Temperature Effects on Development and Reproduction of *Heterodera cajani* on Pigeonpea

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**Abstract:** Effects of constant and fluctuating temperature on development and reproduction of *Heterodera cajani* were studied on pigeonpea cv. ICPL 87 in growth chambers at 10, 15, 20, 25, and 30 C and in a greenhouse fluctuating between 22.2 and 37.8 C. Nematode penetration was greatest (*P* = 0.001) in roots at 25 C; there was no penetration at 10 C. The basal threshold temperature for development was calculated to be 11 C. Completion of one *H. cajani* generation required 17, 28, 35, and 66 days (323, 392, 315, and 264 degree-days) at 30, 25, 20, and 15 C, respectively, and 19 days (356 degree-days) at a fluctuating temperature. Survival was greater at 20 and 25 C than at 15 and 30 C. The greatest (*P* = 0.05) number of females (17.9 females per root) were produced at 25 C, compared with 13.2 at 20 C, 7.9 at 30 C, and 2.5 females at 15 C. Nematode reproduction was 1.6 to 7.1 times greater at 25 C than at other temperatures. Emergence of juveniles from egg sacs and cysts was greater at 25 and 30 C than at 15 and 20 C. Equations were developed to predict nematode development rate, cumulative juvenile emergence from egg sacs and cysts, and population increases as influenced by temperature.

**Key words:** Basal threshold temperature, *Cajanus cajan*, degree-day, development rate, *Heterodera cajani*, nematode reproduction.

The development rate for poikilothermic organisms, including plant-parasitic nematodes, is temperature dependent and can be linearly related to temperature within lower and upper threshold limits (1). This relationship between development rate and temperature is described by physiological-time based models (1,2,4,6,7,21,24). The duration of development is calculated by computing the number of thermal units (degree-days [DD] or degree-hours) accumulated. Temperature requirements for development vary with the nematode species and are useful for predicting nematode multiplication and survival (4,12).

The pigeonpea cyst nematode, *Heterodera cajani* Koshy, is the most important and widely distributed nematode pest of pigeonpea (*Cajanus cajan* (L.) Millsp.) in India, where more than 90% of the world's pigeonpea crop is cultivated (9,17,18). An initial population density of three juveniles (J2) per cm² soil causes 25% suppression in biomass of pigeonpea genotype ICP 2376 in the greenhouse (19).

Pigeonpea cyst nematode completes one generation in 16 days at 29 C, whereas at lower temperatures (10–25 C), it requires between 45 and 80 days (9). Temperature greatly influences emergence of *H. cajani* J2 from cysts and egg sacs (10,18), which occurs between 20–35 C with an optimum temperature of 28 C (18). Slight deviations in temperature from the optimum significantly affect emergence. Information on thermal requirements of *H. cajani* for development and reproduction is limited. Therefore, the influence of temperature on penetration of *H. cajani* J2 into pigeonpea roots, rate of development, survival, reproduction, and emergence of J2 from egg sacs and cysts was investigated.

**Materials and Methods**

Experiments were conducted in growth chambers and in a greenhouse at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India. The culture of *H. cajani* was obtained from a Vertisol (black cotton soil) (44% sand, 16% silt, 40% clay; 0.54% organic carbon) field at the ICRISAT research farm and reared on pigeonpea cv. ICPL 87 in 20-cm-d plastic pots in a greenhouse. Nematode inoculum was obtained by extracting cysts and egg sacs from soil and roots by Cobb's decant-
ing and sieving technique (3). Cysts were then incubated at 25 C to collect emerged J2 in distilled water in 6-cm-d plastic petri dishes. The J2 were collected daily, rinsed with distilled water, and stored at 15 C in glass flasks. Inoculum was not stored longer than 2 days.

**Nematode development at constant temperature:** Six seeds of pigeonpea cv. ICPL 87 were sown in 10-cm-d plastic pots containing a 600-g mixture of autoclaved sand and black cotton soil (3:1 w/w). The pots were placed immediately at 20, 25, and 30 C in growth chambers with a 12-hour photoperiod and 60–75% relative humidity (RH). For 10 and 15 C, pots were kept for 1 week at 25 C for seed germination before transferring them to 10 and 15 C. The plants were irrigated with 50 ml water per pot at 48-hour intervals. After 1 week, four seedlings were retained in each pot. One thousand (+73) viable J2 (emerged within 24 hours) were placed close to the roots by making depressions with a glass rod adjacent to the roots and then pouring in a water suspension of J2. At 24-hour intervals, seedlings from two pots (eight seedlings) at each temperature were removed by gentle tapping of the pot soil. Roots were then washed in water and stained with 0.1% cotton blue lactophenol solution and stored in clear lactophenol. The stained roots were pressed between two glass plates (15 x 10 cm), and the number of juveniles and their respective stages in the roots were counted. For identification of different growth stages, roots with nematodes were mounted in lactophenol and observed with a high resolution microscope (100x). This procedure was continued until egg production was observed. The life stages were identified as third-stage juveniles (J3), fourth-stage male and female juveniles (J4), and adult males and females (9,13). The number of individuals recorded at maximum occurrence of each life stage was used for working out the survival of various life stages at different temperatures. From the 9th day onward, soil from the pots was also washed through a 400-mesh-sieve (38-μm pore size) using Cobb's decanting and sieving technique, followed by a modified Baermann funnel method to collect males and juveniles (3,16). At the end of the experiment, roots were washed on a 80-mesh sieve (850-μm pore size), and the number of cysts (females) per root was counted. The number of eggs in 20 cysts and 20 egg sacs was assessed to estimate the average egg number per cyst and per egg sac.

**Nematode development at fluctuating temperatures:** Development of *H. cajani* was studied at fluctuating temperatures in a greenhouse in June 1991. Temperature in the greenhouse was recorded by a thermograph. Daily minimum and maximum temperature during the study ranged from 22.2 to 26.7 C (mean 25.4 C) and 32.2 to 37.8 C (mean 34.0 C), respectively. Overall mean temperature was 29.7 C. Seeds of pigeonpea cv. ICPL 87 were sown in 10-cm-d plastic pots containing sterilized sand and black cotton soil (3:1 w/w) mixture. The pots were placed in the greenhouse after sowing. The nematode inoculation method and the observation techniques were as described.

**Emergence of juveniles:** Cysts and egg sacs were collected from *H. cajani*-infected roots of 30-day-old pigeonpea cv. ICPL 87 plants by Cobb's decanting and sieving method (3). Newly formed white cysts (females) were selected for the experiment. Seven petri dishes (6-cm-d) each containing 20 cysts in tap water, and five petri dishes each containing 20 egg sacs were incubated at 15, 20, 25, and 30 C in incubators with a 12-hour photoperiod. Initially at 48-hour intervals, emerged juveniles were counted and removed, and fresh water was added to the petri dishes. As juvenile emergence decreased, the observation interval was increased to 3 day after 6 days and to 4 day after 15 days of incubation for recording emerged juveniles from egg sacs, whereas juvenile emergence from cysts was recorded at 7 day intervals after 28 days of incubation at different temperatures. The number of
unhatched eggs and J2 in the egg sacs and cysts were counted after 19 and 70 days of incubation, respectively.

**Statistical analysis:** Data on nematode penetration, survival, reproduction, and emergence were subjected to analysis by ANOVA using the GENSTAT program. The treatment means were compared by least significant difference (LSD) at 5 and 0.1% level of significance. The effect of temperature over time on juvenile emergence was also computed by multiple regression. The reciprocals of the number of days required to complete one generation (proportion of development completed per day) at each temperature were plotted against the temperature, and the basal threshold temperature for development was calculated for the X-intercept. Data were converted to accumulated degree-days (1,2,4,7,24) for each temperature as

\[
DD = (T - t) \times DAI
\]

where \( t \) = calculated basal threshold temperature, \( T \) = temperature in the growth chamber, and \( DAI = \) days after inoculation, as days taken to complete a life stage from inoculation. In the greenhouse experiment, degree days were calculated as follows:

\[
DD = \sum_{i=1}^{n} \left[ \frac{(T_1 + T_2)}{2} - t \right]
\]

where \( T_1 \) and \( T_2 \) are maximum and minimum temperatures on the ith day, \( n \) is days after inoculation and \( t \) is the calculated basal threshold temperature.

### Results

**Nematode penetration:** *Heterodera cajani* J2 penetrated pigeonpea roots at 15, 20, 25, and 30°C but not at 10°C. The number of juveniles in roots differed significantly among temperatures (Table 1). Nematode penetration was greatest (\( P = 0.001 \)) at 25°C. Mean penetration up to 7 days after inoculation was 10.4% at 15°C, 16.2% at 20°C, 24.9% at 25°C, 11.9% at 30°C, and 15.4% at the fluctuating temperature in the greenhouse. Interaction between incubation temperature and incubation period was significant (\( P = 0.001 \)). The number of nematodes per root increased 56.3% at 20°C and 95.8% at 25°C with increase in the incubation period from 3 to 5 days. At 15°C and the fluctuating temperature, penetration did not differ between days. At 30°C, the number of nematodes inside the roots decreased by 38 and 59% on day 5 and day 7 after inoculation (DAI), respectively, as compared with 3 DAI.

**Nematode development:** The relationship between temperature and the reciprocal of days required to complete a life stage was linear (Table 2, Fig. 1). Development was faster at 30°C than at 25, 20, 15°C, and the fluctuating temperature. Completion of one generation was shortest (17 days) at 30°C and longest (66 days) at 15°C. The rate of development of *H. cajani* from penetration to second-generation J2 between 15 and 30°C was described by the equation

\[
DR = -0.0320 + 0.00290 T \quad (A)
\]

\[r^2 = 0.922, \ P = 0.05\]

where DR = predicted rate of development (proportion of development com-
TABLE 2. Cumulative days and accumulated degree-days required to complete various developmental stages of Heterodera cajani at different temperatures.

<table>
<thead>
<tr>
<th>Developmental stage</th>
<th>Cumulative days required</th>
<th>Accumulated degree-days above 11°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 20 25 30 GH†</td>
<td>15 20 25 30 GH†</td>
</tr>
<tr>
<td>J3</td>
<td>19 9 7 5 6</td>
<td>76 81 98 95 107</td>
</tr>
<tr>
<td>J4 male</td>
<td>31 12 10 8 9</td>
<td>124 108 140 152 165</td>
</tr>
<tr>
<td>J4 female</td>
<td>36 14 12 9 10</td>
<td>144 126 168 171 185</td>
</tr>
<tr>
<td>Male</td>
<td>36 15 13 11 12</td>
<td>144 135 182 209 225</td>
</tr>
<tr>
<td>Female</td>
<td>47 20 16 12 13</td>
<td>188 180 224 228 243</td>
</tr>
<tr>
<td>Egg laying</td>
<td>53 27 22 13 15</td>
<td>212 243 308 247 281</td>
</tr>
<tr>
<td>J2 emergence (second generation)</td>
<td>66 35 28 17 19</td>
<td>264 315 392 323 356</td>
</tr>
</tbody>
</table>

†GH = Greenhouse temperatures fluctuating from 22.2–37.8°C.

Accumulated degree-days above 11°C were determined for nematode development at the four constant temperatures and the fluctuating greenhouse temperatures (Table 2). More degree-days were required for development at 25 and 30°C than at 15 and 20°C. Degree-days required to complete a life cycle from J2 to the next generation J2 were 264 at 15°C, 315 at 20°C, 392 at 25°C, and 323 at 30°C. Males required 19 to 45 fewer degree-days for development than females.

Development at varying temperatures: To complete various life stages, H. cajani required between 12 and 34 additional degree-days at a fluctuating greenhouse temperature than at 30°C. One generation was completed in 19 days. It required 107, 165, 185, 225, 243, 281, and 356 degree-days to complete J2 to J3, J4 male, J4 female, adult male, adult female, egg laying and new J2, respectively.

Nematode survival and reproduction: Survival of different stages of H. cajani was greater at 20 and 25°C than at 15 and 30°C (Table 3). The maximum nematode penetration was 12.6% at 15°C, 25.8% at 20°C, 33.8% at 25°C, and 17.6% at 30°C; 47.5–78.4% of penetrated J2 developed to J3. Development from J3 to J4 was greater at 25°C than at 20, 30, and 15°C. The male-to-female ratio was close to 1:1 at 15 and 30°C and greater than 1:2 at 20 and 25°C.
Only 1.0, 3.2, 5.3, and 7.2% of the inoculated J2 developed to females at 15, 30, 20, and 25 C respectively. The average number of eggs produced per female was 90 at 30 C, 109 at 20 C, 124 at 15 C and 125 at 25 C. The reproductive factor was 1.6 to 7.1 times greater at 25 C than at other temperatures (Table 3).

From the data on development, survival, and reproduction, the following exponential equation was developed to predict the temperature-based population increase of *H. cajani* on pigeonpea:

\[ P_f = P_i \times RF^g \]  
(B)

where \( P_f \) = final nematode population density, \( P_i \) = initial nematode population density, and \( g \) = number of generations completed in one season, which can be worked out as total crop season in days \( \times \) DR (nematode development rate per day). \( RF_e \) = reproductive factor, estimated from the equation assuming quadratic (nonlinear regression) of reproductive factor on temperature, and the fitted equation is \( RF_e = -50.3655 + 5.0373T - 0.1083T^2 \), where \( T \) is the mean temperature during the crop season.

In the above equation, temperature accounted for 89% of the variability represented in reproductive factor \( (r = 0.890) \).

**Emergence of juveniles from egg sacs and cysts:** No differences \( (P = 0.05) \) in J2 emergence from egg sacs was recorded at 20, 25, and 30 C up to day 2. From day 4 onwards, the J2 emergence at 25 and 30 C was greater than at 15 and 20 C (Fig. 2A). The differences in juvenile emergence from cysts were not significant \( (P = 0.05) \) at 25 and 30 C, except at day 14, when emergence at 25 C was greater \( (P = 0.05) \) than at 30 C (Fig. 2B). Juvenile emergence remained lower at 15 and 20 C than at 25 and 30 C at all the incubation periods. The juvenile emergence from egg sacs in 19 days of incubation was 32.3% at 15 C, 71.5% at 20 C, 93.2% at 25 C, and 90.4% at 30 C. Emergence of juveniles from cysts incubated for 70 days was 6.2% at 15 C, 36.3% at 20 C, 90.1% at 25 C, and 88.3% at 30 C. More than 90% of the emerged juveniles from cysts at 25 and 30 C were released within 28 days of incubation. Juvenile emergence was faster from egg sacs than from cysts. Juvenile emergence was 93.1% at 25 C and 90.4% at 30 C from egg sacs within 15 days of incubation as compared with 64.1% at 25 C and 48.7% at 30 C from cysts within 14 days of incubation. The relationship between juvenile emergence from egg sacs and cysts and temperature and incubation time is described by the following multiple regression equations:

\[ E_e = -34.2 + 2.56T - 0.34t + 0.1491(T \times t) \]  
(C)

\[ r^2 = 0.753, P = 0.01 \]

\[ E_c = -69.4 + 4.46T - 0.244t + 0.0284(T \times t) \]  
(D)

\[ r^2 = 0.828, P = 0.01 \]

where \( E_e \) = predicted percentage cumulative emergence from egg sacs, \( E_c \) = predicted percentage cumulative emergence from cysts, \( T \) = temperature, and \( t \) = time (incubation days).

**DISCUSSION**

The basal development threshold temperature of 11 C for *H. cajani* is higher than that for other cyst and root-knot nematodes; it is 5.6 C for *Heterodera glycines* (1), 8.0 C for *Heterodera schachtii* (7), 6.7 C for *Meloidogyne hapla* (22), 8.3 C for *Meloidogyne incognita* (22) and 10.1 C for *Meloidogyne arenaria* (5). The higher basal threshold of *H. cajani* reflects adaptation of this species to tropical regions (20).

As reported for other cyst-forming nematodes, fewer degree-days were required to complete development at lower temperatures of 15 and 20 C than at higher temperatures of 25 and 30 C (1, 7, 11). Howe (8) has reported for insects that the number of degree-days required for complete development is too low at temperature near to the lower threshold and too high at or above the optimum. It seems that fluctuating temperature is more favorable for nematode invasion and devel-
TABLE 3. Numbers of second-, third-, and fourth-stage juveniles and adults of <em>Heterodera cajani</em> developed per plant at different temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>J2t</th>
<th>J3</th>
<th>J4</th>
<th>Male</th>
<th>Female</th>
<th>% adults</th>
<th>Male/female ratio</th>
<th>Reproductive factor (RF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>31.4</td>
<td>14.9</td>
<td>7.4</td>
<td>2.4</td>
<td>2.5</td>
<td>2.0</td>
<td>1:1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>20</td>
<td>64.4</td>
<td>50.5</td>
<td>20.5</td>
<td>5.9</td>
<td>13.2</td>
<td>7.6</td>
<td>1:2.2</td>
<td>5.8</td>
</tr>
<tr>
<td>25</td>
<td>84.4</td>
<td>64.1</td>
<td>30.9</td>
<td>6.8</td>
<td>17.9</td>
<td>9.9</td>
<td>1:2.6</td>
<td>9.2</td>
</tr>
<tr>
<td>30</td>
<td>44.1</td>
<td>29.4</td>
<td>13.1</td>
<td>4.4</td>
<td>7.9</td>
<td>4.9</td>
<td>1:1.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

LSD

| P = 0.05 | 25.4 | 20.4 | 10.5 | 1.5  | 4.6    | 2.6     |
| P = 0.001| 46.6 | 37.5 | 19.2 | 2.8  | 8.4    | 5.4     |
| CV (%)    | 43.7 | 49.5 | 56.2 | 29.8 | 30.0   | 33.7    |

Means of eight replications. Adults (%) = number of adults (male + females) per root system + inoculum rate) x 100; reproductive factor = number of eggs produced per root system + inoculum rate.

Fig. 2. Cumulative emergence of <em>Heterodera cajani</em> J2 at different temperatures in petri dishes (A) From egg sacs. (B) From cysts. LSD (P = 0.05) (temperature x incubation period), egg sacs = 7.51, cysts = 11.65.

Development than the same constant temperature. The nematode penetration and development at fluctuating temperature (mean temperature 29.7 °C) are comparable to that at constant temperature of 30 °C. The nematode penetration, as apparent from the number of J2 in roots at 3 DAI, was faster at constant than at fluctuating temperature (Table 1), but nematode mortality in roots was higher at constant than at fluctuating temperature, resulting in significantly lower number of J2 in roots at constant temperature at 5 and 7 DAI. The nematode accumulated 12–34 more degree-days at fluctuating temperature than at constant for completing various life stages. This additional requirement of degree-days at fluctuating temperature should be taken into account when developing models at constant temperatures to simulate natural conditions.

The temperature requirements for...
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hatch, activity, development, and reproduction vary within a species (23). In present studies, the root penetration at 25 C was 1.31, 1.91, and 2.69 times greater than at 20, 30, and 15 C, respectively (Table 3). The percentage development to the adult stage after penetration was almost the same at 20, 25, and 30 C, however, indicating that root penetration is more sensitive to temperature change than development. Roberts et al. (14) also reported that *M. incognita* can develop at lower temperatures, but it requires temperature above 18 C for penetration. More degree-days were accumulated during development from J2 to J3 than subsequent stages of development. This may be due to the time lag between root penetration and establishment of feeding sites.

Nematode development was faster at 30 than at 20 or 25 C, but fewer individuals survived at 30 C. In *H. glycines* also, nematode development was faster at 31 than at 24 C, but fewer adults developed at this temperature (15). The nematode reproduction was 3.3 times greater at 25 than at 30 C, but no significant differences were recorded in juvenile emergence at 25 and 30 C. This may indicate that juvenile emergence can occur at a wider temperature range than development and reproduction. Similarly, more degree-days were accumulated during egg laying and J2 emergence than development of different life stages in pigeonpea roots (Table 2).

More *H. cajani* juveniles emerged at 25 and 30 C than at 15 and 20 C. The optimum temperature for juvenile emergence was 28–29 C (10,18). Juvenile emergence from egg sacs was faster than emergence from cysts (10,18). In these studies, we also observed more rapid juvenile emergence from egg sacs than from cysts. More than 90% of the emerged juveniles from cysts were released within 28 days of incubation; remaining juveniles emerged at a very slow rate. These observations are similar to those of Sharma and Swarup (18). Koshy and Swarup (10) reported 89.5% juvenile emergence from white cysts within 30 days of incubation.

We consider that 25 C is optimum for *H. cajani* development and reproduction. Equations A and B are useful for predicting nematode development rate and population increase as influenced by temperature, whereas equations C and D will help in determining the percentage cumulative emergence of juveniles from egg sacs and cysts at different temperatures and time intervals. Since these equations were developed from laboratory, growth chamber, and greenhouse experiments, they are to be validated under field conditions before using them for predicting *H. cajani* populations in field. However, these equations will form a basis for developing models for predicting population densities in the field.

**LITERATURE CITED**


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