# Interactions between the closely related potato cyst nematode species *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone).

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Voor mijn ouders

## Voorwoord

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Loes den Nijs
Wageningen, april 1992

## Contents

## Introduction

In The Netherlands potatoes are grown on an area of about 175.000 ha (Anonymous, 1991a). One third of this area produces the raw material for the potato starch industry. Ware potatoes and seed potatoes are grown on the remaining area. The crop rotation, especially in the starch potato area, is very narrow: potato once every two or three years. The potato cyst nematodes, *Globodera rostochiensis* (Woll.) and *G. pallida* (Stone), are major pests, particularly when potatoes are grown so frequently.

Currently, control of potato cyst nematodes is based on the use of chemicals in combination with the growing of resistant potato cultivars and crop rotation. However, pesticides still play a dominant role in control. Of all pesticides used in The Netherlands 60% are soil fumigants (13 million kg active material each year, Anonymous, 1991b). To improve the present situation, a 31% reduction of these fumigants in 1995 and a 48% reduction by the year 2000 has been set by the Government. To realize these aims the frequency of applications will be limited and soil disinfection will only be allowed when an infestation has been detected. Routine, preventive soil desinfection will be forbidden in 1997 (Anonymous, 1991b). As a result of this policy the control of potato cyst nematodes will increasingly rely on crop rotation and the use of resistant potato varieties.

Potato cyst nematodes are obligate parasites restricted to solanaceous hosts. Two distinct species of potato cyst nematodes have been described (Stone, 1972). Both species survive in the soil as eggs inside cysts. The life-cycle starts when the host, the potato plant *Solanum tuberosum* L., develops its root-system in the soil and exudates leach from the roots. The root exudates trigger the dormant juveniles which then become activated and emerge from the eggs. The juveniles (J2) penetrate the roots intracellularly and modify the host tissue to form a syncytium. This is the pathogenic stage. The effects on plant growth characteristics are well documented by Trudgill (1986), Seinhorst (1986) and Evans & Haydock (1990). The decrease of photosynthesis and transpiration rates that results from nematode attack is one of the mechanisms by which damage, the reduction of potential yield, is caused (Schans & Arntzen, 1991).

The sedentary juveniles develop through the J3 and J4 stages into adult females or into slender males. The latter leave the roots to fertilize the females on the root surface. Eggs are produced inside the females, the females die and become cysts which detach from the roots when the potatoes are lifted. Unhatched eggs inside cysts can remain alive in the soil for at least ten years (Oostenbrink, 1950). There is an annual decline in the number of living eggs and juveniles of about 30%, presumably caused by natural enemies and spontaneous hatching in the absence of host plants. Reproduction of the nematodes is dependent on environmental conditions, on initial population density and on the genetic constitution of the host plant (e.g. presence of resistance genes).

Resistance is a powerful tool in the control of nematodes. From a nematological point of view resistance describes the following process: nematodes are stimulated to hatch by the root exudates of the host plant, juveniles may be able to penetrate the roots but they cannot reproduce on the host containing specific resistance genes. A gene-for-gene relationship for the H1-gene exists since nematodes which possess the corresponding virulence genes are able to develop on these resistant cultivars (Janssen *et al.*, 1990). The nematode populations can be classified as pathotypes according to their ability to multiply on differential clones with $P_f/P_i \leq 1$ (final population density/initial population density) for avirulent and $P_f/P_i > 1$ for virulent populations. At the moment five pathotypes of *G. rostochiensis* are distinguished (Ro1-5) and three of *G. pallida* (Pa1-3) (Kort *et al.*, 1977).

The predominant source for resistance against *G. rostochiensis* is *Solanum tuberosum* ssp. *andigena* (Huijsman, 1957), which was first discovered by Ellenby (1954) in the Andean area, the gene centre for *Solanum* spp. From the same area came *S. vernei* (Ross & Huijsman, 1969) which is the major source for *G. pallida* resistance but contains also resistance against *G. rostochiensis*. The use of new genetic sources of resistance will probably result in an increase in the number of pathotypes recognised.

The aforementioned definition of pathotypes implies that populations classified as identical pathotypes may vary in the number of virulent individuals for a given differential. This might lead to a different reaction of supposedly similar populations towards new resistant varieties. Therefore, other classification systems, based on the initial introductions from South America resulting in virulence groups, are under consideration (Janssen, 1990, Bakker, 1987).

With regard to infestations, the present situation in The Netherlands shows a wide range of variation. Particularly in the starch potato region, considerable areas are infested with mixtures of both species and various pathotypes. The available resistant potato varieties however, were required to possess resistance genes against the current pathotypes of the potato cyst nematode species. Control of the nematodes based on repeated cropping of such cumulatively resistant varieties results in a selection pressure on the nematode populations leading to an increase in virulence and a progressive loss of resistance (Turner, 1983, Stone, 1985). To prolong the efficient use of resistant varieties, alternate cropping of species-specific resistant varieties instead of cumulatively resistant varieties might prove to be a more successful strategy. In this situation the population development of each species is alternately suppressed and selection for increased virulence will be reduced at the same time (Spitters & Ward, 1988; Perry & Jones, 1981).

To develop rotation schemes with these objectives, information is needed on the actual species composition in the field and on the reproduction of both species on the potato cultivars used. More knowledge is also needed on the interactions between the nematode species, since these may affect both their distribution and reproduction.

Up to now most emphasis in research has been on the distribution, geographically as well as on a field scale (Evans & Stone, 1977; Evans & Brodie, 1980; Schomaker & Been, 1992; Seinhorst, 1986) and on reproduction of the single species (Seinhorst,1982; McKenzie & Turner, 1987, Phillips, 1984; Phillips *et al.*, 1989). With a recently developed serological test the distribution of both species in agricultural fields can be routinely determined (Schots *et al.*, 1989; 1992). Before each variety is included in the Dutch list of recommended varieties ("de Rassenlijst"), the resistance and thus the reproduction of the known pathotypes on this cultivar has to be assessed. However, the available data on interactions between the species are still very limited.

Two species can interact in many ways with various consequences for their populations. Generally, interactions are classified by the effect of the interaction, and not by the mode of interaction itself. Interactions may be beneficial to one or both species, neutral or detrimental. When closely related species exploit the same ecological resource, mutual detrimental interaction -defined as competition- is most likely to occur. Competition takes place when the resources which both populations require, are in short supply. This can be either by direct interaction, e.g aggressive encounters between competitors, and is than called interference competition, or by indirect inhibitory effects, such as shortage of food or space and is than classified as exploitation competition (Pianka, 1978).

Usually the interactions between nematodes are detrimental (antagonistic) to at least one of the species (Eisenback & Griffin, 1987). These authors reviewed the interactions between different groups of nematodes e.g. ectoparasites, ectoparasites versus migratory endoparasites or migratory endoparasites versus sedentary endoparasites. For all these specialized parasites of plants they concluded that interaction between the nematodes may be affected by host suitability and environmental or edaphic factors. Furthermore most interactions seemed to be density dependent as well as time dependent and were of the exploitation competition type *sensu* Pianka, 1978.

The root knot nematodes, *Meloidogyne spp.*, are sedentary endoparasitic nematodes, like the potato cyst nematodes . They also have a highly specialized parasitic relationship with their host. Wajid & Haider (1991) determined interspecific interactions of *M. javanica* with different races of *M. incognita* in a 50% mixture at one density. They found reductions in the reproduction of each species in the presence of the other species in the roots. The interaction was not very pronounced and it was concluded that the species could therefore coexist in mixed populations in agricultural fields. Most other studies on interactions have been on qualitative aspects and few experiments were done on cyst nematodes only (Marshall, 1989).

In case of the two closely related species *G. rostochiensis* and *G. pallida*, which have similar feeding sites on the same host, interactions are generally assumed to be mutually antagonistic because the nematodes compete for the available feeding sites (Norton, 1978). The interactions may affect the host-parasite relationship, fecundity, rate of population increase and the resulting population density of the nematode species.

The objective of this thesis is to describe and quantify the interactions between *G. rostochiensis* and *G. pallida* and the effects on the development of their populations. For the study of interactions it is essential that the proportions of both species can be adequately quantified. *G. rostochiensis* and *G. pallida* are difficult to distinguish morphologically. Methods based on morphology are laborious and not accurate because of variability and overlap of most of the discriminating characters (Franco, 1978). Methods based on biochemical approaches are suitable for distinguishing *G. rostochiensis* from *G. pallida* but they have hardly been used quantitatively. Chapter 2 describes a 1-dimensional gel electrophoresis technique based on the separation of heat stable species specific proteins (Bakker *et al.*, 1988), modified to meet the quantitative demands of the present study.

Pot experiments were carried out to investigate whether interactions between the two potato cyst nematodes do occur and to determine the type of interaction. Potato plants were subjected to various initial nematode densities of the two species in mixtures. Because host plant characteristics may have a strong influence on these interactions, cultivars with different resistance properties were used in this experiment (chapter 3).

The hatching of the eggs in response to root exudates is a critical step in the relationship between the nematode and its host. Differences in nematode behaviour in response to the host plant could explain several aspects of interaction between the species. Therefore the hatching behaviour of the two nematode species was analysed (chapter 4).

According to the definition of biological species, the species *G. rostochiensis* and *G. pallida* are not supposed to interbreed. However, under experimental laboratory conditions hybrids were formed, although they were found to be infertile (Mugniery, 1979) or only a small percentage was fertile (Miller, 1983). In chapter 5 pot experiments are described which were designed to determine whether both species hybridize and, if they do, to what extent this influences the fitness of the offspring in subsequent generations. In these experiments it was also investigated whether, under the influence of possible interactions, both populations can coexist over years.

In chapter 6 the results are discussed in view of the ecology of the species and the implications for control strategies.

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# Quantification of *Globodera rostochiensis* and *G. pallida* in mixed populations using species-specific thermostable proteins.

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## Abstract

A method has been developed to quantify species ratios in mixed populations. The method is based on the separation of species- specific thermostable proteins by SDS-PAGE. Densitometric analyses of the 17 kD protein of *Globodera pallida* and the 18 kD protein of *G. rostochiensis* revealed a high correlation ($R^2 = 0.93$) with the species ratio in the mixed samples. Within the limits of 10 to 90% of each species, one can estimate with 95% reliability the species composition with 3 to 6% deviation.

Additional keywords:gel electrophoresis, potato cyst nematodes, species composition.

## Introduction

Control of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida,* is a major problem in the potato growing areas of the Netherlands. Growing resistant potato cultivars is one of the environmentally sound ways to decrease the nematode populations in the soil and to achieve good yields. A reliable screening test to characterize and monitor field infestations of potato cyst nematodes according to species offers possibilities for optimal use of resistant cultivars because resistance directed to either of the species can be used. In case of mixed populations the effects of resistant cultivars on multiplication rates have to be known for both species. At present we are investigating the effect of growing potato cultivars with species-specific resistance on mixed populations of *Globodera pallida* and *G. rostochiensis* under laboratory conditions and therefore need a reliable method to measure differential population changes.

Various methods are available. Methods based on morphology are laborious and not accurate because of variability and overlap of most of the distinguishing characters (Franco, 1978). Alternative methods are based on biochemical approaches such as disk-electrophoresis (Trudgill et al., 1972), 1-dimensional-electrophoresis (Bakker et al., 1988; Von Stegemann et al., 1982), 2-dimensional-electrophoresis (Bakker and Gommers, 1982), isoelectric focusing (Fleming and Marks, 1982; Ohms and Heinicke, 1983), serology (Schots et al., 1987), DNA-probing (Burrows and Perry, 1988) or combinations of these techniques such as immuno-electrophoresis (Wharton et al., 1983). These methods are suitable for distinguishing *G. rostochiensis* from *G. pallida.* With the exception of IEF by Fleming and Marks (1982; Marks and Fleming, 1985) they have hardly been used quantitatively.

We have analysed the quantitative use of a modified 1-dimensional gel electrophoresis technique based on the separation of heat-stable species specific proteins (Bakker et al., 1988; Schots et al., 1987). For routine ecological research, the method has to be simple, relatively cheap, rapid, sensitive and reproducible. In addition, the method should utilise large samples of cysts because results should reflect accurately the composition of the populations. The work reported here describes the possibilities and drawbacks of this approach.

## Materials and methods

### *Nematodes.*

Plants cv. Bintje, susceptible to all pathotypes, were inoculated with an egg suspension (5 eggs/g of soil) of *Globodera pallida* (Pa3; Research Institute for Plant Protection) and *Globodera rostochiensis* (Ro1; Research Institute for Plant Protection). The plants were grown in pots in an artificial soil mixture ( 30% gravel, 60% silver sand, 10% clay powder) in a greenhouse at 18-22°C with 16 h daylight for about three months. The newly formed cysts were separated from the soil using a cyst-elutriator (Seinhorst, 1964).

### *Preparation of protein extracts.*

To prepare a protein solution about 1000 cysts were incubated in 1.5 ml demineralized water for 24 h. The cysts were crushed and the suspension was purified using a 150 $\mu$m sieve. Eggs were collected by centrifugation (5 min, 2850 g) at room temperature. The pellet was resuspended in 700 ml 10 mM Tris-buffer pH

7.4. Subsamples of about 140 ml were homogenized for 10 sec in a tissue grinder (2 ml mortar, teflon pestel, Heidolph microhomogenizer, 2200 rpm) at 0°C. The egg shells and juvenile cuticles were removed by centrifugation (5 sec, 10.500 g). This solution was heated at 95°C (TCS-Metallblock thermostat) for 5 min and denatured protein was pelleted (15 min, 10500 g). The supernatants with the species-specific heat-stable proteins were stored at -20°C.

The protein concentrations of the solutions were determined with the Proti analyzer (Bradford-type analysis (Bradford, 1976), modified Coomassie Brilliant Blue G250 reagent, extinction measured at 465 and 595 nm), as recommended by the manufacturer Marius (Utrecht, the Netherlands).

### *Gel electrophoresis.*

Electrophoresis samples were prepared from protein stock solutions. For each replicate separate stock solutions of *Globodera rostochiensis* and *G. pallida* were used.

The composite samples covered the species ratios from 100:0% to 0:100% in steps of 10% by adding from both stock solutions the appropriate proportions of protein solution. The amount of protein added to the gel from mixed samples were 5 mg, 8 mg and 10 mg; 1 mg protein from samples with 100% of the individual species were also applied on the gel. Phosphorylase-b (97.4 kD) was used as reference protein.

One dimensional sodium dodecyl sulfate polyacrylamide slab-gel electrophoresis (SDS-PAGE) was carried out essentially as described by Laemmli (1970), with 4% (w/v) polyacrylamide stacking gel and 15% (w/v) polyacrylamide separation gel. Running conditions: 50 V stacking gel (1.5 h) and 150 V separating gel (6.5 h, 4-10 °C.). The gels were stained in 0.2% Coomassie Brilliant Blue R250 (12 h) in fixative solution (25% methyl alcohol, 10% acetic acid) and destained in the fixative solution (48 h).

### *Densitometric analysis.*

Pictures were taken from the gels (Kodak Ektachrome 64) and the colour positives (10.2 x 12.7 cm) were scanned with the LKB Ultrascan XL Laser Densitometer. The scanning data were analysed using an interactive program (LKB 2400 gelscan XL$^{tm}$ software package version 1.2.) to determine the peak area by integration via Gausian curve fitting.

## Results

*Extraction of thermostable species-specific proteins.*

Our approach was based on the isolation procedure of species-specific proteins of Schots et al. (1987). This method however gave low protein yields, inadequate separation of proteins and low reproducibility. Therefore the method was optimized to meet our criteria.

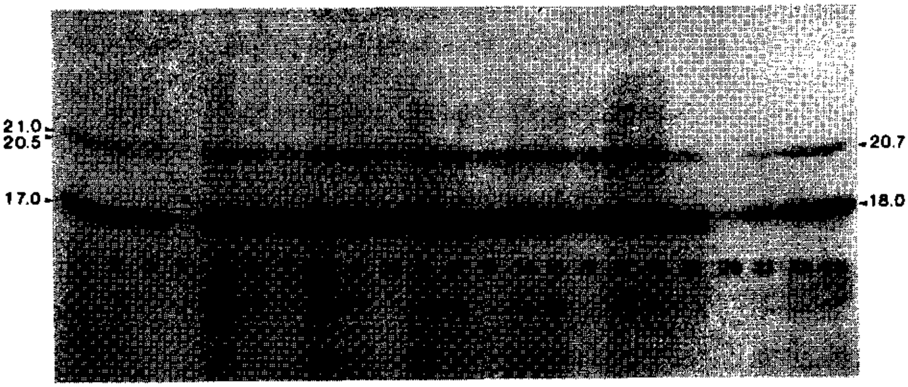

**Fig. 1: Protein pattern of thermostable proteins of *Globodera rostochiensis* (20.8 kD, 20.6 kD and 18 kD) and *G. pallida* (21 kD, 20.5 kD and 17 kD) after SDS-PAGE (15% acrylamide, 50V-150V). Lane 1-4: 100% Pa, 10, 8, 5 and 1 mg respectively; lane 5-7: 90% Pa and 10% Ro, 10, 8 and 5 mg resp.; lane 8-10: 80% Pa and 20% Ro, 10, 8 and 5 mg resp.; lane 11-13: 70% Pa and 30% Ro, 10, 8 and 5 mg resp.; lane 14-16: 60% Pa and 40% Ro, 10, 8 and 5 mg resp.; lane 17-19: 50% Pa and 50% Ro, 10, 8 and 5 mg resp.; lane 20-23: 100% Ro, 10, 8, 5 and 1 mg resp..**

It was established that the egg/larvae suspension should not exceed the equivalent of the contents of 125 cyst per 100 ml to obtain optimal homogenization of eggs and larvae and extraction of proteins. Greater densities resulted in highly viscous solutions with poorly homogenized eggs. Removal of egg shells and debris after homogenisation by centrifugation gave reproducible recovery of the thermostable proteins; however, prolonged centrifugation times resulted in considerable loss of these proteins.

The heating procedure of the protein solution ("au bain Marie") also influenced the reproducibility considerably. Using a heated metal-block greatly improved this step. The heating time could be reduced as well.

Thus, protein solutions with reproducible concentrations of thermostable proteins were obtained (coefficient of variation 9-10%).

*Separation.*

According to Schots et al.(1987) the thermostable species-specific proteins are 21 kD, 20.5 kD and 17 kD in *G. pallida* and 20.8 kD, 20.6 kD and 18 kD in *G. rostochiensis.* We concentrated our work on the 17 kD and 18 kD proteins in *G. pallida* (Pa) and *G. rostochiensis* (Ro) respectively, because these were consistently present (Fig. 1) and their molecular weight difference was relatively greatest. Since these differences are still small, the separation procedure had also to be optimized. SDS-PAGE showed clear band patterns. Optimal resolution was found with 15% (w/v) polyacrylamide (Fig. 1). At percentages of respectively 10% and 20% polyacrylamide the bands became too diffuse or too close for densitometric analysis.

Best separation results were obtained with 8 mg of protein per lane. At 5 mg per lane the minor species was below detectable levels at ratios of 90:10 and 10:90, and at 10 mg overloading occurred at the 90:10 and 10:90 ratios for the dominant species. Fig. 1 shows these effects at ratio 90:10.

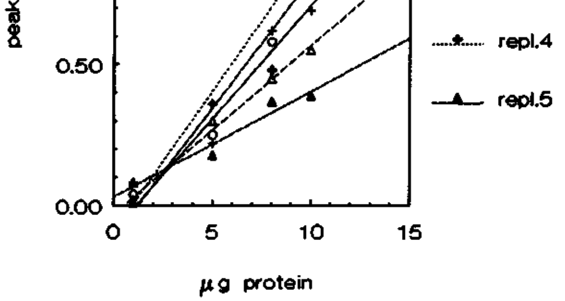

**Fig. 2: Standard curves for the 18 kD thermostable species- specific potein of *G. rostochiensis*, determined by regression analysis ($R^2$= 0.90 to 0.99), five replicates. Proteins separated in 15% polyacrylamide SDS-PAGE, stained with Coomassie Brilliant Blue R250. Densitometric analysis by LKB-Ultrascan 595nm.**

*Quantification.*

The general protein stain Coomassie Brilliant Blue was used as a saturated staining technique. The variability of the stained gels is generally supposed to be negligible. However, analysis of the stain intensity of phosphorylase b, the reference protein, showed considerable variation in separate experiments. Five different gels with 5 mg phosphorylase gave peak area measurements of between 0.32 and 1.54 units and when 0.5 mg was applied the values were between 0.5 and 0.1 units, with variation coefficients of 54% and 38% respectively. Freshly made stain solutions minimize this variability.

Fig. 2 shows the results of five replicate gels of a concentration series of the *G. rostochiensis* stock solution (1, 5, 8, 10 mg per lane). For the 18 kD Ro protein as well as the 17 kD Pa protein a linear relationship was found between the amount of protein and measured peak area with $R^2$ = 0.90 to 0.99. Between the standard curves considerable variance was observed. At 5 and 8 mg of protein the variation coefficient was smallest (respectively 24% and 27%). The high variability (50%) at the 1 mg values probably resulted from the low amount of protein which is close to the detection limits of this method.

The detection level for the 18 kD Ro was reached at 0.8 mg of protein stock solution. The 17 kD protein of Pa was still detectable when 0.5 mg of protein stock solution was applied to the gel. This amount is equivalent to 500 eggs, according to the protein content found in the eggs, respectively 1.09 mg per 1000 eggs for Pa and 1.01mg per 1000 eggs for Ro. Saturation became apparent above 15 mg of protein stock solution.

Five replicate series with nine different Pa - Ro ratios were used for a calibration curve to determine species composition. To circumvent variability between gels due to differences in staining and gel conditions, comparisons between gels were not made. Instead the stain intensities of the bands of the 18 kD of Ro and 17 kD of Pa were compared to each other per lane. The peak area of the Pa protein was divided by the peak area of the Ro protein; the logarithmic value of this quotient was linearly related to the standard ratios of the mixed proteins.

Regression analysis on all data revealed a high correlation ($R^2$ = 0.93) with the linear relation: $y = -0.713 + 0.0182x$. A calibration curve was constructed (Fig.3) with 95% confidence intervals ($P=0.05$) for mean values. Using the mean of five replicates of peak area quotient measurements species composition can be assessed within a range of 10 to 90% of each species with 3 to 6% deviation. To verify this method protein samples with known ratios, based on larvae counts before protein extraction, were analysed on gels. Table 1 shows the counted percentage of Pa and the calculated percentage of Pa in the samples based on the scanning data and using the calibration curve. All numbers, except one, are acceptable according to the above mentioned criteria.

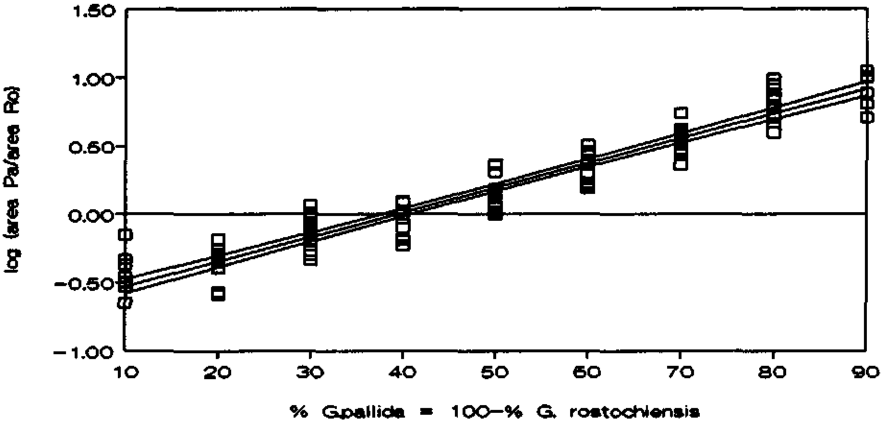

**Fig. 3: Calibration curve for assessing the composition of mixed-species samples of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. Regression analysis ($R^2$=0.93) and 95% confidence interval for m (P=0.05). Proteins separated in 15% polyacrylamide SDS-PAGE, stained with Coomassie Brilliant Blue R250. Densitometric analysis by LKB-Ultrascan 595nm.**

## Discussion

Research on population dynamics of potato cyst nematode species in mixed populations requires quantification of their proportions. Methods based on morphological characters are not suitable because of the overlap in size and shape of most of these characters. Biochemical methods based on intrinsic characters such as thermostable proteins used in this study, depend on presence or absence of a character allowing unambiguous species determination. A routine nematological research and advisory tool for monitoring cyst populations will certainly be based on such an approach (Marks and Flemming, 1985; Schots, 1988). The value of species specific DNA probes, as used for potato cyst nematodes (Burrows and Perry, 1988) and soybean cyst nematodes (Besal et al., 1988), has not yet been developed for routine application but also seems promising.

Because cyst contents vary we used stock solutions of thermostable proteins to prepare composite samples. These samples were made on the basis of protein concentrations, thereby assuming that the thermostable proteins are present in both species in equal amounts. This assumption is realistic in view of the presumed homology of the species specific thermostable proteins (Schots, 1988) and the concentrations values of the protein solutions we found (1.01mg/1000 eggs for *G. rostochiensis* and 1.09mg/1000 eggs for *G. pallida*). The stain capacity however, differs for Pa and Ro (detection level for Ro is 0.8 mg and for Pa 0.5 mg), therefore the intersection of the calibration curve is not found at the expected ratio 50:50 but at the ratio 40:60.

| *% G.pallida* | | |
|---|---|---|
| **Counted** | **Estimated** | **Difference** |
| 24.5 | 27.7 | 3.2 |
| 24.5 | 29.5 | 5.0 |
| 28 | 21.4 | -6.6 |
| 28 | 22.5 | -5.5 |
| 44.7 | 45.1 | 0.4 |
| 51.6 | 48.0 | -3.6 |
| 54 | 51.4 | -2.6 |
| 54 | 50.3 | -3.7 |
| 73.8 | 67.6 | -6.2 |
| 75.1 | 70.5 | -4.6 |
| 76 | 74.0 | -2.0 |
| 76 | 71.7 | -4.3 |
| 86 | 90.2 | 4.2 |
| 86 | 87.3 | 1.3 |

**Table 1: Analysis of composition samples of *Globodera rostochiensis* and *G. pallida* based on counted larvea numbers and estimated by gel electrophoresis and using the calibration curve.**

Proportions of both species in the composition samples were quantified by SDS-PAGE instead of IEF (Marks and Fleming, 1985). The method of Marks and Fleming (1985) allows analysis of up to 30 cysts per sample. These numbers however are too low to reflect the actual composition of populations. Assuming a Poisson distribution one needs to analyse at least 25 to 30 cysts to establish the presence of 10% of either of the species in a population with 95% probability. In this approach, aggregation in the field is not taken into account (Fox and Atkinson, 1986). We therefore use samples of at least 500 cysts to determine species ratios. Furthermore, IEF gives narrow protein bands which are difficult to quantify.

Variation found in assessing standard curves (Fig. 2) was circumvented by taking peak area quotients of the proteins per lane. Because ratios are measured instead of absolute amounts the actual population densities of *Globodera pallida* and *G. rostochiensis* have to be derived from counts of the total eggs and larvae contents of a given sample.

The technique presented here estimates proportions of *Globodera pallida* and *G. rostochiensis* with 3-6% deviation at the 95% probability level within the range of 10-90% of each species. The method is useful in monitoring mixed populations for research purposes. In monitoring routine field populations for advisory purposes the method may be too laborious. Also more information on protein contents of single species populations under field conditions is needed to generalize this method. Tests based on ELISA (Schots, 1988) or DNA-probes (Burrows and Perry, 1988) may offer in the near future better possibilities.

## Acknowledgements

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# Interaction between *Globodera rostochiensis* and *G. pallida* in simultaneous infections on potatoes with different resistance properties.

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## Abstract

Mixtures of *Globodera rostochiensis* (Ro1) and *G. pallida* (Pa3) were reared on susceptible and species specific resistant potato varieties at different densities. The proportion of the newly formed cysts belonging to each species was determined by way of SDS-PAGE and the relative population increase (RPI) of each species was calculated. Comparisons of the RPI of single species populations and of the mixtures revealed that interaction between the two species occurred. The RPI of *G. rostochiensis* was significantly decreased when *G. pallida* was highly dominant in the mixed population. These effects were density independent. The cause of this phenomenon and the implications for integrated pest management (IPM) are discussed.

## Introduction

In integrated control of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, the use of resistant varieties is a major strategy, especially now that the use of agrochemicals is under pressure in The Netherlands. The commercially resistant varieties currently available possess resistance genes against *G. rostochiensis* (pathotype Ro1, Ro1+Ro4, Ro1+Ro4+Ro2/3, Ro1+Ro2/3) or against both potato cyst nematode species (Ro1-4+Pa2). Repeated cropping of varieties with the same or similar resistance genes produces a selection pressure on the nematode populations leading to an increase in virulence and a progressive loss of resistance. Several authors have established this effect (Jones and Kempton (1978), Stone (1985), Mulder (1988), Van der Wal (1987), Jones, 1985)). A simulation model led to similar conclusions (Spitters and Ward, 1988).

Alternate cropping of species-specific resistant varieties is a possible strategy for a more efficient use of resistant varieties. The population development of each species is alternately suppressed and selection for increased virulence will be reduced at the same time (Spitters and Ward, 1988, Perry and Jones, 1981). To develop rotation schemes with these objectives information is needed on the reproduction of the species on the potato cultivars to be used, the actual species composition in the field and the interaction between the nematode species.

In parts of The Netherlands the frequent growing of Ro-resistant cultivars has led to *G. pallida* becoming dominant in fields which were previously thought to be infested only with *G. rostochiensis.* Mulder (in prep.) demonstrated that in such fields *G. rostochiensis* reappeared after two crops of susceptible potatoes and Kort and Bakker (1980) showed that mixed populations were present in some fields in the Netherlands.

Little is known on the interaction between *G. rostochiensis* and *G. pallida.* Stelter (1983) concluded from his experiments with mixtures of *G. pallida* and *G. rostochiensis,* that the changes in the post harvest proportions of the two species resulted from differences in their reproductive rates on the varieties used. No evidence of an interaction was presented. Jones and Perry (1978) concluded from their modelling results that *G. pallida* probably suffered from competition with *G. rostochiensis* when densities of the latter were relatively high. Marshall (1989) concluded, based on a marked change in the proportions of the two species found in pot and field experiments, that interaction was present. However, in both cases comparisons in reproductive rates were not made.

The experiment described in this paper is an attempt to answer the question whether interaction between *G. rostochiensis* and *G. pallida* occurs and to what extent it may influence the population dynamics of each species and the damage to the plants. Because interaction might be influenced by the resistance quality of the plants, the experiment was carried out with fully susceptible and species specific resistant varieties.

## Materials and Methods

### *Hosts and parasites*

The nematode species *G. pallida* (designated as Pa3, Research Institute for Plant Protection) and *G. rostochiensis* (designated as Ro1, Research Institute for Plant Protection) were used. Cv. Elkana (ex andigena CPC 1673) and clone ZB35-29 (ex andigena CPC 2802, Plant Breeding Institute, Cambridge, United Kingdom) were used as the species specific resistant varieties, respectively resistant against *G. rostochiensis* and *G. pallida.* As a standard, the fully susceptible cv. Bintje was used. The plants were grown in small pots (diameter 10 cm, height 20 cm) in an artificial soil mixture (30% hydrocorn (2-4mm), 60% silver sand, 10% clay powder) and kept at a soil moisture level of 15% by weighing and watering every two or three days with an automatic water supply system. The amount of evaporation was determined simultaneously (Lock and den Nijs, in prep.).

To create the different species compositions and densities the appropriate aliquots of eggs and larvae suspensions were mixed which resulted in the settings reflected as follows: the total initial density (Pi(Pa+Ro) in eggs.$g^{-1}$ soil)/species composition (%Pa) 0.7/100, 5.8/100, 25.9/100, 0.7/98.8, 5.9/97.8, 25.1/98.5, 0.4/98.3, 5.3/88.9, 29.3/93.5, 0.7/86.8, 6.7/89.7, 27.3/75.3, 0.7/69.3, 7.4/61.3, 29.7/77.2, 0.8/60.3, 5.5/47.2, 26.3/38.6, 0.7/41.5, 7.2/21.1, 29.7/34.3, 0.8/0, 5.6/0, 30.0/0. Each of the 24 different suspensions of eggs and juveniles were inoculated in fifteen pots using a 20 cm long canula and a syringe. Cv. Bintje, cv. Elkana and clone ZB35-29 were planted on it in five replicates each. The 360 pots were placed on pallets in the glasshouse according to a Randomized Block Design . Growth conditions were 20 °C during daytime, 15°C during the night, 80% relative humidity. The experiment started in April 1987 and ended in July 1987.

*Observations*

The total water consumption per plant and thus the evaporation is related to the growth of the plant. Nematode attack reduces the water consuption (Seinhorst, 1981). The evaporation per plant was measured and used as an estimate for the amount of damage caused by the nematodes. Yield was estimated by determining fresh weight of the tubers.

Cysts were extracted from the soil by the Seinhorst-elutriator (Seinhorst, 1964), and the total final population density was determined by crushing the cysts and counting an aliquot of the suspension of eggs and juveniles (eggs.$g^{-1}$ soil). From the final population 500 cysts were processed for species specific protein extraction and determination. Proteins were separated by SDS-PAGE and species composition was estimated from the calibration curve of log(peak area quotient of Pa/Ro) vs. composition of Pa and Ro (den Nijs and Lock, 1990). From this species ratio, expressed as % of *G. pallida* and the joint final density, the final population density of *G. pallida* and *G. rostochiensis* could be separately calculated.

When one of the species was below detection level (between 0 and 10%), these data were excluded from the analysis.

### *Statistical analyses*

To analyse the influence of species composition and initial density of each species on species interaction the Relative Population Increase (RPI) of each species, defined by Pf/Pi, was used. The means of the five replicates were used for the analysis. The logarithmically transformed data (log RPI) were analysed by multiple regression analysis with initial density (Log(Pi) of Ro or Pa), species composition (%Pa or Ro) and variety as explanatory variables. For a better fit the model includes also linear and quadratic effects of initial density and %Pa, and the interactions of variety with initial density and %Pa.

Yield and evaporation of the plants were also analysed by multiple regression with initial density, species composition and variety as explanatory variables. The shift in composition of the mixed populations was analysed by Student's t-test.

## Results

### *Relative population increase.*

The results of the regression analysis are listed in table 1. Due to the resistance properties of cv Elkana the RPI of *G. rostochiensis* was zero on this variety, consequently the dataset for ANOVA is smaller for this species. The model accounted for 82.8% and 67.7% of the variance for the RPI of *G. pallida* and *G. rostochiensis*, respectively.

| | *G. rostochiensis* | | | *G. pallida* | | |
|---|---|---|---|---|---|---|
| change | df | MS | vr | df | MS | vr |
| species composition (% Pa) | 1 | 0.48410 | 13.39** | 1 | 0.06208 | 2.56 |
| initial density ($\log P_i$) | 1 | 1.14266 | 31.60** | 1 | 2.66665 | 110.03*** |
| $(\% \text{ Pa})^2$ | 1 | 0.38541 | 10.66** | 1 | 0.00030 | 0.01 |
| $(\log P_i)^2$ | 1 | 0.00127 | 0.04 | 1 | 0.52585 | 21.70** |
| variety | 1 | 0.02047 | 0.57 | 2 | 1.34634 | 55.55** |
| % Pa x variety | 1 | 0.39832 | 11.01** | 2 | 0.00474 | 0.20 |
| $\log P_i$ x variety | 1 | 0.08562 | 2.37 | 2 | 0.24481 | 10.10** |
| residual | 23 | 0.03617 | | 43 | 0.02423 | |

Significant at: * 0.05, ** 0.01 and *** 0.001. (F-test)

**Table 1: Analysis of variance of the relative population increase of *G. rostochiensis* and *G. pallida* on cv. Bintje, cv. Elkana and clone ZB35-29 after fitting to the multiple regression model $\log Y = \text{CONSTANT} + \%PA + \log Pi + \%PA^2 + \log Pi^2 + \text{VARIETY} + \%PA \times \text{VARIETY} + Pi \times \text{VARIETY}$.**

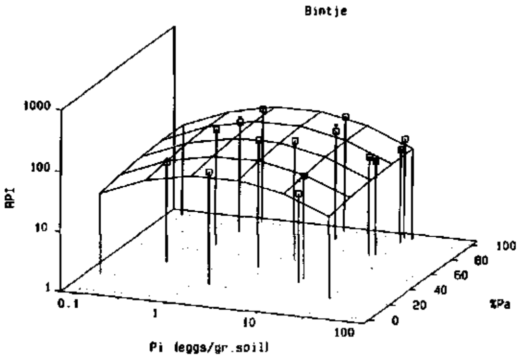
Varying the species composition did not significantly affect the RPI of *G. pallida*. Intraspecific interaction influenced the RPI of *G. pallida*, the effect of initial density (log(Pi) of Pa) was highly significant (Table 1). The levels and slopes of the RPI-curves differed for each variety. RPI was greatest on cv. Bintje, intermediate on cv. Elkana and least on clone ZB35-29, which was expected because of the resistance capacity of this clone. Figure 1 visualizes the model and the observed data.

| initial species composition: %Pa | total initial density (Pa+Ro egg/g.soil) | cv. Bintje |  | clone ZB35-29 |  |
|---|---|---|---|---|---|
|  |  | final % Pa | shift | final % Pa | shift |
| 98.8 | 0.7 | > 90[1] |  | > 90 |  |
| 98.5 | 25.1 | > 90 |  | > 90 |  |
| 97.8 | 5.9 | > 90 |  | > 90 |  |
| 97.3 | 0.5 | > 90 |  | > 90 |  |
| 93.5 | 29.3 | > 90 |  | > 90 |  |
| 89.7 | 6.7 | 95.7 (82.5-100)[2] | + | 87.4 (76.6-98.2) | - |
| 88.9 | 5.3 | > 90 |  | 62.8 (53.5-72.1) | -* |
| 86.8 | 0.7 | 91.3 (10.0-100) | + | 71.7 (67.5-75.9) | -* |
| 77.2 | 29.7 | 73.5 (65.3-81.7) | - | 36.6 (29.6-43.6) | -* |
| 75.3 | 27.3 | 93.9 (91.5-96.3) | +* | 68.5 (53.1-83.9) | - |
| 69.3 | 0.7 | 72.2 (61.8-82.6) | + | 54.0 (51.8-56.2) | -* |
| 61.3 | 7.4 | 69.7 (56.3-83.1) | + | 37.4 (32.9-41.9) | -* |
| 60.3 | 0.8 |  |  | 43.2 (38.6-47.8) | -* |
| 47.2 | 5.5 | 56.6 (50.3-62.9) | +* | 41.5 (31.2-51.8) | - |
| 41.5 | 0.7 | 24.6 (12.9-36.3) | - * | 17.3 (10.5-24.1) | -* |
| 38.6 | 26.3 | 39.6 (32.0-47.2) | + | 37.7 (18.2-57.2) | - |

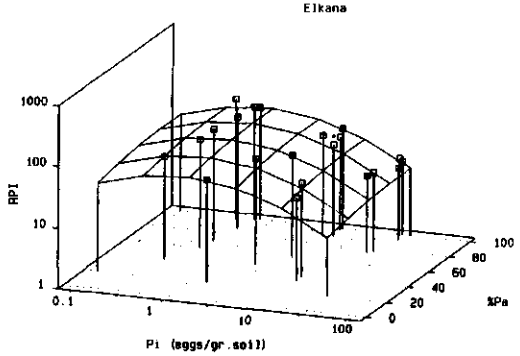
**Table 2: Shifts between the populations of *G. rostochiensis* and *G. pallida*, expressed as the percentage *G. pallida* in the mixed population on cv. Bintje, cv. Elkana and clone ZB35-29.**

The RPI of *G. rostochiensis* was progressively decreased by an increasing Pi and by an increasing proportion of *G. pallida* in the mixture. Significant effects were found for the initial density, species composition (%Ro), %Ro$^2$ and %Ro x variety (Table 1). Interspecific interaction markedly influenced the RPI of *G. rostochiensis* (Table 1 and Fig. 2). The factor variety is not significant, which means that the RPI for *G. rostochiensis* on both varieties is similar. However, the effect of *G. pallida* on the RPI of *G. rostochiensis* depends on the variety. This is ascribed to the resistance properties of the varieties used. The curvatures of the reproduction curves are similar but their slopes depend on the variety (fig.2 A + B).

**A**


Bintje
1000
100
10
1
RPI
0.1
1
10
100
Pi (eggs/gr.soil)
0
20
40
60
80
100
%Pa

**B**


Elkana
1000
100
10
1
RPI
0.1
1
10
100
Pi (eggs/gr.soil)
0
20
40
60
80
100
%Pa

**C**

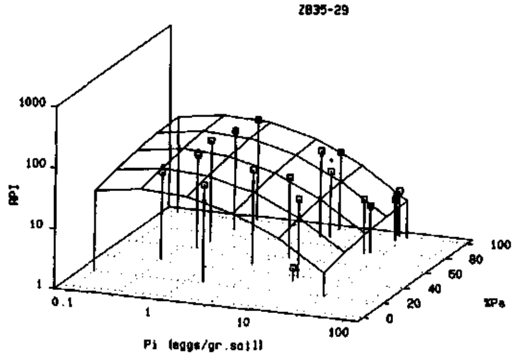

Z835-29
1000
100
10
1
RPI
0.1
1
10
100
Pi (eggs/gr.soil)
0
20
40
60
80
100
%Pa

**Fig. 1: Relationship between the initial density (log Pi) of *G. pallida* and the relative population increase (RPI) of *G. pallida* at various species compositions of *G. rostochiensis* and *G. pallida*, expressed as %Pa, on three varieties. Each point (the big squares) represents the mean of five replicates. The small squares show the intersections with the surface of the fitted model.**

**A) cv. Bintje:**

$\log RPI = 1.701 + 0.00547 \times \%Pa + 0.1567 \times \log(Pi) - 0.0000327 \times \%Pa^2 - 0.2991 \times \log(Pi)^2$,

**B) cv. Elkana:**

$\log RPI = 1.5930 + 0.0057 \times \%Pa - 0.0435 \times \log(Pi) - 0.0000327 \times \%Pa^2 - 0.2991 \times \log(Pi)^2$

**C) clone ZB35-29:**

$\log RPI = 1.287 - 0.0067 \times \%Pa - 0.2301 \times \log(Pi) - 0.0000327 \times \%Pa^2 - 0.2991 \times \log(Pi)^2$.

Table 2 shows the changes in the ratios of the two species; on cv. Bintje from 45% Pa upwards the proportion of *G. pallida* population tended to increase and subsequently the population of *G. rostochiensis* decreased, whereas below 45% Pa the proportion of *G. pallida* population tended to decrease and that of the *G. rostochiensis* population subsequently increased. This is in agreement with the reproduction results; when one species is numerically dominant the RPI of the other species, here *G. rostochiensis*, is reduced. On clone ZB35-29 the shift towards a decreasing *G. pallida* percentage is clear. This was expected because of its partial resistance to *G. pallida*.

*Evaporation and yield.*

The model used for RPI data was also fitted to the evaporation and yield data. Respectively 66.9% and 51.5% of the variance was accounted for by this model. As expected, the total initial density and the variety affected the evaporation and yield significantly (table 3). The species composition influenced the evaporation significantly, which indicates that not only the numbers of nematodes are important in the attack of the plants but also the species. However this effect was mainly observed in the potato clone ZB35-29. Here the amount of *G. rostochiensis* nematodes caused more damage, probably due to a lower tolerance to this species. The significant interaction of species-composition x variety on the yield confirms this (Table 3).

## Discussion

The results of the experiment show clearly that interaction between *G. rostochiensis* and *G. pallida* occurred which resulted in a significant decrease of the relative population increase (RPI) of *G. rostochiensis* when *G. pallida* was dominantly present. This effect was observed on cv. Bintje and clone ZB35-29, which are both susceptible to *G. rostochiensis.* The reciprocal effect of *G. rostochiensis* on the RPI of *G. pallida* was not significant. However, the species ratios in the experiment were not fully complementary for each species.

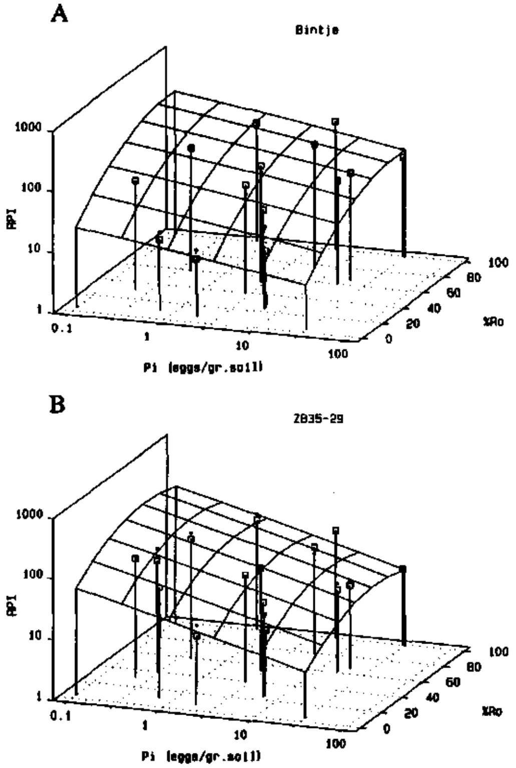


**Fig. 2: Relationship between the initial density (log Pi) of *G. rostochiensis* and the relative population increase of *G. rostochiensis* at various species composition of *G. rostochiensis* and *G. pallida*, expressed as % Ro, on cv. Bintje and clone ZB35-29, according to the model. Each point (the big squares) represents the mean of five replicates. The small squares show the intersections with the surface of the fitted model.**

**A) cv. Bintje :**

**$\log RPI = 1.007 + 0.02399 \times \%Ro - 0.2277 \times \log(Pi) - 0.0001279 \times \%Ro^2 - 0.0131 \times \log(Pi)^2$.**

**B) clone ZB35-29:**

**$\log RPI = 1.296 + 0.2344 \times \%Ro - 0.3907 \times \log(Pi) - 0.0001279 \times \%Ro^2 - 0.0131 \times \log(Pi)^2$.**

The influence of *G. pallida* on the RPI of *G. rostochiensis* on cv. Bintje and clone ZB35-29, as shown in figure 2, was found at all densities. This means that the interaction between the two species is not only competition for food or root space, as suggested by Jones and Kempton (1978) and Kort (1979). Other mechanisms of interaction must be present. It is possible that the lower RPI has been caused by the inefficient (not optimal) mating of the nematodes. In excess of other nematode species interspecific copulations may occur (Mugniery, 1979). If this happened frequently chances of intraspecific fertilization might have been reduced. In field situations species are aggregated due to their occurrence in cysts. When mixed juvenile suspensions are used instead of cysts, as in our experiment, interspecific encounters are more likely to occur. In experiments with two different densities Marshall (1989) found indications of competition effects between *G. rostochiensis* and *G. pallida*. *G. rostochiensis* multiplied at the expense of *G. pallida*, although in the extreme ratios *G. pallida* was able to maintain itself within the population. These effects were independent of density. However, one of these densities was extremely high which may have created intraspecific interactions resulting in a reduced multiplication. So the effects he described are difficult to interpret because of the presence of intra- and interspecific competition.

| | G. rostochiensis | | | *G. pallida* | | |
|---|---|---|---|---|---|---|
| change | df | MS | vr | df | MS | vr |
| species composition (% Pa) | 1 | 88147 | 6.35* | 1 | 0.00005508 | 0.61 |
| initial density (logP$_i$) | 1 | 488828 | 35.23** | 1 | 0.0384898 | 42.82* |
| (% Pa)$^2$ | 1 | 1 | 0.00** | 1 | 0.0007751 | 0.86 |
| (logP$_i$)$^2$ | 1 | 46064 | 3.32* | 1 | 0.0056372 | 6.27* |
| variety | 2 | 471160 | 33.95** | 2 | 0.0033661 | 3.74* |
| % Pa x variety | 2 | 73322 | 5.28** | 2 | 0.0074493 | 8.29* |
| logP$_i$ x variety | 2 | 209485 | 15.10** | 2 | 0.0029105 | 3.24* |
| residual | 61 | 13877 | | 61 | 0.0008989 | |

Significant at: * 0.05 and ** 0.01 (F-test)

**Table 3: Analysis of variance of the evaporation and yield data of cv. Bintje, cv. Elkana and clone ZB35-29, inoculated simultaneous with *G. pallida* and *G. rostochiensis* and in single species inoculations.**

Jones and Perry (1978) found a characteristic delay in the increase of the *G. pallida* population in presence of the *G. rostochiensis* population. They suggested after analysis of the data with their model, that large densities of *G. rostochiensis* suppressed the reproduction of small populations of *G. pallida* until the *G. rostochiensis* population dropped below a certain level. However, since no density estimates for the separate species were available no clear evidence for competition was presented. From our experiment it is obvious that interspecific interaction occurs, but only the negative influence of *G. pallida* on the RPI of *G. rostochiensis* was found to be statistmcallysignificant. Considering the absence of some experimental settings, the tendency of the negative influence of *G. rostochiensis* on the RPI of

*G. pallida* observed and the results of Jones and Perry (1978), the effects of interspecific interaction may be mediated by each of both species, depending on dominancy.

Shifts between the populations we found are in accordance with the findings of Stelter (1983), Kort and Jaspers (1973) and Marshall (1989). The expectation that the reproductive capacity of the nematodes on the plant varieties used is a major factor for population changes was confirmed.

When a rotation scheme is developed with different kinds of resistant potato varieties and with the purpose of manipulating the species populations it is essential that both species are, at least in small amounts, present in the soil. According to the findings of Mulder (pers. comm.) in The Netherlands this will be the case, as he finds *G. rostochiensis* as soon as *G. rostochiensis* susceptible varieties are grown, after years of *G. pallida* infestation on *G. rostochiensis* resistant varieties. Marshall (1989) found that ratios of 1% Pa - 99% Ro and 5% Pa - 95% Ro did not change after one crop of *G. rostochiensis* susceptible potatoes. Stelter (1983) found that after 10 years the species ratio 98% Ro vs. 2% Pa remained the same on a *G. rostochiensis* susceptible potato variety. In our experiment the complementary ratios were present. However, by limitations of the method we were not able to determine if and in what amount *G. rostochiensis* stayed in the population in the mixtures of 95% Pa - 5% Ro and 99% Pa - 1% Ro. More information is needed for long term effects and a better detection method for species present below 10% should be developed.

Jones and Kempton (1978) suggested as one possible effect of the presence of mixed populations that the joint maximum population density and hence the crop damage in mixed populations might be smaller than when each species occurs separately. In our experiment yield was not significantly affected by species composition but the effect of species composition on evaporation was significant. The experiment was carried out in small pots. Due to the limited space the roots might have been attacked by all nematodes present after which recovery of growth rate could take place (Seinhorst, 1982). This might obscured the response of yield to the total initial density and species composition in comparison to the evaporation. The significant effect of the interaction species composition x variety can be ascribed to the tolerance property of these varieties.

Finally, the experiment was carried out under controlled conditions, which ensured synchronous development. In the field the environmental conditions will fluctuate which may have a differential effect on the species. Cysts instead of separate nematodes infest the soil, which ensures a certain amount of aggregation resulting in more intraspecific than interspecific interactions. Furthermore the resistance property of the plants grown will have a strong influence on the population dynamics of the species. For the population dynamics of the species in the field, these considerations imply that, the populations of each species may largely develop independently. Whether the interspecific interactions in extreme situations, which were clearly demonstrated in our experiment, occur in field situations and whether they are strong enough to influence the population dynamics of the species in the field, needs further investigation in both field experiments and simulation modelling.

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# Differential hatching of the potato cyst nematodes *Globodera rostochiensis* and *G. pallida* in root diffusates and water of differing ionic composition.

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## Abstract

The hatching differences of *Globodera rostochiensis* and *G. pallida* were assessed in potato root diffusate (PRD) of cv . Bintje, cv. Elkana and clone ZB35-29. *G. pallida* hatched better in the PRDs than *G. rostochiensis*. It was shown that the experimental test conditions strongly influenced the hatching results. The water type used in the hatching tests had a significant discriminating effect on the species; *G. rostochiensis* reached relative hatch percentages of 60 to 90% in demineralized and tap water, whereas *G. pallida* never exceeded the 15%. These differences were independent of the various batches that were used or the different years the tests were carried out. Silver sand percolate had a inhibiting effect on the hatching of both nematode species. Boron and a high electrical conductivity may be responsible for this. The results are discussed from an ecological point of view as well as for research consequences.

*Additional keywords*: ecological species differences, diapause, potato root diffusate, test conditions.

## Introduction

In plant-nematode interrelationships the hatching process is critical (Perry, 1987). In the framework of research on interactions between the species of potato cyst nematodes, the question arose whether hatching differences play an important role. In mixed populations of *G. rostochiensis* and *G. pallida* interspecific interactions take place resulting in a relatively lower population increase of the minority species (Den Nijs, 1992). It is possible that differences in hatching between the nematode species may influence interspecific interaction.

Most nematodes possess the ability to overcome adverse environmental conditions by arresting their development and becoming dormant. For cyst nematodes, especially potato cyst nematodes, it is not clear whether quiescence or diapause is the adequate term to describe this state of rest of the eggs inside the cyst (Antoniou, 1989; Evans and Perry, 1976; Evans, 1987), although Hominick *et al.* (1985) and Hominick (1986) concluded that diapause was present in *G. rostochiensis* cysts. To activate the nematode juveniles inside the eggs, a hatching agent present in potato root diffusate (PRD) is thought to be necessary. As a result the nematodes hatch from the eggs and penetrate the roots of the host. In the life cycle of potato cyst nematodes the ability to survive in a dormant state, the breaking of this dormancy and the subsequent hatching process are interrelated. They synchronize the development of the nematode with that of its host plant, the potato (Perry, 1989).

Much research has been carried out to unravel the process of hatching, especially for the nematode species *G. rostochiensis*. Clarke and Perry (1977) gave an extensive review of the factors which might influence this process of hatching of cyst-nematodes. Perry and Clarke (1981) and Perry (1986, 1987, 1989) reviewed the mechanisms involved, especially the responses of the juvenile, between the onset of hatching and subsequent eclosion. Since *G. pallida* and *G. rostochiensis* are considered sibling species (Sturhan, 1985) one would expect the process of hatching to be similar. However, the rates of hatching and the reaction of both potato cyst nematode species to PRD of different potato cultivars and to temperature are different (Evans, 1983: Robinson *et al.*, 1987), and suggest essential differences in the ecology of the species.

In experiments to compare hatching activity of *Globodera* species in relation to hatching stimuli, knowledge of the impact of other potential stimulating factors in the experiment is required. To exclude differences in the physiological stages of the nematodes, Hominick *et al.* (1985) suggested that only mature cysts of *G. rostochiensis* of the same age and reared and stored under well defined conditions should be used. Apart from these intrinsic factors, the reaction of the nematodes towards external factors among the test conditions might differ and interfere with the hatching process.

Hatching tests were carried out for the two potato cyst nematode species with the emphasis on experimental test conditions. During the PRD-hatching test it appeared that the nematodes reacted very strongly to the control solution (silver sand percolate, SSP). The water type used also had a strong influence on the hatching results. Clarke and Shepherd (1966) and Clarke and Hennessy (1987) reported the influence of inorganic compounds on the hatching behaviour of cyst nematodes and Clarke *et al.* (1978) showed that osmotic stress can inhibit the hatching of nematodes from the eggs. Therefore apart from the hatching behaviour in PRD, analyses were made of the hatching behaviour of the nematode species in the control solutions and in the different water types used in the experiments. The mineral compositions of the water types were also examined.

## Materials and methods

Vintage cysts of *G. rostochiensis* (Ro1) and *G. pallida* (Pa3), reared on the susceptible potato cv. Bintje in the glasshouse and stored at 4°C for three, four or five years, were used for the experiments. Batches of these cysts were used simultaneously (Table 1).

| | Test 1 | Test 2 | Test 3 |
|---|---|---|---|
| *Nematodes batches:* | | | |
| Ro1-85 | + | + | - |
| Ro1-86 | - | + | + |
| Ro1-87 | - | - | + |
| Pa3-85 | + | + | - |
| Pa3-86 | - | + | + |
| Pa3-87 | - | - | + |
| *Hatching agents:* | | | |
| PRD of cv. Bintje, Elkana, ZB35-29 | | | |
| undiluted | + | - | - |
| diluted with TW | | | |
| 1/4, 1/16, 1/64, 1/256 | + | - | - |
| SHA | | | |
| undiluted | + | + | + |
| diluted with DW | | | |
| 1/4, 1/16, 1/64, 1/256 | + | - | - |
| SSP | | | |
| undiluted | + | + | + |
| diluted with TW | | | |
| 1/4, 1/64 | + | - | - |
| 1/16, 1/256 | + | - | + |
| TW | + | + | + |
| DW | - | + | + |
| TW:DW in ratios | | | |
| 1:255, 1:15, 15:1, 255:1 | - | + | - |

**Table 1: Experimental set-up for the 3 hatching tests. Test 1 was carried out from 31 March until 10 Aug. 1988, test 2 from 28 Februari until 1 May 1990 and the last test from 1 May until 31 July 1990. The abreviations used in the table have the following meaning: PRD= potato root diffusate, SHA= standard hatching agent, SSP= silver sand percolate, DW= demineralized water, TW= tap water.**

For each hatching test the cysts were soaked for one week in tap water and egg suspensions were made. The eggs were then exposed to the various hatching agents.

Hatching tests were performed, essentially according to the method of Den Ouden (1963), by placing a thousand eggs on a 10 μm sieve in a small glass tube with a concave bottom (ϕ 17 mm, height 2.5 cm) in 1.5 ml hatching agent, incubated at 20°C in the dark, with 5 replicates per treatment. Each hatching agent was refreshed 10 times during the experiment, at gradually longer intervals and the hatched juveniles were counted. The accumulated number of hatched juveniles was determined.

For each experiment, the hatching activity of the batches of cysts were determined by assessing the hatch in Standard Hatching Agent (SHA: 15 mg partially purified hatching agent (Janzen and van der Tuin, 1956) + 50 mg streptomycine per liter demineralized water). The highest accumulated number of hatched juveniles in the SHA was assumed to be the maximum hatch of a batch and was set at 100% (Table 2). For each experiment the hatching data were related to the maximum hatch of the batch used in that experiment (Table 1), e.g. maximum hatch of a batch of Pa3 in the undiluted solution of SHA is A juveniles; this is set at 100%. In demineralized water (DW) B juveniles have hatched, which corresponds with (B/A)*100% = X percentage relative hatch in DW. For the hatching data of hatching test 1 the calculations were adjusted with the hatching data of the control solution of the corresponding dilutions, e.g. maximum hatch for Pa3 was A larvae=100%, accumulated hatch in 1/16 diluted SSP (=control) was C hatched larvae (mean of 5 replicates), accumulated hatch in 1/16 diluted PRD of cv. Bintje was B juveniles. The percentage relative hatch in 1/16 diluted PRD was (B-C)/A*100%.

| | accumulated hatched juveniles in SHA | | |
|---|---|---|---|
| Batches | Test 1<br>1988 | Test 2<br>1990 I | Test 3<br>1990 II |
| Ro1-85 | 570 (62.6) | 243 (15.5) | - |
| Ro1-86 | - | 723 (26.9) | 729 (19.3) |
| Ro1-87 | - | - | 797 (53.1) |
| Pa3-85 | 619 (22.5) | 545 (25.0) | - |
| Pa3-86 | - | 561 (26.9) | 311 (29.5) |

**Table 2: Vitality of different batches of *G. pallida* and *G. rostochiensis*, based on the maximum hatch of 1000 eggs in standard hatching agent (SHA). Mean of 5 replicates with standard deviation in brackets.**

*Hatching test 1*

Hatching activity of *G. rostochiensis* and *G. pallida* in the presence of PRD of cv. Bintje, cv. Elkana and clone ZB35-29.

PRD was collected from cv. Bintje, susceptible for both nematode species, cv. Elkana, resistant against *G. rostochiensis* and clone ZB35-29, partial resistant against *G. pallida*. Tuber pieces were placed in small pots (7x7x5cm) in silver sand. For each cultivar or clone 350 pots were used, for the control 250 pots were used. All pots were watered regularly, so that soil moisture level never exceeded the field capacity. After two months the roots had grown throughout the soil and the pots were percolated with tap water. From each pot only the first 2.5 ml of percolate was collected. Percolate collected from pots with silver sand but without plants was used as control solution in the experiment with PRDs (Table 1). All percolates were filtered, streptomycine was added ($0.05\ g.l^{-1}$) and stored at 4°C in the dark.

Three year old cysts of Rol and Pa3 (Rol-85, Pa3-85) were used for this experiment. The hatching agents were the PRDs of cv. Bintje, cv. Elkana and clone ZB35-29, SHA undiluted and diluted 4, 16, 64 and 256 times with tap water and tap water alone (TW). As control SSP was taken with the same dilutions as the PRDs (Table 1). The hatching test was carried out from 31 May until 10 August 1988.

*Hatching test 2*

Hatching activity of *G. rostochiensis* and *G. pallida* in tap water and demineralized water.

Four and five year old cysts of Rol (Rol-85, Rol-86) and Pa3 (Pa3-85, Pa3-86) were subjected to the following treatments: SHA, TW, DW and mixtures of TW and DW with ratios of 1:255, 1:15, 15:1 and 255:1 (TW:DW) respectively (Table 1). The test was carried out from 28 February until 1 May 1990.

*Hatching test 3*

Hatching activity of *G. rostochiensis* and *G. pallida* in silver sand percolate.

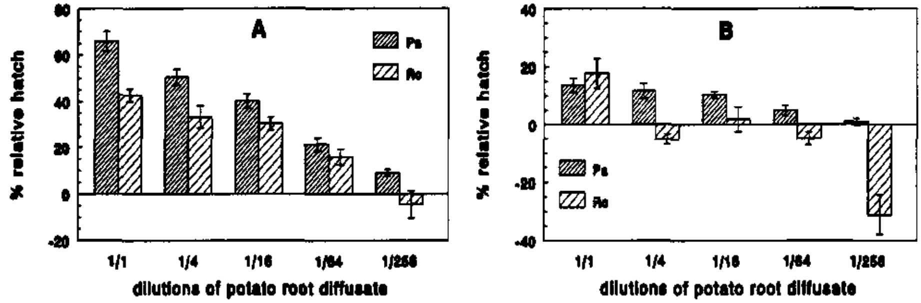
Undiluted and diluted (1/16 and 1/256) SSP, SHA, TW and DW were used for hatching tests. Three and four year old cysts of Rol (Rol-86 and Rol-87) and Pa3 (Pa3-86 and Pa3-87) were used in the test, which was carried out from 1 May until 31 July 1990 (Table 1).

*Mineral analysis*

The control, TW and DW were analysed for macro- and micro-elements using standard procedures of the Laboratory for Soil and Crop Testing, Naaldwijk, The Netherlands.

*Statistical analysis*

The total amounts of hatched nematodes in the different hatching agents were expressed as percentages of the maximum hatch per batch. In this way vitality differences of the batches are excluded and results of different batches are comparable. Statistical analysis (ANOVA) was applied on the results per species.



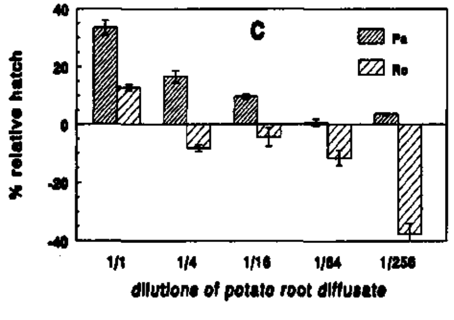


**Figure 1: The relative hatch of *Globodera rostochiensis* (Ro1-85) and *G. pallida* (Pa3-85) in undiluted and diluted potato root diffusates (PRDs). Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and corrected for the control (= silver sand percolate) and are means of 5 replicates. The bars indicate the standard error of means.**

**A: cv. Bintje, B: cv. Elkana, and C: clone ZB35-29.**

## Results

*Hatching test 1*

The differential hatching effect of PRDs on *G. pallida* and *G. rostochiensis* are shown in figure 1A, 1B and 1C. It is clear that hatching of both nematode species decreased when diffusates were diluted. A striking effect however is the seemingly negative hatch of *G. rostochiensis* (figure 1B and 1C). This is caused by the better hatch of this nematode species in the control solution than in the root diffusate solution.

Table 3 shows the hatching data of *G. rostochiensis* and *G. pallida* in the control solution, SSP. Dilution of this percolate with TW increased the hatch of both species significantly. However, the level of hatch which both nematode species reached in the solution diluted 256 times with TW differed strongly, with 11.8% and 62.2% hatch for *G. pallida* and *G. rostochiensis* respectively. These differences in the behaviour of both species were further investigated in hatching test 2 and 3.

| dilution | Pa-85 | Ro-85 |
|---|---|---|
| 1/1 | 6.91 | 6.70 |
| 1/4 | 8.24 | 22.56 |
| 1/16 | 9.31 | 22.67 |
| 1/64 | 14.70 | 32.91 |
| 1/256 | 11.76 | 62.21 |

maximum hatch in SHA:
Pa: 619 lv. = 100%; Ro: 570 lv. = 100%

**Table 3: The relative hatch percentages of *G. rostochiensis* and *G. pallida* in different dilutions of silver sand percolate. (data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates).**

*Hatching test 2*

The hatching behaviour of the two species in TW and DW were considerably different (Table 4). For *G. pallida* the average hatching percentages in pure TW and DW never exceeded 10%, whereas the percentages of hatch for *G. rostochiensis* were always above 45%. The percentage of TW in the water mixtures was used as a factor in the ANOVA.

No significant differences were found between the two batches of each species in their reaction to TW and DW (P=0.580 for *G. rostochiensis* and P=0.198 for *G. pallida*). Further analyses were made on the combined data of the batches (Ro1-85 + Ro1-86 and Pa3-85 + Pa3-86 resp.). The percentage of TW in mixed solutions influenced the hatching rates of both nematode species significantly, mainly due to the relatively low hatch in pure DW (Table 4).

*Hatching test 3*

The influence of SSP on the hatching pattern of both nematode species was examined by using the data of this experiment with the hatching data obtained from the control solution of experiment 1 (=SSP, undiluted, 1/16 and 1/256 times diluted). Analysis of variance on these combined data revealed that the three batches of *G. rostochiensis* did not differ significantly (P=0.967) in their reaction to the SSP.

| % tap water | Pa<br>% hatch | Ro<br>% hatch |
|---|---|---|
| 0.0(DW) | 6.70 | 45.9 |
| 0.4(TW:DW=1:255) | 7.76 | 75.1 |
| 6.3(TW:DW=1:15) | 9.49 | 77.1 |
| 93.8(TW:DW=15:1) | 14.14 | 84.7 |
| 99.6(TW:DW=255:1) | 14.05 | 77.4 |
| 100.0(TW) | 9.14 | 79.0 |
| LSD α=0.05,df=48 | 2.51 | 32.07 |

**Table 4: The hatching reaction of *G. pallida* and *G. rostochiensis* to an increasing percentage of tap water (TW) in a mixture with demineralized water (DW). Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are the mean of 10 replicates, two batches of each species.**

| dilution | batches of *G. pallida* | | | batches of *G. rostochiensis* | | |
|---|---|---|---|---|---|---|
| | Pa85 | Pa86 | Pa87 | Ro85 | Ro86 | Ro87 |
| 1/1 | 6.92 | 7.73 | 7.51 | 6.7 | 7.9 | 8.7 |
| 1/16 | 9.32 | 14.04 | 11.47 | 22.7 | 36.1 | 19.8 |
| 1/256 | 11.78 | 14.49 | 14.39 | 62.2 | 48.8 | 67.3 |
| LSD α = 0.05,df = 36 | | 2.98 | | | 19.61 | |

**Table 5: The relative hatch percentages of three batches of *G. pallida* and *G. rostochiensis* in undiluted and diluted silver sand percolate (data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates).**

| | Batches of *G. pallida* | | Batches of *G. rostochiensis* | |
|---|---|---|---|---|
| | Pa86 | Pa87 | Ro86 | Ro87 |
| DW | 15.97 | 15.51 | 77.0 | 35.6 |
| TW | 13.42 | 14.07 | 9.7 | 54.9 |
| LSD α = 0.05, df = 32 | 3.42 | | 11.30 | |

**Table 6: The relative hatching rates of two batches of *G. pallida* and *G. rostochiensis* in tap water (TW) and demineralized water (DW) (data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates).**

The percentage hatch increased significantly (P<0.001) for the three batches of *G. rostochiensis* when the SSP was diluted with an increasing amount of TW. For the three batches of *G. pallida* it was also found that dilution of the SSP increased the hatching significantly (P<0.001), but the levels of hatch were different for each batch (Table 5). Both species also reacted differently to inhibition of hatch by the undiluted SSP; hatching of *G. pallida* was approximately halved, whereas the hatch of *G. rostochiensis* in the undiluted SSP was approximately one seventh of that in the most dilute SSP (Tables 3 and 5).

Both species reached their maximum hatch in the greatest dilution of SSP (1/256). This solution is almost comparable to TW. The hatching rates of both species in pure TW were not significantly different from the hatching rates in the SSP diluted 256 times (P=0.356 for *G. pallida*, P=0.155 for *G. rostochiensis*).

The hatching reactions of the two batches of *G. pallida* to TW and DW were not significantly different (P=0.102). The mean hatching rate was 14.7%. The water type did not cause a significant difference in the hatching of *G. rostochiensis* (P=0.139), but an interaction of batch with water type was present (P=0.002). Table 6 shows the mean values of the hatching rates of the batches in both water types.

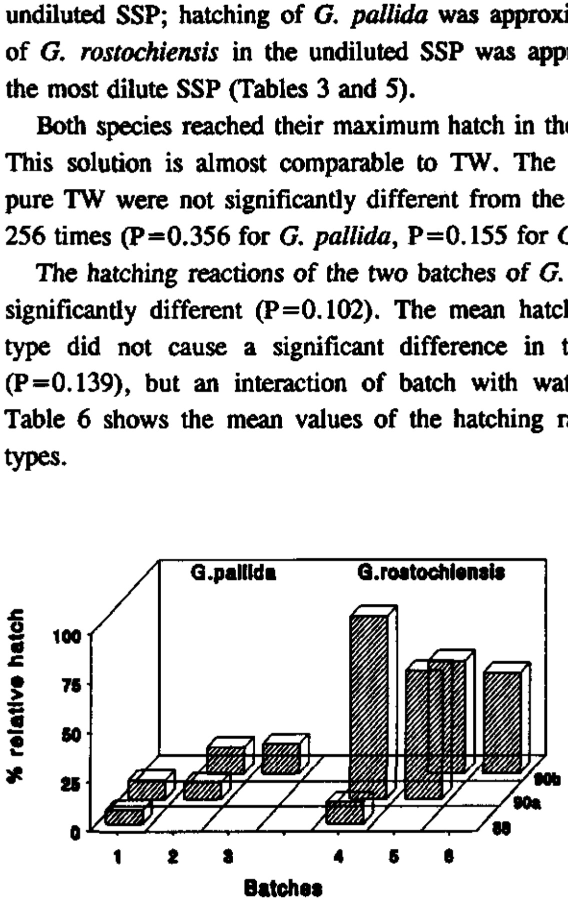


**Figure 2: The relative hatch of *Globodera rostochiensis* and *G. pallida* in tap water (TW). Various batches were used in different years. Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates. Batch 1, 2, 3, 4, 5 and 6 represent *G. pallida* Pa3-85, Pa3-86, Pa3-87 and *G. rostochiensis* Ro1-85, Ro1-86 and Ro1-87 respectively.**

*Effects of water and its ionic composition*

In all three hatching tests, TW was used so the spontaneous hatch in water could be determined. Figure 2 shows the results of this spontaneous hatch of *G. pallida* and *G. rostochiensis* in TW for the different batches used in the tests over the years.

Figure 3 shows the hatching results of both species in DW (hatching tests 2 and 3). The hatch of *G. pallida* remained low for all batches, independent of the date the tests were carried out or the water type and showed little variation. In contrast, the spontaneous hatch of all batches of *G. rostochiensis* was high in both water types and the high levels of hatch, which were more variable than those of *G. pallida*, were reached independent of the date the tests were carried out.

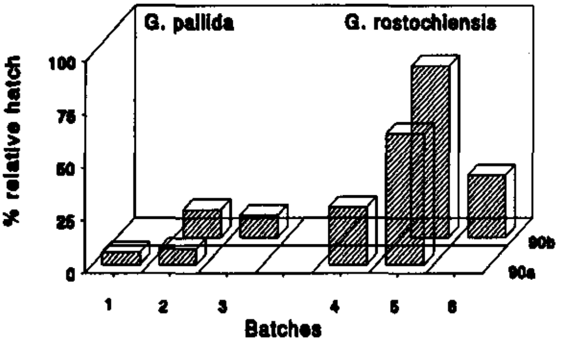


**Figure 3: The relative hatch of *Globodera rostochiensis* and *G. pallida* in demineralized water (DW) in two hatching tests. Various batches were used. Data are expressed as percentages of the maximum hatch per batch in standard hatching agent (SHA) and are means of 5 replicates.**
**Batch 1, 2, 3, 4, 5 and 6 represent *G. pallida* Pa3-85, Pa3-86, Pa3-87 and *G. rostochiensis* Ro1-85, Ro1-86 and Ro1-87 respectively.**

Chemical analysis of the different water types showed strong differences in ion concentrations (Table 7) resulting in different electrical conductivities. The high electrical conductivity of the SSP was partly caused by the high concentration of Na, Ca and Cl. The concentration of Boron in the SSP was also high.

## Discussion

Hatching tests are at present the most reliable method to determine the viability of cyst contents after soil fumigation, or to assess the resistance properties of cultivars. Standard procedures are carried out with emphasis on reproducibility by taking enough replicates, but without taking into account the history of the cysts, the presence or absence of diapause or species differences that might exist and interfere in the hatching results. Hominick *et al.* (1985) for example, showed that environmental conditions during the development of females of *G. rostochiensis* on potato roots influenced the hatching characteristics of the juveniles. In our hatching tests batches of cysts of one population of each species were used, grown and stored under well defined conditions. Because of our interest in the differences in the hatching reaction of the species towards PRD, only the number of hatchable juveniles was investigated. By determining the maximum accumulated hatch in a SHA and expressing the amount of hatch in the unknown hatching agent as a percentage of this maximum hatch we were able to make comparisons between batches (Table 4, 5 and 6) and simultaneously reduced the variability of the hatching trials (Hominick *et al.*, 1985). By assuming that the maximum hatch was reached in the SHA for both species, and all data were related to these numbers, it was possible to express the influence of hatching agents on both species as a relative decrease of hatching. Therefore the changes in hatching pattern of the species may be compared.

The hatching of the nematode species in the PRDs was influenced by the presence of the SSP in the PRDs. The SSP reduced hatching for both species to about 7-8%. Dilution of this SSP with TW increased the hatching of both species significantly, with a final hatching level in the 256 times diluted solution of approximately 13% and 60% for *G. pallida* and *G. rostochiensis*, respectively. In the undiluted PRDs the differential effect of TW on the hatching of the nematode species did not play a role; only the inhibition by the SSP influenced the hatching. The results of the PRDs were adjusted with these control data and related to the hatch in SHA. They show that *G. pallida* hatches better than *G. rostochiensis* in the PRDs (figure 1A, B and C). The non-transformed data of the hatch in undiluted PRDs showed a similar pattern (e.g. for cv. Bintje: 450 juvenile of *G. pallida* and 281 juveniles of *G. rostochiensis* hatched). Apparently PRD is the major factor to induce hatch of *G. pallida*, whereas for *G. rostochiensis* other factors influence the hatching as well.

In experiments on the interaction between the two potato cyst nematodes described by Den Nijs (1992), plants were grown in a soil mixture which contained 60% silver sand. Reproduction was normal indicating that hatching was not influenced by the presence of this silver sand.

Clarke and Shepherd (1966) found that Boron inhibits hatch of *Heterodera avenae*, *H. glycines* and *H. trifolii*. The inhibition by silver sand found in the present work might, among other things, have been caused by the high concentration of this element.

Clarke *et al.* (1978) demonstrated that osmotic stress inhibits the hatch of *G. rostochiensis*. When the osmotic value of the surrounding environment is equivalent or greater than that of the egg contents, water uptake, essential for hatch, stops (Perry and Clarke, 1981). The SSP has an osmotic pressure of 0.24 atmosphere, whereas the osmotic pressure inside eggs is 9.78 atmosphere, namely equivalent to

0.4M sucrose solution (Clarke *et al.*, 1978). So the inhibition of hatching was not caused by an osmotic stress of the solution.

The reaction pattern of the nematode species in both water types differs considerably. Consistently over batches and years few *G. pallida* hatched, whereas *G. rostochiensis* reached high levels of hatching. Water hatch or spontaneous hatch has been used to determine the minimum hatchability of cysts. Most information exists on *G. rostochiensis*: it usually reaches values of 10% hatch (Evans, 1982, 1983; Fenwick, 1952; Shepherd, 1962). Mulder and Vroom-Wolf (1990) found high percentages of hatch in tap water, ranging from 5 to 60%, dependent on the time of the year, in experiments on periodicity with vintage cysts of one population of *G. rostochiensis*. We found in all hatching tests relative hatching rates varying from 60 to 90%. This corresponds with 23 to 47% hatch when expressed as percentages of the total amount of eggs applied. So spontaneous hatch of *G. rostochiensis* in TW was consistently high and independent of the period when hatching tests were performed.

*G. pallida* and *G. rostochiensis* share the same habitat but it appears that they have different ways to ensure that the roots are reached and the population is maintained. By reacting mainly to PRD, *G. pallida* synchronizes its life cycle completely with its host. Robinson *et al.* (1987) found that *G. pallida* had a much slower initial rate of hatch and utilized its lipid reserves more slowly than *G. rostochiensis*. This prolonged hatch and persistence may ensure that root growth is less affected, thus reducing intraspecific competition. From an ecological point of view this seems to be a good strategy.

*G. rostochiensis* shows a more opportunistic behaviour, by reacting to non-specific hatching triggers. Combined with the faster hatch of this species it might diminish interspecific interaction in predictable situations. However, the juveniles ought to posess the ability to survive a certain period in the soil when the host is not present. Indeed Den Ouden (1960) found that *G. rostochiensis* could survive 7 weeks in the soil and Mulder *et al.* (1988) found that *G. rostochiensis* survived longer in soil than *G. pallida*, under the same conditions. Insight in the ecological conditions in the original habitat in Peru, might bring more understanding on this behaviour.

The species specific differences in hatching behaviour have considerable implications for hatching tests. Results from hatching tests obtained from one species may not be relevant to the other species. It seems advisable to choose the test conditions very carefully in order to avoid artefacts which may obscure the effects of interest.

In the hatching tests presented here, egg suspensions instead of cysts were used to avoid a major source of variation. In the field the nematode eggs are protected inside cysts, other factors may therefore influence hatching as well. However, when the high spontaneous hatch of *G. rostochiensis*, we found in water, also takes place in the soil, it can lead to the natural break down of the nematode population of more than 30%, a figure which is generally accepted as being normal. Andersson (1988) and Den Ouden (1960) have found higher rates of 50 to 60%. For *G. pallida*, data on natural break down of populations in the field are not available at the moment.

| Elements | | TW | DW | SSP |
|---|---|---|---|---|
| NH4 | (mmol.$l^{-1}$) | 0.1 | 0.1 | 0.1 |
| K | (mmol.$l^{-1}$) | 0.1 | 0.1 | 0.3 |
| Na | (mmol.$l^{-1}$) | 0.5 | 0.2 | 2.9 |
| Ca | (mmol.$l^{-1}$) | 0.6 | 0.1 | 1.2 |
| Mg | (mmol.$l^{-1}$) | 0.2 | 0.1 | 0.3 |
| NO3 | (mmol.$l^{-1}$) | 0.2 | 0.1 | 0.4 |
| Cl | (mmol.$l^{-1}$) | 0.3 | 0.2 | 2.6 |
| SO4 | (mmol.$l^{-1}$) | 0.2 | 0.1 | 0.7 |
| HCO3 | (mmol.$l^{-1}$) | 1.3 | 0.2 | 1.6 |
| P | (mmol.$l^{-1}$) | 0.01 | 0.01 | 0.01 |
| Fe | ($\mu$mol.$l^{-1}$) | < 0.2 | < 0.2 | < 0.2 |
| Mn | ($\mu$mol.$l^{-1}$) | < 0.2 | < 0.2 | 0.3 |
| Zn | ($\mu$mol.$l^{-1}$) | 0.6 | < 0.2 | 0.5 |
| B | ($\mu$mol.$l^{-1}$) | < 5.0 | < 5.0 | 9.0 |
| Cu | ($\mu$mol.$l^{-1}$) | 1.0 | < 0.5 | 0.6 |
| pH | | 7.9 | 7.4 | 7.4 |
| EC | (mS.$m^{-1}$) | 20 | 10 | 70 |

**Table 7: The results of the chemical analysis on micro- and macro elements in tap water (TW), demineralized water (DW) and silver sand percolate (SSP).**

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

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## Abstract

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 four 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.

## Introduction

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 and Kempton, 1978; Kort and 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 retricted 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 succesful 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* (Franco, 1979; Mugniéry, 1978), the ecological differences might be greater than generally assumed.

Jones and Kempton (1978) used a simulation model of the population dynamics of the potato cyst nematodes 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. 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 the nematode species *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 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 (2-4mm), 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.

| | 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 |

**Figure 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.**

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 electrophoresis according to the method of den Nijs and Lock (1990). 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 Students'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.

| | Pa | Ro | $M_i$-F1 | $M_i$-F2 | P values |
|---|---|---|---|---|---|
| 1985 | 538 | 855 | 676 | | |
| 1988 | 590 | 469 | 530 | 805 | < 0.0001 |
| 1989 | 637 | 647 | 692 | 669 | 0.7019 |
| 1990 | 423 | 376 | 500 | 422 | 0.2152 |
| average over 1988-1990 | | | 574 | 632 | 0.1666 |

**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 ($\alpha=0.05$) was applied on the number of $M_i$-F1 cysts versus the number of $M_i$-F2 cysts, ANOVA ($\alpha=0.05$) was applied on all data of the $M_i$-F1 and $M_i$-F2 over the years 1988, 1989 and 1990.**

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 ofnumber 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.

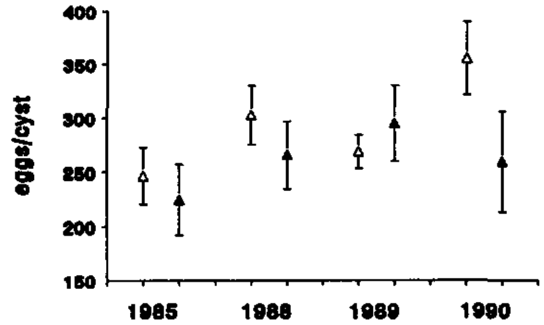

**Figure 2: Mean and confidence intervals ($\alpha=0.05$) of the cyst contents of the $M_i$-F1 cysts (▲) in comparison to the mean cyst contents of the single species populations of *G. rostochiensis* and *G. pallida* (△). Data represent the mean of 5 replicates.**

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.

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

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

**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.**

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 reproducion compared to the singe 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 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.

| generation | 1985-1990 | 1988-1990 | 1989-1990 | 1990 |
|---|---|---|---|---|
| $M_t$ | 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 $\alpha=0.05$.

**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.**

## Discussion

The purpose of the experiments described here was to establish whether intercrossing between the two potato cyst nematodes might take place in the controled 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 Mugniéry (1979), and Franco and Evans (1978) 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 wil 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 and 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 (in press) 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 a, 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.

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## General discussion

### Interactions between the two species

Interactions between species can take place in various ways. In this thesis the term interaction refers to competition, i.e. mutually detrimental exploitation of the same ecological resources. Distinction is made between interference competition (by direct interaction) and exploitation competition (by indirect inhibiting effects) *sensu* Pianka (1978).

When interactions between sibling species are studied it is essential to distinguish these species adequately to make sure that the effects can be ascribed to the right species. In the case of *G. rostochiensis* and *G. pallida*, which look extremely similar, methods based on morphological characters are not suitable (Stone,1975). Biochemical techniques are more useful because of the unambiguous establishment of intrinsic characters. An adapted one-dimensional gel electropheresis of species-specific thermostable proteins was shown to be a valuable method for species identification and quantification (chapter 2).

To create the best opportunity for possible interactions it was essential to optimize the synchronous development of both species and host. By using suspensions of eggs and juveniles of both species as inoculum instead of cysts, it was possible to create similar inoculation conditions such as equal total initial densities, time of inoculation and distribution in the soil. For each species, cysts of the same population, reared and stored under equal conditions, were used.

Competition for food or feeding sites influences the reproduction. According to Jones and Perry (1978) intraspecific competition is the main density-dependent population regulatory mechanism of cyst-nematodes. To make a distinction between intraspecific competition and interspecific competition experiments were conducted with a range of initial densities of both species applied separately and in mixtures. The relative population increase, defined as the final nematode density ($P_f$) devided by the initial nematode density ($P_i$), was used to determine whether interactions were present.

A slight but significant reduction of the relative population increase for *G. rostochiensis* was found when *G. pallida* was present in a much higher relative density. This effect was established in pots with various nematode densities and various species compositions on potato cultivars with different resistance properties (chapter 3). Although situations in which *G. pallida* was in minority were underrepresented in the experiments, the tendency of a slight decrease in the relative population increase of *G. pallida* as a result of interaction with *G. rostochiensis* was likewise apparent. However, the results found for *G. rostochiensis* cannot be translated uncritically to *G. pallida* because the species show considerable differences in their ecology. According to the model the reduction of the relative population increase was not related to absolute density. Therefore it was concluded that the mode of interaction was not primarily competition for food (exploitation competition *sensu* Pianka, 1978).

As an alternative explanation, the lower reproduction might have been caused by intercrossing or by obstructing the intraspecific mating by excess of males of the other species leading to a reduced fertilization of the females.

In the pots with the most extreme species ratios an excess of males of one species may have hampered intraspecific fertilization of the other species, i.e. interference competition for mates, *sensu* Pianka 1978. It has been shown that males of *G. rostochiensis* are attracted to the females of other species of the same genus and of related genera although they prefer their own species (Green & Miller, 1969; Parrott & Berry, 1976). In experiments on the influence of multiple mating on the reproduction on *G. rostochiensis* Green *et al.* (1970) found that males obstructed each other in attemps to copulate. In the experiments decribed in this thesis, the environmental conditions were established to obtain synchronous development of both nematode species. Therefore the appearance of males of both species might well have taken place at the same time creating at those extreme species ratios opportunities to hamper fertilization.

Whether hybridization took place substantially between the species was investigated in the crossing-experiments (chapter 5). In these experiments, carried out over several years, the relative population increase of *G. rostochiensis* and *G. pallida* was examined in single species populations and in 50% mixtures of both species for a number of generations. The relative population increase of *G. pallida* in the mixtures was not significantly different from that of the single species populations and *G. rostochiensis* showed the same pattern. Apparently they reproduced independently in the 50% mixtures. Although the two species had high chances for mixed encounters, intercrossing leading to hybridization did not play any detectable role in these crossing-experiments. The interactions described in chapter 3 were not found in these 50 % mixtures which provides more evidence that the observed effects might be caused by interference competition for mates *sensu* Pianka 1978.

Differences between species might strongly influence the occurrence of interaction or the mode of interaction. The two potato cyst nematode species differ with regard to development (Mugniery, 1978), and hatching behaviour in relation to temperature

(Robinson *et al.*, 1987). In addition, differences in reaction pattern of the species towards the different potato varieties used in this study might interfere with the interactions observed. For instance, Evans (1983) screened root diffusates of 25 different potato cultivars for hatching activity of *G. rostochiensis* and *G. pallida*. Both species reacted differently towards the various root diffusates.

In the present study the hatching behaviour was assessed for both species in the potato root diffusates (PRD) of the cultivars used in the pot experiments. The results showed that *G. pallida* reached higher numbers in the PRDs than *G. rostochiensis* (chapter 4). In the hatching tests it appeared that the method to obtain the PRDs influenced the hatching results considerably. The control solution, silver sand percolate, showed a strong inhibiting effect. Mineral analysis of the solution showed a high concentration of boron, which is known to inhibit hatching (Clarke & Shepherd, 1966). Despite this inhibitory effect it was still possible to compare the hatching activity of both nematode species in undiluted PRD because the hatching data of the PRDs were adjusted with the control data.

It might be questioned whether the better hatching of *G. pallida* than *G. rostochiensis* in the PRDs has affected the results of the interaction experiments. If *G. pallida* did hatch better than *G. rostochiensis*, it might have occupied most of the available sites on the roots. This should, especially at higher densities, influence the reproduction of *G. rostochiensis* by lack of sites to establish and feed. The observed reduction in the relative population increase of *G. rostochiensis*, however, was independent of the initial densities, and for *G. pallida* the same tendency was found (chapter 3). So results on interaction cannot be explained merely by differences in hatching behaviour of the two species. This confirms that interference competition and not exploitation competitionis is the type of interaction which may have taken place here.

Apart from the inhibitory effect of the silver sand another striking effect on egg hatching occurred: hatching in tap water was very high for *G. rostochiensis*, about five times as high as the usually recorded values of approximately 10% (Evans, 1982, 1983; Shepherd, 1962). *G. pallida* however, did not reach such high rates in tap water. Periodicity found by Den Ouden (1960) and Mulder *et al.* (1990) cannot be the cause of this phenomenon, because the hatching tests were carried out in different periods during the year, all with similar results. The different behaviour of the two species in relation to dormancy might be the cause of the established hatching differences in water. After diapause has been broken *G. rostochiensis* eggs hatched easily when conditions were favourable (for instance when water was present) whereas *G. pallida* showed hatching activity only when triggered by PRD (chapter 4). Forrest (1989) also found that *G. pallida* was hatchable in water only when activated by PRD. Bean and Perry (1990) found the same tendency in experiments with herbicides. The hatching behaviour of *G. pallida* suggests that the eggs are in a state of facultative diapause, induced by token stimuli in the environment (Evans, 1987), whereas the hatching of *G. rostochiensis* shows that, apart from the facultative diapause, a substantial proportion of eggs are in another state of dormancy. The latter state might be similar to that occurring in the eggs of *H. schachtii*. According to Zheng & Ferris (1991) the eggs of the latter species can be divided in four types of dormancy, the eggs exhibiting the following behaviour: eggs that hatch rapidly in water, eggs that hatch rapidly in host root diffusate, eggs that hatch over a long period in water and eggs that hatch over a long period in host diffusate.

Another striking difference in both egg hatching and reproduction between the species was apparent in the amount of variance of the experimental data. The hatching of *G. pallida* was consistently low with little variation and its reproduction in both pot experiments also showed consistently low variation, whereas for *G. rostochiensis* the reproduction in both pot experiments varied strongly and the rate of hatching was also much more variable.

The different reaction of the species to the initial hatching trigger, the consistently different behaviour in hatching and reproduction and the absence of hybridization indicate that the species are much more divergent than the often assigned term sibling species suggests. Both species originate from the Andean region. The distribution of *G. pallida* in that area is restricted between the equator and 15.6°S whereas *G. rostochiensis* is only found more southerly (Evans *et al.*, 1975; Canto Saenz & De Scurrah, 1977). The origin of the species is difficult to trace but based on the differences and similarities in protein pattern Bakker (1987) concluded that the species diverged from each other millions of years ago.

So, findings on one of the two species cannot be readily translated to the other species. A thorough study of the biology of the species on natural host plants in the area of origin will give a better understanding of the ecological differences.

## Implications for potato cyst nematode control

One of the experiments in this study was indicative of interference competition between the two species. The other experiments showed no competition whatsoever. The results were obtained under controlled conditions in greenhouse experiments which favoured both species equally well. By using suspensions of eggs and juveniles as inoculum in the limited space of pots the conditions for interaction were highly favourable. In the field, environmental conditions are much more heterogeneous and variable. Nematodes of both species may respond differently to the available microhabitat conditions, which may cause segregation of the species. In addition, nematodes infest the soil as cysts which ensures a certain amount of aggregation of one species. Because of this and the fact that interactions were of minor importance under optimal conditions, it is unlikely that they occur in the field to such an extent that the nematode populations will be influenced significantly. Hence, populations of both species will develop independently.

For control strategies, in particular for using the right resistant varieties, it is essential to know which species is present in the soil and additionally in what species composition they are present. The method to determine species ratios used in this study may not be very practical for monotoring field populations because of some drawbacks such as the limited range of species detection (both species are not detectable below the 10%), and because the method is laborious and technical skill is needed. An ELISA test, recently developed by the Laboratory of Monoclonal Antibodies and the Department of Nematology, Wageningen Agricultural University, based on monoclonal antibodies (Schots *et al.*, 1992), makes it possible to determine whether the cysts are potato cyst nematodes and to identify *G. pallida* in mixtures of cysts. For routine screening this method is more useful than the above mentioned. In the near future such identification tests might also be developed based on the genetic constitution of the nematodes, such as the DNA-probes used by Burrows & Perry (1988).

When the species composition in the field is known, the best choice of resistant varieties can be made. Alternate cropping of species specific resistant potato varieties might well be a succesfull control strategy. The population development of the separate species is alternately suppressed and selection for increased virulence will be slower (Spitters & Ward, 1988; Jones, 1985). To prevent underestimation of the total population increase it is necessary to determine the initial density of both species separately, as nematode reproduction is mainly dependent on its own initial density. Predictions of the population development of the separate species will be straight forward because interactions between the different potato cyst nematode species in the field are of no importance.

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## Summary

In integrated control of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*, the use of resistant varieties is an important strategy, especially now the application of agrochemicals in The Netherlands is increasingly restricted. Repeated cropping of varieties with the same or similar resistance genes produces a selection pressure on nematode populations leading to an increase in virulence and a progressive loss of resistance. To prolong the efficient use of resistant varieties, alternate cropping of varieties with species specific resistance instead of totally resistant varieties might prove to be a more successful strategy. In this situation the population development of each nematode species is alternately suppressed and selection for increased virulence will be reduced at the same time. To develop rotation schemes with these objectives information is needed on the reproduction of the species on the varieties to be used, the actual species composition and distribution in the field, and the interactions between the nematode species. The latter was the subject of this thesis (chapter 1)

A method has been developed to quantify species ratios in mixed populations. The method is based on the separation of species specific thermostable proteins by SDS-PAGE. Densitometric analysis of the 17 kD protein of *G. pallida* and the 18 kD protein of *G. rostochiensis* revealed a high correlation ($R=0.93$) with the species ratio in the mixed samples. Within the limits of 10 to 90% of each species, one can estimate with 95% reliability the species composition with 3 to 6% deviation (chapter 2).

To determine whether interactions between the species takes place mixtures of *G. rostochiensis* (Ro1) and *G. pallida* (Pa3) were reared on susceptible (cv. Bintje) and species specific resistant (cv. Elkana and clone ZB35-29) potato varieties at different nematode densities. The proportion of newly formed cysts belonging to each species was determined by way of SDS-PAGE and the relative population increase of each species, defined as the final population ($P_f$) divided by the initial population ($P_i$), was calculated. Comparisons of the relative population increase of each species in the single populations and in the mixtures revealed that interaction between the two species occurred. The relative population increase of *G. rostochiensis* was significantly decreased when *G. pallida* was highly dominant in the mixed populations. These effects were density independent which makes it unlikely that exploitation competition is responsible for this. It was suggested that interference competition for mates might be responsible for these interaction effects (chapter 3).

Differences in hatching behaviour of the two species in response to the host plant could interfere in the interaction. Therefore, hatching differences of *G. rostochiensis* and *G. pallida* were assessed in potato root diffusate (PRD) of cv. Bintje, cv. Elkana and clone ZB35-29. *G. pallida* hatched better in the PRDs than *G. rostochiensis*. However, the reduced relative population increase of *G. rostochiensis* was found at all densities indicating that *G. pallida* did not reach the roots sooner and occupy all available feeding sites. Therefore, it was concluded that the differences in hatching behaviour did not play any significant role in the interactions.

It was shown that the experimental test conditions strongly influenced the hatching results. The water type used in the hatching tests had a significant discriminating effect on the species; *G. rostochiensis* had a relative hatch of 60 to 90% in demineralized and tap water, whereas the hatch of *G. pallida* never exceded 15%. These differences were independent of the various cyst batches that were used or the different years the tests were carried out. Silver sand percolate had an inhibiting effect on the hatching of both nematode species. The high concentration of boron may explain the latter effect (chapter 4).

An investigation was conducted to study whether both species could hybridize in pots under optimal environmental conditions and to see what consequences this might have for the next generations. Pot experiments were carried out for four years with 50% mixtures of both species and single species populations, reared on the susceptible cv. Bintje. Analysis 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. The fact that hybridization did not take place confirms the idea that the observed interaction is mainly a result of interference competition (chapter 5).

The different reaction of the species to the initial hatching trigger, the consistently different behaviour in hatching and reproduction and the absence of hybridization indicate that the species are much more divergent than the term sibling species suggests. Findings on one of the two species cannot be uncritically translated to the othet species. A thorough study of the biology of the species on natural host plants in the country of origin will give a better understanding of the ecological differences.

The results were obtained under controlled conditions with optimal chances for interspecific encounters leading to interactions. Only in one of the experiments was a significant but slight interaction found. In field situations conditions are much more heterogeneous and variable causing seggregation of the species. Additionally, nematodes infest the soil as cysts ensuring a certain amount of aggregation. It is therefore unlikely that interactions occur in the field, hence both species populations will develop independently.

For control strategies, in particular for using the right resistant varieties, it is essential to know which species inhabits the soil and additionally what species composition is present. For monitoring field populations the method described here has some draw backs, a recently developed ELISA test might be of more use.

To prevent underestimation of the total population increase one ought to determine the initial density of both species separately, as nematode reproduction is dependent on its own population density before planting. Predictions of the population development based the separate species are relatively straight forward because interactions between the different potato cyst nematode species in the field are of no importance (chapter 6).

## Samenvatting

Het telen van resistente rassen is bij de geïntegreerde bestrijding van het aardappelcysteaaltje, *Globodera rostochiensis* en *G. pallida*, een belangrijke maatregel. Doordat het gebruik van chemische middelen door de Nederlandse overheid steeds meer aan banden wordt gelegd, worden resistente rassen zelfs het belangrijkste hulpmiddel in de aaltjesbestrijding. Echter het zeer intensief telen van resistente rassen met dezelfde of vergelijkbare resistentiegenen veroorzaakt een selectiedruk op de aaltjespopulaties resulterend in een toename van virulentie in deze populatie en daardoor een afnemende gebruikswaarde van resistente rassen. Om langer gebruik te kunnen maken van resistente rassen zou het alternerend telen van soortspecifieke resistente rassen (resistentie tegen "pathotypen" van één soort) in plaats van cumulatief resistente rassen (resistentie tegen alle bekende "pathotypen" van beide soorten) een oplossing kunnen bieden. Men bereikt hiermee dat de populatieontwikkeling van de soorten afwisselend geremd wordt. Tegelijkertijd wordt de selectiedruk voor virulentie verminderd. Om voornoemde doelen te bereiken zouden vruchtwisselingsschema's ontworpen moeten worden op basis van informatie over de reproduktie van de soorten op de te gebruiken rassen, de actuele soortensamenstelling en verdeling in het veld en de mogelijke interacties die tussen de soorten kunnen optreden. Dit proefschrift handelt over het laatste onderwerp (hoofdstuk 1).

Allereerst werd een methode ontwikkeld waarmee de soortensamenstelling in een mengsel kwantitatief kan worden bepaald. Deze methode is gebaseerd op het scheiden van soortspecifieke thermostabiele eiwitten met behulp van SDS-PAGE. De verhouding tussen de bandjes op gel van het 17 kD eiwit van *G. pallida* en het 18 kD eiwit van *G. rostochiensis* werd densitometrisch geanalyseerd. Deze verhouding bleek een hoge correlatie ($R = 0{,}93$) te bezitten met de soortensamenstelling in het mengmonster. Tussen de grenzen van 10 tot 90% relatieve vertegenwoordiging van elke soort in een mengsel van soorten kan de soortensamenstelling met 95% betrouwbaarheid op 3 tot 6% nauwkeurig bepaald worden (hoofdstuk 2).

Om vast te stellen of er interacties plaats vinden tussen de soorten werden de twee aaltjessoorten in verschillende verhoudingen en hoeveelheden aangebracht in potten met een vatbaar ras (cv. Bintje) en twee soortspecifieke resistente rassen (cv. Elkana en kloon ZB35-29). Van de nieuw gevormde cysten werd de verhouding tussen de soorten bepaald door gebruik te maken van bovengenoemde methode. Vervolgens kon de einddichtheid per soort bepaald worden waarna de relatieve populatietoename, gedefinieerd als de einddichtheid ($P_f$) gedeeld door begindichtheid ($P_i$), kon worden berekend. Voor elke soort werd de relatieve toename in de monopopulatie vergeleken met die in de mengsels. Hieruit bleek dat de relatieve populatietoename van *G. rostochiensis* significant werd verminderd als *G. pallida* dominant aanwezig was. Deze interactie-effecten bleken onafhankelijk te zijn van de initiële dichtheid, wat het onwaarschijnlijk maakt dat exploitatie-competitie hier een rol speelt. Hier lijkt meer sprake te zijn van interferentie-competitie voor het andere geslacht tussen de mannetjes van beide soorten (hoofdstuk 3).

Verschil in lokgedrag van beide aaltjessoorten naar de waardplant toe zou een rol kunnen spelen bij de gevonden interacties. Daarom is het lokgedrag van *G. rostochiensis* en *G. pallida* onderzocht in de wortelexudaten van de planten die gebruikt werden in de potproeven, te weten cv. Bintje, cv. Elkana en klonn ZB35-29. *G. pallida* bleek beter te worden gelokt in de wortelexudaten dan *G. rostochiensis*. Ondanks de hogere lokpercentages van *G. pallida* resulteerde dit niet tot een duidelijk voordeel van deze soort in de potproeven. Als *G. pallida* door zijn lokgedrag beter de wortels gekoloniseerd zou hebben, dan zou dit vooral bij hogere dichtheid effect hebben gehad. Uit de potproeven bleek dat de interacties onafhankelijk van dichtheid optraden. De verschillen in lokking hebben blijkbaar geen rol van betekenis gespeeld bij de interacties.
De experimentele toetscondities bleken sterk de resultaten sterk te beïnvloeden. *G. rostochiensis* bereikte relatieve lokkingspercentages van 60 to 90% in gedemineraliseerd water en kraanwater, terwijl *G. pallida* in beide situaties nooit hoger kwam dan 15%. Deze verschillen bleken onafhankelijk te zijn van de verschillende gebruikte 'batches' en van het tijdstip, in het jaar en over de jaren heen, waarop de toets werd uitgevoerd. Daarnaast bleek het percolaat van zilverzand (het substraat waarin de planten groeiden) een remmend effect op de lokking van beide aaltjessoorten uit te oefenen. De hoge concentratie aan borium zou hiervoor een oorzaak kunnen zijn (hoofdstuk 4).

Vervolgens is onderzocht of beide aaltjessoorten onder zo gunstig mogelijke omstandigheden in potten met elkaar kruisten en wat voor gevolgen dit had voor de volgende generaties. Gedurende vier jaar werden potproeven uitgevoerd waarbij cv. Bintje als waardplant diende en als inoculum de beide soorten apart en in een 50%-50% mengsel gebruikt werden. Tevens werden de gevormde cysten van de nieuwe generaties van het afgelopen jaar gebruikt als inoculum. Na analyse van het aantal cysten, de cysteinhoud en de relatieve populatietoename van de soorten bleek dat de soorten onafhankelijk van elkaar reproduceren en kruisingen niet waren opgetreden. In de beperkte ruimte van de potten bleken de populaties stabiel naast elkaar te kunnen leven. Het feit dat kruisingen tussen de soorten niet optreden in deze gecontroleerde potomstandigheden ondersteunt de hypothese dat de gevonden interacties voornamelijk te kenschetsen zijn als interferentie-competitie (hoofdstuk 5).

De twee aardappelcysteaaltjes soorten zijn nauw verwant en worden vaak aangeduid als "sibling species". Het verschil tussen de soorten in hun reactie op de lokstimulans, het consistente verschil in de variabiliteit wat betreft het lokgedrag en de reproduktie en het niet kruisen tussen de soorten geven aan dat de soorten veel verder van elkaar verwijderd zijn dan de term "sibling species" veronderstelt. Onderzoeksresultaten die men voor de ene soort vindt, kunnen daarom niet zonder meer vertaald worden naar de andere soort. Een grondig onderzoek naar de biologie van de soorten op hun natuurlijke waardplanten in het oorsprongsgebied zou tot een beter begrip leiden van de ecologische verschillen tussen de soorten.

De resultaten zijn verkregen onder gecontroleerde omstandigheden die zo waren gekozen dat de ontmoetingskansen tussen de soorten zo groot mogelijk waren waardoor interacties zouden kunnen ontstaan. Slechts in één experiment werd een kleine maar significante reductie gevonden in de relatieve populatietoename als resultaat van interacties. In het veld zijn de omstandigheden zeer divers en variabel waardoor een zekere ruimtelijke scheiding van de soorten plaats zal vinden. Daarnaast bevinden de aaltjes zich in cysten in de grond, wat een zekere mate van clustering per soort veroorzaakt. Het is daarom onwaarschijnlijk dat er interacties tussen de soorten zullen plaatsvinden in het veld; beide populaties zullen zich onafhankelijk ontwikkelen.

Bij de bestrijding van het aardappelcysteaaltje, en specifiek bij het gebruik van resistente rassen, is het essentiëel om te weten met welke aaltjessoort de grond besmet is, en in het geval van mengpopulaties, hoe de samenstelling van dit mengsel is. Voor het analyseren van veldpopulaties op grote schaal heeft de bovenbeschreven methode enkele nadelen. Een ELISA toets, recent ontwikkeld bij het Laboratorium voor Monoclonale Antistoffen en de vakgroep Nematologie van de Landbouwuniversiteit van Wageningen, lijkt hiervoor meer geschikt.

Om te voorkomen dat de toename van de totale aaltjespopulatie wordt onderschat is het noodzakelijk dat voor elke soort apart de begindichtheid bepaald wordt, daar de populatietoename hiervan afhankelijk is. Bij het voorspellen van de populatieontwikkeling van de afzonderlijke soorten hoeft men geen rekening te houden met interacties daar gebleken is dat die geen rol van betekenis spelen (hoofdstuk 6).

## Curriculum vitae

Lucia Johanna Maria Francisca (Loes) den Nijs werd op 4 oktober 1956 te Haarlem geboren. In 1975 behaalde zij in dezelfde plaats op het Triniteitslyceum het diploma Atheneum B. Vervolgens begon zij in het zelfde jaar de studie Plantenziektenkunde te Wageningen aan de toenmalige Landbouwhogeschool, nu Landbouwuniversiteit geheten. Tijdens de doctoraalfase werd onderzoek gedaan naar de biologische bestrijding van aardrupsen met behulp van entomofage aaltjes (hoofdvak Entomologie), de reuzecelvorming in de aardappelwortel veroorzaakt door het aardappelcysteaaltje werd met licht- en electronenmicroscoop onderzocht (hoofdvak Nematologie), tijdens haar stage in Engeland werd de biologische bestrijding van het havercysteaaltje door schimmels onderzocht en voor het vak Natuurbeheer werd de hydrobiologie van poldersloten bekeken. De doctoraalfase werd op 28 maart 1983 met lof afgesloten.

Van januari tot juli 1984 heeft zij gewerkt bij het Hilbrands Laboratorium voor bodemziekten aan een project betreffende de mogelijkheden van biologische bestrijding van het aardappelcysteaaltje. In juli 1984 kwam zij in dienst bij het IPO alwaar zij onderzoek opstartte wat geleid heeft tot dit proefschrift.