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Development, Distribution, and Host Studies of the Fungus *Macrobiotophthora vermicola* (Entomophthorales)

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Abstract: The life cycle and host range of *Macrobiotophthora vermicola* were studied. Secondary spores produced from forcibly ejected primary spores adhered to the cuticle of *Cruznema tripartitum*, germinated, and penetrated the cuticle within 30 minutes. New primary spores were produced within 24 hours of initial spore adhesion. In a host range study, species of Rhabditidae, Diplogasteridae, and Aphelenchoidea were hosts, but not species of Bunonematidae, Tripylidae, Cephalobida, or Tylenchina. Numbers of second-stage *Meloidogyne incognita* juveniles were not decreased when added to soil seeded with infected *C. tripartitum*. In six Tennessee soybean fields, *Macrobiotophthora vermicola* was the most commonly encountered nematode-destroying fungus, followed by a sterile, nonseptate fungus and *Arthrobotrys conoides*. Nematophagous fungi were isolated more frequently from silt loam soils than from clay soils. Addition of *C. tripartitum* to soil extract plates as a bait nematode did not increase isolations of nematophagous fungi.

Key words: biological control, Entomophthorales, host range, *Macrobiotophthora vermicola*, nematophagous fungus.

Entomophthora vermicola was described from Queensland, Australia, in 1977 (11). It is characterized by parasitism of nematodes, forcibly discharged primary spores, and production of sticky, passively detached secondary spores. Because of differences between this fungus and other *Entomophthora* spp., it was transferred to the poorly known, tardigrade-parasitic genus *Macrobiotophthora* Reukauf (16). Tucker (17) studied in detail the infection process and means of axenic culture, with nematode–fungus cultures derived from those used for the studies in this paper.

During routine assays of plant-parasitic nematodes from a snapbean (*Phaseolus vulgaris* L.) field in Cumberland County, Tennessee, *Macrobiotophthora vermicola* (McCulloch) Tucker was found in cadavers of rhabditid nematodes. Later, it also was collected from a tomato (*Lycopersicon esculentum* Mill.) field in Cocke County and from soybean (*Glycine max* (L.) Merr.) fields in western Tennessee. The objectives of this study were to determine the abundance of *M. vermicola* in selected west Tennessee soybean fields, to examine its developmental cycle in nematodes, and to assess its potential as a biological control of plant-parasitic nematodes.

MATERIALS AND METHODS

Isolation of nematophagous fungi: All soil samples were processed by means of a semiquantitative method (10), in which a soil suspension was vacuum-concentrated onto a 0.45-μm-mesh filter disc that was then placed in the center of a 2% water agar petri plate. To determine the presence and frequency of nematophagous fungi in soybean field soil, duplicate plates of 73 samples from six fields (three clay, three silt loam) were prepared. One set of plates received an amendment of 10 gravid *Cruznema tripartitum* (Linstow) Zullini as a possible stimulant for the fungi. In the other set, the indigenous nematodes served as the stimulating agent. All plates were examined every 2 or 3 days for 1 month for the appearance of nematophagous fungi. Comparisons of total isolations between plates amended with *C. tripartitum* and unamended plates, and between clay-derived and silt loam-derived plates, were made with a chi-square type goodness-of-fit test. The hypothesis in each case was that total isolations would be equal.

Development of Macrobiotophthora vermicola: Cruznema tripartitum was maintained on 1% plain agar amended with dry pea soup

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mix (2) as the stock host for *M. vermicola.* Every 2–3 weeks, two cubes of agar, one containing fungal spores and one with *C. tripartitum,* were placed adjacent to each other on each of several fresh agar plates to maintain the fungus. To study the rate of penetration and development, groups of 10 adult male nematodes were inoculated with infective spores of the fungus by allowing them to crawl across agar surfaces heavily infested with spores. Males were used because females usually contained numerous eggs that obscured development of the fungus. Nematodes were confined on agar plates in cages made of a plastic cover slip cemented to a plastic ring (3 × 15 mm d). After 0.25, 0.5, 1, 2, 4, 8, 16, and 24 hours, groups of 10 nematodes were placed in a small quantity of water in a 5-ml beaker, killed and fixed by the addition of hot 4% formalin, then processed to anhydrous glycerin by a rapid method (15). These nematodes were mounted on slides for observation of fungal penetration and internal growth. The behavior of other inoculated nematodes was observed until fungal sporulation occurred.

Effects of M. vermicola on other nematodes: Seventeen nematode species of several orders were exposed to infective spores to determine their susceptibility to the fungus (see Table 2). Nematodes were allowed to contact fungal spores by their own movement or were pushed or dragged across fields of spores. Exposed nematodes were observed daily for one week.

The ability of the fungus to destroy *Meloidogyne incognita* (Kofoid and White) Chitwood juveniles in soil was also tested. Numerous *C. tripartitum* inoculated with spores of *Macrobiotophthora vermicola* were added in groups of 400 to each of four plastic bags of 200 ml steam-sterilized loamy sand soil. Four other bags of soil each received 400 noninoculated *C. tripartitum.* Eggs of *Meloidogyne incognita* were collected from tomato roots with a sodium hypochlorite method (7) and 10,000 eggs were added to each bag. The bags were sealed, the soil within was mixed, and the bags were stored in darkness at room temperature. Eight days later, *C. tripartitum* and *M. incognita* were extracted with a sugar-flotation–centrifugation technique and counted.

RESULTS AND DISCUSSION

Extraction of nematophagous fungi: Macrobiotophthora vermicola was the nematophagous fungus most frequently extracted from west Tennessee soybean fields in this study (Table 1), occurring in about half (51.3%) of all plates. Only two other fungi were found frequently (in more than 10% of the plates): a sterile fungus with nonseptate hyphae that exuded a sticky substance when a nematode contacted them and *Arthrobotrys conoides,* a three-dimensional net trapper. Norton (13) also frequently isolated *A. conoides* (56% of 237 samples) and a similar sticky fungus (41%) from forage legume soil samples but did not report any fungi similar to *M. vermicola.*

The widely separated incidence of *M. vermicola* (Queensland, Australia, and Tennessee, U.S.A.) is puzzling because of its distinctive nature and the recent high interest in nematophagous fungi. *Macrobiotophthora vermicola* was not isolated from 69 soil and plant samples in a study of endoparasitic fungi in Ireland (6) or from several hundred samples in New Zealand (4). A similar fungus was reported from Iowa (3), and Amin and Webster (1) described, but did not name, a fungus from Devon, England, very similar to that of McCulloch (11), apparently unaware of the description of *M. vermicola* 3 years earlier. *Macrobiotophthora vermicola*-like fungi thus appear to be widely distributed and possibly have been frequently mistaken for superficially similar *Conidiobolus* spp.

The addition of *C. tripartitum* to the plates had no effect on the appearance of nematophagous fungi, but there were significantly ($P = 0.05$) more isolations from silt loams than from clay soils (Table 1). Gray (5) found that *Panagrellus redivivus* (L.) Goodey was a suitable bait nematode for nearly all of 30 nematophagous fungi and concluded that these fungi displayed little or no prey selectivity. In the present study, *C. tripartitum* also appeared to be a suitable

TABLE 1. Frequency of nematode-destroying fungi in six west Tennessee soybean fields.

Fungus	Isolations (of 146 plates)		Number of isolations with *Cruznema tripartitum*		Number of isolations	
	Number	Percent of total	Added	Not added	Silt loam	Clay
Macrobiotophthora vermicola (McCulloch) Tucker	75	51.3	31	44	43	32
Sterile fungus with sticky hyphae	55	37.6	31	24	33	22
Arthrobotrys conoides Drechsler	19	13.0	7	12	13	6
Meristacrum asterospermum Drechsler	6	4.1	3	3	4	2
Acrostalagmus obovatus Drechsler	3	2.1	2	1	3	0
Harposporium anguillulae (Lohde) Karling	3	2.1	3	0	2	1
Dactylella sp.	1	0.7	0	1	0	1
Haptoglossa heterospora Drechsler	1	0.7	1	0	1	0
Monacrosporium sp.	1	0.7	0	1	0	1
Stylopage hadra Drechsler	1	0.7	1	0	0	1
None	42	28.8	27	15	18	24
Total isolations			79	86	99	66
χ^2			n.s.		$P < 0.05$	

bait nematode, but as with *P. redivivus,* it provided little additional accuracy to the isolations. Factors involved in the greater suitability of silt loam soils may include increased porosity and larger soil pores, which would allow for increased fungal dispersal via actively discharged spores and enhanced chances of contact with passing nematodes.

Development of Macrobiotophthora vermicola: Primary spores developed from infected nematodes in culture ca. 24 hours after inoculation (Fig. 1A). The primary spores were produced on negatively geotropic sporophores projecting from the nematode cadaver and subsequently were forcibly discharged, landing on substrate up to 10 mm from their origin. Primary spores did not adhere to, or infect, nematodes. Within 12 hours, each primary spore gave rise to a single secondary spore (Fig. 1B) elevated on a short stalk above the primary spore. Tucker (17) terms these secondary spores "letiferispores." A small tertiary spore sometimes developed 2 or more weeks after secondary spore formation (Fig. 1C). Secondary and tertiary spores were coated with a sticky substance and functioned as infective units. When a susceptible nematode touched a secondary or ter-

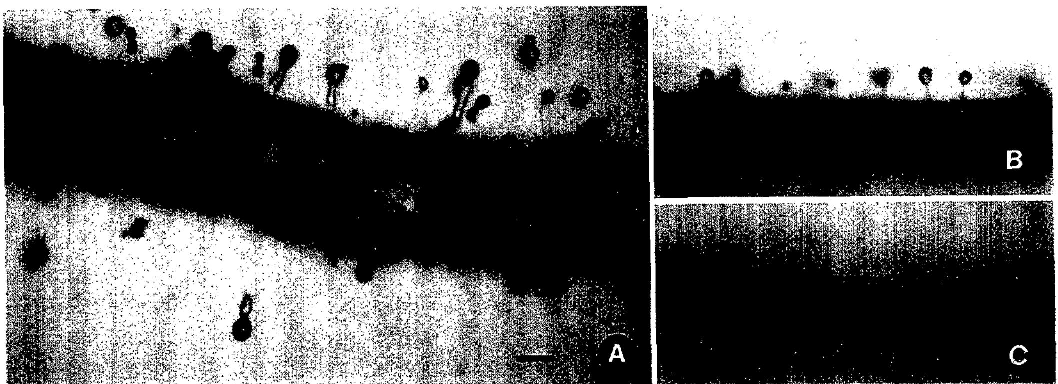

FIG. 1. Spore production of *Macrobiotophthora vermicola.* A) Primary spore production from a *Cruznema tripartitum* cadaver. B) Secondary spores. C) Tertiary spore production from a secondary spore on right; typical secondary spore on left. Scale bars = 25 μm.

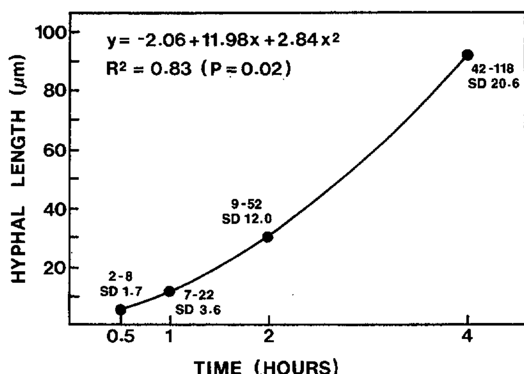

FIG. 2. Growth of *Macrobiotophthora vermicola* hyphae within *Cruznema tripartitum* during the first 4 hours after inoculation.

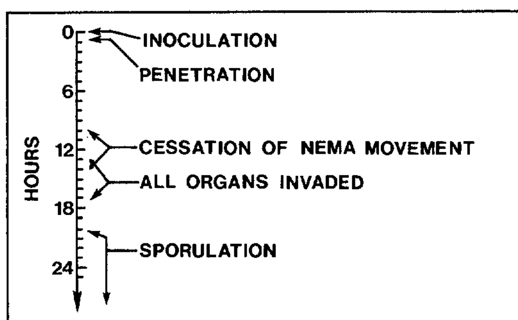

FIG. 3. Time line of *Macrobiotophthora vermicola* development in *Cruznema tripartitum* following inoculation with secondary spores.

tiary spore, the spore instantly adhered to the cuticle.

Adhesion on the head or esophageal region of a nematode immediately elicited a vigorous, thrashing motion and other apparent avoidance movements. The spore usually germinated and began penetration within 30–60 minutes after inoculation. Unless the spore had adhered to the nematode's anterior end, inoculated nematodes continued to feed for several hours. Within 4 hours, invasive hyphae were 50–100 μm long (Fig. 2). Internal hyphal development was so extensive 10–14 hours later that nematodes ceased to move, and shortly afterward most organs had been invaded. However, cuticle, stoma, esophageal valves, male spicules and gubernaculum, and eggs in utero were not consumed. Female cadavers frequently contained juveniles that had hatched after the mother's death; these did not become infected unless they left the cadaver and contacted other infective spores. These events are summarized in Figure 3.

During the development of primary spores, zygospores also formed, the mechanism of which has been described by Tucker (16). Adult *C. tripartitum* yielded 30–70 primary spores each. Cultures containing several hundred nematodes were totally eliminated within four days.

Host range and biocontrol potential of M. vermicola: Macrobiotophthora vermicola exhibited distinct selectivity in its ability to infect nematodes. The fungus successfully destroyed members of the secernentean orders Diplogasterida, Rhabditida, and Tylenchida (Table 2). In the Rhabditida, bunonematid nematodes (*Bunonema husseyi* Bernard, *Rhodolaimus dimorphus* Bernard), to which spores adhered, were not infected, possibly because the minute body size and network covering the projecting tubercles of these species prevented spores from adhering to the cuticle proper (14). In the Tylenchida, aphelenchoid nematodes were successfully parasitized, but not species of *Criconemella, Helicotylenchus, Heterodera,* or *Meloidogyne.* In most of the Tylenchina tested, secondary spores failed to adhere to the cuticle. In the test with inoculated or uninoculated *C. tripartitum* mixed in soil with *M. incognita,* the numbers of *M. incognita* juveniles extracted did not differ between the treatments. However, no *C. tripartitum* were extracted from the soil seeded with inoculated *C. tripartitum.* Thus it appears that *M. vermicola* has limited if any potential as a biological control agent for many plant-parasitic nematodes. It may play a significant role, however, in population regulation of certain microbivorous nematodes or Aphelenchoidea.

The mechanism by which secondary spores adhere to the cuticle is unknown and is worthy of attention. Some nematophagous fungi use lectins that react to nematode surface carbohydrates, leading to trap or spore adhesion and subsequent infection (8,9,12). In an extensive and thorough investigation of *M. vermicola* spore

TABLE 2. Results of tests of various nematodes as hosts of *Macrobiotophthora vermicola*.

Nematode	Spores adhering, nematodes killed	Spores adhering, no infection	Spores not adhering
Diplogasterida			
Mesodiplogaster sp.	+		
Rhabditida			
Bunonema husseyi Bernard		+	
Caenorhabditis elegans (Maupas) Dougherty	+		
C. briggsae (Dougherty & Nigon) Dougherty	+		
Cruznema tripartitum (Linstow) Zullini	+		
Diploscapter coronatus (Cobb) Cobb	+		
Rhodolaimus dimorphus Bernard		+	
Tylenchida			
Aphelenchoides sp.	+		
Aphelenchus avenae Bastian	+		
Criconemella xenoplax (Raski) Luc & Raski			+
Helicotylenchus pseudorobustus (Steiner) Golden			
Heterodera glycines Ichinohe (J2)		+	
Meloidogyne incognita (Kofoid & White) Chitwood (J2)			+
Cephalobida			
Acrobeles sp.			+
Chiloplacus sp.		+	
Cuticonema vivipara Sanwal			+
Panagrolaimus sp.			+
Enoplida			
Tripyla sp.			+

adhesion, Tucker (17) found that incubation of *C. tripartitum* in solutions of lectins, chitinase, or neuraminidase failed to inhibit attachment. Alkaline or acidic hydrolysis of nematodes or oxidation of nematodes in periodic acid also failed to prevent adhesion. Likewise, incubation of nematodes in protease, lipase, or glutaraldehyde solutions failed to inhibit spore adhesion. It appears, then, that the attachment phenomenon in *M. vermicola* is very dissimilar to that reported for trapping fungi and other endoparasitic fungi with adhesive spores.

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