

## Hatching Behavior of Potato Cyst Nematodes from the Canary Islands

J. A. GONZALEZ<sup>1</sup> AND M. S. PHILLIPS<sup>2</sup>

**Abstract:** The present work investigated early hatching differences in naturally occurring field populations and newly reared populations of potato cyst nematodes from the Canary Islands. Hatching behavior of the two species appears to be distinct, with more juveniles hatched from *G. pallida* that hatch earlier and over a shorter time than *G. rostochiensis*. The hatching rate of 3-year-old PCN populations was more than double (mean 44.5% ± 1) that shown by newly reared populations (mean 19.1% ± 12.5), and those that could be classified as pathotype Pa1 (Pa1 and P13) were found to hatch particularly poorly. Significant differences were also observed in the juveniles released in tap water between newly reared populations of both species, with mean hatch significantly higher for *G. rostochiensis*. The results are discussed in relation to the implication that these findings may have for competition between the two species of PCN in the field.

**Key words:** Canary Islands, *Globodera pallida*, *G. rostochiensis*, hatch, persistence, potato cyst nematode.

When potato is grown in a soil infested with the potato cyst nematodes (PCN) *Globodera pallida* and *G. rostochiensis*, chemical substances produced by the growing roots stimulate hatching of the second-stage juveniles enclosed in the eggs inside the cysts (1,2). Sometimes PCN may enter a dormant state due to unfavorable environmental conditions (quiescence) or endogenous factors (diapause) (4). Under such circumstances, and even in the presence of the host, only a small proportion of the total eggs respond to the stimulus of the root diffusate and hatch. After a period of time, the dormancy may disappear due to changes in the environment that become favorable or when endogenous factors make the nematode receptive to the stimulus released from the host plant.

Differences in the hatching behavior of English and Northern Irish populations of PCN have been reported. Both species seem to differ, with *G. rostochiensis* being more ready to hatch than *G. pallida* (9–11).

Also, in populations of PCN that were re-produced simultaneously, *G. pallida* had a much slower initial rate of hatch and used its lipid reserves more slowly than *G. rostochiensis* (17). Depletion of lipid reserves has been associated with a loss in infectivity and delayed development of the third stage of late-hatching juveniles of *G. rostochiensis* (15).

There is evidence that hatching behavior in some cyst nematode species is under genetic control and might be a heritable character ([3,5] for *G. rostochiensis* and [14] for *Heterodera avenae*). In *G. rostochiensis*, lines produced by mating early-hatching nematodes hatched early and showed no signs of dormancy, whereas lines from late-hatched parents produced eggs that showed dormancy before hatching (3). Also, hatch in water was faster from cysts derived from water-hatched juveniles than from cysts derived from diffusate-hatched juveniles. These differences seemed to be related to a difference in resistance to hypertonic solutions and could be genetically controlled (5).

### MATERIALS AND METHODS

**Experiments:** Two experiments were made. The first experiment was conducted to investigate possible differences in early hatching of field populations of the two species of PCN from the Canary Islands and Britain. This experiment included populations from Tenerife representative

Received for publication 20 June 1995.

<sup>1</sup> Dpto de Agroecología, Centro de Ciencias Medioambientales, CSIC, Serrano 115dpdo. E-28006 Madrid (Corresponding author).

<sup>2</sup> Zoology Department, Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, UK.

The authors thank A. Bello and D. L. Trudgill for helpful comments on the manuscript and G. Malloch, A. Holt, and J. Roberts for their technical assistance. This research was supported in part by grants from the Scottish Society for Crop Research and "Dirección General de Investigación Científica y Técnica" to J. A. González.

of both species of PCN, as well as of different environments on the island. The second experiment investigated diapause in newly reared populations from the Canary Islands. In this experiment, PCN populations from Tenerife produced under the same conditions were compared with both newly reared and old cysts of British populations.

*Preparation of potato root diffusate (PRD):* The procedure used was that described by Forrest and Farrer (6): potato sprouts were cut from tubers of cv "Maris Piper" and grown in trays of sand for 1 week at 20°C and in a photoperiod of 16 hours. Immediately prior to an experiment, the roots of four sprouts were cleaned of sand by gentle washing in tap water and then immersed in 100 ml of tap water shaken for 2 hours at laboratory temperature. The rooted sprouts were removed and the diffusate filtered and stored at 4°C. Fresh PRD was made weekly.

*Preparation of samples and experimental conditions:* Well-developed cysts were hand-picked and sealed in polyester 105- $\mu$ m mesh size bags. The bags containing the cysts were placed in 50-ml entomological glass vials and soaked in 10 ml of tap water. After 2 or 3 days, the water was replaced by the same volume of fresh PRD. The experiments were conducted in a cabinet in the dark at a constant temperature of 20°C. Hatched juveniles were counted in methacrylate dishes under a microscope and, after each count, cysts were washed with tap water and the PRD replaced with fresh stock.

*Populations of PCN:* The populations of PCN used represented the two species found on the Island of Tenerife and were chosen from different geographic locations and agricultural environments. For the first experiment, the cysts of PCN from the Canary Islands were reared in May 1988 on the Island of Tenerife from naturally infected potato fields. For the second experiment, all newly reared populations used were multiplied simultaneously on cv. Désirée and under the same conditions in a glasshouse (20°C and 16-

hour daylength) for 90 days. The British PCN populations were from the SCRI PCN collection (Table 1).

*Experiment I:* Batches of 10 cysts per population were soaked for 3 days in tap water, which was then replaced with PRD. The duration of the experiment was 11 days. In this experiment, four *G. rostochiensis* and eight *G. pallida* populations from the Canary Islands were tested. Also, three control populations from Britain (British *G. pallida* from Lindley Pa2/3 and *G. rostochiensis* Ro1, both reared in autumn 2 years previously) and a second British *G. rostochiensis* (CJ1) population reared from a naturally infected field were included. It was presupposed that the majority of juveniles were of an age where they were not in diapause and would respond to the hatching stimulus, except for population CJ1, which was reared only 3 months earlier and therefore was expected to be in diapause.

*Experiment II:* The experiment was started in June 1989 using batches of 50 cysts per population (four *G. rostochiensis* and five *G. pallida*) produced in a glasshouse 2 months earlier under uniform conditions. The cysts were soaked in tap water for 3 days when the water was replaced by PRD. The duration of the experiment was 32 days. As controls, 4-year-old populations (*G. pallida* (Lin Pa2/3), Pa1 and *G. rostochiensis* Ro1) reared in 1985 were included. Four replicates and one control (using tap water instead of PRD) per population were tested. Unhatched eggs were counted after the experiment using the Seinhorst and den Ouden method (18).

*Statistical analysis:* Both experiments were conducted under controlled conditions with the vials randomized in the incubator chamber; thus, a completely randomized one-way ANOVA procedure was used to compare total hatch and percentage of hatch. A Student-Newman-Keuls test with a significance level of 5% was used for mean separation. Factor analysis was used to detect differences in the percentage of hatch between populations.

TABLE 1. Populations of *Globodera rostochiensis* and *G. pallida* used in the experiments.

Populations <sup>a</sup>	Site of origin	Pot/Field	Reared
Experiment I			
Ro1	Scotland	P	Autumn 86
R1	S.J. de la Rambla, CI <sup>b</sup>	F	May 88
R6	Icod, CI	F	May 88
R9	Icod, CI	F	May 88
R10	La Guancha, CI	F	May 88
CJ1	Scotland	F	Autumn 88
Lin	England, Lindley	P	Autumn 86
P2	Los Realejos, CI	F	May 88
P4	San Miguel, CI	F	May 88
P5	Vilaflor, CI	F	May 88
P6	Arico, CI	F	May 88
P7	Arico, CI	F	May 88
P11	Güimar, CI	F	May 88
P13	Fasnia, CI	F	May 88
P14	Granadilla, CI	F	May 88
Experiment II			
Ro1 85	Scotland	P	Autumn 85
Ro1 89	Scotland	P	May 89
R1	S.J. de la Rambla, CI	P	May 89
R6	Icod, CI	P	May 89
R10	La Guancha, CI	P	May 89
Lin 85	England, Lindley	P	Autumn 85
Lin 89	England, Lindley	P	May 89
Pa1 85	Scotland	P	May 89
Pa1 89	Scotland	P	May 89
P4	San Miguel, CI	P	May 89
P5	Vilaflor, CI	P	May 89
P13	Fasnia, CI	P	May 89

<sup>a</sup> *G. rostochiensis* populations = Ro1, R1, R6, R9, R10, and CJ1; *G. pallida* populations = Lin, P2, P4, P5, P6, P7, P11, P13, and P14.

<sup>b</sup> CI = Canary Islands

## RESULTS

At the end of experiment I, significant more juveniles hatched from *G. pallida* populations than from *G. rostochiensis* ( $P = 0.0328$ ) (Fig. 1). Also, the mean percentage of total hatch at 6 ( $P = 0.0213$ ) and 9 ( $P = 0.0150$ ) days was significantly higher for the *G. pallida* populations (Fig. 2).

Factor analysis enables grouping of the populations according to their overall hatching behavior. The analysis shows that the two species of PCN from the Island of Tenerife do not overlap and that hatching behavior appears to be distinct. The two species are separated along the second axis, and the mixed population (M1) shows intermediate behavior being located between the two species groups (Fig. 3). The British control *G. rostochiensis* (Ro1) fits

with the model but not the CJ1 nor the *G. pallida* control (Lin).

In the second experiment, the analysis of variance showed highly significant ( $P < 0.001$ ) difference between populations in

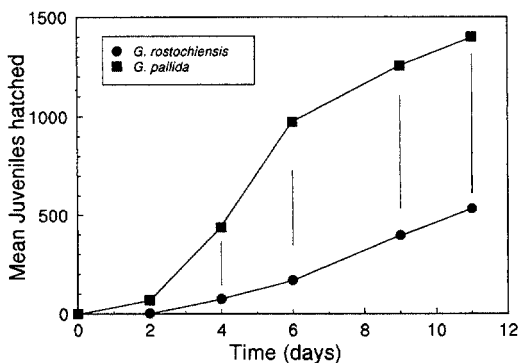


FIG. 1. Mean number of juveniles hatched in experiment I. Bars indicate last significant difference at a level of significance of 5%.

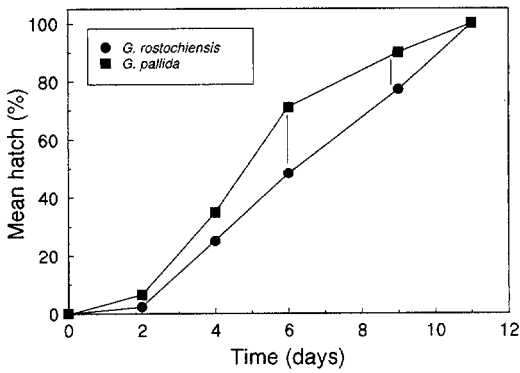


FIG. 2. Juveniles hatched in experiment I as percentage of total hatch. Bars indicate last significant difference at a level of significance of 5%.

their total percentage of hatch in PRD. A Student-Newman-Keuls (SNK) test was used to detect differences ( $P < 0.05$ ) in the average hatch observed between the populations. The results are shown graphically in Figure 4.

The percentage of juveniles hatched from the old *G. pallida* populations tested (Pa1 85' and Lin 85') was significantly higher than that which hatched from the newly reared populations. This indicates that proportionally fewer eggs in these older populations were in diapause. The old *G. rostochiensis* (Ro1 85') population failed to hatch.

A range of variation was observed in the new populations. British populations Lin 89' and Pa1 89' represent the two extremes of the variation, the former showing a relatively high percentage hatch ( $32.7 \pm 5.7\%$ ) and the latter showing a small proportion of juveniles hatched ( $4.1 \pm 1\%$ ). Population Ro1 89' was intermediate ( $23.9 \pm 4.9\%$ ) and significantly different to Pa1 89'.

Within the Canarian *G. pallida* populations, P4 and P5 hatched in a similar manner to Lin 89' (linked by range "B"), and the hatch from the P13 was not significantly different from Pa1 89' (linked by range "G"). Also, significant differences were found between the *G. rostochiensis* populations R1 and R6, R10.

A proportion of juveniles hatched in tap water, the values ranging from 0.1% with

population Ro1 85' to 2.8% with R6. The differences in the percentages of juveniles hatched in tap water between the newly reared populations species was significant ( $P = 0.0401$ ), and the total number of juveniles released from the *G. rostochiensis* populations greater ( $P = 0.023$ ) than that from *G. pallida*. No significant differences were detected in the pattern of hatch between species of the newly reared PCN populations.

## DISCUSSION

When a suitable host is present, early hatching is likely to have biological significance for PCN, mainly because they have a density-dependent population growth that involves mechanisms such as an epigenetic determination of sex that is influenced by the availability of food and (or) root space (12,19). When both species are present in the field, it is likely that the juveniles of the species to first reach the roots will have a biological advantage over the other species.

Diapause represents a biological advantage for PCN, allowing the use of environmental clues as signals to achieve synchronization between nematode and host life cycles (10); this is particularly relevant in temperate climatic conditions. However, in areas where the climatic conditions allow more than one crop and more than one generation of the nematode per year, as occurs in the Canary Islands, the populations of PCN with shorter diapause period may have biological advantage over populations with a longer one.

In Tenerife, PCN appears to have a distinct distribution with both species and mixed populations present in the natural soils of the traditional agrosystems of the north of the island. Seed potatoes are normally exchanged between the two sides of the island, and presumably *G. rostochiensis* has had the opportunity to become established in the south. However, only *G. pallida* has been found in this part of the island (8).

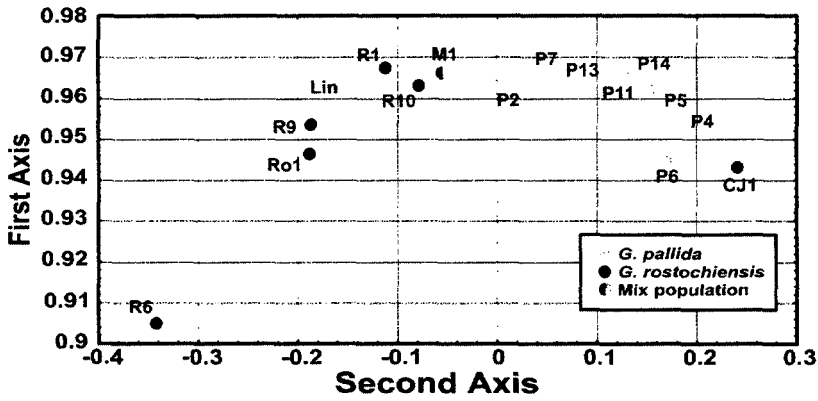


FIG. 3. Factor analysis on percentage of total hatch of populations used in the experiment I. The two axes represented account for 93.98% of total variance.

The differences found between field populations of PCN from the Canary Islands in their hatching behavior over 11 days exposed to the host stimulus (PRD), with *G. pallida* releasing more juveniles, earlier and over a shorter time than *G. rostochiensis*, may explain in part the different distribution of PCN on the Island of Tenerife. Also, when the stimulus of PRD is not present (as may happen in the field between crops or in fallow), more juveniles are released from newly produced *G. rostochiensis* cysts than from those of *G. pallida*.

This may be an additional reason for its success in the low-diversity agrosystems of the southern part of the island, thereby preventing *G. rostochiensis* from establishing and overcoming it when both species are present as a mixture in a field.

Other factors that may have contributed for the non-establishment of *G. rostochiensis* are the use of varieties resistant to *G. rostochiensis* pathotypes Ro1 and Ro4, a more efficient use of lipid reserves by *G. pallida* resulting in higher motility and infectivity (16), and interactions between both species other than competition for food or root space, i.e., inefficient mating of the nematodes, which may occur in the field. Such an effect does occur in pot experiments (13), resulting in a significant decrease of the relative population increase of *G. rostochiensis* when *G. pallida* is dominantly present.

The newly produced *G. pallida* populations showed a lower hatch than the older control populations, which had a hatching rate more than double (mean 44.5% ± 1) that of the newly reared populations (mean 19.1% ± 12.5). The exception was the control Ro1 85' population of *G. rostochiensis*, which had a low hatch rate of 12.5%. This could be due to an overestimate of the viability of the remaining eggs. The new *G. rostochiensis* populations had low rates of hatch similar to those of the new *G. pallida*, suggesting that this species was also in diapause.

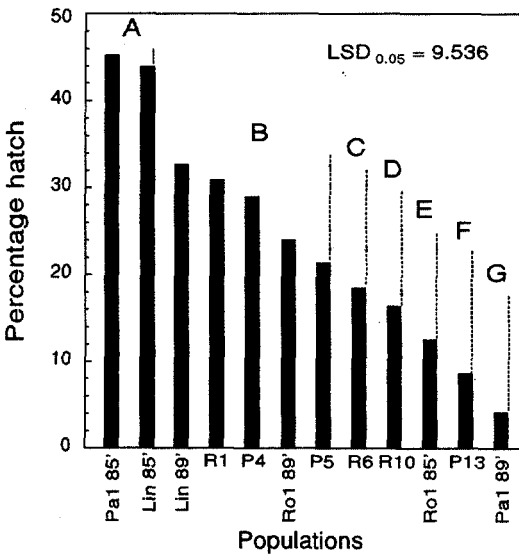


FIG. 4. Percentage of juveniles hatched from populations in experiment II, groups of non-significant ranges at a level of significance of 5%.

Factor analysis showed that the two species of field populations from the Canary Islands hatch in a different manner; however, British controls *G. pallida* Lin and *G. rostochiensis* CJ1 did not fit with the model. Possible reasons are the age of the Lin population (having been reared in autumn 1986, 18 months older than the Canarian populations) and CJ1 reared only 3 months prior to the experiment and perhaps in diapause.

In this study, field populations of *G. pallida* appear to hatch more ready than *G. rostochiensis* populations. This differs from previously reported results for English and Northern Irish PCN populations (9, 11, 17), where *G. pallida* has been found to hatch more slowly than *G. rostochiensis*. These studies were done with newly reared cysts (less than 2 months old) (9, 11) or with laboratory-reared populations (15). Our results show that newly reared populations of pathotype Pa1 (Pa1 and P13 (7)) hatch very poorly. This may explain the findings of Guile (9), who tested a mixture of pathotypes Pa1 and Pa2/3 as representing *G. pallida* population.

The differences observed between and within PCN species may be of biological significance as they may affect interspecific competition in the field and the success of *G. pallida* when both are present in the same field.

#### LITERATURE CITED

1. Antoniou, M. 1989. Arrested development in plant-parasitic nematodes. *Helminthological Abstracts* (Series B) 58:1-19.
2. Clarke, A. J., and R. N. Perry. 1977. Hatching of cyst-nematodes. *Nematologica* 23:350-368.
3. El Shautoury, H. 1978. Genetic control of dormancy in the potato cyst nematode. *Experientia* 34: 448-449.
4. Evans, A. A. F., and R. N. Perry. 1976. Survival strategies in nematodes. Pp. 383-424 in Croll, N. A., ed. *The organization of nematodes*. London: Academic Press.
5. Evans, K. 1979. Is there genetic control of hatching in cyst-nematodes? *Journal of Nematology* 11:297.
6. Forrest, J. M. S., and L. A. Farrer. 1983. The

response of the white potato cyst-nematode *Globodera pallida* to diffusate from potato and mustard roots. *Annals of Applied Biology* 103:283-289.

7. González, J. A., M. S. Phillips, and D. L. Trudgill. 1996. Virulence of Canarian populations of potato cyst nematodes (*Globodera* spp.). *Nematologica* 41:468-479.

8. González, J. A., C. M. Rodríguez, and M. S. Phillips. 1991. *In* Estudio de Fitopatología, del Moral, ed. Nematodos de la papa formadores de quistes (*Globodera* spp.) e influencia del cultivar "Cara" en Canarias. SEF, 254-255.

9. Guile, C. T. 1967. On cyst colour changes, bio-nomics, and distribution of potato cyst eelworm (*Heterodera rostochiensis* Woll.) pathotypes in the East Midlands. *Annals of Applied Biology* 60:411-419.

10. Hominick, W. M., J. M. Forrest, and A. A. F. Evans. 1985. Diapause in *Globodera rostochiensis* and variability in hatching trials. *Nematologica* 31:159-170.

11. McKenna, L. A., and R. D. Winslow. 1972. Differences in hatch and development rates of potato cyst nematode pathotypes. *Annals of Applied Biology* 7:274-278.

12. Mugniery, D., and G. Fayet. 1981. Détermination du sexe chez *Globodera pallida* Stone. *Revue de Nématologie* 4:41-45.

13. Nijs, den L. J. M. F. 1992. Interaction between *Globodera rostochiensis* and *G. pallida* in simultaneous infections on potatoes with different resistance properties. *Fundamental and Applied Nematology* 15: 173-178.

14. Rivoal, R. 1978. Biologie d'*Heterodera avenae* Wollenweber in France I. Différences dans les cycles d'éclosion et de développement des deux races Fr1 et Fr4. *Revue de Nématologie* 1:171-179.

15. Robinson, M. P., H. J. Atkinson, and R. N. Perry. 1985. The effect of delayed emergence on infectivity of juveniles of the potato cyst *Globodera rostochiensis*. *Nematologica* 31:171-178.

16. Robinson, M. P., H. J. Atkinson, and R. N. Perry. 1987. 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*. *Revue de Nématologie* 10:343-348.

17. Robinson, M. P., H. J. Atkinson, and R. N. Perry. 1987. 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*. *Revue de Nématologie* 10: 349-354.

18. Seinhorst, J. W., and H. den Ouden. 1966. An improvement of Bijloo's method for determining the egg content of *Heterodera* cysts. *Nematologica* 12:170-171.

19. Trudgill, D. L. 1967. The effect of environment on sex determination in *Heterodera rostochiensis*. *Nematologica* 13:263-272.