Effect of Soil Temperature on the Life Cycle of *Meloidogyne chitwoodi* Races 1 and 2 and *M. hapla* on Russet Burbank Potato

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Summary – Charchar, J.M. & G.S. Santo. 2009. Effect of soil temperature on the life cycle of *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla* on Russet Burbank potato.

The effect of soil temperature on the development, reproduction and life cycle of *M. chitwoodi* races 1 and 2 and *M. hapla* on Russet Burbank potato seedlings was studied under greenhouse condition. *Meloidogyne chitwoodi* race 1 (MC1) took 93, 54, 39, and 42 days or 651, 702, 741 and 1,050 degree-days (DD); *M. chitwoodi* race 2 (MC2) took 95, 59, 39, and 39 days or 665, 767, 741 and 975 DD; and *M. hapla* (MH) took 106, 72, 44 and 35 days or 212, 576, 616 and 700 DD to complete a life cycle, from J_2 to J_2, respectively, at 12, 18, 24 and 30 °C. None of the nematodes were able to complete a life cycle at 6 ºC in 115 days. MC2 had a wider temperature range for development to adult females than MC1 or MH in the same experimental condition. Optimum temperature determined for reproduction at the end of the first generation was 30 ºC for MH. For MC races 1 and 2, it was situated between 12 and 24 ºC.

Key words: *Solanum tuberosum*, Columbia root-knot nematode, Northern root-knot nematode, development, reproduction.

Resumo – Charchar, J.M. & G.S. Santo. 2009. Efeito da temperatura do solo no ciclo de vida de *Meloidogyne chitwoodi* raças 1 e 2 e *M. hapla* em batata 'Russet Burbank'.

O experimento foi conduzido para determinar o efeito da temperatura do solo no desenvolvimento, reprodução e ciclo de vida de *M. chitwoodi* raças 1 e 2 e *M. hapla* em plantas de batata 'Russet Burbank', em condições de casa de vegetação. *Meloidogyne chitwoodi* raça 1 (MC1) requereu 93, 54, 39 e 42 dias ou 651, 702, 741 e 1.050 graus-dias (GD); *M. chitwoodi* raça 2 (MC2) requereu 95, 59, 39 e 39 dias ou 665, 767, 741 e 975 GD; e *M. hapla* (MH) requereu 106, 72, 44 e 35 dias ou 212, 576, 616 e 700 GD para completar o ciclo de vida de J_2 a J_2, nas temperaturas de 12, 18, 24 e 30 ºC respectivamente. Nenhuma das espécies de nematoide completou o ciclo de vida à temperatura de 6 ºC durante o período vegetativo de 115 dias. Juvenis J_2 de MC2 originaram fêmeas adultas sob maior variação de temperaturas que MC1 e MH, no mesmo experimento. A temperatura ótima determinada para a reprodução de *M. chitwoodi* raças 1 e 2 ao final da primeira geração situou-se entre 12 e 24 ºC, ao passo que para *M. hapla* foi 30 ºC.

Palavras-chaves: *Solanum tuberosum*, nematoide de galhas de Colúmbia, nematoide de galhas do Norte, desenvolvimento, reprodução.

Introduction

Potato (*Solanum tuberosum*) is a very important vegetable crop cultivated in the Pacific Northwest USA, from were the finding of large commercial potato-growing areas infected with *Meloidogyne chitwoodi* and/or *M. hapla* has increased the interest in controlling

the damage caused by root-knot nematodes (Brown & Mojtahedi, 2005). Both nematode species cause economic losses in potato production because they reduce the quality of the potato tuber (Griffin & Jorgenson, 1969b; Santo & O'Bannon, 1981a). *Meloidogyne chitwoodi* causes more severe tuber damage and is more difficult to control than *M. hapla* (Santo *et al.*, 1980; 1981a; O'Bannon & Santo, 1984) and populations of *M. chitwoodi* as low as 1 egg / 250 cm^3 soil cause economic loss in Pacific Northwest (Pinkerton *et al.*, 1987; 1991). Santo & Pinkerton (1985) discovered the *M. chitwoodi* race 2 on potato 'Russet Burbank' in Washington State, which is capable of reproducing on alfalfa (*Medicago sativa* L.), not a suitable host for *M. chitwoodi* race 1. *Meloidogyne chitwoodi* race 3 was found infecting a wild potato (*Solanum bulbocastanum*) in California State, which has resistance to races 1 and 2 (Mojtahedi *et al.*, 1994; 1995).

Studies of Pinkerton *et al.* (1986; 1987; 1991) demonstrated that race 2 causes tuber damage similar to race 1, and it can be found widespread in all the major potato growing regions of the Pacific Northwest. The infection and number of generations of *M. chitwoodi* races 1 and 2 on potato cultivars increase as the temperature also increases, considering that more heat units or degree-days (DD) are accumulated (Pinkerton *et al.*, 1991). The percent of tuber infection with internal symptoms of *M. chitwoodi* increases when the potato tuber is stored at 21 to 24 °C and more than 740 DD is accumulated (David, 2007).

Soil temperature has a marked effect on the development and reproduction of root-knot nematodes (Griffin & Jorgenson, 1969a; Taylor & Sasser, 1978). The dominance of *M. chitwoodi* races 1 and 2 over *M. hapla* is attributed to their ability to develop and reproduce over a wider temperature range. *Meloidogyne chitwoodi* races 1 and 2 develop in potato roots and reproduces at soil temperature as low as 6 ºC (Charchar, 1987), whereas *M. hapla* and other common root-knot nematode species do not reproduce at this low temperature (Finley, 1976; Santo & O'Bannon, 1981b; Inserra *et al.*, 1985). In the Pacific Northwest, *M. chitwoodi* becomes established and populations increase early in the season when soil temperatures are low, from this resulting more generations per year than in *M. hapla* (Santo & O'Bannon, 1981b; O'Bannon & Santo, 1984). Inserra *et al.* (1985) reported the effect of temperature on the life cycle of *M. chitwoodi* on 'Nugaines' wheat (*Triticum aestivum*). The life cycle and reproduction of *M. hapla* have also previously been studied on potato (Thomason & Lear, 1961; Griffin & Jorgenson, 1969a), with development and reproduction occurring at 25 ºC, but not at 15 and 30 ºC (Griffin and Jorgenson, 1969a). *Meloidogyne chitwoodi* race 3 seems not to be spread around the potato producing areas of the United States since no damage of this race on the commercial potato cultivars has been reported (Mojtahedi *et al.*, 1994).

Considering that comparable information on the effect of soil temperature on the life cycle of *M. chitwoodi* races 1 and 2 and *M. hapla* on potato were not available, the objective of this paper was to determine the effect of soil temperature on development and reproduction of these root-knot nematodes on 'Russet Burbank' potato seedlings under greenhouse conditions.

Material and Methods

Populations of *Meloidogyne chitwoodi* race 1 (MC1) from Washington and race 2 (MC2) from Oregon were isolated from potato 'Russet Burbank' and maintained on wheat 'Nugaines' and alfalfa 'Thor', respectively. A population of *M. hapla* (MH) from Washington was isolated from alfalfa and maintained on pepper (*Capsicum annuum*) 'California Wonder', which served as differential host. These three nematode populations were reared on tomato (*Lycopersicon esculentum*) 'Columbia' on separate benches maintained at 20 to 26 ºC under greenhouse conditions. Periodically, they were checked for possible contamination through a differential host test on alfalfa 'Thor', wheat 'Nugaines', pepper 'California Wonder', and carrot (*Daucus carota*) 'Chantenay Red Cored' (Mojtahedi *et al.*, 1988).

Plants were watered as needed and fertilized monthly with a granulated Osmote formulation (Joe Berger & Co., Renton, Washington), 14-14-14 (10 g / pot). Nematode eggs for inocula were extracted from galled roots of 55 to 65 day-old tomato plants with sodium hipochloride (NaOCl) for four minutes

(Hussey & Barker, 1973). An inoculum of second-stage juveniles (J_2) was obtained by placing highly concentrated egg suspensions on 25 µm (500 mesh) screens and incubated at 24º C for 24 hours.

Single-eye seed pieces of 'Russet Burbank' potato were planted in methyl bromide fumigated sand in metal flats in the greenhouse. After five weeks, the original seed pieces were removed and a single plant was transplanted into an eight cm-diameter plastic cup containing 250 cm^3 of methyl bromide fumigated sandy loam soil. The plants were held in a greenhouse for one week. The cups were inserted into empty cups of the same size, placed in 25 cm-diameter glass jars, three cups per jar. Interspaces between cups were filled with methyl bromide fumigated soil to the top edge of the cups, which was four cm from the top of the jar. The jars were immersed in controlled water temperature tanks in a greenhouse (Charchar, 1987). After an additional week, inoculations were made by pipetting a suspension of 150 freshly hatched J_2 of MC1 and MC2, or MH in 5 m*l* of water around the exposed roots of the plants in each cup. Plants were randomized in eight replicates and grown at constant soil temperatures of 6, 12, 18, 24 and 30 ºC (± 1 ºC). Extra inoculated 'Russet Burbank' potato seedlings served as indicators for the detection of nematode phases, reflecting time of harvest of the experimental seedlings. Plant foliage was kept at ambient temperature maintained at 22 to 26 ºC. The light source was a series of VHO cool white fluorescent bulbs with an intensity of 4.8 *x* 103 *lux*, positioned 91 cm above the temperature tanks. During the experimental period, plants received regular water and were fertilized once with a granulated Osmote formulation 14-14-14 at a rate of 5 g / cup.

A complete randomized block design with eight replicates for each nematode population was used to compare developmental stages of the nematode populations at the same temperature. A split-plot design with a separate tank for each temperature was used to compare phases of the nematode populations at different temperatures (Gomez & Gomez, 1969). The experiment was terminated at the respective temperatures six days prior to egg mass initiation (beginning of egg production) of the second generation, or when plants died. One plant for each nematode population from each temperature was harvested periodically and analysed for development and reproduction as characterized by the following three stages: **a)** egg mass initiation (beginning of egg production); **b)** root invasion of first generation J_2 (beginning of the second generation); and **c)** six days prior to egg mass initiation of the second generation. The three reproductive stages were assessed after 22 to 106 days, when indicator potato plants showed presence of egg mass initiation and invasion of the first generation of J_2 at all temperatures.

The reproduction at the completion of the first generation was assessed for all nematode populations at all temperatures at least six days before the second generation of egg masses was established on the potato root system to avoid mixture of eggs between two generations of the nematodes. Roots were cut off the plant, washed, and stained individually with 2 % phloxin B for 15 minutes for quantification of egg mass matrices (Charchar *et al.*, 1984).

Following the phloxin B staining, the numbers of eggs per egg mass and the total egg production for each nematode population were determined by shaking the masses in 1 % NaOCl (Hussey & Barker, 1973) for separating and accounting eggs. Finally, the roots were stained with acid-fuchsin NaOCl technique (Byrd *et al.*, 1983) to assess the total number of nematodes that invaded the root systems.

Results and Discussion

The number of days (D) and of degree-days (DD) to egg mass initiation (beginning of egg production) and completion of the first generation, from J_2 to J_2, were determined (Table 1) for MC1, MC2 and MH. To reach egg mass initiation, MC1 required 91, 49, 33, 22 and 28 D or 91, 343, 429, 418 and 700 DD (base 5 ºC) and MC2 required 74, 49, 33, 22 and 22 D or 74, 343, 429, 418 and 550 DD (base 5 ºC), respectively, at 6, 12, 18, 24 and 30ºC. MH required 59, 39, 27, and 22 D or 118, 312, 378 and 440 DD (base 10 ºC) at 12, 18, 24 and 30 ºC, respectively. MH was not able to initiate egg masses at 6 ºC.

To complete its life cycle, MC1 required 93, 54, 39, and 42 D or 651, 702, 741 and 1,050 DD; MC2 95, 59, 39, and 39 D or 665, 767, 741 and 975 DD; and MH 106, 72, 44 and 35 D or 212, 576, 616 and

700 DD, respectively, at 12, 18, 24 and 30 ºC. At 6ºC none of the nematodes were able to complete a life cycle (Table 1).

The initial population of J_2 of MC1, MC2 and MH developing to adult females and females producing egg mass were significantly ($P \leq 0.05$) affected by soil temperature at egg mass initiation (Table 2). Egg mass initiation ranged from 22 to 91 days after inoculation depending on the nematode species and temperature (Table 1). The percent of MC1 J_2 developing to adult females was highest at 12ºC, followed by 18, 24, 30, and 6 ºC. More MC2 females developed at 30 ºC than at 6 or 12 ºC, but was not different from 18 or 24 ºC. The optimal developmental temperature for MH females was 30 ºC. Also more females were found at 12, 18, and 24 ºC than at 6 ºC, where none of the J_2 developed to females.

No differences ($P \leq 0.05$) were observed between MC1 and MC2 at 12, 18 and 24 ºC, but more females of MC2 were found at 6 and 30 ºC. No differences were observed in the percent of adult females between MH and MC1 or MC2 at 18 and 24 ºC. At 30 ºC more MH J_2 developed to females than MC1, but did not differ from MC2. Likewise, at 12 ºC MH did not differ from MC2, but infected roots contained fewer females than roots inoculated with MC1. Although no MH females developed at 6 ºC, it did not differ statistically from MC1 or MC2 (Table 2).

More than 88 % of the females at egg mass initiation produced egg masses at all temperatures, except at 6 ºC, where MH did not produce egg masses (Table 2). Significantly fewer egg masses were produced by MC1 and MC2 females at 6 ºC than at all other temperatures. No differences were observed among the other temperatures, except for MH, where females at 12 ºC produced fewer egg masses than at 18, 24 and 30 ºC. A higher percentage of MH females produced egg masses at 30 ºC than MC1 and MC2, and more than MC2 at 18 and 24 ºC. Conversely, fewer egg masses were produced by MH than MC1 and MC2 at 6 and 12 ºC. No differences were observed between MC1 and MC2, except at 6 ºC, where MC2 produced more egg masses than MC1 (Table 2).

Eggs produced per egg mass by MC1, MC2 and MH (none produced) at egg mass initiation were lowest at 6 ºC than at the other temperatures (Table 3). No significant differences were observed from 12 to 30 ºC for MC1 and MC2. More eggs / egg mass were recorded for MH at 30 ºC compared to 12 and 18 ºC, but did not differ from 24 ºC. Likewise, at 24 ºC MH females produced more eggs / egg mass than at 12 ºC, but not at 18 ºC. *Meloidogyne chitwoodi* race 1 produced more eggs / egg mass than MC2 at 24 ºC, and MH more than MC1 at 30 ºC (Table 3).

No differences were found among the three populations at 6, 12 and 18 ºC. Egg production for MH was not observed at 6 ºC. In terms of total egg production more MC1 eggs were recovered at 12 ºC than at 6 or 30 ºC, but did not differ from 18 and

Table 1 - Numbers of days and of degree-days required for the development of *Meloidogyne chitwoodi* races 1 (MC1) and 2 (MC2) and *M. hapla* (MH) from second stage juveniles (J_2) to egg mass initiation (beginning of egg production) and completion of the first generation, from J_2 to J_2.

Temp. (ºC)	Days						Degree-days[1]					
	Egg mass initiation			First generation			Egg mass initiation			First generation		
	MC1	MC2	MH	MC1	MC2	MH	MC1	MC2	MH	MC1	MC2	MH
6	91	74	-[2]	—[3]	—	—	91	74	-	—	—	—
12	49	49	59	93	95	106	343	343	118	651	665	212
18	33	33	39	54	59	72	429	429	312	702	767	576
24	22	22	27	39	39	44	418	418	378	741	741	616
30	28	22	22	42	39	35	700	550	440	1,050	975	700

[1]Degree-days were calculated by subtracting the constant temperature from the base temperature (base temperature used for *M. chitwoodi* was 5 ºC and for *M. hapla* 10 ºC) times the number of days required for the appearance of the egg masses and for presence of J_2 from the first generation.
[2]No egg mass production.
[3]First generation not completed in 115 days.

Table 2 - Effect of temperature on the percent of *Meloidogyne chitwoodi* races 1 (MC1) and 2 (MC2) and of *M. hapla* (MH) adult females and females producing egg masses on Russet Burbank potato seedlings at the time of egg mass initiation (beginning of egg production) after inoculation with 150 second stage juveniles (J_2).

Nematode species/ race	Temperature (ºC)				
	6	12	18	24	30
Percent of adult females					
MC1	2.1 Db	29.7 Aa	19.9 Ba	19.8 Ba	11.6 Cb
MC2	12.5 Ca	17.7 BCab	21.3 ABa	25.5 Aa	29.7 Aa
MH	0.0 Cb	11.6 Bb	11.5 Ba	18.5 Ba	39.6 Aa
Percent of females producing egg mass					
MC1	41.0 Bb	99.1 Aa	97.7 Aab	97.0 Aab	88.6 Ab
MC2	56.5 Ba	96.5 Aa	92.7 Ab	94.8 Ab	92.2 Ab
MH	0.0 Cc	88.4 Bb	98.4 Aa	99.6 Aa	99.1 Aa

Values are means of eight replicates. Values followed by the same low case letter in columns or values followed by the same capital letter in rows are not significantly different ($P \leq 0.05$), according to Duncan's Multiple Range Test, based on arcsine-transformed data.

Table 3 - Numbers of eggs per egg mass and total egg production of *Meloidogyne chitwoodi* races 1 (MC1) and 2 (MC2) and *M. hapla* (MH) on Russet Burbank potato seedlings at the time of egg mass initiation (beginning of egg production).

Nematode species/ race	Temperature (ºC)				
	6	12	18	24	30
Eggs / egg mass					
MC1	2 Ba	136 Aa	68 Aa	98 Aa	81 Ab
MC2	5 Ba	81 Aa	74 Aa	57 Ab	86 Aab
MH	0 Da	50 BCa	54 Ba	91 ABab	184 Aa
Total egg production					
MC1	13 Ca	3,744 Aa	1,083 ABa	1,061 ABa	549 Bb
MC2	45 Ca	584 Ab	655 Aa	434 Ab	304 Bb
MH	0 Da	250 Cb	689 BCa	1,012 Ba	8,403 Aa

Values are means of eight replicates. Values followed by the same low case letter in columns or values followed by the same capital letter in rows are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test, based on log-transformed data.

24 ºC (Table 3). *Meloidogyne chitwoodi* race 2 produced more eggs at 12, 18 and 24 ºC than at 6 or 30 ºC. Total egg production for MH increases as temperature increased with the highest number at 30 ºC. The lowest number of eggs produced for all three populations was at 6 ºC.

Comparisons among the populations at each temperature showed that MC1 produced more eggs at 12 ºC; MH at 30 ºC; and MC1 and MH at 24 ºC. No differences among the populations were observed at 6 and 18 ºC (Table 3).

Egg production was also observed at the completion of the first generation (Table 4). The completion of the first generation for MC1, MC2 and MH at the different temperatures ranged from 35 to 106 days (Table 1). Egg production was not recorded at 6 ºC because, as the plants died after 115 days, the nematodes did not complete a life cycle. The lowest number of eggs / egg mass and total egg production of MC1 and MC2 was observed at 30 ºC and no significant differences occurred at 12, 18 and 24 ºC. In terms of eggs / egg mass no differences were recorded for MH from 12 to 30 ºC (Table 3).

However, at the completion of the first generation, MH produced more total egg production at 30 ºC with sequentially lower production at 24, 18 and 12 ºC (Table 4). *Meloidogyne hapla* produced more eggs / egg mass than the MC races 1 and 2 at 24 and 30 ºC. No other differences were observed among the three populations. Total egg production at the completion of the first generation for MC1 was greater than that of MC2 or MH at 12 ºC; MH was better than MC1 and MC2 at 24 and 30 ºC, and MC2 was better than MC1 at 30 ºC. No differences were observed at 18 ºC (Table 4).

Six days prior to initial egg mass production of the second generation, total egg production for the first generation was determined (Table 5). Egg production per egg mass was similar to that observed at the end of the first generation (Table 4). The number of total eggs recovered was also comparable among the treatments, but with some statistical differences. Less total eggs of MC1 and MC2 were recovered at 30 ºC than at 12, 18 and 24 ºC. At 12 ºC MC2 and MH produced less egg than MC1. No differences in egg production were observed for MH at 18 and 24, and 24 and 30 ºC. *Meloidogyne chitwoodi* race 1 produced more eggs at 18 ºC than MH; MC2 and MH produced more at 24 ºC, and MC1 and MC2 did not differ at 30 ºC (Table 5).

All three nematode populations were able to complete one life cycle at all temperatures, except at 6 ºC (Table 1). The number of days required for MH to complete a life cycle decreased with the increase in temperature, with a minimum of 35 days at 30 ºC. A similar pattern was observed with MC1 and MC2, which took 39 days to complete a life cycle at 24 ºC, the same period required by MC2 at 30 ºC. The *M. chitwoodi* races were able to complete a life cycle faster at the lower temperatures (12 to 24 ºC) than MH, and MH was faster at 30 ºC. However, at 24 and 30 ºC all three populations were able to complete one life cycle within five days of each other. At 12 and 18 ºC MH required at least 11 days longer than the MC races. Although none of the populations were able to complete a life cycle at 6 ºC, MC1 and MC2 were able to produce egg masses (Table 3). However, no reinfection of roots by J_2 was observed after 115 days, when the experiment was terminated due to the death of the plants. The MC races may have been able to complete a life cycle if the plants were able to survive longer at 6 ºC.

Preliminary studies conducted on the effect of

Table 4 - Numbers of egg per egg mass and total egg production of *Meloidogyne chitwoodi* races 1 (MC1) and 2 (MC2) and *M. hapla* (MH) on Russet Burbank potato seedlings at the completion of the first generation from J_2 to J_2.

Nematode species/ race	Temperature (ºC)				
	6	12	18	24	30
		Eggs / egg mass			
MC1	-[1]	641 Aa	596 Aa	515 Ab	152 Bb
MC2	-	534 Aa	606 Aa	434 Ab	164 Bb
MH	-	596 Aa	776 Aa	835 Aa	860 Aa
		Total egg production			
MC1	-	15,972 Aa	15,438 Aa	15,116 Ab	661 Ba
MC2	-	8,875 Ab	12,430 Aa	10,643 Ab	4,591 Bb
MH	-	4,961 Cb	8,673 Ca	21,020 Ba	46,568 Ac

Values are means of eight replicates. Values followed by the same low case letter in columns or values followed by the same capital letter in rows are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test, based on log-transformed data.
[1]Generation not completed in 115 days.

Table 5 - Number of egg per egg mass and total egg production of *Meloidogyne chitwoodi* races 1 (MC1) and 2 (MC2) and *M. hapla* (MH) on Russet Burbank potato seedlings at least 6 days prior to egg mass production of the second generation.

Nematode species/ race	Temperature (ºC)				
	6	12	18	24	30
		Eggs / egg mass			
MC1	-[1]	559 Aa	739 Aa	764 Aa	73 Bb
MC2	-	416 Aa	780 Aa	770 Ab	99 Bb
MH	-	438 Aa	763 Aa	952 Ab	1,013 Aa
		Total egg production			
MC1	-	15,021 Aa	12,121 Aa	11,821 Ab	1,344 Bb
MC2	-	2,965 Bb	9,226 Aab	17,377 Aa	1,135 Cb
MH	-	3,642 Cb	8,497 Bb	19,089 ABa	55,438 Aa

Values are means of eight replicates. Values followed by the same low case letter in columns or values followed by the same capital letter in rows are not significantly different ($P \leq 0.05$) according to Duncan's Multiple Range Test, based on log-transformed data.
[1]Generation not completed in 115 days.

temperature on the development duration of MC1 and MH on potato roots were not consistent with results reported in these studies (Charchar *et al*., 1984). The inconsistencies were due to several factors that occurred in the initial studies: **1)** egg maturation was not taken into account, which would have lengthened the life cycle; **2)** undesirable fluctuations in temperature were observed at 7 ºC during the experiment; and **3)** confusion occurred in distinguishing between matrix material and actual egg production. Thus, when conducting life cycle studies, it is important that the exact stage of nematode development is known and can be easily recognized.

The number of degree-days required for the nematodes to produce egg masses and complete a life cycle tended to increase with temperature (Table 1). Because the MC races 1 and 2 began accumulating heat units at lower temperatures, they required more DD than MH at each temperature. The determination of DD for a nematode to complete a life cycle is important in establishing predictive models (Ferris, 1976). In the Pacific Northwest potato tubers are not infected until J_2 from the first generation appears. Thus, being able to predict the time of egg mass initiation in the roots and emergence of J_2 will facilitate timing of nematode chemical control to protect the tubers from nematode invasion.

Although MH (isolate WAMH) J_2 were able to penetrate roots at 6 ºC, they were not able to develop to adult females within 115 days (Tables 2 and 3). The most favorable temperature for development of MH females was at 30 ºC. The highest number of MC1 females was observed at 12 ºC and 18 to 30 ºC for MC2. This indicates that MC2 has a higher temperature tolerance than MC1 for female development. Development rate of MC2 was greater than MC1 at 6 and 30 ºC indicating that MC2 has a wider temperature range than MC1. The development rate of MH and MC2 did not differ at 12 to 30 ºC, which indicates a close similarity in the biological behavior of these two isolates at egg mass initiation. Soil temperatures did not seem to affect egg mass production as it did female development. Egg masses were produced by at least 88 % of the females at all temperatures, except at 6 ºC where it was considerable less (Table 2), indicating that the J_2 populations were synchronized before inoculations.

Under the temperature regime of this study, the optimum temperature for total egg production of MH was at 30 ºC (Tables 3, 4 and 5). It is possible that the optimum temperature for reproduction of MH may be higher than 30 ºC. In addition, MH produced more eggs / egg mass at 24 and 30ºC, and more total eggs at 30 ºC than both MC races 1 and 2 at the end of the first generation. These results obtained with MH were consistent with previous studies using the same isolate (Santo & O'Bannon, 1981b; O'Bannon & Santo, 1984). However, Griffin and Jorgensen (1969a) reported that the optimum temperature for MH reproduction was at 25 ºC and was partially inhibited at 30 ºC. These differences may be related to the abilities of the different isolates to tolerate higher and lower temperatures (Stephan & Trudgill, 1982). The MC races 1 and 2 had a wider optimum temperature range for the total egg production at the end of the first generation (Table 4). Both races produced eggs equally well at 12, 18 and 24ºC.

In relation to *M. chitwoodi*, MC1 produced more total eggs than MC2 at 12 ºC, but no differences were observed in female development and the number of eggs / egg mass at 12 ºC; MC2 had a higher reproductive potential at 30 ºC, indicating again a higher temperature tolerance than MC1. Comparing total egg production at egg mass initiation (Table 3) to completion of the first generation (Table 4), it is interesting to note that MC2 was able to produce eggs more rapidly than MC1 from 12 to 30 ºC. From egg mass initiation to the completion of the first generation, the total egg production of MC2 compared to MC1 increased from a low of 5-fold at 18 ºC to a high of 14-fold at 30 ºC. Even though the ability of MH to penetrate roots and the length of its life cycle were affected at temperatures of 12 to 24 ºC, it did not have a comparable effect on total egg production compared to MC1 and MC2.

Egg production of the first generation females at least six days prior to egg mass initiation of the second generation (Table 5) generally followed the same pattern observed at the end of the first generation (Table 4). The females continued to lay eggs for a period of time after completion of a life cycle. It should be noted that the completion of the life cycle

was determined from inoculated J_2 to the detection of J_2 in the roots and the end of egg production was not taken into account. It was demonstrated here that the number of degree-days for the three nematode populations to complete their life cycles increased by increasing the soil temperature. These findings were confirmed posterioly by Pinkerton *et al.* (1991) and David (2007).

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