egg and cyst to survice in a dormant state (Ibrahim & Perry, 1992). The host plant affects the physiology of cyst nematodes (Perry, 1989) and modifications to plant growth, for example, may interfere with the ability of encysted nematodes to become dormant at the end of the host growing season. Thus, there is a need to determine the variations in hatching patterns of eggs in cysts produced at different phases of host growth. The present work examines the hatch in rice root diffusate, banana root diffusate, soil leachate and distilled water from cysts of *H. sacchari* and *H. oryzicola* which had been harvested from rice plants at monthly intervals. These two species were chosen for comparison because of their differences in eggsac production and their contrasting dependency on root diffusates for hatching from cysts.

Materials and methods

Stock cultures of *H. sacchari* and *H. oryzicola* from upland rice in Côte d'Ivoire and Kerala, India, respectively, were maintained routinely on rice cvs IR 36 and Upl Ri-5 in 14 cm diameter free-draining plastic pots in a heated glasshouse (25-35 °C) with a minimum photoperiod of 10 h. Cysts were extracted from soil using a fluidising column (Trudgill *et al.*, 1973).

Five seeds of rice, cv. Upl Ri-5, were sown in a clay loam soil in each of 40 14 cm diameter plastic pots and 24 100 ml volume plastic pots (« vacapots » : H. Smith Plastics). Fourteen days after sowing, batches of 35 cysts, packaged with moist sand in 45 μm mesh nylon netting, were placed immediately below the soil surface adjacent to the seedlings. Twenty pots and twelve vacapots were inoculated for each species and cysts used for inoculation were removed after ten days. Seedlings in vacapots were used to monitor nematode development.

On five occasions, at intervals of 30 days, mature, new (mid-brown colour) cysts were recovered (Trudgill *et al.*, 1973) from pots containing rice plants. Hatching bioassays were done on four batches of 25 cysts per species in 2 ml of each test solution in excavated glass blocks at 25 °C, except for *H. sacchari* where limited numbers of cysts after 30 days restricted the bioassays on this first extraction to four batches of 16 cysts per test solution.

Five solutions were used: glass distilled water (GDW), soil leachate (SL), full strength solutions of banana root diffusate (BRD) and rice root diffusate (RRD) and a 10 % solution (v/v) of RRD in GDW (RRD 1:9). SL, BRD (from one, two month old sword sucker cv. Dwarf Cavendish per 25 cm diameter pot) and RRD (from five, one month old plants of cv. Upl Ri-5 per 14 cm diameter pot) were collected in a similar manner to the method of Fenwick (1949).

Counts of hatched second stage juveniles (J2) were made weekly for 20 weeks. At each count, J2 were removed and fresh solutions were added from stocks held at 4 °C. At the end of each test, cysts were broken open and the number of unhatched J2 were counted to determine the percentage hatch. Data were analysed by two way analysis of variance after logit transformation of percentages. Treatment effects were split into three contrasts representing a comparison between the average of GDW and SL and root diffusates overall, a comparison between GDW and SL, and a comparison between the root diffusates.

Results

Five successive extractions at 30 days intervals of *H. sacchari* and *H. oryzicola* cysts were completed during the life of the host plants; these are referred to as extractions 1 to 5. There were marked differences between the two species in the variation in cyst content and the hatching response of successive batches.

The mean number of eggs per cyst of the two species at each 30 day period of plant growth (Fig. 1) was determined at the end of each series of hatching tests. The number of eggs per cysts of *H. sacchari* reached a maximum at extraction 2 and declined thereafter with the fewest eggs per cysts being recorded from cysts produced on senescing plants; cyst contents ranged from 91 to 222 eggs per cyst. By contrast, cysts of *H. oryzicola* contained fewest eggs at the first extraction time (91 eggs per cyst) with the maximum number at extraction 4 (175 eggs per cyst).

The mean number of eggs per cyst of the two species of *H. sacchari* (Fig. 2 A) and *H. oryzicola* (Fig. 2 B) from each of the five sampling intervals shows marked differences between the species in their hatching response to the five test solutions. The hatch from cysts of *H. sacchari* from the first extraction was negligible; in all treatments the hatch was less than 15 %. Cysts from extractions 2 and 3 gave substantially increased hatches of between 40 % and 58 % in soil leachate and diluted

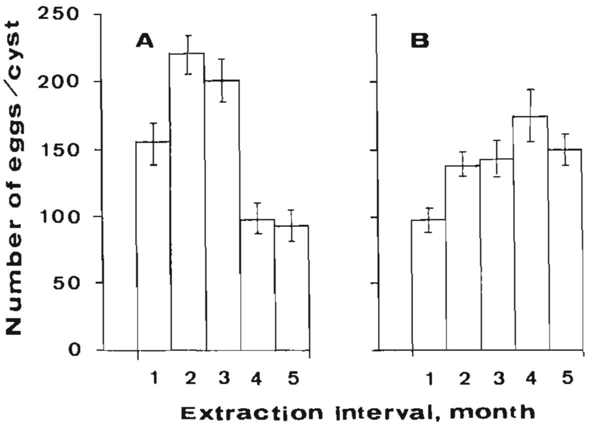
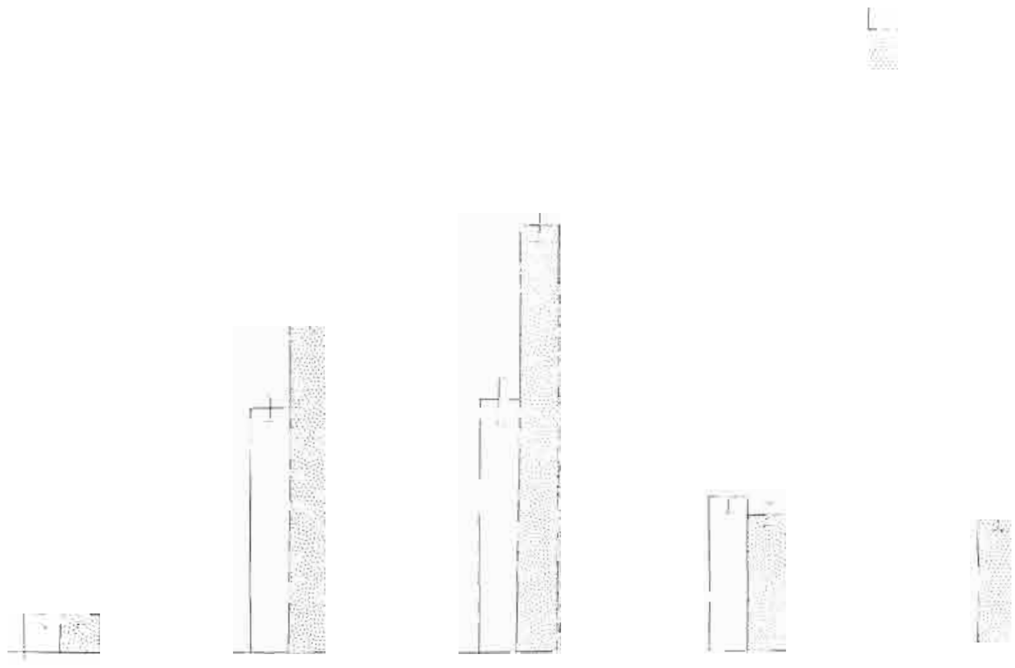


Fig. 1. The number of eggs per cyst at each extraction time (see text) of *Heterodera sacchari* (A) and *H. oryzicola* (B).



and undiluted diffusates; the hatch in GDW (30 % and 32 % in extractions 2 and 3, respectively) was markedly less than in other treatments. Overall hatch from cysts of extraction 4 and 5 was significantly less ($P < 0.001$) than from the previous two batches and there is an indication that a proportion of eggs in these cysts, produced on senescing plants, is dependent on hatch stimulation by root diffusates. In addition, a large percentage of the contents of these cysts are refractory to hatch stimulation. There was no indication that diluting RRD had any effect on the hatch from cysts of *H. sacchari*.

One notable feature of the hatching behaviour of *H. sacchari* was the long period over which hatching took place. For example, few juveniles emerged from cysts of the third extraction during the first five weeks of the hatching tests (Fig. 3 B); the rate of hatch increased markedly between weeks 5 and 8 and then declined. Thus, the majority of juveniles hatched after 5 weeks in

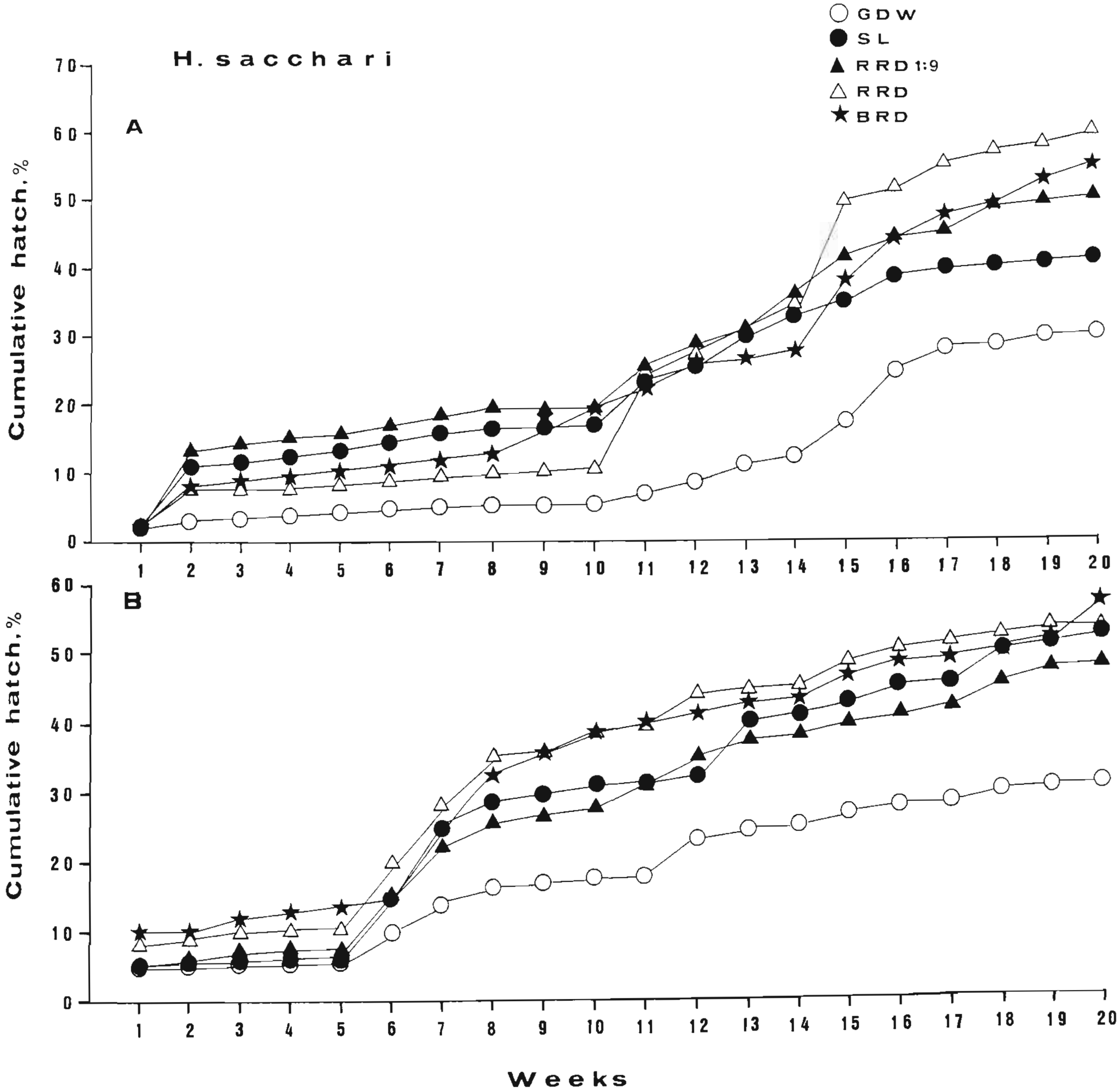


Fig. 3. The cumulative percentage hatch over 20 weeks from cysts of *Heterodera sacchari* obtained at the second extraction (A) and the third extraction (B) and set to hatch in rice root diffusate (RRD), diluted rice root diffusate (RRD 1:9), banana root diffusate (BRD), soil leachate (SL) and glass distilled water (GDW).

the test solutions. Such a delay would have been too long for these juveniles to have been responsible for the new cysts extracted 30 days later; thus, the 30 days extraction interval cannot be correlated with generation time. Hatch from cysts of the second extraction (Fig. 3 A) was also slow during the first ten weeks and subsequently the rate of emergence increased.

At each extraction time, the hatch from cysts of *H. oryzicola* (Fig. 2 B) followed a similar pattern. The hatch in GDW or SL never exceeded 15 % and, at each extraction, the average hatch in GDW and SL was significantly less ($P < 0.001$) than the average hatch in diluted and undiluted root diffusates; there were no significant differences ($P > 0.05$) between hatches in diffusate treatments. Although the total hatch in all test did not exceed 50 % of the viable cyst contents, the hatch was consistent in all batches and there was no evidence that the age of the host plant influenced the hatch from cysts of *H. oryzicola.*

The rate of hatching of *H. oryzicola* was slow except in BRD. Fig. 4 shows the hatching profile for *H. oryzicola* cysts from the fourth extraction; while the hatch in RRD remained nearly constant, the hatch in BRD was rapid between weeks 3 and 5 and slowed thereafter.

Discussion

Heterodera sacchari and *H. oryzicola* have markedly different hatching behaviours. Irrespective of the age of the host plant producing cysts, *H. oryzicola* is dependent on root diffusates to induce substantial hatch. Apart from cysts produced after 30 days, diffusates from banana and rice stimulated hatch equally well; banana has been recorded as a host for *H. oryzicola* (Charles & Venkitesan, 1984). By contrast, the dependence of *H. sacchari* on diffusates is less easily defined. For cysts extracted 60 days or more after inoculation, hatch in GDW was always significantly less than in diffusates; however, soil leachate elicited substantial hatch from cysts of the second and third extraction. A small proportion of eggs in cysts from extraction 4 and 5 are dependent on root diffusates for hatch and the total percentage hatch from these cysts was considerably less than from the earlier extractions. Comparison between cysts produced on healthy and senescing plants indicates that cysts from senescing plants contain approximately 20 % more eggs which are refractory to hatching stimuli and an additional 10-15 % which require diffusate stimulation for hatch.

These 30-35 % of viable J2 probably ensure persistence of the species between host crops. Juveniles which require diffusate to stimulate hatch but hatch immediately on stimulation are quiescent, whereas those juveniles which are refractory to host stimuli, even when favourable conditions are present, are likely to be in a state of diapause (Evans & Perry, 1976). Diapause is an effective method of ensuring synchrony between host

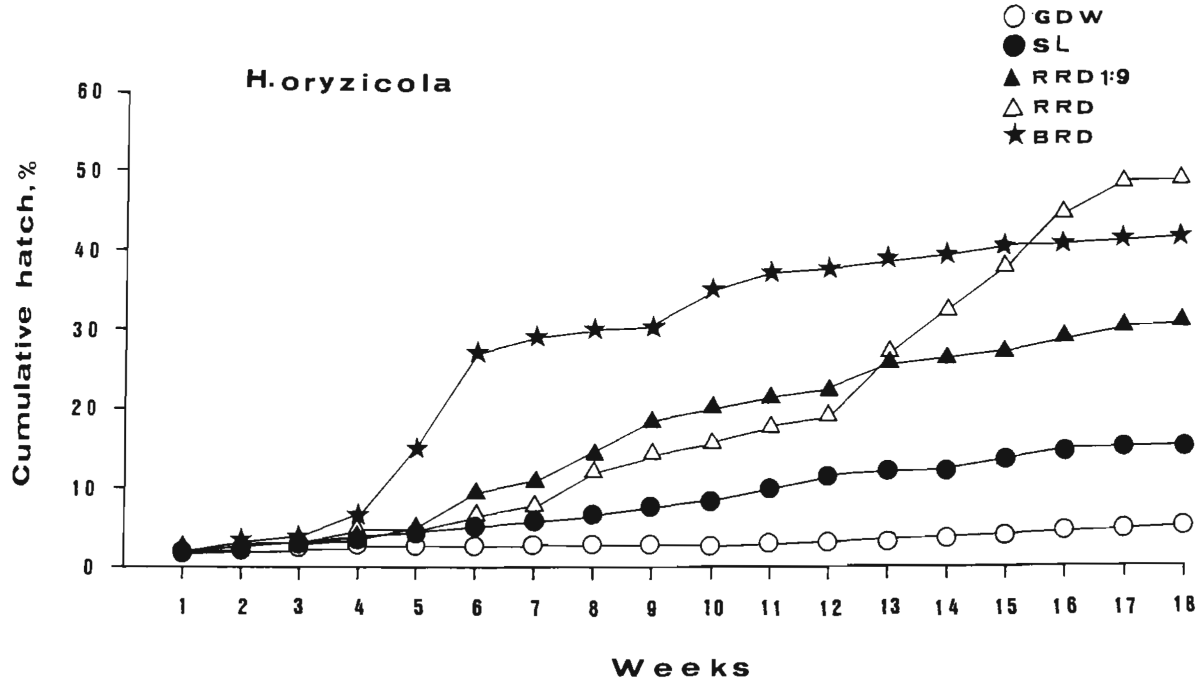


Fig. 4. The cumulative percentage hatch over 20 weeks from cysts of *Heterodera oryzicola* obtained at the fourth extraction and set to hatch in rice root diffusate (RRD), diluted rice root diffusate (RRD 1:9), banana root diffusate (BRD), soil leachate (SL) and glass distilled water (GDW).

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