Mycophagous Amoeboid Organisms From Soil that Perforate Spores of Thielaviopsis basicola and Cochliobolus sativus

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ABSTRACT

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Direct visual evidence in laboratory culture chambers showed that certain free-living, soil-inhabiting, amoeboid organisms perforated and fed on chlamydospores and mycelium of *Thielaviopsis basicola* and conidia of *Cochliobolus sativus*. The annulations and performations of fungal spores observed in the culture chambers were similar to those found in spores incubated in field soils obtained from various locations in Canada. The systematic position of the mycophagous amoebae seems to be with the protozoan order Proteomyxida and the family Vampyrellidae, but species identification cannot be made with certainty because the taxonomy is still controversial. Based on their widespread distribution in arable soils and their marked predacious activity it is postulated that the mycophagous soil amoebae may play a significant role in the ecology of soilborne fungal plant pathogens and their biological control.

Additional key words: mycophagous Vampyrellidae.

Various investigators (4, 17, 20) have reported that pigmented conidia of *Cochliobolus sativus* (Ito and Kurib) Drechs. ex Dastur, chlamydospores of *Thielaviopsis basicola* (Berk. and Br.) Ferr. and spores of other species of fungi (21) often exhibit perforations in their walls after incubation in natural soils for several weeks. The size of the perforations usually ranged from 0.2 to 6 μm in diameter. Annular depressions on the wall surface are also frequently observed on the spores and are apparently the initial stages in the formation of the larger holes (20). Scanning and transmission electron microscopy shows the details of the perforations but they are also visible with the light microscope, especially with phase-contrast and Nomarski interference-contrast light microscopy.

Although perforation of spores can be readily observed when spores are incubated for several weeks in moist arable soils obtained from many locations, attempts to isolate specific causal agents and carry out "Koch's postulates" have been unsuccessful (5, 6, 20). Recently Anderson and Patrick (1) and Old (18, 19) presented direct visual and other evidence showing that soil-inhabiting amoeboid organisms were capable of causing the characteristic annular depressions and perforations in spores of *C. sativus* and *T. basicola*. These studies (1, 18, 19) appear to be among the first to describe perforation and parasitism of spores of higher fungi by amoeboid organisms. There have been reports, however, of similar amoeboid organisms causing perforations and feeding upon *Oedogonium* sp. in the wall of which "a beautiful round hole was bored in the membrane" (23), and various algae including *Spirogyra* sp. (12, 13). There have also been reports of similar amoeboid organisms from soil feeding on bacteria (24, 25) and on various species of nematodes (22, 26). The purpose of the present study is to present additional information on this association, with emphasis on means of isolating the amoebae, their distribution in soil, activity, and cultural characteristics.

MATERIALS AND METHODS

Isolation of the amoebae.—Soil suspensions were prepared by mixing 5 g of soil (Vineland clay loam from field plots of Horticultural Research Institute, Vineland Station, Ontario), with 5 ml of sterile distilled water (SDW) in 150 × 15-mm test tubes. The numerical increase of the perforating amoebae was favored by the addition of 1 ml suspension of spores of *C. sativus* or chlamydospores of *T. basicola* (approximately 50,000 spores/ml) to each test tube of soil suspension. After 3 wk of incubation at 22 C, samples of the suspension containing spores with perforations were transferred to suspensions of spores without perforations and incubated for an additional 3 wk. After this second period, cysts of the amoebae were evident with the aid of a stereomicroscope at ×100. These cysts were isolated with a micropipette and serial washed in SDW. Single cysts were isolated and cultured in SDW in 250-ml flasks at 20-22 C, using fungal spores as food. After 3-4 wk of incubation the cultures were stored at 5 C. To establish new cultures of the amoebae, cysts or the perforated fungal spores containing cysts, were transferred from the above to a fresh suspension of fungal spores. This method has been used successfully to maintain the amoebae in culture since December 1975.

Bioassays for the amoebae in field soil were conducted

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by serial dilution and most probable number techniques (16). Soil samples (5 g dry weight) were mixed in a blender with 45 ml tap water for 5-10 sec prior to serial dilution. One-milliliter samples from 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions were transferred to test tubes. Spores of *T. basicola* or *C. sativus* (1 ml of 5×10^5 spores/ml per tube) were used as an indicator of perforating activity in the soil. Each dilution series was replicated five times. After 6 wk incubation at 20 C soil smears were prepared from each test tube and microscopic observations were carried out at ×100 and ×250. Readings were based on presence or absence of perforations in 100 fungal spores.

Observations of mycophagous activity of the amoebae.—The various stages of mycophagous activity of amoebae, from attack of fungus spores through perforation, feeding, and encystment, were studied on microscope slides with and without cover slips. Undisturbed cultures in plastic petri dishes and in specially constructed well slides also were studied by means of an inverted microscope (Model, Reichert) equipped with phase-contrast optics. The well slides were prepared by drilling two holes, 6 mm in diameter, through a plastic slide (6 × 3 × 4 cm) and sealing one side of each hole with No. 1 cover slip (22 mm diameter) and candle wax. A plastic ring 1.5 cm in diameter and 1 cm high was glued permanently around the top of each hole and removable plastic caps were fitted over each ring to prevent desiccation. These units are referred to hereafter as well-slide chambers. The amoebae and spores were placed in these wells. They could be located readily in the limited area.

TABLE 1. Populations[a] of mycophagous amoebae (capable of causing perforations of spore walls of *Thielaviopsis basicola* or *Cochliobolus sativus*) found in cultivated and sod soil as affected by sampling date and soil depth at Vineland, Ontario

Sample date	Mycophagous amoebae in: Cultivated soil 0-5 cm (no./g)	Cultivated soil 5-15 cm (no./g)	Cultivated soil 15+ cm (no./g)	Sod soil 0-5 cm (no./g)	Sod soil 5-15 cm (no./g)	Sod soil 15+ cm (no./g)
May 1976	15.0	9.0	0	1.0	1.0	0
May 1977	3.5	3.0	7.0	2.5	2.0	2.5
Oct 1977	11.3	4.0	0	4.5	1.0	0.5
Means	9.9	5.3	2.3	2.7	1.3	1.0

[a]Estimated by most probable number method (O. C. Maloy and M. Alexander, Phytopathology 48:126-128).

Effect of temperature on excystment.—The effect of temperature on excystment of the amoeba was studied as follows. Cysts of the amoeba were washed once by centrifugation and added to small plastic petri dishes containing 5 ml of 10% soil extract (SE). Individual cysts were placed in notches scratched in the base of each dish. Approximately 25 notches per dish were made by scratching with a scalpel. The dishes were incubated at temperatures of 5, 10, 15, 20, 25, 30, and 35 C (±1 C). The soil extract was prepared by steaming (100 C) 100 g of field soil in 100 ml of distilled water (DW) for 1 hr. The supernatant was diluted 1:10 with DW and autoclaved. All treatments were replicated four times and the experiment was repeated once.

Multiplication of the amoebae.—The rate of multiplication of the amoebae was studied in the well slide chambers in 10% SE. Each chamber was inoculated with a single amoeba cyst and 500−1,000 chlamydospores of *T. basicola* or conidia of *C. sativus*. The fungus spores were produced on V-8 Juice agar and washed three times by centrifugation in SDW. The chambers were incubated at 20 C and observations were made using an inverted microscope at ×100 and ×250 magnification. Active and encysted organisms were counted. As controls, the cysts were incubated under similar conditions but without the fungal spores.

OBSERVATIONS AND RESULTS

Occurrence of the mycophagous amoebae in soil.—The mycophagous amoebae were found in seven of the ten arable soils sampled in southwestern Ontario. In one test 13 amoebae were found per gram of a well-drained clay loam and 11 per gram were found in poorly drained sandy loam, based on estimates by the most probable number method.

TABLE 2. Effect of incubation time and food source on increase of mycophagous amoebae in culture and number of fungal spores perforated

Food sources[a]	Data[b]	Amoebae and perforated spores per chamber after incubation at 20 C: 0 day (no.)	1 day (no.)	2 days (no.)	3 days (no.)	4 days (no.)	5 days (no.)	6 days (no.)	7 days (no.)
C. sativus conidia	A	1	1.2	3.2	7.6	10.3	24.5	42.7	77
	B		0.9	2.8	10.5	20.5	51.9	144.8	276.5
T. basicola chlamydospores	A	1	1.1	4.5	8.3	7.9	12.0	19.4	25.5
	B		0.3	19.0	34.0	42.5	95.3	175.8	386.3
Bacterial species	A	1	1.1	1.4	1.1	1	1	1	1

[a]Food sources: conidia of *Cochliobolus sativus*, chlamydospores of *Thielaviopsis basicola*, and large numbers of unidentified species of soil bacteria.

[d]Data row identification: A = number of amoebae per chamber and B = number of perforated spores per chamber. Data are the means of counts from 10 chambers.

Soil samplings were conducted in 1976 and again in the same locations in 1977. In 1976 the amoebae were most numerous in the top 5 cm of soil but in 1977 they were more evenly distributed to a depth of 20 cm (Table 1). The deeper distribution of the amoebae in 1977 may have been due to less rain during the 2 mo prior to sampling.

Cysts of the perforating amoeba were observed most frequently in association with or actually inside fungal

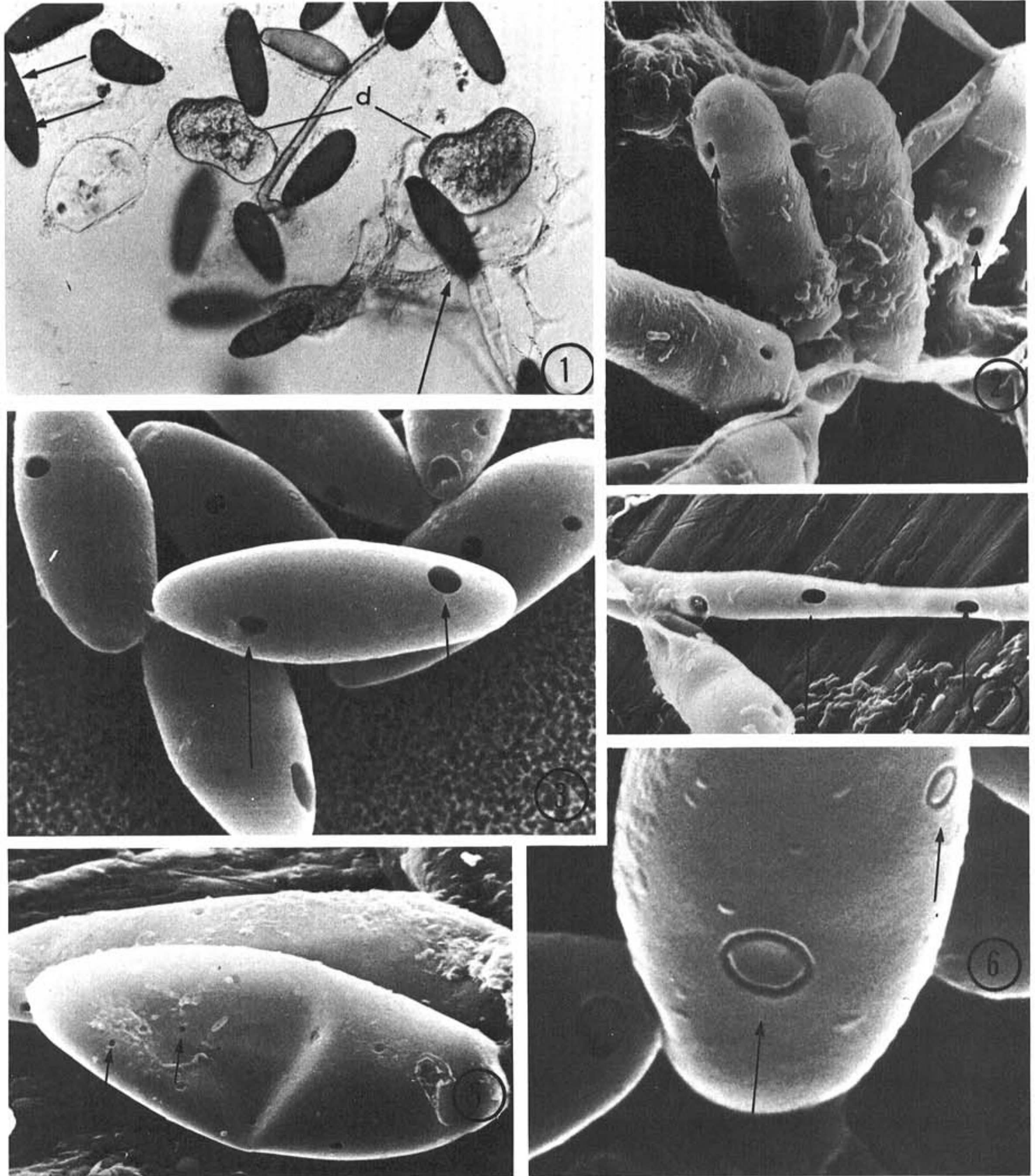

Fig. 1–6. Kinds and stages of perforations caused by mycophagous amoebae in spores of *Cochliobolus sativus* and *Thielaviopsis basicola*. **1**) Conidia of *C. sativus* with perforations in their walls (short arrows) and mycophagous amoeba (long arrow) attacking other conidia; digestive cysts of the amoeba also are shown (d), ×250. **2**) Scanning electron micrograph (S.E.M.) of chlamydospores of *T. basicola* with perforated walls (arrows), ×1,000; **3**) S.E.M. of *C. sativus* conidia with perforations (arrows) ×1,000. **4**) S.E.M. of hypha and chlamydospore of *T. basicola* with perforations (arrows), ×1,000. **5**) S.E.M. of *C. sativus* conidia with smaller perforations (0.5 to 1 μm diameter) (arrows), ×1,500. **6**) S.E.M. of *C. sativus* conidia with annulations (arrows), ×2,500.

spores with large perforations (1-7 μm in diameter) in their walls. In some of the bioassay tests, cysts of the amoebae were not seen; however, when fresh fungal spores were added as bait to these soils and the soils incubated in petri dishes, active amoebae were often seen perforating the spores after 1 to 2 wk. In some tests we also observed amoebae of *Mayorella* sp. (14) which appeared to be consuming the species responsible for

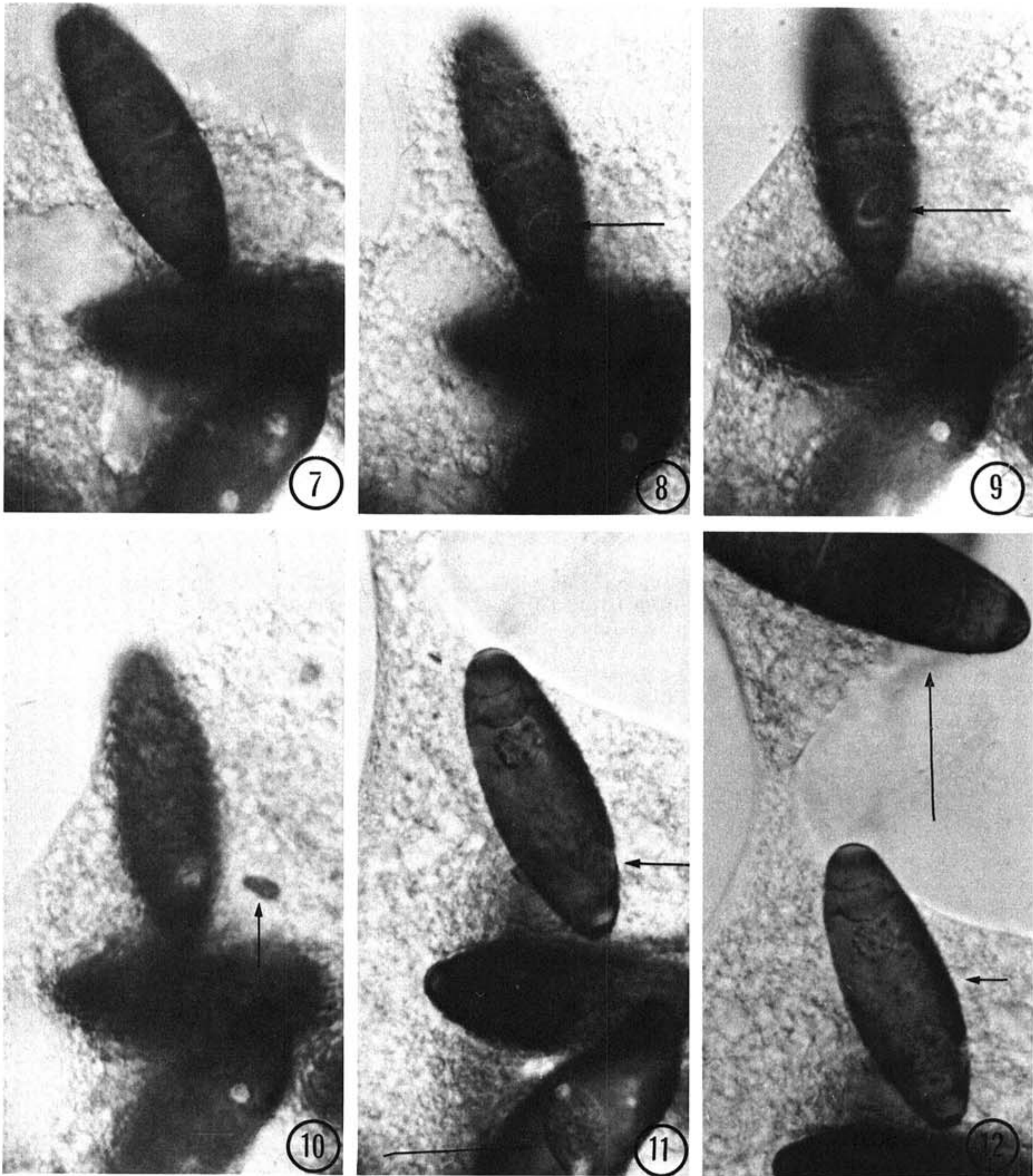

Fig. 7–12. Nomarski interference-contrast light micrographs showing selected sequences of the perforation of *Cochliobolus sativus* conidia by the mycophagous amoeba; **7**) amoeba partially surrounds the conidium, time 0; **8**) annulation on conidial wall becoming evident (arrow), time 10-15 min; **9**) loosening of annular disk (arrow), time 15-20 min; **10**) annular disk expelled (arrow), perforation in conidial wall visible, time 20-25 min; **11**) conidial contents partially emptied by the amoeba, perforation is to one side (arrow) due to shifting of spore, time 30-40 min, note spore at bottom with digestive cyst (arrow); **12**) original spore almost empty of contents (short arrow) and the amoeba begins to attack another spore (long arrow), time 40-50 min, ×800.

spore perforations. The parasitism of one amoeba species by another may explain an apparent loss of perforating ability noted with some soils with incubation time in our laboratory studies. We also observed several other species of soil amoebae which completely engulfed the fungal spore and produced smaller diameter (0.2–1.0 μm) perforations (Fig. 5).

Feeding and mode of penetration of fungus spores.—The amoeboid organism fed and multiplied readily on melanized fungal spores but were not observed to feed on the soil bacteria present in the cultures as contaminants (Table 2). During 10 days of incubation some 276 conidia of *C. sativus* and 386 chlamydospores of *T. basicola* were perforated and emptied of cytoplasm from an initial inoculation of one amoeba into the culture chamber. Feeding and multiplication of the amoebae did not occur when incubated under similar conditions in the presence of large populations of soil bacteria. In the culture chambers containing the bacteria the average diameter of the cysts decreased from 36 μm to 17.9 μm after 10 days of incubation. The average cyst diameter of amoebae feeding on fungal spores remained constant or decreased only slightly during the 10-day incubation period (Table 3).

The amoebae also were observed to perforate and feed on chlamydospores of *Fusarium solani*: (Mart.) App. & Wr. em. Snyd. & Hans., as well as spores of *Alternaria tenuis* Auct., and on endoconidia of *T. basicola*. In cultures containing algae and other protozoa, the amoeba also engulfed and digested small chlorophyll-bearing flagellates and consumed the contents of small cysts belonging to soil amoebae collectively known as the *limax* amoebae (14). The amoebae also perforated and consumed quiescent nematodes belonging to *Meloidogyne, Rhabditis,* and *Mononchus*. The amoebae did not immobilize active nematodes or form digestive cysts around them as described for *Theratromyxa weberi* Zwillenberg (22,26). The feeding process associated with nematodes, including formation of digestive cysts and excystment was similar to that described for fungal spores (Fig. 20). Perforations approximately 5 μm in diameter were clearly visible in the empty cuticles of the affected nematodes.

The amoeba appeared to locate the fungal spores by chance encounters. Generally the organism was in contact with the spore for several minutes before feeding occurred. The first indication of feeding was the flowing and clumping of the amoeba around the spore but the spore rarely was enclosed completely. The filopodia gradually contracted and large vesicles up to 7 μm appeared in the cytoplasm which then appeared to flow back and forth over the spore wall. Eventually a few vacuoles became stationary and an annulation in the spore wall became visible at the periphery of the vacuoles. To produce an annulation, a vacuole of the amoeba remained stationary and in contact with that section of the spore wall for approximately 5 min or longer. In profile the cytoplasm within the amoeba became more concentrated over the annulation. Eventually the annulation was complete and the disk within the annulation area became detached from the spore wall (Fig. 7 to 12). The detached disk was drawn into the amoeba or was lifted and moved to one side. Shortly, the plasmalemma of the attacked spore burst and the cytoplasm bubbled violently and flowed into the amoeba. Excess water in the spore cytoplasm was gradually expelled by a number of contracting vesicles formed at the periphery of the amoeba. The organism entered the spore through the one or two perforations it had made and removed the last traces of protoplasm. The spore then appeared to be empty and the perforations (1–7 μm in diameter) in its wall were visible (Fig. 11, 12). The perforation and feeding process may take only 15 min but may last up to 30 to 60 min. The perforation and feeding process was similar on spores of *C. sativus, T. basicola,* and *Fusarium solani*. A typical perforation and feeding sequence is shown in Fig. 7 to 12.

Activity and morphology of the mycophagous amoebae.—The amoebae responsible for spore perforations in our studies were similar to those described by Goodey (8) and others (3, 11). In the creeping or trophic extended state the individuals often exceeded 500 μm in length and sometimes fused with adjacent organisms to produce reticulate plasmodia, 1 to 2 mm in length (Fig. 13, 14). In a contracted state prior to encystment, the organisms were approximately 50 μm in diameter. Extremely fine filopodia were the only type of pseudopodial structures produced (Fig. 14). The filopodia, which are very active, commonly were produced at the extremities but were produced from any portion of the body. Anastomoses between adjacent filopodia or with filopodia from different parts of the organism were common. The organism moved by flowing between or behind the main filopodia or over a broader front behind the shorter filopodia. The protoplasm of the organism often became concentrated in two or more areas

TABLE 3. Effect of incubation time and food source on cyst size of the mycophagous amoebae

Food source	Cyst size after incubation at 20 C		
	0 day (μm)	6 days (μm)	10 days (μm)
C. sativus conidia.	39.6[a] ± 9.3	36.6 ± 6.9	35.2 ± 10.0
T. basicola chlamydospores	37.5 ± 7.4	31.8 ± 8.3	31.4 ± 9.5
Soil bacteria	36.0 ± 7.3	24.7 ± 1.7	17.9 ± 1.7

[a]Cyst diameter (μm); for *Cochliobolus sativus* and *Thielaviopsis basicola,* 25 cysts were measured; 10 cysts were measured from cultures with bacteria as the food source.

connected by extremely fine threads of protoplasm. The protoplasm was dense and faintly brownish in transmitted light. It contained numerous small granules and contractile vesicles 1-2 μm in diameter (Fig. 13, 14). Clear protoplasm often was observed between adjacent filopodia, but this could be due to the extreme thinness of

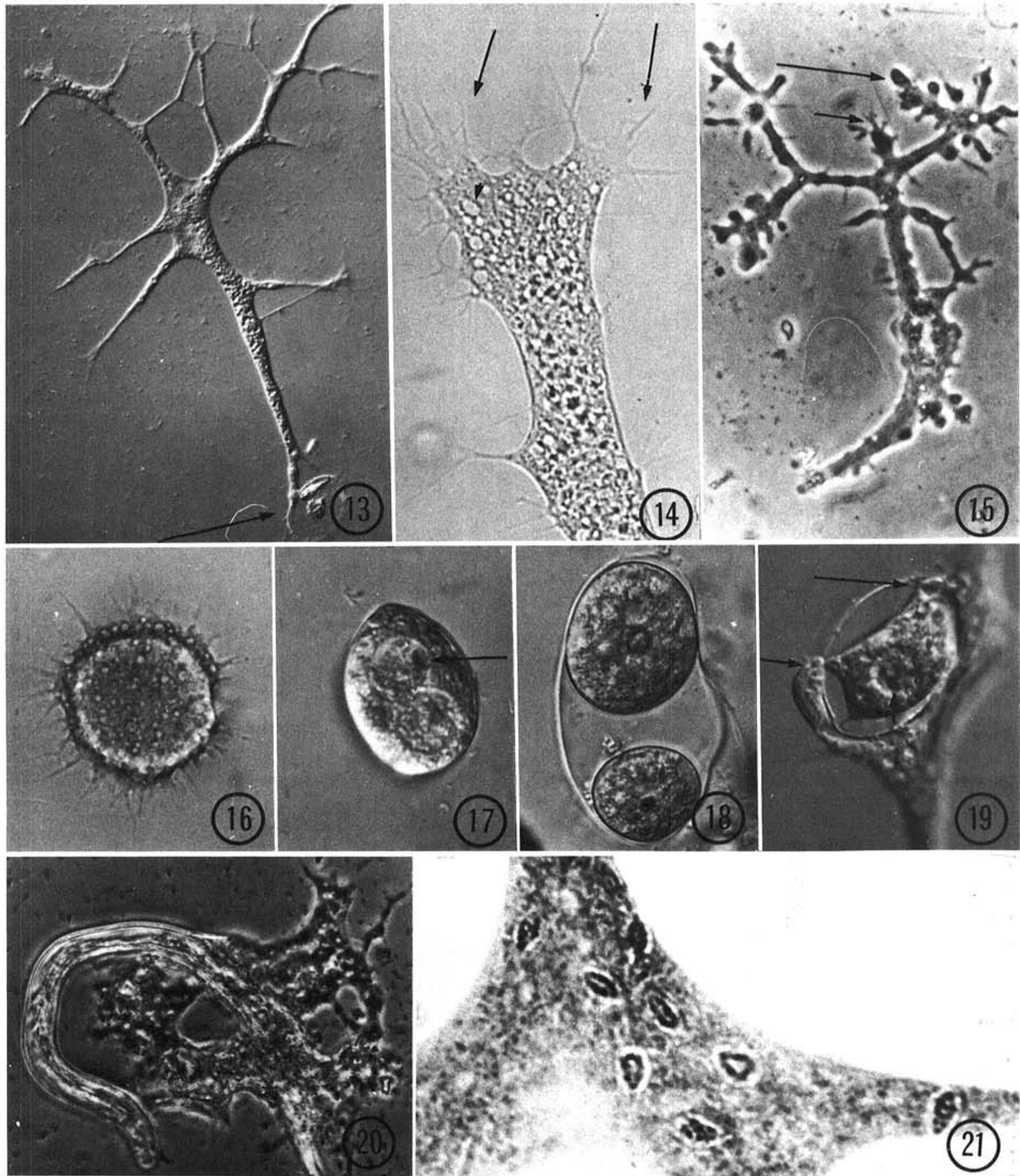

Fig. 13–21. Nomarski interference-contrast light micrographs of mycophagous amoebae showing various stages. **13)** Trophic mycophagous amoeba [note characteristic branching and cyst (arrow)] ×270; **14)** terminal portion of amoeba showing characteristic filopodia (arrows) and small contractile vesicles (arrow), ×700; **15)** *Leptomyxa reticulata* [note lobopodia (large arrow) and filopodia (small arrow)] ×375. **16)** Mycophagous amoeba during cyst formation, ×700; **17)** digestive cyst of the amoeba containing two digestive vacuoles (note spore wall disk within vacuole (arrow) ×700; **18)** two resting cysts within a larger digestive cyst, ×700; **19)** excystment, note three excystment holes (large arrow) and vacuole containing spore wall disks and other undigested residue (arrow), ×700. **20)** Nematode attacked by mycophagous amoeba, ×375. **21)** Nuclei of mycophagous amoeba, ×2,000.

the body. Some of the observed stages in the life cycle of the mycophagous soil amoeba are illustrated in Fig. 13 to 19 and schematically in Fig. 22.

The organism was multinucleate, but nuclei were not visible in living specimens. The nuclei were seen readily in stained preparations using Schaudinn's fixative (15) and Delafield's haematoxylin stain (14). The nuclei were oblong, spindle shaped, eucaryotic, and 2-4 μm long (Fig. 21). The number of nuclei per organism varied and in large individuals up to 30 nuclei were counted.

Multiplication encystment and excystment of the amoebae.—The amoebae reproduced by simple division of the protoplasm into two or three individuals; these resembled the original amoeboid form except that they were smaller. No differentiation of the protoplasm was observed prior to division. The division took place before encystment or directly following excystment. Fusion between adjacent individuals was observed with no apparent change in subsequent activity. Occasionally in large individuals the protoplasm became concentrated into several masses which are connected by fine protoplasmic threads; these masses often became reunited again into one undifferentiated body.

Shortly after an amoeba had perforated and fed on a fungal spore it became progressively sluggish and less extended. The filopodia were gradually withdrawn and the organism assumed a disk or spherical shape (Fig. 16). The volume of the protoplasm seemed to diminish probably due to water loss from the many contracting vesicles found at the periphery of the organism. A smooth cyst wall developed in 1 to 3 hr (Fig. 17, 18). During this time a large central digestive vacuole was formed. This vacuole contained the fungus protoplasm and the disks from the fungal spore walls. In older cultures the cyst contents continued to contract as digestion took place and a second and third cyst wall was formed. Occasionally large cysts, containing two to five secondary cysts, were observed (Fig. 18). Their occurrence likely

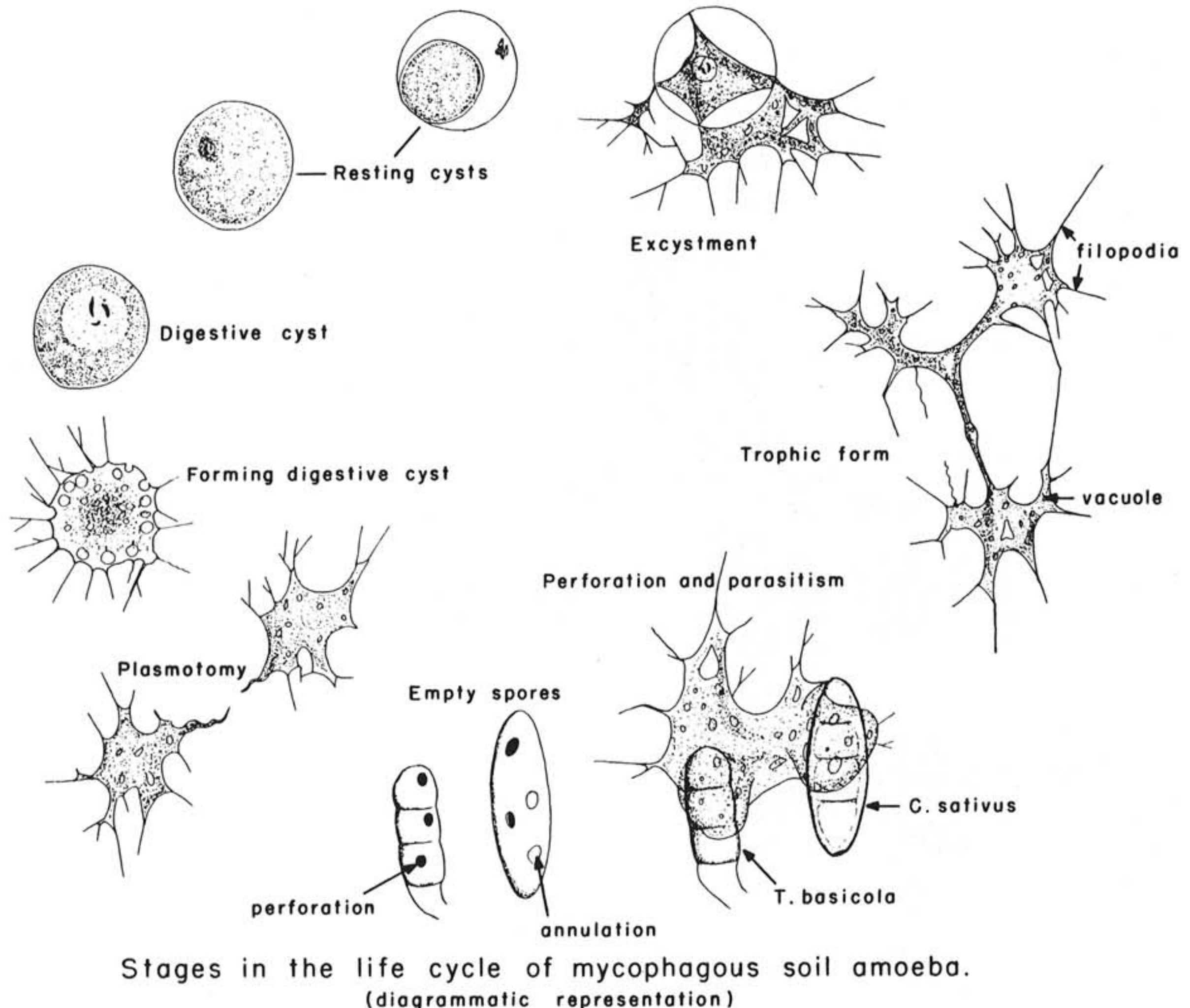

Fig. 22. Schematic representation of the various stages in the life cycle of the mycophagous soil amoeba.

depended on the size of the original amoeboid mass and the number of digestive vacuoles present at encystment.

In freshly established cultures containing abundant fungal spores excystment occurred after 12 to 24 hr (Fig. 19). The conditions bringing about excystment were not studied extensively. Soil extract seemed to enhance the process (Table 4). Before excystment, cytoplasmic activity within the cyst increased until the entire contents began rotating. Excystment occurred through three or four minute holes in the cyst wall; these holes were evident only during excystment. The nondigested residue remained within the cyst wall. The excystment process, from the time the organism began to emerge until it was free of the cyst, took approximately 15-30 min. In an aqueous medium containing 10% soil extract the maximum, minimum, and optimum temperatures for excystment were 30-35 C, 5 C, and 15-20 C, respectively (Table 5).

DISCUSSION

Some of the free-living amoebae in soil have the ability to perforate, penetrate, and feed on chlamydospores and mycelium of *T. basicola* and conidia of *C. sativus*. Except for our earlier report (1) and those of Old (18, 19) on *C. sativus*, this appears to be among the first studies describing a new association of organisms, namely that of soil amoebae predacious on plant pathogenic fungi. The significance of these findings, however, are difficult to assess at this time but we believe that they have important implications in the study of the ecology and the biological control of soilborne plant pathogens.

In laboratory culture chambers the amoebae appear to be very effective in reducing populations of viable spores. It is significant too that the amoebae appear to feed selectively on pigmented spores, the type of fungal propagules that are believed to be most resistant to biodegradation in soil (2). At present, however, we cannot postulate the effectiveness of the mycophagous amoebae under natural field conditions nor their relative importance in the biological control of soilborne fungal plant pathogens. Preliminary studies in greenhouse pot tests showed that populations of *T. basicola* and *C. sativus* were reduced by the addition of cysts and trophic forms of the amoebae to the soil.

Our preliminary surveys showed that the mycophagous amoebae are widely distributed in agricultural soils in Canada. They also occur in Holland and Scotland (17,18). Therefore they are likely to be a normal component of most soils. Based on their widespread distribution and their marked predacious activity it is highly probable that the mycophagous soil amoebae may have considerable potential in the biological control of soilborne fungal plant pathogens. Considerably more study is needed, however, to determine their significance and effectiveness in nature. Their distribution in soil and in the rhizosphere of infected plants and the determination whether they are an important component of the so-called "pathogen-suppressive soils" (2) also merits attention.

The systematic position of the perforating mycophagous amoebae is in the protozoan order Proteomyxida Lankester (9) and the family Vampyrellidae Doflein (10). This family is characterized by naked amoeba with anastamosing filopodia. This group of organisms has not been studied in detail and descriptions of many of its members are inadequate to permit assured identification. *Leptomyxa reticulata* Goodey (8) is perhaps the best known member of the family. On numerous occasions we observed an organism in soil dilution plates that is *L. reticulata* (8, 24, 25). In 10% soil extract solution the plasmodium of *L. reticulata* was either spreading and sheet-like or reticulate and somewhat angular (Fig. 15). Movement was accomplished by a gliding motion along a broad front or by means of short lobopodia. Although short filopodia were present these normally were inactive or in the posterior portion of the organism. In contrast, the amoeba observed in our studies as responsible for spore perforations (Fig. 13) characteristically took the form of an elongated protoplasmic chord with slender filopodia at the terminal portions. Lobopodia were not produced and movement took place by means of the extremely long filopodia (Fig. 14). *Leptomyxa reticulata* is essentially a bacterial feeder (24,25) and only rarely digests small fungal spores (7). *Leptomyxa reticulata,* was never observed to perforate or parasitize the contents of *C. sativus* or *T. basicola* spores.

The morphology and movement of the amoeba responsible for spore perforations in arable soils in Ontario closely resembles that of an organism described by Leidy in 1876 (11) as *Biomyxa vagans* Leidy; the description is incomplete it thus is difficult to use for accurate identification. Leidy (11) apparently saw diatoms within the cytoplasm of *B. vagans,* but did not observe ingestion or digestion or any other feeding. In our studies, the mycophagous amoeba was parasitic primarily on fungal spores. This activity resembles members of the

TABLE 4. Effect of culture medium on excystment of the mycophagous ameobae

Culture medium	Cysts excystment after incubation at 20 C			
	12 hr (%)	24 hr (%)	48 hr (%)	72 hr (%)
Distilled water (DW)	0	10	18	20
DW + fungus spores	1	30	38	43
Soil extract (10%)	9	54	84	89
Soil extract + spores	12	54	88	92

TABLE 5. Effect of temperature on excystment of the mycophagous amoebae in 10% soil extract

Temperature (C)	Cyst excystment after incubation			
	1 day (%)	2 days (%)	3 days (%)	4 days (%)
5	1	2	6	20
10	8	17	21	33
15	23	47	70	84
20	68	82	90	91
25	31	41	51	66
30	14	18	24	31
35	0	0	0	0

genus *Vampyrella* Cienkowski which parasitized filamentous algae by first causing a perforation of the cell wall (12, 13). *Vampyrella* also is characterized by short pinhead pseudopodia which also were observed in our studies.

The organism observed in our studies also bears some resemblance to *Theratromyxa weberi* Zwillenberg (27), a parasite of nematodes (22, 26). However, *T. weberi* forms a digestive cyst around the nematode and excystment results in the release of a number of smaller individuals. This mode of multiplication and excystment was not observed in our studies. The mycophagous amoeba in our studies causes perforations in the cuticle of the affected nematodes and this was not reported for *T. weberi* (22, 26). Old (18, 19) reported that the large soil amoeba which caused perforations of conidia of *C. sativus,* in soils in Scotland resembled *L. reticulata*. The organism which we found causing perforations in spores of *C. sativus* and *T. basicola* in arable soils of southwestern Ontario, Canada resembled that described by Old (18, 19) but we believe the organism in Ontario soils has morphological and biological features that distinguish it from the typical *L. reticulata* as described by Goodey (8).

Although the systematics of this interesting group of mycophagous soil amoebae are inadequate and considerably more research is needed on their biology and taxonomy, we believe that those causing the large perforations in spores of *C. sativus* and *T. basicola* belong to the genus *Vampyrella* (12, 13).

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