COMPARATIVE THERMAL-TIME REQUIREMENTS FOR DEVELOPMENT OF *MELOIDOGYNE ARENARIA, M. INCOGNITA*, AND *M. JAVANICA*, AT CONSTANT TEMPERATURES

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ABSTRACT

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Understanding thermal-time relationships of plant-parasitic nematodes is necessary to predict their geographical distributions, population dynamics and potential for decreasing crop yields. Our objective was to compare the thermal-time requirements for development of three major species that affect Florida agriculture, namely *Meloidogyne arenaria* race 1, *M. javanica*, and *M. incognita* race 4. The base temperature (T_b) and heat units expressed as degree-days (DD) required for nematode development from second-stage juveniles (J2) to egg-laying females were determined at constant temperatures ranging from 12 to 35°C. Freshly hatched J2 of the three species were inoculated on okra (*Abelmoschus esculentus*) and placed in growth chambers. Data were subjected to regression analysis to estimate the base temperature for each species. The shortest time and average DD above base temperatures 10.3, 9.8, and 10.6ºC for development were 19 days and 316 DD, 15 days and 300 DD, and 17 days and 334 DD, for *M. arenaria, M. incognita*, and *M. javanica*, respectively.

Key words: base temperature, degree days, root-knot nematodes, thermal-time requirements.

RESUMEN

Dávila-Negrón, M., D.W. Dickson. 2013. Comparación de requisitos térmicos para el desarrollo de Meloidogyne arenaria, M. incognita, and M. javanica a temperaturas constantes. Nematropica 43:152-163.

La información sobre relaciones térmicas para los nematodos fitoparasíticos es necesaria para predecir la distribución geográfica de las especies, dinámica poblacional y pérdida en rendimiento de los cultivos. Esta investigación tiene como objetivo el determinar los requisitos térmicos para las especies *Meloidogyne arenaria* raza 1, *M. javanica*, and *M. incognita* raza 4, a temperaturas constantes. Los estudios realizados para determinar la temperatura base (T_b) necesaria para el desarrollo de las especies y las unidades de calor (DD) requeridas para completar el desarrollo desde el segundo estadio juvenil (J2) a hembras con masas de huevos, fueron a temperaturas constantes en un rango de 12 a 35°C. Juveniles de segundo estadio y recién eclosionados, pertenecientes a las tres especies, fueron inoculados en plántulas de okra (*Abelmoschus esculentus*) y colocados en las cámaras de crecimiento. A través de un análisis de regresión se estimó la temperatura base (T_b) para el desarrollo de cada una de las especies. El tiempo mínimo requerido para completar el desarrollo y el promedio de DD sobre 10.3, 9.8, y 10.6ºC fue 19 días y 316 DD, 15 días y 300 DD, y 17 días y 334 DD, para *M. arenaria, M. incognita,* y *M. javanica*, respectivamente.

Palabras clave: días grado, nemátodo nodulador, requisitos térmicos, temperatura base.

INTRODUCTION

Temperature is one of the major factors affecting growth and development of root-knot nematodes (Trudgill and Perry, 1994; Trudgill *et al*., 2005). It is a major environmental factor influencing their distribution, survival, and rate of development (Bergeson, 1959; Bird and Wallace, 1965; Daulton and Nusbaum, 1961; Ferris *et al*., 1978; Madulu and Trudgill, 1994; Trudgill *et al*., 2005; Vrain *et al*., 1978). A direct relationship was demonstrated to exist between temperature and the time required for development of J2 to mature egg-laying females (Tyler 1933). A measure of the physiological time required for the completion

of the developmental process can be expressed in degree-days (DD) or degree-hours (Arnold, 1960). The summation of the physiologically effective temperature (i.e., T_e minus T_b) over the duration of the process, and expressing time in thermal units, removes the time-dependency of biological processes where this is due to temperature changes (Trudgill *et al*., 2005).

Thermal-time requirements were used by Trudgill and Perry (1994) to compare tropical and temperate species of plant-parasitic nematodes. These authors established that the thermal time requirements of many poikilothermic organisms are fundamental to their ecological strategies. The T_b values for plant-parasitic nematodes reflect their adaptation to the temperature regimes they experience during growth and development and that heat units required for life processes or a complete life cycle reflects their ecological strategies within different environments. It also provides useful practical information in the development of nematode population models (Duncan and McSorley, 1987; Ferris *et al*., 1978; Moorhead *et al*., 1987; Starr and Jeger, 1985).

There is good reason to establish the relevance of the thermal time relationship to nematodes of agricultural importance and to understand the requirements for the most important species. Comparative data are not available on the number of heat units required for development of the three most important root-knot nematodes, *M. incognita, M. arenaria* and *M. javanica* that infect vegetables. It is expected that their rate of development may be similar but no direct comparisons have been made.

The objective of this study was to determine the thermal-time requirements of Florida populations of *M. arenaria* race 1, *M. incognita* race 4, and *M. javanica* in growth chambers. To accomplish this objective we determined: the shortest time to reach different developmental stages at different temperatures, the basal temperature for development of the three *Meloidogyne* spp., the rate of development under constant temperature on a favorable host, and the number of heat units (expressed in degree-days) that each species requires to develop from an infective J2 stage to a J3, J4, female and an egg-laying female.

MATERIALS AND METHODS

Nematode Isolates and Inoculum Preparation

The isolate of *M. arenaria* (Neal) Chitwood race 1 was obtained from peanut (*Arachis hypogaea* L.) grown at the former University of Florida Green Acres Research Farm, Alachua Co., FL. Isolates of *M. incognita* (Kofoid and White) Chitwood race 4 and *M. javanica* (Treub) Chitwood were obtained from infected roots of tomato (*Solanum esculentum* Mill. cv. Rutgers) maintained in a greenhouse at the Entomology and Nematology Department, University of Florida. Single egg mass isolates of each of these species were increased on tomato cv. Rutgers and kept in a greenhouse at 25 ± 5°C. Species and race identification was confirmed using perineal patterns, differential host tests, and isozyme phenotypes (Harris and Hopkinson, 1976; Hartman and Sasser, 1985).

Eggs of the three nematode species were extracted from galled tomato roots by immersing in 0.5% NaOCL solution (Boneti and Ferraz, 1981). The eggs were collected on a sieve with 25-µm pore openings and then placed on a modified Baermann funnel (Rodríguez-Kábana and Pope, 1981) at 27°C to allow them to hatch. Second-stage juveniles collected during the first 24 h were discarded and the subsequent 48-h-old J2 were used for the experiments.

Post-infection Development at Constant Temperatures

The post-infection development of each of the three species was evaluated on okra (*Abelmoschus esculentus* L. Moench) cv. Clemson Spineless at 12, 15, 18, 21, 24, 27, 30, 33, and 35°C. Okra seeds were germinated in a commercial growing mix (Fafard, BWI, Plymouth, FL), maintained at 21 ± 8°C in a greenhouse and the seedlings were fertilized weekly with 100 ml of a 20-20-20 (N-P_2O_5-K_2O) solution with micronutrients. One month after emergence, the seedlings were transferred to insulated polystyrene foam cups (250 ml) containing autoclaved builder's sand (97% sand and 3% clay) and placed in an environmental control chamber (Walker *et al*., 1993) at 25°C with 16 hours of light/day and a light intensity of 30 µmol/m^{-2} s^{-1}.

Each nematode species was tested separately on different dates to determine their basal threshold temperature (T_b) for development and the heat units required for their development. The 48-h-old J2 were concentrated in 5 ml water to deliver ca. 500/seedling. The J2 were poured in 2.5-cm deep holes around the root system of 45-d-old okra seedlings. Seedlings were placed in the environmental control chambers for 48 hr at 25°C to allow J2 penetration. After 48 h, the okra roots were removed from the sand and washed thoroughly with tap water to remove any J2 that had not penetrated. The seedlings then were transplanted into sand in the insulated foam cups and distributed to different temperature chambers. Thirty inoculated plants were placed in each of nine environmental chambers set to give a constant temperature. Seedlings were watered every other day with 40 ml of a fertilizer solution containing 0.21 g/liter of a soluble 20-20-20 (N-P_2O_5-K_2O) with micronutrients. Plants at temperatures lower than 21°C were watered with only 20 ml of the fertilizer solution to prevent waterlogging of the sand.

Development was determined by sequential sampling. Two seedlings of each temperature treatment were harvested starting 3 d after inoculation (DAI) and then at 2-d intervals until 31 DAI. Roots of each plant were washed, weighed, and 25% of the total root system was chosen arbitrarily. These roots were

rewashed, cleared, and stained with acid fucshin (Byrd *et al*., 1983). The root pieces were placed individually between two glass microscope slides (25 × 75 × 1 mm) and pressed. Developmental stages of nematodes were observed with the aid of a light microscope at a magnification of 40× and identified (Triantaphyllou and Hirschmann, 1960) (Figs. 1 to 3). When immature females were observed, the plant root systems were first stained with 15 mg/liter Phloxine B solution to detect egg mass formation (Dickson and Struble, 1965) before clearing and staining with acid fucshin. Numbers of nematodes at different developmental stages were counted and expressed as a percentage of the total numbers that penetrated roots.

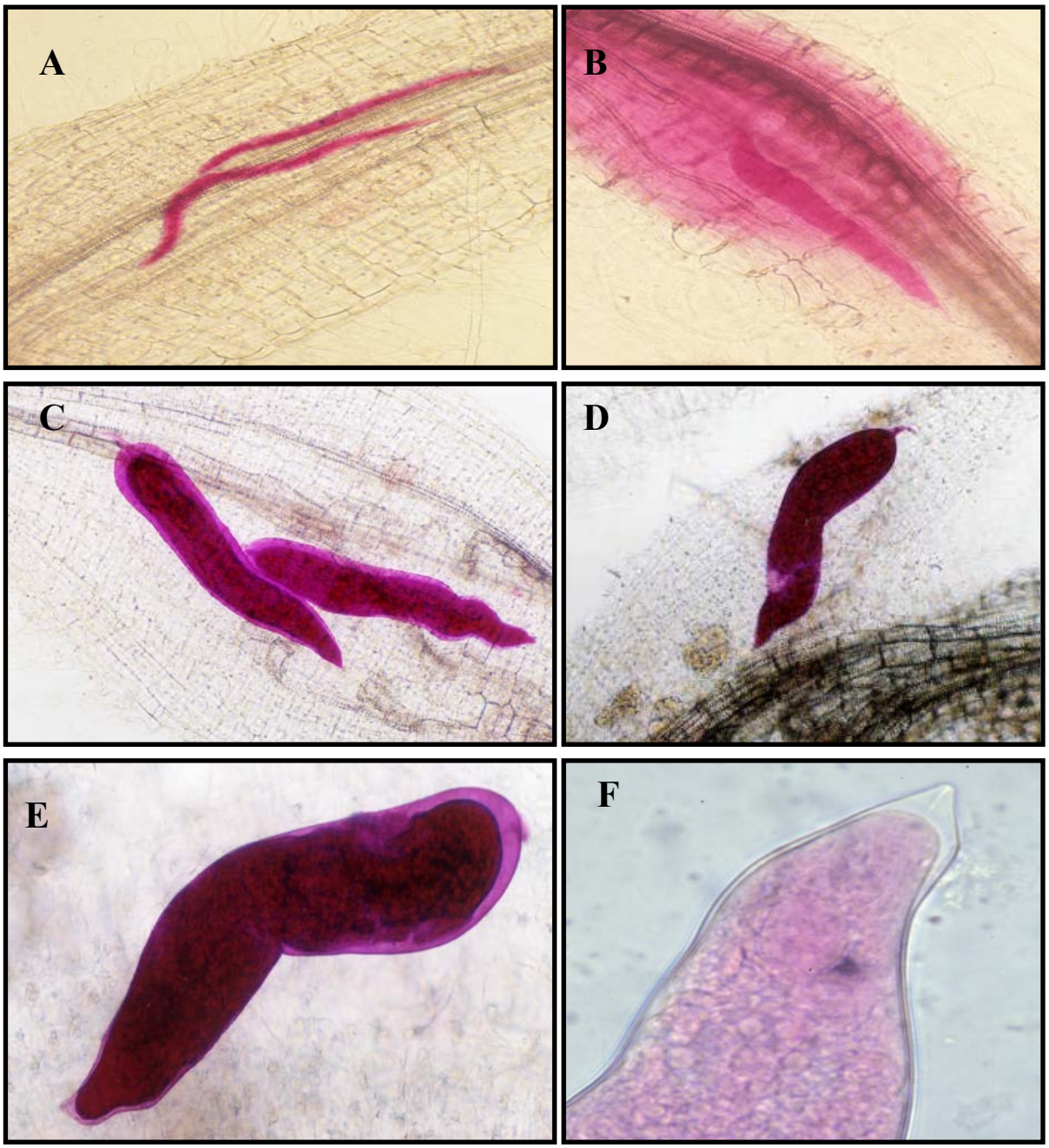

Fig 1. Developmental stages of *Meloidogyne* spp. [A = second-stage (J2) infective juvenile; B = J2 developed, sexually undifferentiated; C, D = J2 sexually differentiated; E = third-stage juvenile (J3); F = anterior portion of a J3].

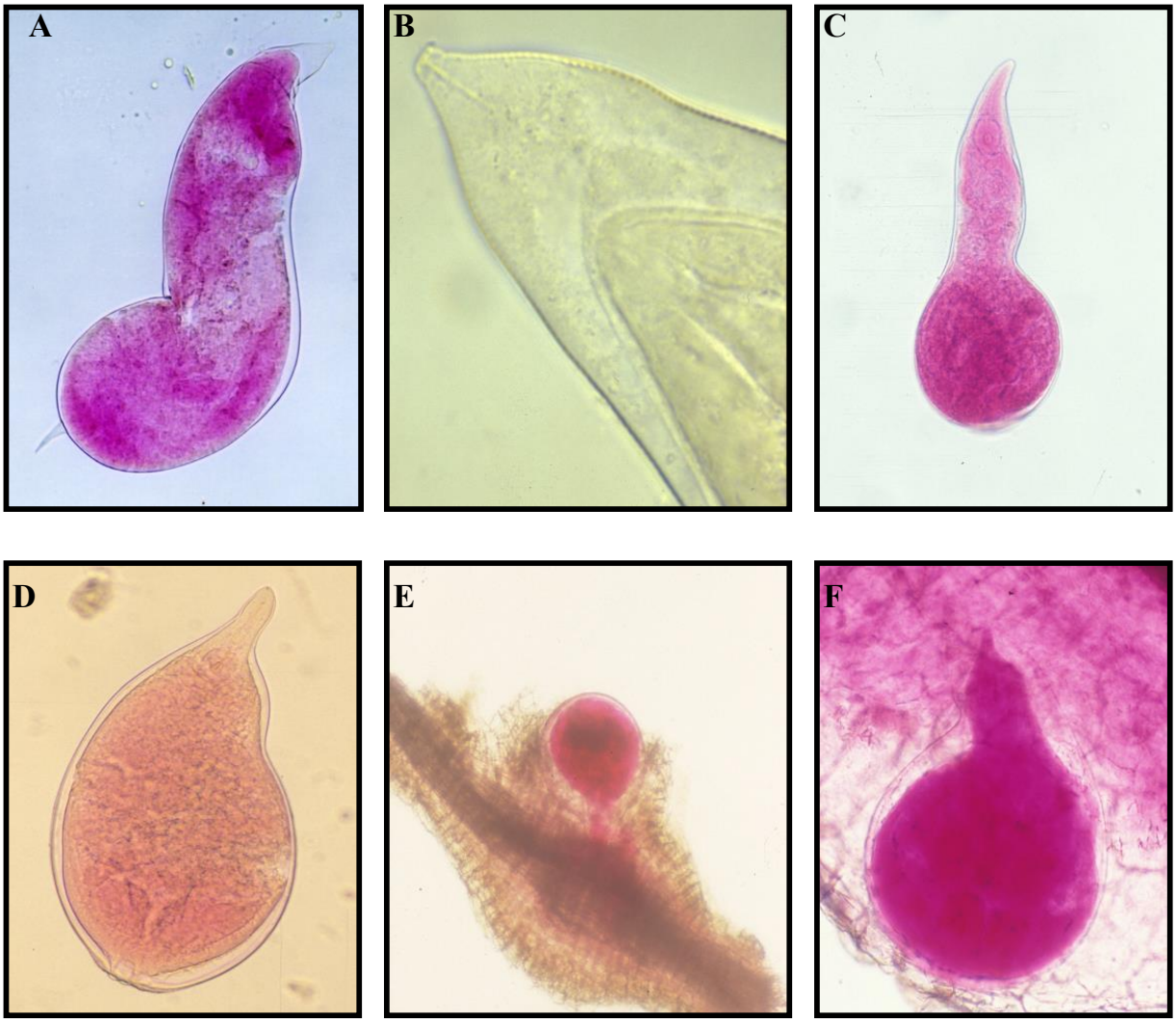

Fig 2. Developmental stages of *Meloidogyne* spp. [A = fourth-stage juvenile (J4); B = anterior part of J4; C, D = non-egg-laying female and E, F = egg-laying female].

Base Temperature for Development and Degree-days

Developmental rate of different stages for each nematode species was related to temperature by regression (Trudgill and Perry, 1994). The equation for a linear relationship between T_e (environmental temperature) and rate of development (for values of T_e between T_b [base temperature] and T_o [optimum temperature]) is: $y=ax + b$ where y is the development rate (1/duration of development), x is the environment temperature, b is the intercept on the y axis, and a is the temperature coefficient (slope) of the regression line (Trudgill *et al*., 2005). Base temperature for development (T_b) was determined by solving the regression equation for the x-intercept. The average of the T_b estimates for each developmental stage was designated as the T_b for the nematode species. Degree-days (accumulated daily mean temperature above T_b) required to complete each developmental stage were calculated for each root-knot nematode species based on the equation DD = (Tmax + Tmin)/2 - T_b × number of days required to reach a developmental stage (Arnold, 1960). Because the temperatures were constant, the simplified equation became DD = constant temperature - T_b × number of days required to reach a developmental stage for each temperature. The average of the number of degree-days required for each stage to develop under different temperatures was designated as the number of degree-days required to reach each developmental stage.

RESULTS

Post-infection Development at Constant Temperatures

Cumulative days and accumulated degree-days required for development of the various life stages from J2 to egg-laying females of *M. arenaria, M. incognita*, and *M. javanica* on okra at a constant temperature are reported in Table 1. None of the species developed further than a J2 at 12°C and 15°C after 31 d. At 18°C, *M. incognita* and *M. arenaria* developed faster than *M. javanica* from J3 stage to non-egg laying females. None of the species reached the egg-laying stage at 18°C. At 21°C, *M. incognita* reached all developmental stages faster than *M. arenaria* and *M. javanica*. However, the percentage of non-egg-laying females of *M. arenaria* and *M. javanica* 31 DAI was higher than 90% whereas, *M. incognita* was 40% (data not shown). Furthermore *M. incognita* and *M. arenaria* females were observed laying eggs 25 and 29 DAI, respectively. *M. javanica* females were never observed laying eggs at 21°C or below.

In the intermediate temperature range (24 to 30°C) the rate of development of the three species increased (Table 1). The earliest observation of a J3 at 30°C was 7 DAI for *M. incognita* and 9 DAI for *M. arenaria* and *M. javanica*, whereas at 24 and 27°C, J3 were observed 11 and 9 DAI, respectively, for all three species.

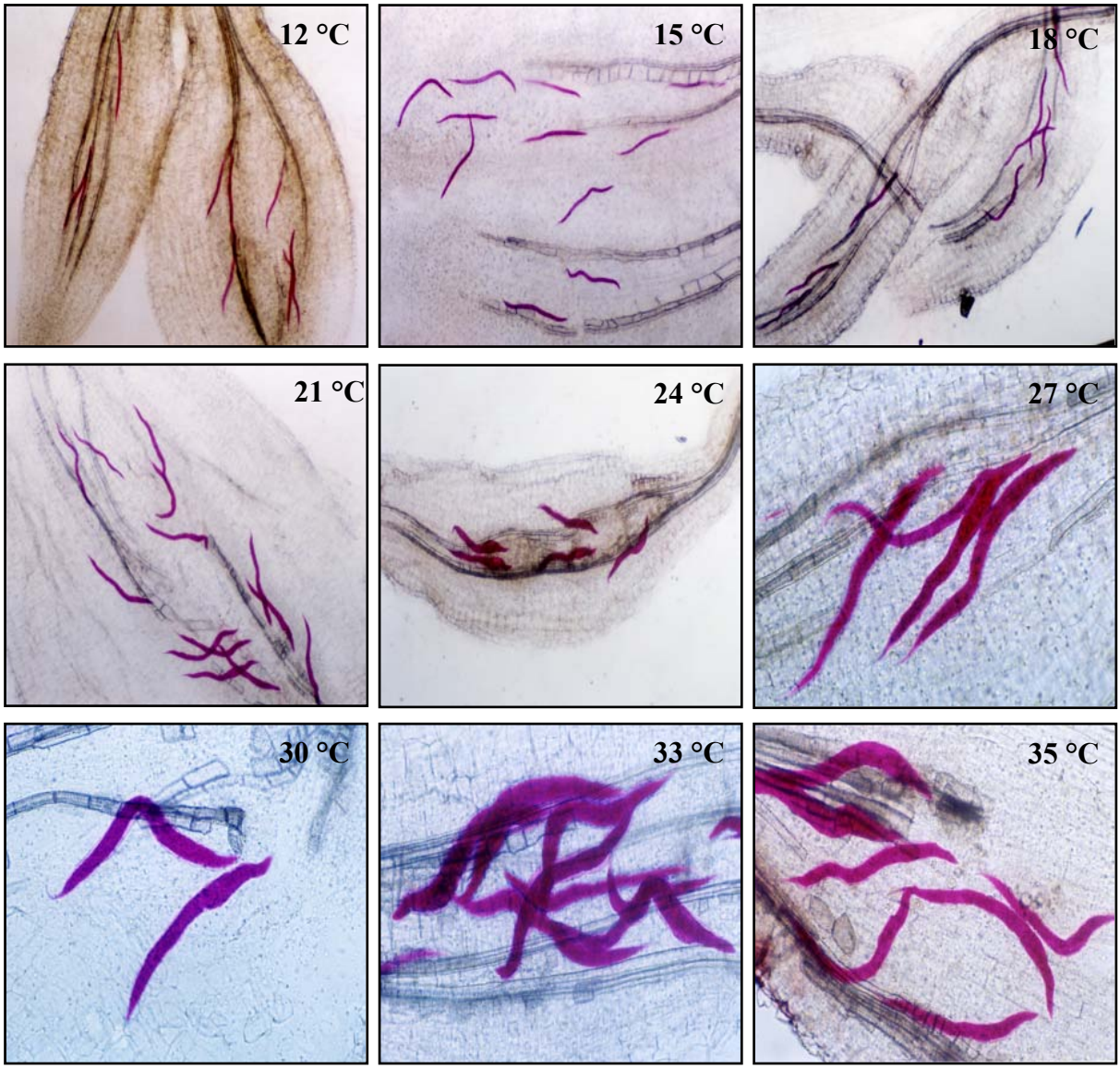

Fig 3. Development of *Meloidogyne arenaria* second-stage juveniles growing on okra in a growth chamber at constant temperatures 5 DAI.

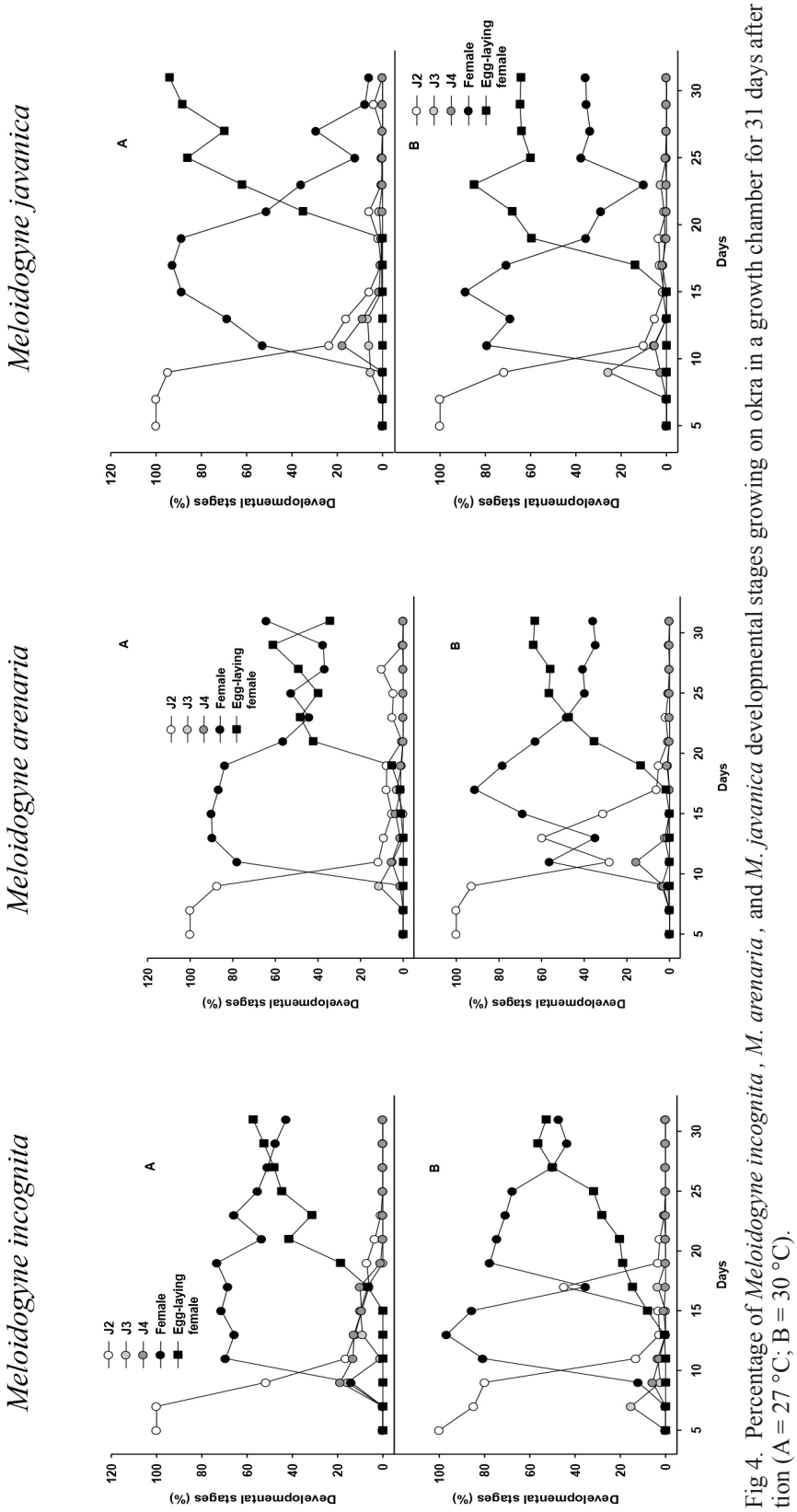

Fig 4. Percentage of *Meloidogyne incognita* , *M. arenaria* , and *M. javanica* developmental stages growing on okra in a growth chamber for 31 days after inoculation (A = 27 °C; B = 30 °C).

Fourth-stage juveniles were observed 1 or 2 days later. *M. incognita* was the first to reach the non-egg laying female stage (shortly after the fourth molt). At 24°C, *M. incognita, M. arenaria*, and *M. javanica* non-egg-laying females were observed 11, 13, and 15 DAI, respectively. At 27 and 30°C, *M. incognita* non-egg-laying females were observed 9 DAI, whereas for *M. arenaria* and *M. javanica* it was 11 DAI. The percentage of *M. incognita* females increased from 20% to 100% from 9 to 13 DAI at 27 and 30°C (Fig. 4). However, it was not until 11 DAI that *M. javanica* reached 60% to 80% at these temperatures. Conversely, 11 DAI *M. arenaria* reached 80% and declined to 60% at 27ºC and 30ºC, respectively (Fig 4). *M. incognita* females were the first to start egg-laying 17 and 15 DAI at 27 and 30°C, respectively. At these same temperatures, *M. javanica* started egg laying at 21 and 17 DAI, whereas *M. arenaria* required 19 DAI. The percentage of egg-laying females increased over time at 27 and 30°C in all species (Fig. 4).

In the high temperatures ranges (33 and 35°C) the rate of development of *Meloidogyne* spp. did not increase linearly (Table 1). These high temperatures also affected the growth of okra seedlings and some plants died before the experiment was completed.

Base Temperature for Development and Degree-days

The relationship between temperature and rate of development (reciprocal of time required to complete a developmental stage) was linear for all *Meloidogyne* spp. grown from 18 to 30°C (Tables 1-3). Temperature-dependent equations of development were derived for *M. arenaria, M. incognita*, and *M. javanica* (Table 2). The regression line fitted to the data between 18 to 27°C for *M. arenaria* J3 to egg-laying females accounted for 95% or more of the variation and between 18 to 30°C for *M. incognita* and *M. javanica* accounted for 93% or more of the variation.

The base temperatures (T_b) for development of J3 to egg-laying females are shown in Table 3. The T_b for each species was calculated from a range of threshold temperatures averaging 10.3°C for *M. arenaria*, 9.8°C for *M. incognita*, and 10.6°C for *M. javanica* (Table 3).

The number of heat units or degree-days required for the three species to reach different stages of development under each temperature is shown in Table 1. At temperatures as low as 12 and 15°C, the degree-days accumulated were not enough for the development of J3 to egg-laying females. At temperatures ranging from 18 to 30°C, the degree-days accumulated over time for the formation of different developmental stages were slightly different among temperatures (Table 1). At 33 and 35°C there was no increase in development regardless of the accumulation of additional heat units, thus it was assumed that the requirement of degree-days was the same as that for the optimum temperature of 30°C.

The average number of degree-days required for the three species is shown in Table 1. The degree-days required for development of J3 and J4 for *M. arenaria* were similar and approximately half the amount time as required for reaching the egg-laying stage. *M. incognita* had similar requirements for the development of J4 and female stage, and required fewer DD to complete all developmental stages when compared with *M. arenaria* and *M. javanica*.

DISCUSSION

Comparative Development of Three Meloidogyne spp.

The rate of development of *M. arenaria, M. incognita*, and *M. javanica* was slow at temperatures below 18°C and egg-laying females were not observed. No development further than a J2 was observed at 12 and 15°C during the 31 day period after inoculation. It is likely that given more time further development would have occurred. Extrapolation of the line from the regression analysis shows that theoretically, *M. incognita, M. arenaria* and *M. javanica* will need 35, 40, and 50 d, respectively, to begin egg laying at 18°C. Confirmation of these extrapolations was not possible because of the limited period of observations. The delay in the development of root-knot nematodes at low temperatures has been observed previously (Davide and Triantaphyllou, 1967; Madulu and Trudgill, 1994; Tyler, 1933; Vrain *et al*., 1978). Slower development at low temperatures also has been observed on free-living nematodes (Vancoppenolle *et al*., 1999). At 21°C, *M. javanica* did not reach the egg-laying stage within 31 days of observation; however, Madulu and Trudgill (1994) reported that it completed its life cycle after 44 days at the same temperature. In our study, extrapolation of the regression line showed that theoretically *M. javanica* should start to lay eggs after 34 d. Egg-laying females of *M. arenaria* and *M. incognita* were observed at 21°C, which was the same as reported previously (Davide and Triantaphyllou, 1967; Yeon *et al.*, 2003).

In the intermediate temperature range (24 to 30°C) the rate of development of the three species increased. Third and fourth-stage juveniles, non-egg-laying females, and egg-laying females developed faster than at low temperatures. For all three species, the number of days required to reach the next developmental stage decreased as temperature increased. The number of days required for the three species to begin laying eggs was similar to other reports (Tyler, 1933; Triantaphyllou and Hirschmann, 1960; Tzortzakakis and Trudgill, 1996; Yeon *et al*., 2003). We observed that at temperatures above 27°C for *M. arenaria* and above 30°C for *M. incognita* and *M. javanica*, the rate of development slowed down instead of accelerating in response to the higher temperature. Above these temperatures, the rate of development declined sharply indicating that those are the optimum temperatures (T_o) for these species. The decline in development was observed by Tyler (1933) at temperatures above 28°C for *Meloidogyne* sp.

Table 1. Cumulative days and accumulated degree-days (DD) required for first observation of different developmental stages of *Meloidogyne arenaria* race 1 (Ma1), *M. incognita* race 4 (Mi4), and *M. javanica* (Mj) on okra (*Abelmoschus esculentus*) inoculated with freshly hatched second-stage juveniles and held at a constant temperature.

Meloidogyne Spp.	Developmental Stages[x]	Temperature °C																
		12	15	18		21		24		27		30		33		35		µ
Ma1	J3	0	0	19[y]	(146)[z]	15	(161)	11	(151)	9	(150)	9	(150)	9	(150)	9	(150)	151
	J4	0	0	21	(162)	15	(161)	13	(178)	9	(150)	9	(150)	9	(150)	9	(150)	157
	Female	0	0	23	(177)	17	(182)	13	(178)	11	(184)	11	(184)	9	(204)	11	(184)	185
	Egg-laying female	0	0	0		29	(310)	23	(315)	19	(317)	19	(317)	19	(317)	19	(317)	316
Mi4	J3	0	0	17	(139)	11	(123)	11	(156)	9	(155)	7	(141)	7	(141)	7	(141)	142
	J4	0	0	21	(172)	13	(146)	11	(156)	9	(155)	9	(155)	9	(155)	9	(155)	156
	Female	0	0	23	(189)	13	(146)	11	(156)	9	(155)	9	(155)	9	(155)	9	(155)	159
	Egg-laying female	0	0	0		25	(280)	23	(327)	17	(292)	15	(303)	15	(303)	17	(292)	300
Mj	J3	0	0	19	(141)	13	(135)	11	(147)	9	(148)	9	(148)	9	(148)	9	(148)	145
	J4	0	0	25	(185)	15	(156)	13	(174)	11	(180)	9	(175)	9	(175)	9	(175)	174
	Female	0	0	27	(200)	17	(177)	15	(201)	11	(180)	11	(180)	11	(180)	11	(180)	185
	Egg-laying female	0	0	0	0	0	0	25	(335)	21	(344)	17	(330)	17	(330)	19	(330)	334

DD = degree days above a threshold temperature specific for each nematode (Ma1 = 10.3 ºC, Mi4 = 9.8 ºC, and Mj = 10.6 ºC).
[x]J3 = third-stage juvenile; J4 = fourth-stage juvenile.
[y]Cumulative days.
[z]Accumulated degree-days

Table 2. Effect of temperature on the reciprocal time in days (day^{-1}) for development from a second-stage juvenile to different developmental stages (J3, J4, female and egg-laying female) of *Meloidogyne* spp.

Meloidogyne Spp.	J3	J4	Female	Egg-laying female
Ma	$y = 0.0067 \times -0.0694$	$y = 0.0067 \times -0.075$	$y = 0.0053 \times -0.0528$	$y = 0.003 \times -0.0291$
	$r^2 = 0.9902$	$r^2 = 0.9478$	$r^2 = 0.9978$	$r^2 = 1.0$
	$x = 10.4$°C	$x = 11.2$°C	$x = 10.0$°C	$x = 9.7$°C
Mi	$y = 0.0063 \times -0.0517$	$y = 0.0068 \times -0.0717$	$y = 0.0072 \times -0.0821$	$y = 0.0032 \times -0.0288$
	$r^2 = 0.9291$	$r^2 = 0.9794$	$r^2 = 0.9684$	$r^2 = 0.9508$
	$x = 8.2$°C	$x = 10.5$°C	$x = 11.4$°C	$x = 9.0$°C
Mj	$y = 0.0063 \times -0.0592$	$y = 0.0055 \times -0.056$	$y = 0.0056 \times -0.0637$	$y = 0.0031 \times -0.0359$
	$r^2 = 0.9902$	$r^2 = 0.9782$	$r^2 = 0.968$	$r^2 = 0.988$
	$x = 9.4$°C	$x = 10.2$°C	$x = 11.4$°C	$x = 11.6$°C

Table 3. Base and optimum temperature for development of third (J3) and fourth (J4) stage juveniles, females (F), and egg-laying females (EF) of *Meloidogyne arenaria* race 1, *M. incognita* race 4, and *M. javanica* on okra (*Abelmoschus esculentus*) at a constant temperature.

	Temperature °C		
Developmental stage	*M. arenaria*	*M. incognita*	*M. javanica*
J3	10.4	8.2	9.4
J4	11.2	10.5	10.2
F	10.0	11.4	11.4
EF	9.7	9.0	11.6
Mean	10.3	9.8	10.6
Optimum temperature	27.0	30.0	30.0

At average temperatures of 16.2, 19.5, 25.0, and 30.0°C *M. incognita* completed the life cycle (from juvenile to juvenile) on tomato plants in 63, 44, 27, and 20 d, respectively (Ploeg and Maris, 1999). In their study, no reproduction occurred at 35.4°C; therefore, the authors concluded that the To for life cycle completion lies between 30 and 35.4°C. Although our study differed from their experiment in the temperatures evaluated, the host, and the ending point of observations, extrapolations from the regression line showed that at the same constant temperatures, *M. incognita* should start egg laying at 45, 30, 20 and 15 d, respectively. Differences in number of days can be attributed to additional days required for embryogenesis and eggs hatch. Contrary to their observations we observed females laying eggs at 35°C but the rate of development was almost constant at temperatures above 30°C as they reported. A decrease in the rate of development of *M. javanica* at temperatures between 27 and 31°C has been reported (Trudgill, 1995). The rate of development was almost the same and the life cycle was not completed on tomato plants at a constant temperature of 35°C (Trudgill, 1995).

Our results confirmed previous reports of the linear relationship between temperature and rate of development for these nematode species (Trudgill and Perry, 1994; Trudgill *et al*., 2005; Tyler, 1933). The reproduction of *M. incognita* and *M. javanica* on wheat was directly proportional to temperatures between 14 and 30°C and between 18 and 26°C, respectively (Roberts and Van Gundy, 1981). The rate of development of *M. javanica* increased linearly between 18 and 27°C and decreased at 30°C (Madulu and Trudgill, 1994). In their study, the life cycle from J2 to newly hatched J2, was completed in tomato plants after 67, 44, 32, 25, and 24 d at 18, 21, 24, 27, and 30°C, respectively. Our results are close to their findings considering that the ending point for our observations was the development of egg-laying females and not the development of newly hatched J2.

In our experiments, development at high temperatures (33 and 35°C) was adversely affected in all three species. As discussed above, the rate of development reached a plateau at temperatures greater than T_o, before decreasing as the maximum temperature was approached. At 33 and 35°C, the death of plants inoculated with *M. arenaria* at 23 DAI did not allow observations beyond this date; however, 70% and 30%, of females, respectively, began egg laying 19 DAI. Plants inoculated with *M. javanica* and *M. incognita* also were affected by high temperatures, 33 and 35°C but egg-laying females were observed 15 and 17 DAI, respectively, prior to death of the plants. Previous research showed that *M. incognita* laid eggs 13 DAI at 30 and 35°C (Davide and Triantaphyllou, 1967). Our results differ from that observed by Trudgill (1995) at 35°C. The difference in the host used for our experiment can explain partially these results. Although both tomato and okra are reported as good hosts for the common root-knot nematodes species, okra is a crop that can tolerate higher temperatures (Wilson, 1996). Reduced rates of food uptake will decrease rates of growth and egg laying and this might be associated with the condition of the host plant (Tyler, 1933). Anwar *et al*. (1994) reported that different host crops affected the rate of J2 penetration, development, and fecundity of *M. incognita* females.

Our results show the possibility of reproduction of these species at higher temperatures. It is important to consider the reproduction of root-knot nematodes at temperatures above 30°C since "resistance-breaking" races or pathotypes of root-knot nematodes have been reported at high soil temperatures (Noling, 2003; Roberts, 1992).

Base Temperature (T_b) for Development and Degree-days (DD)

The high r^2 (93-95%) of the regression analysis indicate reasonably accurate T_b estimate. The T_b of *M. arenaria* (10.3°C) determined in this study is similar for *in-vitro* egg development of *M. arenaria*, which had a linear relationship with temperature above 10.1 oC (Ferris *et al*., 1978). However, it is different than

reported for the development of *M. arenaria* on oriental melon under glasshouse conditions (Yeon *et al*., 2003). The authors determined a Tb of 12.2°C based on the lowest coefficient of variation among data sets of egg mass formation. It is likely that differences between our results are based on the methodology used. On their estimation of the Tb the regression line had only three data points and different temperatures were means of fluctuating soil temperature. Despite differences in the T_b for their population and the population evaluated in our study, at a mean soil temperature of 24.8°C, *M. arenaria* required 24 d to lay eggs and this is close to our results at a constant 24°C.

The methodology used in our experiment provided an accurate measurement at a series of points of precise constant temperatures as suggested by Trudgill *et al*., 2005. Plants were observed every other day and a possible delay of up to 48 h could have occurred in observations of the first specimen of each stage which could have affected the T_b estimation.

Also seasonal or diurnal variations in the environmental temperatures complicate the interaction between organisms and their environment and in some species there may be a lack of linearity close to T_b. It is important to consider that the T_b determined in our experiment is an average of different T_b values obtained for each developmental stage. There is evidence that differences in T_b for populations of a given species are a result of adaptation to specific environments (Milne and Du Plessis, 1967; Trudgill, 1995; Trudgill *et al*., 2005).

The T_b of *M. incognita* (9.8°C) determined in our study is similar to that reported by Vrain *et al*., (1978) and Ploeg and Maris, (1999). However, others have reported slightly higher value for development (Davide and Triantaphyllou, 1967)

Apparently, it is possible to observe different T_b values for different developmental life stages (Tyler, 1933). The T_b value for embryogenesis of *M. incognita* was different than juvenile development (Vrain and Barker, 1978). However, this T_b is almost identical to the T_b observed in our study for the development of *M. incognita* from J3 stage. Another example of different T_b for different developmental life stages is for *M. hapla*, which has a T_b for embryogenesis of 6.74°C (Vrain and Baker, 1978), whereas for juvenile development it is 8.8°C (Vrain *et al*., 1978). Tyler (1933) observed that apparently the T_b for maturity and egg-laying tends to be higher than for early stages of the life cycle which agrees with Vrain and Baker (1978). Similar to their report, in our study *M. incognita* and *M. javanica* showed lower T_b for early stages of development than for non-egg-laying and egg-laying females.

The T_b estimated for *M. javanica* in our study (10.6 °C) is different from 12.9°C, as previously reported (Madulu and Trudgill, 1994) for a population from Tanzania growing on tomato plants. *M. javanica* grown on tobacco under fluctuating temperatures had a lower T_b (7.5°C) for development from J2 to egg-laying females compared with our results (Milne and Du Plessis, 1967). They reported that the rate of development of *M. javanica* was less on lucerne than on several other crops. As discussed previously herein, the differences on T_b for the same species can be due to their adaptation to specific environments (Milne and Du Plessis, 1967; Trudgill, 1995; Trudgill *et al*., 2005) and host status differences (Trudgill, 1995). Differing from *M. arenaria* and *M. incognita,* similar T_b values for embryogenesis and juvenile development have been reported for *M. javanica*. The T_b value reported by Trudgill (1995) was 13.0°C and Tzortzakakis and Trudgill (2005) reported 12.66°C and 12.94°C for two different populations of *M. javanica.*

Although the three root-knot nematode species evaluated in this study showed different base temperatures for development their T_b values differed by less than 0.8°C. There is evidence that T_b values are used to indicate adaptations of poikilothermic organisms to different geographical regions (Trudgill *et al*., 2005). Also, for both nematodes and plants, the temperate species mainly have lower T_b than comparable tropical species (Trudgill, 1995). Our populations showed differences in T_b values with other populations from different geographical regions, grown on different hosts and in some cases the methodology used was not similar. These aspects need to be considered when comparisons are made.

Meloidogyne incognita, M. javanica, M. arenaria and *M. exigua* were described as thermophiles that do not have extended survival in soils below 10.0°C (Van Gundy, 1985). The order of tolerance to cold is *M. chitwoodi* >*M. hapla* >*M. incognita* >*M. arenaria* >*M. javanica*. If we compare the three *Meloidogyne* spp. in this study, their T_b values are in the same order of cold tolerance (9.8 >10.3 >10.6 for *M. incognita, M. arenaria*, and *M. javanica*, respectively). This supports the idea that species with lower T_b are adapted to cold climates and it will give them a competitive advantage for development at lower temperature whereas species with higher T_b values will compete better at higher temperatures.

Meloidogyne incognita required fewer degree-days than *M. arenaria* and *M. javanica* to reach all the developmental stages. In our experiment *M. incognita* required an average number of 300 $DD_{9.8}$ to complete a life cycle from J2 to egg-laying female, whereas, Ploeg and Maris (1999) estimated 400 $DD_{10.1}$ for development from J2 to J2 in tomato plants. They reported that J2 were first observed 20 DAI at 30°C. In our experiment, egg-laying females were first observed 15 DAI at 30°C. Because our end point was not J2, the additional 5 d reported by Ploeg and Maris were probably enough for egg development and hatching of J2. Tyler (1933) reported that egg development and hatching for Meloidogyne spp. took 9 d at 27°C. If we assume a T_b of 8.26 for embryogenesis (Vrain and Barker, 1978), in 5 d 109 DD would have been accumulated at 30°C and the DD values are even more similar (400 and 409 DD).

The DD required for *M. arenaria* (316 $DD_{10.3}$) for development from juvenile to egg-laying females were similar to that reported on four oriental melon crops grown in a glasshouse (Yeon *et al*., 2003). They reported an average of 313 DD accumulated above 12.2°C (T_b). In their experiment the start and end point for observations were the same as ours. Regardless of the difference in the T_b, the number of DD is similar to our results. We should expect that, since this population has a higher T_b than ours, the effective units accumulated daily would be less, and, therefore, a smaller DD value. Based on a linear relationship, there is an inverse relationship between Tb and DD associated with latitude and (or) habitat that adapts each species to its thermal environment (Trudgill *et al*., 2005).

Our results showed that *M. javanica* required an average of 334 $DD_{10.6}$ to develop from J2 to egg-laying females. Embryogenesis studies were not conducted in our research but if we add 138 DD for embryogenesis, it gives a total of 472 DD for one generation and this value is higher than that previously reported (Madulu and Trudgill, 1994; Trudgill, 1995).

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