Only Parrott and Trudgill (1970) reported resistance to some *G. pallida* populations in two cultivars, which have been derived from CPC 1673. Multa has no other known source of PCN resistance in its pedigree.

In this paper, the results are presented of further investigations into the PCN resistance of Multa. The level of resistance of Multa to various potato cyst nematode populations was determined, and the inheritance of the resistance and its origin were investigated.

Materials and methods

EXPERIMENTAL PROCEDURES

Resistance tests were carried out with tubers, planted in 240 ml pots, containing 120 g peaty soil, or with rooted stem cuttings, planted in 150 ml pots, filled with 75 g peaty soil. The tubers had been presprouted and the stem cuttings had been grown for about three weeks and had a small root system before transplanting. Pots were inoculated at planting with a preset volume of cysts (Vinke *et al.,* 1992), containing on average 29 cysts, with an average total weight of 1.18 mg. Pots were placed in a greenhouse, with an average temperature of 20 °C, and were watered daily. After 6 to 8 weeks, when cysts were well developed, rootballs were removed from the pots and the number of newly formed cysts, visible on the rootball, was counted. This number is representative for the total number of newly formed cysts (Forrest & Holliday, 1979). A score (1-5) was given for the size of the root system.

Numbers of cysts were transformed to the logarithm with base 10 of the numbers of cysts plus 1, to obtain homogeneity of variance, necessary for analysis of variance. Correlations between data, which did not show a normal distribution, were determined by Spearman's rank correlation coefficient r_s.

RESISTANCE IN RELATION TO VARIATION IN THE PATHOGEN

Rooted stem cuttings of Multa and the susceptible standard cultivar Maritta were tested with twelve Dutch PCN populations, four of *G. rostochiensis* and eight of *G. pallida* (Table 1), with twenty replicates (Experiment 1). The populations varied in pathotype designation, place of origin, and protein pattern, as shown with two dimensional gel electrophoresis by Bakker (1987). Only *G. rostochiensis* populations C258 and G1510 have not been investigated by Bakker.

INHERITANCE OF RESISTANCE

Multa was crossed with the susceptible standard cultivar Maritta, and with the diploid *S. phureja* 81-1886-524, known to have some resistance to *G. pallida* (Dellaert *et al.,* 1988) and to produce 2^n-gametes. In addition, Multa was selfed. Tubers of 81 genotypes from the cross Maritta × Multa, 85 genotypes from the cross Multa × 81-1886-524, which were identified as hybrids by the presence of seed spots (Hermsen & Verdenius, 1973), and 90 genotypes obtained from selfing of Multa, were used in a resistance test with *G. pallida* population D236, with three replicates (Experiment 2).

Table 1. Details of potato cyst nematode (PCN) populations, numbers of cysts on Multa and on the susceptible standard cultivar Maritta, and numbers of cysts on Multa relative to Maritta, when tested with these populations (Experiment 1). Relative numbers of cysts carrying the same letter are not significantly different from each other (LSD; P < 0.05).

PCN populations	Species	Pathotype designation	Origin of populations	Number of cysts on		Number of cysts on Multa relative to the number on Maritta
				Multa	Maritta	
Mierenbos A	*G. rostochiensis*	Ro1	Wageningen	132*	202	0.65 *de*
C258	*G. rostochiensis*	Ro3	Odoorn	140*	233	0.60 *de*
F515	*G. rostochiensis*	Ro4	Emmen	115	150	0.76 *e*
G1510	*G. rostochiensis*	Ro5	Norg	96	140	0.69 *de*
D234	*G. pallida*	Pa2	Smilde	3*	117	0.03 *a*
D236	*G. pallida*	Pa2	Anlo	3*	129	0.02 *a*
P2-22	*G. pallida*	Pa2	Coevorden	64*	161	0.39 *c*
Rookmaker	*G. pallida*	Pa3	Valthe	63*	156	0.40 *c*
(Coll.) 1077	*G. pallida*	Pa3	Anjum	68*	140	0.49 *cd*
74-768-20	*G. pallida*	Pa3	Sleen	73*	221	0.33 *c*
75-884-4	*G. pallida*	Pa3	Vriezenveen	63*	144	0.44 *c*
(Coll.) 1112	*G. pallida*	Pa3	Westerbork	29*	121	0.24 *b*

* Indicates significantly fewer cysts on Multa than on Maritta, tested with the same population (LSR; P < 0.05).

Rooted stem cuttings of the same 81 genotypes of the cross Maritta × Multa were tested with three *G. pallida* populations, D236, P2-22 and Rookmaker, with five replicates (Experiment 3). Parent genotypes were also included.

Rooted stem cuttings of the same 85 genotypes from the cross Multa × 81-1886-524, and the parents, were tested with two *G. pallida* populations, D236 and Rookmaker, with three replicates (Experiment 4).

ORIGIN OF RESISTANCE

Solanum tuberosum ssp. *andigena* CPC 1673 was a seed sample, from which various genotypes have been used in potato breeding (Huijsman, 1957). These genotypes, from which Multa and various other cultivars have been derived, do not exist anymore. Tubers of the other ancestors of Multa, Record and Oberarnbacher Frühe, were tested for resistance, together with Multa and the susceptible standard cultivar Maritta, with *G. pallida* populations D236 and P2-22 (Experiment 5). Furthermore, tubers of eleven cultivars with resistance to *G. rostochiensis*, derived from *S. tuberosum* ssp. *andigena* CPC 1673, were also included in this experiment. The cultivars originate from three genotypes, CPC 1673-1, CPC 1673-11 and CPC 1673-20, backcrossed two or three times with *S. tuberosum* ssp. *tuberosum*. One of the cultivars, Amigo, has been derived from a cross between genotypes, originating from two backcrosses of different CPC 1673 genotypes with *S. tuberosum* ssp. *tuberosum*. The mentioned cultivars have been released between 1960 and 1970. Experiment 5 was carried out with five replicates.

Results

Significant differences in resistance levels were found in all experiments. Correlations (r_s) between log transformed number of cysts and root scores were below 0.10 and not significant, except in Experiment 3.

RESISTANCE TO A RANGE OF PCN POPULATIONS

In Experiment 1, Multa appeared to have a high level of resistance to two *G. pallida* populations, D234 and D236, as indicated by the formation of very few cysts (Table 1). Apart from the high level resistance to D234 and D236, the cultivar showed a lower level of resistance to most other PCN populations, characterized by the formation of significantly fewer cysts than formed on the susceptible standard cultivar Maritta. The level of this resistance varied with PCN populations (Table 1). The resistance level in Multa was significantly higher to most *G. pallida* populations than to the *G. rostochiensis* populations.

INHERITANCE OF RESISTANCE

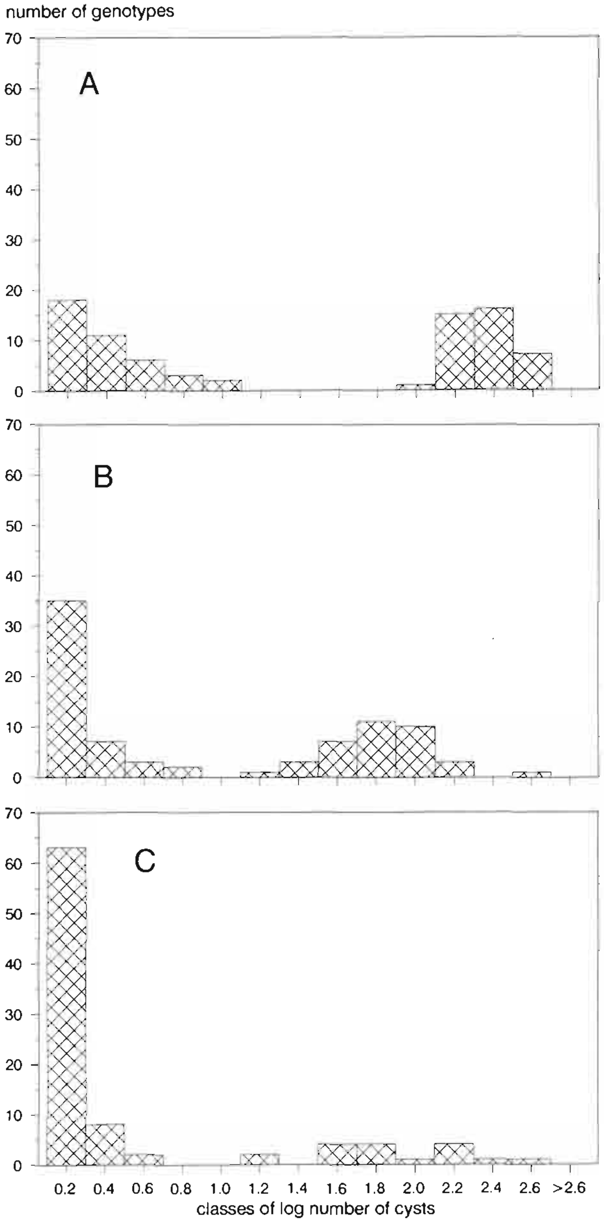

Fig. 1. Frequency distributions of genotypes for log transformed numbers of cysts, tested with *Globodera pallida* population D236 in Experiment 2. A : progeny of Maritta × Multa; B : progeny of Multa × 81-1886-524; C : progeny of Multa selfed. Only upper limits of classes are indicated.

In Experiment 2, tests with *G. pallida* population D236 showed a clear distinction between groups of highly resistant and susceptible genotypes for the Maritta × Multa progeny (Fig. 1 A). For the Multa × 81-1886-524 progeny (Fig. 1 B) and for Multa selfed (Fig. 1 C) also, although less clear, groups of highly resistant and susceptible genotypes could be distinguished. The first two progenies were retested in Experiments 3 and 4. The results were very similar. The log numbers of cysts after retesting was highly correlated

Table 2. Segregation of Multa progenies into genotypes, highly resistant or susceptible to *Globodera pallida* population D236 (Experiments 2 and 3). Genotypes with different classification after retesting, have been listed as unclear.

Progeny	Number of genotypes			Chi-square value with expected segregation pattern		Probability of a larger chi-square value
	Highly resistant	Susceptible	Unclear			
Maritta × Multa	40	39	2	(1:1)	0.006	> 0.995
Multa × 81-1886-524	47	36	2	(1:1)	1.467	> 0.100
Multa selfed	73	17		(3:1)	1.793	> 0.100

with the log numbers of cysts in Experiment 2, as presented ($r_s = 0.94$ and 0.96 respectively; $P < 0.01$).

Based on these results, the hypothesis that the resistance to D236 in Multa is based on a single, dominant gene, was tested. Assuming that Multa is heterozygous (simplex) for the resistance, then in case of the first two progenies, a 1:1 segregation would be expected, and with Multa selfed a 3:1 segregation. The number of genotypes per group of highly resistant and susceptible genotypes was determined with a criterion of the log transformed number of cysts being > 1 or < 1, based on the frequency distributions as presented in Figure 1A-C. Four genotypes showed a different classification after retesting. These have been listed separately in Table 2. The deviations from the expected segregation patterns were low and not significant (Table 2).

The earlier reported resistance of 81-1886-524 to *G. pallida* populations (Dellaert *et al.,* 1988) was confirmed (Table 3). The level of this resistance was about equal to that of Multa to P2-22 and Rookmaker in Experiment 3 (Table 3), and again about equal to that of Multa to Rookmaker in Experiment 4 (Fig. 2 C).

Table 3. Number of cysts on three genotypes, used as parents of progenies, and the number of cysts relative to the number on Maritta (Experiment 3). Numbers of cysts, found with the same PCN population and carrying different letters are significantly different from each other (LSR; $P < 0.05$).

PCN population	Genotype	Number of cysts	Number of cysts relative to Maritta
P2-22	Multa	64 *a*	0.22
	81-1886-524	61 *a*	0.21
	Maritta	297 *b*	
Rookmaker	Multa	49 *a*	0.27
	81-1886-524	48 *a*	0.26
	Maritta	185 *b*	
D236	Multa	0 *a*	0.00
	81-1886-524	45 *b*	0.26
	Maritta	175 *c*	

The distribution of genotypes within the Maritta × Multa progeny for log number of cysts numbers with P2-22 and Rookmaker (Experiment 3) is shown in Figure 2 A, B. Very few genotypes as resistant as Multa were found. Tested with these populations, only 9 and 7, respectively, of the 79 genotypes had a significantly lower number of cysts than the susceptible parent Maritta. Only within this progeny and with P2-22 and Rookmaker, low but significant correlations between log numbers of cysts and root score were found ($r_s = 0.32$ and 0.26 respectively). Within the Multa × 81-1886-524 progeny, tested with Rookmaker in Experiment 4, several genotypes were found with the same number or fewer cysts than both parents (Fig. 2 C). Within this progeny, tested with Rookmaker, 55 of the 83 genotypes had significantly fewer cysts than Maritta. The on average higher level of resistance of this progeny, compared to that of the Maritta × Multa progeny, is likely to be due to the earlier mentioned resistance of 81-1886-524.

Genotypes with high level resistance to D236 were on average not more resistant to *G. pallida* populations P2-22 and Rookmaker than genotypes without high level resistance to D236 (Table 4). This indicates that there is no relationship between the presence of the monogenic resistance and the low level resistance to these populations, and that these populations are highly virulent to the monogenic resistance.

ORIGIN OF RESISTANCE

The cultivars Record and Oberarnbacher Frühe, ancestors of Multa, did not show any resistance to *G. pallida* populations D236 or P2-22 (Table 5). However, high level resistance to D236 was found in eight other cultivars, derived from *S. tuberosum* ssp. *andigena* CPC 1673 (Table 5). Only Saturna, Aurora and Provita were found to be highly susceptible to D236. Resistant cultivars were derived from all three CPC 1673 genotypes, used as parent (Table 5). The results indicate that the resistance to D236, as found in Multa, is likely to have been derived from *S. tuberosum* ssp. *andigena* CPC 1673. The probability of the number of cultivars with resistance to D 236 was calculated. Considering only the cultivars that were derived from three backcrosses with presumably susceptible *S. tuberosum* ssp. *tuberosum* cultivars, seven out of nine cultivars were found to be resistant. Even when assuming quadruplex resistance to D236 in CPC 1673, the probability of such a high number of resistant cultivars after three backcrosses without selection was only 0.051.

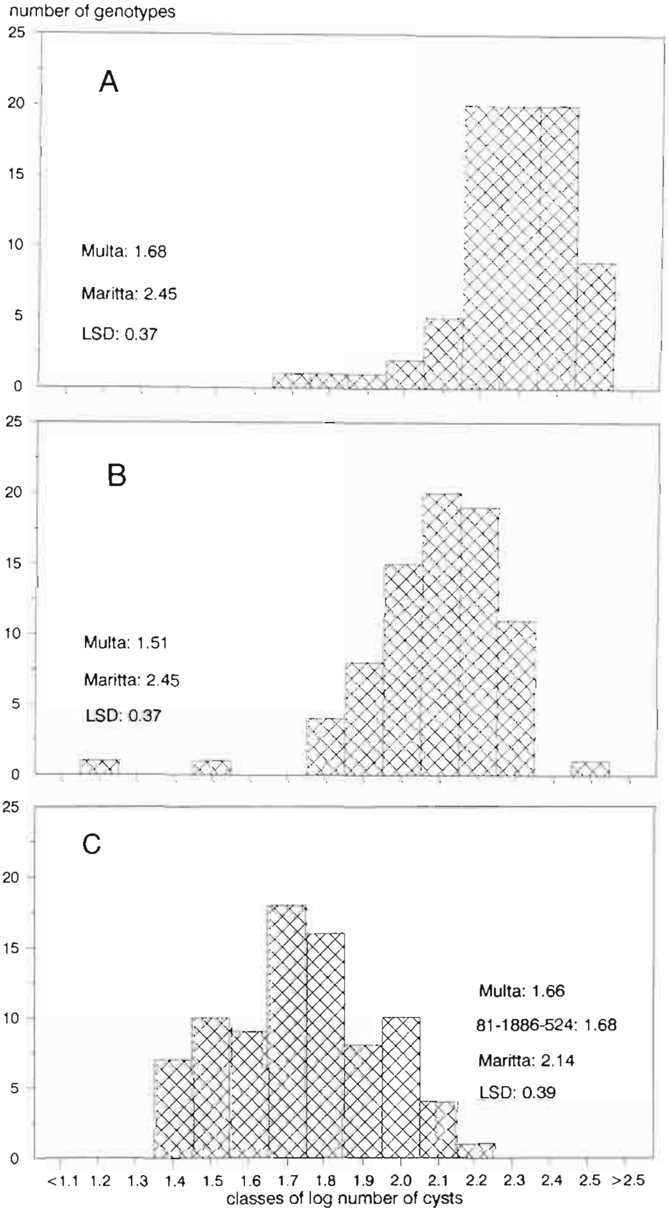

Fig. 2. Frequency distributions of genotypes for classes of log transformed numbers of cysts. A : Progeny of Maritta × Multa, tested with *Globodera pallida* population P2-22 in Experiment 3; B : Progeny of Maritta × Multa, tested with *G. pallida* population Rookmaker in Experiment 3; C : Progeny of Multa × 81-1886-524, tested with Rookmaker in Experiment 4. Only upper limits of classes are indicated. Values for parents and LSD's ($P < 0.05$) are presented in the figures.

Three out of eleven cultivars, Ehud, Prevalent and Marijke, also showed a significantly lower number of cysts than Maritta with P2-22 (Table 5).

Discussion

The results presented here give evidence of an until now unknown high level, monogenic resistance to some *G. pallida* populations in the potato cultivar Multa, unnoticed since its release more than 25 years ago. After Dunnett (1962) found monogenic resistance in *S. multidissectum* to virulence group Pa 1, this is only the second report of monogenic resistance to *G. pallida*. Resistance to *G. pallida* from *S. tuberosum* ssp. *andigena* CPC 2802, originally attributed to a single H3 gene (Howard *et al.*, 1970), was later found to show polygenic inheritance (Dale & Phillips, 1982).

A clear distinction between resistance and susceptibility in the frequency distributions of the Multa progenies was observed, which formed a good basis for assessing segregation patterns. The arbitrary division of a continuous distribution for numbers of cysts into categories of resistant and susceptible genotypes may lead to wrong conclusions on numbers of genes involved (Luedders, 1989).

The high level resistance of Multa was found to be effective to two populations of *G. pallida*, D234 and D236. Two dimensional gel electrophoresis (Bakker, 1987) showed that these two populations have highly similar protein patterns, and that their protein patterns

Table 4. Mean log number of cysts for the Maritta × Multa progeny in Experiment 3, and the Multa × 81-1886-524 progeny in Experiment 4, and for genotypes of these progenies, separated into two groups, either highly resistant or susceptible to D236.

Progeny	Number of genotypes	Log number of cysts with		
		D236	P2-22	Rookm.
Maritta × Multa (Expt 3) :				
All genotypes	79	1.15	2.24	2.05
Resistant to D236	40	0.28	2.23	2.04
Susceptible to D236	39	2.05	2.25	2.06
Multa × 81-1886-524 (Expt 4) :				
All genotypes	83	0.87		1.69
Resistant to D236	47	0.15		1.69
Susceptible to D236	36	1.81		1.69

differ more from various other *G. pallida* populations investigated than many of those populations amongst each other. It is interesting to note that these differences in protein pattern are reflected by differences in virulence characteristics, as we found these two populations to be avirulent to the reported single resistance gene, while at least two other *G. pallida* populations, P2-22 and Rookmaker, were found to be highly virulent to this gene.

Low level resistance in Multa was found to all other *G. pallida* populations, used in our experiments, but its level varied between *G. pallida* populations. The low level resistance might be explained by these *G. pallida* populations being a mixture of nematodes, avirulent and virulent to the monogenic resistance. However, with the *G. pallida* populations P2-22 and Rookmaker, this explanation was not applicable, as no effect of the monogenic resistance on the resistance level to these populations was found within the Multa derived progenies (Table 4). The low level resistance to these populations thus presumably has a different genetic basis. This resistance seems to be mainly recessively inherited, as very few genotypes within the Maritta × Multa progeny were found, which were equally resistant as Multa, while several genotypes were found, which were as susceptible as Maritta. In this case, root system size may have had a small effect on numbers of cysts found, as a low but significant correlation with root score was observed.

Although the original *S. tuberosum* ssp. *andigena* CPC 1673 ancestors of Multa and related cultivars have been lost, it is clear that they have been the source of the major gene *G. pallida* resistance. Other ancestors of Multa did not show this resistance while many of the other *G. rostochiensis* resistant cultivars, derived from three different CPC 1673 genotypes, were found to possess the high level resistance to D236. The high number of cultivars, derived from CPC 1673, with resistance to D236 may be explained by fairly close linkage of this resistance gene with the H1 gene, conferring resistance to *G. rostochiensis* virulence group Ro 1, or by such a linkage with some agronomical trait, for which cultivars were selected. Linkage to the H1 gene of some unknown factor increasing *G. pallida* resistance was suggested by Dale and Phillips (1984). An explanation by assuming quadruplex resistance in the CPC 1673 sources is highly improbable, but could not be rejected, with a probability

Table 5. Details of pedigree of Multa and its ancestors and eleven *Globodera rostochiensis* Ro1 resistant cultivars, derived from three *Solanum tuberosum* ssp. *andigena* CPC 1673 genotypes, the number of backcrosses with *S. tuberosum* ssp. *tuberosum*, and the number of cysts and the number of cysts relative to Maritta, with *G. pallida* populations D236 and P2-22 in Experiment 5. Numbers of cysts for each population carrying the same letter are not significantly different from each other (LSR; $P < 0.05$).

Potato cultivar	CPC 1673 genotype and number of backcrosses			Numbers of cysts with *G. pallida* populations		Number of cysts relative to Maritta with	
	CPC 1673-1	CPC 1673-11	CPC 1673-20	D236	P2-22	D236	P2-22
Multa	2			0 *a*	100 *b*	0.00	0.44
Multa ancestors							
Oberarnb. Frühe				175 *b*	156 *bc*	0.78	0.68
Record				154 *b*	201 *bc*	0.69	0.88
Other cultivars							
Saturna	2			193 *b*	173 *bc*	0.86	0.75
Aurora	3			280 *b*	161 *bc*	1.25	0.70
Ehud	3			4 *a*	86 *b*	0.02	0.38
Element	3			0 *a*	123 *b*	0.00	0.54
Prevalent		3		1 *a*	116 *b*	0.00	0.51
Provita		3		174 *b*	155 *bc*	0.78	0.67
Amigo		2	2	1 *a*	183 *bc*	0.00	0.80
Alcmaria			3	1 *a*	185 *bc*	0.00	0.81
Amaryl			3	0 *a*	195 *c*	0.00	0.85
Bellona			3	0 *a*	149 *bc*	0.00	0.65
Marijke			3	0 *a*	25 *a*	0.00	0.11
Susceptible standard							
Maritta				224 *a*	229 *c*		

only just above 0.05. Possibly, the resistance gene found in Multa may have also been incorporated in some cultivars, derived from CPC 1673, in which Parrott and Trudgill (1970) found resistance to some *G. pallida* populations.

The source of the low level resistance in Multa remains unclear, but the mainly recessive mode of inheritance does suggest that various ancestors may have been heterozygous for this resistance. The source of the low level resistance, found in some other CPC 1673 derived cultivars, also remains unclear. Low level resistance has been reported also in other cases of major gene resistances to PCN. Dunnett (1962) found with *S. multidissectum* hybrids, that apart from the H2 resistance gene to the PCN population Duddingston, also a low level of resistance to Duddingston was found in part of the progeny, lacking the H2 gene. This low level resistance thus has a genetic basis, different from the H2 gene. In cultivars with *G. rostochiensis* resistance from *S. tuberosum* ssp. *andigena* CPC 1673, apart from high level resistance to virulence group Ro1, a varying, lower level of resistance to several other *G. rostochiensis* populations has been found but it is not known whether this low level resistance is caused by the H1 gene, or has a different genetic basis (Janssen *et al.,* 1990).

With the high level *G. pallida* resistance to populations D234 and D236, a new virulence group within *G. pallida* may be distinguished in the way of Arntzen and van Eeuwijk (1992). However, the frequency of populations, avirulent for this resistance, is presumably not very high, since the resistance to D236 has not been reported before, even although many cultivars derived from CPC 1673 have been grown now for more than 20 years.

This research has led to the distinction of a single gene conferring high level resistance to *G. pallida* and of low level resistance to *G. pallida* with a mainly recessive type of inheritance. The discovery of the single gene for resistance to *G. pallida* may stimulate research for single genes with resistance to a wider spectrum of *G. pallida* populations. Such resistance genes can be incorporated by breeders in new potato cultivars more easily than the polygenic resistance from *S. vernei,* as commonly used now.

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