Durability of Resistance to *Globodera pallida*

I. Changes in Pathogenicity, Virulence, and Aggressiveness During Reproduction on Partially Resistant Potato Cultivars

Henk J. Schouten and Johanna E. Beniers

Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, 6700 AA Wageningen, the Netherlands.
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ABSTRACT

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We studied changes in pathogenicity, virulence, and aggressiveness of *Globodera pallida* populations over time. As a measure for pathogenicity, the reproduction factor on a partially resistant host was used; for aggressiveness, the reproduction factor on a susceptible reference host was used; and, for virulence, the ratio pathogenicity/aggressiveness was used. The *G. pallida* populations were reared in a glasshouse for four generations on potato cultivars with different levels of resistance. The cultivar Elkana did not increase pathogenicity significantly, but the more resistant cultivars

Karakter and Darwina did. This increase in pathogenicity was caused by an increase in virulence, whereas aggressiveness generally was not altered significantly. The increase in virulence appeared to be caused by an enhanced ability of eggs to develop into cysts, and not by an increase in egg production per new cyst. The observed changes in virulence could be predicted reasonably well by a simple numerical model. The rate of selection depended strongly on the nematode population. Rearing a mix of two different populations on a susceptible host decreased the virulence strongly, as predicted by the Hardy-Weinberg equilibrium, but increased the aggressiveness because of heterosis.

Additional keyword: potato cyst nematodes.

Around 1984, the first potato cultivars with resistance to the potato cyst nematode *Globodera pallida* were introduced onto the market in western Europe (25). These cultivars have a major drawback, i.e., they are resistant to the so-called Pa2 group of *G. pallida*, but much less resistant to the Pa3 group (13). As a result, the Pa2 group has been strongly reduced in the Netherlands, but not the Pa3 group (25). Fortunately, new cultivars have been introduced since 1994 that have high levels of resistance to both the Pa2 and Pa3 groups (4).

Our objective was to determine whether these new cultivars are able to suppress *G. pallida* permanently, or whether virulent *G. pallida* nematodes will emerge because of selection pressure. For this objective, we reared nematode populations for several generations on cultivars with different levels of resistance (i.e., ability to suppress nematode reproduction) and determined whether the reproduction rates of the nematode populations increased over the successive generations. Also, we modeled the virulence courses and compared the model's predictions with the observed courses.

Jones et al. (11) performed pioneering research on selection to virulence. They showed that the reproduction rate of *G. rostochiensis* populations increased when these populations were reared repeatedly on resistant hosts. The rate of selection depended strongly on the nematode population and host genotype (11). Based on these observations, they developed analytical models (7-9), explaining theoretically the rate of selection. When they assumed that virulence was inherited recessively and that resistance inhibited the development of female nematodes, but not of the males, their selection models explained the observed changes in reproduction rate in pot experiments rather well (8,9). From the analytic mod-

els, they deduced simulation models for the population density and genetics over time in a field situation (8-12).

Whereas Jones and his coworkers (7-12) performed their selection experiments mainly with *G. rostochiensis*, and only during three generations, Turner and her coworkers (23,24) performed selection experiments with *G. pallida* populations for up to 11 generations. They demonstrated unequivocally that selection can occur in *G. pallida* populations (24).

Our experiments deviated in several respects from Turner's experiments. The most prevalent differences are the following. (i) We added a cultivar that shows resistance to both the Pa2 and the Pa3 groups. (ii) We reared nematodes not only on a resistant host, but also in a parallel series on a susceptible host, starting with the same *G. pallida* population. The two parallel series yielded a selected and a nonselected population. The reproduction factors of these two populations were measured under almost identical environmental conditions and compared statistically. Jones and Turner studied only selected populations. (iii) A difficulty in validating selection models with experimental results usually is the obscurity of the initial frequency of virulent nematodes in the population before selection starts. We solved this problem, to some extent, by adding a selection experiment that started with a mixture of an avirulent population and a highly virulent population put together in a known ratio.

MATERIALS AND METHODS

Selected population and nonselected population. The reproduction rate may vary over different generations, because of changing environmental conditions and possible differences in quality of the mother tubers. These changes in reproduction rate must not be ascribed to selection (2). Therefore, we decided to measure not only the reproduction rate of a population that was reared for several generations on a partially resistant host, but also of the

Corresponding author: H. J. Schouten; E-mail address: h.j.schouten@cpo.dlo.nl

same starting population that was reared for the same number of generations on a susceptible host. As the partially resistant host might have exerted a selection pressure, the nematode population reared on this host was called the selected population, whereas the population from the susceptible host was called the nonselected population. The populations were produced in 'production series.' Besides these production series, there were 'measurements series,' in which the reproduction rates on the susceptible host and on partially resistant hosts were determined. The reproduction rates of the selected and the nonselected populations, determined at the same time in the same glasshouse, on roots from tubers from the same potato stocks, were compared statistically.

Nematode populations. The *G. pallida* populations Coll. 1112 (Pa3) and D383 (Pa2) were used. They had previously been multiplied in a glasshouse on the susceptible cultivar Irene in order to produce harvested eggs of equal ages and similar physiological conditions. After multiplication, some of the eggs from the Pa2 population were mixed with eggs from the Pa3 population in the proportion of four to one (80% Pa2 and 20% Pa3). This mixed population is called the 'Pa2/3 population'. The Pa2/3 population was not reproduced again on 'Irene' before starting the selection experiment.

Potato cultivars. The cultivars used are listed in Table 1. 'Irene' was used as a susceptible reference cultivar, and 'Karakter' as a representative of the cultivars with strong resistance to both the Pa2 and the Pa3 groups. The cultivars Elles and Darwina have descended from the host that was proposed as the differential to separate Pa2 from Pa3, i.e., SVP (Vt^h)² 62.33.3 (13). As appears from Table 1, they inherited this ability to differentiate. For some experiments, dormancy of the potatoes was ended by applying gibberellins (Boom BV, Meppel, the Netherlands).

Production of the successive nematode generations. The soil was a mixture of silver sand, hydro-gravel, and clay powder in the proportion of 12:3:2. Steiner solution (20 ml/kg of dry soil;

electric conductivity = 2) and fertilizer (1 g/kg of dry soil; N-P-K 12:10:8) were added to the soil. Each pot was filled with 5 kg of dry weight of soil.

Cysts of the nematode populations were crushed (21), and a suspension of eggs injected into the soil, using 20-cm-long needles, to give four eggs per gram of dry soil. After inoculation, two potato sprouts were planted per pot. The day length in the glasshouse was 16 h and the temperature was 15°C, controlled by air-

Fig. 1. Pathogenicity (number of newly formed eggs per inoculated egg [P_f/P_i]) of a *Globodera pallida* A, Pa3 and B, Pa2/3 population to a partially resistant cultivar. The *G. pallida* population was reared for several generations on the susceptible cultivar Irene providing a nonselected population (dotted lines), or on a partially resistant cultivar providing a selected population (solid lines). The symbols refer to the partially resistant cultivars Elles (○), Elles (—), Darwina (×), and Karakter (Δ).

TABLE 1. Number of new eggs per inoculated egg on the test cultivar relative to the number on the susceptible cultivar Irene in glasshouse tests prior to selection

Potato cultivar	Resistance sources ^a	Resistant to pathotypes ^b	Reproduction (%) of		
			Pa2	Pa3	Pa2/3
Irene	...	...	100 (34) ^c	100 (50)	100 (35)
Elkana	1, 2	Ro1, Ro2/3	70	65	67
Elles	1, 2, 3	Ro1, Ro2/3, Pa2	0.17	30	8.5
Darwina	1, 2, 3	Ro1, Ro2/3, Pa2	0.086	20	5.3
Karakter	1, 2, 3, 4	Ro1, Ro2/3, Pa2, Pa3	0.47	2.6	1.1

^a Sources of resistance to *Globodera*: 1 = *Solanum tuberosum* ssp. *andigena* CPC 1673, 2 = *S. vernei* ssp. *balsii* 2/1, 3 = SVP (Vt^h)² 62.33.3 (= *S. vernei* 2488-3 × *S. vernei* CPC 2487-3), and 4 = *S. vernei* LGU8 × *S. oplocense* EBS 1786.

^b As described in Duringhof (3) and Ebskamp and Bonthuis (4).

^c The absolute number of new eggs per inoculated egg on 'Irene' ($n = 5$; $\sigma = 3$) is given in parentheses.

TABLE 2. Aggressiveness (P_f/P_i ^a on the susceptible cultivar Irene) and virulence ($[P_f/P_i]_{\text{resistant}}/[P_f/P_i]_{\text{susceptible}}$) of *Globodera pallida* populations reared for several generations on a partially resistant cultivar, relative to aggressiveness and virulence of the populations reared for the same number of generations on 'Irene'

Population	Reared on potato cultivar	Relative aggressiveness ^b				Relative virulence ^b			
		Generation				Generation			
		1	2	3	4	1	2	3	4
Pa3	Elkana	1.02	1.28	...	0.80	1.17	0.90	...	1.28
	Elles	1.04	1.37	0.94	0.82	0.78	0.83	1.05	1.26
	Darwina	0.80* ^c	1.11	0.91	0.99	1.38	1.08	1.52*	1.44*
	Karakter	1.22	0.85	1.06	...	1.14	3.71*	4.02*	...
Pa2/3	Elkana	0.74*	1.57*	...	0.73	1.42	0.78	...	1.32
	Elles	1.36	1.59*	1.09	0.95	4.12*	12.2*	18.1*	29.0*
	Darwina	1.26	1.34	1.00	1.03	4.72*	17.9*	33.0*	59.3*

^a P_f/P_i = the number of newly formed eggs per inoculated egg.

^b Pathogenicity is composed of two components, aggressiveness and virulence, with pathogenicity = aggressiveness × virulence.

^c A significant effect ($P < 0.05$) of rearing the *G. pallida* population on a partially resistant host is indicated by *.

conditioning. Twice a week, the water contents of the pots were adjusted to approximately 15% by weighing some pots.

When the majority of the new cysts had turned brown, 17 weeks after inoculation, watering of the plants was stopped. Ten days later, the crops were removed and the soil allowed to dry gradually. The cysts were elutriated from the soil, dried, and stored for several weeks at approximately 20°C. From pilot studies, it appeared that storage at low temperatures (4°C) would have enhanced diapause. We obtained two generations per year. During this experiment, we did not detect diminished reproduction of the nematodes by diapause.

The Pa3 population was reproduced in this way in parallel series on all cultivars listed in Table 1 for four generations. However, the reproduction rate on 'Karakter' was low and, therefore, it was necessary to multiply this population on 'Irene' after two generations on 'Karakter', in order to gain enough inoculum for the next generation. Consequently, we obtained one cycle less on 'Karakter'. The Pa2/3 population was reared on 'Irene', 'Elkana', 'Darwina', and 'Elles'; 'Karakter' was too resistant to this population for successive generations.

Measurement of the reproduction factors. Apart from pots for the production series, pots for the measurement series were used in four replicates in a fully randomized design. The same inoculation procedure, pot size, soil, and growing conditions were applied as for production of the successive generations, but only one sprout per pot was planted, and the moisture contents of the pots were adjusted more accurately by weighing all pots individually. To diminish position effects in the glasshouse, the pots changed place twice a week, allowing the pots to stay at the outer, more-light-receiving rows for a few days only (1). The number of cysts per pot and the number of eggs in the new cysts were estimated (1,18,19,21). From these data, the reproduction factor for each pot was calculated, i.e., the number of newly formed eggs per inoculated egg (P_f/P_i).

Working definitions. Van der Plank (26) split pathogenicity into two components, aggressiveness and virulence. We used his definitions and adapted them to make the terms quantitatively:

$$\text{Pathogenicity} = \text{aggressiveness} \times \text{virulence}$$

Pathogenicity equals here the reproduction factor (P_f/P_i) on a partially resistant host. Aggressiveness equals the reproduction factor on the susceptible reference host. Turner used the word 'fitness' for aggressiveness (23). However, as fitness usually refers not only to the ability to reproduce on a host, but also to the ability to survive, we prefer the term aggressiveness, according to van der Plank (26). Pathogenicity and aggressiveness are expressed in

the number of new eggs per inoculated egg. Virulence equals here the reproduction factor on a partially resistant host relative to the reproduction factor on the susceptible host, using the same nematode population as inoculum at P_i = four eggs per gram of dry soil. In this working definition, virulence of the nematode population equals the relative susceptibility of the host. Virulence is expressed in percentages.

Statistical analysis. As pathogenicity, aggressiveness, and virulence may have varied over generations, because of variability of the mother tubers and environmental conditions, these parameters were analyzed statistically by using reproduction factors within one generation and within one glasshouse. We did not test changes over generations, but rather compared selected and nonselected populations within a generation.

The data were transformed using log-transformations in order to obtain homogeneous variances and normal distributions. Within a generation, a pooled variance was estimated for the reproduction factors. This pooled variance was used in Student's *t* test for paired comparison of a selected population with the analogous nonselected population. Effects of selection on pathogenicity and aggressiveness were tested in this way.

For virulence, however, an extra step was needed, as virulence was not estimated directly from the reproductions factors, but from a quotient, i.e., reproduction factor on the test host/reproduction factor on the susceptible reference host. For estimation of the pooled variance of virulence, the following statistical rule was applied (14):

$$\text{var}\left(\frac{a}{b}\right) \approx \frac{\text{var}(a)}{(\bar{b})^2} + \frac{(\bar{a})^2}{(\bar{b})^4} \text{var}(b)$$

in which, for *a* and *b*, pathogenicity and aggressiveness were substituted, respectively.

The statistical package GENSTAT (Rothamsted Experimental Station, Harpenden, United Kingdom) was used.

RESULTS

Pathogenicity. Figure 1A shows the pathogenicity (P_f/P_i) of the selected Pa3 populations and of the nonselected Pa3 population. Reproduction of the Pa3 nematodes on 'Elkana' did not increase the pathogenicity to 'Elkana' significantly. Reproduction on the more resistant 'Elles' did not raise the pathogenicity to 'Elles' either. However, in the case of 'Darwina', the pathogenicity increased significantly after the second generation. After three generations, the pathogenicity to 'Darwina' was 1.4 as high as of the non-selected population ($P < 0.05$). Selection was faster on the most resistant cultivar Karakter; whereas it took three generations on 'Darwina' to raise the pathogenicity with a factor of 1.4, on 'Karakter', this took only one generation. After three generations on 'Karakter', the pathogenicity of the selected population was 4.2 times higher than the nonselected population ($P < 0.01$).

Like the Pa3 population, the Pa2/3 population showed no significant change in pathogenicity after reproduction on 'Elkana' (Fig. 1B). But on 'Elles', the selected Pa2/3 population was very significantly ($P < 0.001$) more pathogenic than the nonselected population. After four generations, the Pa2/3 population reared on 'Elles'

TABLE 3. Effect of selection on components of virulence of the fourth nematode generation reared on partially resistant hosts, i.e., the proportion of inoculated eggs that are able to grow into cysts (C), and the number of new eggs per new cyst (E)

<i>Globodera pallida</i> population	Potato cultivar	Components of virulence ^a	
		RVC ^b	RVE ^b
Pa3	Elkana	1.11	1.19
	Elles	1.62*	0.80
	Darwina	1.38*	1.07
	Karakter	3.72*	1.11
Pa2/3	Elkana	1.19	1.11
	Elles	24.2*	1.21
	Darwina	62.2*	0.97

^a Significant effects ($P < 0.05$) are indicated by *.

^b C on the partially resistant host was divided by C on the susceptible cultivar Irene, giving virulence for cyst formation (VC). Subsequently, VC of the selected population was divided by VC of the nonselected population (relative virulence for cyst formation; RVC). The same approach was used for the second virulence component, the relative virulence for cyst content (RVE). The products of the two components approximately equal the virulence data of the last generation in Table 2.

TABLE 4. Mixing of the genotypes of Pa2 and Pa3 after one generation on 'Irene', assuming random mating

Generation	Pa2	Pa3	Σ
Zero			
Frequency	80%	20%	100%
	Pa2 × Pa2	Pa2 × Pa3 ^a	Pa3 × Pa3
After one generation			
Frequency	0.8 × 0.8 = 64%	32%	0.2 × 0.2 = 4%
			100%

^a In this progeny, heterosis may occur.

was 28 times as pathogenic to ‘Elles’ as the nonselected population. The effect was even stronger on ‘Darwina’. The pathogenicity of the selected Pa2/3 population was 61 times higher than that of the nonselected population ($P < 0.001$).

The Pa2/3 population could not be tested on ‘Karakter’, as the initial pathogenicity was too low on this cultivar for production of enough inoculum for the next generation.

Aggressiveness. To measure for aggressiveness, the reproduction factor P_f/P_i on ‘Irene’ was used. From Table 2, it appears that selection generally did not affect the aggressiveness of the Pa3 or Pa2/3 population. Although the aggressiveness fluctuated to some extent over time, probably because of changes in environmental conditions or quality of the tubers, the aggressiveness of the selected populations did not deviate significantly from the aggressiveness of the analogous nonselected populations within a generation of either Pa2/3 or Pa3, measured in the same season in one glasshouse. In Table 2, there are some significant effects, but these effects do not show a trend and might have been caused by chance. Apparently, increased levels of pathogenicity after selection, shown in Figure 1, are not based on increased aggressiveness.

Virulence. Table 2 shows the virulence of the selected populations relative to the nonselected populations. Comparing Table 3 with Figure 1 reveals that changes in pathogenicity are caused by changes in virulence.

We have subdivided the virulence into two components by splitting the reproduction into two phases, the development from egg into cyst and the production of new eggs within the new cyst. Thus, the first component of virulence is the fraction of inoculated eggs that were able to develop into cysts on a partially resistant host, relative to that fraction on the susceptible host. The second component is the number of new eggs per new cyst on the partially resistant host divided by that number on the susceptible host. The product of these two components equals the virulence.

From Table 3, it appears that the increased virulence caused by selection resulted from an increased success of eggs to become cysts, but not by increased numbers of eggs in the new cysts.

DISCUSSION

Heterosis can be defined as the superiority of heterozygous genotypes in comparison with the corresponding homozygotes, and is the contrast of inbreeding depression (15). It is a common feature in higher organisms and might play a role in *Globodera* too. As the Pa2 and Pa3 populations were mixed, heterosis might have occurred in the Pa2/3 population. By comparing the level of aggressiveness of the Pa2/3 population with the levels of the Pa2 and Pa3 populations, heterosis can be quantified.

It was assumed that the Pa2 juveniles and Pa3 juveniles of the Pa2/3 population developed normally during the first generation on ‘Irene’. Then, in 32% of the offspring, the genomes of Pa2 and Pa3 were mixed, possibly leading to heterosis (Table 4). The remaining 68% of the offspring resulted from Pa2 $\times$ Pa2 and from Pa3 $\times$ Pa3, giving no mixing of gene pools from different populations and, therefore, no heterosis effect.

If there would have been no heterosis, the aggressiveness of the Pa2/3 population would have been the weighted mean of the levels of aggressiveness of the Pa2 population and the Pa3 population (38 and 69 new eggs per inoculated egg, respectively, giving 44 as weighted mean). However, the measured aggressiveness (59 new eggs per inoculated egg) was 33% higher than the weighted mean ($P < 0.005$). This increased aggressiveness was presumably caused by the offspring that occurred from mixing Pa2 and Pa3, which equaled 32% of the total offspring (Table 4). This means that heterosis increased the aggressiveness of these nematodes by approximately a factor of 2.

Heterosis affected aggressiveness and, therefore, pathogenicity. The analysis of changes in pathogenicity of Pa2/3 due to selection was, therefore, complicated by heterosis. However, the other component of pathogenicity, i.e., virulence, was not affected by heterosis, as changes in aggressiveness do not play a role for this parameter. For this reason, selection was further analyzed using the parameter virulence.

Similar to previous models (8,10,12,16,17,20,22), the courses of virulence of the Pa2/3 population can be modeled, as shown in Table 5. The following assumptions were made for this model. (i) Resistance inhibited the development of females, but not of males. (ii) Mating was random. (iii) Avirulence was dominant over virulence (6). (iv) The difference in virulence between Pa2 and Pa3 to ‘Elles’ and ‘Darwina’ was caused by one virulence gene only. (v) Pa2 was fixed (‘pure’) with respect to the avirulence allele (AA). The low reproduction rate of Pa2 on ‘Elles’ and ‘Darwina’ (Table 1) was negligible. (vi) Pa3 was fixed for the virulence gene (aa). The small increase in pathogenicity of Pa3 on ‘Darwina’ (Fig. 1A) was negligible. (vii) The resistance of ‘Elles’ and ‘Darwina’ to the Pa3 population is assumed to have been caused by partial resistance genes, to which the Pa2 and Pa3 populations harbored no virulence genes. Although we are aware that this model oversimplifies the real situation, we still made the simplifications and their attendant faults, as the model appears to be helpful in understanding.

Figure 1 shows that the rearing of Pa2/3 on the susceptible cultivar decreased pathogenicity to ‘Elles’ and ‘Darwina’. This decrease was caused by an significant decrease in virulence ($P < 0.005$), although the susceptible cultivar presumably exerted no selection pressure to decrease virulence. This decrease in viru-

TABLE 5. Modeled changes in genotype frequencies and virulence when a mixture of a virulent (aa) and an avirulent (AA) *Globodera pallida* population is reared on a susceptible or a resistant cultivar^a

Generation		Reared on a susceptible cultivar					Reared on a resistant cultivar					Relative virulence ^b
		P(AA)	P(A)	P(Aa)	P(a)	P(aa)	P(AA)	P(A)	P(Aa)	P(a)	P(aa)	
0	Juveniles and males	0.8		0.0		<u>0.2</u> ^c	0.8		0.0		<u>0.2</u> ^c	1.0
	Females	0.8		0.0		0.2	0.0		0.0		1.0	
	Male gametes		0.8		0.2			0.8		0.2		
	Female gametes		0.8		0.2			0.0		1.0		
1	Juveniles and males	0.64		0.32		<u>0.04</u>	0.0		0.8		<u>0.2</u>	5.0
	Females	0.64		0.32		0.04	0.0		0.0		1.0	
	Male gametes		0.8		0.2			0.4		0.6		
	Female gametes		0.8		0.2			0.0		1.0		
2	Juveniles and males	0.64		0.32		<u>0.04</u>	0.0		0.4		<u>0.6</u>	15.0

^a Assumptions are as follow. (i) Resistance inhibited the development of females, but not of males. (ii) Mating was random. (iii) Virulence was dominant over avirulence (6). (iv) The difference in virulence between Pa2 and Pa3 to ‘Elles’ and ‘Darwina’ was caused by one virulence gene only. (v) Pa2 was fixed (‘pure’) with respect to the avirulence allele (AA). The low reproduction rate of Pa2 on ‘Elles’ and ‘Darwina’ (Table 1) was negligible. (vi) Pa3 was fixed for the virulence gene (aa). The small increase in pathogenicity of Pa3 on ‘Darwina’ (Fig. 1) was negligible. (vii) The resistance of ‘Elles’ and ‘Darwina’ to the Pa3 population is assumed to have been caused by partial resistance genes, to which the Pa2 and Pa3 populations harbored no virulence genes.

^b The ratio of the eighth and fifth column.

^c The underlined figures show the modeled change in virulence to the resistant host.

lence can be understood from Table 5. Although the frequency of the alleles *A* and *a* did not alter during rearing on the susceptible cultivar, these alleles were rearranged over the different genotypes *AA*, *Aa*, and *aa* according to the Hardy-Weinberg equilibrium. Consequently, virulence decreased.

The rearrangement of the alleles to the Hardy-Weinberg equilibrium explains, to some extent, the reduction in virulence. If the difference between the selected and nonselected Pa2/3 populations would have been ascribed to selection for virulence solely, neglecting the Hardy-Weinberg equilibrium on the susceptible cultivar, then the rate of selection for virulence would have been overestimated dramatically. It should be realized that this overestimation of selection may also occur if a field sample that contains cysts from different populations is reared in glasshouses. This effect is even stronger if virulence is determined by more than one gene, especially if these genes are located on different chromosomes.

The rate of selection for virulence on a resistant cultivar can be modeled too (Table 5). In Figure 2, the modeled courses are compared with the observed courses. Strikingly, the observed and modeled courses are rather similar, although the model is simple and starts from rigid assumptions (listed above). The similarity between the predicted courses and the observed courses indicates that the explanation from the model for the observed changes in virulence is plausible. Apparently, the model predicted rather well the reduction in virulence caused by the Hardy-Weinberg equilibrium during rearing on a susceptible host. Also, the model predicted the selection to virulence during rearing on a resistant host rather well. The similarity, however, does not prove that all assumptions in the model were realistic. The minor deviations in Figure 2 underline this restriction.

Conclusions that can be drawn from this study include the following:

- Virulence to 'Karakter' exists in the Netherlands, although 'Karakter' has been denoted officially (3) to be resistant to both pathotypes of *G. pallida* that are present in this country (Pa2 and Pa3). Apparently, the pathotype classification of *G. pallida* is more complicated than the scheme of Kort et al. (13) has suggested.
- 'Karakter' caused significant selection for virulence, although its resistance is based on at least several genes (Table 1).
- Turner (23) and Turner et al. (24) showed that the potato clone SVP (Vt^3)² 62.33.3 can provoke selection for virulence. We confirmed this with cultivars derived from this genitor, i.e., with the 'Elles' and 'Darwina'.
- The increase in pathogenicity was not caused by selection

Fig. 2. The solid lines represent the frequency of virulent nematodes *P(aa)*, according to the model described in Table 5. The dotted lines represent the observed virulence of the Pa2/3 population relative to the Pa3 population ('Elles', —; 'Darwina', ×). The upwards lines refer to rearing on a resistant host, and the downward lines to rearing on a susceptible host.

for aggressiveness, but by selection for virulence. The increase in virulence was caused by an increasing proportion of eggs that were able to develop into cysts, and not by an increasing number of new eggs per new cyst.

- Increase in virulence did not cause loss of aggressiveness.
- The rate of selection depended strongly on the *G. pallida* population.
- The Pa2/3 population had a high within-population variance with respect to virulence and, therefore, was more sensitive to selection than the Pa3 population. Analogously, Fisher (5) stated in his 'fundamental theorem of natural selection' that the rate of increase of mean fitness is equal to the genetic variance in fitness within that population.
- Rearing of the mixed population on the susceptible cultivar changed its virulence, probably because of rearrangement of the virulence alleles among the different genotypes according to the Hardy-Weinberg equilibrium.
- Heterosis occurred after mixing of the Pa2 and Pa3 population.
- The observed changes in virulence corresponded reasonably well to a simple population genetic model.

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LITERATURE CITED

1. Beniers, J. E. 1995. Methode voor Bepaling van de Relatieve Vatbaarheid van Aardappelrassen voor Aardappelcysteaaltjes in de Kas. Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Wageningen, Holland.
2. Caligari, P. D. S., and Phillips, M. S. 1984. A re-examination of apparent selection in *Globodera pallida* on *Solanum vernei* hybrids. Euphytica 33:583-586.
3. Düringhof, H. A. 1995. Naamlijst A.M.-resistente aardappelrassen 1995. Nederlandse Staatscourant 28(8 febr.):12.
4. Ebskamp, A. G. M., and Bonthuis, H. 1996. 71st List of Varieties of Field Crops 1996. De Boer, Hilversum, Holland.
5. Fisher, R. A. 1930. The Genetical Theory of Natural Selection. 1st ed. Clarendon, Oxford.
6. Janssen, R., Bakker, J., and Gommers, F. J. 1992. The effect of heterozygosity for virulence on the development of the potato cyst nematode, *Globodera rostochiensis*. Fundam. Appl. Nematol. 15:463-466.
7. Jones, F. G. W. 1979. The problems of race-specificity in plant resistance breeding. Pages 741-752 in: Proc. 1979 Br. Crop Prot. Conf. Pests Dis., Vol. 3. British Crop Protection Council, London.
8. Jones, F. G. W. 1985. Modelling multigenic resistance to potato cyst nematodes. EPPO (Eur. Mediterr. Plant Prot. Organ.) Bull. 15:155-166.
9. Jones, F. G. W., Parrott, D. M., and Perry, J. N. 1981. The gene-for-gene relationship and its significance for potato cyst nematodes and their solanaceous hosts. Pages 23-36 in: Plant Parasitic Nematodes. Vol. III. B. M. Zuckerman and R. A. Rohde, eds. Academic Press, Inc., New York.
10. Jones, F. G. W., Parrott, D. M., and Ross, G. J. S. 1967. The population genetics of the potato cyst-nematode *Heterodera rostochiensis*: Mathematical models to simulate the effects of growing eelworm-resistant potatoes bred from *Solanum tuberosum* ssp. *andigena*. Ann. Appl. Biol. 60: 151-171.
11. Jones, F. G. W., and Pawelska, K. 1963. The behaviour of populations of potato-root eelworm (*Heterodera rostochiensis* Woll.) towards some resistant tuberous and other *Solanum* species. Ann. Appl. Biol. 51:277-294.
12. Jones, F. G. W., and Perry, J. N. 1978. Modelling populations of cyst-nematodes (Nematoda: Heteroderae). J. Appl. Ecol. 15:349-371.
13. Kort, J., Ross, H., Rumpenhorts, H. J., and Stone, A. R. 1977. An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. Nematologica 23:333-339.
14. Mood, A. M., Graybill, F. A., and Boes, D. C. 1974. Introduction to the Theory of Statistics. 3rd ed. McGraw-Hill, New York.
15. Rieger, R., Michaelis, A., and Green, M. M. 1991. Glossary of Genetics. Classical and Molecular. 5th ed. Springer-Verlag, Berlin.
16. Schouten, H. J. 1994. Preservation of avirulence genes of potato cyst nematodes through environmental sex determination: A model involving complete, monogenic resistance. Phytopathology 84:771-773.

17. Schouten, H. J. 1994. Don't shut the door on avirulent nematodes. Durability of resistance to potato cyst nematodes. *Prophyta* 48(2):26-27.
18. Seinhorst, J. W. 1964. Methods of extraction of *Heterodera* cysts from not previously dried soil samples. *Nematologica* 10:87-94.
19. Seinhorst, J. W. 1974. Separation of *Heterodera* cysts from dry organic debris using ethanol. *Nematologica* 20:367-369.
20. Seinhorst, J. W. 1995. Selection by resistant potato cultivars in populations of *Globodera rostochiensis*, pathotypes Ro 1 and Ro 3. *Nematologica* 41:67-79.
21. Seinhorst, J. W., and Den Ouden, H. 1966. An improvement of Bijloo's method for determining the egg content of *Heterodera* cysts. *Nematologica* 12:170-171.
22. Spitters, C. J. T., and Ward, S. A. 1988. Evaluation of breeding strategies for resistance to potato cyst nematodes using a population dynamic model. *Euphytica* 42(Suppl.):87-98.
23. Turner, S. J. 1990. The identification and fitness of virulent potato cyst-nematode populations (*Globodera pallida*) selected on resistant *Solanum vernei* hybrids for up to eleven generations. *Ann. Appl. Biol.* 117:385-397.
24. Turner, S. J., Stone, A. R., and Perry, J. N. 1983. Selection of potato cyst-nematodes on resistant *Solanum vernei* hybrids. *Euphytica* 32:911-917.
25. van der Burgt, A. M., Schouten, H. J., and Bakker, J. 1995. Virulentie van aardappelcysteaaltjes en teelt van resistente rassen in N.O.—Nederland van 1973 tot 1991. *Gewasbescherming* 26:3-9.
26. van der Plank, J. E. 1969. Pathogenic races, host resistance, and an analysis of pathogenicity. *Neth. J. Plant Pathol.* 75:45-52.