MICROSPORIDIOSIS IN MASS-REARINGS OF THE
PREDATORY MITES *AMBLYSEIUS CUCUMERIS* AND
*A. BARKERI* (ACARINA: PHYTOSHEIIDAE)

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SUMMARY
The commercial production of the predatory mites *Amblyseius cucumeris* and *A. barkeri*, used for biological control of thrips in vegetables in greenhouses, is at stake, because of a reduction in production and quality of these predatory mites in mass-reearings, due to the action of a pathogen (Microspora: Pleistophoridae). Aspects of the pathology and transmission are discussed.

INTRODUCTION
The predatory mites *Amblyseius cucumeris* (Oudemans) and *Amblyseius barkeri* (Hughes) are used for biological control of the thrips species *Frankliniella occidentalis* Pergande and *Thrips tabaci* Lindeman, both serious pests in greenhouse vegetables. The predators are part of the integrated pest management programmes of several vegetables in greenhouses in the Netherlands. For the commercial production of the predators, a mass rearing method is used, originally developed by Ramakers and van Lieburg (1982).

The predators are reared in large aerated containers, which are filled with hundreds of liters of wheat bran. A temperature of approximately 22 °C and a high relative humidity (~90%) allows the growth of fungi on the bran. The fungi serve as food for stored product mites (*Acarus siro* L. or *Tyrophagus putrescentiae* (Schrank) and *Tyrolichus casei* (Schrank)), which in turn are fed upon by the predatory mites.

Recently, the productivity of the mass rearing decreased drastically as a consequence of a disease in these *Amblyseius* species (Ramakers et al., 1989; M. Dissevelt & W. Ravensberg (Koppert B.V., pers. comm.). Not only productivity, but also quality of the predators produced may have been reduced. For these reasons, the disease is a threat for the biological control of thrips. If pesticides (such as pyrethroids) have to be used instead, the full program of integrated pest control measures is at stake, since the pesticides may interfere with the effectiveness of other natural enemies.

DIAGNOSIS
The pathogen causing the disease belongs to the Microspora. Ramakers et al. (1989) suspected that the pathogen is a member of the genus *Nosema*, but more detailed investigations showed that we are probably dealing with a new species belonging to the genus *Pleistophora* (A.M. Huger (Institut für Schädlingsbekämpfung, Darmstadt, B.R.D., pers. comm.).

Microsporidia belong to the phylum Microspora, which is one of the 7 recently erected phyla within the Protozoa (Levine et al., 1980). They are common pathogens of invertebrates, but can be found in vertebrates as well (Canning, 1971; Hazard et al., 1981).
BIOLGY OF MICROSPORIDIA

Microsporidia are obligatory intracellular pathogens; the only way to survive outside the host cell is in the spore stage (Vavra, 1976). The spores have a thick wall, which protects the sporoplasm (the infective germ) against unfavourable environmental conditions and makes it well suited for its function in transmission (Huger, 1961). Spores ingested by a host, germinate in the gut by extruding the so-called polar filament, which injects the sporoplasm into a host cell (Canning, 1971; Vavra, 1976). In the vegetative phase of the life cycle, the germ undergoes several cellular divisions, and finally sporulation takes place (Vavra, 1976). The spores are released into the environment by excretion (with faeces or other excretion products), or when infected hosts die and desintegrate.

**Figure 1:** Disease-free A. barkeri under light microscope, magn. 1270 x; g: gut.

**Figure 2:** A. barkeri with microsporidiosis under light microscope, magn. 1270 x.

**Figure 3:** Squash preparation of A. barkeri with microsporidiosis (same individual as in fig. 2) under phase-contrast light microscope, magn. 1270 x; s: spores.
PATHOLOGY

Microsporidiosis in the predatory mites *A. cucumeris* and *A. barkeri* is only recognizable in heavily infected mites when the disease is in an advanced stage and when many spores have been produced. Such diseased mites have a swollen and whitish appearance and are sluggish in their movements. The symptoms are even more striking when the mites are observed under the light microscope: several organs (e.g., gut; see figure 1) are easily recognized in disease-free individuals, while internal structures in infected mites are hardly visible (figure 2). Squash preparations of mites in an advanced stage of the disease show numerous spores which have been formed in the cells of the mites, leaving hardly any healthy tissue fragments behind (figure 3).

In the mass-rearing, also infected prey mites have been observed. The symptoms of microsporidiosis in these mites are comparable to those in the predatory mites, although somewhat less conspicuous. Disease-free flour mites (and the other stored product mites) already have a whitish appearance and are slower in their movements than predatory mites.

QUESTIONS

In order to prevent future infestations in mass-rearings of predatory mites with microsporidia (or to cure already infected ones), it is important to know how the microsporidium persists in the culture. In other words: (i) Which conditions make it possible for the spores to survive outside the host? and (ii) How do mites become infected (or: how is the microsporidium transmitted)? The importance of the first question with respect to persistence of the disease in culture, depends on the number of free spores and on their contribution to transmission of the disease.

PERSISTENCE OF THE SPORES

Spores excreted (with faeces or other excretion products) by diseased mites, together with spores released after death of their hosts, form the "free-spore pool". On one hand, the pool increases in size by accumulation of spores; on the other hand it decreases since spores become inactivated by several factors. For many microsporidia ultraviolet light is one of the more destructive environmental factors (Ignoffo et al., 1977; Kaya, 1975). In mass-rearings, spores are not directly exposed to sunlight, which means that UV-light does not play a prominent rôle in the inactivation of the spores. Temperature and relative humidity, on the other hand, may be important factors for survival of the free spores in mass-rearings. With increasing temperature and relative humidity, reduced spore viability has been observed (Gardner et al., 1977), but tests on the persistence of spores under stored conditions, often vary with species of microsporidium: some microsporidia cannot even survive short periods of desiccation, while others require dry storage to prevent germination (Canning, 1982).

It is not known how the mass-rearing conditions (relative humidity ~90%; temperature ~22 °C) affect the virulence of the spores. Also other conditions of the mass-rearings (e.g., CO₂ concentration) may be of importance for the longevity of the spores. In addition, conditions that are negative for spore persistence, may well be positive for the development of microsporidia in mites, and vice versa. For example, a high temperature might reduce the virulence of spores, and at the same time it might accelerate the development of the microsporidiosis in the host.

TRANSMISSION OF THE MICROSPORIDIUM

Since the microsporidium occurs at two trophic levels (the predator and the prey), the possible ways by which the microsporidium may be transmitted are more extended than in case of a restriction to one host species. In our opinion, five possible ways of transmission of microsporidia may be distinguished. In addition to vertical transmission (from parent(s) to offspring) (v), four ways of horizontal transmission (h) may occur: by predation (h1), by contact with the free-spore pool (h2), by contact with the other
species or with conspecifics (other than feeding or mating) (h3), and by mating (h4) (see figure 4).

Since in the rearing not only infected predators but also infected flour mites (or other stored product mites) can be found, a possible way of transmission is ingestion of the spores when predatory mites are feeding on infected flour mites (h1a). Predatory mites are known to be cannibalistic, which means that the infection may be established by consuming diseased conspecifics as well (h1b). It seems less likely that flour mites become infected by diseased predatory mites; however, prey sometimes manages to escape from a predatory mite that started feeding on it (pers. obs. E.B.), and this may be just sufficient to transmit the microsporidium to the flour mite (h1a).

Contact with the free-spore pool may be an important way of transmission (h2), since the wheat bran and the fungi are contaminated by spores. By eating, or even tasting, all kinds of substances (like fungi), flour mites, and also predatory mites, may ingest spores. Even by cleaning their legs or mouthparts, infection might occur.

A male may transfer spores along with sperm during mating, or might obtain spores from a diseased female (h4). Many microsporidia are transmitted to the offspring of the host by means of vegetative phases (v) (Canning, 1971), and this may well be the case with the microsporidium in the mass-rearing. The host may be either or both of the parents.

Physical contact with other mites (other than mating or feeding) (h3) may be considered as a fifth possibility, but one should realize that spores probably have to be ingested to become infective, and that it is not likely that diseased mites carry any other spores on the cuticula than those obtained from the free-spore pool (= transmission by contact with free-spore pool).

![Figure 4: Possible ways of transmission of a microsporidium in the mass-rearing of the predatory mites Amblyseius cucumeris and A. barketi on the flour mite Acarus siro (or Tyrophagus putrescentiae and Tyrophagus casei). h1a: horizontal transmission by predation h1b: horizontal transmission by cannibalism h2: horizontal transmission by contact with free-spore pool h3: horizontal transmission by contact with conspecifics or other species (not feeding or mating) h4: horizontal transmission by mating v: vertical transmission f: contribution to free-spore pool by excretion by and death of diseased mites](image-url)
Transmission probably depends on the incubation time and the infective dose. Once a host has been infected by a microsporidium, it takes time before spores are formed, before the infection has spread to the reproduction organs. Only then the microsporidium will be available for (horizontal or vertical) transmission. The rate at which the disease develops in a host, is probably set by the infective dose, i.e., the number of spores that caused the infection.

In theory, it is possible the mites become immune to the microsporidium, especially when the infective dose is low. Once immune, they may become susceptible again. However, a memory component to the defence system, is thought to depend on life expectancy of animals. For short-lived organisms like mites, memory would be of limited value to their fitness (Anderson, 1986).

In case of transmission by predation (or cannibalism) the feeding behaviour of the mites has to be taken into account. Young predatory mites were found to eat only juvenile stages of flour mites or eggs, while the older predatory mites also preyed on the older ones (pers. obs. E.B.). These observations combined with the incubation time and infective dose dependency of the transmission, may very well restrict or even inhibit the transmission by predation.

CURRENT AND FUTURE RESEARCH

Currently we focus on sorting out which of the transmission mechanisms hold true. To do so, an efficient biological assay method is indispensable. Recently, Beerling (unpublished) developed such a method for predatory mites and flour mites. By means of a bioassay, several aspects of the microsporidiosis can be studied; isolated mites are fed known dosages of spores, and the effect can be followed in time. Changes in behaviour and appearance can be observed, and the development of the disease can be studied histologically by making sections at different moments after infection. The virulence of the microsporidium (LD<sub>50</sub>, LT<sub>50</sub>) can be tested for different mite stages and for different mite species.

This bioassay method may also be used to test spores which have been exposed to viability-reducing treatments (e.g., high and low temperatures and relative humidities; chemicals), or to study the effect of different environmental conditions on the development of the microsporidium in the mites.

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REFERENCES


