Report on Short Term Scientific Mission
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Host: Ralf Ehlers, Institute of Phytopathology, University of Kiel
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Place: Kiel
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By Muzeyen Mohammed, 2005-06-03

Purpose of the visit

To learn in vivo and in vitro production techniques of entomopathogenic nematodes,
To get acquainted with handling and storage methods of entomopathogenic nematodes,
To set an experiment on control of sciarid flies by entomopathogenic nematodes at temperatures above 30°C.

Title:
Control of Sciarid flies by Entomopathogenic nematodes at temperatures above 30°c

Introduction

Insects cause considerable damage to plants and human possessions. In some instances the extent of damage to plants is high and it is difficult to get net return. In general, most of the insects are controlled by chemical insecticides. In the excitement and euphoria surrounding the use of these power full tools, the natural laws of ecology were all but forgotten. In recent years many unfavourable side effects are recognized with the use of chemical insecticides, viz. insect resistance, toxicity to beneficial organisms and environmental pollution. This has led researchers to adopt integrated approaches to manage pest population below injury level with out disturbing the biotic balance. Integrated pest management (IPM) is the intelligent selection and use of pest control actions that will ensure favourable economic, ecological and sociological consequences. Biological control is one of the most important tools in the IPM.

Sciarid flies commonly infest glass house ornamentals where they rapidly increase to levels where serious damage may occur. The larvae feed on organic matter, fungi or algae (Freeman, 1983) also on the roots of cuttings and small plants, tunnelling into their stems (Binns, 1973). Sciarid larvae can damage mature plants and are vectors of bacterial and fungal pathogens (Leath & Newton,1969).

Many growers are satisfied with *S. feltiae* as a biological control agent for sciarids, however there has been concern that poor control is achieved when temperatures approach 30°C or more in glasshouses. *Steinernema feltiae* is a cold-adapted nematode species with infection occurring between 8° and 30°C and reproduction between 10° and 25°C (Grewal et al., 1994). Poor persistence and lack of reproduction of *S. feltiae* at warm temperatures poses a serious constraint for the use of this species in the greenhouses where temperatures often exceed 30°C during the summer. Gouge and Hague (1994) reported that the efficacy of the cold-adapted *S. feltiae* against fungus gnats was reduced if soil temperatures in the greenhouse remained above 25°C for prolonged period of time and they suggested that *S. feltiae* should be used

against sciarids at temperatures between 15° and 26°C for most satisfactory results. In a subsequent study these researchers demonstrated that the warm-adapted *Heterorhabditis* spp., *S. anomali* and *S. riobrave* provided better control of sciarids than *S. feltiae* at 30°C (Gouge and Hauge, 1995a or b).

Therefore this study was conducted to investigate the potential of EPN as biocontrol of insects at higher temperatures with the following objectives:

1) To select warm active entomopathogenic nematode species
2) To find out species of entomopathogenic nematodes that could control sciarid flies at temperatures above 30°C.

MATERIALS AND METHODS

Culturing nematodes

The greater wax moth was used for multiplication of nematodes. Nematode species cultured: *H.indica, H.bacteriophora, H.mareelatus, S.feltiae, S.glaseri, S.riobravis*

Rearing sciarid insects

Sciarids were reared at 21°C in ventilated plastic boxes(28x16x9cm) filled to a depth of about 5cm with peat. About 10ml of water was mixed into the peat so that the mixture will be moist to the touch and 1g of cereal was sprinkled over the surface of peat as food source for the larvae. Adult sciarids were put into the boxes and every 3-4 days a further 1g of the cereal was added together with a cotton wool plug soaked in a 20 percent glucose which boosted the number of eggs laid by the sciarid females (Gouge,1994).

Temperature gradient and water trap apparatus

A temperature gradient with water traps was used to test for high temperature activity of nematodes according to Ehlers et al.(2005).

Experiment 1
Selection of warm active nematodes

To screen for nematode species with high temperature activity DJ were pipetted on a filter paper (18x18mm). The filter paper with nematodes accumulated in the middle was placed on aluminium plate form into a chamber filled with water and left inside the incubator for 2 hrs. The filter paper was also placed in the counting dishes to get the total count of nematodes. Then water inside the chambers was pipetted into a counting dish to count nematodes moved into the water. The filter paper was also placed in the counting dishes to get the total count of nematodes. The percentage of nematodes moved to water was calculated. Detailed information about the method is published in Ehlers et al. (2005).

Experiment 2
Infective potential of EPN nematodes at different temperature

Larvae of *Tenebrio melitor* (Colepotera: Tenebrionidae) were used for screening for nematode species with high infective potential at different temperatures. Nematode species tested: *H.bacteriophora, H.marelatus, S.feltiae, S.glaseri, S.riobravis*. Fine course dry sand was mixed with water so that the mixture content will be 10% and placed in Petri dishes to a depth

of about 0.5cm. Ten insect larvae were placed in Petri dishes on top of the sand. Suspensions of nematodes of each of the 5 species were inoculated at a rate of 20 nematodes per larvae. Untreated larvae received only water. Replication was 4 folds. The Petri dishes were then sealed with parafilm and incubated at 25°, 30° & 35°C. After 48 hrs mortality was recorded and the number of nematodes infecting the larvae counted by dissecting the larvae in Ringers solution.

Experiment 3
Control of sciarids with EPN at temp. above 30°C.

Those nematode species and strains, which proved to be active or caused high mortality with a higher invasive potential in Tenebrio larvae were used to test for their efficacy against sciarid flies. Two nematode species: *H. indica* and *S. riobravis* together with *S.feltiae* for comparison were used. An insect trap consisting of a dark (10x10x10 cm) pot and a clear plastic box fixed with a tube to the dark pot was constructed in such a way that emerging adult flies can move from the dark pot to the bright box which was painted with a sticky substance so adult flies would get stuck in the upper chamber. Peat soil was moistened to the touch and filled in the pot to about 4 cm depth. 35 sciarid larvae were placed in each trap. The suspensions of each of the species of nematodes were inoculated at a rate of 25 per 1 cm^2 area of top surface of the pot. Untreated pots received only water. All the treated pots for each species together with control were incubated at 25°, 30°, & 35°C.The number of sciarid flies emerged with in 15 days were recorded.

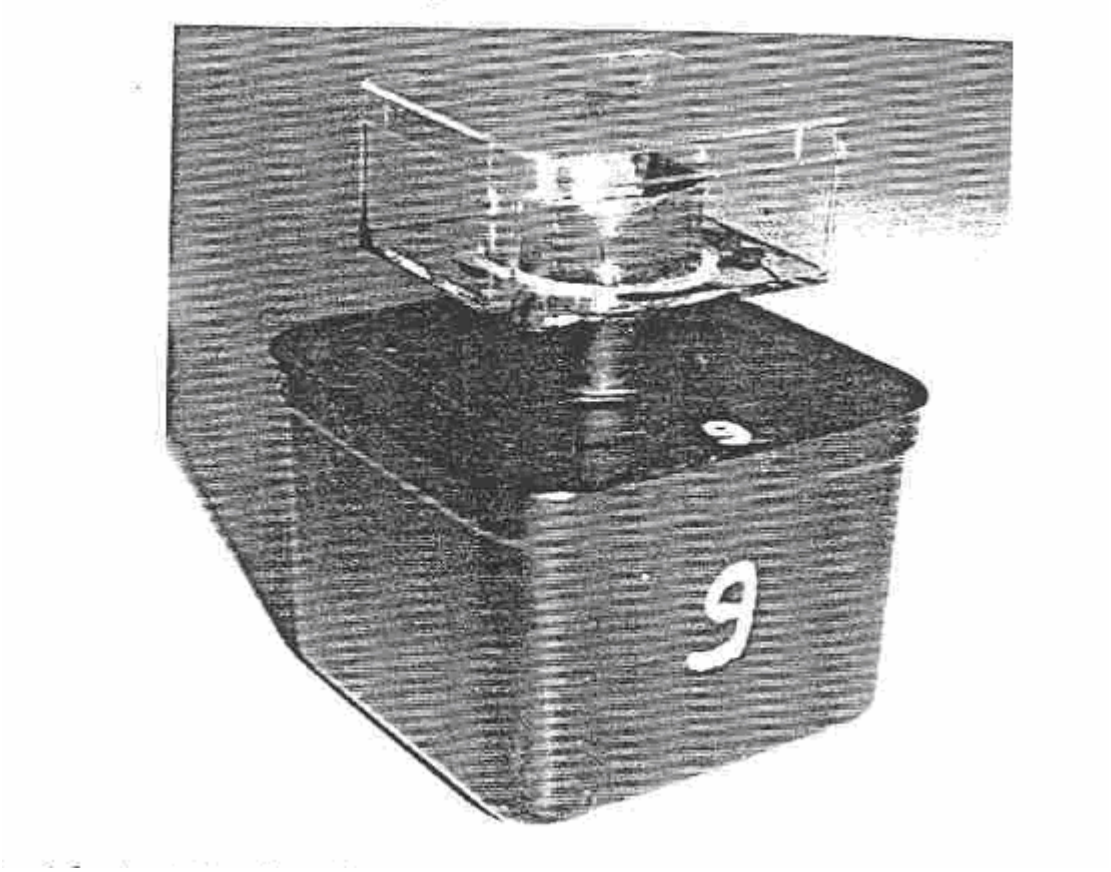

Figure 1. Insect trap

RESULT AND DISCUSSION

Selection of warm active nematodes

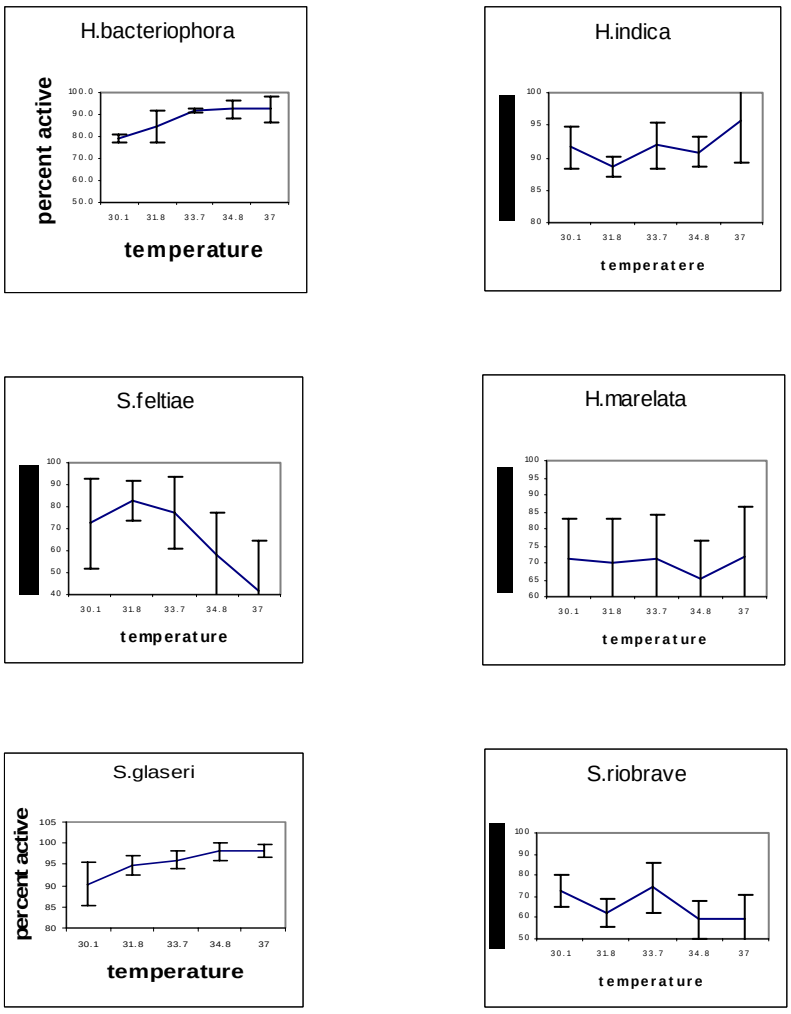

Figure 2 . Mean percent activity of nematode species across temperature gradients

Above 80% of the total nematode population of *S.glaseri*, *H.indica* and *H.bacteriophora* were active at each point of temperature gradients. *S.feltiae* showed a drastic decrease in activity at higher temperature, whereas *H.marelatus* and *S.riobravis* were less activity (60%).

MORTALITY

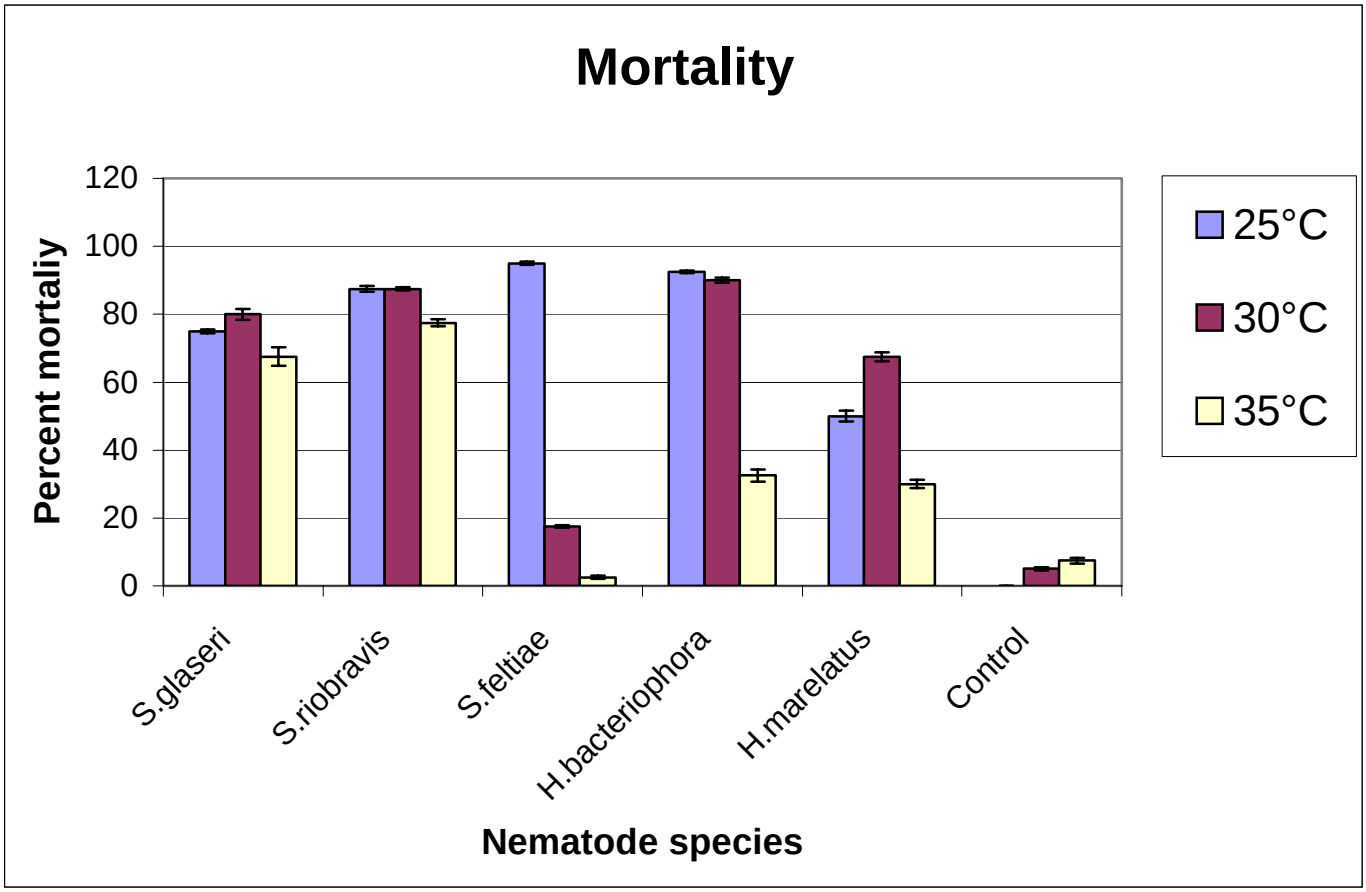

Figure 3 Mortality of 4 replicates at differnt temperatures. Bars indicate standard deviation.

At 25°C, *H. marelatus* was the least effective: There was no significance difference among all other species in causing mortality at 30°C, *S.feltiae* was the least effective among all other species and no significant difference from control. There was no significance difference among all the other species in causing mortality. At 35°C,

S.riobravis was the most effective but there was no significant difference compared to *S.glaseri. S.feltiae, H.bacteriophora* & *H.marelatus* each of them showed no significant difference in causing mortality compared to control

INFECTIVITY

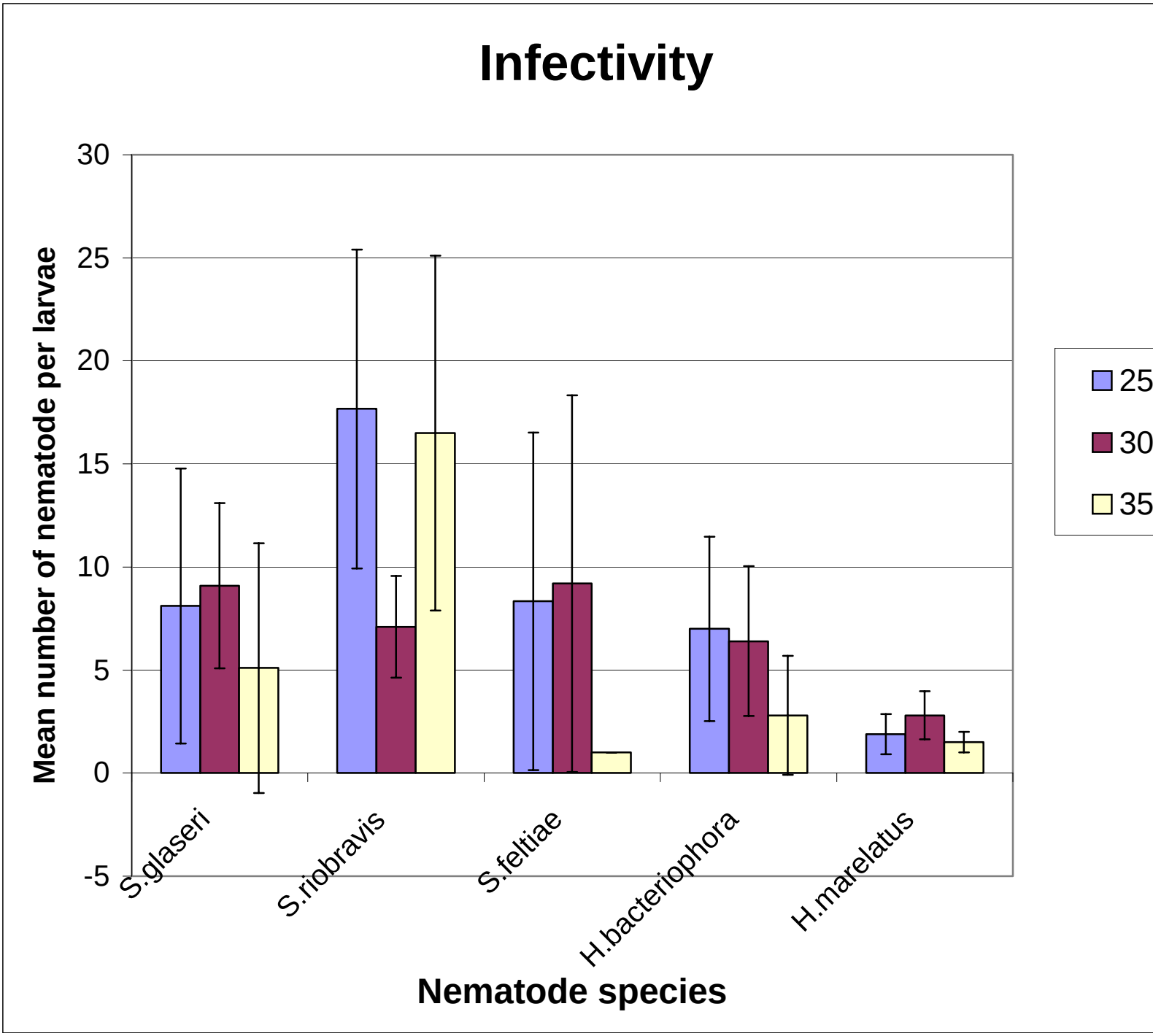

Figure 4 .Mean invasion of 10 nematodes at different temperatures

At 25°C, *S.riobravis* was the most invasive while *H. marelatus* is the least. The other 3 species showed a similar invasive potential of 50% of *S.riobravis*
At 30°C, the invasive potential of *S. riobravis* decreased. *H.marelatus* is the least invasive. And all the other species were better in infectivity more or less similarity. At 35°C, *S.riobravis* was the most invasive nematode followed by *S.glaseri*.

CONCLUSION

H.indica ,H.bacteriophora and *S.glaseri* could be potential candidates to be tested for control of sciarids at higher temperatures. But it has no sense to test *S. glaseri* against sciarid flies because of its big size compared to the insect. To see the clear difference in warm activity among the nematode species the range of temperature gradient should be increased.

At 25°C, *S.riobravis* and *H.bacteriophora* can be tested and used for control of sciarids at equal control rates obtained with *S.feltiae*. At 30°C, *S.riobravis* and *H.bacteriophora* could be good candidates to be further tested against sciarids. At 35°C, *S.riobravis* could be the best candidate to be tested for its efficacy against sciard flies.

NOTE:
EXPERIMENT ON CONTROL OF SCIARIDS AT TEMPERATURES ABOVE 30°C IS STILL ONGOING

References

Binns, E.S. (1973).Fungus gnats (Diptera: Mycetophilidae, Sciaridae) and the role of mycophagy in soil: a review .Revue Ecol. Boil .sol.,18:77-90.

Ehlers, R.-U., O*estergaard,* H*ollmer, S.,* W*ingen, M.* and S*trauch, O. (2005).* Genetic selection for heat tolerance and low temperature activity of the entomopathogenic nematode-bacterium complex *Heterorhabditis bacteriophora - Photorhabdus luminescens*. BioControl 50, in press.

Freeman, P. (1983). Sciarid flies, Diptera; Sciaridae. Hand books for the identification of British insects 9, part 6.London, Royal Entomological Society.68 p

Gouge, D.H. and Hague, N.G.M. (1994). Development of Steinernema feltiae (Steinernematidae: Nematoda) in Bradysia paupera (Sciaridae: Diptera). Bulletin IOBC / WPRS 17, 132–135.

Gouge, D.H. and Hague, N.G.M. (1995a). The susceptibility of different species of sciarid flies to entomopathogenic nematodes. Journal of Helminthology 69, 313-318.

Gouge, D.H. and Hague, N.G.M. (1995b). Glasshouse control of fungus gnats, Bradysia paupera, on fuchsias by Steinernema feltiae. Fundamental and Applied Nematology 18, 77-80.

Grewal, P.S., Selvan, S., Gaugler, R., (1994). Thermal adaptation of entomopathogenic nematodes: Niche breadth for infection, establishment, and reproduction. Journal of Thermal Biology 19, 245-253.

Leath, K.T. & NEWTON, R.C. (1969). Interaction of a fungus gnat,Bradysia sp.(Sciaridae0 with Fusarium spp.on alfalfa and red clover.Phytopathology,59:257-258.