Turkish Journal of Agriculture and Forestry

Volume 39 | Number 1 Article 9

1-1-2015

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İLKER KEPENEKCİ

SELÇUK HAZIR

AYŞE ÖZDEM

TÜBİTAK

Turkish Journal of Agriculture and Forestry

http://journals.tubitak.gov.tr/agriculture/

Research Article

Turk J Agric For
(2015) 39: 74-79

doi:10.3906/tar-1403-96

Evaluation of native entomopathogenic nematodes for the control of the European cherry fruit fly *Rhagoletis cerasi* L. (Diptera: Tephritidae) larvae in soil

İlker KEPENEKCİ[1], Selçuk HAZIR[2,*], Ayşe ÖZDEM[3]

[1]Department of Plant Protection, Faculty of Agriculture, Gaziosmanpaşa University, Tokat, Turkey
[2]Department of Biology, Faculty of Arts and Sciences, Adnan Menderes University, Aydın, Turkey
[3]Fruit Pests Laboratory, Department of Entomology, Plant Protection Central Research Institute, Ankara, Turkey

Received: 17.03.2014 • **Accepted:** 27.09.2014 • **Published Online:** 02.01.2015 • **Printed:** 30.01.2015

Abstract: Turkey is the world's largest producer of sweet cherries. The European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae), is a major pest of sweet cherry crops in Europe and Turkey. The detection of only one larva in the fruit by the processor can result in the rejection of the entire crop of that orchard and/or farm as "wormy" and unmarketable. The main control tactic for *R. cerasi* is to prevent the females from laying eggs in the fruit. Currently, only a few insecticides are being used, and their application is debatable due to problems with residual ecotoxicity for humans and beneficial organisms. As an alternative to using insecticides for the reduction of adult populations, 4 indigenous entomopathogenic nematode species, *Steinernema carpocapsae* (Anamur isolate), *S. feltiae* (Rize isolate), *Heterorhabditis bacteriophora* (Ekecik isolate), and *H. marelatus* (Ankara isolate), were bioassayed against last-instar *R. cerasi* larvae at different temperatures (10, 15, and 25 °C) and nematode concentrations (0, 100, 500, and 1000 IJs/larva). Temperature and nematode concentration had a significant effect on the efficacy of nematode species. *S. feltiae* was the most virulent species at all temperatures and nematode concentrations. Only *S. feltiae* showed higher than 40% mortality at low temperatures (10 and 15 °C). At 25 °C, *S. feltiae* caused 95% mortality, followed by *H. marelatus* (82%) and *H. bacteriophora* (76%), at 1000 IJs/larva concentration. Our results indicate that *R. cerasi* larvae are highly susceptible to entomopathogenic nematode infection. In particular, *S. feltiae* has high potential for reducing last-instar larval populations, thus decreasing the adult population in the spring.

Key words: Entomopathogenic nematodes, *Steinernema*, *Heterorhabditis*, *Rhagoletis cerasi*, biological control

1. Introduction

Sweet cherry, *Prunus avium* L. (Rosales: Rosaceae), is one of the most important export products of Turkey, the country with the highest amount of cherry production in the world at 470,887 t annually (Başaran and Özdem, 2013). The European cherry fruit fly, *Rhagoletis cerasi* L. (Diptera: Tephritidae), is a major pest of sweet cherry crops in Europe and Turkey. Adults appear from May to July depending on soil temperature and moisture, and the female flies oviposit their eggs into the ripening fruits. Eggs are laid individually, and each egg is inserted singly beneath the skin of the fruit. The larva feeds on the flesh of the developing fruit for approximately 4 weeks. The last third-instar larva emerges from the fruit and pupates in the ground, a few centimeters below the surface, approximately 5–6 h after penetration into the soil (Özdem and Kılınçer, 2008). It is difficult to determine whether a fruit is infested until the larva emerges by chewing a hole or the fruit is cut open to reveal the larva inside. For processed cherries, the detection of only one larva by the processor can result in the fruit being classified as "wormy", which renders the entire crop from that orchard and/or farm unmarketable. The best management strategy is to prevent fruit infestation by the adult female fly. Thus, the main target for the control of *R. cerasi* is to prevent females from laying eggs in the fruit.

Because the larvae of the fly develop within the fruit, they are protected from most insecticides such as carbaryl, malathion, and synthetic pyrethroids. Growers spray trees with a pesticide every 5–10 days during the time flies are present (Stark and Lacey, 1999). The zero-tolerance level of fruit flies in processed cherries has made absolute control a necessity. However, European Union directives prohibit the use of many of the "older", harsher insecticides such as imidacloprid, thiametoxam, carbaryl, carbofuran, and malathion. The application of the "newer" and/or remaining "older" insecticides is less effective against this pest, resulting in higher fruit infestation unless there is frequent application. Thus, the application of the currently available insecticides, such as dimethoate, is subject to debate due to continuing problems with residual

* Correspondence: selcuk.hazir@gmail.com

ecotoxicity against humans and nontarget organisms (Daniel and Wyss, 2009).

Yellow sticky traps combined with attractants are effective for the monitoring and mass trapping of adults, which frequently rest on the leaves of cherry trees (Katsoyannos, 1976; Özdem and Kılınçer, 2009). Suggested control options include soil plowing in the fall or spring to destroy fly pupae and timed insecticide applications against adults using a degree-day model (Kovanci and Kovanci, 2006). However, this control strategy does not produce satisfactory results in practice because the damages exceed the established thresholds. Therefore, recent research has focused on alternative control methods against *R. cerasi*, with satisfactory results being reported for entomopathogenic fungi in the laboratory (Daniel and Wyss, 2009) and for entomopathogenic nematodes (EPNs) in laboratory and field conditions (Köppler et al., 2003; Herz et al., 2006).

EPNs have great potential to control third-instar *R. cerasi* larvae because they emerge from the fruits and enter the soil to pupate (Köppler et al., 2003; Herz et al., 2006). EPNs in the families Steinernematidae (genus *Steinernema*) and Heterorhabditidae (genus *Heterorhabditis*) are obligate insect-parasitic organisms and are mutualistically associated with bacteria from the genera *Xenorhabdus* (steinernematids) and *Photorhabdus* (heterorhabditids) (Kaya and Gaugler, 1993). The infective juvenile (IJ) stage of the nematodes searches for an adequate host in the soil and enters the insect host through natural openings (mouth, anus, and spiracles) or through the cuticle (mostly in *Heterorhabditis*). The symbiotic bacteria are released into the insect hemocoel when the IJ enters the target insect host (Dowds and Peters, 2002). The bacteria multiply and produce toxins in the hemocoel, killing the insect within 48 h by septicemia and toxemia. The developing nematodes feed on the bacteria and degraded host tissues (Kaya and Stock, 1997). Once nutrients are exhausted in the insect cadaver, progeny nematodes develop into the IJ stage and emerge from the cadaver into the soil to search for another host (Griffin et al., 2005).

Entomopathogenic nematodes have been used against soil pests such as white grubs, root weevils, rootworms, sciarid flies, cutworms, and armyworms (Hazır et al., 2003). A number of soil pests are potential candidates for biological control in Turkey using EPNs. One of them is the cherry fruit fly, *R. cerasi*. Hence, we evaluated the control potential of 4 indigenous EPN species against the third-instar larvae of *R. cerasi* under laboratory conditions.

2. Materials and methods

2.1. Nematodes and insects

The Turkish EPNs *Steinernema carpocapsae* (Weiser) (Anamur isolate), *S. feltiae* Filipjev (Rize isolate), *Heterorhabditis bacteriophora* Poinar (Ekecik isolate), and *H. marelatus* Liu & Berry (Ankara isolate) were obtained from the Department of Entomology of the Plant Protection Central Research Institute, Ankara, Turkey. Nematodes were reared in last-instar *Galleria mellonella* L. (Lepidoptera: Pyralidae) at 25 °C as described by Kaya and Stock (1997). The nematode-killed *G. mellonella* larvae were placed on a white trap, and the IJs emerging from the cadavers were collected from the water and stored at 10 °C in tissue culture flasks. IJs were used within 2 weeks for the experiments.

Cherries that were soft with dark brown spots were handpicked in June and July from an untreated orchard in the Ayaş district of Ankara, Turkey. From previous experience, such cherries had high probability of being infested by the fruit fly. Moreover, because no pesticides had been used in this orchard for several years, the infestation of the fruits ranged from 45% to 50%. The collected infested fruits were transferred to the lab and kept at 25 °C and 65% humidity until use.

2.2. Effect of temperature and nematode concentration on *R. cerasi* larval mortality

To determine optimum nematode application rate and temperature, the experiments were conducted with 0, 100, 500, and 1000 IJs at 10, 15, and 25 °C. Plastic cups (6.5 cm in depth and 6 cm in diameter; surface area approximately 28 cm^2) were used for the experiments (Kepenekci et al., 2004). Fifty cubic centimeters (approximately 70 g) of autoclaved and air-dried loam-sandy soil (70% sand and 30% silt) was placed into each cup. The soil moisture level was adjusted to 10% (w/w) by adding distilled water. The cups were left overnight at the targeted temperature (10, 15, or 25 °C) to equilibrate before nematodes were introduced. Four different nematode concentrations in 0.5 mL of distilled water were applied to the soil surface of each cup. One infested fruit was placed at the top of the soil in each cup after 24 h. The stalk of each fruit was covered by wet cotton to preserve freshness. Control cups were prepared as above, except that only water was added to them. Each fruit was checked for larval emergence 10 days after nematode treatment, and the soil in each cup was sieved to determine larval/pupal mortality by dissecting each individual for the presence of nematodes. Ten replicates were used for each nematode concentration and temperature regime. Fifteen cups were prepared for each treatment, and when the cups for each treatment were examined, the first 10 that had a larva or a pupa in the soil constituted the 10 replicates for that treatment. The experiments were conducted in parallel and repeated 5 times during the same season. The experiments were carried out in June and July.

2.3. Statistical analyses

Data were analyzed using one-way ANOVA followed by Tukey's test, where P = 0.05 was used to separate means.

Percentage data were arcsine-transformed before statistical analysis. Two-way ANOVA was used to analyze the interactions between nematode species, concentrations, and temperatures with SPSS (SPSS Inc., USA, 1999).

3. Results

The data showed that the third-instar *R. cerasi* larvae were susceptible to all tested EPN species, and the temperature, nematode species, and concentration had a significant effect on mortality (Table). Control mortality was minimal at all temperatures, ranging from 0% to 2% (Figures 1a–1c).

At 10 °C, the mortality caused by the nematodes varied from 1.6% to 23.4%, and *S. feltiae* and *H. bacteriophora* showed significantly higher mortality than *S. carpocapsae* and the control. On the other hand, *H. marelatus* demonstrated an intermediate level of virulence at 100 IJ concentration ($F = 8.51$; $df = 4, 24$; $P < 0.05$) (Figure 1a). At 500 IJs/larva, the lowest larval mortality was obtained with *S. carpocapsae*. There was a statistical lower difference between *S. carpocapsae* and the other nematode treatments, but this was not observed in the control group. *S. feltiae* showed significantly higher mortality than *H. marelatus* and *S. carpocapsae* ($F = 19.08$; $df = 4, 24$; $P < 0.05$) (Figure 1a). At the highest nematode concentration (1000 IJs), *S. feltiae* showed numerically higher mortality than the other nematode treatments. Except for *H. bacteriophora*, S. *feltiae* showed significantly higher mortality than all other nematode species ($F = 33.29$; $df = 4, 24$; $P < 0.05$) (Figure 1a)

Nematode species generally displayed increased virulence in parallel with increasing temperatures. At 15 °C, all 4 species of EPNs caused greater mortality than the control treatment at 100 ($F = 11.76$; $df = 4, 24$; $P < 0.05$) and 500 ($F = 14.17$; $df = 4, 24$; $P < 0.05$) IJ concentrations. However, no statistically significant differences were observed among the nematode species (Figure 1b). At 1000 IJ concentration, there was a significant difference between species *S. feltiae* and *H. marelatus*. *H. bacteriophora* and *S. carpocapsae* showed an intermediate level of virulence ($F = 40.51$; $df = 4, 24$; $P < 0.05$) (Figure 1b). Compared to 10 and 15 °C, higher larval mortality was obtained at 25 °C for all nematode species. At 100 IJs/larva, no significant differences were observed among the nematode species at 25 °C ($P > 0.05$) (Figure 1c). At the intermediate IJ concentration (500 IJs), *S. carpocapsae* showed the lowest virulence among the nematode species. Significant difference was observed only between *S. carpocapsae* and the other nematode treatments ($F = 199.69$; $df = 4, 24$; $P < 0.05$) (Figure 1c). At the highest nematode concentration (1000 IJs), *S. carpocapsae* induced the lowest mortality (47%), whereas *S. feltiae* was the most virulent species in causing 95% mortality, followed by *H. marelatus* (82%) and H. *bacteriophora* (76%). Except for *H. marelatus*, there was a significant difference between *S. feltiae* and the other nematode species ($F = 102, 71$; $df = 4, 24$; $P < 0.05$) (Figure 1c).

4. Discussion

This study demonstrated that the virulence of native EPN species against *R. cerasi* larvae was strongly influenced by temperature and nematode concentration. As a general tendency, *S. feltiae* was the most efficacious species at all temperatures and nematode concentrations tested. At lower temperature (10 and 15 °C), *S. feltiae* produced more than 40% mortality at 1000 IJs/larva. It is known that *S. feltiae* is adaptable to lower temperatures (Grewal et al., 1994; Hazir et al., 2001). Even the tropical isolate of *S. feltiae* (MG-14) does well at low temperatures (Hazir et al., 2001). On the other hand, *Heterorhabditis* species are known to be adaptable to warm temperatures (Grewal et al., 1994; Karagoz et al., 2009a). In our study, *S. carpocapsae* was the least virulent species against *R. cerasi* larvae at all temperatures. However, Köppler et al. (2003) tested the efficacy of 15 isolates belonging to *H. bacteriophora*; *H. megidis*; *S. feltiae*; *S. carpocapsae*; *S. affine* (Bovien, 1937) Wouts, Mracek, Gerdin & Bedding; 1982, *S. bicornutum* Tallosi, Peters & Ehlers, 1995; and *Heterorhabditis* sp. Poinar against European cherry fruit fly larvae and pupae and reported that *S. carpocapsae* and *S. feltiae* were the most successful species in laboratory,

Table. Interactions between nematodes, concentrations, and temperatures on the larval mortality of *Rhagoletis cerasi.*

Source	Sum of squares	df	F	P
Nematode	540.29333	4	208.8763	<0.0001
Concentration	77.76889	2	60.1306	<0.0001
Temperature	447.58222	2	346.0687	<0.0001
Nematode–Concentration	25.92	8	5.0103	<0.0001
Nematode–Temperature	189.57333	8	36.6443	<0.0001
Concentration–Temperature	19.37778	4	7.4914	<0.0001

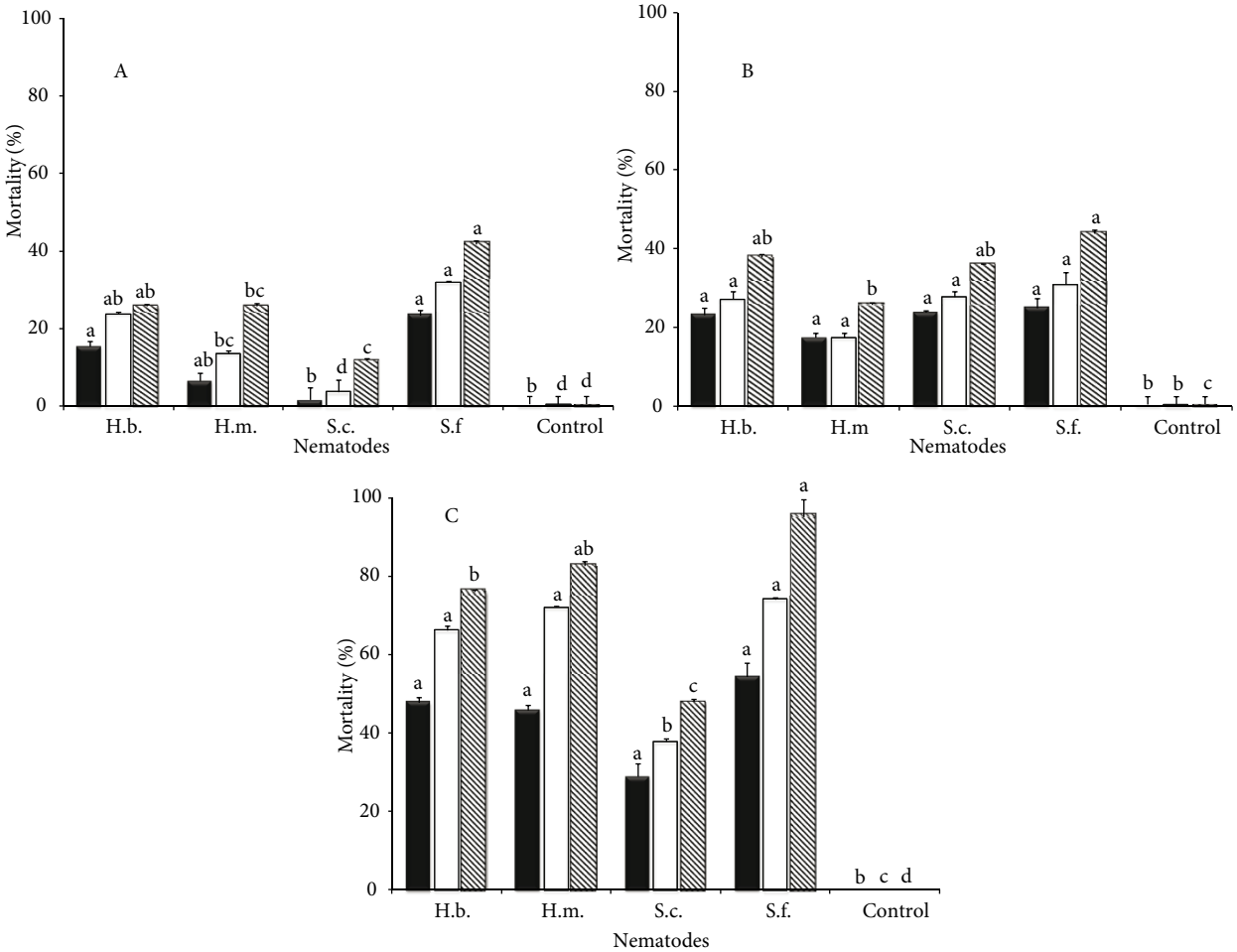

Figure 1. Mortality (%) of *Rhagoletis cerasi* third-instar larvae following the application of entomopathogenic nematodes *Heterorhabditis bacteriophora* (*H.b.*), *H. marelatus* (*H.m.*), *Steinernema carpocapsae* (*S.c.*), and *S. feltiae* (*S.f.*) at 10 °C **(A)**, 15 °C **(B)**, and 25 °C **(C)**. Data are expressed as mean ± SEM. Different lowercase letters above the bars indicate significant differences at $P < 0.05$. ■100 IJ, □ 500 IJ, ▤1000 IJ.

semifield, and field conditions with 88%, 78%, and 88% larval mortality, respectively. No pupal mortality was observed in any nematode treatments. In another study conducted by Stark and Lacey (1999), *S. carpocapsae* caused more infectivity (65%) than *S. feltiae* (35%), *H. bacteriophora* (50%), and *H. marelatus* (15%) against larvae of the western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae). They conducted the experiments in 100 × 15 mm petri dishes at 25 °C with a nematode concentration of 6360 IJs/20 larvae. When we compared their results to our intermediate concentration (500 IJs) at 25 °C, *S. feltiae* showed the highest larval mortality (76%), followed by *H. marelatus* (72%), *H. bacteriophora* (66%), and *S. carpocapsae* (37%), respectively. The difference may be attributed to nematode strain difference, host species difference, and experimental design.

Karagoz et al. (2009b) tested the efficiency of 5 native Turkish EPN species against last-instar *C. capitata* larvae under laboratory conditions. The *S. feltiae* 09-31 Aydın isolate was highly virulent and caused 78% mortality, whereas *S. weiseri* and *S. carpocapsae* killed 50% and 56% of the larvae, respectively. The 2 different *H. bacteriophora* strains that were tested caused less than 50% mortality. Sirjani et al. (2009) reported that *S. feltiae* was the most efficacious against third-instar larvae of olive fruit fly *Bactrocera oleae* (Rossi) compared to *S. carpocapsae*; *S. riobrave* Cabanillas, Poinar & Raulston; *S. glaseri* (Steiner); *H. bacteriophora*; and *H. marelatus*. These data from other studies support the conclusion that *S. feltiae* is adaptable to dipterous larvae (Lewis et al., 2006).

The infectivity of nematode species to hosts has been correlated with their foraging behavior and responses to host cues (Lewis, 2002; Griffin et al., 2005). It is known that *R. cerasi* larvae emerge from the fruit to pupate in the top few centimeters of the soil surface (Özdem and Kılınçer, 2008). *S. feltiae* has an intermediate foraging strategy,

seeking its hosts on or just beneath the soil surface (Lewis et al., 2006), and will infect a number of tephritid larval species (Stark and Lacey, 1999; Sirjani et al., 2009). In a field study, *S. feltiae* caused 79% mortality against larvae of the western cherry fruit fly, *R. indifferens* (Yee and Lacey, 2003). In another study, Herz et al. (2006) applied *S. feltiae* by tractor-mounted spray boom under the canopy area of cherry trees and monitored nematode persistence in the field after application. The activity of EPNs in soil samples was high after application but dropped to 60% of the initial activity within 1 week.

In conclusion, among the tested Turkish nematodes, the most promising results showed that *S. feltiae* (Rize isolate) had great control potential against *R. cerasi* larvae. However, the efficacy of our *S. feltiae* isolate alone or in combined application with another biocontrol agent, such as an entomopathogenic fungi, should be tested against *R. cerasi* larvae and pupae to reduce the adult generations under field conditions.

Acknowledgments

We thank Dr Harry K Kaya from the University of California, Davis, for editing the manuscript. We also thank Erdoğan Ayan and Engin Tülek (Plant Protection Central Research Institute, Department of Entomology, Fruit Pests Laboratory and Nematology Laboratory) for their valuable assistance.

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