BioControl **45:** 337–343, 2000.

RESEARCH NOTE

Infectivity of a Bulgarian and an American strain of *Steinernema carpocapsae* against codling moth

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Received 5 October 1999; accepted in revised form 7 February 2000

Abstract. A strain of *Steinernema carpocapsae* obtained from overwintering codling moths collected in Bulgaria was identified to species using the polymerase chain reaction. The infectivity of this strain towards codling moth prepupae was compared to that of a US strain at three temperatures (11, 15 and 20 °C). Infectivity was low at 11 °C with 8% mortality recorded for both strains. Mortality was 61% and 62% at 15 °C, and 82% and 81% at 20 °C for the Bulgarian and US strain, respectively. The mean percent mortality at each individual temperature was significantly different from each other when mortalities of the two strains were combined. Thus, the Bulgarian strain did not provide a low temperature infectivity advantage compared to the US strain.

Key words: Biological control, codling moth, entomopathogenic nematodes, *Cydia pomonella*, *Steinernema carpocapsae*

Introduction

The codling moth, *Cydia pomonella* (L.), is a serious pest of apples and pears throughout the world (Barnes, 1991). Eggs are laid on leaves or on the fruit; upon hatching, larvae bore directly into the fruit, causing damage that reduces its quality, or renders the fruit unusable. Chemical insecticides are regularly used to control codling moths, but resistance to azinphos-methyl, the most

commonly used insecticide, has been reported, as well as cross resistance to other insecticides (Varela et al., 1993; Knight et al., 1994; Sauphanor et al., 1998). These broad spectrum insecticides also harm beneficial insects. The development of resistance to insecticides, the resurgence of secondary pests, and the environmental problems caused by the use of insecticides are the main reasons for the 'Pesticide Environmental Stewardship Program', an initiative whose objective is to reduce insecticidal use in American agriculture (EPA, 1994). One way of achieving this goal is through increased use of biological control agents such as entomopathogens.

Among the many possible candidates for biological control of the codling moth, entomopathogenic nematodes are very promising. The application of entomopathogenic nematodes would be ideal for controlling overwintering codling moth larvae, which would be possible from the time the majority of the larvae leave the fruit and begin searching for sites in which to construct hibernacula. However, the ideal window of opportunity in terms of optimal temperature may be fairly narrow. Temperatures below 15 °C (during late fall, winter and early spring) limit the infectivity of the nematode, *Steinernema carpocapsae* (Weiser). Of the five species of entomopathogenic nematodes that were evaluated for infectivity by Lacey and Unruh (1998) and Lacey and Chauvin (1999), *S. carpocapsae* was consistently the most active under simulated operational conditions. One of its main limitations, however, is the lack of activity at and below 10 °C (Lacey and Unruh, 1998). Even at 15 °C, activity is severely reduced. Discovery of strains that are highly active at temperatures below 15 °C would increase the non-pesticidal options for treatment of diapausing prepupae in cryptic habitats in the fall when most larvae have exited the fruit or in the spring before emergence of adults takes place.

In an attempt to find natural enemies that can be used in integrated pest management programs in the USA, the European Biological Control Laboratory (EBCL) has been conducting foreign explorations in different countries. Nematodes were found infecting overwintering codling moths collected in Bulgaria; here we report on the identification of the Bulgarian nematode species and subsequent tests for infectivity at three different temperatures.

Materials and methods

Nematode collection

In October 1998, corrugated cardboard bands that had been placed around the trunk of apple trees in four locations in Bulgaria on 10 August, and containing overwintering stages of *C. pomonella* were brought back to the quarantine

facility at EBCL with the objective of isolating any pathogens that were present. Several specimens collected from bands placed in Jabalkovo (42° 19.17 N; 22° 43.45 E; ∼ 5 km N of Kjustendil) were infected with nematodes. These specimens were individually placed in Petri dishes containing water agar and kept in the dark at room temperature. Infective juvenile (IJ) nematodes from four different codling moth larvae (4 replicates) were used to infect *Galleria mellonella* (L.) to obtain sufficient IJ progeny numbers that could be used for genomic analysis and bioassays.

Genomic analysis

Genomic DNA was isolated from approximately 100 µl IJs using a Phytopure DNA extraction kit (Nucleon Biosciences, Bucks, UK; for details, see Reid and Hominick, 1992). Vrain's et al. (1992) primers (18S forward primer 5′ TTG ATT ACG TCC CTG CCC TTT 3′; 26S reverse primer 5′ TTT CAC TCG CCG TTA CTA AGG 3′) were used for polymerase chain reaction (PCR). The reaction tubes were placed in a thermocycler pre-heated to 94 °C and incubated for 2 min at 94 °C followed by 40 cycles of 94 °C for 30 sec, 50 °C for 1 min, 72 °C for 1.5 min. The final cycle was for 5 min at 72 °C; then, samples were held at 4 °C. From each reaction tube, 4 µl were removed and run on a 1% agarose gel in 0.5 × TBE to check for PCR product. The PCR products were then digested with the following enzymes at 37 °C overnight: Alu I, Dde I, Hinf I, and Rsa I. Digestion products were separated by electrophoresis in 1.5% (w/v) agarose gels in 0.5 × TBE at 100 V for 3.5 hours. Molecular weight marker band sizes were as follows: 2000, 1200, 800, 400, 200, 100 base pairs. A standard of *Steinernema feltiae* (Filipjev) internal transcribed spacer (ITS) region of the ribosomal DNA repeat unit cut with the appropriate restriction enzymes was also included. Restriction fragment length polymorphisms (RFLPs) were visualized by ethidium bromide staining and photographed. The RFLP profiles were compared to those obtained from 17 entomopathogenic nematode species in order to identify the unknown strain from Bulgaria.

Infectivity

Infectivity of the Bulgarian isolate of *S. carpocapsae* was compared with that of the Sal strain (originating in an apple orchard in Indiana, USA) at 11, 15 and 20 °C using the bioassay method described by Lacey and Unruh (1998). Briefly, the method consisted of allowing mature larvae to spin cocoons within perforated corrugated cardboard strips (15.2 cm^2). These cocooned larvae (prepupae) were exposed to IJs of the two *S. carpocapsae* isolates; these were produced in *G. mellonella* (L.) at 25 °C, harvested and quantified

using procedures described by Kaya and Stock (1997). Aqueous suspensions of the IJs were diluted to 152 viable IJs/ml prior to application. One ml of IJ suspension was added to the surface of each cardboard strip and the liquid was evenly distributed over the surface using a plastic bacterial spreader resulting in 10 IJs/cm^2. Control strips were treated with one ml of deionized water. Four replicate strips were used for each isolate and control for each of the three test temperatures on each of three test dates. After treatment, the strips were placed in Petri plates lined with moist filter paper as described by Lacey and Unruh (1998). The plates were sealed with parafilm and incubated for 24 hours at the appropriate temperature after which the parafilm was removed and the plates were incubated for 6 days when mortality was assessed. Both strains of nematodes were also grown at 15 °C and assayed in an identical manner as those grown at 25 °C; this was done to determine whether rearing at lower temperatures enhances the IJs activity at lower temperature. All treatments and controls for all temperatures were compared using the General Linear Models program (SAS, 1996).

Results and discussion

The upper panels in Figure 1 show the ITS RFLP profiles for 19 isolates of 17 species cut with the four restriction enzymes used in this study. The lower panel shows the same digests of the four samples from Bulgaria along with *S. feltiae* as a control. The banding patterns obtained from the Bulgarian nematodes are consistent with those of *S. carpocapsae* (Figure 1, lane 9 in the upper gels, marked with an arrow). *S. carpocapsae* has been previously reported infecting *C. pomonella* in the Czech Republic (Weiser, 1955), the USA (Dutky and Hough, 1955; Poinar, 1991), Italy (Vinciguerra and Tacconi, 1984), Mexico and Poland (Poinar, 1991). This is the first report of *S. carpocapsae* infecting *C. pomonella* in Bulgaria.

To compare infectivity between the Bulgarian and the Sal strains, an initial 3 × 3 factorial ANOVA was conducted in which the analysis compared mortalities in control larvae and that produced by the two nematode isolates at the three temperatures. The interaction term from this analysis was highly significant ($F = 13.7$, df = 4,16, $p < 0.001$). Simple effects contrasts were conducted to compare the control mortality with that produced by the nematode treatments at each temperature separately. At 15 and 20 °C, mortality was significantly higher in the nematode treatments than in the controls ($p < 0.001$ for both contrasts), whereas at 11 °C nematodes had no significant effect on mortality ($F = 0.9$, df = 1,16, $p = 0.36$).

To examine for possible isolate differences, we ran a 2 × 3 ANOVA (isolate × temperature) that excluded control data. The interaction effect (F =

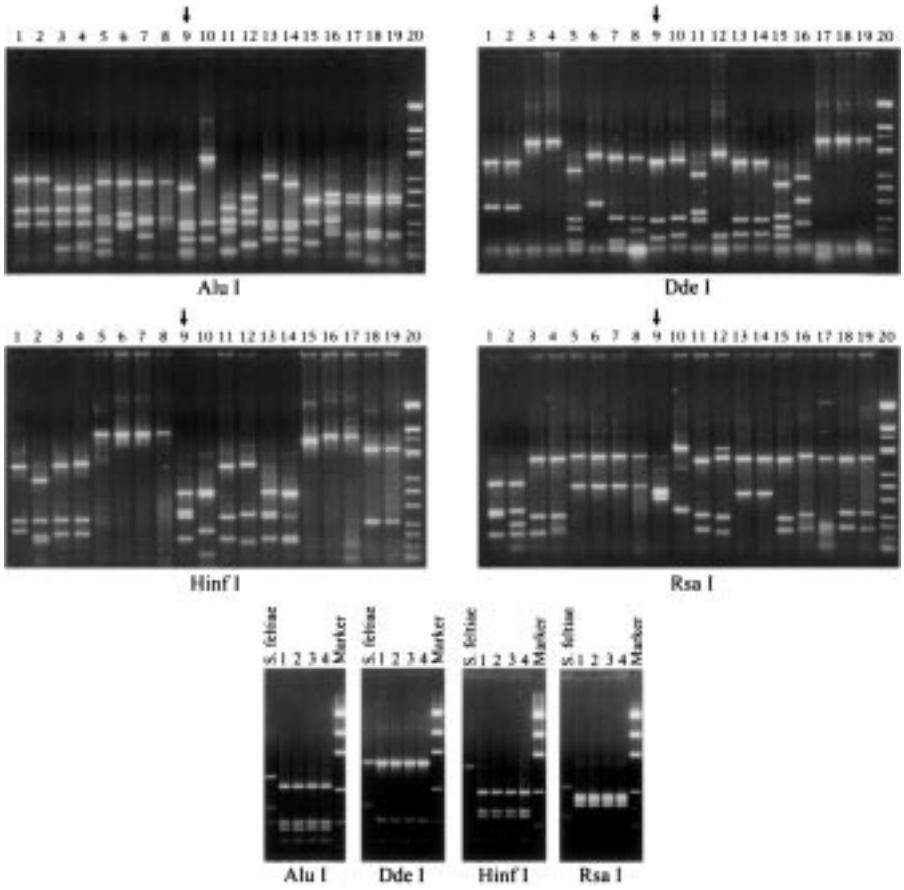

Figure 1. ITS RFLP profiles for 19 isolates of 17 nematode species cut with 4 restriction enzymes (Alu I, Dde I, Hinf I, and Rsa I). The lower panel shows the same digests for 4 nematode samples from Bulgaria along with *S. feltiae* as a control.
Lane 1, *S. feltiae* A1 RFLP type; 2, *S. feltiae* A2 RFLP type; 3, *S. kraussei* (German); 4, *S. kraussei* (UK); 5, *S. affine*; 6, *S. intermedium*; 7, species C1 (UK); 8, species E1 (UK); 9, *S. carpocapsae*; 10, *S. bicornutum*; 11, *S. scapterisci*; 12, species SSL1 (Sri Lanka); 13, species SSL2 (Sri Lanka); 14, species Malaysia; 15, *S. karii*; 16, *S. arenarium*; 17, *S. glaseri*; 18, species NC513 (USA); 19, *S. cubanum*; 20, molecular weigh markers in four upper panel photographs represent the following band sizes 2000, 1200, 800, 750, 500, 400, 300, 200, 150, 100, 50 base pairs. MW markers in the four smaller lower panels: 2000, 1200, 800, 400, 200, and 100 bp.

Table 1. Larvicidal activity of the Bulgarian and Sal strains of *Steinernema carpocapsae* (10 IJs/cm^2) at various temperatures

Temperature (°C)	Mean% mortality ± s.e.			
	Controls	Bulgarian	Sal	Bulgarian and Sal combined
11	1.2 ± 1.4	8.1 ± 5.3	8.0 ± 2.6	8.0 ± 4.0 a
15	1.7 ± 1.0	60.8 ± 15.6	62.4 ± 16.1	61.6 ± 15.9 b
20	1.2 ± 0.9	82.1 ± 4.4	81.1 ± 3.9	81.6 ± 4.2 c

Data presented is for nematodes reared at 25 °C. No significant differences in prepupae mortality were found between nematodes reared at 15 °C and 25 °C.
Means followed by the same letter are not significantly different using ANOVA and Tukey's test for mean separation (F = 67.7, df = 2,10, p = 0.0001).

0.02, df = 2,10, p = 0.98) and isolate effect (F = 0.0, df = 1, 10, p = 0.98) were not significant. When mortalities observed at each of the 3 temperatures were averaged and analyzed with ANOVA followed by Tukey's test, the differences between temperatures were highly significant (F = 67.7, df = 2,10, p = 0.0001; Table 1). Assays of both strains of nematodes produced at 15 °C revealed no difference in mortality compared to that produced by infective juveniles grown at 25 °C; therefore, rearing at lower temperatures did not enhance IJs activity at lower temperature as has been reported for other entomopathogenic nematodes (Grewal et al., 1996).

The infectivity of *S. carpocapsae* is optimal at 25 °C and this nematode species does well as a parasite of codling moth and other hosts between 20 and 30 °C (Lacey and Unruh, 1998). The Bulgarian strain did not cause higher mortality than the Sal strain in our tests. Thus, exploration for entomopathogenic nematodes will continue with the objective of identifying strains that cause higher mortality than the Sal strain at low temperatures.

Acknowledgments

We thank Pedro Hernández-Crespo (Université de Montpellier) and Patrick F. Dowd (USDA, Peoria) for their comments on a previous draft of this manuscript; and David Horton (Yakima Agricultural Research Laboratory, USDA) for statistical assistance.

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