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# EFFECT OF TEMPERATURE ON THE POTENTIAL OF THREE HETERORHABDITID NEMATODES FROM THE CARIBBEAN BASIN AND NORTH AMERICA AS BIOLOGICAL CONTROL AGENTS OF THE SWEET POTATO WEEVIL, *Cylas formicarius*(Fabricius) (Coleoptera: Apionidae)

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## ABSTRACT

Two heterorhabditid entomopathogenic nematode species from the Caribbean (*Heterorhabditis* sp. D1 - JAM34, *Heterorhabditis* sp. - El Yunque) and one heterorhabditid nematode from North America (*H. bacteriophora*, HP88) were evaluated for their ability to survive in soil at temperatures between 11 and 35 °C and subsequently infect and reproduce in late instar larvae of the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Apionidae). The tolerance of the three nematode species to soil temperature appeared to be related to their climatic origin. One of the species from the Caribbean, *Heterorhabditis* sp. D1 strain (JAM34), was more tolerant of warmer temperatures than the North American nematode *H.bacteriophora* HP88 strain. HP88 was also better adapted to cooler temperatures than JAM34. *Heterorhabditis* sp. El Yunque, a novel species, was isolated from higher elevations (600 m) in Puerto Rico and was least tolerant to test temperatures. These data indicate that interspecific differences may occur among heterorhabditid species in their tolerance to soil temperatures. The importance of these data in developing an integrated pest management strategy for the sweet potato weevil in the Caribbean is discussed.

## INTRODUCTION

Sweet potato, *Ipomoea batatas* Lam., is an important source of food and an income generator for many small farmers within the Caribbean. One of the major constraints of its production is the sweet potato weevil, *Cylas formicarius* (Fabricius) (Coleoptera: Apionidae). Losses as high as 50% of harvested roots have been observed (unpublished data). The cryptic nature of weevil life stages that develop and feed within sweet potato roots and vines has made management of this pest with chemical insecticides ineffective. Moreover, cultural practices which have been recommended for weevil management have not been adopted by farmers as they find these techniques time consuming, labour-intensive and costly. As a result, appreciable losses in yield and revenue continue to occur.

As export markets for sweet potato expand, local farmers are being encouraged to increase their production of sweet potato and to make the transition from small subsistence farming to large producers and/or exporters of the crop. Thus the need exists to develop a

low-input pest management programme which is cost-effective, environmentally safe and suitable for farmer adoption.

Recently, new biological approaches to manage the weevil have been explored, including the use of steinernematid and heterorhabditid entomopathogenic nematodes. In laboratory and field evaluations, these nematodes have suppressed weevil populations and reduced root damage and are therefore considered potential biological control agents for the sweet potato weevil (Jansson et al., 1990; Mannion and Jansson, 1992, 1993).

For entomopathogenic nematodes to be effective at managing weevil infestations, third stage juveniles 'infectives' must move through the soil environment, enter infested roots and infect weevils (Mannion and Jansson, 1992). Within the soil environment infective juveniles are subjected to a wide range of temperatures which affect their efficacy. The tolerance of infective juveniles to temperature varies with species and/or strain (Molyneux, 1985, 1986; Kung et al., 1991) and as such, before field applications are conducted the tolerance of infective juveniles to various temperatures needs to be determined, so that nematode species/strains best suited to withstand the soil conditions at the site of introduction are selected. This study was therefore undertaken to determine the short term survival of two heterorhabditid nematodes from the Caribbean Basin and one heterorhabditid from North America in soil at various temperatures and their subsequent infectivity and reproduction in sweet potato weevil larvae.

## MATERIALS AND METHODS

The selection of the three heterorhabditid nematodes for this study was based on their climatic origins and species characterization (Table 1). Nematodes were reared on greater wax moth, *Galleria mellonella* (L.), larvae as described by Woodring and Kaya (1988). Infective juveniles less than 10 days old were used in all experiments.

Table 1 Sources of heterorhabditid nematodes

| Climatic origin | Neotropical | Neotropical | Temperate |
|---|---|---|---|
| Species | *Heterorhabditis* sp. D1 | *Heterorhabdits* sp. D1 | *Heterorhabdits bacteriophora* |
| Species group | I | III | |
| Isolate/strain | JAM34[a] | E1 Yunque[a] | HP88[b] |
| Origin | White Horses, Jamaica | Palmer, Puerto Rico | Utah, USA (Poinar, 1991) |
| EE[c] | 0.54 | 0.47 | 0.73 |

[a]Nematodes were isolated from soil baited with *Galleria mellonella* larvae

[b]Orginally obtained from a commercial source (Biosys Inc. Palo alto, CA)

[c]EE, mean extraction efficiency; total number nematodes recovered/total number nematodes applied (Kung *et al.*, 1990)

Sweet potato weevils were originally obtained from a laboratory colony at the University of Florida, Tropical Research and Education Center (TREC), Homestead. Weevils were reared on 'Jewel' sweet potato as described by Jansson and Hunsberger (1991).

This experiment modified the methods of Kung et al. (1991). Polyvinyl chloride (PVC) pipe sections (4 cm diam. x 5 cm length) were filled with 50 g of gravelly loam soil which was sieved (2 mm mesh sieve), dried at 120 °C for 24 h and moistened with 1.5 mL of sterilized water. PVC pipe sections were sealed with parafilm and placed in humidity chambers to minimize water loss. Plastic boxes (16.5 x 31 x 8.5 cm) filled to a depth of 2.5 cm with deionized water served as humidity chambers. Soil-filled pipe sections were placed 1.5 cm above the water on a hardware cloth table (15 x 29 cm) within the plastic boxes. Humidity chambers were sealed and placed in environmental chambers at 11, 18, 26, 30, and 35 ± 2 °C and 60% R.H. for 24 h to equilibrate. Boxes were then removed and 3,200 infective juveniles suspended in 1.5 mL of distilled water added to each pipe section; pipe sections were resealed with parafilm and returned to their respective chambers. Infective juvenile survival, infectivity, and reproductive potential were determined after 1, 2, 4, and 8 wk. Each nematode x temperature x time combination was replicated six times.

A centrifugal flotation technique was used to extract nematodes from the soil (Kung et al., 1990). Soil from each plastic pipe section was placed in a 1000-mL beaker and filled with 700 mL of deionized water. The soil suspension was allowed to settle for 10 min, rinsed once through a series of mesh sieves (25, 150, 300 µm openings) and the filtrate collected in six 15-mL centrifuge tubes. Tubes were spun at 4,800 rpm for 10 min and the supernatant discarded. Ten millilitres of a sucrose solution (453 g/L distilled water) was added to each centrifuge tube, vortexed for 1 min, and recentrifuged at 3,840 rpm for 5 min. The supernatant from each tube was poured into a 100-mL graduated cylinder and diluted with distilled water; a 2-mL aliquot was removed and further diluted with distilled water. The numbers of viable infective juveniles within the suspension were then counted.

The extraction efficiency of each nematode was determined by comparing the total number of nematodes recovered from a sample to the number of nematodes applied (Table 1). For each nematode, six extractions were conducted to determine efficiency. Percentage survival of each nematode was calculated by the following equation (Kung et al., 1991):

$$\frac{\text{Number of viable nematodes extracted}}{\text{Number of nematodes inoculated x mean extraction efficiency}}$$

Nematode-inoculated soil was removed from three PVC pipes and poured into Petri plates (9cm diam). Nematodes were reconstituted by adding 3 mL of deionized water to each plate and then incubated at 26 °C. After 24 h, the soil was mixed and 20 late instars of sweet potato weevil placed in the centre of each dish. In the remaining three soil samples, eight G. mellonella larvae were added to the plates to confirm the presence of viable infective juveniles. Dishes were covered and incubated for 7 days in the dark at 26 °C and 60%

RH. Nematode-killed larvae were determined by the cadaver colours brick-red, brown, green-brown, and green.

Nematode-infected weevil cadavers were removed from the soil and placed in modified White traps (Mannion and Jansson, 1992). Twenty days after larvae were introduced into modified White traps the numbers of cadavers producing progeny and the numbers of infective juveniles produced per cadaver were recorded. For each nematode species, reproductive potential was determined by counting the progeny from no more than 20 nematode-killed cadavers randomly selected from the infectivity samples. If the the number of nematode-killed cadavers was less than 20 then progeny from all cadavers wascounted. Sample sizes ranged between 3 and 20 cadavers.

All data sets were analyzed by analysis of variance (ANOVA) (Sokal and Rolf, 1981) and means were separated by Tukey's Studentized Range Test (Tukey, 1953).

## RESULTS

In the absence of an insect host, there was a significant interaction between temperature and incubation time for all heterorhabditid nematodes ($F = 4.95$; $df = 24$; $P \leq 0.0001$). In general, significant differences were observed among the nematodes in their abilities to withstand temperature conditions ($F \geq 5.69$; $df = 2,15$; $P \leq 0.05$) (Figure 1).

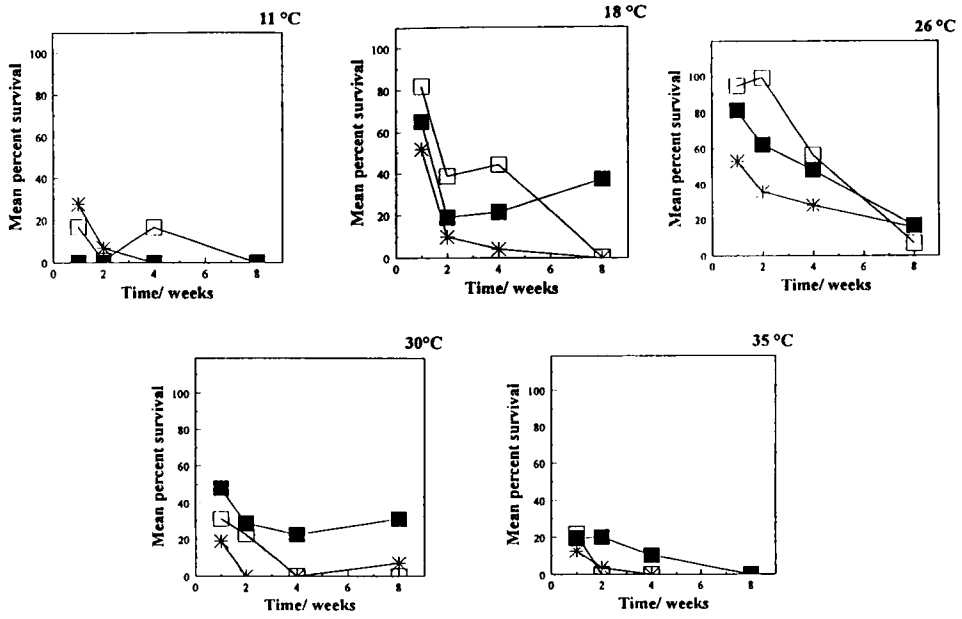


Figure 1 The effect of temperature on the survival of *Heterorhabditis* sp. D1 JAM34 (■), *Heterorhabditis* sp. El Yunque (*), and *H. bacteriophora* HP88 (□)

The numbers of infective juveniles surviving declined over time for all nematodes. JAM34 achieved optimal survival at 26 °C followed by 18 and 30 °C. Poor survival was recorded at 35 °C; the cool temperature of 11°C was lethal to infectives. In contrast, HP88 survived best at 26 °C followed by 18°C; poor survival occurred at the extreme temperatures, 11, 30, and 35 °C. Percentage survival of El Yunque was lower than that for HP88 and JAM34, and never exceeded 53%; survival of El Yunque was also highest at 26 °C. Incubation of El Yunque at temperatures of 11, 30, and 35 °C was lethal to infectives after 2 weeks.

Infectivity of the three heterorhabditid nematodes to the sweetpotato weevil was significantly affected by temperature and time interactions ($F = 3.12$; $df = 24$; $P \leq 0.0001$) and closely followed the trends observed for survival (Figure 2). Within most temperature/time regimes, significant differences in the infectivity were observed among nematodes ($F \geq 8.21$; $df = 2,6$; $P \leq 0.05$). JAM34 infected most weevil larvae at 26 °C followed by 18, 30 and 35 °C; no infection occurred at 11 °C. HP88 and El Yunque were most infectious at 26 and 18 °C and least infectious at 30 and 35 °C. A temperature of 11 °C was lethal to El Yunque and no infection occurred. However, considerable infection by HP88 occurred at 11 °C. At 18 °C, HP88 was more infectious than El Yunque. Similar trends in parasitism were observed between sweet potato weevil larvae and wax moth larvae.

Reproduction occurred over the same temperature range as infectivity; however, not all nematode-infected sweet potato weevil cadavers produced infectives. For all the nematodes, no clear trends in the percentage cadavers producing infectives were observed at the test conditions (Table 2). Temperature significantly affected the number of progeny produced within weevil cadavers ($F = 13.37$; $df = 5$; $P \leq 0.0001$). At a few test conditions, there were significant differences in the numbers of infectives produced among the nematodes ($F \geq 4.53$; $P \leq 0.05$); however, no clear trends were observed in the numbers of infectives produced under each temperature/time regime. Progeny production within sweet potato weevil cadavers ranged from 8 to 7,790, 7 to 9,003, and 7 to 10,008 infectives for El Yunque, HP88, and JAM34, respectively.

## DISCUSSION

Infective juveniles rely on their food reserves to provide energy to seek and infect hosts and subsequently reproduce within host cadavers. Temperature influences the motility of infectives and consequently the rate at which their food reserves are utilized (Molyneux, 1985). Many nematodes, in order to survive changes in environmental temperatures adjust their metabolic activities by entering a state of dormancy or modifying their behaviour (Lee and Atkinson, 1977; Ishibashi and Kondo, 1990). Thus, differential tolerance of heterorhabditid species JAM34, El Yunque and HP88 to soil temperatures of 11 and >30 °C may have been due to differences in strategies employed by infectives to survive. The poor survival of the nematodes at high temperatures may have been due, in part, to the increased motility and respiration of infective juveniles resulting in the rapid depletion of their food reserves (Burman and Pye, 1980; Kaya, 1990 and references therein). Moreover, at high

temperatures (35 °C), the soil moisture content may have been reduced resulting in desiccation and death of infective juveniles.

Several studies have shown that moisture is critical for infective juvenile survival and infectivity (Schmidge, 1963; Molyneux and Bedding, 1984).

Differential progeny production under different temperature conditions may have been due in part to the growth rate of the bacterial symbiont, *Photorhabdus luminescens*. Wright (1992) investigated reproduction of steinernematids and heterorhabditids at cool temperatures and found that their ability to reproduce was related to the growth rate of the bacterial symbiont.

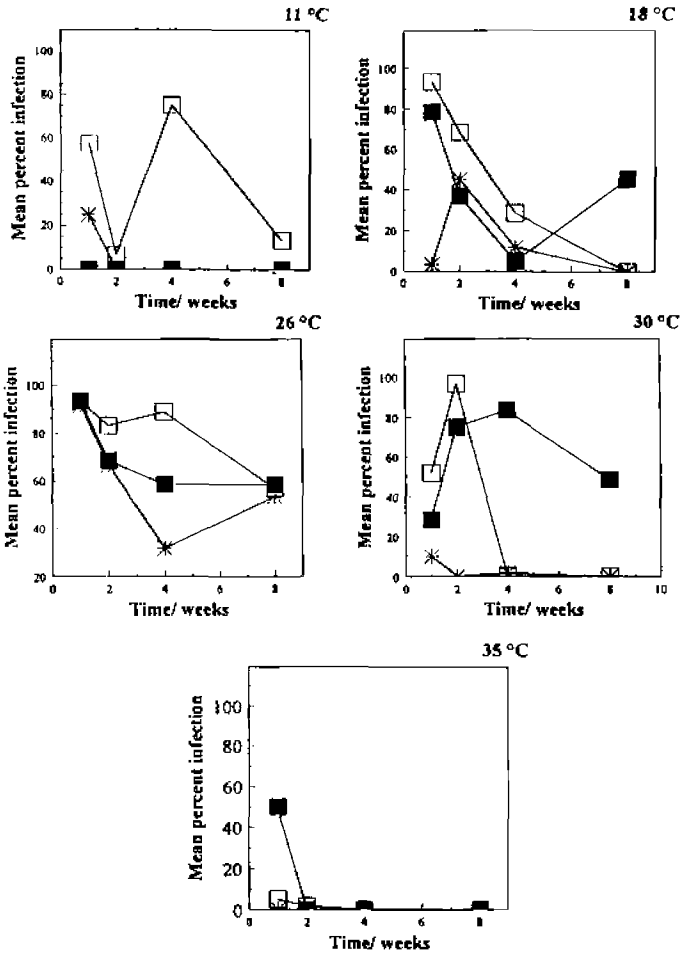


Figure 1 The effect of temperature on infectivity the of *Heterorhabditis* sp. D1 JAM34 (■), *Heterorhabditis* sp. El Yunque (*), and *H. bacteriophora* HP88 (□) to late instar sweetpotato weevil, *C. formicarius*

Table 2 Percentage nematode-killed sweetpotato weevil, Cylas formicarius, larval cadavers producing infectives under various incubation conditions

| Temperature (°C) | Time (weeks) | Nematode-killed cadavers producing infectives HP881 | El Yunque | JAM34 |
|---|---|---|---|---|
| 11 | 1 | 89.1 | 59.2 | * |
| | 2 | 100.0 | * | * |
| | 4 | 88.9 | * | * |
| | 8 | 87.5 | * | * |
| 18 | 1 | 80.3 | 100.0 | 82.2 |
| | 2 | 36.5 | 92.6 | 68.1 |
| | 4 | 100.0 | 100.0 | 100.0 |
| | 8 | * | * | 74.0 |
| 26 | 1 | 62.5 | 70.9 | 91.2 |
| | 2 | 77.0 | 100.0 | 66.6 |
| | 4 | 92.6 | 100.0 | 39.6 |
| | 8 | 61.8 | 87.5 | 72.0 |
| 30 | 1 | 83.8 | 66.7 | 94.1 |
| | 2 | 82.7 | * | 42.2 |
| | 4 | * | * | 36.0 |
| | 8 | * | * | 62.0 |
| 35 | 1 | 100.0 | * | 86.7 |
| | 2 | * | * | * |
| | 4 | * | * | * |
| | 8 | * | * | * |

1 HP88, Heterorhabditis bacteriophora; El Yunque, Heterorhabditis sp. D1; JAM34, *Heterorhabditis sp. D1*

* no nematode-killed cadavers were recorded under incubation conditions

Moreover, temperature may have affected the bacterial phase present within weevil cadavers and concomitantly affected infective juvenile production. A further possible source of variation in infective juvenile production may have been thermal damage to the reproductive organs of the nematode vector and/or the bacterial symbiont at the highest temperatures. Kaya (1977) reported that *S.carpocapsae* developed but did not reproduce when cultured at 30 °C. He suggested that the lack of reproduction may have been due to the lack of viable sperm and ova in the nematode.

Differential tolerance of nematodes to environmental temperatures has been related to their climatic origin or habitat (Molyneux, 1985; Kung et al., 1991). The temperature-related differences in survival and infectivity among the three heterorhabditid species may therefore be due, in part, to adaptations to the climatic conditions of the habitats of these nematodes (Molyneux, 1985). The warm climatic conditions of the Caribbean probably influenced the higher levels of survival and subsequent infectivity, and reproduction of JAM34 at warm temperatures. HP88, which was isolated from a cooler region (Utah, USA) may have been better adapted to survive, infect, and reproduce at cooler temperatures. Although El Yunque was isolated from the tropics, it was poorly adapted to withstand the warmer temperatures (30 and 35 °C). JAM34 was isolated adjacent to a beach and El Yunque was isolated at approximately 600 m elevation. These observations might suggest that El Yunque occupied a niche which had a cooler temperature range than JAM34.

Consistent with earlier studies (Jansson et al., 1990; Mannion and Jansson, 1992, 1993), *H. bacteriophora* HP88 is a potential biological control agent for the sweet potato weevil. *Heterorhabditis* sp. D1 isolate JAM34 also appears to be a potential biological control agent for this pest. The deleterious effects of low temperatures on infectives of *Heterorhabditis* sp. D1 (JAM34) suggest that this nematode should not be introduced in locales experiencing low temperatures. In contrast, the tolerance of HP88 to low temperatures and partial tolerance to warm temperatures suggests that this nematode may be effective in areas experiencing cool and moderately warm temperatures. The short term and low levels of persistence of El Yunque coupled with its reduced virulence observed in an earlier study suggest that El Yunque would not be a good biological control agent of *C. formicarius*.

The results of the present study suggest that *Heterorhabditis* sp. D1, JAM34 should be considered as a potential biological control tactic in designing an integrated pest management (IPM) programme for the sweet potato weevil within the Caribbean. However, for the full potential of JAM34 to be realized in an IPM programme, investigations of the interactions of temperature with other abiotic factors (moisture, pH, chemistry of the soil solution, soil texture) which affect nematode persistence should be conducted (Kaya, 1990 and references therein). Moreover, the compatibility of these nematodes with other control tactics currently being employed (e.g. cultural practices, female weevil sex pheromone, chemical treatment of planting materials) should be evaluated so that a combination of tactics which effectively reduce weevil populations below an economic injury level can be selected.

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