# Notes brèves

## INFLUENCE OF THE NUMBER OF PARASITIZING CONIDIA OF HIRSUTELLA RHOSSILIENSIS ON THE MORTALITY OF DITYLENCHUS DIPSACI

## Jean-Claude CAYROL\* and Jean-Pierre FRANKOWSKI\*

Many endoparasitic nematophagous fungi have adhesive spores which adhere to cuticle of the nematode host, germinate and penetrate through the cuticle to invade the body cavity. Among the better known species are Meristracum asterospermum (Drechs., 1940), Meria coniospora (Dreschs., 1941), Catenaria anguillulae (Couch, 1945), Myzocitium lenticulare (Baron, 1976a); Myzocitium anomalum (Barron, 1976b); Hirsutella heteroderae (Sturhan & Schneider, 1980) and H. rhossiliensis (Jaffee & Zehr, 1982). The conidia often attach to the cuticle in localized areas. For example, the zoospores of C. anguillulae are attracted to the body orifices of nematodes by the released exudate gradients and encyst at or near such openings (e.g., anus, vulva, excretory pore, mouth) (Sayre & Keeley, 1969; Boosalis & Mankau, 1970). Most infections by the adhesive conidia of *M. coniospora* are in the head region near the buccal cavity. With the genus Hirsutella, we have also observed that the conidia at first adhere to the head region, then in the tail region and finally cover the entire surface of the cuticle when nematodes are exposed to the fungus. If nematodes are placed in contact with sporulating mycelia for increasing periods of time, it is possible to obtain individuals with differing quantities of adhering spores. The objective of this study was to establish the relationship between the number of attached spores and nematode mortality.

A strain of *H. rhossiliensis* (No. 195.81, C. B. S. Baarn) was cultured on potato dextrose agar and tested against the nematode *Ditylenchus dipsaci* obtained from heavily infested garlic bulbs. Three hundred *D. dipsaci* were added to sporulating cultures of *H. rhossiliensis* in each of six Petri dishes. The nematodes were collected after migrating over the fungus for ten minutes from three of the plates and after one hour from the other three plates by rinsing each plate with 5 ml water. From nematodes exposed to the fungus for ten minutes two groups of 50 nematodes were hand-picked, one group with one to five adhering conidia and another with five to ten conidia. From nematodes exposed for one hour a group of

50 nematodes containig 30-40 adhering conidia were selected. Each three groups of 50 nematodes were transferred to a separate Petri dish of 1 % water agar. By regular observations it was possible to follow the development of parasitism and note the time of death among the different groups of nematodes. Nematodes were considered dead if they did not move when probed with a fine needle. All nematodes which had one to five conidia attached to the cuticle were killed after five days and the body cavity invaded by the fungus after the 6th day. Those with five to ten attached conidia were killed after four days and the body cavity invaded by the fungus. The group with 30-40 attached conidia were killed after only two days and then invaded by the fungus

*D. dipsaci* individuals were always killed irrespective of the number of adhering conidia; a single attached conidium was sufficient. The only difference between the three categories of the nematodes observed was that with increasing numbers of attached conidia death occurred more quickly.

Our results correlate with those of Jaffee and Zehr (1982) who observed that *Criconemella xenoplax* juveniles with ten adhering *H. rhossiliensis* conidia were killed by the fungus three to four days after the attachment of spores to their cuticles. The results also indicate very efficient parasitic behavior by the fungus with promising implications for applied biological control of certain nematodes.

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<sup>\*</sup> INRA, Station de Recherches de Nématologie et de Génétique moléculaire des Invertébrés, 123, boulevard Francis-Meilland, B.P. 2078, 06606 Antibes Cedex, France.

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## COMPARATIVE ACTIVITY OF DIFFERENT HIRSUTELLA SPECIES TOWARDS THREE PLANT PARASITIC NEMATODES

## Jean-Claude CAYROL\*, Régine CASTET\* and Robert A. SAMSON\*\*

Nematophagous fungi have often been tested for biological control of plant-parasitic nematodes but have only occasionally given encouraging results (Jatala et al., 1981; B'chir, Horrigue & Verlodt, 1983; Cayrol, 1983). Very little is known, however, about endoparasitic fungi which infect nematodes with their conidia. Some studies have indicated that the endoparasite Meria coniospora is very agressive (Jansson, Jeyaprakash & Zuckerman, 1985a) and able to reduce Meloidogyne sp. numbers in soil (Jansson, Jeyaprakash & Zuckerman, 1985b). The endoparasitic fungi of the genus Hirsutella are mainly insect and mite parasites; there are few studies concerning nematophagous activity. Sturhan and Schneider (1980) described a new species, H. heteroderae, which parasitized the hop cyst nematode, Heterodera humuli. Later, Eavre, Jaffee and Zehr (1983) indicated that H. rhossiliensis suppressed populations of Criconemella xenoplax. Our objective was to test the activity of several different species of Hirsutella against three plant-parasitic nematodes.

### Material and methods

Fungi

All of the isolates tested were obtained from Centraalbureau voor Schimmelcultures, Baarn, Netherlands and included the known insect or mite parasites, *H. thompsoni*, *H. satumaensis*, and *H. subulata*; as well as five different strains of *H. rhossiliensis* isolated from nematodes. The origin of isolates tested is indicated in Table 1. All fungi were cultured on potato dextrose agar (Difco 0013 - 01-4) in Petri dishes at 20°. All strains sporulated abundantly after 14 days and were ready for testing.

#### Nematodes

The species tested included *Aphelenchoides fragariae* obtained from monoxenic cultures on balsam seedlings, *Ditylenchus dipsaci* extracted from infested garlic bulbs and *Meloidogyne incognita* from egg masses excised from roots of tomato.

About 300 nematodes of each species were introduced in a small drop of sterile water into separate Petri dishes of each of the test fungi. The nematodes had been surface sterilized by washing in three successive five min baths of mercurothiolic acid 1:1000, streptomycin sulfate 7 : 1 000 and sterile water. The dishes were examined regularly until some conidia adhered to the cuticles of the introduced nematodes, then individuals with about 20 attached conidia were hand-picked and transferred to Petri dishes containing 2 % water agar. One hundred nematodes of each species were so transferred from each fungus. The nematodes could be easily observed on the agar and nematodes mortality estimated in comparison with control plates containing non-infected nematodes. Nematodes were considered dead when there was no reaction to stimulation with a fine needle.

\* INRA, Station de Recherches de Nématologie et de Génétique moléculaire des Invertébrés, 123, boulevard Francis-Meilland, B.P. 2078, 06606 Antibes Cedex, France.

<sup>\*\*</sup> Centraalbureau voor Schimmelcultures, 3740 AG Baarn, Nederland.