cenococcum geophilum*, *Hyphodontia* sp. and the basidiomycete with wide hyphae which was associated with pink ectomycorrhizae. The latter fungus was also observed at the inside of scalariform tracheids, although it had not been observed in pine tracheids, maybe due to their much thinner section.

Birch (*Betula pubescens* Ehrh.)

Plant material of birch origin was but poorly represented: it was comprised of seeds (achenes) and small fragments of foliar limbs.

Leaves

Fragments of foliar limbs were thinned down and discoloured. Veins were intact, as well as the upper epidermis. Other leaf tissues had disappeared, probably eaten by invertebrates, to the exception of some remnants of palissadic parenchyma. Stomata were scarcely encountered, since most of them pertained to the lower epidermis, which has been lost (consumed). Traces of past intense fungal development were observed in the epidermis and in parenchymatous remains, in the form of empty hyphae and fructifications (ascocarps? pycnidia?) nibbled by fauna. Bacteria were present but not abundant. Birch leaf fragments were embedded by 'ectomycorrhizal' fungi in the same way as other plant litter debris. It seemed that the decomposition of birch leaves was arrested (when not consumed by fauna) at a stage where plant and fungal cytoplasm had totally disappeared but cell walls still remained untouched.

Achenes

Some achenes seemed intact and were probably viable. Others showed signs of recent fungal attacks (little penetration, living hyphae). From these features I hypothesized that birch achenes fell recently to the ground and rapidly crept down to the F₁ layer under the influence of summer rains. Decay of achenes started by the development of hyaline and melanised hyphae (*Cenococcum geophilum*) at the surface of the wings (Fig. 280). When sectioned, some seeds showed a few melanised hyphae (*Cenococcum geophilum*?) but bacteria were never found.

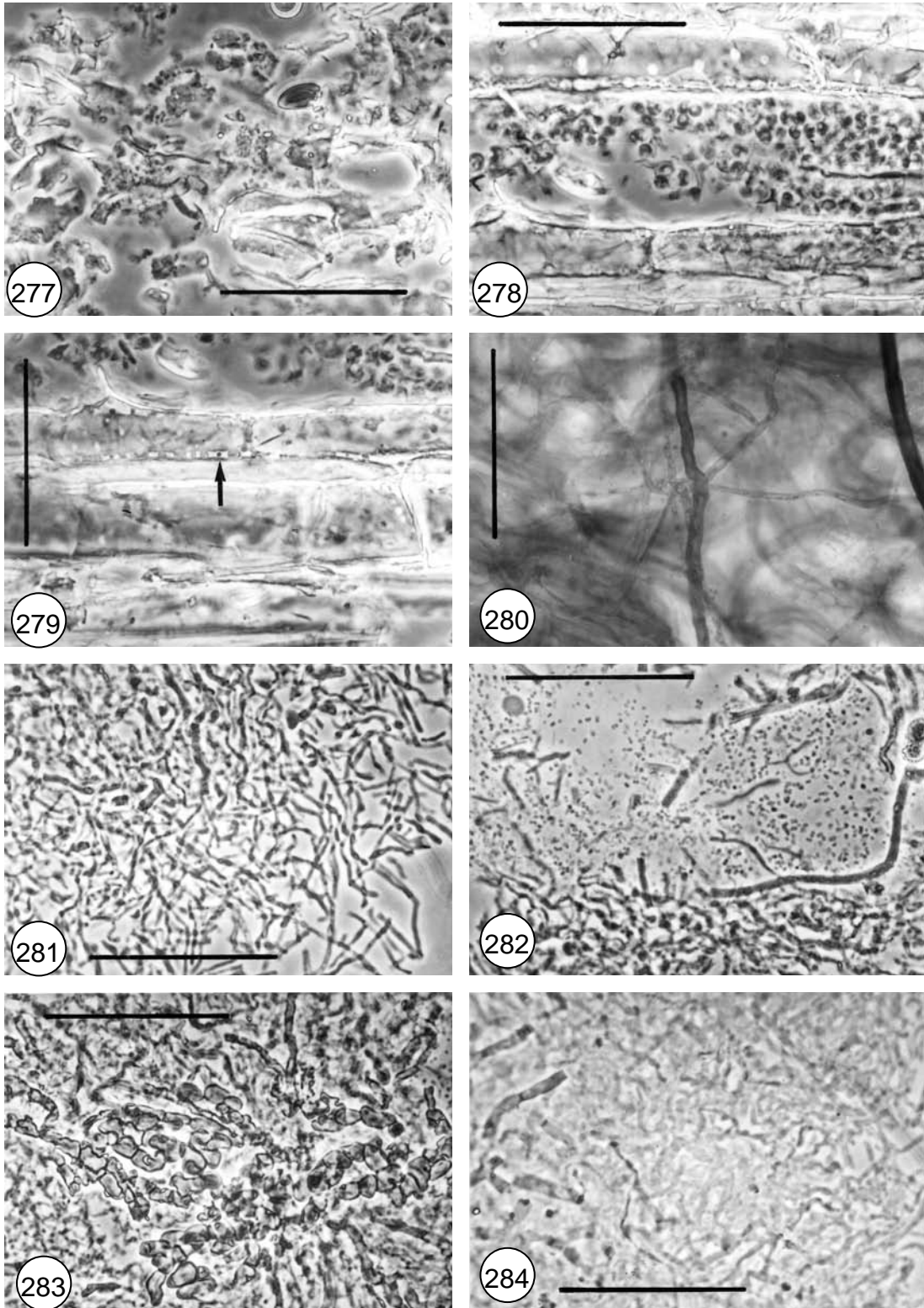


Fig. 277: Bracken rachis, section showing parenchyma invaded by bacteria (CP); Fig. 278: Idem, showing unidentified microorganisms in parenchymatous cells (CP); Fig. 279: Idem, showing ancient soft-rot type attacks of plant cell walls (arrow = living micro-hypha in transversal section, stained blue) (BMSL); Fig. 280: Birch achene, hyaline hyphae of fungi imperfecti and melanised hyphae of *Cenococcum geophilum* at the surface of seed wing (BMSL); Fig. 281: Mucus, detail showing living hyaline hyphae (CP); Fig. 282: Idem, showing pullulating bacteria in the peripheral part of mucus deposit (CP); Fig. 283: Idem, zone with melanised hyphae (CP); Fig. 284: Idem, hyaline hyphae in way to be degraded (CP)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; length of segment = 50 μm

Mucus

Preliminary remarks

Mucus was deposited either by slugs or by earthworms against a piece of plant material (more generally pine bark), along which animals crept. Mucus deposits where the seat of intense microbial development, but two stages were identified. In every case, they were embedded in melanised hyphae of *Cenococcum geophilum* (most often with encrusted walls as in Figure 242) and in hyaline hyphae of *Hyphodontia* sp. Many bacteria and green algae (Chlorophyceae) were seen at the surface of mucus deposits.

Fungal development

This fungus could not be identified, since it did not resemble any of the other mycelia found in the studied profile. Its irregular hyaline hyphae formed a dense felt (Fig. 281). They were living, as attested by their opacity in phase contrast. At the inside of this felt, there were zones where hyphae of the same fungus became melanised and fragmented, indicating incipient sporulation (Fig. 283). Bacteria pullulated in the peripheral part of mucus deposits (Fig. 282).

Decay of the fungus

Hyphal contours became fuzzy and fungal cytoplasm lost its opacity under phase contrast (Fig. 284). Echinulate arthrospores accumulated in melanised zones (Fig. 285). At this stage, mucus deposits were colonised by hyphae of *Cenococcum geophilum* and the whole material was invaded by bacteria (Fig. 285). However, it must be noticed that dense networks of decaying hyaline hyphae (Fig. 284) remained devoid of bacteria. I suggest that the incipient decay of hyaline hyphae was the result of an autogenic process and thus was not microbially mediated: bacterial invasion occurred only after fungal death. Mucus seemed an attractive, although selective substrate for microorganisms.

Rhizomorphs

White rhizomorphs were also observed protruding from bark fragments, twigs, and macroinvertebrate faeces. In transversal section (Fig. 286) no differentiation appeared between external and internal hyphae, contrary to brown rhizomorphs of *Marasmius androsaceus* in L₂ (Fig. 79). The presence of clamp connections and oxalic-acid excreting cystidia of particular form (Fig. 287) allowed identifying this fungus as *Odontia bicolor* (Alb. & Schwein.) according to Nobles (1953, 1965). Given that

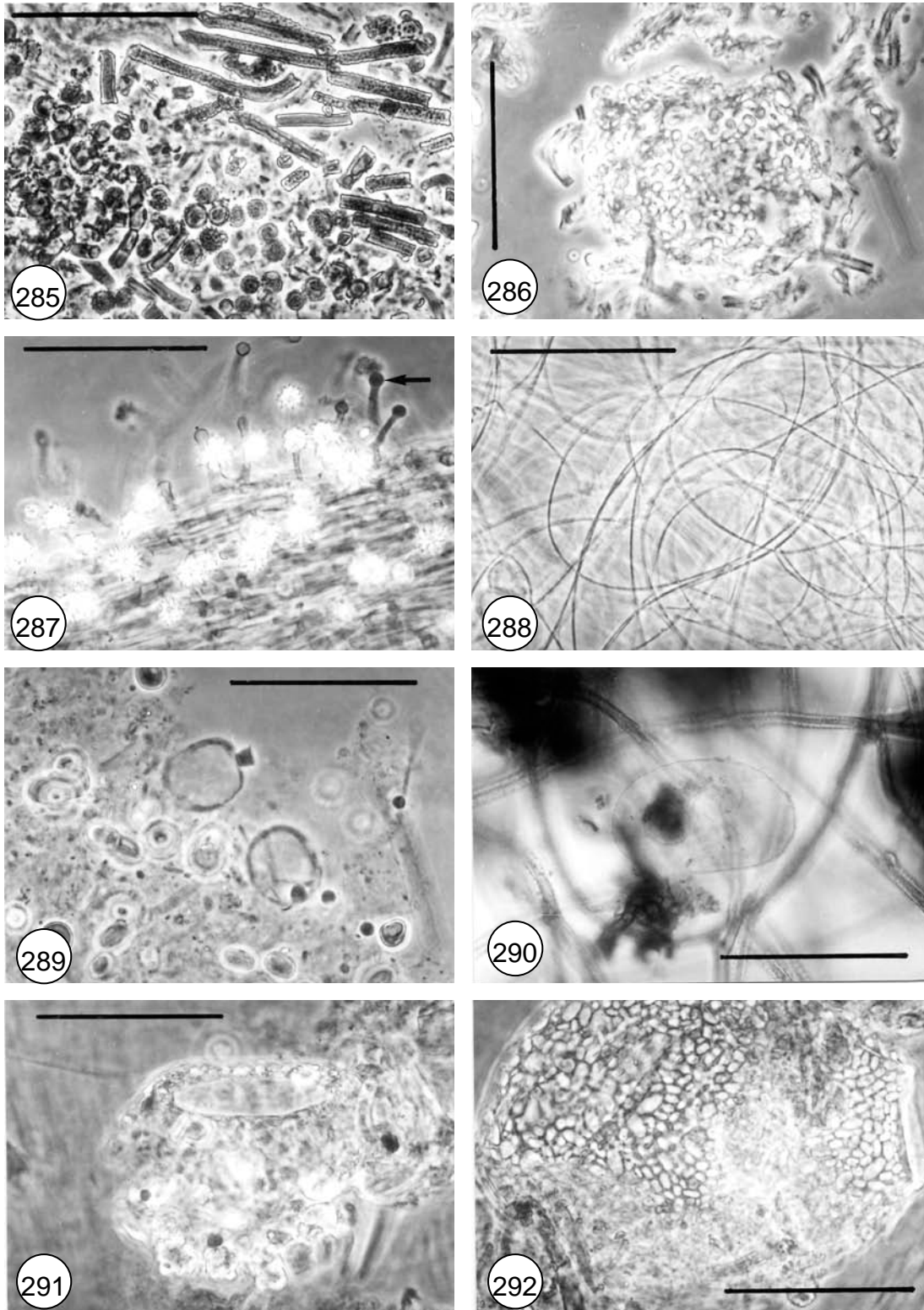


Fig. 285: Mucus, detail showing arthrospores, melanised encrusted hyphae of *Cenococcum geophilum* and bacteria (CP); Fig. 286: White rhizomorph protruding from pine bark, transversal section (CP); Fig. 287: Rhizomorph of *Odontia bicolor* with cystidia (arrow = cystidia having lost its oxalate crystals) (CP); Fig. 288: Spider silk cocoon (CP); Fig. 289: Shells of golden algae and bacteria carried on a springtail antenna (CP); Fig. 290: Trophozoite of naked amoeba in the mycelium embedding enchytraeid faeces (BMSL); Fig. 291: Cyst of *Phryganella acropodia* (Thecamoeba) in an enchytraeid intestine (CP); Fig. 292: Cyst of *Nebela collaris* (Thecamoeba) in an enchytraeid intestine

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; length of segment = 50 μ m

cystidia and clamp connections were not found on rhizomorphs of similar aspect and anatomy, all white rhizomorphs present in the same sample cannot be attributed with certitude to *Odontia bicolor*. In the same way, hyphae of *Odontia bicolor* resembled to a great extent to the mycelium of pink mycorrhizae, but no connection was observed between both mycelia and no cystidia of similar morphology were found around the mycorrhiza, letting the problem unsolved.

Quartz grains

Quartz grains were observed from place to place. Most of them were probably transported by soil animals. In comparison with L₂, more and bigger (sand size) quartz particles were found in F₁, thereby indicating prominent macrofaunal activity (mainly of earthworms). These grains were clean, indicating they provided mainly from the leached A₁₂ horizon (Table 1). No particular deposit was registered at the surface of quartz grains. Many sand grains were also present in animal faeces with mineral content as will be seen below.

Spider cocoons

Wefts made of non-cellular threads without any microbial colonisation (Fig. 288) were interpreted as empty spider cocoons.

Colonies of Chrysophyceae (golden algae)

I often observed gelatinous masses rich in bacteria, which revealed themselves, once mounted under a light microscope, to be palmelloid colonies of encysted golden algae or Chrysophyceae (Fig. 289). Each cyst was made of a rigid shell with a plug-like appendix. I was unable to identify them with certainty, but they probably pertained to primitive forms such as *Chrysapis* according to Hollande (1952). Such gelatinous masses were often encountered on the antennae of springtails, but cysts of golden algae were also found at the surface of pine bark fragments.

Fauna

Inventory

Table 8 classifies all living specimens which were found free in the environment or after dissecting pine needles and miscellaneous debris. Nematodes (many specimens of which were found when sectioning plant material) and probably also rotifers were largely underestimated, as this has been already noticed. Protozoa were not searched for at all, although they were frequently encountered in microscopic slides. At last, some specimens of mesofauna were probably not recovered in entirety, such as oribatid box mites of the genus *Phthiracarus*, most larval development of which took place at

Table 8. Taxonomic name, abundance and estimated biovolume of living animals found in the F₁ layer

Taxonomic groups and species	Abundance	Upper estimate of biovolume (μL)	Lower estimate of biovolume (μL)
Rotifers	3	0.82 10 ⁻³	0.21 10 ⁻³
Nematodes	42	0.19 10 ⁻¹	0.49 10 ⁻²
<i>Eudorylaimus sp.</i>	26	0.11 10 ⁻¹	0.29 10 ⁻²
<i>Prionchulus punctatus</i>	10	0.8 10 ⁻²	0.2 10 ⁻²
<i>Dorylaimus sp.</i>	3	0.21 10 ⁻³	0.52 10 ⁻⁴
<i>Anaplectus sp.</i>	2	0.18 10 ⁻³	0.44 10 ⁻⁴
<i>Cephalobus sp.</i>	1	0.53 10 ⁻⁴	0.13 10 ⁻⁴
Enchytraeids	83	4.6	1.1
Oribatid mites	58	11	2.8
<i>Rhysotritia duplicata</i>	14	3.5	0.88
<i>Phthiracarus sp.</i>	10	7.2	1.8
<i>Oppia subpectinata</i>	9	0.17 10 ⁻¹	0.44 10 ⁻²
<i>Oppiella nova</i>	7	0.57 10 ⁻²	0.14 10 ⁻²
<i>Nothrus sylvestris</i>	5	0.37	0.92 10 ⁻¹
<i>Nanhermannia nana</i>	3	0.3 10 ⁻¹	0.75 10 ⁻²
<i>Suctobelba subcornigera</i>	3	0.16 10 ⁻²	0.39 10 ⁻³
Oppiidae (nymphs)	3	0.76 10 ⁻³	0.19 10 ⁻³
<i>Oribatula tibialis</i>	2	0.14 10 ⁻¹	0.35 10 ⁻²
<i>Ceratozetes sp.</i>	1	0.18 10 ⁻²	0.46 10 ⁻³
<i>Odontocephus elongatus</i>	1	0.58 10 ⁻²	0.15 10 ⁻²
Miscellaneous mites	38	0.41	0.1
Actinedida	17	0.18 10 ⁻¹	0.45 10 ⁻²
Uropodina	9	0.76 10 ⁻¹	0.19 10 ⁻¹
Acaridida	7	0.26 10 ⁻²	0.66 10 ⁻³
Gamasina	5	0.31	0.77 10 ⁻¹
Spiders	1	0.1	0.26 10 ⁻¹
Woodlice	1	0.65 10 ⁻¹	0.16 10 ⁻¹
<i>Trichoniscus sp.</i>		0.65 10 ⁻¹	0.16 10 ⁻¹
Springtails	67	0.2	0.5 10 ⁻¹
<i>Folsomia manolachei</i>	23	0.28 10 ⁻¹	0.7 10 ⁻²
<i>Parisotoma notabilis</i>	15	0.24 10 ⁻¹	0.61 10 ⁻²
<i>Pseudosinella terricola</i>	8	0.92 10 ⁻¹	0.23 10 ⁻¹
<i>Willemia anophthalma</i>	8	0.31 10 ⁻²	0.78 10 ⁻³
<i>Isotomiella minor</i>	4	0.54 10 ⁻²	0.14 10 ⁻²
<i>Lepidocyrtus lanuginosus</i>	4	0.26 10 ⁻¹	0.65 10 ⁻²
<i>Entomobrya sp.</i>	2	0.71 10 ⁻²	0.18 10 ⁻²
<i>Megalothorax minimus</i>	2	0.21 10 ⁻²	0.52 10 ⁻³
<i>Pogonognathellus flavescens</i>	1	0.11 10 ⁻¹	0.28 10 ⁻²
Fly larvae	14	1.4	0.36
Sciaridae	13	1.4	0.36
Cecidomyiidae	1	0.16 10 ⁻²	0.41 10 ⁻³
Beetle larvae	1	0.14 10 ⁻¹	0.34 10 ⁻²
Cantharidae	1	0.14 10 ⁻¹	0.34 10 ⁻²
Fly nymphs	2	0.42	0.1
Insect imagos	1	0.47 10 ⁻²	0.12
Thripsidae	1	0.47 10 ⁻²	0.12

the inside of pine needles. Only part of pine material was dissected, letting this group underestimated, too.

Within these limits, some comparisons could be done with overlying layers. Enchytraeids were still the dominant group in number in F₁, like they were in L₂ (Table 5), but this was no longer true in biovolume, because of the large size of box mites (*Phthiracarus sp.*, *Rhysotritia duplicata*), the reason why oribatid mites became the dominant group in biovolume in F₁. Enchytraeids and oribatids showed a contrasted evolution according to depth in the three studied litter layers. Enchytraeids showed a continuous increase with depth in density (7, 39 then 83 specimens) and biovolume (0.22, 2.6 then 4.6 µL for the upper estimate), while oribatids showed a trough in L₂ both in density (46, 12 then 58 specimens) and biovolume (0.95, 0.092 then 11 µL), with a considerable shift in species composition in F₁ (see below). The second more important group in density (although poorly represented in biovolume) was Collembola (springtails). They were more and more numerous with depth (9, 12 then 67 specimens). Although largely underestimated, nematodes increased in abundance (1, 21 then 42 specimens). Fly larvae were a little more abundant in F₁ (14 specimens) compared to L₂ (12 specimens) but their biovolume dramatically increased (1.4 against 0.33µL) because of the lower contribution of small-sized cecidomyiids. More generally whole fauna increased with depth both in density (76, 106 then 311) and biovolume (1.96, 3.4 then 18 µL), but also in diversity, in particular at a lower taxonomic level.

Protozoa

This taxonomic group could not be explored in its entirety, because of its small size, transparency and concealed way of life. However, many protozoa could be observed while sectioning and mounting decaying plant material.

Trophozoits of naked amoebae were observed at the inside of decaying plant material, in particular pine needles, but also at the inside of pine bark and moss stems (Fig. 269), and more rarely at the surface of plant material (bracken leaflets, pine bark) and in the mycelium embedding plant debris and enchytraeid faeces (Fig. 290). It should be noticed that naked amoebae seemed absent from animal corpses.

Cyst amoebae (in the form of empty cysts or, more rarely, of living specimens) occupied a variety of environments. They were frequently observed, both at the inside and at the outside of decaying material. However, different thecamoeban species did not seem to occupy the same niches. *Phryganella acropodia* (Fig. 291) was abundantly and exclusively found in arthropod corpses (mostly in oribatid mites) while other abundant species, such as *Nebela collaris* (Fig. 292), *Euglypha sp.*, and *Corythion dubium* (Fig. 293) colonised indifferently plant debris and corpses. It should be noticed that *Nebela collaris* was most often seen at the inside of decaying plant material (pine needles, bracken veins, bark). Other species, such as *Pseudodifflugia gracilis* var. *aerophila* Bonnet & Thomas, *Nebela militaris* Penard, and *Trinema complanatum* var. *aerophila* (Decloître), were found too infrequently for discerning their preferences. All these species were actively consumed by enchytraeids.

Among flagellates, colonies of chrysomonadins (also called Chrysophyceae or golden algae when classified among algae), with many encysted specimens, were found on springtail antennae (Fig.

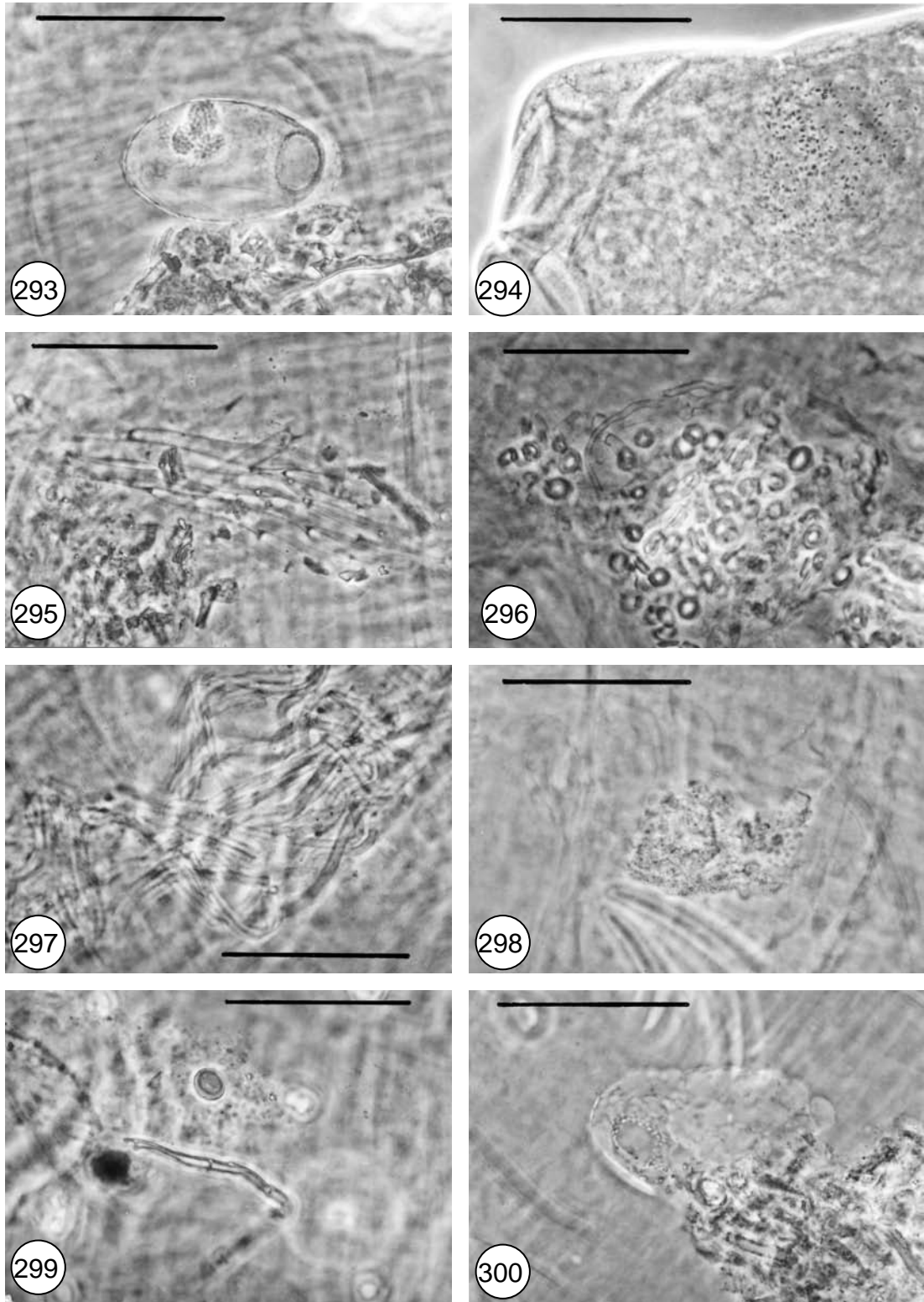


Fig. 293: Cyst of *Corythion dubium*, with bacterial colonies, at the inside of an enchytraeid intestine (CP); Fig. 294: Rotifer observed by transparency, showing its intestinal microflora (CP); Fig. 295: Enchytraeid gut contents observed by transparency, moss leaf fragments showing cell walls in way to be degraded (CP); Fig. 296: Idem, pine tracheid areolae in aggregates and empty basidiomycetous hyphae (CP); Fig. 297: Idem, hyaline hyphae in way to be degraded (CP); Fig. 298: Idem, oxalate crystals in aggregates, coming from hyphae of *Hyphodontia sp.* (CP); Fig. 299: Idem, bacterial colony in way to be degraded (CP); Fig. 300: Idem, cyst of *Trinema sp.* in way to be degraded

CP = phase contrast; length of segment = 50 μm

289) and at the surface of pine bark. Gregarines were present in the form of dense masses of sporocysts at the inside of some living animals, in particular enchytraeids, but a gregarine colony was also observed at the inside of an arthropod leg (corpse). Infections by gregarines have been frequently observed in soil invertebrates and were well described by Purrini (1983).

Protozoan cysts, which could not be identified at a lower taxonomic level, were observed at the inside of decaying plant material, such as bracken veins and moss stems (Fig. 268).

Rotifers

Although this group was but badly recovered in studied samples, rotifers were observed during sorting under the dissecting microscope (three specimens in enchytraeid faeces between bark and wood in a pine twig), in sections done in the decaying material (inside of a pine needle, surface of a type A excrement) and at the inside of a *Phthiracarus* corpse. The observation of gut contents did not reveal anything else than the intestinal microflora (Fig. 294), which was present in all collected specimens.

Nematodes

Dorylaimidae (*Eudorylaimus* and *Dorylaimus*) were dominant. The same dominant group had been registered in L₂ (Table 5) but here much more specimens were collected, either under the dissecting microscope (26 specimens, against 8 in L₂) or under the light microscope (two specimens in arthropod corpses). The observation under phase contrast of dorylaimid intestines did not reveal any other contents than bacterial masses, often in way to be degraded (fuzzy contour, lack of opacity), thereby supporting what had been observed in L₂. The bacteriophagous behaviour of Dorylaimidae in an environment favourable to fungi is worth to notice, inasmuch as this group was not considered to have specialised food requirements (Arpin, personal communication). However, I cannot discard the possibility that they also used their buccal stylet to perforate fungal hyphae, swallowing bacteria being only part of their food habits.

The mononchid *Prionchulus punctatus* came in second position, both in density and in volume. I saw in its gut small more or less digested nematodes (*Cephalobus*, unidentified Tylenchinae) and chitinous fragments of unknown animal origin, thereby testifying for predatory activity (Arpin 1980). As in L₁ (Figs. 58-60), bacterial colonies in way to be degraded were observed in its gut, confirming observations in electron microscopy by Arpin & Kilbertus (1981), which established that the food habits of this 'predatory' nematode had a bacteriophagous component.

Other nematodes not indicated in Table 8, not found during scrutiny of the whole material under the dissecting microscope (and thus not measurable), were observed while mounting corpses during investigations in light microscopy: one Cephalobinae, one Acrobelinae and three unidentified specimens. A Tylenchinae (mycophagous species-group) was found appressed to the mantle of an orange-brown ectomycorrhiza which had been sectioned and stained.

Infestations of nematodes by bacteria and flagellates were observed, too.

Enchytraeid worms

- Preliminary remarks

Enchytraeids were extremely abundant in the F₁ layer. In addition to entire specimens, many fragments, issued from the asexual reproduction of adults, were found. Fragmentation seems to be the rule in *Cognettia sphagnetorum* (Standen 1973), which was probably the dominant species in the studied site. Shortest specimens issued from fragmentation had not fully achieved their regeneration and thus were still starving. Over the 83 enchytraeid specimens studied, only 2 seemed to pertain to another species: they were slender, with a straight intestine, contrary to the dominant species (Figs. 145 & 146), and they harboured a different intestinal microflora.

- Generalities

The food diet of *Cognettia sphagnetorum* was highly variegated, although some choice could be evidenced. Table 9 shows the frequency of the 10 food categories which were found in the 61 non-starving specimens studied, expressed as per cent individuals showing the category in their intestine. Plant material (pine, moss, miscellaneous) was made of fragments of plant tissues, which may occupy a large volume in the intestine, while bacteria could be frequently ingested without making a significant contribution to the food bolus. Estimates of ingested volumes could not be done with means at my disposal, unfortunately. Within these limits, it can be said that the food diet of this species was shared between plant material ($\frac{1}{3}$ non-starving specimens had it in their gut) and microbial material (mainly fungi, bacteria to a weaker extent, algae still less present).

Table 9. Percent occurrence of main food categories ingested by *Cognettia sphagnetorum* in the F₁ layer

Food categories	Frequency (%)
Fungi	90
Bacteria	66
Plant material (pine)	34
Plant material (moss)	34
Cysts of Thecamoeba	31
Pine pollen grains	28
Quartz grains	8
Mesofaunal faeces	8
Algae (including cyanobacteria)	5
Miscellaneous plant material	3

Upon reading Table 9 it appeared that thecamoeban cysts (impossible to know whether cyst amoebae had been ingested dead or alive) and pollen exines (same uncertainty as for cyst amoebae) were overrepresented in enchytraeid guts, and thus were probably actively searched in the environment. On the opposite side, animal faeces seemed neglected, at least those of mesofauna, the only ones which could be identified as such: plant material was poorly transformed in macrofaunal faeces, as will be seen below, thus it could not be distinguished from the material directly ingested by enchytraeids. However, it must be highlighted that enchytraeids were never found tunnelling through faeces of macroinvertebrates, while they abounded in pine needles, pine twigs and moss stems.

- Moss material

Moss tissues were frequently encountered in enchytraeid intestines, to the same extent as pine material, which was a novelty compared to the L₂ layer. It is worth to notice that moss material was consumed in L₁, where moss was living (green, meaning herbivory), was neglected in L₂, where moss was dead but with its internal tissues still not processed by fungi, and was consumed again in F₁, where moss cells were colonised by fungi and their walls attacked incipiently (microbivory and saprophagy). This can be explained if only cytoplasm (whether of plant or microbial origin) was attractive to these animals. Moss tissues observed by transparency in the gut of enchytraeids showed some degradation of cell walls, wall contours appearing fuzzy under phase contrast (Fig. 295). Fungal hyphae, which were present at the inside of moss cells (Figs. 266 & 267), were no longer visible. It can be suspected that enchytraeid worms were able to digest cytoplasmic contents, mainly responsible for attractiveness of food, but also fungal cell walls and, at least partly, moss cell walls. However, concerning the latter substrate, it should be highlighted that its attack has been largely prepared beforehand by fungi (see above) and thus is only in progress in enchytraeid intestines. Observations done in L₁ did not show any sign of attack of cell walls in the ingested moss fragments (Fig. 52).

- Pine tissues

Ingested tissues were of varying origin, as this had been already observed in L₂ (Figs. 161-163). The following tissues could be identified in F₁, thanks to their recalcitrant biochemical composition: epidermis, hypodermis, stomatal guard cells, tracheids with bordered pits, pieces of resin. Cell walls were still intact, except in cases where a severe microbial attack occurred before ingestion. Figure 296 shows an aggregation of areolae (from pitted cells of transfusion tissue) in the midst of a half-digested network of basidiomycetous hyaline hyphae. Given that the only recognizable remains of pine tissues were hard lignified structures, it is highly probable (but without any certitude) that enchytraeids were able to digest mesophyll cells and parenchymatous cells of transfusion tissue.

- Fungal material

Like in L₁ (Fig. 53) and L₂ (Figs. 148-153), enchytraeids ingested in F₁ a mixture of hyaline hyphae (mostly coming from basidiomycetous rhizomorphs and mycelia) and melanised hyphae (mostly from the ascomycete *Cenococcum geophilum*). Melanised hyphae seemed intact, except in abovementioned cases where they had been previously attacked by bacteria and fauna. However, hyaline material was always in way to be degraded (Fig. 297). During digestion, hyphal walls became thicker and took a fuzzy contour. In the case of *Hyphodontia sp.* associated with orange-brown ectomycorrhizae, hyphae of which were most often covered with minute oxalate crystals, aggregates of these crystals were observed, attesting for the total digestion of the hyaline fungal wall, to the exception of calcium oxalate (Fig. 298). It is thus highly probable that *Cognettia sphagnetorum* was able to digest fungal cell walls, to the exception of those impregnated with melanin.

- Bacteria

Bacterial colonies appeared in way to be degraded, according to current features observed under phase contrast such as fuzzy contours of their walls (Fig. 299).

- Thecamoeban cysts

Still intact cysts could be found in enchytraeid intestines (Figs. 291-293), but degradation patterns were observed in *Trinema sp.* (Fig. 300), *Euglypha sp.* (Fig. 301) and *Phryganella acropodia* (Fig. 302). In the latter case, quartz particles and miscellaneous debris, which were incorporated in the cyst, subsisted alone. Thorough scrutiny of the 63 specimens allowed attributing with certitude all quartz particles found in enchytraeid intestines to the digestion of cysts of *Phryganella acropodia* and not to the direct ingestion of mineral particles.

- Pine pollen

Pollen exine was sometimes still recognizable, in particular the sculptured exine of lateral bladders (Fig. 303), but most time only granular masses were observed (Fig. 304), indicating at least partial digestion of sporopollenin.

- Mesofaunal faeces

Oribatid faeces (Fig. 305) kept intact their shape, contrary to other faeces (most probably of springtails) the content of which more or less dispersed in enchytraeid intestines (Fig. 306). In the latter case, bacteria, which abound in those faeces (see below), were in way to be degraded.

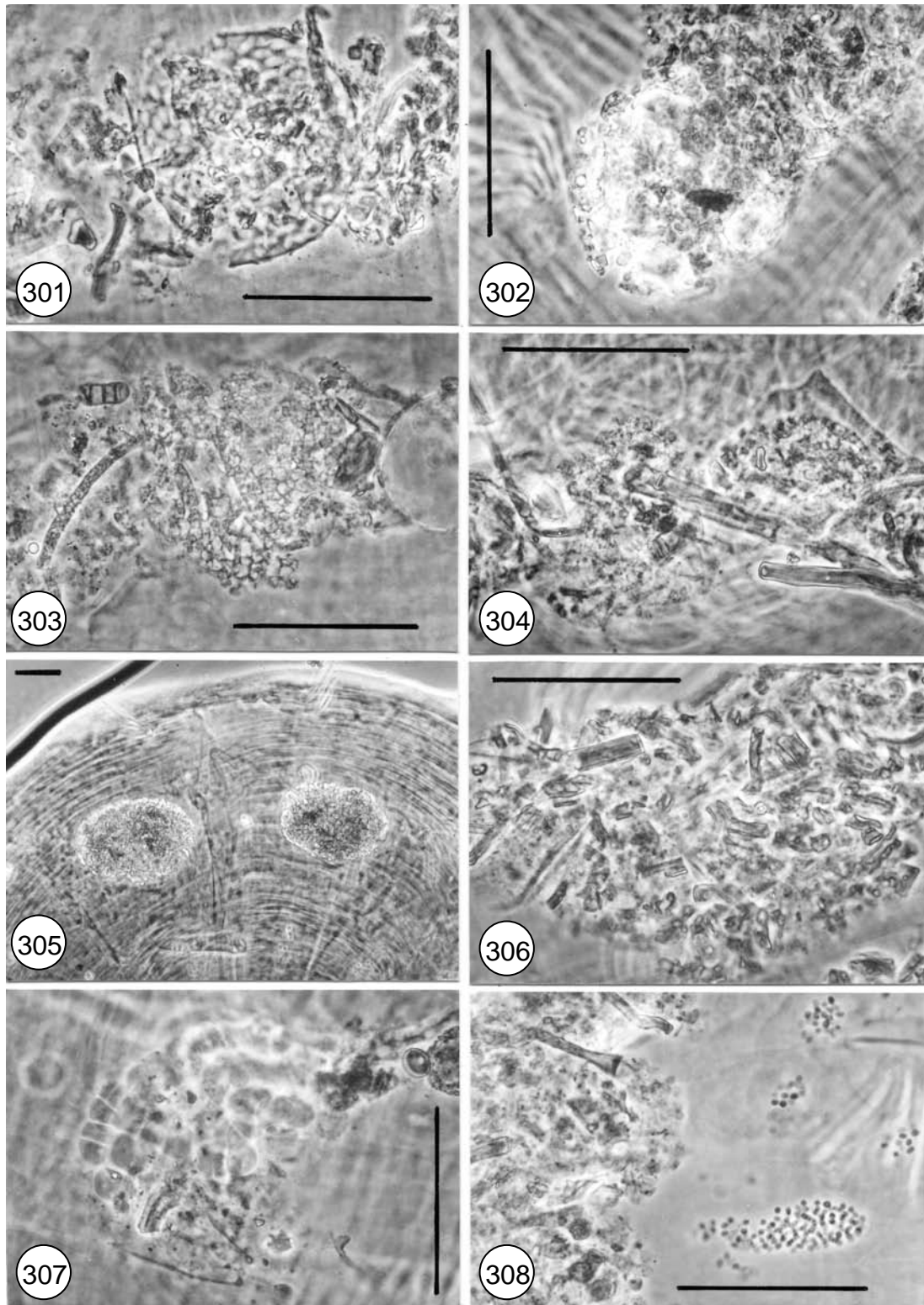


Fig. 301: Enchytraeid gut contents observed by transparency, cyst of *Euglypha* sp. in way to be degraded (CP); Fig. 302: Idem, cyst of *Phryganella acropodia* in way to be degraded, with quartz particles standing in place (CP); Fig. 303: Idem, remains of a pollen pine, with exine still recognizable in bladders (CP); Fig. 304: Idem, granular mass issued from the degradation of pollen exine, with fragments of melanised hyphae of *Cenococcum geophilum* (CP); Fig. 305: Idem, mite faeces (CP); Fig. 306: Idem, other mesofaunal faeces dispersed in the intestine (CP); Fig. 307: Idem, viable colony of Chlorophyceae (CP); Fig. 308: Idem, intestinal microflora of a morphological type different from that found in *Cognettia sphagnetorum* (CP)

CP = phase contrast; length of segment = 50 μ m

- Algae

Algae might be filamentous or unicellular Cyanophyceae (cyanobacteria), which were generally degraded in enchytraeid intestines, as observed in L₂ (Fig. 160), or Chlorophyceae, which seemed to remain viable, as ascertained by the opacity of their cytoplasm (Fig. 307). The poor digestibility of green algae had been already noted in L₂ (Fig. 157).

- Intestinal microflora

The intestinal microflora of *Cognettia sphagnetorum* was comprised of two morphological types which had been already described in L₂ (Figs. 170 & 171). The other enchytraeid species observed in F₁ displayed only one morphological type of intestinal microflora, at least judging from the only two specimens present in the sample (Fig. 308). This observation points to some kind of specificity in the bacteria hosted by enchytraeid intestines.

- Transport on the tegument

Few remarks have to be added to observations done in L₁ (Figs. 45-50) and L₂ (Fig. 154). The abundance of mineral particles on the tegument of enchytraeids, despite their organic-only diet (to the exception of cysts of *Phryganella acropodia*), points to the importance of vertical movements, probably of daily periodicity, exerted by these animals in the soil (Springett et al. 1970). Fungal spores were always actively transported on the tegument (Fig. 309), attesting for a prominent role of enchytraeid worms in the dissemination of fungal propagules.

Oribatid mites

- Generalities

Oribatids were mostly represented by box mites, in abundance (41%) and even more in biovolume (97%). These animals feed mainly on plant material. They were absent from overlying layers, to the exception of a single specimen found in L₁. This holds true also for *Nothrus sylvestris* (Koch), which was absent in L₁ and L₂. Other species, for the main, had already been observed in overlying layers.

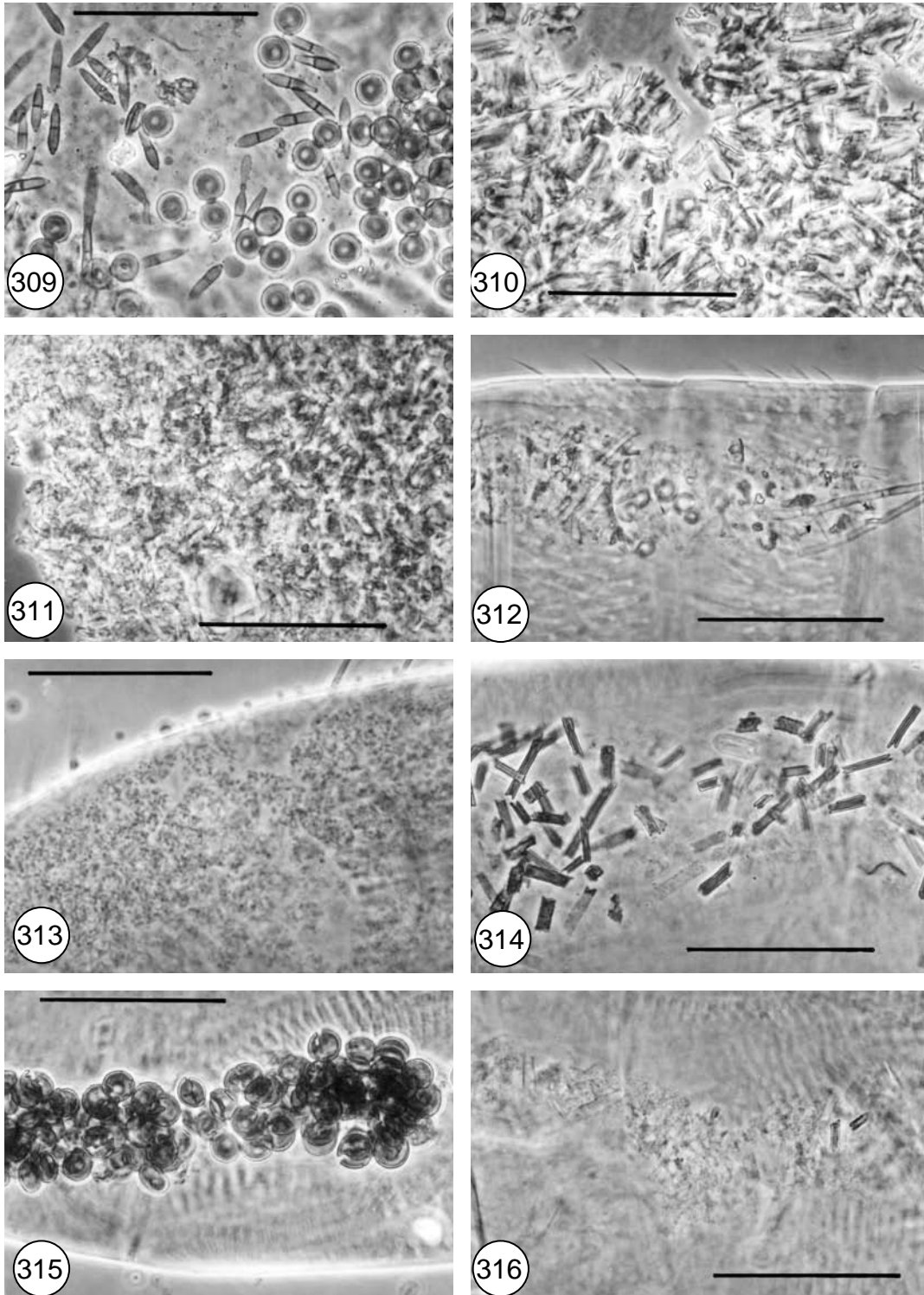


Fig. 309: Fungal spores transported on the tegument of an enchytraeid (CP); Fig. 310: *Phthiracarus sp.*, detail of second-order faecal pellet showing crushed plant cell walls with neat contours and strong refringence (CP); Fig. 311: Idem, detail of the third-order faecal pellet, showing crushed plant cell walls with fuzzy contours and browning of most internal parts (CP); Fig. 312: *Folsomia manolachei*, intestine observed by transparency showing hyaline hyphae in way to be degraded and areolae from pine tracheids (CP); Fig. 313: *Parisotoma notabilis*, observation of the body by transparency showing septicemia (CP); Fig. 314: *Pseudosinella terricola*, intestine observed by transparency showing hyaline fungal hyphae (in way to be degraded) and fragmented melanised fungal hyphae (with intact walls) (CP); Fig. 315: Idem, melanised fungal spores, more or less broken (CP); Fig. 316: Idem, hyaline hyphae in way to be degraded and aggregates of oxalate crystals (CP)

CP = phase contrast; length of segment = 50 μ m

- Box mites

This taxonomically (although not morphologically) heterogeneous group was represented by the genera *Rhysotritia* and *Phthiracarus*, which did not occupy the same micro-niches, at least when taking into account their ontogenetic stages or ‘stases’ (Table 10).

Table 10. Micro-niches occupied by box mites in the F1 layer

Stase	Genus	Needle	Environment
Larva	<i>Phthiracarus</i>	3	
Nymph	<i>Phthiracarus</i>	2	
Adult	<i>Phthiracarus</i>	1	4
Larva	<i>Rhysotritia</i>	2	9
Nymph	<i>Rhysotritia</i>		1
Adult	<i>Rhysotritia</i>		2

If adults of both genera were near always found outside of pine needles, this did not hold for larvae: while larvae of *Phthiracarus* (together with their nymphs) were always found at the inside of pine needles, those of *Rhysotritia duplicata* were mostly found in the environment. The presence of chorion debris around larvae of *Rhysotritia duplicata* found in the environment, sometimes still embedding their whole body, attested for hatching (and thus egg deposition) taking place outside of pine needles, penetration of pine needles occurring in a further step. A great number (13) of pre-larvae of this species were found in the environment: they were not accounted for in my census because they were not active. Eggs and pre-larvae of *Rhysotritia duplicata* were easy to recognize by their externally sculptured chorion. The differential ecology of *Phthiracarus* and *Rhysotritia* larvae was explained by the lack of chorion in *Phthiracarus* eggs, which are deposited in cavities created by females at the inside of pine needles, an observation firstly done by Grandjean (1940).

Feeding habits of box mites were based on the consumption of pine plant material. Larvae ground finely plant cells, thanks to the combined movement of rutellum and chelicerae (Dinsdale 1974b). The anatomical structure of pine tissues was no longer recognizable in larval guts, due to fine grinding of cell walls, but adults and nymphs, which ground more coarsely plant material, showed some recalcitrant remnants of pine tissues, such as tracheid areolae, attesting for the pine origin of gut contents. Fungal hyphae (basidiomycetous hyaline hyphae or melanised hyphae of *Cenococcum geophilum*) were sometime present, but only in little amount and in adults only. The presence of an intestinal microflora was detected only in *Rhysotritia duplicata*: however, it must be noticed that bacteria were found associated with post-colon microvilli of *Phthiracarus sp.* by Dinsdale (1974a, 1975).

An interesting phenomenon was the observed change in the aspect of the ingested plant material in the course of intestinal transit. As this is the rule in oribatid mites, two or three faecal

pellets can be seen to be moulded in the gut, as this has been observed in *Adoristes ovatus* feeding on fungi in L₁ (Fig. 63). Anatomical reasons for this particular ontogenetic pattern have been detailed by Woodring & Cook (1962), Hoebel-Mävers (1967) and Tarman (1968). The last faecal pellet, located in the rectum and ready to be excreted, was always more compact and darker than the preceding one, which was still located either in the colon or in the mesenteron. Compaction of the food bolus in the colon has been described by Dinsdale (1974a, 1975). Comparative observations on second- and third-order faecal pellets showed that plant cell wall debris were still intact in the second faecal pellet while their contours became fuzzy in the third pellet, and that the more when observing inner parts (Figs. 310 & 311). Thus changes similar to what had been observed during ageing of faeces made of melanised fungal material, i.e. fuzzy contour of cell debris and darkening (Figs. 210 & 211), occurred at the inside of mite guts filled with cellulosic plant material. The transformation of plant organic matter into a dark amorphous mass strongly suggests that the formation of humus, i.e. inherited humin in the sense of Duchaufour (1977), occurs in the intestine of box mites.

- *Nothrus sylvestris*

The particular abundance of fungal hyphae of *Cenococcum geophilum* was noticeable: hyphae were cut into small pieces. Encrusted hyaline hyphae of *Hyphodontia sp.* were present, too, although in smaller amounts and always in the company of *Cenococcum geophilum*, as well as some debris of cell walls which could come from pine roots. It could be hypothesized that *Nothrus sylvestris* browsed the fungal mantle of jet-black ectomycorrhizae formed by *Cenococcum geophilum*, and ingested accidentally some cortical tissue. The attractiveness of the root system of pine for this species could explain why *Nothrus sylvestris* was absent from L₁ and L₂ where fungal mycelia abounded but roots were absent. Gut contents of *Nothrus sylvestris* in F₁ resembled those of *Platynothrus peltifer*, another Camisiidae, in L₁ (Fig. 62). The vertical segregation observed between these two species, which were phylogenetically related and had similar feeding habits, was reminiscent of the segregation observed between *Nothrus sylvestris* and *Hermaniella granulata* (Nicolet), which had been experimentally reproduced by Anderson (1978).

Contrary to *Platynothrus peltifer*, no intestinal microflora was observed in *Nothrus sylvestris*. A particular phenomenon needs to be noted. Encrusted hyaline hyphae of *Hyphodontia sp.* remained always intact (at least their walls) while melanised hyphae of *Cenococcum geophilum* appeared to be degraded. This unexpected result shows that the enzymic armature of soil animals, in particular of oribatid mites, may vary to a great extent according to the food ingested. Stefaniak & Seniczak (1976, 1981, 1983) and Seniczak & Stefaniak (1978) showed on several oribatid species that individuals selected in their gut associated bacterial strains which varied according to the food to which they had been accustomed in culture.

- Oppiidae

Oppiella nova (Oudemans) fed on the encrusted hyaline basidiomycete *Hyphodontia sp.* which was associated with orange-brown ectomycorrhizae. Hyphae were cut into small pieces and

agglomerated, then degraded in the digestive tract. Melanised hyphae, probably coming from *Cenococcum geophilum*, were sometimes present, but their walls remained intact. In one case, I observed melanised fungal material in a highly degraded state, but the concomitant abundance of bacteria let me suppose that faecal material had been ingested.

Feeding habits of *Oppia subpectinata* (Oudemans) did not differ to a great extent from those of *Oppiella nova*, with similar degradation of hyphae of *Hyphodontia sp.* and recalcitrance of *Cenococcum geophilum*. However, the latter fungus was more abundant, which could be explained by the larger size of *Oppia subpectinata* and associated mouth parts, making more easy for these animals to cut thick recalcitrant hyphae of *Cenococcum geophilum*.

Nymphs of Oppiidae displayed the same strictly mycophagous feeding habits, with degradation of hyaline hyphae. Observations done on Oppiidae in F₁ strongly support those done on the genus *Oppia* in L₂ (Fig. 174).

- Other species

If I except *Suctobelba subcornigera* (Oudemans), which did not display any gut contents, other studied species, *Oribatula tibialis* (Nicolet), *Nanhermannia nana*, *Odontocephus elongatus* (Michael) and *Ceratozetes sp.*, fed on a mixture of hyphae of *Hyphodontia sp.* and *Cenococcum geophilum*. It should be noticed that the degradation of hyaline hyphae was observed in *Ceratozetes sp.* and that melanised hyphae were dominant in *Oribatula tibialis*.

- Conclusions

At the inside of the oribatid group, box mites seemed original with their ‘macrophytophagous’ feeding habits in the sense of Luxton (1972). Hayes (1963) showed that different species of box mites could prefer different stages of pine needle decomposition, which was not confirmed in the present study, at least concerning *Phthiracarus* and *Rhysotritia*. However present results confirmed observations by Hayes (1963) that this animal group exhibited a clear preference for more advanced stages of pine needle decomposition, which might explain its absence from L₁ and L₂ layers. Other species were mycophagous, i.e. ‘microphytophagous’ in the sense of Luxton (1972), or exhibited a definite preference for fungi when and where they were abundant. The transfer from non-exclusive phytophagy to mycophagy when fungal material is abundant in the environment has been observed to affect a great number of oribatid species (Hartenstein, 1962a, b; Luxton, 1972; Haq, 1981; Behan-Pelletier & Hill, 1983).

Miscellaneous mites and spiders

Other mites and spiders did not possess any solid matter in their gut, which did not indicate their feeding habits. However, near all of them are known as predators which did not swallow their prey but rather suck their haemolymph and some internal tissues (after pre-oral digestion in the case of spiders). A unique specimen of Acaridida showed fragments of basidiomycetous hyphae and bacteria in its gut.

Woodlice

Two juvenile specimens of *Trichoniscus* were observed, tentatively identified as *Trichoniscus pusillus* (Dallens, personal communication). Their gut contents appeared to be composite and highly degraded: fungal material (broken spores, long hyphal fragments of *Cenococcum geophilum*), mineral material (quartz grains), animal material (fragments of arthropod cuticles, broken cysts of *Thecamoeba*) and amorphous organic matter. This was reminiscent of enchytraeid variegated feeding habits and I hypothesized that these specimens had fed on enchytraeid faeces. The composition of their gut contents strongly differed from the composition of type B faeces (Fig. 224), which were made of fragmented but poorly transformed pine needles (see below).

Springtails

Isotomidae were the dominant family, at least from the point of view of densities. *Folsomia manolachei*, *Parisotoma notabilis* and *Isotomiella minor* (Schäffer) were represented by 23, 15 and 4 specimens, respectively, totalling 42 specimens among the 67 springtails collected in F₁. *Folsomia manolachei* dominated the community in density, as in the overlying L₂ layer, but it was accompanied by a much higher number of species. The presence of poduromorph species, such as *Willemia anophthalma* Börner, a species typical of very acid humus (Ponge, 1980, 1983; Pozo, 1986), and of neelid species such as *Megalothorax minimus* (Willem), was noticeable, as was that of the acidophilic entomobryid *Pseudosinella terricola* Gisin. These species were absent from overlying layers, indicating a shift towards a deeper-living community (Ponge 1980). However, some epigeic species were still present, such as juveniles of *Entomobrya sp.*, and *Pogonognathellus flavescens*.

Folsomia manolachei ingested mainly a mixture of hyaline and melanised fungal hyphae, cut in small irregular pieces and mixed with numerous bacteria. Hyaline encrusted hyphae of *Hyphodontia sp.* displayed a variety of degradation stages, some hyphae becoming hardly discernible. Less frequently, tracheid areolae attested for the consumption of pine tissues, probably issuing from needles (Fig. 312). As for observations done in L₂ (see above), the interpretation was that *Folsomia manolachei* ingested invertebrate faeces (mite or more probably enchytraeid faeces because of the irregular size of hyphal fragments) which had been invaded by bacteria previous to ingestion. The direct ingestion of strongly decayed pine needles was not supported, because fragments of needle epidermis and hypodermis and melanised stromata of *Verticicladium trifidum* should be present in addition to tracheid areolae. The observation of enchytraeid faeces, which were accumulating in the F₁ layer (see below), let me suppose that hyaline basidiomycetous hyphae and bacteria which colonised these faeces were the main food resource of *Folsomia manolachei*, in addition to miscellaneous degradation products which could be present in enchytraeid faeces, too.

Quite similar observations were done in *Isotomiella minor*, which exhibited pine areolae mixed with highly transformed fungal and bacterial material. Gut contents of *Parisotoma notabilis* were similarly comprised of bacteria and highly transformed fungal fragments, but the high proportion of starving specimens (12 among 15, i.e. 80%) should be noticed, together with several specimens which were seriously infected by bacteria (Fig. 313). This indicated that the population of *Parisotoma notabilis* was in a bad condition and poorly active trophically. I was unable to explain this phenomenon, but I want to note that cultures of this species were submitted to wide fluctuations (personal observations).

Feeding habits of *Pseudosinella terricola* were quite different. This species, which was dominant in volume within the springtail group, belongs to the *terricola-mauli* species group (Ponge 1980, 1983). It fed exclusively on fungi, like the congeneric *Pseudosinella alba* (Packard) (Arpin et al., 1980; Ponge & Charpentié, 1981). Contrary to the three abovementioned isotomid species *Pseudosinella terricola* ingested only fungal material, without any bacteria in admixture: hyaline and melanised hyphae, cut regularly in small pieces (Fig. 314), and spores (Fig. 315). Melanised hyphae kept their walls intact, contrary to hyaline hyphae (Figs. 314 & 316). In the case of hyaline hyphae with encrusted walls, oxalate crystals remained and agglomerated once fungal walls had been degraded (Fig. 316), as this had been observed in enchytraeids (Fig. 298). When the digestive process was incipient, well delimited zones could be found in the intestine where fungal hyphae were degraded while the rest of the food bolus was still intact (Fig. 317). Humbert (1974) showed the coexistence of zones with strongly different pH values in the mesenteron, where the food bolus remains a long time, the passage from a zone to another shifting forward during digestion. This could explain transitional patterns such as that depicted in Figure 317. The biggest spores (basidiospores?) appeared to be broken, but their wall was not degraded. In this case only fungal cytoplasm was digested, probably as it happens in *Pseudosinella alba* (Ponge & Charpentié, 1981).

Feeding habits of *Willemia anophthalma*, a species living in very acid and waterlogged soils (Ponge, 1980, 1983; Pozo, 1986), did not differ to a great extent from the previous species, being exclusively mycophagous. However, some differences deserve to be highlighted. Dematiaceous (melanised) material was absent, only fragments of hyaline basidiomycetous hyphae being found (Fig. 318). Contrary to *Pseudosinella terricola*, oxalate crystals seemed to have been dissolved in the gut, as this had been observed in *Oppia* sp. in L₂ (Fig. 174). In the course of the digestion of basidiomycete hyphae, intra-hyphal septa seemed to resist more than external walls (Fig. 318). At last, another difference with *Pseudosinella terricola* was the presence of bacteria in the gut of *Willemia anophthalma* (Fig. 319). These bacteria were in way to be degraded and were absent from starving specimens, thus they did not belong to symbiotic microflora and should be considered as a food resource.

Food habits of *Lepidocyrtus lanuginosus* (Gmelin), a species belonging to a genus brother to *Pseudosinella*, were mainly based on unicellular Chlorophyceae, mixed or not with fungi but always dominant (Fig. 320). However, observations on gut contents could only be made on two specimens, the other two being starved. The cytoplasm of algal cells generally disappeared (no opacity in phase contrast) and some walls appeared in way to be degraded. Thus the enzymic equipment of this species seemed to be adapted to an algal diet, even if algae were not the only food resource used by these animals, contrary to most animal groups prospected.

Observing two specimens belonging to the genus *Entomobrya* showed that their gut contents were comprised of melanised fungal spores, broken when of a big size, mixed with bacteria, and pine

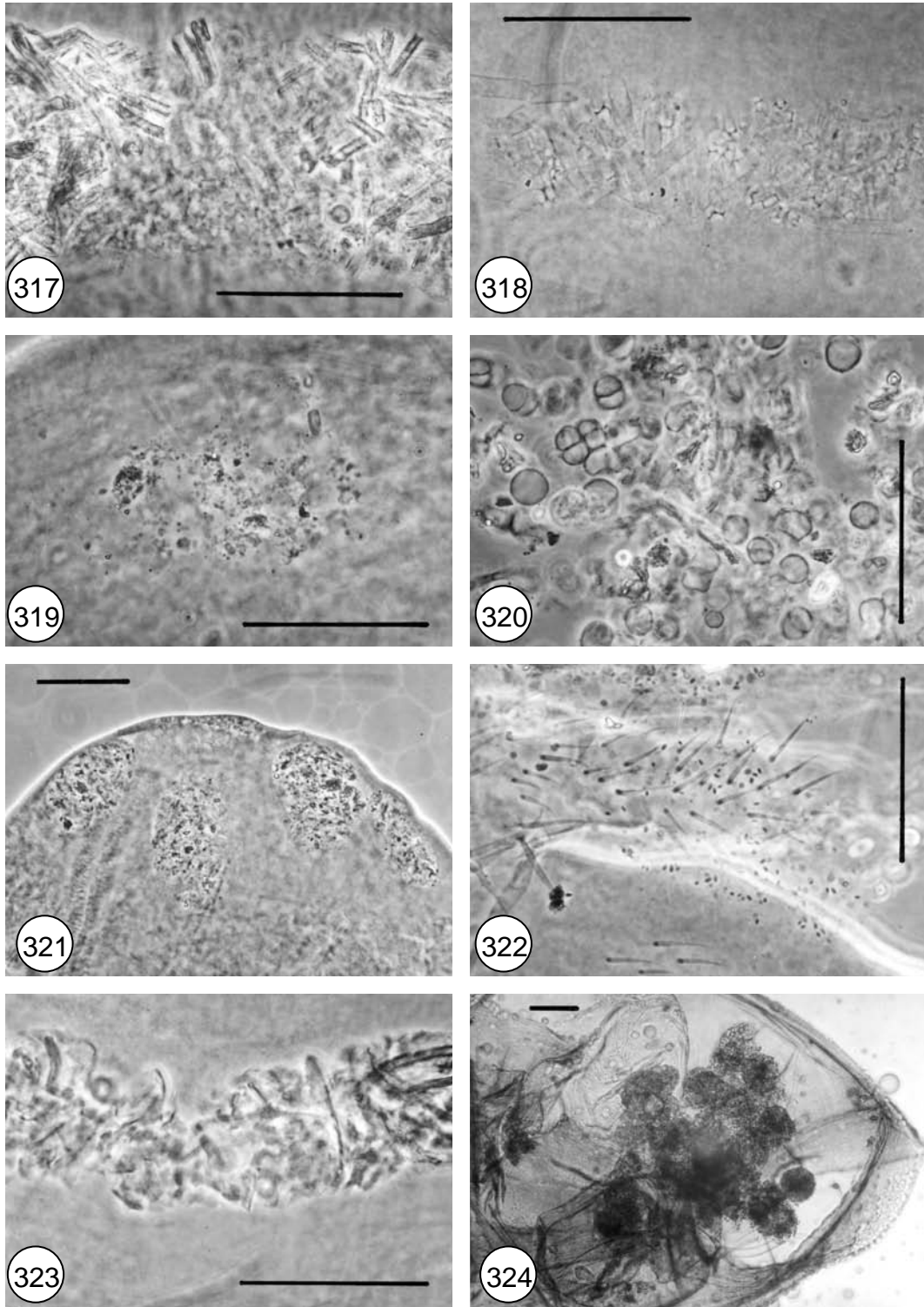


Fig. 317: *Pseudosinella terricola*, intestine observed by transparency showing the passage from a zone with degraded hyphal walls (centre) to two zones with intact walls (right and left) (CP); Fig. 318: *Willemia anophthalma*, intestine observed by transparency showing hyaline basidiomycetous hyphae in way to be degraded (CP); Fig. 319: Idem, bacteria in way to be degraded (CP); Fig. 320: *Lepidocyrtus lanuginosus*, intestine observed by transparency showing Chlorophyceae (empty walls) mixed with bacteria (CP); Fig. 321: *Megalothorax minimus*: intestine observed by transparency showing faecal pellets in way to be moulded in the intestine (CP); Fig. 322: *Parisotoma notabilis*, bacteria transported on a leg (CP); Fig. 323: Sciarid larvae, intestine observed by transparency showing pine material (see areolae) in way to be degraded (CP); Fig. 324: Faeces of minute oribatid mites at the inside of a corpse of *Phthiracarus* sp. (CP)

CP = phase contrast; length of segment = 50 μ m

pollen grains, broken in small pieces. The absence of fungal hyphae, although dominant in the environment, was remarkable.

Megalothorax minimus was particular, because of the presence of several faeces in formation in the intestine (Fig. 321), resembling what had been observed in oribatid mites (Fig. 63), but without any browning and compaction of the food bolus. Gut contents were comprised of bacteria and minute unidentifiable debris. This species, which belongs to a group (Neelidae) which is quite original by its anatomical features within the Class Collembola (Massoud, 1971; Dallai, 1979), was unique in feeding only on food items of micrometric size. By its feeding habits, this minute springtail pertains rather to microfauna than to mesofauna.

The unique specimen observed belonging to *Pogonognathellus flavescens* showed hyaline hyphae (in way to be degraded) and melanised hyphae (with intact walls) in its gut. They were cut into pieces and mixed with bacteria.

A great variety of feeding habits was thus observed in Collembola, but without any definite sharing of food resources. Apart from typified feeding behaviour of *Pseudosinella alba*, *Willemia anophthalma*, *Lepidocyrtus lanuginosus* and *Megalothorax minimus*, other species displayed a diet made of a mixture of fungal and bacterial material, probably issued from the consumption of invertebrate faeces (coprophagy). Fungal material and bacteria were degraded, melanised items (hyphae, spores) being either cut into pieces or broken but without any apparent degradation of cell walls. Many authors, faced to the strongly degraded aspect of collembolan gut contents, spoke of humified or 'amorphous' organic matter (Poole, 1959; Knight & Angel, 1967; Gilmore & Raffensperger, 1970; Bödvarsson, 1970; Marshall, 1978). The comparative study of all types of animal faeces and gut contents found in the sample gives weight to coprophagy as the dominant feeding behaviour in Collembola, a hypothesis which has been already emitted by Poole (1959). The great amount of starving individuals (29%, if one discards the particular case of *Parisotoma notabilis*) is worth to notice, too. This is special to springtails and is explained by repetitive moulting all along the life of these non-metamorphosing animals (Singh, 1964; Jooisse, 1975).

Among items transported on the tegument of Collembola, in addition to already described chryomonadin colonies (Fig. 289), the mucilage of which favoured the development of bacteria, numerous bacterial colonies were seen adhering to antennae and legs (Fig. 322). Carrying and dispersal of microbial propagules, even though of lesser extent than in enchytraeids, is thus another aspect of the role of springtails in the biological functioning of the soil.

Insect larvae

Even though the total number of fly larvae did not change to a great extent when compared to L₂ (14 in F₁ against 12 in L₂), the community change which had been already mentioned in L₂ still continued in F₁, i.e. Cecidomyidae were replaced by Sciaridae as far as litter became more aged. There was practically no longer Cecidomyidae in F₁ (1 against 13 Sciaridae). It was a pity that the absence of any solid matter in cecidomyid guts prevented me to discern their favoured food, which could have explained, at least partly, the niche segregation observed within fly larvae. Chironomidae were totally absent from F₁, but they were also poorly represented in the overlying layers.

Sciaridae ingested a noticeable amount of plant tissues issuing from pine needles. It must be mentioned that two specimens were directly picked off from the inside of pine needles while dissecting them. The fungal material seemed intact, at least concerning hyphal walls, whether hyaline or melanised, as this had been already observed in L₁ (Fig. 67) and L₂ (Figs. 178 & 179). Contrary to fungal material, plant material (pine tissues) was strongly degraded in sciarid intestines (Fig. 323). This contrast is remarkable, inasmuch as it seems to be the contrary of what has been observed in most other groups, where hyphal walls (at least those of hyaline hyphae) were more easily digested than recalcitrant lignocellulosic pine cell walls. Quite similar to enchytraeids (see above), a revival of macrophytophagous feeding behaviour was observed in sciarid larvae: mosses were consumed in L₁, pine needles in F₁, but plant material was abandoned in the intermediate layer L₂ to the benefit of fungi. However, and contrary to enchytraeids, only very few moss material was consumed by sciarid larvae in F₁. Other food items must be noticed, too, such as pine pollen grains, bacteria, cysts of Thecamoeba (*Euglypha* spp., *Nebela collaris*), sometimes but very rarely quartz grains. Ingested items, in particular fungal hyphae, were of a greater length than in microarthropods (springtails, oribatid mites) but of a shorter length than in enchytraeid worms.

The other two families of insect larvae, Cecidomyiidae (1 specimen) for fly larvae, Cantharidae (1 specimen) for beetle larvae, did not possess any particulate matter in their intestines, thus nothing can be said about their food regime. However, it should be noticed that Cantharidae are known to be carnivorous (Paulian, 1949).

Animal corpses

Preliminary remarks

Table 11 shows densities and nature of the different corpses and animal fragments which were found when sorting litter components under the dissecting microscope. Among miscellaneous arthropod fragments there were many isolated insect legs or wings, which could come from the amputation of living imagos. In the ignorance of their living or dead origin they were counted as corpses. No arthropod exuviae were found at all.

There was a dramatic increase in the number of corpses compared to the overlying layer. This increase was much higher than that of living specimens (4, 15 then 336 corpses against 75, 85 then 226 living specimens in L₁, L₂ then F₁, respectively, to the exclusion of microfauna). This means that accumulation took place, mainly in the form of oribatid exoskeletons (74% of censused corpses). Springtail corpses were totally absent from the F₁ layer, despite of a dramatic increase in abundance. Most oribatid corpses were of adults, suggesting that this group was not submitted to a high level of predation, in particular at adult stage: long duration of life and low reproduction rates are known in this group (Norton, 1985). However, this author mentioned the existence of external and internal parasites in adults, which might result in the production of intact exoskeletons when adults die from parasitism, while younger stages are swallowed by predators and do not accumulate exoskeletons.

Table 11. Number of animal corpses and corpse fragments found under the binocular microscope in the F₁ layer

Enchytraeids	13
Oribatid mites	248
<i>Rhysotritia duplicata</i>	50
<i>Platynothrus peltifer</i>	35
<i>Phthiracarus sp.</i>	29
<i>Oribatula tibialis</i>	23
<i>Ceratoppia bipilis</i>	21
<i>Chamobates sp.</i>	21
<i>Oppia ornata</i>	11
<i>Oppia subpectinata</i>	11
Unidentified Oppiidae	10
<i>Oppiella nova</i>	5
<i>Micreremus brevipes</i>	4
<i>Nanhermannia nana</i>	3
<i>Adoristes ovatus</i>	3
Unidentified oribatid mites	3
<i>Nothrus sylvestris</i>	2
<i>Parachipteria punctata</i>	2
<i>Suctobelba</i> group <i>sarekensis</i>	2
<i>Oppiella obsoleta</i>	2
Unidentified Pelopsidae	1
<i>Suctobelba</i> group <i>trigona</i>	1
<i>Suctobelba</i> group <i>nasalis</i>	1
<i>Scheloribates latipes</i>	1
Miscellaneous mites	40
Gamasina	24
Uropodina	12
Actinedida	3
Acaridida	1
Spiders	2
Miscellaneous arthropod fragments	29
Eggs (dead)	4

Enchytraeid worms

Dead enchytraeid worms were in the form of brown fragments, the inside of which was hard to observe because of the opacity of animal tissues. They appeared to be tanned (mummified), without any trace of microorganisms. Given their small size and the intensity of fragmentation in the F₁ layer of the dominant species, *Cognettia sphagnetorum*, I hypothesized they were unregenerate, non-viable fragments.

Oribatid mites

Box mites were dominant (79 specimens, i.e. 32% of oribatid corpses), which was expected given the numerical dominance of this group. However, *Platynothrus peltifer* corpses were abundant in F₁ while this species was numerically dominant in L₁. This witnessed the past history of the F₁ layer, which was in a more superficial position and at a lesser advanced stage of decomposition during previous winter or spring. The presence of *Micreremus brevipes* (Michael) is worth to notice, since this species had not been found as living specimens in the three layers investigated. Corpses of *Micreremus brevipes* probably fell from tree foliage, where this species is known to live (Travé, 1963).

It was not possible to discern differences in the fate of corpses belonging to different species. However, an observed relationship between adult mortality and larval development should be noticed. A majority (80%) of corpses of *Rhysotritia duplicata* harboured one, two or more rarely three eggs which were seemingly intact, often with pre-larvae at the inside, sometimes reduced to an empty chorion. Such a phenomenon was not retrieved in *Phthiracarus*, where only one corpse was seen with an egg at the inside. Larvae or larval exuviae were retrieved in some corpses of *Ceratoppia bipilis* and more rarely in *Oribatula tibialis*. From these observations it can be concluded that ecdysis (hatching) occurred once female mother was dead or that it caused its death. In this case the plant or fungal material which had been ingested by the female before it died could offer a food resource for the young larvae which hatched in its body. It was not uncommon to see the food bolus, normally in the form of two or three big pellets, pulverised in numerous much smaller pellets of similar size, seemingly excreted by oribatid larvae (Fig. 324) or enchytraeids (Fig. 325) which penetrated the dead body of an adult oribatid. Larvae of *Rhysotritia duplicata* were never retrieved at the inside of dead female bodies, only an empty chorion being observed. It is highly probable that mortality was at a high rate in egg-carrying females, or more exactly in females carrying pre-larvae, as observed by Grandjean (1940). In this case hatching occurs at the inside of mother body if pre-larval development was achieved, larvae soon coming out from the corpse of their parent. This stage could be protracted in *Ceratoppia bipilis* and *Oribatula tibialis*, being seemingly achieved at the following moult only. These hypotheses, coherent with my personal observations, need to be verified in culture, which is out of the scope of the present study.

Corpses observed in the F₁ layer displayed a variety of decay patterns, without any general or specific trend which could be discerned. Similarly, no successional trend between fungi and bacteria was discernable. However, it should be remarked that the most advanced stage of decomposition of corpses was a complete cleaning by animals which penetrated at the inside, either enchytraeids (Fig. 325) or oribatids (Fig. 324), which were identified by the typical shape and size of their faeces, when present. In this case, there were no longer microorganisms in the cleaned corpses, to the exception of a film on the inner side of the cuticle.

The inside of corpses was colonised by several fungi, which were often seen to cohabitate. An unidentified hyphomycete was repeatedly encountered, which had not been observed in decaying plant material or in faeces. It was present as a network of hyaline hyphae, weakly septate (Fig. 326), part of which could be dead (empty hyphae or 'ghosts') and mixed with bacteria (Fig. 327). Mycorrhizal fungi were also present. *Cenococcum geophilum* was often present, alone (Fig. 328) or in the company of bacteria (Fig. 329). This fungus attacked the cuticle, too. Its melanised hyphae could be seen running at the surface of the cuticle, with a decay zone a few μm wide on both sides of hyphae (Fig. 330). The attack of the cuticle was also observed to be caused by other unidentified fungi imperfecti (Fig. 331). *Hyphodontia sp.*, the basidiomycete associated with orange-brown mycorrhizae, was also

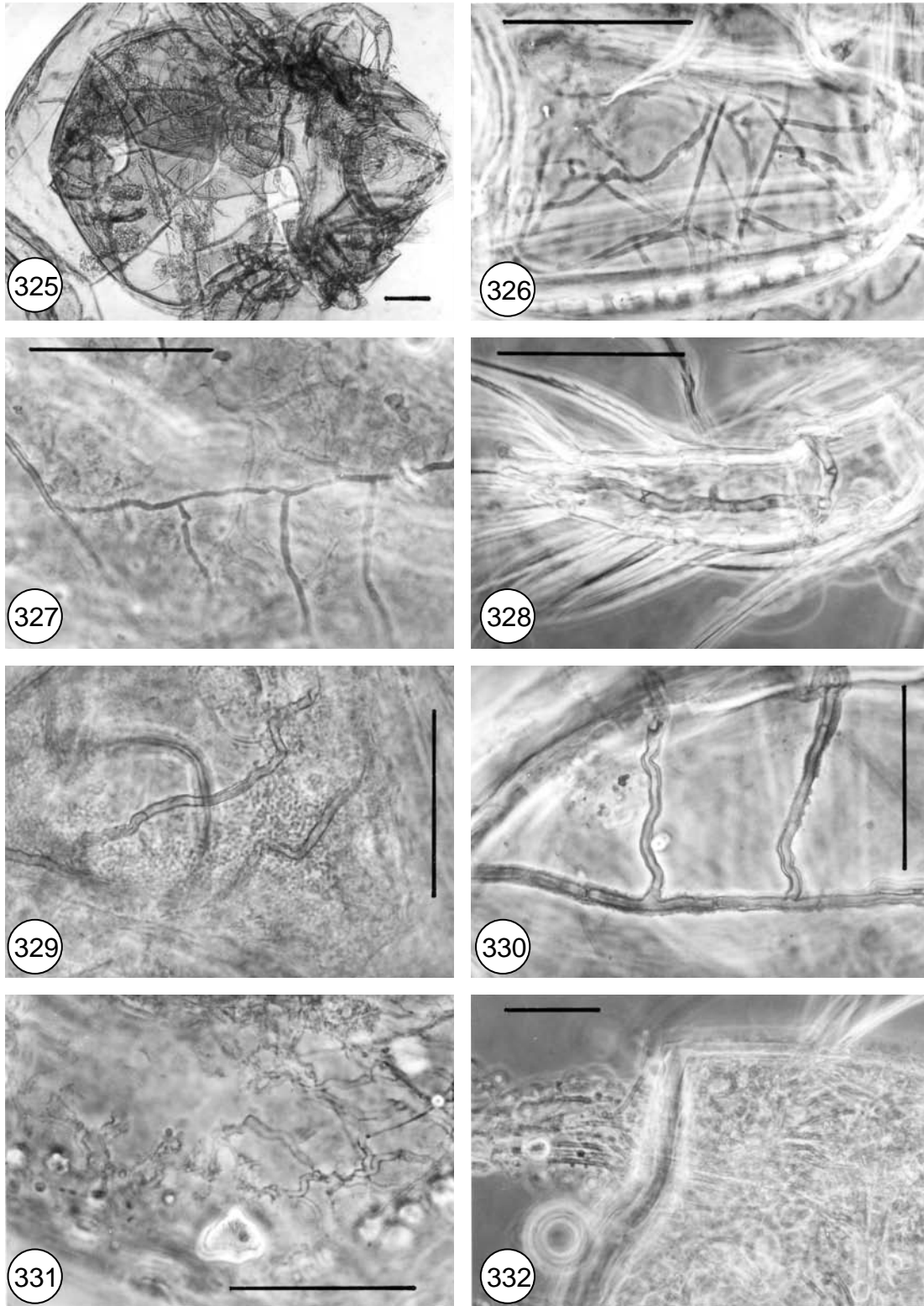


Fig. 325: Faeces of enchytraeid worms at the inside of a corpse of *Chamobates* sp. (CP); Fig. 326: Living hyaline hyphae at the inside of a corpse of *Oppia subpectinata* (CP); Fig. 327: Living and dead hyaline hyphae at the inside of a corpse of *Platynothrurus peltifer* (CP); Fig. 328: Melanised hyphae of *Cenococcum geophilum* at the inside of a leg from a corpse of *Rhysotritia duplicata* (CP); Fig. 329: Melanised hyphae of *Cenococcum geophilum* and bacteria at the inside of a corpse of *Rhysotritia duplicata* (CP); Fig. 330: Corpse of *Oribatula tibialis*, attack of the cuticle by hyphae of *Cenococcum geophilum* (CP); Fig. 331: Corpse of *Chamobates* sp., attack of the cuticle by hyaline hyphae; Fig. 332: Development of basidiomycetous hyphae of *Hyphodontia* sp. at the inside of a corpse of *Rhysotritia duplicata*

CP = phase contrast; length of segment = 50 μ m

seen penetrating oribatid corpses (Fig. 332). I found cystidia of this fungus, quite similar to those already described in L₂ (Figs. 120 & 121) and F₁ (Fig. 241), at the inside of two oribatid corpses, further attesting for the variegated way of life of *Hyphodontia sp.* Ectomycorrhizal fungi were also observed embedding corpses, as they did for the rest of decaying material.

Bacteria living at the inside of oribatid corpses were curiously similar to those living symbiotically in living specimens (intestinal microflora), as for example in *Platynothrus peltifer* (Fig. 333). Actinomycetes were also observed (Fig. 334), although less frequently.

Many animals seemed to live, at least temporarily, in oribatid corpses. Enchytraeids and oribatids (larvae and adults of small species) deposited faeces and, in the case of enchytraeids, pollen grains and quartz particles transported on the tegument. In addition, numerous cyst amoebae were observed, most of them (62%) being *Phryganella acropodia*, as mentioned above. Nematodes and rotifers were also observed, although less frequently.

Other arthropods

The colonisation of other arthropod corpses did not differ from that of oribatid mites. Fungal development, mostly of ectomycorrhizal fungi, was observed outside and inside decaying bodies. The basidiomycete associated with velvety pink ectomycorrhizae was observed at the inside of a gamasid leg (Fig. 335). The more corpses or fragments of legs were of a big size, the more frequently I observed traces of the passage of miscellaneous invertebrates: nematodes, cyst amoebae, mites, enchytraeid worms.

Animal faeces

Type A faeces (slugs?)

The examination of type A faeces under the dissecting microscope (Fig. 223) revealed that they were comprised of long fragments of pine needles, without any apparent change in their aspect. The microscopic examination of sections confirmed that point, showing unaltered fragile tissues such as mesophyll (Fig. 336). Fungal hyphae could be seen, attesting from previous fungal colonisation of pine needles, but all cells, whether of pine or fungi, were devoid of cytoplasm. Microbial development was only rarely observed and was localised to some micro-sites: bacteria (Fig. 337), chroococcal Cyanophyceae (Fig. 338), and very rarely fungi. These faeces, which have been tentatively attributed to slugs, did not seem to offer good conditions for the development of microflora, probably because of the absence of any plant or fungal cytoplasm. As a consequence, the pine material which was present at the inside of type A faeces seemed to be arrested at an earlier stage of decay than that of other pine needles found in F₁.

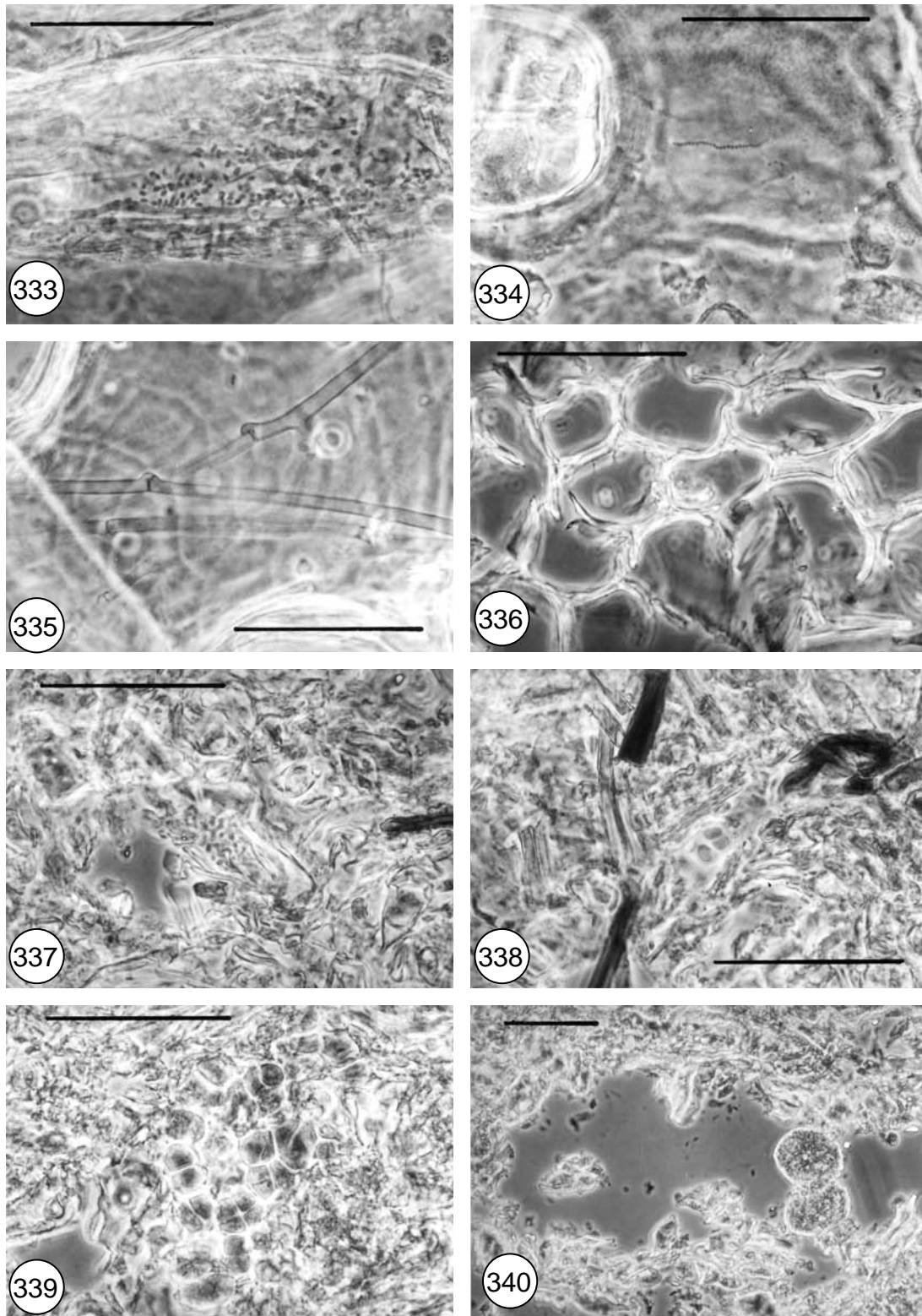


Fig. 333: Bacteria at the inside of a corpse of *Platynothus peltifer*, resembling those of intestinal microflora (CP); Fig. 334: *Oribatula tibialis*, actinomycetes on the inner surface of the cuticle (CP); Fig. 335: Hyphae with clamp connections of the basidiomycete associated with pink ectomycorrhizae, at the inside of a gamasid corpse (CP); Fig. 336: Section through type A excrement (slug?), detail showing pine needle mesophyll with empty cells but intact walls (CP); Fig. 337: Idem, showing bacteria in a micro-site (CP); Fig. 338: Idem, showing Cyanophyceae (cyanobacteria) in a micro-site (CP); Fig. 339: Section through type B excrement (woodlouse?), detail showing Chlorophyceae in a micro-site (CP); Fig. 340: Idem, detail showing oribatid faeces in a cavity (CP)

CP = phase contrast; length of segment = 50 μ m

Type B faeces (woodlice?)

Although fragments of pine needles found in type B faeces (Fig. 224) were shorter than in type A, they exhibited similar features when sectioned and examined under the light microscope: plant and fungal cell walls were intact, cytoplasm was absent, bacterial and algal colonies (Fig. 339) were limited to some micro-sites. It should be highlighted that micro-sites with microbial colonies were more abundant when not far from the surface of type B faeces, pointing to difficulties for colonising internal parts of faeces. Reasons for this limitation could be manifold: absence of interconnected pores, absence of vectors (fauna), anaerobic conditions. The same hypotheses have been already erected to explain particular features of soil micro-aggregates (Kilbertus, 1980). However, faeces of type B were penetrated by mesofauna, as attested by the deposition of oribatid faeces in some cavities (Fig. 340). The question thus remains quite open.

There was a great variation in the density of micro-sites colonised by bacteria from faeces to faeces. Decay of bacteria (fuzzy contour under phase contrast) was observed in some faeces which exhibited a high number of micro-sites (Fig. 341). This was interpreted as an indication of ageing.

The ingested material (pine needles) could be mixed with faecal material from animals having previously browsed the ingested pine needles. For instance, Figure 342 shows in a section the abrupt passage from transfusion tissue to a mass of finely ground plant material. This was interpreted as the presence of faecal material (probably from type C, i.e. earthworm faeces) applied against a pine needle which had been nibbled up to the central cylinder.

As in the previous case (type A faeces) the absence of any penetration by ectomycorrhizal fungi was remarkable. The absence of any moss material should be highlighted, too, indicating a clear trophic choice.

Type C faeces (earthworms)

Two major differences with the previous two types of macroinvertebrate faeces (types A and B) should be noted. First, type C faeces were comprised of small, although irregular particles (Fig. 343). This resulted from the ingested material being ground in the muscular gizzard of earthworms, hence the irregular size of particles, contrary to arthropods which cut the material with their mouthparts before swallowing it. Second, the content of type C faeces was composite, being an intimate mixture of plant cells, fungal hyphae and stromata, pollen grains, resin and oribatid faeces (Fig. 344). The fact that oribatid faeces were retrieved intact was indicative of their strong cohesion. Some quartz grains were found but only scarcely. Fungal hyphae, mostly represented by *Hyphodontia sp.*, the basidiomycete associated with orange-brown ectomycorrhizae, were abundant, which was not the case in faecal types A and B. The variegated nature of type C faeces strongly suggested the absence of definite trophic choice from the part of the earthworms which produced them.

When they were free in the environment (not attached to a plant substrate) type C faeces did not exhibit any intense bacterial and algal development. As in type B faeces, micro-sites filled with micro-organisms, such as chroococcal Cyanophyceae, were observed (Fig. 345). Sometimes bacterial

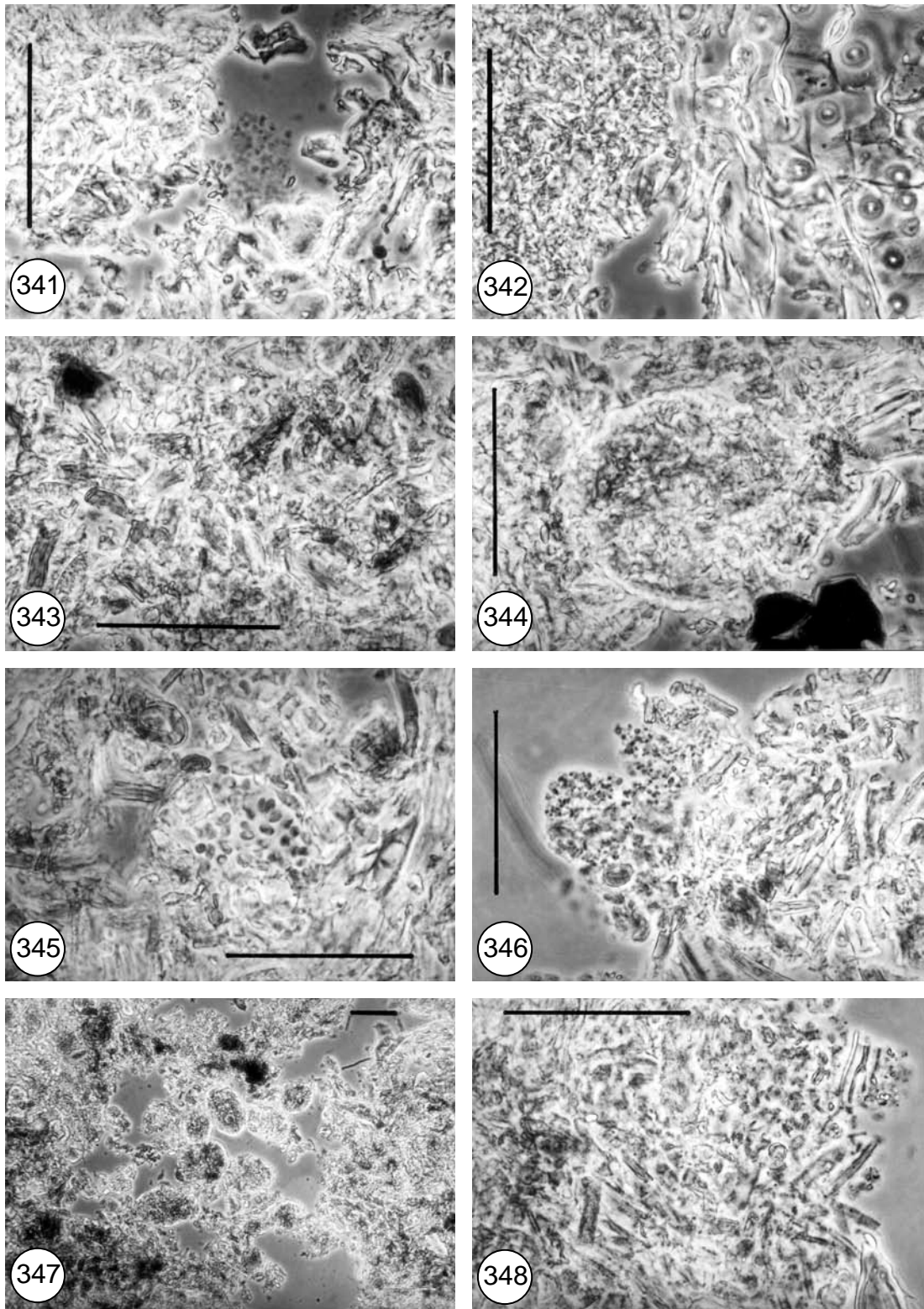


Fig. 341: Section through type B excrement (woodlice?), detail showing a bacteria colony in way to be degraded (CP); Fig. 342: Idem, detail showing the passage from transfusion tissue from pine needles (right side) to finely ground plant material from earthworm faeces (CP); Fig. 343: Section through type C excrement (earthworms), detail showing finely but irregularly ground pine tissues (CP); Fig. 344: Idem, detail showing an oribatid excrement (CP); Fig. 345: Idem, detail showing a micro-site with chroococcal Cyanophyceae (CP); Fig. 346: Idem, detail showing bacterial development at the surface of the excrement (CP); Fig. 347: Idem, detail showing box mite faeces deposited in a gallery (CP); Fig. 348: Idem, detail showing hyaline hyphae in way to be degraded (CP)

CP = phase contrast; length of segment = 50 μ m

development occurred at the surface of excrements (Fig. 346). However, type C faeces were intensely penetrated by fungi, either *Cenococcum geophilum* (Fig. 230) or basidiomycetous rhizomorphs. These faeces were also penetrated by animals, such as box mites, which bore galleries (Fig. 347). The tunnelling activity of an adult *Rhysotritia duplicata* was ascertained through the deposition among its faeces of an egg with a typical chorion in a gallery.

A great number of type C faeces were tightly appressed against fragments of plant organs (pine needles or bark, moss stems, etc...) or squashed between two distinct plant debris. Movements of animals were thought responsible for such deformation of freshly deposited lumbricid faecal material. Bacterial development was more intense than in free faeces and reached the inner part of the excrement, in addition to still colonised micro-sites. The external origin of microbial colonisation was attested by the more advanced stage of decomposition in the periphery of the faeces, in particular concerning hyaline fungal hyphae (Fig. 348). Several hypotheses were erected to explain differences in bacterial development between free and appressed type C faeces, none of them being fully supported by observations, (i) differences in ageing state, free faeces being more recent than appressed ones (but the latter were penetrated by slow-growing fungi such as *Cenococcum geophilum*), (ii) further contamination by animal movements, (iii) alleviation of anaerobiosis, etc...

It must be noticed that type C faeces, which were attributed to earthworms, did not exhibit any sign of mixing of mineral with organic matter. I hypothesized that they were produced by epigeic earthworms. Only one species was collected in the study site (Bouché, personal communication): *Dendrobaena octaedra*, an epigeic earthworm associated to very acid humus (Satchell, 1967; Bouché, 1972; Martinucci & Sala, 1979).

Mineral faeces

- Preliminary remarks

A weak number of faeces, instable when manipulated with forceps, exhibited a mineral content which attested for the deeper origin of their content. Their size and aspect was reminiscent of type C (earthworm) faeces, although *Dendrobaena octaedra* was classified as an epigeic. However, Martinucci & Sala (1979) considered this species as a weak burrower, not living exclusively in litter. It might be supposed that this animal moved down, probably for physiological reasons (diapause or quiescent stage), at some periods of the year (Satchell, 1967). I did not observe any mixing of mineral with organic matter, since the uppermost majority of earthworm faeces were purely organic, while a minority was purely or near-purely mineral.

- White mineral faeces

Their content probably came from the A₁₂ horizon (see Table 1) which was very poor in organic matter. They were comprised of quartz grains (of sand size), silt and clay particles. Traces of microflora (probably issued from deeper horizons) were observed: bacteria at the surface of quartz grains (Fig. 349), and empty and collapsed hyaline hyphae aggregating mineral masses (Fig. 350). These hyaline hyphae seemed to have a strong aggregative power: they were often seen covered with clay and silt mineral particles (Fig. 351). It has been verified experimentally that fungi were able to synthesize organic compounds with a high aggregative power for fine (< 50µm) particles (Martin et al., 1959).

- Grey mineral faeces

In addition to colonising hyaline hyphae which had been described in white mineral faeces, grey mineral faeces displayed fragments of empty sclerotia and hyphae of *Cenococcum geophilum* (Fig. 352) and opaque dark brown organic fragments (humus) of unknown origin. Despite of the grey colour of these faeces, which was due to the admixture of small organic debris (melanised fungal material, humified organic matter), quartz grains seemed clean, not covered with organic matter, if I except bacteria as depicted in white mineral faeces (Fig. 349). Grey mineral faeces came probably from the A₁₁ horizon, which was richer in organic matter and dark-coloured (see Table 1).

Enchytraeid faeces

These faeces were assembled in light-brown masses, free in the environment, at the inside of pine needles (Fig. 222) or between bark and wood in fallen branches and twigs of pine. Faeces were agglomerated by a dense network of fungal hyphae, which ensured the cohesion of faecal assemblages (Fig. 353). The composition of enchytraeid faeces was quite similar to their gut contents, plant material (mostly from pine) being dominant. Fungal material, which had been probably near totally digested (Fig. 297), was no longer observed in the excrements. On the contrary, plant cell walls were still intact. No bacterial development was visible, but faecal masses were abundantly colonised by hyphae of ectomycorrhizal fungi: *Cenococcum geophilum*, *Hyphodontia* sp., encrusted (Fig. 354) or not, and the basidiomycete associated with pink ectomycorrhizae (Fig. 355). Filamentous hormogonal (*Nostoc*-like) Cyanophyceae with heterocysts were often observed in the mycelium embedding enchytraeid faeces (Fig. 354). Cyst amoebae were very abundant, as well as naked amoebae (Fig. 290). Colonies of chroococcal Cyanophyceae were scarcely observed, too (Fig. 356).

Oribatid faeces

Whether they were found at the inside of pine needles (Fig. 221), bark, faeces of macroinvertebrates (Figs. 340 & 347) or free in the environment, most oribatid faeces were comprised of plant material, the pine origin of which was deduced from the presence of areolae. Sometimes some amount of fungal

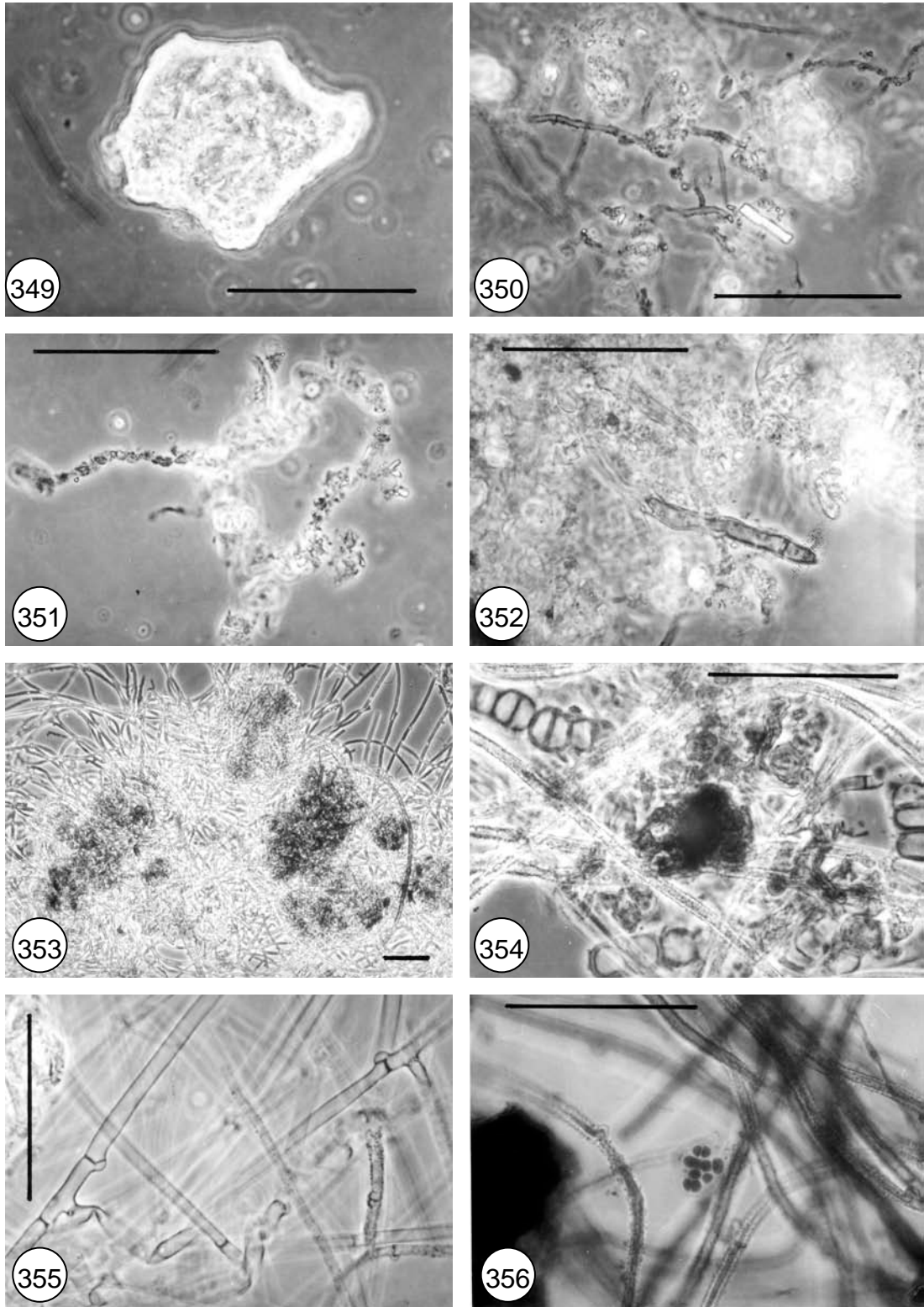


Fig. 349: Mineral faeces, detail showing bacteria at the surface of a quartz grain (CP); Fig. 350: Idem, detail showing empty hyaline hyphae aggregating mineral particles (CP); Fig. 351: Idem, detail showing the agglutination of clay and silt particles around dead hyphae (CP); Fig. 352: Idem, fragment of hypha of *Cenococcum geophilum* (CP); Fig. 353: Enchytraeid faeces embedded in the secondary mycelium of *Hyphodontia* sp. (CP); Fig. 354: Idem, detail showing encrusted hyphae of *Hyphodontia* sp. and filamentous Cyanophyceae (CP); Fig. 355: Idem, detail showing hyphae of the basidiomycete associated with pink ectomycorrhizae in the mycelium embedding enchytraeid faeces (CP); Idem, detail showing a colony of chroococcal Cyanophyceae (BMSL)

CP = phase contrast; BMSL = methyl blue-Sudan III-lactophenol coloration; length of segment = 50 μ m

material was visible, only hyphae of *Cenococcum geophilum* being still recognizable (Fig. 357). Given their plant content, the majority of oribatid faeces observed in the F₁ layer came from box mites, smaller faeces being attributed to larvae, faeces of intermediate size to nymphs and larger faeces to adults. The degree to which pine material was ground differed according to the size of excrements and thus to 'stases' of the species, as explained above. There was always a contrast between the outer and the inner part of the excrement (Fig. 358), the outer part being lightly coloured while the inner part was of a brown colour. Observations on large faeces, where the plant material was coarsely ground (Fig. 359), showed transparent, refringent cell remains in the periphery while the inner part was made of an amorphous mass, brown opaque, without any recognizable item. It had been previously observed that this phenomenon was not due to ageing of faeces, but could be followed at the inside of oribatid guts (Fig. 311). Brown spherules, which had been already observed at the inside of pine needles (Fig. 235), were retrieved intact in oribatid faeces (Fig. 360). They can be suspected to be highly resistant to digestive enzymes, whether of microbial or animal origin.

Microbial colonisation of oribatid faeces was feeble in the F₁ layer, being often limited to the surface of excrements, where bacterial and algal development could be observed (Fig. 361). However, some faeces, of small size and lightly coloured, maybe older (?), displayed an intense bacterial development over their whole thickness (Fig. 362).

Sciarid faeces

Sciarid faeces were identified as such when larvae were still present among their own faeces, otherwise they were not classified. Their content was variegated. Consumption of pine needles was attested by the presence of epidermic remains (Fig. 363) or xylem tracheids, the same for bracken fern, but fungal material was largely dominant: hyphal walls of *Cenococcum geophilum* or *Hyphodontia* sp., fragments of sclerotia or of fungal mantles. Moss fragments were also found. However, the absence of soft tissues of pine (transfusion tissue, mesophyll, root cortex) and bracken (fundamental parenchyma), and the observation of living specimens (Fig. 323), let me suppose that most soft plant tissues were entirely digested, cell walls comprised. However, fungal walls remained intact, hence the apparent dominance of fungal material.

Bacterial development was never observed at the inside of sciarid faeces, only chroococcal cyanobacteria (Cyanophyceae) were observed at the surface of some faeces (Fig. 324).

Conclusions about the F₁ layer

Compared to L₁ and L₂, it must be noticed that faunal activity dramatically increased in the F₁ layer. Several zoological groups, phylogenetically remote, participated to the fragmentation of pine needles: earthworms (Annelida), woodlice (Crustacea), slugs (Mollusca) for macrofauna, enchytraeids (Annelida), box mites (Arachnida) and sciarid larvae (Insecta) for mesofauna. Microfauna were also involved in the internal colonisation of pine needles: cyst and naked amoebae (Protozoa), nematodes (Nematoda), rotifers (Rotifera). Among these groups, the feeding activity of earthworms and box

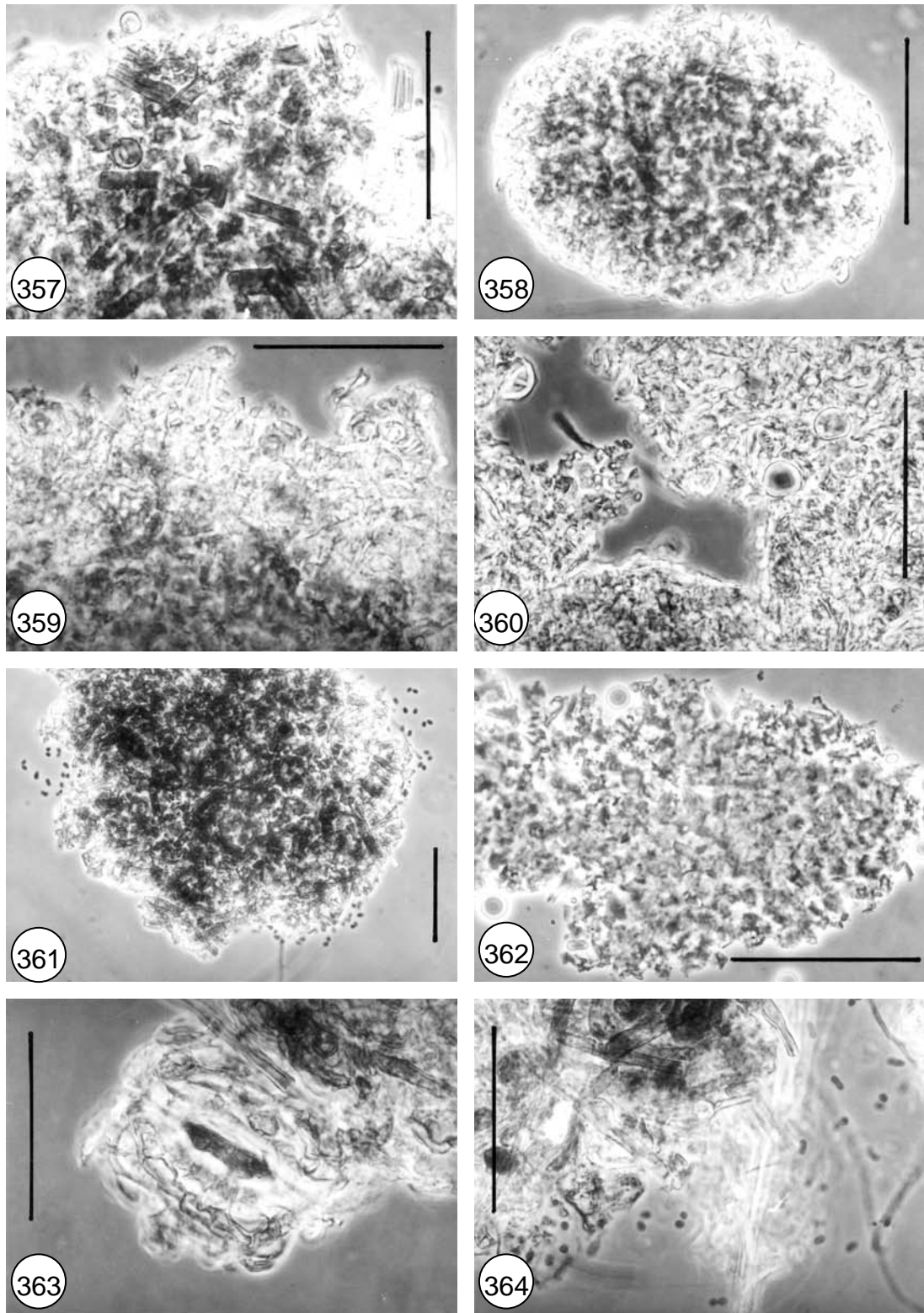


Fig. 357: Oribatid faeces, detail showing persistence of hyphae of *Cenococcum geophilum* (CP); Fig. 358: Idem, overview of a small excrement showing browning of finely ground pine material in the inner part (CP); Fig. 359: Idem, detail of a large excrement showing more coarsely ground pine material (CP); Fig. 360: Idem, detail showing the persistence of brown spherules from pine needle tissues (CP); Fig. 361: Development of chroococcal Cyanophyceae at the periphery of an oribatid excrement (CP); Fig. 362: Bacterial development at the inside of a small oribatid excrement (CP); Fig. 363: Sciarid faeces, detail showing remains of pine epidermis (stomatal guard cells) (CP); Fig. 364: Idem, detail showing chroococcal Cyanophyceae at the periphery (CP)

CP = phase contrast; length of segment = 50 μm

mites was dominant. Both groups did not process pine needles in the same manner. While earthworms incorporated pine needle material with moss, pollen, and fungal material (among others) in their faeces, which were deposited in the environment, box mites accumulated their faeces at the inside of pine needles, only internal tissues being consumed.

There was a neat impact of animals on cytoplasm, whether of plant or microbial origin. This was not the case for cell walls. Hyaline fungal walls were digested by all animal groups, to the exception of sciarid larvae, but plant cell walls were degraded only by sciarid larvae and box mites. Hydrolysis of cellulose could be suspected to occur in sciarid larvae, but box mites transformed cellulose by another process, without solubilising it. In the latter case cellulose became brown and lost its transparency and refringence, but did not disappear as solid matter. Since this was the result of observations, without any dosage or chemical characterisation, it cannot be concluded definitely that there was digestion of cellulose in the former case (sciarids) and humification in the latter case (oribatids), although this was highly probable. Anyhow, a strong impact of both groups on cellulosic cell walls is beyond doubt. Concerning melanised fungal walls (*Cenococcum geophilum* for the main) it should be noted that only a limited array of oribatid mites belonging to the Camisiidae family (*Platynothrus peltifer*, *Nothrus sylvestris*) were able to transform this highly recalcitrant material.

Despite of the fact that animal activity was much higher in F₁ than in the overlying layer L₂, it should be noted that most plant debris, when not consumed by soil animals, did not change further. Most transformations of the plant material took place in animal guts and faeces. A great variety of microorganisms was observed (bacteria, algae, fungi), with an accumulation of empty fungal walls, whether hyaline or melanised. However, most microbial development was that of ectomycorrhizal fungi of pine. Bacteria were in lower numbers in F₁, and animal faeces did not seem to be a favourable micro-habitat for them. Concerning algae, Cyanophyceae were in lower numbers compared to overlying layers, but this decrease was less pronounced for Chlorophyceae. A negative impact of ectomycorrhizal fungi on bacterial populations could be suspected from their concomitant increase and decrease with depth, respectively.

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