N O T E

Defensive Interaction of *Heterorhabditis bacteriophora* (Rhabditida: Heterorhabditidae) Infective Juvenile Nematodes against *Metarhizium anisopliae* (Hypocreales: Clavicipitaceae)[1]

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Entomopathogenic nematodes offer an environmentally safe and IPM- compatible alternative to conventional insecticides (Georgis et al. 1991, Environ. Entomol. 20: 815 - 822) but do not always provide adequate pest control in practical field applications (Georgis and Gaugler 1991, J. Econ. Entomol. 84: 713 - 720; Georgis et al. 2006, Biol. Control. 38: 103 - 123; Klein 1990, In: R. Gaugler and H. K. Kaya (Eds.), Entomopathogenic Nematodes in Biological Control, pp. 195 - 214; Klein 1993, In: R. Bedding et al. (Eds.), Nematodes and the Biological Control of Insect Pests, pp. 49 - 58). The combined use of the nematodes with other biological control agents like entomopathogenic fungi is thus considered for management of insect pests (Anbesse et al. 2008, Nematol. 10: 701 - 709; Ansari et al. 2006, Biol. Control. 39: 453 - 459; Barbercheck and Kaya 1991, Environ. Entomol. 20: 707 - 712; Choo et al. 2002, BioControl. 47: 177 - 192). The study reported herein showed interesting interactions between the nematode *Heterorhabditis bacteriophora* Poinar and the fungus *Metarhizium anisopliae* (Metschn.) Sorokin. *Heterorhabditis bacteriophora* exhibited a defensive response when its infective juveniles (IJs) were mixed with *M. anisopliae* (strain F52 in oil formulation) in distilled water at the rate of 390 IJs/ml and 2.5×10^7 conidia/ml, respectively. We observed nematode IJs being trapped by *M. anisopliae* conidia within 96 h of contact with the fungus.

The IJs, also called 'dauer juveniles', are the 3rd stage juvenile nematodes (J3) that retain the 2nd stage (J2) cuticle as a protective sheath against environmental extremes and parasitism by nematophagous fungi (Timper and Kaya 1989, J. Invertebr. Pathol. 54: 314 - 321). Under natural conditions, the IJs of *Heterorhabditis* spp. do not

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cast off the outer sheath (J2 cuticle) before entering a potential host. In the current study, a hypha tube germinating from a conidium adhered to the outer sheath, but did not penetrate the inner cuticle of IJs, as shown by the arrow in Fig. 1 A & B. After 192 h, the IJs shed the outer sheath to be free from the fungal trap (Fig. 1 C & D I-III). In addition, although the mortality of IJs increased over time (F=13.89, df=2, P = 0.0008), it was not significantly affected by the presence of the fungus within 192 h after treatment (F=2.92, df=1, P = 0.1131) (two-way ANOVA, α=0.05) (Fig. 2). Finally, when mixed with *H. bacteriophora*, *M. anisopliae* was still capable of growing colonies in Veen's medium 192 h after treatment. Similar symptoms also were observed in a repeated experiment, in which the IJs exsheathed at 11 days after treatment.

Nematode-trapping fungi are common in soil habitats (Gray 1987, Biol. Rev. Cambridge Philos. Soc. 62: 245 - 304; Jaffee et al. 1996, Mycologia. 88, 554 - 564), but impact of entomopathogenic fungi on parasitism by entomopathogenic nematodes is unknown. Trapping is commonly used by nematophagous fungi as a tactic to capture and infect nematodes. In the current study, however, *M. anisopliae* readily adhered to

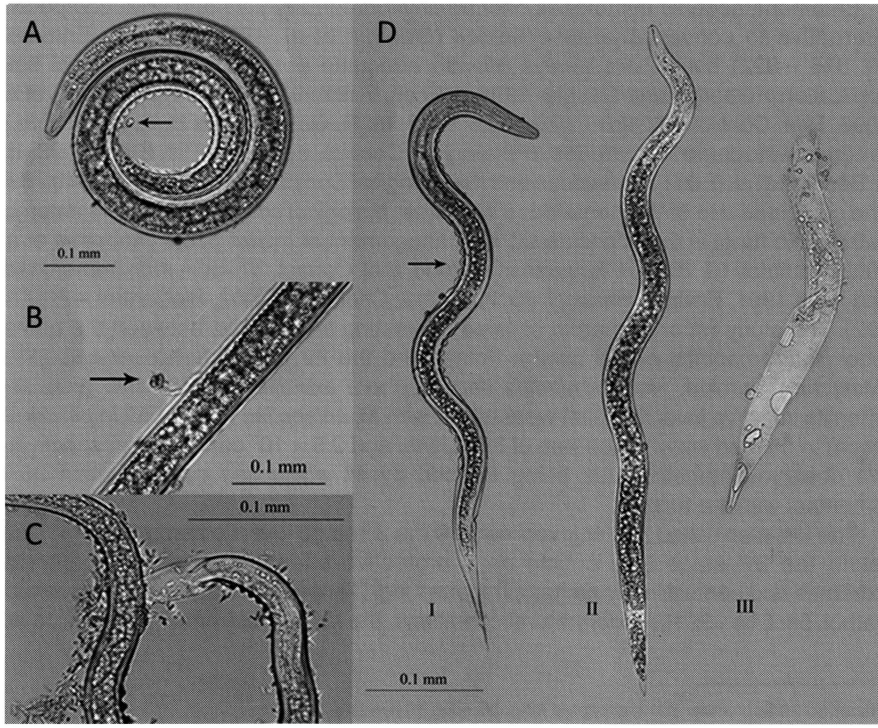

Fig. 1. *Metarhizium anisopliae* conidia germinated and adhered to the outer sheath of *Heterorhabditis bacteriophora* infective juveniles (IJs) 96 h after treatment (indicated by arrows in A & B); IJs exsheathed to detach from *M. anisopliae* 192 h after treatment (C); IJs before exsheathing (D I: sheath wrinkles pointed by an arrow), after exsheathing (D II), and the shed cuticle (D III).

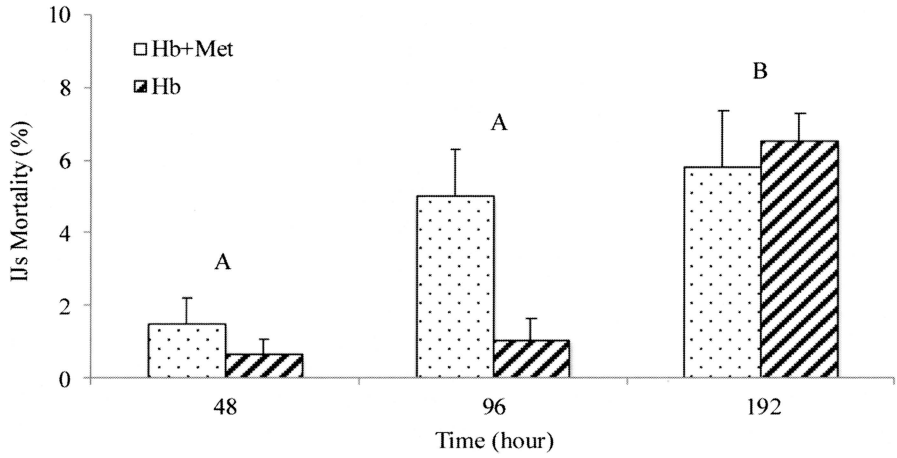

Fig. 2. Mortality of *Heterorhabditis bacteriophora* IJs 48, 96, and 192 h after treatment with *H. bacteriophora* alone (Hb), or mixed with *Metarhizium anisopliae* (Hb+Met) (mean±SEM). Different letters indicate significant differences between times after treatment (treatments combined for analysis) (Tukey's HSD, α=0.05).

but did not kill *H. bacteriophora* IJs, which cast off the J2 cuticle and were free from the fungal trapping. Our observations agree with those of Poinar and Jansson (1986, Rev. Nematol. 9: 241 - 244) who found that entomopathogenic nematode dauers trapped by the fungus *Monacrosporium ellipsosporum* (Grove) escaped infection by exiting their J2 cuticle. Also, Timper and Kaya (1989) reported that conidia of endoparasitic fungi *Hirsutella rhossiliensis* Minter & Brady and *Drechmeria coniospora* (Drechsler) adhered to the J2 cuticle of *Heterorhabditis* spp. but infected <0.7% of the dauer juveniles. Exsheathing due to conidial adhesion in *Heterorhabditis* spp., however, was not reported with either fungus, and conidia usually did not germinate or germinated but only penetrated the J2 cuticle and grew between J2 and J3 cuticles without infecting the dauer. The interactive response between the nematode and the fungus observed in our study may indicate potential for their combined use in managing pest insects. The impact of *M. anisopliae*-induced exsheathing on the physiological fitness and virulence of nematode IJs merits further evaluation.

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