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Pathogenicity, reproductive potential, and reproductive success of isolates of *Heterorhabditis bacteriophora* Poinar 1975 (Rhabditida: Heterorhabditidae) from Italy**

ABSTRACT

Entomopathogenic nematodes of the genus *Heterorhabditis* usually turn insect larvae brick red after infection. During a soil survey for entomopathogenic nematodes in southern Italy, several isolates of *Heterorhabditis bacteriophora* were found, three of which turned *Galleria mellonella* larvae green upon infection ("green strains"). The aim of this study was to compare pathogenicity, reproductive potential, and reproductive success of two of the three "green" (strains IH-C3 nd IH-C13) and two "red" (strains IH-MR4 and IH-MR8) *H. bacteriophora* from Italy. IH-C13 was most pathogenic (LC_{50} = 9 IJs/larva) and IH-MR4 was least pathogenic (LC_{50} = 29 IJs/larva). *G. mellonella* larvae infected with IH-C13 or IH-MR8 produced more infective juveniles (3.8 and 3.9 million IJs/10 *Galleria* larvae, respectively) than those infected with IH-C3 or IH-MR4 (2.8 and 3.5 million IJs/10 *Galleria* larvae, respectively). Reproductive success increased as concentrations of IJs/larva increased. IH-C13 and IH-MR8 reproduced at lower IJ concentrations than IH-C3 and IH-MR4.

Key words: bioassays, entomopathogenic nematodes, heterorhabditid red and green strains, infectivity comparison.

INTRODUCTION

Entomopathogenic nematodes are obligate parasites of insects. Infection of insect hosts begins when the nonfeeding infective juveniles (IJs) of entomopathogenic nematodes enter the host through natural openings or integument membranes. Once inside the host, nematodes release symbiotic bacteria from their intestines that cause septicemia and kill the host, usually within 48 hr. The bacteria transform the host into suitable food for the nematodes. Nematodes feed on the bacteria, as well as on nutrients of the host, and mate and reproduce until resources are depleted. IJs are produced when food becomes scarce inside the dead host and they leave the host to seek a new host (POINAR, 1979, 1990).

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Entomopathogenic nematodes have been isolated from many regions of the world (POINAR, *l.c.*; SHAMSELDEAN *et al.*, 1994; HOMINICK *et al.*, 1996; LIU & BERRY, 1995; DE DOUCET *et al.*, 1999; GRIFFIN *et al.*, 1999). Nematode species and isolates differ in survival, pathogenicity, reproductive potential, and efficacy against particular insect pests (KUNG *et al.*, 1990; BEDDING *et al.*, 1983). Many new nematode isolates have been recovered from soil by using *Galleria mellonella* L. (Lepidoptera: Galleriidae) larvae as bait (BEDDING and AKHURST, 1975). Some of which may have high potential in biological control for a variety of crop pests.

Heterorhabditis bacteriophora was first described from Utah (POINAR, 1975), but has since been collected from many parts of the world. Tarasco and Triggiani (1997, 1998) found several isolates of *H. bacteriophora* during soil surveys in southern Italy. Among the isolates were three that turned *G. mellonella* larvae gray-green, rather than the brick red usual with heterorhabditid species.

The objective of this study was to compare pathogenicity, reproductive potential, and reproductive success of two of the "green" isolates with two "red" isolates of *H. bacteriophora*, also collected from southern Italy.

MATERIALS AND METHODS

NEMATODES

The four isolates of *H. bacteriophora* (2 green and 2 red) used in the study were collected by the *Galleria* baiting (BEDDING and AKHURST, *l.c.*) during a soil survey in southern Italy (TARASCO and TRIGGIANI, *l.c.*). Cultures were maintained on the last instars of *G. mellonella*. The green isolates were collected from a pine forest (IH-C3) and an olive grove (IH-C13). The red isolates were collected from uncultivated land (IH-MR4) and a vineyard (IH-MR8).

A stock suspension of infective juveniles of each nematode isolate was obtained using the standard method developed by DUTKY *et al.* (1964). To obtain fresh infective juveniles (IJs), ten *Galleria* larvae were placed in a 100 x 10 mm Petri dish on a 90 mm filter paper and were treated with about 2,000 IJs in 1.5 ml of tap water. Two weeks after treatment, waxworms were put on modified White traps (WHITE, 1927) to recover of infective juveniles (IJs). IJs were kept at 4°C and used within ten days after harvesting.

Pathogenicity test

Galleria larvae were inoculated on filter paper with 1, 2, 4, 8, 16, 32, 64, 128, and 256 IJ(s)/larva in 0.5 ml of tap water in 60 x 10 mm Petri dishes. IJs were counted directly under a stereomicroscope for lower concentrations (1, 2, 4, 8, 16, and 32 IJ(s)) or diluted to determine higher concentrations (64, 128, and 256 IJ(s)). One *Galleria* larva was placed in each Petri dish. There were 4 blocks of 10 replications (n = 40 per concentration per isolate). Dishes were covered with a lid and put in a container (15 x 32 x 40 cm) wrapped in 2 garbage bags to minimize desiccation. Larval mortality was recorded after 96 hr at 22 ± 1°C. Data were pooled and analyzed by probit analysis to determine LC_{50} and LC_{90}, confidence intervals, and slopes.

Reproductive potential

One 90-mm Whatman filter paper placed in a 100 x 15 mm Petri dish was treated with 2,000 IJs in 1.5 ml distilled water, ten *Galleria* larvae (250-350 mg each) were then added and the dish was covered. There were 6 replications for each isolate. Two weeks after treatment, dead larvae were transferred to White traps to collect emerging IJs. IJs were counted every 3 days until no more IJs emerged. Reproductive potentials among the isolated were compared with Duncan's multiple analysis.

Reproductive success

After mortality had been recorded for the pathogenicity test, dishes were left in the containers for another 96 hr at 22 ± 1°C to allow the nematodes to reproduce in the *Galleria* larvae. Eight days after the initial treatment, *Galleria* larvae were dissected to determine the production of IJs.

RESULTS

All nematode isolates killed the hosts. The lethal concentrations (LC) of IJs were lowest in the green isolate IH-C13 and highest in the red isolate IH-MR4 (tab. 1). IH-C13 was the most virulent isolate and IH-MR4 the least.

Tab. 1. LCs of IJs causing mortality in four isolates of *H. bacteriophora* from Italy.

Strains	LC_{50} (95% CI)	LC_{90} (95% CI)	Slope ± SE
C 3	24 (6-35)	104 (66-557)	2.000 ± 0.511
C 13	9 (5-12)	36 (26-64)	2.093 ± 0.326
MR 4	29 (19-39)	138 (88-339)	1.880 ± 0.321
MR 8	21 (15-26)	59 (45-92)	2.814 ± 0.491

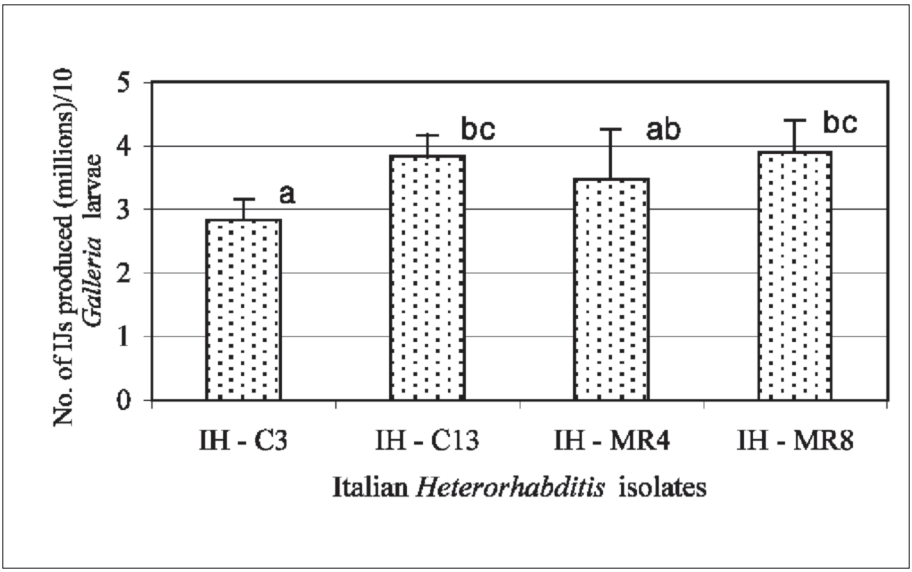

Fig. 1 - Mean ± SE number of IJs produced per 10 *Galleria* larvae. Bars with the same letters not differ significantly (P>0.05).

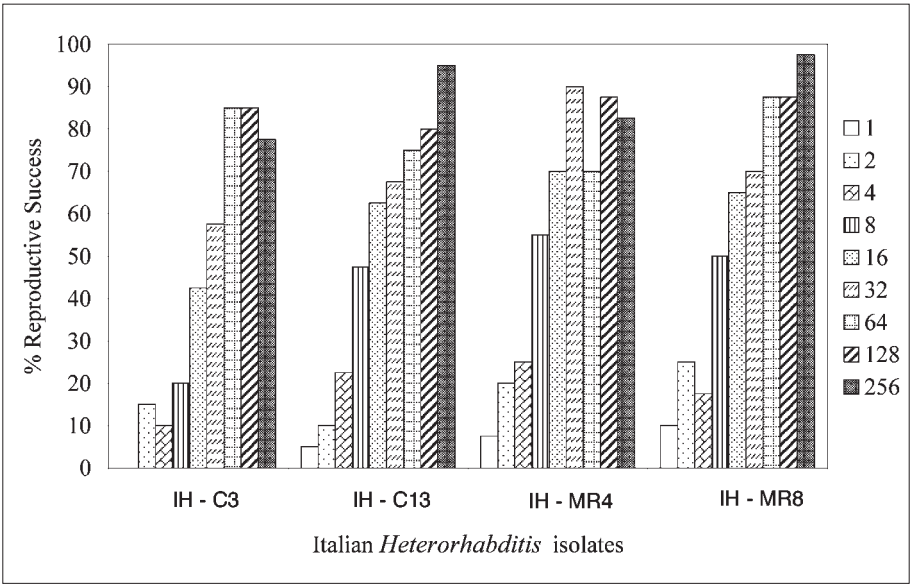

Fig. 2 - Reproductive success rate with different numbers of IJ(s)/*Galleria* larva.

IH-C13 and IH-MR8 produced more IJs per *Galleria* larva than did IH-C3 and IH-MR4 (Fig. 1).

Reproductive success rates increased with nematode concentration in all isolates. For IH-C13 and IH-MR8, only 16 and 32 IJs/larva gave 90% reproductive success, whereas IH-C3 needed 128 IJ/larva to achieve the same reproductive success. IH-MR4 did not reach 90% reproductive success within the experimental ranges (Fig. 2). When reproductive success is compared at different rates, IH-C13 and IH-MR8 consistently performed better than IH-C3 and IH-MR4 at most rates except at 4 IJs/larva. Reproductive success of IH-MR4 was the least at every concentration.

DISCUSSION

Although *Galleria* larvae were susceptible to each isolate, the four isolates differed appreciably in their pathogenicity, reproductive potential, and reproductive success. IH-C13 and IH-MR8 were more pathogenic and produced more IJs/*Galleria* larva than IH-C3 and IH-MR4, indicating that the color of the cadaver is not related to the pathogenicity and reproductive potential of the nematodes.

Reproductive potential of entomopathogenic nematodes is important to reinfest the habitat (Morris *et al.*, 1990). Our data suggest that IH-C13 and IH-MR8 would reinfest better than IH-C3 or IH-MR4.

Reproductive success was similar among the isolates. There were significant differences among the isolates for each IJs concentration and these differences are consistent with different concentrations of IJs. Conservation of different isolates of the same species is important because they might have different pathogenicity or reproductive potential. Green isolates of *H. bacteriophora* from southern Italy are worth studying further to understand their potential as biological control agents.

RIASSUNTO

Patogenicità, potenziale riproduttivo e successo riproduttivo di ceppi italiani di *Heterorhabditis bacteriophora* Poinar, 1975

I nematodi entomopatogeni del genere *Heterorhabditis* insieme ai batteri simbionti *Photorhabdus*, generalmente conferiscono una colorazione rossa alle larve che infestano. Durante un'indagine sulla presenza di nematodi entomopatogeni in Italia meridionale, sono stati isolati diversi ceppi di *Heterorhabditis bacteriophora*, 3 dei quali coloravano di verde le larve di *Galleria mellonella* infestate.

È stata comparata la patogenicità, il potenziale riproduttivo ed il successo riproduttivo di 2 dei 3 ceppi "verdi" (IH-C3 e IH-C13) con 2 ceppi normali "rossi" (IH-MR4 e IH-MR8) di *H.*

bacteriophora isolati anch'essi nel meridione d'Italia. IH-C13 è risultato il più infettivo (LC_{50} = 9 IJs/larva) e IH-MR4 il meno virulento (LC_{50} = 29 IJs/larva). Le larve di *G. mellonella* infestate con IH-C13 o IH-MR8 hanno prodotto più stadi giovanili infettivi (IJs) (3.8 e 3.9 milioni di IJs/10 larve di *Galleria*, rispettivamente) in confronto alle larve infettate con IH-C3 o IH-MR4 (2.8 e 3.5 milioni IJs/10 larve di *Galleria*, rispettivamente). Il successo riproduttivo è aumentato in relazione all'aumento della concentrazione di infestazione di IJs/larva. IH-C13 e IH-MR8 hanno evidenziato una capacità riproduttiva migliore a basse concentrazioni di IJs rispetto a IH-C3 e IH-MR4.

Parole chiave: biosaggi, nematodi entomopatogeni, ceppi rossi e verdi, eterorabditidi, confronto di patogenicità

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