# VIRULENCE OF THREE SPECIES OF ENTOMOPATHOGENIC NEMATODES TO THE CHESTNUT WEEVIL, *CURCULIO ELEPHAS* (COLEOPTERA: CURCULIONIDAE)

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## ABSTRACT

Kepenekci, I., A. Gokce, and R. Gaugler. 2004. Virulence of three species of entomopathogenic nematodes to the chestnut weevil, *Curculio elephas* (Coleoptera: Curculionidae). Nematropica 34:199-204.

Indigenous entomopathogenic nematodes were evaluated in laboratory soil cup experiments as candidates for management of the chestnut weevil, *Curculio elephas* (Coleoptera: Curculionidae), the most severe insect pest of chestnut in Turkey. Three entomopathogenic nematode species, *Steinernema carpocapsae* (Anamur strain), *S. feltiae* (Tur-S3 strain), and *Heterorhabditis bacteriophora* (Tur-H1 and Tur-H2 strains) (Rhabditida: Steinernematidae, Heterorhabditidae) were bioassayed against last-instar weevils at different temperatures (10, 15, and 25°C) and nematode concentrations (0, 100, 500, and 1000). The steinernematid species were unable to cause lethal weevil infections at 10°C whereas the heterorhabditid strains still induced 21-22% host mortality. The Tur-H2 strain of *H. bacteriophora* was the most virulent nematode at all temperatures tested, most notably killing 96.5% of weevil larvae at 25°C. $LC_{50}$ values for the Tur-H2 and Tur-H1 strains of *H. bacteriophora* at 15°C, the most probable field application temperature, were 266 and 494 infective juveniles, respectively.

*Key words:* chestnut weevil, *Curculio elephas*, efficacy, *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*.

## RESUMEN

Kepenekci, I, A. Gokce, y R. Gaugler. 2004. Virulencia de tres especies de nemátodos entomopatógenos al picudo del castaño *Curculio elephas* (Coleoptera: Curculionidae). Nematropica 34:199-204.

Se evaluaron nemátodos entomopatógenos indígenos como candidatos para el manejo del picudo del castaño, *Curculio elephas* (Coleoptera: Curculionidae), la plaga de insecto más severo en el castaño en Turquía. Tres especies de nemátodos entomopatógenos, *Steinernema carpocapsae* (raza Anamur), *S. feltiae* (raza Tur-S3), y *Heterorhabditis bacteriophora* (razas Tur-H1 y Tur-H2) (Rhabditida: Steinernematidae, Heterorhabditidae) fueron ensayados contra picudos en el último estado de "instar" a diferentes temperaturas (10, 15 y 25°C) y concentraciones de nemátodos (0, 100, 500 y 1000). Las especies que pertenecen a la familia Steinernematidae no causaron infecciones letales en el picudo a 10°C, mientras que las razas que pertenecen a la familia Heterorhabditidae indujeron una mortalidad del huésped de 21-22%. La raza Tur-H2 de *H. bacteriophora* era el nemátodo más virulento a todas las temperaturas ensayadas, más notablemente matando el 96.5% de larvas del picudo a 25°C. Valores de $LC_{50}$ para las razas Tur-H2 y Tur-H1 de *H. bacteriophora* a 15°C, la cual es la temperatura más probable en el campo, fueron 266 y 494 de juveniles infectivos respectivamente.

*Palabras clave: Curculio elephas*, eficacia, *Heterorhabditis bacteriophora*, picudo del castaño, *Steinernema carpocapsae, Steinernema feltiae.*

## INTRODUCTION

The chestnut weevil, *Curculio elephas* (Curculionidae: Coleoptera), is a major pest of chestnut, *Castenea sativa*, throughout the Black Sea region of Turkey (Tuncer and Serdar, 1996). The weevil has four larval stages. Embryonic and larval develop-

ment requires 35-40 days. The last-instar larvae drop to the ground in early fall and burrow into the soil to a depth of 3-15 mm, where an earthen cell is constructed for overwintering. Most larvae pupate in late June of the following year but some may remain as larvae in the soil for 2-4 years. Adults emerge from mid-August to the end of September and feed for a week before ovipositing into chestnut fruit. Damaged fruit drops prematurely but damage is variable depending on degree of infestation (up to 8-10 larvae per acorn) and chestnut variety (Anonymous, 1995). This pest causes a 20-25% annual crop loss in Turkey (Yaman *et al.*, 1999). Current control relies on chemical insecticides but asynchronous emergence and the prolonged larval diapause limit success. Thus, an alternative control method in addition to chemical control is desirable.

Entomopathogenic nematodes—steinernematid and heterorhabditid nematodes containing mutualistic bacteria—are extraordinarily lethal to many insect pests, including several weevils. Infective-stage juvenile nematodes enter insects through the mouth, anus, spiracles, or areas of thin cuticle. After penetrating to the haemocoel the nematodes release their bacteria which quickly multiply and overwhelm the hosts, usually within 24 to 48 hr. The developing nematodes feed upon the bacteria and liquefying host tissues, mate, and produce two or more generations before emerging as infective juveniles from the depleted insect cadaver in search of fresh hosts.

Entomopathogenic nematodes possess impressive attributes for the biological control of many soil-inhabiting insects in addition to their high lethality, ease of culture and application, and high safety level (Gaugler, 2002). Consequently, these nematodes have been commercially available in the U.S., Western Europe, Japan, and China and applied against pests of cranberries, turfgrass, mushrooms, apples, peaches, ornamentals, citrus, and other insect pests in horticulture, agriculture, and home and garden (Georgis, 2002). They have been applied with greater frequency and success against weevil larvae than any other group of insects. The largest use in Europe has been applications against black vine weevil, *Otiorhynchus sulcatus*, whereas most use in the U.S. has been to control the citrus weevil, *Diaprepes* spp. (R. Georgis, pers. comm.). In an analysis of the reasons responsible for the successful use of nematodes against *Diaprepes* weevils, Shapiro-Ilan *et al.* (2002) noted that "if a nematode does not possess a high level of virulence toward the target pest there is little hope of success." Georgis and Gaugler (1991) further stress that the choice of nematode species is the most critical aspect to achieving satisfactory field results.

The goal of the present study is to ultimately extend the entomopathogenic nematode successes noted in Europe and the U.S. to Turkey, in this case with control of chestnut weevil. The first step in achieving this end has been achieved by the isolation of several indigenous species and strains (Susurluk *et al.*, 2001; Kepenekci, 2002; Kepenekci and Susurluk, 2003). We assessed the virulence of three species of entomopathogenic nematodes from Turkish soil against chestnut weevil larvae at different concentrations and temperatures.

## MATERIALS AND METHODS

Entomopathogenic nematodes and wax moth larvae, *Galleria mellonella*, were obtained from stock cultures maintained at the Plant Protection Central Research Institute of Ankara, Turkey. Our *S. carpocapsae* strain was isolated from soil in a forest area of Anamur (Içel) (Kepenekci, 2002), whereas *S. feltiae* (Tur-S3), *H. bacteriophora* (Tur-H1), and *H. bacteriophora*

(Tur-H2) were isolated from soil samples taken at the Agricultural Faculty of Ankara (Susurluk *et al.*, 2001; Kepenekci and Susurluk, 2003). Nematodes were reared on last-instar wax worms at 25°C according to methods outlined by Woodring and Kaya (1988). After harvesting, the nematodes were stored at 5°C for 2 wk before testing.

Last-instar chestnut weevils for bioassays were field collected from the chestnut growing area of Ordu Province, Turkey. Soil for the bioassays was taken where the insects were collected. The soil was a loamy sand (sand:silt:clay, 70:15:15), pH 6, and organic matter 2% by dry weight. Soil was autoclaved and dried at room temperature before testing.

Nematode-host exposures were carried out in plastic cups, 6.5 diam × 6 cm deep, according to bioassay procedures developed by Shapiro *et al.* (1999) for weevil larvae. A single last-instar chestnut larva was placed at the bottom of each cup and the cup was filled with 200 $cm^3$ of soil. Cups were left overnight at the test temperature to equilibrate before nematodes were introduced.

Optimal temperature experiments were conduced in constant temperature incubators set at 10, 15, or 25°C. Approximately 500 nematodes in 5 ml water were transferred by pipette onto the soil surface of each cup such that the final soil moisture was standardized at field capacity. In controls, 5 ml of distilled water was applied to each cup. The cups were placed in incubators and larval mortality was recorded after three weeks of incubation. Ten cups were used for each treatment and the experiment was repeated three times at ten day intervals. Data were transformed by arcsine before analysis. Analysis of variance (ANOVA) and Fisher's multiple range test (MINITAB, 1998) was used to test for differences between treatments.

The relation between nematode concentration and weevil larval mortality was tested in *H. bacteriophora* Tur-H2 and Tur-H1 at 15°C. The procedure was as described above except that a range of concentrations was applied: 0, 100, 500, and 1000 infective juveniles. Ten cups were used for each treatment and the experiment was repeated three times at ten day intervals. Data were analyzed using POLO-PC (LeOra Software, 1994) which uses the maximum likelihood ratio test according to Finney (1971).

## RESULTS

All nematode species and strains displayed increased virulence in parallel with rising temperature [$P < 0.05$ (Fig. 1)]. At the lowest temperature tested, 10°C (Fig. 1A), the steinernematid species were unable to initiate lethal host infections, whereas the heterorhabditid strains displayed significant virulence ($F = 4.77$; df = 4,10; $P < 0.05$) in killing 21-22% of weevil larvae. At 15°C (Fig. 1B), the mortality caused by the nematodes varied from 16.9 to 70.2% and *S. carpocapsae* and *H. bacteriophora* Tur-H2 were significantly different from the control ($F = 5.82$; df = 4,10; $P < 0.05$). The Tur-H2 strain of *H. bacteriophora* was more than twice as virulent at any other nematode at this temperature, killing 70.2% of larvae. At 25°C (Fig. 1C), *S. feltiae* killed 40.2% of larvae, whereas an intermediate level of virulence was demonstrated by *S. carpocapsae* and *H. bacteriophora* Tur-H1 in causing mortalities of 53.2 and 72.1% respectively. The Tur-H2 strain of *H. bacteriophora* was the most virulent nematode at 25°C, killing 96.5% of exposed weevils.

In concentrations tests conducted at 15°C comparing the Tur-H1 and Tur-H2 strains of *H. bacteriophora* showed that there was no significant difference in virulence of strains. The $LC_{50}$ for the Tur-H2 strain was 265.67 nematodes, with upper and

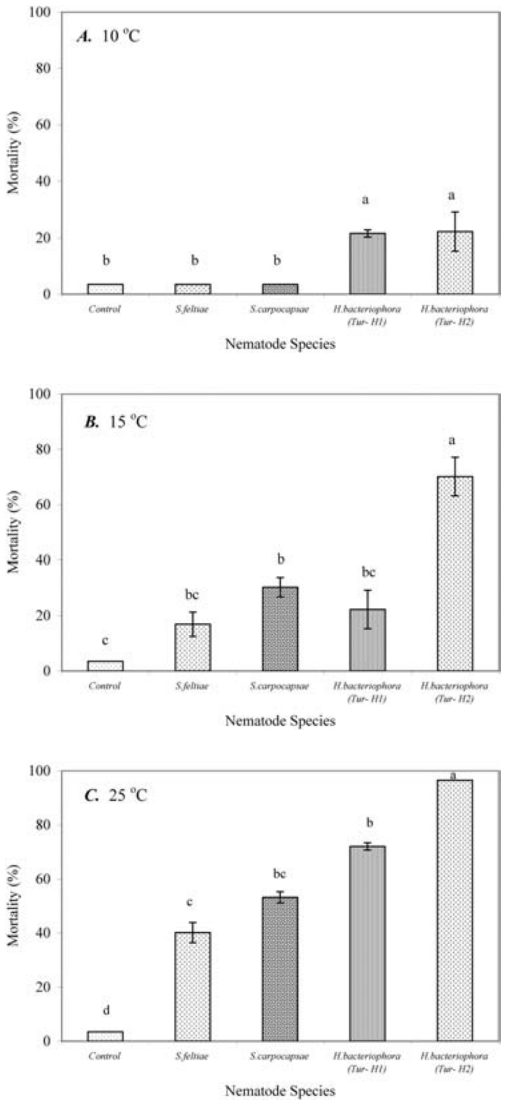


Fig. 1. Mortality of *Curculio elephas* caused by *Steinernema feltiae, Steinernema carpocapsae,* and *Heterorhabditis bacteriophora* (Tur-H1 and Tur-H2 strains) at (A) 10°C, (B) 15°C, and (C) 25°C. Bars (mean ± SEM) with different letters are significantly different from each other ($P < 0.05$; One-way ANOVA, Fisher test).

lower fiducial limits of 139.25 and 434.07, regression slope of 2.89 and -7.01 intercept. By contrast, the $LC_{50}$ for the Tur-H1 strain of *H. bacteriophora* was 493.97, with fiducial limits of 243.18 and 919.17, regression slope of 3.11, and intercept of -8.39.

## DISCUSSION

Entomopathogenic nematode intra- and interspecific virulence against the chestnut weevil was strongly influenced by temperature. Both *H. bacteriophora* strains retained significant if modest virulence to weevil larvae at 10°C, whereas the two steinernematid species were non-infective at this temperature. Weevil mortality induced by all nematode species and strains was greatest at 25°C, and three of the four strains tested were more than twice as virulent at 25°C than 15°C. Other workers have also reported that infective juvenile ability to induce insect mortality increases sharply at temperatures above 15°C (Gaugler, 1981; Grewal *et al.*, 1994; Menti *et al.*, 2000). *S. feltiae* tends to perform well at cool temperatures (Grewal *et al.*, 1994), which was why it was included in our assays, but this species displayed poor virulence against chestnut weevils regardless of test temperature. This finding is consistent with *S. feltiae's* standing as being most specific for and virulent against dipteran hosts (Peters and Ehlers, 1994). *S. carpocapsae* and the Tur-H1 strain of *H. bacteriophora* showed an intermediate level of virulence. Although Tur-H1 out performed *S. carpocapsae* at 10°C the level of control achieved was too small to be useful. The Tur-H2 strain of *H. bacteriophora* was overall the most virulent strain. This was most apparent at the most important temperature, 15°C, where only the Tur-H2 strain showed acceptable virulence. This temperature is similar to soil temperatures in the Black Sea region of Turkey in autumn, when the weevil is in the soil and most vulnerable to entomopathogenic nematode attack.

Our results agree with earlier studies by Selvan *et al.* (1994), Klein (1990), and Jack-

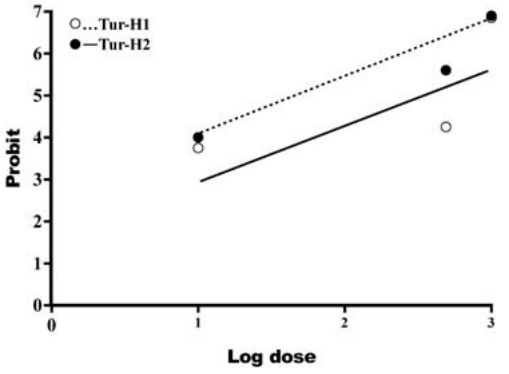


Fig. 2. Dose-mortality response of *Curculio elephas* exposed to *Heterorhabditis bacteriophora* (Tur-H1 and Tur-H2 strains) at 15°C.

son and Brooks (1989). Shapiro and McCoy (2000) tested nine entomopathogenic nematodes against the citrus weevil, *Diaprepes abbreviatus*, at 20, 24, and 29°C and found that *S. riobrave* produced the greatest mortality at all tested temperatures. Smith *et al.* (1993) tested *S. carpocapsae*, *S. feltiae*, and *H. bacteriophora* on the pecan weevil and reported that *S. carpocapsae* was the most virulent species among the tested nematodes.

The Tur-H2 strain of *H. bacteriophora* was the most virulent of the four nematodes assayed. Shapiro-Ilan (2001) screened nine nematode species, including *H. indica*, *H. marelatus*, *H. bacteriophora*, *S. carpocapsae*, and *S. feltiae* against pecan weevil larvae and found that only the three heterorhabditid species caused significant mortality. *H. marelatus* was the most virulent species assayed against black vine weevil larvae (*Otiorhynchus* spp.) (Berry *et al.*, 1997; Kakouli-Duarte *et al.*, 1997).

Dose-mortality assays with *H. bacteriophora* Tur-H2 showing an $LC_{50}$ of 266 infective juveniles and regression line slope of 2.86 are similar to the results of Jackson and Brooks (1989). These workers tested four *S. carpocapsae* strains against the corn rootworm, *Diabrotica virgifera virgifera*, and reported $LC_{50}$ values for the Breton and Agriotos strains of 222 and 325 infective juveniles and slopes of 2.04 and 1.32 respectively.

We conclude that the Tur-H2 strain of *H. bacteriophora* is the best candidate for further tests against the chestnut weevil. Only this strain possesses the capability to cause a high proportion of lethal infections at the target temperature of 15°C. The basis for the high virulence of Tur-H2, remains unclear but may include factors such as superior host searching capability, adaptations to overcome the weevil immune response, or good bacterial growth at this temperature. Nevertheless, we have identified a locally adapted nematode strain possessing high virulence for the target insect. We are now prepared for the third stage of our research: validating our laboratory soil tests under field conditions. Further studies on the Tur-H2 strain will focus on field efficacy, persistence, and interaction with environmental factors.

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