

The effect of brief exposures to potato root diffusate on the hatching of *Globodera rostochiensis*

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SUMMARY

Hatching from free eggs of *Globodera rostochiensis* was stimulated by immersion in potato root diffusate for only 5 min per week for 5 weeks; increasing the period of stimulation up to 24 h did not significantly increase hatch. Repeated stimulation and the use of fresh diffusate significantly enhanced hatch when compared to the hatch resulting from single exposures to diffusate or to the hatch obtained when the same diffusate was used each week.

RÉSUMÉ

L'effet d'immersions brèves dans l'exsudat radiculaire de pomme de terre sur l'éclosion de Globodera rostochiensis

L'éclosion d'œufs libres de *Globodera rostochiensis* est stimulée par une immersion limitée à 5 minutes par semaine pendant 5 semaines dans un exsudat radiculaire de pomme de terre; l'allongement de la durée d'immersion jusqu'à 24 heures n'entraîne aucune augmentation significative du taux d'éclosion. Une immersion répétée de même que l'emploi d'exsudat frais améliorent significativement l'éclosion, comparativement à celle résultant d'une immersion unique ou à celle obtenue en employant le même exsudat pendant 5 semaines d'expérience.

The hatching mechanisms of nematodes have been reviewed (Perry & Clarke, 1981) and one aspect that was apparent was the rapid action of root diffusate in initiating the hatching of *Globodera rostochiensis* (Woll.) and *G. pallida* Stone. During the first 24 h after the hatching stimulus physiological changes in the unhatched *G. rostochiensis* juvenile, such as an increase in the oxygen uptake (Atkinson & Ballantyne, 1977a), a decrease in the adenylate energy charge (Atkinson & Ballantyne, 1977b) and an increase in water content (Ellenby & Perry, 1976) occur 3-4 days prior to movement and hatching (Doncaster & Shepherd, 1967). A 5 mn exposure each week is sufficient to induce a 43% hatch of juveniles from free eggs of *G. pallida* (Forrest & Perry, 1980). Similar data are lacking for the hatch of *G. rostochiensis* so we investigated the response of *G. rostochiensis* to brief exposures of potato root diffusate to learn more about the hatching mechanism of this species.

Materials and Methods

Cysts of *G. rostochiensis* Ro1, grown on potato cv. Arran Banner, in pot cultures were from a single generation harvested in 1979 and had been subsequently stored at room temperature (20°). They were used in batches of 25 and soaked in artificial tap water (Greenaway, 1970) for four days at 20°.

They were then cut, the eggs were freed from the cyst and any free juveniles were removed. The suspension of eggs and cyst debris from each batch was added to 6 mm diameter glass cylinders with 45 µm mesh nylon netting fixed over the ends (Forrest & Perry, 1980). Soil leachate and potato root diffusate were obtained by the method of Fenwick (1949) and the latter was diluted with artificial tap water 1 in 4 by volume. Four different experimental protocols were followed, each involving exposure of the eggs to root diffusate for seven different periods from 5 mn to 24 h (Fig. 1) at 20°.

Tests were also carried out to determine the percentage hatch over a five week period from hatches of eggs kept continuously in potato root diffusate and soil leachate. Counts of hatched juveniles were made weekly when diffusate and leachate were replenished from stock solutions kept at 5°.

EXPERIMENT 1 : REPEATED STIMULUS USING FRESH DIFFUSATE EACH WEEK

Each cylinder was placed in a 9 ml capacity vial containing 3 ml of diffusate, for the appropriate time. After treatment the cylinders were rinsed under running tap water for 30 mn then placed in a vial containing 3 ml of artificial tap water. Previous tests showed that washing for 30 mn completely removed the hatching stimulus. The above procedure was repeated with fresh diffusate from the stock solution each week for 5 weeks and counts of hatched juveniles were taken weekly. Each test was repeated three times at the end of which the number of unhatched eggs was counted and the percentage hatch was determined over the test period.

EXPERIMENT 2 : REPEATED STIMULUS USING THE SAME DIFFUSATE EACH WEEK

The above procedure was followed except that, for each period of exposure, the same root diffusate was retained throughout the 5 weeks.

EXPERIMENT 3 : SINGLE STIMULUS

In these tests, for each time period, root diffusate was used for the initial stimulus only. Counts were taken at weekly intervals when the tap water in the vials was changed ; the eggs were neither re-exposed to diffusate nor rinsed for 30 mn.

EXPERIMENT 4 : SINGLE STIMULUS WITH RINSING

To check whether the extra mechanical stimulation and oxygenation associated with rinsing enhanced hatch, the single stimulus procedure was followed but each week the cylinders were flushed with tap water for 30 mn before being placed in 3 ml of fresh artificial tap water.

Results

The mean number of free eggs recovered from a batch of 25 cysts was $4\ 945 \pm 247$. The total hatch

for eggs kept continuously in root diffusate and soil leachate for 5 weeks was 69% and 2%, respectively. The total percentage hatch of juveniles for each exposure to root diffusate is given in Fig. 1. Results were analysed using two way analysis of variance after arcsin transformation of percentages.

A 5 mn exposure to fresh root diffusate each week induced a 51% hatch and there was no significant increase or decrease in hatch with increase in the period of stimulation ($P > 0.05$). Using the same diffusate each week for re-stimulation gave less consistent results with the greatest hatch (36%) after 15 mn exposure each week and the least (14%) after 1 h exposure each week. The variation in hatch with period of exposure was not statistically significant ($P > 0.05$). However, for each period of exposure the percentage hatch was much greater when fresh diffusate was used each week and the difference between results for the repeated stimulation tests using the same diffusate and fresh diffusate is highly significant ($P < 0.01$). Even weekly exposures of up to 24 h to the same diffusate did not elicit as great a hatch as 5 mn per week of fresh diffusate (28% and 51% respectively) even though the total time exposed to diffusate in the latter test was only 25 mn. Thus, the use of fresh diffusate from stock solutions for re-stimulation is of greater importance than the duration of stimulation.

Using root diffusate for the initial stimulation without any subsequent rinsings elicited hatches which did not exceed 15% (Fig. 1). The smallest hatch was after 24 h exposure (9%) and the largest after 30 mn (15%). Including weekly rinsing, with the concomitant mechanical stimulation, did not significantly increase hatch ($P > 0.05$). For both sets of data an increase in the period of exposure did not significantly affect the total percentage hatch ($P > 0.05$). For each time period the hatches from both tests using single stimulation was lower than hatches from experiments on repeated exposure to diffusate and this difference was statistically significant ($P < 0.01$). Thus, repetition of the stimulus, even with the same diffusate and for short periods, stimulated greater hatch.

Discussion

All treatments with potato root diffusate increased hatch from free eggs when compared to the hatch induced by soil leachate. However, a single stimulation, whether or not it was accompanied by weekly rinsing, enhanced hatch only slightly. A much greater hatch was obtained with repeated stimulations especially if fresh diffusate was used each time. Duration of stimulation is of only marginal import-

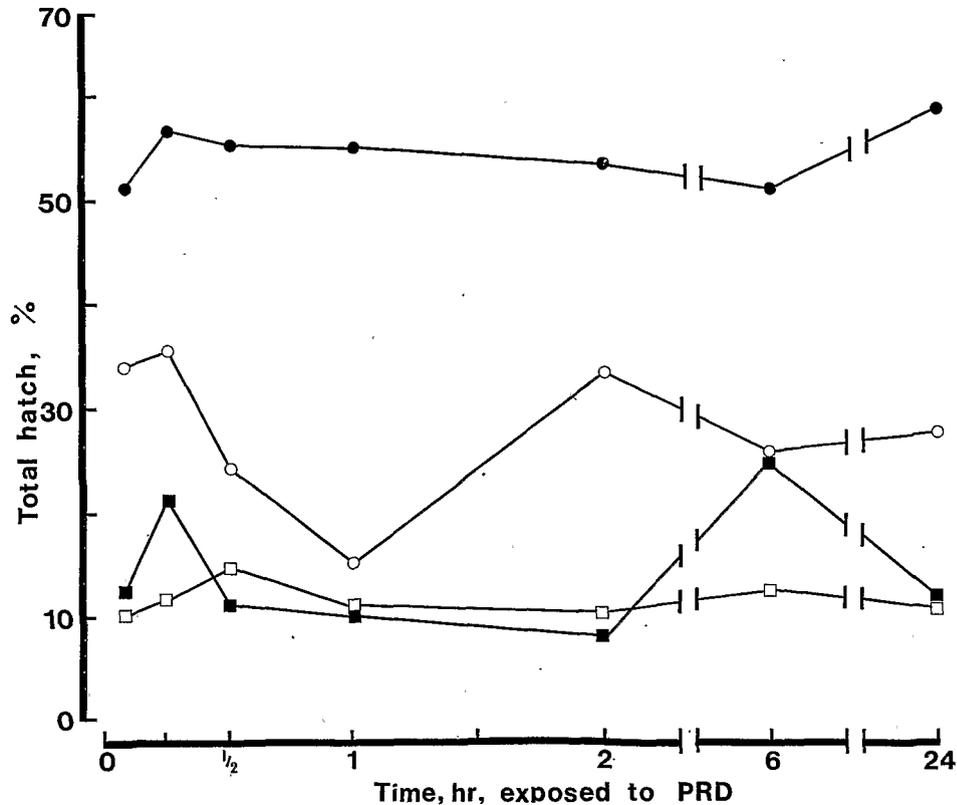


Fig. 1. The total percentage hatch of free eggs of *Globodera rostochiensis* exposed to potato root diffusate (PRD) for various periods : black circles : repeated weekly exposure to fresh diffusate ; white circles : repeated weekly exposure to the same diffusate ; black squares : single exposure with subsequent weekly rinsings ; white squares : single exposure with no rinsings.

ance in hatching ; this work shows that repetition of the stimulation and fresh diffusate are of overriding importance in stimulating hatch from free eggs of *G. rostochiensis*.

Clarke, Perry and Hennessy (1978) proposed that a change in permeability of the *G. rostochiensis* egg shell allowed the diffusion of egg fluid solutes out of the egg, which removed the osmotic stress on the juvenile with concomitant increase in juvenile water content. This increase allows the juvenile to become fully mobile and start the behavioural sequence leading to eclosion. The change in permeability has yet to be established although it is known to occur in *Ascaris suum*, a species whose hatching mechanism (Clarke & Perry, 1980) shows some similarities to that of *G. rostochiensis*. Permeability change could be caused directly or indirectly by the hatching agents (Clarke & Perry, 1977 ; Perry, 1978) and it is possible that the juvenile could be involved in the hatching process at an early stage.

Fisher (1966) found that exposing fourth stage juveniles of *Paratylenchus nanus* to root diffusate induced moulting 8-13 days later but it was not necessary to expose them to diffusate all the time. Exposure for 24 h "triggered" moulting and Rogers and Sommerville (1968) suggested that root diffusate could stimulate a receptor which in turn restarted internal mechanisms governing moulting or could replace a missing link in the sequence of internal secretions. The present work on *G. rostochiensis* shows that very brief exposures to root diffusate are sufficient to "trigger" the sequence of events leading to hatch. The enhancement of hatch by repeated stimulation may indicate a receptor threshold level being reached whereas an equivalent period of single stimulation does not reach the threshold level and the receptor, therefore, does not "fire". Ellenby and Perry (1976) suggested that the *G. rostochiensis* juvenile could be involved in the initial stages of the hatching sequence, as the

hatching factors may initiate a neurosecretory response from the juvenile.

Although of importance, repetition of the stimulus is not the only significant factor; using fresh diffusate each week, even for periods as short as 5 mn, significantly increased hatch almost to the level obtained with eggs continuously in diffusate. The reasons for this are unknown but it is unlikely that the diffusate activity had been reduced when the same diffusate had been used weekly for 5 weeks because afterwards the diffusate used for all seven time periods was pooled and 3 ml of this diffusate for 3 weeks elicited a 63% hatch.

In soil conditions there is a 35-40% "carry over" of viable cyst contents from one year to the next (Jones & Parrott, 1969). This would seem unlikely if hatching is elicited by very short exposures to root diffusate which may persist in the soil for some time (Tsutsumi, 1976; Perry, Hodges & Beane, 1982). However, under field conditions not all cysts would be reached by root diffusate; the cyst as a hatching unit (Ellenby, 1956) is obviously a significant factor and, as pointed out elsewhere (Clarke & Perry, 1977), tests on free eggs may ignore important factors such as inhibition of hatch or distribution of eggs within cysts. Changes in the physiology of the unhatched juvenile (associated with dormancy, for example) may block the sequence of events leading to eclosion.

Soaking for 5 mn in diffusate is also very effective in stimulating hatch of juveniles in whole cysts of *G. pallida* (Forrest in Forrest & Perry, 1980) and *G. rostochiensis* (Perry, unpubl.) but with these experiments it was not certain that rinsing after the exposure period effectively removes all the diffusate from within the cyst.

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