*Plant Pathology* (2012) **61**, 1002–1010 Doi: 10.1111/j.1365-3059.2011.02576.x

# Thermal requirements for the embryonic development and life cycle of *Meloidogyne hispanica*

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The life cycle of a Portuguese *Meloidogyne hispanica* isolate on susceptible cv. Easypeel and resistant (*Mi-1.2* gene) cv. Rossol tomato plants was studied in growth chambers at constant temperatures (10–35°C). The development within the egg and hatching were compared to those of a Portuguese *M. arenaria* isolate. The base temperature was 10·11 and 8·31°C with 179·5 and 235·3 thermal units for *M. hispanica* and *M. arenaria*, respectively, suggesting better potential adaptation to low temperatures by *M. arenaria* than *M. hispanica*. No egg development occurred at 10 or 35°C. An increase in invasion of tomato roots by *M. hispanica* second-stage juveniles (J2s) was correlated with an increase in temperature on both tomato cultivars. Tomato cv. Rossol limited *M. hispanica* development at 20, 25 and 30°C, but not at 35°C, indicating that these high temperatures blocked the resistance mechanism provided by the *Mi-1.2* gene. At 15°C, J2s penetrated tomato cv. Rossol roots, but failed to develop and establish feeding sites. On tomato cv. Easypeel, nematode development and reproduction occurred at 20, 25 and 30°C, but at 20°C the life cycle was 1·5 and 2·0 times longer than at 25 and 30°C, respectively. No egg production was observed at 15°C. The results of this study showed that *M. hispanica* is most suited to soil temperatures around 25°C. Predicted climate change might favour the spread of this nematode species into southern Europe and northwards. The thermal requirements for *M. hispanica* development are analysed and compared with those of *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*.

*Keywords*: base temperature, ontogenesis, postembryonic development, root penetration, root-knot nematodes, thermal time

## Introduction

Data on the thermal time requirements for the development of plant-parasitic nematodes are very useful for predicting nematode geographical distributions, population dynamics and resulting crop yield losses.

Root-knot nematodes (RKN) are poikilothermic organisms and their development is usually dependent on temperature (Trudgill *et al.*, 2005). Since the first studies on the linear relationship between thermal time and the rate of nematode development which analysed the effects of the temperature on the life cycle of an unknown *Meloidogyne* species (Tyler, 1933), extensive research has shown that rates of RKN embryonic and postembryonic development and life cycle are strongly influenced by temperature and vary with the species of *Meloidogyne*. The linear relationship between temperature and the rate of development allows an estimation of the lower threshold temperature value and the thermal environment to which these nematodes are adapted and also the thermal constant that shows relative rate of development (Lahtinen *et al.*, 1988; Madulu & Trudgill, 1994; Zhang & Schmitt, 1995; Ploeg & Maris, 1999; Yeon *et al.*, 2003; Charchar & Santo, 2009; Strajnar *et al.*, 2011).

The higher values of lower threshold temperature are usually associated with tropical species and the lower values with temperate ones. The optimum temperature range for *M. hapla* and some other cold-climate-adapted species is 15–25°C, whereas for *M. javanica* and other warm-climate species it is 25–30°C. Above 40°C and below 5°C very little activity or no development occurs in any *Meloidogyne* species (Taylor & Sasser, 1978).

*Meloidogyne hispanica* is one of the lesser-known species of RKN, which parasitizes several economically important plant species and cultivars from dicotyledons and monocotyledons in the families Alliaceae, Apiaceae, Asteraceae, Brassicaceae, Caryophyllaceae, Chenopodiaceae, Cucurbitaceae, Fabaceae, Poaceae and Solanaceae (Maleita *et al.*, 2011, 2012). This species has been recorded in all continents, and in Portugal was found alone or in mixed populations of RKN in different regions of the centre and south, associated with several cultivated plants (Abrantes *et al.*, 2008; Conceição *et al.*, 2009; Maleita *et al.*, 2011). However, there is no information on the thermal requirements for development of *M. hispanica*.

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*Published online 19 January 2012*





Plant Pathology

The present research was undertaken to evaluate the effects of temperature on the embryonic development of *M. hispanica* and *M. arenaria*, and also on the penetration and postembryonic development of *M. hispanica* on tomato plants of cvs Easypeel and Rossol. The duration of ontogenetic development at various temperatures was expressed in days and also in degree-days (DD) or thermal units to remove the time dependency of temperature changes. The results are compared to data published for *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica*. The possible effects of climate change on the aggressiveness and spatial distribution of *M. hispanica* are analysed.

## Materials and methods

### Nematode isolates

An isolate of *M. hispanica* obtained from fig (*Ficus carica*) roots in Odeceixe, Faro, Portugal, and one of *M. arenaria* obtained from tomato (*Solanum lycopersicum*) roots in Coimbra, Portugal, were reared on tomato cv. Easypeel and kept at 25 ± 2°C in a growth chamber.

### Embryogenesis

The *M. arenaria* isolate was included in this experiment for comparison. Egg masses of both RKN isolates, containing a relatively large proportion of eggs in their earliest stages of development, were collected from infected tomato roots. Thirty eggs (1- or 2-cell stage) of each isolate/treatment were carefully selected from the egg masses, washed in sterilized distilled water and transferred into sterilized Petri dishes containing 2% water agar. The eggs were then incubated at 10, 15, 20, 25, 30 or 35°C and observed every 6 h in the first day and then every 24 h until hatching of second-stage juveniles (J2s). The developmental stages considered were two, four, six and eight cells, multicellular, gastrula, embryo, first-stage juvenile (J1), J2 within the egg and hatched J2.

### Penetration, postembryonic development and life cycle of *M. hispanica*

The tomato plants used in these experiments were cv. Easypeel, highly susceptible, and cv. Rossol, which has the RKN resistance *Mi-1.2* gene (Maleita *et al.*, 2011). Tomato seedlings, germinated at 26–27°C on moist filter paper in Petri dishes, were transplanted singly into 5·5-cm-diameter plastic pots containing 100 $cm^3$ steam-sterilized mixture of sandy loam soil and sand (1:3).

Three-week-old Easypeel and Rossol seedlings were inoculated with freshly hatched J2s of *M. hispanica* obtained from egg masses (150 J2s per seedling). The plants were transferred to their respective growth chambers (15, 20, 25, 30 and 35°C) with a 12-h photoperiod. Nematode penetration (four seedlings per treatment) was monitored at 3, 6, 9, 12, 18 and 24 h after inoculation (HAI) and every 12 h in the following 2 days. At 15°C, nematode root penetration was monitored at the same intervals for 7 days. Roots were washed and stained with acid fuchsin (Byrd *et al.*, 1983) and the number of J2s inside the roots was recorded.

To study postembryonic development, the seedlings were removed 3/7 days after inoculation (DAI) and the root systems washed gently to remove all J2s from the root surface. Single seedlings were transplanted into 14·5-cm-diameter Petri dishes with a hole laterally, containing a mixture of sandy loam soil and sand (1:1), and maintained vertically. Seedlings grown at 15, 20, 25, 30 and 35°C (four seedlings per treatment) were harvested daily between 3/7–10 days; at 2-day intervals for 20 days and at 5-day intervals thereafter until the end of the experiment. Roots were washed and stained with acid fuchsin (Byrd *et al.*, 1983) and nematodes were removed from the root tissues, mounted in glycerin and observed microscopically. The various developmental stages of the nematode (vermiform J2; swollen and sexually undifferentiated J2; early swollen J2 differentiating into a female/male; J2 female/male shortly before moulting; fourth-stage female/male juvenile; and adult female/male shortly after fourth moult) were identified on the basis of body shape and gonad development (Triantaphyllou & Hirschmann, 1960). As soon as egg masses were produced, they were removed and placed in glass blocks containing tap water and maintained at the same temperature to detect the first newly hatched J2s. The life cycle was considered complete when the first hatched J2s were found in the newly formed egg masses.

### Thermal time requirements

The recorded length of time (days) taken at different temperatures for the stages of ontogenetic development (embryogenesis, development within the egg until J2 hatching, postembryonic development and life cycle completion) was expressed as the reciprocal of this time ($days^{-1}$) to obtain the development rate ($R$) at each temperature. The value of the base temperature for development (Tb, $R = 0$) that reflects the thermal environment (Te) to which the nematode is adapted was obtained by linear regression of $R$ on the temperature and extrapolating the regression line to the abscissa. The thermal constant (S) which represents the number of heat units above the Tb required for completion of the different phases of the ontogenetic development was obtained by the reciprocal of the temperature coefficient (slope) of the regression and at each temperature according to: $S = (Te - Tb)/R$ expressed in degree (°C) days (DD) or thermal units (Trudgill, 1995; Trudgill *et al.*, 2005).

The regression lines expressing the relationship between temperature and rate of development of *M. hispanica* were compared with those reported for temperate climate RKNs *M. arenaria*, *M. hapla, M. incognita* and *M. javanica* (Bird, 1972; Ferris *et al.*, 1978; Inserra *et al.*, 1983; Lahtinen *et al.*, 1988; Madulu & Trudgill, 1994; Ploeg & Maris, 1999; Yeon *et al.*, 2003; Tzortzakakis & Trudgill, 2005; Davila Negrón, 2006).

### Data analysis

Statistical analysis was performed using STATSOFT STATISTICA version 7 for Windows. Data for the effect of temperature and tomato cultivars on nematode penetration were checked for evidence of a normal distribution using the Kolmogorov–Smirnov test, and for variance homogeneity using Levene's test. Transformation of the data was performed using the formula $[\sqrt{(x + 0{\cdot}5)}]$. Following ANOVA, treatment means within each factor were compared using Fisher's LSD test ($P < 0{\cdot}05$).

## Results

### Embryogenesis

The relationship between temperature and nematode embryonic development for *M. hispanica* was similar to that of *M. arenaria*. At 10 and 35°C, no embryonic development was observed in either species. At 15, 20, 25 and 30°C, the rate of egg development increased with temperature, but more slowly in *M. arenaria* than in *M. hispanica* (Figs 1 and 2).

The optimal temperatures for egg development in both species were 25 and 30°C. At the temperature range 15–30°C, the egg developmental phases from first cleavage to embryo formation and differentiation into the J1 were slightly shorter for *M. hispanica* than for *M. arenaria*. A similar trend was also observed for the duration of postembryonic development within the egg from the J1 until its moult into a J2 and the hatching process time of the J2 (Figs 1 and 2).

The thermal requirement analysis of the embryonic development (until J1) rate yielded a positive linear function with temperature. A linear regression model was obtained for both species and was used to determine Tb for the studied temperatures (Fig. 2). The Tb for the embryonic development of *M. hispanica* was 11·49°C and that of *M. arenaria* 10·24°C, with S values of 76·92 and 100 DD, respectively. Thermal constants were estimated for all temperatures (Table 1). For the embryonic and postembryonic development within the egg including hatching, the Tb of *M. hispanica* was 10·11°C ($y = 0{\cdot}0056x - 0{\cdot}0563$, $R^2 = 0{\cdot}9979$) and 8·31°C for *M. arenaria* ($y = 0{\cdot}0043x - 0{\cdot}0353$, $R^2 = 0{\cdot}9934$) with S of 179·5 and 235·3 DD, respectively (Fig. 5a).

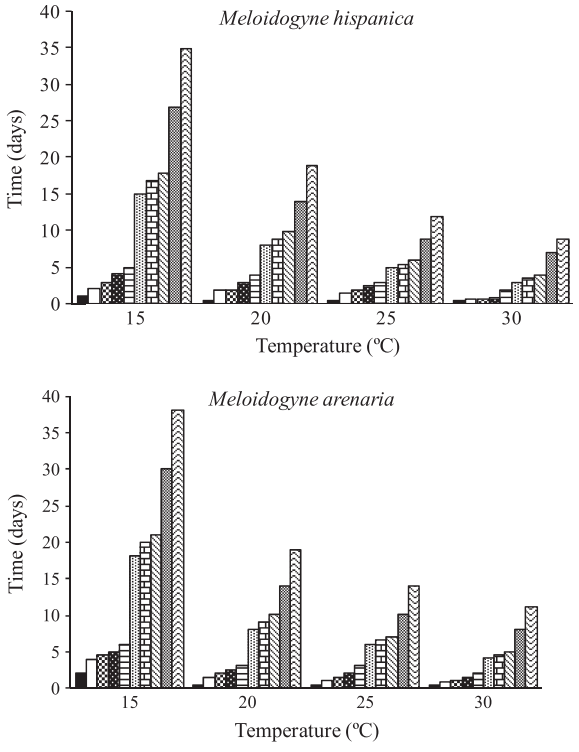


Figure 1 Embryogenesis and postembryonic development within the egg until hatching of *Meloidogyne hispanica* and *M. arenaria* at four temperatures. Duration, in days, of the two- (■), four- (□), six- (▩) and eight-cells stages (▩), multicellular stage (▤), gastrula stage (▦), embryo (▩), first-stage juvenile (▨), second-stage juvenile within the egg (■) and hatched juvenile (▨).

### Penetration, postembryonic development and life cycle of *M. hispanica*

*Penetration*

Eighteen HAI, J2s were found in the root tissues of both tomato cultivars at 20–35°C, suggesting successful root penetration. However, the number of J2s inside the roots was significantly different between temperatures and between the tomato cultivars at certain temperatures (Table 2). At 15°C, root invasion by J2s was delayed in both cultivars compared to that observed at higher temperatures. The delay was more accentuated in the susceptible cv. Easypeel. *Meloidogyne hispanica* was able to penetrate the roots of the two tomato cultivars at all temperature regimes regardless of the presence or absence of genes conferring resistance to RKN. Details of the root penetration by the J2s on the two cultivars at different times and temperatures are shown in Table 2.

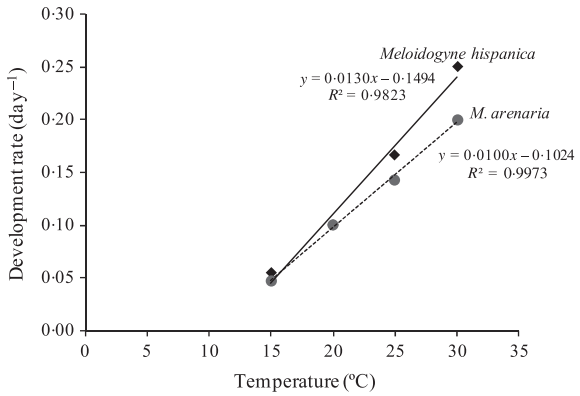


Figure 2 Relationship between embryonic development rate and temperature for *Meloidogyne hispanica* and *M. arenaria*. Rate of development for each temperature = 1/total of days required to occur for development. *R* = predicted rate of embryonic development with the limits $15 \leq T \leq 30$°C.

Table 1 Thermal constants for one- or two-cell stage to first-stage juvenile development of *Meloidogyne hispanica* and *M. arenaria* at four constant temperatures, when considering the base temperature as 11·49°C for *M. hispanica* and 10·24°C for *M. arenaria*

| Temperature | | *M. hispanica* | | | *M. arenaria* | | |
|---|---|---|---|---|---|---|---|
| | | Development | Thermal constant (S) | | Development | Thermal constant (S) | |
| °C | °F | period (days) | °C | °F | period (days) | °C | °F |
| 10 | 50 | ND[a] | — | — | ND | — | — |
| 15 | 59 | 18 | 63·2 | 110·5 | 21 | 100·0 | 188·2 |
| 20 | 68 | 10 | 85·1 | 151·4 | 10 | 97·6 | 179·6 |
| 25 | 77 | 6 | 81·1 | 144·8 | 7 | 103·3 | 188·7 |
| 30 | 86 | 4 | 74·0 | 132·6 | 5 | 98·8 | 179·8 |
| 35 | 95 | ND | — | — | ND | — | — |

[a]No data, no development occurred.

*Postembryonic development and life cycle*

The development of J2s in roots was influenced by tomato cultivar and temperature. The effect of temperature regime on *M. hispanica* development is presented as the percentage of each developmental stage found in the roots at each observation (Figs 3 and 4).

At 15°C, an unfavourable temperature for tomato growth, the plants were small and root development was slow. At 26 DAI, only vermiform J2s were found in roots of cv. Easypeel. On this cultivar, the females of these J2s developed and attained egg-laying adult stage at 75 DAI (Fig. 3a). On cv. Rossol, the J2s were able to invade the root tissues, but did not develop. At 24 DAI, all the J2s were associated with necrotic cells (data not show), which prevented the establishment of feeding sites and nematode development. Necrotic cells were also observed 5 DAI, at 20, 25 and 30°C, with fewer at 30°C than at 20 or 25°C (Fig. 4a–c).

At 20°C, the entire life cycle (from J2 to J2) was completed in approximately 53 and 62 days on cvs Easypeel and Rossol, respectively. Adult females were detected at 26 DAI for both tomato cultivars (Figs 3b and 4a). Deposition of gelatinous matrix was recorded at 45/50 DAI and eggs were laid at 45 DAI on tomato cv. Easypeel and 5–10 days later on cv. Rossol.

The duration of the life cycle of *M. hispanica* on cv. Easypeel at 25°C was shorter than at 20°C (35 vs. 53 days). The development of the juveniles required 10 days and adult females were first observed at 12 DAI (Fig. 3c). The gelatinous matrix, eggs and newly hatched J2s were observed 22, 24 and 35 DAI, respectively. At this temperature (25°C), there was no *M. hispanica* reproduction on the resistant cv. Rossol. More than 90% of the juveniles were associated with necrotic cells and only a few females were found (Fig. 4b).

Development of *M. hispanica* J2s on both tomato cultivars was similar at 30 and 35°C. Adult females and gelatinous matrix were observed 10 and 16–18 DAI, respectively, with the presence of some necrotic tissue, on tomato cv. Rossol at 30°C. At 30 and 35°C egg production was observed 18–20 and 22 DAI on cvs Easypeel and Rossol, respectively. However, J2s from these eggs hatched only at 30°C on both cultivars 26–27 DAI. No hatching occurred at 35°C (Figs 3d,e and 4c,d).

Thermal requirements for postembryonic development and life cycle completion were determined on the basis of the results for tomato cv. Easypeel. There was a linear relationship between temperature and rate of nematode postembryonic development (from J2 to adult stage, $y = 0{\cdot}0061x - 0{\cdot}0784$, $R^2 = 0{\cdot}9722$) and life cycle completion (from J2 to J2, $y = 0{\cdot}0019x - 0{\cdot}0198$, $R^2 = 0{\cdot}9999$, Fig. 5b). The Tb for postembryonic development and the life cycle of *M. hispanica* was 12·85 and 10·22°C, respectively, with S of 163·93 and 515·46 DD, respectively. Thermal constants for life cycle were estimated for all the temperatures (Table 3).

## Discussion

The results of this study indicate that *M. hispanica* is a temperate-climate species like *M. arenaria*, *M. incognita* and *M. javanica*. This species developed poorly at 15°C on tomato. However, the possibility cannot be excluded that *M. hispanica* would invade and reproduce at this temperature on hosts (Alliaceae and Poaceae) better adapted to low temperatures than tomato, because egg development and root invasion occurred at 15°C.

The thermal optimum for *M. hispanica* and *M. arenaria* embryogenesis and development within the egg, until hatching, lies between 25 and 30°C, and the minimal temperature under the conditions of this experiment was 15°C. The thermal optimum for *M. javanica* embryogenesis also lies between 25 and 30°C, the rate of development being extremely slow at 15°C (Bird, 1972). By contrast, although very slow, development within the eggs until emergence of J2s occurred at 10°C for the temperate RKN species *M. hapla* (95–97 days) (Inserra *et al.*, 1983). The calculated Tb values obtained for embryogenesis were 11·49 and 10·24°C for *M. hispanica* and *M. arenaria*, respectively. These results agree with those of Davila Negrón (2006) and Ferris *et al.* (1978), who obtained Tb values of 10·3 and 10·11°C for *M. arenaria* life cycle and embryonic development, respectively. The regression lines of development within the egg until hatching for *M. hispanica* and *M. arenaria* intersected at 15·9°C (Fig. 5a). The better adaptation and competitiveness of *M. arenaria* than *M. hispanica* at low temperatures, indicated by lower Tb for both embryogenesis and

**Table 2** Number of second-stage juveniles (J2s) of *Meloidogyne hispanica* inside the roots of tomato cultivars Easypeel and Rossol, after inoculation with 150 J2s, under five temperatures (15, 20, 25, 30 and 35°C)

| Hours after inoculation | Temperature | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 15°C | | 20°C | | 25°C | | 30°C | | 35°C | |
| | Easypeel | Rossol | Easypeel | Rossol | Easypeel | Rossol | Easypeel | Rossol | Easypeel | Rossol |
| 3 | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·3 ± 0·5b |
| 6 | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·0 ± 0·0a | 0·8 ± 1·0a | 0·5 ± 0·6a | 3·5 ± 2·9b |
| 9 | 0·0 ± 0·0ab | 0·0 ± 0·0ab | 0·0 ± 0·0ab | 0·0 ± 0·0ab | 0·0 ± 0·0ab | 1·8 ± 2·1a–d | 3·5 ± 3·1b–f | 5·5 ± 2·9cef | 2·5 ± 3·0b–df | 5·3 ± 3·8c–f |
| 12 | 0·0 ± 0·0a–c | 0·0 ± 0·0a–c | 0·0 ± 0·0a–c | 2·8 ± 2·2bdf | 0·5 ± 1·0a–c | 1·3 ± 1·3a–cf | 6·8 ± 4·5bd–f | 12·8 ± 5·3de | 6·3 ± 7·5b–df | 13·3 ± 6·7de |
| 18 | 0·0 ± 0·0a | 0·0 ± 0·0a | 1·5 ± 1·7a | 10·0 ± 4·2bc | 8·5 ± 5·8bc | 11·0 ± 6·2bce | 14·8 ± 10·3bce | 26·5 ± 5·3d | 20·5 ± 10·1c–e | 59·8 ± 16·5f |
| 24 | 0·0 ± 0·0a | 0·5 ± 1·0a | 6·5 ± 2·1bc | 17·5 ± 19·7b–d | 16·3 ± 6·8b–d | 24·5 ± 9·3cd | 19·0 ± 10·4cd | 51·5 ± 5·1e | 23·8 ± 13·4cd | 65·8 ± 24·1e |
| 36 | 0·0 ± 0·0a | 9·0 ± 3·0b–d | 15·0 ± 5·5b–e | 21·0 ± 10·0b–f | 58·3 ± 23·9gh | 57·5 ± 17·4gh | 36·0 ± 15·3d–f | 59·8 ± 7·3g–i | 28·0 ± 17·9c–f | 84·3 ± 7·8hi |
| 48 | 0·0 ± 0·0a | 9·8 ± 4·35b | 40·3 ± 7·4de | 26·0 ± 15·3e | 67·0 ± 16·8c | 65·3 ± 15·0c | 66·5 ± 16·5c | 71·3 ± 18·4c | 30·3 ± 5·6de | 83·8 ± 4·6c |
| 60 | 0·0 ± 0·0c | 19·0 ± 4·3a | 61·8 ± 18·6b | 31·8 ± 3·4a | 80·8 ± 12·2b | 74·3 ± 14·5b | 73·5 ± 34·0b | 66·8 ± 11·8b | 34·5 ± 16·1a | 68·5 ± 16·3b |
| 72 | 0·5 ± 1·0a | 12·8 ± 7·6b | 69·8 ± 7·3c–g | 35·5 ± 7·1h | 83·3 ± 15·7c–f | 65·5 ± 12·5ce–g | 75·0 ± 10·2c–g | 59·8 ± 12·0cfg | 34·8 ± 2·1h | 85·3 ± 9·2c–e |
| 96 | 10·8 ± 6·2a | 14·8 ± 4·1a | — | — | — | — | — | — | — | — |
| 120 | 5·3 ± 4·99a | 15·8 ± 9·8a | — | — | — | — | — | — | — | — |
| 144 | 17·0 ± 10·6a | 31·8 ± 4·0a | — | — | — | — | — | — | — | — |
| 168 | 33·0 ± 7·35a | 28·0 ± 7·0a | — | — | — | — | — | — | — | — |

Data are means of four replicates ± standard deviation. Means in each row followed by same letter do not differ significantly at $P > 0{\cdot}05$, according to Fisher's LSD test.

development within the egg until J2 hatching (10·24 and 8·31°C vs. 11·49 and 10·11°C, respectively), was confirmed by comparing the intersections of regression lines for the development of *M. incognita* (16·75°C) and *M. javanica* (20·04°C) with the values obtained for *M. hispanica*. This comparison shows that the embryonic and postembryonic development in the eggs of *M. arenaria* and *M. hispanica* are faster at lower temperatures, giving a competitive advantage (Fig. 5a). In mixed populations of *M. hispanica* and *M. javanica* or *M. incognita*, *M. hispanica* J2s will invade the roots earlier than the J2s of the other two species, although higher temperatures are more suitable for postembryonic development of *M. javanica* and *M. incognita* (Fig. 5a,b).

Root invasion by *M. hispanica* J2s was influenced by temperature and was faster at 35°C (3 HAI) than at 15°C (72 HAI), which is the minimum temperature for nematode root penetration. Similar results were obtained for penetration by *M. hapla* J2s on alfalfa, with penetration greatest at 24 and 28°C, slightly lower at 20 and 32°C and lowest at 12 and 16°C (Griffin & Elgin, 1977). Other studies conducted with Florida isolates of the closely related species *M. arenaria*, *M. incognita* and *M. javanica* showed that J2s were not able to colonize okra roots at temperatures below 18°C (Davila Negrón, 2006). In the present study, at 3 DAI there was no significant difference between the numbers of J2s that penetrated the roots of the cultivars at 25 and 30°C, but in tomato cv. Rossol the number was significantly higher at 15 and 35°C and lower at 20°C. These results differ from those of Griffin & Elgin (1977), who stated that penetration of *M. hapla* J2s was not statistically different between resistant and susceptible plants.

The development of *M. hispanica* from J2 to J2 was influenced by temperature and tomato cultivar. The base temperature required for life cycle completion in tomato cv. Easypeel (10·22°C) is analogous to that estimated for egg development until hatching of the J2s (10·11°C), in agreement with the findings of Trudgill (1995) for Tb values calculated for the development within the egg and the entire life cycle.

*Meloidogyne hispanica* invaded the root system of tomato cv. Easypeel and reproduced at a faster rate at 30°C than at 25 and 20°C. Although *M. hispanica* J2s were able to penetrate roots at 15°C, they did not develop within 80 days, probably because of the unfavourable conditions for root development, which reduced the availability of the nutrients necessary for nematode development. The effect of low temperature on nematode physiology cannot be excluded (Cardin, 1979). No hatching occurred at 35°C, indicating that this temperature limited life cycle completion. These results were similar to those obtained for *M. javanica* and *M. incognita*, which did not develop at 34·7 and 35·4°C, respectively (Trudgill, 1995; Ploeg & Maris, 1999). Comparing available data on the effects of temperature on the duration of *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* life cycles with those obtained for *M. hispanica*, the Tb for *M. hispanica* (10·22°C) lies between values for *M. incog-*

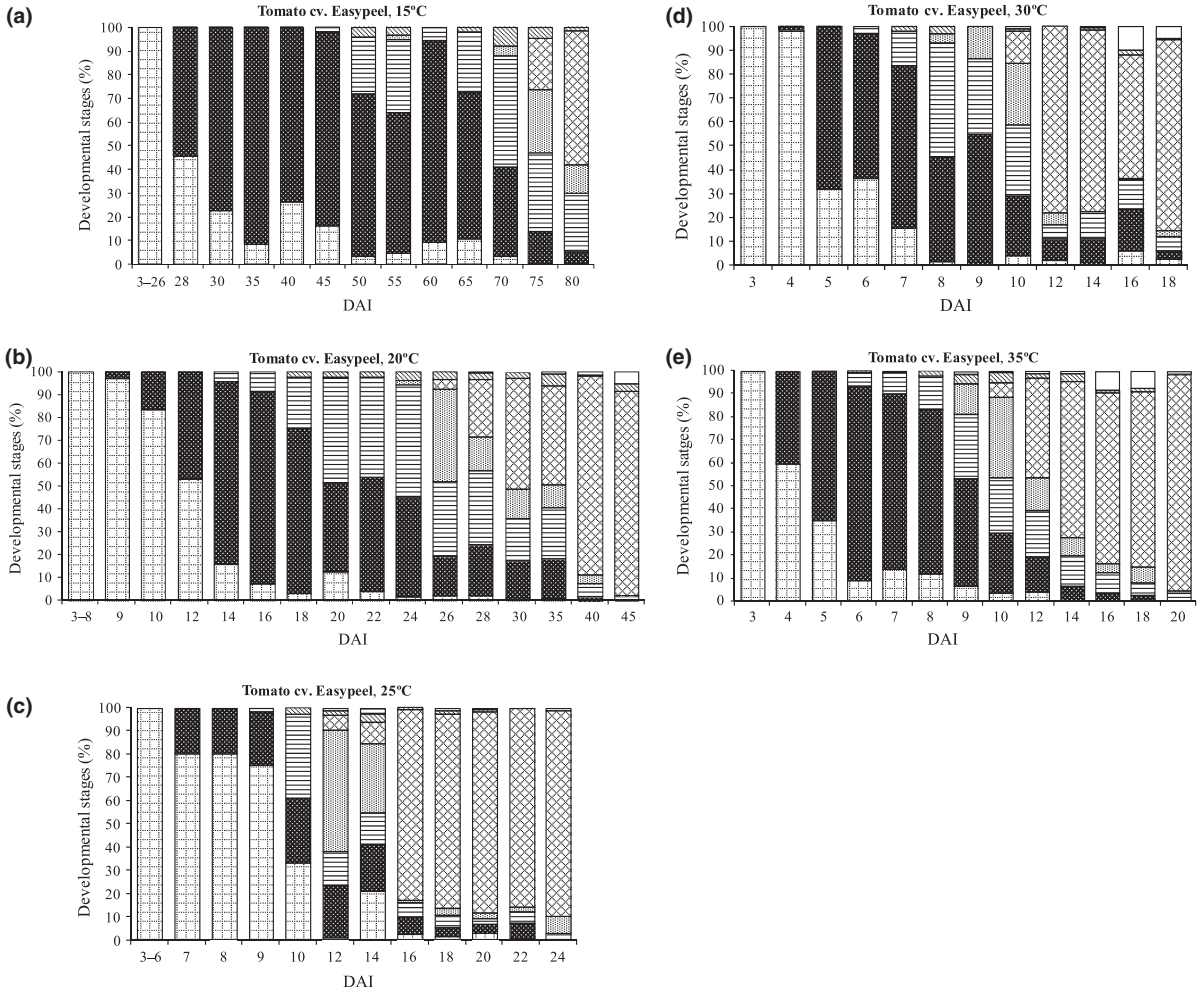


Figure 3 Postembryonic development of *Meloidogyne hispanica* in roots of tomato cv. Easypeel from 3 days after inoculation (DAI) until deposition of eggs in gelatinous matrix, at 15°C (a), 20°C (b), 25°C (c), 30°C (d) and 35°C (e). (▦) Vermiform second-stage juvenile (J2); (▩) swollen and sexually undifferentiated J2; from earliest sexually differentiated J2 until fourth-stage female (▤) and male (▧) juvenile; adult female (▣) and male (□) shortly after fourth moult; (▣) adult female (based on Triantaphyllou & Hirschmann, 1960); data are means of four replicates).

*nita* (Tb 10·10°C and S 400 DD) and *M. arenaria* (Tb 12·20°C and S 313 DD). However, the S value for *M. hispanica* (515·46 DD) lies between those of *M. incognita* (400 DD) and *M. hapla* (553 DD) (Lahtinen *et al.*, 1988; Madulu & Trudgill, 1994; Ploeg & Maris, 1999; Yeon *et al.*, 2003). Analysis of the relationship between the rate of development and temperature for these species revealed that, at temperatures below 13·89 and 18·78°C, *M. hispanica* has a shorter life cycle than *M. arenaria* and *M. javanica*, respectively, and that *M. hapla* and *M. incognita* have shorter life cycles than *M. hispanica* at every temperature studied (Fig. 5b).

*Meloidogyne hispanica* development on the resistant tomato cv. Rossol was seriously compromised and affected by temperature. Following the initial nematode penetration at temperatures below 35°C, several J2s failed to establish and develop in roots of this cultivar. At temperatures between 15 and 30°C from 5 DAI, the necrotic cells observed prevented feeding site establishment and further development. At 25°C no reproduction occurred. However, the life cycle was completed in 62 days at 20°C and the proportion of adult females recorded on roots was significantly lower than on cv. Easypeel. Increasing the level of initial inoculum, *M. hispanica* is a RKN able to overcome the resistance mechanism conferred by the gene *Mi-1.2*, but the resistant cultivar retains some capability to limit nematode development and reproduction (Maleita *et al.*, 2011). At 30 and 35°C *M. hispanica* development was similar on both tomato cultivars because the tomato *Mi-1.2* gene only confers resistance at soil temperatures below 28°C for some *Meloidogyne* spp. (Williamson, 1998). The ability of *M. hispanica* to develop and reproduce at 30°C on the resistant tomato cv. Rossol was the result of the

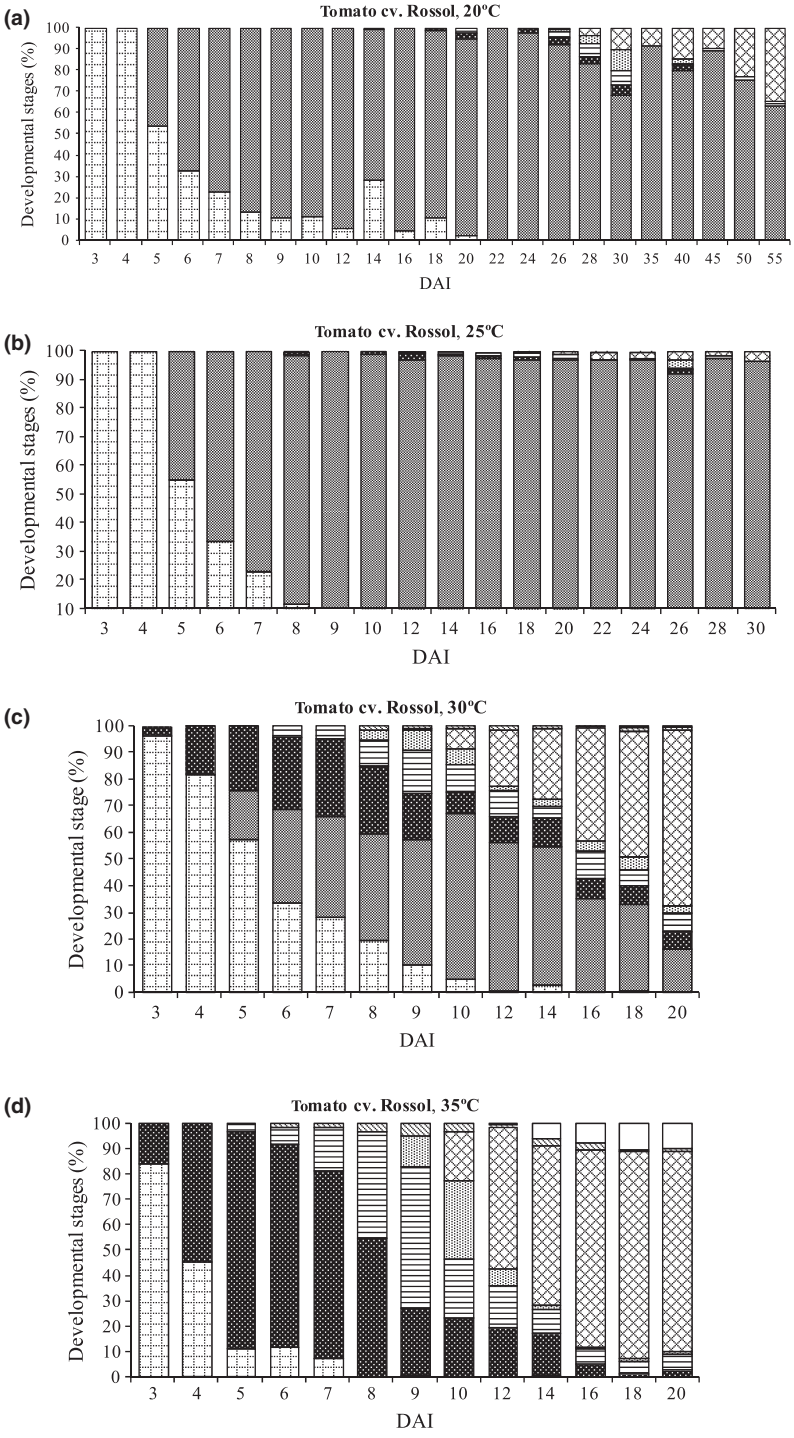


Figure 4 Postembryonic development of *Meloidogyne hispanica* in roots of tomato cv. Rossol from 3 days after inoculation (DAI) until deposition of eggs in gelatinous matrix, at 20°C (a), 25°C (b), 30°C (c) and 35°C (d). (▦) Vermiform second-stage juvenile (J2); (▩) J2 associated with necrosed cells; (▩) swollen and sexually undifferentiated J2; from earliest sexually differentiated J2 until fourth-stage female (▤) and male (▧) juvenile; adult female (▦) and male (□) shortly after fourth moult; (▨) adult female (based on Triantaphyllou & Hirschmann, 1960); data are means of four replicates.

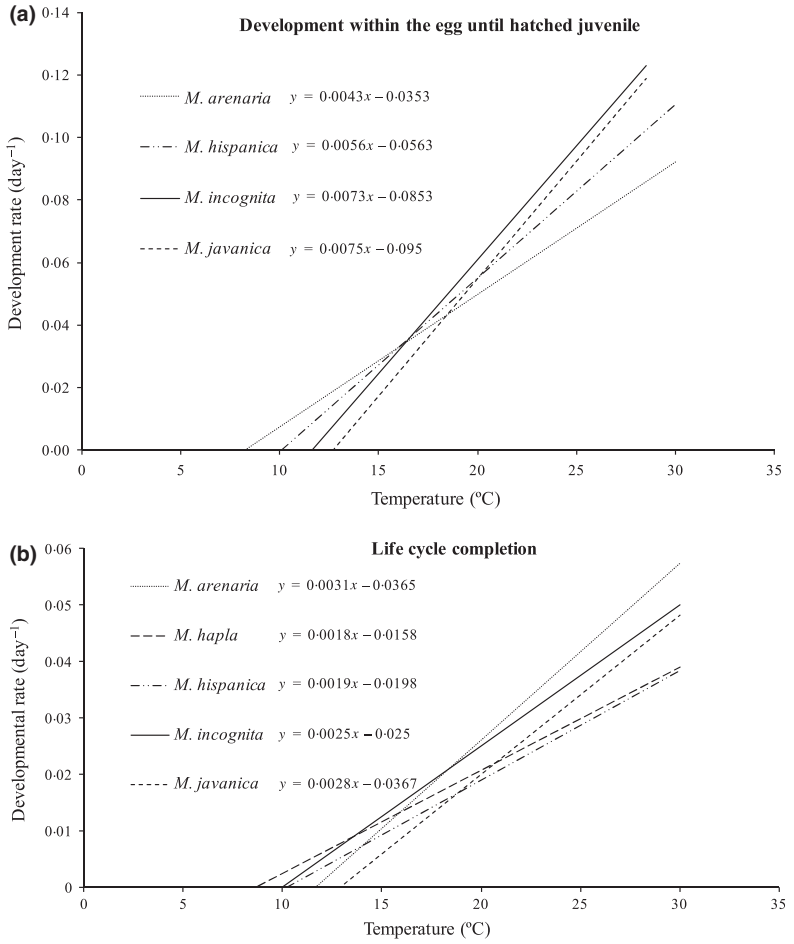


Figure 5 Relationship between *Meloidogyne* species development rate and temperature. (a) Regression lines for *M. incognita* and *M. javanica* development within the egg until hatched juvenile, calculated from Tzortzakakis & Trudgill (2005). (b) Regression lines for *M. arenaria*, *M. hapla*, *M. incognita* and *M. javanica* life cycle completion (from J2 to J2) calculated from Lahtinen *et al.* (1988), Madulu & Trudgill (1994), Ploeg & Maris (1999) and Yeon *et al.* (2003).

Table 3 Thermal constants for the life cycle of *Meloidogyne hispanica* on tomato cv. Easypeel at three constant temperatures, when considering the base temperature as 10·22ºC

| Temperature | | Development period (days) | Thermal constant (S) | |
|---|---|---|---|---|
| °C | °F | | °C | °F |
| 20 | 68 | 53 | 513·5 | 928·2 |
| 25 | 77 | 35 | 517·3 | 933·8 |
| 30 | 86 | 26 | 514·3 | 927·7 |

inactivation of the resistance mechanism conferred by the gene *Mi-1.2* under these high-temperature conditions.

According to these results, *M. hispanica* is a species most suited to soil temperatures around 25°C. The Tb and S data, combined with information on soil temperatures and cropping practices indicate that predicted climate changes would favour *M. hispanica* spreading into southern Europe and moving northwards as a temperate-climate RKN.

## Acknowledgements

This research was supported by FEDER funds through the *Programa Operacional Factores de Competitividade – COMPETE* and by national funds through FCT – *Fundação para a Ciência e a Tecnologia* – under the project PTDC/AGR-AAM/103873/2008. CM was supported by a FCT-MCTES grant (SFRH/BD/24275/2005) supported by QREN funds through the *Programa Operacional Potencial Humano*. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

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