

OBSERVATIONS ON THE INFLUENCE OF TEMPERATURE OF SECOND STAGE JUVENILES (*Meloidogyne* spp.) IN THE ABSENCE OF THE HOST PLANT

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Abstract

The knowledge of root-knot nematodes behavior in different habitats and especially the knowledge of their reaction to major ecologic factors (humidity, temperature) are important for the assessment of pathogen potential in a specific area, being at the same time an important criterion for understanding the behavior towards host plants.

*The temperature is the most important factor, not just in the development of the entire biological cycle of root-knot nematodes, but at the same time it also affects their distribution, spread and survival under thermic stress conditions (excessive heat or cold). Alongside with the water and oxygen, the temperature is the triggering factor of hatching and running all stages until the adult stage. The articles aims to determine the survival of juveniles stages for *Meloidogyne* species, in the absence of host plants, and by exposing second stage juveniles to different temperatures.*

The juveniles survival and mobility decreases quite much at relative low temperatures (4°C and 10°C). Forward movements, mobility, hatching and lifetime were accentuated at higher temperatures (20°C) for a few weeks, so that after a period to decrease drastically (in 10 and 12 week) for the species considered for the study.

Keywords: galls, root-knot nematodes (*Meloidogyne* spp.), second stage juveniles, temperature.

INTRODUCTION

The genus *Meloidogyne* Göldi 1892 or the root-knot nematodes (RKN) consist of sedentary, polyphagous root endoparasites (Sharon *et al.*, 2007). Plant nematodes are present in almost any type of habitat, the only survival factors are the food and humidity resources. A few species have developed survival skills even in extreme conditions, such as desert conditions, for a period long enough of rest by reducing the consumed energy.

These nematodes re-enter in the normal activity state when the environment conditions become favorable. Plant nematodes represent approximately 20% from the descriptions of entire phylum (Ferraz and Brown, 2002).

The easiest mechanism involving the immediate answer to environment stress factors is the partial decrease of metabolic activity of nematodes and their entering into a dormant status. If unfavorable conditions keep going or

are getting worse, the nematodes end their movements and enter into a cryptobiosis status. This alternation of different metabolic statuses may take days, weeks, months and even years, becoming reversible as soon as the environmental conditions become favorable (Ferraz and Brown, 2002; Das *et al.*, 2011).

The objective of this experiment was to follow the three species of root-knot nematodes (*Meloidogyne hapla*, *Meloidogyne incognita* and *Meloidogyne javanica*) at 4°C, 10°C and 20°C on different periods of time (2, 4, 6, 8, 10 and 12 weeks), in the absence of host plants, where the survival of all species was correlated with the temperature.

MATERIALS AND METHODS

The material infected (represented by second stage-juveniles) was collected from the nematodes reserve of the Regional Laboratory of Nematology of Brașov. The nematodes stock

from which the extraction took place, it was maintained on *Lycopersicum esculentum* and *Cucumis sativus* plants in controlled conditions of 14 h light/day, in flower pot of 12cm Ø from one sub-layer made of 50% river sand and 50% clay soil, tight fitted at 120°C for 20 minutes. When the seedlings had three leaves, they have been inoculated with juveniles. After 35 days, the collection of egg masses took place and their setting in the incubator for hatching the juveniles (hatching chamber) and their collection (McKenry and Roberts, 1985; Southey, 1985). In order to study the survival of infested juveniles fresh hatched, in the absence of host plants of the three species, they have been placed in plastic cylinders of 8 cm height and 3 cm diameter with an addition of distilled water. In every plastic cylinder ampoule, 200 second stage juveniles have been placed from every species, which would be analyzed further. Before we start the experiment properly, a witness lot was observed in order to check the impact between the running water and distilled water, on the survival of juveniles. Wright (1998) noticed that distilled water is not a favorable environment for plant nematodes, existing the possibility of some ionic and osmotic pressure which might affect severely the survival (Das *et al.*, 2011).

Therefore, the observations have been provided on juvenile stages of *Meloidogyne hapla*, *M. incognita* and *M. javanica*. After five weeks, there was observed no difference in their morphology or survival, so that the distilled water was used, without concerns, as environment for the maintenance and observance of nematodes. The juveniles from the three species have been exposed to 4°C, 10°C and 20°C because these temperatures correspond to the averaged tempered season, specific to our country. The ampoules have been placed in

incubators and the tests consisted of three repetitions. The volume of distilled water from ampoules was maintained at 5 ml and the plug of containers has been perforated in order to permit the vaporization of water. In order to avoid the anoxic conditions for nematodes, the fluids have been bubbled once at 2-3 days, for 1hour, with an aquarium pump. The survival was observed by checking the juveniles' mobility under binocular stereomicroscope (20x) and then at light microscope (60x) Leica MZ95. Before checking the survival of juveniles kept at 4°C, and at 10°C, they have been stored at room temperature for 4 h. The examination should, this way, emphasize their recovery of mobility in a big way. Motionless and dying nematodes have been "stimulated" using a nematological needle in order to check the mobility respons.

RESULTS AND DISCUSSION

There have been noticed significant differences between the three species of nematodes exposed to three different temperatures for 12 weeks concerning the survival.

The survival was calculated for the most important statistical parameters expressed in environments, mean, mean deviation and dispersion for temperatures of 4°C (Table 1), 10°C (Table 2) and 20°C (Table 3). For the proper statistical analysis the program XLSTAT (AddinSoft) was used, version 7,5 functioning under Excel.

The difference between species (percent) was observable at the extreme temperatures (4°C and 20°C) in comparison to the intermediate level of temperature (10°C) (Table 4). The survival of *M. hapla* species was severely affected at 4°C whilst at 10°C and 20°C the survival was quite constantly.

Table 1. The effects of nematodes interaction (minimum no., maximum no., mean, mean deviation and dispersion) at temperatures of 4°C, for 12 weeks for the species *M. hapla*, *M. incognita* and *M. javanica*

Temperature/species	4°C <i>Meloidogyne hapla</i>					4°C <i>Meloidogyne incognita</i>					4°C <i>Meloidogyne javanica</i>				
	Min.	Max.	Mean	Mean deviation	Dispersion	Min.	Max.	Mean	Mean deviation	Dispersion	Min.	Max.	Mean	Mean deviation	Dispersion
Week 2	90	102	95,33	6,11	37,33	56	61	58,67	2,51	6,33	18	25	20,67	3,78	14,33
Week 4	61	69	67,6	4,04	16,33	40	45	43	2,67	7	12	14	12,67	1,15	1,33
Week 6	19	22	20,33	1,52	2,33	25	39	32	7	49	2	2	2	0	0
Week 8	0	2	1,33	1,15	1,33	10	14	11,33	2,30	5,33	0	0	0	0	0
Week 10	0	0	0	0	0	0	1	0,33	0,57	0,33	0	0	0	0	0
Week 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2. The effects of nematodes interaction (minimum no., maximum no., mean, mean deviation and dispersion) at temperatures of 10°C, for 12 weeks for the species *M. hapla*, *M. incognita* and *M. javanica*

Temperature/species	10°C <i>Meloidogyne hapla</i>					10°C <i>Meloidogyne incognita</i>					10°C <i>Meloidogyne javanica</i>				
	Min.	Max.	Mean	Mean deviation	Dispersion	Min.	Max.	Mean	Mean deviation	Dispersion	Min.	Max.	Mean	Mean deviation	Dispersion
Week 2	187	198	193,3	5,68	32,33	144	158	150,7	7,02	49,33	183	191	188	4,35	19
Week 4	174	189	183	7,93	63	100	108	103	4,35	19	179	182	180,3	1,52	2,33
Week 6	161	163	162	1	1	90	92	91,33	1,15	1,33	170	172	170,7	1,15	1,33
Week 8	100	107	102,3	4,08	16,33	81	88	83,67	3,78	14,33	126	142	136	8,71	76
Week 10	24	49	40	13,89	193	72	81	77,67	4,93	24,33	80	88	85	4,35	19
Week 12	23	35	31	6,92	48	12	112	45,67	57,44	33,33	21	26	23	2,64	7

Table 3. The effects of nematodes interaction (minimum no., maximum no., mean, mean deviation and dispersion) at temperatures of 20°C, for 12 weeks for the species *M. hapla*, *M. incognita* and *M. javanica*

Temperature/species	20°C <i>Meloidogyne hapla</i>					20°C <i>Meloidogyne incognita</i>					20°C <i>Meloidogyne javanica</i>				
	Min.	Max.	Mean	Mean deviation	Dispersion	Min.	Max.	Mean	Mean deviation	Dispersion	Min.	Max.	Mean	Mean deviation	Dispersion
Week 2	186	198	191,7	6,02	36,33	157	188	175,3	16,25	264,33	190	195	193	2,64	7
Week 4	140	164	148,3	13,57	184,33	64	183	76,33	10,69	114,33	102	104	103	1	1
Week 6	52	86	72,67	18,14	329,33	21	25	23	2	4	4	9	5,67	2,88	8,33
Week 8	5	8	6,33	1,52	2,33	2	10	4,67	4,61	21,33	0	1	0,33	0,57	0,33
Week 10	0	1	0,33	0,57	0,33	0	1	0,33	0,57	0,33	0	0	0	0	0
Week 12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4. The percentage of nematodes survival at temperatures of 4 °C, 10 °C and 20 °C, for 12 weeks for the species *M. hapla*, *M. incognita* and *M. javanica*

Percent of survival	<i>Meloidogyne hapla</i>						<i>Meloidogyne incognita</i>						<i>Meloidogyne javanica</i>					
	Week						Week						Week					
	2	4	6	8	10	12	2	4	6	8	10	12	2	4	6	8	10	12
4 °C	47,67	32,34	10,17	0,67	0	0	29,34	21,5	16	5,67	0,17	0	10,34	6,34	1	0	0	0
10 °C	96,67	91,50	81	51,17	20	15,5	75,34	51,5	45,67	41,84	38,89	22,84	94	90,17	85,34	68	42,5	11,5
20 °C	95,84	74,17	36,34	3,17	0,17	0	87,67	38,17	11,5	2,34	0,17	0	96,5	51,5	2,84	0,17	0	0

At 4°C the survival of this species was of 0 % in week 10 and 12. At this species was also observed a similar type of survival in week 10 and 12 at 20°C. Many authors, such as Towson and Apt (1983) state that the survival duration of root-knot nematodes close related to humidity and especially to temperature. The low survival (under 50% in the first four weeks) at 4°C of *M. hapla* species, in the present experiment, shows that the nematodes are not getting through the biological cycle in good conditions, although the species is known to be cryophilic, able to survive in soil at temperatures under 10°C during several weeks (Karssen and Moens, 2013).

Some authors show that *Meloidogyne hapla* species may survive at temperatures lower than - 8°C and this tolerance to low temperatures varies according to different growing stages (Vrian *et al.*, 1978). It seems that the eggs are better adapted to low temperatures than the juveniles recently hatched are.

In fact, in 10 and 12 week, the survival at 4°C was not possible at any of the three species of root-knot nematodes.

As opposed to inferior temperatures, at 10°C the survival was almost 23% in the last week, for *Meloidogyne incognita* species, species to which the juveniles still presented intense forward wave movements. It is noticed a constant survival of juveniles at the temperature of 10°C during 12 weeks, at all three species.

However, at 20°C the juveniles of the three species have survived in high percentage during the four weeks (over 74% *Meloidogyne hapla*), drops under 5% in 8th week, while in the last week the survival percentage is absent at all three species.

The juveniles from *Meloidogyne javanica* species proved to be sensitive to low temperatures of 4°C, so that they survive only in ratio of 10, 34% in the 2nd week, that in the 6th week to survive only 1% of them. The constant survival during the study was registered at 10°C, due to the high percentage (over 60% survival) in the 8th week at this species. Not the same thing happened at the temperature of 20°C when the juveniles *Meloidogyne javanica* have survived little over 50% in the first 4

weeks, after which the death rate increased suddenly. The differentiation between the alive and dead juveniles is possible by their coloration with potassium permanganate (Jatala, 1975) or by the verification of nematodes fluorescence in UV light after their immersion in fluorescein diacetate (Bird, 1979).

These methods are though very useful when a small number of nematodes is processed. (<100 second stage juveniles). At the same time, we noticed the following aspects encountered in the laboratory:

Meloidogyne incognita causes big galls, isolated or under agglomerated form, which sometimes look a strand of pearls on the entire length of root (Figure 1).



Figure 1. *Meloidogyne incognita* – galls merged on old roots (*Lycopersicum esculentum*)

Meloidogyne hapla causes relatively small galls, positioned sometimes on the secondary roots or on terminal roots (Figure 2).

Meloidogyne javanica causes relatively small galls, as opposed to *Meloidogyne incognita* species. At tomatoes, the galls appear frequently on thin roots and on lateral roots (Figure 3).



Figure 2. *Meloidogyne hapla* – galls on terminal roots (*Cucumis sativum*)



Figure 3. *Meloidogyne javanica* – galls present at the soil surface (*Lycopersicum esculentum*)

CONCLUSIONS

After hatching, at 20°C, the fresh *Meloidogyne hapla* juveniles should be able to find a host plant shortly, because after four weeks their survival decreases drastically. The species is known as northern root knot nematode and at 10°C or beyond this thermic level, *M. hapla* begins to develop. Although the species was discovered in the temperate climate, also considered resistant to low temperatures, nematologists are still not sure of its origin.

A more constant evolution, more tolerant in relation to and averaged temperature of the experiment (10°C), was the species *M. incognita* explaining this way a wider distribution of this nematode and would not have excluded a potential threat for multiple cultures with this species.

Nevertheless, exploring these results *in vitro* is difficult to be done. However, they may have an orientation character, mostly, because the soil is a complex system in which the effects of seasons, daily rhythm, changes of temperature, fluctuation of humidity and many other factors would have a bigger impact on the survival and infectiousness of juveniles from second-stage stage.

In this experiment, the survival was verified by the observation of forward wave movements. It is known that in unfavorable situations of environment, *Meloidogyne* juveniles may enter into diapause state. It is possible that one part of the nematodes to be already in this state and during the experiment to be considered dead, especially the juveniles exposed at 4°C. One important conclusion, regarding the *M. javanica* species, recently identified in

Romania, shows that the survival has the highest level in the first weeks (96.50 % in the first two weeks), while in the 10th week, the juveniles of this species had 0% of survival.

One explanation of this low percentage for the survival of these juveniles would be that the loss of lipids from their body is accelerated in the first weeks, which would suggest higher metabolic activities, in comparison to the juveniles of the other two species.

Most probably, these higher metabolic activities have as purpose the search for the host plant together, on which are overlapping the unfavorable thermic conditions. The biological cycle is strongly influenced by the dynamics of ecologic factors, especially by temperature, but also by the trophic support on which the root-knot nematodes species are developing.

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