

The influence of soil moisture and storage time on the motility, infectivity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*

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SUMMARY

A range of sand moisture contents and storage times were used to examine the effect of these parameters on lipid utilization and invasion by hatched juveniles of *Globodera rostochiensis* and *G. pallida*. Motility and lipid depletion were fastest at pF 1.66 which corresponded to the point of inflection of the sand moisture characteristic curve. *G. rostochiensis* utilized its lipid reserves significantly more rapidly than *G. pallida* at all sand moisture contents. Depletion of lipid reserves during storage led to reduced motility and infectivity of hatched *G. rostochiensis* juveniles. This effect became significant when lipid reserves fell below approximately 65 % of the original level. The results indicate that the infective life of *Globodera* juveniles ranges from 6-11 days under optimal conditions for motility and enhanced persistence is probably related to the ability to conserve food reserves under unfavourable conditions such as high or low soil moisture contents.

RÉSUMÉ

Influence de la température sur l'éclosion, l'activité et l'utilisation des lipides chez les juvéniles de deuxième stade des nématodes à kystes de la pomme de terre Globodera rostochiensis et G. pallida

Une gradation dans l'humidité du milieu (sable) et le temps de stockage a permis d'étudier l'influence de ces deux paramètres sur l'utilisation des lipides par les juvéniles de *Globodera rostochiensis* et *G. pallida*, ainsi que sur la pénétration de ces mêmes juvéniles dans les racines de l'hôte. La mobilité et l'épuisement des lipides sont plus rapides à un pF de 1,66, qui correspond à l'inflexion de la courbe caractéristique d'humidité du sable. *G. rostochiensis* utilise ses réserves lipidiques significativement plus rapidement que *G. pallida* quelle que soit l'humidité du sable. L'épuisement des lipides pendant le stockage des juvéniles de *G. rostochiensis* réduit leur mobilité et leur pouvoir infestant. Ce phénomène devient significatif lorsque les réserves lipidiques descendent en dessous de 65 % de leur niveau originel, approximativement. Ces résultats indiquent que l'infestivité des juvéniles de *Globodera* dure de six à onze jours dans des conditions optimales de mobilité, et qu'une persistance plus grande dans le sol est probablement en relation avec la faculté de conserver des réserves nutritives dans des conditions adverses, telles une humidité du sol très forte ou très faible.

The survival of infective, non-feeding stages of certain plant and animal parasitic nematodes depends upon neutral lipid reserves (Barrett, 1976; Lee & Atkinson, 1976) and their depletion has been correlated with a reduction in motility (Van Gundy, Bird & Wallace, 1967; Croll & Matthews, 1973; Reversat, 1980) and infectivity (Wallace, 1966; Reversat, 1980; Storey, 1984; Robinson, Atkinson & Perry, 1986). The second stage juveniles (J2) of certain plant parasitic nematodes can remain infective in soil for up to four months (Van Gundy, Bird & Wallace, 1967) and during this period food reserves are depleted at a rate that depends upon various biotic and abiotic factors such as temperature, soil moisture and the influence of the host plant. Previous work has examined the effects of temperature on hatching and motility aspects of the infectivity of the potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* (Robinson, Atkinson & Perry, 1987) but

little is known about the effects of soil moisture on the survival of these two species.

Abiotic factors, such as soil structure, aeration and moisture, influence nematode activity in soil (Jones, 1975). Fine soil particle size and close packing, as in dense clays, results in nematodes traversing only the macropores between aggregates rather than the pore spaces between particles. In coarse sand, pore spaces are usually larger than the diameter of most nematodes and thus movement of nematodes through the pores is possible. However, movement is also dependent upon water films around the soil particles (Wallace, 1958a). In a water-saturated sandy soil, pore spaces are blocked and oxygenation prevented, leading to reduced motility. As the sand drains, movement and activity of individuals increases until the point of inflection of the moisture characteristic curve is reached when many of the available pores are emptying. Further drainage results in a

decline in motility as the forces retaining water increase and the water film thickness in the pore spaces is insufficient for rapid movement of nematodes. Thus the moisture content of the soil affects the rate of movement of nematodes and it is of interest to examine whether there are related changes in the rate of neutral lipid utilization and survival of PCN.

This work examines the relationship between soil moisture and depletion of food reserves for both species of PCN. Once this is established, *G. rostochiensis* is used as a model to determine the effect of lipid depletion on motility at different moisture levels and on the ability of juveniles to invade host roots.

Materials and methods

Cysts of *G. rostochiensis* (population Ballycastle, 1981; pathotype Rol) and *G. pallida* (population Woburn, 1982; pathotype Pa3) were reproduced on pot grown potato plants cv. Arran Banner, extracted by standard methods (Shepherd, 1970) and stored dry at room temperature for nine months until the start of experiments. Hatched J2 were obtained by soaking cysts for one week in glass distilled water (GDW) at 20° before replacing the GDW with potato root diffusate (PRD). PRD was obtained from potato cv. Arran Banner as described by Shepherd (1970) and diluted 1 in 4 by volume with GDW. Only J2 which hatched during the first week after exposure to PRD were used for experimentation.

DETERMINATION OF THE MOISTURE CHARACTERISTIC OF SAND

Sand of particle size 150-400 µm was used for all experiments. This size range is optimal for movement of J2 of PCN (Wallace, 1958b). The moisture characteristic curve of the sand was determined by applying suction of up to pF 1.95 using the sintered glass funnel apparatus described by Wallace (1954).

EFFECT OF SOIL MOISTURE AND STORAGE TIME ON THE LIPID DECLINE OF HATCHED J2

Hatched J2 of *G. rostochiensis* and *G. pallida* were stored in sand at pFs 1.66, 1.81 and 1.95 for up to 18 days to study the effect of soil moisture on the rate of neutral lipid utilization. Lipid reserves of active individuals were stained with Oil Red O (G.T. Gurr, Ltd.) according to the methods of Storey (1984) and the lipid content of at least 40 single juveniles was quantified with a Vickers M86 scanning microdensitometer (Croll, 1972).

Approximately 180-250 freshly hatched J2 were stored in 3.5 cm diameter plastic pots filled with 40 g of sand. Each pot was fitted with a fibre wick in its base

and placed in a large sand-filled container joined to a sintered glass disc which allowed the sand moisture to be varied. After saturating the sand with fresh PRD, the suction pressure of three identical set-ups were adjusted to the above pFs. Pots containing J2 were then removed after 3, 6, 9 and 18 days and the neutral lipid reserves of at least 40 individuals were determined.

Data from lipid analyses were logarithmically transformed and subjected to regression analysis (Genstat Manual, 1977) to estimate the times taken to utilise 50 % of lipid reserves (T50) at each of the soil moistures. Analysis of parallelism (Ross, 1980) was used to determine the significance of differences in the rates of lipid utilization.

EFFECT OF SOIL MOISTURE AND STORAGE TIME ON THE MOTILITY OF J2

The movement of J2 of *G. rostochiensis* in sand at different moisture contents was measured in motility tubes containing cores of sand divided into 1 cm sections (Evans, 1969). After adjusting suction pressures to pFs 1.48, 1.66, 1.81 and 1.95 using freshly collected PRD, three replicates of approximately 200 hatched J2 were introduced into the tubes and left for 48 h at 20°. The sand cores were then divided and the J2 in each section were counted. A motility index (M.I.) was determined for each replicate using the formula of Townsend and Webber (1971) :

$$M.I. = \frac{(No. \text{ in } 1 \text{ cm} \times 0.5) + (No. \text{ in } 2 \text{ cm} \times 1.5) + (No. \text{ in } 3 \text{ cm} \times 2.5)}{\text{Total number recovered}}$$

Motility assessments using the same apparatus were carried out on J2 of *G. rostochiensis* which had been stored in PRD for 5, 10, 15 and 20 days after hatching. The moisture content of sand saturated with PRD was adjusted to pF 1.66, which preliminary experiments had shown to be the point of inflection of the moisture characteristic curve and optimum for nematode motility. Nematodes were introduced into the tubes, left for 48 h and motility assessed as before.

Motility indices from both assessments were subjected to analysis of variance (Genstat Manual, 1977) which was weighted according to total number of nematodes recovered in each tube.

EFFECT OF STORAGE TIME ON THE RELATIVE INFECTIVITY OF J2

Hatched J2 of *G. rostochiensis* which had been stored for up to 25 days were used to infect tomato seedlings to investigate their infectivity following starvation. Groups of three seedlings, cv. Moneymaker, were transplanted from potting compost to pots containing 40 g

of acid washed sand and maintained at pF 1.66 using the apparatus described above. Fifteen days after seedling emergence, a series of pots was inoculated with three replicates of approximately 200 hatched J2 of *G. rostochiensis* which had been stored for 5, 10, 15, 20 and 25 d at 20° in PRD. Prior to inoculation, aliquots of at least 40 J2 were stained and neutral lipid content determined as described above. Fourteen days after inoculation, the roots of each tomato plant were stained in boiling 0.1 % cotton blue in lactophenol (Hooper, 1970) and the nematodes counted.

Data were subjected to analysis of variance of percentage invasion transformed to angles of equal information (α); $\alpha = \arcsin \sqrt{(\% \text{ invasion}/100)}$.

Results

LIPID UTILIZATION

The rates at which J2 of *G. rostochiensis* and *G. pallida* used their neutral lipid reserves during storage at three suction pressures are shown in Figure 1. In both species, lipid depletion to 50 % of initial reserves was fastest at pF 1.66 and least at pF 1.95. The times taken to utilize 50 % of lipid reserves (T50) ranged from 9.7 to 17.8 days for *G. pallida* and 6.7 to 10.9 days for *G. rostochiensis*. J2 of *G. rostochiensis* used their lipid reserves more rapidly than *G. pallida* at all suction pressures even though initial lipid reserves were not significantly different ($P > 0.05$). Analysis of parallelism confirmed that intraspecific rates of lipid utilization at the three suction pressures were significantly different ($P > 0.01$).

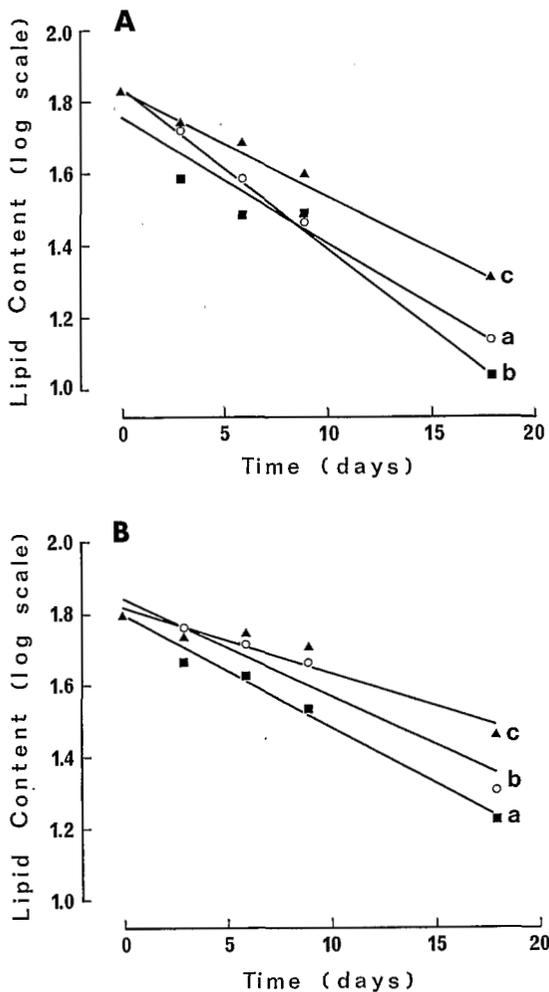


Fig. 1. Declines in neutral lipid reserves for hatched juveniles of *G. rostochiensis* (A) and *G. pallida* (B) stored at 20° in sand at pF 1.66 (a), 1.81 (b) and 1.95 (c). Lipid content in microdensitometer units is shown on a logarithmic scale.

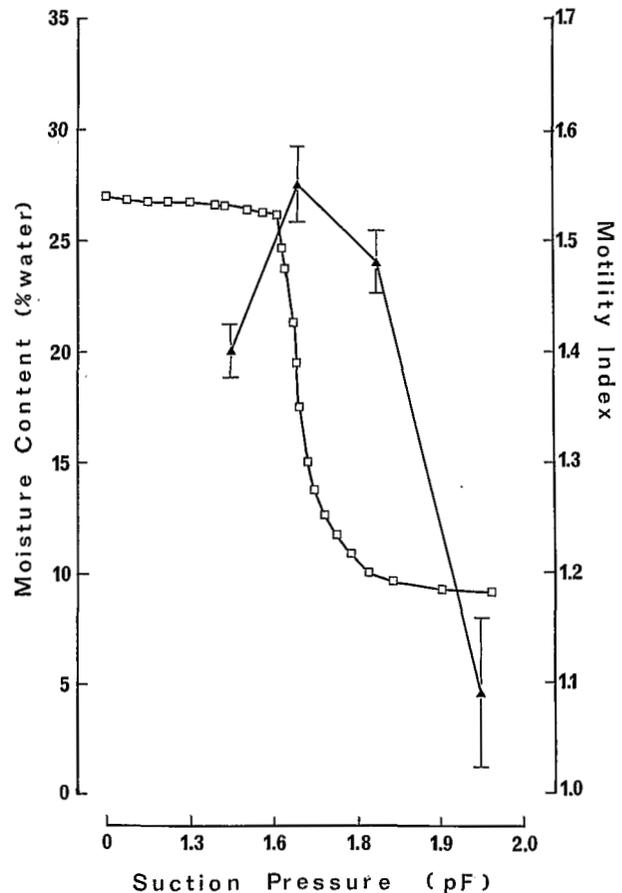


Fig. 2. Motility index (▲) of *G. rostochiensis* juveniles in sand at four moisture contents, superimposed on the moisture characteristic curve for that sand (□). Vertical lines represent the standard error of the mean.

MOTILITY

Figure 2 shows the motility index of J2 of *G. rostochiensis* after 48 h in sand at four suction pressures, superimposed on the moisture characteristic curve for the sand used in the experiments. The motility index was greatest at pF 1.66 and lowest at pF 1.95. Analysis of variance of weighted indices showed no significant differences between indices at pF's 1.66 and 1.81 ($P > 0.05$) but the indices at pF's 1.48 and 1.95 were significantly lower than these ($P < 0.05$).

The motility of starved J2 of *G. rostochiensis* is given in Table 1. Motility indices decreased with increase in duration of storage from 1.72 ± 0.07 with no storage to 1.12 ± 0.11 after 20 days storage. Analysis of variance showed that differences between the means were significant ($P < 0.001$) but examination of the LSDs indicated that the significant difference occurred between the 5 and 10 days storage times; starvation for periods longer than 10 days did not further reduce motility ($P > 0.05$).

INVASION

The relative invasion of J2 of *G. rostochiensis* decreased with storage as a percentage of the value at the start of the experiment (Tab. 1). Analysis of variance showed that storage had no significant effect on invasion until between 5 and 10 days ($P > 0.05$). It subsequently influenced invasion significantly ($P < 0.05$) until, after 25 days, only 4.3 ± 2.6 % of the J2 invaded the plants.

Discussion

Various suction pressures were used to examine the rate of neutral lipid utilization and motility and, in both species of *Globodera*, the rate of lipid depletion was greatest at pF 1.66 and was significantly reduced in sand with less water. However, there is an important difference between the two species. Initial lipid contents were similar, but *G. rostochiensis* utilized its lipid reserves significantly more rapidly than *G. pallida* at all sand moisture contents. This may be an important factor contributing to the greater persistence of hatched juveniles of *G. pallida* (Robinson, Atkinson & Perry, 1987). Lipid utilization by hatched J2 of *Globodera* spp. follows a semi-logarithmic decline which is similar to the pattern of lipid utilization in infective juveniles of *Ancylostoma tubaeforme* (Croll, 1972), *Meloidogyne naasi* (Ogunfowora, 1979), *Heterodera oryzae* and *M. javanica* (Reversat, 1980; 1981). The fall in the rate of lipid utilization with time may enhance persistence of juveniles as suggested for *A. tubaeforme* (Croll & Matthews, 1973).

The sand used in this work was saturated with PRD and the times taken for J2 to utilize 50 % of their lipid reserves (T50) were between 10-18 days for *G. pallida* and 7-11 days for *G. rostochiensis* depending on sand matric potential. These periods are considerably shorter than T50 values for J2 in watch glasses containing water (Storey, 1984) or PRD (Robinson, Atkinson & Perry, 1987). Lipid utilization is also faster than for juveniles of *M. oryzae* and *M. javanica* stored in deionized

Table 1

The relationship between the length of storage in potato root diffusate at 20° for hatched juveniles of *G. rostochiensis* and their motility index at pF 1.66 and ability to invade host roots; the corresponding neutral lipid content of juveniles is also shown.

length of storage (days)	motility index \pm SEM ¹	% invasion \pm SEM	relative % invasion \pm SEM	lipid content ² \pm SEM
0	1.72 ± 0.07	57.5 ± 22.5	100.0	236.2 ± 14.2
5	1.53 ± 0.13	35.0 ± 5.5	60.9 ± 9.6	204.7 ± 11.7
10	1.29 ± 0.11	16.3 ± 7.2	28.3 ± 12.5	159.2 ± 8.5
15	1.20 ± 0.07	7.5 ± 3.2	13.0 ± 5.6	131.4 ± 10.7
20	1.12 ± 0.11	20.0 ± 5.5	34.8 ± 9.6	116.0 ± 13.9
25	—	2.5 ± 1.5	4.3 ± 2.6	—
LSD ³	0.311	29.0	50.4	28.9

(1) Standard Error of the Mean.

(2) Neutral lipid content measured in microdensitometer units.

(3) Least Significant Difference ($P = 0.05$).

water or phosphate buffer (Reversat, 1980; 1981). Host root diffusate is known to stimulate movement of J2 (Clarke & Hennessy, 1984) and increased oxygenation in sand may provide an additional stimulus. Wallace (1966) suggested that juveniles utilize their sources of energy at a rate dependent upon the degree of activity and this hypothesis gains some support from the present work. Regression analysis established that in *G. rostochiensis* there is a highly significant correlation between motility and rate of lipid utilization ($P < 0.001$).

The maximum rate of nematode movement in soil occurs near the point of inflection of the moisture characteristic curve for that soil (Wallace, 1958a) and at a soil particle diameter of about one third to one half the length of the nematode (Wallace, 1958b). The particle size of sand used in the present work (150-400 μm) meets the size criterion for optimum movement of *Globodera* spp. and maximum motility occurred at pF 1.66 which is at the inflection point of the moisture characteristic curve for this sand (Fig. 2).

Storing J2 of *G. rostochiensis* for between 5-10 days significantly reduced their motility in sand compared to unstored J2. The effects on motility became significant when lipid reserves fell below approximately 65 % of the original level; invasion was also significantly reduced below this lipid level. These results support the suggestion that motility and invasion are independent of lipid reserves until a certain critical level is reached (Van Gundy, Bird & Wallace, 1967; Storey, 1984; Robinson, Atkinson & Perry, 1986). The subsequent concurrent declines in invasion and motility indicate a relationship between the minimum energy reserves necessary for invasion and the propulsive forces required.

This work shows that both *G. rostochiensis* and *G. pallida* remain infective in soil for relatively short periods compared to *M. javanica* and *Tylenchulus semipenetrans* (Van Gundy, Bird & Wallace, 1967). The rate of lipid utilization is influenced by physical factors and the presence or absence of host root diffusates. Enhanced persistence and prolonged infective periods are probably related to the ability to conserve food reserves under conditions that are not optimal for motility and invasion such as, in this case, high or low soil moisture contents.

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