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Effect of storage *in vitro* and in soil on the hatch from cysts of the pigeonpea cyst nematode, *Heterodera cajani*

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Summary – Hatching and infectivity of second stage juveniles (J2) from cysts of the pigeonpea cyst nematode, *Heterodera cajani*, were examined after cysts had been exposed to 0, 60, 80 and 100 % relative humidity (RH) for up to 3 weeks or had been stored in moist or air dried soil for up to 12 months. A delay in hatch was related to storage time and RH; 80 and 100 % RH had less effect on hatch than 0 and 60 % RH when cysts were subsequently placed in cowpea root diffusate (CRD). Desiccation of cysts reduced but did not completely inhibit hatch. Hatch was always greatest in CRD, compared to soil leachate (SL) and distilled water, irrespective of the desiccation treatment but there were indications that desiccation increased the percentage hatch in CRD relative to that in SL. Eggs within cysts were able to withstand extremes of desiccation; *in vitro* desiccation gave a maximum reduction in viable eggs after 3 weeks exposure to 0 % RH of approximately 30 %, which is similar to the reduction found in dry soil after storage of between 2 to 12 months. Cysts stored in moist soil for up to 12 months gave a greater percentage hatch in CRD than those from air dried soil but the actual number of J2 emerging per cyst was lower. Eggs hatched during the first 4 months of storage in moist soil but only during the first 2 months in air dried soil. The modifications of the hatching pattern of *H. cajani* in response to desiccation stress is discussed in relation to dormancy and survival of this species during the intercrop period.

Résumé – ***Influence du stockage* in vitro *et dans le sol sur l'éclosion des kystes du nématode à kyste du pois d'Angole*, Heterodera cajani** – L'éclosion et l'infestivité des juvéniles de deuxième stade (J2) issus de kystes du nématode à kyste du pois d'Angole, *Heterodera cajani*, ont été testées après que les kystes ont été exposés à une humidité relative (RH) de 0, 60, 80 et 100 % pendant 3 semaines ou après avoir été stockés pendant au moins 12 mois dans un sol séché à l'air. Un retard dans l'éclosion a pu être mis en relation avec le temps de stockage et la RH. Les RH 80 et 100 % ont moins d'influence sur l'éclosion que les RH 0 et 60 % lorsque les kystes sont ensuite placés dans du diffusat radiculaire de niébé (CRD). La dessiccation des kystes réduit mais n'annule pas l'éclosion. Celle-ci est toujours plus élevée dans le CRD que dans le percolat de sol (SL) ou l'eau distillée quel que soit le traitement de dessiccation, mais des indications existent suivant lesquelles la dessiccation accroîtrait le pourcentage d'éclosion dans le CRD par rapport à celui observé dans le SL. Les œufs contenus dans les kystes sont capables de supporter des valeurs extrêmes de dessiccation. La dessiccation *in vitro* produit la réduction maximum du nombre d'œufs viables après une exposition de 3 semaines à 0 % RH; cette réduction, 30 %, est similaire à celle observée après un stockage de 2 à 12 mois dans du sol sec. Dans le CRD, le pourcentage d'éclosion des kystes stockés dans du sol humide pendant au moins 12 mois est plus élevé que celui des kystes stockés dans le sol séché à l'air, mais le nombre réel de J2 issus de chaque kyste est plus faible. Dans le sol humide, les œufs éclosent pendant les 4 premiers mois de stockage mais seulement pendant les 2 premiers dans le cas de sol séché à l'air. Les modifications des modalités d'éclosion de *H. cajani* en réaction à une contrainte de dessiccation sont discutées en relation avec la dormance et la survie de cette espèce pendant l'intersaison culturale.

Key-words : Desiccation, hatching, *Heterodera cajani*, root diffusate, storage, survival.

The pigeon-pea cyst nematode, *Heterodera cajani* Koshy, 1967, has a short life cycle of 17-22 days at 29 °C and completes several generations in a crop growing season (Koshy & Swarup, 1971 *a, b;* Gaur & Singh, 1977). In a study of hatching from cysts and eggsacs of six successive generations of *H. cajani* produced on cowpea during a single growing season, Gaur *et al.* (1992) found that, irrespective of generation, eggs in eggsacs hatched in water without the need for stimulation by host root diffusates; in contrast, although hatch from cysts of the first four generations was similar in water and diffusates, eggs in cysts from the fifth and sixth generations showed a significant dependence on diffusates for a substantial hatch. These last two generations were produced on senescing plants and the dependence on host diffusate ensures that the second stage juveniles (J2) do not hatch in the absence of host plants. Juveniles within eggs in cysts from the last two generations are also prepared for a survival period, for they have greater lipid reserves than juveniles in the eggsacs (Gaur *et al.*, 1992). Dependence on diffusates for hatch, protection within the eggshell and cyst and high energy reserves all enhance the chances of survival of unhatched J2 during the intercrop period.

Mechanisms involved in nematode survival are the subject of detailed study which needs to be supported by

information on abilities of individual species to survive in the soil and under controlled experimental conditions. In common with other tropical species of cyst nematodes in the absence of a host crop, cysts of *H. cajani* remain in the soil subjected to varying degrees of desiccation. However, although this damaging pest occurs commonly in the semi-arid regions of India and Egypt (Sharma *et al.*, 1992), information about its survival is largely anecdotal. As part of a study on the biology of *H. cajani*, this paper reports on the desiccation survival of unhatched J2 and the effect of host root diffusates on hatching and infectivity of J2 from cysts exposed *in vitro* to varying relative humidities or stored in dry or moist soil.

Materials and methods

Nematode cultures

Cowpea plants, *Vigna unguiculata* International Institute of Parasitology Acc. No. 588/272, were grown singly in 15 cm diameter plastic pots filled with steam sterilized sand/loam mix, 1:4 v/v (which had been stored for several months after sterilisation) in a controlled temperature glasshouse (26-30 °C) with daily 14 h light period. Ten days after germination, 1000 freshly hatched J2 of *H. cajani* were inoculated around the roots of each seedling.

Storage of cysts : *in vitro* treatment

Five months after inoculation brown cysts were extracted by standard methods (Shepherd, 1986) and batches of approximately 400 cysts were prepared for desiccation. Excess water was removed from around the cysts using strips of filter paper and each batch of cysts was spread evenly to form a single layer in a Petri dish lid. Lids were transferred into constant humidity chambers, volume 550 cm^3, at 0, 60, 80 and 100 % relative humidity (RH). Freshly activated silica gel was used as the desiccant for 0 % RH; glycerol/water solutions (Grover & Nicol, 1940) were used for 60 and 80 % RH and distilled water (DW) was used for 100 % RH. To check humidity, paper hygrometers were placed inside the chambers. Desiccation was done at 20 °C and batches of cysts were removed after 0 h (control), 3 h, 3 days and 3 weeks for hatching tests.

Storage of cysts in dry and moist soil

A separate batch of cowpea plants, inoculated and grown as above, were cut at soil level after 6 months. The pots containing infested soil and roots were kept at 26 °C in two groups; one group was watered regularly to keep the soil moist (moisture content over 10 % w/w, equivalent to over 30 % of field capacity) for a 12 month period, while pots in the second group were left to air-dry to a moisture level of 2-3 % w/w. Cysts were extracted from two pots from each group at intervals up to 12 months and used in hatching tests.

Hatching tests

Three batches of 30 cysts for each treatment from the humidity chamber studies and four batches of 25 cysts from the stored soil studies were placed in excavated glass blocks containing 2 ml of either DW, soil leachate (SL) or cowpea root diffusate (CRD) at 28 °C. CRD was obtained (Fenwick, 1949) from 4 week-old cowpea plants grown in steam sterilized sand/loam with four plants per 15 cm diameter pot; SL was collected at the same time from pots containing only sand/loam.

Counts of hatched juveniles were made after 3 and 7 days and at weekly intervals thereafter up to 8 weeks, except for cysts that had been stored in soil for 12 months where hatching tests were continued up to 12 weeks. At each count, J2 were removed and fresh solutions added from stock solutions kept in polythene bottles at 2 °C. At the end of each test, cysts were broken open and the number of eggs containing unhatched J2 were counted to determine the percentage hatch. The data were subjected to a two way analysis of variance after arcsin transformation of percentages. The level of significance selected for comparisons using the LSD test is the 5 % level; results are reported as significant or non-significant with reference to this level only. The hatch curves over the total incubation period were also compared by paired t-tests.

Infectivity tests

Four 9 cm diameter pots, each containing approximately 350 g of the infested moist soil and four similar pots containing the dry soil, were sown with a germinated cowpea seed; the dry soil had been moistened for 2 days before planting. Both dry and moist soil contained 675 ± 4.5 cysts per 100 g moist soil, with a mean egg content of 122 ± 14.5. After 4 weeks, the seedling in each pot was washed free of soil, roots and shoots were weighed and the number of juveniles (all stages) per g root was estimated after staining in 0.05 % acid fuchsin in glycerol (Bridge *et al.*, 1982).

Results

In vitro storage of cysts

The cysts used in this study had a mean of 129 ± 17 eggs containing juveniles (termed viable eggs/cyst). Exposure of these cysts to 100 % RH for up to 3 weeks did not affect this number. Keeping the cysts for 3 h at 0, 60 and 80 % RH also had no effect on viable egg numbers, whereas 3 days at 0 and 60 % RH decreased the numbers of eggs/cyst to 110 ± 12 and 114 ± 12, respectively, and 3 weeks exposure to 0, 60 and 80 % RH gave a decrease to 85 ± 10, 102 ± 10 and 118 ± 12, respectively (Fig. 1).

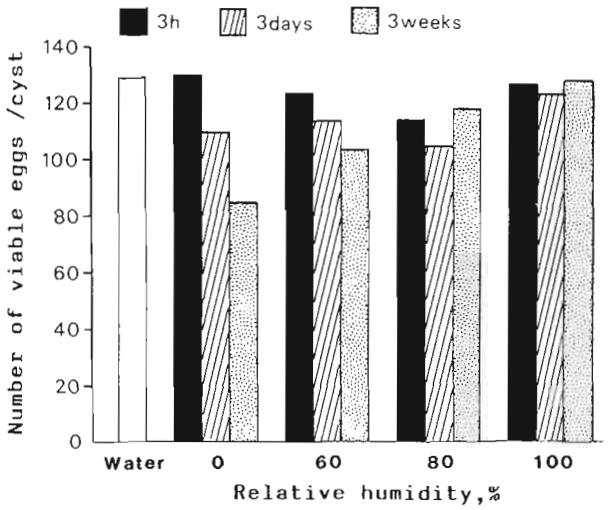

Fig. 1. *The effect of storage for 3 h, 3 days and 3 weeks on the number of viable eggs/cyst of* Heterodera cajani *at varying relative humidities; water control : no desiccation.*

Hatch from cysts after *in vitro* storage

Cumulative hatches from cysts in CRD, DW and SL after exposure to all levels of RH for 3 h, 3 days and 3 weeks are given in Fig. 2 and Table 1. Irrespective of prior treatment, CRD gave a higher percentage hatch than DW or SL. After 60 days, cumulative hatch in untreated cysts was 51.2 % in CRD compared to 22.3 % (SL) and 32.0 % (DW). Exposure for 3 h to 80 and 100 % RH did not significantly affect hatch in CRD with a total hatch of 54.7 % and 56.5 %, respectively; after exposure for 3 h to 0 and 60 % RH, hatch in CRD was reduced significantly to 47.7 % and 41.1 %, respectively (Table 1). Hatches in SL and DW were 6.8 % and 16.5 % from cysts previously exposed to 0 % RH, and 3.6 % and 5.3 % from cysts previously exposed to 60 % RH, respectively. A similar decline in hatch was found from cysts after 3 days and 3 weeks exposure. The longest period (3 weeks) of storage gave a slightly greater reduction in hatch at all humidities except for exposure to 0 and 60 % RH which gave a significantly greater hatch in CRD, compared to SL and GDW, of 52.8 and 44.4 %, respectively.

In cysts kept at 100 % RH for 3 h, 3 days and 3 weeks, total hatch in CRD was 56.5, 40.8 and 37.3 %, respectively. The minimum percentage hatch in CRD, 31.9 %, was from cysts exposed to 60 % RH for 3 days; total hatch from cysts at 60 % RH was consistently lower than those at 0 % RH (Table 1).

The statistical comparison of the hatch curves by paired t-tests showed that hatch from cysts exposed to humidities lower than 100 % followed by incubation in SL were not significantly different; however, hatch was significantly less than that for controls. Cysts incubated in DW after exposure to 0 and 60 % RH gave hatch curves significantly lower than those for cysts exposed to 80 % and 100 % RH or from undesiccated controls (Fig. 2 A, B, C).

Incubation of cysts in CRD after exposure to different RHs gave some inconsistencies. At 60 % RH, the hatch curve was 8 % lower than that at 0 % RH, after 3 days exposure (Fig. 2 H); the curves for the hatch from cysts exposed to 0 % RH were the same as those for controls and only 3.7 and 5.5 % lower than those for cysts exposed to 80 and 100 % RH. The hatch curves for control cysts and those exposed for 3 days to 80 and 100 % RH did not differ significantly (Fig. 2). Similar hatching patterns were noted for the 3 h and 3 weeks exposure times, except that the hatch curves were significantly higher for 3 weeks at 0 and 60 % RH (Fig. 2 G, I).

A comparison of the hatch curves in SL and CRD after 3 days exposure shows that in undesiccated controls, the curve for CRD was 13 % higher than in SL while it was higher by 22.3, 14.8, 26.2 and 26.7 % at 0, 60, 80 and 100 % RH, respectively, thus indicating that prior desiccation can cause significantly enhanced hatch in response to CRD. It is also evident that hatch in SL is

Table 1. *Effect of* in vitro *storage of* Heterodera cajani *cysts at different relative humidities on their total cumulative hatch after 60 days in cowpea root diffusate (CRD), soil leachate (SL) and distilled water (DW).*

Time	Hatching medium	Relative humidity (%)				
		Water	0	60	80	100
3 h	CRD	51.2 *46.12*	47.7 *44.08*	41.1 *38.98*	54.7 *48.20*	56.5 *48.70*
	SL	22.3 *29.18*	6.8 *15.12*	3.6 *11.54*	15.5 *22.64*	9.8 *18.53*
	DW	32.0 *36.40*	16.5 *24.30*	5.3 *13.70*	15.3 *22.86*	27.9 *32.94*
3 days	CRD	51.2 *46.12*	37.8 *40.10*	31.9 *35.21*	44.2 *42.33*	40.8 *38.62*
	SL	22.3 *19.18*	4.3 *12.23*	3.3 *10.94*	4.1 *11.98*	5.1 *13.70*
	DW	32.0 *36.40*	9.1 *17.32*	9.8 *17.68*	34.8 *37.24*	38.8 *38.94*
3 weeks	CRD	51.2 *46.12*	52.8 *44.55*	44.4 *43.98*	40.5 *39.67*	37.3 *40.42*
	SL	22.3 *29.18*	2.1 *8.55*	2.2 *6.50*	8.5 *21.38*	2.5 *8.90*
	DW	32.0 *36.40*	15.0 *23.09*	2.7 *10.91*	37.6 *37.27*	5.9 *14.19*
LSD $P \leq 0.05$				*7.45*		

Means of three batches; figures in italics are angular transformed values.

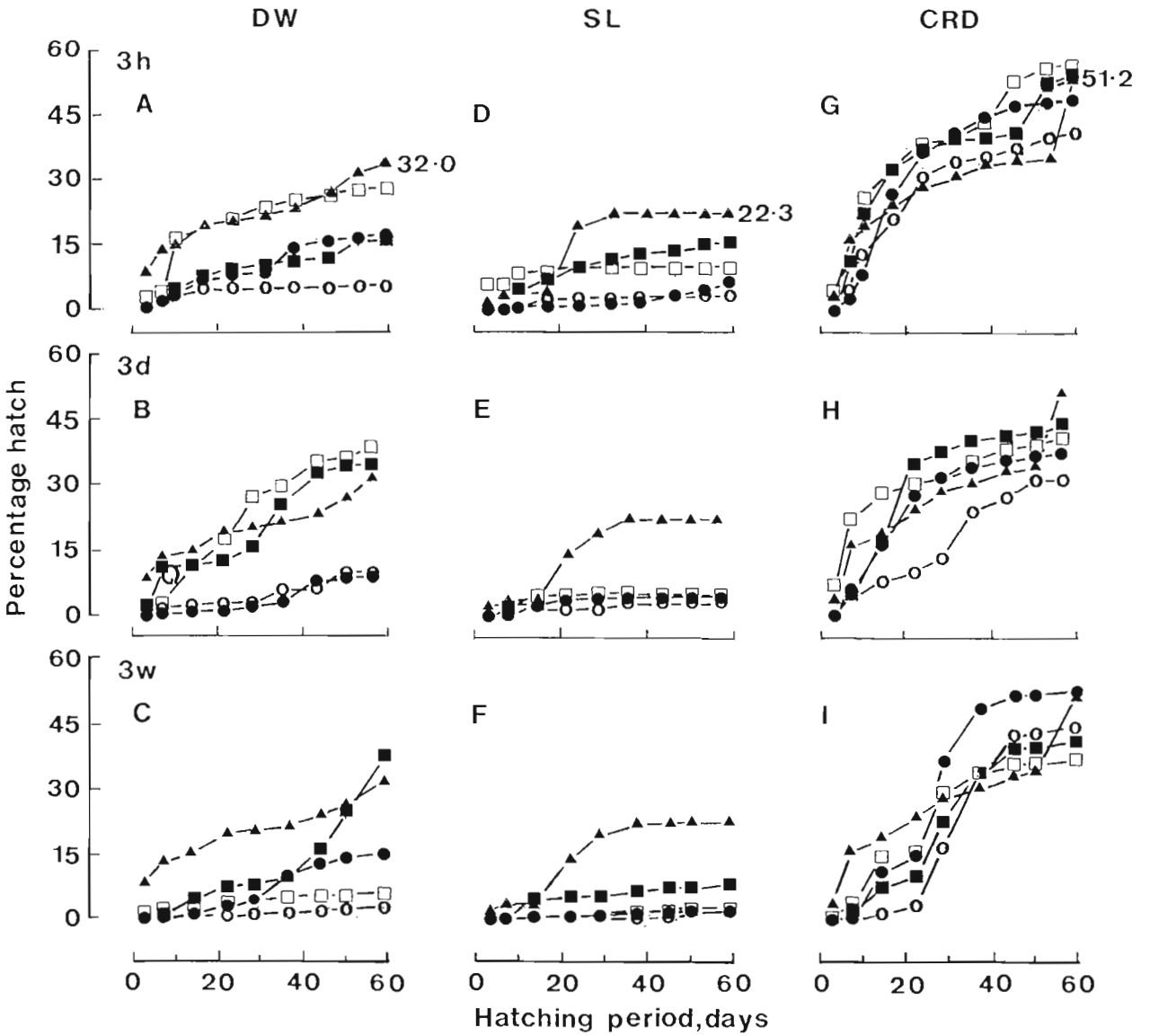

Fig. 2. *Percentage hatch in distilled water (DW), soil leachate (SL) and cowpea root diffusate (CRD) from cysts of* Heterodera cajani *previously subjected to various relative humidities for 3 hours (3 h), 3 days (3 d) and 3 weeks (3 w).* (●), *0 %,* (○), *60 %;* (■), *80 % and* (□), *100 % R.H.,* (▲) *water control; figures are the final cumulative percentage hatch in water.*

inhibited when compared to hatch in DW.

VIABILITY OF EGGS IN CYSTS STORED IN DRY OR MOIST SOIL

Survival of viable eggs in dry soil was greater than that in moist soil. From an initial mean egg content of 122 ± 14 eggs/cyst the number of viable eggs/cyst was reduced to about 80 in both soils at 2 months; no further reduction occurred in cysts in dry soil but after 4 months in moist soil numbers had reduced to 47 eggs/cyst, a figure which was constant for up to 12 months storage (Table 2).

HATCH FROM CYSTS STORED IN DRY OR MOIST SOIL

After 2 months storage, cysts extracted from moist soil gave a negligible hatch in SL (2.0 %) and DW (4.9 %) compared to 73.5 % in CRD; after 4 months storage, hatch in SL, DW and CRD was 5.2, 23.3 and 87.0 %, respectively. A similar difference in hatch was noted after 6, 8 and 12 months storage (Table 2). Hatch

Table 2. *Total cumulative percentage hatch in cowpea root diffusate (CRD), soil leachate (SL) and distilled water (DW) from* Heterodera cajani *cysts extracted from moist and dry soil stored up to twelve months.*

Time (months)	Soil	CRD	SL	DW	Viable eggs/cyst ± SE	Mean no. J2 hatched/cyst ± SE in CRD
0		53.5 *47.02*	22.3 *28.14*	33.8 *35.52*	122 ± 14.5	65 ± 6.0
2	moist	73.5 *58.68*	2.0 *8.03*	4.9 *12.68*	80 ± 3.7	59 ± 5.1
4		87.0 *67.70*	5.2 *13.1*	23.3 *29.25*	47 ± 5.2	41 ± 4.0
6		53.7 *46.35*	0.8 *4.87*	18.0 *25.47*	56 ± 6.3	29 ± 2.3
8		51.5 *45.43*	0.4 *3.74*	7.7 *15.76*	49 ± 3.3	25 ± 3.2
12		61.2 *51.80*	0.7 *4.61*	16.7 *22.97*	48 ± 2.2	29 ± 5.0
2	dry	61.3 *51.44*	2.1 *8.22*	5.1 *13.18*	79 ± 2.8	48 ± 5.2
4		59.2 *50.34*	4.1 *11.55*	6.9 *14.81*	92 ± 2.6	54 ± 5.1
6		50.3 *45.12*	4.0 *11.13*	9.7 *19.96*	83 ± 4.0	42 ± 3.4
8		35.4 *36.49*	1.7 *7.41*	6.6 *14.70*	90 ± 4.4	31 ± 2.2
12		50.0 *44.37*	1.7 *7.61*	9.9 *18.19*	87 ± 4.8	43 ± 6.0
LSD $P \leq 0.05$ (for interaction)			*4.25*			

Means of four replicates; figures in italics are angular transformed values.

from cysts extracted from the dry soil gave a similar pattern to that from cysts from moist soil : CRD gave a much greater hatch than either SL or DW (Fig. 3). Statistical analysis confirms that the percentage hatch in CRD was significantly greater than the hatch in SL and DW, irrespective of storage time. Overall, the percentage hatch was significantly greater in cysts from moist soil; rate of hatch differed as cysts from dry soil commenced hatching one week later than those from moist soil.

Infectivity tests

Juveniles from cysts stored in moist soil invaded seedlings after each storage time although mean numbers/g root varied (Table 3). Storage in moist soil enhanced invasion and after each storage period the number of

Table 3. *Infectivity of* Heterodera cajani *after storage in host-free moist and dry soil four weeks after sowing cowpea.*

Period of storage (months)	Moist soil			Dry soil		
	Shoot fresh wt. (g)	Root fresh wt. (g)	Nematodes/ g root	Shoot fresh wt. (g)	Root fresh wt. (g)	Nematodes/ g root
0	3.6	2.2	620			
2	3.7	2.3	713	1.5	0.2	81
4	3.2	1.9	1 150	1.8	0.1	244
6	1.3	1.3	1 586	1.5	0.4	229
8	5.0	1.9	1 102	1.9	0.5	427
12	2.2	1.3	724	1.4	0.2	250
LSD ≤ 0.05	1.1	0.7	353.1	0.5	NS	184.3

Means of four replicates.

J2/g root that had invaded was greater than in controls which had not been stored; maximum invasion (1586 J2/g) occurred after cysts had been stored in moist soil for 6 months and was more than twice the invasion from cysts which had not been stored (620 J2/g). In the soil which had been dry, seedlings grew poorly, possibly due to the adverse effects of drying on soil structure, and root invasion by *H. cajani* juveniles was consistently less than in moist soil (Table 3).

Discussion

The cysts of *H. cajani* used for this study were extracted from cowpea plants five months after inoculations. This was to ensure that tests were done on cysts which would persist after crop harvest and thus provide ecologically relevant information about survival abilities. The majority of cysts used were from the fifth and sixth generations as eggs in cysts from earlier generations hatch well in water and are unlikely to persist in the soil (Gaur *et al.,* 1992). This is confirmed by the mean number of eggs per cyst and the hatch from control cysts. The mean of 122 eggs/cyst is close to the results of Gaur *et al.* (1992) who found approximately 140 eggs/cyst in cysts from the fifth and sixth generations; the hatching pattern of untreated control cysts in the present work is also comparable to the hatch found previously in these two generations (Gaur *et al.,* 1992), with about 20 % of the cyst contents being dependent on root diffusate for hatch stimulation.

The dependence on host root diffusates for hatch by a proportion of eggs, the greater lipid reserves of encysted J2 and the increased proportion of eggs retained in the cysts were all features of the sixth generation which were interpreted by Gaur *et al.* (1992) as factors which contribute to the survival of *H. cajani* populations during the intercrop period. In areas of the world where *H. cajani* is

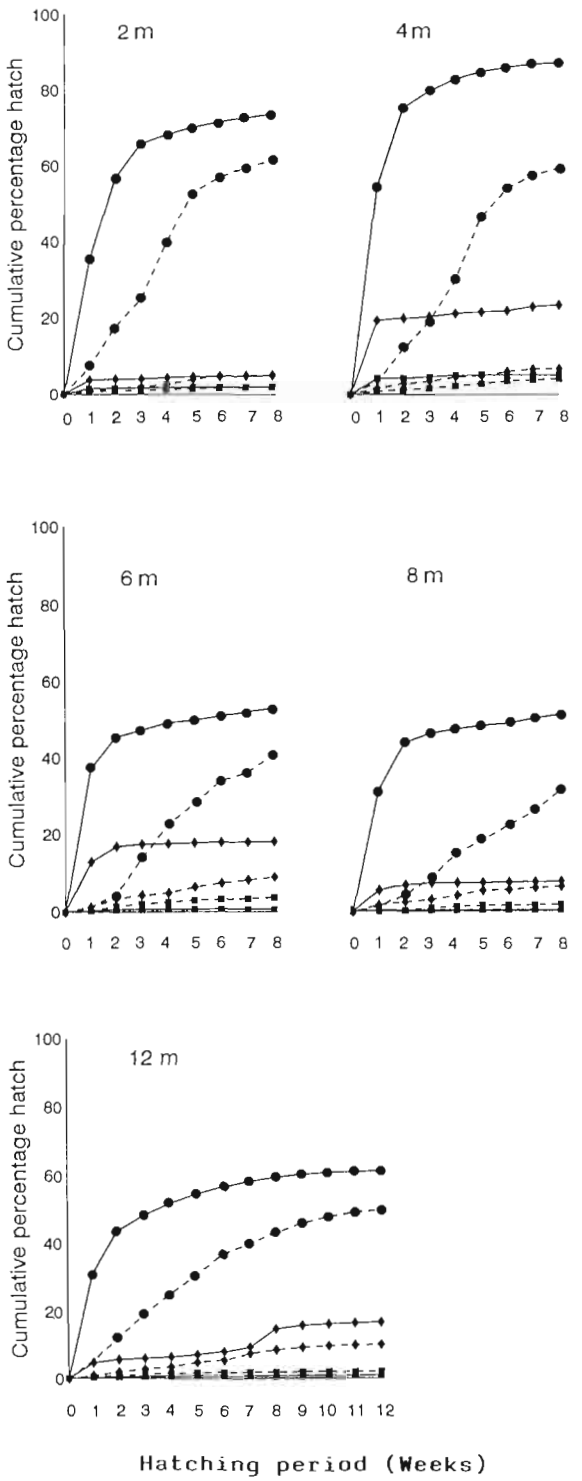

Fig. 3. *Percentage hatch from cysts of* Heterodera cajani *stored in moist (_) and dry (_ _ _ _) soil for up to 12 months (m); (●) cowpea root diffusate, (♦) distilled water and (■) soil leachate.*

endemic, this period is characterised by lack of moisture and high temperature. The present work has demonstrated that cysts are able to withstand extreme conditions of desiccation. *In vitro* desiccation of cysts gave approximately 30 % reduction in viable eggs after 3 weeks exposure to 0 % RH; 0, 60 and 80 % RH provide very severe desiccation not related to ecological conditions likely to be experienced by *H. cajani*. However, the results do indicate the ability of the eggshell and cyst to protect the unhatched J2. This is supported by the survival in dry soil, where a reduction in viable eggs of about 30 % was found (Table 2). The decrease in viable egg content during the first 2 months in dry soil indicates that some eggs hatched while the soil was drying; after 4 months the soil was very dry and the remaining two thirds of the eggs remained unhatched and viable even after one year.

The reduction in viable egg content in moist soil was much greater (approximately 60 %); this is probably because some J2 hatched and others were metabolically active and used energy reserves with a concomitant adverse effect on survival. Dry soil favours long term survival of cysts because the J2 are quiescent, a state associated with lowered metabolism and conservation of energy reserves (Evan & Perry, 1976). This is further supported by the results of *in vitro* tests : at 0 % RH, there was no significant effect on percentage hatch in root diffusate whereas exposure to higher humidities adversely affected final percentage hatch. This is similar to the greater decline in viability of eggs of *Globodera rostochiensis* exposed to 88 % RH compared to 7.5-78.7 % RH (Mai & von Machow, 1952; Lewis *et al.*, 1960). By contrast, cysts of *H. schachtii* are vulnerable to drying. Hatching from cysts of this species stored in dry sand ceased after 11 months, whereas eggs in cysts stored in moist sand remained viable and over 30 % hatched in root diffusate after 47 months storage (Caubel, 1993).

The hatching behaviour of cysts of *H. cajani* subjected to rapid desiccation at low RH was different from undesiccated cysts. There was a delay of approximately a week in the commencement of hatch from rapidly dried cysts which may be due to the time required for rehydration and resumption of full metabolism.

Although hatch was always greatest in CRD irrespective of the storage treatment, the results indicated that after severe desiccation fewer eggs hatch in soil leachate whilst hatch in host root diffusate was enhanced. The modification in hatching behaviour induced by desiccation was also evident in results from experiments with stored soil. The hatch from cysts stored in dry and moist soil was dependent on stimulation by host root diffusates to a much greater extent than hatch in control cysts or in previously reported work (Gaur *et al.*, 1992). This aspect requires further investigation. The difference could be due to desiccation-induced changes in juvenile physiology making more of them dependent on root diffu-

sates for hatch; this would not be termed diapause, as the juveniles readily hatch under favourable conditions (Evans & Perry, 1976). Alternatively, desiccation may result in a differential death of cyst contents; eggs which hatch in water may be more vulnerable to desiccation than those requiring root diffusate. The latter explanation appears more likely as dependence on host root diffusates for hatch in species such as *G. rostochiensis* is associated with eggshell permeability characteristics and the presence of trehalose in the perivitelline fluid, which can also enhance desiccation survival (Perry, 1983, 1989). J2 of the rice cyst nematodes *H. sacchari, H. oryzae* and *H. oryzicola* show no intrinsic ability to survive desiccation (Ibrahim & Perry, 1992) and require the protection of the egg for survival. The cyst as an ecological unit (Ellenby, 1946) is central to the survival of cyst nematodes whether from temperate or tropical regions.

Infectivity tests demonstrated that cysts stored in moist and dry soil contained infective J2. The number of J2 per g of fresh cowpea root 4 weeks after germination was fewer in plants grown in the soil previously stored dry than in that stored moist, probably because of poor plant growth in the dried soil; decreased rhizobial nodulation was noted on the roots of plants from dry soil whilst plants from moist soil had good nodulation.

The modified hatching pattern of *H. cajani* in response to desiccation stress and to plant senescence (Gaur *et al.*, 1992) demonstrates effective adaptation to ensure survival of the species during periods of adverse environmental conditions without a suitable host. This work forms the basis for future studies to determine whether juveniles of *H. cajani* and *H. sorghi* (Gaur *et al.*, 1995) which do not hatch immediately on stimulation are in diapause and whether exposure to desiccation triggers diapause in further unhatched juveniles. An understanding of the changes in hatching behaviour of these nematodes under conditions of frequent dry periods after crop harvest in the tropics and subtropics, may assist in the development of management strategies which limit nematode population densities, such as modifications to moisture regimes and cropping systems.

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