# STUDIES OF PARASITIC FUNGI ON THE CANE PEST *NUMICIA VIRIDIS* MUIR

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*Numicia viridis* Muir, otherwise known as the green leaf-sucker, is an important pest of sugarcane in South Africa (Anon.[2, 3]; Dick[5]). It is capable of causing considerable damage to this crop and has therefore been the subject of repeated pest population surveys. Studies of its life history, pest-host plant-relationships and means of biological control, are all currently receiving considerable attention (Anon.[1]; Carnegie[5]).

Studies, carried out by the Entomology Department of the Experiment Station of the South African Sugar Association, have revealed that natural populations of *Numicia* sometimes fall quite dramatically. The dead insects are usually blown away by the wind, and those recovered from the soil are normally too contaminated to yield information on the cause of death. However, a limited number of dead *Numicia* can sometimes be found loosely attached to cane foliage, and these have been used to determine which organisms were the cause of death.

## Material and Methods

Adult *Numicia* used in these studies were collected during entomological surveys in cane fields in various districts both in South Africa and Swaziland. Both live and dead specimens were secured. Live specimens were used for studies of disease infection and they were reared in cylindrical glass jars, 3 x 18 inches in diameter, the open ends of which were covered with a fine mesh and a perforated lid. A rooted cane sett bearing healthy green leaves was placed in each cylinder, the young plant having been reared beforehand in nutrient solution for approximately 6 weeks.

Dead specimens were classified into four groups:

(a) specimens showing no visible symptoms of deterioration.

(b) specimens which had shrivelled but showed no other sign of decomposition.

(c) specimens showing external symptoms of fungus growth.

(d) specimens in an advanced stage of decomposition, the body being distended and liable to disintegrate.

The dead *Numicia* in their separate groups were stored under both dry and moist atmospheric conditions, and were compared with killed healthy specimens treated in the same way. Superficial and internal microflora from previously healthy and diseased specimens were examined histologically and by culturing on various media. The internal microflora were kept distinct from the external by culturing samples only after they had been carefully surface sterilized and then rinsed with sterile water.

Culturing was carried out in petri dishes, using the following media, the pH of which varied from 4.5 to 7.0: sterilized slices of potato; liquid and semi liquid forms of a mixture comprising 10g maltose, 8g peptone, and 25g glycerol; brain-heart infusion gelatine; blood agar and gelatine; potato dextrose agar; and Czapak's agar. Finely pulverised bodies of *Numicia* were spread over the surface of these media, and in some cases fungal mycelium was transferred from the insect to the culture media, using a needle.

## Identification of Micro-Organisms

Healthy *Numicia* were found to be contaminated with very many different types of saprophytic fungi, many of which are also found on plants and in the soil on decaying vegetable matter. A wide range of bacteria were also found associated with the insect, all of which appeared to be non pathogenic in character. These fungi and bacteria were also present on diseased specimens, but no association was found between them and the state of health of the *Numicia*.

Cultures of inoculum obtained from the external and internal parts of dead and dying insects, yielded quite a number of fungal species which were not present in cultures derived from healthy specimens. The frequency of their occurrence eliminated the possibility of their being caused by secondary contamination. However, in view of the multiplicity of organisms found, an experiment was put down, designed to clarify the association between specific organisms and *Numicia* disease. Fifty six *Numicia* which were dying or had recently died of disease, and 20 healthy specimens, were each used to inoculate ten culture plates. This provides for duplication of the 5 culture media and gives a total of 760 plates. The organisms associated with the diseased insects are listed in Table I.

TABLE I

**Parasites associated with natural mortality of *Numicia viridis* Muir**

| Parasite | Contamination | | | |
|---|---|---|---|---|
| | Healthy insects | | Diseased insects | |
| | Number | % | Number | % |
| *Beauveria bassiana* .. | 3 | 1.5 | 94 | 16.8 |
| *Microsporum* sp. .. .. | 0 | 0 | 58 | 10.4 |
| *Fusarium semitectum* .. | 7 | 3.5 | 172 | 31.5 |
| *Dactylium fusarioides* .. | 0 | 0 | 35 | 6.2 |
| *Fusarium poae* .. .. | 0 | 0 | 126 | 22.5 |
| *Entomophthoraceae* .. | 0 | 0 | 542 | 96.6 |
| *Mucor* sp. .. .. .. .. | 2 | 1 | 314 | 56.0 |
| Nematodes .. .. .. | 0 | 0 | 69 | 12.2 |
| Total number of insects examined .. .. .. | 200 | | 560 | |

Eight parasites are listed, only three of which were found in cultures derived from healthy insects, and then in only a very few cases. In contrast, cultures from the dead or dying insects revealed relatively high populations of each parasite and a particularly high incidence of a fungus of the family *Entomophthoraceae*. Ninety-six per cent of these cultures contained this group of fungi, 56% were infected with a species of *Mucor*, 31.5% with *Fusarium semitectum* and 22.5% with *Fusarium poae* (Peck) Wollenweber. In addition, in 10.4% of the specimens examined, *Microsporum* sp. was found associated with the wing parts, while a small percentage of the diseased insects also yielded *Dactylium fusarioides* Frag. and Cif. Nematodes were found infecting about 12% of the diseased specimens.

## Histological Studies

Isolation of fungi from the internal body of the insect for purposes of identification, was followed up by histological studies to determine what parts of the insect were infected. Healthy and diseased adults were compared, the specimens being fixed in Romeis solution (25 ml saturated $HgCl_2$ + 20 ml of 5% Trichloro-acetic acid + 5 ml formalin), and embedded in paraffin wax. Rotary and freezing microtomes and razors were used to provide sections, which were then stained. No evidence of fungal growth was found on the bodies of any freshly killed healthy insects. When, however, healthy specimens were put into a petri dish containing cane leaves, for eight hours, and allowed to mix with *Numicia* which had died from disease, microscopic examination revealed that they in turn had become infected. This infection apparently occurred when the healthy specimens crawled over the bodies of their dead companions, the latter being covered with abundant conidia.

The newly infected *Numicia* were returned to the glass jars in which they were being reared on sugarcane. In those where humidity was constantly maintained at levels in excess of 90%, many of the insects died within 48 hours. Where relative humidity was maintained at 85%, no such mortality occurred but the *Numicia* did start to die after the lapse of a further 60 hours. Within two weeks all the *Numicia* which had been exposed to infection had died, while only 10% mortality occurred among non-infected and isolated control samples.

Observation of infected insects in their glass jars shows that in the hours preceding death they are unusually active, crawling up and down the sides of the jar for periods varying from 4 to 7 hours. They then hide themselves before they actually die, and it is then quite difficult to find them. At no time before or immediately after death can any external symptoms of infection be seen with the naked eye. (Fig. 1). However, microscopic examination reveals the presence of an internal fungal mycelium, and hyphae sometimes protrude through the external membrane (Fig. 2).

The fungal growth occurs mainly in the abdominal and thoracic region. Where relative humidity is high (95-100%), the body swells to about twice its size within 24 hours of death, and hyphae grow through the external membranes. Thereafter, under these conditions, the mycelium develops until it eventually covers the whole body with a mass of sporulating hyphae and conidiophores, the latter bearing the infective conidia (Fig. 3, 4 and 5). Microtome sections, shown in Fig. 6, reveal that internally the mycelium extends right through the body tissue and that it bears zygospores. Infective conidia are not normally produced on the body when humidity is low, and it is therefore assumed that a hot humid climate is necessary for rapid spread of natural infection.

## Artificial Infection

Two of the fungi isolated in vitro were found to be closely associated with mortal infections of *Numicia*, namely the genera *Mucor* and *Entomophthora*. Logically, pure cultures of both *Entomophthora* and *Mucor* should be tested to confirm that these are in fact primary parasites of *Numicia*. Unfortunately, cultures of *Entomophthora* have not so far been induced to sporulate, but *Mucor* has been used successfully in this manner. Thus, healthy adult *Numicia* were placed in petri dishes containing a pure culture of the isolated species of *Mucor* for 3 hours. They were then transferred to cages attached to cane plants. Histological examination of specimens showed that infection occurred after 3 days, the insects dying soon after. Similar confirmation of the infectiveness of *Entomophthora*, the apparently more important parasite, will have to await the development of techniques to induce sporulation of the cultured fungus.

## **Entomophthora** sp.

Natural infection of *Numicia* by the entomogenous fungus *Entomophthora* has been established, even though it has not been possible to produce suitable pure cultures for artificial inoculation. The vegetative characteristics of this fungus are very variable, but consist of a more or less closed cluster of short, curved tubular branches of hyphae (Fig. 3 and 5), which originate from a common point. These clusters of cells, comprising the thallus, give the surface of the insect a pock-marked appearance (Fig. 2 and 3).

The peripheral cells of the thallus produce tubular structures which are capable of penetrating the adjacent host cells. The hyphae themselves are limited in length and tend to break at their septa into component cells (Fig. 5). These hyphal bodies multiply rapidly by dividing and budding, and any one of them may act as a conidiophore, discharging a conidium from its terminal end (Fig. 4 and 5). The conidia are large, colourless, multinucleate bodies, which vary in shape and size. They sometimes resemble sporangia produced by other genera (Fig. 7), and are formed in millions on the surface of the dead insects. Each conidium is capable of germinating and

it was found that germination was stimulated by intervention of dry conditions for 5 or 6 hours, followed by a period of high humidity. The germ tube which is then formed (Fig. 8-12) is able to penetrate the cuticle of *Numicia* on its soft abdomen, whereupon the multinucleate cytoplasma becomes disseminated in the insect's body. In some cases, the germ tube branches immediately to form a mycelium (Fig. 13 and 14). In other instances a second or even a third conidium was formed before a mycelium was produced (Fig. 15 and 16). Hyphal bodies are sometimes produced within individual hyphae (Fig. 14) and they are eventually released as independent bodies (Fig. 17). They multiply by simple division and, when conditions are appropriate, develop into sporangioles. These sporangioles, in turn, may develop by further modification to form conidia (Fig. 18).

Sexual reproduction was observed as a union of mycelial segments or free hyphal bodies, forming thick-walled cells or resting spores (Fig. 19-24). Fusion of hyphal bodies may result in immediate outgrowth of a germ tube (Fig. 25) or in the formation of zygospores (Fig. 26). These are formed from one of the fusing hyphal cells, at a site which is usually distinct from the point of fusion. The nuclei and most of the cytoplasm from both cells pass into an outgrowth, or budding zygote which then separates from the fused parent cells, so creating an independent zygospore (Fig. 26). Zygospores are thick-walled bodies which germinate after a short rest period (Fig. 27). During this rest period they can survive otherwise critical climatic changes, so preventing the fungus dying out.

## Discussion and Conclusion

It has been shown from a limited number of trials that several fungi parasitise *Numicia viridis*, and are the cause of mortal infections of the pest. These pathogenic fungi infect healthy *Numicia* when they crawl over the corpses of victims of the disease. Infection is almost certainly contracted by penetration of germ tubes from the conidia through the soft abdomen of the insect, but it is possible that there are other, as yet unknown means of infection. It is also possible that certain environmental or climatic conditions will reduce insect resistance to infection due perhaps to encouragement of germ tube penetration by enzymes, or weaknesses in the body surface. It is, however, quite certain that given favourable climatic conditions, namely hot humid weather, that infection can spread rapidly, causing very heavy mortality of *Numicia*.

Further studies will be needed to provide information on modes of infection and conditions governing this. In addition, culturing techniques for *Entomophthora* will have to be improved, and means will have to be found for producing infective material in bulk for field experiments. There is, however, good reason to believe that artificial stimulation of some of the factors limiting the spread of natural infection may conceivably provide practical means of ensuring biological control of *Numicia*.

## Summary

Micro-organisms associated with dead and dying specimens of the cane pest *Numicia viridis* Muir, have been examined. Seven fungi were isolated, two genera of which appear to be of particular importance, namely *Mucor* sp. and *Entomophthora* sp. These organisms kill their hosts and, in hot humid conditions, continue to develop saprophytically on the corpse, producing conidia which, on contact, can infect healthy specimens. *Mucor* sp. has been raised in pure culture and used to reinfect *Numicia*. So far, pure cultures of the even more virulent *Entomophthora* have not been induced to sporulate, but work on this is to be continued.

### References

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4. Carnegie, A. J. M. (1966). "The progress of an untreated outbreak of *Numicia viridis* Muir." Proc. Annual Cong. S. Afr. Sugar Tech. Ass. 40: 319-327.
5. Dick, J. (1964). "Numicia—The green leaf-sucker of sugarcane". Bull. No. 15. S. Afr. Sugar Ass. Expt. St.

## Discussion

**Mr. Carnegie:** Two of the fungi mentioned as possibly being useful for control of Numicia, namely *Beauveria* and *Fusarium*, have been worked on extensively in the last hundred years. There have been some successes, which have been well publicised, but also a lot of failures.

**Dr. Roth:** I did not claim Beauveria could successfully control Numicia. The promising fungi are *Entomophthora* and *Mucor*.

**Dr. Dick:** Mr. Carnegie's remarks apply to any fungi that have been tried for the purposes of controlling insects.

**Mr. du Toit:** Numicia appears to be under control in the coastal areas. Dr. Roth implies that these fungi need a certain amount of humidity and therefore Numicia populations may in fact already be controlled in the coastal areas by these fungi.

**Dr. Roth:** In a dry irrigated area, the fungus would try and develop from the resting, or zygospore stage during irrigation. But when irrigation ceases, and arid conditions again prevail, it will die immediately. This may be why in irrigated areas biological control by these fungi has not been successful.

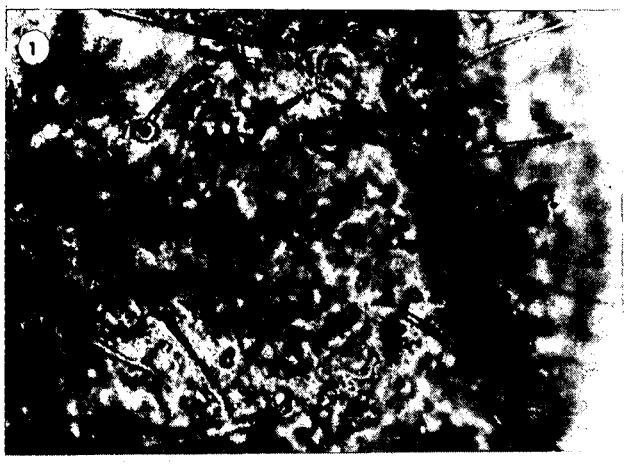
**Mr. Date:** Under the canopy is not the humidity as high in irrigated areas as it is in the coastal belt?

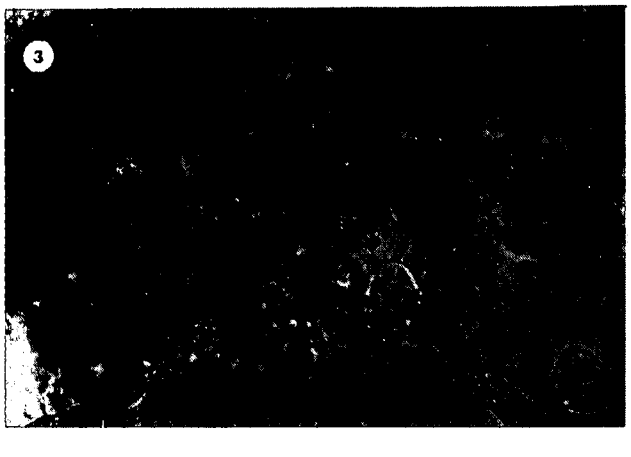
**Mr. Glover:** Even after showers of rain the humidity under the cane plant remains high for some time under certain conditions.

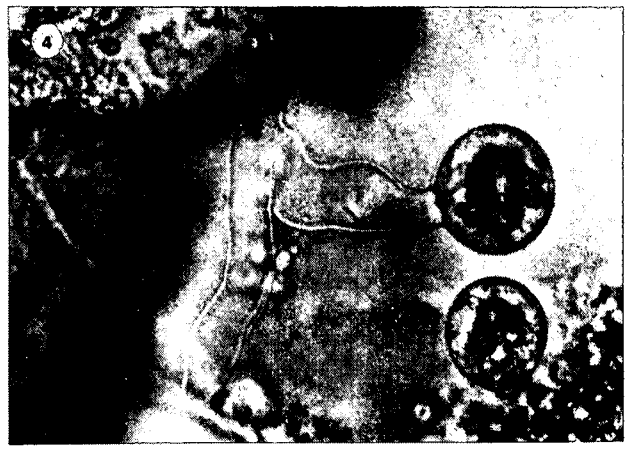
**Mr. Harris:** Other factors, including temperature and humidity, must be taken into account. In Florida attacks of a certain fungus take place only every three years, despite efforts to start attacks in the intervening periods by spraying spores over the area.

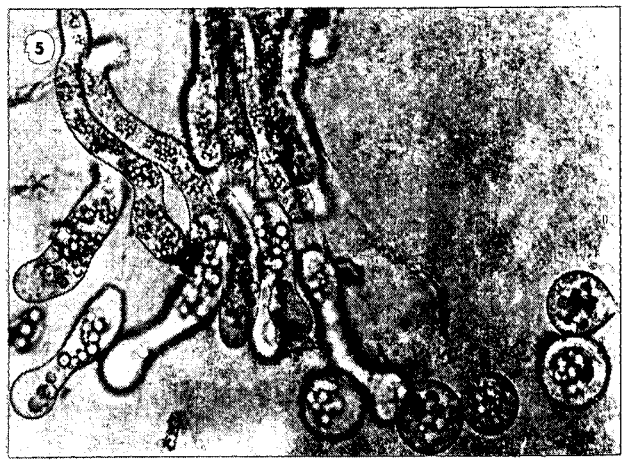
It appears that fungi are always present but will only attack insects under certain conditions.

**Dr. Roth:** Under very hot, humid conditions, which are not dissipated by wind, temperature is unlikely to rise high enough to harm the fungus.









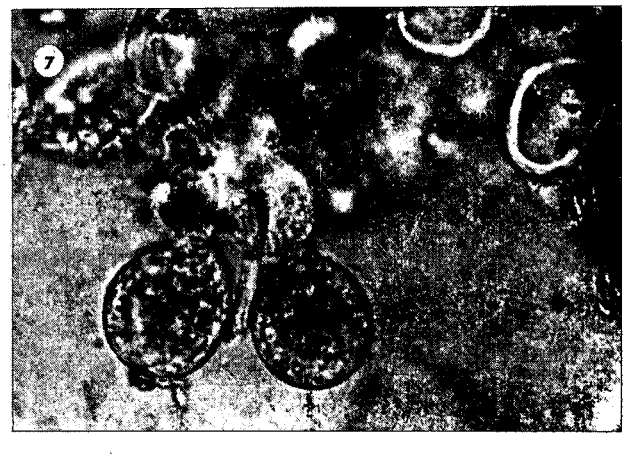


FIGURE 1: Part of the distended body surface of Numicia, three days after death. (x 450).

FIGURE 2: The fungal thallus covering the exoskeleton of Numicia. Note the protruding conidiophores. (x 750).

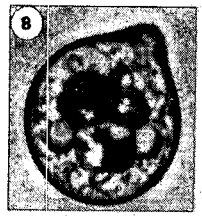
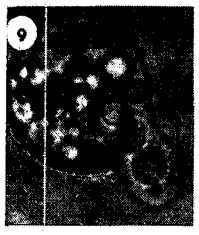
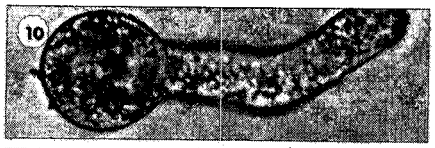
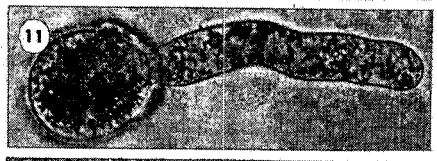
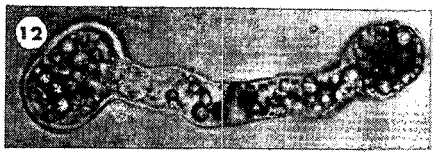
FIGURE 3: Mass of germinating conidia and free hyphal bodies. Note the tubular structure of the peripheral cells of the thallus. (x 480).

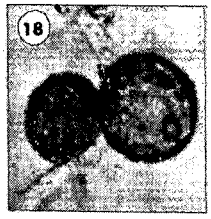
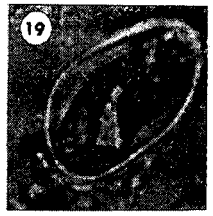
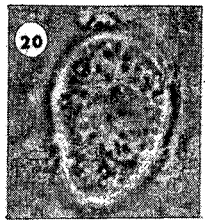
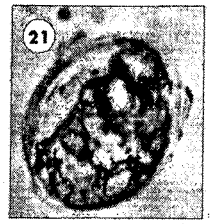
FIGURE 4: Branched conidiophores, each of which terminates in a single conidium. (x 750).

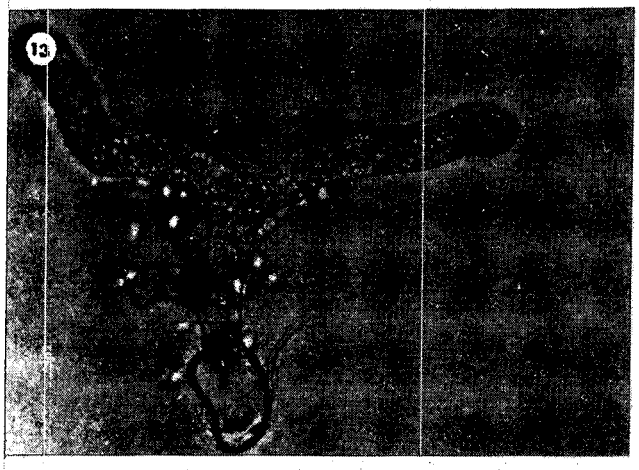
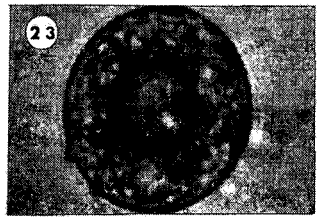
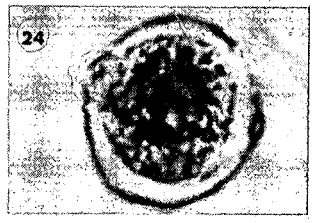
FIGURE 5: Typical short curved hyphae of the entomogenous fungus Entomophthora. Note the formation and release of conidia. (x 550).

FIGURE 6: Development of mycelium, and the formation of zygospores of Entomophthora sp. within the body of Numicia. (x 450).

FIGURE 7: Conidia which differ in shape from those in Fig. 4. Produced on club-shaped conidiophores they resemble sporangia in other genera. (x 850).

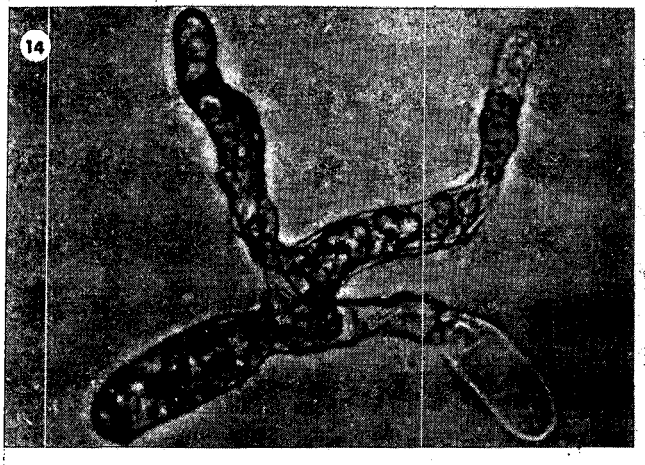
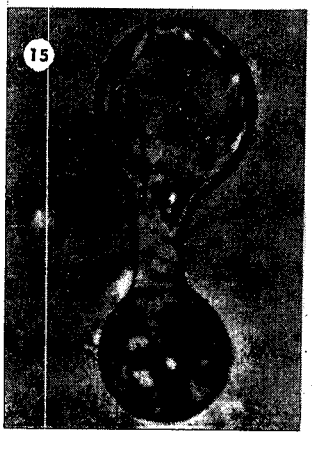
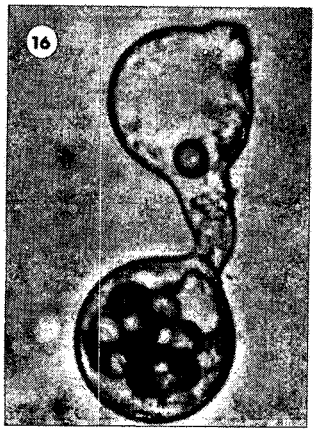
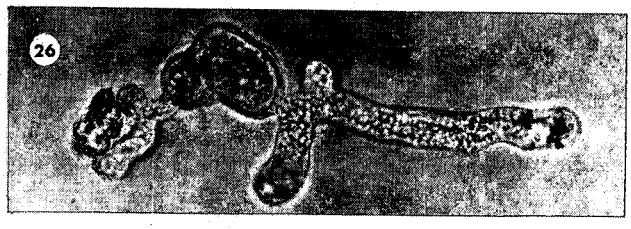






FIGURE 8: Conidium of Entomophthora sp. before germination. (x 750).
FIGURE 9: Conidium which is just starting to germinate. (x 800).
FIGURES 10 and 11: Increasingly advanced stages in the germination of a conidium. (x 600).
FIGURE 12: Germination of a conidium terminating with the formation of another conidium at the apex of the germ tube. (x 600).
FIGURE 13: Germination resulting in the formation of several branches of the germ tube. (x 600).
FIGURE 14: Very young mycelium formed from a germinating conidium, containing hyphal bodies. (x 600).
FIGURE 15: Germinating conidium producing a second conidium at the end of a short germ tube. (x 650).
FIGURE 16: Germinating conidium, producing a second conidium and starting to form a third. (x 700).
FIGURES 17 and 18: Development of conidia from hyphal bodies. (x 600).
FIGURES 19 to 24: Different stages in the development of resting spores or zygospores. (x 850).
FIGURE 25: Sexual fusion of two hyphal bodies and development of a germ tube. (x 750).
FIGURE 26: Sexual fusion of hyphal bodies resulting in the formation of a zygospore (early stage) and its further germination. (x 650).
FIGURE 27: Germination of a zygospore after a period of rest. (x 850).