Influence of temperature on the development of the temperate root-knot nematodes *Meloidogyne chitwoodi* and *M. fallax*

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Summary. Hatching, migration, invasion and post-penetration development of *Meloidogyne chitwoodi* and *M. fallax* were examined at 15, 20 and 25°C. The optimum temperature for hatching of second-stage juveniles (J2) of *M. chitwoodi* and *M. fallax* was 20°C. However, temperatures above 20°C and no higher than 25°C were more favourable for hatching of *M. chitwoodi* than *M. fallax* J2, which in contrast hatched in greater number at temperatures below 20°C and no lower than 15°C. *Meloidogyne chitwoodi* J2 migrated farther and in higher numbers than those of *M. fallax* in sand columns. The optimum temperature for migration was 20°C for *M. chitwoodi* and 25°C for *M. fallax*. Invasion of roots by both nematodes was higher on potato than maize at all temperatures. For both nematodes the degree-days (DD_5, base temperature 5°C) required for completing their life cycle were 555-740 DD_5 on potato and 705-740 DD_5 on maize. Temperature played a more important role in post-penetration development than the host plant. The behavioural differences found between *M. chitwoodi* and *M. fallax* are discussed in relation to different survival strategies of the two species.

Key words: degree-days, development, hatching, invasion, life cycle, maize, migration, potato.

Temperature has a direct impact on nematode biology by influencing processes such as growth and reproduction, sex determination and survival (Madulu & Trudgill, 1994; Trudgill, 1995). Temperature also influences the length of embryogenesis of root-knot nematodes. Higher temperatures significantly shortened the duration of the embryonic development of *Meloidogyne chitwoodi* Golden *et al.*, 1980 and *M. hapla* Chitwood, 1949 (Inserra *et al.*, 1983). At 20°C, the duration of embryogenesis did not differ between both nematode species; however, at 10°C, embryogenesis of *M. hapla* took 13 days longer than that of *M. chitwoodi*. At 12°C, embryonic development up to hatching of *M. hapla* required 5 days more than *M. chitwoodi*, whereas at 24°C, embryonic development up to hatching of *M. hapla* took only 2 days longer (Charchar & Santo, 2001).

Temperature also impacts on processes of post-embryonic development of root-knot nematodes like hatching, migration and root invasion of second-stage juveniles (J2). Low temperature (7°C) significantly reduced hatching of both *M. chitwoodi* and *M. hapla*, but J2 of *M. chitwoodi* hatched seven times more than J2 of *M. hapla* (Inserra *et al.*, 1983). Pinkerton *et al*. (1987) demonstrated that migration of *M. chitwoodi* and *M. hapla* was greater at 18°C than at 12°C; overall J2 of *M. chitwoodi* migrated further and in greater numbers than J2 of *M. hapla* at 12, 18 and 24°C. Optimal temperatures for root invasion have been reported for *M. hapla* (Wong & Mai, 1973) and *M. chitwoodi* (Umesh & Ferris, 1994). Compared to *M. hapla*, *M. chitwoodi* invaded roots in greater numbers at lower temperatures, although a crop effect could have influenced the results.

The host crop also affects the post-penetration development and the length of the life cycle of root-knot nematodes. At 15, 20 and 25°C, *M. chitwoodi* developed and completed its life cycle faster on

potato than on corn and wheat. When *M. chitwoodi* and *M. hapla* were inoculated simultaneously on potato roots, *M. chitwoodi* developed and reproduced more rapidly than *M. hapla* at the same temperatures (O'Bannon & Santo, 1984). By contrast, at 30°C, significantly more *M. hapla* than *M. chitwoodi* females were found 14 days after penetration. Although both species are temperate root-knot nematodes, it seems that *M. chitwoodi* is better adapted to lower temperatures. *Meloidogyne chitwoodi* is a temperate root-not nematode of great economic importance for the potato industry in the Pacific North West of the United States (USA), where it was discovered and described by Golden *et al.* (1980). This species was later found in Europe, where it was considered a predominant parasite of potato in many northern European countries, until Karssen (1996) discovered that the European populations of *M. chitwoodi* were associated with another closely related root-knot nematode that he described and named *M. fallax.* This species does not occur in the USA and has great economic relevance for the potato and field vegetable industries in north Europe.

Meloidogyne chitwoodi and *M. fallax* have a wide host range including economically important crops such as potato, sugar beet, carrot, cereals and maize (O'Bannon *et al.*, 1982; Umesh & Ferris, 1994; Karssen, 1996; Den Nijs *et al.*, 2004; Wesemael & Moens, 2008; Wesemael *et al*., 2011). Both species can have multiple generations during a crop-growing season (Pinkerton *et al*., 1991; Brinkman *et al*., 1996; Karssen, 1996; Wesemael & Moens, 2008). In 1998, both species were listed as quarantine pests in the EU. Knowledge of their biology and life cycle at different temperature regimes is required to enable scientifically sound detection and control strategies. Information on the thermal optima for *M. fallax* is not available. This paper reports the results of comparative studies on the effects of temperature on hatching, migration, invasion and life cycle duration of *M. chitwoodi* and *M. fallax*.

MATERIALS AND METHODS

Preparation of egg suspension. Pure stock cultures of both *M. chitwoodi* (Postel, Belgium) and *M. fallax* (Baexem, The Netherlands) were established on tomato plants (*cv.* Tiny Tim) grown in the glasshouse (25±2°C). Mature egg masses were collected from the tomato roots 45 days after nematode inoculation. The egg masses were macerated for 30 s at 14,500 g in a Waring blender and sieved (200 µm) to separate the eggs from the gelatinous matrix and root debris. The eggs were then transferred onto a 5-µm floating sieve to separate hatched J2 from eggs. The eggs remaining on the sieve were collected in distilled water and an approximate concentration of 250 eggs ml^{-1} was used for the hatching experiment.

Influence of temperature on hatching. A suspension of 250 eggs at different stages of embryonic development (1 ml) of each nematode species was transferred onto 5-µm mini sieve tubes. These were made of two small diameter tubes open at one end and inserted into each other. The base of the smaller diameter (2 cm) tube was sealed with a 5-µm sieve cloth and was suspended inside the wider tube, which had a solid plastic base. Whilst a 5-µm sieve cloth retains all eggs, it allows quite a large number of J2 to pass because of the irregularity of the apertures of the cloth (unpublished information). The height of the inner tube could be adjusted as required. The sieves were kept in temperature controlled growth chambers in the dark at 15, 20 or 25°C. Each observation was repeated four times with four different batches. From the 3rd day after the setup of the experiment, hatched J2 were collected from the outer tube at one-day intervals during three successive weeks and counted with the aid of a dissecting microscope. At each observation the water level in the mini sieve tubes was adjusted with distilled water. At the end of the experiment all the eggs containing developing embryos and with unhatched J2 were counted to determine the percentage hatch. Numbers of hatched J2, expressed as a percentage of the initial egg population, were used for statistical analysis. The hatching data were fitted to the logistic model $y = c/(1+\exp(-b\times(time-m)))$, where y is the cumulative percentage hatch, m – the time, expressed in days, at which 50% hatch is reached, b – the hatching rate (% day^{-1}), and c – the final hatching percentage. The parameters of the logistic model (m, b and c) were calculated with an iterative process to maximize the R^2 value.

Collection of inoculum. Second-stage juveniles of *M. chitwoodi* and *M. fallax* were obtained from the stock culture. The plants were uprooted and their roots were washed and cut into pieces (±0.5 cm). Eggs and juveniles were liberated by maceration (14,500 g for 10 s in a Waring blender). The macerated root suspension was then poured on a cotton tissue filter and left in the dark at 20±1°C. Hatched J2 were collected on a daily basis during 10 successive days; J2 were pooled and stored at 4°C. Before use, the J2 (1-10 days old) were placed on a 5-µm sieve in a modified Baermann funnel to concentrate and to separate living from dead J2. Freshly collected J2 were used for migration,

invasion and post-penetration development experiments.

Influence of temperature on migration of J2. Moist sand columns were used for the experiment on the migration of J2. Sand columns consisted of plastic tubes (10 cm long, 1 cm diam.) filled with sand under negative pressure. A flexible tube was inserted at one end of the plastic tube and the open end of the flexible tube was kept at the same height of the top of the plastic tube kept in vertical position. The plastic tube was filled with sand for up to 9 cm and constantly vibrated during filling with sand and water to avoid air bubbles in the column. Then it was kept horizontally. The open end of the flexible tube was kept at 49 cm under the level of the horizontally placed plastic tube to remove excess water. The tube was kept in this position until equilibrium was reached (*ca* 5 min). Then the bottom end of the plastic tube was closed with a rubber cork. One drop of a suspension containing approximately 200 J2 (1-10 days old) of either *M. chitwoodi* or *M. fallax* was released at the top end of the tube, which was then corked. Columns containing nematodes were placed horizontally and kept at 15, 20 and 25°C in the dark for 7 days with 5 replications for each temperature. Seven days after inoculation, the columns were cut into 1-cm long sections starting from the point of introduction of the J2. Sand in each section was washed with a small volume of water. For this purpose, each section was placed in a vial and agitated. The water was gently decanted onto a counting dish while the sand and the piece of tube were left in the vial. Nematodes were counted under a dissecting microscope.

Influence of temperature on invasion of roots by J2 and their post-penetration development. Single eyes of potato (*cv.* Gloria) and single maize seedlings were grown in small plastic pots (5 cm diam. × 5 cm high) filled with heat sterilised river sand. The plants were grown for 35 days in the glasshouse (fluctuating temperature) before they were inoculated with 1-10 days-old J2 of *M. chitwoodi* or *M. fallax*. 150 J2 suspended in 1 ml water were inoculated in three holes of 2 cm depth around each plant, for a total of 450 J2 pot^{-1}. Immediately after inoculation, the pots were placed in growth chambers at 15, 20 and 25°C. Seven days later, all plants were gently uprooted and washed to remove non-penetrated free J2. To observe the root invasion, four plants of each treatment (temperature × host) were stained with acid fuchsin (Daykin & Hussey, 1985). The number of juveniles in the roots was counted under the microscope. The remaining uprooted plants were transplanted into 10-cm diam. plastic pots containing heat sterilised garden soil and

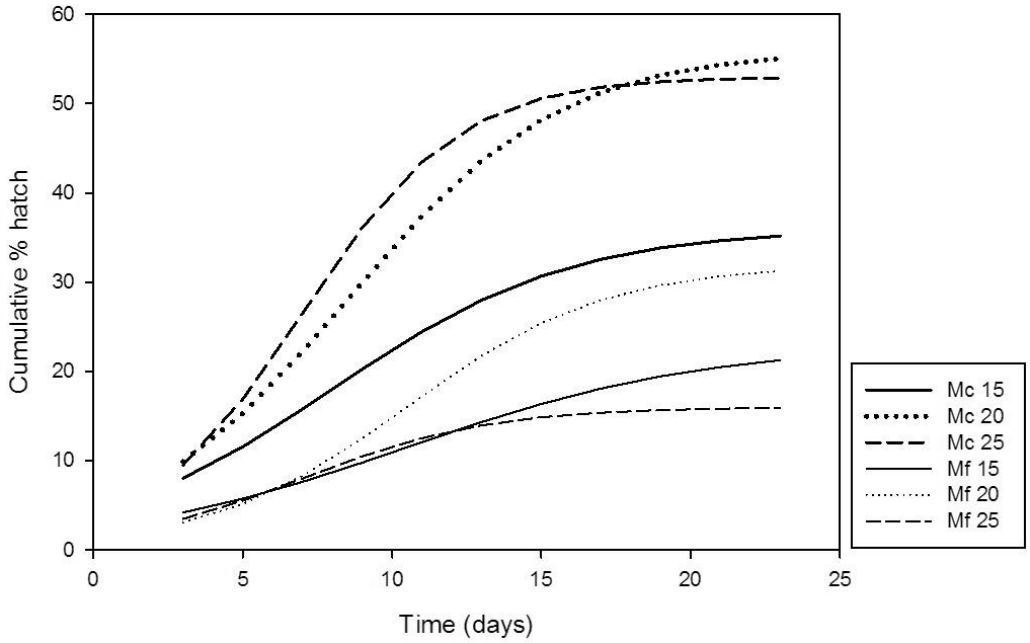

Fig. 1. Fitted curves showing the expected cumulative percentage hatch of *Meloidogyne chitwoodi* (Mc) and *M. fallax* (Mf) at three different temperatures (15, 20 and 20°C) in distilled water.

placed in the growth chambers (post-penetration development experiment) at the original temperature. At 17, 27, 37, 43, 47, 52, 57, 62, 67, 71 and 75 days after inoculation (DAI), plants were uprooted, washed, and the nematodes inside the roots were stained as above. The numbers of vermiform and swollen juveniles, adult males and females in the roots were counted under the microscope. No attempt was made to determine the life stages of the swollen juveniles inside the roots. Nematode J2 were extracted from the soil by the centrifugal-floatation method (Coolen & D'Herde, 1972) and counted under the dissecting microscope. For observations at 17, 27 and 37 DAI, four plants (replicates) were examined for each temperature. For the other DAI only one plant for each temperature was considered. The degree-days for *M. chitwoodi* and *M. fallax* to complete their life cycle were calculated using a base temperature (minimum temperature for development) of 5°C. When calculating the thermal time (degree days), knowledge on the base temperature (Tb) is important (Trudgill *et al.*, 2005). Pinkerton *et al.* (1991) estimated from field studies on potato a Tb of 5°C for *M. chitwoodi*. For *M. fallax* the Tb is unknown but we assumed it to be similar to *M. chitwoodi*.

Experimental design and data analysis. All laboratory experiments were set up in a completely randomised design (factorial). Analysis of variance (ANOVA) was carried out for hatching, migration and invasion experiments. T-tests separated between the mean values ($P < 0.01$).

RESULTS

Influence of temperature on hatching. The optimum temperature for hatching of J2 for both *M. chitwoodi* and *M. fallax* was 20°C (Fig. 1, Table 1). At this temperature the maximum percentage of hatched J2 (c) after 23 days was 56 and 32 for *M. chitwoodi* and *M. fallax*, respectively (Table 1). The cumulative percentage of hatched J2 of *M. chitwoodi* was higher than *M. fallax* at all examined temperatures over the whole incubation period. *Meloidogyne chitwoodi* J2 started hatching sooner than those of *M. fallax* (Fig. 1). Compared with *M. fallax*, 50% of *M. chitwoodi* J2 hatching (m), at 15 and 20°C, was reached 2.5 and 2 days earlier, respectively (Table 1). At 15°C and 25°C, the rate of hatching (b) was higher for *M. chitwoodi* than for *M. fallax*; there was no difference between both species at 20°C (Table 1). At 15 and 20°C, J2 of both *M. chitwoodi* and *M. fallax* continued hatching until the last observation day (day 23). When incubated at 25°C, J2 of both species stopped hatching after 21 days. Overall, J2 of *M. chitwoodi* hatched in higher numbers at 20 and 25°C, whereas J2 of *M. fallax* hatched better at 15 and 20°C.

Table 1. Parameters of the logistic curve $y = c/(1 + \exp(-b\times(\text{time}-m)))$ describing hatching of second-stage juveniles of *Meloidogyne chitwoodi* and *M. fallax* at different temperatures and corresponding R^2-values. The results are the means of four replicates of the time at which 50% of the total hatching is reached (m), the hatching rate (b) and the maximum hatching percentage (c). Significant differences (paired t-test, $P < 0.01$) between treatments are marked with a different letter.

Temperature (°C)	m (days)	b (%/day)	c (%)	R2
M. chitwoodi				
15	8.0b	0.25b	36d	0.99
20	8.5b	0.28b	56e	0.99
25	7.0a	0.38d	53e	0.99
M. fallax				
15	10.5c	0.20a	23b	0.99
20	10.5c	0.30bc	32c	0.99
25	7.0a	0.32c	16a	0.99

Influence of temperature on migration of J2. Seven days after the inoculation, the cumulative

Table 2. Percentage of migrated *Meloidogyne chitwoodi* and *M. fallax* second-stage juveniles in moist sand columns at different distances from the inoculation point 7 days after inoculation at different temperatures.

Temperature (°C)	Distance from inoculation point (cm)						
	0 to 1	1 to 2	2 to 3	3 to 4	4 to 5	5 to 6	6 to 7
M. chitwoodi							
15	46.47a*	25.99a	14.52a	8.10b	3.71b	1.09b	0.12a
20	53.31a	21.02a	13.17a	7.07b	3.56b	1.55b	0.32a
25	51.60a	23.69a	12.05a	6.64b	3.54b	1.83b	0.65a
M. fallax							
15	62.67a	23.40a	10.53a	3.40a	0.00a	0.00a	0.00a
20	61.08a	22.43a	11.26a	4.26a	0.97a	0.00a	0.00a
25	41.08a	31.32b	14.89b	7.76b	3.43b	1.10b	0.42a

* Data are the means of five replications. Significant differences (paired t-test, $P < 0.01$) between treatments per column are marked with a different letter.

percentage of inoculated J2 that had migrated in the sand columns was 53.5 (15°C), 46.7 (20°C) and 48.4 (25°C) for *M. chitwoodi* and 37.3 (15°C), 38.9 (20°C) and 58.9 (25°C) for *M. fallax*. Table 2 shows the percentage of the retrieved J2 at each distance from the inoculation point. None of the J2 of either species migrated beyond 7 cm. Overall, the *M. chitwoodi* J2 migrated further and in greater numbers than those of *M. fallax* at all three temperatures. Migration of *M. chitwoodi* J2 was significantly higher than *M. fallax* at 15 and 20°C, but there was no significant difference at 25°C.

The temperature did not influence migration of *M. chitwoodi* J2. *Meloidogyne fallax* migrated furthest and highest in numbers at 25°C than at other temperatures. The J2 of this species moved no further than 4 and 5 cm at 15°C and at 20°C, respectively.

Table 3. Invasion at different temperatures of *Meloidogyne chitwoodi* and *M. fallax* second-stage juveniles in maize and potato roots seven days after inoculation (450 J2 plant^{-1}).

Temperature (°C)	Maize		Potato	
	M. chitwoodi	*M. fallax*	*M. chitwoodi*	*M. fallax*
15	39a*	61c	185d	145a
20	51b	66c	174c	170c
25	55b	50b	168c	159b

* Data are the means of four replications. Significant differences per host (paired t-test, $P < 0.01$) are marked with a different letter.

Influence of temperature on root invasion by J2 and their post-penetration development. Invasion and post-penetration development of *M. chitwoodi* and *M. fallax* was strongly influenced by both temperature and crop. The attractiveness of potato was markedly higher than maize for both *M. chitwoodi* and *M. fallax* (Table 3). Root invasion of both crops by *M. fallax* J2 was highest at 20°C. By contrast, root invasion by *M. chitwoodi* J2 was highest at 25°C on maize and 15°C on potato.

At 20 and 25°C both *M. chitwoodi* and *M. fallax* completed their life cycle (from J2 to J2) between 27 and 37 DAI on potato (Table 4). At 25°C egg masses of both species were observed. The presence of a larger number of vermiform J2 of both nematodes in roots at 37 DAI compared to 27 DAI indicated that many of these J2 had hatched from the eggs deposited by the females that were observed at 27 DAI. At 43 DAI, J2 from the second generation were found freely in the soil. At 15°C, development of *M. chitwoodi* and *M. fallax* was monitored up to 75 DAI (data not shown). Both nematode species were unable to complete their life cycle on potato at this temperature in less than 75 days (end of the observations). Adult females were detected but no egg masses were observed.

On maize both nematodes completed their life cycle at 37 DAI at 25°C and 47 DAI at 20°C (Table 5). At 15°C development of *M. chitwoodi* and *M. fallax* was monitored up to 75 DAI (data not shown). Both nematode species were unable to complete their life cycle on maize at this temperature in less than 75 days. At 15°C females of *M. chitwoodi* and *M. fallax* were found from 52 DAI and 57 DAI onwards, respectively. No egg masses were observed during the observation period (end 75 DAI).

The degree-days required to complete the life cycle of both *M. chitwoodi* and *M. fallax* were calculated (base temperature = 5°C) as 555-740 DD_5 on potato and at 705-740 DD_5 on maize. The number of females of *M. chitwoodi* and *M. fallax* was greater in potato than in maize for both nematodes at all the observation days at all temperatures.

DISCUSSION

Nematodes are poikilothermic animals; temperature is one of the most important abiotic factors in their development and biology (Perry, 2002). The data obtained in this study clearly demonstrate that temperature is an important criterion differentiating *M. chitwoodi* and *M. fallax* at different stages of their biology. The optimum temperature for hatching of both species was 20°C. However, J2 of *M. chitwoodi* hatched more at high (20 and 25°C) than low (15°C) temperatures, whereas *M. fallax* J2 hatched better at low (15 and 20°C) than higher (25°C) temperatures. This suggests that *M. fallax* has a lower temperature optimum for hatching than *M. chitwoodi*. These results confirm those obtained by Inserra *et al.* (1983) for *M. chitwoodi*. In our experiment, hatching of J2 of both nematodes ceased after 3 weeks. These findings differ from those of Wesemael *et al*. (2006) who reported hatching from undisturbed egg masses up to 12 and 14 weeks after the start of incubation for *M. chitwoodi* and *M. fallax*, respectively. Moreover, they found a delay in hatching for *M. chitwoodi* compared with *M. fallax*. In the present work, the number of *M. fallax* J2 that hatched was lower than that of *M. chitwoodi* J2 at all temperatures including the lowest (15°C). The protective role of the egg sac can be an explanation for the differences between our results and those obtained by Wesemael *et al*. (2006). Wallace (1968) found that at a relative humidity of 98%, hatch of *M. javanica* from egg sacs remained constant, whereas hatch from free eggs declined markedly and eventually

Table 4. Influence of temperature on the development of *Meloidogyne chitwoodi* and *M. fallax* on potato at different days after inoculation (DAI) with 450 J2 plant^{-1}.

DAI	Temperature (°C)	Potato									
		M. chitwoodi					*M. fallax*				
		Vermiform juveniles	Swollen juveniles	Females	Males	2nd generation J2**	Vermiform juveniles	Swollen juveniles	Females	Males	2nd generation J2**
17	15	40*	83	0	0	0	32	139	0	0	0
	20	4	111	4	0	0	0	131	44	0	0
	25	0	77	76	0	0	0	56	69	12	0
27	15	6	139	0	0	0	2	159	2	0	0
	20	4	135	11	0	0	0	2	149	15	0
	25	0	5	165	12	0	0	9	114	13	0
37	15	0	46	117	0	0	0	7	137	11	0
	20	17	3	139	13	0	44	2	152	19	0
	25	218	0	71	7	0	178	6	71	2	0
43	15	3	17	129	0	0	9	36	161	0	0
	20	14	2	186	1	280	265	0	162	3	260
	25	96	0	33	1	160	38	0	53	3	138
47	15	0	16	0	0	0	0	29	131	22	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
52	15	0	24	154	0	0	0	4	169	0	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
57	15	0	6	169	0	0	0	124	251	0	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
62	15	0	5	138	0	0	0	5	171	0	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–

*Data are means of four replicates. Observations were ended when the first J2 of second generation were detected.
**J2 found in soil.

stopped after 10 days. Total percentage of cumulative hatch was low for both *M. chitwoodi* (56% at 20°C) and *M. fallax* (32% at 20°C) suggesting uncompleted embryonic development. Although eggs used in our experiments were collected from egg masses of the same age, they may have differed in their embryonic development.

Second-stage juveniles of *M. chitwoodi* migrated further and in greater numbers than those of *M. fallax* at both 15 and 20°C. However, at 25°C, *M. fallax* J2 migrated over a long distance like those of *M. chitwoodi*. Pinkerton *et al.* (1987) found that *M. chitwoodi* migrated in greater numbers than *M. hapla* and furthest at 18°C compared with 12°C and 24°C. The results from this study indicate that migration of *M. fallax* requires higher temperatures. Migration of *Meloidogyne* spp. from deeper soil layers can play an important role in the infection of host plants (Johnson & Mckeen, 1973; Prot & van Gundy, 1981; Mojtahedi *et al*., 1991).

Comparing effects of the host on invasion, we observed that J2 of both *M. chitwoodi* and *M. fallax* penetrated potato roots in much greater numbers than maize roots at all three temperatures tested. This indicates that potato is a better host than maize for both species as the rate of invasion decreases on poor hosts (Anwar *et al.*, 1994). These results are in agreement with the results obtained by other authors for *M. chitwoodi* (Santo & O'Bannon, 1981; O'Bannon *et al*., 1982) and *M. fallax* (Karssen, 1996). Comparing nematode-temperature combinations, *M. chitwoodi* invaded potato roots in higher numbers at 15°C, whereas in maize invasion was highest at 25°C. The opposite pattern, but less pronounced, was found for *M. fallax*. These differences could not be attributed to differences in migration with different temperatures. It appeared from our work that for *M. chitwoodi* and *M. fallax* optimum temperature for invasion depends on both nematode and host species. Root diffusates can play an important role in host location and the condition and age of the host plant influences their activity (Perry, 1997). Possibly temperature can also have an effect on root diffusates and this might explain the

Table 5. Influence of temperature on the development of *Meloidogyne chitwoodi* and *M. fallax* on maize at different days after inoculation (DAI) with 450 J2 plant^{-1}.

DAI	Temperature (°C)	Maize									
		M. chitwoodi					*M. fallax*				
		Vermiform juveniles	Swollen juveniles	Females	Males	2nd generation J2**	Vermiform juveniles	Swollen juveniles	Females	Males	2nd generation J2**
17	15	40*	0	0	0	0	56	1	0	0	0
	20	14	34	0	0	0	29	35	0	0	0
	25	24	26	0	0	0	15	37	0	0	0
27	15	30	9	0	0	0	28	20	0	0	0
	20	14	41	0	0	0	10	37	2	0	0
	25	15	35	10	0	0	15	30	3	0	0
37	15	15	21	0	0	0	7	33	0	0	0
	20	3	8	2	11	0	15	24	14	12	0
	25	169	4	14	2	0	181	2	5	3	0
43	15	6	1é	0	0	0	8	24	0	0	0
	20	0	14	26	0	0	0	12	29	0	0
	25	31	10	11	0	218	28	14	8	2	238
47	15	15	18	0	0	0	15	17	0	0	0
	20	20	6	0	-	88	12	3	6	0	60
	25	–	–	–	–	–	–	–	–	–	–
52	15	10	18	154	0	0	14	29	0	0	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
57	15	13	10	169	0	0	31	12	11	0	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–
62	15	17	15	138	0	0	13	15	10	0	0
	20	–	–	–	–	–	–	–	–	–	–
	25	–	–	–	–	–	–	–	–	–	–

*Data are means of four replicates. Observations were ended when the first J2 of second generation were detected.
**J2 found in soil.

differences we found. However, we did not differentiate host location and subsequent invasion and more research is required on this aspect.

Both temperature and crop have a great influence on the post-penetration development and the length of the life cycle of *M. chitwoodi* and *M. fallax*. Once the juveniles penetrated the roots, *M. fallax* tended to develop and complete its life cycle faster than *M. chitwoodi* on both hosts. This was also reported by van der Beek *et al*. (1998) on potato. Taking 5°C as base temperature for calculating the degree-days required to complete the life cycles of both root-knot nematodes, we found for *M. chitwoodi* and *M. fallax* 555-740 DD_5 on potato and 705-740 DD_5 on maize, respectively. Obviously, the number of degree-days was not different between *M. chitwoodi* and *M. fallax* in this study. At 20°C both *M. chitwoodi* and *M. fallax* required 10 days more to complete their life cycle on maize compared with potato. At 25°C both nematodes completed their life cycle within the 37th day after inoculation on both crops. This suggests that temperature is a more important factor than the host plant for the development of *M. chitwoodi* and *M. fallax* inside the roots. At 15°C both nematodes did not complete their life cycle on either crop within 75 days after inoculation. This result confirms findings from other authors who observed that low temperature inhibits the rate of development of *M. chitwoodi* (O'Bannon *et al.*, 1982; O'Bannon & Santo, 1984).

Wesemael *et al*. (2006) discussed a different survival strategy of *M. chitwoodi* and *M. fallax* suggesting the ability of hatched J2 of *M. fallax* to survive in the soil, whereas *M. chitwoodi* developed quiescent J2. The lower temperature optimum for hatching of *M. fallax* supports this strategy. Due to the limited migration at lower temperatures, J2 of *M. fallax* can restrict their energy utilisation and this

can enable them to survive longer in the soil. Further research on energy reserves, survival and infectivity of these two nematode species is required to develop effective management programmes.

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A. Khan, W. Wesemael, M. Moens. Влияние температуры на развитие галлообразующих нематод умеренного климата: *Meloidogyne chitwoodi* и *M. fallax*.

Резюме. Процессы отрождения личинок из яиц, миграций личинок 2-й стадии, их проникновения в растения и последующего развития у галлообразующих нематод *Meloidogyne chitwoodi* и *M. fallax* исследовали при температурах 15, 20 и 25°C. Оптимальной для отрождения личинок 2-й стадии *M. chitwoodi* и *M. fallax* температурой оказалось 20°C. Было отмечено, что температуры выше 20°C, но не более 25°C были более благоприятны для отрождения личинок 2-й стадии *M. chitwoodi*, чем *M. fallax*. Личинки этого вида вылуплялись в больших количествах при температурах ниже 20°C, но не менее 15°C. Личинки 2-й стадии *M. chitwoodi* мигрировали в колонках, наполненных песком, быстрее и в больших количествах, чем личинки *M. fallax*. Оптимальной температурой для миграции была 20°C для *M. chitwoodi* и 25°C для *M. fallax*. Проникновение внутрь растения у обоих видов нематод происходило успешнее на корнях картофеля, чем на корнях кукурузы. Для этих двух нематод значение параметра DD_5 (при базовой температуре 5°C), необходимое для завершения жизненного цикла, составляло 555-740 DD_5 на картофеле и 705-740 DD_5 на кукурузе. В развитии нематод после проникновения в корень растения температура играла большую роль, чем вид растения-хозяина. Обсуждаются поведенческие различия между *M. chitwoodi* и *M. fallax* в связи с различными стратегиями выживания этих двух видов.