Bulletin of Entomological Research (2005) **95**, 473–482 DOI: 10.1079/BER2005379

Screening of entomopathogenic nematodes for virulence against the invasive western corn rootworm, *Diabrotica virgifera virgifera* (Coleoptera: Chrysomelidae) in Europe

S. Toepfer[1], C. Gueldenzoph[1], R.-U. Ehlers[2] and U. Kuhlmann[1]*

[1]CABI Bioscience Switzerland Centre, Rue des Grillons 1, 2800 Delémont, Switzerland: [2]Institute for Phytopathology, Christian-Albrechts-University Kiel, Klausdorfer Street 28, 24223 Raisdorf, Germany

Abstract

Entomopathogenic nematode species available in Europe were screened for their efficacy against both the root-feeding larvae and silk-feeding adults of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte. Laboratory screening tests were aimed at the selection of candidate biological control agents for the management of this invasive alien pest in Europe. *Steinernema glaseri, S. arenarium, S. abassi, S. bicornutum, S. feltiae, S. kraussei, S. carpocapsae* and *Heterorhabditis bacteriophora* were studied to determine their virulence against third instar larvae and adults of *D. v. virgifera* in small-volume arenas (using nematode concentrations of 0.5, 0.8, 7.9 and 15.9 infective juveniles cm^{-2}). All nematode species were able to invade and propagate in *D. v. virgifera* larvae, but adults were rarely infected. At concentrations of 7.9 and 15.9 cm^{-2}, *S. glaseri, S. arenarium, S. abassi* and *H. bacteriophora* caused the highest larval mortality of up to 77%. *Steinernema bicornutum, S. abassi, S. carpocapsae* and *H. bacteriophora* appeared to have a high propagation level, producing 5970 ± 779, 5595 ± 811, 5341 ± 1177 and 4039 ± 1025 infective juveniles per larva, respectively. *Steinernema glaseri, S. arenarium, S. feltiae, S. kraussei* and *H. bacteriophora* were further screened at a concentration of 16.7 nematodes cm^{-2} against third instar larvae in medium-volume arenas (sand-filled trays with maize plants). *Heterorhabditis bacteriophora*, *S. arenarium* and *S. feltiae* caused the highest larval mortality with $77 \pm 16.6\%$, $67 \pm 3.5\%$, and $57 \pm 17.1\%$, respectively. In a next step, criteria for rating the entomopathogenic nematode species were applied based on results obtained for virulence and propagation, and for current production costs and availability in Europe. These criteria were then rated to determine the potential of the nematodes for further field testing. Results showed the highest potential in *H. bacteriophora*, followed by *S. arenarium* and *S. feltiae*, for further testing as candidate biological control agents.

*Author for correspondence
Fax: 0041 32 4214871
E-mail: u.kuhlmann@cabi.org

Keywords: *Diabrotica virgifera virgifera*, *Steinernema*, *Heterorhabditis*, entomopathogenic nematodes, Europe, biological control, virulence, screening

Introduction

The western corn rootworm, *Diabrotica virgifera virgifera* LeConte (Coleoptera: Chrysomelidae) is the most destructive pest of maize, *Zea mays* L. (Poaceae), in North America (Krysan & Miller, 1986). Since the 1990s, *D. v. virgifera* has become a serious invasive pest in Europe (Baca, 1994; Sivcev *et al.*, 1994). The majority of yield loss attributed to this univoltine pest is due to larval feeding on maize roots, which ultimately results in plant lodging. In addition, *Diabrotica* adults occasionally cause yield losses due to intensive silk-feeding (Chiang, 1973). As of 2004, the western corn rootworm has invaded almost all maize production areas within central Europe (Kiss *et al.*, 2005), and significant economic damage is foreseen if control measures are not implemented (Baufeld & Enzian, 2005).

In a sustainable and integrated pest management approach in Europe, biological control could play an important role for managing this pest (Kuhlmann & van der Burgt, 1998) in combination with other control strategies, such as tolerant maize hybrids or crop rotation (Toepfer & Kuhlmann, 2004a). A 3-year field survey conducted in the focal points of the invasion (Hungary, Croatia and Yugoslavia) revealed that effective indigenous natural enemies do not attack any of the life stages of *D. v. virgifera* in the European maize agro-ecosystem (Toepfer & Kuhlmann, 2004b). Based on these findings, two options are available for biological control of *D. v. virgifera* in Europe. The first option is a classical biological control approach involving the selection and introduction of specific natural enemies from the area of origin of *D. v. virgifera* (Kuhlmann *et al.*, 2005). The second option (to be used either alone or in combination with the first option) is an inundative biological control approach using commercially available natural enemies, such as entomopathogenic nematodes (Kuhlmann & van der Burgt, 1998; Ehlers, 2003). In general, soil-dwelling entomopathogenic nematodes have demonstrated great potential as biological control agents of arthropod pests (Gaugler, 2002), and their success stories share two basic characteristics: (i) the selection of a nematode species with known effectiveness against the target host; and (ii) favourable economic feasibility of nematode mass production (Gouge & Shapiro-Ilan, 2003).

Kuhlmann & van der Burgt (1998) reviewed previous North American studies evaluating the potential of entomopathogenic nematodes as biological control agents for a number of *Diabrotica* species. Members of the Steinernematidae and Heterorhabditidae were the most studied entomopathogenic nematode species. In general, although laboratory tests have demonstrated high *Diabrotica* mortality, the results from field tests appear to be highly dependent on environmental conditions and application techniques (Barbercheck, 1993; Jackson & Brooks, 1995). Previous research activities in North America on the suppression of *D. v. virgifera* populations using entomopathogenic nematodes focused on *Steinernema carpocapsae* (e.g. Nickle *et al.*, 1994; Jackson & Brooks, 1995), *S. feltiae* (Gaugler, 1981; Poinar *et al.*, 1983) and *Heterorhabditis bacteriophora* (e.g. Jackson, 1996; Jackson & Heseler, 1996). However, the susceptibility of *D. v. virgifera* to these nematode species appears variable, and controlled performance studies under field conditions produced conflicting results (Georgis *et al.*, 1991; Wright *et al.*, 1993; Jackson & Brooks, 1995; Riga *et al.*, 2001). Based on these studies, the following factors influencing the efficacy of entomopathogenic nematodes must be considered: (i) the origin and specificity of the nematode strain (Jackson & Brooks, 1995; Gouge & Shapiro-Ilan, 2003); (ii) their host finding strategy; (iii) their ability to survive, move and persist in the soil; (iv) the environmental conditions in the soil (Georgis *et al.*, 1991; Barbercheck, 1993); and (v) the application timing must coincide with moist soil surface (Gaugler, 2002) and with the most susceptible larval instars of *D. v. virgifera* (Jackson & Brooks, 1995).

Taking these concerns into consideration, entomopathogenic nematode species available in Europe, many of which have not previously been investigated for corn rootworm control, were screened for their efficacy against *D. v. virgifera*. These screening tests were aimed at the selection of candidate biological control agents for future field tests for the sustainable management of the alien invasive pest *D. v. virgifera* in Europe. For the purpose of this study, the virulence of eight entomopathogenic nematode species applied at low concentrations (0.5 to 15.9 nematodes cm^{-2}) against *D. v. virgifera* larvae and adults was investigated in small-volume arenas. In addition, the propagation of entomopathogenic nematodes in *D. v. virgifera* larvae and adults was evaluated to determine the efficiency of reproduction in the target pest. Based on the outcome of these experiments, selected nematodes were further tested in medium-volume arenas containing maize plants in order to simulate more natural conditions. In order to evaluate which nematode species merited further study in the field, a rating system was devised to jointly consider the data obtained for virulence and propagation in this study, as well as current production costs associated with each individual nematode species (Ehlers, 2001, 2003).

Materials and methods

Sources and rearing of nematodes and D. v. virgifera

Sources and origin of strains of *S. glaseri*, *S. arenarium*, *S. abassi*, *S. bicornutum*, *S. feltiae*, *S. kraussei*, *S. carpocapsae* (all Rhabditida: Steinernematidae) and *H. bacteriophora* (Rhabditida: Heterorhabditidae) used in this study are summarized in table 1. Nematodes were reared in last instar larvae of *Galleria mellonella* (Linnaeus) (Lepidoptera: Pyralidae) according to Kaya & Stock (1997). All nematodes were stored for some days in sterile water in the dark at a constant temperature of 4°C until used for experiments with the exception of *S. abassi*, which was kept at 14°C as it prefers warmer temperatures (according to recommendations of the provider e-nema GmbH, Raisdorf, Germany). To ensure maximum infectivity of the nematodes used during experiments, a quality control was conducted with a fraction of

https://doi.org/10.1079/BER2005379

Table 1. Origin and source of tested strains of entomopathogenic nematode species.

Nematode species[1]	Strain	Origin	Source
Steinernema glaseri (Steiner)	NC	USA	R. Gaugler, New Brunswick, NJ, USA
S. arenarium (Artyukhovsky)		Poland	M. Tomalak, Poznan, Poland
S. abassi Elawad, Ahmad, Reid		Palestine	N. Iraki, Bethlehem, Palestine
S. bicornutum Tallosi, Peters, Ehlers		Serbia	R.U. Ehlers, Kiel, Germany
S. feltiae (Filipjev)	Hybrid	European strains	e-nema GmbH, Raisdorf, Germany
S. kraussei (Steiner)	Bellin	Germany	R.U. Ehlers, Kiel, Germany
S. carpocapsae (Weiser)	All	USA	Biosys, Columbia, MD, USA
Heterorhabditis bacteriophora Poinar	Hybrid	European and US strains	e-nema GmbH, Raisdorf, Germany

[1]All nematodes were provided by e-nema GmbH, Raisdorf, Germany.

each species following standard protocols, i.e. the determination of LD_{50} in *G. mellonella* (Kaya & Stock, 1997).

In order to ensure permanent availability of *D. v. virgifera* for experiments, a non-diapause strain was continuously reared under quarantine laboratory conditions (25°C day, 15°C night, 14L:10D, 40 to 60% r.h.). Eggs of this strain were obtained from the USDA-ARS Northern Grain Insect Research Laboratory at Brookings, South Dakota, United States. Prior to the emergence of first instar *D. v. virgifera* larvae, eggs were incubated in plastic trays (300 mm × 200 mm × 50 mm) containing sterilized soil and an abundant root mass of 4- to 5-day-old maize plants. After approximately 14 days, third instar larvae were collected from the trays for use in experiments. The mean fresh weight of *D. v. virgifera* larvae reached 116.1 ± 5.9 mg ($n = 30$), and the mean dry weight was 24.3 ± 1.4 mg ($n = 30$). A fraction of the insects was reared to the adult stage for experimental use. *Diabrotica v. virgifera* adults were kept in gauze cages (300 × 300 × 550 mm) with a water source, maize leaves and artificial diet (Singh & Moore, 1985). Experiments were carried out under quarantine laboratory conditions at CABI Bioscience Switzerland Centre.

Screening for nematode virulence against D. v. virgifera *larvae*

Eight nematode species were screened in small-volume arenas, i.e. 63 cm^2 Petri dishes (diameter 90 mm), filled to 10 mm with sterile sand adjusted to 15% water content. Each Petri dish was supplied with two 2-day-old germinated maize seeds (var. Magister, Novartis Seeds SA, France) as a food source for individual *D. v. virgifera* larva. Nematode concentrations of 30 (0.5 cm^{-2}), 50 (0.8 cm^{-2}), 100 (1.6 cm^{-2}), 500 (7.9 cm^{-2}) and 1000 infective juveniles (15.9 cm^{-2}) in 1 ml sterile water were applied to the centre of each Petri dish. Individual third instar larvae of *D. v. virgifera* were then added to each Petri dish and the dishes covered with a lid and kept in the dark for seven days at 25°C day/15°C night. Thirty replicates were made for each nematode concentration, plus a non-treated control group. After seven days, Petri dishes were destructively sampled for larvae in the sand and mortality was determined. The mean mortality of *D. v. virgifera* larvae was compared among nematode species and among nematode concentrations by Fisher's PLSD posthoc Test after an ANOVA (Kinnear & Gray, 2000).

Steinernema glaseri, S. arenarium, S. feltiae, S. kraussei and *H. bacteriophora* were selected for further testing in medium-volume arenas to mimic semi-natural conditions. For this experiment, plastic trays (240 cm^2; 190 × 120 × 45 mm) were filled up to 30 mm with sterile sand (15% RH) and 15 maize seeds were evenly distributed on the surface. After four or five days, once the seeds had germinated, each tray was infested with ten third instar larvae of *D. v. virgifera*, followed by the application of nematodes at a concentration of 4000 infective juveniles per 240 cm^2 tray (or 16.7 infective juveniles cm^{-2}), in 10 ml sterile water. The infective juveniles were evenly distributed over the surface of the sand with a pipette. The control group received only water. The 15 mm rim of each tray was painted with 'Fluon' (= fluid tetrafluoroethelene co-polymer) to prevent *Diabrotica* larvae from escaping over the edge and out of the experimental tray. Twenty replicates were carried out for each of the nematode species tested. Trays were kept in the dark for seven days (25°C day/15°C night) and then screened manually for dead and living larvae in the sand. The mean larval mortality was compared between nematode species using an independent sample *t*-test at a significance level of $P < 0.05$ (Kinnear & Gray, 2000).

Propagation of nematode species in D. v. virgifera *larvae*

Dead *D. v. virgifera* host larvae obtained from each small-volume arena were placed individually in a nematode emergence trap as described in Kaya & Stock (1997). After three weeks, emigrating infective juveniles in the suspension were transferred into a small vial, which was then filled to 10 ml with sterile water. Two 100 µl aliquots were taken from the vials and the number of nematodes contained in 100 µl was determined under stereo-microscope. The mean number of nematodes in these two aliquots was multiplied by 100 to estimate the total number of infective juveniles that had exited the host cadaver, which provided the propagation rate per infected *D. v. virgifera* larva. The propagation data were averaged for each nematode species from all application concentrations as the variation in larvae weight was small (see above). An independent sample Mann-Whitney U test was used with a significance level of $P < 0.05$ to test for differences between the propagation of nematode species in *D. v. virgifera* larvae.

https://doi.org/10.1079/BER2005379

Screening for virulence against D. v. virgifera *adults*

Indirect application

Petri dishes (63 cm^2; diameter 90 mm) were filled to 10 mm with sterilized sand adjusted to 15% water content. Each Petri dish was supplied with a single drop of artificial diet for the *D. v. virgifera* adults (for diet see Singh & Moore, 1985). Nematode concentrations of 50 (0.8 cm^{-2}) and 100 infective juveniles (1.6 cm^{-2}) of *S. glaseri, S. arenarium, S. abassi, S. bicornutum, S. feltiae, S. kraussei, S. carpocapsae* and *H. bacteriophora* in 1 ml of sterile water were applied to the centre of individual Petri dishes. An individual adult *D. v. virgifera* was then added to each Petri dish. The dishes were then covered with a lid and kept for seven days at 25°C day/15°C night (14L:10D). Thirty replicates were made for each nematode species tested and for each nematode concentration, plus a control with sterile water only. After seven days, dead adults were individually placed in nematode traps (following the method described above) for three weeks to assess nematode infection and propagation.

Direct applications

Adult *D. v. virgifera* were placed individually into a small container (diameter 60 mm, 80 mm high). Using a pipette, suspensions with 50 or 100 infective juveniles of each of the eight aforementioned nematode species were applied in 1 ml sterile water directly onto the adult body (van der Burgt *et al.*, 1998). After nematode application, each adult of *D. v. virgifera* was placed in a Petri dish (diameter 9 cm) supplied with moist filter paper and a drop of diet and reared for eight days (14L:10D; 25°C day, 15°C night). After eight days, dead adults were individually placed in nematode traps for one week to assess nematode infection.

Rating of the biological control potential of entomopathogenic nematodes

In order to determine their potential for further field testing as biological control agents, the entomopathogenic nematode species were rated with regard to the results obtained from each experiment. Virulence in small- and medium-volume arenas, propagation in larvae, as well as current production costs and availability in Europe were used as criteria for rating the nematode species investigated (Ehlers, 2001, 2003). The significant differences displayed in figs 2 to 4 were rated such that each significant difference (a, b, c and d) was given a full value (1, 2, 3 and 4), and intermediate significance letters (ab, bc, and cd) received corresponding numerical values (1.5, 2.5 and 3.5). The commercial feasibility was rated such that large-scale European rearing capabilities in liquid culture were ranked the highest, and assigned a value of 3, small-scale liquid culture were ranked as 2, and solid-state culture were ranked the lowest with a corresponding value of 1. The rating of the virulence results from the medium-volume arenas was weighed by four in order to give it the same weight as the virulence results of the four nematode concentrations in the small-volume arena. The arithmetic mean of all ratings provided the mean rating of the nematode species for further field testing recommendations.

Results

Virulence of nematode species against D. v. virgifera *larvae*

The infection rates of *D. v. virgifera* larvae by the different nematodes are compared among the four different nematode concentrations (fig. 1). For the two lowest nematode concentrations (0.5 and 0.8 cm^{-2}), data were pooled because there was no significant difference in mortality between these concentrations (independent sample *t*-test, $F = 0.07$, $t = 0.21$, $df = 15$, $P = 0.084$). At low concentrations of 0.5–0.8 or 1.6 cm^{-2}, *S. glaseri, S. arenarium, S. kraussei* and *S. feltiae* caused a mortality of 15% to 40%. In contrast, *S. abassi, S. bicornutum, S. carpocapsae* and *H. bacteriophora* were only effective at higher concentrations of 7.9 or 15.9 cm^{-2}. *Steinernema glaseri, S. arenarium, S. bicornutum, S. kraussei, S. carpocapsae* and *H. bacteriophora* showed a significant increase in virulence with increasing nematode concentration (fig. 1).

In fig. 2, the infection rates of *D. v. virgifera* larvae are compared among the different nematodes screened. At the lowest nematode concentration (0.5 to 0.8 $cm^{-2)}$, *S. glaseri*, *S. kraussei* and *H. bacteriophora* were the most virulent, causing mortality of 23.3%, 14.5% and 7.8%, respectively (fig. 2). At nematode concentrations of 1.6 cm^{-2}, *S. glaseri, S. feltiae* and *S. arenarium* caused highest mortality with 40%, 25% and 20%. At higher concentrations of 7.9 and 15.9 cm^{-2}, *S. glaseri, S. arenarium, S. abassi* and *H. bacteriophora* caused the highest mortality, and *S. bicornutum* and *S. kraussei* caused moderate mortality (fig. 2). At those concentrations, *S. feltiae* and *S. carpocapsae* caused significantly lower mortality than the other nematode species.

The five species that were tested further, *S. glaseri, S. arenarium, S. feltiae, S. kraussei* and *H. bacteriophora*, demonstrated the ability to infest the larvae of *D. v. virgifera* in medium-volume arenas with maize plants (fig. 3). *Steinernema arenarium* infected 67 ± 3.5% of the *D. v. virgifera* larvae; *S. feltiae* infected 57 ± 17.1%, *H. bacteriophora* 77 ± 16.6% and *Steinernema kraussei* 53 ± 5.5% (unpaired *t*-test with $P > 0.005$). *Steinernema glaseri* infected significantly less host larvae (23 ± 4.3%; unpaired *t*-test at $P < 0.05$).

Propagation of nematode species in D. v. virgifera *larvae*

Infective juvenile production of entomopathogenic nematodes within *D. v. virgifera* larvae is summarized in fig. 4. High numbers of infective juveniles per host larva were found in *S. bicornutum* (5970 ± 779 juveniles $n = 15$), *S. abassi* (5595 ± 811, $n = 10$), *S. carpocapsae* (5341 ± 1177; $n = 16$), and *H. bacteriophora* (4039 ± 1025, $n = 9$). Despite being significantly less than most of the aforementioned species, a moderate number of infective juveniles were produced by *S. arenarium* (3336 ± 234, $n = 35$) (Mann-Whitney U test: *S. arenarium* vs. *S. bicornutum*, $P = 0.016$; *S. arenarium* vs. *S. abassi*, $P = 0.005$; *S. arenarium* vs. *S. carpocapsae*, $P = 0.028$; *S. arenarium* vs. *H. bacteriophora*, $P = 0.909$). Significantly fewer infective juveniles were produced by *S. glaseri* (1598 ± 223, $n = 29$), *S. feltiae* (2204 ± 268, $n = 12$), and *S. kraussei* (1954 ± 315, $n = 14$) (Mann-Whitney U test: *S. glaseri*, *S. feltiae* and *S. kraussei* vs. *S. arenarium*, $P = 0.00$, $P = 0.005$ and $P = 0.003$).

https://doi.org/10.1079/BER2005379

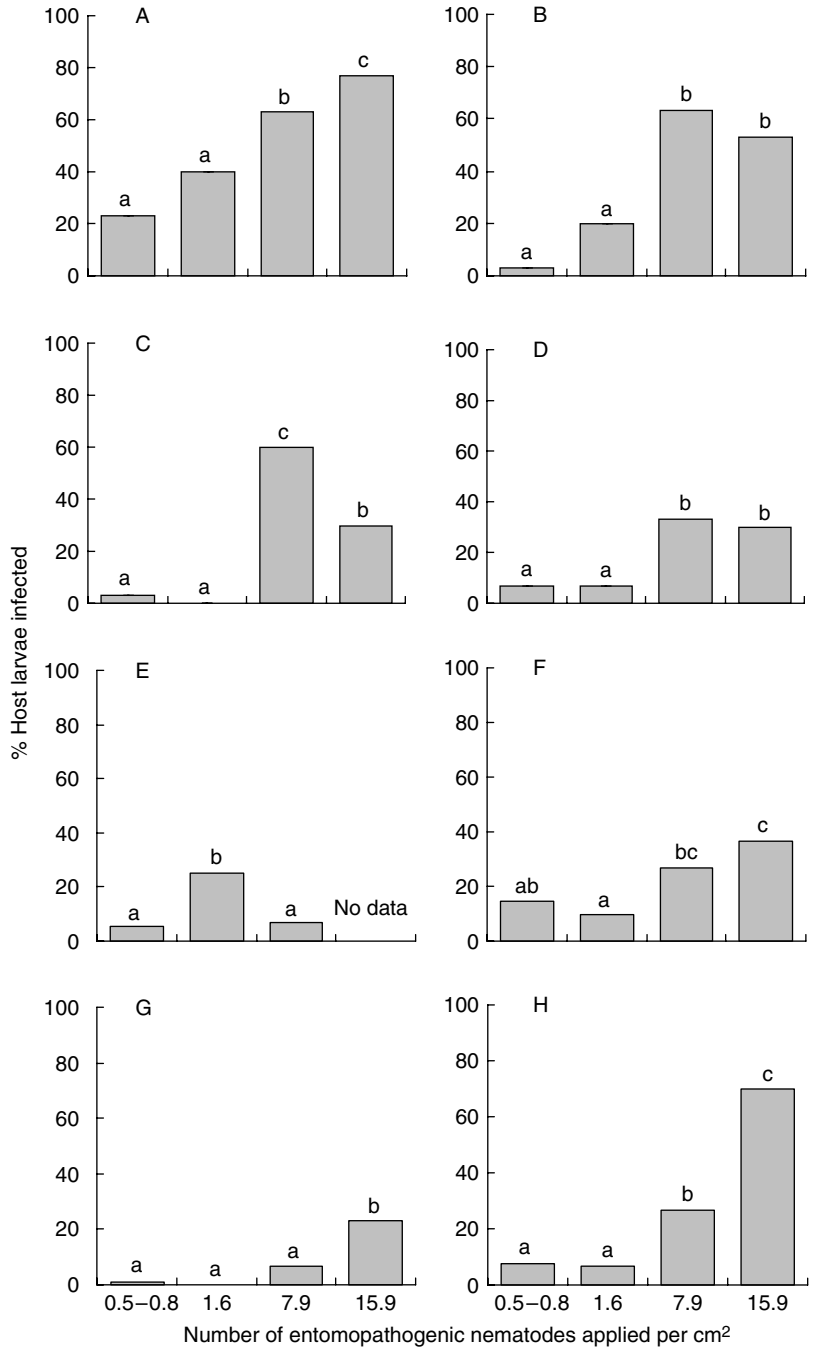

Fig. 1. Virulence of *Steinernema glaseri* (A), *S. arenarium* (B), *S. abassi* (C), *S. bicornutum* (D), *S. feltiae* (E), *S. kraussei* (F), *S. carpocapsae* (G) and *Heterorhabditis bacteriophora* (H) on *Diabrotica v. virgifera* larvae at different nematode concentrations in small-volume arenas. Percent infection of *Diabrotica v. virgifera* larvae seven days after nematode application; 30 arenas per nematode species and nematode concentration; letters on bars indicate significant differences at $P < 0.05$ using multiple comparisons with ANOVA posthoc test, Fishers PLSD.

Virulence of nematode species against D. v. virgifera *adults*

Regardless of whether nematodes were applied directly or indirectly, *Diabrotica v. virgifera* adults were generally not infected. Only a single *Diabrotica* adult out of all treatments was infected by *H. bacteriophora*. The natural mortality of *D. v. virgifera* adults in both the test and control groups was low, ranging from 6% to 7% (two out of 30 beetles tested).

https://doi.org/10.1079/BER2005379

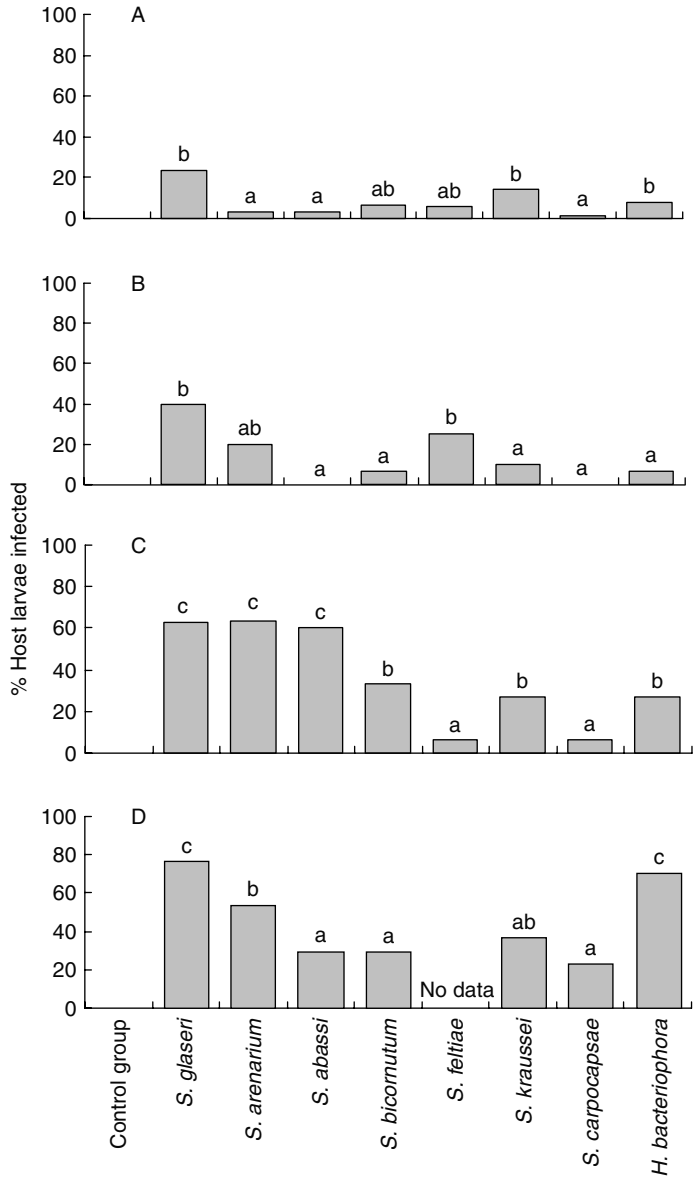

Fig. 2. Comparison of virulence among entomopathogenic nematode species on *Diabrotica v. virgifera* larvae in small-volume arenas. Percent infection of *Diabrotica v. virgifera* larvae seven days after application of nematodes at concentrations of 0.5–0.8 cm^{-2} (A), 1.6 cm^{-2} (B), 7.9 cm^{-2} (C) and 15.9 cm^{-2} (D); 30 arenas per nematode species and nematode concentration; letters on bars indicate significant differences at $P < 0.05$ using multiple comparisons with ANOVA posthoc test, Fishers PLSD.

Rating of the biological control potential of entomopathogenic nematodes

Heterorhabditis bacteriophora, with a mean value of 2.9, showed the highest potential for further testing as a candidate biological control agent under European field conditions (table 2). This was followed by *S. arenarium* with a value of 2.3, and *S. feltiae* with a value of 2.1. *Steinernema kraussei* and *S. glaseri* were of secondary priority for further testing whereas *S. abassi, S. carpocapsae* and *S. bicornutum* were ranked lowest.

Discussion

From this study it can be concluded that all the entomopathogenic nematode species tested showed the ability to infest the larval stages of *D. v. virgifera* and to produce offspring, whereas adults were generally not attacked. This reflects very much the natural situation in North and South America, where entomopathogenic nematodes have been reported to infest the larval stages of *Diabrotica* species almost exclusively (Elsey, 1977). A single study by van der Burgt *et al.* (1998) reports the successful

https://doi.org/10.1079/BER2005379

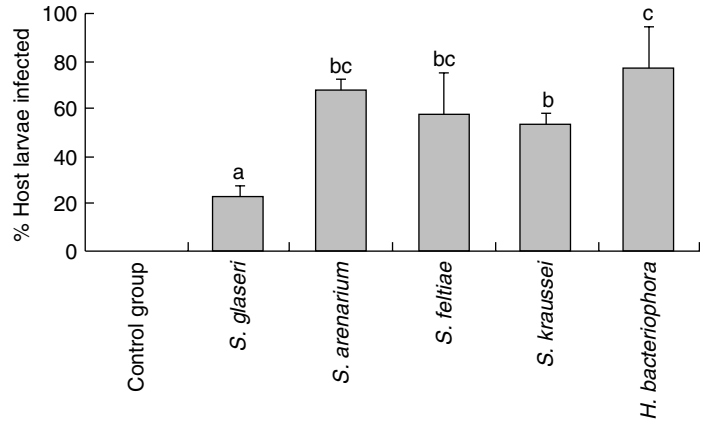

Fig. 3. Comparison of virulence among entomopathogenic nematode species on *Diabrotica v. virgifera* larvae in medium-volume arenas with maize plants. Percent infection of *Diabrotica v. virgifera* larvae seven days after nematode application, at a concentration of 16.7 cm^{-2}; 20 arenas per nematode species; letters on bars indicate significant differences at $P < 0.05$ using an independent sample t-test.

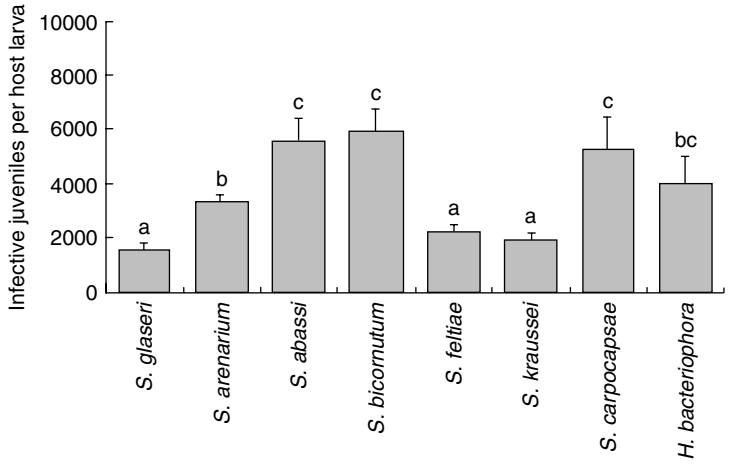

Fig. 4. Infective juveniles of entomopathogenic nematodes per individual larvae of *Diabrotica v. virgifera* in small-volume arenas. Propagation rates at different nematode concentrations 0.5, 0.8, 1.6, 7.9 and 15.9 nematodes cm^{-2} are averaged (30 replicates each); letters on bars indicate significant differences at $P < 0.05$ using an independent sample Mann-Whitney U-test).

Table 2. Mean rating of the potential of entomopathogenic nematodes tested as biological control agents against *Diabrotica v. virgifera* larvae based on commercial feasibility of entomopathogenic nematodes and according to significant differences shown in figs 2 to 4. These significant differences were rated such that each significant difference (a, b, c and d) was given a full value (1, 2, 3 and 4), and intermediate significance letters (ab, bc and cd) received corresponding numerical values (1.5, 2.5 and 3.5). The commercial feasibility was rated such that large-scale European rearing capabilities in liquid culture were ranked the highest, and assigned a value of 3, small-scale liquid culture were ranked as 2, and state solid culture were ranked the lowest with a corresponding value of 1.

Factor	Nematodes cm^{-2}	*H. bacteriophora*	*S. arenarium*	*S. feltiae*	*S. kraussei*	*S. glaseri*	*S. abassi*	*S. carpocapsae*	*S. bicornutum*
Virulence									
Small-volume arena	0.5/0.8	2	1	1.5	2	2	1	1	1.5
	1.6	1	1.5	2	1	2	1	1	1
	7.9	2	3	1	2	3	3	1	2
	15.9	3	2	–	1.5	3	1	1	1
Medium-volume arena*	16.7	3	2.5	2.5	2	1	–	–	–
Propagation	Pooled	2.5	2	1	1	1	3	3	3
Commercial feasibility		3	1	3	3	2	1	3	1
Mean rating		2.85	2.30	2.10	2.05	1.80	1.67	1.67	1.58

*Weighed by four in order give the results from the medium-volume arena the same weight as the results from the small-volume arena.

https://doi.org/10.1079/BER2005379

infection of Hungarian *D. v. virgifera* adults with three European strains of *H. bacteriophora* (at rates of 80–95%) and two European strains of *S. feltiae* (at rates of 50–65%). However, in comparison to the present study, van der Burgt *et al.* (1998) applied twice the number of nematodes per adult, potentially resulting in higher infections. Additionally, van der Burgt *et al.* (1998) reported low propagation rates of 3 to 200 infective juveniles per *Diabrotica* adult, suggesting that adult *D. v. virgifera* are not optimal hosts for entomopathogenic nematode reproduction. Furthermore, Jackson & Brooks (1989) reported that *Diabrotica* adults have an immune response to entomopathogenic nematode infective juveniles, resulting in encapsulation and death of invading nematodes. Thus, the biological control of adult *D. v. virgifera* using entomopathogenic nematodes does not appear to be a promising management option (Levine & Oloumi, 1991).

Despite the lack of success using entomopathogenic nematodes against adult western corn rootworm, all eight nematode species tested were able to infest *D. v. virgifera* larvae and propagate successfully. The mortality in *D. v. virgifera* larvae reached up to 23% at low nematode concentrations of 0.5–0.8 cm^{-2} and up to 77% at concentrations of 15.9 cm^{-2} in small-volume arenas. Virulence varied among the nematode species used, the concentrations applied, and the volume of experimental arenas. Therefore, differences in the virulence and propagation of different species were used to rate their efficacy. A high efficacy of an entomopathogenic nematode species is one important characteristic required for the successful biological control of a pest using nematodes; however, commercial feasibility (with respect to the potential of mass propagation) is also important (Gouge & Shapiro-Ilan, 2003). Thus, the commercial feasibility of each nematode species tested was rated. The arithmetic mean of those ratings of virulence, propagation, and commercial feasibility of each nematode species was used as a tool to better judge its potential for further field testing as a biological control agent (table 2). The results of this rating are discussed species by species in descending order of importance:

1. *Heterorhabditis bacteriophora* appeared to be the most promising candidate for biological control of *D. v. virgifera* in Europe, as this species: (i) caused the highest mortality in *D. v. virgifera* larvae in the medium volume arenas (70–100%); (ii) showed increased virulence in small-volume arenas as nematode concentrations increased (up to 70% infected host larvae at 15.9 nematodes cm^{-2}); (iii) resulted in 4039 ± 1025 infective juveniles per host larva; and (iv) is commercially available at relatively low production costs in liquid culture (Ehlers, 2001).

2. *Steinernema arenarium*, which is closely related to *S. glaseri*, has been isolated from several European countries (A. Peters, personal communication, 2004). *Steinernema arenarium* appeared to be highly virulent in medium-volume arenas (60–80% mortality) as well as in small-volume arenas (up to 50% mortality), and performed a moderate propagation of 3000 infective juveniles per *D. v. virgifera* larva. This species occasionally showed incomplete propagation at high application concentrations, which was probably the result of limited nutrition available in single *D. v. virgifera* larvae. A detriment to the use of *S. arenarium* in field trials is that it is not easily mass produced in liquid culture, and is thus less economically and commercially feasible (Ehlers, 2001).

3. *Steinernema feltiae* demonstrated variable virulence in both medium and small volume arenas. Its propagation was relatively low (2204 ± 268 infective juveniles per host larva). *Steinernema feltiae* is known to be a generalist, with only moderate efficacy against specific insect targets (Peters *et al.*, 1996). In addition, previous research on *Diabrotica barberi* Smith & Lawrence has shown that *S. feltiae* may not be able to reach larvae that are hidden in maize roots (Thurston & Yule, 1990). Therefore, it is difficult to predict its biological control potential against *D. v. virgifera*. However, commercial production of *S. feltiae* can be done at comparatively low costs, and thus it is recommended for further testing against *D. v. virgifera* under field conditions.

4. *Steinernema kraussei* infected *D. v. virgifera* larvae at moderate levels in small-volume arenas with relatively low propagation rates, and infected about 50% of the *D. v. virgifera* larvae in medium volume arenas. *Steinernema kraussei* is only of secondary priority for testing as a biological control agent in the field as this species prefers relatively low soil temperatures (D. Sturhan, personal communication, 2004), which is in conflict with the thermal requirements of *D. v. virgifera* and maize (Toepfer & Kuhlmann, 2004a).

5. *Steinernema glaseri* was the only species that resulted in 20–40% mortality of *D. v. virgifera* larvae at low nematode concentrations in the small-volume arena. Moreover, it highly increased its virulence with increasing nematode concentrations (up to 77% infected host larvae at 15.9 nematodes cm^{-2}). For unknown reasons, *S. glaseri* infected significantly fewer larvae in the medium-volume arenas compared to the other nematode species; despite this, approximately 20% of *D. v. virgifera* larvae still died due to infection by *S. glaseri*. Similar results have been reported by Riga *et al.* (2001) in the control of *D. v. virgifera* larvae with *S. glaseri* in greenhouse experiments in Canada. A detriment to the use of *S. glaseri* in field trials is the fact that it is not easily mass produced in liquid culture (Ehlers, 2001), and that it is still being debated as to whether this species naturally occurs in Europe (A. Peters & D. Sturhan, personal communication, 2004).

6. *Steinernema abassi* was not tested in medium-volume arenas as it originates from the Middle East (Palestine) and is therefore likely to be restricted for field use in some European countries. However, this species showed a fairly rapid and high infection level of *D. v. virgifera* larvae, with propagation rates of up to 6000 infective juveniles per larva. This species might also be adapted to warm and dry regions, and therefore suited to the current major *D. v. virgifera* population areas in central and south-eastern Europe such as Hungary, Romania, Serbia and Croatia (Kiss *et al.*, 2005).

7. *Steinernema carpocapsae* has been investigated in North America as a potential biological control agent against *D. v. virgifera* (Gaugler, 1981; Jackson & Brooks, 1989, 1995; Riga *et al.*, 2001). *Steinernema carpocapsae* has been applied together with liquid fertilizers at the time of maize sowing, and resulted in significantly fewer *Diabrotica* beetles in treated areas compared with untreated and clopyrifos treated areas (Poinar *et al.*, 1983). *Steinernema carpocapsae* was found to complete its life cycle in second and third instar larvae, as well as in pupae of *D. v. virgifera* in the USA (Jackson & Brooks, 1995). Journey & Ostlie (2000) reported acceptable root protection in Minnesota, USA, when 10^6 to 10^7 *S. carpocapsae* per 30.5 cm row were applied when second or third instar larvae of *D. v. virgifera* were present in the

field; conversely early applications during the egg stage or first instar larvae were less effective. In the present study, *S. carpocapsae* applied to third instar larvae resulted in the lowest mortality among all nematodes tested in the small-volume arena and was therefore not tested further. This is in contrast to the high infection levels of up to 90% found under laboratory conditions in the United States (Nickle *et al.*, 1994), indicating that the origin of the strains tested may affect their virulence. However, as this nematode species typically displays an ambusher behaviour (Gaugler, 2002), it is likely that this species is less adapted to control *Diabrotica* larvae under field conditions, as it fails to actively search the soil for potential hosts.

8. *Steinernema bicornutum* was not tested in medium-volume arenas as its virulence in the small-volume arenas resulted in a moderate efficacy, making this species a less suitable candidate for biological control purposes.

In conclusion, *H. bacteriophora* demonstrated the highest potential for further field testing as a biological control agent against *D. v. virgifera* larvae in Europe (table 2), followed by *S. arenarium* and *S. feltiae*. Adding to their appeal as potential biological control agents is the fact that *Heterorhabditis bacteriophora* and *S. feltiae* are available at reasonable product costs as they have high reproductive capacities in liquid culture (Ehlers, 2001). Open field tests to control *Diabrotica* species with nematodes have often yielded variable results in North America (Kuhlmann & van der Burgt, 1998). Based on the results obtained in North American studies using entomopathogenic nematodes against *D. v. virgifera*, and based on the fact that the screenings in this study were carried out in sand and under laboratory conditions, it is clear that a thorough understanding regarding the establishment parameters of each nematode in field soil following application and regarding the nematodes' host finding strategies in the soil and in the maize root are required (Thurston & Yule, 1990; Levine & Oloumi, 1991). It is therefore recommended to test the aforementioned 'promising' nematode species at different soil-surface moistures and soil texture conditions (Barbercheck & Warrick, 1997) as well as with different application techniques and timing (Jackson & Brooks, 1995; Journey & Ostlie, 2000). In the event that an inoculative control strategy using entomopathogenic nematodes for the control of *D. v. virgifera* is considered, the nematodes' potential to persist and the role of alternative hosts in the soil food-web (Brust, 1991) would become essential and would need to be addressed.

Acknowledgements

The authors gratefully acknowledge the continuous supply of *D. v. virgifera* eggs by Chad Nielson (USDA-ARS Northern Grain Insect Research Laboratory, Brookings, South Dakota, USA). We thank Virginia Larraz and Dr Feng Zhang for technical assistance during the experiments. Regina Burger (Landi REBA, Switzerland) gave input on commercial aspects in potentially applying nematodes against *D. v. virgifera*. We thank Dr Stefan Kuske (Agroscope, FAW Waedenswil, Switzerland) and Dr Arne Peters (e-nema GmbH, Raisdorf, Germany) for their detailed and helpful comments on the manuscript draft. We also thank Tara Gariepy (Saskatoon, Canada) and Lars Andreassen (Winnipeg, Canada) for reviewing the English text. This research was funded by Landi REBA, Basel, Switzerland, CABI Bioscience, Delémont, Switzerland, and e-nema GmbH, Raisdorf, Germany.

References

Baca, F. (1994) New member of the harmful entomofauna of Yugoslavia *Diabrotica virgifera virgifera* LeConte (Coleoptera, Chrysomelidae). *Zastita Bilja* **45**, 125–131.

Barbercheck, M.E. (1993) Tritrophic level effects on entomopathogenic nematodes. *Environmental Entomology* **22**, 1166–1171.

Barbercheck, M.E. & Warrick, W.C. Jr. (1997) Evaluation of trap cropping and biological control against southern corn rootworm (Coleoptera: Chrysomelidae) in peanuts. *Journal of Entomological Science* **32**, 229–243.

Baufeld, P. & Enzian, S. (2005) Maize high-risk areas and potential yield losses due to western corn rootworm (*Diabrotica. v. virgifera* LeConte) damage in selected European countries. pp. 285–302 *in* Vidal, S., Kuhlmann, U. & Edwards, C.R. (*Eds*) *Western corn rootworm: ecology and management*. Wallingford, Oxon, CABI Publishing.

Brust, G.E. (1991) Augmentation of an endemic entomogenous nematode by agroecosystem manipulation for the control of a soil pest. *Agriculture Ecosystems and Environment* **36**, 175–184.

Chiang, H.C. (1973) Bionomics of the northern and western corn rootworms. *Annual Review of Entomology* **18**, 47–72.

Ehlers, R.U. (2001) Mass production of entomopathogenic nematodes for plant protection. *Applied Microbiology and Biotechnology*. **56**, 623–633.

Ehlers, R.U. (2003) Entomopathogenic nematodes in the European biocontrol market. *Communications in Agriculture and Applied Biological Sciences* **68**, 3–16.

Elsey, K.D. (1977) Parasitism of some economically important species of Chrysomelidae by nematodes of the genus *Howardula*. *Journal of Invertebrate Pathology* **29**, 384–385.

Gaugler, R. (1981) Biological potential of neoplectanid nematodes. *Journal of Nematology* **14**, 126–131.

Gaugler, R. (2002) *Entomopathogenic nematology*. Wallingford, Oxon, CABI Publishing.

Georgis, R., Kaya, H.K. & Gaugler, R. (1991) Effect of steinernematid and *Heterorhabditis* nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) on non-target arthropods. *Environmental Entomology* **20**, 814–822.

Gouge, D.H. & Shapiro-Ilan, D.I. (2003) Case studies in cotton and citrus: use of entomopathogenic nematodes. *Indian Journal of Nematology* **33**, 91–102.

Jackson, J.J. (1996) Field performance of entomopathogenic nematodes for suppression of western corn rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* **89**, 366–372.

Jackson, J.J. & Brooks, M.A. (1989) Susceptibility and immune response of western corn rootworm larvae (Coleoptera: Chrysomelidae) to the entomogenous nematode, *Steinernema feltiae* (Rhabditida: Steinernematidae). *Journal of Economic Entomology* **82**, 1073–1077.

Jackson, J.J. & Brooks, A. (1995) Parasitism of western corn rootworm larvae and pupae by *Steinernema carpocapsae*. *Journal of Nematology* **27**, 15–20.

Jackson, J.J. & Hesler, S.L. (1996) Placement and application rate of the nematode *Steinernema carpocapsae* (Rhabditida: Steinernematidae) for suppression of the western corn

rootworm (Coleoptera: Chrysomelidae). *Journal of the Kansas Entomological Society* **68**, 461–467.

Journey, A.M. & Ostlie, K.R. (2000) Biological control of the western corn rootworm (Coleoptera: Chrysomelidae) using the entomopathogenic nematode, *Steinernema carpocapsae*. *Environmental Entomology* **29**, 822–831.

Kaya, H.K. & Stock, S.P. (1997) Techniques in insect nematology. pp. 281–324 *in* Lacey, L.A. (*Ed.*) *Manual of techniques in insect pathology*. London, Academic Press.

Kinnear, P.R. & Gray, C.D. (2000) *SPSS for windows made simple*. East Sussex, Psychology Press.

Kiss, J., Edwards, C.R., Berger, H., Cate, P., Cean, M., Cheek, S., Derron, J., Festic, H., Furlan, L., Igrc-Barcic, J., Lammers, W., Omelyuta, V., Princzinger, G., Reynaud, P., Sivcev, I., Sivicek, P., Urek, G. & Vahala, O. (2005) Monitoring of western corn rootworm (*Diabrotica v. virgifera* LeConte) in Europe 1992–2003. pp. 29–40 *in* Vidal, S., Kuhlmann, U. & Edwards, C.R. (*Eds*) *Western corn rootworm: ecology and management*. Wallingford, Oxon, CABI Publishing.

Krysan, J.L. & Miller, T.A. (1986) *Methods of study for pest* Diabrotica. New York, Springer.

Kuhlmann, U. & van der Burgt, W.A.C.M. (1998) Possibilities for biological control of the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, in Central Europe. *Biocontrol News and Information* **19**, 59–68.

Kuhlmann, U., Toepfer, S. & Zhang, F. (2005) Is classical biological control against western corn rootworm in Europe a potential sustainable management strategy? pp. 263–284 *in* Vidal, S., Kuhlmann, U. & Edwards, C.R. (*Eds*) *Western corn rootworm: ecology and management*. Wallingford, Oxon, CABI Publishing.

Levine, E. & Oloumi, S.H. (1991) Management of diabroticite rootworms in corn. *Annual Review of Entomology* **36**, 229–255.

Nickle, W.R., Connick, W.J. Jr. & Cantelo, W.W. (1994) Effects of pesta-pelletized *Steinernema carpocapsae* (All) on western corn rootworms and Colorado potato beetles. *Journal of Nematology* **26**, 249–250.

Peters, A., Huneke, K. & Ehlers, R.U. (1996) Host finding by the entomopathogenic nematode *Steinernema feltiae*. *IOBC/WPRS Bulletin* **19**, 99–102.

Poinar, G.O. Jr., Evans, J.S. & Schuster, E. (1983) Field test of the entomogenous nematode, *Neoaplectana carpocapsae*, for control of corn rootworm larvae (*Diabrotica* sp., Coleoptera). *Protection Ecology* **5**, 337–342.

Riga, E., Wistlecraft, J. & Potter, J. (2001) Potential of controlling insect pests of corn using entomopathogenic nematodes. *Canadian Journal of Plant Sciences* **81**, 783–787.

Singh, P. & Moore, R.F. (1985) *Handbook of insect rearing*. Amsterdam, Elsevier Science.

Sivcev, I., Manojlovic, B., Krnjajic, S., Dimic, N., Draganic, M., Baca, F., Kaitovic, Z., Sekulic, R. & Keresi, T. (1994) Distribution and harmful effect of *Diabrotica virgifera* LeConte (Coleoptera, Chrysomelidae), a new maize pest in Yugoslavia. *Zastita Bilja* **45**, 19–26.

Thurston, G.S. & Yule, W.N. (1990) Control of larval northern corn rootworm (*Diabrotica barberi*) with two steinernematid nematode species. *Journal of Nematology* **22**, 127–131.

Toepfer, S. & Kuhlmann, U. (2004a) IPM strategies for the western corn rootworm (Coleoptera: Chrysomelidae): current status and potential adoptations to the European maize productions system. *Mitteilungen der Deutschen Gesellschaft für Allgemeine und Angewandte Entomologie* **14**, 391–398.

Toepfer, S. & Kuhlmann, U. (2004b) Search for natural enemies of the invasive alien chrysomelid, *Diabrotica virgifera virgifera*, in Central Europe. *BioControl* **49**, 385–395.

van der Burgt, W.A.C.M., Budai, C., Fodor, A. & Lucskai, A. (1998) Parasitism of western corn rootworm adults by *Steinernema* spp. and *Heterorhabditis* spp. *Proceedings of the Society for Experimental and Applied Entomology* **9**, 165–170.

Wright, R.J., Witkovski, J.F., Echtenkamp, G. & Georgis, R. (1993) Efficiency and persistence of *Steinernema carpocapsae* (Rhabditida: Steinernematidae) applied through center-pivot irrigation system against larval corn rootworms (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* **86**, 1348–1354.

(Accepted 17 May 2005)