

The influence of temperature on the hatching, activity and lipid utilization of second stage juveniles of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida*

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SUMMARY

The hatching and persistence of eight populations of *Globodera rostochiensis* and *G. pallida* were examined at six different temperatures. Interspecific comparison showed that *G. pallida* had a much slower initial rate of hatch and utilized its lipid reserves more slowly than *G. rostochiensis*. There was no difference between activity coefficients of the species at each temperature but the results do suggest that *G. pallida* is the species better adapted to hatch at lower temperatures. The results are discussed in relation to the effects of these factors on competition between the two species.

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

L'éclosion et la persistance de huit populations de *Globodera rostochiensis* et *G. pallida* ont été étudiées à six températures différentes. Les comparaisons entre espèces montrent que *G. pallida* présente un taux initial d'éclosion plus faible et utilise ses réserves lipidiques plus lentement que *G. rostochiensis*. Il n'apparaît pas de différences entre espèces dans les coefficients d'activité quelle que soit la température, mais les résultats obtenus suggèrent que *G. pallida* est mieux adapté à une éclosion aux températures basses. Ces résultats sont essentiellement discutés en fonction des effets des facteurs étudiés sur la compétition entre les deux espèces.

Within Britain, *G. rostochiensis* is the dominant species of potato cyst nematode (PCN) in Scotland and South-Eastern England whereas *G. pallida* is prevalent in the Humber basin and increasing in East Anglia. The two occur together in varying proportions but in pot tests it has been shown that there is a tendency for one to become predominant (Parrott, Berry & Farrell, 1975). Lane and Holliday (1974) found that *G. pallida* replaced *G. rostochiensis* on susceptible potatoes in soils infested with both species and suggested it did so faster in cooler soils. Similar results were found in New Zealand (Foot, 1978). *G. pallida* has a lower optimum temperature for hatching (Franco, 1979) and may undergo female development and egg production at lower temperatures than *G. rostochiensis* (Berry *et al.*, 1977). However, it is not known at what stage from hatching to maturity *G. pallida* secures its advantage over *G. rostochiensis* and whether differences in the hatching or persistence of hatched juveniles, as affected by temperature, contribute to differences in the competitiveness of the two species.

The prevalence of either one of the two species of PCN is also affected by cultural practices. Early harvesting of potato crops may favour *G. pallida* since females of this species were found to develop slightly faster than those of *G. rostochiensis* (Webley & Jones, 1981). The widespread cultivation of "Maris Piper" potatoes which are resistant to *G. rostochiensis* Ro1 but susceptible to *G. pallida* has led to the increased incidence of the latter species. Therefore, nematicides, especially aldicarb and oxamyl, are being increasingly used to control *G. pallida*. In field experiments, however, oxamyl controlled *G. pallida* less than *G. rostochiensis*, especially in peaty soils (Whitehead *et al.*, 1984). It was suggested by these authors that the inadequate control of *G. pallida* by oxamyl may be due to differences in the hatching characteristics of the two species. It may also be due to differences in the persistence of hatched juveniles and these aspects are examined in this work.

The persistence of juveniles of *Globodera* spp. has

been correlated with the extent of their neutral lipid reserves (Storey, 1984; Robinson, Atkinson & Perry, 1986). Robinson, Atkinson and Perry (1986) showed that lipid utilization by unhatched juveniles of *G. rostochiensis* following stimulation by potato root diffusate resulted in a reduction in the infectivity of late hatching individuals. Therefore, differences in the rate of hatching of populations of PCN may affect their persistence. Increased persistence of hatched juveniles in the soil would favour an ability to prolong the invasion period and be of value if hatching was mistimed relative to root growth.

The combined effect of differences in hatching rate of juveniles and their survival in the soil and the influence of temperature on these parameters has not been studied for PCN. This has now been examined for eight populations of *G. rostochiensis* and *G. pallida* as part of a larger study of the infection process of these nematodes (Robinson, Atkinson & Perry, 1986, 1987).

Materials and methods

Four populations of each *Globodera* species were examined (Tab. 1). These were reproduced on pot grown potato plants cv. Arran Banner in 1983, extracted by standard methods (Shepherd, 1970) and the cysts stored dry until use in the spring of 1984.

Table 1

Details of the initial populations of *Globodera rostochiensis* and *G. pallida* used in these experiments before these were reproduced under standard conditions

Species	Site of origin	Patho-type	Pot/ Field grown	Year
<i>G. rostochiensis</i>				
A	Ballycastle	Ro1	P	1983
B	Feltwell	Ro1	P	1983
C	Dundonald	Ro1	P	1983
D	Rothamsted	Ro1	P	1983
<i>G. pallida</i>				
E	Bridgeground	Pa2/3	F	1982
F	Woburn	Pa3	P	1983
G	Rothamsted	Pa2/3	P	1983
H	Dunminning	Pa1	P	1983

HATCHING TESTS

Five replicates of 20 cysts of each population were soaked for seven days in distilled water at 5, 10, 15, 20, 25 and 30° before second stage juveniles (J2) were stimulated to hatch at these temperatures by replacing the water with potato root diffusate (PRD); hatching

was negligible at 5 and 30° so these treatments were discontinued. The PRD was obtained from potato plants cv. Arran Banner as described by Shepherd (1970) and diluted with distilled water (1 in 4) to give an optimum dilution for hatching. Numbers of J2 emerging were recorded at regular intervals and the PRD was replaced after each count with fresh stock kept at 5°. After 23 days, the cysts were broken open and the number of unhatched J2 counted in order to obtain the total percentage hatch over the trial period.

ACTIVITY COEFFICIENT

All the J2 of each population hatching within four days exposure of cysts to a hatching stimulus were transferred to fresh PRD. Five and 20 days later, the activity coefficients of each population were determined. The activity coefficient is the product of the percentage active J2 and the average number of undulations per minute (Croll & Matthews, 1973). Juveniles were counted as inactive if they had not completed one full undulation over a one minute period.

LIPID UTILIZATION

The lipid content of a sample of at least 30 hatched J2 of each population was measured for those individuals hatching over the first four days after PRD stimulation. Further samples were taken for lipid analysis after storing the early hatching J2 in PRD for 20 days. Neutral lipids were stained with Oil Red O (G.T. Gurr) according to the methods of Storey (1984) and the lipid content of individual juveniles was quantified with a Vickers M86 scanning microdensitometer (Croll, 1972).

Results

HATCHING

Figure 1 shows the proportions of viable cyst contents which hatched over 23 days at 10, 15, 20 and 25°. Analyses of variance (Genstat Manual, 1977) were carried out on percentages transformed to probits (Finney, 1971). A significant species effect indicated that *G. rostochiensis* hatched more freely than *G. pallida* at all temperatures ($P = 0.05$). Maximum hatch for both species occurred between 15 and 20°, with a significant peak at 20° for *G. rostochiensis*. Within each species, three of the four populations of *G. rostochiensis* showed a significant increase in percentage hatch from 15 to 20° whereas only one population of *G. pallida* hatched more freely at the higher temperature ($P = 0.05$). This indicates that *G. pallida* has a slightly lower optimum hatching temperature than *G. rostochiensis* although the relative reduction in hatch at 10 and 25° was similar for both species.

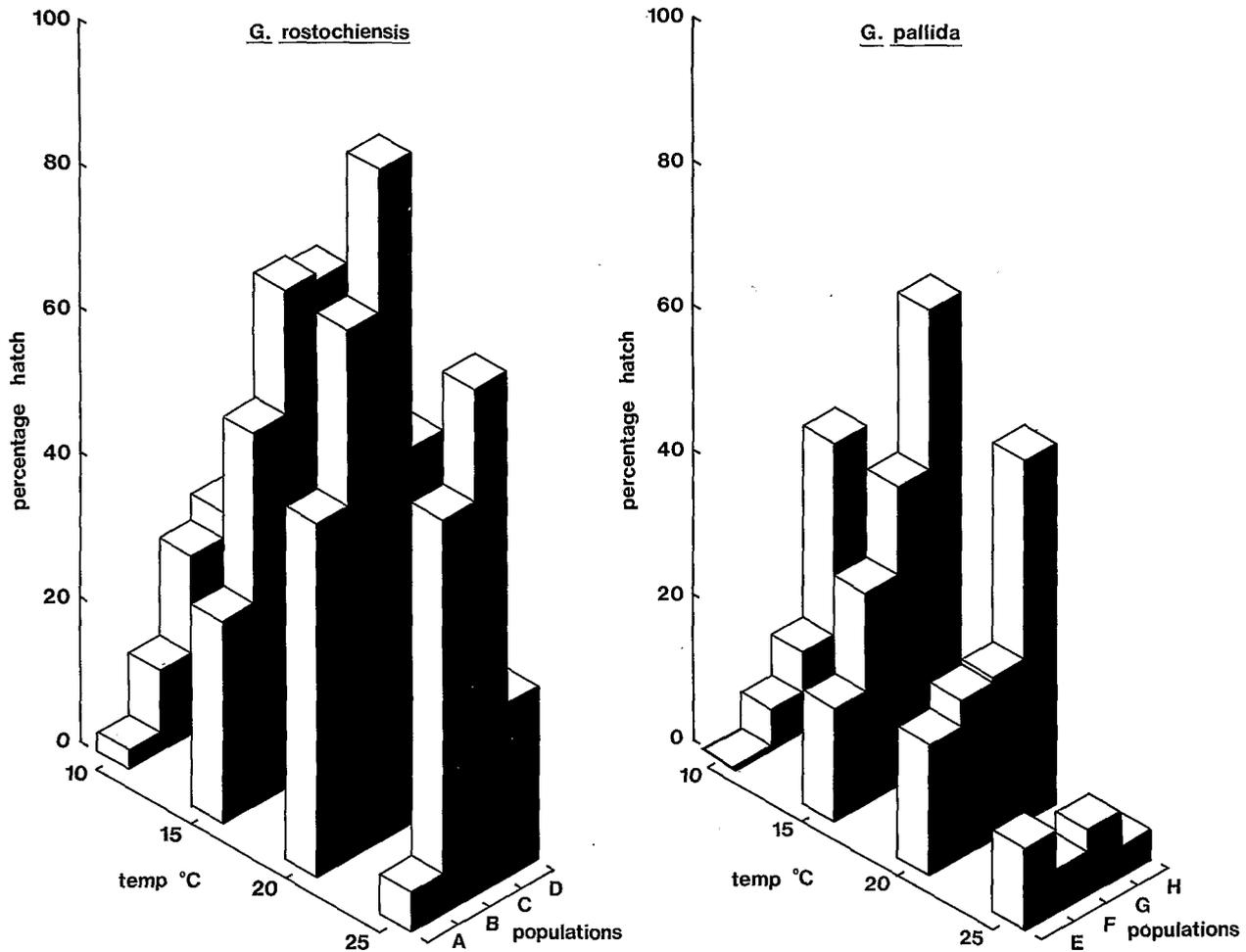


Fig. 1. Percentages of juveniles of four populations of *G. rostochiensis* and of four populations of *G. pallida* emerging from cysts at four different temperatures over a 23 day period.

ACTIVITY

The activity coefficients for both species at five and 20 days after hatch are given for all four temperatures in Figure 2. Both species were less active at 25° than at lower temperatures at five days and analysis of variance revealed no significant differences between the species at this time. Overall, activity coefficients declined for both species after 20 days and only *G. rostochiensis* continued to be significantly less active at 25° than at the other temperatures ($P < 0.05$).

LIPID UTILIZATION

The percentage of initial lipid content that was depleted by each population at the four temperatures after 20 days is shown in Figure 3. Differences between

species and between temperatures were examined by analysis of variance of percentage lipid depletion transformed to angles of equal information (Genstat Manual, 1977). At 15 and 20°, *G. rostochiensis* used significantly more lipid than *G. pallida* ($P < 0.05$). The former showed a higher rate of lipid utilization at 20° than at the other temperatures whereas temperatures of between 10 and 20° had no significant effect on the percentage lipid depletion by *G. pallida* ($P = 0.05$). Both species utilized less lipid at 25° than at other temperatures.

Discussion

The hatching trials showed differences between *G. rostochiensis* and *G. pallida* both in the percentage hatch over 23 days and in the response to a range of tempera-

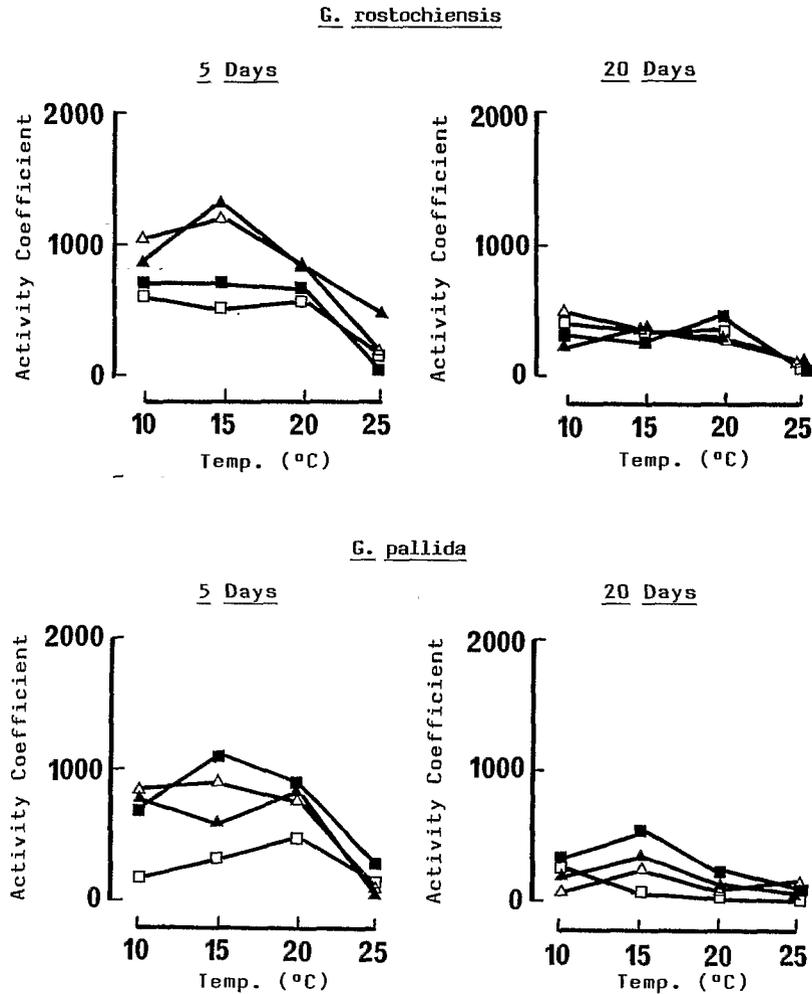


Fig. 2. Activity coefficients at five and twenty days in potato root diffusate for *G. rostochiensis* and *G. pallida*; symbols: *G. rostochiensis* Δ = population A, \blacktriangle = B, \square = C, \blacksquare = D; *G. pallida* \square = population E, \blacksquare = F, \triangle = G, \blacktriangle = H.

tures. *G. rostochiensis* overall hatched more freely than *G. pallida*, suggesting a greater initial rate of juvenile emergence from cysts. In general, the hatching responses to different temperatures were dissimilar with *G. pallida* having a slightly lower thermal optimum than *G. rostochiensis*. This is in agreement with previous findings (Parrott & Berry, 1976) although adaptation to hatching at low temperatures in the field by previous generations may also have led to this effect (Hominick, 1979).

The optimum temperatures for activity in both species of PCN were similar. Maximum activity coefficients were recorded at temperatures below 25° and they decline to approximately 30 % of initial values after 20 days storage. Similar optima for motility between 10 and 15° have also been found for *Meloidogyne javanica* (Thomason, Van Gundy & Kirkpatrick, 1964) and *Heterodera avenae* (Davies & Fisher, 1976).

Rates of lipid utilization by the populations of *G. rostochiensis* showed a significant thermal optimum at 20° which did not occur for *G. pallida*. At 20° the estimated times taken by juveniles to utilize 50 % of their lipid reserves (T50) in PRD were 22 days for *G. pallida* and 15 days for *G. rostochiensis*. A reduction in the lipid content of juveniles has been correlated to a loss in infectivity (Storey, 1984; Robinson, Atkinson & Perry, 1986) and therefore *G. pallida* may remain infective in the soil for longer periods than *G. rostochiensis*. The T50 values for these species at 20° are considerably less than recorded previously (Storey, 1984) and may be due to the stimulatory effect of PRD on the motility of juveniles (Clarke & Hennessy, 1984). The reduced lipid utilization at 25° was unexpected and it is associated with a decline in motility. Possibly it represents a quiescent response to high temperature which may be related to

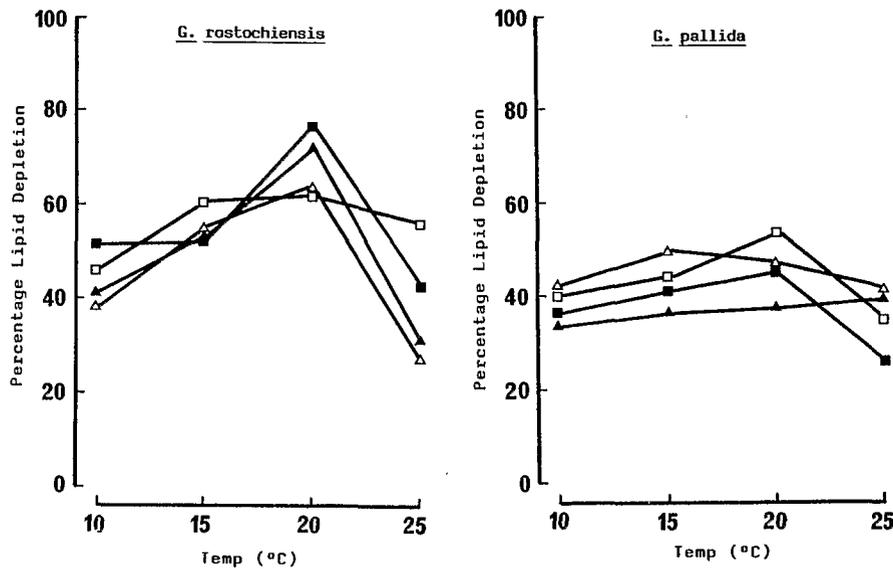


Fig. 3. Percentage lipid depletion in four populations of *G. rostochiensis* and *G. pallida* at four temperatures over a 20 day period (see Fig. 2 for symbols).

the dormant state that occurs during survival of the unhatched of these two species. Such a quiescent response may be a form of the metabolic compensation shown by poikilotherms to higher temperatures (Bullock, 1955). This is reflected in the Q_{10} for *G. rostochiensis* calculated according to the Arrhenius formula (Von Brand, 1960) based on lipid utilization rates; between 5 and 20° the Q_{10} was 2.7 whereas between 20 and 25° it was reduced to 1.7. The latter is similar to values obtained for *Heterodera oryzae* (Reversat, 1977) which may also show some form of acclimation to increasing temperature.

Our results suggest that *G. pallida* is well adapted to low temperatures in terms of hatching and persistence and this is consistent with its ability to gain an advantage in competition with *G. rostochiensis* in cool soils (Foot, 1978). The prolonged hatch and persistence of *G. pallida* may ensure that root growth is less affected by the parasite, thus reducing intraspecific competition among females. These same attributes may also partly explain the poor control of *G. pallida* by oxamyl in the field (Whitehead *et al.*, 1984) where juveniles may persist and remain infective for longer than the effective life of the nematicide. By contrast to *G. pallida*, the rapid initial rate of hatch of *G. rostochiensis* favours early establishment in roots and may even partially exclude *G. pallida*. In mixed populations, *G. pallida* may be favoured by low soil temperatures and densities that do not alter early root growth whereas *G. rostochiensis* is more likely to become predominant in relatively warmer soils and at high densities. In Britain, the low soil temperature and

typical densities of less than 15 eggs/g of soil at planting may favour *G. pallida* more frequently than *G. rostochiensis* on ware potatoes, whereas the position might be reversed elsewhere, particularly with early potato production in warmer soils. The widespread use of potatoes with gene H1 resistance to *G. rostochiensis* but susceptibility to *G. pallida* in short rotations, however, is creating a more significant increase of *G. pallida*. Further work is required on the biological differences between the species in order that the efficacy of nematicide control of *G. pallida* can be improved.

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