Plant Pathology Bulletin 17: 203-208, 2008

Effect of temperature on the survival of *Meloidogyne incognita*

Bie Yun Tsai

Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan
E-mail: bieyntm@ntu.edu.tw; Fax: +886-2-2363-6490
Accepted for publication: February 25, 2008

ABSTRACT

Tsai, B. Y. 2008. Effect of temperature on the survival of *Meloidogyne incognita*. Plant Pathol. Bull. 17: 203-208.

The temperatures tested were 5, 10, 15, 20, 25, 30, 35, 40, and 45℃. The longest survival of *Meloidogyne incognita* second-stage juveniles was 380 days at 15℃ and the shortest was less than 4 h at 45℃. At 45℃, 98.8% nematodes were dead after 3.5 h. At 40℃, all the nematodes were dead after 6 days. At 35℃, all the nematodes were killed after 60 days. After 25 days, only few nematodes survived at 30℃ and none survived at 25℃. There were only small percentages of nematodes survived at 20℃ and 10℃ after 60 days. The mortality at 5℃ was 99.3% after 20 days. The nematodes survived at 15℃after 180, 220, 260, 320, and 380 days were capable of infecting mung bean roots and produced galls and egg masses, although the infectivity of those survived nematodes were all significantly lower than the fresh nematode. The present findings can be used as the basis for evaluating the control strategies such as solarization, crop rotation, and fallow practice. The data showed that control strategies based on starvation of the nematodes, such as crop rotation and fallow, would not be effective in the cool seasons in certain areas, never the less, in Taiwan, fallow or crop rotation in the early summer would be more effective than in the winter for the control of *M. incognita*.

Key words: crop rotation, fallow, *Meloidogyne*, solarization, survival, temperature

INTRODUCTION

Temperature is an important environmental factor for organisms. Different organisms have their own optimal temperatures for normal activities and high and low temperature limits they can tolerate. This is also true for nematodes. Some traditional ways of nematode management strategies, such as solarization, crop rotation, and fallow, may be useful alternatives to chemical nematicides. Solarization controls nematodes by the heat from solar energy. It requires information on the response of nematodes to high temperatures. Crop rotation reduces nematode population by growing non-host or poor host plants to limit accessibility of food to nematodes. The duration nematodes can survive without food is also influenced by temperature because consumption of the reserved energy is dependent on the level of activity which is influenced by temperature.

While there were studies on the influence of temperature on reproduction and pathogenicity of nematodes [15, 16, 17], very few information was available on the longevity of nematode when influenced by temperature. Although effects of storage temperature on the recovery of nematodes from soil has been studied [2, 3], the results could be interfered by other factosr which was

not planned in the experiments, i.e., the nematodes could have entered anhydrobiosis in the soil due to slow desiccation after long term storage and extended their duration of survival [18, 19]. Therefore, research on the longevity of nematodes stored in water is necessary to assess the influence of temperature on the survival of the nematodes.

Different nematodes may respond to temperature differently even within the same genus. Finnegan, *et al.* [6] reported that *Heterorhabditis bacteriophora* was more temperature tolerant than the North-West European and Irish types of *Heterorhabditis*. Even different strains of nematodes within the same species may respond to temperature differently [1]. Therefore, it is necessary to study the response of the target nematodes to temperature instead of basing on generalization. The root-knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood is an important plant-parasitic nematode in Taiwan. The control has been mainly dependent on chemical nematicides. With the concern for pesticide residues in our food and water, it is necessary to find alternative control measures. The traditional way of control with solarization, crop rotation, and fallow practice is worth reevaluating. The present research was designed to obtain basic information on the effect of temperature on the survival of *M. incognita* to be used as the basis for evaluating the above mentioned control strategies.

MATERIALS AND METHODS

Preparation of nematodes

A population of *M. incognita* was originally collected from the experimental station of the National Taiwan University and cultured on mung bean (*Vigna radiata* (L.) Wikzek) seedlings grown in Seed-Pack Growth Pouch (Mega International of Minneapolis). Egg masses were dissected from roots and hatched in a hatching chamber. The hatching chamber was made of a small Petri dish with lid (5.4 cm in diam.) containing a thin layer of distilled water over two layers of facial tissue supported by a compressed-styrofoam disposable sauce dish which was carved many rectangular holes to function like a screen. Fresh second-stage juveniles were collected every day and kept at 15℃. They were used within three days. The concentration of nematodes was adjusted to approximately 100 nematodes per ml.

Temperature treatments

The aliquot of 20 ml of the nematode suspensions was loaded into a small flask and sealed with saran wrap and a rubber band. Four flasks were sampled from each temperature treatment each time. The temperatures tested were 5, 10, 15, 20, 25, 30, 35, 40, and 45℃. The duration of the treatment for different temperatures was estimated based on preliminary tests. In the 45℃ treatments, samples were counted at 0, 0.5, 1, 1.5, 2, 2.5, 3, 3.5 , and 4 h interval. In the 40℃ treatments, samples were counted at 0, 0.5, 1, 2, 4, 24, 48, 96, and 144 h. Samples were counted at 0, 10, 20, 25, 35, 45, and 60 days in the 35, 30, 25, 20, 10 and 5℃ treatments. In the 15℃ treatment, samples were taken at 0, 10, 20, 40, 60, 120, 180, 220, 260, 320, and 380 days. At each sampling time, the nematode suspension was well mixed in the flask and three aliquots of 1 ml were taken for counting the nematodes. The nematode counts were the average of the three samples. Nematodes were counted in three categories: active, paralyzed, and dead. The immobile nematodes that responded to the touch of a nematode pick were counted as paralyzed and those did not were counted as dead. The experiments, including the infectivity tests listed below, were repeated in one month with another batch of nematodes.

Infectivity and reproduction of the long term surviving nematodes

Mung bean seeds were surface sterilized with 70% ethanol for 4 minutes and sown in Seed-Pack Growth Pouch (Mega International of Minneapolis) and kept at 28℃ in a growth chamber with 16 h photoperiod. The seedlings were used five days after sowing. The surviving nematodes from 180, 220, 260, 320, and 380 days at 15℃ treatments were inoculated to the mung bean roots. The paralyzed nematodes were combined with active nematodes in the inoculation as they could possibly regain their activity with the stimulation of food signal from the plants. The number of galls and egg masses per root system was counted under the dissecting microscope at 30 days after inoculation. The females were also counted after teasing the root tissue to expose the nematode bodies. The

% infectivity was calculated based on the number of females per root system divided by the number of active and paralyzed nematodes inoculated. There were four replicates per treatment.

Arcsine square root transformation was performed for percentages of all the above experiments before statistical analysis. One-way analysis of variance was carried out with SAS 9.1 software (SAS Institute, Cary, NC, USA) and treatment means were compared with Duncan's multiple range tests at the 95% level of confidence. In the repeated experiment, the data were combined with the first one because similar results were observed in the two experiments.

RESULTS

At 45℃, none of the nematodes were moving after 0.5 h treatment, however, 68.5% of them regained activity after being at room temperature (around 21℃) for one day, 28.8% remained paralyzed, and 2.7% died (Table 1). After 1 h treatment at 45℃, no nematodes regained activity after being at room temperature for one day, 96.4% nematodes were paralyzed and 3.6% died. The % mortality increased with time in 45℃ treatment, and all the nematodes died after 4 h. At 40℃, paralyzation of nematodes increased within 24 h but very little mortality (Table 1). They were motionless but responded to the touching of a nematode pick. After 4 h exposure to 40℃, 97.8% of the nematodes were paralyzed by heat and only 2.2% of nematodes were dead. By 48 h at 40℃, 44.3% of nematodes were dead and the paralyzed percentages dropped to 55.8%. After 6 days at 40℃, all the nematodes were dead. At 5℃, the nematodes almost all died by 20 days (Table 2). After 25 days, almost no nematodes survived at 30℃ and none survived at 25℃. After 60 days, all of the nematodes were

Table 1. The effect of high temperatures on *Meloidogyne incognita* second-stage juveniles

Exposure time (h)	40℃		45℃	
	% Paralyzation[1]	% Mortality	% Paralyzation	% Mortality
0	0.0 e	0.0 e	0.0	0.0 e
0.5	2.0 d	0.3 e	28.8 d	2.7 d
1	61.0 b	1.4 d	96.4 a	3.6 d
1.5	-[2]	-	60.3 b	39.7 c
2	97.2 a	0.8 d	63.9 b	36.1 c
2.5	-	-	47.5 c	52.5 b
3	-	-	0.6 e	99.4 a
3.5	-	-	1.2 e	98.8 a
4	97.8 a	2.2 d	0.0 f	100.0 a
24	98.0 a	2.0 d		
48	55.8 b	44.3 c		
96	5.3 c	96.5 b		
144	0.0 e	100.0 a		

[1] Means within each column with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range tests after arcsine square root transformation.

[2] Not done.

Table 2. The mortality of *Meloidogyne incognita* second-stage juveniles in regular and cold temperatures treatments

Time (days)	% Mortality[1]					
	35℃	30℃	25℃	20℃	10℃	5℃
0	0.0 f	0.0 d	0.0 d	0.0 e	0.0 e	0.0 c
10	2.9 e	9.4 c	7.1 c	3.3 d	1.3 e	75.8 b
20	40.0 d	78.2 b	70.5 b	1.7 d	41.3 d	99.3 a
25	61.0 c	99.6 a	100.0 a	-[2]	-	
35	90.4 b			55.0 c	72.5 c	
45	95.0 b			82.2 b	87.0 b	
60	100.0 a			97.5 a	95.1 a	

[1] Means within each column with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range tests after arcsine square root transformation.

[2] Not done.

dead at 35℃ while there were a few nematodes survived at 20℃ and 10℃.

At 15℃, most of the nematodes remained active within 40 days (Table 3). By 60 days after treatment the active nematodes decreased to 72.2%. Only a small percentage of nematodes remained active after 180 days. At 380 days, 97.6 % of nematodes were dead. There were only small percentages of nematodes paralyzed throughout the experiment.

The nematodes survived at 15℃ after 180, 220, 260, 320, and 380 days treatments were all capable of infecting mung bean roots and produced galls and egg masses (Table 4), although the infectivity of the long term surviving nematodes were all significantly lower than the fresh nematode used in control. The infectivity of the 380-days-old second-stage juveniles was 12.9%, significantly lower than the fresh nematodes which was 25.7%. The numbers of galls per root system of the surviving nematodes were all significantly lower than the fresh nematodes, and 380-days-old nematodes was 48 which was the lowest. The numbers of egg masses produced by the surviving nematodes per root system were all significantly lower than the fresh nematode control. There were variations of the reproductivity among the surviving second-stage juveniles of different ages.

Table 3. The percentages of mortality and paralyzed *Meloidogyne incognita* second-stage juveniles after storage at 15℃ for different length of time

Time (days)	% Nematodes[1]		
	Active	Paralyzed	Dead
0	100.0 a	0.0 e	0.0 g
10	98.6 b	0.0 e	1.4 f
20	96.3 b	2.2 d	1.5 f
40	93.8 c	2.7 c	3.5 e
60	72.2 d	3.5 b	24.3 d
120	25.7 e	3.0 b	71.3 c
180	5.9 f	2.0 d	92.2 b
220	3.8 f	3.8 b	92.4 b
260	4.5 f	0.9 d	94.6 b
320	0.7 g	6.3 a	92.9 b
380	0.4 g	1.3 d	97.6 a

[1] Means within each column with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range tests after arcsine square root transformation.

Table 4. Infectivity and reproduction of *Meloidogyne incognita* second-stage juveniles after storage at 15℃ for different length of time

Source of inoculum	No. Galls[1]	No. Egg Masses[1]	% Infection[1]
180-days-old	72.0 b	20.8 e	19.0 b
220-days-old	62.5 b	45.3 c	16.7 c
260-days-old	67.1 b	71.0 b	21.9 b
320-days-old	52.2 c	35.1 d	20.3 b
380-days-old	48.0 c	27.3 e	12.9 d
Fresh control	79.0 a	85.2 a	25.7 a

[1] Means within each column with different letters are significantly different at $P \leqq 0.05$ according to Duncan's multiple range tests after arcsine square root transformation.

DISCUSSION

Studies on *Caenorhabditis elegans* showed that starvation could increase the life span of the nematodes [10, 11]. Although the present research involved the starvation of *M. incognita* second-stage juveniles, it was difficult to assess the effect of starvation on their life span. Because *M. incognita* is an obligate parasite, consequently, the observation on the nematode is made difficult by the host tissue. However, the effect of temperature on the life span of *M. incognita* second-stage juveniles was clearly demonstrated in this study. At regular temperature (25-35 ℃), the life span of *M. incognita* second-stage juveniles without food was around 25 days, but at 15℃, it could be as long as 380 days, increased 15 folds. On the contrary, high temperature treatments at 40℃and 45℃, shortened the life span by 76% and 99.3%, respectively.

Bergeson [3] reported that the larvae of *M. incognita* acrita died in 14 days at 4.4℃. This agreed with the results that there was almost 100% mortality of *M. incognita* second-stage juveniles treated with 5℃ after 20 days. There was 70.5% mortality of *M. incognita* second-stage juveniles at 25℃ after 20 days. The result was similar to previous report on *M. javanica* where 76% mortality was found after storing at 25℃ for 13 days [13].

It seems logical that the nematodes survived longer at 20℃ and 10℃ than 30℃ and 25℃ as the energy consumption would be slower at lower temperatures. It is surprising that *M. incognita* second-stage juveniles survived longer at 35℃ than at 25℃ and 30℃. The same trend was observed in the repeated experiment.

The lesion nematode *Pratylenchus jordanensis* was reported to survive in the alfalfa field soil stored in polyethylene bags for 124 days at 30℃ [12]. The fourth stage juveniles of *Xiphinema index* survived 4 years in the soil stored at 7℃ and 20℃ [4]. Since the moisture content

of the soil after 4-years storage was not reported, it was not clear weather the soil desiccated gradually during the long term storage and provided opportunity for the nematodes to enter the state of anhydrobiosis and prolonged their survival. It is very likely that *X. index* have more energy reserves in their bodies than *M. incognita* second-stage juveniles because the former are much larger nematodes. Assuming they are both in the inactive state to save energy consumption, *X. index* will survive longer than *M. incognita* second-stage juveniles.

The present findings not only provided information on the biology of the nematodes but also basic information for nematode control strategies, such as solarization, crop rotation, and fallow practice.

Solarization has been used for nematode control [7, 9, 14]. However, soil temperature under solarization mulch varies considerably in different areas. In southern Georgia, Johnson III, *et al.* [9] reported that soil temperatures at 10 cm under solarization mulch averaged 48℃ compared to 41℃ in non-covered control plots. In central Mississippi, Egley [5] reported that soil temperature under solarization mulch averaged 58℃ compared to 35℃ in non-covered plots. In Israel, Horowitz *et al.* [8] reported that soil temperatures at 15 cm averaged 36℃under solarization mulch and 28℃ in bare ground plots. It is clear that the soil temperature under solarization mulch can vary a great deal in different areas due to different climates. Therefore, it is important to know the response of the nematodes to high temperatures and their duration in order to estimate the efficacy of solarization.

The present studies showed that the heat of 40℃ rendered the nematodes unconscious at the beginning, and then almost half of the paralyzed nematodes died as the heat continued for 48 h. After 4 days, only 5.3% of nematodes remained paralyzed and the rest of them were all dead. By 6 days, all the nematodes were killed at 40℃. At 45℃, the treatment time between 2.5 h and 3 h appeared to be the critical point because the mortality rate increased from 52.5 to 99.4%. All of the nematodes died in 4 h at 45℃. This is useful information for using solarization as nematode control strategy. In areas where there is strong sunlight, solarization of *M. incognita* second-stage juveniles can be achieved within a week. More time should be allowed for solarization if the soil temperature can not reach 40℃. Higher temperature or longer exposure was needed to kill the eggs with the protection of the gelatinous matrix. To overcome this drawback, irrigation of the soil to stimulate the hatching of eggs can be combined with solarization.

Crop rotation and fallow practice controls nematodes based on starvation of the nematodes. The duration of crop rotation and fallow can be deducted based on the present findings. At regular temperatures, 25℃ and 30℃, *M. incognita* second-stage juveniles died in 25 days without food (Table 2). Providing sufficient irrigation to stimulate the hatching of eggs at the beginning of crop rotation or fallow, and allow three weeks for the hatching of eggs (unpublished data), could control *M. incognita* effectively in a period of one and a half months

The surviving *M. incognita* second-stage juveniles retained their infectivity and reproduction ability after surviving at 15℃ without food for 380 days (Table 4). The results showed that control strategies based on starvation of the nematodes, such as crop rotation and fallow, would not be effective in the cool seasons in certain areas. In Taiwan, it can be predicted that fallow or crop rotation in the early summer would be more effective than in the winter for the control of *M. incognita* based on the present findings.

LITERATURE CITED

1. Anderson, J. L., Albergotti, L., Proulx, S., Peden, C., Huey, R. B., and Phillips, P. C. 2007. Thermal preference of *Caenorhabditis elegans*: a null model and empirical tests. J. Experi. Biol. 210: 3107-3116.
2. Barker, K. R., Nusbaum, C. J., and Nelson, L. A. 1969. Effects of storage temperature and extraction procedure on recovery of plant-parasitic nematodes from field soils. J. Nematol. 1: 240-247.
3. Bergeson, G. B. 1959. The influence of temperature on the survival of some species of the Genus *Meloidogyne*, in the absence of a host. Nematologica 4: 344-354.
4. Demangeat, G., Voisin, R., Minot, J. C., Bosselut, N., Fuchs, M., and Esmenjaud, D. 2005. Survival of *Xiphinema index* in vineyard soil and retention of *Grapevine fanleaf* virus over extended time in the absence of host plants. Phytopathology 95: 1151-1156.
5. Egley, G. H. 1983. Weed seed and seedling reductions by soil solarization with transparent polyethylene sheets. Weed Sci. 31: 404-409.
6. Finnegan, M. M., Downes, M. J., O'Regan, M., and Griffin, C. T. 1999. Effect of salt and temperature

stresses on survival and infectivity of *Heterorhabditis* spp. Nematology 1: 69-78.
7. Giannakou, I. O., Anastasiadis, I. A., Gowen, S. R., and Prophetou-Athanasiadou, D. A. 2007. Effects of a non-chemical nematicide combined with soil solarization for the control of root-knot nematodes. Crop Prot. 26: 1644-1654.
8. Horowitz, M., Roger, Y., and Herlinger, G., 1983. Solarization for weed control. Weed Sci. 31: 170-179.
9. Johnson III, W. C., Davis, R. F., and Mullinix Jr., B. G. 2007. An integrated system of summer solarization and fallow tillage for *Cyperus esculentus* and nematode management in the southeastern coastal plain. Crop Prot. 26: 1660-1666.
10. Kaeberlein, T. L., Smith, E. D., Tsuchiya, M., Welton, K. L., Thomas, J. H., Fields, S., and Kennedy, B. K. 2006. Lifespan extension in *Caenorhabditis elegans* by complete removal of food. Aging Cell 5: 487-494.
11. Malene, H., Stefan, T., Douglas, C., Nataliya, L., Seung-Jae, L., and Cynthia, K. 2007. Lifespan extension by conditions that inhibit translation in *Caenorhabditis elegans*. Aging Cell 6: 95-110.
12. Mani, A. 1999. Survival of the root-lesion nematode *Pratylenchus jordanensis* Hashim in a fallow field after harvest of alfalfa. Nematology 1: 79-84.
13. Moje, W., and Thomason, I. J. 1963. Toxicity of mixtures of 1,2-dibromo-3-chloropropane and 1,2-dibromoethane to the root-knot nematode, *Meloidogyne javanica*. Phytopathology 53: 428-431.
14. Oka, Y., Shapira, N., and Fine, P. 2007. Control of root-knot nematodes in organic farming systems by organic amendments and soil solarization. Crop Prot. 26: 1556-1565.
15. Santo, G. S., and O'Bannon, J. H. 1981. Effect of soil temperature on the pathogenicity and reproduction of *Meloidogyne chitwoodi* and *M. hapla* on Russet Burbank potato. J. Nematol. 13: 483-486.
16. Thomason, I. J., and Lear, B. 1961. Rate of reproduction of *Meloidogyne* spp. as influenced by soil temperature. Phytopathology 51: 520-524.
17. Thomason, I. J., Van Gundy, S. D., and Kirkpatrick, J. D. 1964. Motility and infectivity of *Meloidogyne javanica* as affected by storage time and temperature in water. Phytopathology 54: 192-195.
18. Towson, A. J., and Apt, W. J. 1983. Effect of soil water potential on survival of *Meloidogyne javanica* in fallow soil. J. Nematol. 15: 110-15.
19. Tsai, B. Y. 1978. Anhydrobiosis of the reniform nematode: survival and coiling. M. S. Thesis. University of Hawaii. 63 pp.

摘 要

蔡碧雲. 2008. 溫度對 *Meloidogyne incognita* 存活之影響。植病會刊 17：203-208. (台北市 國立台灣大學植物病理與微生物學系；電子郵件：bieyntm@ntu.edu.tw；傳眞：+886-2-2363-6490)

供試溫度爲 5, 10, 15, 20, 25, 30, 35, 40 及 45℃。根瘤線蟲 *Meloidogyne incognita* 二齡幼蟲最長之存活爲 15℃ 處理之 380 天，最短之存活爲 45℃ 處理之 4 小時；在這個 溫度下處理到 3.5 小時 98.8% 線蟲已死亡。在 40℃ 之處理到第六天線蟲已全部死亡，35℃ 之處理到第六十天線蟲全部死亡。30℃ 處理 25 天後，線蟲幾乎已全部死亡，而 25℃ 者已全部死亡。處理 60 天後，在 20℃ 及 10℃ 之線蟲僅少數存活。 低溫的 5℃ 之處理到第二十天線蟲之死亡率已達 99.3%。在 15℃ 存活 180, 220, 260, 320 及 380 天之線蟲皆能侵入綠豆苗根部造成根瘤並產生卵塊，然而此等長期存活之線蟲侵入率較新鮮之線蟲爲低。本研究之結果可作爲評估太陽能消毒法、輪作、及休耕等線蟲防治策略之參考。實驗數據顯示以餓死線蟲爲策略之防治方法；如輪作及休耕等，在某些地區之較冷季節可能無效，而在台灣，輪作及休耕可能在夏初開始進行者比在冬天有效。

關鍵詞：溫度、存活、太陽能消毒法、輪作、休耕、*Meloidogyne*