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Selection for virulence of *Globodera pallida* by potato cultivars

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Summary – In two fields infested with *Globodera pallida*, plots were laid out in which the susceptible potato cultivar Mentor, the slightly resistant cv. Elkana, or the resistant cv. Darwina were grown continuously. At the start of the experiment no significant differences in virulence were found within the fields. After 8 years, the relative susceptibility of cv. Darwina was on average 8.5 % to the *G. pallida* populations from the Mentor-plots, but 30 % to the populations from the Darwina-plots. The virulence of the *G. pallida* populations from the Darwina-plots had increased also significantly to SVP $(Vt^n)^2$ 62.33.3, one of the parents of cv. Darwina. Continuous cultivation of cv. Elkana did not increase virulence significantly. There was no significant correlation between virulence and reproduction rate on a susceptible cultivar.

Résumé – ***Sélection de* Globodera pallida *en vue de la virulence au moyen de cultivars de pomme de terre*** – Dans deux champs infestés par *Globodera pallida* des parcelles ont été établies sur lesquelles ont été cultivés en continu les cultivars de pomme de terre suivants : Mentor (sensible), Elkana (légèrement résistant) et Darwina (résistant). Au début de l'expérience, aucune différence significative de virulence n'est observée entre les deux champs. Après 8 années, la sensibilité relative du cv. Darwina correspondait à 8,5 % en moyenne de la population de *G. pallida* des parcelles du cv. Mentor, mais à 30 % de la population des parcelles du cv. Darwina. La virulence des populations de *G. pallida* des parcelles du cv. Darwina a également augmenté significativement par rapport à SVP (VT^m) 62.33.3, l'un des parents du cv. Darwina. La culture continue du cv. Elkana n'augmente pas significativement la virulence. Il n'apparaît pas de corrélation significative entre la virulence et le taux de reproduction sur un cultivar sensible.

Key-words : *Globodera pallida*, resistance, virulence.

Since 1968 starch potato cultivars with resistance to the potato cyst-nematode *Globodera rostochiensis* have been grown in the northeastern Netherlands. However, these cultivars appeared to have hardly any resistance to the potato cyst-nematode *G. pallida*. Therefore, new cultivars, derived from the *Solanum vernei*-hybrid SVP $(Vt^n)^2$ 62.33.3, were introduced. These cultivars have resistance to a restricted range of *G. pallida*-populations in the Netherlands.

In order to gain insight into the durability of resistance to *G. pallida* in the field an experiment was conducted to test whether cultivation of resistant cultivars gave rise to an increase in virulence of *G. pallida*, and thus to an increase in susceptibility of the cultivars. Further, it was examined whether increased virulence was associated with decreased reproduction on a completely susceptible cultivar.

In 1983 the experiment was started in eight fields that were infested with *G. pallida* but not with *G. rostochiensis* (Hendriks, 1988). In each field plots were laid out, in which one potato cultivar was grown continuously in a randomized block design with four replicates per field, and ten to twelve cultivars per replicate. After four years, pathotype tests indicated that in one of the fields selection for increased virulence had occured. Before the continuous cultivations started, no significant differences ($P < 0.05$) in virulence occurred between populations in the Mentor-plots, Elkana-plots, and Darwina-plots within a field. Therefore the difference in virulence after the continuous cultivations can be ascribed to difference in selection pressure by the three cultivars. Because further selection to virulence was thought to be hazardous, the field experiment was stopped (Hendriks, 1988).

Materials and methods

CONTINUOUS CULTIVATION

However, for two fields in which the *G. pallida* populations did not show a significant increase in virulence, the continuous cultivation, in 1983 initiated by Hendriks, was carried on as follows : from the two fields, soil was dug from the plots in which the cvs Mentor, Elkana, and Darwina had been grown continuously. The sources of resistance of these cultivars are given in Table 1. The soil was put into 1 m^2 boxes, thus creating small new plots in order to continue the cultivation of the cultivars outdoors. The soil was kept separate by field and cultivar (two fields × three cvs), but the replicates were mixed. From 1988 till 1990 the cvs Mentor, Elka-

Table 1. *The cultivars used for determination of the virulence of the* Globodera pallida *populations. The sources of resistance are indicated.*

Cultivar	Source of resistance			
	1	2	3	4*
Irene	–	–	–	–
Mentor	–	–	–	–
Elkana	×	×	–	–
SVP $(Vt^n)^2$ 62.33.3	–	×	×	–
Darwina	×	×	×	–
Karakter	×	×	×	×

– = source not used, × = source used

* 1 = *Solanum tuberosum* spp. *andigena* CPC 1673
2 = *S. vernei* spp. *balsii* 2/1
3 = *S. vernei* CPC 2488-3 × *S. vernei* CPC 2487-3
4 = *S. vernei* LGU8 × *S oplocense.*

na, and Darwina were grown in the six microplots, one cultivar per microplot.

Virulence assessments

In the spring of 1991 cysts were sampled from the microplots for accurate estimations of virulence and fitness. The reproduction rates of the six *G. pallida* populations on a series of cultivars were determined according to the method described by Seinhorst (1984). The experiment was made with four replicates in a glasshouse. The soil was a mixture of silver sand, hydrogravel, and clay powder in the proportion of 12 : 3 : 2. To the soil Steiner nutrition solution (ec = 2) and fertilizer (NPK 12 : 10 : 18) were added. The pots were filled with 10 kg of soil each, and inoculated with an egg suspension of a *G. pallida* population by means of 20 injections per pot. The inoculation density P_i was five eggs per g dry soil for the populations from the Mentor boxes and Elkana boxes. However, there were not enough cysts available from the boxes in which the more resistant cultivar Darwina had grown. Therefore, P_i equalled two eggs per g dry soil for the population from the Darwina boxes. Then in each pot one potato sprout was planted and one stem was allowed to grow. The cultivars used are indicated in Table 1. The day length was 16 hours, daily temperature was 20 to 25 °C and the temperature at night approximately 15 °C. For 14 weeks the moisture content of all pots was adjusted to 15 % twice a week by weight. The number of cysts per inoculated egg, and the number of eggs per new cyst were estimated according to Seinhorst and Oostrom (1984). These date allowed calculation of the number of new eggs per inoculated egg.

The multiplication rate on a resistant cultivar was expressed as a percentage of the multiplication rate on the susceptible cultivar Irene. This percentage is called relative reproduction of the population, or relative susceptibility of the cultivar. The relative susceptibility does not depend on inoculum density if the inoculum density of the resistant cultivar and susceptible cultivar are the same, and if the inoculum density equals 0.1 to 6 eggs per g soil (Seinhorst & Oostrom, 1984).

Fitness

The absolute reproduction rate on the susceptible cultivar Irene was used as measure of fitness.

Results

Selection for virulence

From Table 2 it appears that the *G. pallida* populations from the Darwina-plots were significantly more virulent than the populations from the Mentor-plots and Elkana-plots. The relative susceptibility of Darwina to

Table 2. *Susceptibility of six cultivars to* Globodera pallida *populations from plots in which three potato cultivars had been grown continuously during eight years. The number of new eggs per inoculated egg on the susceptible cultivar Irene is italicized. The relative susceptibility (%) compared to Irene is printed in roman. The standard deviations are put in brackets. The susceptibility refers to the number of new eggs per inoculated egg.*

	Field 1					
Cultivar	Mentor		Elkana		Darwina	
Irene	*18*	*(1)*	*17*	*(3)*	*2.3*	*(0.1)*
Irene	100	(5)	100	(15)	100	(3)
Mentor	61	(12)	58	(10)	59	(12)
Elkana	42	(8)	43	(9)	53	(7)
SVP $(Vt^n)^2$ 62.33.3	13	(3)	15	(3)	44**	(8)
Darwina	7.9	(1.8)	6.5	(1.5)	31**	(1.4)
Karakter	1.1	(0.2)	1.0	(0.7)	2.3	(1.7)
	Field 2					
Cultivar	Mentor		Elkana		Darwina	
Irene	*22*	*(3)*	*17*	*(4)*	*21*	*(7)*
Irene	100	(13)	100	(21)	100	(18)
Mentor	50	(9)	70	(15)	62	(13)
Elkana	42	(6)	46	(11)	56	(14)
SVP $(Vt^n)^2$ 62.33.3	16	(2)	18	(5)	33*	(7)
Darwina	9.1	(1.8)	12	(3)	28**	(6)
Karakter	0.35	(0.12)	0.45	(0.19)	0.67	(0.35)

** ($P < 0.01$), * ($P < 0.05$)
The *G. pallida*-populations from plots in which continuously cv. Darwina was grown were significantly more virulent to SVP $(Vt^n)^2$ 62.33.3 and to cv. Darwina than the populations from the plots in which cv. Mentor or cv. Elkana was grown. Differences in virulence were tested by means of Student's t-test after $\sqrt{}$-transformation, but in case of cv. Karakter the Wilcoxon signed rank test was applied.

the populations from the Darwina-plots was for Field 1 four times greater and for Field 2 three times greater than the populations from the Mentor and Elkana-plots. These populations had also become significantly more virulent to SVP $(Vt^n)^2$ 62.33.3. This *Solanum vernei*-hybrid is a parent of cv. Darwina. The populations from the Darwina-plots showed no significant increase in virulence to the other test cultivars.

The populations from the Elkana-plots did not differ significantly ($P = 0.05$) in virulence from the populations from the Mentor-plots.

Components of virulence

For both fields the increase in virulence of the populations from the Darwina-plots was due to an increase in the percentage of eggs that developed into cysts ($P < 0.01$) (Table 3). For Field 1, but not for Field 2, the virulence had increased additionally by an increase in number of eggs per cyst (Table 4).

Fitness

The more virulent populations from the Darwina-plots and the less virulent populations from the Mentor and Elkana-plots did not differ significantly in fitness in the case of Field 2 ($P < 0.05$) : the absolute numbers of new eggs per inoculated egg on the susceptible cultivar Irene were not significantly different (Table 2, second italicized line).

However, in the case of Field 1 it looks from Table 2 as if the fitness of the populations from the Darwina-plots was far lower than the fitness of the populations from the Mentor and Elkana-plots : the virulent populations from the Darwina-plots produced 2.3 eggs per inoculated egg on cv. Irene, but the populations from the Mentor and Elkana-plots produced 18 new eggs per egg on cv. Irene. After one generation on cv. Irene, however, the reproduction rates no longer differed significantly (Table 5). Probably, the cysts that were sam-

Table 3. *Like Table 2. In this table susceptibility refers to the number of cysts per inoculated egg.*

	Field 1					
Cultivar	Mentor		Elkana		Darwina	
Irene	*0.11*	*(0.01)*	*0.076*	*(0.005)*	*0.011*	*(0.001)*
Irene	100	(8)	100	(7)	100	(4)
Mentor	72	(7)	78	(7)	70	(7)
Elkana	48	(7)	50	(9)	51	(5)
SVP $(Vt^n)^2$ 62.33.3	16	(2)	21	(2)	46**	(4)
Darwina	10	(2)	11	(2)	32**	(2)
Karakter	1.6	(0.2)	1.2	(0.4)	7.4	(5.6)
	Field 2					
Cultivar	Mentor		Elkana		Darwina	
Irene	*0.11*	*(0.01)*	*0.10*	*(0.01)*	*0.11*	*(0.03)*
Irene	100	(8)	100	(12)	100	(9)
Mentor	65	(8)	76	(13)	80	(10)
Elkana	49	(4)	46	(7)	62[1]	(7)
SVP $(Vt^n)^2$ 62.33.3	23	(2)	21	(4)	44	(6)
Darwina	13	(2)	14	(2)	35**	(5)
Karakter	0.51	(0.10)	0.48	(0.16)	0.89	(0.31)

** ($P < 0.01$), [1] ($P < 0.05$); only difference in virulence between the populations from cv. Elkana and from cv. Darwina.
Differences in virulence were tested by means of Student's t-test after log-transformation, but in the case of cv. Karakter the Wilcoxon signed rank test was applied.

Table 4. *Like Table 2. In this table absolute number of eggs per cyst is given.*

	Field 1		
Cultivar	Mentor	Elkana	Darwina
Irene	169 (16)	224 [3] (19)	196 (12)
Mentor	138 (23)	164 (7)	170 (19)
Elkana	147 (15)	194 [3] (12)	213 [1] (4)
SVP $(Vt^n)^2$ 62.33.3	134 (14)	161 (11)	197 [1] (20)
Darwina	127 (15)	136 (5)	191 [1,2] (6)
Karakter	118 (6)	150 (48)	280 (15)
	Field 2		
Cultivar	Mentor	Elkana	Darwina
Irene	204 (13)	173 (24)	184 (31)
Mentor	157 (13)	166 (22)	146 (12)
Elkana	177 (13)	175 (15)	168 (16)
SVP $(Vt^n)^2$ 62.33.3	140 (7)	149 (14)	137 (7)
Darwina	137 (8)	148 (10)	150 (18)
Karakter	129 (27)	150 (20)	116 (19)

[1] ($P < 0.01$), difference in virulence between populations from cv. Mentor and from cv. Darwina.
[2] ($P < 0.05$), difference in virulence between populations from cv. Elkana and from cv. Darwina.
[3] ($P < 0.05$), only difference in virulence between populations from cv. Mentor and from cv. Elkana. Student's *t*-test was applied without data transformation.

However, in the case of Field 1 it looks from Table 2 as if the fitness of the populations from the Darwina-plots was far lower than the fitness of the populations from the Mentor and Elkana-plots : the virulent populations from the Darwina-plots produced 2.3 eggs per inoculated egg on cv. Irene, but the populations from the Mentor and Elkana-plots produced 18 new eggs per egg on cv. Irene. After one generation on cv. Irene, however, the reproduction rates no longer differed significantly (Table 5). Probably, the cysts that were sam-

Table 5. *Reproduction rate on cv. Irene of the* Globodera pallida *populations from Field 1 after eight growing seasons of cv. Mentor, or cv. Elkana, or cv. Darwina, and one extra generation on cv. Irene in pots. The standard deviations are put in brackets.*

	cv. Mentor	cv. Elkana	cv. Darwina
New eggs per inoculated eggs	33.6 (4.1)	33.4 (3.5)	30.1 (3.2)
Cysts per inoculated egg	0.11 (0.01)	0.10 (0.003)	0.089 (0.007)
New eggs per cyst	316 (11)	320 (27)	340 (24)

Student's t-test without transformation was applied. No significant differences were found.

pled from the Darwina-plot contained a high frequency of old eggs. The vitality was recovered by producing new cysts on the susceptible cv. Irene. This indicates that, also in the case of Field 1, there was no genetically determined decrease in fitness.

Discussion

In the breeding programme that gave rise to cv. Mentor no sources of resistance to potato cyst-nematodes were used (Table 1). Therefore cv. Mentor is considered to be susceptible to *G. pallida,* and consequently unlikely to exert a strong selection pressure for virulence during the 8 years of cultivation.

Cultivar Elkana was bred to have resistance to *G. rostochiensis* (Table 1), but this resistance also affects the reproduction of *G. pallida.* In our experiment, the populations reared on cv. Elkana showed no significant increase in virulence. This cv. probably suppressed the reproduction of the different genotypes of *G. pallida* approximately to the same extent. Then, selection for virulence cannot be fast (Spitters & Ward, 1988; Schouten, 1995).

The *S. vernei*-hybrid SVP $(Vt^n)^2$ 62.33.3 differentiates strongly between *G. pallida* populations. For that reason Kort *et al.* (1977) used this genotype as differential within *G. pallida,* and defined the pathotypes Pa2 and Pa3 on the basis of reproduction on SVP $(Vt^n)^2$ 62.33.3. Cv. Darwina, which has SVP $(Vt^n)^2$ 62.33.3 as a parent, has inherited this ability to differentiate. This led to an increase in virulence in both fields. Apparently, in both fields virulence genes and avirulence genes to cv. Darwina were present, and the frequency of virulence genes increased at the expense of the avirulence genes.

Selection by cv. Darwina also increased virulence to SVP $(Vt^n)^2$ 62.33.3. This indicates that alternation in time of two different potato cultivars that have identical resistance genes, does not retard selection for virulence. Alternation of cultivars that have different resistance genes, in contrast, may retard the selection rate considerably (Spitters & Ward, 1988).

Turner (1990) pursued selection experiments for eleven generations with six *G. pallida* populations on SVP $(Vt^n)^2$ 62.33.3. She raised the virulence of several populations to nearly full compatibility with this *S. vernei*-hybrid. In her experiments, every new generation started with newly formed cysts. In contrast, in our experiment the old cysts remained longer in the population, thus reducing the selection rate. As in our experiments, Turner found no decrease in fitness with increasing virulence.

Whitehead (1991) detected selection for increased virulence of some but not all *G. pallida* populations, although the same cultivar was grown. The rate of selection to virulence depends not only on differentiating ability of the cultivar, but also on the frequency of the virulence genes in the *G. pallida* population (Turner *et al.,* 1983; Schouten, 1995).

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