

A ZYGOMYCETOUS FUNGUS AS A MORTALITY FACTOR IN A LABORATORY STOCK OF SPIDERS

The first instars of our laboratory stocks of several spider species are usually fed with fruit flies, *Drosophila melanogaster*. In 1987 and 1988, we noticed a disease in several hatchling groups of *Cupiennius salei* Keyserling (Ctenidae) and *Ischnothele guyanensis* Walckenaer (Dipluridae). The spiders did not accept food and did not move very much. They sat most of the time on the bottom of the box (instead of hanging under the lid) and their appearance became dark and wet. Such spiders died 2-6 weeks after these symptoms were recognized.

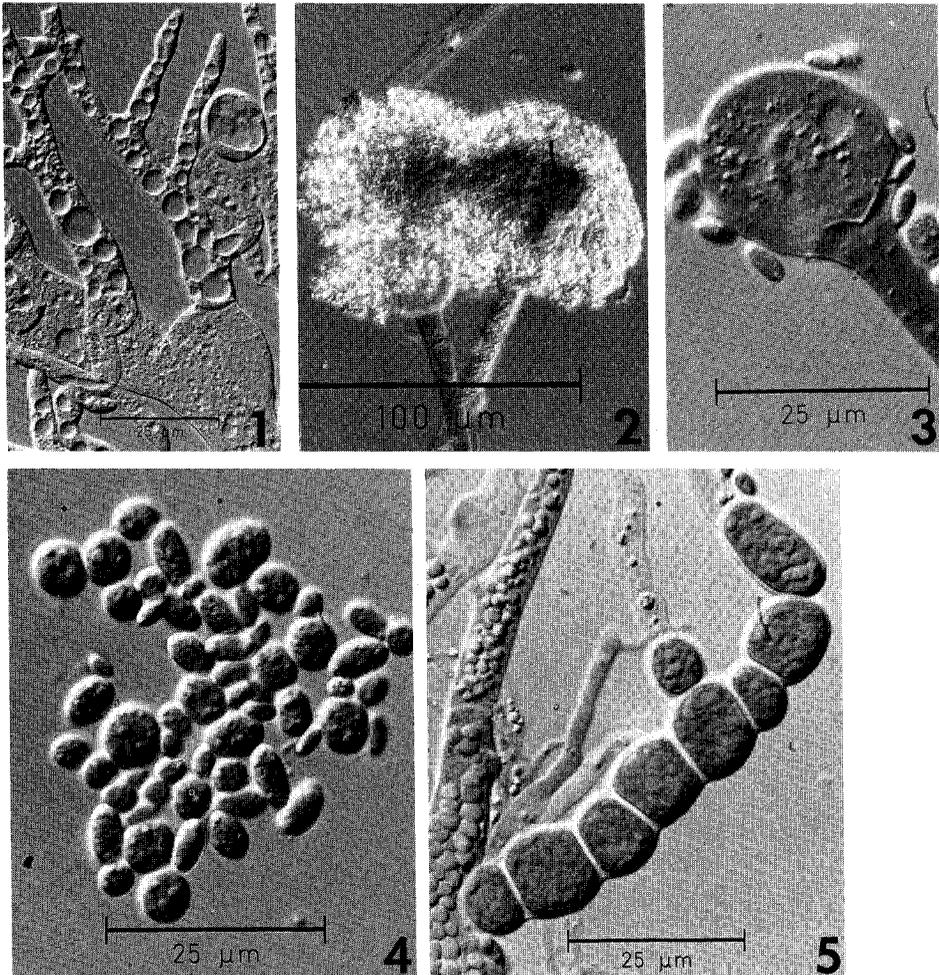
The infection rate of a given hatchling group (50-100 spiderlings) was about 90-100% and probably all infected spiders died (total *N* of dead spiders >500). We do not know whether the surviving spiders had not been infected or whether they successfully fought the infections. When the disease was recognized at an early stage, some techniques could increase the survival rate to approximately 20-30%. We tried several breeding techniques and found the following methods to lower spider mortality: Low air humidity (<70%), no free water, cleaning the box once a week, lids with additional slits to provide a better air circulation and no *Drosophila* food. The relative success of our changed breeding technique indicated that our spiders had probably been infected by a pathogen which originated from our *Drosophila* culture. Since *Drosophila* vials house a wide range of fungi in the food medium of the larvae, it is possible that the flies function as a vector for these pathogenic fungi when fed to the spiders.

To test this assumption we anaesthetized a total of 22 *Ischnothele* and 8 *Cupiennius* from different breeding groups by CO₂, cut off the opisthosoma under sterile conditions, disinfected the cuticle with ethanol (70%), opened the body ventrally with fine scissors and took a tissue sample with a sterile needle. The tissue was inoculated on Petri dishes and cultured on malt agar at 20° C. After 1-2 days the first fungal colonies could be detected. For further identification some fungus colonies were selectively transferred to new Petri dishes and cultured and propagated as above.

From all spider samples we were able to isolate the zygomycete *Mucor hiemalis* f. *hiemalis* (Figs. 1-5). This identification was confirmed by W. Gams and M. A. A. Schipper. This fungus is distributed worldwide and common in the soil or on plants (Zycha et al. 1969). It is known to kill honey bees (Burnside 1935) and several Lepidoptera, Coleoptera and Diptera species (Heitor 1962), but causes also a tomato disease (Zycha et al. 1969).

From some spider samples we could further isolate an unidentified fungus imperfectus. In nearly all spiders high numbers of bacteria were found. A microscopic examination of the tissue sample soon after the dissection of the spider revealed that the intestinal tract of most spiders contained up to three different bacterial forms. We did not make further efforts to identify them.

How does the fungus infect the spider? Since the spiders feed on infected *Drosophila* flies, we first thought that the fungus enters the spider's body via spores which survive the extraoral ingestion and pass through the prosoma filter system. An inhibition test with a suspension of *M. hiemalis* spores (10⁶ spores/ml) on agar plates and 2µl digestive fluid of *Cupiennius* did not prevent the



Figures 1-5.—The zygomycete *Mucor hiemalis f. hiemalis* Wehmer, isolated from a laboratory stock of spiders (interferential contrast microscope): 1, the primitive siphonal mycelium, grown from sporangiospores in a submers culture; 2, two sporangia with a dissolved sporangial wall; 3, empty sporangium, columella with remnants of the sporangium wall; 4, sporangiospores of different sizes; 5, spherical gemmae at older mycelium (slide culture).

spores from germinating. This indicates that *M. hiemalis* spores could survive the ingestion by a spider, although the digestion of fungus spores could be shown for orb-weaving spiders (Smith and Mommsen 1984). But could the spores pass through the prosoma filter? Though particles $>1 \mu\text{m}$ are normally retained by the effective filter system, larger particles such as pollen or spores can pass it as well (Collatz 1987). To test this assumption, we injected $20 \mu\text{l}$ of a spore suspension (10^6 spores/ml) into crickets which were fed to spiders. We chose spores of varying size (from 1 to $10 \mu\text{m}$) from three fungus species: two tropical fungi (to exclude possible error and interpretation problems) and *M. hiemalis*. The spiders ($N = 15$) were killed and tissue samples from the opisthosoma and prosoma (behind the filter) were inoculated on malt agar. In no case could fungal growth be observed. This indicates that the infection by *M. hiemalis* spores probably does not occur during the normal feeding procedure.

Greenstone et al. (1987) succeeded in infecting spiders with the pathogenic hyphomycete *Nomuraea atypicola* by topical application of a spore suspension and Heitor (1962) mentions that *M. hiemalis* can infect insects through injuries. So it is possible that the infection by this fungus occurs through microscopic lesions of the cuticle or other sensitive openings (book lungs?).

Is the infection of spiders by *M. hiemalis* a mere laboratory effect caused by contact with infected food items or does it occur regularly among free-living spiders as well? To answer this question we collected 10 spiders representing 10 different species from other parts of the building where our laboratory spiders were bred (*Pholcus phalangioides* (Fuesslin) (Pholcidae), *Dysdera crocota* C. L. K. (Dysderidae) and *Tegenaria* sp. (Agelenidae)) and from nearby parts of the campus (*Argiope bruennichi* (Scopoli), *Larinioides cornutus* (Clerck) *Araneus diadematus* Clerck (Araneidae), *Pisaura mirabilis* (Clerck) (Pisauridae), *Linyphia triangularis* (Clerck) (Linyphiidae), *Clubiona* sp. (Clubionidae) and *Xysticus* sp. (Thomisidae)). The spiders were treated as mentioned above and malt agar Petri dishes were inoculated. In no case could any fungal growth be found. This probably indicates that the infection by *M. hiemalis* is restricted to our laboratory stock, although the wide dispersion of the fungus could enable it to be a more common pathogen of spiders.

At the end of 1988, the complete laboratory stock of *Cupiennius salei* was moved from Regensburg to Bern. The spiders were housed in rooms where no *Drosophila* have been bred before. All plastic containers were replaced by new materials and the spiders were exclusively fed with crickets. Under these conditions no fungal disease of the previous epidemic dimension could be observed and the survival rate of hatchlings was about 90-100% during the first 3-4 instars ($N > 800$). This can be understood as a further argument for a correlation between *Drosophila* food, fungal infection and spider mortality (though it does not prove a cause and effect relationship).

Until now true pathogenic fungi of spiders were only known from Ascomycetes (the genera *Cordyceps* and *Torrubiella*, Clavicipitales) and from their hyphomycete anamorphs (*Gibellula*, *Nomuraea* and 7 other genera), the imperfect fungi (Nentwig 1985; Evans & Samson 1987). No fungi pathogenic to spiders are known from the Myxomycetes or from the Basidiomycetes. The herein reported case of *M. hiemalis* is probably the first observed pathogenic example from the Zygomycetes. Although we present here only a laboratory case, it is possible that Zygomycetes infect spiders under natural conditions as well. An interesting feature of the zygomycete pathogens is the apparent lack of host specificity. According to our knowledge, pathogenic fungi of spiders do not infect insects and the insect pathogenic fungi (e.g., Entomophthorales) do not infect spiders (Evans and Samson 1987). In contrast to this, *M. hiemalis* seems to have a wide host range and includes insects and spiders.

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