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Nematode-fungal interactions. Structure-function relationships.

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Summary of this thesis

Nematophagous fungi can infect and digest nematodes and use the contents of the prey for development of fungal biomass. Generally, these organisms encounter the nematode host in two ways either by means of trapping devices formed on an extended mycelium (predatory or nematode-trapping fungi) or by means of conidia (endoparasitic fungi). Currently, the structure/function relationships during the complex nematode/fungus interaction of the free-living bacteriovorous nematode *Panagrellus redivivus* and the predatory fungus *Arthrobotrys oligospora* were thoroughly investigated by means of different light- and electron-microscopical methods. For endoparasitic fungi, however, knowledge about these aspects of infection, at the onset of our studies, was comparatively small.

The latter group of fungi are more dependent on nematodes as a nutrient source than the facultative predatory fungi and therefore may be possible candidates for the biological control of plant-parasitic fungi which can cause severe damage to agricultural crops. The main aim of the work presented in this thesis is a more thorough investigation of these fungi on a fundamental descriptive level. It was a logical step to use the fungus *Drechmeria coniospora* as a model organism; knowledge of this fungus was already available with respect to its host-specificity and life-cycle. The vegetative mycelium is localized inside the nematode and produces numerous conidia outside the dead nematode.

In **chapter 1** the state of the art with respect to physiological aspects and structure/function relationships of nematophagous fungi is reviewed including the different studies presented in this thesis. **Chapters 2** and **3** describe in detail the infection process of *P. redivivus* and the fungus *D. coniospora*. Nematode infection was initiated with adhesion of mature conidia to the nematode cuticle by means of an adhesive knob; to this purpose an adhesive layer located at the distal end of the spore was present. In case of *P. redivivus* adherence preferentially occurred near the sensory-organs. It was somewhat unexpected to find that the initial hyphal outgrowth, developing on the adhesive knob following adhesion, was not immediately dedicated to penetration of the nematode prey. This structure designated "appressorium", however was not a virtual waste of energy, but in fact appeared a prerequisite to achieve proper penetration. In this way an intimate contact between fungal cell wall and nematode cuticle is realized, which was initially not effectuated due to the

relatively rigid adhesive layer on the knob. The actual penetration of the cuticle was subsequently realized by means of a very thin penetration tube (with a diameter of less than $0.5 \mu\text{m}$) formed on the appressorium. Penetration invariably occurred on living nematodes. Our combined observations strongly suggest that penetration occurs by mechanical force and (local) enzymic softening of the cuticle. The very close contact between fungal cell wall and nematode cuticle after penetration suggest an effective sealing mechanism thus preventing leakage of nematode contents into the environment. Subsequently, trophic hyphae invaded the nematode via the pseudocoel; penetration of the internal organs was never observed. The sinusoidal hyphal growth pattern typical for this stage of infection is probably functional in that it prevents rupture of these hyphae due to the movements of the nematode prior to its death. In the course of infection, nematode movements gradually slowed down and 40-48 hr after the onset of infection the majority of nematodes were moribund or dead. Almost simultaneously conidiophore development through the cuticle started. At this stage the trophic hyphae accumulated storage materials in the form of lipid droplets together with glycogen. Lipid droplets were often associated with microbodies (peroxisomes) characterized by the presence of catalase and β -oxidation enzymes. Conidiophores were formed on tips of trophic hyphae appressed to the inner side of the cuticle. The process of outgrowth is most probably similar to that of the initial penetration in that it occurred via enzymic action and mechanical force. After outgrowth conidiophores rapidly developed, became septated and pegs were formed near the septa and hyphal tips. The conidiophores possessed an electron dense layer at the outside of their wall. On individual pegs numerous conidia rapidly developed, one after the other, resulting in the formation of clusters of spores. Production of conidia already started when colonization of the nematode body by trophic hyphae was not completed.

Approximately 5000 - 10,000 spores were formed at the expense of a single nematode. All observations favour the idea that the main strategy of the fungus is not the production of vegetative hyphal mass but instead to produce conidia at the highest possible numbers.

In order to study whether the above infection mechanisms are unique for *D. coniospora* or more widely spread among the endoparasitic fungi, we investigated the infection process of the fungus *Verticillium balanoides* using the nematode *P. redivivus*, which is described in **chapter 4**. These studies showed that initial mechanisms of infection (namely adhesion, the formation of an appressorium and subsequent penetration) together with the overall subcellular morphology of the trophic

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hyphae and the production of conidiophores and conidia, are very similar to those observed for *D. coniospora*. Besides these similarities a number of differences were observed. Firstly, conidia of *V. balanoides* adhered randomly to the nematode cuticle. Secondly, the fungus formed an infection bulb upon penetration. Thirdly, the cellular membranes of the nematode were not immediately ruptured as a result of hyphal invasion by *V. balanoides*, this occurred at a later phase of infection. Finally, bulk of the conidia formed adhesive layers following liberation from the phialide. In case of *D. coniospora* maturation (that is the formation of an adhesive knob) occurred in only part of the conidia produced after liberation of the conidiiferous pegs.

To further investigate the metabolic function of the microbodies inside trophic hyphae biochemically, the nematode-fungus system was inappropriate. One of the major problems was to obtain sufficient biological mass. Therefore we have sought conditions to induce the proliferation of these organelles in vegetative cells of *A. oligospora* by growing the organism in media supplemented with growth substrates known to induce microbodies in other fungi. The results indicated that *A. oligospora* is able to grow on oleic acid or D-alanine as the sole carbon source. In glucose-grown cells of *A. oligospora* microbodies were scarce and small in size. However, after a shift of glucose-grown cells to oleic acid-containing media many microbodies developed. The overall morphology of oleic acid-grown hyphae was largely comparable to that of trophic hyphae during infection of nematodes. Activities of the enzymes of the β -oxidation pathway, but not catalase, were induced during these growth conditions. Similarly, microbodies were induced during growth of hyphae on D-alanine albeit smaller in size and number; both D-amino acid oxidase and catalase activities were strongly enhanced. Biochemically and ultrastructurally, the above enzymes were located inside microbodies. These results suggest a key role for microbodies in both oleic acid and D-alanine metabolism (**chapter 5**).

A. oligospora and *D. coniospora* are representatives of two major groups of nematophagous fungi (facultative and obligate parasites, respectively). The possible competitive interactions between these fungal species during infection of one and the same nematode may reflect the ecological status of the organisms in nature and are studied in **chapter 6**. For this purpose, individuals of *P. redivivus* were infected with conidia of *D. coniospora* and subsequently at different stages of the infection manipulated in "trap"-cells of *A. oligospora*. At early stages of infection with *D. coniospora*, both fungi penetrated the cuticle, developed trophic hyphae and used nematode contents for the development of fungal biomass outside the nematode.

However, in the zone in which the two fungi met invariably dead hyphae of *A. oligospora* were observed, which indicated that *D. coniospora* acts as an antagonist against *A. oligospora* under these conditions. At later stages of infection of *D. coniospora*, infection of the predatory fungus often failed as judged by restricted development of trophic hyphae and death of the infection bulb. The interaction of trophic hyphae of both fungi included, i) contact or close vicinity of hyphae of both species, ii) thickening of the cell wall of *A. oligospora* at the sites of contact followed by, iii) degradation of hyphal compartments and shrivelling of the cell wall of *A. oligospora*. Invariably, *D. coniospora* hyphae remained virtually unaffected.

Adhesion of conidia of *D. coniospora* not always resulted in effective infection as has been observed with different nematode species. We studied this phenomenon using the free-living nematode *Acrobeloides buetschilii* as a model organism (**chapter 7**). Under optimal conditions for conidial adhesion approximately 70% of the nematodes was carrying one or more spores 16 hr after addition of the organisms to a fungal culture. Nearly all these nematodes had spores adhering to the head region; approximately half of them carried also spores on the body. Furthermore, conidial attachment was relatively more pronounced on younger nematodes. However, successful penetration of the cuticle in the majority of cases failed. In the case of effective infection the production of conidiophores and conidia was observed, albeit at very low frequencies. Not all conidia germinated within 16 hr after adhesion. If so, penetration of the host cuticle often failed. In such cases often a second adhesive knob was formed on the appressorium (even more knobs could be formed on one conidium). A substantial part of the conidia was subsequently removed, probably due to moulting of the nematodes. Successful penetration occurred from appressoria formed on primary or secondary adhesive knobs and was associated with a local separation of the layers of the cuticle by the developing penetration tube. Often electron dense material was observed near the penetration site. Failure of infection most probably is not the result of loss of virulence of a laboratory strain. This view was based on the finding that attempts to improve the infection frequencies by passing the fungus several times through the host were not successful. We therefore conclude that specific properties of the cuticle of *A. buetschilii* prevent a proper penetration in the majority of cases.

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