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Emergence of *Heterodera cajani* Juveniles from Cysts and Egg Sacs

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Abstract

The effects of temperature, host root exudates, host age, cyst maturity, and cyst wall fragments on emergence of *Heterodera cajani* juveniles from egg sacs and cysts were investigated in laboratory and greenhouse experiments. Emergence from egg sacs was greater and more rapid ($P = 0.05$) than from white (young) or brown (mature) cysts. From white cysts, 50-80% of the juveniles emerged within 1 month at 25-30°C. Emergence of the remaining juveniles was not entirely temperature dependent and was slow and gradual. Fifty-three percent of the juveniles from brown cysts emerged at 25° C, 52% at 20 ° C, and 5% at 15° C. About 48% of the juveniles did not emerge even after incubation for 525 days at 25° C. These dormant juveniles were either free or within eggs in cysts. Juvenile emergence was greater from cysts produced on 30-day-old pigeonpea (cv. ICPL 87) plants than from cysts produced on 60-, 90-, 120-, and 150-day old plants. Root exudates from 15-day-old pigeonpea cv. ICPL 87 stimulated emergence of 15-25% dormant juveniles in mature cysts. Emergence from free eggs was about 14-times greater than from encysted eggs. Cyst wall fragments and other cyst contents repressed emergence of juveniles from eggs. The pattern of second-stage juvenile emergence in *H.cajani* is complex and temperature is a major, but not the only important factor. A part of the encysted juvenile population undergoes diapause.

Introduction

Pigeonpea cyst nematode (PPCN), *Heterodera cajani* Koshy, is an important nematode pest of pigeonpea (*Cajanus cajan* (L.) Millsp.). It is widely distributed in the major pigeonpea-producing states of Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Maharashtra, Punjab, Rajasthan, Tamil Nadu and Uttar Pradesh in India, and in parts of Egypt (Sharma, *et al.*, 1992). The host range of PPCN is largely confined to species of Leguminosae, Pedaliaceae, and Euphorbiaceae (Koshy & Swarup, 1972; Sharma *et al.*, 1992). It reduces foliage production and grain yield of pigeonpea (Sharma *et al.*, 1993). Nematicides are not commonly used to control PPCN and high yielding PPCN resistant cultivars have not been developed. It is possible that weak links in the PPCN-pigeonpea relationship could be manipulated to develop tactics for the management of PPCN and an understanding of the nature of juvenile emergence from cysts and egg sacs would aid in development of management tactics for controlling PPCN. It may be possible to arrest juvenile emergence during the initial stages of crop growth when pigeonpea is very vulnerable to PPCN damage or enhance emergence in the absence of host plants (Clarke & Perry, 1977; Evans, 1979; Perry & Clarke, 1981; Shepherd, 1962).

Many biotic and abiotic factors regulate emergence of second-stage juveniles of *Heterodera spp.* (Koshy & Swarup, 1971; Sharma & Swarup, 1984; Singh & Sharma, 1994). The objectives of this research were to study the effect of temperature, incubation period, host age, host root exudates, cyst maturity, and cyst wall on juvenile emergence of PPCN.

Materials and Methods

A population of PPCN was collected from a pigeonpea field at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Andhra Pradesh, India and raised on pigeonpea cv. ICPL 87 in 20-cm diameter plastic posts containing a mixture of autoclaved sand and black-cotton soil (3:1, w/w) in a greenhouse. White (young) and brown (mature) cysts, and egg sacs were collected from pigeonpea roots using a 80-mesh (180-pm-pore size) sieve (Cobb, 1918; Sharma & Nene, 1986). Cysts and egg sacs were sorted and separated under a stereo microscope.

Effect of temperature

Emergence of second-stage juveniles (J2) from white cysts, brown cysts, and egg sacs collected from roots of pigeonpea (cv. ICPL 87) plants was studied in seven batches at 15, 20, 25, and 30° C

in incubators with 12-h photoperiod. Each batch consisted of 20 egg sacs, 20 white cysts, or 20 brown cysts in tap water in 6-cm diameter plastic Petri dishes incubated at one of the four temperatures. The emerged J2 were counted at 2-day intervals for 21 days. Then, the cysts were gently cut open and J2 in cysts, J2 in eggs, and eggs in embryonic stages counted. The egg sacs were treated with 0.25% sodium hypochlorite to release the eggs (Sharma & Nene, 1987).

The effect of temperature on J2 emergence from brown cysts was studied in a second experiment at 15, 20, 25, and 30° C in incubators with a 12-h photoperiod. Cysts extracted from roots of pigeonpea (cv. ICPL 87) plants were carefully sorted and picked to avoid empty and partially-filled cysts. Ten 6-cm diameter plastic Petri dishes, each containing 20 cysts in tap water, were incubated at each temperature. The emerged juveniles were counted and removed at 2-day intervals for the first 85 days of incubation, and then at 7 or 14-day intervals. After 525 days of incubation, the cysts were gently cut open and J2 in cyst, J2 in eggs, eggs in embryonic stages, and diseased eggs were counted.

Effect of host age

The effect of plant age on J2 emergence from white cysts, collected from roots of 30-, 60-, 90-, 120-, and 150-day old pigeonpea plants, was studied at four incubation temperatures. Seeds of pigeonpea cv. ICPL 87 were sown in 15-cm diameter plastic pots (four for each plant age) containing 1.5 kg of a mixture of autoclaved sand and black-cotton soil (3:1, w/w) and inoculated at 5 different intervals. The pots were maintained in a greenhouse (ambient temperature 25-30° C; relative humidity 65 ± 5%) and 2,500 J2 were placed close to seeds (day 0), or roots of 30-, 60-, 90-, and 120- day old plants. Four weeks after inoculation, the plants were harvested and cysts were collected from soil and roots by sieving (Sharma & Nene, 1986). Five batches of 20 cysts in 6-cm diameter Petri dishes were incubated at 15, 20, 25, and 30°C as previously described. Emerged J2 were counted and removed initially at 2-day interval for 30 days, and then at 7-, and eventually 14-day intervals until 280 days after inoculation. The number of eggs and J2 remaining in cysts were counted.

Effect of root exudates

The effect of root exudates of pigeonpea cv. ICPL 87 on J2 emergence from brown cysts was studied at 25°C. Pigeonpea seeds were surface-sterilized with 2.5% sodium hypochlorite for five minutes, rinsed in sterile water, and allowed to germinate on a moist blotting paper in Petri dishes for three days at 25°C. The radicles of 25 germinating seedlings, introduced through holes made in sterilized styrofoam sheet placed over a 250 ml beaker, were immersed in 50 ml sterilized water. The beakers were covered with a black paper and incubated at 25° C. After 7 days, root exudate in the beakers was passed through Whatman No. 1 filter paper, and the filtrate was tested for its effect on the emergence of J2 from cysts.

Ten batches of 20 brown cysts were placed in 6-cm diameter Petri dishes containing 5-ml root exudates at 25°C. Cysts placed in 5-ml water served as control. The emerged juveniles were counted and removed at 7-day interval. Root exudates and sterilized water were added to the Petri dishes after each observation. The cysts in a batch were gently cut open 36 days after incubation and eggs and juveniles were counted.

In a second experiment, the cysts already incubated in water at 15, 20, 25 and 30°C for 525 days (see effect of temperature for more details) were placed in root exudates of 14-day old pigeonpea plants and incubated at 25°C. After 21 days, the numbers of juveniles which had emerged from these cysts were counted as described.

Effect of cyst wall

The influence of cyst wall fragments and cyst contents was studied at 25°C in Petri dishes in five batches. The treatments were: free eggs obtained from 15 cysts plus fragments of cyst wall and other cyst contents; free eggs from 15 cysts without fragments of cyst wall and cyst

contents; and 15 cysts. The emerged juveniles were counted at 7-day intervals. The numbers of unhatched eggs in the cyst and in other treatments were counted under stereo microscope 36 days after incubation.

Statistical analysis

The percentage emergences of *H. cajani* J2 were calculated in each experiment. Data were arcsin transformed and subjected to two way or one way analysis of variance depending on the number of factorial treatments. The treatment means were compared by least significant difference technique at 5% and 1% levels of significance. Original (in parentheses) and arcsin transformed data are in Tables 1 to 5.

Results and discussion

Effect of temperature

In the first experiment, J2 emergence was significantly greater from egg sacs (P =0.01) than from white or brown cysts (Table 1). Juvenile emergence from egg sacs and white cysts was significantly greater at 25 and 30°C than at 15 and 20°C. However, J2 emergence from brown cysts was greater at 20 and 25°C than at 15 and 30°C.

Table 1. Effect of four incubation temperatures on emergence of *H. cajani* juveniles from egg sacs, and white and brown cysts after 21 days.

Cysts/ Egg sacs	% juvenile emergence at (°C) 15	20	25	30
Egg sac	34.6 (32.3)	57.8 (71.5)	75.1 (93.2)	72.3 (90.4)
White cyst	1.1 (0.1)	26.7 (21.4)	60.6 (75.1)	57.8 (71.3)
Brown cyst	2.6 (0.3)	20.4 (12.4)	18.3 (10.8)	8.4 (2.2)
LSD (cysts/egg sac x temperature)		P= (0.05) 4.29	P =(0.01) 7.42	

In the second experiment, the rate of emergence of J2 from brown cysts differed with incubation temperature (Fig. 1). Emergence of J2 continued throughout the entire period of 525 days. Initially, emergence was quicker at 20°C than at 25 and 30°C. However, a sudden increase in emergence occurred at 30°C after 65 days of incubation and number of emerged J2 at 30°C after 250 days of incubation was significantly greater than at other temperatures. The rate of emergence at 25°C increased gradually after 365 days of incubation and by the 490th day the number of emerged J2 at 25°C was greater P= 0.05) than that at 20°C. Emergence at 15, and 20°C continued steadily. Cumulative emergence after 525 days was slightly above 50% at 25 and 30°C, 45% at 20°C, and 5% at 15°C (Table 2). After 525 days of incubation, 41-93% juveniles, 0.1-2.1% eggs in embryonic stages were still found within cysts, and 5-10% eggs had fungus infection.

Table 2. Effect of incubation temperature on juvenile (J2) emergence from *H. cajani* cysts over a 525 day period.

Temperature (°C)	J2 emerged (%)	J2 in cysts (%)	J2 in eggs (%)	Eggs in embryonic stages (%)
15	12.7 (5.0)	52.8 (63.0)	32.7 (29.8)	0.0 (0.0)
20	41.9 (44.6)	12.7 (6.3)	42.9 (46.4)	2.4 (0.6)
25	46.9 (53.3)	31.9 (28.6)	19.5 (12.6)	6.2 (2.0)
30	46.1 (51.9)	29.7 (25.7)	26.8 (21.0)	0.7 (0.1)
LSD (P=0.05)	4.89	7.44	6.32	3.20

Effect of host age

There was a significant interaction between plant age and incubation temperature for emergence of J2 from cysts. A greater number of J2 emerged at 25 and 30°C from cysts produced on 30-day old plants than from cysts produced on older plants (Table 3). Juveniles emerged (P= 0.05) in greater

Table 3. Effect of pigeonpea host age and cyst incubation temperature on emergence of *H. cajani* juveniles

Host age (days)	% juvenile emergence at (°C) 15	20	25	30
30	16.9 (8.6)	37.3 (37.2)	73.2 (91.1)	71.3 (89.0)
60	8.6 (2.3)	47.8 (54.7)	53.4 (64.1)	51.0 (60.2)
90	7.7 (1.8)	45.5 (50.8)	56.8 (69.3)	49.2 (57.3)
120	3.0 (0.4)	53.5 (64.4)	59.1 (72.4)	66.0 (83.0)
150	11.2 (4.1)	45.1 (50.0)	59.8 (74.5)	52.8 (63.1)
LSD		$P = 0.05$	$P = 0.01$	
Host age x temperature		7.31	12.46	

numbers at 25 and 30°C than at 20°C from cysts produced on 30-day old plants, whereas emergence from cysts produced on older plants was less temperature sensitive. Greater number of J2 emerged within 30 days from cysts produced on very young (30-day old) and old (120-, and 150 day old) plants than from cysts produced on young (60-day old) and maturing (90-day old) plants within this period. Interaction between temperature and incubation period was, however, not significant 30 days after incubation.

Effect of root exudates

Root exudates stimulated J2 emergence (Table 4). Exposure of cysts to root exudates for about two weeks was required to enhance the rate of emergence. The number of emerged J2 was 4.5- times greater (P=0.05) from cysts incubated in root exudates for 15-36 days than from cysts kept in water.

In the second experiment, root exudate triggered emergence of J2 from cysts already incubated for 525 days in water. About 5% juveniles from cysts incubated at 15°C and 20°C, 6% from cysts incubated at 25°C, and 22% from cysts incubated

Table 4. Effect of pigeonpea root exudates on the emergence of *H. cajani* juveniles from brown cysts incubated at 25°C for 36 days.

Treatment	J2 emerged (%)	J2 in cysts (%)	J2 in side eggs (%)	Eggs in embryonic stages (%)
Root exudate	29.9 (25.0)	53.1 (63.8)	17.9 (9.9)	0.0 (0.0)
Water	12.9 (5.6)	56.9 (69.7)	25.2 (18.8)	5.1 (1.6)
LSD (P=0.05)	4.08	NS	5.27	3.71

at 30°C emerged within three weeks of exposure to the root exudate. No juvenile emergence occurred in water during this period. Interestingly, emergence was influenced by the temperature at which the cysts were previously incubated.

Effect of cyst wall

Emergence of juveniles from free eggs was 2.9 and 13.9 times greater compared with emergence from free eggs with fragments of cyst wall plus other cyst contents in the Petri dish, and from encysted eggs, respectively (Table 5). Juveniles continued to emerge from free eggs over the 36 day incubation period, but J2 did not emerge from eggs within cysts or free eggs plus cyst wall fragments after 13 days of incubation (Fig. 2).

Table 5. Effect of cyst wall fragments and cyst contents on emergence of *H. cajani* juveniles.

Treatment	Juveniles emerged (%)	J2 in-cysts (%)	J2 in eggs (%)	Eggs in embryonic stages (%)
Encysted eggs	9.9 (3.6)	57.7 (69.1)	24.4 (24.5)	0.0 (0.0)
Free eggs + cyst contents	24.6 (17.4)	0.0 (0.0)	76.2 (78.6)	3.7 (2.1)
Free eggs - cyst contents	45.1 (50.2)	0.0 (0.0)	36.4 (35.0)	13.7 (7.1)
LSD (P=0.05)	17.79		9.49	7.53

Our results suggest that several factors, including temperature, host age, cyst wall and cyst contents, regulate J2 emergence in PPCN. Emergence behaviour is complex and is apparently governed by interactions of many factors. Emergence of J2 is also regulated by host age (Ellenby & Smith, 1697; Gaur *et al.*, 1992, Hill & Schmitt, 1989; Singh, 1993; Tefft & Bone, 1985) and cyst contents (Kaul, 1962; Okada, 1971; Okada, 1972; Shepherd, 1962) in other cyst nematode species, and our data indicated that PPCN cyst contents contained emergence inhibitors.

We observed that some encysted PPCN juveniles were stimulated by host root exudates, while some were stimulated neither by optimum temperature nor by root exudates and emerged gradually at a slow rate with passage of time. There is little information on physiology of hatching in PPCN. However, these results and previous reports indicate that the emergence pattern in PPCN is influenced considerably by environmental stimuli perceived by the female. Greater numbers of eggs are laid in the egg sacs when environmental conditions of temperature, moisture, nutrition etc. are favourable. For example, it has been observed that PPCN cysts produced on pigeonpea plants grown under favourable conditions in the glasshouse are usually smaller than cysts produced on pigeonpea plants growing under low-input rainfed conditions in the field, and that large proportions of the eggs are laid in the egg sacs (Gaur *et al.*, 1992; Singh, 1993). Eggs laid in the cysts have significance for survival under prolonged unfavourable conditions (Sharma & Swarup, 1984). Regardless of optimum conditions for emergence, a limited number of juveniles would not emerge from cysts; if all juveniles emerged at the same time and no host roots were available, the nematode might become locally extinct. All these mechanisms of temperature-, host-, and time-mediated delays in emergence of PPCN juveniles have survival value and adaptive significance. Such mechanisms must have evolved gradually but have persisted because of their survival value. Arrested juvenile emergence under favourable environmental conditions is evidence of diapause in PPCN (Sharma & Swarup, 1984). This behaviour of arrested juvenile emergence has been reported in other cyst nematode species (Hominick *et al.*, 1985; Ishibashi *et al.*, 1973; Okada, 1971; Okada, 1972; Yeates, 1973), and in cyst nematode species occurring in India (Sharma & Swarup, 1984). PPCN has incorporated these strategies to survive under semi-arid tropical conditions. Further studies on the chemistry and genetics of emergence behaviour will strengthen our understanding of emergence of juveniles of PPCN.

References

Clarke .A .J., Perry, R.N. 1977. Hatching of cyst nematodes, *Nematologica* 23:350-368.

Cobb, N.A. 1918. Estimating the nematode population of soil. U.S. Government Printing Office; Washington, D.C. U.S. Department of Agriculture Technology Circular I.

Ellenby, C. and Smith, L. 1967. Emergence of larvae from new cysts of the potato root eelworm, *Heterodera rostochiensis. Nematologica* 13:273-278.

Evans, K. 1979. Is there genetic control of hatching in cyst nematodes? *Journal of Nematology* 11: 293-317.

Gaur, H.S., Perry, R.N., and Beane, J. 1992. Hatching behaviour of six successive generations of the pigeonpea cyst nematode, *Heterodera cajani*, in relation to growth and senescence of cowpea, *Vigna unguiculata. Nematologica* 38: 190-202.

Hill, N.S., and Schmitt, D.P. 1989. Influence of temperature and soybean phenology on dormancy induction of *Heterodera glycines. Journal of Nematology* 21:361-369.

Hominick, W.M., Forrest, J.M.S., and Evans, A.A.F. 1995. Diapause in *Globodera rostochiensis* and variability in hatching trials. *Nematologica* 31:159-170.

Ishibashi, N., Kondo, E., Muraoka, M. and Yokoo, T. 1973. Ecological significance of dormancy in plant-parasitic

nematodes; 1. Ecological difference between eggs in gelatinous matrix and cyst of *Heterodera glycines*. *Applied Entomology and Zoology* 8:53-63.

Kaul, R. 1962. Untersuchungen uber einen ans Zysten des kartoffelnematoden (*Heterodera rostochiensis* Woll.) isolienten phenolischen Kompelx. *Nematologica* 8:288-292.

Koshy, P.K., and Swarup, G. 1971. Factors affecting emergence of larvae from cysts of *Heterodera cajani* Koshy 1967. *Indian Journal of Nematology* 1:209-219.

Koshy, P.K. and Swarup, G.1972. Susceptibility of plants to pigeonpea cyst nematode. *Indian Journal of Nematology* 2:1-6.

Okada, T. 1971. The hatching response of the soybean cyst nematode, *Heterodera glycines* Ichinohe (Tylenchida: Heteroderidae). *Applied Entomology and Zoology* 6:91-93.

Okada, T. 1972. Hatching inhibitory factor in the cyst contents of the soybean cyst nematode, *Heterodera glycines*. *Applied Entomology and Zoology* 7:99-102.

Perry, R.N. and Clarke, A.J. 1981. Hatching mechanisms of nematodes. *Parasitology* 83:435-449.

Sharma, S.B. and Nene, Y.L. 1986. Extraction of *Heterodera cajani* cysts using modified sieving technique. *Indian Journal of Nematology* 16:123-124.

Sharma, S.B. and Nene, Y.L. 1987. A technique to extract *Heterodera cajani* eggs from egg-sacs. *International Pigeonpea Newsletter* 3:11-13.

Sharma, S.B., Nene Y.L., Reddy, M.V. and Mc Donald, D. 1993. Effect of *Heterodera cajani* on biomass and grain yield of pigeonpea on vertisol in pot and field experiments. *Plant Pathology* 42:163-167.

Sharma, S.B., Smith, D.H. and McDonald, D. 1992. Nematode constraints of chickpea and pigeonpea production in the semi arid tropics. *Plant Disease* 76:868-874.

Sharma, S.B. and Swarup, G. 1984. *Cyst forming nematodes of India.* New Delhi: Cosmo Publications. 152 pp.

Shepherd, A.M. 1962. The emergence of larvae from cysts in the genus *Heterodera.* Commonwealth Agricultural Bureaux, St. Albans, U.K. Technical Communication No. 32.

Shepherd, A.M. and Cox, P.M. 1967. Observations on periodicity of hatching of eggs of the potato cyst nematode (*Heterodera rostochiensis* Woollenweber). *Annals of Applied Biology* 60:143-150.

Singh, M. 1993. *Management of pigeonpea cyst nematode, Heterodera cajani.* Legumes Pathology Report 21. India, Andhra Pradesh: International Crops Research Institute for the Semi-Arid Tropics. 144 pp.

Singh, M. and Sharma, S.B. 1994. Temperature effects on development and reproduction of *Heterodera cajani* on pigeonpea. *Journal of Nematology* 26:241-248.

Tefft, P.M. and Bone, L.W. 1985. Plant induced hatching of eggs of the soybean cyst nematode, *Heterodera glycines*. *Journal of Nematology* 17:275-279.

Yeates, G.W. 1973. Annual cycle of root nematodes on white clover in pasture. I. *Heterodera trifolii* in a yellow-grey earth. *New Zealand Journal of Agricultural Research* 16: 569-574.

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