Persistence and foraging behaviour of heat tolerant *Heterorhabditis bacteriophora* strain in soil

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Summary. Entomopathogenic nematodes (EPN), *Heterorhabditis* and *Steinernema* genera, have considerable biological control potential against soil-inhabiting insect pests. One of the most important factors for sustainable and successful biological control is their heat tolerance in released areas. To determine persistence abilities and foraging behaviour characteristics of infective juveniles (IJ) of *H. bacteriophora* strains having tolerance to heat, one tolerant *H. bacteriophora* strain (HIZ) was compared with a non-tolerant strain (Hb11). The strain having more than 80% survival capability at the temperatures of 40ºC was used as the tolerant strain; the other strain with less than 30% survival rate at 40ºC was evaluated as the non-tolerant strain. An outdoor experiment to evaluate persistence was carried out for 4 months. Vertical PVC columns filled with soil were used to detect foraging behaviour of the strains. The results showed that there are significant differences of persisted IJ numbers (except for one sampling) and foraging behaviour between heat tolerant and non-tolerant strains. According to the results, the tolerant strain was more persistent in soil and had better foraging behaviour than the non-tolerant strain. This study also revealed that the heat toleration ability of *H. bacteriophora* was positively linked to its persistence and foraging behaviour characteristics.

Key words: Biological control, entomopathogenic nematode, host seeking, temperature, survival.

Entomopathogenic nematodes (EPN) of the families Steinernematidae and Heterorhabditidae are safe biological control agents as a bioinsecticide for several economically important insect pests (Ehlers & Peters, 1995; Ehlers & Hokkanen, 1996; Ehlers *et al*., 2006). EPN can be used to control soil dwelling insects in particular and have substantial advantages over chemical insecticides as they are environmentally benign. *Heterorhabditis bacteriophora* Poinar, 1975 (Rhabditida: Heterorhabditidae) has been used against many insect pests on orchards, vegetables and nurseries. The species can be produced in large scale liquid culture (Ehlers, 2001), and has recently been sequenced (Ciche, 2007). One of the most important stresses the species has to overcome is heat in the soil. Temperatures above 40ºC in areas where the EPN is released destroy crucial proteins of some EPN (Griffin, 1993; Grewal *et al*., 1994). However, infective juveniles (IJ) have more tolerance to lower than higher temperatures (Kaya, 1990). The temperature tolerance of the IJ depends on their geographical origins; moreover, extreme temperatures are generally unfavourable for the persistence in soil (Kaya, 1990). EPN from warmer areas survive better at higher temperatures, whilst nematodes from cooler regions survive better at cooler temperatures (Kaya, 1990; Curran, 1993). This feature of EPN, especially strains of *H. bacteriophora*, has been used to obtain hybrid tolerant strains by crossing strains and selective breeding of tolerant strains (Mukuka *et al*., 2010a). Heritability of the heat tolerance trait is relatively high in *H. bacteriophora* (Glazer *et al*., 1991; Ehlers *et al*., 2005; Mukuka *et al*., 2010b, c). In addition to heat tolerance capabilities, persistence and foraging behaviour are especially important for sustainable *H. bacteriophora* application in field conditions. In field crops, EPN usually persist not much longer than 1 year, whereas a longer persistence of 23 months was detected for *H. bacteriophora* after release in beans followed by wheat as cover crop over red clover and pasture (Susurluk & Ehlers, 2008). The foraging behaviour of *H. bacteriophora* plays a key role in effective biological control (Csontos, 2002; Susurluk *et al*., 2003). The control

potential of *H. bacteriophora* is increased if it can efficiently search into deeper of the soil after application onto the soil surface.

The aim of the present study was to compare persistence and foraging behaviour capabilities of one heat tolerant strain and one non-heat tolerant strain of *H. bacteriophora* isolated from different climatic conditions.

MATERIALS AND METHODS

***Heterorhabditis bacteriophora* strains, insect and soil.** Nematode strains used in the experiment were HIZ (Heterorhabditid from Izmir) isolated from Izmir and Hb11 (11th isolate) from Erzurum. Izmir has a warm climate and 80% of the HIZ strain survived at 40°C (unpublished data) and, thus, is designated as heat tolerant. Erzurum is a cooler region in Turkey and the Hb11 is not heat tolerant. Survival rate of the non-tolerant strain was less than 30% at 40°C (unpublished data). Both strains were identified by PCR-RFLP.

Greater wax moth larvae, *Galleria mellonella* L. (Lepidoptera: Pyralidae), were reared in 1500 ml volume glass containers at 30±2°C on artificial medium according to Wiesner (1993). The weight of larvae used in the experiment was 250±30 mg. IJ of the strains produced *in vivo* on *G. mellonella* were used for persistence and foraging behaviour experiments. Both experiments were carried out in clay-loam soil (35% sand, 39% silt, and 26% clay).

Persistence experimental design. As the aim was to detect persistence directly in the soil, the experimental plots had no crops or weeds. In addition, insects were not observed on any plots during the experimental period. Experimental plots were separated by gaps of 50 cm. Application of EPN against soil dwelling insect pests in the field is generally made in March or April in Turkey. Thus, in the first half of April, *H. bacteriophora* was applied onto soil at a dose of 0.5 million IJ in 0.5 l water m^{-2} (e-nema GmbH-Germany, unpublished data). Then, each plot was watered with 5 l tap water immediately after nematode application. The experiment was arranged in a random plot design (2 × 5 m) with three replications for each strain. The experiment was repeated the following year in different plots on other side of the same field.

In order to detect naturally occurring EPN in the experimental area, ten soil samples from each plot were collected prior to application of *H. bacteriophora* and each sample taken randomly from the plots was baited with five *G. mellonella* larvae for 3 days at 25°C. Soil temperatures between 0 and 10 cm depth were recorded from the plots at the time of nematode application and soil sampling in each year.

Soil sampling and insect baiting. Soil samples were collected with a soil borer (3 cm diam. and 15 cm long) once every 2 weeks between the second half of April and August. Five soil samples (each soil sample approximately 50 g) were taken from each plot. Each sample was examined individually.

The soil samples were baited with three last instar larvae of *G. mellonella* at 25ºC for 3 days. After 3 days, dead larvae were taken from the soil. The dead larvae were dissected in order to count the number of penetrated IJ. Living larvae were also dissected in order to detect IJ that had penetrated but not yet killed the larvae.

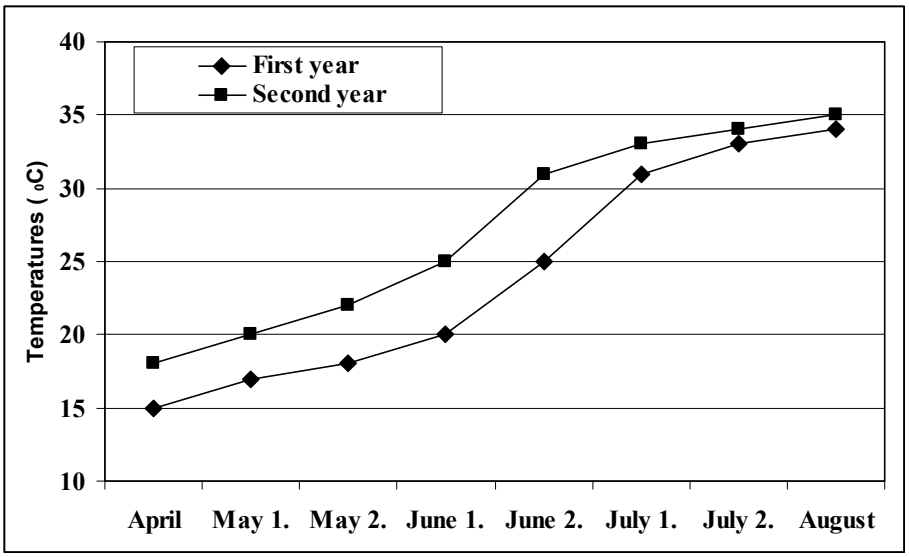
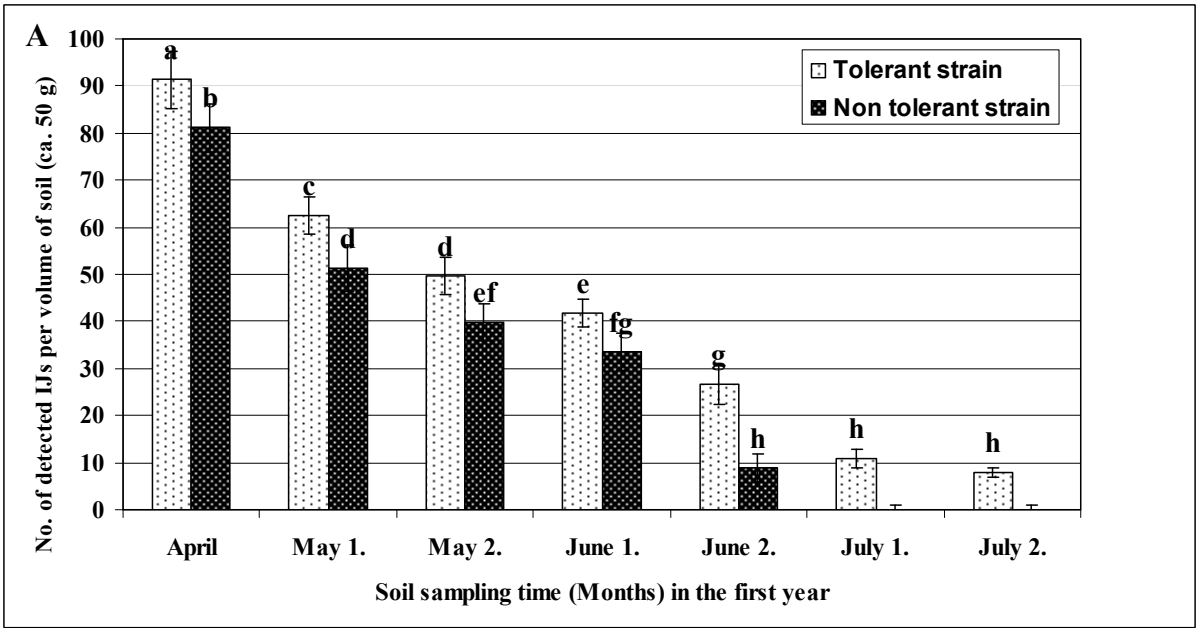


Fig. 1. Soil temperatures (ºC) at soil sampling time in the first and second years.



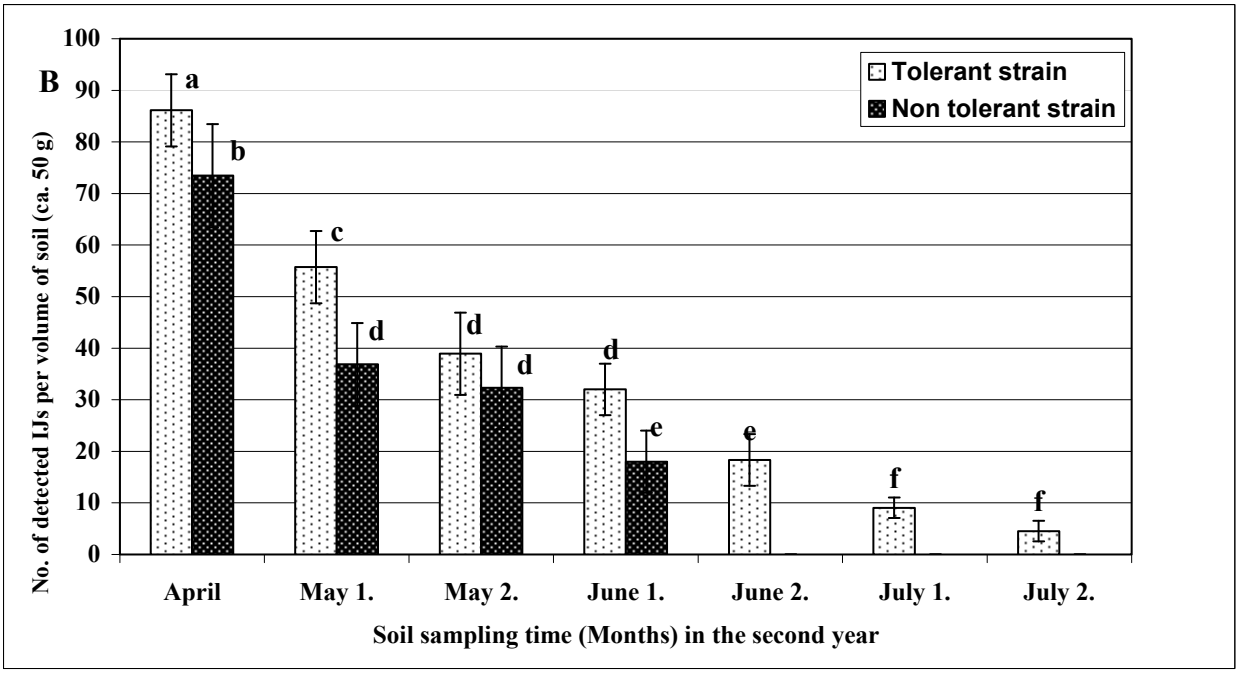


Fig. 2. Persistence of tolerant and non-tolerant *Heterorhabditis bacteriophora* strains as detected infective juveniles from each sample collected from plots between April and July in the first year (A) and the second year (B); 1 and 2 indicate first half and second half of the month, respectively. Means ± SE followed by the same letters are not significantly different ($P < 0.05$).

Foraging behaviour experimental design. Vertical PVC columns (5 cm diam. and 9 cm high) divided into six sections, of 1.5 cm height each were used for the experiment. The columns were assembled with adhesive tape and filled with moist (10% w/v) soil (content described above) (Susurluk *et al.*, 2003; Susurluk, 2008). A single *G. mellonella* larva as the host insect was placed at the bottom of each plastic column. A metal screen was glued to the bottom section to prevent the larva from moving within the sand column. Each strain was applied to the top of the columns at the dose of 1000 ± 45 IJ in 1 ml of distilled water. The control group consisted of columns without the insect. Immediately after the process, the columns were sealed with parafilm, in order to avoid evaporation. The vertical distribution of both *H. bacteriophora* strains was observed at six different depths: 0-1.5, 1.5-3.0, 3.0-4.5, 4.5-6.0, 6.0-7.5 and 7.5-9.0 cm at 25°C for 6 days (Susurluk *et al.*, 2003; Susurluk, 2008).

After 6 days, each section was carefully separated and IJ were isolated from the experimental soil using a modified Cobb's decanting and sieving method and then counted under a binocular microscope (Klein Beekman *et al.*, 1994). The *G. mellonella* placed at the bottom of each column were dissected in Ringer's solution and the IJ or other stages of *H. bacteriophora* were counted. There were five replicates for each treatment and the experiment was once repeated.

Statistical analyses. The number of IJ from the *G. mellonella* larvae in each parcel in the persistence experiment, and the number of detected IJ in each column section in the foraging behaviour experiment were analysed by one-way ANOVA, and the means were compared by least significant difference (LSD) test for post-hoc comparisons. Data obtained from replicates of the foraging behaviour experiment were pooled and analysed together, although data from the persistence experiments were analysed separately for each year. The minimum level of significance was taken as $P < 0.05$ (JMP 7.0).

RESULTS

Persistence experiment. No endemic EPN were found in the experimental plots in the field. Soil temperatures at the time of application were 15ºC and 18ºC in the first and second years, respectively. Soil temperatures at the time of soil samples collection are given in Fig. 1. The results indicated that soil temperatures in the second year were generally cooler than those in the first year. Differences in persistence between tolerant and non-tolerant strains were statistically significant in all months, except in the second half of May in the second year. Although between 8 and 11 IJ of the tolerant strain were found in July in the first year, no non-tolerant strain was detected during this month. Fewer than 10 IJ of the non-tolerant strain were detected in the second half of June (Fig. 2A).

Similar to the first year results ($F = 92.89$, df = 13, 196; $P < 0.001$) were obtained in the second year ($F = 103.23$, df = 13, 196; $P < 0.001$). However, differences between tolerant and non-tolerant strains in numbers of IJ from plots were not statistically significant in May 2. Moreover, no non-tolerant strain IJ was detected from the second half of June to August. The number of IJ of the tolerant strain in July was fewer than 10 in the second year (Fig. 2B). In August of both years, soil samples from the experimental plots yielded no nematodes.

Monitoring of foraging behaviours of the strains. At the end of the experiment, *ca* 75% and 60% of inoculated IJ were recovered in treated columns with *G. mellonella* larva and control columns, respectively. After 6 days, no nematodes for either strain were detected between 6 and 9 cm sections in control columns ($F = 92.45$; df = 5, 54; $P < 0.001$); they could reach 6 cm depth only. Although IJ of the non-tolerant strain were only found down to depth of 7.5 cm in treated columns, 13.52 ± 4 IJ of tolerant strain were detected in *G. mellonella* larva at 9 cm depth ($F = 114.45$; df = 6, 63; $P < 0.001$). IJ of the non-tolerant strain could not reach *G. mellonella*. There was a significant difference between the number of IJ of both strains from 4.5 cm to 7.5 cm in treated columns (Table 1A), while differences in number of IJ between tolerant and non-tolerant strains were not statistically significant in each section of control columns (Table 1B).

In addition to comparisons between tolerant and non-tolerant strains, all depth layers were also compared between treated columns with *G. mellonella* larva ($F = 101.74$; df = 5, 54; $P < 0.001$) and control columns (without *G. mellonella* larva) ($F = 81.73$; df = 5, 54; $P < 0.001$) for each strain. According to the results, IJ of both strains moved faster (*ca* 1.5 cm in 6 days) in columns with insect larva than the IJ in control columns (Tables 1A and 1B).

DISCUSSION

Factors with influence on EPN during the time of application and over the following few hours are most critical for their persistence in the soil (Smits, 1996). Environmental conditions can have major effects on establishment and persistence of EPN (Susurluk & Ehlers, 2008). In the range of 15-25°C, higher temperatures increase the rate of metabolism

Table 1. Numbers of tolerant and non-tolerant *Heterorhabditis bacteriophora* strains moving to the bottom of the column without (A) and with (B) *Galleria mellonella* larva as control. All depths were compared between tolerant and non-tolerant strains, and differences indicated with small letters. Moreover, all depths were also compared between with and without *G. mellonella* in each strain indicated with capital letters. Means ± SE followed by the same letters are not significantly different ($P < 0.05$).

(A) Soil depths (cm)	*H. bacteriophora* (tolerant strain)	*H. bacteriophora* (non-tolerant strain)
0-1.5	410.51 ± 22 a /A	409.76 ± 24 a /H
1.5-3.0	158.12 ± 16 b /B	147.89 ± 15 bc /I
3.0-4.5	33.00 ± 10 bc /C	27.33 ± 13 c /J
4.5-6.0	10.85 ± 2 d /D	13.97 ± 3 d /J
6.0-7.5	—	—
7.5-9.0	—	—

(B) Soil depths (cm)	*H. bacteriophora* (tolerant strain)	*H. bacteriophora* (non-tolerant strain)
0-1.5	220.42 ± 22 ab /E	251.76 ± 18 b /H
1.5-3.0	193.20 ± 19 ac /F	227.89 ± 17 ab /K
3.0-4.5	144.14 ± 21 d /B	173.33 ± 23 cd /I
4.5-6.0	120.55 ± 12 e /B	67.97 ± 11 fd /J
6.0-7.5	55.65 ± 9 f /G	25.90 ± 8 g /J
7.5-9.0	30.60 ± 10 g /C	—
in *Galleria mellonella* larva at 7.5-9.0	13.52 ± 4 h /D	—

and shorten the life span (Kaya, 1990; Grewal *et al*., 1994).

Susurluk & Ehlers (2008) stated that *H. bacteriophora* applied in the field persisted for over 24 months in Germany. However, in the present study, no nematode was detected after 4 months in experimental plots after nematode application. The differences may be the result of differences in climatic conditions, plant design on the field and occurrence of host insects in the soil. Persistence of *H. bacteriophora* in soil depends on several factors and the resistance to heat can vary considerably between EPN species, and even between strains of *H. bacteriophora* (Susurluk *et al.*, 2001; Susurluk & Ehlers, 2008; Mukuka *et al.*, 2010a, b). The more important question is whether or not there is a difference in persistence between heat tolerant and non-tolerant strains of *H. bacteriophora*. According to the present results, the persistence capacity of the heat tolerant strain was generally higher than that of non-tolerant strain. In July in both years, the non-tolerant strain was not found in experimental plots, while the tolerant strain was still present in July for both years. The results showed that tolerant strain HIZ was more persistent than non-tolerant strain Hb11 in warmer months such as July. However, no IJ of either strain could be detected in the plots in August in both years. The present study also showed that, in general, more IJ were detected in the first year than the second year for both strains; this may be explained by the fact that soil temperatures were lower in the second year than in the first year; lower soil temperature were considered more suitable for the strains. This agrees with Susurluk & Ehlers (2008), who stated that cooler soil temperatures enhanced the persistence of IJ. The heat tolerance ability of *H. bacteriophora* also increases the capacity for persistence in soil.

There are different host foraging behaviours of EPN. The species of EPN are classified as cruisers, ambushers or intermediates (Csontos, 2002). Campbell & Gaugler (1993) designated *S. glaseri*, *S. feltiae* and *H. bacteriophora* as cruisers. However, there are also differences between the same species.

Susurluk *et al*. (2003) concluded that two different *H. bacteriophora* strains isolated from the same area have different foraging tactics. Georgis & Poinar (1983) stated that the presence of *G. mellonella* larvae stimulates the movement of *Heterorhabditis* isolates. Westermann (1995) reported that 95 to 99% of *Heterorhabditis* isolates in the presence of *G. mellonella* left the top layer of sand columns and were recovered from the bottom (9 cm) layer 6 h later, and at 8 h 59 and 68% of *H. megidis* HO1 had left the top layer in the absence and presence of *G. mellonella*, respectively. His results showed that in its host searching behaviour, *H. megidis* is different from *H. bacteriophora.* In the present study, the tolerant *H. bacteriophora* strain (HIZ) showed a greater tendency to move towards the host than did non-tolerant *H. bacteriophora* (Hb11). Both strains showed a reduced tendency to move downwards in control columns, when compared with treated columns. Moreover, differences in movement of IJ in columns treated with *G. mellonella* larva and in columns without the larva indicate a positive relationship between heat tolerance and distance travelled; there is a possible link between heat tolerance and host searching behaviour in *H. bacteriophora* HIZ strain.

Although very important characters, such as virulence, host penetration and reproductive potential of heat tolerant strains of *H. bacteriophora* have been studied, persistence and foraging behaviour capacities of the species have not been yet investigated. Thus, the present study is the first attempt to examine these characters in heat tolerant and non-tolerant *H. bacteriophora* strains. However, the conclusions belong only to one tolerant strain (HIZ) and one non-tolerant strain Hb11 of *H. bacteriophora*. Therefore, further studies are needed with different *H. bacteriophora* strains in order to justify the conclusions.

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I. A. Susurluk. Выживаемость в почве и поисковое поведение изолята *Heterorhabditis bacteriophora*, толерантного к повышенным температурам.

Резюме. Энтомопатогенные нематоды (EPN) родов *Heterorhabditis* и *Steinernema* обладают значительным потенциалом для использования их в качестве биологического агента в борьбе с почвообитающими насекомыми-вредителями. Одним из важнейших факторов для успеха биологической борьбы является устойчивость нематод к высоким температурам. Чтобы выявить способность инвазионных личинок (IJ) EPN к выживанию и особенности поискового поведения, сравнивали характеристики одного устойчивого (HIZ) и одного неустойчивого к жаре (Hb11) штаммов *H. bacteriophora.* Устойчивым считался штамм с более чем 80%-ной способностью к выживанию при 40ºC, а неустойчивым – с менее чем 30%-ной выживаемостью при 40ºC. Чтобы определить способность к выживанию, в течение 4-х месяцев проводили полевой эксперимент. Для определения поискового поведения использовали поливинилхлоридные вертикальные колонки, заполненные почвой. Результаты эксперимента показали, что устойчивый и неустойчивый штаммы различаются поисковым поведением и количеством выживших IJ (кроме одной пробы). Устойчивый штамм показал более высокую выживаемость и более успешное поисковое поведение по сравнению с неустойчивым. Результаты показывают также, что устойчивость к высоким температурам *H. bacteriophora* положительно связана с выживаемостью и особенностями поискового поведения.