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Efficacy of the entomopathogenic nematodes *Steinernema kraussei* and *Heterorhabditis megidis* against the black vine weevil *Otiorhynchus sulcatus* in open field-grown strawberry plants

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Abstract

1 Entomopathogenic nematodes are commercially available for inundative biological control of many insects, including the black vine weevil *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae). Currently, there is a lack of commercial application tests in field-grown crops comparing the efficacy of different species of entomopathogenic nematodes.

2 Field trials were carried out under different growing conditions in Ireland and Norway to evaluate the efficacy of two commercially available nematode species on the market for control of the black vine weevil *Heterorhabditis megidis* and *Steinernema kraussei.*

3 *Heterorhabditis megidis* was evaluated not only at temperatures ideal for this species (soil temperatures above 10 °C), but also in the low temperature trials with *S. kraussei* as a 'positive control'. *Steinernema kraussei* is sold as a cold active product and was therefore evaluated at low soil temperatures (below 10 °C).

4 The overall results indicated that *H. megidis* was effective as long as temperatures were optimum (not dropping below 10 °C). For *S. kraussei*, the results obtained were rather disappointing, where control barely reached 50% in the trial with the coldest temperature. Temperature and soil type appeared to be a major limiting factor for the efficacy of both nematode species.

5 On the basis of the results and experience obtained in these trials, the future implications for biological control of *O. sulcatus* with entomopathogenic nematodes in commercial field-grown strawberry production are discussed.

Keywords Biological control, black vine weevil, Coleoptera, Curculionidae, entomopathogenic nematodes, *Heterorhabditis megidis*, *Otiorhynchus sulcatus*, soft fruit, *Steinernema kraussei*, strawberry.

Introduction

The black vine weevil *Otiorhynchus sulcatus* (F.) (Coleoptera: Curculionidae) is a serious pest of many economically important plants, including annual and perennial ornamentals and small soft fruit crops in many parts of the world, (Smith, 1932; Masaki *et al*., 1984; Moorhouse *et al*., 1992). It is endemic to temperate areas of Europe and has spread to the U.S.A., parts of temperate South America and Australasia, mainly as a result of the movement of infested plants. The adults feed on leaves during the summer months and deposit eggs on host plants such as strawberry. Developing larvae cause serious economic damage by feeding on plant roots. Larvae are usually present from July/August to May/June. All adult weevils are parthenogenetic females, whereby a single female can establish a new population reaching high densities in just a few generations. On strawberry plants, the damage threshold is estimated to be between two and eight larvae per plant, where a single larva at the base of the stem may cause more damage than several larvae at the periphery (Evenhuis, 1978). Larval development is influenced by temperature and several reports show that larvae younger than in their third instar do not survive low winter temperatures (Garth & Shanks, 1978; Hesjedal, 1982; Stenseth,

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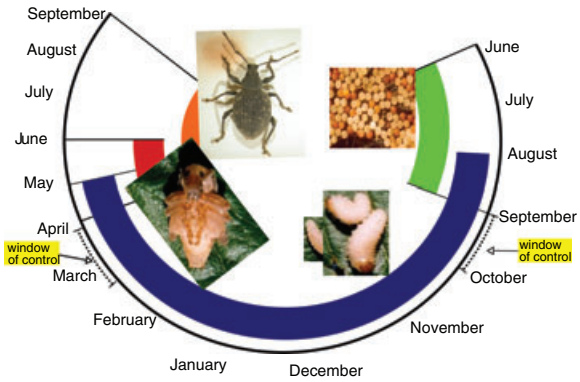

Figure 1 Generalized life cycle of the black vine weevil *Otiorhynchus sulcatus*, indicating two windows of control of larvae using entomopathogenic nematodes.

1987; Moorhouse *et al*., 1992; S. Haukeland, unpublished data). *Otiorhynchus sulcatus* appears to have adapted to this by vigorous egg production early in the season, thereby increasing the overwintering success of larvae (Son & Lewis, 2004). For direct control of larvae, there are two windows for control (Fig. 1), either targeting the larvae in autumn to reduce the population at an early stage before overwintering and/or targeting the larvae in spring before they resume feeding, ahead of pupation. Reaching the larval stages in the root zone of strawberry plants is a challenge for any control measure and usually involves large quantities of liquid or the use of more persistent pesticides (Moorhouse *et al*., 1992). In addition, larvae are often more difficult to reach when plants are grown under plastic mulch. Currently, most countries have restricted the use of toxic pesticides as a soil-drench due to increased pesticide regulations (Karabelas *et al*., 2009). The withdrawal of organochlorines (e.g. aldrin and dichlorodiphenyltrichloroethane) and a number of modern husbandry practices, such as black polythene mulch and the expansion of horticultural production, are partly responsible for the increased frequency of *O. sulcatus* infestations subsequent to the late 1980s (Lovelidge, 2008). Current control strategies include entomopathogenic nematodes (EPNs) (Kakouli-Duarte *et al*., 1997; Wilson *et al*., 1999; Fitters *et al*., 2001a, b; Willmott *et al*., 2002; Bruck *et al*., 2005; Lola-Luz *et al*., 2005; Lola-Luz & Downes, 2007; Susurluk & Ehlers, 2008), entomopathogenic fungi (Moorhouse *et al*., 1992; Bruck, 2004; Shah *et al*., 2007, 2008; Ansari *et al*., 2008) and chemical control (Neiswander, 1953; Cross *et al*., 1995; Shah *et al*., 2007; Reding & Persad, 2009).

EPNs in the families Heterorhabditidae and Steinernematidae are currently used as biological control agents against many pests, including *O. sulcatus* (Gaugler, 2002; Cowles *et al*., 2005; Georgis *et al*., 2006). EPNs have been successfully used to control *O. sulcatus* larvae in potted plants and glasshouse crops (Bedding & Miller 1981; Simons, 1981; Dolmans, 1983; Georgis & Poinar, 1984; van Tol, 1993; Cowles *et al*., 2005; Lola-Luz & Downes, 2007; Susurluk & Ehlers, 2008). In Europe, *Heterorhabditis megidis*, *Heterorhabditis bacteriophora* and *Steinernema kraussei* are sold for control of *O. sulcatus* in ornamentals and strawberry. *Heterorhabditis* species are not considered effective at low temperatures, whereas *S. kraussei* is cold active and considered effective at temperatures well below 10 °C (Long *et al*., 2000; Willmott *et al*., 2002). In the present study, we report the results obtained from field trials conducted in Norway and Ireland using two commercial nematode products, *H. megidis* and *S. kraussei,* against *O. sulcatus* larvae in field-grown strawberry plants. In Norway, two trials (1999 and 2002) aimed to test the only available nematode product available at the time (*H. megidis*) and four trials (2003–2005) aimed to evaluate the efficiency of *S. kraussei* at low temperature. In Ireland, two trials, in two separate years (2002–2003), were conducted outdoors under black polythene mulch, aiming to evaluate the efficacy of *H. megidis* for the control of *O. sulcatus*.

Materials and methods

Norway

All field trials were conducted in southern Norway in strawberry fields with a natural infestation of *O. sulcatus*. This was determined by visual observation of plants before the start of each trial. The experimental design was randomized blocks. Treatment plots consisted of single rows of 16 strawberry plants cv Polka with two buffer plants on either side. Plants were grown under black polythene mulch. In each row, plants were 25 cm apart and the distance between the rows was 1.3 m. Each treatment plot was replicated four times.

Steinernema kraussei and *H. megidis* treatments were applied as a drench either as a single or double application. The double application was performed with an approximately 1-week interval between each drench. Applications varied with suspensions of 15 000–50 000 nematodes applied as a drench in 100 mL of water per plant depending on the trial. Nematodes were supplied by Becker Underwood (U.K.). Before application, nematodes were checked for activity under a stereo microscope (MZ75; Leica, Germany). Control plots consisted of untreated plants. Soil temperatures were recorded using a Tinytag (Tinytag Plus TG12-0020; Gemeni Data Loggers, U.K.) logger placed 5–10 cm in the soil for trials 3–6. The soil type in each trial was also recorded. An overview of the experiments is provided in Table 1.

For treatments and assessments in trials 1–6: trials 1 and 2 consisted of single and double applications of *H. megidis* to target *O. sulcatus* larvae in the same season as oviposition (summer and autumn applications). Trials 3, 4 and 5 were spring treatments (April) comparing *H. megidis* and *S. kraussei* targeting larvae that had overwintered. Trial 6 was conducted as a late autumn treatment (October, *H. megidis* and *S. kraussei*), with an additional EPN treatment the next spring (Table 1). For all the autumn treatments, plants were assessed the next spring (trials 1, 2 and 6). For the summer treatment (trial 2), plants were assessed after approximately 2 months and, for the spring treatments (trials 3, 4, 5 and 6), plants were assessed 1 month after EPN application. Trials were assessed by destructive sampling of at least eight plants per plot and the number of live *O. sulcatus* larvae and pupae per plant was recorded. For trials 2–6, soil samples were collected from the root zone of each sampled plant in each plot for analysis of nematode persistence. Soil samples were mixed to give one sample per plot (four

samples per treatment). In the laboratory, three sub-samples per plot (sample) were placed in 500-mL plastic containers with perforated lids (width 6 cm, height 10 cm; Dumaplast, Belgium) and baited with three late instar *Galleria mellonella* L. (Lepidoptera: Pyralidae) larvae (Bedding & Akhurst, 1975). The containers were incubated in the dark at 17 °C for 12 days. Nematode induced mortality for the baited *G. mellonella* larvae was recorded after 6 and 12 days.

Ireland

Strawberry plants of cv Elsanta were planted in March 2002, on raised beds at Teagasc Clonroche Co., Wexford, and in March 2003 at Teagasc Kinsealy, Co. Dublin. Strawberry beds were 20 cm in height and each bed accommodated two rows of plants, each row comprising 150 plants. The distance between the rows and between plants within the row was 30 cm. Three raised polythene beds were used, with each treatment replicated ten times. A replicate consisted of 20 plants, made up of ten plants in each of the raised rows on the same bed. Each plot was separated from the next by four buffer plants on either side. The experimental design was a complete randomized block design with ten replicates of each of the five treatments. The treatments were application regimes of *H. megidis* (UK211), supplied by Becker Underwood. The five treatments were: Single (September), Single (October), Double (September and October) Triple (September, October and April) and Water only as control (four water applications). The nematode tested in both years was *H. megidis* (UK211) applied as a drench comprising of 25 000 IJs in 50 mL of water per plant. The suspension was applied around the crown of each plant and control plants received 50 mL of water in the same way. Approximately 10–15 days after the last application, all 20 plants were examined destructively and all stages of live and dead *O. sulcatus* were recorded.

In both years, *O. sulcatus* adults were collected from an infested strawberry field in County Wexford and later released on the ten experimental plots. The area where the experiments were carried out was isolated with no other immediately adjacent crops. On 29 June 2002, 750 newly-hatched *O. sulcatus* adults were released on the ten experimental plots. A second release of 100 adults was carried out on 6 July 2002. In addition on 3 September 2002, each plot was inoculated with five melanized eggs per plant. Eggs were not tested for their viability but melanized eggs were considered to be viable (Moorhouse *et al*., 1992). The eggs were obtained from David Deakin (The Old Shambles, Wellington, Herefordshire, U.K.). In 2003, a total of 3700 *O. sulcatus* adults were released on the experimental plots. In addition, each plot received 20 melanized eggs per plant. Blocks 1–5 were inoculated with eggs on 15 July 2003. The remaining blocks (6–10), were inoculated with eggs on 24 July 2003. In 2003, the eggs were obtained from a culture of newly-emerged adult *O. sulcatus* that was maintained in the laboratory by T.L.L. Briefly, 150 adult weevils were placed in each of two perforated plastic food storage containers (premier food container, 30 × 30 × 25 cm). Sand was placed at the bottom of the container and water was provided through wet cotton wool placed on Petri dishes (diameter 3 cm). Adult weevils were fed a combination of *Euonymous fortunei* and yew (*Taxus baccata*) branches. Fresh food was offered to the weevils every 3 days, when cages were cleaned, and all eggs collected. Melanized eggs were placed in Petri dishes (diameter 9 cm) and were maintained at 9 °C until used in the trials.

Statistical analysis (Ireland and Norway)

Experimental data (comparing the mean number of surviving larvae per plant for each of the treatments) were analyzed using MINITAB, release 15 (Minitab Inc., State College, Pennsylvania) and subjected to nonparametric tests because the data would not support analysis by analysis of variance (ANOVA).

Nematode persistence was expressed as mean percentage infected *G. mellonella* bait per plot. The percentage means in the different treatments were compared using ANOVA and a general linear model in MINITAB, release 15. Further comparisons were made when a significant difference was found using pairwise comparisons (Tukey's 95% simultaneous confidence intervals).

Results

Norway

The results obtained are summarized in Table 1. Table 2 shows the soil type for each field trial and Fig. 2 shows the mean daily soil temperatures that were recorded for trials 2–6. It was clear that trials 1 and 4 gave the best results when evaluating *H. megidis* under field conditions. In trial 1, the number of larvae per plant was significantly reduced with the double application of *H. megidis* compared with the untreated control (Friedman's test: $S = 1.8$, d.f. $= 3$, $P < 0.05$). The single treatment was significantly less effective than the double treatment ($P < 0.05$) and not significantly different from the control ($P > 0.05$). The soil temperature recorded directly at the time of treatment was 14 °C. The autumn was mild until the end of September and beginning of October (mean daily soil temperature above 10 °C; J. K. Henriksen, personal communication). The soil type in trial 1 was sandy loam. Trial 4 was conducted in spring where the double application of *H. megidis* caused a significant reduction in the average number of larvae at $P < 0.05$ (Friedman's test: $S = 9.89$, d.f. $= 4$). The mean daily soil temperature during the whole trial was above 10 °C, in the range 11.5–17 °C (Fig. 2b). The cold activity of *S. kraussei* was not tested as intended in trial 4 because soil temperatures were unusually high for the time of year. In trial 4, the soil type was also sandy loam. In the remaining four trials (trials 2, 3, 5 and 6), *H. megidis* and *S. kraussei* did not reduce the number of larvae significantly compared with the untreated control ($P > 0.05$ for all treatments). The soil type in trials 2 and 3 was silty loam sand (5–10% clay) and, in trials 5 and 6, silty clay loam (10–25% clay content). In trial 2, average daily temperatures in the soil at a depth of 10 cm (recorded at the nearest representative meteorological station) were in the range 15–20 °C throughout the treatment time from July to September (Fig. 2a). For the autumn treatment, mean daily soil temperatures dropped to 10 °C approximately 8 days after the nematode application and stayed below 10 °C for the remaining trial period until the spring assessment. For trial 3, mean daily

Table 1 Results from Norwegian trials 1–6 evaluating the efficacy of *Heterorhabditis megidis* and *Steinernema kraussei* against *Otiorhynchus sulcatus* larvae in field-grown strawberrys

Trial	Treatments	Application rate per plant	Time of application	Mean ± SEM number *Otiorhynchus sulcatus* larvae per plant[a]	EPN persistence % mean ± SEM infected bait[a]
1	Untreated control	0	Autumn	1.8 ± 0.6^{1}	Not sampled
	Heterorhabditis megidis	25 000 × 2	Autumn	0.1 ± 0.1^{2}	—
	Heterorhabditis megidis	25 000	Autumn	0.9 ± 0.4^{1}	—
2	Untreated control	0	Summer + autumn	3.9 ± 0.7^{1}	0 ± 0^{1}
	Heterorhabditis megidis	50 000	Summer	2.1 ± 0.5^{1}	92 ± 8^{2b} and 5 ± 5^{1c}
	Heterorhabditis megidis	50 000	Autumn	4.5 ± 1.2^{1}	69.5 ± 8^{2}
	Heterorhabditis megidis	50 000 × 2	Summer + autumn	4.8 ± 1.4^{1}	63.8 ± 13^{2}
3	Untreated control	0	Spring	3.2 ± 0.8^{1}	25 ± 18^{d1}
	Heterorhabditis megidis	30 000 × 2	Spring	1.0 ± 0.3^{1}	63 ± 24^{1}
	Steinernema kraussei	30 000 × 2	Spring	1.4 ± 0.6^{1}	92 ± 8^{2}
	Steinernema kraussei	30 000	Spring	2.0 ± 0.7^{1}	83 ± 17^{2}
	Steinernema kraussei	15 000 × 2	Spring	2.0 ± 0.3^{1}	92 ± 8^{2}
4	Untreated control	0	Spring	5.5 ± 3.3^{1}	33 ± 24^{d1}
	Heterorhabditis megidis	30 000 × 2	Spring	0.6 ± 0.5^{2}	42 ± 25^{1}
	Steinernema kraussei	30 000 × 2	Spring	1.3 ± 0.9^{1}	75 ± 16^{1}
	Steinernema kraussei	30 000	Spring	2.8 ± 1.6^{1}	67 ± 24^{1}
	Steinernema kraussei	15 000 × 2	Spring	6.3 ± 1.7^{1}	75 ± 25^{1}
5	Untreated control	0	Spring	1.7 ± 0.7^{1}	0 ± 0^{1}
	Heterorhabditis megidis	25 000 × 2	Spring	2.1 ± 0.5^{1}	33 ± 24^{1}
	Heterorhabditis megidis	25 000	Spring	1.3 ± 0.5^{1}	58.5 ± 21^{1}
	Steinernema kraussei	25 000 × 2	Spring	0.9 ± 0.2^{1}	100 ± 0^{2}
	Steinernema kraussei	25 000	Spring	1.0 ± 0.3^{1}	83 ± 17^{2}
6	Untreated control	0	Late autumn + spring	9.4 ± 2.4^{1}	0 ± 0^{1}
	Heterorhabditis megidis	30 000 × 2 + 25 000	Late autumn + spring	8.3 ± 1.6^{1}	33 ± 25^{1}
	Steinernema kraussei	30 000 × 2 + 25 000	Late autumn + spring	4.3 ± 0.2^{1}	100 ± 0^{2}
	Steinernema kraussei	30 000 + 25 000	Late autumn + spring	7.3 ± 2.8^{1}	100 ± 0^{2}
	Steinernema kraussei	15 000 × 2 + 25 000	Late autumn + spring	4.3 ± 0.7^{1}	100 ± 0^{2}

[a]Means followed by the same superscript number are not significantly different ($P < 0.05$).
[b]Sampled the same year as the treatment.
[c]Sampled the next spring.
[d]Presence of indigenous *Steinernema* sp.
EPN, entomopathogenic nematode.

Table 2 Soil type in Norwegian field trials 1–6 on evaluating the efficacy of entomopathogenic nematodes against *Otiorhynchus sulcatus*

Trial[a]	Soil type (% clay)
1	Sandy loam (0–5)
2	Silt loam sand (5–10)
3	Silt loam sand (5–10)
4	Sandy loam (0–5)
5	Silt clay loam (10–25)
6	Silt clay loam (10–25)

[a]For details, see Table 1.

soil temperatures were in the range 5–11 °C in the first 13 days after nematode applications. Subsequently, temperatures rose gradually to 10 °C, and were in the range 10–16 °C for the remaining trial period (11 days) (Fig. 2b). Trial 5 was carried out in a similar manner to trial 4 but in a different field with a different soil type and level of *O. sulcatus* infestation. Mean daily soil temperatures were in the range 6–8 °C for the first 8 days after nematode applications, then rose to above 10 °C (up to 12 °C) for a short period (4–5 days). For most of the remaining trial period, the temperature dropped to below 10 °C, except for 4–5 days when it rose to 11–12 °C (Fig. 2b). In trial 6, the mean daily soil temperatures were just above 10 °C (range 11–13 °C) for the first 12 days after the first nematode application, and remained under 10 °C for the rest of the trial period (range 8–0.5 °C) (Fig. 2b).

Nematode persistence

Persistence of the applied EPNs in soil is shown in Table 1. In general, there was a tendency toward poorer persistence for *H. megidis* at low temperature, as indicated in trials 5 and 6, where persistence was only 33% (as measured by infected bait per plot), which was significantly lower than for all the *S. kraussei* treatments (trial 5, $F = 6.2$, d.f. $= 4$, $P = 0.004$; trial 6, $F = 29.67$, d.f. $= 4$, $P < 0.001$). In trial 2, where only *H. megidis* was used, it was clear that long-term survival of this species was poor. Two months after the July treatment, nematode persistence was high at 92%, whereas, when the same plots were sampled the next spring, only 5% persistence was observed ($F = 29.38$, d.f. $= 3$, $P < 0.001$). In trials 3 and 4, a low level of *Steinernema* spp. was isolated in the untreated control plots, indicating the presence of indigenous EPNs.

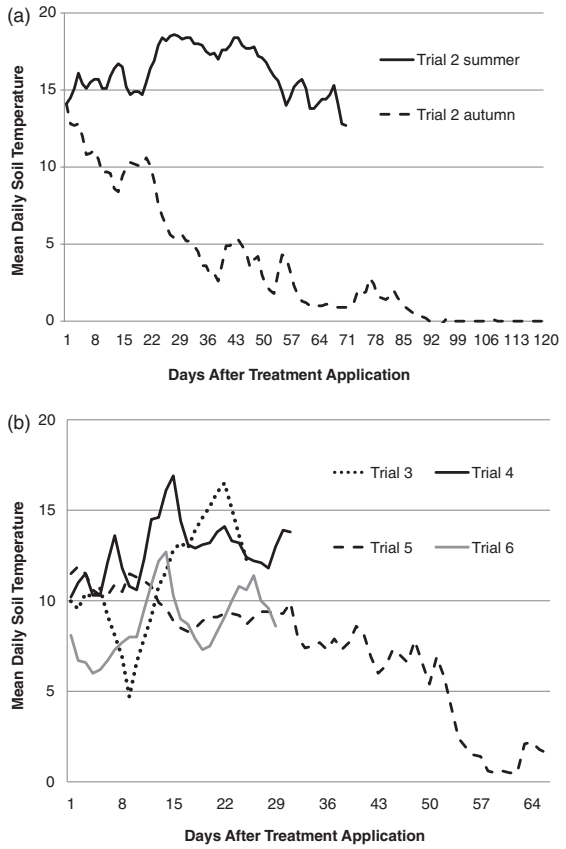

Figure 2 (a) Mean daily soil temperatures (°C) recorded for field trial 2 (summer and autumn applications) conducted in Norway, evaluating the efficiency of *Heterorhabditis megidis* against *Otiorhynchus sulcatus*. (b) Mean daily soil temperatures (°C) recorded for field trials 3, 4, 5 and 6, conducted in Norway, evaluating the efficiency of the cold active entomopathogenic nematode species *Steinernema kraussei* against *Otiorhynchus sulcatus*.

Ireland

Despite the high number of BVW released in the 2002 planting, larval establishment was not high. Only six out of the ten blocks were assessed in the 2002 planting because most data consisted of zeros. An average of 0.9 larvae/pupae per plant were found in the control treatments (water only). The nematode treated plots had 0.4–1.2 larvae/pupae per plant (Table 3). During September 2003, the average soil temperature was 15.9 °C and, in October, it was 10.4 °C. However, because EPN application in October took place early in the month, temperatures were higher than the monthly average, fluctuating in the range 11–14 °C. Significantly more live *O. sulcatus* larvae were found in the control treatments compared with the plots that had received nematode application (Friedman's test: $S = 27.06$, d.f. $= 4$, $P < 0.001$; Table 3). When the test was repeated, omitting the control treatment, there were significant differences detected among the EPN treatments (Friedman's test: $S = 15.69$, d.f. $= 3$, $P < 0.05$). The output of the statistical test indicated that this was attributable to the triple treatment. When this treatment and the control were omitted from the test, no significant differences were found between the single and double application (Friedman's test: $S = 2.15$, d.f. $= 1$, $P > 0.05$).

Table 3 Mean ± SEM number of live *Otiorhynchus sulcatus* larvae after one, two and three applications of *Heterorhabditis megidis* from field experiments in Ireland

	Insects per plant	
Treatment	2002 planting	2003 planting
Control	0.9 ± 0.51	20.9^{a} ± 1.92
Single (September)	1.1 ± 0.71	11.2^{b} ± 1.62
Single (October)	1.2 ± 0.47	10.1^{b} ± 3.33
Double (September + October)	0.4 ± 0.5	8.2^{b} ± 2.16
Triple (September, October, April)	0.7 ± 0.4	1.8^{c} ± 0.68

Different superscript letters indicate statistical differences (Friedman's test: $S = 27.06$, d.f. $= 4$, $P < 0.05$).

Discussion

EPNs are effective against a number of soil dwelling pests, including *O. sulcatus*. Many studies to date have demonstrated the effectiveness of EPNs for the control of *O. sulcatus* under protection and in the field (Kakouli-Duarte *et al*., 1997; Fitters *et al*., 2001a; Gill *et al*., 2001; Booth *et al*., 2002; Willmott *et al*., 2002; Lola-Luz *et al*., 2005; Lola-Luz & Downes, 2007). In Norway, only two out of the six field trials (trials 1 and 4) resulted in acceptable curative control of *O. sulcatus* in field-grown strawberry plants (Table 1). Temperature and soil type are limiting factors for use of entomopathogenic nematodes against *O. sulcatus* (Long *et al*., 2000; van Tol *et al*., 2004) and the results obtained from our field trials support this claim. Kung *et al*. (1990) showed that nematode efficacy decreases with increasing clay content. In the field trials conducted in Norway, nematode efficacy was poorest in soils with higher clay content.

In Ireland in 2003, a higher infestation rate was achieved, and entomopathogenic nematode (*H. megidis*) application to control *O. sulcatus* in the field indicated that effective control of this pest is possible in outdoor crops in Ireland, with multiple application being more effective. The highest mortality was recorded in the triple application (86%). This is in agreement with previous experiments carried out for the control of *O. sulcatus*, where multiple nematode applications are the most effective (Lola-Luz *et al*., 2005). Similar results were reported by Fitters *et al*. (2001a), who recorded the control of *O. sulcatus* in field-grown strawberries of 76%. Fitters *et al*. (2001a) found no statistically significant differences between a single application in October and a single application in September. In trials 1 and 4 in Norway, the double application of *H. megidis* gave significantly better control of *O. sulcatus*, and similar results were reported by Lola-Luz and Downes (2007). It is likely that a double application increases the nematode-target contact over time and thereby nematode efficacy. However, multiple applications are not considered to be viable by all growers (Gary McCarthy, personal communication).

van Tol and Raupp (2006) reported a lack of continuous efficacy when nematodes were applied in the summer. They attributed this to low nematode recycling as a result of a

lack of available hosts and poor persistence, resulting in few nematodes being present to be effective against developing hosts (later instars of *O. sulcatus* larvae) later in the season. This may explain the poor results obtained in trial 2, given that the experiment was conducted during the summer, targeting young *O. sulcatus* larvae. Although nematodes were shown to persist well in the soil, after 3 months they did not have a significant effect on the developing *O. sulcatus* larvae. Higher mortality of *O. sulcatus* was anticipated with the added application in autumn; however a sudden drop in temperature a few days after nematode application contributed to the reduced nematode efficacy. This indicates that a cold autumn and spring limits the use of *H. megidis* against *O. sulcatus* infestations.

The field efficacy of commercial *S. kraussei* (Norway only) was also tested for the first time under grower conditions. The effect of *S. kraussei* was rather disappointing, especially when comparing the results of the present study with those achieved by Willmott *et al*. (2002), who conducted a controlled field trial where strawberry potted plants were artificially infested with *O. sulcatus* eggs in autumn. Nematode treatments (*S. kraussei* and *S. carpocapsae*) were applied in a similar manner to those in the present study and also with the same application regimes (single, double and half dose). Assessments were made after 14 weeks, which is comparable with trial 6 in the present study. The prevailing temperatures in the soil were well below 10 °C for the whole experimental period. The results reported by Willmott *et al*. (2002) showed a very low survival rate of *O. sulcatus* larvae for all the *S. kraussei* treatments, which was only 18% at the highest dose (i.e. significantly lower than in untreated control pots). The *S. carpocapsae* treatments had no effect on *O. sulcatus* larvae, similar to the poor effect of *H. megidis* in the trials at low temperature in the present study. It is likely that the conditions under which the experiment was set up in the study by Willmott *et al*. (2002) favoured nematode activity against the weevil larvae as a result of the small scale of each experimental unit, the use of compost and the limited interactions with soil (predatory, infectious) biota in the field. Another factor influencing the results obtained by Willmott *et al*. (2002) was the uniform distribution of *O. sulcatus* infestation, thus reducing the variability of the results that can affect statistical analysis.

The present studies show quite clearly that it is a challenge to control larvae of *O. sulcatus* using EPNs under field conditions at low temperatures. The developmental threshold temperature for *O. sulcatus* larvae, estimated by Stenseth (1979) to be in the range 2–6 °C, appears to be below temperatures where nematodes can be infective. *Otiorhynchus sulcatus* larval activity in the spring is associated with increasing temperatures; the larvae break out of their overwintering cells and start feeding voraciously (Evenhuis, 1978). Larval activity probably commences when soil temperatures approach 6 °C. Thus, there is little time for nematode host finding and infection during spring. At lower temperatures, nematode activity (and their bacterial symbionts) slows down, requiring more time for infection (Long *et al*., 2000). High levels of *O. sulcatus* mortality may be reached if the spring is warm (as observed for trial 4). If larval feeding started earlier than nematode infections, however, a spring application may not always prevent root damage.

We conclude that entomopathogenic nematodes can be a successful component of an integrated pest management programme for *O. sulcatus* control in field-grown strawberries. The results obtained in our field trials indicate that *H. megidis* remains the ideal nematode for use in soils with low clay content and at optimal temperatures. We recommend repeated autumn applications of *H. megidis* for curative control of *O. sulcatus*. Nematode applications in the autumn can prevent any damage the next spring and also reduce future *O. sulcatus* populations. Growers need to be aware of *O. sulcatus* activity early in the season with respect to preventing heavy infestations. Field observations should be carried out in areas of the field where there is suspicion of *O. sulcatus* activity (i.e. feeding notches on leaves, parts of the field with smaller plants) and plants can be pulled out and examined thoroughly for larvae. Nematode applications are a challenge in fields using black polythene mulch because the application needs to be very precise to reach the root zone. Thus, apart from temperature and soil type as limiting factors in the use of EPNs, further work aiming to improve the delivery of EPNs to plants, both in the field and in indoor crops, is required.

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