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Vine weevil control in Ireland with entomopathogenic nematodes: optimal time of application

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Outdoor trials with potted primroses (1997 and 1998) and field-grown strawberries (1998) artificially infected with vine weevil (*Otiorhynchus sulcatus*) were conducted in Ireland to assess the best time of nematode (*Heterorhabditis megidis*, isolate UK211) application in autumn. Approximately 75 to 85% control was obtained in primrose in both years when nematodes were applied at the end of September to mid-October. The 1997 primrose trial showed that an early September nematode application helped to avoid plant damage (important when protecting young and vulnerable plants), but that October and November nematode applications gave a higher (95%), but delayed, control level when left until spring. Control in strawberry was similar to that in primrose, with maximum 76% control. Maximum control levels obtained with nematodes were similar to those obtained with chlorpyrifos (Dursban) in strawberry and gamma HCH (Lindane) in primrose. These results clearly show that nematodes can be applied successfully outdoors, under Irish weather conditions, until mid-October.

Keywords: Biocontrol; *Fragaria sp.*; *Heterorhabditis sp.*; *Otiorhynchus sulcatus*; *Primula spp.*

Introduction

The vine weevil, *Otiorhynchus sulcatus,* is a serious pest of soft fruit and hardy ornamental nursery stock. Both adults and larvae feed on a wide range of plant species from the families Rosaceae, Ericaceae and Taxaceae, which include many economically important plants. Most damage is done by the larvae feeding on the

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roots (Moorhouse, Charnley and Gillespie, 1992; Smith, 1932) but adult feeding can be a problem in ornamental plants. Adult beetles are parthenogenetic flightless females that emerge from soil from the end of May until mid-July, depending on climate and feed at night on foliage (Smith, 1932). Egg-laying starts in mid-June and ceases in October with peak egg laying in August. Adults lay between 200 to 500 eggs (Smith, 1932). Larvae feed on roots during autumn and spring, their activity being temperature dependent. Larvae pupate in May and adults emerge about a month later.

Until recently, *O. sulcatus* was controlled by the incorporation of the persistent organochlorine insecticide Aldrin into the potting compost. This gave effective and lasting control, but the routine incorporation of Aldrin is no longer permitted in the EU and the USA in the 1990's for environmental and toxicological reasons (Cross *et al.*, 1995). Since the banning of Aldrin, many other chemicals have been tested for the control of larvae in the soil, the best being a slow release granular formulation of chlorpyrifos, but control in commercial practice has not been as good when compared to Aldrin (Cross *et al.*, 1995). The failure of chemical pesticides, the banning of persistent chlorinated hydrocarbons and the increasing dispersal of the insect by infested potted plants has led to an urgent need to develop better and safer pest control methods (Zimmermann, 1996).

An environmentally friendly way of controlling insect pests is by the use of natural enemies such as entomopathogenic nematodes (EPN). The soil dwelling stage, the infective juvenile, of the nematode actively searches the soil for host insects. After penetration of the insect, the nematodes release symbiotic bacteria into the heamocoel. This causes septicemia followed by death of the insect within a day or so and the nematodes reproduce within the cadaver. There are two main EPN families, Steinernematidae and Heterorhabditidae, that are currently used for biological control of insect pests. Although there are many similar characteristics between the two EPN families, members of the Heterorhabditidae (*Heterorhabditis* spp.), especially *Heterorhabditis megidis*, are considered more suitable for the control of *O. sulcatus* due to their higher pathogenicity to the larvae (Georgis and Poinar, 1984; Schirocki and Hague, 1993; van Tol, 1993a, 1996).

One of the problems of using infective *Heterorhabditis* spp. against *O. sulcatus* is that the weevil larvae are not present when soil temperatures are optimal for nematode efficacy (i.e. 20 °C), but are present late in the year when soil temperatures are often below 12 °C. This limits the use of *Heterorhabditis* spp. as they generally do not work well below 12 °C (e.g. van Tol, 1996). The use of nematodes is usually recommended between August and late September when the majority of weevil eggs have hatched and before larvae do significant damage. Outdoor experiments in The Netherlands from 1991 to 1994 have shown that nematode application up to the beginning of October gives reasonable control levels if the temperature remains higher than 12 °C; a maximum of 80% control was obtained in *Thuja* and *Waldsteinia* spp. when the nematodes were applied at the end of September (van Tol, 1996). Another problem with nematode use is the limited persistence of applied nematodes in soil. Due to environmental and biotic factors, numbers decline rapidly (days) post application to less than 1% of the initial population (Ishibashi and Kondo, 1986; Smits, 1996). However, long

term (years) persistence in soil of entomopathogenic nematodes has also been found (Smits, 1996 and references therein) suggesting that at least low numbers of nematodes persist in soil, through either survival or recycling in host insects.

Irish winters are moderate compared with those in mainland Europe, and this may have an effect on vine weevil populations and on optimal time of nematode applications in both autumn and spring. Complete control of naturally vine-weevil infested primroses was obtained outdoors at Kinsealy Research Centre after treatment with 50,000 *H. megidis* infective juveniles per pot (Dunne, 1990). However, application dates were not given and nematode application rates were very high. Mid-August and mid-October nematode applications (25,000 per plant) in the field to control vine weevils in strawberry (in three locations in Ireland; Clonroche, Adamstown and Kinsealy) gave no significant reduction in larval numbers (Dunne, 1990). It was suggested that nematodes may be more effective when applied in September when the pest is present and the soil temperatures are higher. The main objective of this work was to assess the best time of application of nematodes under field conditions in Ireland in both primrose and strawberry. Primrose was used as a model plant as they are easy to grow in containers and are highly susceptible to vine weevil larvae. The questions addressed were: firstly, how late in autumn can nematodes be applied for optimal efficacy against vine weevil larvae and with minimal damage to the plants and, secondly, would double nematode application improve vine weevil control levels under field conditions. Persistence of applied nematodes in peat in potted primroses was also assessed.

Materials and Methods

Nematode culture and storage conditions

Nematodes (North West European *H. megidis* isolate UK211) were cultured on the wax moth *Galleria mellonella* at 20 °C. The emerging infective juveniles were washed three times by sedimentation in tap water and stored in plastic food containers (Bellaplast Polarcup, Roundstone, UK) at a concentration of 1000 nematodes/ml (50 ml/container). Prior to use in the experiment, the containers with nematodes were stored for 1 week at 20 °C and subsequently at 9 °C for 4 weeks. A storage period at 9 °C has been shown to improve infectivity of the nematodes at 9 °C (Fitters, Dunne and Griffin, 2001). A separate batch of nematodes was cultured and stored in this way for each application date. In the 1998 primrose and strawberry outdoor trial, two sources of nematodes were used: 1) *H. megidis* (UK211) reared in *G. mellonella* in the laboratory and, 2) commercially produced *H. megidis* (UK211) sold as Nemasys-H (MicroBio, UK). The commercial nematodes were obtained by post and stored at 9 °C for up to 4 days before use.

Primrose outdoor pot trial – 1997

Primroses (*Primula*, cv. Charisma) were grown in Irish Peat Moss (Shamrock®) mixed with slow release fertiliser (Osmocote®) and lime in plastic pots (diameter 12 cm, volume 0.5 l) in the summer of 1997. The plants were infected with *O. sulcatus* eggs, obtained from a laboratory culture, on 27 August. To facilitate egg application, the eggs were mixed with sieved dry peat. Each plant received approximately 1.5 g of egg-infested peat (about 16 eggs/plant), which was placed in a small indentation in the soil underneath the primrose leaves. The pots were placed outside on a

gravel bed at Kinsealy Research Centre. The plot was surrounded by wooden planks that were covered in plastic, the top of which was coated with Fluon GP1 (Whitford Plastics Ltd. Runcorn, Cheshire, England), to prevent natural infestations with *O. sulcatus* or other beetles.

The experiment was set up in four randomised blocks. Each block contained 10 vine weevil treatments with 12 plants each plus an additional 30 pots without vine weevil eggs for assessment of nematode survival (see below). Nematodes were added to the potted primroses at five dates in autumn (22 September, 6 and 20 October and 3 and 17 November). The nematodes were applied to the soil at a concentration of 8000 nematodes/pot (in 8 ml of tap water). Control pots received 8 ml of tap water on the same application days (untreated pots). Four application treatments (untreated pots, 1st, 3rd and 5th nematode application dates) were set up in duplicate, one replicate was harvested in December and the second was harvested the following May. Each block was surrounded by a row of pots with untreated primroses. Plants were harvested and vine weevil larvae counted in December 1997 and May 1998. Because many primroses died due to extensive vine weevil feeding, carrot disks (5 cm length, variable width) were added to the soil (three disks per pot to all pots that had missing or yellowing plants; that is more than 50% of pots) as an additional feed source and replaced monthly between the December and May harvest. The number of healthy (properly rooted) plants (December harvest only) was determined.

Primroses without vine weevil larvae were used to assess persistence of nematodes in the compost. On each of the five application dates, 8000 nematodes/pot were applied to seven of these pots per block. Nematode persistence was assessed by baiting the soil with the wax moth larvae (*G. mellonella*). For each nematode application date, one pot per block was taken to the laboratory at 2-week intervals (up to 12 weeks), starting on the day of application of the nematodes. The pots were placed at room temperature overnight. The next day the primrose was removed and the soil was thoroughly mixed and weighed. Ten percent of the soil (w/w) was placed in a plastic food container (Bellaplast Polarcup, Roundstone, UK) and 10 *G. mellonella* larvae were added. After 3 days the *G. mellonella* larvae were replaced by 10 fresh ones, left for another 3 days and replaced a third time. The infected *G. mellonella* larvae were dissected after 5 days and the number of nematodes that had developed into adults was counted. Assessments of persistence were performed at 20 °C.

Strawberry and primrose outdoor trials – 1998

In 1998, control of *O. sulcatus* with nematodes was tested in both strawberries in the field and in potted primroses outdoors. Both trials were manually weeded and no herbicides were used.

Primroses (*Primula* cv. Quantum) were planted in June 1998 in plastic pots, similar to the 1997 primrose experiment. *O. sulcatus* eggs were obtained from laboratory culture and mixed with dried sieved peat. Approximately 18 eggs/pot were added to the primroses on 1 September, placed in a small indentation in the peat.

Nematodes were applied (8000 nematodes/pot in 8 ml of tap water) on four different dates in the autumn of 1998: a) 16 September, b) 29 September, c) 13 October and, d) 28 October. The seven nematode treatments were a single ne-

matode application on the above four dates and three double applications on combinations of two of those dates (ac, ad and bd). In total there were 14 treatments, as both commercial nematodes (Nemasys-H, MicroBio Ltd, UK) and laboratory-reared nematodes were applied. Due to a supply problem with Nemasys-H, the fourth commercial batch was replaced by laboratory-reared nematodes consisting of a mix of the nematodes used for applications a, b and c. These nematodes were stored at 9 °C for 6 to 10 weeks. As a control, 8 ml of tap water was applied (untreated pots). Gamma HCH (80% active ingredient) was used as a chemical treatment (used at 15 ml/100 l; applied at 100 ml/pot) and was applied on 23 September. The experiments consisted of four blocks. Each block contained 16 treatments (rows) of 10 pots each. This experiment was harvested in February 1999 and the number of live *O. sulcatus* larvae was assessed.

Strawberry plants (*Fragaria* sp., cv. Cambridge Favourite) were planted in medium loam in raised beds (September 1997) at Kinsealy Research Centre. The plants were planted on 80 cm spaced drills with 40 cm between plants. On 2 September, the strawberry plants were infested with approximately 55 eggs (mixed with peat) per plant. The eggs were added on three sides of each plant in a small indentation made in the soil close to the roots and subsequently covered with soil to prevent the eggs from drying out.

In the strawberry field, only commercial nematodes were applied (125,000 nematodes/plant in 125 ml) at the same dates as in the 1998 primrose experiment (see above). A chlorpyrifos drench (Dursban, 2 ml 48% EC/l) was used as a chemical treatment (250 ml/plant) applied on 23 September. The strawberry experiment was set up in four blocks. Each block contained nine treatments, with 13 plants each. The experiment was harvested in May 1999. Each plant was lifted carefully, so as not to disturb the root ball, placed on a plastic bag and the soil was crumbled and searched for live *O. sulcatus* larvae.

Nematode quality

An estimation of nematode quality was obtained by testing all nematode batches in the laboratory in a close contact bioassay with *Tenebrio molitor* (Fitters *et al.*, 2001). Wells of a Multiwell plate (24 well, Nunc, Denmark) were filled with 1 g of oven-dried sand and one *T. molitor* larva and the plates were thermo-equilibrated at 9 °C overnight. The next day, 50 nematodes per well were added in 50 μl of water using a multi-Pipette (Finnpipette). The plates were closed with Parafilm and placed at 9 °C in the dark. After 72 h, the *T. molitor* larvae were retrieved from the wells, washed, surface dried with tissue paper to remove potentially externally adhering nematodes from their cuticles, placed (collectively per multiwell plate) in a plastic food container (125 ml; Bellaplast Polarcup, Roundstone, UK) lined with filter paper and stored at 20 °C. Mortality was assessed after 5 days by counting the number of dead larvae. On each assessment day and for each nematode batch, four replicate plates were used.

Statistics

Statistical analyses were performed using Minitab (release 13.1) (2000) or Sigma Stat for Windows 1.0 (1993). Primrose and strawberry experimental data were analysed using an ANOVA on the means per block (square root transformed) followed by Tukey's pair-wise comparison. Nematode quality was assessed by sub-

jecting the *T. molitor* mortality data to ANOVA, followed by an all pair-wise comparison (Student-Newman-Keuls) and t-tests. Standard error values were calculated from the percentage control data.

Results

Primrose outdoor pot trial – 1997

On average 3.1 *O. sulcatus* larvae were recovered in December in the untreated (water only) pots and between 0.7 and 1.6 larvae in the nematode-treated pots. All nematode applications had significantly fewer larvae compared to the untreated pots, but did not differ from each other ($P < 0.05$). Percentage control was calculated relative to the December untreated pots with maximum control of 77% for the 20 October nematode application (Figure 1).

There were, on average, 3.4 live vine weevil larvae in the untreated pots that were harvested in May 1998. This number was not significantly different from the December untreated pots. All three nematode treatments of the May harvest had significantly less vine weevil larvae compared with the December untreated pots, with 1.4, 0.2 and 0.3 larvae/pot in the 22 September, 6 and 20 October application dates, respectively ($P < 0.05$). The September application date had significantly higher vine weevil larvae numbers when compared to the October applications dates when harvested in May. When compared with the December harvests, there were significantly fewer live vine weevil larvae harvested in May for the infection date 17 November ($P < 0.05$). Percentage control was calculated relative to the May untreated pots. The control levels of the October and November nematode application dates increased, when compared with the December harvest, to 93 and 91%, respectively (Figure 1).

The primroses used in this experiment were not fully-grown at the time of infection with vine weevil eggs. As a result many of them died due to the better than

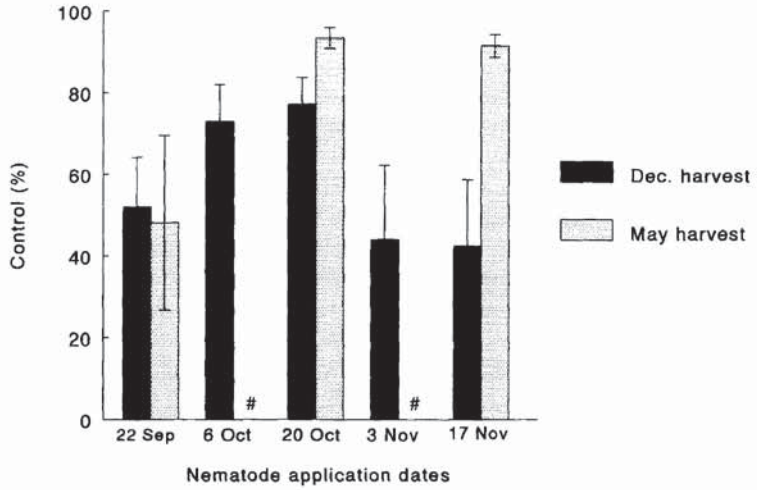

Figure 1: Percentage Otiorhynchus sulcatus control in potted primroses (Primula) after application of nematodes (Heterorhabditis megidis, isolate UK211; 8000 / pot) at five dates in autumn. Bars represent s.e. # = Assessment not done.

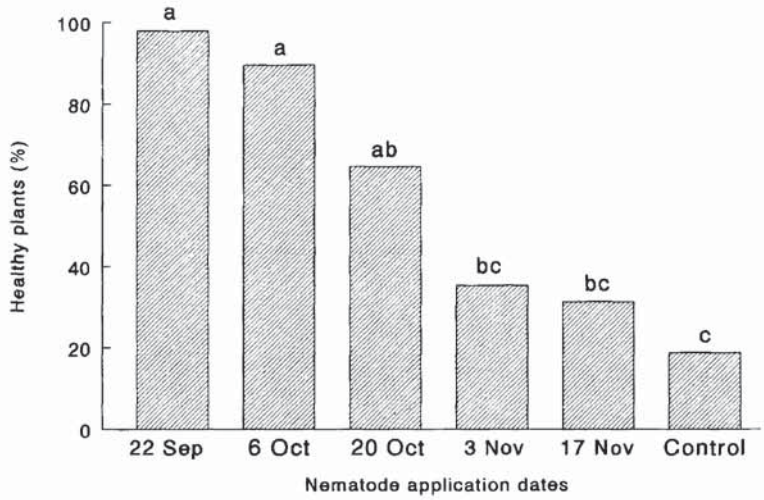

Figure 2: Primrose (Primula) plant quality (% healthy plants, i.e. properly rooted) in December 1997 after application of nematodes (Heterorhabditis megidis, isolate UK211; 8000 / pot) at five dates in autumn. Same letter indicates no significant difference between treatments (ANOVA, Tukey's all pair-wise comparison, $P < 0.05$).

anticipated establishment of vine weevil larvae. The plant damage was considerable with the late nematode application dates and the untreated pots (35% or less of the plants were properly rooted). There was a significant difference in the number of healthy plants between the different nematode application dates ($P < 0.001$; Figure 2), with the least damage (more than 90% healthy plants) when the nematodes were applied on 29 September or 6 October. Plant damage was even more widespread in May and was therefore not assessed.

Many *Tipula* sp. larvae (leather-jackets) were also found in the pots when harvested. However, no block or treatment effect was found on occurrence of these insect larvae and no nematode infected *Tipula* was found.

The soil temperature (10 cm depth) at the site was below 12 °C from the end of October onwards (Figure 3a). Nematode persistence, measured as infectiousness when baited with *G. mellonella* larvae, in the compost dropped significantly between week 0 (plants taken in directly after nematode application) and the next assessment ($P < 0.05$; Figure 4) and remained at a low level thereafter. On average, approximately 28% of the applied nematodes were recovered when the soil was baited with *G. mellonella* in the week following nematode application. After 2 weeks this number had dropped to about 10%. The nematodes used for the last applications dates (3 and 17 November) show higher recovery rates than the other batches after 4 to 10 weeks. In general, the later the nematodes were applied, the higher the nematode recovery after 6 to 10 weeks.

Strawberry and primrose experiment – 1998

Between 0 and 15 living vine weevil larvae per pot were retrieved in the prim-

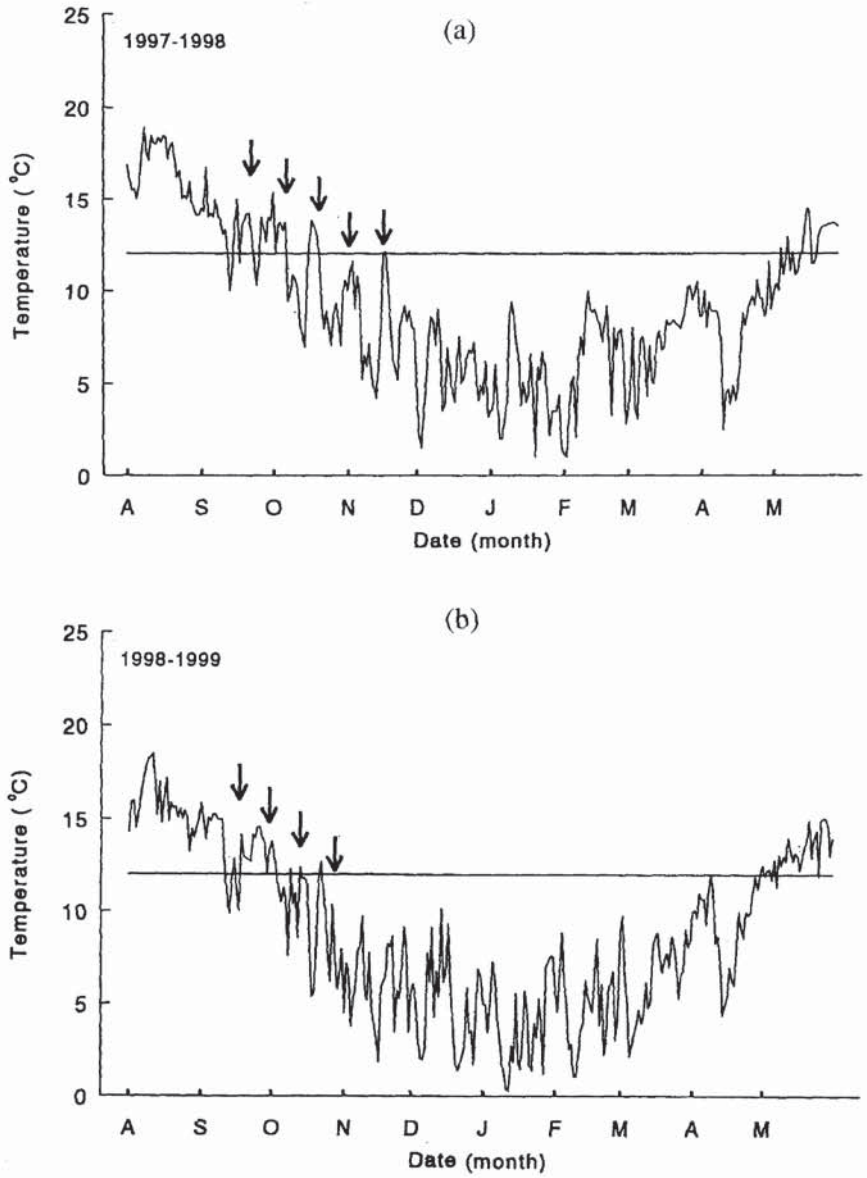

Figure 3: Soil temperature at 10 cm depth at 0900 h at Kinsealy Research Centre in (a) autumn 1997 to spring 1998 and (b) autumn 1998 to spring 1999. Arrows indicate dates of nematode application. Horizontal lines represent 12 °C.

rose experiment, with on average 6.8 larvae per pot in the untreated pots and between 0.9 and 7.3 larvae per pot in the other treatments. All nematode treat-

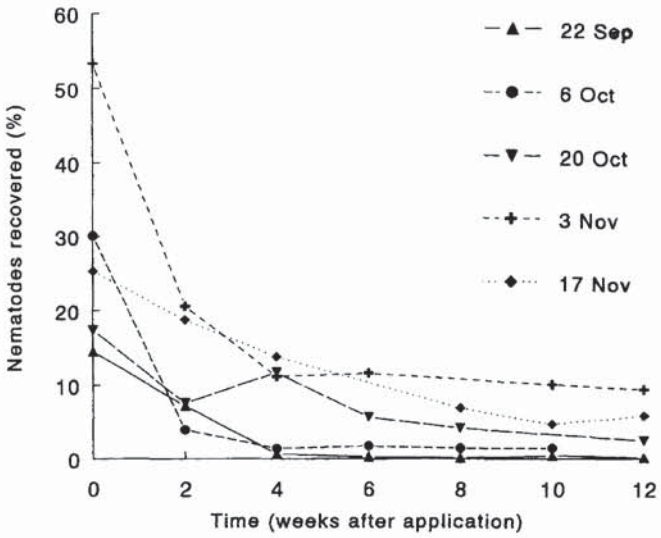

Figure 4: Percentage of nematodes (Heterorhabditis megidis, isolate UK211) recovered from soil by baiting with G. mellonella larvae at 2-week intervals from application date of the nematodes. Each point represents the mean of four replicate soil samples baited with 30 G. mellonella larvae each.

ments, except applications on 28 October, had significantly lower vine weevil larvae per pot ($P < 0.05$) when compared to the untreated pots. The lowest vine weevil numbers per pot for a single nematode application were obtained by application on 29 September for both Nemasys-H and laboratory-reared nematodes (1.2 and 1.4 larvae/pot) and for the 13 October application of the laboratory-reared nematodes (0.9 larvae/pot). These vine weevil numbers were not significantly different to treatment with gamma HCH. Within application dates, treatments with Nemasys-H and laboratory-reared nematodes only gave significantly different vine weevil numbers when applied on 13 October. Double nematode applications were in no case significantly different from the best single nematode application. Percentage control was calculated relative to the untreated pots value and is given in Figure 5.

The temperature, 10 cm below soil surface, in the experimental area is shown in Figure 3b. No plant losses occurred in the 1998 primrose experiment as the plants were more mature at the time of vine weevil egg application than in the 1997 primrose experiment.

The average number of *O. sulcatus* larvae recovered from the individual strawberry plants was very low; 1.0 (ranging between 0 and 8 larvae per plant). On average 1.6 vine weevil larvae per plant were recovered in the untreated pots and between 0.4 and 1.8 larvae per plant were found in the nematode or chemical treatments. An abundance of ground beetles was observed while harvesting the plot and these may have contributed to the low numbers by predation. Many *O. sulcatus* larvae had already pupated at this

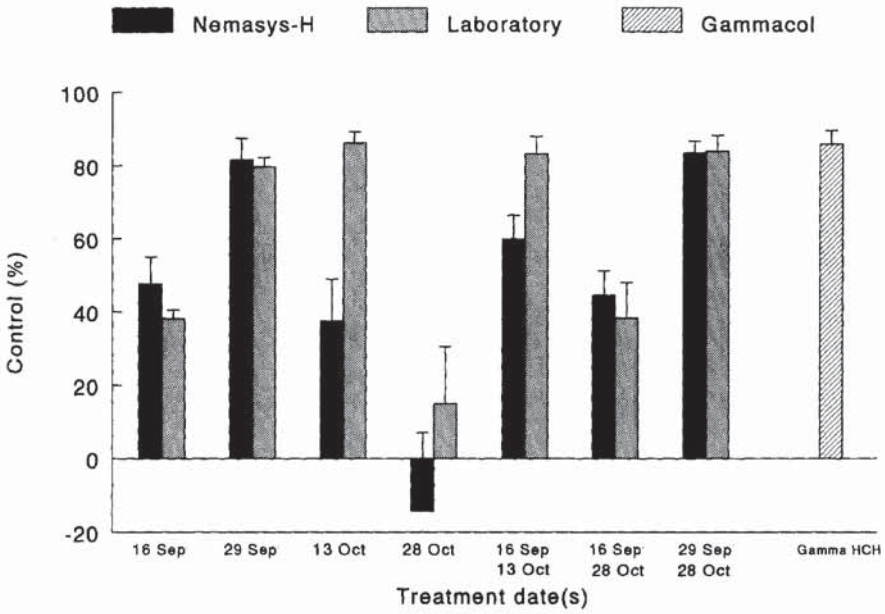

Figure 5: Percentage Otiorhynchus sulcatus control levels after nematode (Heterorhabditis megidis, isolate UK211) application. Both commercial (Nemasys-H) and laboratory-grown nematodes were applied in the autumn of 1998 to potted primroses (Primula sp.) on 16 and 29 September and 13 and 28 October and multiple nematode applications. Gamma HCH (Lindane) was used as a chemical treatment. Percentage control was calculated relative to the untreated pots. Bars represent s.e.

time and were found close to the soil surface. The ranking of treatments was similar to that in the primrose experiment but differences were not significant. Percentage control was calculated relative to the untreated pots and is given in Figure 6. Highest control levels were obtained by a single nematode application made on 16 and 29 September, all double applications and the chemical treatment with chlorpyrifos.

In the infectivity bioassays significantly higher ($P < 0.05$) mortality levels in weevil larvae were found using the laboratory-reared nematodes when compared to the commercial (Nemasys-H) nematodes (Table 1). Quality of the nematodes also varied significantly between batches within the two nematode sources in the *T. molitor* bioassay, with a tendency for nematodes used in later application dates to have lower quality.

Discussion

Nematodes gave up to 93% control of vine weevil larvae in both potted primroses and field grown strawberries under outdoor conditions and this control level was often better than or equal to that obtained with chemicals. In the 1998 experiment, optimal time of nematode application was at the end of September and beginning of October in both the primrose and the strawberry experiment. This confirms the results of the previous semi-field trial with primroses in 1997. It

is possible that maximum vine weevil mortality in the 1998 primrose experiment was not reached at the time of harvest in February as the primrose experiment in 1997 showed an increase in mortality between the December and May harvest. However, the strawberry experiment gave a similar pattern of results and was harvested in May, confirming the preferred timing of application.

Although the *O. sulcatus* control levels of the different treatments in the strawberry experiment were not significantly different from each other, the relative order of control of the various treatments are an almost perfect image of that obtained in the primrose experiment, where significant differences were recorded. This would suggest that labour intensive trials with strawberries in the field could be replaced by easier and cheaper primrose experiments in pots. Although strawberry plants can suffer severely from vine weevil attack, there is evidence that strawberry plants are not the best choice for vine weevil experiments as there can be a high natural larval mortality and the plants are not attractive to adult beetles (van Tol, Visser and Sabelis, 2000).

Double nematode applications were not found to enhance vine weevil control levels in the 1998 experiment. Control levels of double nematode applications always equalled, but did not exceed, the highest control level of the individual applications. Similar findings were obtained in field trials in The Netherlands (R. van Tol, personal communication).

In the 1997 primrose trial, the lowest levels of vine weevil larvae were recorded

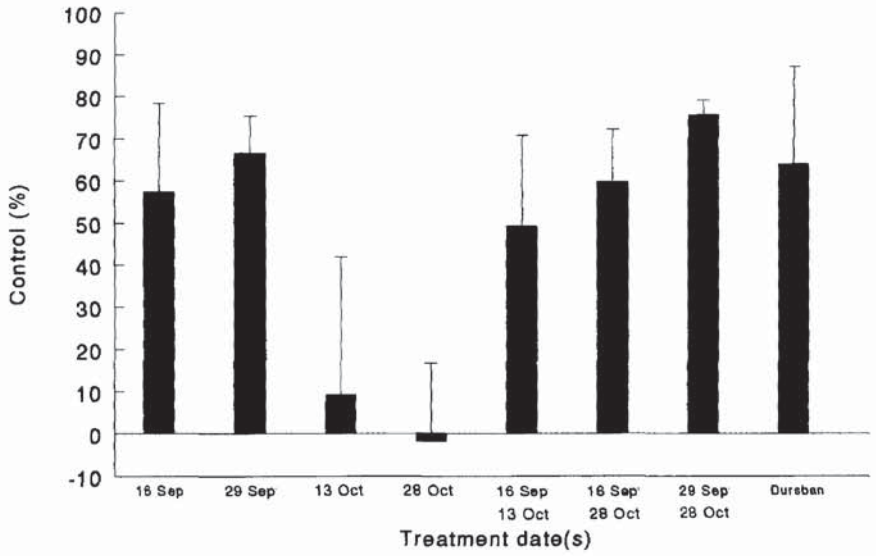

Figure 6: Percentage Otiorhynchus sulcatus control levels after nematode (Heterorhabditis megidis, isolate UK211) applications in the autumn of 1998 to strawberry (Fragaria sp.) on 16 and 29 September and 13 and 28 October and multiple nematode applications. Chlorpyrifos (Dursban) was used as a chemical treatment. Percentage control was calculated relative to the untreated pots. Bars represent s.e.

(December harvest) in the treatments where the nematodes were applied on 6 and 20 October. However, when plant damage was taken into account, an earlier nematode treatment at the beginning of October would in this case be preferred. Severe plant damage in the two early nematode application dates (22 September and 6 October) was restricted when compared to the later applications and this could be due to having killed some weevils at an early stage before they had done much feeding. These results clearly indicate that an early nematode application can be very effective in protecting young and vulnerable plants from vine weevil attack, thus helping them through the vital establishment period.

Difference in plant damage levels between the 1997 and 1998 primrose experiments was most likely caused by the size of the plants at time of vine weevil egg inoculation. In the 1998 experiment the plants were more established at time of inoculation and could therefore harbour more larvae without visibly suffering. It is not likely that the difference in plant damage between the 2 years was due to different primrose cultivars used, as in our experience all primrose cultivars are susceptible to vine weevil larvae.

In the 1997 primrose experiment, the later nematode-application dates (20 October and 17 November) gave far better control levels when the pots were harvested in spring than the same treatments did when harvested in December. This would suggest that the nematodes do not kill the vine weevil larvae instantly when applied in October or November, when the temperatures are often below 12 °C, but that eventually they do. Carrot disks were added to the soil as an additional food source, therefore, vine weevil larvae did not die due to a lack of food in pots where the primroses were gone or nearly gone. Moreover, because number of larvae alive in the untreated pots (no nematodes) of the December and May harvest were not significantly different from each other, it is concluded that the vine weevil larvae did not die of causes other than nematodes between December and May. Several mechanisms may have contributed to this delayed, but increased control level. Firstly, the nematodes may have persisted in soil and penetrated when the temperature rose. Although nematode persistence in soil declined rapidly after application, after 4 weeks hardly any nematodes were recovered with the *G. mellonella* bait method for the early applications, the nematodes of the later treatments persisted longer,

Table 1. Infectivity assessment of *Heterorhabditis megidis* (UK211) nematodes, from commercial (Nemasys-H) and laboratory cultures, in a close contact bioassay with *Tenebrio molitor* at 9 °C for 3 days

Application dates 1998	*T. molitor* mortality (%)		
	Commerical	Laboratory	
		6 to 10 weeks old	4 weeks old
16 September	54.2aA[1]	–	60.4aA
29 September	10.4bA	–	59.4aB
13 October	1.0bA	–	49.0aB
28 October	–	1.0A	18.8bB

[1]Within columns, different letters (lower case) indicate significant differences between application dates (one-way ANOVA, Student Newman Keuls, $P < 0.05$). Within rows, different letters (upper case) indicate significant differences between nematode sources (t-test, $P < 0.05$).

possibly due to the lower temperatures and therefore lower overall biological activity in the soil. Secondly, the nematodes may have persisted in the insect. Schirocki and Hague (1996) found that *Steinernema carpocapsae* that had just penetrated, but not killed, *O. sulcatus* larvae, survived inside the larvae for up to 40 days when placed at 5 or 10 °C, after which period the nematodes were still capable of killing the host when the temperature was increased. It is possible that the same occurred for *H. megidis* UK211 nematodes used in this experiment. Apart from, or additional to the delayed mortality due to low temperature, insect cuticle perforation by the nematode in autumn could also cause secondary infections that cause the insects to die, without signs of nematode or associated bacterial development later in spring. Finally, increased mortality in spring could also be achieved by recycling of nematodes in vine weevil pots (from infected *O. sulcatus* larvae). It was not possible to distinguish what part each of these mechanisms played in this experiment, as few dead or infected larvae were recovered in spring.

In the 1997 primrose experiment, most pots (> 50%) were given carrot disks as an additional food source for the vine weevil larvae and this could have affected the natural mortality levels in that possibly more larvae survived the winter than would have done otherwise. Comparison with the control could therefore be unfair as without feeding, more vine weevil larvae could have died in the control relative to the nematode treated pots. However, both the untreated pots and the 3 and 17 November nematode treated pots had low levels of healthy rooted plants (< 33%) and were not significantly different from each other when harvested in December. As an equal number of pots were given additional carrots, mortality values can thus be compared. The absolute mortality levels due to nematodes would most likely be even higher when no carrot disks were added.

Around 50% control was obtained at the last two application dates in the 1997 primrose outdoor pot trial when harvested in December, despite the low soil temperatures during and after nematode application. Soil temperatures at 10 cm depth were measured daily at 0900 h close to the experimental site. However, pot temperatures could differ from soil temperatures as pots are more exposed. Comparison of open-ground and container soil temperatures in The Netherlands showed that container temperatures were generally lower or equal to those in the open ground (peat soil) from September onwards (van Tol, 1993b). On the other hand, although the measured morning temperatures did not exceed 12 °C, it is likely that at least at some days the temperature during daytime would exceed 12 °C, especially when exposed to direct sun light. A temporary increase in temperature up to 12 °C can influence the efficacy of nematodes against vine weevil larvae (van Tol, 1993a). In a climate room experiment, *H. megidis* reduced the number of vine weevil larvae by 80 to 100% after 6 h of 12 °C soil temperature followed by 9 °C until the end of the experiment. The same nematode isolates gave no control at continuous 9 °C but achieved total control at continuous 12 °C (van Tol, 1993a). This could explain the relatively high control levels when measured soil temperatures remained below 12 °C.

In the 1998 primrose trial, the nematode batches (commercial and laboratory reared) used for 29 September application gave no significant difference in con-

trol levels despite differences in quality in the bioassay. This would suggest that the bioassay conditions do not reflect actual field conditions. This was the case on 29 September, as directly after nematode application in the field the temperature rose to 14 °C (Figure 3b). However, with the 13 October application, a difference was found between the two types of nematodes in both the field and the bioassay. This could mean that a minimum infectivity in the bioassay is required, say more than 10% mortality, otherwise the batch should be discarded. More research needs to be done to test reliability of this hypothesis.

The results of the present studies clearly demonstrate that nematodes can be applied successfully to control vine weevil larvae in Ireland until mid October. Application at the beginning of October is advised to get best control levels, but only in well-established plants. In young and vulnerable plants an early nematode application could help them safely to get through the first vital establishment period (see also van Tol, 1996). A delayed control effect, which only manifests itself in spring, can be obtained by late October nematode applications in potted plants. In strawberry, nematode delivery can be aided with the use of a drip irrigation system (T-tape®) (Kakouli-Duarte, Labuschagne and Hague, 1997) and perhaps an additional spring nematode application, although financially not very attractive, in the beginning of May just before the new adults emerge.

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