

Effects of daylength during cyst formation, storage time and temperature of cysts on the *in vitro* hatching of *Globodera rostochiensis* and *G. pallida*

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Summary – Cysts from two potato cyst nematode populations were produced in three seasons with differing daylength and hatching of juveniles was stimulated with potato root diffusate after storage at 13 or 22 °C. Behaviour of the progenies differed. Long-day conditions produced rapid high hatching rates (61-97 %) in both populations, but progenies obtained under the shortest-day regime had lower hatching rates (12-45 %) when cysts were stored at 13 °C and no hatching at 22 °C. With moderately short days, increase of the storage temperature from 13 to 22°C increased the hatching of the *Globodera rostochiensis* population from 1 to 9 % and from 1 to 34 % and reduced hatching of the *G. pallida* population from 30 to 0 % and from 87 to 8 % (6 and 12 weeks' storage, respectively). *G. rostochiensis* juveniles hatched quicker than *G. pallida* at 22 °C, but not if storage temperature was 13 °C. Variations in hatching behaviour between seasonal progenies were more marked with *G. rostochiensis* than with *G. pallida*.

Résumé – Effets de la photopériode pendant la formation des kystes, de la température et du temps de stockage des kystes sur l'éclosion *in vitro* de *Globodera rostochiensis* et *G. pallida* – Des kystes de *Globodera rostochiensis* et *G. pallida* (une population chacun) sont produits pendant trois périodes de l'année et, après stockage à 13 et 22 °C, l'éclosion des J2 est stimulée par des exsudats radiculaires de pomme de terre. Les différentes générations montrent des comportements différents lors de l'éclosion. Les jours longs conduisent à une éclosion rapide et forte (61-97 %), alors que les générations obtenues pendant les jours très courts ont des taux d'éclosion faibles (12-45 %) lorsque les kystes sont stockés à 13 °C tandis qu'il n'y a aucune éclosion à 22 °C. Avec des jours modérément courts, l'augmentation de la température de stockage de 13 à 22 °C accroît l'éclosion de *Globodera rostochiensis* de 1 % à 9 % et de 1 % à 34 % et réduit celle de *G. pallida* de 30 % à 0 % et de 87 % à 8 % (6 et 12 semaines de stockage, respectivement). A 22 °C la population de *G. rostochiensis* éclot plus rapidement que celle de *G. pallida*, ce qui n'est pas le cas à 13 °C. Les variations sont plus marquées pour *G. rostochiensis* que pour *G. pallida*.

Key-words : *Globodera*, hatching, diapause, daylength, root diffusate, potato.

Nematodes can increase their survival rates by arresting development under unfavourable conditions. These periods of inactivity are called "dormancy", which may be divided into quiescence and diapause. Quiescence is a hypometabolic stage directly initiated by adverse environmental conditions and finishes when favourable conditions return. The ability of certain plant nematodes to synchronize their life cycles to that of the host is related to diapause. Obligate diapause seems to be initiated by internal factors while environmental factors promote the dormancy in the case of facultative diapause, acting as a signal for the receptive stage (Antoniou, 1989). Potato cyst nematodes (PCN) consecutively exhibit an obligate diapause which is initiated immediately after formation of the cysts and which has a limited duration – first season diapause (Ellenby & Smith, 1967 – and a facultative one, so far demonstrated for *Globodera rostochiensis* (Hominick *et al.*, 1985). In any situation, juveniles normally need an adequate chemical stimulus from a host root diffusate to hatch (Perry & Charke, 1981).

The knowledge of factors which induce facultative diapause can be very important in pest control (Sikora, 1984) and in resistance-screening tests (Janssen *et al.*,

1987). Some studies showed the influence of light (Franco & Evans, 1979; Hominick, 1986) and low temperatures (Hominick, 1979) on the receptive stages determining facultative diapause in *Globodera rostochiensis*. Janssen *et al.* (1987) found that diapause can be bypassed by avoiding desiccation of the cysts. Phenology of the host plant during cyst formation has been related to hatching success in some *Heterodera* species (Gaur *et al.*, 1992).

The aim of the present work is to investigate facultative diapause in *G. rostochiensis* and *G. pallida* by studying the hatching behaviour of juveniles stored for 6 and 12 weeks at 13 and 22 °C, after different daylength regimes during the formation of the cysts.

Material and methods

Two populations – P and R – were chosen for the experiment. The first, pathotype Pa2 of *G. pallida*, was obtained from the collection of the Centro Internacional de la Papa (Lima, Peru) in October 1986. The *G. rostochiensis* population was recovered from a field in Northern Spain and was virulence group Ro1/Ro4 (Salazar, 1991).

Table 1. Production of populations of *Globodera rostochiensis* and *G. pallida* on cv. Arran Banner for standardised hatching tests on F2 progenies.

Production of the parental populations	Seasonal multiplication periods and daylengths	Beginning of the stimulation after 6 (a1) and 12 (a2) weeks of storage	
September (year 0)	S1 21.6 to 12.9 (year 1) Daylengths 15 h 04' - 12 h 38'	6 weeks	24.11 (year 1)
			12 weeks
	S2 3.10 to 23.12 (year 1) Daylengths 11 h 43' - 9 h 17'	6 weeks	6.3 (year 2)
			12 weeks
	S3 13.2 to 9.6 (year 2) Daylengths 10 h 35' - 14 h 59'	6 weeks	6.9 (year 2)
			12 weeks

PRODUCTION OF THE ORIGINAL POPULATIONS

For each population an inoculum of 10 viable eggs per gram of soil (25 to 30 cysts) was put in muslin bags in 12 cm diam. clay pots with sprouted tubers cv. Arran Banner. Their progenies (F1) were recovered in summer 1987, processed by counting and estimating viable cyst contents (Shepherd, 1986) and stored at 4-8 °C in darkness. The parental generations were rejected.

PRODUCTION OF THE SEASONAL PROGENIES

Three seasonal inoculations of the F1 generations were performed on cv. Arran Banner. The first, S1, took place 9 months after the recovery of the F1 and then at 4-month intervals, S2 and S3 respectively. The timetable of this study is shown in Table 1. The first and second multiplication periods correspond to a summer (S1) and an autumn (S2) season, respectively. The third

multiplication season (S3) includes the last part of the winter and the whole spring. Temperature in the greenhouse was maintained at 20-25 °C and relative humidity at 80 %. Irrigation was by sprinkling when required. The F2 generation cysts were processed as for the F1. After this time the F2 generations were stored at 13 ± 0.5 °C and 22 ± 1 °C in darkness.

HATCHING

After 6 and 12 weeks' storage (age 1 and age 2 respectively), hatching was stimulated at the original F2 storage temperatures. Three replicates of 25 intact cysts per group were taken and placed on sieves in histology blocks in darkness. After soaking for one week, potato root diffusate (PRD) was added, obtained from cv. Arran Banner using the method of Evans (1983).

The numbers of juveniles hatched in each histology block were counted weekly and PRD was replaced at this time. Stimulation continued for 2 weeks after the juveniles stopped hatching. After crushing the cysts from each replicate and counting the remaining eggs and unhatched juveniles, the percentage of hatched juveniles based on the estimated total content (hatching rate) was calculated.

Results

Daylength decreases during multiplication season S1 (moderately short days) and S2 (short days) and increases during season S3. Root weight and tuber weight varied within seasons as expected and reached a maximum in season S3. The highest multiplication rates of nematodes were obtained in season S1 (Table 2). No correlation between root weight and multiplication rates were observed.

TOTAL HATCH

The percentages of hatched juveniles from the F2 generations after different storage temperatures and times are presented in Table 3 and the analysis of variance for each season in Table 4. The nematode population significantly influenced hatching rate for S1, S2

Table 2. Effects of daylength and hours of sunshine on root and tuber weight and nematode multiplication on cv. Arran Banner.

Season	Average daylength (h)	Average sunshine hours per day (h)	Root dry weight per plant (g)	Tuber fresh weight per plant (g)	Multiplication of nematodes (number of cyst × viable contents)	
					P	R
S1	14 h, Decreasing	7	0.07	9.3	55	74
S2	10 h, Decreasing	4	0.02	14.5	13	24
S3	13 h, Increasing	6	2.08	64.7	20	16

and S3, as did storage temperature on the S1 and S2 progenies, and storage time (age) on S1 and S3 progenies. Temperature and age did not interact, whereas there was a significant interaction between population in all cases. The other interactions were significant only for S1 and S2 progenies.

Hatching of the progenies from season S3 was much higher than for the other two progenies and much less variable (61-97 %) between the different storage conditions. The hatching rates for the season S2 were the smallest (0-45 %) and no hatching was recorded in the cases in which cysts were stored at 22 °C. For S1 progenies, the longer storage period (12 weeks) increased

hatching if stored at appropriate storage temperatures. Thus, for population R hatching increased from 9 to 34 % if storage temperature was 22 °C, while for population P hatching reached a high level (30 to 87 %) if stored at 13 °C.

Increasing the storage time of the cysts generally increased the hatching rates, population P from season S2 being a clear exception.

Generally, under optimal storage conditions, the hatching rates of population P were greater than those of population R for seasons S1 and S2 while for season S3 the opposite was the case.

Table 3. Mean hatching rates of the F2 progenies after 6 and 12 weeks of storage at 13 and 22 °C.

Season	Hatching (%)						
	S1		S2		S3		
Population	P	R	P	R	P	R	
Storage Time	Temperature						
6 weeks (age 1)	13 °C	30	1	45	12	82	85
	22 °C	0	9	0	0	61	96
12 weeks (age 2)	13 °C	87	1	22	21	87	86
	22 °C	8	34	0	0	85	97
LSD (p < 0.05)		2	15	2	1	5	1

Table 4. Analyses of variances for the hatching rates of juveniles from each seasonal progeny.

Source of variation	d.f.	Mean squares		
		S1	S2	S3
Replication	2	0.0052 n.s.	0.0002 n.s.	0.0062 n.s.
Population	1	0.4255***	0.0579***	0.2019***
Temperature	1	0.2400**	1.5819***	0.0157 n.s.
Age	1	0.5674***	0.0065 n.s.	0.0693*
Population * Temperature	1	1.7689***	0.0532***	0.1645***
Population * Age	1	0.952*	0.0532***	0.0346 n.s.
Temperature * Age	1	0.0001 n.s.	0.0050 n.s.	0.0244 n.s.
Population * Temperature * Age	1	0.1891**	0.0487***	0.0157 n.s.
Error	14	0.0187	0.0022	0.0080
Mean hatching		0.385	0.259	1.198
CV		36 %	18 %	7 %

Values have been transformed using arc sine \sqrt{x}

SPEED OF HATCHING

In Figure 1 the numbers of hatched juveniles per cyst over the hatching period are presented for the two progenies obtained in each season.

In general, population P hatched quicker at 13 °C than at 22 °C, but the opposite was true for population R. For each population, hatching of the S3 progeny was quicker than the hatching recorded from S1 and S2 progenies with respect to the same storage temperature.

In the case of the progenies from season S3, storage time did not act on speed of hatching since curves recorded after 6 and 12 weeks storage run in parallel for the same population and temperature. When storage temperature was 13 °C, the S3 progenies of populations R and P had a similar hatching behaviour, characterized by a high initial hatching which decreased as the stimulation period progressed. Nevertheless, at 22 °C the hatching of juveniles of population R started very early, with 25 to 50 % of the juveniles hatched after only one week of stimulation, whereas the hatching velocity of S3 progeny of population P stored at 22 °C was almost constant.

More than 50 % of the hatchable juveniles of population R from season S1 stored at 22 °C hatched during the first and second week of stimulation but, in spite of that, the S1 and S2 progenies of population R hatched very slowly. Hatching was also slow for the S1 progeny of population P stored at 22 °C despite a slight increase after 7 weeks of stimulation. When stored at 13 °C, juveniles of S1 and S2 progenies of population P hatched much more rapidly than those of population R although normally they did not start hatching immediately after stimulation, and one (S2, 6 weeks storage) and two (S1, 12 weeks storage) peaks of hatching were recorded over the stimulation period.

Discussion

Several authors have reported the influence of day length and light intensity during cyst formation on the hatching of juveniles in *G. rostochiensis* and *G. pallida*. For example, Franco and Evans (1979) found that more juveniles hatched from cysts obtained under 16 h than under 12 h days, and Hominick (1986) found that hatching responses depended on the amount and/or intensity of light on the plants. From our results it is also evident that juveniles from cysts produced under increasing daylength conditions (S3) hatched with no apparent limitation while the shorter days were the more variable and lower hatching rates were recorded. Since the presence of quiescence must be ruled out looking at the storage conditions which were the same for all progenies, we can state that increasing daylength during multiplication prevented the establishment of diapause.

Short days seemed to affect *G. rostochiensis* more than *G. pallida*, since the average hatching rates of *G. pallida*

progenies were higher than those of *G. rostochiensis* for seasons S1 and S2.

In the presence of diapause, a high variability in the hatching rates was found depending on the storage conditions and on the nematode population. As shown by the significant interactions in the analysis of variance in Table 4, it is difficult to draw general conclusions but nevertheless some trends are noticeable. The progenies obtained under shortest days (S2) are only able to hatch partially after storage at 13 °C and not at all after storage at 22 °C (Table 3). The hatching rate is greater for *G. pallida* than for *G. rostochiensis* but only for the shorter storage period. In contrast for the progenies obtained under moderate daylength conditions (S1), storage at 13 °C is effective with P population, *G. rostochiensis* progeny from S1 needing storage at 22 °C to hatch. Generally the temperature effects within the S1 progenies were emphasized after a longer time of action.

Although less noticeable, differences in hatching behaviour related to temperature occurred for the S3 progenies in the same way as described for the S1 progenies. The preference of *G. rostochiensis* for higher temperatures than *G. pallida* is widely reported (Parrot & Berry, 1976). Franco (1979) found that *G. rostochiensis* populations hatch better at temperatures between 15 and 20 °C, while the optimal temperatures for the hatching of *G. pallida* were somewhat lower, between 10 and 20 °C. Stanton and Sartori (1990) specified 22 °C as the optimum temperature for the hatching of *G. rostochiensis*.

It has also been reported that under the same conditions, juveniles of *G. pallida* need more time to hatch than those of *G. rostochiensis* (McKenna & Winslow, 1972; Parrot & Berry, 1976; Robinson *et al.*, 1987; Salazar, 1991). This is supported by our results (Fig. 1). While the hatching curves of the progenies subject to an intensive diapause are greatly dependent on the storage conditions, the R population from the S3 season tended to hatch quicker, and reached full relative hatching in a shorter period than the P population, especially for the 22 °C storage temperature.

The present results obtained for these populations and under these trial conditions confirm that *G. rostochiensis* and to some less extent *G. pallida*, are subject to a facultative diapause, which is fixed during the formation of the population and can be prevented under increasing long-day conditions. Such diapause can be considered gradual, since it does not imply an all-or-nothing response. Population specific temperatures and storage time can modify to a certain degree the hypoactive stage by increasing the hatchability of *Globodera* spp. juveniles. In situations where diapause induction is missing or only partial, the populations behave according to the ecophysiological characteristics of each species.

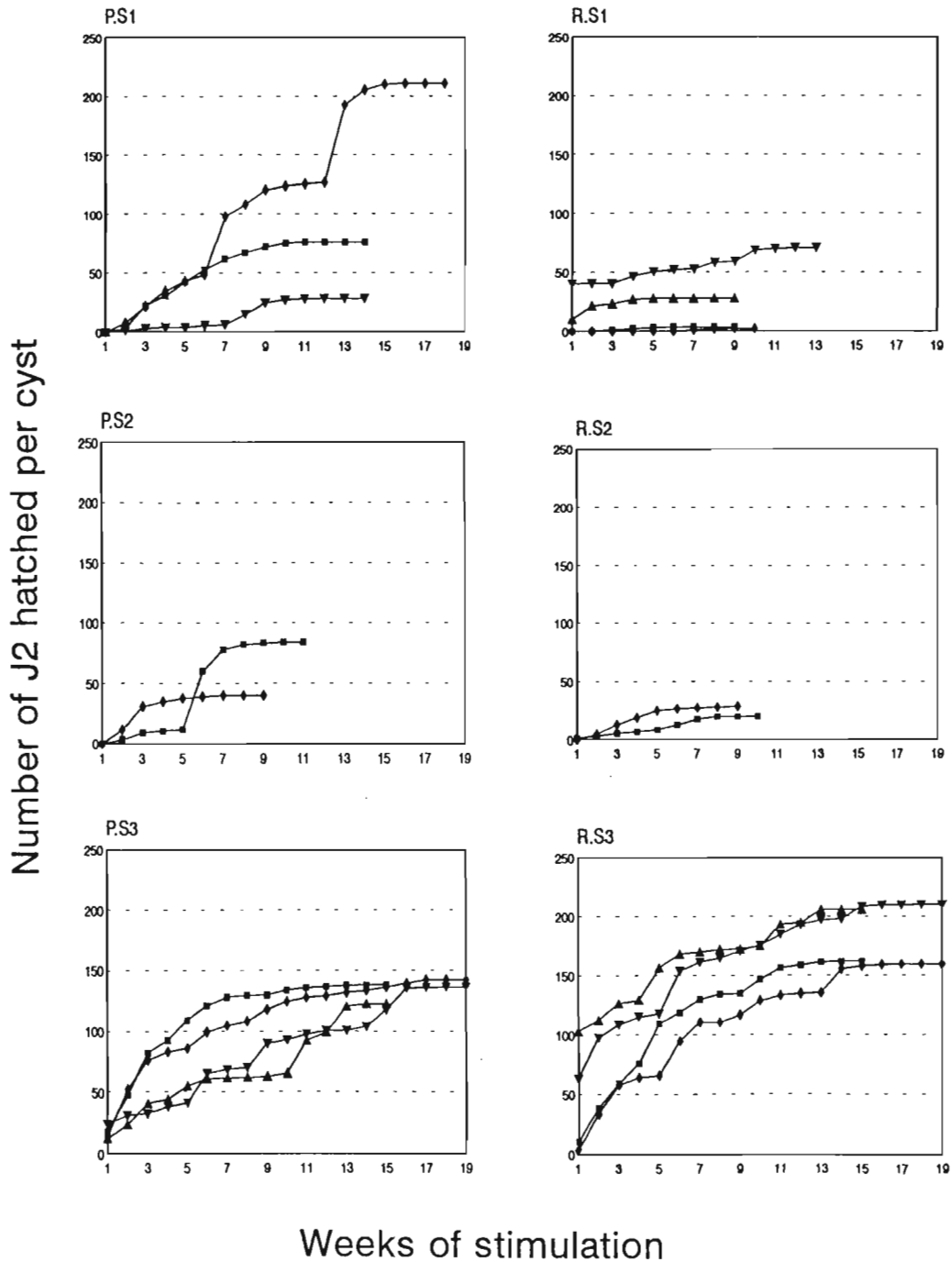


Fig. 1 : Cumulative weekly totals of hatched juveniles from two different PCN populations, P (*Globodera pallida* Pa2) and R (*G. rostochiensis*, virulence group Ro1/Ro4). Cysts were obtained under different daylength conditions (S₁, S₂, S₃) and stored for different periods (age 1, age 2) under different temperatures (13 °C, 22 °C). (See Table 1 for key to multiplication periods and storage conditions ; ■ = 13 °C, age 1 ; ◆ = 13 °C, age 2 ; ▲ = 22 °C, age 1, ▼ = 22 °C, age 2).

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