SELECTION OF GENETIC LINES OF *GLOBODERA PALLIDA* WITH DIFFERENT LEVELS OF VIRULENCE TO RESISTANT POTATO GENOTYPES

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Summary. Potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, are major pests of the potato crop. Knowledge of species and virulence groups present in field populations is important in the control of these nematodes by means of resistant cultivars. The choice of appropriate potato genotypes to classify the degree of virulence of PCN populations would be made easier if nematode populations with known levels of virulence could be selected. The main goal of the present work was to select and characterize lines of *G. pallida*, using controlled matings. Lines were selected from three populations of *G. pallida* by making controlled crosses between individuals raised on two partially resistant potato genotypes (cv. Vantage and clone 12380) and the susceptible cv. Désirée. From a total of 531 controlled matings, about 40% fertilized females were obtained. The variation in virulence present in 24 of these lines and two parent *G. pallida* populations was evaluated by measuring their multiplication rates on several host differential genotypes. Reproduction rates were different in terms of both the numbers of eggs and the numbers of cysts produced. It was possible to select a range of "populations" (i.e. lines) with different virulence levels.

Since the introduction of an international pathotype scheme for potato cyst nematodes (PCN) (Kort *et al*., 1977), there have been many evaluations of the shortcomings of the scheme, such as that by Bakker *et al.* (1993). While some nematode pathotypes can be clearly defined, on the basis of their reaction with major resistance genes (Anderson and Anderson, 1982), and a gene-for-gene interaction of a nematode virulence gene with a plant resistance gene has been demonstrated for the H1 resistance gene and a pathotype of *Globodera rostochiensis* (Janssen *et al*., 1991), the situation with the virulence of PCN towards the minor resistance genes, with which plant breeders must work to achieve *G. pallida* resistance, is more complicated. It is now accepted that, in genotypes of *Solanum* spp. that exhibit polygenic resistance to PCN, there is a continuum of resistance matched by a corresponding range in virulence in PCN (Fleming and Powers, 1998). A possible solution to the problem of classifying the degree of virulence of PCN populations would be to assess the virulence on a range of clones that includes standard clones with well-characterized but different degrees of resistance (Mugniery *et al*., 1989). However, the choice of appropriate potato genotypes would, in turn, be made easier if nematode populations with known and stable levels of virulence could be selected.

There have been a number of attempts to select virulent PCN populations. The most successful has been the selection of lines of *G. rostochiensis* completely virulent and completely avirulent towards the H1 gene (Janssen *et al*., 1990). Selection of PCN lines with virulence towards particular partially resistant potato genotypes is a more difficult and time-consuming process. Turner (1990) selected six *G. pallida* populations on two potato genotypes with partial resistance and, by starting each new generation with newly formed cysts, raised the degree of virulence to almost 100% within eleven generations or less. Beniers *et al.* (1995) grew a non-resistant cultivar and two partially resistant cultivars (one with a low and the other with a high degree of resistance) in field plots, infested with *G. pallida*, for eight years. The virulence of the population in plots that grew the highly resistant cultivar had increased towards that cultivar but only to 30% of the value towards the non-resistant cultivar. The results of these experiments reflect the problem of crosses occurring during the selection process between virulent and non-virulent individuals, a problem that is more pronounced in field-based selection, where unselected individuals survive between crops in old cysts (Beniers *et al*., 1995). Selection by Turner (1990) used only new cysts for each generation but did not exclude the possibility that some non-virulent individuals were present within cysts.

Schouten (1994) made a theoretical analysis of the selection for virulence of PCN on partially resistant hosts, suggesting that females would develop only from juveniles fully compatible with the host. Males, on the other hand, will occur more frequently when the proportion of avirulent individuals in a population is greater. This will slow the selection for virulence in a population or even prevent complete selection for virulence.

As a consequence of the conclusions reached by others, it can be reasoned that completely virulent popula-

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tions could only arise when fully virulent females were mated by fully virulent males. As it is likely that females are mated by many males (Evans, 1970), the likelihood of a virulent female being mated only by virulent males would be greater if access to the female were restricted to one male. Further, as the sex ratio in PCN is shifted towards maleness when the density of nematodes in roots is high (Trudgill, 1967), it is more likely that males of virulent genotypes will be produced when males are reared under crowded conditions in potato roots. The use of a non-resistant cultivar will remove selection pressure, so that virulent individuals are as likely to become male as female. If virulence occurs at a low frequency in a population, and the population is exposed to a resistant cultivar, then all the virulent individuals will become female and none will become male, hence the importance of using a non-resistant cultivar for the production of virulent males. Using a high population density increases the number of individuals that become male, overcoming the tendency to become female in individuals where virulence is linked to this tendency and thereby increasing the number of males with virulent genotypes. These hypotheses were tested in the production of lines of *G. pallida* from single male × single female crosses, having raised the males and females under conditions likely to maximize the frequency with which individuals fully virulent towards partially resistant potato clones occur.

MATERIALS AND METHODS

Nematode populations

Three populations of *G. pallida* provided by Rothamsted Research, Harpenden, UK, were used: Woodwalton not selected (WNS) from Cambridgeshire, UK; Woodwalton selected on ZC 83/6 (WZC, the Woodwalton population after exposure to four successive crops in four successive years of potato clone ZC 83/6, with resistance from *Solanum tuberosum* ssp. *andigena* CPC 2802); and Luffness (L) from Scotland, UK. The selection of the Woodwalton population on ZC 83/6 was done in microplots and had increased its virulence to 50% towards this clone (Whitehead, 1991a). Luffness has an intrinsically high level of virulence towards potato genotypes with partial resistance to *G. pallida* (Blok *et al.*, 1997). The three populations were multiplied on the non-resistant cultivar Désirée to provide a stock of more than 1,000 cysts of each population.

Potato genotypes

For the selection procedure, three potato genotypes were used: Désirée, the standard non-resistant genotype; Vantage with moderate resistance to *G. pallida*; and 12380 with high levels of resistance to *G. pallida* (Mugniery *et al.*, 1989). Both of the resistant lines derive their resistance from *S. vernei*. The choice of genotypes was conditioned by the EPPO recommendations suggesting that certain potato cultivars and clones should be adopted as international standards for characterisation of PCN virulence (Mugniery *et al.*, 1989). Clone ZC 83/6 was not included in the selection procedure because this potato clone has been lost.

Production of females

Plants of the two partially resistant potato genotypes (cv. Vantage and clone 12380) were grown in 10-cm diameter plastic pots (500 cm^3) with a mixture of sterilised soil and sand. For each population, several samples of 50 cysts each were kept for 7 days in water and then transferred to potato root diffusate to obtain hatched second stage juveniles (J2). About eight days after planting, the soil around each of 30 plants was inoculated with 1,800 freshly hatched J2 of one of the three PCN populations. Eighteen days after inoculation, the plants were carefully removed from the pots, washed free of soil and transferred to 15-cm diameter glass funnels filled with tap water and covered with aluminium foil to exclude light and prevent the growth of algae. The water was aerated by means of an aquarium pump. In these conditions, the males fall away from the roots as soon as they emerge and are unable to fertilise the females (Green *et al.*, 1970). The water was replaced every two days and the males removed. From 24 days onwards, females were removed from the roots with fine tweezers and washed three times with sterile distilled water before use.

Production of males

A similar system to that for the production of females was used for the production of males. Fifteen plants of the genotype Désirée were used and each plant was inoculated with 6,000 freshly hatched J2, to increase the density of nematodes within roots and thereby increase the number of males with virulent genotypes. Males were collected daily, to avoid loss of vitality in potentially non-aerated water at the bottom of the stems of the funnels, and washed three times with sterile distilled water before use.

Single male × single female crosses

Agar plates were prepared in sterile 10-cm diameter plastic Petri dishes, from a 2% solution of Difco Agar Noble. The plates were poured in a laminar flow cabinet to maintain sterility and, after setting, a small depression was made in the centre with a heated sterile glass rod. The dishes were sealed with Parafilm® and kept at ambient temperature until used (within two or three days).

The unfertilised females removed from roots were placed, one per dish, in the small central depression that had been made in the agar. A single male was placed next to each female with a fine nylon bristle attached to a mounted needle. The dishes were then re-sealed and kept in an incubator at 20 °C, in the dark. Any dishes that developed bacterial or fungal contamination were

Table I. Numbers of females obtained from crosses between *Globodera pallida* females, raised either on Vantage or 12380 genotypes inoculated with 1,800 second stage juveniles (J2), and males, raised on Désirée inoculated with 6,000 J2.

Host and Population	Females obtained (Nº)	Females	
		with eggs Nº (%)	without eggs Nº (%)
Vantage			
WNS	33	15 (45)	18 (55)
WZC	79	34 (43)	45 (57)
L	31	14 (45)	17 (55)
Total	143	63	80
12380			
WNS	53	36 (68)	17 (32)
WZC	215	47 (22)	168 (78)
L	120	63 (52)	57 (48)
Total	388	146	242
GRAND TOTAL	531	209 (40)	322 (60)

WNS - Woodwalton not selected
WZC - Woodwalton selected on ZC83/6
L - Luffness

discarded. Three months after making the crosses, the females that contained eggs were transferred individually to glass blocks containing tap water. After a week, the water was replaced by potato root exudate. The hatched J2 were multiplied on the cultivar Désirée, to establish individual genetic lines from each fertilised female, for as many generations as were required to provide more than 1,000 cysts for subsequent experimental work. The host plants were grown either on agar in Petri dishes (Mugniery and Person, 1976) or in closed plastic containers containing a mixture of sterile soil and sand (Foot, 1977).

The virgin females, obtained from the two partially resistant potato genotypes, were mated with males from the same population that had been raised on Désirée. A total of 531 individual crosses were made, 143 with females from Vantage and 388 with females from 12380 (Table I). About 40% of the crosses were successful, yielding 209 females with eggs, 63 from Vantage and 146 from 12380 (Table I). Multiplication of these 209 lines on Désirée resulted in further losses due to fungal contamination or other reasons. Losses were particularly high when Petri dishes were used (76%), and somewhat lower when plastic containers were used (62.5%). Only 42 lines were multiplied successfully (for up to six generations) to yield the target of 1,000 cysts for further experiments: 29 from females raised on 12380 (twelve from WNS, twelve from WZC, and five from L), and thirteen from females raised on Vantage (one from WNS, six from WZC, and six from L).

Assessment of degree of virulence of genetic lines

From the 42 lines (Table II), only 24 were used for the virulence assay because of the available number of cysts. Some of the remaining lines started to decline after the third or fourth generation, possibly due to inbreeding (Janssen, 1990). It had been intended to use a balanced sub-set of populations for this work but losses were heavier than expected. The assay was performed on plants of the genotypes Désirée, Vantage and 12380 grown in plastic pots containing 800 g of sterile sandy soil. A single potato sprout on a hemispherical piece of tuber cut from a seed tuber was planted in each pot. Each pot was inoculated with cysts contained in a small polyester bag, to give approximately 5 eggs/g of soil. The Pi was assessed by counting five replicates of a suspension of eggs and J2 obtained by crushing 50 cysts of each line/population in water. Pots were arranged in a randomized complete block design with fourfold replication of each population × host combination and kept at 18 °C, with a photoperiod of 16 hours. Fertiliser was added to the pots and, fifteen weeks after planting, the tops of the plants were removed and the soil dried. The bags containing the inoculum were removed and the new cysts were extracted with a modified Fenwick can (Shepherd, 1986), counted, crushed, and the number of eggs per plant estimated only if more than 25 cysts were produced in the four replicates. The virulence of the original populations was also assessed using the same

Table II. Codes of the genetic lines raised from single male × single female crosses and subsequently used for the virulence assay.

Original population	Genetic line codes	
	Females raised on 12380	Females raised on Vantage
WNS	WNS 7, WNS 12, WNS 13, WNS 20, WNS 25, WNS 27, WNS 31, WNS 33	WNSV 13
WZC	WZC 12, WZC 31, WZC 34, WZC 36, WZC 39	WZCV 4, WZCV 11, WZCV 26, WZCV 41
L	L 36, L 63	LV 5, LV 13, LV 16, LV 17

WNS - Woodwalton not selected
WZC - Woodwalton selected on ZC 83/6
L - Luffness

methodology. Unfortunately, no results were obtained for the Luffness population because three of the four replicate plants died during the experiment.

Statistical analysis

The cyst and egg counts were subjected to logarithmic transformation to normalise the variances. Cyst production on the two partially resistant genotypes was also calculated as a percentage of that produced on Désirée and analysed after angular transformation. Analyses of variance were performed after appropriate transformations of data from cysts, eggs, fecundity, mean multiplication rate (calculated for cysts and eggs) and absolute multiplication rates (calculated for cysts and eggs) using *STATISTICA*, version 5.0 (Statsoft, 1997).

RESULTS

Significant differences were found between the nematode lines and the potato genotypes, the variation being much higher in the genotypes (Table III). The dissimilarities observed in the potato genotypes were expected due to their large differences in terms of resistance. There was also much variation between the nematode lines, and the differences observed were related to the potato genotype used because the lines reacted differently to the different potato genotypes.

A great variation in the numbers of cysts among *G. pallida* lines was observed over the three potato genotypes (Table IV). All the lines produced cysts on all the genotypes. As expected, it was on Désirée that most cysts were produced, except for LV 13, where the number of cysts on Désirée was similar to that obtained on Vantage. The lowest values overall were found on 12380, the genotype with the highest resistance, and on which the numbers of cysts were lower or similar to those produced on Vantage (Table IV).

The numbers of eggs produced ranked in the same order as did the numbers of cysts, i.e. most on Désirée and fewest on 12380 (Table IV). In nine lines, the number of eggs obtained on 12380 was less than the number of eggs inoculated (Pi = 4,000). On Vantage, only WNS 27 produced fewer eggs than were inoculated, although in WNS 31 and WZCV 11 the numbers of eggs produced were only slightly higher than the Pi. On Vantage, the number of eggs produced by WNS 7 was higher than on Désirée.

Fecundity (the number of eggs per cyst) differed significantly among *G. pallida* lines on all potato genotypes (Table V). On Désirée, it ranged from 62 (WNS 13) to 214 (WZC 36); on Vantage from 67 (WZCV 11) to 238 (WNS); and on 12380 from 48 (WZC 31) to 199 (L 63).

For all lines, nematode increase was higher on Désirée because many more cysts were produced on this genotype. On Vantage, only WNS 27 had a reproduction rate less than one. On 12380, lines selected from Luffness (L 36 and LV 13) had the highest values and only one (LV 17) had a reproduction rate slightly below one (Table VI). The lowest nematode reproduction on 12380 generally was observed in lines selected from WZC, except for WZC 34 and WZCV 4, which had greater reproduction than the original WZC population. Reproduction rates based on cysts ranged from 10.1 (WZC 12) to 41.8 (WNS 12) on Désirée; from 1.0 (WNS 20) to 19.7 (LV 13) on Vantage; and from 0.1 (WZCV 26) to 3.0 (LV 13) on 12380 (Table VI). Multiplication rates based on eggs varied from 4.0 (WZCV 11) to 53 (LV 5) on Désirée; from 0.6 (WNS 27) to 14.5 (LV 13) on Vantage; and from 0.1 (WZC 31) to 3.8 (LV 5) on 12380 (Table VI). On 12380, the reproduction rate based on the number of eggs was not determined in some lines due to the small numbers of cysts produced (<25 cysts/4 replicates).

The multiplication rates for cysts relative to the number produced on Désirée ranged from 3.2 (WNS 27) to 92.2 (LV 13) on Vantage, and from 0.3 (WNSV 13) to 14.2 (LV 13) on 12380 (Table VII). Comparing the behaviour of the lines with the original populations, the results revealed that, for WNS, only three lines from Vantage had higher (but not significantly) values (WNS 7, WNS 12 and WNS 25) and, for WZC, all the lines

Table III. Percentage of variation explained by the analysed factors (nematode populations and potato genotypes) after ANOVA.

Parameter	Nematode line/population (%)	Potato genotypes (%)	Interaction line/population ×genotype (%)
Nº of cysts	12.61	74.63	12.76
Nº of eggs	22.39	45.97	31.64
Fecundity	26.32	14.26	59.42
Mean multiplication rate (cysts)	6.88	83.93	9.19
Mean multiplication rate (eggs)	12.19	74.18	13.63
Relative multiplication rate (cysts)	28.83	48.01	23.16
Relative multiplication rate (eggs)	39.73	31.75	28.52

Table IV. Numbers of cysts and eggs produced by *G. pallida* lines/populations on three differential potato genotypes inoculated with 4,000 second stage juveniles/pot.

Line/population codes	Differential potato genotypes					
	Désirée		Vantage		12380	
	cysts	eggs	cysts	eggs	cysts	eggs
WNS 7	392 ab	28,110 b	157 bcde	32,239 mn	31 cdefg	5,140 gh
WNS 12	1,422 b	158,099 opqr	502 e*	47,590 op	34 bcdefg	5,333 hi
WNS 13	463 ab*	28,912 b	49 abcd	5,255 bc	36 cdefg	7,149 jl
WNS 20	719 ab*	55,794 defg	42 abc	5,729 bcd	49 cdefg	8,924 l
WNS 25	560 ab	50,131 def	152 bcde	25,039 lm	15 bcdefg	1,581 d
WNS 27	610 ab	101,180 jlm	20 a	2,346 a	5 abc*	— a
WNS 31	751 ab*	73,115 ghi	59 abcd	4,728 b	46 defg	7,762 jl
WNS 33	400 ab	46,201 cd	43 ab	7,616 ef	16 bcdefg	1,528 d
WNSV 13	915 ab	176,277 qr	76 abcde*	13,609 hi	3 ab	— a
WZC 12	444 ab	34,898 bc	160 bcde	18,087 ij	11 abcdef	682 c
WZC 31	861 ab	118,405 lmno	206 bcde	33,044 mn	10 abcd*	462 b
WZC 34	753 ab*	152,150 hopq	159 bcde	36,518 no	60 fg	6,381 hij
WZC 36	426 ab*	91,320 ijl	74 abcde	11,437 gh	29 cdefg	3,039 ef
WZC 39	528 ab	34,954 bc	117 bcde	16,217 ij	14 abcde	1,496 d
WZCV 4	585 ab	42,201 cd	106 abcde	8,946 fg	60 cdefg	7,464 jl
WZCV 11	193 a	16,116 a	72 abcde	4,791 b	3 ab*	— a
WZCV 26	409 ab	47,415 cde	69 abcde	7,480 def	2 a	— a
WZCV 41	1,061 b	162,970 pqr	140 bcde	19,956 jl	23 bcdefg	2,558 e
L 36	707 ab*	134,754 mnopq	70 abcde	10,383 gh	51 defg*	6,820 ijl
L 63	977 b	114,251 lmn	78 abcde	18,076 ij	61 fg	12,089 m
LV 5	1,462 b	211,918 r	82 abcde*	6,521 cde	92 g	15,377 m
LV 13	406 ab	62,888 efgh	375 de	57,973 p	58 efg	3,604 f
LV 16	1,005 ab*	129,885 mnop	253 cde	33,345 n	64 fg	7,905 jl
LV 17	626 ab	75,776 hij	190 bcde	17,837 ij	26 bcdefg	3,883 fg
WNS	396 ab	67,162 fgh	65 abcde	15,364 ij	3 ab	— a
WZC	799 ab	135,766 mnopq	342 de	41,314 no	42 cdefg	3,343 ef

*The numbers of cysts are means of three (*) or four replicates. The numbers of eggs are calculated from the means of five counts.
After logarithmic transformation [$\log_{10}(x+1)$] figures in the same column sharing a letter are not significantly different ($P<0.001$) according to Tukey's test (Honestly significant Difference Test).
— not determined.

had lower values. For Luffness, all the lines except LV 5 produced fewer cysts on 12380 than on Vantage, and LV 5 had a relative multiplication rate inferior to WNS and WZC on Vantage; LV 16 and LV 17 on Vantage had relative multiplication rates inferior to WZC but higher than WNS; and LV 13 had values much higher than the original populations tested.

The relative multiplication rates based on the number of eggs ranged from 2.3 (WNS 27) to 114.7 (WNS 7) on Vantage and from 0.4 (WZC 31) to 24.7 (WNS 13) on 12380 (Table VII). The results obtained on Vantage were similar to those obtained for cysts, with only WNS 7, WNS 12 and WNS 25 showing higher values than WNS and only WZC 12 and WZC 39 showing higher values than WZC; for Luffness, LV 13 on Vantage had a value (91.8%) close to that on Désirée. On 12380, all the lines from Luffness had higher values than the original populations (WNS and WZC). Those lines produced fewer cysts (Table IV) but the fecundity was greater on 12380 when compared to the other two genotypes (Table V).

Populations with a multiplication rate higher than one were considered as virulent. Therefore, all lines were virulent on Désirée; only WNS 27 was avirulent on Vantage and WNS 7, WNS 13, WNS 20, WNS 31, WZC 34, WZC 36, WZCV 4, L 36, LV 5, LV 13 and LV 16 were virulent on 12380 (Table VI).

On Vantage only WNS 27 had a multiplication rate lower than 5%, although, WNS 20 and LV5 had values around five (5.8 and 5.6, respectively). LV 17 revealed a higher multiplication rate on Vantage (92.2 for cysts and 91.8 for eggs) and a low multiplication rate on 12380 (14.2 for cysts and 5.7 for eggs). On 12380 WNS 7, WNS 13, WNS 20, WNS 31, WZC 34, WZC 36, WCZV 4 and all lines from Luffness, except LV17, had a multiplication rate higher than 5% (Table VII).

Table V. Fecundity* of *G. pallida* lines/populations on three differential potato genotypes inoculated with 4,000 second stage juveniles/pot.

Line/population codes	Differential potato genotypes		
	Désirée	Vantage	12380
WNS 7	72 ab	206 bcdefg	166 ghij
WNS 12	111 def	95 cdefg	158 ghij
WNS 13	62 a	108 abcdef	197 ij
WNS 20	78 abc	136 defg	181 ij
WNS 25	90 bcd	165 fghi	109 ef
WNS 27	166 ijlm	119 bcdefg	— a
WNS 31	97 cde	80 abc	171 hij
WNS 33	119 efg	179 ghi	99 de
WNSV 13	193 lmn	178 ghi	— a
WZC 12	79 abc	113 bcdefg	61 bc
WZC 31	138 fghij	161 fghi	48 b
WZC 34	202 mn	230 hi	107 ef
WZC 36	214 n	156 efghi	105 def
WZC 39	66 a	139 efg	111 ef
WZCV 4	72 ab	84 abcd	124 efg
WZCV 11	97 cde	67 a	— a
WZCV 26	116 efg	109 abcdefg	— a
WZCV 41	154 hijl	143 efgh	110 ef
L 36	191 lmn	149 efghi	135 fgh
L 63	117 efg	231 hi	199 j
LV 5	145 ghij	79 ab	168 hij
LV 13	155 hijl	155 efghi	62 bc
LV 16	129 fghi	132 defg	124 efg
LV 17	121 efgh	94 abcde	151 ghi
WNS	170 jlmn	238 i	— a
WZC	170 jlmn	121 bcdefg	80 cd

* Fecundity = Number of eggs produced/cyst.
After logarithmic transformation [($\log_{10}$(x+1)] figures in the same column sharing a letter are not significantly different (P<0.001) according to Tukey's test (Honestly significant Difference Test).
— not determined.

The analysis of variance showed that there were significant main effects of lines and potato genotypes and also significant effects of the interactions between lines and potato genotypes.

DISCUSSION

Relatively few females for crosses were obtained on Vantage (Table I). These plants grew poorly compared to the other clones and had few roots, small aerial parts and root systems that frequently rotted. Rotting of these plants was aggravated when the roots were kept in water inside the funnels.

The percentage of fertilized females (40%) was similar to that obtained by Green *et al.* (1970) (Table I). However, higher values (63%) were obtained in 1990 by Janssen *et al.* (1990). This disparity can in part be explained by differences in the methodology. Janssen *et al.* (1990) made the crosses with females on roots of sprouts grown on agar plates. In our work the females were removed from the roots and placed on agar. Females removed from roots could be injured by handling and/or deprived of food. Another explanation could be that the females were collected too early from roots. Green *et al.* (1970) noted that immature females are un-

Table VI. Mean reproduction rates* of *G. pallida* lines/populations on three differential potato genotypes inoculated with 4,000 second stage juveniles/pot.

Line/population codes	Differential potato genotypes					
	Désirée		Vantage		12380	
	cysts	eggs	cysts	eggs	cysts	eggs
WNS 7	17.8 a	7.0 b	5.4 abcde	8.1 fghij	1.1 abcdef	1.3 fg
WNS 12	41.8 a	39.5 opq	14.8 de	11.9 ij	1.0 abcdef	1.3 g
WNS 13	14.0 a	7.2 b	1.6 ab	1.3 abc	1.2 abcdef	1.8 h
WNS 20	37.8 a	14.0 def	1.0 a	1.4 efghi	1.2 abcdef	2.2 i
WNS 25	21.5 a	12.5 de	5.8 bcde	6.3 abcd	0.6 abcd	0.4 c
WNS 27	29.1 a	25.3 ijl	0.9 a	0.6 a	0.2 abc	— a
WNS 31	26.8 a	18.3 fgh	1.7 ab	1.2 ab	1.3 abcdef	1.9 hi
WNS 33	25.0 a	11.5 cd	2.7 abc	1.9 abcde	1.0 abcdef	0.4 c
WNSV 13	38.1 a	44.1 qr	1.7 ab	3.4 bcdefg	0.1 ab	— a
WZC 12	10.1 a	8.7 bc	5.0 abcde	4.5 cdefghi	0.3 abc	0.2 b
WZC 31	22.1 a	29.6 hlmno	4.2 abcde	8.3 fghij	0.3 abc	0.1 ab
WZC 34	37.6 a	38.0 opq	4.1 abcde	9.1 abcdef	3.0 f	1.6 gh
WZC 36	22.4 a	22.8 hij	3.9 abcde	2.9 abcdef	1.5 cdef	0.8 de
WZC 39	19.6 a	8.7 bc	1.9 ab	4.1 bcdefgh	0.5 abc	0.4 c
WZCV 4	19.5 a	10.6 cd	3.5 abcd	2.2 abcde	2.0 cdef	1.9 hi
WZCV 11	10.7 a	4.0 a	4.0 abcde	1.2 ab	0.2 abc	— a
WZCV 26	16.4 a	11.9 cde	2.8 abcd	1.9 abcde	0.1 a	— a
WZCV 41	37.9 a	40.7 pqr	4.7 abcde	5.0 defghi	0.8 abcd	0.6 d
L 36	33.7 a	33.7 nopq	3.3 abcd	2.6 abcde	2.4 def	1.7 h
L 63	24.4 a	28.6 lmn	2.0 ab	4.5 cdefghi	1.5 cdef	3.0 j
LV 5	36.5 a	53.0 r	2.1 abc	1.6 abcd	2.3 def	3.8 l
LV 13	21.4 a	15.7 efg	19.7 e	14.5 j	3.0 ef	0.9 de
LV 16	18.3 a	32.5 mnop	4.6 abcde	8.3 ghij	1.2 abcdef	2.0 hi
LV 17	21.6 a	18.9 ghi	6.5 bcde	4.5 cdefghi	0.9 abcde	1.0 ef
WNS	13.7 a	16.8 fg	2.2 abc	3.8 abcdefgh	0.1 a	— a
WZC	28.5 a	33.9 mnopq	12.2 cde	10.3 hij	1.5 bcdef	0.8 de

* Reproduction rate (Pf/Pi) = final population (Pf) (number of cysts or eggs)/ initial population (Pi) (number of cysts or eggs).
After logarithmic transformation [$\log_{10}(x+1)$] figures in the same column sharing a letter are not significantly different ($P<0.001$) according to Tukey's test (Honestly significant Difference Test).

attractive to males or infertile. However, later collection ran the risk that, because of the rapid rotting of the roots, the females would not be in good condition.

Several authors have drawn attention to the fact that, when resistant cultivars are used continuously to control PCN populations, without previous study of the virulence of the populations concerned, there is the risk of selection for virulence in those populations (Turner, 1990; Whitehead, 1991a). WZC generally produced more females than WNS on both of the resistant potato genotypes, suggesting that there was a selection for virulence during the development of the four generations on ZC 83/6.

During the initial multiplication phase, several nematode lines were lost due to the low number of eggs found inside the cysts (e.g. line WNSV 2 with just 8), probably caused by the methodology used. In such cases the number of inoculated J2 was not enough to obtain males or the females had insufficient food reserves to produce many eggs.

Multiplication in Petri dishes allowed cysts to be recovered before dormancy of the J2 set in (Janssen *et al.*,

Table VII. Relative reproduction rate* of *G. pallida* lines/populations on three differential potato genotypes inoculated with 4,000 second stage juveniles/pot.

Line/population codes	Differential potato genotypes			
	Vantage		12380	
	cysts	eggs	cysts	eggs
WNS 7	40.0 bcd	114.7 n	7.9 def	18.3 j
WNS 12	35.3 abcd	30.1 ij	2.4 abcde	3.4 def
WNS 13	10.5 abc	18.2 efg	7.8 cdef	24.7 l
WNS 20	5.8 ab	10.3 cd	6.9 bcdef	16.0 j
WNS 25	27.1 abcd	50.0 l	2.6 abcde	3.2 de
WNS 27	3.2 a	2.3 a	0.8 abcd	— a
WNS 31	7.9 abc	6.5 abc	6.1 bcdef	10.6 i
WNS 33	10.6 abc	16.1 def	3.9 abcdef	3.2 de
WNSV 13	8.3 abc	7.7 bc	0.3 ab	— a
WZC 12	35.9 abcd	51.8 l	2.5 abcde	2.0 cd
WZC 31	23.9 abc	27.9 ij	1.1 abcd	0.4 b
WZC 34	21.1 abc	24.0 ghij	7.9 def	4.2 efg
WZC 36	17.2 abc	12.5 cde	6.8 cdef	3.3 def
WZC 39	22.1 abc	46.4 l	2.6 abcde	4.3 efg
WZCV 4	18.2 abc	21.2 ghi	10.3 ef	17.7 j
WZCV 11	37.2 bcd	29.7 ij	1.6 abcde	— a
WZCV 26	16.8 abc	15.8 def	0.4 a	— a
WZCV 41	13.2 abc	12.3 cde	2.2 abcde	1.6 c
L 36	9.8 abc	7.7 bc	7.2 cdef	5.1 fgh
L 63	8.0 abc	15.8 def	6.2 cdef	10.6 i
LV 5	5.6 abc	3.1 ab	6.3 cdef	7.3 h
LV 13	92.2 d	91.8 m	14.2 f	5.7 gh
LV 16	25.2 abc	25.7 hij	6.3 cdef	6.1 gh
LV 17	30.3 abcd	23.5 ghij	4.1 abcdef	5.1 fgh
WNS	16.3 abc	22.9 ghij	0.8 abc	— a
WZC	42.8 cd	30.5 j	5.3 abcdef	2.5 cd

* Relative reproduction rate = final population (cysts or eggs) (Pf) in the potato genotype/final population (cysts or eggs) (Pi) in the susceptible genotype, expressed as a percentage. After angular transformation (arcsin & 8730) figures in the same column sharing a letter are not significantly different ($P<0.001$) according to Tukey's test (Honestly significant Difference Test).

1987), which permits the production of more generations in a short period of time. The higher percentage of multiplication failure (78%) in this method was caused by the low number of J2s inoculated per plant and not by the method itself. Overall, however, multiplication in the closed containers containing the soil and sand mixture was faster.

Désirée is a good genotype to use as a susceptible control since all lines and populations had high multiplication rates on it. However, WNS 7 produced three times more eggs per cyst on Vantage than on Désirée (Tables IV and V). This could be explained by differences in the environmental conditions or in plant development but, when environmental conditions were kept rigorously the same, it was always Vantage that showed the worst plant development compared to the other genotypes. Thus, it is perhaps more correct to ascribe the results with WNS 7 to the genetic characteristics of that line.

Consistent differences in the evaluation of the reproduction/virulence of nematodes based on cysts or eggs

were observed (Tables VI and VII). In general, the relative reproduction rates were higher when considering egg values (Table VII). This disparity results from differences observed in fecundity between the lines: more cysts do not necessarily imply more eggs. When the resistance of potato genotypes is evaluated only by the numbers of cysts, it is possible that they may be considered more resistant than they really are, or to underrate the virulence of PCN populations (Phillips and Trudgill, 1983; Whitehead, 1991b; Cunha, 2001). On the other hand, there are others who refute this idea, and claim that there are no differences in cyst contents produced on susceptible and partially resistant hosts if the plants are maintained in the same conditions (Turner, 1990; Bendezu *et al.*, 1998). In our tests, the environmental conditions were kept rigorously the same throughout the experimental process for all the genotypes used. Therefore, our results emphasize the need to use both cyst and egg counts for the evaluation of the virulence of PCN populations.

Analysis of variance of the fecundity revealed that there were significant differences between PCN lines/populations and that sometimes fecundity was higher when the number of cysts was lower (Tables IV and V). There were no great differences in nematode fecundity on the different genotypes (Table V). Similar results were found by Turner (1990): the fecundity of *G. pallida* populations tested on partially resistant clones was not different from the fecundity on the susceptible host. Partial resistance probably affects mainly the number of females rather than the number of eggs/cyst. On the other hand, resistant cultivars with the H1 gene affect not only the number of females that are formed but also the number of eggs/cyst (Mullin and Brodie, 1988).

Several authors have suggested that mean reproduction rates should not be used to evaluate the virulence of populations. However, when susceptible and partially resistant potato genotypes were used, such data were useful for the evaluation (Bendezu *et al.*, 1998).

The data on mean reproduction rates were, in some ways, different from what was expected. Although not all potato genotypes allowed reproduction rates of the nematode greater than one, all allowed the reproduction of at least a few nematode lines (Table VI). It was also observed that all nematode lines produced at least some cysts on the potato genotype 12380, which demonstrated that at least some of the individuals of each line have genes for virulence towards this genotype. Significant differences were always found between the lines and the potato genotypes, with the variation being much higher among genotypes (Table III). The dissimilarities observed in the potato genotypes were expected due to their large differences in terms of resistance. With regard to the nematode lines, the differences observed revealed a high variability in virulence related to each genotype used.

The variation found between the multiplication rates of populations WNS and WZC could be due to the fact that WZC was selected on ZC 83/6 (with high resistance derived from *Solanum tuberosum* ssp. *andigena*) over four generations. WZC will have a higher frequency of virulent alleles compared to WNS. These results confirm that selection for virulence on one potato genotype with partial resistance does not imply selection for virulence towards other genotypes, with their partial resistance derived from other sources. It was observed that a higher multiplication rate on Vantage did not correspond to a higher multiplication rate on 12380 (e.g. LV 13) and vice versa (e.g. WZCV 4). Thus, it is again confirmed that the virulence variation related to genotypes derived from *S. vernei* is polygenic (Bendezu *et al.*, 1998). Similar results were found by Phillips and Dale (1992), who observed that populations selected on ex *S. vernei* were more virulent on the selecting clone but were also more virulent towards some other *S. vernei* clones and some ex CPC 2802 (*S. t. andigena*) clones.

On Vantage, some lines showed relative multiplication rates (eggs) much lower than the original populations (e.g. WNS 27 and LV 5), so these lines could have potential as sources of molecular markers for avirulence (Table VII). WNS 7 and LV 13 (with 40 to 60% relative reproduction rates) overcame the resistance that this genotype has to *G. pallida* populations. For the majority of the lines, the resistance of Vantage was superior to that expected (Table VII), probably because there was no selection for virulence towards this potato genotype.

On 12380, considered to have resistance higher than 95% to the majority of *G. pallida* populations, its resistance to WNS 7, WNS 13, WNS 20, WNS 31, WZCV 4 and L 63, was lower than 95% (Table VII). Lines selected from WNS presented, in general, a higher relative reproduction rate than the original populations, confirming that there was a selection for virulence. It is interesting to note that lines selected from WZC usually presented lower values than other lines. These results suggest that the selection on ZC 83/6 over several generations (Whitehead, 1991a) caused a loss of the alleles responsible for resistance breaking on genotype 12380. Therefore, the selected lines that have the greatest probability of yielding molecular markers for avirulence on 12380 are: WNS 27, WNSV 13, WZCV 11 and WZCV 26; and of yielding markers for virulence are: WNS 7, WNS 13, WNS 20, WNS 31, WZCV 4 and L 63.

Virulence variation found among the selected lines could also be due to the genetical characteristics of the males used in the crosses. The probability of males being virulent is less than for females, except when pressure is exerted in the way described above. Thus, many of the crosses could have been between a virulent female and an avirulent male, which would be obvious when the virulence of following generations is assessed. Another possibility, when there were only one or two cysts per pot, is that, sometimes, even when the nematodes are avirulent for a particular gene, the mechanisms of resistance conferred by the genes of the host do not act (Janssen, 1990).

This study revealed that it is possible to produce lines from a single population, using controlled single matings, with differences in virulence comparable to those observed among the majority of European *G. pallida* populations. The lines obtained showed variation in virulence comparable to that observed between field populations (Bendezu *et al*., 1998; Cunha, 2001). Although lines of *G. pallida* that were 0 and 100% (a)virulent were not obtained, this study suggests that a larger experiment might have success. Since WZC has been selected on potato cultivars with resistance derived from *Solanum tuberosum* ssp. *andigena* (clone ZC 83/6), additional investigations are needeed to test the lines on cultivars derived from that source of resistance.

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