# SUSCEPTIBILITY OF CABBAGE ROOT MAGGOT, *DELIA RADICUM*, TO ENTOMOPATHOGENIC NEMATODES (STEINERNEMATIDAE AND HETERORHABDITIDAE)

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**Summary.** Susceptibility of last instar maggots of *Delia radicum* to 13 isolates of five *Steinernema* (*S. feltiae*, *S. carpocapsae*, *S. affine*, *S. arenarium* and *S. glaseri*) and four isolates of *Heterorhabditis* (*H. megidis* and *H. bacteriophora*) was evaluated in the laboratory. Each of the populations controlled the maggot to some extent but differed in efficiency. When exposing one maggot to ten infective juveniles (IJs), *S. feltiae* isolates showed the highest efficiency: 12-32% mortality at two days after infection (dai) and 26-73% mortality at four dai. *S. glaseri* had the lowest pathogenicity (5.6% mortality at four dai), whereas *S. carpocapsae*, *S. affine*, *S. arenarium*, *H. megidis*, and *H. bacteriophora* were intermediately active (8-20% mortality at four dai). Comparisons of *S. feltiae* populations showed Lxm31 as the best performing one. Experiments in which last instar maggots were exposed during 48 hrs to 12, 25, 50, 100, or 200 IJs yielded a $LD_{50}$ of *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium*, *H. megidis* Sn5 and *H. bacteriophora* NJ-hbr of 12, 102, 258, 196, and 344 IJs per larva, respectively. As the effect of *S. glaseri* on maggots was too weak, a linear relation between dose and mortality was not found. The $LT_{50}$ for *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium*, *H. megidis* Sn5 and *H. bacteriophora* (NJ-hbr) was calculated at 17, 82, 192, 77, and 104 hours per larva, respectively.

The cabbage root maggot, *Delia radicum* (Diptera: Anthomyiidae), is a destructive pest of cruciferous crops throughout Europe and North America (Finch, 1989). It is the most serious pest of cauliflower and cabbage but also damages Brussels sprout, radish, turnip and garden brassica (McKinlay, 1992). The adult fly lays eggs mainly at the base of the plant stem but also at other plant parts such as the fold of the enveloping leaflets of the Brussels sprout buttons. The hatched maggots burrow into the roots or the sprouts and plants may be attacked at all growth stages. The most serious damage, however, is usually observed on young plants, which can be killed if severely attacked in the seedbed or shortly after transplanting.

Insecticides are frequently used to control the cabbage root fly. Increased awareness of environmental hazards and toxicity of insecticides as well as the resistance of insects to insecticides has prompted the search for alternative means of pest control. Biological control using entomopathogenic nematodes (epn) is one possibility. Species of the Steinernematidae and Heterorhabditidae are lethal insect parasites that have a mutualistic association with bacteria of the genera *Xenorhabdus* and *Photorhabdus*, respectively. They are successful in the control of many insects (Klein, 1990).

When searching for effective nematodes, it is preferable to screen species rather than isolates, because there is greater variation between species than between isolates (Bedding, 1990). Besides the intrinsic qualities of the species, epn efficiency is highly affected by the dose (Hominick and Reid, 1990). There is a positive correlation between the insect mortality and the dose of nematodes applied (Forschler and Nordin, 1988; Glazer and Navon, 1990; Peters and Ehlers, 1994).

Knowing and understanding the differences in infectivity between epn species and isolates is necessary. Prediction of the insect mortality by the establishment of a relation between the nematode dose and efficacy, and the exposure time and efficacy, provides vital information for making strategic decisions in biological control. In this paper we report on a series of experiments to determine the efficacy of different epn species and isolates – mainly collected in Belgium – against *D. radicum*.

## MATERIALS AND METHODS

**Nematodes.** The nematode species and isolates used in this study were selected on a geographical basis and are shown in Table I. They were cultured in last-instar larvae of *Galleria mellonella* L. (Woodring and Kaya, 1988) and stored in distilled water at 5 °C for a period of no longer than two weeks. The nematodes were kept at room temperature (18-22 °C) for 24 hours before using.

**Insect.** *Delia radicum* Bouché maggots were collected from the edges of cauliflower fields in the province of West Flanders in Belgium and reared on rutabaga (*Brassica napus napobrassica* L. Rchb.) at 20 °C according the method described by Harris and Svec (1966).

**Susceptibility of *D. radicum* to different species and isolates.** The susceptibility of last stage larvae of *D. radicum* to epn was studied in 24-well plates (Falcon, Le Pont de Claix, France). Each well was filled with 2 g sterilised sand (225-425 µm) and incubated at room temperature for 24 hours before use. In each well one last instar larva of *D. radicum* was exposed to ten infective juveniles (IJs) of one of the isolates. The insect lar-

**Table I.** Species and isolates of entomopathogenic nematodes used in the study.

| Species | Isolate | Origin |
|---|---|---|
| *Steinernema feltiae* (Filipjev, 1934) Wouts, Mracek, Gerdin and Bedding, 1982 | Lxm31, Rs51, VBM5, Lxm16, Rs50, Nm30 and Ma41 | Belgium (Miduturi *et al.*, 1997) |
| | D-hbr | R.-U. Ehlers |
| | NeS | Nemasys |
| *S. affine* (Bovien, 1937) Wouts, Mracek, Gerdin and Bedding, 1982 | Hm13 and Z15 | Belgium (Miduturi *et al.*, 1997) |
| *S. carpocapsae* (Weiser, 1955) Wouts, Mracek, Gerdin and Bedding, 1982 | All | R.-U. Ehlers |
| *S. arenarium* (Artyukhovsky, 1967) Wouts, Mracek, Gerdin and Bedding, 1982 | | S.E. Spiridonov |
| *S. glaseri* (Steiner, 1929) Wouts, Mracek, Gerdin and Bedding, 1982 | | S.E. Spiridonov |
| *Heterorhabditis bacteriophora* Poinar, 1976 | NJ-hbr | R.-U. Ehlers |
| *H. megidis* Poinar, Jackson & Klein, 1987 | Sn5 and VBM30 | Belgium (Miduturi *et al.*, 1997) |
| | NeH | Nemasys |

vae that were used had been collected close to pupation; consequently there was no or very low natural larval mortality. If in a separated control treated with distilled water, insect mortality exceeded 5%, the experiment was repeated. One hundred *D. radicum* larvae were examined for each epn isolate. The plates were incubated at 20 °C and insect mortality was checked at two and four days after infection (dai). Dead larvae were dissected to ascertain that their death was caused by the epn.

**Relation between nematode dose, infectivity and *D. radicum* mortality.** *Steinernema feltiae* Lxm31, *S. carpocapsae* All, *S. glaseri*, *S. arenarium*, *Heterorhabditis megidis* Sn5, and *H. bacteriophora* NJ-hbr (Table I) were selected for the experiment conducted at 20 °C in 24-well plates (preparation as above). Each last instar larva of *D. radicum* was exposed to 12, 25, 50, 100 or 200 IJs. For each combination of nematode species and dose, 24 insect larvae were examined. Two dai, the insect larvae were washed free of nematodes and incubated on dry filter paper in a Petri dish for another two days at 20 °C. At the end of this period, dead insects were recorded and dissected in Ringer solution (Woodring and Kaya, 1988) to count the number of nematodes that had penetrated.

**Relation between exposure time, infectivity and *D. radicum* mortality.** *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium*, *H. megidis* Sn5, and *H. bacteriophora* NJ-hbr were also used in an exposure time experiment done in 24-well plates prepared as described above. In each well one last instar larva of *D. radicum* was exposed to 100 IJs for 3, 12, 24, 36, 48, 72, 96 or 120 hours. After these periods, the larvae were washed free of nematodes and incubated on dry filter paper in a Petri dish (20 °C) for the remainder of the 120 hours. Insect mortality was recorded after that period and dead insects were dissected in Ringer solution. The number of nematodes that penetrated into the insect was counted. Each combination of epn species/isolate and exposure time was examined on 24 larvae.

**Data analysis.** NCSS software package was used for all data analysis. $\chi^2$ test by making a contingency table was used to check significant differences ($P<0.05$) in susceptibility of *D. radicum* to the epn species. $LD_{50}$ and $LT_{50}$ values were calculated by probit analysis. Invasion rate of nematode species at different doses and exposure time was compared with the $\chi^2$ test.

## RESULTS

**Susceptibility of *D. radicum* to different nematodes species and isolates.** All *S. feltiae* isolates killed *D. radicum* larvae (Fig. 1). The mortality obtained with this species depended on the isolate and varied between 12 and 32% at two dai and between 26 and 73% at four dai. At both observations, insect mortality differed significantly between populations ($P<0.01$). Isolates Lxm31 and D-hbr caused the highest mortality; NeS and Rs50 caused the lowest.

Significant ($P<0.01$) differences in *D. radicum* mortality were also found between epn species (Fig. 2). At two dai all species except *S. glaseri* killed a proportion (1-17%) of *D. radicum* larvae. At four dai, *S. feltiae*, *H. megidis*, *H. bacteriophora* and *S. carpocapsae* killed 44.35%, 18.2%, 16.5% or 15.5% of the *D. radicum* larvae, respectively. The least infective species were *S. affine*, *S. arenarium* and *S. glaseri* causing mortalities of

8.3%, 8.3% and 5.6%, respectively. Dissection of dead insects showed that all species, except *S. affine*, produced offspring (data not shown).

**Relation between nematode dose, infectivity and *D. radicum* mortality.** The median lethal dosage ($LD_{50}$) of *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium*, *H. megidis* Sn5 and *H. bacteriophora* NJ-hbr was calculated at 12, 102, 258, 196 and 344 IJs per larva, respectively (Table II). For the same series of species, the $LD_{95}$ was 119, 1709, 14414, 5881 and 16583 IJs per larva. The 95% fiducial limit of both the $LD_{50}$ and $LD_{95}$ demonstrated significant differences between *S. feltiae* Lxm31 and the other species. The $LD_{50}$ and $LD_{95}$ of *S. carpocapsae* All, *S. arenarium*. *H. bacteriophora* NJ-hbr and *H. megidis* Sn5 were not significantly different.

For all epn species, the mean number of IJs that established in *D. radicum* larvae increased with increasing inoculation dose. The linear relationship between the number of nematodes established in *D. radicum* larvae and the number of nematode applied is shown in Table III. The $\chi^2$-test showed no significant differences between the mean percentages of nematodes obtained after different inoculation doses ($P>0.05$).

**Relation between the nematode exposure time, infectivity and *D. radicum* mortality.** The number of IJs extracted from *D. radicum* larvae increased with increasing exposure time (Fig. 3). The majority of IJs, however, penetrated insect larvae during the first 48 hours. The number of IJs dissected from the insects was significantly influenced by the epn species ($P<0.01$) and exposure time ($P<0.01$). The fastest infection was observed with *S. feltiae* Lxm31; it was the only species that infected *D. radicum* within three hours. *H. megidis* Sn5 infected *D. radicum* after 12 hours. *S. carpocapsae* All, *S. arenarium* and *H. bacteriophora* NJ-hbr were only detected at 24 hours.

The mortality of *D. radicum* larvae increased with increasing exposure time for all species (Fig. 4). After 24 hours *S. feltiae* Lxm31, *S. carpocapsae* All and *S. arenarium* killed more than 62.5%, 12.5% and 4.2%, respectively. For *H. megidis* Sn5 and *H. bacteriophora* NJ-hbr a mortality of 16.7 and 12.5% was observed. The $\chi^2$ test

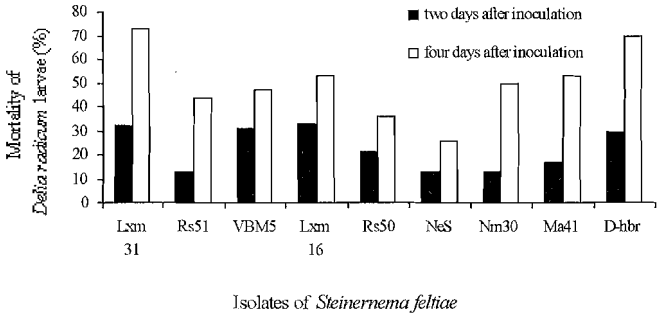


**Fig. 1.** Mortality of *Delia radicum* last instar larvae after exposure to ten infective juveniles of *Steinernema feltiae* isolates at 20 °C for two (black columns) or four days (white columns).

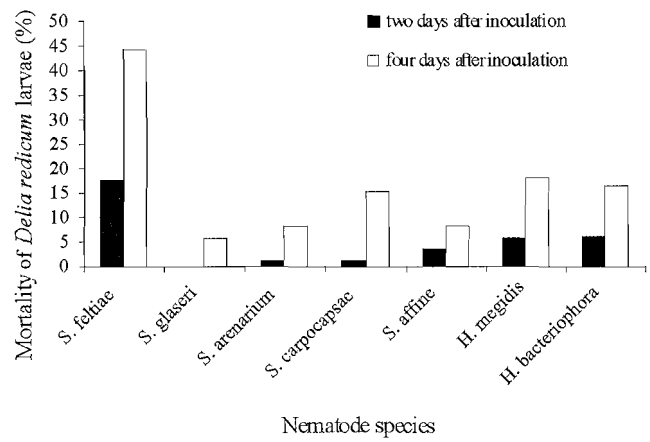


**Fig. 2.** Mortality of *D. radicum* last instar larvae after exposure to 10 infective juveniles of different species of entomopathogenic nematodes at 20 °C for two (black columns) or four days (white columns). The result for *S. feltiae, S. affine* and *Heterorhabditis megidis* is the average of 9, 2 and 3 isolates, respectively.

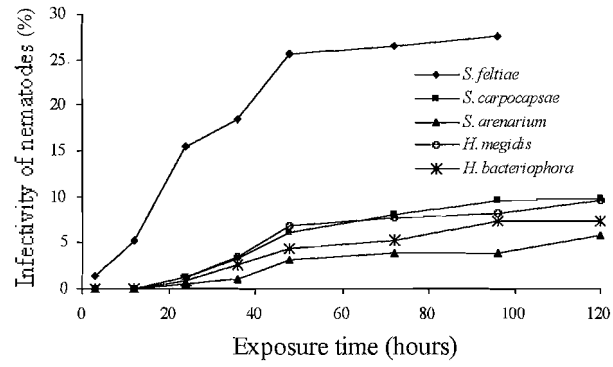


**Fig. 3.** Mean number of infective juveniles that established in the last instar larvae of *D. radicum* after exposure to 100 infective juveniles of *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium*, *H. megidis* Sn5 or *H. bacteriophora* NJ-hbr during different times. The numbers of *S. feltiae* could not be determined after 120 hours as the species developed a second generation.

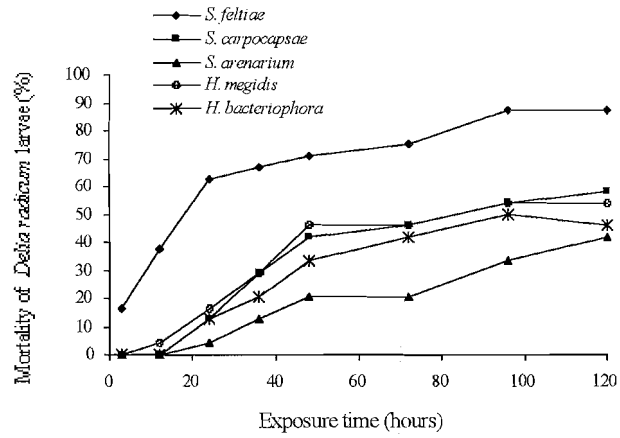


**Fig. 4.** Evolution of mortality of *D. radicum* last instar larvae after exposure to 100 infective juveniles of *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium*, *H. megidis* Sn5 or *H. bacteriophora* NJ-hbr.

**Table II.** The $LD_{50}$ and $LD_{95}$ and their fiducial limit of entomopathogenic nematode species to *Delia radicum* last stage larvae.

| Nematode species | Isolate | $LD_{50}$ (95% fiducial limit) | $LD_{95}$ (95% fiducial limit) |
|---|---|---|---|
| *Steinernema feltiae* | Lxm31 | 12 (6-21) | 119 (57-245) |
| *S. carpocapsae* | All | 102 (63-167) | 1709 (358-8142) |
| *S. arenarium* | | 258 (57-529) | 14414 (415-499997) |
| *Heterorhabditis megidis* | Sn5 | 196 (74-384) | 5881 (456-75864) |
| *H. bacteriophora* | NJ-hbr | 344 (98-1201) | 16583 (433-634453) |

**Table III.** Relationship between the number of infective juveniles (y) infecting *D. radicum* last instar stage larvae and the nematode doses (x) applied for five species of entomopathogenic nematodes.

| Nematode species | Isolate | Regression | $R^2$ |
|---|---|---|---|
| *Steinernema feltiae* | Lxm31 | y = 0.2914x + 1.6141 | 0.9913 |
| *S. carpocapsae* | All | y = 0.0735x - 0.1519 | 0.9968 |
| *S. arenarium* | | y = 0.0487x - 0.1686 | 0.9967 |
| *Heterorhabditis megidis* | Sn5 | y = 0.0543x - 0.0391 | 0.9971 |
| *H. bacteriophora* | NJ-hbr | y = 0.0385x + 0.0859 | 0.9938 |

**Table IV.** The $LT_{50}$ and $LT_{95}$ and their fiducial limit of entomopathogenic nematode species to *Delia radicum* last instar stage larvae.

| Nematode species | Isolate | $LT_{50}$ (95% fiducial limit) | $LT_{95}$ (95% fiducial limit) |
|---|---|---|---|
| *Steinernema feltiae* | Lxm31 | 17 (11-25) | 295 (89-978) |
| *S. carpocapsae* | All | 82 (60-113) | 688 (190-2490) |
| *S. arenarium* | | 192 (96-385) | 2286 (197-26591) |
| *Heterorhabditis megidis* | Sn5 | 77 (56-105) | 702 (190-2602) |
| *H. bacteriophora* | NJ-hbr | 104 (70-155) | 1020 (205-5066) |

showed that for the same nematode species *D. radicum* mortality was significantly different between different exposure times ($P<0.01$). The longer the exposure time, the higher the insect mortality. The $LT_{50}$ for *S. feltiae* Lxm31, *S. carpocapsae* All, *S. arenarium, H. megidis* Sn5 and *H. bacteriophora* NJ-hbr was 17, 82, 192, 77, and 104 hours, respectively (Table IV).

## DISCUSSION

Screening of biological pesticides in the laboratory is a standard procedure before a new antagonist is developed and used in the field. Bedding (1990) underlined the importance of testing a number of epn species against any particular insect before initiating field evaluation for biological control. Laboratory studies are essential steps for maximising the chances for success and labour saving in the field experiments.

Differences in infectivity between nematode isolates have been documented for many insect hosts (Forschler and Nordin, 1988; Griffin *et al.*, 1989). Our study confirms results obtained in previous studies (Bracken, 1990; Sulistyanto *et al.*, 1994) by demonstrating that *S. feltiae* is much more virulent against *D. radicum* than other epn species. Isolates Lxm31 and D-hbr were the most virulent. Behavioural differences or differences in the number of bacteria per IJs may account for differences in virulence among different species or isolates (Dunphy and Webster, 1986).

Pathogenicity of epn is a complex process of attraction, penetration and multiplication. Epn migrate to the host by responding to host stimuli of various natures: contact cues (Lewis *et al.*, 1992), volatile cues (Lewis *et al.*, 1993), culture temperatures (Burman and Pye, 1980) or $CO_2$ (Gaugler *et al.*, 1980). Once they enter the host's body, epn are exposed to the immune reaction of the host. If the nematode can overcome this reaction and release its symbiotic bacteria, the host will die within 48 hours. In the 24-well plates used in our study, the nematodes had no distance to overcome. Differences in attraction to the host may thus be excluded. We never observed any IJ encapsulated in insect larvae. A single nematode present in the haemocoel always caused the death of the insect (S. Chen, unpublished). As these criteria do not differentiate between the isolates, differences in pathogenicity might be explained by differences in ability to penetrate the insect body. We observed that the cuticle of *D. radicum* was difficult to penetrate directly by *Heterorhabditis* spp. On *G. mellonella* most IJs of these species accumulated at the inter-segments and there caused many black spots. On *D. radicum*, however, we observed only a few black spots. Compared to the small size of the insect host, IJs of *S. glaseri* and *S. arenarium* may be too large, which may partially explain their low infectivity. *S. carpocapsae* got

easily in contact with the insect, but only a few IJs penetrated the insect (S. Chen, unpublished).

The results of the dose-response experiments demonstrated a positive correlation between the dosage of epn and the level of control of *D. radicum*. Similar results have been found for other combinations of the epns and insects (Kaya, 1978; Simons, 1981; Molyneux *et al.*, 1983). As the slope of the regression line is an indication of the virulence of the nematode species – because it measures the infection by all individuals capable of invading and establishing within insects (Hominick and Reid, 1990) – our study proved *S. feltiae* to be the most virulent species against *D. radicum*. This agrees with most of the previous studies (Sulistyanto *et al.*, 1994; Schroeder *et al.*, 1996).

The number of nematodes that established in the host was in proportion to the dose applied. Mraček (1982) estimated that 10-20% of a dose of *S. carpocapsae* applied to 0.3 $dm^3$ of soil was recovered from twenty *G. mellonella* larvae. Fan and Hominick (1991) reported that 36% of *Steinernema* spp. (Nash isolate) could penetrate and establish in *G. mellonella*; this percentage was independent of the dose. In our study, 29% of the IJs of *S. feltiae* were recovered after 48 hours. However, only 7.4% and 5.4% of the initial dose applied were recovered for *S. carpocapsae* and *H. megidis*, respectively (Table II). Obviously, the percentage of nematodes recovered vary with both the epn and the insect species.

The $LT_{50}$ is another parameter for measuring the performance of epn. A good isolate should have a short $LT_{50}$ as after their release epn are immediately exposed to unfavourable environmental factors. The shorter the $LT_{50}$, the higher the chance to survive and to infect the host. *S. feltiae* was the only species that penetrated *D. radicum* larvae in three hours.

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