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Short note

HATCHING OF FOUR SUCCESSIVE GENERATIONS OF *HETERODERA SORGHI* IN RELATION TO THE AGE OF SORGHUM, *SORGHUM VULGARE*

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The contrasting hatching behaviour of cyst nematodes may be related to different strategies for survival in the absence of a host crop. Many species of cyst nematodes from tropical regions complete several generations during the host growing season and demonstrate variations in hatching behaviour. The hatching response of cysts of *Heterodera cajani* changes with the age of the host plant on which they are produced; as the host plant begins to senesce, a greater percentage of the eggs in the cysts depend on root diffusates to initiate hatch (Gaur *et al.,* 1992). With the onset of plant senescence, females of *H. sacchari* develop into cysts containing a large proportion of J2 which do not hatch and some which depend on root diffusate for hatch stimulation (Ibrahim *et al.,* 1993). Thus, in later generations of *H. cajani* and *H. sacchari,* a large proportion of J2 do not hatch ensuring that these dormant individuals survive the intercrop period. By contrast, J2 of *H. oryzicola* are always dependent on host diffusate stimulation for substantial hatch, irrespective of generation (Ibrahim *et al.,* 1993).

The sorghum cyst nematode, *H. sorghi,* has a life cycle of 20-24 days at 28-36°C (Srivastava, 1985) and produces up to four generations per season; small eggsacs may be present but they contain few, if any, eggs (Srivastava & Chawla, 1993). Cysts of *H. sorghi* hatch readily in water (Srivastava, 1985) although, in preliminary tests, we found considerable variation in the hatch from different batches of cysts. The present study investigated the hatching behaviour of successive generations of *H. sorghi* in response to standard sorghum root diffusate.

Materials and methods

A population of *H. sorghi* from New Delhi was cultured on *Sorghum vulgare* cv. Lindse 555 in a glasshouse at 26 ± 2°C with 14 h daylight. Cysts from 90 day old plants were placed in glass distilled water (GDW) at 28°C and hatched J2 were used as the inoculum within 72 h of hatching. J2 were inoculated, at the rate of 1000 J2 per pot, around the roots of 10 day-old seedlings of sorghum growing in a steam sterilised sand/loam mix in 15 cm diameter plastic pots. Inoculated pots were kept in the glasshouse under the same conditions as described above. Cysts were extracted using a fluidising column (Trudgill *et al.,* 1973) at intervals of approximately 5 weeks, at the completion of each generation. Only cysts just beginning to tan were selected to avoid those from an earlier generation; any eggsacs found were carefully removed and discarded. Cysts were stored in GDW at 15-20°C for about 10 days until they were fully tanned; there was no hatch during this period.

Sorghum root diffusate (SRD) and soil leachate (SL) were collected from 10 cm plastic pots of sterile sand/loam with and without plants, respectively, after 4 weeks (Fenwick, 1949) and stored at 2°C. Pilot experiments indicated that 4 week-old plants gave SRD with optimum hatching activity. Batches of ten cysts were placed in excavated glass blocks containing 2 ml of either GDW, SRD, 10 % SRD in GDW (0.1 SRD) or SL and kept at 28°C; the experiment was replicated three times. Hatched juveniles were removed and counted at 3 and 7 days and then at weekly intervals until the weekly hatch rate was less than one J2 per cyst per week. At each counting interval fresh test solution was added. At the end of the test, the cysts were broken open, remaining unhatched viable eggs were counted and percentage hatch was calculated; the mean number of eggs per cyst for each generation was also recorded. The same procedure was followed for each generation. The percentage hatch data were subjected to factorial analysis of variance for incubation period, test solutions and generations after arcsin transformation of percentages. The means were compared for significant differences at the 5 % level of probability.

Results and discussion

Four successive generations were completed during the life of the host plants. The nematode life cycle of about 5 weeks is longer than the 20-24 days reported by Srivastava (1985); the difference is probably due to the lower temperature at which the plants were grown in the present work. The mean number of eggs per cyst increased from 224 in the first generation (G1) to 314 in G2 and reached a maximum of 501 in G3; the number declined to 281 in cysts of G4 produced on senescing plants.

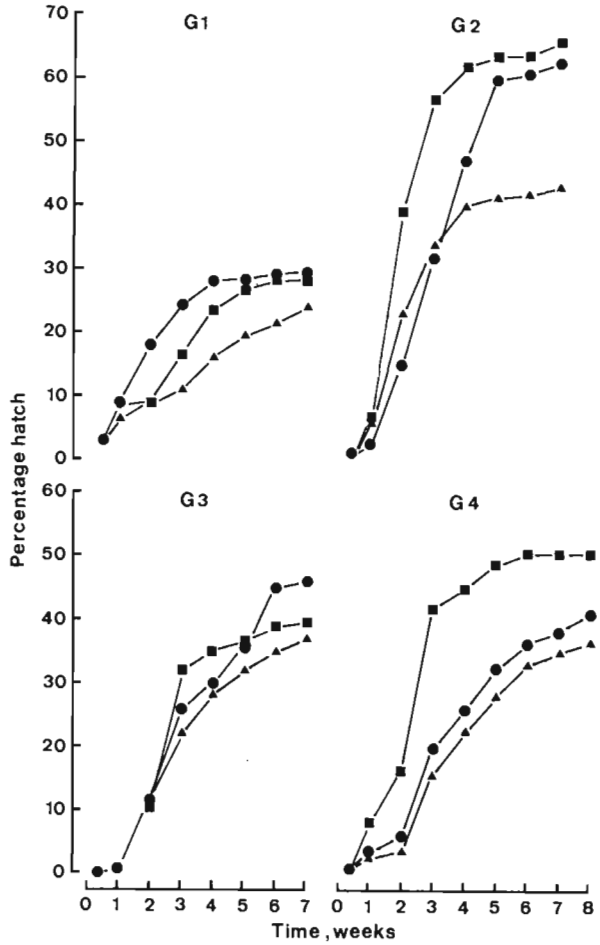

Fig. 1. *Cumulative percentage hatch from cysts of four successive generations (G1 - G4) of* Heterodera sorghi *exposed to sorghum root diffusate* (●), *0.1 sorghum root diffusate* (■) *and soil leachate* (▲).

There were differences between treatments and generations in the percentage hatch (Fig. 1). The poor hatch of *H. sorghi* in G1 of less than 30 % in any treatment was also found with cysts of *H. sacchari* from young plants (Ibrahim *et al.*, 1993). Results for the hatch in GDW did not differ significantly from the hatch in SL so only the data for hatch in SL are presented; Gaur *et al.* (1992) noted that SL is the more appropriate control for comparison with root diffusates. The generation giving the maximum hatch in SL was G2 with 43.2 % compared with 23.8 % (G1), 36.8 % (G3) and 36.1 % (G4). Similarly, the maximum hatch in host root diffusate was from G2 cysts, with 65.7 % hatch in 0.1 SRD and 62.0 % hatch in SRD after 7 weeks. These data confirm that *H. sorghi* does not require stimulation by host root diffusates for substantial hatch. Although hatch of this species is not dependent on stimulation by diffusates and in G1 there is no significant difference between treatments, the use of SRD significantly enhanced hatch in G2, G3 and G4 by 18.3, 9.3 and 4.6 %, respectively, compared to hatch in SL. Hatching of *H. cajani* was significantly increased by host root diffusates only in the fifth and sixth generations, produced on senescing plants (Gaur *et al.*, 1992); this was interpreted as part of a survival strategy with a percentage of the cyst contents prepared for survival in the absence of a host.

In the first three generations of *H. sorghi* there were no significant differences between the hatch in diluted and undiluted SRD but, in G4, hatch was significantly greater in 0.1 SRD than in SRD with total cumulative hatches of 50.7 % and 40.7 %, respectively. The SRD used was the same for each generation so the 10 % enhancement of hatch in G4 by dilution of diffusate is likely to be associated with some, as yet unexplained, change in the eggs. As with *H. goettingiana* (Perry *et al.*, 1981) and *H. cajani* (Gaur *et al.*, 1992) there does not appear to be any major hatch inhibitor in host root diffusates.

There was no clear pattern in the rate of hatch although, in general, the maximum rate in all treatments occurred during the second week in G2 but was delayed until the third week for G3 and G4 (Fig. 1). This may be related to increasing host maturity but the influence of the plant is unlikely to be adverse up to and including G3 since the mean number of eggs per cyst increased up to G3. In G4, the egg content of the cysts was considerably reduced and this may be associated with plant senescence; a similar increase in cyst content on actively growing plants followed by a decline on senescing plants was found with successive generations of *H. cajani* on cowpea (Gaur *et al.*, 1992).

From 57 to 76 % of eggs in cysts of *H. sorghi* did not hatch in SL in any generation; even when stimulated by SRD 34 to 60 % of eggs did not hatch. Thus, there is clearly a large " carry-over " of unhatched J2 to the next host crop. In tropical and subtropical regions, where sorghum, maize and other host crops are grown, there are usually very dry and/or cold periods following harvest. The J2 of *H. sorghi* that are refractory to hatching stimulation are presumably in diapause, although this needs to be confirmed using experimental protocols recommended by Hominick *et al.* (1985). The phenomenon of dormancy is well documented (Evans & Perry, 1976; Perry, 1989; Zheng & Ferris, 1991) and this appears to be an example of a host mediated effect as found by Hominick (1986) in cysts of *G. rostochiensis*.

The present work indicates the presence of three kinds of eggs in *H. sorghi* cysts : ones that hatch freely in soil leachates, those that require stimulation from host root diffusates to hatch and a large percentage which do not hatch immediately; the proportions of these three types of eggs change with successive generations. The host plant influence on hatching of cyst nematodes needs to be examined in detail, firstly, to determine whether hatching response of J2 is a consequence of earlier changes in the plant which may have affected, for

example, the nutrient balance of the nematode syncytium and thus the food uptake of the female producing the J2 and, secondly, to determine the changes in the plant which lead to production of active diffusate at only certain stages of plant growth (Perry *et al.*, 1981).

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