EFFICACY OF *HETERORHABDITIS BACTERIOPHORA* (STRAIN OLI) IN RELATION TO TEMPERATURE, CONCENTRATION AND ORIGIN OF THE INFECTIVE JUVENILE

M. M. A. de Doucet, M. B. Miranda, M. A. Bertolotti, and K. A. Caro

Centro de Zoología Aplicada, Cátedra de Parasitología, Facultad de Ciencias E. F. y Naturales, Universidad Nacional de Córdoba, CC 122, 5000 Córdoba, Argentina.

ABSTRACT

Doucet, M. M. A. de, M. B. Miranda, M. A. Bertolotti, and K. A. Caro. 1996. Efficacy of *Heterorabditis bacteriophora* (strain OLI) in relation to temperature, concentration and origin of the infective juvenile. Nematropica 26:129-133.

The effects of temperature and inoculum density on the biocontrol efficacy of *Heterorhabditis bacteriophora* were studied. Efficacy of infective juveniles from hermaphroditic females and amphimictic females were compared using *Galleria mellonella* as hosts. The infective juveniles were placed in contact with insects at concentrations of 2, 4, 8, 16 and 32 individuals per host. Temperatures varied between 12°C and 36°C at intervals of 2°C. Temperature, concentration of infective juveniles and origin of juveniles had significant influences ($P \leq 0.01$) on host mortality. Highest mortality was recorded at 26°C. Infective juveniles of the hermaphrodites killed more *G. mellonella* than did those of the amphimictic females.

Key words: Heterorhabditis bacteriophora, infective juveniles, inoculum density, temperature.

RESUMEN

Doucet, M. M. A. de, M. B. Miranda, M. A. Bertolotti y K. A. Caro. 1996. Eficacia de *Heterorabditis bacteriophora* (cepa OLI) en relación a temperatura, concentración y el origen del juvenil infeccioso. Nematrópica 26:129-133.

Se analizó el efecto que ejercen la temperatura y la densidad de inóculo en la infectividad de *Heterorhabditis bacteriophora* así como la agresividad de los juveniles infectivos originados de generaciones hermafrodita y anfimictica. Los juveniles infectivos se pusieron en contacto con una larva de insecto en concentraciones de: 2, 4, 8, 16 y 32 individuos. Las temperaturas evaluadas variaron entre 12°C y 36°C (a intervalos de 2°C). Temperatura, cantidad y tipo de juvenil infectivo influyeron significativamente ($P \leq 0.01$) sobre la mortalidad del huésped. La mayor capacidad infectiva se registró a 26°C; las concentraciones del inóculo compensaron el efecto de la temperatura; los juveniles de la generación hermafrodita se manifestaron más infectivos que aquellos de la anfimíctica.

Palabras clave: capacidad infectiva, *Heterorhabditis bacteriophora,* juveniles infectivos, temperatura.

INTRODUCTION

The efficacy of entomopathogenic nematodes such as *Heterorhabditis bacteriophora* (Poinar) depends partly on the ability of their infective juveniles (IJ) to locate, penetrate and kill the host (Gaugler and Kaya, 1990; Ishibashi and Kondo, 1990). Diverse strains of these nematodes are known to differ in their aggressiveness toward target insects (Doucet *et al.,* 1992b). Additional factors limiting efficacy include temperature, which affects all the vital processes (Molyneux, 1984; Molyneux 1985; Molyneux, 1985; Gaugler, 1988; Kaya, 1990), and the inoculum density available for infecting the host (Doucet *et al.,* 1992b; Griffin and Downes, 1994; Molyneux, 1984).

When *H. bacteriophora* penetrates an insect, all IJ develop into first generation

hermaphrodites despite originating from either hermaphroditic or amphimictic females (Doucet *et al.*, 1996). Individuals that mature in the first generation produced by these hermaphrodites become amphimictic and mate to produce a second generation of IJ. Infective juveniles from either generation are distinguishable based on morphometry (Doucet *et al.*, 1992a) suggesting that they may have different biological characteristics including infectivity. Accurate knowledge of these characteristics is important, because only the hermaphrodites can reproduce in a liquid medium on a large scale (Gaugler and Kaya, 1990; Strauch *et al.*, 1987).

The goals of this work were to assess: 1) if the efficacy of the IJ of *H. bacteriophora* is different according to its generation of origin, and 2) effects of temperature and inoculum density on biological control efficacy of the nematode to *Galleria mellonella* (L.) (Lepidoptera).

MATERIALS AND METHODS

H. bacteriophora (OLI) was obtained from the Laboratory of Nematology at the Center for Applied Zoology, Universidad Nacional de Córdoba, Argentina. This nematode was isolated from Oliva, Córdoba, which is a temperate region. The nematodes were increased and maintained following conventional techniques (Dutky *et al.*, 1964). *G. mellonella,* the host insect used for the experiments, was reared in the laboratory on pollen and wax.

Tests were conducted with insect larvae weighing between 0.13 and 0.20 g. Single insects were placed into Eppendorf tubes containing sand with either 0, 2, 4, 8, 16, or 32 infective juveniles produced from either hermaphroditic (HIJ) or amphimictic (AIJ) females (Doucet *et al.*, 1992b). The tubes were maintained in incubators at 2-degree increments from 12-36°C, and each treatment was replicated 20 times.

The percent mortality of *G. mellonella* was calculated on the third (T1) and fifth (T2) day after inoculation. Results were analyzed separately for each time of observation by multiple logistic regression (Hosmer and Lemeshow, 1989). Only data from those temperatures at which mortality occurred were used for this analysis.

RESULTS AND DISCUSSION

No mortality was observed in any of the control treatments. The regression analysis indicated major effects ($P \leq 0.0001$) of all three independent variables on the mortality of *G. mellonella.*

The optimum temperature at which both HIJ and AIJ killed *G. mellonella* was 26°C (Figs. 1 and 2), which coincides with that reported for other populations of *H. bacteriophora* from temperate regions (Molyneux, 1986; Blackshaw and Newell, 1987). Moreover, at high inoculum densities and with increased time, HIJ killed most of the insects at temperatures between 18-30°C (Figs. 1A and 2A). In contrast to the vaulted shape of the surface responses for HIJ, those for AIJ more sharply peaked at 26°C, even at high inoculum densities. The most noticeable result of these population differences was that HIJ's killed *G. mellonella* more effectively than AIJ's at temperatures above and below optimum (Fig. 3).

The temperature ranges at which IJ's kill insect hosts vary for different species and strains of *Heterorhabditis* (Molyneux, 1984; Molyneux, 1985; Molyneux, 1986; Griffin and Downes, 1994). This study extends these observations to include origin of infective juveniles. Processes such as dispersal, penetration, development of associated bacteria and death of insects occur slowly at low temperatures (Griffin

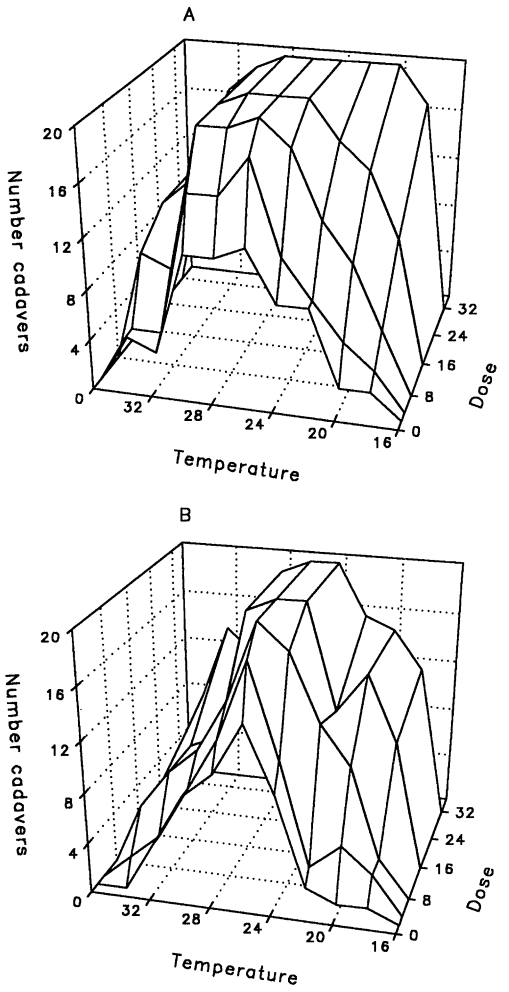

Fig. 1. Mortality of *Galleria mellonella* during 3 days caused by *Heterorhabditis bacteriophora* OLI at different temperatures and inoculum densities. Infective juveniles were from the hermaphroditic generation (A) or the amphimictic generation (B).

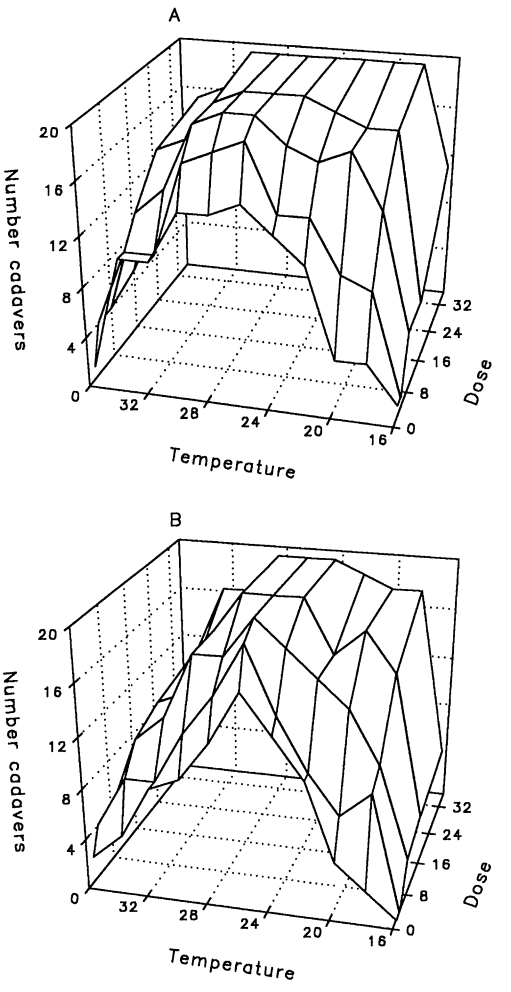

Fig. 2. Mortality of *Galleria mellonella* during 5 days caused by *Heterorhabditis bacteriophora* OLI at different temperatures and inoculum densities. Infective juveniles were from the hermaphroditic generation (A) or the amphimictic generation (B).

and Downes, 1994) and explain the limited ability of HIJ and AIJ to kill hosts at temperatures of 18°C by T1 and 16°C by T2. At the opposite extreme, high temperatures increase nematode respiration (Griffin, 1993) and the rate at which energy reserves are exhausted, thus reducing IJ survival and activity (Molyneux, 1984). Nevertheless, under the highly favorable conditions for infecting insects in the present study, both types of IJ were able to cause some insect mortality at the fairly high temperature of 36°C.

The reasons for morphometric and behavioral differences in IJ of different generations are unknown. The fact that they develop sequentially following infection suggests the possible involvement of

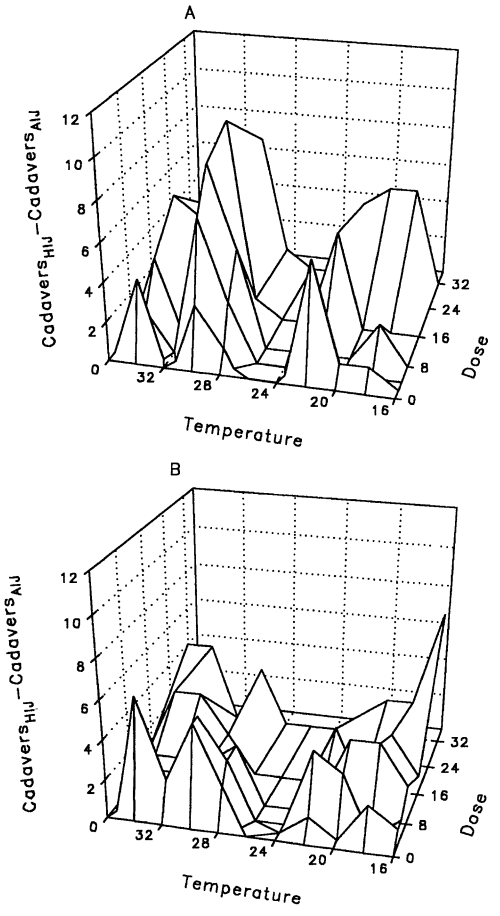

Fig. 3. The difference in mortality of *Galleria mellonella* at different temperatures and inoculum densities caused by *Heterorhabditis bacteriophora* infective juveniles from either the hermaphroditic generation (HIJ), or the amphimictic generation (AIJ) during three (A) or five (B) days post-inoculation.

changes in the media in which they develop (Poinar and Hansen, 1983; Doucet *et al.,* 1992b). Similarly, the changing habitat may influence the interaction between the nematode and its associated bacteria, or the development of the latter within the insect (Boemare and Doucet, 1996).

These results provide new information regarding important behavioral differences in *H. bacteriophora* IJ produced by amphimictic or hermaphroditic parents. Our findings support the continued mass production of these organisms in liquid medium which should favor the production of IJ with the greatest biocontrol potential (Strauch *et al.,* 1994).

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