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Research Papers

Virulence assessment of Portuguese isolates of potato cyst nematodes (*Globodera* spp.)

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Summary. Identification of species and virulence groups of potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis*, present in field populations is important in the control of these nematodes by means of resistant cultivars. In order to characterize the virulence of *Globodera* spp. isolates from Portugal, 43 *G. rostochiensis* and three *G. pallida* isolates were evaluated by measuring their multiplication rates on a susceptible potato cultivar and five differential potato genotypes in a growth chamber pot experiment. Principal Component Analysis and Hierarchical Cluster Analysis showed that the reproduction rates were different in terms of both the numbers of eggs and the numbers of cysts produced. Portuguese isolates of PCN were more virulent on genotypes derived from *Solanum vernei* than on genotypes derived from other *Solanum* resistance sources, and there was a significant nematode isolate × host genotype interaction. The virulence bioassay clearly distinguished the two PCN species but failed to differentiate isolates into pathotypes. There was a wide and continuous range of virulence to the resistant genotypes, especially in *G. rostochiensis* isolates.

Key words: fecundity, genetic diversity, *Globodera pallida*, *Globodera rostochiensis*, IPM.

Introduction

Potato is an important staple crop in Portugal and, according to the INE 2009 Agricultural Census (INE, 2010), an average of 40,000 ha of potatoes are grown every year. The potato yield average is around 15.5 t ha^{-1}, compared to an average of 27.6 t ha^{-1} in the European Union and 43 t ha^{-1} in the United Kingdom. Such differences in yield are attributed, to a great extent, to the presence of potato cyst nematodes (PCN), *Globodera* spp., present in all main potato production areas, with a national average of 50% of fields infested, and 100% of fields in some areas (Cunha *et al*., 2004). PCN invasion limits the extent of potato root systems, decreasing the efficiency of nutrient uptake and the rate of crop growth and canopy expansion. PCN damage can also reduce the efficiency of photosynthesis and water use by potato plants, and is sometimes associated with premature plant senescence (Lane and Trudgill, 1999). In virtually all countries where potatoes are grown, PCN, *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 and *G. pallida* (Stone, 1973) Behrens, 1975 cause damage to potato crops and have quarantine status (Santos *et al*., 1995; Turner and Evans, 1998). Management of these nematodes usually involves crop rotation and nematicides. The most practical alternative to the use of nematicides is the exploitation of host resistance as a component of integrated pest management (IPM).

Development of resistant cultivars requires knowledge of the variability of populations of pathogen species. The success of this control measure may be limited by the presence in the field of mixtures of species and mixtures of pathotypes or virulence

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groups within the populations. Therefore, it is important to know the genetic diversity present in field populations and to define the virulence groups that may exist within PCN populations.

Several schemes for identifying PCN pathotypes, based on the differential responses of potato clones, have been proposed (Canto Saenz and De Scurrah, 1977; Kort *et al*., 1977; Nijboer and Parlevliet, 1990). These schemes were not satisfactory because many differential potato clones have polygenicallybased resistance. In 1984, at the European and Mediterranean Plant Protection Organization (EPPO) Workshop on Cyst Nematodes, it was concluded that only *G. rostochiensis* pathotypes classified as Ro1 (including Ro4) and *G. pallida* classified as Pa1 could be regarded as distinct pathotypes in the strict sense. The other pathotypes were regarded as being a continuum of populations of individuals carrying different virulence genes that should be regarded as virulence groups distinguished by their behaviour on standard clones or commercial cultivars of potato (Anon, 1985). Mugniéry *et al*. (1989) considered that, although virulence groups cannot be accurately defined, the concept may be helpful when describing differences in virulence in relation to quantitative (partial) resistance.

The main objective of the present study was to assess the virulence of a large set of PCN Portuguese isolates, 43 *G. rostochiensis* and three *G. pallida*, in terms of quantitative reproduction, using several host genotypes with different sources of resistance, compared with reproduction on a cultivar susceptible to both nematode species.

Materials and methods

Nematode isolates

The *G. rostochiensis* isolates (43) were selected from a survey in various districts of Portugal, and the three *G. pallida* isolates used in the virulence assay were the only ones collected from potato fields in the survey (Table 1). The low number of *G. pallida* isolates found reflects the known natural distribution of PCN in Portugal, where this species is much less widespread than *G. rostochiensis* (Santos *et al*., 1995; Cunha *et. al*., 2004). The isolates were multiplied on the susceptible potato cv. Désirée to provide a stock of more than 1000 cysts of each.

Potato genotypes

The clones/cultivars used and their effectiveness and source of resistance are given in Table 2. Cultivar Désirée was used as the standard susceptible genotype. The choice of potato genotypes was conditioned by the EPPO recommendations suggesting that

Table 1. Portuguese isolates of potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*), their codes (PT, H: continental Portugal; M: Madeira Island) and origin.

Species/Isolate codes	Origin
G. rostochiensis	
PT1, PT14, PT45, PT99, PT111, PT150, PT225	Aveiro
PT126, PT3638, PT3639, PT3798	Bragança
PT93, PT070	Coimbra
M9, M54, M55	Funchal
PT32, PT64, PT66, PT69, PT70, PT74, PT75, PT79, PT81	Guarda
H3448, H3781, H4044, H4046, H4066, H4166	Porto
PT219, PT220, PT221, PT222	Setúbal
PT125, PT567, PT811, PT897, PT5695, PT5698, PT5699, PT5700	Vila Real
G. pallida	
PT104, PT116	Aveiro
PT5701	Vila Real

Table 2. Potato genotypes used as differential hosts to determine potato cyst nematode virulence.

Potato genotype	Source of resistance	Resistance genes	Resistance
Désirée	None	None	Susceptible
Corsair	*Solanum multidissectum* and *S. vernei*	H2	Resistant to Pa1 Partially resistant to other *G. pallida* populations (1; 2) [a]
Morag	*S. vernei* CPC 2488 and CPC 2487	Polygenic	Partially resistant to Pa2/3 *ca.* 80% resistant to most of *G. pallida* populations Partially resistant to *G. rostochiensis* populations (1; 2)
Vantage	*S. vernei*	Polygenic	Moderate resistance to *G. pallida* populations Partially resistant to *G. rostochiensis* populations (1)
12674	*S. tuberosum andigena* CPC 2802	H3	Resistance to Pa3 Resistance to *G. rostochiensis* European populations (3; 4)
Santé	*S. tuberosum andigena* and *S. vernei*	Polygenic	Partially resistant to Pa2/3 Resistant to *G. rostochiensis* populations (5)

[a] References: 1, Mugniéry *et al.* (1989); 2, Gonzalez *et al.* (1996); 3, Phillips and Trudgill (1998); 4, Bendezu *et al.* (1998); 5, Whitehead (1991).

certain partially resistant potato cultivars and clones should be adopted as international standards for characterisation of PCN virulence (Mugniéry *et al*., 1989).

Virulence assessment

One goal of the experiment was to compare the intrinsic virulence of nematode isolates on a set of genotypes with different levels of resistance to PCN, so only new cysts were used. Because *G. pallida* may hatch more slowly than *G. rostochiensis* and this may affect nematode increase (Whitehead *et al*., 1984, 1987; Robinson *et al*., 1987) cysts were used for inoculum in preference to hatched juveniles. Potato genotypes were 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. The inoculum (initial population density = Pi) was assessed by counting five replicates of a suspension of eggs and second-stage juveniles (J2) obtained by crushing 50 cysts of each isolate in water. Each pot was then inoculated with cysts contained in a small polyester bag, to give approximately 5 eggs g^{-1} of soil. Pots were arranged in a randomized complete block design with fourfold replication of each isolate × host combination and kept at 18°C, with a photoperiod of 16 h. Fertilizer was added to the pots, and 15 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 extracted with a modified Fenwick can (Shepherd, 1986) and counted. The cysts from the four replicates were mixed, and 50 were randomly picked and crushed and the eggs + J2 counted to estimate the number of eggs + J2/plant. The eggs and J2 were counted only if more than 25 cysts were produced in the four replicates. When only 25 cysts or less were produced in the four replicates, the value was considered not significant and the number of eggs + J2 less than the Pi (4,000 eggs).

The final population density (Pf) was expressed as the total number of newly formed cysts or the total number of newly formed eggs + J2.

Virulence of the isolates was assessed by calculating the absolute reproduction rate based on cysts and on eggs + J2 (Pf/Pi). The relative reproduction rates based on cysts and on eggs + J2 (expressing the reproduction rates on the resistant cultivars as a percentage of the reproduction rate on the susceptible cultivar Désirée) were also calculated to assess differences in virulence and to rank populations. The percentage of resistance was calculated at 100 minus the percentage of relative reproduction on the genotype, considered the percentage of susceptibility.

Statistical analyses

The data (numbers of cysts/pot and eggs + J2/pot) were analysed using standard analyses of variance. The data were subjected to logarithmic trans-

formation (log_{10}) to normalise the variances. Analyses of variance were performed and analysed after appropriate transformations of data by angular transformation, using Statistica version 5.0 (Statsoft, 1997). The numbers of cysts/pot and eggs + J2/pot were also analysed using Hierarchical Cluster Analysis (HCA) to construct dendrograms by means of the NTSYS program, version 2.0 (Rohlf, 1998). The analyses of the isolate × genotype interaction effects were also displayed using a Principal Component Analysis (PCA) of the interaction effects (Kempton, 1984; Phillips and McNicol, 1986). This analysis removes the average differences between hosts and isolates and shows the relative specific effects of each isolate × genotype combination (Phillips *et al*., 1998). The results are displayed in biplots, in which the isolates are represented by lines and the potato genotypes by points. The angle formed between the lines represents the interaction and the length of the line the magnitude of the interaction effects. The potato genotypes furthest from the origin have the greatest interaction with nematode isolates. The interaction is positive if a line can be drawn, at right angles, from a genotype point to an isolate line. The interaction is negative if the line can only be made to a projection of the isolate line back through the origin. The PCA was also performed using NTSYS, version 2.0.

Results

A great variation in the numbers of cysts and eggs + J2 among *G. rostochiensis* and *G. pallida* isolates was observed over the six potato genotypes (Tables 3 and 4). As expected, it was on Désirée that most cysts and eggs were produced by both species. On average, cyst production on Désirée averaged 812.3 for all isolates, with values of 804.5 for *G. rostochiensis* and 923.3 for *G. pallida*, indicating good conditions for nematode reproduction. Although all PCN isolates reproduced well on Désirée, the number of new cysts produced ranged from 101 (PT1) to 1,723 (PT222) (Table 3). The number of eggs + J2 produced by all isolates, with an average of 92,886, was 92,045 for *G. rostochiensis* and 104,949 for *G. pallida*. The number of eggs produced on Désirée ranged from 7,100 (PT3638) to 238,739 (PT222) (Table 4), indicating that all isolates produced more eggs than were inoculated (4,000), i.e. have Pf/Pi > 1. However, the rates of multiplication of each isolate on this susceptible cultivar differed significantly, ranging from 1.8 (PT3638) to 59.7 (PT222) for *G. rostochiensis* and from 19.4 (PT5701) to 37.0 (PT116) for *G. pallida* isolates (Table 5). The number of eggs + J2 produced did not rank in the same order as did the number of cysts, because the fecundity (the number of eggs + J2/cyst) differed significantly among the PCN isolates. On Désirée, mean fecundity ranged from 8.4 (PT3638) to 205.0 (P070) for *G. rostochiensis* and from 75.6 (PT5701) to 158.8 (PT116) for *G. pallida* (Table 6).

Differences in virulence towards cvs Corsair, Morag and Vantage were observed among the 43 isolates of *G. rostochiensis* (Tables 3, 4, 5 and 6). Corsair was the most resistant to *G. rostochiensis* isolates, followed by Vantage and Morag. On these cultivars, the mean numbers of new cysts ranged from 21.5 (H4066) to 584.8 (PT222) for Morag, 15 (PT3798) to 495.3 (PT222) for Vantage and from 0.5 (PT897) to 332.3 (PT222) for Corsair. The numbers of eggs + J2, when determined, ranged from 391 (H4066) to 104,861 (PT126) for Morag, 1,017 (PT3798) to 112,638 (PT222) for Vantage and from 258 (PT3639) to 76,152 (PT222) for Corsair. For Corsair, the numbers of eggs from three isolates (PT897, PT3798 and PT5695) were not determined due to the low numbers of new cysts. The rates of multiplication of the isolates on these cultivars differed significantly. On Corsair, the numbers of eggs + J2 produced by 22 PCN isolates was less than the Pi (4,000). On Vantage and Morag, only eight and four isolates, respectively, produced few eggs with Pf/Pi <1 (Table 5). The three isolates of *G. pallida* reproduced well on these three cultivars, with mean numbers of new cysts ranging from 197.3 (PT104) to 291.5 (PT5701) for Morag, 63.8 (PT104) to 136.3 (PT5701) for Vantage and from 87.8 (PT104) to 240.3 (PT5701) for Corsair (Table 3). The numbers of eggs + J2 ranged from 17,634 (PT104) to 54,860 (PT5701) for Morag, 7,803 (PT104) to 29,238 (PT116) for Vantage and from 9,793 (PT104) to 37,431 (PT5701) for Corsair. As in Désirée, the numbers of eggs + J2 produced in these three genotypes did not rank in the same order as did the numbers of cysts, because the fecundity differed significantly among the isolates. For *G. rostochiensis* mean fecundity ranged from 11.6 (PT3638) to 264.8 (PT126) on Morag, from 17.0 (PT3638) to 258.2 (PT219) on Vantage and from 13.2 (H4044) to 300.0 (PT81) on Corsair. For *G. pallida,* mean fecundity ranged from 89.4 (PT104) to 188.2 (PT5701) on Morag, from 122.4 (PT104) to 181.6 (PT116) on Vantage and from 111.6 (PT104) to 155.8 (PT5701) on Corsair (Table 6).

Table 3. Numbers of cysts produced by *Globodera* spp. isolates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot[a].

Species/ Isolate code[b]	Potato genotype					
	Désirée	Corsair	Morag	Vantage	12 674	Santé
G. rostochiensis						
PT1	100.8	14.3	25.8	25.0	0.0	2.0
PT14	831.3	96.5	138.8	104.8	0.0	6.5
PT32	274.0	22.3	91.0	22.3	0.0	0.0
PT45	540.3	22.0	102.3	76.5	0.0	0.8
PT64	677.0	25.0	87.8	77.5	0.0	0.0
PT66	480.8	9.5	78.0	46.5	0.0	0.0
PT69	269.5	8.8	73.0	27.3	0.0	0.3
PT70	561.3	66.3	106.3	158.3	0.0	2.5
PT74	361.7	38.3	35.3	66.0	0.0	0.0
PT75	331.3	20.8	111.5	52.0	0.0	0.0
PT79	1643.3	80.0	282.0	121.5	0.0	1.8
PT81	515.8	61.0	76.5	107.3	0.0	0.0
PT93	636.3	160.8	230.5	179.8	0.0	0.0
PT99	1275.3	250.8	235.5	294.3	0.0	0.3
PT111	593.0	24.5	133.5	36.3	0.0	0.0
PT125	327.0	29.7	80.0	121.5	0.0	1.0
PT126	1285.0	127.0	396.0	415.0	0.0	0.0
PT150	628.0	44.0	161.5	151.8	0.0	0.5
PT219	1283.0	252.5	229.3	267.8	0.0	0.0
PT220	888.5	212.8	173.0	324.5	0.0	0.0
PT221	713.3	62.8	172.5	48.3	0.0	0.0
PT222	1722.5	332.3	584.8	495.3	0.0	0.0
PT225	1243.3	133.3	123.8	87.0	0.3	2.3
PT567	1476.8	25.0	94.3	48.3	0.0	0.0
PT811	430.0	57.5	66.3	100.0	0.0	6.3
PT897	1290.5	0.5	247.8	156.5	0.0	0.0
PT3638	845.3	83.3	81.0	102.5	0.0	0.0
PT3639	768.0	6.8	66.5	59.3	0.0	0.8
PT3798	399.5	1.5	105.0	15.0	0.0	0.3
PT5695	1436.3	2.0	210.8	115.5	0.0	0.0
PT5698	762.5	16.5	48.8	137.5	0.0	3.8
PT5699	938.0	70.5	95.8	75.0	0.0	9.8
PT5700	433.0	81.8	52.8	78.8	0.0	0.0
H3448	689.0	21.8	88.3	87.5	0.0	10.3
H3781	677.3	42.3	136.5	114.8	0.0	3.0
H4044	982.8	199.5	145.3	129.8	0.0	0.0
H4046	929.8	32.5	76.3	100.0	0.0	0.0
H4066	1423.5	13.8	21.5	184.0	0.0	0.3
H4166	1293.3	66.0	144.5	110.3	0.0	5.0
M9	221.0	11.5	34.3	30.8	0.0	0.0
M54	947.0	12.8	264.5	99.3	0.0	1.5
M55	995.3	82.8	317.0	167.0	0.0	1.8
P070	473.3	9.5	123.3	81.0	0.0	3.3
G. pallida						
PT104	811.5	87.8	197.3	63.8	24.5	266.5
PT116	934.0	126.8	147.8	161.0	70.3	171.0
PT5701	1024.5	240.3	291.5	136.3	22.3	362.0

[a] The numbers of cysts are means of four replicates.
[b] For isolate codes and origin see Table 1.

(*Continued*)

Globodera rostochiensis isolates did not multiply on potato genotypes 12674 and Santé. Only one of the isolates (PT225) produced a few cysts (0.3, mean of four replicates) on genotype 12674, and 22 isolates produced a few cysts (0.3–10.3, means of four replicates) on Santé. Genotype 12674 was the most resistant to *G. pallida* isolates (Tables 3, 4, 5 and 6). The virulence of *G. pallida* isolates also showed some variation but a value of Pf/Pi <1 was only recorded on genotype 12674 inoculated with the isolate PT104. In general, the reproduction of isolate PT5701 was greater than that of PT116, which in turn was greater than that of PT104 on the six potato cultivars (Table 5). The mean reproduction rate (20.7) of isolate PT5701 on Santé was greater than on Désirée (19.4), despite the lower number of cysts on Santé. The variation detected, once more, is related to the differences in fecundity of the isolates (Table 6).

Table 4. Numbers of eggs + second-stage juveniles produced by *Globodera* spp. isolates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot[a].

Species/ Isolate code[b]	Potato genotype					
	Désirée	Corsair	Morag	Vantage	12 674	Santé
G. rostochiensis						
PT1	7620	1001	2208	260	0	nd
PT14	42228	13452	14930	7898	0	377
PT32	28222	2141	8973	1615	0	0
PT45	109897	2319	13074	15836	0	nd
PT64	49286	3660	13900	9982	0	0
PT66	73082	490	9391	9998	0	0
PT69	36760	649	4541	1239	0	nd
PT70	70718	7606	11539	12917	0	nd
PT74	65822	8453	4332	14375	0	0
PT75	51609	2673	16547	9516	0	0
PT79	76575	8352	23744	7460	0	nd
PT81	91185	18300	17289	20313	0	0
PT93	91493	39223	46284	18227	0	0
PT99	71417	22773	21525	25840	0	nd
PT111	73058	2852	9532	2262	0	0
PT125	58402	3982	14688	18055	0	nd
PT126	214081	22123	104861	102422	0	0
PT150	52626	5078	20123	19673	0	0
PT219	215287	60398	38390	69133	0	0
PT220	145003	47443	30206	59448	0	0
PT221	59347	5024	22667	1546	0	0
PT222	238739	76152	102448	112638	0	0
PT225	118362	14476	10449	15817	nd	nd
PT567	212652	2565	8520	5124	0	0
PT811	58136	7324	7765	11300	0	316
PT897	164926	nd	42126	10986	0	0
PT3638	7100	2883	940	1743	0	0
PT3639	89549	258	4017	8693	0	nd
PT3798	47061	nd	12327	1017	0	nd
PT5695	122373	nd	19942	12035	0	0
PT5698	105835	2148	5645	24118	0	nd
PT5699	62658	4526	7032	3225	0	251
PT5700	44512	5072	7265	4366	0	0
H3448	105693	1723	9919	19005	0	1103
H3781	95221	6794	19165	19852	0	nd
H4044	47764	2633	7468	8048	0	0
H4046	48536	3367	5951	5780	0	0
H4066	133809	593	391	18768	0	nd
H4166	180545	8395	28409	10760	0	nd
M9	19183	1412	3726	4213	0	0
M54	119890	809	26662	12849	0	nd
M55	154662	7630	43366	12391	0	nd
P070	97026	903	16047	12101	0	nd
G. pallida						
PT104	89427	9793	17634	7803	2538	29368
PT116	147946	15317	15253	29238	4780	16416
PT5701	77452	37431	54860	21295	4810	82970

[a] The numbers of eggs + second-stage juveniles are means of five counts.
[b] See Table 1.
nd, Not determined due to the low number of cyst obtained.

The mean multiplication rates based on cysts of *G. rostochiensis* isolates on the five host genotypes, relative to the number produced on Désirée, ranged from 1.5 (H4066) to 36.2 (PT93) on Morag, from 3.3 (PT567) to 37.2 (PT125) on Vantage and from 0.1 (PT897) to 25.3 (PT93) on Corsair (Table 7). On the genotypes 12674 and Santé the greatest mean multiplication rate value was only 2.0 (PT1 on Santé). For the *G. pallida* isolates, the values ranged from 15.8 (PT116) to 28.5 (PT5701) on Morag, from 7.9 (PT104) to 17.2 (PT116) on Vantage and from 10.8 (PT104) to 23.5 (PT5701) on Corsair. On the genotype 12674 all the *G. pallida* isolates presented relatively low mean values (2.2 for PT5701 to 7.5 for PT116) and on Santé relatively high values (18.3 for PT116 to 35.3 for PT5701) (Table 7).

Table 5. Mean reproduction rates of *Globodera* spp. isolates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot[a].

Species/ Isolate code[b]	Potato genotype					
	Désirée	Corsair	Morag	Vantage	12 674	Santé
G. rostochiensis						
PT1	1.9	0.3	0.6	0.5	0.0	nd
PT14	10.6	3.4	3.7	2.0	0.0	0.1
PT32	7.1	0.5	2.2	0.4	0.0	0.0
PT45	27.5	0.6	3.3	4.0	0.0	nd
PT64	12.3	0.9	3.5	2.5	0.0	0.0
PT66	18.3	0.1	2.4	2.5	0.0	0.0
PT69	9.2	0.2	1.1	0.3	0.0	nd
PT70	17.7	1.9	2.9	3.2	0.0	nd
PT74	16.5	2.1	1.1	3.6	0.0	0.0
PT75	12.9	0.7	4.1	2.4	0.0	0.0
PT79	19.1	2.1	5.9	1.9	0.0	nd
PT81	22.8	4.6	4.3	5.1	0.0	0.0
PT93	22.9	9.8	11.6	4.6	0.0	0.0
PT99	17.9	5.7	5.4	6.5	0.0	nd
PT111	18.3	0.7	2.4	0.6	0.0	0.0
PT125	14.6	1.0	3.7	4.5	0.0	nd
PT126	53.5	5.5	26.2	25.6	0.0	0.0
PT150	13.2	1.3	5.0	4.9	0.0	nd
PT219	53.8	15.1	9.6	17.3	0.0	0.0
PT220	36.3	11.9	7.6	14.9	0.0	0.0
PT221	14.8	1.3	5.7	0.4	0.0	0.0
PT222	59.7	19.0	25.6	28.2	0.0	0.0
PT225	29.6	3.6	2.6	4.0	nd	nd
PT567	53.2	0.6	2.1	1.3	0.0	0.0
PT811	14.5	1.8	1.9	2.8	0.0	0.1
PT897	41.2	nd	10.5	2.8	0.0	0.0
PT3638	1.8	0.7	0.2	0.5	0.0	0.0
PT3639	22.4	0.1	1.0	2.2	0.0	nd
PT3798	11.8	nd	3.1	0.3	0.0	nd
PT5695	30.6	nd	5.0	3.0	0.0	0.0
PT5698	26.5	0.5	1.4	6.0	0.0	nd
PT5699	15.7	1.1	1.8	0.8	0.0	0.1
PT5700	11.1	1.3	1.8	1.1	0.0	0.0
H3448	26.4	0.4	2.5	4.8	0.0	0.3
H3781	23.8	1.7	4.8	5.0	0.0	nd
H4044	11.9	0.7	1.9	2.0	0.0	0.0
H4046	12.1	0.8	1.5	1.5	0.0	0.0
H4066	33.5	0.2	0.1	4.7	0.0	nd
H4166	45.1	2.1	7.1	2.7	0.0	nd
M9	4.8	0.4	0.9	1.1	0.0	0.0
M54	30.0	0.2	6.7	3.2	0.0	nd
M55	38.7	1.9	10.8	3.1	0.0	nd
P070	24.3	0.2	4.0	3.0	0.0	nd
G. pallida						
PT104	22.4	2.5	4.4	2.0	0.6	7.3
PT116	37.0	3.8	3.8	7.3	1.2	4.0
PT5701	19.4	9.4	13.7	5.3	1.2	20.7

[a] Reproduction rate (Pf/Pi) = final population (Pf) (number of eggs + second-stage juveniles) / initial population (Pi) (number of eggs + second-stage juveniles).
[b] See Table 1.
nd, See Table 4.

The mean relative multiplication rates based on the numbers of eggs + J2 produced by the *G. rostochiensis* isolates on the five host genotypes ranged from 4.0 (PT567) to 50.6 (PT93) on Morag, from 2.2 (PT3798) to 47.8 (PT126) on Vantage and from 0.3 (PT3639) to 42.9 (PT93) on Corsair (Table 8). On Corsair this relative multiplication rate was not determined for three isolates (PT897, PT3798 and PT5695) due to the low numbers of cysts obtained. On the genotypes 12674 and Santé the highest value was 1.1 (H3448 on Santé). For the *G. pallida* isolates, the values ranged from 10.3 (PT116) to 70.8 (PT5701) on Morag, from 8.7 (PT104) to 27.5 (PT5701) on Vantage and from 11.1 (PT116) to 107.1 (PT5701) on Corsair. As for relative multiplication rates for cysts, on the genotype 12674 all the *G. pallida* isolates presented

Table 6. Fecundity of *Globodera* spp. isolates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot[a].

Species/ Isolate code[b]	Potato genotype					
	Désirée	Corsair	Morag	Vantage	12 674	Santé
G. rostochiensis						
PT1	75.6	70.0	85.6	86.4	0.0	nd
PT14	50.8	139.4	107.6	75.4	0.0	58.0
PT32	103.0	96.0	98.6	72.4	0.0	0.0
PT45	203.4	105.4	127.8	207.0	0.0	nd
PT64	72.8	146.4	158.4	128.8	0.0	0.0
PT66	152.0	51.6	120.4	215.0	0.0	0.0
PT69	136.4	74.2	62.2	45.4	0.0	nd
PT70	126.0	114.8	108.6	81.6	0.0	nd
PT74	182.0	221.0	122.6	217.8	0.0	0.0
PT75	155.8	128.8	148.4	183.0	0.0	0.0
PT79	46.6	104.4	84.2	61.4	0.0	nd
PT81	176.8	300.0	226.0	189.4	0.0	0.0
PT93	143.8	244.0	200.8	101.4	0.0	0.0
PT99	56.0	90.8	91.4	87.8	0.0	nd
PT111	123.2	116.4	71.4	62.4	0.0	0.0
PT125	178.6	134.2	183.6	148.6	0.0	nd
PT126	166.6	174.2	264.8	246.8	0.0	0.0
PT150	83.8	115.4	124.6	129.6	0.0	nd
PT219	167.8	239.2	167.4	258.2	0.0	0.0
PT220	163.2	223.0	174.6	183.2	0.0	0.0
PT221	83.2	80.0	131.4	32.0	0.0	0.0
PT222	138.6	229.2	175.2	227.4	0.0	0.0
PT225	95.2	108.6	84.4	181.8	nd	nd
PT567	144.0	102.6	90.4	106.2	0.0	0.0
PT811	135.2	125.8	117.2	113.0	0.0	50.6
PT897	127.8	nd	170.0	70.2	0.0	0.0
PT3638	8.4	34.6	11.6	17.0	0.0	0.0
PT3639	116.6	38.0	60.4	146.6	0.0	nd
PT3798	117.8	nd	117.4	67.8	0.0	nd
PT5695	85.2	nd	94.6	104.2	0.0	0.0
PT5698	138.8	130.2	115.8	175.4	0.0	nd
PT5699	66.8	64.2	73.4	43.0	0.0	25.6
PT5700	102.8	62.0	137.6	55.4	0.0	0.0
H3448	153.4	79.2	112.4	217.2	0.0	107.6
H3781	140.6	160.8	140.4	173.0	0.0	nd
H4044	48.6	13.2	51.4	62.0	0.0	0.0
H4046	52.2	103.6	78.0	57.8	0.0	0.0
H4066	94.0	43.0	18.2	102.0	0.0	nd
H4166	139.6	127.2	196.6	97.6	0.0	nd
M9	86.8	122.8	108.8	137.0	0.0	0.0
M54	126.6	63.2	100.8	129.4	0.0	nd
M55	155.4	92.2	136.8	74.2	0.0	nd
P070	205.0	95.0	130.2	149.4	0.0	nd
G. pallida						
PT104	110.2	111.6	89.4	122.4	103.6	110.2
PT116	158.4	120.8	103.2	181.6	68.0	96.0
PT5701	75.6	155.8	188.2	156.2	216.2	229.2

[a] Fecundity = Number of eggs + second-stage juveniles produced/cyst.
[b] See Table 1.
nd, See Table 4.

(*Continued*)

lower mean values (2.8 for PT104 to 6.2 for PT5701) and on Santé higher values (11.1 for PT116 to 107.1 for PT5701) (Table 8).

According to the EC Potato Cyst Nematode Directive 1969 (Anon., 1969), PCN isolates with a multiplication rate greater than 1 should be rated as virulent. Therefore, all the PCN isolates were virulent on Désirée (Table 9) but five increased less than 10fold on this cultivar. Considering the 43 *G. rostochiensis* isolates, 38 were virulent on Morag, 35 on Vantage and 21 on Corsair. Five of the virulent isolates (PT93, PT126, PT222, PT897 and M55) increased more than 10fold on Morag, four (PT126, PT219, PT220 and PT222) on Vantage and three (PT219, PT220 and PT222) on Corsair. On the genotypes 12674 and Santé, all isolates were classified as avirulent (Table

Table 7. Relative reproduction rates (cysts) of *Globodera* spp. isolates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot[a].

Species/ Isolate code[b]	Potato genotype				
	Corsair	Morag	Vantage	12 674	Santé
G. rostochiensis					
PT1	14.2	25.6	24.8	0.0	2.0
PT14	11.6	16.7	12.6	0.0	0.8
PT32	8.1	33.2	8.1	0.0	0.0
PT45	4.1	18.9	14.2	0.0	0.2
PT64	3.7	13.0	11.5	0.0	0.0
PT66	2.0	16.2	9.7	0.0	0.0
PT69	3.3	27.1	10.1	0.0	0.1
PT70	11.8	18.9	28.2	0.0	0.5
PT74	10.6	9.8	18.3	0.0	0.0
PT75	6.3	33.7	15.7	0.0	0.0
PT79	4.9	17.2	7.4	0.0	0.1
PT81	11.8	14.8	20.8	0.0	0.0
PT93	25.3	36.2	28.3	0.0	0.0
PT99	19.7	18.5	23.1	0.0	0.0
PT111	4.1	22.5	6.1	0.0	0.0
PT125	9.1	24.5	37.2	0.0	0.3
PT126	10.0	30.8	32.3	0.0	0.0
PT150	7.0	25.7	24.2	0.0	0.1
PT219	19.7	17.9	20.9	0.0	0.0
PT220	23.9	19.5	36.5	0.0	0.0
PT221	8.8	24.2	6.8	0.0	0.0
PT222	19.3	34.0	28.8	0.0	0.0
PT225	10.7	10.0	7.0	0.0	0.2
PT567	1.7	6.4	3.3	0.0	0.0
PT811	13.4	15.4	23.3	0.0	1.5
PT897	0.1	19.2	12.1	0.0	0.0
PT3638	9.9	9.6	12.1	0.0	0.0
PT3639	0.9	8.7	7.7	0.0	0.1
PT3798	0.4	26.3	3.8	0.0	0.1
PT5695	0.2	14.7	8.0	0.0	0.0
PT5698	2.2	6.4	18.0	0.0	0.5
PT5699	7.5	10.2	8.0	0.0	1.1
PT5700	18.9	12.2	18.2	0.0	0.0
H3448	3.2	12.8	12.7	0.0	1.5
H3781	6.2	20.2	16.9	0.0	0.5
H4044	20.3	14.8	13.2	0.0	0.0
H4046	3.5	8.2	10.8	0.0	0.0
H4066	1.0	1.5	12.9	0.0	0.0
H4166	5.1	11.2	8.5	0.0	0.4
M9	5.2	15.5	13.9	0.0	0.0
M54	1.4	27.9	10.5	0.0	0.2
M55	8.3	31.9	16.8	0.0	0.2
P070	2.0	26.0	17.1	0.0	0.7
G. pallida					
PT104	10.8	24.3	7.9	3.0	32.8
PT116	13.6	15.8	17.2	7.5	18.3
PT5701	23.5	28.5	13.3	2.2	35.3

[a] Relative reproduction rate = final population (cysts) (Pf) in the potato genotype / final population (cysts) (Pf) in the susceptible genotype expressed as a percentage.
[b] See Table 1.

9). For the virulent isolates, Morag showed greater variation in resistance than the other cultivars. For numbers of cysts produced, the resistance ranged from 63.8 to 98.5% on Morag, from 62.8 to 96.7% on Vantage and from 74.7 to 99.9% on Corsair. For numbers of eggs + J2 produced, the resistance of the genotypes ranged from 49.4 to 96.0% on Morag, from 52.2 to 98.8% on Vantage and from 57.1 to 99.7% on Corsair. The *G. pallida* isolates were virulent towards all genotypes, with the exception of isolate PT104 on 12674. This genotype was the most resistant, and the resistance, based on the numbers of cysts ranged from 92.5 to 97.8%, or numbers of eggs + J2 produced, ranged from 93.8 to 97.2%. The three *G. pallida* isolates increased more than 10fold on Désirée but all of them increased less than 10fold, with the

Table 8. Relative reproduction rates (eggs + second-stage juveniles) of *Globodera* spp. isolates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles / pot[a].

Species/ Isolate code[b]	Potato genotype				
	Corsair	Morag	Vantage	12 674	Santé
G. rostochiensis					
PT1	13.1	29.0	28.3	0.0	nd
PT14	31.9	35.4	18.7	0.0	0.9
PT32	7.6	31.8	5.7	0.0	0.0
PT45	2.1	11.9	14.4	0.0	nd
PT64	7.4	28.2	20.3	0.0	0.0
PT66	0.7	12.9	13.7	0.0	0.0
PT69	1.8	12.4	3.4	0.0	nd
PT70	10.8	16.3	18.3	0.0	nd
PT74	12.8	6.6	21.8	0.0	0.0
PT75	5.2	32.1	18.4	0.0	0.0
PT79	10.9	31.0	9.7	0.0	nd
PT81	20.1	19.0	22.3	0.0	0.0
PT93	42.9	50.6	19.9	0.0	0.0
PT99	31.9	30.1	36.2	0.0	nd
PT111	3.9	13.1	3.10	0.0	0.0
PT125	6.8	25.2	30.9	0.0	nd
PT126	10.3	50.0	47.8	0.0	0.0
PT150	9.7	38.2	37.4	0.0	nd
PT219	28.1	17.8	32.1	0.0	0.0
PT220	32.7	20.8	41.0	0.0	0.0
PT221	8.5	38.2	2.6	0.0	0.0
PT222	32.0	42.9	47.2	0.0	0.0
PT225	12.2	8.8	13.4	nd	nd
PT567	1.2	4.01	2.4	0.0	0.0
PT811	12.4	13.4	19.4	0.0	0.5
PT897	nd	25.5	6.7	0.0	0.0
PT3638	40.6	13.2	24.5	0.0	0.0
PT3639	0.3	4.5	9.7	0.0	nd
PT3798	nd	26.2	2.2	0.0	nd
PT5695	nd	16.3	9.8	0.0	0.0
PT5698	2.0	5.3	22.8	0.0	nd
PT5699	7.2	11.2	5.2	0.0	0.4
PT5700	11.4	16.3	9.8	0.0	0.0
H3448	1.6	9.4	18.0	0.0	1.1
H3781	7.1	20.1	20.9	0.0	nd
H4044	5.5	15.6	16.9	0.0	0.0
H4046	6.9	12.3	11.9	0.0	0.0
H4066	0.4	0.3	14.0	0.0	nd
H4166	4.7	15.7	6.0	0.0	nd
M9	7.4	19.4	22.0	0.0	0.0
M54	0.7	22.2	10.7	0.0	nd
M55	4.9	28.0	8.0	0.0	nd
P070	0.9	16.5	12.5	0.0	nd
G. pallida					
PT104	11.0	19.7	8.7	2.8	32.8
PT116	10.4	10.3	19.8	3.2	11.1
PT5701	48.3	70.8	27.5	6.2	107.1

(*Continued*)

[a] Relative reproduction rate = final population (eggs + second-stage juveniles) (Pf) in the potato genotype / final population (eggs + second-stage juveniles) (Pf) in the susceptible genotype expressed as a percentage.
[b] See Table 1.
nd, See Table 4.

exception of PT5701, on Morag and Santé. The least resistant genotype was Santé.

The isolates ranked the resistance of the genotypes with a high level of consistency and this was confirmed by the analysis of variance of the cyst and egg data (Tables 10 and 11). The analysis of variance showed that there were significant main effects among isolates and among potato genotypes and also significant effects of the interactions between nematode isolates and potato genotypes ($P<0.001$). Significant differences were found among the nematode isolates and the potato genotypes, the variation being much greater in the genotypes.

Results of the Hierarchical Cluster analyses (HCA) (Figures 1 and 2) and of the Principal Component Analysis (PCA) (Figures 3 and 4), for cysts and

Table 9. Virulence responses of *Globodera* spp. isolates based on reproduction rates on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles /pot[a].

Species/ Isolate code[b]	Potato genotype[c]					
	Désirée	Corsair	Morag	Vantage	12674	Santé
G. rostochiensis						
PT1, PT3638	+	-	-	-	-	-
PT14, PT70, PT74, PT79, PT81, PT93, PT99, PT126, PT150, PT219, PT220, PT222, PT225, PT567, PT811, PT5700, H3781, H4166, M55	+	+	+	+	-	-
PT32, PT69, PT111, PT3798	+	-	+	-	-	-
PT45, PT64, PT66, PT75, PT125, PT897, PT5698, PT5695, H3448, H4044, H4046, P070, M54	+	-	+	+	-	-
PT221, PT5699	+	+	+	-	-	-
PT3639, H4066, M9	+	-	-	+	-	-
G. pallida						
PT104	+	+	+	+	-	+
PT116, PT5701	+	+	+	+	+	+

[a] Reproduction rate (Pf/Pi) = final population (Pf) (number of eggs + second-stage juveniles) / initial population (Pi) (number of eggs + second-stage juveniles).
[c] +, Pf/Pi>1; -, Pf/Pi≤1.
[b] See Table 1.

Table 10. Analysis of variance of cyst numbers (after logarithmic transformation) produced from 43 isolates of *Globodera rostochiensis* and three of *G. pallida* on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot.*

Source of variation	Degrees of freedom	Mean Square	Variance ratio
Nematode isolates	45	1.145440	1453.67*
Host genotypes	5	50.749770	64405.87*
Isolates × genotypes interaction	225	0.240790	305.59*
Residual	1104	0.000788	
Total	1379		

*Significance for $P<0.001$.

Table 11. Analysis of variance of egg+ second-stage juveniles numbers (after logarithmic transformation) produced from 43 isolates of *Globodera rostochiensis* and three of *G. pallida* on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot.*

Source of variation	Degrees of freedom	Mean Square	Variance ratio
Nematode isolates	45	2.039500	1257.9*
Host genotypes	5	196.995100	121494.5*
Isolates × genotypes Interaction	225	0.840700	518.5*
Residual	1104	0.001621	
Total	1379		

*Significance for $P<0.001$.

eggs + J2 produced, clearly distinguished the two PCN species, but grouping of isolates according to geographic origin was not possible (Table 1). Isolates clustered differently on HCA when cysts and eggs + J2 produced were considered. The interactions revealed by the analysis of variance are also revealed in the biplots of PCA. The first axis, accounting for 50 of the variation for cysts and 53% of the variation for

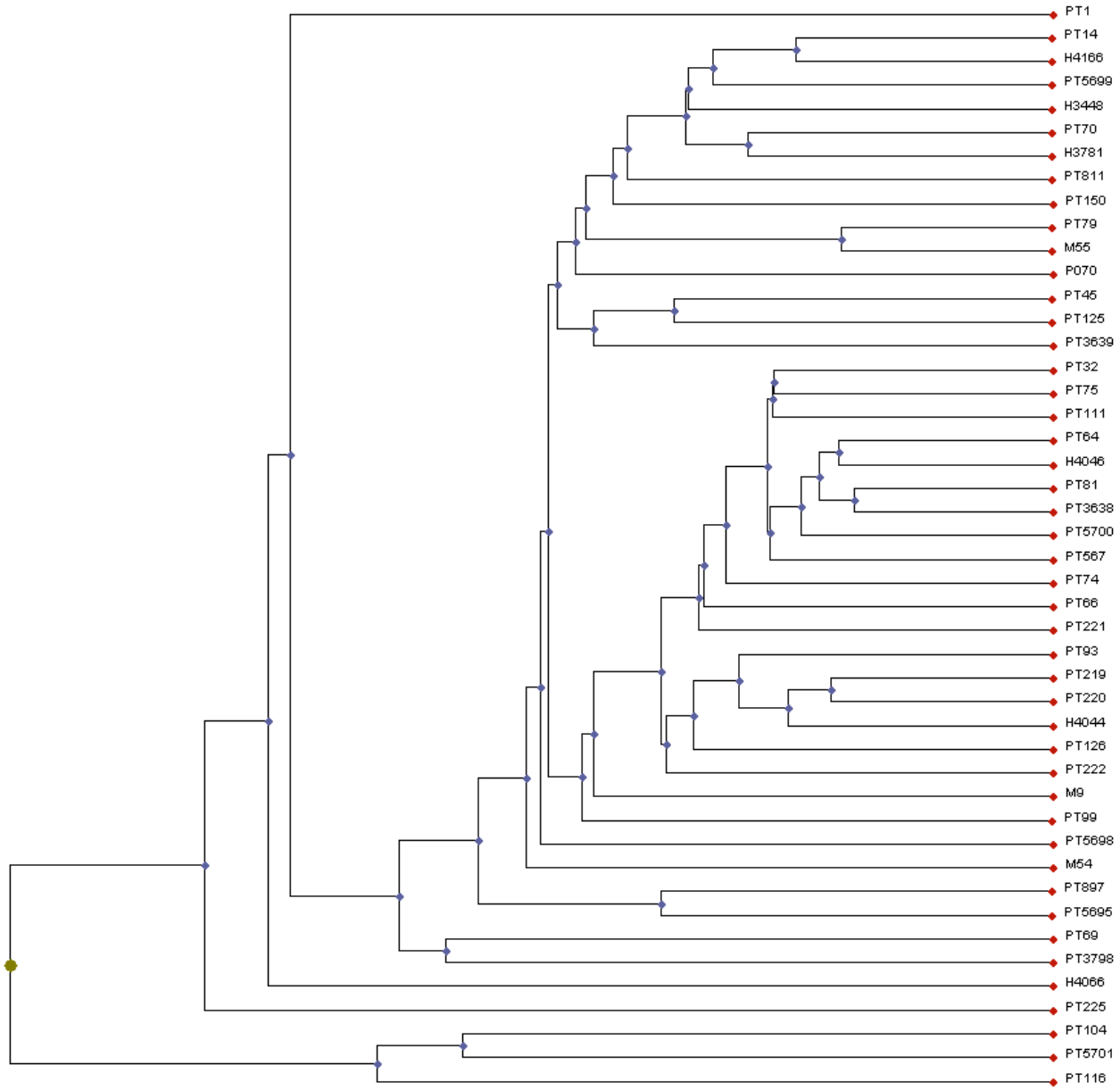

Figure 1. Relationships of 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on Hierarchical Cluster Analyses of the cyst numbers produced on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles / pot. For isolate codes see Table 1.

eggs + J2, clearly differentiated two groups of genotypes and the isolates of the two species. The second axis, accounting for 28 of the variation for cysts and 30% of the variation for eggs + J2, clearly showed the difference between the two groups of genotypes and among the three *G. pallida* isolates. The *G. rostochiensis* isolates appeared almost together but with the exception of isolates PT126 and PT222 for cysts, and PT220, PT219, PT126 and PT222 for eggs + J2. The PCA showed that, for both, cysts and eggs + J2, there

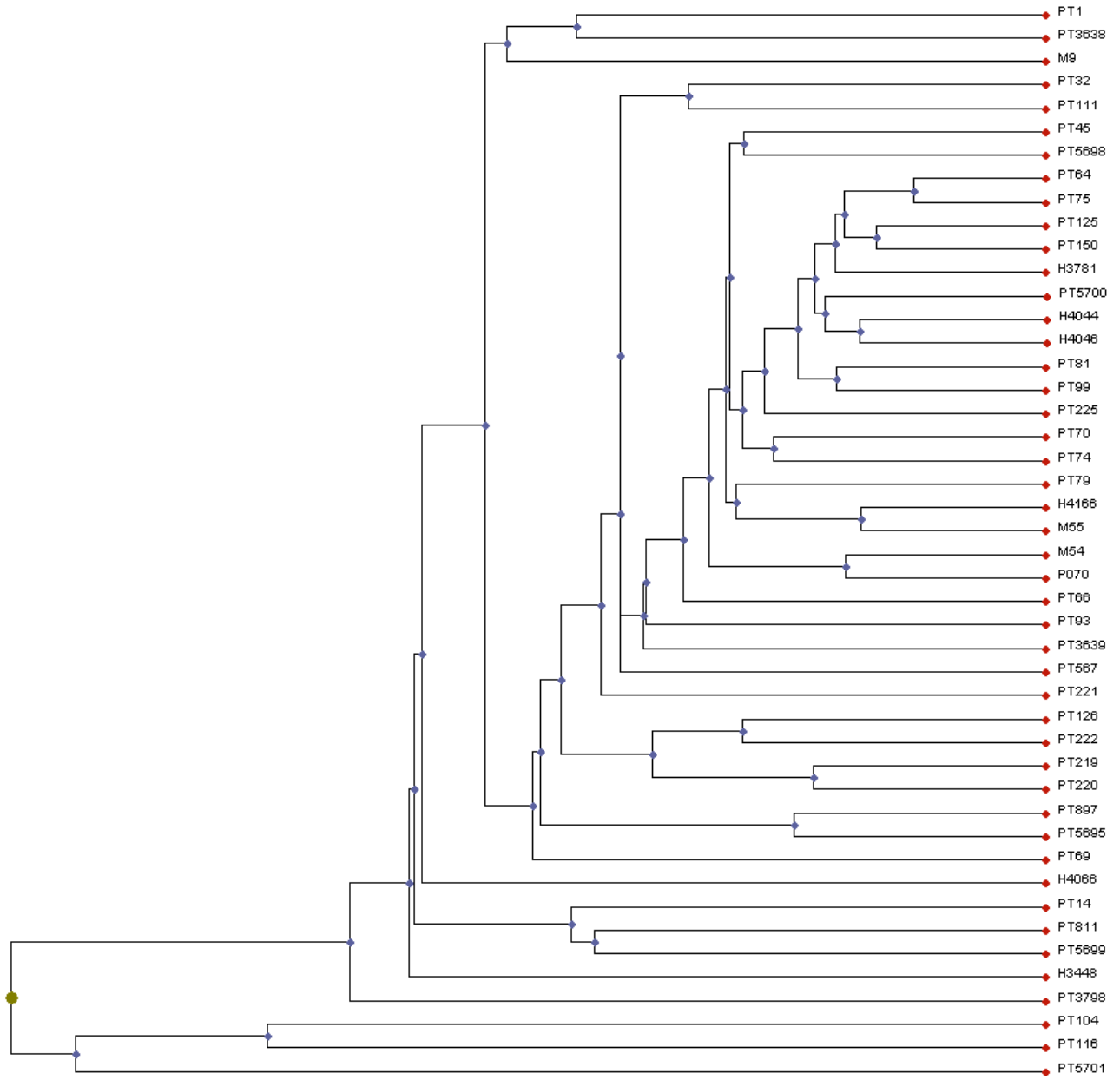

Figure 2. Relationships of 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on Hierarchical Cluster Analyses of the egg + second-stage juveniles numbers produced on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot. For isolate codes see Table 1.

were significant interactions among the genotypes Désirée, Corsair, Morag and Vantage, and almost all isolates of *G. rostochiensis*, and there were also significant interactions with all potato genotypes and the *G. pallida* isolates.

Discussion

The pathotype classifications of the isolates used in this experiment were not identified, not only because all the differential clones needed in the interna-

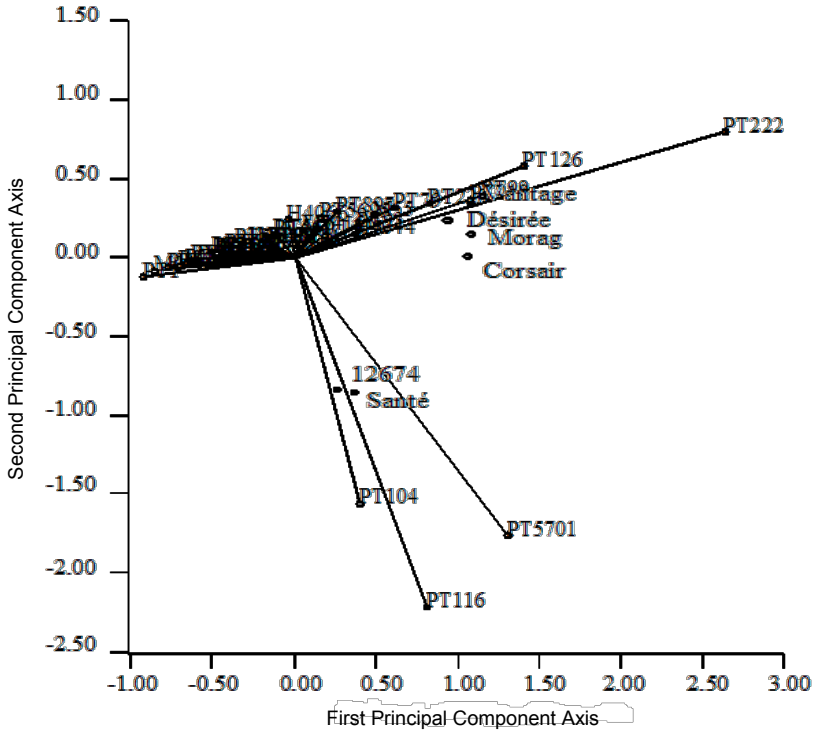

Figure 3. Principal Component Analysis of the host genotypes x nematode isolates interaction effects for 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on the cyst numbers produced on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot. For isolate codes see Table 1.

tional pathotyping scheme of Kort *et al*. (1977) were not available but also because of the serious doubt that has been cast on the validity of this scheme (Phillips and Trudgill, 1983; Mugniery *et al*., 1989).

Désirée was a good genotype to use as a susceptible control since all isolates had good reproduction on this cultivar (Tables 3 and 4). Although both species of *Globodera* increased most on Désirée, there were significant differences in increase (Pf/Pi) among isolates of both species. For one isolate of *G. pallida* (PT7501), the nematode increase was greater on Santé than on Désirée when the number of eggs + J2 was considered (Table 5). Dale and Phillips (1985) have already shown that susceptibility to *G. pallida* varies significantly among susceptible cultivars of *Solanum tuberosum tuberosum* and, for this reason, the value of expressing Pf/Pi as a percentage of that on Désirée or other susceptible control is doubtful. However, when susceptible and partially resistant potato genotypes were used, such data were useful for the evaluation, because it was observed that the resistance of partially resistant potato genotypes and virulence of the nematode isolates generally were ranked in similar order (Mugniéry *et al*., 1989).

Consistent differences in the evaluation of the reproduction of both nematode species based on cysts or eggs + J2 were observed (Tables 3 and 4). In general, the relative multiplication rates were greater when considering eggs + J2 values (Tables 7 and 8). The disparity of the results is due to the differences observed in fecundity, which differed significantly among all isolates: more cysts do not necessarily imply more eggs. Sometimes fecundity was greater when the number of cysts was lower, which is in agreement with Whitehead (1991) who considered that cysts/pot should not be used as a direct measure of nematode increase. Resistance to *G. pallida* resulted both in fewer cysts/pot and in fewer eggs + J2 within cysts, as can be seen on genotype 12674 (Table 6). On the other hand, resistance to *G. rostochiensis* was expressed in fewer cysts/pot but with cysts containing as many or more eggs as

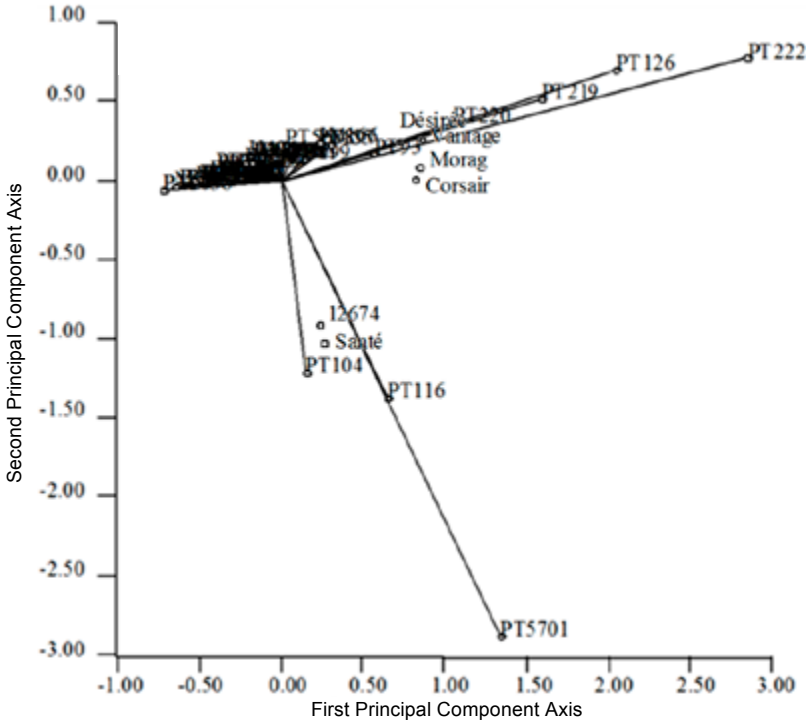

Figure 4. Principal Component Analysis of the host genotypes x nematode isolates interaction effects for 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on the egg + second-stage juveniles numbers produced on six differential potato genotypes inoculated with 4000 eggs+ second-stage juveniles/pot. For isolate codes see Table 1.

in cysts on the susceptible cultivars. This can be explained by the longer period of hatching in *G. pallida* (Whitehead *et al*., 1987), which results in those cysts developing late having less egg laying capacity. The period of hatching in *G. rostochiensis* is shorter, so all the females have longer periods to lay eggs. Therefore, when the resistance of potato genotypes is evaluated only by the numbers of cysts, they may be classified as more or less resistant, than in reality, and the virulence of PCN isolates may be underrated (Phillips and Trudgill, 1983; Whitehead, 1991). For example, if there were more cysts but with few eggs/cyst, using cyst numbers alone would tend to classify genotypes as less resistant than they really are. On the other hand, some authors have refuted 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 assays, to minimize the impact of environmental parameters, the environmental conditions were kept rigorously the same throughout the experiment for all the genotypes.

Our results emphasize the need to both use cyst and egg counts for the evaluation of the virulence of PCN isolates, and that this approach can be very useful to define the partial resistance of potato genotypes. In our biplots, the *G. pallida* isolates were more separated than *G. rostochiensis* (Figures 3 and 4) but on clusters they are not distinct. On clusters they are separated from *G. rostochiensis* but clustered differently when cysts or eggs were considered (Figures 1 and 2).

PCN reproduction on cultivars with partial or quantitative resistance was significantly less than that on a fully susceptible cultivar. The virulence behaviour of the Portuguese isolates showed great variability and for both cysts/pot and eggs/pot the analyses of variance showed highly significant effects of the genotypes and of the nematode isolates. The effect of the genotype × isolate interaction was also highly significant, which is in agreement with previous reports (Gonzalez *et al*., 1996; Bendezu *et*

al., 1998). The dissimilarities observed in the potato genotypes were expected to be due to the large differences in terms of their resistance and to the variability of the reactions of the potato genotypes to the PCN isolates. Multiplication on resistant genotypes revealed clear differences of virulence among isolates, reflected in interactions between the isolates and the sources of resistance tested.

Results obtained with Portuguese isolates on potato cultivar Morag confirmed that this genotype is partially resistant to *G. rostochiensis* but not *ca.* 80% resistant to *G. pallida* isolates, as reported by Gonzalez *et al*. (1996). Resistance to PT5701 was only 29.2% when eggs were considered, or 71.5%, when cysts were considered. The Portuguese isolates probably had very high frequencies of the virulence alleles that interact with the genes for resistance inherited by Morag from *Solanum* vernei, as suggested by Bendezu *et al*. (1998). On *S. vernei* genotypes, where the resistance is predominantly additive (Gonzalez *et al*., 1996), the cv. Vantage appears to have more genes for resistance than Morag. Corsair, with genes for resistance derived from *S. vernei* and *S. multidissectum*, was the most resistant of the three cultivars. These cultivars were better hosts for *G. pallida* populations than for *G. rostochiensis*. As the *G. rostochiensis* isolates did not reproduce on the potato genotypes derived from *S. andigena* (12674 and Santé), they can be included in the Ro1/Ro4 group. The variation of virulence detected in *G. rostochiensis* isolates indicates a continuum of virulence, rather than discrete pathotypes. This may reflect a lower degree of genetic diversity, compared with *G. pallida,* or that *G. rostochiensis* isolates are more closely related. Either way, these results support a range of observations by other research, suggesting that there is less heterogeneity in European *G. rostochiensis* than in *G. pallida*, as a result of a greater founder effect for *G. rostochiensis* than for *G. pallida* in Europe. These results also support the view that such isolates derive from the same introduction. The continuous range of virulence revealed by the *G. rostochiensis* isolates to resistant genotypes makes the management of PCN by IPM more difficult.

The division of *G. pallida* isolates into pathotypes Pa2/3 seems not very useful and the Portuguese isolates do not fit in any of the pathotypes considered. These isolates reproduced on Corsair (with the H2 resistance gene that confers resistance to Pa1), on Morag and Santé (partially resistant to Pa2/3) and poorly on 12674 (resistant to Pa3). The virulence of *G. pallida* isolates on Morag and on genotype 12674 was similar to United Kingdom *G. pallida* isolates (Phillips and Trudgill, 1998; Phillips *et al*., 1998). Although isolate PT5701 of *G. pallida* appeared to be more virulent than the others, the results on Désirée suggest that the isolates do not have a very diverse genetic composition. This can be partially explained by the fact that the degree of virulence of an isolate depends on the interaction of several genes and the expression of the resistance is ruled not only by the nematode genes but also by their interaction with the environment. This produces a range of values difficult to separate into categories. Our findings are in agreement with those obtained in other studies with PCN isolates from the Canary Islands and the United Kingdom, where the *G. rostochiensis* isolates had less genetic variability than *G. pallida* isolates (Gonzalez *et al.,* 1996; Bendezu *et al*., 1998).

The results with genotype 12674 for Portuguese isolates confirms the results obtained by Howard *et al.* (1970), in which the gene H3 was found to confer high levels of resistance to most European isolates of PCN.

The differences found in the virulence of Portuguese PCN isolates from the same geographic origin indicated that there is no apparent relationship between the levels of virulence and the geographic origin of the isolates.

The virulence towards the potato genotypes was compared with the results obtained with TwoDimensional Gel Electrophoresis, RAPD analysis and SDSCapillary Gel Electrophoresis (Cunha *et al*., 2000, 2006; Conceição *et al*., 2003). All the studies revealed that: i) all Portuguese isolates of *G. rostochiensis* are Ro1/Ro4; ii) *G. pallida* isolates could have come from two different introductions, one for the isolates from Aveiro (PT104 and PT116) and another for the isolate from Vila Real (PT5701); and (iii) there is no relationship between the protein patterns or the virulence behaviour of the isolates and their geographic origin within Portugal. This lack of differentiation may be because Portugal has imported seed potatoes from Ireland, The Netherlands and France for all of the potato producing areas. PCN introductions could have been made in different regions from the same origin or to the same region from different origins.

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