Diapause in the nematode *Globodera pallida*

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Accepted 23 June 1994

**Key words:** *Globodera pallida, G. rostochiensis*, hatching, diapause, emergence, infectivity

**Abstract.** Over a period of 12 months 'new' and 'old' cysts of *Globodera pallida* were hatched in potato root diffusate according to a novel 'nematode-response' hatching protocol. In this protocol, cysts were set to hatch at the beginning of autumn and then left to indicate when their hatching ability was exhausted (when less than 100 juveniles/replicate/week emerged) before another batch of cysts was set to hatch. At any time of the year for the 12 months this experiment was conducted there were cysts hatching. After 12 months of hatching, eight hatching curves were obtained. Based on the hatching curves of the 'new' and 'old' cysts, diapause was shown to be present in 'new' cysts in autumn, winter and early spring. However, diapause was absent in late spring and summer.

Infertility assays to distinguish between juveniles obtained in the periods when cysts were in diapause and when cysts had overcome their diapause failed to show any significant difference in their infectivity. There was no significant difference in the number of eggs in 'new' and 'old' cysts. Based on this observation, it was suggested that high emergence in 'old' cysts may not be a result of few eggs in the cyst but rather due to absence of diapause. Also the presence of large numbers of eggs in 'old' cysts even after being stored for 12 months outdoors in the soil does not support the theories of spontaneous hatching, micro-organism induced hatching or persistence of hatching factors in the soil.

**Introduction**

*Globodera rostochiensis* cysts were found to contain viable eggs after eight years in soil [Franklin, 1937]. Various suggestions have been made to explain why some encysted eggs do not hatch, including the presence of inhibiting substances produced as a result of metabolic activities of the juveniles within the eggs during process of hatching [Ellenby, 1946], oxygen deficiency in the hatching medium [Wallace, 1959], seasonal variation [Calam et al., 1949] and genetic inheritance [El-Shatoury, 1978].

Shepherd and Cox [1967] and Oostenbrink [1967] considered that *G. rostochiensis* eggs which failed to hatch under optimum conditions were in diapause, and they compared the phenomenon with that found in insects. However, their evidence was confusing due to lack of fundamental information on (a) history of cysts, (b) species involved (prior to the work of Stone [1972] *G. pallida* was not distinguished as a separate species from
G. rostochiensis), (c) storage conditions of cysts, (d) hatching conditions of cysts, (e) hatching medium used, (f) how the hatching medium was produced and (g) host plants on which cysts were raised. Hominick et al. [1985] considered all these lapses in designing their experiments and concluded that one population of G. rostochiensis exhibited diapause.

In the work on G. pallida reported in this paper, the protocols of Hominick et al. [1985] are used, but with modifications in the hatching protocol. In this work, a ‘nematode-response’ hatching protocol was used as opposed to fixed calendar hatching times used by Hominick et al. [1985]. In the ‘nematode-response’ approach, cysts were set to hatch at the beginning of autumn (October, 1987) and then left to indicate when their hatching ability is exhausted (when less than 100 juveniles/replicate/week emerged), before another batch of cysts was set to hatch. The advantage of this approach is that, at any time of the year for the period this experiment was conducted (12 months) there are cysts hatching. Also ‘new’ and ‘old’ cysts were compared to elucidate diapause in G. pallida. The ‘new’ cysts are those extracted soon after maturity on host roots and ‘old’ cysts are those that were stored for one calendar year outdoors in a gravel plunge. The overall aim of the experiments is to examine whether or not there is a diapause in G. pallida.

Materials and methods

Setting up cultures

Cysts of G. pallida (Pa 2/3) were isolates that had been continuously grown outdoors on potato plants cv. Pentland crown for a number of years at the Scottish Crop Research Institute (SCRI) at Invergowrie, Dundee, UK. The cysts had been harvested from the 1985 season, extracted from soil and stored at SCRI at room temperature in the dark. On arrival at Silwood Park (IC), Ascot, UK on 29:2:87, these cysts were counted randomly into batches of 100, placed in glass vials and stored in the dark at 20 °C.

On 2:3:87 certified potato seed tubers cv. Pentland crown were set to sprout in greenhouse. Thirty 18 cm plastic pots with the drainage holes covered with fine muslin cloth were filled with steam sterilized loam: sand (2:1). A sprouting tuber of potato was planted into each pot and kept for 3 days in a greenhouse at 18–23 °C.

On 28:4:87, thirty batches of 100 cysts were soaked in sterilized tap water (STW) for seven days in the dark at 20 °C. Then, on 5:5:87 each of the thirty pots containing a sprouting tuber of potato was inoculated with one batch of 100 pre-soaked cysts. Pots were maintained outdoors in a gravel plunge and watered when necessary throughout the growing period.

About three months later, as the potato foliage was senescing, the 30 pots were removed from the gravel plunge. The soil and the roots were