Comparison of the rate of embryogenic development of *Globodera rostochiensis* and *G. pallida* using flow cytometric analysis

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The potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, are sibling species, yet there are marked physiological differences between them, especially in the hatching response and utilisation of energy reserves (Perry, 1998). Whitehead (1997) and Perry (1998) have suggested that the prolonged hatch and relatively slow use of energy reserves of *G. pallida*, leading to enhanced persistence, may partly explain the poor field control of *G. pallida* by non-fumigant nematicides. In general, *G. pallida* has a slightly lower thermal optimum for hatching than *G. rostochiensis* (Franco, 1979; Robinson et al., 1987), although the optimum temperatures for activity of hatched juveniles are similar for both species (Robinson et al., 1987). In experiments using constant temperatures rather than more realistic fluctuating environmental regimes, the rate of post-embryonic development after invasion of host plants by second stage juveniles (J2) was more rapid for *G. pallida* than for *G. rostochiensis* at 9.5 and 11.5°C but slower at 15°C and above (Mugniéry, 1978). There is no information on the comparative rate of embryogenic development of the two species.

Flow cytometry is a technique for measuring several characteristics of biological particles such as cells. Light energy scattered by a particle when it intersects a laser beam is converted to electrical impulses that can be quantified and analysed. Tylka et al. (1993) have used flow cytometry for sorting large numbers of nematode eggs into development categories. The technique has many potential applications, including analysis of the rate of embryogenic development. In the present work, this aspect of the development of PCN to unhatched J2 was determined on a comparative basis under glasshouse conditions.

**Materials and methods**

**Nematodes for inoculation**

Cysts of *G. rostochiensis* Ro1 and *G. pallida* Pa1 were from a single generation grown on potato cv. Désirée in pots and stored dry for at least 1 year at approximately 4°C after extraction. To obtain J2 for inoculation, cysts were pre-soaked in glass distilled water (GDW) at room temperature for 7 days before being placed in potato root diffusate (PRD) obtained from 6 week-old potato plants, cv. Désirée (Perry & Beane, 1989); PRD was diluted 1 in 4 with GDW.

**Pot trial**

To examine the rate of development of *G. rostochiensis* and *G. pallida* on a comparative basis, a pot experiment was set up in May, close to main season planting time, in a glasshouse. Temperatures were recorded over the 14 week experiment. The minimum was 14°C and weekly maxima varied between 18.5°C (week 7) and 24.8°C (week 3), averaging 21.2°C. A chitted tuber piece, cv. Désirée, was planted in each of 20 pots (10 cm diam.) containing steam sterilised sand : loam mix (4 : 1). When plants had grown to a height of 10-12 cm (four-five leaf stage), ten pots were each inoculated with 5000 J2 of *G. rostochiensis* and the remaining ten were each inoculated with 5000 J2 of *G. pallida*. White females were harvested at 6, 8, 10, 12 and 14 weeks after inoculation. Females were collected from two pots at each time interval to give a pooled total of 150 females per sample. Females were stored in GDW at 4°C until used for flow cytometric analysis.
FLOW CYTOMETRIC TECHNIQUE TO DETERMINE EMBRYOGENIC DEVELOPMENT

The use of this technique for sorting nematode eggs into development categories has been described in detail by Tylka et al. (1993). In excess of 35,000 eggs were obtained (Boerma & Hussey, 1984) from each sample of 150 females and were suspended in 0.1% xanthan gum. They were agitated manually to prevent settling. Suspensions were loaded into a Coulter EPICS 753 flow cytometer (Beckman Coulter, CA, USA) with a 150 μm flow cell tip and analysed at a wavelength of 488 nm. A minimum of 3000 eggs was analysed from each sample and there were three sub-samples for each sample date-species combination. The percentage of eggs that contained vermiform juveniles (Tylka et al., 1993) was determined for each sample.

STATISTICAL ANALYSIS

Data were analysed using two factor ANOVA (species × sample date) after arcsin transformation of percentages (SAS, 1987, Cary, NC, USA). Also, data for both nematode species were sorted by week and differences between species were assessed using a one-factor ANOVA.

Results

The percentage of eggs containing J2 during the sampling period (Fig. 1) demonstrates a marked difference between the two species of PCN in the rate of development. Apart from the first sampling date (6 weeks), the percentage of eggs that contained J2 was always greater from females of G. pallida than of G. rostochiensis. This was already evident at the 8 weeks sampling date, when the percentages of eggs containing J2 were 40.7 and 28.5% for G. pallida and G. rostochiensis, respectively. The greatest difference was at the 10 weeks sampling date, when 76.7% of G. pallida eggs contained J2 whereas the percentage for G. rostochiensis was 50.8%. It took a further 4 weeks before the percentage of G. rostochiensis eggs containing J2 was greater than 75% and by this time about 80% of eggs of both species contained J2. Thus, the final number of unhatched J2 from females produced on plants 14 weeks after inoculation was similar for both species, but the rate of development of females produced on younger plants was more rapid for G. pallida than for G. rostochiensis. Statistical analysis of the data showed that there was a significant interaction between species and sample date ($P < 0.0001; F = 177.8$), indicating that the rates of embryological development to J2 of the two species were different. There were no differences between the species in the mean number of eggs per female at each sampling interval (data not shown).

Discussion

The difference in rate of embryogenic development is a further example of physiological differences between the two species of PCN. Under field conditions in Europe, PCN completes only one full generation during the host growing season. At harvest, a large percentage of unhatched J2 will be in diapause (Perry, 1998) and it is unlikely that differences in the rate of development will have any influence on subsequent hatch after diapause has ended.

By contrast, it is possible that differences in development may have survival implications. In some animal-parasitic nematodes, the eggshell undergoes a permeability change during development to establish the resistant characteristics associated with the eggshell surrounding the fully formed infective juvenile (Matthews, 1986). The eggshell surrounding the unhatched J2 of PCN protects the J2 from adverse environmental conditions. However, during the early stages of embryogenesis of PCN, the eggshell may not be in this final resistant form. If the permeability characteristics of the eggshell change in tan-