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# Infectivity, Development, and Reproduction of *Heterodera cajani* on Pigeonpea: Influence of Soil Moisture and Temperature[1]

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*Abstract:* The effect of soil moisture on penetration, development, and reproduction of *Heterodera cajani* on pigeonpea (cv. ICPL 87) was investigated in growth chambers held at 20 and 25 C, and in a greenhouse where temperature fluctuated between 25 and 32 C. Averaged across temperatures, the percentage of juveniles that penetrated roots was 34.3, 31.8, 8.8, and 3.7% at 24, 32, 16, and 40% soil moisture levels, respectively. Numbers of females per root system 4 weeks after infesting soil with second-stage juveniles was 79.6 at 24%, 65.3 at 32%, 26.1 at 16%, and 2.9 at 40% soil moisture. Nematode reproduction was greatest ($P = 0.001$) at 24% soil moisture and 25 C. Reproductive factor was 19.4 at 24%, 15.2 at 32%, 5.7 at 16%, and 0.5 at 40% soil moisture level. Nematode penetration, development, and reproduction at different moisture levels were greater ($P = 0.01$) at 25 and 25–32 C than at 20 C. Plant growth was retarded at 40% soil moisture and 20 C in comparison to that at 24 and 32% moisture levels and 25 C. This information on influence of temperature and soil moisture will be helpful in developing models for predicting changes in *H. cajani* densities in pigeonpea fields during rainy and postrainy dry seasons in the semi-arid tropics.

*Key words: Cajanus cajan,* cyst nematode, development, *Heterodera cajani,* nematode, penetration, pigeonpea, reproduction, temperature, soil moisture.

Soil moisture affects the movement and behavior of nematodes. Water films in soil provide a habitat for most species of plant-parasitic nematodes and an integral medium for nematode migration, survival, and host invasion. Soil moisture levels optimum for nematode movement, infectivity, and development vary from one nematode species to another (2,6,14,17). However, moisture near field capacity is usually favorable for invasion and development of plant-parasitic nematodes (8,13,15). Understanding the responses of nematode species to low and high moisture levels is crucial in developing strategies for nematode management. Moisture and temperature influence each other, and their effects are not readily separated. Since water has one of the largest heat-holding capacities of any substance, wet soil warms up more slowly than dry soil (6). Khan et al. (3) reported that fluctuations in densities of *Hoplolaimus indicus, Hemicriconemoides mangiferae,* and *Helicotylenchus erythrinae* and decline in nematode densities during summer (26–34 C), as well as in winter (8–15 C), were largely due to low soil moisture.

The pigeonpea cyst nematode *Heterodera cajani* Koshy is the key nematode pathogen of pigeonpea (*Cajanus cajan* (L.) Millsp.) in India (11). The nematode is widespread on Vertisols (Typic Pellusterts) in southern India. Temperature greatly influences the biology of *H. cajani,* and temperatures between 25 and 29 C are optimum for nematode development and reproduction (4,11, 12). Soil moisture is a limiting factor for crop production in the semi-arid tropics, and information is lacking about its influence on the disease cycle of *H. cajani.* The objective of this study was to determine the effects of soil moisture levels at different temperatures on infectivity, development, and reproduction of *H. cajani* infecting pigeonpea.

## Materials and Methods

A population of *H. cajani* was obtained from an infested Vertisol soil (44% sand, 40% clay, and 16% silt, with a water-holding capacity of 32.5%, pH 7.0–8.5, and cation exchange capacity of 43–58 meq/100 g) at the International Crops Re-

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search Institute for the Semi-Arid Tropics (ICRISAT) Center, Patancheru, Andhra Pradesh, India. Nematode inoculum was increased on pigeonpea cv. ICPL 87 in 20-cm-d pots containing a mixture of autoclaved sand and a Vertisol (3:1 w/w) in a greenhouse (12). Nematode inoculum was collected by extracting cysts and egg sacs from soil and roots by Cobb's decanting and sieving technique (1) and incubating them at 25 C. Emerged second-stage juveniles were collected in 6-cm-d plastic petri dishes and stored at 15 C.

Experiments were conducted in growth chambers at constant temperatures of 20 and 25 C, and in a greenhouse where temperature fluctuated between 25 and 32 C. Seeds of pigeonpea cv. ICPL 87 were sown in autoclaved soil with known moisture contents in 12.5-cm-d pots. Four moisture levels (40, 32, 24, and 16% by weight) were maintained by adding the required amount of water through PVC tubes (12.5 cm long, 2.5 cm d) of known weight, inserted in each pot. Twenty additional pots without plants were maintained at each moisture × temperature level and weighed to determine evaporation losses and to allow adjustments for plant weight. Based on the evaporation loss estimates, the required quantity of water was added to each pot every day. Once a week, each pot was weighed individually and its weight was adjusted by adding water to the moisture treatment level.

The pots were arranged in a randomized block design in the growth chambers and greenhouse. Soil temperature in pots was recorded daily with a soil thermometer buried 5-cm deep in four pots representing each soil moisture treatment. The average soil temperature was 26.8 ± 0.4 C in the greenhouse and 24.7 ± 0.2 C and 20.2 ± 0.3 C in growth chambers. Relative humidity was 65 ± 5% in the greenhouse and 70 ± 2% in growth chambers. A light source was placed 80 cm from the growth chamber bench, providing 156 $\mu E/m^2/s$ intensity for 12 hours/day. Sun light was the light source in the greenhouse. One week after germination, seedlings were thinned to four plants per pot and 2,000 recently emerged juveniles (500 J2/plant) in 4 ml water were placed close to the roots in each pot. Pots without plants received 4 ml tap water. Gross weight of the pots was then maintained by adding the required amount of water.

*Penetration and development:* Eight seedlings from each moisture level were harvested 1 and 2 weeks after inoculation, and the roots were stained in boiling 0.1% cotton blue lactophenol. The stained roots were pressed between two 15 cm × 10 cm glass plates and number of juveniles in the roots and their respective stages of development were counted with the aid of a stereoscopic microscope. For identification of different growth stages, roots with nematodes were mounted in lactophenol and observed with a compound microscope (×100). The life stages were identified as second-(J2), third-(J3), and fourth-stage (J4) juveniles, males, and females (4,7). Percentage penetration was calculated from the 1-week sample as the total number of juveniles in roots of four plants ÷ number of juveniles added × 100. Percentage of each life stage inside the root was calculated 1 and 2 weeks after inoculation as (number of individuals in a particular development stage ÷ total number of nematodes in the root) × 100. Three weeks after inoculation, the plant roots and soil from five pots at each moisture × temperature level were processed by Cobb's decanting and sieving method (1) and the number of females per root recorded.

*Nematode reproduction and plant growth:* The effect of soil moisture levels and temperature on *H. cajani* reproduction and pigeonpea growth was assessed 4 weeks after inoculation. Roots were washed free of soil, and fresh root and shoot weights were recorded. Plant roots and soil were processed through 180-μm (80-mesh) and 38-μm pore size (400-mesh) sieves to count the soil population of J2 and females (cysts) produced per root (1,9). Egg sacs were treated with 0.25% NaOCl to release the eggs (10), and the average number of

eggs produced per cyst and egg sac was estimated by counting the number of eggs in 20 cysts and 20 egg sacs.

*Statistical analysis:* Analysis of variance was performed using the GENSTAT computer package. The experimental design for penetration and development was a factorial with four moisture levels, three temperatures, and eight replications. The experimental design for reproduction and plant growth was a factorial with four moisture levels, three temperatures, and five replications. The treatment means were compared by least-significant-difference analysis at 0.05 and 0.01 levels of significance. The relationship of number of females per root, number of eggs per root, and reproductive factor (final egg and J2 number ÷ initial J2 inoculum) of *H. cajani* to soil moisture levels was described by a quadratic regression model.

## Results

*Penetration:* Soil moisture and temperature influenced the penetration of J2 into the roots, but the interaction between temperature and moisture was not significant. Greater ($P = 0.001$) number of J2 penetrated roots at 32% and 24% than at 40% and 16% moisture levels (Table 1). The percentage of initial inoculum extracted from roots was 34.3 at 24%, 31.8 at 32%, 8.8 at 16%, and 3.7 at 40% soil moisture levels. The average percentage penetrating roots was 22.1% at 25 C, 21.7% at 25–32 C, and 15.2% at 20 C.

*Development:* Juvenile development was more rapid at 16% and 24% moisture levels than at 32% and 40%, the latter being most unfavorable. Development, as measured by the percentage of J2 developed to J3 within a week of inoculation, was lower at 32% soil moisture, and little development beyond J2 was seen during this period at 40% soil moisture (Table 1). The number of females and males in roots was also greatest at 24% soil moisture. Percentage females in root were 18.9 at 24%, 9.2 at 16%, 3.6 at 32%, and 1.4 at 40% moisture levels. About 46% of the juveniles in roots were still in the second developmental stage 2 weeks after inoculation in pots maintained at 40% soil moisture (Fig. 1). Differences among moisture treatments persisted 3 and 4 weeks after inoculation (Table 2). The average number of females per root 3 weeks after inoculation was 59 at 24%, 34 at 32%, 10 at 16%, and 1.0 at 40% soil moisture. Four weeks after inoculation, the number of females per root at 24% soil moisture was 80, which was 1.2, 3.1, and 27.4 times higher than the numbers for the 32%, 16%, and 40% soil moisture treatments, respectively (Table 2). The relationship of number of females per root to soil moisture levels was nonlinear (Table 3).

Table 1. Penetration of *Heterodera cajani* juveniles in roots of pigeonpea cv. ICPL 87 1 week after inoculation at four moisture levels and three temperature regimes.

| Moisture level (%) | Number of J2/root | | | | % J2 in root[a] | | | | % J3 in root at[a] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25–32 C | 25 C | 20 C | Mean | 25–32 C | 25 C | 20 C | Mean | 25–32 C | 25 C | 20 C | Mean |
| 40 | 18.8 | 20.4 | 16.1 | 18.4 | 100.0 | 94.7 | 100.0 | 98.2 | 0.0 | 5.4 | 0.0 | 1.8 |
| 32 | 188.3 | 180.8 | 108.1 | 159.0 | 77.4 | 36.9 | 100.0 | 71.4 | 22.6 | 63.1 | 0.0 | 28.6 |
| 24 | 171.0 | 187.6 | 156.5 | 171.7 | 26.4 | 42.7 | 100.0 | 56.4 | 72.6 | 57.3 | 0.0 | 43.3 |
| 16 | 55.4 | 53.1 | 22.9 | 43.8 | 42.9 | 45.9 | 100.0 | 62.9 | 57.1 | 54.2 | 0.0 | 37.1 |
| Mean | 108.3 | 110.5 | 75.9 | | 61.7 | 55.0 | 100.0 | | 38.1 | 45.0 | 0.0 | |
| LSD, $P = 0.001$ | | | | | | | | | | | | |
| | Temperature (T) | | | 25.9[b] | | | | 7.5 | | | | 7.8 |
| | Moisture (M) | | | 52.2 | | | | 8.7 | | | | 9.0 |
| | T × M = | | | NS | | | | 15.1 | | | | 15.6 |

[a] Percentage J2 or J3 = (Number of J2 or J3 in roots ÷ total number of juveniles in roots) × 100. Data values are mean of eight plants.

[b] Significant at 5% level.

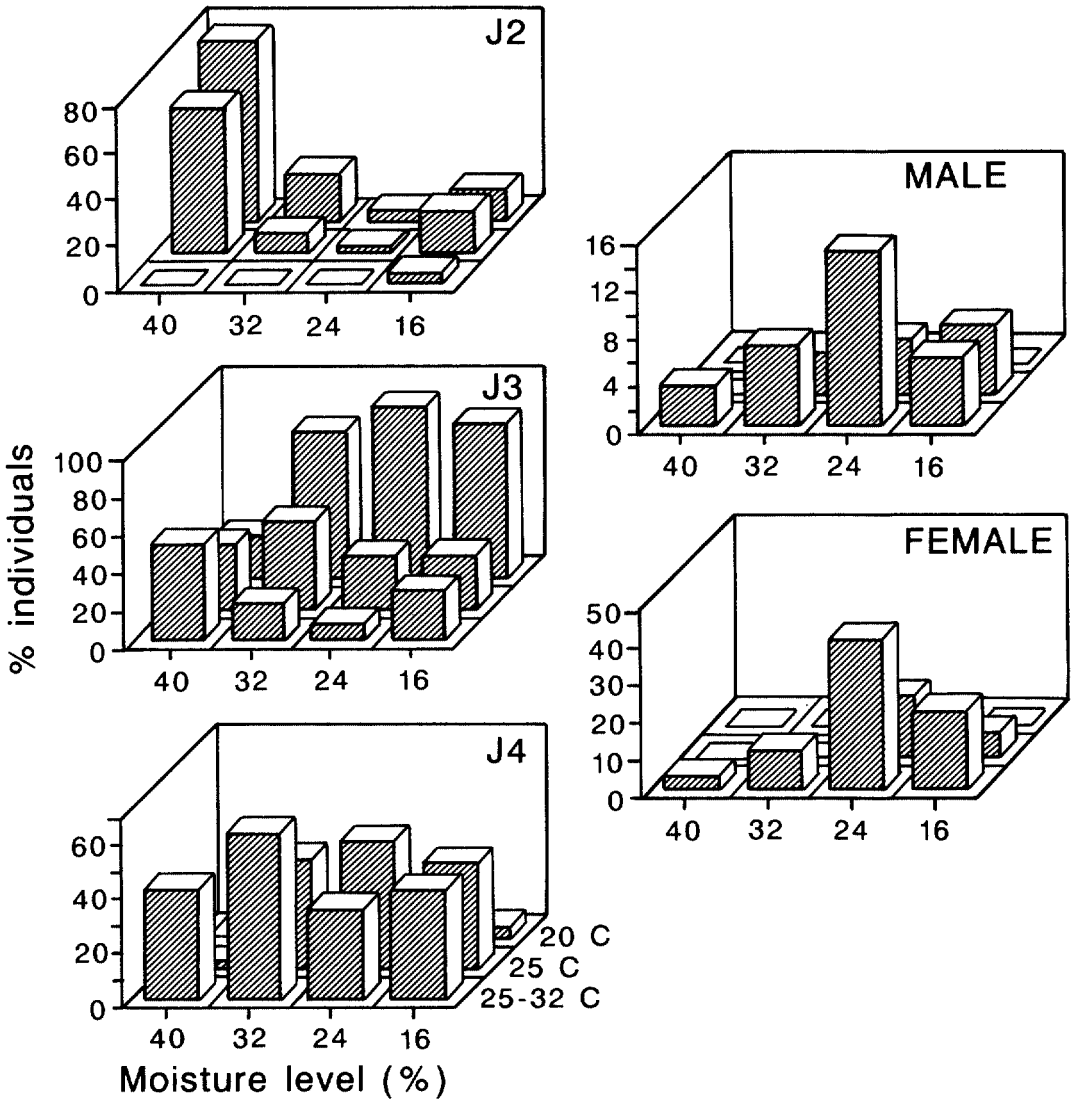


FIG. 1. Proportions of second (J2), third (J3), and fourth-stage juveniles (J4), males, and females of *Heterodera cajani* in pigeonpea roots 2 weeks after inoculation at four moisture levels and three temperatures. LSD ($P$ = 0.001) (temperature × moisture) = J2 – 28.4, J3 – 30.5, J4 – 16.7, male – 6.9, female – 10.5.

Numbers of adult and J4 in the roots were greater ($P$ = 0.05) at 25–32 C in the greenhouse than at 25 C in the growth chamber (Table 1). Adults were not formed at 20 C until 2 weeks of inoculation (Fig. 1). Greater ($P$ = 0.001) numbers of females were produced at 25 C and 25–32 C than at 20 C for 3–4 weeks after inoculation (Table 2).

*Reproduction:* Egg numbers at 16% and 24% moisture were 10–15% greater ($P$ = 0.05) than those at 32% and 40% soil moisture (Table 4). The reproductive factor at 24% soil moisture was 19.4, which was 3.4, 1.3, and 38.8 times that at 16%, 32%, and 40% soil moisture levels, respectively. The number of eggs at 25 C was nearly 10 times greater ($P$ = 0.001) than at 20 C. At 20 C, the number of eggs per female did not vary among moisture treatments. The reproductive factor was 13.3 at 25–32 C, 15.8 at 25 C, and 1.5 at 20 C. Regression models were significant at 25–32 C and 25 C (Table 3) and showed a nonlinear rela-

TABLE 2. Number of females produced per root at 3 and 4 weeks after inoculation at four moisture levels and three temperature regimes.

| Moisture levels (%) | At 3 weeks (C) | | | | At 4 weeks (C) | | | |
|---|---|---|---|---|---|---|---|---|
| | 25–32 | 25 | 20 | Mean | 25–32 | 25 | 20 | Mean |
| 40 | 2.8 | 0.0 | 0.0 | 0.9 | 8.2 | 0.2 | 0.2 | 2.9 |
| 32 | 63.4 | 37.2 | 0.4 | 33.7 | 80.6 | 76.8 | 38.6 | 65.3 |
| 24 | 78.4 | 97.2 | 0.5 | 58.7 | 76.8 | 95.8 | 66.2 | 79.6 |
| 16 | 15.9 | 12.7 | 0.3 | 9.6 | 26.6 | 22.2 | 29.6 | 26.1 |
| Mean | 40.1 | 36.8 | 0.3 | | 48.0 | 48.8 | 33.7 | |
| LSD, $P = 0.001$ | | | | | | | | |
| | Temperature (T) | | | 14.9 | | | | 13.4 |
| | Moisture (M) | | | 17.1 | | | | 15.4 |
| | T × M | | | 29.7 | | | | 26.8 |

Data values are means of eight plants.

tionship between soil moisture levels and number of eggs per root. The interactive effect of temperature and moisture on nematode reproduction was significant ($P = 0.05$). The number of females and eggs markedly increased with decrease in soil moisture from 32% to 24% at 25 C. The reproductive factor did not differ between temperatures at 40% moisture, and at 20 C it did not differ between moisture levels. Nematode reproduction at 24% soil moisture was 1.4 times higher at 25 C in the growth chamber than at 25–32 C in the greenhouse.

*Plant growth:* The growth of ICPL 87 was better at 24% and 32% soil moistures and 25 C temperature than at other moisture levels and temperatures (Table 5). The root, shoot, and total fresh weights were lowest ($P = 0.001$) at 40% soil moisture and 20 C.

## DISCUSSION

In our experiments, 24% soil moisture and 25 C temperature were conducive to the infectivity, development, and reproduction of *H. cajani* on pigeonpea. The soil moisture tension at 24% moisture level is between −1 and −5 bars, which is an unsaturated soil moisture situation for the Vertisols; field capacity ranges between 32% and 35%, and wilting range is between 17% and 19% (P. Singh, Senior Scientist [Soil Science], ICRISAT, pers. comm.). Inhibition of penetration, devel-

TABLE 3. Regression equations for reproductive parameters of *Heterodera cajani* at four moisture levels and three temperature regimes.

| Moisture effect on | 25–32 C | 25 C | 20 C |
|---|---|---|---|
| Number of females per root | $Y = -271.1 + 26.18\ x^{a} - 0.4789\ x^{a}$ ($R^2 = 0.86$) | $Y = -334.5 + 31.79\ x^{a} - 0.5867\ x^{2a}$ ($R^2 = 0.96$) | $Y = -132.1 + 14.96\ x^{b} - 0.293\ x^{2b}$ ($R^2 = 0.67$) |
| Number of eggs per root | $Y = -34895.0 + 3432.0\ x^{a} - 63.48\ x^{2b}$ ($R^2 = 0.76$) | $Y = -50029.0 + 4852.0\ x^{a} - 90.26\ x^{2a}$ ($R^2 = 0.95$) | $Y = -4125.00 + 429.00\ x - 8.28\ x^{2}$ ($R^2 = 0.47$) |
| Reproductive factor | $Y = -73.1 + 7.202\ x^{b} - 0.1334\ x^{2a}$ ($R^2 = 0.79$) | $Y = -101.26 + 9.815\ x^{a} - 0.1826\ x^{2a}$ ($R^2 = 0.94$) | $Y = -8.36 + 0.870\ x - 0.01680\ x^{2}$ ($R^2 = 0.49$) |

Y = number of females or eggs or reproductive factor, x = moisture level.
[a] Significant at 1% level.
[b] Significant at 5% level.

TABLE 4. Reproduction of *Heterodera cajani* on pigeonpea cv. ICPL 87 at four moisture levels and three temperature regimes.

| Moisture level (%) | Number of eggs/female (C) | | | | Number of J2 in soil (C) | | | | Total number of eggs produced/root[a] (C) | | | | Reproductive factor[b] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25–32 | 25 | 20 | Mean | 25–32 | 25 | 20 | Mean | 25–32 | 25 | 20 | Mean | 25–32 | 25 | 20 | Mean |
| 40 | 73 | 173 | 0 | 82 | 5 | 1 | 0 | 2 | 660 | 30 | 0 | 230 | 1.3 | 0.1 | 0.0 | 0.5 |
| 32 | 129 | 152 | 13 | 98 | 89 | 1 | 5 | 32 | 10,600 | 11,670 | 510 | 7,593 | 21.2 | 23.3 | 1.0 | 15.2 |
| 24 | 133 | 164 | 31 | 109 | 843 | 115 | 1 | 320 | 11,200 | 15,830 | 2,060 | 9,697 | 22.4 | 31.7 | 4.1 | 19.4 |
| 16 | 150 | 184 | 14 | 116 | 105 | 21 | 3 | 43 | 4,060 | 4,100 | 420 | 2,860 | 8.1 | 8.2 | 0.8 | 5.7 |
| Mean | 121 | 168 | 14 | | 261 | 35 | 2 | | 6,630 | 7,908 | 748 | | 13.3 | 15.8 | 1.5 | |
| LSD, $P = 0.001$ | | | | | | | | | | | | | | | | |
| | Temperature (T) | | | 11.0 | | | | 123.9 | | | | 1,696.9 | | | | 3.39 |
| | Moisture (M) | | | 12.7 | | | | 143.2 | | | | 1,959.6 | | | | 3.92 |
| | T × M | | | 21.8 | | | | 248.3 | | | | 3,393.9 | | | | 6.79 |

Data values are mean of five replications.

[a] Total eggs/root = (number of females/root × number of eggs/female) + J2 in soil.

[b] Reproductive factor = ([number of females/root × number of eggs/female] + J2 in soil) ÷ no. of J2 inoculated.

TABLE 5. Fresh weights (g) of pigeonpea cv. ICPL 87 plants grown for 4 weeks at four moisture levels and three temperatures regimes.

| Moisture levels (%) | Root (C) | | | | Shoot (C) | | | | Total plant (C) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 25–32 | 25 | 20 | Mean | 25–32 | 25 | 20 | Mean | 25–32 | 25 | 20 | Mean |
| 40 | 1.05 | 0.81 | 0.47 | 0.78 | 0.85 | 0.71 | 0.47 | 0.67 | 1.90 | 1.50 | 0.94 | 1.45 |
| 32 | 1.74 | 2.05 | 0.78 | 1.52 | 1.13 | 1.80 | 0.71 | 1.21 | 2.86 | 3.89 | 1.49 | 2.75 |
| 24 | 1.57 | 1.87 | 0.97 | 1.47 | 0.84 | 1.13 | 0.67 | 0.88 | 2.42 | 2.85 | 1.64 | 2.31 |
| 16 | 1.37 | 1.78 | 0.68 | 1.28 | 0.74 | 1.33 | 0.62 | 0.90 | 2.11 | 3.04 | 1.30 | 2.15 |
| Mean | 1.43 | 1.63 | 0.73 | | 0.89 | 1.24 | 0.62 | | 2.32 | 2.82 | 1.34 | |
| LSD, $P$ = 0.001 | | | | | | | | | | | | |
| | Temperature (T) | | | 0.31 | | | | 0.22 | | | | 0.44 |
| | Moisture (M) | | | 0.36 | | | | 0.25 | | | | 0.51 |
| | T × M | | | 0.35[a] | | | | 0.43 | | | | 0.89 |

Data values are means of five replications.
[a] Significant at 5% level.

opment, and reproduction of *H. cajani* at 40% soil moisture could be due to low nematode activity and survival due to reduced oxygen-diffusion rates in soil, accumulation of carbon-dioxide, and (or) production of toxins (8,16,17). Low soil-oxygen levels in wet soil also reduce root growth and water uptake, resulting in lower plant biomass (5,8) to sustain nematode populations. Pigeonpea is sensitive to waterlogging, and even a moderate excess of soil moisture may cause general yellowing of the crop and a decline in crop vigor. At 16% soil moisture, reduced motility of the nematodes due to adverse dry conditions likely contributed to poor root penetration, development, and reproduction (3,16,17). Survival of *Heterodera glycines* was also adversely affected in soils that were too wet or too dry (13). Root penetration, development, and reproduction of *H. cajani* in pigeonpea roots were greater at 25 and 25–32 C than at 20 C, confirming earlier studies in which 25 C to 29 C was optimum and low numbers of females and eggs were produced at 20 C (4,11,12).

This study is the first report of the influence of soil moisture on *H. cajani*. Pigeonpea is a rainy-season, rain-fed crop in India. The soil moisture in pigeonpea fields generally recedes gradually from sowing time to harvest. This study will be useful for developing models of nematode population dynamics in pigeonpea fields between rainy and dry seasons, but further studies under field conditions are needed to validate the results before they can be used for predictive models.

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