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Hatching behaviour of the rice cyst nematodes *Heterodera sacchari* and *H. oryzicola* in relation to age of host plant

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Summary – Hatching of the rice cyst nematodes, *Heterodera sacchari* and *H. oryzicola,* has been examined in rice root diffusate, banana root diffusate, soil leachate and distilled water using cysts which had been collected from rice plants at five successive monthly intervals. The two species 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. The dependence of *H. sacchari* on diffusates is less easily defined; it is only with cysts from the last two extractions that a small proportion of eggs were dependent on root diffusates for hatch and the total percentage hatch from these cysts was considerably less than from cysts collected from younger plants. The hatching behaviour of the two species are discussed in the context of their contrasting strategies for survival in the absence of a host crop.

Résumé – ***Modalités de l'éclosion chez les nématodes à kystes du riz* Heterodera sacchari *et* H. oryzicola *en fonction de l'âge de la plante hôte*** – L'éclosion des nématodes à kystes du riz *Heterodera sacchari* et *H. oryzicola* a été observée dans un diffusat radiculaire de riz, un diffusat radiculaire de bananier, un percolat de sol et de l'eau distillée en utilisant des kystes récoltés sur des pieds de riz à cinq périodes séparées par un intervalle d'un mois. Les deux espèces ont un comportement très différent. Quel que soit l'âge de la plante sur laquelle les kystes ont été produits, *H. oryzicola* est sous la dépendance des diffusats radiculaires pour parvenir à une éclosion importante. La dépendance de *H. sacchari* vis-à-vis des diffusats est moins aisée à définir; chez les kystes provenant des deux dernières extractions, une faible proportion des œufs dépend des diffusats radiculaires pour éclore, et le taux d'éclosion total de ces kystes est considérablement plus faible que celui des kystes récoltés sur des plantes plus jeunes. Le comportement de ces deux espèces lors de l'éclosion est discuté dans l'optique des différences de stratégie assurant leur survie en l'absence de plante hôte.

Key-words : Nematodes, *Heterodera,* rice, hatching, relation to host.

Four species of cyst nematodes infect rice. *Heterodera oryzicola* Rao & Jayaprakash, 1978 and *H. elachista* Ohshima, 1974 are found on upland rice only in Kerala, India and Japan respectively, while *H. oryzae* Luc & Berdon Brizuela, 1961 and *H. sacchari* Luc & Merny, 1963 are more widespread, occurring on upland and lowland rice, principally in West Africa (Bridge *et al.,* 1990). Banana is also a host for *H. oryzae* (Taylor, 1978) and *H. oryzicola* (Charles & Venkitesan, 1984).

Individual females of *H. oryzicola, H. elachista* and *H. oryzae* deposit many eggs into a large eggsac from which the juveniles hatch freely in water. Evidence suggests that juveniles in eggs retained in the cysts require host root diffusates to stimulate hatch (Merny, 1966, 1972; Jayaprakash & Rao, 1982). By contrast, *H. sacchari* rarely has an eggsac and encysted eggs hatch readily in water without the need for hatch stimulation by rice root diffusate (Bridge *et al.,* 1990).

The life cycle of each species of rice cyst nematode is completed in 24-30 days and there are several generations during the host growing season. Merny (1966) considered that the rapid emergence of juveniles from eggsacs of *H. oryzae* caused reinfestation during a rice season. Changing conditions during the growing season could influence the hatching patterns of successive generations. For example, in recent work on the hatching behaviour of the pigeon-pea cyst nematode, *H. cajani* Koshy, 1967, eggs in eggsacs hatched in water and there was no difference between generations; however, the hatch from cysts was similar over the first four generations but the fifth and sixth generations, produced on senescing plants, showed a marked dependency on host root diffusate for hatch (Gaur *et al.,* 1992). The dependency on host diffusates ensures that the juveniles do not hatch in the absence of suitable hosts; they are able to survive between host crops with the protection against environmental extremes afforded by the eggshell and the cyst wall.

The cyst as an ecological unit is clearly essential for survival of cyst nematodes. Juveniles of *H. sacchari, H. oryzae* and *H. oryzicola* have no intrinsic ability to survive desiccation; they require the protection of the

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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

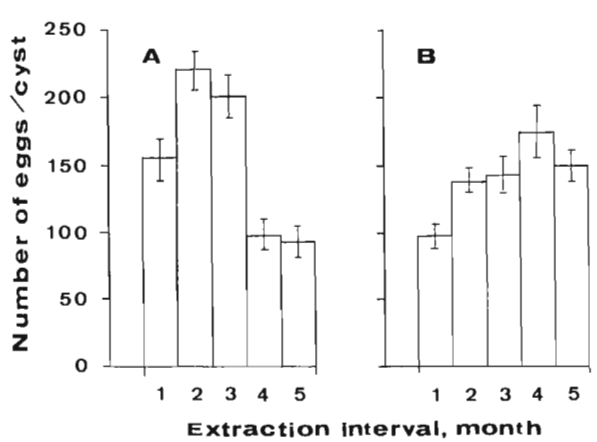

Fig. 1. The number of eggs per cyst at each extraction time (see text) of *Heterodera sacchari* (A) and *H. oryzicola* (B).

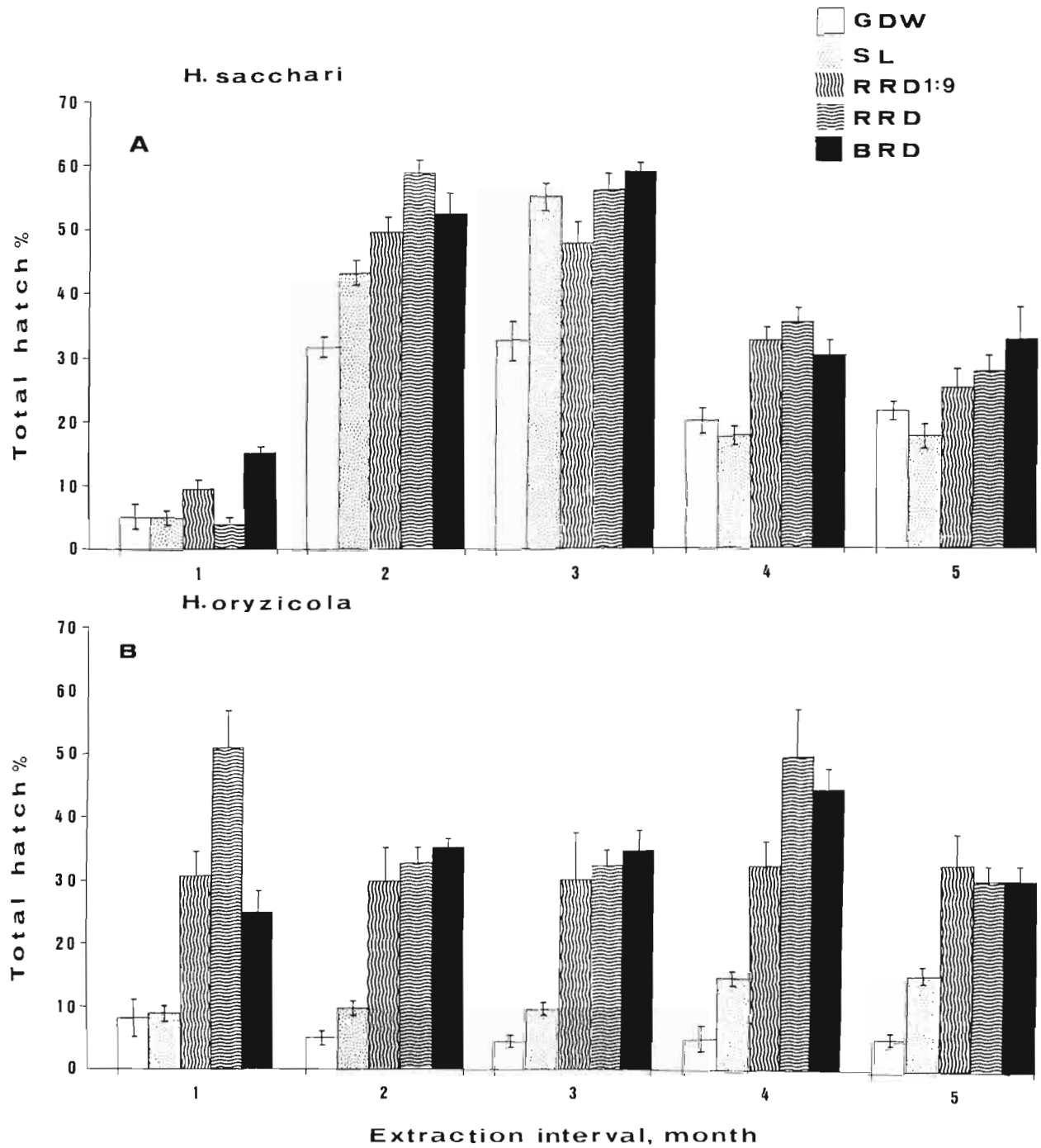

Fig. 2. The total percentage hatch after 20 weeks from cysts of *Heterodera sacchari* (A) and *H. oryzicola* (B) at each extraction time (see text) in rice root diffusate (RRD), diluted rice root diffusate (RRD 1:9), banana root diffusate (BRD), soil leachate (SL) and glass distilled water (GDW).

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

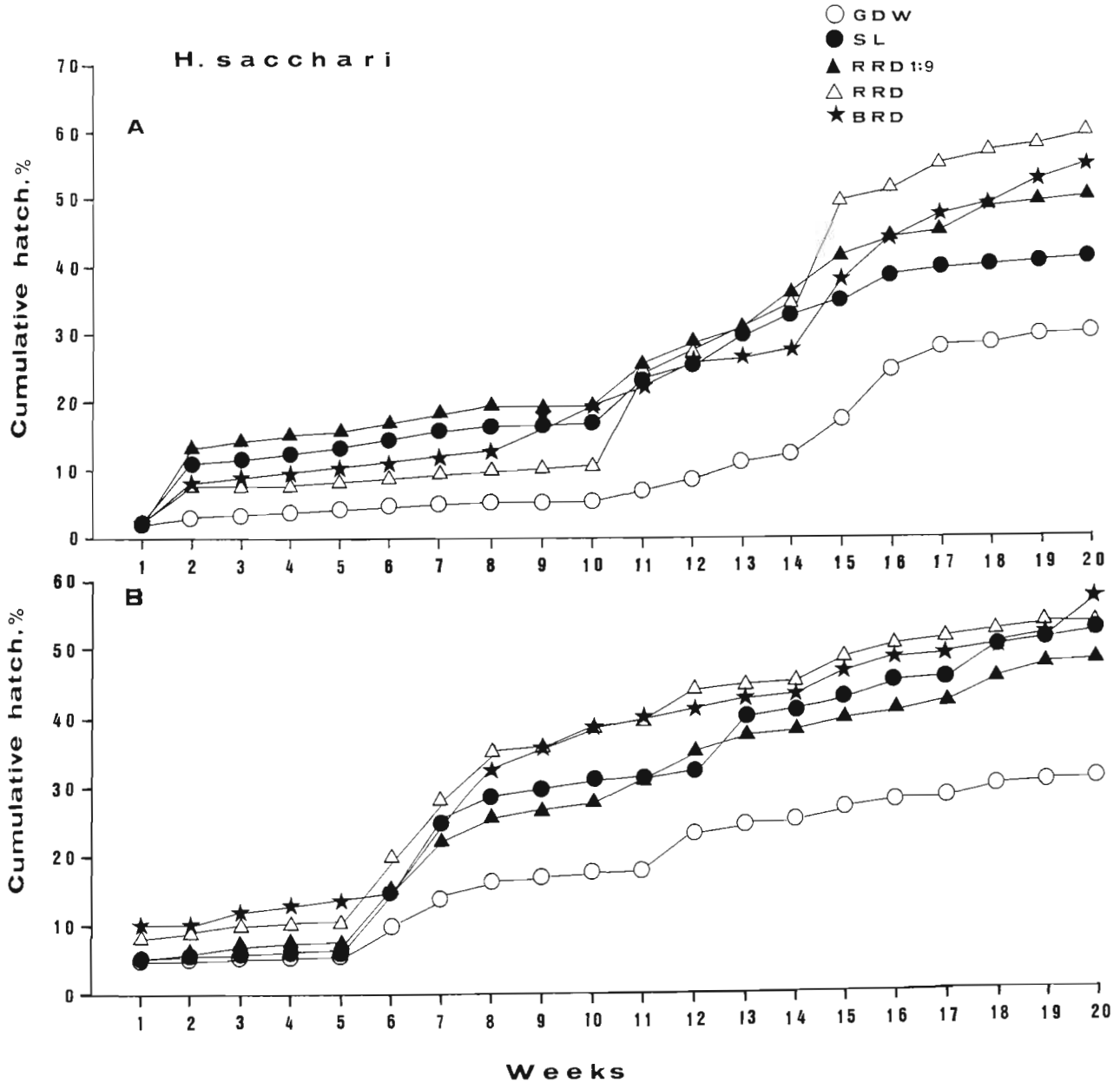

Fig. 3. The cumulative percentage hatch over 20 weeks from cysts of *Heterodera sacchari* obtained at the second extraction (A) and the third extraction (B) and set to hatch in rice root diffusate (RRD), diluted rice root diffusate (RRD 1:9), banana root diffusate (BRD), soil leachate (SL) and glass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

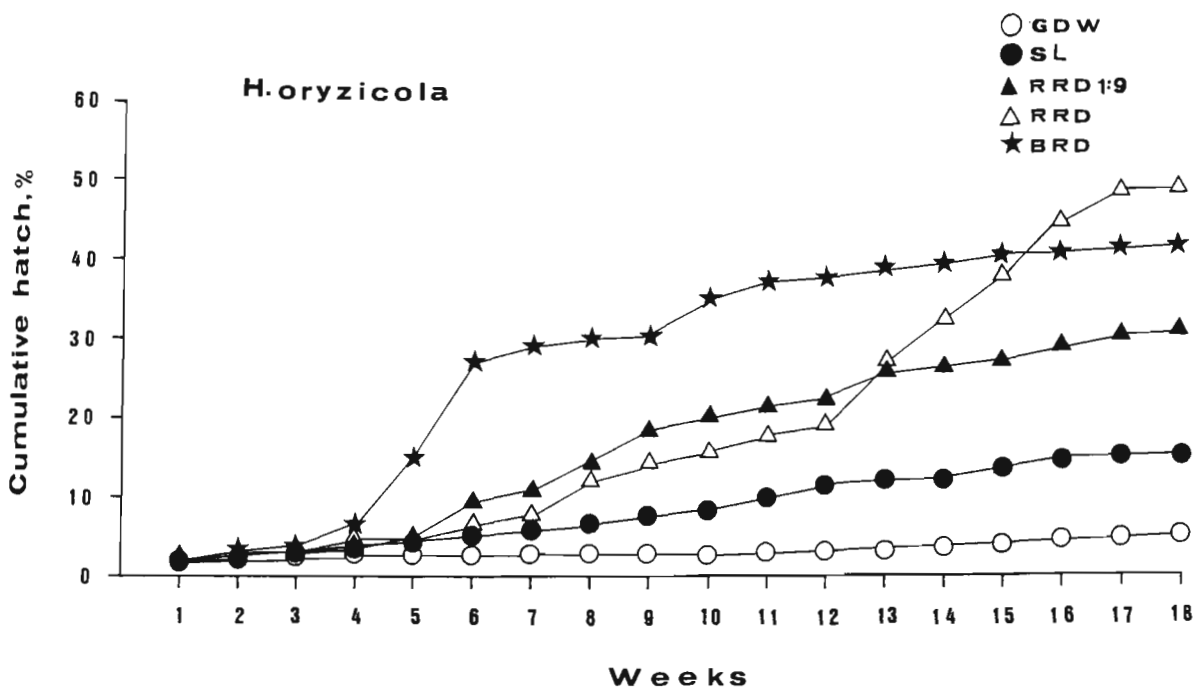

Fig. 4. The cumulative percentage hatch over 20 weeks from cysts of *Heterodera oryzicola* obtained at the fourth extraction and set to hatch in rice root diffusate (RRD), diluted rice root diffusate (RRD 1:9), banana root diffusate (BRD), soil leachate (SL) and glass distilled water (GDW).

and parasite life cycles and has been demonstrated in *Globodera rostochiensis* by Hominick *et al.* (1985) Diapause appears to be initiated by signals passed to the nematode from the plant during the growing season (Hominick, 1986). Similar studies on *H. sacchari* are needed to confirm whether diapause occurs and to determine the factors responsible for its induction.

In these experiments, cysts of both species were exposed to standard root diffusates from one month old rice plants. It is possible that the root difusate activity declines as the rice plant senesces, as has been noted with other host root diffusates (see Perry *et al.*, 1980, for example). If this is the case, then more eggs would remain unhatched at the end of the host's growing season. This aspect warrants investigation as it is likely that the unhatched juveniles could survive in the absence of host crops with the additional protection of the egg shell and the cyst wall. Free juveniles of both *H. sacchari* and *H. oryzicola* are unable to survive severe drying regimes and can only survive as unhatched juveniles (Ibrahim & Perry, 1992). Similarly, the survival of encysted J2 of *H. oryzae* is considerably enhanced compared with free J2 or J2 in eggsacs (Merny, 1972).

In hatching tests with *G. rostochiensis,* inhibitors may be present in root diffusates and dilution of the diffusate reduces the influence of the inhibitors, resulting in increased hatch. The present work indicates that there are no inhibitors present in diffusate from rice plants and, in this respect, diffusate effect on hatching of the two rice cyst nematodes is similar to that of *H. goettingiana* (Perry *et al.*, 1980).

The poor hatch from cysts of *H. sacchari* extracted after 30 days was unexpected. At each extraction, cysts were used at the same stage of development and this was checked on control cysts from vacapot cultures to ensure that eggs contained viable juveniles. However, cysts extracted on day 30 may have had a larger proportion of incompletely embryonated eggs. The hatching tests ran for sufficient time to enable juveniles to hatch if they were in a suitable physiological state; perhaps the unhatched juveniles were in diapause although, where this has been shown in cyst nematodes, diapause usually occurs in J2 in cysts produced on senescing plants. Some cysts from the first extraction had egg sacs, which is unusual for *H. sacchari;* these may provide juveniles for rapid reinfection and formation of the subsequent generation while a proportion of the encysted eggs are refractory to hatch stimulation. The cysts of this first extraction contained very few eggs.

The extended hatching period for *H. sacchari* and *H. oryzicola* means that the extraction intervals cannot be correlated with generations. The extraction interval was 30 days and, although the life cycle has been reported to take 24-30 days, there will be considerable overlaps as juveniles hatch over periods extending to 20 weeks. This is in contrast to *H. cajani,* which has a short development time of 17-22 days and hatching is very rapid, with the majority of J2 hatched by the second week (Gaur *et al.*, 1992); the sampling interval in the work on *H. cajani* was sufficient to allow for the development of the next generation and this could also be defined by the marked flush of new cysts at each successive generation.

Heterodera oryzicola and *H. sacchari* have contrasting hatching behaviours to enhance survival in the absence of a host crop. Host age appears not to affect *H. oryzicola* but is alters the hatching characteristics of *H. sacchari.* With the onset of plant senescence, females of *H. sacchari* develop into cysts containing a large proportion of juveniles which do not hatch and some which depend on root diffusate for hatch stimulation. Thus, to survive the intercrop period, the hatching behaviour of *H. sacchari* changes to ensure that a large proportion of J2 do not hatch; these dormant individuals are either quiescent, requiring host stimulation by the following host crop, or in diapause. Irrespective of the age of the host plant on which it develops, encysted eggs of *H. oryzicola* are always dependent on host diffusate stimulation for substantial hatch and can remain dormant until the subsequent growing season. The large eggsac, associated with cysts of this species, may contain eggs which hatch readily in water and provide J2 for reinvasion of the host during the growing season, as occurs, for example, in *H. oryzae* (Merny, 1966), *H. cajani* (Gaur *et al.*, 1992) and *H. glycines* (Ishibashi *et al.*, 1973).

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