

The effect of potato root diffusate on the desiccation survival of unhatched juveniles of *Globodera rostochiensis*

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SUMMARY

Exposing cysts and free eggs of *Globodera rostochiensis* to potato root diffusate before subjecting them to desiccation decreased hatch, compared to controls exposed to soil leachate, when the cysts and eggs were stimulated to hatch after rehydration. Loss of trehalose and alteration of egg-shell permeability is thought to be associated with exposure to potato root diffusate, rendering the unhatched juvenile more susceptible to desiccation.

RÉSUMÉ

Effet des diffusats de racines de pomme de terre sur la résistance à la dessiccation des juvéniles non éclos de Globodera rostochiensis

Des kystes et des œufs libres de *Globodera rostochiensis*, primitivement mis pendant 24 heures en contact avec un diffusat de racine de pomme de terre, ont été soumis à la dessiccation et ensuite mis à nouveau en contact avec du diffusat de racine pour provoquer l'éclosion. Dans ces conditions, l'éclosion a été plus faible que chez des kystes ou des œufs qui avaient été primitivement mis en contact avec des percolats de sol. L'auteur pense que l'exposition au diffusat de racine cause une perte en trehalose et une altération de la perméabilité du tégument de l'œuf qui rendraient le juvénile non éclos plus sensible à la dessiccation.

Some plant-parasitic nematodes have structural and behavioural adaptations that enhance desiccation survival (Evans & Perry, 1976). The unhatched second-stage juvenile of *Globodera rostochiensis* is protected from desiccation by the egg-shell which becomes increasingly impermeable to water as it dries (Ellenby, 1968), and probably by the trehalose solution surrounding the juvenile (Clarke, Perry & Hennessy, 1978) in which loss of water would be opposed by increasing concentration of the egg fluid solutes. Both the egg-shell, especially the inner lipo-protein membrane (Perry, Wharton & Clarke, 1982), and trehalose are thought to be involved in the hatching mechanism of *G. rostochiensis* (Clarke & Perry, 1977). Clarke, Perry and Hennessy (1978) suggested that exposure of the egg to potato root diffusate (PRD) caused a change in permeability of the egg-shell permitting leakage of trehalose from the perivitelline space thus allowing the juvenile to hydrate (Ellenby & Perry, 1976) and subsequently hatch; recently Clarke (pers. comm.) demonstrated the release of carbohydrate from cysts treated with PRD before the juveniles emerged.

I have examined the possibility that desiccation survival of the unhatched juveniles of *G. rostochiensis* is affected by exposure to PRD.

Materials and methods

Cysts of *G. rostochiensis* Rol, grown on potato cv. Arran Banner in pots, were from a single generation harvested in 1979 and stored at room temperature (20°) after extraction from the soil. Ten replicates of 20 cysts each were placed in glass staining blocks and soaked in artificial tap water at 20° (Greenaway, 1970). After seven days the tap water was removed and PRD or soil leachate were added each to five replicates. Soil leachate and PRD were obtained by the method of Fenwick (1949) and PRD was diluted with artificial tap water one in four by volume. Cysts were kept in the solutions for 24 h, which is long enough for PRD to initiate the hatching process but too soon for any hatching (Perry & Beane, 1982); cysts were then desiccated, ten replicates of 20 cysts being used for each of seven desiccation

periods (Fig. 1). Batches of 20 cysts were transferred to separate cells on leucocyte migration plates (Sterilin Ltd.). Solutions surrounding the cysts were removed using filter paper slivers and each plate was placed in a small 550 cm³ desiccation chamber containing coarse silica gel to give 0% relative humidity. After desiccation at 20° each batch of cysts was returned to the staining blocks and the cysts rehydrated for one week in artificial tap water. PRD was then added to the cysts and hatching tests were conducted at 20° for five weeks; hatched juveniles were counted weekly and total percentage hatch determined at the end of each test. PRD and soil leachate were replenished weekly.

Similar experiments were carried out on free eggs. Ten cysts were soaked for one week in artificial tap water and the eggs freed by cutting the cysts open with a scalpel. Approximately equal numbers of eggs were transferred to two staining blocks using a 5 λ microcapillary. Soil leachate was added to one group of eggs and PRD to the other and left for 24 h at 20°. Solutions were then removed, eggs washed in distilled water, transferred to migration plates and desiccated for various periods (Fig. 2) at 0% relative humidity. After desiccation, eggs were soaked in artificial tap water for one day and then set to hatch in PRD, counts of hatched juveniles

being taken at weekly intervals for four weeks. Using counts of the number of eggs immediately before desiccation, total percentage hatch was determined at the end of each test.

The increase in metabolism that occurs before eclosion (Atkinson & Ballantyne, 1977a, b) may make juveniles exposed to PRD more susceptible to desiccation by reducing their food reserves, especially lipids. To check this, eggs were hatched, by slight mechanical pressure, in artificial tap water and the juveniles were separated into two groups in leucocyte migration plates, one group being transferred to PRD and the other to soil leachate. After two days these solutions were removed and the nematodes desiccated at 20° using techniques similar to those of Perry (1977). All superficial water was removed from the nematodes using filter paper slivers and the nematodes were dried for various periods in desiccation chambers containing glycerol solutions (Gröver & Nicol, 1940) giving 45% relative humidity. After desiccation, artificial tap water was added to the nematodes and revival checked at intervals up to seven days; the criterion for revival was movement of the nematodes. Tests were repeated with juveniles from both the PRD and soil leachate treatments to determine the time of exposure which 50% of the juveniles would survive (S_{50}).

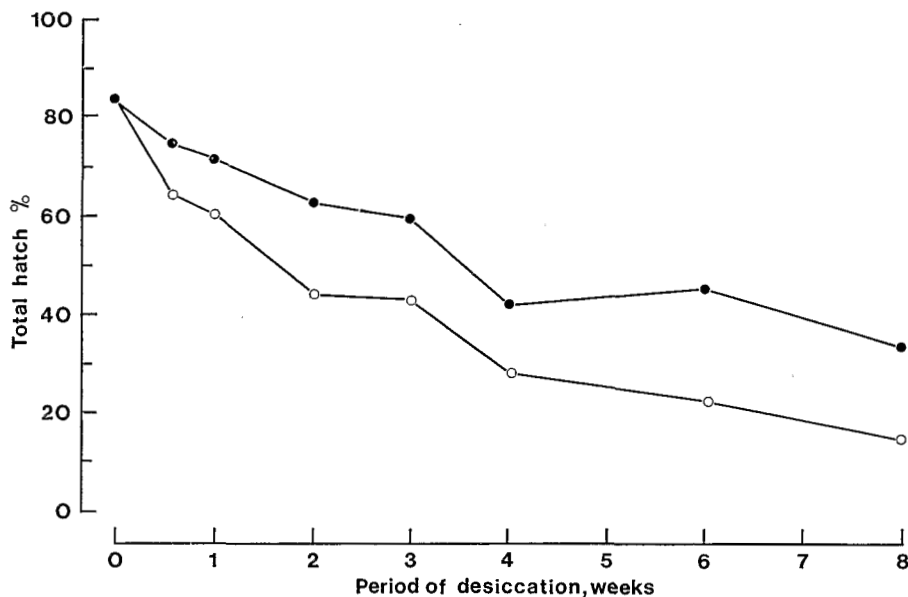


Fig. 1. Hatching of *Globodera rostochiensis* from cysts in potato root diffusate; cysts had previously been subjected to various periods of desiccation at 0% relative humidity and 20° after an initial exposure of 24 h to potato root diffusate (white circles) or soil leachate (black circles).

Results

Fig. 1 shows the total percentage emergence of juveniles of *G. rostochiensis* from cysts after desiccation for various periods after exposure to PRD and soil leachate. There is a decrease in the hatch from cysts from both treatments with increase in the period of desiccation but the hatch from cysts treated with PRD was always at least 10% less than the hatch from cysts exposed to soil leachate. Analysis of variance after arcsin transformation of percentages confirms that the difference between the PRD and soil leachate pre-treatments is highly significant ($P < 0.01$). Even after eight weeks desiccation at 0% relative humidity the hatch from cysts previously exposed to soil leachate was greater than the hatch from the PRD treated cysts which had been desiccated for only half that time (33% and 28% respectively). In both groups of cysts, most of the hatch occurs during the first three weeks of the hatching trials and there is no difference in the rate of hatch between groups: the difference is in the total hatch.

The pattern is the same in experiments with free eggs (Fig. 2). Indeed, the adverse effect on hatching of exposing the eggs to PRD before desiccation is even more marked. The hatch from eggs previously exposed to PRD is less than 15% after only three days

desiccation, whereas a desiccation period of four weeks is required before the hatch from eggs treated with soil leachate declines to this level; the hatch from the soil leachate group desiccated for three days is 62%. At all periods of desiccation up to and including three weeks the hatch from eggs treated with soil leachate before desiccation is at least 20% greater than the hatch from the PRD treated groups. The difference is not so great at the four weeks desiccation period only because the hatch from the PRD treated eggs was already almost zero. Analysis of variance, shows that the difference between the PRD and soil leachate pre-treatments is highly significant ($P < 0.01$). As with experiments on cysts, there is a decrease in hatch with increase in the period of desiccation for both groups of eggs and it is clear from a comparison of Figs 1 and 2 that the cyst itself affords the unhatched juveniles protection against desiccation, as observed by Ellenby (1946, 1968).

The S_{50} value for artificially hatched juveniles exposed to PRD or to soil leachate before drying at 45% relative humidity was 19 min for each group which is very close to the S_{50} value of 17 min found by Ellenby (1968) for a different population of *G. rostochiensis* dried under the same conditions.

Discussion

Free juveniles of *G. rostochiensis* can survive drying for minutes only (Kämpfe, 1959; Ellenby, 1968) yet unhatched juveniles inside cysts will remain viable for at least eight years (Franklin, 1937). Ellenby (1968) has shown that the egg-shell, independently as well as in conjunction with the cyst wall, helps the unhatched juveniles to survive drying by becoming increasingly impermeable and slowing down the rate of drying of the enclosed juvenile; a slow rate of water loss is associated with enhanced survival in many species of nematodes (Evans & Perry, 1976). In addition Clarke, Perry and Hennessy (1978) have suggested that the trehalose solution surrounding the juvenile would protect it from desiccation as loss of water would be opposed by increasing concentration of the egg fluid solutes. The alteration of the permeability characteristics of the egg-shell and the loss of trehalose would render the unhatched juvenile more susceptible to desiccation. Such changes were considered by Clarke and Perry (1977) to be among the initial stages in the hatching mechanism of *G. rostochiensis* after exposure to PRD. So by exposure of unhatched juveniles to PRD for a period long enough to initiate the hatching response but too short for hatching to have occurred, the physical protection that the

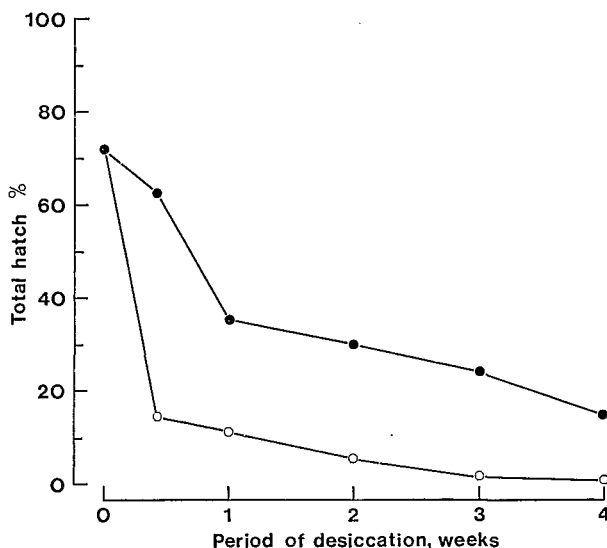


Fig. 2. Hatching of *Globodera rostochiensis* from free eggs in potato root diffusate; eggs had previously been subjected to various periods of desiccation at 0% relative humidity and 20° after an initial exposure of 24 h to potato root diffusate (white circles) or soil leachate (black circles).

juvenile relies upon to enable it to survive desiccation has been altered. Subsequent desiccation causes greater mortality in the PRD treated groups which is reflected in a lower hatch when the eggs or cysts are rehydrated and placed in PRD.

The present work with cysts and free eggs shows that desiccation significantly decreases hatch of juveniles exposed to PRD. Control experiments using artificially hatched juveniles suggest that the effect is not due to any changes induced in the juveniles themselves, but controls may not be perfect because release of juveniles from eggs could result in increased metabolism. Therefore, it is possible that the increase in metabolism of unhatched juveniles exposed to PRD may make them more susceptible to desiccation. However, this aspect is likely to be of only marginal importance as juveniles have almost no intrinsic ability to survive desiccation; they rely on external factors for their protection. It is the removal or alteration of these factors which will have most effect on survival. Although the cyst wall is involved in protecting the unhatched juvenile from desiccation, comparison of the results from experiments with free eggs and cysts shows that changes in the cyst wall induced by PRD are, at most, of minor importance.

Although it is not absolutely certain that the results reported can be attributed solely to changes in egg-shell permeability and trehalose content of the egg, they seem the most likely causes. In the field some eggs may remain unhatched in cysts in soil in which potatoes have grown. Exposure to the PRD may deleteriously affect the subsequent chances of survival of such juveniles.

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