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Istituto di Nematologia Agraria, C.N.R. - 70126 Bari, Italy
and
Istituto Tossine e Micotossine da parassiti vegetali, C.N.R..
70126 Bari, Italy

PARASITISM OF *XIPHINEMA DIVERSICAUDATUM* BY THE FUNGUS *HIRSUTELLA RHOSSILIENSIS*.

by
A. Ciancio, A. Logrieco and F. Lamberti

Parasitism of nematodes by different species of fungi is frequent in nature. Many species of endoparasitic fungi belonging to the group Hyphomycetes have been studied and the mechanism of parasitism discussed by several authors (Drechsler, 1941; Duddington, 1957; Aschner and Kohn, 1958).

Work has been concentrated on the bio-control of cyst-nematodes (Kerry, 1980) or root-knot nematodes (Stirling and Mankau, 1979), because of their economic importance in reducing yields, whereas there is little information about parasitism of other genera of plant parasitic nematodes.

During a survey on the presence of nematophagous fungi in southern Italy, specimens of *Xiphinema diversicaudatum* (Micoletzky) Thorne were observed to be infected by *Hirsutella rhossiliensis* Minter *et* Brady.

This is the first report of this fungus from Italy and its first record on *X. diversicaudatum*.

Materials and Methods

Specimens of *X. diversicaudatum* were extracted from the rhizosphere of a peach orchard at Capua (Caserta), in May 1984. A few of them were infected by hyphae filling the body content and emerging from the cuticle (Fig. 1, a-d). Naturally infected nematodes were washed in sterile distilled water with antibiotics and successively incubated for 3 weeks in the dark on potato dextrose agar (PDA) with streptomycin at 20°C ± 2.

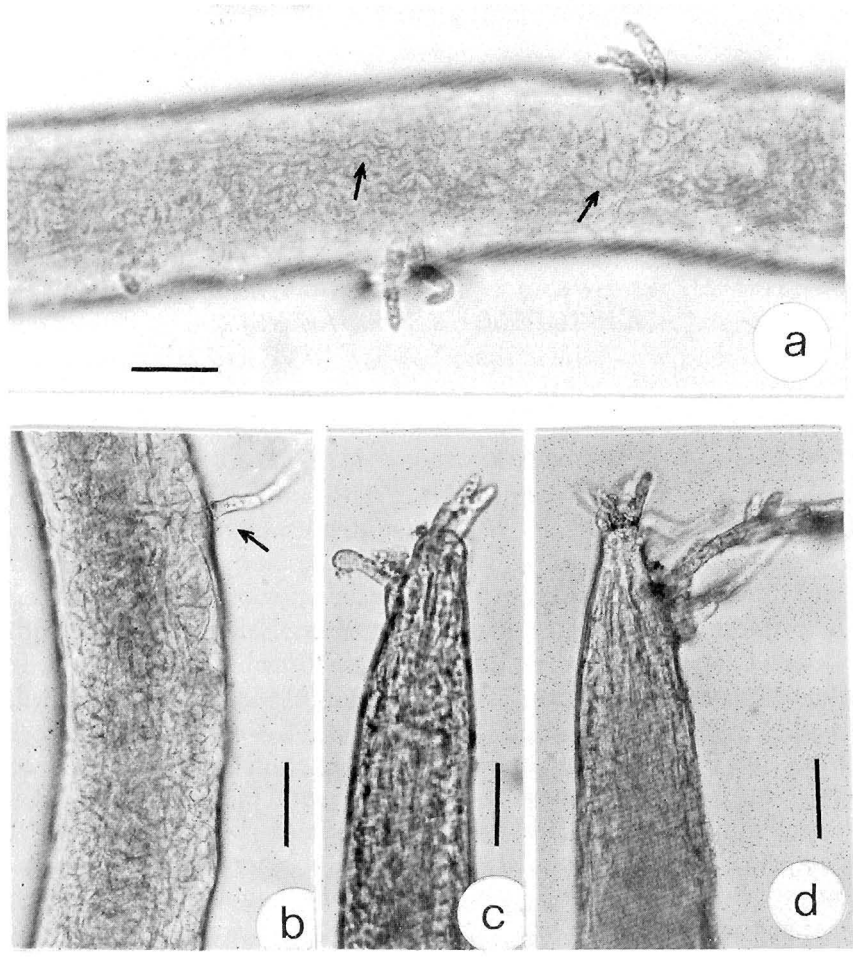

Fig. 1 - Specimens of naturally infected *Xiphinema diversicaudatum* showing hyphae inside the body and emerging through the cuticle in median (a, b) and cephalic regions (c, d). Scale bar = 25 μm.

Among the fungi isolated, *H. rhossiliensis*, identified according to Minter and Brady (1980), was the most common. Some *Fusarium* and *Penicillium* spp. which were present were considered to be saprophytes or opportunists.

Different nutritive media (potato dextrose, oat meal, potato carrot,

tomato juice and yeast extract agars) were used to test the growth rate of *H. rhossiliensis* by transferring hyphal tips to agar plates. To test whether metabolites or toxins lethal to the nematodes were produced, filtrates of the fungus were prepared by homogenizing and filtering 30 days old cultures growing in semi-synthetic liquid medium (Nachmias *et al.*, 1977).

Parasitism on *X. diversicaudatum* was studied by extracting adults and juveniles from the soil by Cobb's sieving method, sterilizing hand-picked individuals by immersion for 20 seconds in 0.01% NaOCl solution, rinsing in sterile 0.01% streptomycin solution, followed by two rinses in sterile distilled water, and inoculating by direct contact of a pure *H. rhossiliensis* culture. Infected nematodes were incubated in watch glasses at room temperature in sterile saturated soil extract (Jaffee and Zehr, 1983). Non-inoculated nematodes were incubated in sterile saturated soil extract at room temperature as control. The test was replicated three times; an average of 15 nematodes was used for each replication.

Results and Discussion

In the *in vitro* test, 31.8% of the inoculated *X. diversicaudatum* were infected after incubation for 1 week at room temperature. The mortality rate was 18.5%. Nematodes were checked whether they were dead by adding to the medium a drop of 0.4% acid lactic solution, which induced live nematodes to move rapidly. Specimens with conidia of *H. rhossiliensis* attached to the cuticle were considered as infected. Hyphae emerging from dead infected nematodes produced the typical phialides of *H. rhossiliensis* on the surface of the liquid medium. Uninoculated nematodes did not show any sign of fungal parasitism nor conidia attached to the cuticle; their mortality was less than 1%.

The filtrate of *H. rhossiliensis* had no effect on *X. diversicaudatum:* after 24 hours exposure, both in the presence or absence of soil extract, all the individuals were still viable.

As shown in table I, the best growth rate for the fungus was obtained by using oat meal agar. Optimum growth of the fungus was obtained at 20°C (Table II).

The genus *Hirsutella* Patouillard belongs to the group of fungi Imperfecti, class Hyphomycetes (Speare, 1920; Mains, 1951) and includes species specialized in parasitism of arthropods (Minter and Brady, 1980). *H. thompsonii* is used in commercial conidial formulations in integrated pest

Table I - *Growth of colonies of* Hirsutella rhossiliensis *on different media.*

Days	Diameter (cm)				
Medium	PDA	PCA	YEA	V8A	OA*
10	0.7	0.5	0.4	0.6	1
20	1.4	1.5	1.2	1.6	2.2

* PDA = potato dextrose agar; PCA = potato carrot agar; YEA = yeast extract agar; V8A = tomato juice agar; OA = oat meal agar.

Table II - *Growth of colonies of* H. rhossiliensis *on PDA at different temperatures.*

Days	Diameter (cm.)			
	10°C	20°C	25°C	30°C
10	0.2	1	0.7	—
20	0.8	1.8	1.4	0.1
25	1.3	2.8	2.3	0.2

management systems in the USA for the control of the citrus rust mite and other Acarina (McCoy, 1980). *H. rhossiliensis* was recently found associated with *Criconemella xenoplax* (Raski) Luc *et* Raski (Jaffee and Zehr, 1982), whereas *H. heteroderae,* supposed to be a synonym of *H. rhossiliensis* (Jaffee and Zehr, 1982), was found to parasitize *Heterodera humuli* Filipjev and others tylenchids (Sturhan and Schneider, 1980).

Absence of toxic activity of culture filtrates suggests that *H. rhossiliensis* mainly parasitizes host nematode by mechanical penetration: as shown in Fig. 2a, *H. rhossiliensis* conidia adhere to the nematode cuticle. Germinating conidia of *H. rhossiliensis* produce a germ tube which penetrates the cuticle (Jaffee and Zehr, 1982). After penetration, mycelia develop inside the nematode, apparently without preference for specific organs, eventually emerging mainly from the anterior region (Fig. 1 a-d; Fig. 2 c, g, h).

Although the rate of parasitism in soil cannot easily be determined, as it is the result of a complex of interactions involving several factors (Mankau, 1980; Jaffee and Zehr, 1982), our results indicate that *X. diversicaudatum* can be included in the list of those nematodes parasitized in nature by *H. rhossiliensis.*

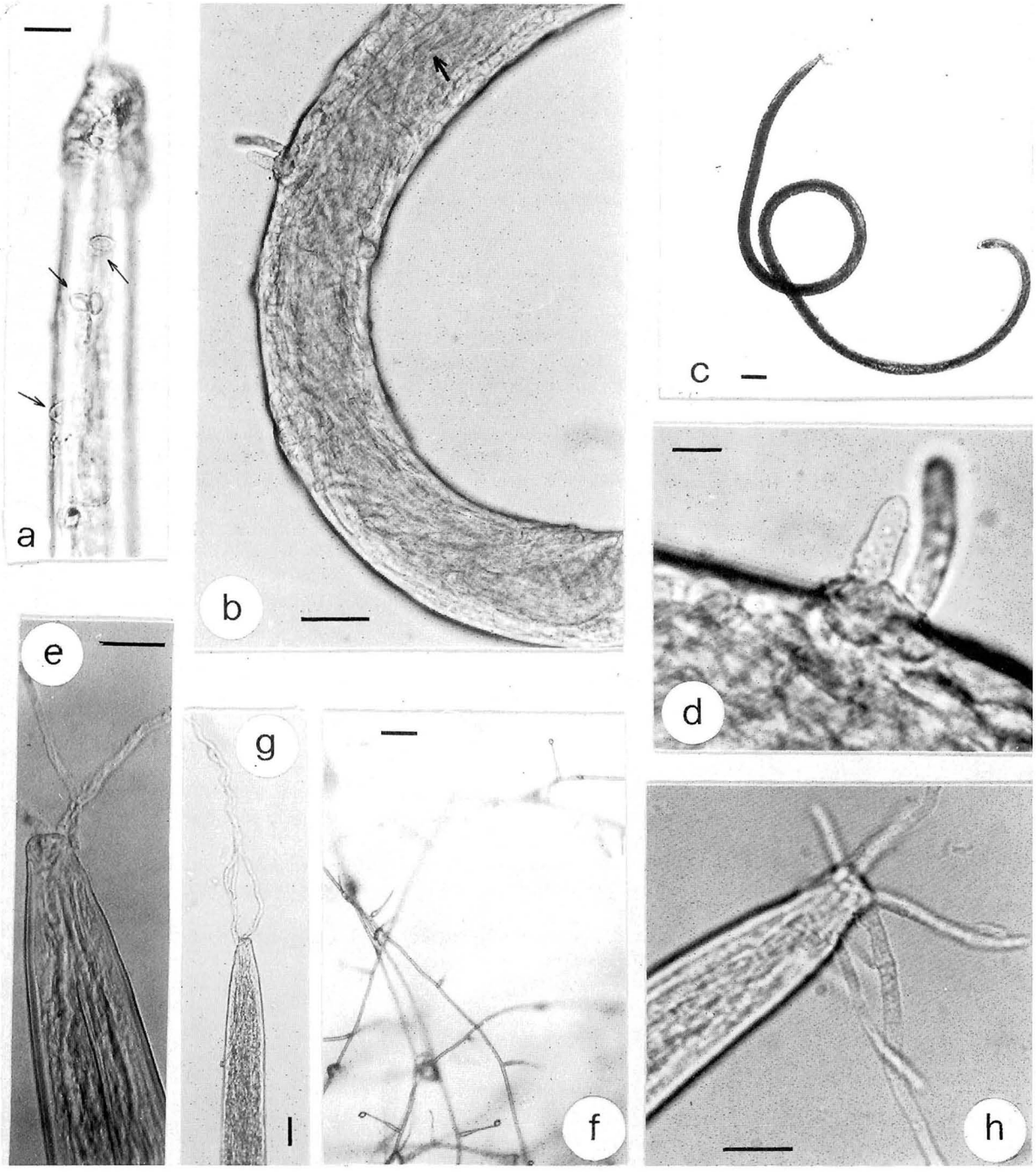

Fig. 2 - Conidia of *Hirsutella rhossiliensis* adhering to the cuticle of artificially inoculated *X. diversicaudatum* (a, arrowed); naturally infected *X. diversicaudatum* showing hyphae emerging from the head (c) and from the caudal region (b, d), by cuticle disruption; (e, g, h) hyphae emerging from artificially infected *X. diversicaudatum*; phialides and conidia of *H. rhossiliensis* (f); scale bar:b, e, h = 25 μm; a = 15 μm; d = 5μm; f, g = 30 μm; c = 100 μm.

SUMMARY

The fungus *Hirsutella rhossiliensis* was isolated in southern Italy from specimens of *Xiphinema diversicaudatum*. In *in vitro* tests *H. rhossiliensis* parasitized adult and juvenile nematodes by contact with conidia. No toxic activity of culture filtrates was observed.

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