Fundam. appl. Nematol., 1992, **15** (6), 545-550

Do *Globodera rostochiensis* and *G. pallida* interbreed in semi-natural conditions? An examination of the next generations

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Accepted for publication 12 February 1992.

Summary — An investigation was conducted to study whether the species *Globodera rostochiensis* and *G. pallida* could hybridize in pots under environmental conditions favourable to both species and to see what consequences this might have for the offspring. Pot experiments were carried out for 4 years with 50 % mixtures of both species and single species populations, reared on the susceptible cv. Bintje. Analyses of the cyst production, the cyst contents and the relative population increase of each species show that the two species do not interbreed. They reproduce independently and are in stable coexistence in these circumstances.

Résumé — Globodera rostochiensis *et* G. pallida *se croisent-ils en conditions semi-naturelles? Examen de leur descendance* — Des recherches ont été conduites pour connaître *i)* si *Globodera rostochiensis* et *G. pallida* pouvaient se croiser lors d'élevages en pots dans des conditions favorables à l'une et l'autre espèces, et *ii)* les conséquences éventuelles sur leur descendance. Les expériences en pots ont duré 4 ans et ont comporté des mélanges à 50 % des deux espèces et des élevages séparés de l'une et l'autre, sur le cv. sensible Bintje. Les analyses concernant la production des kystes, le contenu de ceux-ci et l'accroissement relatif de population pour chacune des espèces démontrent que celles-ci ne se croisent pas : elles se reproduisent indépendamment et, dans les conditions de l'expérience, coexistent de façon stable.

Key-words : *Globodera rostochiensis*, *G. pallida*, interbreeding.

Potato cyst nematodes (= PCN), *Globodera rostochiensis* and *G. pallida*, are one of the best studied plant parasitic nematodes. Many papers have been published on subjects such as ecology, damage, control, the distribution on a world scale and in small fields (Evans & Stone, 1977; Clarke & Perry, 1977; Seinhorst, 1986; Whitehead, 1986). The fact that both species coexist in the same area (Jones & Kempton, 1978; Kort & Bakker, 1980) without apparent hybridization confirms that they are well defined species, according to Mayr (1942). However, it has been shown (Franco & Evans, 1978; Mugniéry, 1979; Miller, 1983) that *G. pallida* and *G. rostochiensis* can be crossed under experimental laboratory conditions. Hybrids were formed, but these were infertile (Mugniéry, 1979) or partly fertile (Miller, 1983). Sturhan (1985) considered that although *G. rostochiensis* and *G. pallida* are clearly separate species, a restricted exchange of gene material between certain populations cannot be excluded. Stone (1983) suggests that because these species may occupy the same ecological niche and are very closely related this enhances the chance of successful recombination of genotypes. However, if frequently interspecific mating takes place resulting in non-fertile hybrids it may reduce the reproductive success of the species, which is not functional in terms of maximizing fitness on an evolutionary time-scale.

Mixed populations exist in the field but whether under these natural conditions matings take place between the species and whether this will lead to hybridizations on a substantial scale, is unknown. The coexistence of the species might partly be explained by the ecological differences between the species such as the slower initial rate of hatch of *G. pallida* (Robinson *et al.*, 1987), and the higher optimum temperature for development of *G. rostochiensis* (Mugniéry, 1978; Franco, 1979), the ecological differences might be greater than generally assumed.

Jones and Kempton (1978) used a simulation model of the population dynamics of PCN to assess the different possibilities of coexistence to non-coexistence of both species. They found in preliminary pot experiments that in all circumstances *G. pallida* replaced *G. rostochiensis*. In pot experiments with mixed populations and with environmental conditions favourable for both species den Nijs (1992) showed that an interaction took place between the nematode species resulting in a reduction of the relative population increase of *G. rostochiensis* when *G. pallida* was present in majority.

ISSN 1164-5571/92/06/545 06 $ 3 / © Gauthier-Villars - ORSTOM

She suggested that the intraspecific mating of *G. rostochiensis* was hampered by an excess of *G. pallida* males.

The purpose of this study was to determine whether *G. rostochiensis* and *G. pallida* hybridize in pots and if they do, to what extent this influences the fitness of the offspring in the subsequent generations. Another aim of these experiments was to investigate whether both populations can coexist in the limited space of pots over generations.

Material and methods

Cysts of *G. rostochiensis* (designated as Ro1 at the Research Institute for Plant Protection) and *G. pallida* (designated as Pa3, Research Institute for Plant Protection), reared on cv. Irene in 1984 were used as the initial populations. For all experiments suspensions of eggs and juveniles were used as inoculum. The initial density was approximately 5 eggs per gram of soil, inoculated in the pots using a 20 cm long canula and a syringe.

Cv. Bintje was used as the host plant during the experiments. Small pieces of tuber were placed in pots (diam. = 10 cm; h = 20 cm) in an artificial soil mixture [30 % hydrocorn (2-4 mm), 60 % silver sand, 10 % clay powder, Steiner solution]. The soil moisture level was kept at 15 % by use of an automatic water supply system (Lock & den Nijs, unpubl.). The pots were placed on pellets in a completely randomized design in the glasshouse and rotated twice a week. Growth conditions were 20 °C during day time and 15 °C during the night, the relative humidity was kept at 80 %.

In 1985 the experiment started with single species populations and a 50 % mixture (M1) of the species as inoculum, each treatment in five replicates. From each pot a soil sample of 500 g was taken for nematodes analysis. The remaining newly formed cysts of the five M1-replicates, the so-called M1-F1 cysts, were put together and stored. The cysts of the single species populations were treated likewise. The experiment was interrupted in 1986 and 1987. In 1988 the eggs and juveniles of the stored cysts were used as inoculum (eggs and juvenile suspension, 5 egg·g^{-1}) to form the second generation (M1-F2). The newly formed cysts of the single species populations were used for the new 50 % mixture (M2) and for the continuation of the single species populations. In 1989 and 1990 the same procedure was followed, eventually leading to the forming of the third and fourth generation cysts. For a plan of the sequence of generations in the experiment see Figure 1.

Cysts were extracted from the soil by the Seinhorst elutriator, the final population density was determined by crushing the cysts and counting an aliquot of the suspension (eggs·g^{-1} soil). Subsequently, from the final mixed populations, species composition was determined by way of SDS-polyacrylamide gel electropheresis according to the method of den Nijs and Lock (1990).

Fig. 1. The formation of the generations of *Globodera rostochiensis* (Ro) and *G. pallida* (Pa) in single species populations and in the mixture (M_i) over the years.

	Pa	Ro	M_1	M_2	M_3	M_4
1985	F1	F1	F1			
	↓	↓	↓			
1988	F2	F2	F2	F1		
	↓	↓	↓	↓		
1989	F3	F3	F3	F2	F1	
	↓	↓	↓	↓	↓	
1990	F4	F4	F4	F3	F2	F1

From this species ratio, expressed as % of *G. pallida*, and the joint final density the Relative Population Increase (RPI), defined as Pf/Pi, of *G. rostochiensis* and *G. pallida* could be calculated separately.

Data analysis

It is assumed that if mating between the species takes place this should result in hybridization and the formation of sterile juveniles in the M_i-F1 cysts. Continuation of these M_i-F1 cysts into the M_i-F2 should give a decrease of reproduction and hence the total number of cysts in the M_i-F2 in comparison to the number of M_i-F1 cysts. Student-t test was applied on the number of cysts of M_i-F1 and M_i-F2 per year. To exclude the year effect, ANOVA was also applied on the number of cysts of M_i-F1 and M_i-F2 over the years 1988, 1989 and 1990 with year as block factor.

It is hypothesized that if the species do not hybridize they might hamper each other during the mating process. This can lead to a reduced fertilization resulting in smaller or empty cysts. The average cysts' content is expected to be lower than that of the 50 % mixture of both species. This was tested with the Student's t-test. If, however, the hypothesis is true that the species are acting independently of each other, then the RPI of each species in the mixed population should be similar to that in the single species population. ANOVA was applied on the data of both species separately, followed by the Tukey HSD test for interval comparisons.

To determine if there is a stable coexistence between the species in each mixture (M1-4) the shifts between the two species, expressed in percentage of *G. pallida* present, were tested for their significant deviations from the initial 50 % mixture, over the years. Data were analysed by ANOVA followed by Tukey HSD test for interval comparison.

Results

Table 1 shows the number of cysts, expressed per 500 gram of soil, of the single species populations and the ones formed in the first (M_i-F1) and second (M_i-F2) generation of the mixed populations. If mating between the species took place and non-fertile hybrids were formed, the number of cysts in the second generation should be lower. This was tested in repeated experiments for three years (Table 1); no significant reduction of number of F2 cysts was found. So it seems unlikely that non-fertile hybrids were formed. The reason for the higher number of cysts in the F2 formed in 1988, in comparison to the F1, is difficult to explain.

Table 1. Total number of cysts of *Globodera pallida* (Pa) and *G. rostochiensis* (Ro) in single species populations, in the 50 % mixture (M_i-F1) and its second generation (M_i-F2) per 500 gr of soil. Data represent the mean of five replicates. Student-t test (α = 0.05) was applied on the number of M_i-F1 cysts versus the number of M_i-F2 cysts, ANOVA (α = 0.05) was applied on all data of the M_i-F1 and M_i-F2 over the years 1988, 1989 and 1990.

	Pa	Ro	M_i-F1	M_i-F2	P values
1985	538	855	676		
1988	590	469	530	805	< 0.001
1989	637	647	692	669	0.7019
1990	423	376	500	422	0.2152
1988-1990 (average)	—	—	574	632	0.1666

The number of eggs and juveniles per cyst for F1 were compared to the average number for the single species populations of *G. pallida* and *G. rostochiensis*. Figure 2 shows that in three of the four comparisons the cyst contents did not differ significantly from each other. Only in 1990 was the number of eggs and juveniles per F1 cyst significantly lower than the average of *G. pallida* and *G. rostochiensis*. So there is no consistent evidence that the species might interfere with each other in the mating process eventually resulting in lower cyst contents.

The data of the RPI of both nematode species in the single species populations and in the mixed populations are shown in Table 2. Analyses for each year and each species were conducted to test for their similarity in RPI, because if the species behave independently from each other the RPI should be similar regardless of the population mixtures in which the species is present. For *G. pallida* this was found to be true in most cases. However, the data of *G. rostochiensis* are much more variable. In 1990 this species reproduced similarly in all situations, but in contrast in 1988 nematode reproduction compared to the single species population was lower in the F1 but higher in the F2. Because of this variability for *G. rostochiensis* one cannot conclude that intercrossing, if occuring, affects the reproductive success noticeably. This and the results for *G. pallida* support the hypothesis that intercrossing does not take place and consequently the species reproduce independently from each other. Table 3 shows the shifts which have taken place between the species in the mixtures over the years. The starting point was always 50 %. In all cases, except one, the percentage *G. pallida* did not deviate from the starting situation. This implies that the species can

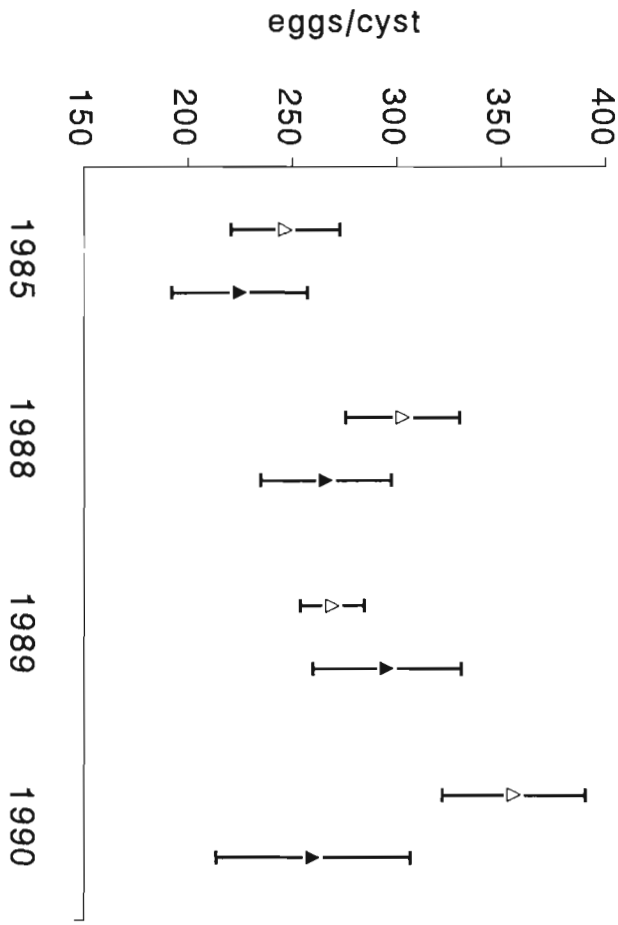

Fig. 2. Mean and confidence intervals (α = 0.05) of the cyst content of the M_i-F1 cysts (▲) in comparison to the mean cyst contents of the single species populations of *G. rostochiensis* and *G. pallida* (△). Data represent the mean of five replicates.

Table 2. The relative population increase (RPI = Pf/Pi) of *Globodera pallida* (Pa) and *G. rostochiensis* (Ro) reared on cv. Bintje in single species populations (s.s.p.), in 50 % Pa/Ro mixtures (M_i-F1) and in the following generations over the years. Data represent the means of five replicates.

	1985	1988	1989	1990
G. pallida				
s.s.p.	38.6 *a* *	77.7 *a*	76.5 *a*	53.5 *ab*
M_i-F1	41.1 *a*	80.6 *a*	73.1 *ab*	44.9 *b*
M_i-F2		88.4 *a*	78.4 *a*	58.3 *ab*
M_i-F3			57.0 *b*	46.2 *ab*
M_i-F4				59.1 *a*
G. rostochiensis				
s.s.p.	51.1 *a*	69.9 *b*	55.5 *b*	52.9 *a*
M_i-F1	33.6 *b*	47.3 *c*	79.1 *a*	41.7 *a*
M_i-F2		94.7 *a*	78.5 *a*	45.2 *a*
M_i-F3			77.4 *a*	37.7 *a*
M_i-F4				44.7 *a*

(*) ANOVA, followed by Tukey HSD interval test. Within the column same letters indicate no significant difference at $\alpha = 0.05$.

Table 3. Shifts between *Globodera pallida* (Pa) and *G. rostochiensis* in mixed populations, starting from 50 % Pa, over years. Data are expressed as % Pa and represent the means of five replicates.

Generation	1985-1990	1988-1990	1989-1990	1990
M_i	50.0 *ab* *	50.0 *a*	50.0 *ab*	50.0 *a*
F1	55.7 *b*	65.0 *b*	48.0 *a*	52.2 *a*
F2	52.0 *ab*	64.8 *b*	54.2 *b*	
F3	44.3 *a*	69.3 *b*		
F4	51.1 *ab*			

(*) ANOVA, followed by Tukey HSD interval test. Within the column same letters indicate no significant difference at $\alpha = 0.05$.

coexist and that, on average, they have equal rates of reproduction in the situations tested here. The mixed population started in 1988 made a shift from 50 % towards 65 % in that year. The following years the mixture did not change significantly and showed a stable coexistence of the species.

It is not known why this population deviated from the others.

Discussion

The purpose of the experiments described here was to establish whether intercrossing between the two potato cyst nematodes might take place in the controlled conditions of pot experiments. The data have clearly shown that hybridization between the two species does not play any significant or detectable role in the test conditions. In these pot experiments suspensions of eggs and juveniles were used as inoculum, which should ensure the best chances for mixed encounters. Nevertheless, the nematodes apparently preferred to mate with one of its own species. Green and Miller (1969) already found that quantitative differences in the male-attractants occurred between closely related species of the genus *Globodera*. Such a mechanism enhances the likelyhood of inbreeding but does not exclude outbreeding (Green, 1980). The experiments of Franco and Evans (1978), and Mugniéry (1979) showed, *in vitro*, that hybrids could be formed when one male was placed with one female of the other species, however, when males could chose between females of both species, the males aggregated around the females of their own species (Parrot & Berry, 1976).

In the field the conditions for the nematode species to encounter each other are completely different. The soil is infested with cysts, which ensures a certain amount of aggregation of juveniles of the same species. Furthermore independant introductions of infestation of both species take place which ensures spatial separation of the species, although soil tillage will cause secondary spread (Kort & Bakker, 1980). Therefore the chances for intraspecific encounters might be greater than for interspecific encounters. More information on the microdistribution of the species is needed, however. Additionally, in the field environmental conditions vary during the growing season. This will have a differentiating effect on the development and behaviour of the nematode species (Parrot & Berry, 1979; Robinson *et al.*, 1987). This might lead to a different phenology of the males and sexual maturity of females. Although overlap in the presence of maturity stages of both species might be possible, the probability of encountering will be smaller.

Den Nijs (1992) found in pot experiments with varying mixtures of the potato cyst nematode species interactions between these species. The experimental conditions for the experiments described here were identical, with the exception that only the susceptible cv. Bintje and a 50 % mixture of the species was used. In this 50 % mixture the nematodes reproduced independently from each other, and interactions that lead to a decrease of the relative population increase were not found. However, it might still be possible that intercrossing takes place or that intraspecific fertilization might be hindered in extreme situations when one of the species is present in excess compared to the other species, as suggested by den Nijs (1992). The absence of significant shifts from the starting point of the mixed populations over the years supports the idea that the species can coexist independently as Jones and Kempton (1978) suggested. Apparently the species occupy different

niches and they do not interfere with each other in this 50 % situation. The stable coexistence of the populations is caused by the similar relative population increase of both species. However it is interesting to note that the reproduction of *G. rostochiensis* is much more variable than that of *G. pallida*. This phenomenon has also been observed in the hatching pattern of these nematodes as mentioned by den Nijs and Lock (1992) and is apparently a species specific characteristic.

In the experiments described here the environmental conditions, e.g. temperature, cultivar, initial nematode density, were carefully chosen to avoid unwanted discriminating effects on the species. The fact that both species reproduced equally well and stayed in equilibrium over the years showed that we succeeded in this. However, in field experiments in less controlled conditions, the nematode species vary in their reproduction capacity as noted by Jones and Perry (1978) who gave a list of the parameter values for the maximum reproduction as found in the literature for both species. The different responses of the nematode species to temperature, soil type, initial nematode density and cultivar, for instance, has created the situation that in some areas *G. pallida* is the most common species, whereas in other areas *G. rostochiensis* is dominant (Robinson *et al.*, 1987; Marshall, 1989).

The potato cyst nematodes *G. rostochiensis* and *G. pallida* are clearly two separate species. They do not interbreed in pots, as these experiments have shown, and it seems highly unlikely that they do so in the field when environmental conditions vary and favour the species differently.

Acknowledgments

The author wishes to thank Clasien Lock and Anne-Marie de Heij for technical assistance, and Kees Booij and Lijbert Brussaard for useful discussions and critical comments on the manuscript.

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