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Infectivity comparison between "red" and "green" isolates of *Heterorhabditis bacteriophora* Poinar 1976 (Rhabditida: Nematoda) in relation to temperature*

ABSTRACT

A study was conducted to evaluate the effects of temperature on the infective efficacy of 3 isolates of *Heterorhabditis bacteriophora* (IH-C3, IH-C6, IH-C13) associated with green strains of *Photorhabdus luminescens* (infected larvae of *Galleria mellonella* L. turn green) in comparison with 3 isolates of *H. bacteriophora* (IH-ME2, IH-LU1, IH-CE1) associated with red strains of *Photorhabdus luminescens* (infected larvae turn red).

The percentage of larval mortality was recorded after a 72-h exposure period to the infective juvenile nematodes (Ijs) at various temperatures between 10°C and 35°C, at intervals of 5°C.

For all nematode strains the highest larval mortality was recorded at 25-30°C. The data showed that temperature had a significant influence on host mortality for all *H. bacteriophora* isolates tested. Strain IH-CE1 showed the highest larval mortality percentage for the range 20-35°C.

There were no significant differences among red and green isolates at the same temperatures except for one case: at 20°C the red isolates were more virulent than green ones.

Key words: entomopathogenic nematodes, pine forest, olive-grove, maize field, bioassay.

INTRODUCTION

Entomopathogenic nematodes of the genera *Steinernema* (Steinernematidae: Rhabditida) and *Heterorhabditis* (Heterorhabditidae: Rhabditida) are obligate and lethal parasites of insects (POINAR, 1990). Their infective juveniles (IJs), usually soil dwelling, penetrate an insect host through natural openings (mouth, anus and spiracles), or through the integument in the case of *Heterorhabditis,* and release symbiotic bacteria, held in their foregut, which ultimately kill the insect from septicemia (POINAR, 1979).

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° The first author has planed the research. The second author has collaborated in the gathering, breeding and identification of the species of nematodes. Both authors have collaborated on processing the data and in layout of the paper.

During the last 3 years a survey was conducted on the occurrence of Steinernematid and Heterorhabditid entomopathogenic nematodes in samples of soil collected from cultivated and uncultivated habitats in Southern Italy (TARASCO & TRIGGIANI, 1997).

Among the Heterorhabditids we have, so far, collected 11 isolates of *Heterorhabditis bacteriophora* Poinar 1976; 3 of them were identified as *H. bacteriophora* with green strains of *Photorhabdus luminescens* (Enterobacteriacae) (Poinar, pers. com.); the *Galleria mellonella* L. (Lepidoptera: Galleriidae) larvae infected with these *Heterorhabditis* turn green (green *H. bacteriophora* isolates). Green were isolated also in Ireland (Griffin, pers. com.) and mention about this *P. luminescens* was reported by KAYA (1997).

The aim of this study was to evaluate the pathogenic behaviour of different Italian *H. bacteriophora* isolates in relation to temperature by compareing the infective efficacy of the green isolates with 3 red isolates.

MATERIALS AND METHODS

The *H. bacteriophora* isolates were collected from different biotopes (tab.1).

Tab. 1 - Characteristics of the sites with *Heterorhabditis* strains collected in Apulia Region

Nematode	Locality	Altitude (m a.s.l.)	Time	Habitat	Soil texture	pH	Strains
H. bacteriophora	Castellaneta (TA)	0	IX - 1996	pine forest	sand	7.6	IH-C3
H. bacteriophora	Castellaneta (TA)	50	IX - 1996	olive	sand	7.7	IH-C13
H. bacteriophora	Castellaneta (TA)	50	IX - 1996	maize field	clay	8.0	IH-C6
H. bacteriophora	Mellitto (BA)	350	IX - 1996	olive	silt	9.4	IH-ME2
H. bacteriophora	Cerignola (FG)	120	X - 1996	artichoke	loamy sand	8.0	IH-CE1
H. bacteriophora	Lucera (FG)	70	X - 1996	uncultivated land	clay	7.9	IH-LU1

The 3 green isolates were collected in the same area from a pine forest heavy infested with *Thaumetopoea pityocampa* (Den. et Schiff.) (Lepidoptera: Thaumetopoeidae) is (IH-C3), olive-grove (IH-C13) and maize field (IH-C6) habitats, while the red isolates (fig. 1) were collected in different areas of Apulia; the 3 red isolates used for the bioassay, IH-ME2, IH-CE1, IH-LU1, were obtained respectively from an olive-grove, artichoke field and uncultivated land habitats.

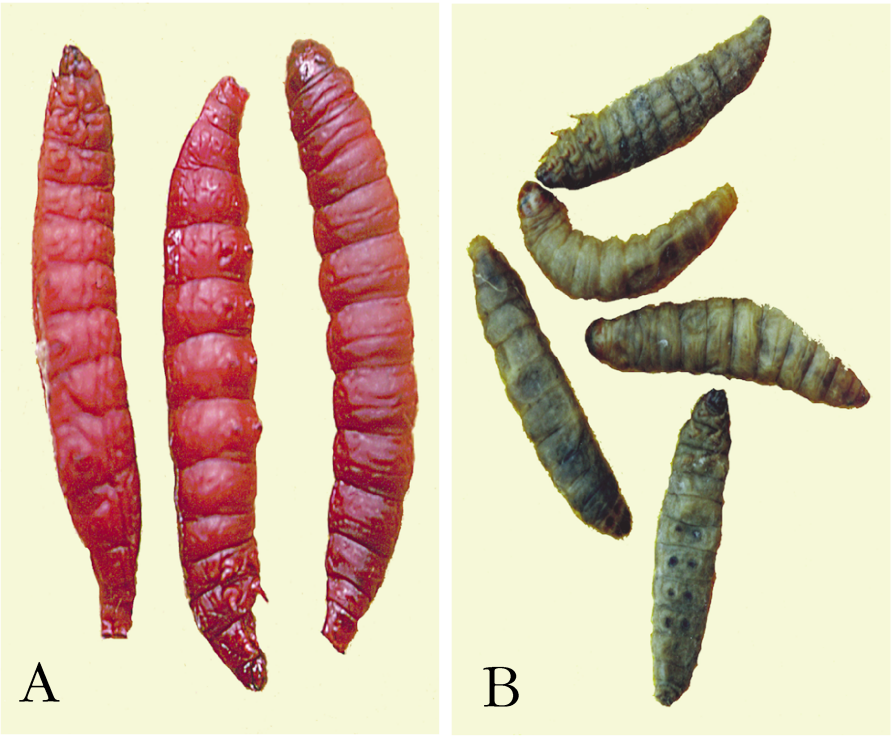


Fig. 1 - Last instars of *Galleria mellonella* infected with: A- red isolates of *Heterorhabditis bacteriophora*; B- green isolates of *Heterorhabditis bacteriophora*.

The nematodes were cultured in the laboratory in late instar larvae of *G. mellonella* at 24 °C and IJs were harvested from dead *Galleria* larvae using the White traps (WHITE, 1927). The IJs were used for experimental purposes within two weeks of harvesting.

The infectivity of *H. bacteriophora* isolates was compared in a laboratory bioassay against *G. mellonella* larvae. Infectivity was determined by larval mortality rate assay.

For each *H. bacteriophora* isolate the IJs were placed in contact with *G. mellonella* larvae at the same concentration: one milliliter of distilled water containing approximately 1000 IJs was applied in small plastic boxes (3,2 cm high x 9,5 cm inner diameter) each holding 40 g of sterilized peat (75% degree of humidity) and 10 *G. mellonella* final instar larvae; there were 3 replicates for each treatment and 3 boxes without nematodes served as control. Temperatures ranged between 10°C and 35°C at intervals of 5°C. The

bioassay was repeated 3 times. The percentage of larval mortality was recorded after 72-h exposure to IJs.

A general linear model procedure and Tukey's multiple range test (SAS Institute, 1987) was performed to analyze the virulence data. An arcsin transformation of the square root was used on data presented in percentages. All comparison were made at the 0.05 level of significance.

RESULTS

No mortality was observed in any of the control treatments. For all *H. bacteriophora* isolates tested, the data showed the limited ability of all IJs to kill hosts below a temperature of 20°C. The highest larval mortality was recorded at 25-30°C and only the IH-CE1 (a red isolate) showed good results at 20°C (graf. 1, graf. 2).

The development of associated bacteria and death of insects occured generally in a similar way and there were no significant differences ($P>0.05$) among the isolates at 10 and 15°C; while significant differences ($P<0.05$) were found at 20-25-30-35°C (graf. 1, graf. 2).

At low temeperatures (10-15°C) pathogenic activity occurs slowly; very low larval mortality was found at 10°C for IH-C3 (10%) and at 15°C for IH-C3, IH-CE1 (14%), IH-ME2 (10%) and IH-C6 (6%) (graf. 3).

At 20-25°C, IH-C6 showed a percentage of larval mortality significantly lower (36,7% and 90% respectively) in comparison with the other isolates (graf. 3, graf. 4).

A relatively lower percentage of larval mortality occurred at 30°C for IH-C3 (90%) (graf. 4).

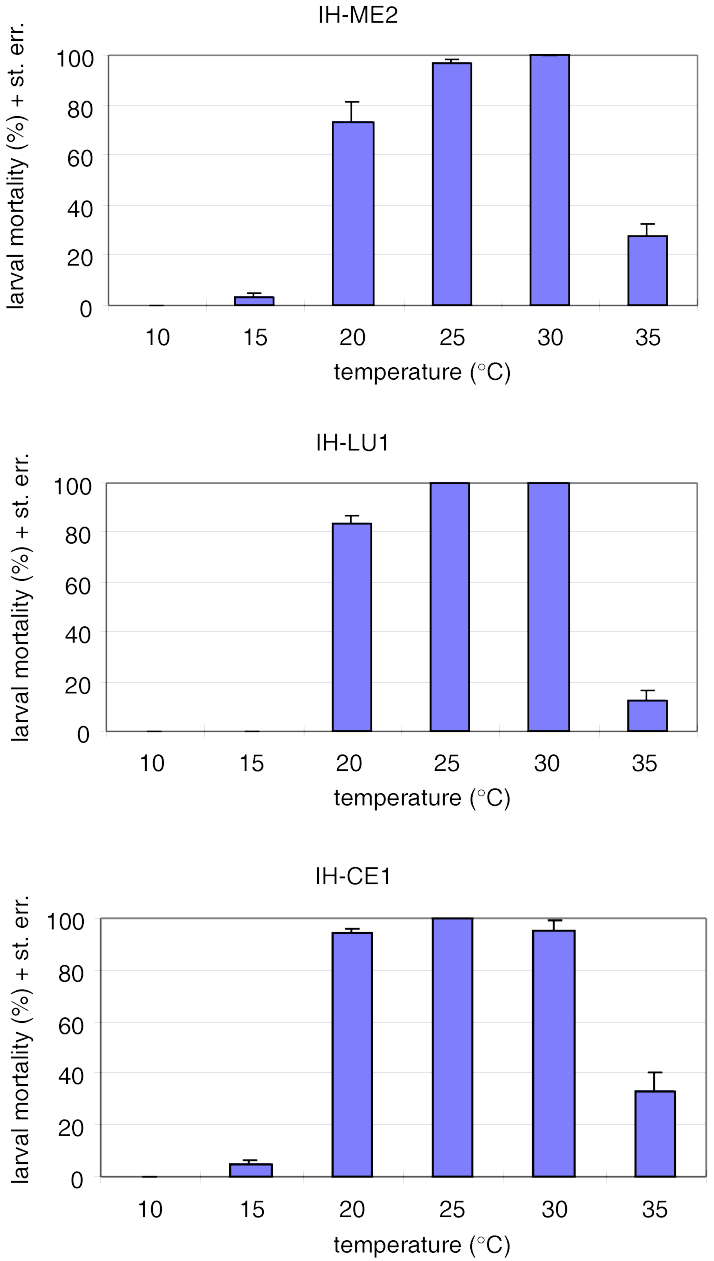
The increase of temperature (35°C) caused a reduction of virulence in all the italian *Heterorhabditis* tested, as pointed out in graf. 4.

DISCUSSION AND CONCLUSIONS

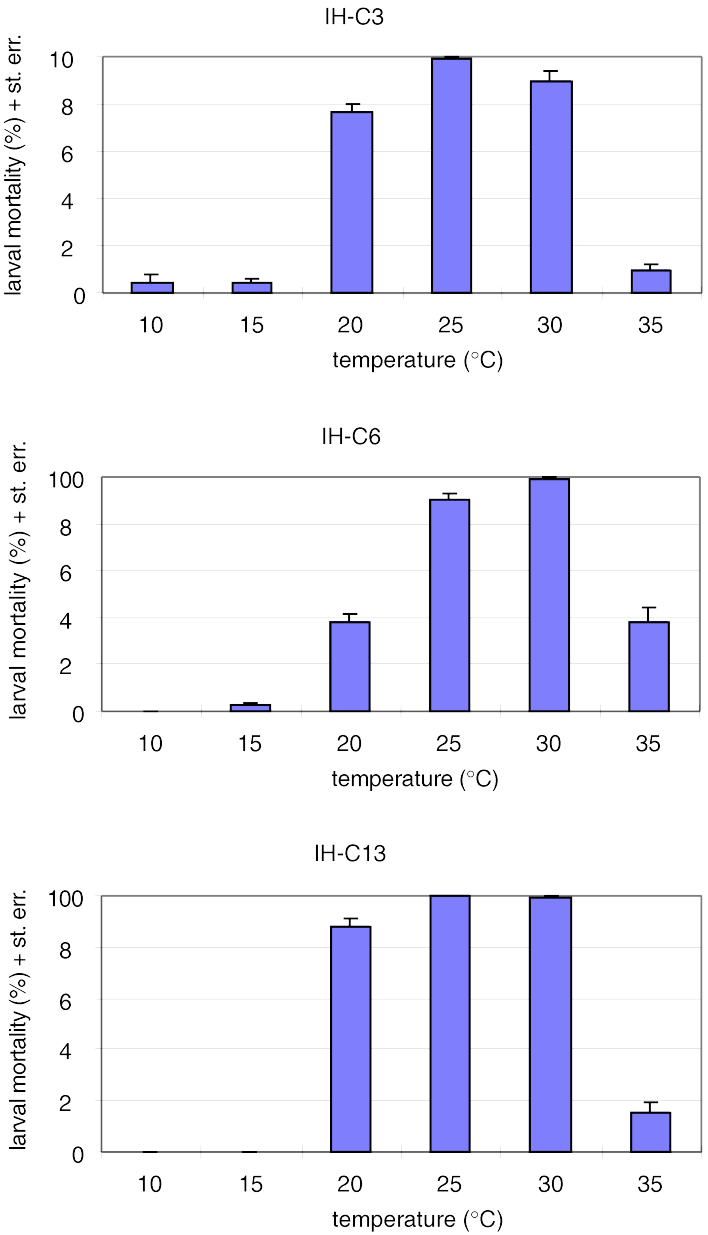
Our results indicate that 25-30°C is the optimum temperature range for the activity of all red and green isolates. These results are similar to those reported for other populations of *H. bacteriophora* from temperate regions (Molyneux, 1986; Blackshaw and Newell, 1987; Doucet *et al.*, 1996).

High (>30°C) and low temperatures (<20°C) caused a reduction of nematode infectivity and this reduction seems to be more evident in green *H. bacteriophora*, above all at high temperatures.

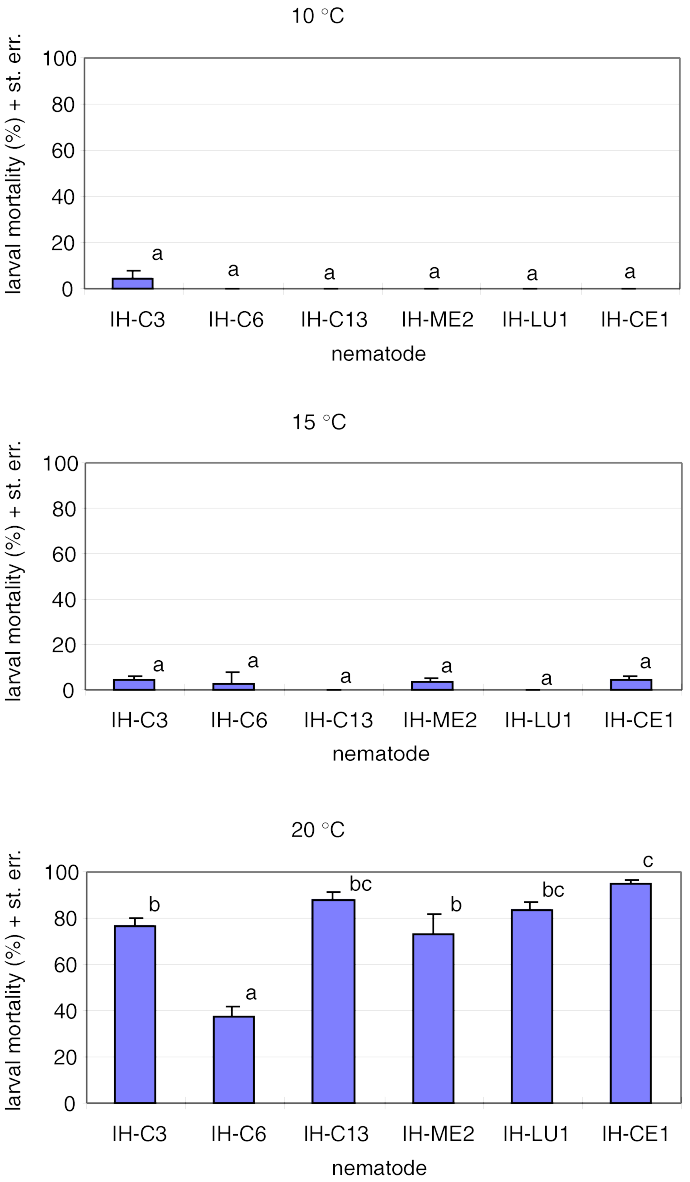
Among red and green isolates there were no significant differences at the same temperature values. The only significant difference was found at 20 °C



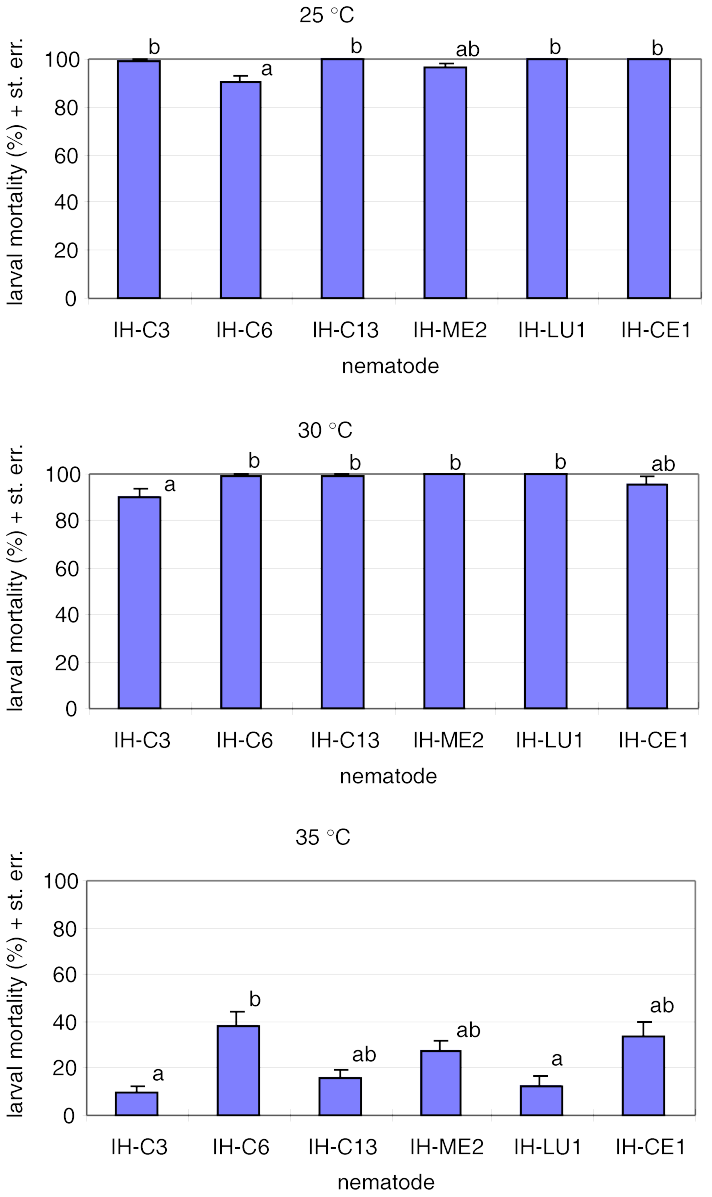
Graf. 1 - Larval mortality rate assay: percentage mortality of *Galleria mellonella* larvae following 72 h of exposure to individual IJs of red (IH-ME2, IH-LU1, IH-CE1) Italian isolates of *Heterorhabditis bacteriophora*, at 6 different temperature values.



Graf. 2 - Larval mortality rate assay: percentage mortality of *Galleria mellonella* larvae following 72 h of exposure to individual IJs of green (IH-C3, IH-C13, IH-C6) Italian isolates of *Heterorhabditis bacteriophora*, at 6 different temperature values.



Graf. 3 - Larval mortality rate assay: comparison at the same temperature (10-15-20°C) of percentage mortality of *Galleria mellonella* larvae among green (IH-C3, IH-C6, IH-C13) and red (IH-ME2, IH-LU1, IH-CE1) Italian isolates of *Heterorhabditis bacteriophora*. Means with the same letter are not significantly different ($P<0.05$, Tukey's multiple range test).



Graf. 4 - Larval mortality rate assay: comparison at the same temperature (25-30-35°C) of percentage mortality of *Galleria mellonella* larvae among green (IH-C3, IH-C6, IH-C13) and red (IH-ME2, IH-LU1, IH-CE1) Italian isolates of *Heterorhabditis bacteriophora*. Means with the same letter are not significantly different ($P<0.05$, Tukey's multiple range test).

($P<0.05$): regarding the infective efficacy the red isolates showed a better performance than green ones.

A noticeable result of these populations was also that red IJs killed *G. mellonella* larvae more effectively then green ones at high temperatures (above the optimum 25-30°C).

It is important to point out that the coloring of the infected *Galleria* was not influenced by temperature variation. The different temperatures only influence the intensity of coloration.

The attempt to examine behavioral differences between red and green Italian *H. bacteriophora* populations by using *Galleria* larval mortality rate assays has provided some useful information on infectivity behavior.

Further investigations are required to characterize other biological aspects of Italian heterorhabditids and understand their ecological role in controlling insect populations.

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RIASSUNTO

Confronto di patogenicità tra ceppi "rossi" e "verdi" di *Heterorhabditis bacteriophora* Poinar 1976 (Rhabditida: Nematoda) in relazione alla temperatura.

È stata valutata la patogenicità di 3 isolati Italiani di *Heterorhabditis bacteriophora* (IH-C3, IH-C6, IH-C13), il cui batterio simbionte *Photorhabdus luminescens* fa diventare le larve test di colore verde, con 3 isolati di *H. bacteriophora* (IH-ME2, IH-LU1, IH-CE1) che colorano le larve infestate di rosso.

I dati sulla mortalità sono stati rilevati 72 ore dopo aver sottoposto larve di *Galleria mellonella* ad una sospensione acquosa di 1000 Ijs/ml. a 10-15-20-25-30-35 °C.

Per tutti gli isolati il tasso di mortalità più elevato si è verificato tra i 25 e i 30°C. IH-CE1 ha ucciso un maggior numero di larve di *G. mellonella* tra i 20 e i 35°C. Alle diverse temperature testate non si sono avute differenze significative tra gli "isolati rossi" e quelli "verdi"; soltanto a 20°C gli "isolati rossi" hanno mostrato una maggiore patogenicità nei confronti con gli " isolati verdi".

Parole chiave: nematodi entomopatogeni, pineta, oliveto, campo di mais, test di patogenicità.

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