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Differential hatching of the potato cyst nematodes Globodera rostochiensis and G. pallida in root diffusates and water of differing ionic composition

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Abstract

The hatching differences of *Globodera rostochiensis* and *G. pallida* were assessed in potato root diffusate (PRD) of cv . Bintje, cv. Elkana and clone ZB35-29. *G. pallida* hatched better in the PRDs than *G. rostochiensis*. It was shown that the experimental test conditions strongly influenced the hatching results. The water type used in the hatching tests had a significant discriminating effect on the species; *G. rostochiensis* reached hatch percentages of 60 to 90% in demineralized and tap water, whereas *G. pallida* never exceeded the 15%. These differences were independent of the various batches that were used or the different years the tests were carried out. Silver sand percolate had a inhibiting effect on the hatching of both nematode species. Boron and a high electrical conductivity may be responsible for this. The results are discussed from an ecological point of view as well as for research consequences.

Additional keywords: ecological species differences, diapause, potato root diffusate, test conditions.

Introduction

In plant-nematode interrelationships the hatching process is critical (Perry, 1987). In the framework of research on interactions between the species of potato cyst nematodes, the question arose whether hatching differences play an important role. In mixed populations of *G. rostochiensis* and *G. pallida* interspecific interactions take place resulting in a relatively lower population increase of the minority species (Den Nijs, 1992). It is possible that differences in hatching between the nematode species may influence interspecific interaction.

Most nematodes possess the ability to overcome adverse environmental conditions by arresting their development and becoming dormant. For cyst nematodes, especially potato cyst nematodes, it is not clear whether quiescence or diapause is the adequate term to describe this state of rest of the eggs inside the cyst (Antoniou, 1989; Evans and Perry, 1976; Evans, 1987), although Hominick et al. (1985) and Hominick (1986) concluded that diapause was present in *G. rostochiensis* cysts. To activate the nematode juveniles inside the eggs, a hatching agent present in potato root diffusate (PRD) is thought to be necessary. As a result the nematodes hatch from the eggs and penetrate the roots of the host. In the life cycle of potato cyst nematodes the ability to survive

in a dormant state, the breaking of this dormancy and the subsequent hatching process are interrelated. They synchronize the development of the nematode with that of its host plant, the potato (Perry, 1989).

Much research has been carried out to unravel the process of hatching, especially for the nematode species *G. rostochiensis*. Clarke and Perry (1977) gave an extensive review of the factors which might influence this process of hatching of cyst-nematodes. Perry and Clarke (1981) and Perry (1986, 1987, 1989) reviewed the mechanisms involved, especially the responses of the juvenile, between the onset of hatching and subsequent eclosion. Since *G. pallida* and *G. rostochiensis* are considered sibling species (Sturhan, 1985) one would expect the process of hatching to be similar. However, the rates of hatching and the reaction of both potato cyst nematode species to PRD of different potato cultivars and to temperature are different (Evans, 1983; Robinson et al., 1987), and suggest essential differences in the ecology of the species.

In experiments to compare hatching activity of *Globodera* species in relation to hatching stimuli, knowledge of the impact of other potential stimulating factors in the experiment is required. To exclude differences in the physiological stages of the nematodes, Hominick et al. (1985) suggested that only mature cysts of *G. rostochiensis* of the same age and reared and stored under well defined conditions should be used. Apart from these intrinsic factors, the reaction of the nematodes towards external factors among the test conditions might differ and interfere with the hatching process.

Hatching tests were carried out for the two potato cyst nematode species with the emphasis on experimental test conditions. During the PRD-hatching test it appeared that the nematodes reacted very strongly to the control solution (silver sand percolate, SSP). The water type used also had a strong influence on the hatching results. Clarke and Shepherd (1966) and Clarke and Hennessy (1987) reported the influence of inorganic compounds on the hatching behaviour of cyst nematodes and Clarke et al. (1978) showed that osmotic stress can inhibit the hatching of nematodes from the eggs. Therefore apart from the hatching behaviour in PRD, analyses were made of the hatching behaviour of the nematode species in the control solutions and in the different water types used in the experiments. The mineral compositions of the water types were also examined.

Materials and methods

Vintage cysts of *G. rostochiensis* (Ro1) and *G. pallida* (Pa3), reared on the susceptible potato cv. Bintje in the glasshouse and stored at 4 °C for three, four or five years, were used for the experiments. Batches of these cysts were used simultaneously (Table 1). For each hatching test the cysts were soaked for one week in tap water and egg suspensions were made. The eggs were then exposed to the various hatching agents. Hatching tests were performed, essentially according to the method of Den Ouden (1963), by placing a thousand eggs on a 10 μm sieve in a small glass tube with a concave bottom (∅ 17 mm, height 2.5 cm) in 1.5 ml hatching agent, incubated at 20 °C in the dark, with 5 replicates per treatment. Each hatching agent was refreshed 10 times during the experiment, at gradually longer intervals and the hatched juveniles were counted. The accumulated number of hatched juveniles was determined.

For each experiment, the hatching activity of the batches of cysts were determined by assessing the hatch in Standard Hatching Agent (SHA: 15 mg partially purified

Table 1. Experimental set-up for the 3 hatching tests. Test 1 was carried out from 31 March until 10 Aug. 1988, test 2 from 28 Februari until 1 May 1990 and the last test from 1 May until 31 July 1990.
PRD = potato root diffusate, SHA = standard hatching agent, SSP = silver sand percolate, DW = demineralized water, TW = tap water.

	Test 1	Test 2	Test3
Nematodes batches:			
Ro1-85	+	+	−
Ro1-86	−	+	+
Ro1-87	−	−	+
Pa3-85	+	+	−
Pa3-86	−	+	+
Pa3-87	−	−	+
Hatching agents:			
PRD of cv. Bintje, Elkana, ZB35-29			
undiluted	+	−	−
diluted with TW 1/4, 1/16, 1/64, 1/256	+	−	−
SHA			
undiluted	+	+	+
diluted with DW 1/4, 1/16, 1/64, 1/256	+	−	−
SSP			
undiluted	+	+	+
diluted with TW 1/4, 1/64	+	−	−
1/16, 1/256	+	−	+
TW	+	+	+
DW	−	+	+
TW : DW in ratios			
1:255, 1:15, 15:1, 255:1	−	+	−

hatching agent (Janzen and Van der Tuin, 1956) + 50 mg streptomycine per liter demineralized water). The highest accumulated number of hatched juveniles in the SHA was assumed to be the maximum hatch of a batch and was set at 100% (Table 2). For each experiment the hatching data were related to the maximum hatch of the batch used in that experiment (Table 1), e.g. maximum hatch of a batch of Pa3 in the undiluted solution of SHA is A juveniles; this is set at 100%. In demineralized water (DW) B juveniles have hatched, which corresponds with (B/A) * 100% = X percentage relative hatch in DW. For the hatching data of hatching test 1 the calculations were adjusted with the hatching data of the control solution of the corresponding dilutions, e.g. maximum hatch for Pa3 was A larvae = 100%, accumulated hatch in 1/16 diluted SSP (=control) was C hatched larvae (mean of 5 replicates), accumulated hatch in 1/16 diluted PRD of cv. Bintje was B juveniles. The percentage relative hatch in 1/16 diluted PRD was (B-C)/A * 100%.

Table 2. Vitality of different batches of *G. pallida* and *G. rostochiensis*, based on the maximum hatch of 1000 eggs in standard hatching agent (SHA). Mean of 5 replicates with standard deviation in brackets.

Batches	Accumulated hatched juveniles in SHA		
	Test 1 1988	Test 2 1990 I	Test 3 1990 II
Rol-85	570 (62.6)	243 (15.5)	–
Rol-86	–	723 (26.9)	729 (19.3)
Rol-87	–	–	797 (53.1)
Pa3-85	619 (22.5)	545 (25.0)	–
Pa3-86	–	561 (26.9)	311 (29.5)
Pa3-87	–	–	316 (30.2)

Hatching test 1: Hatching activity of G. rostochiensis and G. pallida in the presence of PRD of cv. Bintje, cv. Elkana and clone ZB35-29. PRD was collected from cv. Bintje, susceptible for both nematode species, cv. Elkana, resistant against *G. rostochiensis* and clone ZB35-29, partial resistant against *G. pallida*. Tuber pieces were placed in small pots (7 × 7 × 5 cm) in silver sand. For each cultivar or clone 350 pots were used, for the control 250 pots were used. All pots were watered regularly, so that soil moisture level never exceeded the field capacity. After two months the roots had grown throughout the soil and the pots were percolated with tap water. From each pot only the first 2.5 ml of percolate was collected. Percolate collected from pots with silver sand but without plants was used as control solution in the experiment with PRDs (Table 1). All percolates were filtered, streptomycine was added (0.05 g l^{-1}) and stored at 4 °C in the dark. Three year old cysts of Rol and Pa3 (ROl-85, PA3-85) were used for this experiment. The hatching agents were the PRDs of cv. Bintje, cv. Elkana and clone ZB35-29, SHA undiluted and diluted 4, 16, 64 and 256 times with tap water and tap water alone (TW). As control SSP was taken with the same dilutions as the PRDs (Table 1). The hatching test was carried out from 31 May until 10 August 1988.

Hatching test 2: Hatching activity of G. rostochiensis and G. pallida in tap water and demineralized water. Four and five year old cysts of Rol (ROl-85, ROl-86) and Pa3 (PA3-85, PA3-86) were subjected to the following treatments: SHA, TW, DW and mixtures of TW and DW with ratios of 1:255, 1:15, 15:1 and 255:1 (TW:DW) respectively (Table 1). The test was carried out from 28 February until 1 May 1990.

Hatching test 3: Hatching activity of G. rostochiensis and G. pallida in silver sand percolate. Undiluted and diluted (1/16 and 1/256) SSP, SHA, TW and DW were used for hatching tests. Three and four year old cysts of Rol (ROl-86 and ROl-87) and Pa3 (PA3-86 and PA3-87) were used in the test, which was carried out from 1 May until 31 July 1990 (Table 1).

Mineral analysis. The control, TW and DW were analysed for macro- and micro-

elements using standard procedures of the Laboratory for Soil and Crop Testing, Naaldwijk, the Netherlands.

Statistical analysis. The total amounts of hatched nematodes in the different hatching agents were expressed as percentages of the maximum hatch per batch. In this way vitality differences of the batches are excluded and results of different batches are comparable. Statistical analysis (ANOVA) was applied on the results per species.

Results

Hatching test 1. The differential hatching effect of PRDs on *G. pallida* and *G. rostochiensis* are shown in Figures 1A, 1B and 1C. It is clear that hatching of both nematode species decreased when diffusates were diluted. A striking effect however is the seemingly negative hatch of *G. rostochiensis* (Figures 1B and 1C). This is caused by the better hatch of this nematode species in the control solution than in the root diffusate solution. Table 3 shows the hatching data of *G. rostochiensis* and *G. pallida* in the control solution, SSP. Dilution of this percolate with TW increased the hatch of both species significantly. However, the level of hatch which both nematode species reached in the solution diluted 256 times with TW differed strongly, with 11.8% and 62.2% hatch for *G. pallida* and *G. rostochiensis* respectively. These differences in the behaviour of both species were further investigated in hatching test 2 and 3.

Hatching test 2. The hatching behaviour of the two species in TW and DW were considerably different (Table 4). For *G. pallida* the average hatching percentages in pure TW and DW never exceeded 10%, whereas the percentages of hatch for *G. rostochiensis* were always above 45%. The percentage of TW in the water mixtures was used as a factor in the ANOVA. No significant differences were found between the two batches of each species in their reaction to TW and DW ($P = 0.580$ for *G. rostochiensis* and $P = 0.198$ for *G. pallida*). Further analyses were made on the combined data of the batches (Rol-85 + Rol-86 and Pa3-85 + Pa3-86 resp.). The percentage of TW in mixed solutions influenced the hatching rates of both nematode species significantly, mainly due to the relatively low hatch in pure DW (Table 4).

Hatching test 3. The influence of SSP on the hatching pattern of both nematode species was examined by using the data of this experiment with the hatching data obtained from the control solution of experiment 1 (= SSP, undiluted, 16 and 256 times diluted). Analysis of variance on these combined data revealed that the three batches of *G. rostochiensis* did not differ significantly ($P = 0.967$) in their reaction to the SSP. The percentage hatch increased significantly ($P < 0.001$) for the three batches of *G. rostochiensis* when the SSP was diluted with an increasing amount of TW. For the three batches of *G. pallida* it was also found that dilution of the SSP increased the hatching significantly ($P < 0.001$), but the levels of hatch were different for each batch (Table 5). Both species also reacted differently to inhibition of hatch by the undiluted SSP; hatching of *G. pallida* was approximately halved, whereas the hatch of *G. rostochiensis* in the undiluted SSP was approximately one seventh of that in the most dilute SSP (Tables 3 and 5). Both species reached their maximum hatch in the greatest dilution of SSP (1/256). This solution is almost comparable to TW. The

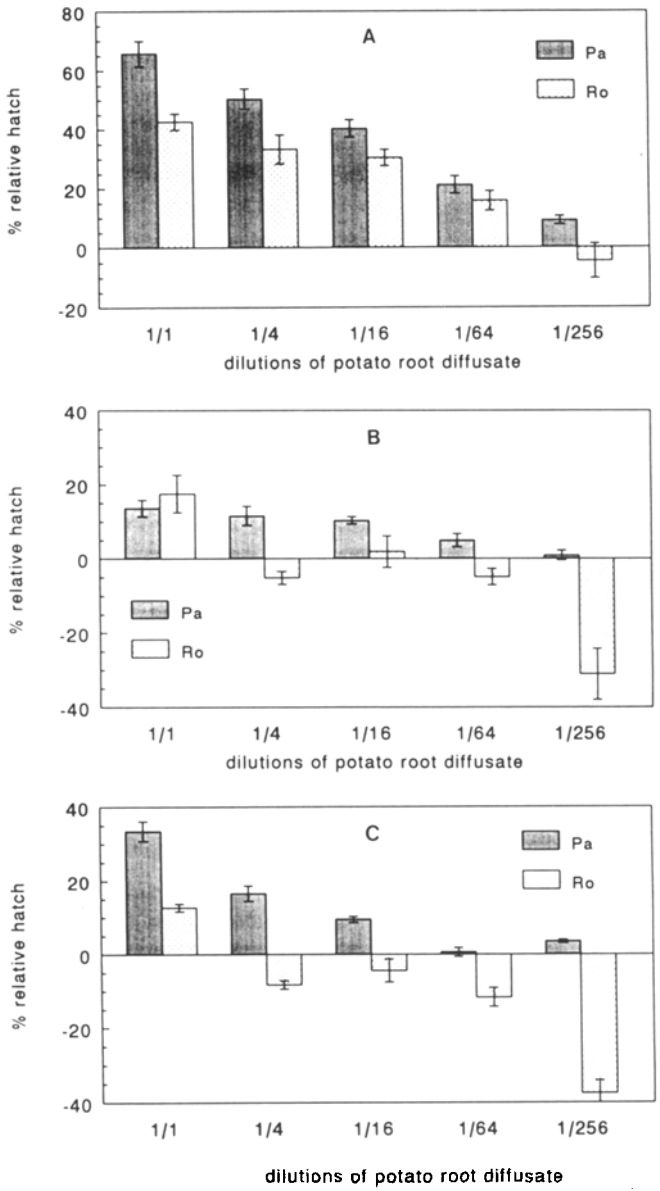

Fig. 1. The relative hatch of *Globodera rostochiensis* (Ro1-85) and *G. pallida* (Pa3-85) in undiluted and diluted potato root diffusates (PRDs). Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and corrected for the control (= silver sand percolate) and are means of 5 replicates. The bars indicate the standard error of means. A: cv. Bintje, B: cv. Elkana, and C: clone ZB35-29.

hatching rates of both species in pure TW were not significantly different from the hatching rates in the SSP diluted 256 times ($P = 0.356$ for *G. pallida*, $P = 0.155$ for *G. rostochiensis*). The hatching reactions of the two batches of *G. pallida* to TW and

Table 3. The relative hatch percentages of *G. rostochiensis* and *G. pallida* in different dilutions of silver sand percolate. (Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates).

Dilution	Pa-85	Ro-85
1/1	6.91	6.70
1/16	8.24	22.56
1/16	9.31	22.67
1/64	14.70	32.91
1/256	11.76	62.21

Maximum hatch in SHA: Pa: 619 lv. = 100%; Ro: 570 lv. = 100%

Table 4. The hatching reaction of *G. pallida* and *G. rostochiensis* to an increasing percentage of tap water (TW) in a mixture with demineralized water (DW). Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are the mean of 10 replicates, two batches of each species.

% tap water	Pa % hatch	Ro % hatch
0 (DW)	6.70	45.9
0.4 (TW : DW = 1 : 255)	7.76	75.1
6.3 (TW : DW = 1 : 15)	9.49	77.1
93.8 (TW : DW = 15 : 1)	14.14	84.7
99.6 (TW : DW = 255 : 1)	14.05	77.4
100 (TW)	9.14	79.0
LSD α = 0.05, df = 48	2.51	32.07

Table 5. The relative hatch percentages of three batches of *G. pallida* and *G. rostochiensis* in undiluted and diluted silver sand percolate. (Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates).

Dilution	Batches of *G. pallida*			Batches of *G. rostochiensis*		
	Pa85	Pa86	Pa87	Ro85	Ro86	Ro87
1/1	6.92	7.73	7.51	6.7	7.9	8.7
1/16	9.32	14.04	11.47	22.7	36.1	19.8
1/256	11.78	14.49	14.39	62.2	48.8	67.3
LSD α = 0.05, df = 36		2.98			19.61	

DW were not significantly different (P = 0.102). The mean hatching rate was 14.7%. The water type did not cause a significant difference in the hatching of *G. rostochiensis* (P = 0.139), but an interaction of batch with water type was present (P = 0.002). Table 6 shows the mean values of the hatching rates of the batches in both water types.

Table 6. The relative hatching rates of two batches of *G. pallida* and *G. rostochiensis* in tap water (TW) and demineralized water (DW). (Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates).

	Batches of *G.pallida*		Batches of *G. rostochiensis*	
	Pa86	Pa87	Ro86	Ro87
DW	15.97	15.51	77.0	35.6
TW	13.42	14.07	9.7	54.9
LSD α = 0.05, df = 32	3.42		11.30	

Effects of water and its ionic composition. In all three hatching tests, TW was used so the spontaneous hatch in water could be determined. Figure 2 shows the results of this spontaneous hatch of *G. pallida* and *G. rostochiensis* in TW for the different batches used in the tests over the years. Figure 3 shows the hatching results of both species in DW (hatching tests 2 and 3). The hatch of *G. pallida* remained low for all batches, independent of the date the tests were carried out or the water type and showed little variation. In contrast, the spontaneous hatch of all batches of *G.rostochiensis* was high in both water types and the high levels of hatch, which were more variable than those of *G. pallida*, were reached independent of the date the tests were carried out.

Chemical analysis of the different water types showed strong differences in ion concentrations (Table 7) resulting in different electrical conductivities. The high electrical conductivity of the SSP was partly caused by the high concentration of Na, Ca and Cl. The concentration of boron in the SSP was also high.

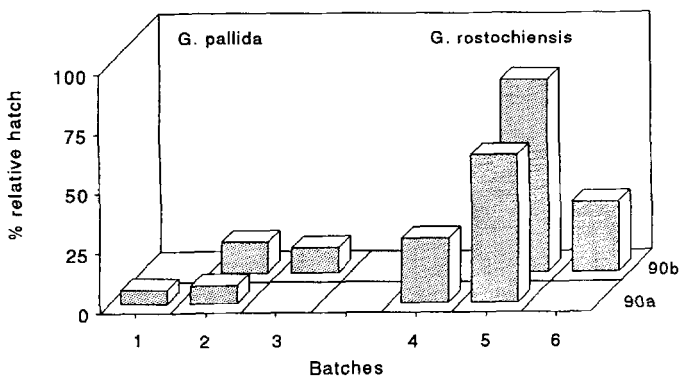

Fig. 2. The relative hatch of *Globodera rostochiensis* and *G. pallida* in tap water (TW). Various batches were used in different years. Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates.
Batch 1, 2, 3, 4, 5 and 6 represent *G. pallida* Pa3-85, Pa3-86, Pa3-87 and *G. rostochiensis* Rol-85, Rol-86 and Rol-87 respectively.

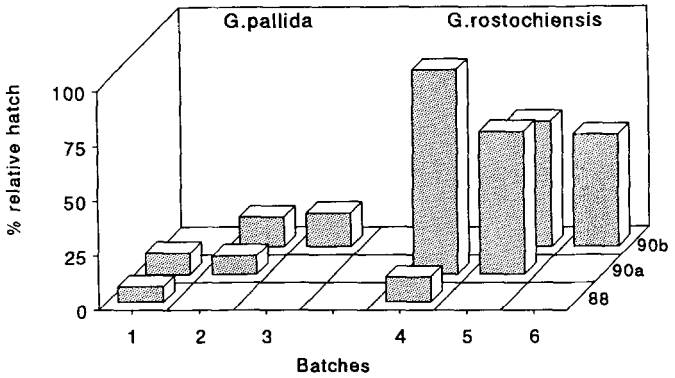

Fig. 3. The relative hatch of *Globodera rostochiensis* and *G. pallida* in demineralized water (DW) in two hatching tests. Various batches were used. Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates. Batch 1, 2, 3, 4, 5 and 6 represent *G. pallida* Pa3-85, Pa3-86, Pa3-87 and *G. rostochiensis* Ro1-85, Ro1-86 and Ro1-87 respectively.

Discussion

Hatching tests are at present the most reliable method to determine the viability of cyst contents after soil fumigation, or to assess the resistance properties of cultivars. Standard procedures are carried out with emphasis on reproducibility by taking enough replicates, but without taking into account the history of the cysts, the presence or

Table 7. The results of the chemical analysis on micro- and macro elements in tap water (TW), demineralized water (DW) and silver sand percolate (SSP).

Elements		TW	DW	SSP
NH_4	(mmol l^{-1})	0.1	0.1	0.1
K	(mmol l^{-1})	0.1	0.1	0.3
Na	(mmol.l^{-1})	0.5	0.2	2.9
Ca	(mmol.l^{-1})	0.6	0.1	1.2
Mg	(mmol.l^{-1})	0.2	0.1	0.3
NO_3	(mmol.l^{-1})	0.2	0.1	0.4
Cl	(mmol.l^{-1})	0.3	0.2	2.6
SO_4	(mmol.l^{-1})	0.2	0.1	0.7
HCO_3	(mmol.l^{-1})	1.3	0.2	1.6
P	(mmol.l^{-1})	0.01	0.01	0.01
Fe	(μmol.l^{-1})	< 0.2	< 0.2	< 0.2
Mn	(μmol.l^{-1})	< 0.2	< 0.2	0.3
Zn	(μmol.l^{-1})	0.6	< 0.2	0.5
B	(μmol.l^{-1})	< 5.0	< 5.0	9.0
Cu	(μmol.l^{-1})	1.0	< 0.5	0.6
pH		7.9	7.4	7.4
EC	(mS.m^{-1})	20	10	70

absence of diapause or species differences that might exist and interfere in the hatching results. Hominick et al. (1985) for example, showed that environmental conditions during the development of females of *G. rostochiensis* on potato roots influenced the hatching characteristics of the juveniles. In our hatching tests batches of cysts of one population of each species were used, grown and stored under well defined conditions. Because of our interest in the differences in the hatching reaction of the species towards PRD, only the number of hatchable juveniles was investigated. By determining the maximum accumulated hatch in a SHA and expressing the amount of hatch in the unknown hatching agent as a percentage of this maximum hatch we were able to make comparisons between batches (Tables 4, 5 and 6) and simultaneously reduced the variability of the hatching trials (Hominick et al., 1985). By assuming that the maximum hatch was reached in the SHA for both species, and all data were related to these numbers, it was possible to express the influence of hatching agents on both species as a relative decrease of hatching. Therefore the changes in hatching pattern of the species may be compared.

The hatching of the nematode species in the PRDs was influenced by the presence of the SSP in the PRDs. The SSP reduced hatching for both species to about 7-8%. Dilution of this SSP with TW increased the hatching of both species significantly, with a final hatching level in the 256 times diluted solution of approximately 13% and 60% for *G. pallida* and *G. rostochiensis*, respectively. In the undiluted PRDs the differential effect of TW on the hatching of the nematode species did not play a role; only the inhibition by the SSP influenced the hatching. The results of the PRDs were adjusted with these control data and related to the hatch in SHA. They show that *G. pallida* hatches better than *G. rostochiensis* in the PRDs (Figures 1A, B and C). The non-transformed data of the hatch in undiluted PRDs showed a similar pattern (e.g. for cv. Bintje: 450 juvenile of *G. pallida* and 281 juveniles of *G. rostochiensis* hatched). Apparently PRD is the major factor to induce hatch of *G. pallida*, whereas for *G. rostochiensis* other factors influence the hatching as well.

In experiments on the interaction between the two potato cyst nematodes described by Den Nijs (1992), plants were grown in a soil mixture which contained 60% silver sand. Reproduction was normal indicating that hatching was not influenced by the presence of this silver sand.

Clarke and Shepherd (1966) found that boron inhibits hatch of *Heterodera avenae*, *H. glycines* and *H. trifolii*. The inhibition by silver sand found in the present work might, among other things, have been caused by the high concentration of this element.

Clarke et al. (1978) demonstrated that osmotic stress inhibits the hatch of *G. rostochiensis*. When the osmotic value of the surrounding environment is equivalent or greater than that of the egg contents, water uptake, essential for hatch, stops (Perry and Clarke, 1981). The SSP had an osmotic pressure of 0.24 atmosphere, whereas the osmotic pressure inside eggs is 9.78 atmosphere, namely equivalent to 0.4M sucrose solution (Clarke et al., 1978). So the inhibition of hatching was not caused by an osmotic stress of the solution.

The reaction pattern of the nematode species in both water types differs considerably. Consistently over batches and years few *G. pallida* hatched, whereas *G. rostochiensis* reached high levels of hatching. Water hatch or spontaneous hatch has been used to determine the minimum hatchability of cysts. Most information exists on *G.*

rostochiensis: it usually reaches values of 10% hatch (Evans, 1982, 1983; Fenwick, 1952; Shepherd, 1962). Mulder and Vroom-Wolf (1990) found high percentages of hatch in tap water, ranging from 5 to 60%, dependent on the time of the year, in experiments on periodicity with vintage cysts of one population of *G. rostochiensis*. We found in all hatching tests relative hatching rates varying from 60 to 90%. This corresponds with 23 to 47% hatch when expressed as percentages of the total amount of eggs applied. So spontaneous hatch of *G. rostochiensis* in TW was consistently high and independent of the period when hatching tests were performed.

G. pallida and *G. rostochiensis* share the same habitat but it appears that they have different ways to ensure that the roots are reached and the population is maintained. By reacting mainly to PRD, *G. pallida* synchronizes its life cycle completely with its host. Robinson et al. (1987) found that *G. pallida* had a much slower initial rate of hatch and utilized its lipid reserves more slowly than *G. rostochiensis*. This prolonged hatch and persistence may ensure that root growth is less affected, thus reducing intraspecific competition. From an ecological point of view this seems to be a good strategy.

G. rostochiensis shows a more opportunistic behaviour, by reacting to non-specific hatching triggers. Combined with the faster hatch of this species it might diminish interspecific interaction in predictable situations. However, the juveniles ought to posess the ability to survive a certain period in the soil when the host is not present. Indeed Den Ouden (1960) found that *G. rostochiensis* could survive 7 weeks in the soil and Mulder et al. (1988) found that *G. rostochiensis* survived longer in soil than *G. pallida*, under the same conditions. Insight in the ecological conditions in the original habitat in Peru, might bring more understanding on this behaviour.

The species specific differences in hatching behaviour have considerable implications for hatching tests. Results from hatching tests obtained from one species may not be relevant to the other species. It seems advisable to choose the test conditions very carefully in order to avoid artefacts which may obscure the effects of interest. In the hatching tests presented here, egg suspensions instead of cysts were used to avoid a major source of variation. In the field the nematode eggs are protected inside cysts, other factors may therefore influence hatching as well. However, when the high spontaneous hatch of *G. rostochiensis*, we found in water, also takes place in the soil, it can lead to the natural break down of the nematode population of more than 30%, a figure which is generally accepted as being normal. Andersson (1988) and Den Ouden (1960) have found higher rates of 50 to 60%. For *G. pallida,* data on natural break down of populations in the field are not available at the moment.

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