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Location of Project:	University of Reading Department of Agriculture
Project Co-ordinator:	Chris Allhusen
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1 PRACTICAL SECTION FOR GROWERS

1.1 APPLICATION

The objective of the project was to establish temperature limits necessary for the successful control of black vine weevil (BVW) larvae using entomopathogenic nematodes. It was found that the soil temperature has to be at least 13°C at the time of nematode application. Since BVW larvae are present in the soil from about August/September to May the next year, nematode application should be done in autumn before the soil temperature decreases or in spring after the temperature has reached 13°C.

1.2 SUMMARY

Scope and objective of the project

Low soil temperatures are often the major factor responsible for unsatisfactory control of *Otiorhynchus sulcatus* (BVW) with entomopathogenic nematodes in the UK. Research was carried out to establish the temperature range in which nematode species are able to invade and kill BVW larvae. Emphasis was put on the lower temperature range and nematode species commercially available in the UK.

It was investigated how long the temperature has to be above the temperature limit to achieve sufficient control and if higher temperatures at a later point after nematode application would still enhance nematode efficacy. Finally, experiments were carried out to investigate if a nematode application in the autumn could have a residual effect on larval mortality in the next spring by nematodes surviving within the host when the temperature is below requirements for nematode development.

Summary of results

The most effective nematodes for the control of the BVW are *Steinernema carpocapsae* and *Heterorhabditis* sp. The temperature limit for host invasion was very similar for all species tested, about 13°C. Extended exposure time at temperatures below 13°C did not enhance larval penetration. Nematode invasion and larval mortality increased with temperature above 13°C.

A daily temperature increase from 10 to 15°C for up to 5 hours had no effect on nematode invasion. Best results were obtained when nematodes and larvae were kept at 15°C directly after nematode application before the temperature dropped to 10°C. A temperature increase from 10 to 15°C after 5 or 10 days after nematode application had little or no effect on nematode efficacy. To obtain satisfactory penetration of BVW larvae 3 to 5 days at 15°C directly after nematode application were necessary.

Infective juveniles (IJs) of *S. carpocapsae* which were able to penetrate a host but are unable to develop because the temperature decreases, are able to survive in a living insect and start to develop and kill the host when the temperature increases. However, some nematodes disappeared from the host during extended low temperature periods and it is therefore unlikely that nematode survival in an insect after application in the autumn is a major factor contributing to larval mortality in the next spring.

Furthermore, experiments have shown that the temperature limit for nematode and bacterial development of *S. carpocapsae* is lower than for nematode penetration. Hence, nematodes which were able to invade BVW larvae at temperatures above 13°C could cause mortality when the temperature drops to 10°C.

Action points for growers

- All UK nematodes tested have similar temperature profiles confirming low establishment and therefore poor efficacy below 13°C.

- Application should be in September or April when soil temperatures are above 12°C with a preference for a late summer treatment against small larvae before damage can occur.

- Measurements of the soil temperature might be necessary to ensure that the temperature is constantly above 13°C.

- Continuous monitoring of the vine weevil population is necessary and repeated treatments are required to keep the pest below the economic threshold.

Practical and financial anticipated benefits

Temperature limits for the efficacy of nematode species in the control of the BVW are necessary information to make decisions about the timing of a nematode application. Nematodes should only be applied when temperatures are high enough to ensure that control is possible. Costs arising from unsuccessful applications can be avoided if temperature recommendations are followed.

2. SCIENCE SECTION

2.1 INTRODUCTION

Monoculture cropping systems, as used in intensive agriculture, provide a ideal environment for the rapid spread of pest and diseases. To keep crop protection problems under control and obtain high yields, chemical pesticides have been used at an increasing rate since the second world war. The world food production increased as well and the ultimate solution for pest and disease problems was thought to have been found. However, over the decades the negative sides of intensive pesticide usage became obvious. Target organisms developed resistance to frequently used pesticides. Additionally, the growing awareness of health hazards to the consumer due to pesticide treatments led to the ban of a variety of chemicals, ultimately resulting in a new way of thinking whereby biological control methods were more important (Hassall, 1990).

Entomopathogenic nematodes are one of the possible biocontrol agents. They are important insect antagonists and encompass a variety of nematode families. However, only rhabditid nematodes of the families Steinernematidae and Heterorhabditidae have emerged as biocontrol agents with the possibility for economical applications in intensive agriculture (Ehlers and Peters, 1995).

Steinernematid and heterorhabditid nematodes combine many characteristics which facilitate their use and commercialisation. The infective juveniles (IJs) are the free-living nematode stage which can be produced *in vitro* on a large scale. They are the active ingredient of the nematode product. The product formulation supports the survival of the IJs, thus, extending shelf live and facilitating handling and application of the nematodes (Georgis and Poinar, 1994).

Steinernematid and heterorhabditid nematodes have a wide host range. This is possible because they are associated with a bacterium which the IJs carry around in their intestine and it is actually this bacterium which causes the rapid death of the insect. These bacteria are non-host specific and because of their rapid action they do not need to be adapted to a specific insect life cycle (Poinar, 1966).

Although entomopathogenic nematodes have a wide host range, they are safe to vertebrates and plants and so far infection of non-target invertebrates under field conditions has not been reported (Akhurst, 1990; Poinar, 1990).

Steinernematid and heterorhabditid nematodes are indigenous to all continents. Their widespread appearance supports their usage all over the world (Poinar, 1990). In spite of their positive characteristics as a biological control agent, it has to be recognised that they are living organisms and successful pest control is only possible if optimal environmental conditions for the IJs can be provided after application. Moisture and temperature are the most important factors influencing nematode efficacy. High moisture requirements make them most successful in the soil environment. Low or very high temperatures can be an other restricting factor for successful pest control (Georgis and Poinar, 1994; Kaya, 1985).

The black vine weevil, *Otiorhynchus sulcatus*, is one target pest which has successfully been controlled by entomopathogenic nematodes in cranberries in the USA (Shanks and Agudelo-Silva, 1990). It is also a pest to many fruit and ornamental crops in the temperate climate of Europe (Simons, 1981; Smith, 1932). However, so far control levels in this environment have been rather moderate due to low temperatures at times when larvae are in the soil (Kakouli, 1995; Sampson, 1994).

Mean soil temperatures in the summer are around 15°C within the top 5 cm of the soil in Britain (Hominick and Briscoe, 1990). In autumn soil temperatures decrease. They drop below 10°C in October and do not exceed 10°C before May the next year (Gwynn, 1993). The BVW larvae, which cause the major damage by feeding on the rootsystem, are present in the soil from late summer to spring the next year (Moorhouse *et al.*, 1992). Hence, nematode application is restricted to the autumn and spring when soil temperatures are above the limits for nematode efficacy.

Nematode species and isolates vary in their temperature requirements and for their appropriate use it is important to know the nematode specific temperature restrictions (Grewal *et al.*, 1994). For the control of *O. sulcatus* nematodes effective at low temperatures are of special interest. Hence, in this study a variety of nematode species

and isolates of the genus *Steinernema* and *Heterorhabditis* were tested against *O. sulcatus* larvae to establish temperature limits and maxima for nematode invasion and pathogenicity.

Soil temperatures are not only influenced by seasons but also by short term climatic changes. Deeper soil layers are well buffered against temperature changes but the top layer changes with atmosphere (Kaya, 1990). A few sunny days may increase soil temperatures for a few hours during the day or keep them at a constant higher level for a longer period. These changes are more noticeable when plants are on raised beds as is done with strawberries. This cultural method increases the soil surface and facilitates the warming up of the soil.

Experiments were carried out to investigate the influence of changing soil temperature on nematode efficacy. Two commercially available nematode species were tested at alternating temperatures between 10 and 15°C.

After nematode application in the autumn, Oakley (1994) found living BVW larvae during the winter which died of nematode infection when transferred to higher temperatures in the laboratory. Grewal et al. (1994) and Molyneux (1986) suggested that IJs which were able to enter an insect but could not start to develop because temperatures dropped soon afterwards are able to survive in a host during low temperature periods and start to develop and kill the insect when the temperature increase. Hence, experiments were carried out to investigate if nematodes which were able to penetrate into an insect after nematode application in autumn could have an effect on larval mortality in the spring.

2.2 EFFECT OF TEMPERATURE ON THE SUSCEPTIBILITY OF *OTIORHYNCHUS SULCATUS* TO ISOLATES OF *STEINERNEMA* AND *HETERORHABDITIS*

2.2.1 Material and methods

Steinernema species

Petri-dishes (3.5 x 1.5 cm) were filled with moist sterile sand and 200 IJs were pipetted evenly onto the sand. One late instar larva of *O. sulcatus* was added to each dish and sealed dishes were placed on a two-dimensional temperature gradient plate (Murdoch *et al.*, 1989) the overall dimension of which were 711 x 711 cm. The area of the plate was sub-divided into 169 small squares by a 13 x 13 cm polystyrene matrix so that each cell could contain a single Petri-dish which was placed directly on the copper plate. To prevent loss of heat to the surrounding environment the whole of the polystyrene matrix was covered with a triple-glazed perflex lid.

Nematodes tested were:
S. carpocapsae UK, ALL
S. feltiae V17E, OBSIII, Nemasys
S. kraussei Nash

O. sulcatus larvae were exposed to nematodes for 6 days at 13 temperatures within the range from 8 to 35°C. Additionally, both *S. carpocapsae* isolates were exposed to the same temperature range for only 4 days and to a temperature range from 6 to 18°C for 10 days. The replication was 6-fold. At the end of the exposure period, larval mortality was recorded and number of nematodes per larva determined.

Heterorhabditis species

Using the same methodology as described for *Steinernema* (section 4.2.1) the following nematodes were tested within the temperature range from 8 to 35°C and an exposure period of 6 days:

Heterorhabditis sp. Fargro, HW79

H. megidis Nemasys H

Nematode counts were log transformed before analysis of variance.

2.2.2 Results

***Steinernema* species**

Independent of exposure period, nematode invasion of both isolates of *S. carpocapsae* was significantly influenced by temperature (4 days exposure: UK isolate $P< 0.01$, ALL isolate $P<0.05$; 6 and 10 days exposure: $P<0.001$) and occurred between 13 and 32°C (Fig. 1, 2, 3). Optimum temperature for nematode invasion was 22 to 30°C. The temperature profiles of both *S. carpocapsae* isolates were very similar. Larval mortality increased with temperature reflecting number nematodes per larva (Fig. 1, 2, 3). However, after 4 and 6 days nematode exposure, the ALL isolate caused higher larval mortality at the lower temperature range than the UK isolate.

Invasion rates of all *S. feltiae* isolates were variable and low over the temperature range tested (Fig. 4). *S. feltiae* Nemasys was the poorest performing isolate and invasion was not significantly affected by temperature. *S. feltiae* OBSIII invaded *O. sulcatus* larvae between 8 and 26°C but the mean number of nematodes never exceeded 2.2. However, invasion was significantly affected by temperature ($P< 0.05$). Invasion of the V17E isolate occurred from 6 to 28°C and was higher than for the other *S. feltiae* isolates. However, invasion was not significantly affected by temperature because the number of nematodes per larva was variable. Larval mortality due to *S. feltiae* Nemasys and *S. feltiae* OBSIII was low below 30°C (Fig. 4). *S. feltiae* V17E caused higher larval mortality than the other *S. feltiae* isolates at a temperature range from 21 to 28°C.

Fig. 1. Mean number of nematodes of *S. carpocapsae* (UK, ALL) having invaded *O. sulcatus* larvae (A), and percentage larval mortality (B) after 4 days nematode exposure

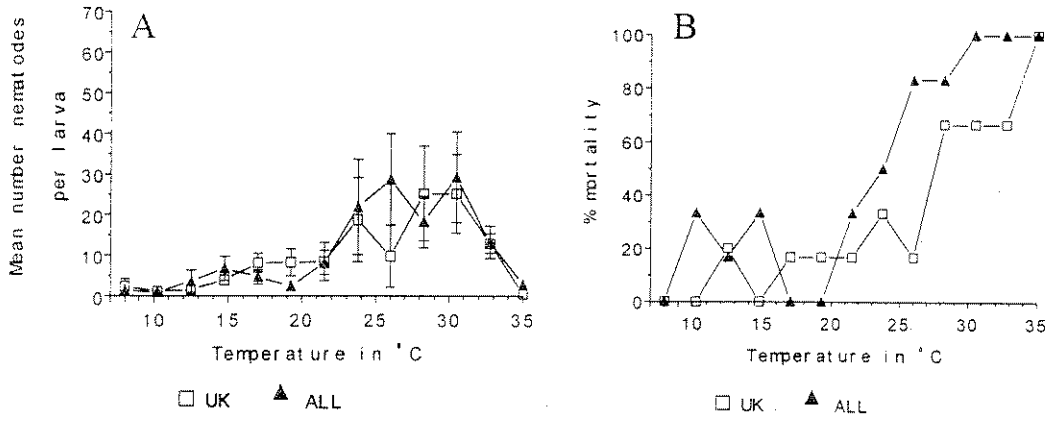


Fig. 2. Mean number of nematodes of *S. carpocapsae* (UK, ALL) having invaded *O. sulcatus* larvae (A), and percentage larval mortality (B) after 6 days nematode exposure

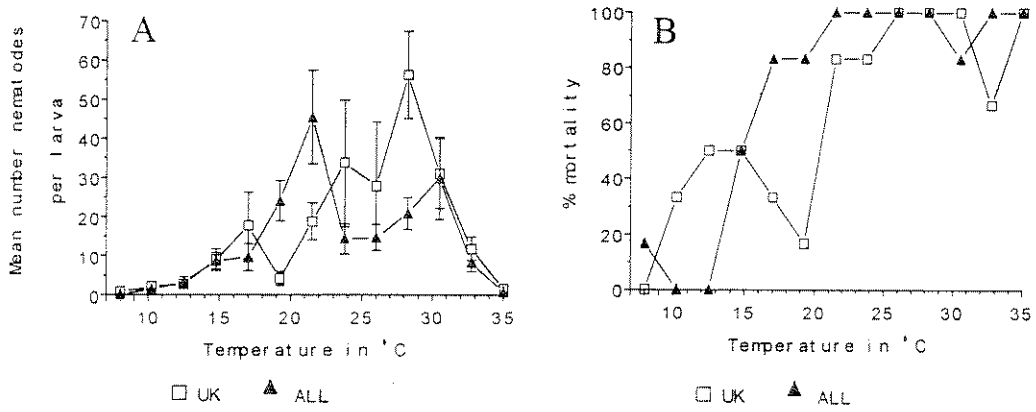
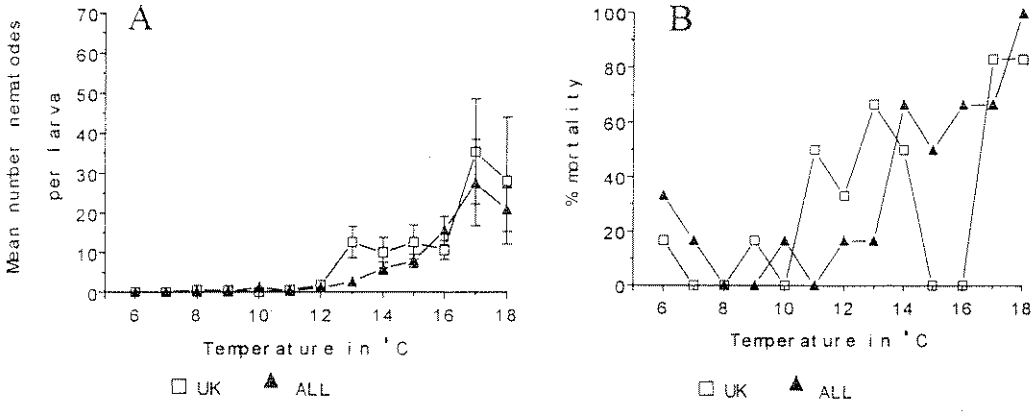


Fig. 3. Mean number of nematodes of *S. carpocapsae* (UK, ALL) having invaded *O. sulcatus* larvae (A), and percentage larval mortality (B) after 10 days nematode exposure



S. kraussei Nash invaded *O. sulcatus* larvae from 10 to 24°C (Fig. 5). Although mean number of nematodes per larva never exceeded 1.5, invasion was significantly affected by temperature ($P<0.05$). Larval mortality was constant and only exceeded 33% at the highest temperature (Fig. 5).

Fig. 4. Mean number of nematodes of *S. feltiae* (V17E, OBSIII, Nemasys) having invaded *O. sulcatus*, larvae (A) and percentage larval mortality (B) after 6 days nematode exposure

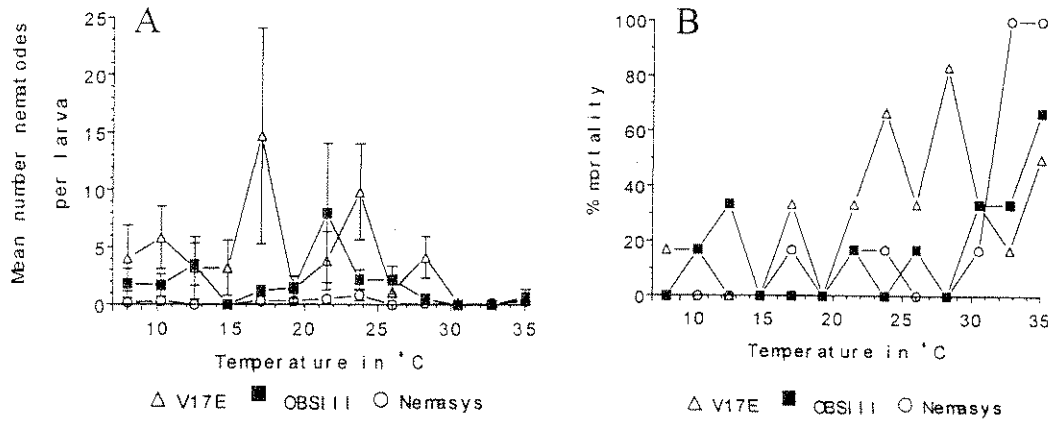
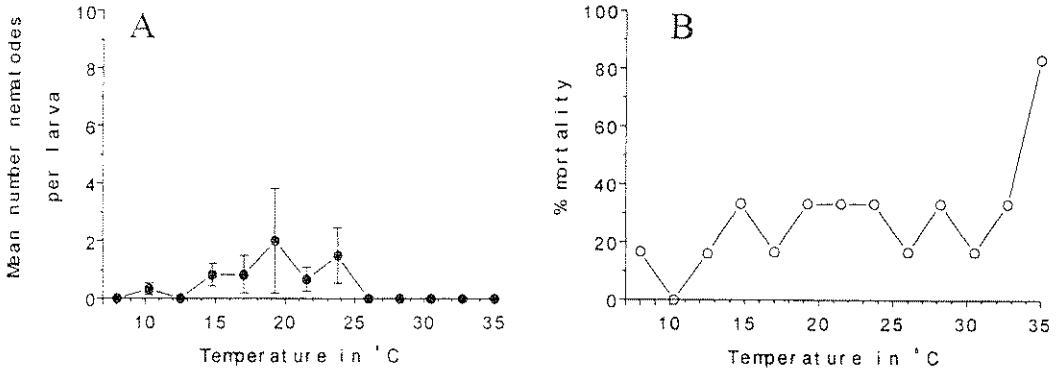


Fig. 5. Mean number of nematodes of *S. kraussei* (Nash) having invaded *O. sulcatus* larvae (A) and percentage larval mortality (B) after 6 days nematode exposure

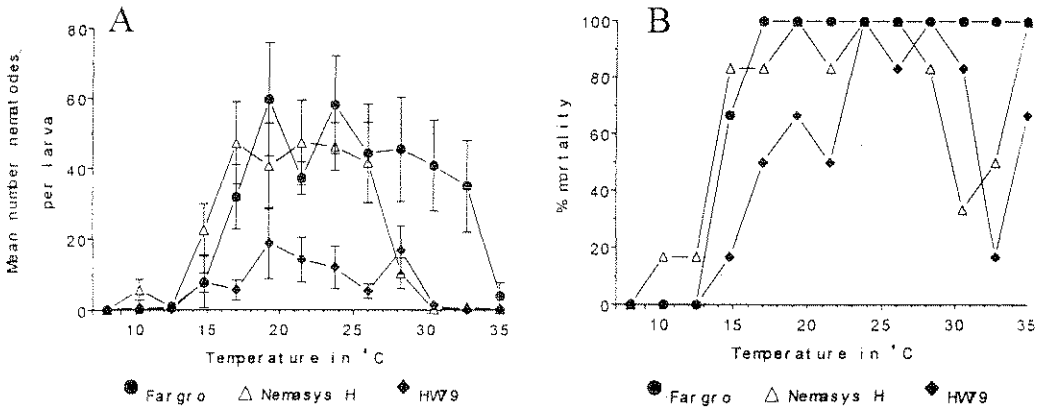


***Heterorhabditis* species**

Nematode invasion by *Heterorhabditis* sp. HW79 and *H. megidis* Nemasys H occurred between 15 and 28°C and was significantly affected by temperature ($P<0.001$) (Fig. 6). Optimum temperature for invasion was 15 to 26°C for Nemasys H and 19 to 28 °C for HW79. However, the mean number of nematodes per larva was lower for HW79 than for Nemasys H. *H. bacteriophora* Fargro entered *O. sulcatus* larvae between 15 and 33°C with the optimum between 19 and 33°C. Within the temperature range of 15 to 26°C, nematode invasion rate was similar for *Heterorhabditis* sp. Fargro and *H. megidis* Nemasys H. More nematodes of *Heterorhabditis* sp. Fargro than of *H. megidis* Nemasys H entered *O. sulcatus* larvae at the temperature range from 28 to 33°C.

For all *Heterorhabditis* isolates tested, larval mortality occurred between 15 and 35°C (Fig. 6). Larval mortality caused by *Heterorhabditis* sp. Fargro was 100 % from 17 to 35°C. *H. megidis* Nemasys H caused larval mortality between 83 and 100 % at the temperature range from 15 to 28 °C. *Heterorhabditis* sp. HW79 killed fewer larvae at lower temperatures than the other nematode isolates and reached 83 to 100 % between 24 and 30°C.

Fig. 6. Mean number of nematodes of *Heterorhabditis* sp. (Fargro, HW79) and *H. megidis* (Nemasys) having invaded *O. sulcatus* larvae (A) and percentage larval mortality (B) after 6 days nematode exposure



2.3 THE EFFECT OF ALTERNATING TEMPERATURES ON INVASION AND PATHOGENICITY OF *STEINERNEMA CARPOCAPSAE* AND *HETERORHABDITIS MEGIDIS*

2.3.1 The effect of a daily temperature increase from 10 to 15°C on nematode invasion and pathogenicity

2.3.1.1 Material and methods

Petri-dishes (3.5 x 1.5 cm) were filled with moist sterile sand and 200 IJs of *S. carpocapsae* UK or *H. megidis* Nemasys H were pipetted evenly onto the sand. One late instar *O. sulcatus* larva was placed in each dish and dishes were sealed with parafilm. Following temperature treatments were applied: constant temperature of 10 or 15°C or 10°C with a daily temperature increase to 15°C for 1 hour, 3 hours or 5 hours. The replication was 12-fold. After 10 days, larval mortality was recorded, the number of nematodes per larva counted.

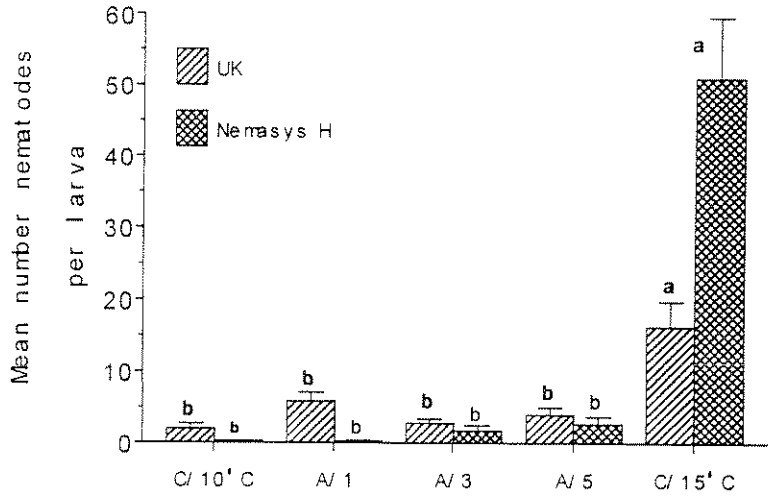
Nematode counts were square root transformed before analysis of variance. For the comparisons of more than two variables the Student-Newman-Keuls Test was applied and significant differences were established on the 5 % ($P<0.05$) or 1 % ($P<0.01$) level.

2.3.1.2 Results

A daily temperature increase from 10 to 15°C for 1 to 5 hours did not significantly affect invasion of *S. carpocapsae* UK and *H. megidis* Nemasys H in comparison to the temperature remaining constant at 10°C (Fig. 7). At a constant temperature of 15°C significantly more nematodes of both species invaded *O. sulcatus* larvae than in the other temperature treatments ($P<0.01$).

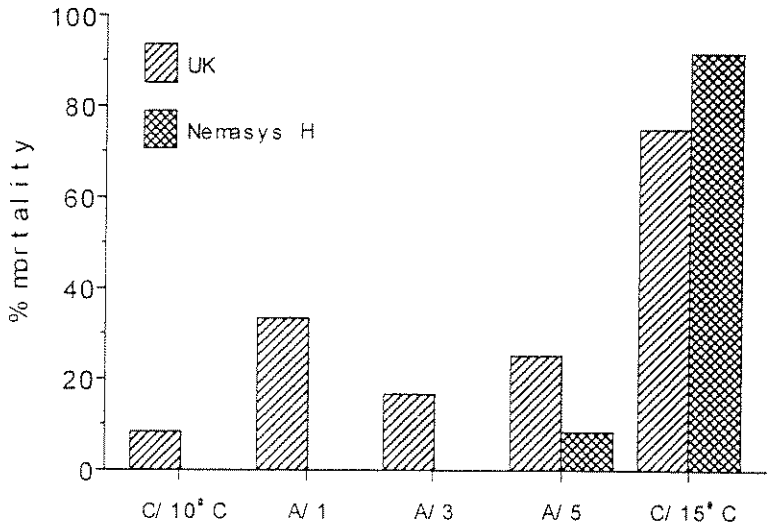
A daily temperature increase to 15°C for up to 5 hours did not result in larval mortality exceeding 33.3 % for *S. carpocapsae* UK and 8.3 % for *H. megidis* Nemasys H (Fig. 8). After exposure to a constant temperature of 15°C, larval mortality was 75.0 and 61.7 % for *S. carpocapsae* UK and *H. megidis* Nemasys H respectively.

Fig. 7. Invasion of *S. carpocapsae* UK and *H. megidis* Nemasys H into *O. sulcatus* larvae during 10 days nematode exposure at constant 10 and 15°C (C/10°C; C/15°C) or at 10°C with a daily temperature increase to 15°C for 1 (A/1), 3 (A/3) and 5 hours (A/5)



Bars marked with the same letter are not significantly different between temperature treatments for the same nematode species ($P<0.01$).

Fig. 8. Larval mortality of *O. sulcatus* exposed to *S. carpocapsae* UK and *H. megidis* Nemasys H for 10 days at constant 10 and 15°C (C/10°C; C/15°C) or at 10°C with a daily temperature increase to 15°C for 1 (A/1), 3 (A/3) and 5 hours (A/5)



2.3.2 The effect of a temperature increase from 10 to 15°C for 5 days at different times after the onset of the experiment on nematode invasion and pathogenicity

2.3.2.1 Material and methods

To evaluate nematode invasion into *O. sulcatus* larvae and larval mortality a number of Petri-dishes were prepared with sand and IJs of *S. carpocapsae* UK and *H. megidis* Nemasys H as described in section 2.2.1.1. The replication was 12-fold. The following temperature treatments were applied:

1. constant 10°C for 15 days,
2. constant 15°C for 15 days,
3. 15°C from day 1 to day 5 then 10°C from day 6 to day 15,
4. 10°C from day 1 to day 5 then 15°C from day 6 to day 10 then 10°C from day 11 to day 15,
5. 10°C from day 1 to day 10 then 15°C from day 11 to day 15.

Mean number of nematodes per larva and larval mortality were recorded.

2.3.2.2 Results

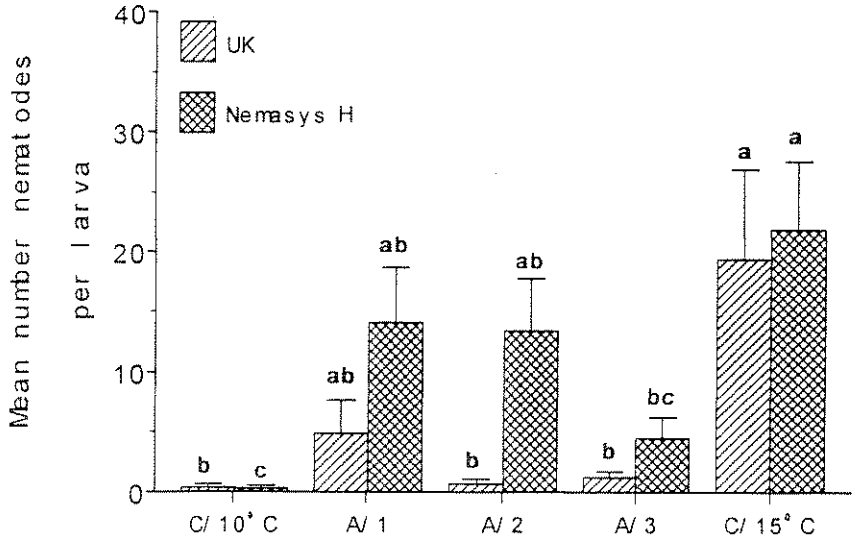
More IJs of *S. carpocapsae* UK invaded *O. sulcatus* larvae when the incubation temperature was 15°C from day 1 to day 5 than at a later temperature increase or at constant 10°C (Fig. 9). However, the difference was not significant. Highest nematode invasion of *S. carpocapsae* UK was recorded at constant 15°C. Nematode invasion was lower when the incubation temperature was 15°C from day 1 to day 5 compared to treatment at a constant 15°C but the difference was not significant.

Nematode invasion by *H. megidis* Nemasys H was similar when the temperature was kept at 15°C from day 1 to day 5 or from day 6 to day 10: nematode invasion was lower, but not significantly, when the temperature was increased to 15°C from day 11 to day 15. The highest invasion rate for *H. megidis* Nemasys H was recorded at a constant temperature of 15°C. However there was no significant difference between

the invasion at a constant temperature of 15°C and a temperature increase from day 1 to day 5 or day 6 to day 10.

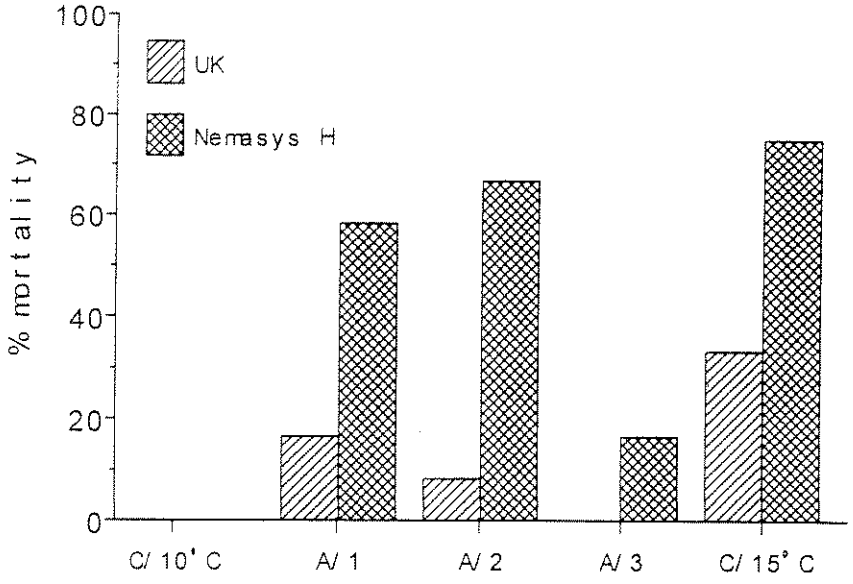
After exposure to *S. carpocapsae* UK, larval mortality did not exceed 33 % for any temperature treatment (Fig. 10). After exposure to *H. megidis* Nemasys H larval mortality reached 58.3 and 66.7 % after a temperature increase from day 1 to day 5 and day 5 to day 10 respectively. Larval mortality was 75 % at a constant 15°C.

Fig. 9. Invasion of *S. carpocapsae* UK and *H. megidis* Nemasys H into *O. sulcatus* larvae during 15 days nematode exposure at constant 10 or 15°C (C/10°C; C/15°C) or at 10°C with a temperature increase to 15°C from day 1 to day 5 (A/1), day 6 to day 10 (A/2) and day 11 to day 15 (A/3)



Bars marked with the same letter are not significantly different between temperature treatments for the same nematode species ($P<0.01$).

Fig. 10. Larval mortality of *O. sulcatus* exposed to *S. carpocapsae* UK and *H. megidis* Nemasys H for 15 days at constant 10 or 15°C (C/10°C; C/15°C) or at 10°C with a temperature increase to 15°C from day 1 to day 5 (A/1), day 6 to day 10 (A/2) and day 11 to day 15 (A/3)



2.3.3 The effect of 15°C for up to 5 days from the onset of the experiment followed by 10°C on nematode invasion and pathogenicity

2.3.3.1 Material and methods

To evaluate nematode invasion into *O. sulcatus* larvae and larval mortality, a number of Petri-dishes were prepared with sand and IJs of *S. carpocapsae* UK and *H. megidis* Nemasys H as described in section 2.2.1.1. Following temperature treatments were applied:

1. constant 10°C for 15 days,
2. constant 15°C for 15 days,
3. 15°C for 1 day then 14 days 10°C,
4. 15°C for 3 days then 12 days 10°C,
5. 15°C for 5 days then 10 days 10°C.

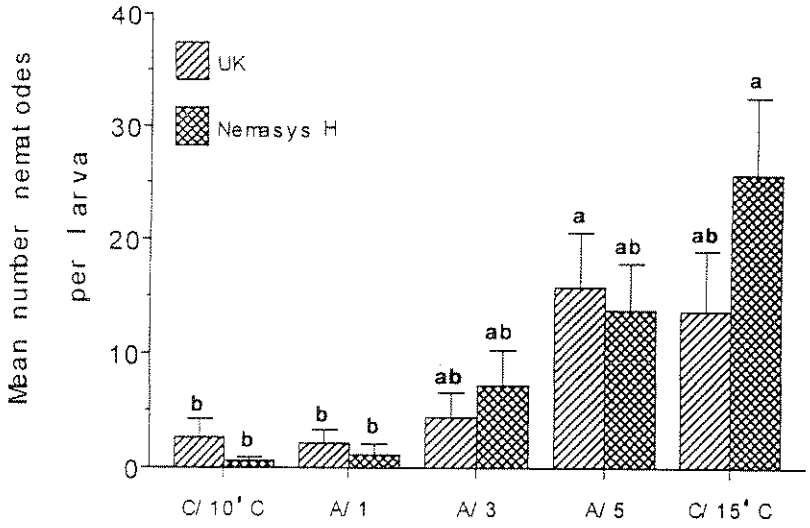
Mean number of nematodes per larva and larval mortality were recorded.

2.3.3.2 Results

Nematode invasion by *S. carpocapsae* UK and *H. megidis* Nemasys increased with exposure time from 1 to 5 days at 15°C (Fig. 11). The invasion rate of *S. carpocapsae* UK after 5 days at 15° C was similar to the temperature treatment of constant 15°C. The invasion rate of *H. megidis* Nemasys H was higher after incubated at constant 15°C than after incubation at 15°C for only 5 days followed by 10°C. However, the difference was not significant.

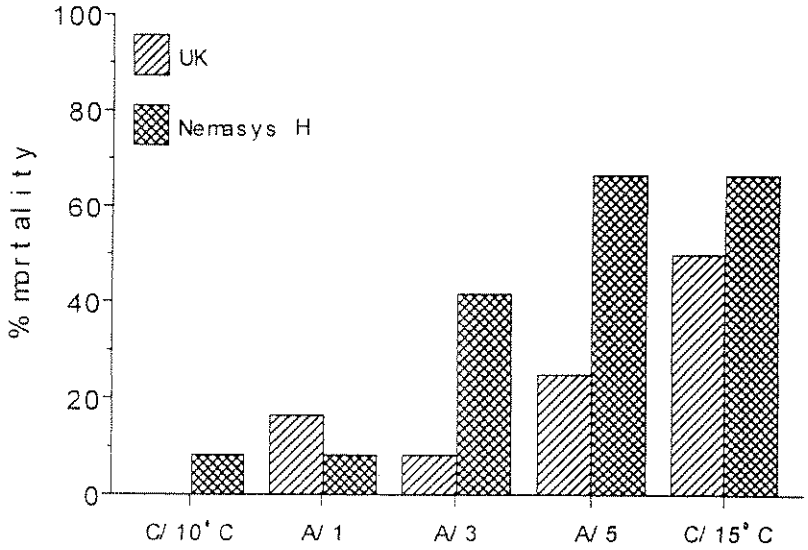
Larval mortality did not exceed 25 % for *S. carpocapsae* UK when the temperature was kept at 15°C for up to 5 days (Fig. 12): at constant 15°C larval mortality was 50 %. Mortality of BVW larvae exposed to *H. megidis* Nemasys H increased with prolonged exposure to 15°C. After 5 days at 15°C larval mortality was 66.7 % which was identical with the mortality rate at constant 15°C.

Fig. 11. Invasion of *S. carpocapsae* UK and *H. megidis* Nemasys H into *O. sulcatus* larvae during 15 days nematode exposure at constant 10 or 15°C (C/10°C; C/15°C) or at 15°C for 1 (A/1); 3 (A/3) or 5 (A/5) days from the onset of the experiment followed by 10°C



Bars marked with the same letter are not significantly different between temperature treatments for the same nematode species (P<0.01).

Fig. 12. Percentage larval mortality of *O. sulcatus* exposed to *S. carpocapsae* UK and *H. megidis* Nemasys H for 15 days at constant 10 or 15°C (C/10°C; C/15°C) or at 15°C for 1 (A/1); 3 (A/3) or 5 (A/5) days from the onset of the experiment followed by 10°C



2.4 THE EFFECT OF LOW TEMPERATURE ON SURVIVAL OF *STEINERNEMA CARPOCAPSAE* IN *GALLERIA MELLONELLA* AND *OTIORHYNCHUS SULCATUS* LARVAE

2.4.1 Material and Methods

Survival of *S. carpocapsae* in *G. mellonella* larvae

A dosage of 400 IJs of *S. carpocapsae* (UK isolate) was applied in 1 ml of water to filter paper in 9 cm Petri dishes. Dishes used for the control received water only. One *G. mellonella* larva was placed in each dish and kept at 22°C for 2 hours. The replication was 12-fold. Larvae were then rinsed with water and transferred to dry filter paper in multi-well tissue culture plates 2.5 cm in diameter, and treated as follows:

Treatment A: exposure at 22°C for 48 hours

Treatment B: exposure at 10°C for 5, 10 or 20 days

Treatment C: exposure at 10°C for 5 or 10 days then 22°C for 48 hours

Larval mortality was recorded at the end of each treatment and the number of nematodes establishing counted. The number of dead nematodes per larva was determined in those treatments where there was 100 % mortality.

Survival of *S. carpocapsae* in *O. sulcatus* larvae

A dosage of 400 infective juveniles (IJs) of *S. carpocapsae* (UK isolate) was applied in 0.5 ml of water to 4 g sterile moist sand in multi-well tissue culture plates 2.5 cm in diameter. Dishes used for the control received water only. One *O. sulcatus* larva was placed in each dish and kept at 22°C for 3 hours. Larvae were then rinsed with water and transferred to nematode-free wells filled with moist sterile sand. Replication was 12-fold; treatments were as followed:

Treatment A: exposure at 5 or 10°C for 10, 20, 30 or 40 days

Treatment B: exposure at 5 or 10°C for 10, 20, 30 or 40 days then at 22°C for 120 hours

Larval mortality was recorded after the completion of each treatment. The number of nematodes infecting the vine weevil larvae was determined for treatment B. Control larval mortality refers to treatment A only.

2.4.2 Results

S. carpocapsae inside *G. mellonella* larvae was able to cause host mortality at 10°C (Table 1): after 20 days, all larvae were dead (treatment B). When larvae were transferred from 10°C to 22°C after 5 and 10 days, larval mortality was 100 % (treatment C). All larvae in the control survived.

After an initial nematode exposure for 2 hours at 22°C and subsequent treatment at 22°C for 48 hours the number of nematodes found in *G. mellonella* larvae was greater than in treatment B or C (Table 1) which suggests that invading nematodes had disappeared when larvae were exposed to 10°C. The percentage of nematodes dead in treatment B and C were significantly greater than in treatment A (Table 1).

S. carpocapsae did not kill *O. sulcatus* larvae during exposure at 5°C except for one larva after 20 days (Table 2). However, mortality increased when larvae were transferred to 22°C. BVW larvae died during exposure to 10°C; after increasing the temperature to 22°C for 3 days there was only a minimal increase in larval mortality. Larval mortality did not exceed 50 % in any treatment because of low initial nematode infection.

The number of *S. carpocapsae* surviving in *O. sulcatus* larvae treated at 5 and 10°C declined with time of exposure to these temperatures (Figure 13; $P<0.05$), indicating that nematodes had disappeared after initial invasion. Exposure temperature did not significantly influence the mean number of nematodes per larva.

Table 1. The percentage mortality of *G. mellonella* larvae, the number of nematodes established and the percentage of dead nematodes in cadavers after initial exposure to *S. carpocapsae* at 22°C for 2 hours followed by treatments at: A, 22°C for 48 hours; B, 10°C for 5, 10 or 20 days; C, 10°C for 5 or 10 days followed by 48 hours at 22°C.

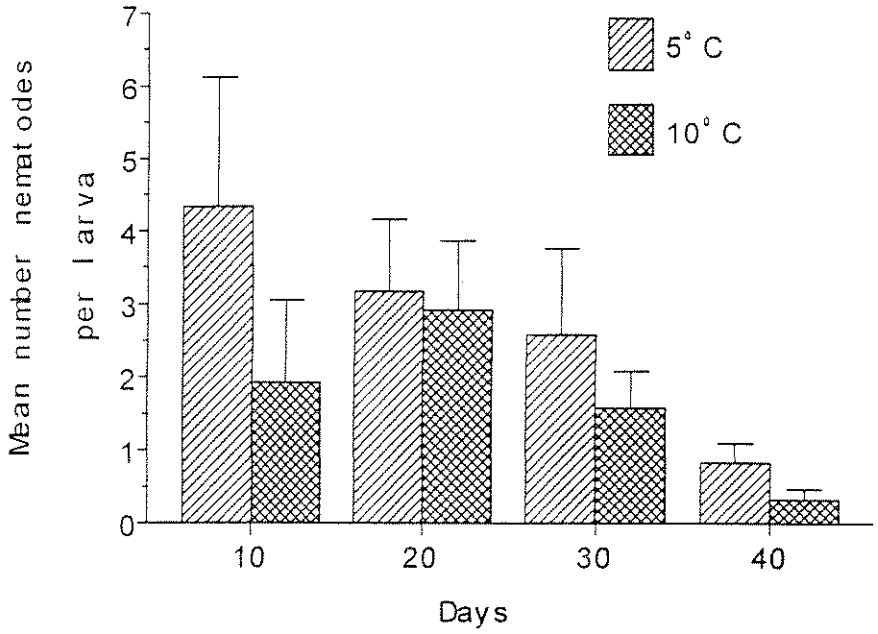
Treatment	Days at 10°C	% larval mortality	No. nematodes established (±SEM)	% dead nematodes
A	0	100	21.5 ±8.3	4.2
B	5	0	--	
	10	17	--	
	20	100	6.3 ±1.4	40.3
C	5	100	10.6 ±3.0	20.6
	10	100	5.7 ± 1.6	40.3

SEM = standard error of the mean

Table 2. The percentage mortality of *O. sulcatus* larvae after initial exposed to *S. carpocapsae* at 22°C for 3 hours followed by treatments at: A, 5°C or 10°C for 10 to 40 days; B, 5 or 10°C for 10 to 40 days followed by 120 hours at 22°C.

	5°C		10°C	
	Treatment		Treatment	
Days	A	B	A	B
10	0.0	25.0	0.0	16.7
20	8.3	50.0	16.7	25.0
30	0.0	33.3	50.0	50.0
40	0.0	33.3	16.7	8.3

Figure 13. Mean number of nematodes per *O. sulcatus* larva after initial exposure to *S. carpocapsae* at 22°C for 3 hours followed by treatments at 5 or 10°C for up to 40 days followed by 22°C for 120 hours



3 CONCLUSIONS

1. Temperature limits for nematode invasion and pathogenicity were above 13°C for all nematode species tested. An extended exposure period below 13°C did not increase invasion of *S. carpocapsae*.

2. Alternation of temperature: There was no significant effect of alternating the temperature daily between 10 and 15°C for up to 5 hours on invasion and pathogenicity of *S. carpocapsae* and *H. megidis*. Host invasion was poor when the temperature was 10°C at the time of nematode application and did not significantly increase when raised to 15°C at a later time. Nematodes were most effective when the temperature was suitable for invasion at the time of application and when it was maintained for 3 to 5 days.

3. Survival: IJs of *S. carpocapsae* which were able to invade BVW larvae at temperatures above 13°C can survive in the living insect at temperatures below 10°C and resume development and kill when the temperature increases. If the temperature decreased not below 10°C nematodes and bacteria development continued at a low rate and larvae died eventually.

4 GLOSSARY

BVW = Black vine weevil, *Otiorhynchus sulcatus*

IJs = Infective juveniles: the non-feeding, free-living nematode stage in the soil and active ingredient of nematode products

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UNIVERSITY OF READING
Contract Accounting
Whiteknights
Tel: x 8106

5 October 1992

DEPARTMENT: AGRICULTURE

INVESTIGATOR: DR N.G.M. HAGUE

HEAD OF DEPARTMENT: PROFESSOR R.J. SUMMERFIELD

FROM: JILL KNIGHT

AWARD OF RESEARCH GRANT OR CONTRACT

the attached are details of an award which have been accepted on behalf of the University. You will have received a copy of the conditions of the award which have also been accepted and confirmation has been given to the sponsor that these will be compiled with by all staff working on the project. Where any arrangements involving staff are concerned, will you kindly ensure that the Personnel Department have full details, in particular if a work permit may be necessary, early contact is essential. Any variation of the agreed budget attached must be agreed in advance.

As you are aware, research grants are awarded to the University, though in practice the **Head of Department** is ultimately responsible for the funds being administered by the named researcher. When a grant comes to an end and it has been established by the Contracts Accounting Office that there is some residual money available, it is the Head of Department who approves of its ultimate use, though obviously the wishes of the sponsor would have to be considered. Conversely, if an award becomes overdrawn, it is the **Department's responsibility** to make good the shortfall. Where there are surplus funds and the work is continuing with an extension from the same sponsor, such funds should be transferred to a new account or alternatively the new grant should go into the old account number.

Distribution: Head of Department
Personnel Department
Standing Committee
Mrs M. Newbery

Contract between University of Reading (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT **Contract No: HNS/SF36**

ENTOMOPATHOGENIC NEMATODES: EFFICACY AT DIFFERENT TEMPERATURES

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Entomopathogenic nematodes have been used commercially in the UK to a limited extent for a number of years.

The principle host insects have been Vine Weevil, *Otiorhynchus sulcatus* in ornamentals (controlled by Heterorhabditid nematodes, eg Nemasys H [AGC] and Fightagrub [Fargro]) and Sciarids in glasshouses/ propagation houses (Nemasys [AGC] and Fightagrub).

The major market segments in the UK for control of vine weevil are strawberries and blackcurrants, both outdoor crops where treatment has to be done in autumn or spring when low temperature is known to influence efficacy.

The commercial objective is to study in detail the efficacy of nematodes at temperatures where control is failing, principally at 15°C or below for field crops but also at temperatures above 28°C in glasshouses/propagation houses.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

Biosys has pioneered the production of entomopathogenic nematodes in fermenters and will support the work in two ways:

1) by making a contribution to the support of the postgraduate student and

2) by supplying nematodes for field trials.

The commercial benefit to the horticultural industry will be the introduction of nematode products into the UK horticulture market, particularly in outdoor crops of strawberries and blackcurrants, which are perceived to be the major market segments at risk to vine weevil (other than ornamentals).

The aim is to introduce products which are competitive both in price and application to conventional insecticides. (If required, it would be possible to produce some pricing structures from the USA for certain market segments).

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

One of the major limiting factors related to the efficient use of entomopathogenic nematodes is the lack of knowledge relating to efficacy at both high temperatures, 28°C or above, and below 15°C.

There are two specific target organisms in British horticulture:

1) The Vine Weevil (*Otiorhynchus sulcatus*) which lays its eggs in July-November. Much of the larval life cycle takes place at temperatures below 15°C and timing of nematode treatment in relation to temperature is critical.

2) Sciarids - fungus gnats are a major problem of glasshouse ornamentals and in propagation houses but there is some evidence from growers that at temperatures exceeding 28°C control becomes inefficient.

It is important to know the reasons for this observed breakdown in control.

Is it caused by the inability of the nematode to invade the host? Or are the lethal bacteria unable to colonise the host haemocoel?

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

The proposer is aware of two projects:

1) Financed by AGC/EEC - which appears to be concerned with discovering "cold tolerant nematodes". This work is being done in Ireland and Netherlands.

2) Financed by MAFF at Littlehampton (HRI) - again is concerned with "cold tolerant nematodes" sampled in the UK. Species being looked at are *S.feltiae* and *S.affinis*. The proposer has knowledge of this work as the person conducting the research is registered for a PhD at Reading.

6. DESCRIPTION OF THE WORK

The experimental work will be divided into three phases:

1) <u>Laboratory</u>

The Crops Research section of the Department of Agriculture has developed a very useful piece of apparatus to measure seed germination over a range of temperatures from 6°C to 40°C. The apparatus can be immediately modified to test various nematode species/strains against Vine Weevil larvae, Sciarid larvae and Wax Moth larvae (the laboratory

test insect).

Host insect would be exposed to a known number of infective juveniles for 4 days and then estimates would be made of (a) Mortality, (b) Invasion and (c) Nematode development within the host.

These results would enable us to pinpoint the temperature range for each nematode strain which needs further investigation to find out why control has not been achieved.

The nematodes to be investigated would be :-

1. *S.feltiae* - two strains, Nemasys and a strain from Reading University;.

2. *S.carpocapse* UK strain.

3. *Heterorhabditis megadis* (Nemasys H/AGC).

4. *Heterorhabditis bacteriophora* (Fargro/ Fightagrub).

We would also include other UK strains/species as they became available.

2) Field investigations

Having established the range of temperature at which control breaks down, we would wish to investigate this further under field conditions.

a) Low Temperatures - strawberries grown under black polythene with irrigation - we would integrate this research with work being done by Bunting Biological Control in Kent.

b) High Temperatures - on ornamentals being propagated in glasshouses during the summer - this would be integrated with other work already being done at Hillier Bros at Winchester, where we are studying persistence of nematodes in compost.

3) Studies on Bacterial Development

It is envisaged that one of the possible reasons for breakdown of control is related to non-reproduction of the lethal bacterium which is responsible for the death of the insect host.

We would wish to investigate the development of bacteria at the critical temperatures resulting from the laboratory experiments.

We would consult with the Microbiology Department at the

University of Reading regarding techniques.

Summary

Year 1 (a) Laboratory investigations including a start with the work on bacteria at low and high temperatures.

(b) Initial field studies on efficacy at high temperature in glasshouses/ propagation houses.

Year 2 Initial field investigations on strawberries through the winter to spring/early summer.

Continue high temperature work in propagation houses and laboratory work on temperature efficacy.

Year 3 Complete work on temperature efficacy of nematodes and bacterial work.

Continue the field studies on strawberries through winter.

7. COMMENCEMENT DATE AND DURATION

Starting 1 October 1992.
Duration 3 years.

8. STAFF RESPONSIBILITIES

Project Leader/Supervisor: Dr N G M Hague

Other Staff

Postgraduate PhD student.

9. LOCATION

The principle work would be done in laboratories of the Crop Protection Research Unit in the Department of Agriculture at the University of Reading.

Other sites where field work would be done are:

a) Field sites to be arranged with Bunting Biological Control Ltd.

b) Propagation houses at Hilliers at Winchester.

10. COSTS

The cost per annum for a postgraduate student at Reading in

1992/93 is £7,500. We would expect the total cost to go up by about 10% per annum.

I would expect the breakdown of costs to be as follows:-

	1992/93	1993/94	1994/95
HDC	4,000	4,500	5,000
Biosys	2,000	2,500	2,750
CPRU	1,500*	1,250*	1,325*
	7,500	8,250	9,075

* The CPRU would be responsible for providing consumables and finance for the travel for field work.

11. PAYMENT

On each quarter day the Council will pay to the Contractor in accordance with the following schedule:

Quarter/Year	1992	1993	1994	1995
1	-	1000	1125	1250
2	-	1000	1125	1250
3	-	1000	1125	1250
4	1000	1125	1250	-

Contract No: HNS/SF36

TERMS AND CONDITIONS

The Council's standard terms and conditions of contract shall apply.

Signed for the Contractor(s)

Signature.....K. LOBLEY.....

Position.....INDUSTRIAL CONTRACTS OFFICER UNIVERSITY OF READING.....

Date.....- 9 SEP 1992.....

Signed for the Contractor(s)

Signature.....

Position.....

Date.....

Signed for the Council

Signature.....

Position.....CHIEF EXECUTIVE.....

Date.....12.6.92.....