BIOLOGICAL CONTROL

Influence of Soil Temperature and Moisture on the Infectivity of Entomopathogenic Nematodes (Rhabditida: Heterorhabditidae, Steinernematidae) against Larvae of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

Cristhiane Rohde, Alcides Moino Jr, Marco A T da Silva, Fabiano D Carvalho, Cleidson S Ferreira

Depto de Entomologia, Univ Federal de Lavras/UFLA, CP 3037, 37200-000 Lavras, MG, Brasil; crisrohde@yahoo.com.br

Edited by Ítalo Delalibera Jr – ESALQ/USP

Neotropical Entomology 39(4):608-611 (2010)

ABSTRACT - The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is considered one of the main pests that affect fruit production in the world. This insect spends part of its life cycle in the soil, making it a target for entomopathogenic nematodes. This work aimed at evaluating the influence of soil temperature and moisture on the infectivity of *Heterorhabditis* sp. RSC01 and *Steinernema carpocapsae* ALL to third-instars of *C. capitata*, and to compare the efficiency of these isolates at five different soil temperatures (19, 22, 25, 28, and 31°C) and three levels of relative soil moisture (100, 75, and 50% of field capacity). Ten *C. capitata* larvae were transferred to plastic jars (12 cm × 6 cm) containing 100 g soil, followed by the application of an aqueous suspension containing 125 infective juveniles (IJ)/cm^2. In the control treatment, 3 ml of distilled water was applied. Mortality evaluations were made five days later and were confirmed by observations of the characteristic symptoms and cadaver dissection. The infectivity was directly proportional to temperature increase, with maximum percent mortality of 86.7% and 80.0% for *S. carpocapsae* and *Heterorhabditis* sp., respectively, at 31°C. At 25°C, the highest mortality for both species was obtained at 75% of field capacity (96.7% and 26.7% for *S. carpocapsae* and *Heterorhabditis* sp., respectively).

KEY WORDS: Microbial control, fruit fly, abiotic factor

Fruit flies (Diptera: Tephritidae) cause significant fruit losses worldwide due to damages resulting from their oviposition into fruits and from pulp consumption by their larvae. Apart from direct losses, they are considered quarantine pests, which create limitations for the transit of fresh fruit due to restrictions imposed by quarantine measures from importing countries (White & Elson-Harris 1992, Bueno 2000, Malavasi 2000). The main genera of agricultural importance are *Anastrepha* Schiner, *Bactrocera* Macquart, *Ceratitis* MacLeay, *Ragholetis* Loew, *Dacus* Fabricius, and *Toxotrypana* Gerstaecker (Korneyev 1999). In Brazil, only the first four genera are economically important, particularly *Anastrepha* and *Ceratitis* (Zucchi 2000).

Ceratitis comprises approximately 65 species, which occur mainly in tropical Africa. From an agricultural point of view, the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is particularly important as it is recognized as one of the most serious pests for fruit production worldwide. *Ceratitis capitata* is polyphagous, distributed across different biogeographic regions, is highly adapted to different ecosystems and can cause different damages to fruits (Zucchi 2000).

According to Nascimento & Carvalho (2000), chemical control is the most frequently used control method against this fly; however, this form of control markedly contributes to agroecosystem imbalance, and non-target effects especially against natural enemies. In addition, this method leaves toxic residues on the fruits, and is detrimental to the consumption of fresh fruit.

While attempting to find an effective and safer alternative to the control of the Mediterranean fruit fly, several studies have been carried out to evaluate the efficacy of entomopathogenic nematodes against this insect pest. High insect susceptibility has been observed, indicating that nematodes are potential biological control agents against this pest. Besides, *C. capitata* spends part of its life cycle in the soil, where entomopathogenic nematodes are found (Lindegren *et al* 1989, Gazit *et al* 2000). However, these entomopathogens can have their survival, infectivity, development, and reproduction affected when exposed to adverse environmental conditions, such as temperature and soil moisture.

Soil temperature can greatly affect the activity of entomopathogenic nematodes. Optimal temperatures for locomotion, infection, and reproduction vary among nematode species and isolates (Grewal *et al* 1994). In general, extreme temperatures of 0 and 40°C are lethal to these entomopathogens; on the other hand, temperatures below 10-15°C can restrict their

mobility, while temperatures higher than 30-40°C can inactivate them. However, some species and isolates can be better adapted to heat, such as *Heterorhabditis megidis*, *Steinernema feltiae*, and *Heterorhabditis merelatus*, which maintain their virulence at temperatures equal to or higher than 29°C (Kung *et al* 1991, Grewal *et al* 1994, Berry *et al* 1997).

Entomopathogenic nematodes also require adequate soil moisture levels for their survival and locomotion, which may vary among nematode species and isolates and among different soil types. Low soil moisture levels can be lethal to these entomopathogens. However, some species develop survival strategies under water stress conditions, by reducing the body surface area exposed to the air and their cell metabolism. This process, known as anhydrobiosis, allows the nematode to become resistant to desiccation, and it can be reversible when the soil becomes wet again. On the other hand, high soil moisture levels can cause oxygen depletion and restrict the entomopathogen's mobility (Koppenhöfer *et al* 1995, Patel *et al* 1997).

The objective of this study was to evaluate the influence of soil temperature and soil moisture on the infectivity of *Heterorhabditis* sp. RSC01 and *Steinernema carpocapsae* ALL to *C. capitata* larvae, and to compare the virulence of these isolates.

Material and Methods

***Ceratitis capitata* rearing.** The colony was started with pupae obtained from the Entomology Laboratory at Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical, Empresa Brasileira de Pesquisa Agropecuária - Embrapa. The colony was maintained under controlled conditions (25 ± 2°C, 70 ± 10% RH, and a 12h photophase), according to Silva (1990).

Obtaining entomopathogenic nematodes. Bioassays were carried out with isolates of *Heterorhabditis* sp. RSC01 (from Amazonas, Brazil) and *S. carpocapsae* ALL (from Georgia, USA), stored at the Banco de Patógenos, Laboratório de Patologia de Insetos, Universidade Federal de Lavras – UFLA. Nematodes were multiplied by the *in vivo* method adapted from Woodring & Kaya (1988), using last-instars of *Galleria mellonella* L. (Lepidoptera: Pyralidae).

Influence of soil temperature on *S. carpocapsae* and *Heterorhabditis* sp. infectivity to *C. capitata* larvae. The efficiency of *S. carpocapsae* and *Heterorhabditis* sp. against third-instars of *C. capitata* was studied under five different soil temperatures (19, 22, 25, 28, and 31°C), in a completely randomized design with three replicates each. Treatments were arranged in a 2 × 5 factorial combination. Ten late-third-instars of *C. capitata* were transferred to plastic pots (12 cm × 6 cm) containing 100 mL soil (oxisol) with 25% moisture (water volume/soil volume) and an aqueous suspension with 125 infective juveniles (IJ)/cm^2. The control treatment received 3 mL of distilled water. The plastic pots were covered and maintained in incubators under the various temperatures at 70 ± 10% relative humidity and a 12h photophase. Soil temperature was measured daily with a thermometer, and remained equal to the temperature at which the incubator was adjusted. Evaluations were made five days later, and mortality was confirmed by means of symptom observations (cadavers infected by *Steinernema* sp. exhibit dark brown color and those infected by *Heterorhabditis* sp. exhibit red color) and cadaver dissections. Data was submitted to analysis of variance (F test) and the means were compared by Scott-Knott's means comparison test or by polynomial regression ($P \leq 0.05$), using the Sisvar statistical software (Ferreira 2000).

Influence of soil moisture on *Steinernema carpocapsae* and *Heterorhabditis* sp. infectivity to *C. capitata* larvae. The efficiency of *S. carpocapsae* and *Heterorhabditis* sp. against *C. capitata* larvae was investigated under three levels of relative soil moisture (100, 75, and 50% of soil field capacity). Initially, a soil sample was sent to the Laboratório de Física do Solo, Departamento de Ciências do Solo, Universidade Federal de Lavras - UFLA, in order to determine the soil field capacity and moisture. The field capacity in the soil samples used in the experiment was determined at 33.4% soil moisture. Therefore, in the treatment at 100% field capacity, soil moisture was standardized at 33.4%; in the 75% treatment, moisture was standardized at 25.0%; and in the 50% treatment, moisture was standardized at 16.7%. The bioassay was carried out following the same experimental procedure described for the temperature study, and was maintained in an incubator at 25 ± 1°C, 70 ± 10% relative humidity, and a 12h photophase. A completely randomized experimental design was used, with three replicates, and treatments were arranged in a 2 × 3 factorial combination. The data was submitted to analysis of variance (F test) and the means were compared by Scott-Knott's means comparison test, using the Sisvar statistical software (Ferreira 2000).

Results and Discussion

Influence of soil temperature on *S. carpocapsae* and *Heterorhabditis* sp. infectivity to *C. capitata* larvae. A direct relationship was observed between the mortalities of both isolates and the increase in soil temperature between 19°C and 31°C, with maximum mortalities of 86.7% and 80.0% for *S. carpocapsae* ($Y = -101.777778 + 6.444444X$, $R^2 = 84.35\%$) and *Heterorhabditis* sp. ($Y = -39.111111 + 3.777778X$, $R^2 = 83.05\%$), respectively, at 31°C (Table 1). There was no mortality in the control.

There were no differences between mean mortality values caused by both nematodes within each temperature, except at 25°C, where *S. carpocapsae* was more efficient than *Heterorhabditis* sp., with 80.0% and 43.3% mortality, respectively. The higher efficiency of *S. carpocapsae* at higher temperatures has already been reported (Grewal *et al* 1994, Brown *et al* 2002). The high efficiency of both species at high temperatures is probably related to the fact that they originated from regions characterized by mild winters and hot summers, although reports indicate *S. carpocapsae* can cause mortality on *Galleria* between 10º and 32ºC (Grewal *et al* 1994, Brown *et al* 2002).

Several studies have demonstrated the influence of temperature on the infectivity of entomopathogenic

Table 1 Mean mortality (± SE) of third instars of *Ceratitis capitata* inoculated with *Steinernema carpocapsae* ALL and *Heterorhabditis* sp. RSC01, maintained under different soil temperatures.

Temperature (°C)	Mortality (%)	
	S. carpocapsae ALL	*Heterorhabditis* sp. RSC01
19	16.7 ± 3.33 ns	40.0 ± 15.27
22	30.0 ± 10.00 ns	40.0 ± 5.77
25	80.0 ± 10.00 a	43.3 ± 6.67 b
28	83.3 ± 8.82 ns	73.3 ± 6.67
31	86.7 ± 6.67 ns	80.0 ± 10.00
CV = 26.83%		

Means followed by the same letter in the row are not different by the Scott-Knott test ($P \leq 0.05$); nsnon significant.

Table 2 Mean mortality (± SE) of third instars of *Ceratitis capitata* inoculated with *Steinernema carpocapsae* ALL and *Heterorhabditis* sp. RSC01, maintained under different soil moisture.

Moisture (% of field capacity)	Mortality (%)	
	S. carpocapsae ALL	*Heterorhabditis* sp. RSC01
100	13.3 ± 3.33 Ab	20.0 ± 0.00 Aa
75	96.7 ± 3.33 Aa	26.7 ± 3.33 Ba
50	16.7 ± 6.67 Ab	10.0 ± 0.00 Ab
CV = 20.41%		

Means followed by the same lower case letter in the same column, and upper case letter, in the same row, are not different by the Scott-Knott test ($P \leq 0.05$).

nematodes (El-Sadawy 2001, Hazir *et al* 2001, Ebssa *et al* 2003, Subramanian & Senthamizh 2004). In this respect, Yul *et al* (2002) observed that *S. carpocapsae* (Pocheon) was pathogenic to *G. mellonella* caterpillars at temperatures of 13, 18, 24, 30, and 35°C, with higher mortality at 24°C, and with lethal time being directly proportional to temperature increase. Hussaini *et al* (2005) also observed a direct relationship between lethal time and temperature and they verified that between 25°C and 32°C, *S. carpocapsae* (PDBC EM 6.11) caused 100% mortality of *G. mellonella* and *Agrotis ipsilon* (Hufnagel) larvae (Lepidoptera: Noctuidae). At lower temperatures (8 and 18°C), however, no infection occurred, or there was a low mortality. These results indicate that this entomopathogen can be used to control insect pests that occur in tropical climate regions.

Influence of soil moisture on *S. carpocapsae* and *Heterorhabditis* sp. infectivity on *C. capitata* larvae. *Steinernema carpocapsae* induced greater host mortality when soil moisture was at 75% of field capacity, causing 96.7% mortality, with low efficiency in the other treatments. On the other hand, *Heterorhabditis* sp. was more effective in the treatments at 100% and 75% of field capacity, causing 20.0% and 26.7% mortality, respectively (Table 2). No mortality was observed in the control.

The efficiency of both nematodes was similar at 100% and 50% of field capacity, but at 75% of field capacity, *S. carpocapsae* was more effective than *Heterorhabditis* sp.

Several studies indicate that soil moisture influence infectivity of entomopathogenic nematodes, demonstrating, in general, a decrease in infectivity as soil moisture decreases (El- Sadawy 2001, Grant & Villani 2003 a, b, 2004, Alekseev *et al* 2006). Similar studies conducted with *H. bacteriophora* DI and *S. glaseri* KG (Molyneux & Bedding 1984) and with *S. glaseri* NC and *S. carpocapsae* ALL (Koppenhöfer *et al* 1995) also resulted in lower nematode infectivity in extreme, low and high (near the saturation point) soil moistures.

The low *S. carpocapsae* infectivity at the highest moisture can be explained by the fact that soil saturation with water reduces oxygen concentration and restricts nematode mobility, which is required to infect the host (Glazer 2002). On the other hand, the low infectivity of both nematodes at the lowest moisture content is probably related to the lack of water between the pores, which is also limiting for nematode locomotion. Another possibility for the low infectivity at the lowest moisture content is that these nematodes have developed physiological and behavioral adaptations that allow them to reduce their metabolism, entering a state of anhydrobiosis (Grewal 2000, Glazer 2002). Anhydrobiosis can be reversed by wetting the soil, causing a recovery of nematode infectivity and virulence. Studies have demonstrated that some species of the *Steinernema* have the ability to enter a state of anhydrobiosis when exposed to low moisture contents (Koppenhöfer *et al* 1995), but nothing is clear on this issue regarding to *Heterorhabditis* sp. Our data demonstrated that the mortality caused by the entomopathogenic nematodes studied is negatively affected by extreme soil temperature and moisture conditions. Studies under field conditions are necessary to determine strategies for the application of nematode-based biocontrol programs for the Mediterranean fruit fly.

Acknowledgments

To Dr Rômulo da Silva Carvalho, Laboratório de Entomologia, Centro Nacional de Pesquisa de Mandioca e Fruticultura Tropical/Embrapa, for supplying the insects to start a lab colony, and to Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, for the scholarship to the first author.

References

Alekseev E, Glazer I, Michael S (2006) Effects of soil texture and moisture on the activity of entomopathogenic nematodes against female *Boophilus annulatus* ticks. BioControl 51: 507-518.

Berry R E, Liu J, Groth E (1997) Efficacy and persistence of *Heterorhabditis marelatus* (Rhabditida: Heterorhabditidae)

against root weevils (Coleoptera: Curculionidae) in strawberry. Environ Entomol 26: 465-470.

Bueno L N (2000) Las moscas de las frutas: importancia económica, aspectos taxonómicos, distribución mundial de los géneros de importancia económica. In Primer Seminario Taller Sobre el Manejo de las Moscas de las Frutas en el Departamento de Arauca. Saravena, Colombia, p.1-19.

Carvalho R S, Nascimento A S (2002) Criação e utilização de *Diachasmimorpha longicaudata* para controle biológico de moscas-das-frutas (Tephritidae), p.165-179. In Parra J R P (ed) Controle biológico no Brasil: parasitóide e predadores. São Paulo, Manole, 626p.

Ebssa L, Borgemeister C, Poehling H M (2003) Effects of host density and temperature on the efficacy of entomopathogenic nematodes for the control of western flower thrips (*Frankliniella occidentalis*). DGaaE-Nachrichten 17: 25-26.

EL-Sadawy H A (2001) Effect of temperature and soil moisture on the infectivity of some entomopathogenic nematodes against larvae of the rice moth and flesh fly. International J Nematol 11: 58-62.

Ferreira D F (2000) Análise estatística por meio do SISVAR (Sistema para Análise de Variância) para Windows versão 4.0. In Anais da Reunião Anual da Região Brasileira da Sociedade Internacional de Biometria. São Carlos, UFSCar, p.255-258.

Gazit Y, Rossler Y, Glazer I (2000) Evaluation of entomopathogenic nematodes for the control of Mediterranean fruit fly (Diptera: Tephritidae). Biocontrol Sci and Technol 10: 157-164.

Glazer I (2002) Survival biology, p. 205-220. In Gaugler R (ed) Entomopathogenic nematology. Wallingford, CABI Publishing UK, 400p.

Grant J A, Villani M G (2003a) Soil moisture effects on entomopathogenic nematodes. Environ. Entomol 32: 80-87.

Grant J A, Villani M G (2003b) Effects of soil rehydration on the virulence of entomopathogenic nematodes. Environ. Entomol 32: 983-991.

Grewal P S (2000) Anhydrobiotic potential and long-term storage of entomopathogenic nematodes (Rhabditida: Steinernematidae). Int J Parasitol 30: 995-1000.

Grewal P S, Selvan S, Gaugler R (1994) Thermal adaptation of entomopathogenic nematodes: niche breadth for infection, establishment, and reproduction. J Thermal Biol 19: 245-253.

Hazir S, Stock S P, Kaya H K, Koppenhöfer A M, Keskin N (2001) Developmental temperature effects on five geographic isolates of the entomopathogenic nematode *Steinernema feltiae* (Nematoda: Steinernematidae). J Invertebr Pathol 77: 243-250.

Hussaini S S, Shakeela V, Dar M H (2005) Influence of temperature on infectivity of entomopathogenic nematodes against black cutworm, *Agrotis ipsilon* (Hufnagel and greater wax moth, *Galleria mellonella* (Linnaeus) larvae. J Biol Control 19: 51-57.

Koppenhöfer A M, Kaya H K, Taormino S P (1995) Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. J Invertebr Pathol 65: 193-199.

Korneyev V A (1999) Phylogenetic relationships among higher groups of Tephritidae, p.73-113. In Aluja M, Norrbom A L (ed) Fruit flies (Tephritidae): phylogeny and evolution of behavior. Washington, CRC Press, 156p.

Kung S P, Gaugler R, Kaya H K (1991) Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. J Invertebr Pathol 57: 242-249.

Lindegren J E, Wong T T, Mcinnis D O (1989) Response of Mediterranean fruit fly (Diptera: Tephritidae) to the entomogenous nematode *Steinernema feltiae* in field tests in Hawaii. Environ Entomol 19: 383-386.

Malavasi A (2000) Áreas – livres ou de baixa prevalência, p.175-181. In Malavasi A, Zucchi R A Moscas-das-frutas de importância econômica no Brasil: conhecimento básico e aplicado. Ribeirão Preto, Holos, 324p.

Molyneux A S, Bedding R A (1984) Influence of soil texture and moisture on the infectivity of *Heterorhabditis* sp. Dl and *Steinernema glaseri* for larvae of the sheep blowfly, *Lucilia cuprina*. Nematology 30: 358365.

Nascimento A S, Carvalho R S (2000) Manejo integrado de mosca-das-frutas p.169-174. In Malavasi A, Zucchi R A Moscas-das-frutas de importância econômica no Brasil: conhecimento básico e aplicado. Ribeirão Preto, Holos, 324p.

Patel M N, Perry R N, Wright D J (1997) Desiccation survival and water content of entomopathogenic nematodes, *Steinernema* spp. (Rhabditida: Steinernematidae) response to soil texture and bulk density. Environ Entomol 28: 1021-1035.

Silva A C (1990) Efeito inseticida, deterrente e supressor alimentar de alguns extratos vegetais sobre *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae) e *Ascia monuste orseis* (Latreille, 1819) (Lepidoptera: Pieridae) em laboratório, Tese de doutorado em Entomologia. Universidade Federal de Lavras, Lavras, 129p.

Subramanian S, Senthamizh K (2004) Effects of moisture on the efficacy of nematodes. Current Nematol 15: 65-67.

White I A, Elson-Harris M M (1992) Introduction, p.1-14. In White I A, Elson-Harris M M Fruit flies of economic significance: their identification and bionomics. Wallinford, CAB International, 601p.

Woodring J L, Kaya H K (1988) Steinernematidae and Heterorhabditidae nematodes: a handbook of techniques southern cooperative. Series Bulletin. Arkansas, Arkansas Agricultural Experiment Station Falleteville, 88p.

Yul C, Wonn L, Sook Y, Myeong L, Thi H (2002) Effects of temperature and nematodes concentration on pathogenicity and reproduction of entomopathogenic nematode, *Steinernema carpocapsae* Pocheon strain (Nematode: Steinernematidae). Korean J Appl Entomol 41: 269-277.

Zucchi R A (2000) Taxonomia, p.13-24. In Malavasi A, Zucchi R A Moscas-das-frutas de importância econômica no Brasil: conhecimento básico e aplicado. Ribeirão Preto, Holos, 324p.

Received 14/XI/08. Accepted 01/II/10.