Zbornik predavanj in referatov s 4. Slovenskega posvetovanja o varstvu rastlin, Portorož, 3.-4. marec 1999 str. 469-478

THE EFFECT OF ECOLOGICAL FACTORS ON PREDATORY NEMATHODS

Miklos NÁDASY[1], Attila LUCSKAI[2], Gábor HARMAT[3]

Pannon University of Agricultural Sciences, Georgikon Faculty of Agriculture, Keszthely, Hungary

ABSTRACT

In our institute it has been taken several exams with entomopathogenic nematodes. The successfully pest controls proved that the insect killing nematodes could be a promising control agents for the plant protection against the insects above and also under ground.
We searched the reply for ecological factors resulting maximal insect killing effect for the nematodes. The first examined factor was the temperature. The larvae of two insects (*Pieris brassicae* and *Melolontha melolontha*) were infected with *Steinernema feltiae* A4 and *Heterorhabditis bacteriophora* HH. The above mentioned nematode strains were isolated from the Hungarian soils. This experiment was carried out at three different temperature, at 17°C, 23°C and 28°C. The highest (100 %) insect mortality was observed at 23°C and at 28°C and only 47 % insect mortality was achieved at 17°C in the case of both insects.
The infecting exams effected much better insect mortality (100 %) in sandy soil than in loamy one. The soil moisture is very important factor in the life of nematodes. Our experiments proved that the nematodes are most effective with 100 % insect mortality, in the soil with 60-70 % soil humidity.

IZVLEČEK

VPLIV EKOLOŠKIH DEJAVNIKOV NA PREDATORSKE OGORČICE

V našem inštitutu so bile opravljene različne raziskave z entomopatogenimi ogorčicami. Uspešni poskusi zatiranja škodljivcev so potrdili, da so entomopatogene ogorčice lahko obetajoči zatiralni agensi za varstvo nadzemnih in podzemnih delov rastlin pred škodljivimi žuželkami.
Raziskovali smo ekološke dejavnike, ki bi imeli največji vpliv na zatiralne posebnosti teh ogorčic. Prvi raziskovani dejavnik je bila temperatura. Ličinke dveh žuželk, kapusovega belina (*Pieris brassicae*) in majskega hrošča (*Melolontha melolontha*) smo okužili s *Steinernema feltiae* A4 in *Heterorhabditis bacteriophora* HH. Omenjena ogorčična seva sta bila izolirana iz madžarskih tal. Ta poskus je bil opravljen pri treh različnih temperaturah, pri 17, 23 in 28 °C. Najvišja, stoodstotna smrtnost žuželk je bila dosežena pri 23 in 28 °C, le 47 odstotna smrtnost pa pri 17 °C in sicer pri obeh žuželkah.
Ogorčice so povzročile mnogo boljšo smrtnost žuželk v peščenih kot pa v ilovnatih tleh. Talna vlažnost je zelo pomemben dejavnik v življenju ogorčic. V naših poskusih so bile ogorčice najbolj, stoodstotno, učinkovite pri talni vlažnosti med 60 in 70 odstotkov.

1 INTRODUCTION

Nowadays those plant protection methodes come to the limelight, which are able to reduce the number of pests without chemicals.

[1] PhD, H-8361 Keszthely, Deák Ferenc Str. 57
[2] PhD student, prav tam
[3] student, prav tam

One make such examinations in the frames of Hungarian-American Search Program in our country for years.
On the part of American the Ohio University Searching Institute Wooster and on the part of Hungarian the Genetical Department of Eötvös Lóránt University and the Plant Protection Institute of PATE take part.
Among the entomopathogen nematods we are employed with the different species and phylums of *Steinernema* sp. and *Heterorhabditis* sp. (1. table).

Table 1: Used species of entomopathogenic nematodes in our experiments

Heterorhabditis bacteriophora HH
Heterorhabditis bacteriophora AZ 32
Heterorhabditis bacteriophora Cserszeg
Heterorhabditis bacteriophora USA
Steinernema feltiae A 53S
Steinernema feltiae A 62S
Steinernema feltiae B 04S
Steinernema feltiae USA
Steinernema feltiae 1048
Steinernema feltiae A4
Steinernema feltiae 1052
Steinernema glaseri
Steinernema riobravis
Steinernema scapterisci
Steinernema carpocapsae

We began with the expriments in 1995. We got good results against the larvas of *Leptinotarsa decemlineata*, *Athalia rosae* and *Scotia segetum* in 1995, the *Melolontha melolontha* and *Helicoverpa armigera* in 1996, the *Pieris brassicae*, *Mamestra brassicae* and *Diabrotica virgifera* in 1997, the *Blatta germanica* and *Tenebrio molitor* in 1998.
We gave an account of the results of these experiments on many international conferences (Portorož 1997, Gent 1997, 1998, Kreta 1997, Skierniewice 1998).

We completed the above mentioned examinations with ecological experiments in 1998. The aim of examinations was to observe the effect of some ecological factor (temperature, soil humidity, soil type) on the behaviours of predatory nematods. In our experiments we are looking for the answer to the question beside which environmental factors can nematods explain their maximal effect.

2 LITERARY SUMMARY

2.1 Temperature

SCHMIEGE (1963) put infective nematodes into waterdrops, kept them on different temperature and found, that the nematode mortality is high until 1 hour exposition time and on 35 degrees Celsius.

Those nematodes, which survived the examination didn't get back their normal activity. 16 hour long exposition on 37 degrees Celsius and 1 hour long on 41 degrees Celsius caused a 100 per cent mortality. Schmiege showed, that the species *Steinernema carpocapsae* don't cause damages in warmblood animals and in a human body.
Those larvas, which are able to infect can be kept for years in water on 50 degrees Celsius, but the infectivity of them decreases gradually.
SCHMIEGE (1963) examined, that 70% of the infective larvas kept in a fridge on 10°C 18 hours long died, but the survivors were able to infect.
The examinations showed, that in the high of St. Paul Minnesota the infective larvas spend the winter in soil. Their activity is between 15 and 28°C the highest.
KAYA (1977) examined the effect of temperature on the reproduction and growth of DD-136 nematode line and found, that the optimal temperature for the growth of this nematode is between 23 and 28°C. He didn't see any development, below 10°C and above 33°C. Kaya's examinations came to the condusion, that these parasites aren't able to live in warmblood creatures.

2.2 Humidity

Humidity is for the most nematodes an essential condition of existence and dryness is one of those important factors, which limitate the activity of steinernematid nematodes.
DUTKY (1959) showed, that the *Steinernema carpocapsae* isn't resistant for dryness, so it dies.
SCHMIEGE (1963) showed, that beside the RH near 100% the infective larvas are able to live. Beside RH 26-27% on 22°C could they just some more than 3 hours survive. WELCH and BRIAND (1961) came to the conclusion, that half ot the *Steinernema carpocapsae* infective larvas died beside RH 80% after 26 minutes and beside RH 90% after 38 minutes. It is evident, that on a such living - place like soil the infective *Steinernema carpocapsae* larvas can resist low RH.
MOORE (1965) wrote, that soil containing infective *Steinernema carpocapsae* larvas dried under to RH 70%, the nematodes stayed 20 days active.

SIMON and POINAR (1973) proved, that the gradual desiccation of nematodes is important for them to stay alive. They put the infective larvas into waterdrop and they experienced with the examination of animals after the evaporation of water that all nematodes died. Almost the half of the nematodes lived among that, which were put into a graduate wetdrawaway chamber for 16 hours even than, when they were put on roomtemperature for days. This longed death shows exactly what happens in nature. It showes the behaviour of nematodes beside the dry condition of soil. Poinar and Simons showed with this technic that 90% of nematodes are able to live beside RH 79.5% even after 12 days. This RH is equivalent of 5.5 PF, which is below the permanent fadepoint of plants.
Even after 4 days with an exposition of 48.4% which is equivalent of very dry soil 80% of nematodes stayed alive. This shows that the infective *Steinernema carpocapsae* larvas are able to live a relative long time in dry period even than, if the RH falls much below the fadepoint of plants.
It seems, that the animals in the state of anabiosis don't move, shriveled coil up and every sign shoves, that they are practically dead. But those, which stayed one night long in water, get easily alive again.

Many poets tried to decrease the steaming of nematode suspension used above the top of sil in freeland examinations.
WELCH and BRIAND (1961) examined glycerin, honey, glucose, sorbitol, urea and agar like possible steaming decreaser, but found, that these are just in high concentration effective. In such concentration have these chemicals fitotoxical and nematicid attributes, they can even increase the development of mycosis. WEBSTER and BRONSKILL (1968) reached successes in the lengthening of life of infective *Steinernema carpocapsae* larvas with the mixture of Gelgard M (0.13%) waterthickener, Folicot 351 (0.2%) steaming - retardent and ufrlatone T (0.1%), which is decreasing the surface tension. Beside such circumstances the mortality of *Pristiphora erichsonii* increased from 24% to 90%.

2.3 Migration in soil

When one mixed nematodes with some soil and in a short term of time those came to the surface and stayed on their tail. This special moving to surface is a natural attribute of more lines of *Steinernema carpocapsae*. REED and CARNEI (1967) examined this attribute and said, that the DD-136 doesn't penetrate deep in soil. They write about 3 type of moving of larvas in infective stadium: slipping, bridginig and jumping. They wrote about slipping like the most general wormmoving, which is typical for DD-136, when he comes to surface. After reaching surface came bridging moving which comes straightly from the waving moveness of the front side of nematode. If the head of the nematode reaches a piece of soil, the animal pulls itself through to the other particulum. Jumping moving can be made like this: the nematode coils up to a waterdrop and suddenly coils out and jumps maybe 10 mm above the medium.
REED and CARNE (1967) came to the conclusion, that the DD-136 nematode has adaptable moving to surface and it is rather there like in the deeper stratum.

EL-SHERIF (in POINAR, 1979) examined the vertical moving of *Steinernema carpocapsae* DD-136 and the line of *Agriotis* is sandy loamsoil columns.
Each column was 30 cm high and they had 7.5 cm diameter and they had 6 parts (each part 5 cm thick).
They put the nematode suspension in the middle of the column for definite time. They devorced the columnparts from eachother and devorced the nematodes with riddles and amended Baermann-funnel technology. They won all of the nematodes back from the soilparts of column. The results showed, that the most of moving DD-136 line moved to the surface (1), the down and up migration is equal with the *Agriotis* line (2) and by both of the lines most of nematodes stayed in the middle of the coloumn.

3 MATERIAL AND METHOD

We made the experiments in the Entomological Laboratory of Plant Protection Institute of PATE in the spring and autumn of 1998. The used insects were the larvas of *Melolontha melolontha* and *Pieris brassicae*. We gathered the wheat grubs from fruuland (Forestry Lábod), the *Pieris brassicae* larvas came from laboratory culture. The used nematodes were Hungarian phylums, *Heterorhabditis bacteriophora* HH and *Steinernema feltiae* 1052. The examinations were made in laboratory culturepots. We put in one pot one larva and 100 gramm soil (blackearth and sand mixture in 50%). The nematode concentration was 1000 and 3000 IJ/ml.
We valued the experiments once a day. We made sure of dying of larvas because of the nematodes through the method of WHITE (1927).

3.1 Examination of effect of temperature

We adjusted the examinations in the spring of 1998 with the larvas of *Pieris brassicae* (1000 IJ/ml), in the autumn of 1998 with the larvas of *Melolontha melolontha* (3000 IJ/ml). We completed the examinations in climatepantries on 17, 23 and 28°C. The experiments were made 14 days long. To keep the soil permanent wet, we put 10 ml water to it a day. We can see the results of examinations in table 2-3 and in figure 1-2.

3.2 Examination of different soiltypes

We adjusted the examinations in climatepartries on 23°C. We used two kind of soil: sand and blacksoil (flowersoil). We added to each soil 10 ml water a day. We finished the examinations on the 14th day. We can, see the results of examinations in table 4 and in figure 3.

3.3 Examination of wetness of soil

We know, that the most important ecological factor is the wetness of soil. That is why we examined the effects of nematodes on wheat grubs in soils with different wetness. We added to soil (sand, flowersoil) 10, 15, 20, 25, 30 ml water. We didn't poor water to the soils between the time of examinations. We made the experiments 14 days long. We can see the results of examinations in table 5 and in figure 4.

4 THE RESULTS OF EXPERIMENTS

4.1 Examination of effect of temperature

We can establish from the dates of table 2 and 3 the following.

Table 2: The effect of temperature on entomopathogen nematodes (*Pieris brassicae*)

1000 IJ/ml	Temperature (°C)	4 day	7 day	14 day
Heterorhabditis bacteriophora	28	93	100	100
	23	52	85	100
	17	11	38	54
Steinernema feltiae	28	90	96	100
	23	48	89	100
	17	15	32	47

Table 3: The effect of temperature on entomopathogen nematodes (*Melolontha melolontha*)

3000 IJ/ml	Temperature (°C)	4 day	7 day	14 day
Heterorhabditis bacteriophora	28	60	100	100
	17	10	35	50
Steinernema feltiae	28	50	70	100
	17	10	20	40

Heterorhabditis bacteriophora (1000 nema/ml)

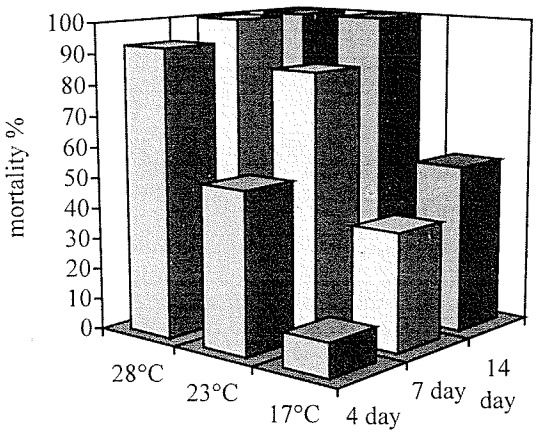

Fig. 1a: Effect of temperature on entomopathogen nematodes (With *Pieris brassicae* larvas)

Steinernema feltiae (1000 nema/ml)

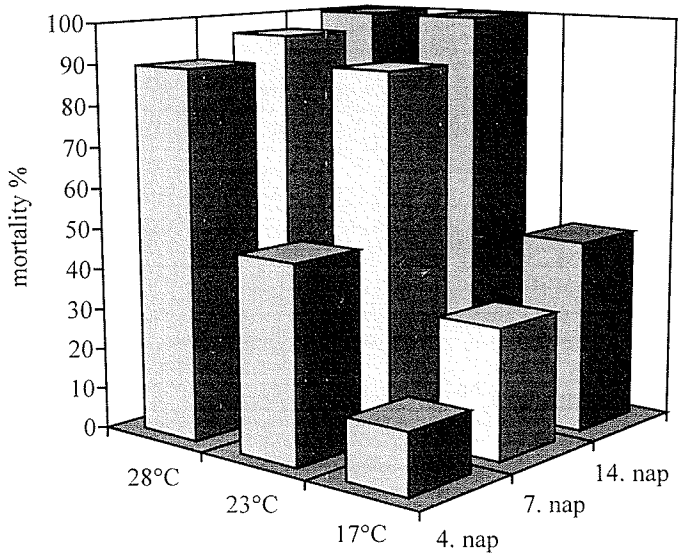

Fig. 1b: Effect of temperature on entomopathogen nematodes (With *Pieris brassicae* larvas)

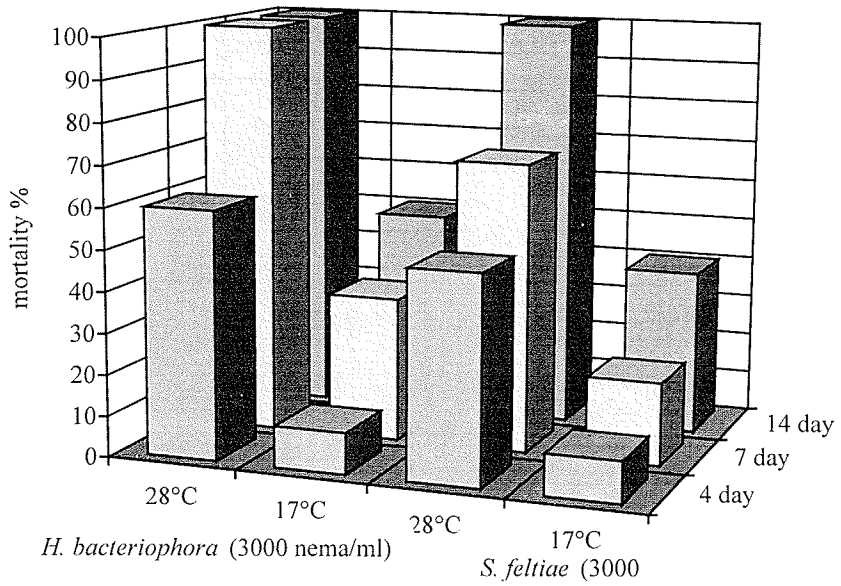

Fig. 2: Effect of temperature on larvae *Melolontha melolontha*

1. Temperature influences the efficiency of nematodes. On a high temperature of 28°C the mortality reached 90% on the 4th day and on the 7th day was destruction of larvas 100%.
   Larvas destructed slower on a lower temperature and even on the 14th day was mortality just 50%.
2. The nematodes were more resultful on *Pieris brassicae* larvas than on wheat grubs.
3. The effect of the two examined nematodes (*H. bacteriophora* and *S. feltiae*) was similar to *P. brassicae* larvas. But the *H. bacteriophora* destructs wheat grubs more.

4.2 Examination of effect of soil types

We can establish the followings from the results showed by the dates in table 4.

Table 4: The effect of soil on predatory nematodes

1000 IJ/ml		4 day	7 day	14 day
Heterorhabditis bacteriophora	Sansdoil	33	73	100
	Flowersoil	13	40	53
Steinernema feltiae	Sansdoil	20	46	80
	Flowersoil	10	20	33

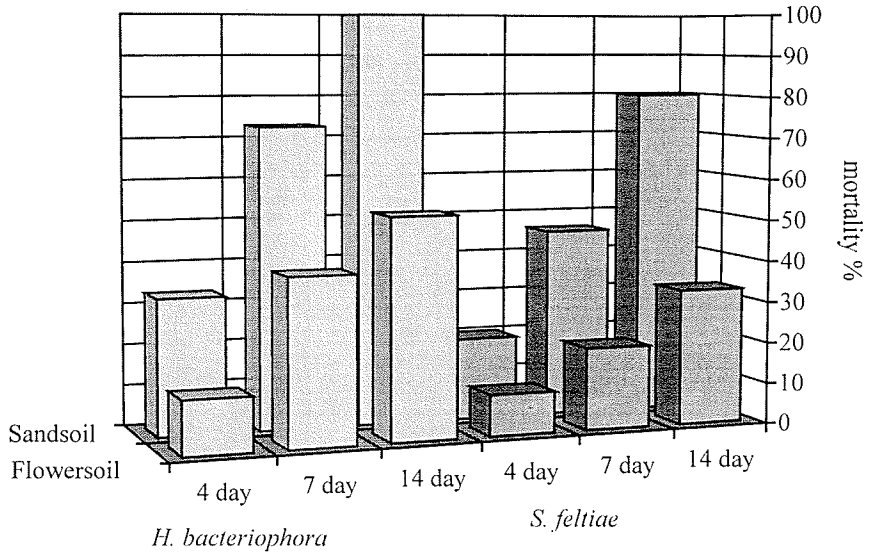

Fig. 3: Effect of soil on entomopatogen nematodes (3000 nema/ml)

1. The predatory nematodes destructed wheat grubs in a soil with sand in a shorter term of time than in flowersoil. The mortality reached on the 7th day 70% and on the 14th day all of them died, but we exprienced in flowersoil just 50% mortality on the 14th day. The explanation is probably that in the case of soils with similar wetness the nematodes moved faster in soil with sand and they infected the animals.
2. Between the two examined nematode species was *H. bacteriophora* more effective on wheat grubs in this examination.

4.3 The effect of wetness of soil

We can establish from the dates of table 5 the followings.

Table 5: The effect of wetness of soil on predatory nematodes

	4 day	**7 day**	**14 day**
10 ml	5	5	25
15 ml	5	10	25
20 ml	25	25	50
25 ml	25	25	50
30 ml	50	75	100

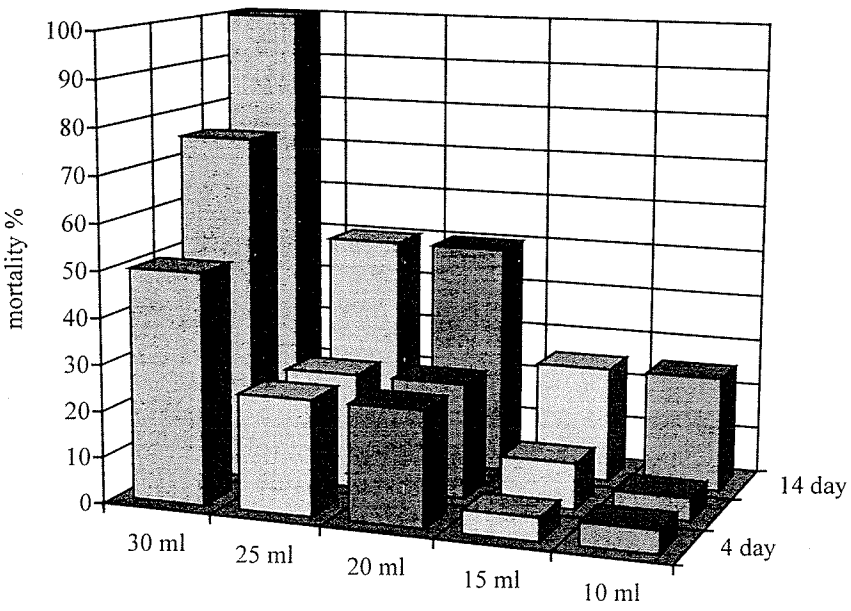

Fig. 4: Connection of *Heterorhabditis bacteriophora* and wetness of soil (3000 nema/ml)

1. Wetness of soil influences the speed of effect of nematodes, if the soil is wetter, the nematode acts faster on wheat grubs. Mortality reached 100% on the 14th day when we added 30 ml water, but when we added 10 ml water, the mortality was just 25%.
2. Wetness of soils influence speed of effect, because destruction comes earlier in wet soil than in dry soil (4, 14 days).
3. We adjusted the examination just with *H. bacteriophora* nematode.

5 SUMMARY

We were looking for the answer in our examinations for the question beside which enviromental factors display nematodes their maximal effect. We examined effect of temperature in climatepantries. We used *Pieris brassicae* and *Melolontha melolontha* larvas, we treated them with *Steinernema feltiae* A4 and *Heterorhabditis bacteriophora* HH nematode species. We put the examined material into climatepantry on 17°C, 23°C and 28°C. We can establish from the dates of experiment, that on the 14th day 28°C, on 23°C the mortality was 100%, and on 17°C 47%. We can establish this tendence in the case of both nematode species and both examined animals. So we can say, that nematodes like high temperature (23-28°C).

In the case of soiltype examination (tied and sand) we came to the conclusion, that nematodes like slack, sandy soil (100% mortality).

In the life of nematodes and so in their practical adjustment the most important factor is soil wetness. Our experiments in this line show, that we can reach 100% mortality with nematodes in a soil with 60-70% wetness. In a soil with lower wetness the mortality percent decreased (32%).

6 LITERATURE

Dutky, S. R. (1959): Insect microbiology.- Adv. Appl. Microbiol., 1: 175.

Kaya, H. (1977): Development of the DD-136 strain of *Neoaplectana carpocapsae* at constant temperatures.- J. Nematol., 9: 346.

Moore, G. E. (1965): The bionomics of an insect-parasitic nematode.- J. Kans. - Entomol. Soc., 38: 101.

Poinar, G. O. Jr. (1979): Nematodes for biological control of insects.- CRC Press, Inc. Boca Raton, Florida, 1-249.

Reed, E. M. / Carnei, P. B. (1967): The suitability of a nematode (DD-136) for the control of some pasture insects.- J. Invertebr. Pathol., 9: 196.

Schmiege, D. C. (1963): The feasibility of using a neoaplectanid nematode for control of some forest insect pests.- J. Econ. Entomol., 56: 427.

Simons, W. R. / Poinar, G. O. Jr. (1973): The ability of *Neoaplectana carpocapsae* (Steinernematidae: Nematodea) to survival extended periods desiccation.- J. Invertebr. Pathol., 22: 228.

Webster, J. M. / Bronskill, J. F. (1968): Use of Gelgard M and an evaporation retardant to facilitate control of larch sawfly by a nematode-bacterium complex.- J. Econ. Entomol., 61: 1370.

Welch, H. E. / Briand, L. J. (1961): Field experiment on the use of a nematode for the control of vegetable crop insects.- Proc. Entomol. Soc. Ont., 91: 197.