



## 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 (2-4 mm), 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 & den Nijs, unpubl.).

To create the different species compositions and densities the appropriate aliquots of eggs and larvae suspensions were mixed, which resulted in the following settings, represented as  $P_i/\% Pa$ , where  $P_i$  = total initial density of Pa and Ro in  $\text{eggs} \cdot \text{g}^{-1}$  soil and  $\% Pa$  = species composition : 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 day-time, 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 consumption (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 ( $\text{eggs} \cdot \text{g}^{-1}$  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 & Lock,

1990). From this species ratio, expressed as % of *G. pallida* and the joint final density, the final population densities 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  $P_f/P_i$ , 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 [ $\log (P_i)$  of Ro or Pa], species compositions ( $\% Pa$  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

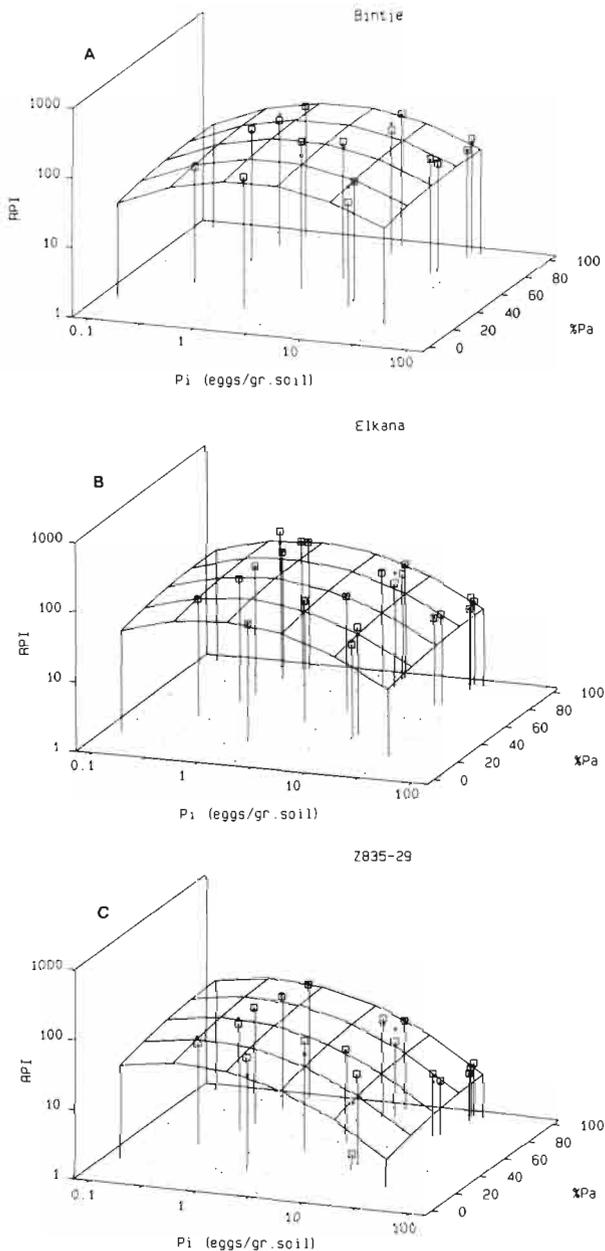
### Relative population increase

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, con-

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

Change	<i>G. rostochiensis</i>			<i>G. pallida</i>		
	df	MS	vr	df	MS	vr
Species composition ( $\% Ro$ or 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***
( $\% Ro$ or Pa) <sup>2</sup>	1	0.38541	10.66**	1	0.00030	0.01
( $\log P_i$ ) <sup>2</sup>	1	0.00127	0.04	1	0.52585	21.70**
Variety	1	0.02047	0.57	2	1.34634	55.55**
$\% Pa \times$ variety	1	0.39832	11.01**	2	0.00474	0.20
$\log P_i \times$ 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).



**Fig. 1.** Relationship between the initial density ( $\log P_i$ ) of *Globodera 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 :  $\log RPI = 1.701 + 0.00547 \times \% Pa + 0.1567 \times \log (P_i) - 0.0000327 \times \% Pa^2 - 0.2991 \times \log (P_i)^2$ ; B : cv. Elkana :  $\log RPI = 1.5930 + 0.0057 \times \% Pa - 0.0435 \times \log (P_i) - 0.0000327 \times \% Pa^2 - 0.2991 \times \log (P_i)^2$ ; C : clone ZB35-29 :  $\log RPI = 1.287 - 0.0067 \times \% Pa - 0.2301 \times \log (P_i) - 0.0000327 \times \% Pa^2 - 0.2991 \times \log (P_i)^2$ .

sequently 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.

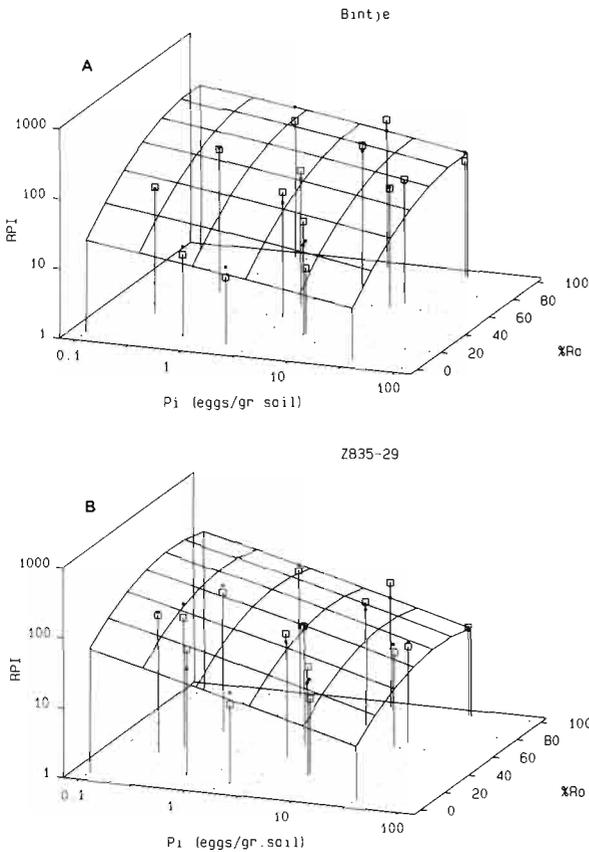
Varying the species composition did not significantly affect the RPI of *G. pallida*. Intraspecific interaction influenced the RPI of *G. pallida*, the effect of initial density [ $\log (P_i)$  of *Pa*] was highly significant (Table 1). The levels and slopes of the RPI-curves differed for each variety. 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.

The RPI of *G. rostochiensis* was progressively decreased by an increasing  $P_i$  and by an increasing proportion of *G. pallida* in the mixture. Significant effects were found for the initial density, species composition (% Ro), % Ro<sup>2</sup> and % Ro  $\times$  variety (Table 1). Interspecific interaction markedly influenced the RPI of *G. rostochiensis* (Table 1; 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).

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  $\times$  variety on the yield confirms this (Table 3).



**Fig. 2.** Relationship between the initial density (log  $P_i$ ) of *Globodera rostochiensis* and the relative population increase of *G. rostochiensis* and *G. pallida*, expressed as %  $R_o$ , 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 :  $\log RPI = 1.007 + 0.02399 \times \% Ro - 0.2277 \times \log (P_i) - 0.0001279 \times \% Ro^2 - 0.0131 \times \log (P_i)^2$ ; B : clone ZB35-29 :  $\log RPI = 1.296 + 0.2344 \times \% Ro - 0.3907 \times \log (P_i) - 0.0001279 \times \% Ro^2 - 0.0131 \times \log (P_i)^2$ .

**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.

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 compe-

**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.

Initial species composition : % Pa	Total initial density (Pa + Ro egg/gr. soil)	Cv. Bintje		Clone ZB35-29	
		final % Pa	Shift	final % Pa	Shift
98.8	0.7	> 90 <sup>1</sup>		> 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) <sup>2</sup>	+	87.4 (76.6-98.2)	—
88.9	5.3	> 90		62.8 (53.5-72.1)	—*
86.8	0.7	91.3 (10-100)	+	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)	—
34.3	29.7	21.2 (3.0-39.4)	—	4.7 (0.0-11.0)	—*
21.1	7.2	17.0 (8.7-25.3)	—	17.0 (10.3-23.7)	—

1 : mean of five replicates; 2 : 95 % confidence interval; \* : significant at  $\alpha = 0.05$ .

**Table 3.** Analysis of variance of the evaporation and yield data of cv. Bintje, cv. Elkana and clone ZB35-29, inoculated simultaneously with *Globodera pallida* and *G. rostochiensis* and in single species inoculations. (Accumulated analysis of variance.)

Change	Evaporation			Yield		
	df	MS	vr	df	MS	vr
Species composition (% Pa)	1	88147	6.35*	1	0.00005508	0.61
Initial density (log Pi)	1	488828	35.23**	1	0.0384898	42.82*
(% Pa) <sup>2</sup>	1	1	0.00	1	0.0007751	0.86
(log Pi) <sup>2</sup>	1	46064	3.32*	1	0.0056372	6.27*
Variety	2	471160	33.95**	2	0.0033661	3.74*
% Pa × variety	2	73322	5.28**	2	0.0074493	8.29*
log Pi × 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).

tion 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 (Mugniéry, 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 inter-specific competition.

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 statistically significant. 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 dominance.

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 whether *G. rostochiensis* remained present in the population in the mixtures of 95 % Pa — 5 % Ro and 99 % Pa — 1 % Ro and, if so, to what extent. 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 obscure 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  $\times$  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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