

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 polygenically based 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 |
|--|-----------|
| <i>G. rostochiensis</i> | |
| 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 |
| <i>G. pallida</i> | |
| 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 | <i>Solanum multidissectum</i> and <i>S. vernei</i> | H2 | Resistant to Pa1 Partially resistant to other <i>G. pallida</i> populations (1; 2) ^a |
| Morag | <i>S. vernei</i> CPC 2488 and CPC 2487 | Polygenic | Partially resistant to Pa2/3 ca. 80% resistant to most of <i>G. pallida</i> populations Partially resistant to <i>G. rostochiensis</i> populations (1; 2) |
| Vantage | <i>S. vernei</i> | Polygenic | Moderate resistance to <i>G. pallida</i> populations Partially resistant to <i>G. rostochiensis</i> populations (1) |
| 12674 | <i>S. tuberosum andigena</i> CPC 2802 | H3 | Resistance to Pa3 Resistance to <i>G. rostochiensis</i> European populations (3; 4) |
| Santé | <i>S. tuberosum andigena</i> and <i>S. vernei</i> | Polygenic | Partially resistant to Pa2/3 Resistant to <i>G. rostochiensis</i> 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 \times 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 \times 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 |
| <i>G. rostochiensis</i> | | | | | | |
| 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 |
| <i>G. pallida</i> | | | | | | |
| 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 |
| <i>G. rostochiensis</i> | | | | | | |
| 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 |
| <i>G. pallida</i> | | | | | | |
| 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 |
| <i>G. pallida</i> | | | | | | |
| 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.

(Continued)

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 |
| <i>G. rostochiensis</i> | | | | | | |
| 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 |
| <i>G. pallida</i> | | | | | | |
| 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 |
| 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.

(Continued)

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 |
| <i>G. rostochiensis</i> | | | | | | |
| 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 |
| <i>G. pallida</i> | | | | | | |
| 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 | | | | | Species/ Isolate code ^b | Potato genotype | | | | | | | | | | |
|--|-----------------|-------|---------|--------|-------|---|-----------------|-------|---------|--------|-------|--------|------|------|------|-----|------|
| | Corsair | Morag | Vantage | 12 674 | Santé | | Corsair | Morag | Vantage | 12 674 | Santé | | | | | | |
| <i>G. rostochiensis</i> | | | | | | PT897 | 0.1 | 19.2 | 12.1 | 0.0 | 0.0 | PT3638 | 9.9 | 9.6 | 12.1 | 0.0 | 0.0 |
| PT1 | 14.2 | 25.6 | 24.8 | 0.0 | 2.0 | PT3639 | 0.9 | 8.7 | 7.7 | 0.0 | 0.1 | PT3798 | 0.4 | 26.3 | 3.8 | 0.0 | 0.1 |
| PT14 | 11.6 | 16.7 | 12.6 | 0.0 | 0.8 | PT5695 | 0.2 | 14.7 | 8.0 | 0.0 | 0.0 | PT5698 | 2.2 | 6.4 | 18.0 | 0.0 | 0.5 |
| PT32 | 8.1 | 33.2 | 8.1 | 0.0 | 0.0 | PT5699 | 7.5 | 10.2 | 8.0 | 0.0 | 0.0 | PT5700 | 18.9 | 12.2 | 18.2 | 0.0 | 0.0 |
| PT45 | 4.1 | 18.9 | 14.2 | 0.0 | 0.2 | H3448 | 3.2 | 12.8 | 12.7 | 0.0 | 1.5 | H3781 | 6.2 | 20.2 | 16.9 | 0.0 | 0.5 |
| PT64 | 3.7 | 13.0 | 11.5 | 0.0 | 0.0 | H4044 | 20.3 | 14.8 | 13.2 | 0.0 | 0.0 | H4046 | 3.5 | 8.2 | 10.8 | 0.0 | 0.0 |
| PT66 | 2.0 | 16.2 | 9.7 | 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 |
| PT69 | 3.3 | 27.1 | 10.1 | 0.0 | 0.1 | M9 | 5.2 | 15.5 | 13.9 | 0.0 | 0.0 | M54 | 1.4 | 27.9 | 10.5 | 0.0 | 0.2 |
| PT70 | 11.8 | 18.9 | 28.2 | 0.0 | 0.5 | M55 | 8.3 | 31.9 | 16.8 | 0.0 | 0.2 | P070 | 2.0 | 26.0 | 17.1 | 0.0 | 0.7 |
| PT74 | 10.6 | 9.8 | 18.3 | 0.0 | 0.0 | <i>G. pallida</i> | | | | | | | | | | | |
| PT75 | 6.3 | 33.7 | 15.7 | 0.0 | 0.0 | PT104 | 10.8 | 24.3 | 7.9 | 3.0 | 32.8 | PT116 | 13.6 | 15.8 | 17.2 | 7.5 | 18.3 |
| PT79 | 4.9 | 17.2 | 7.4 | 0.0 | 0.1 | PT5701 | 23.5 | 28.5 | 13.3 | 2.2 | 35.3 | | | | | | |
| PT81 | 11.8 | 14.8 | 20.8 | 0.0 | 0.0 | ^a Relative reproduction rate = final population (cysts) (Pf) in the potato genotype / final population (cysts) (Pf) in the susceptible genotype expressed as a percentage. | | | | | | | | | | | |
| PT81 | 11.8 | 14.8 | 20.8 | 0.0 | 0.0 | ^b See Table 1. | | | | | | | | | | | |
| 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 | | | | | | | | | | | | |

(Continued)

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

(Continued)

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 |
| <i>G. rostochiensis</i> | | | | | | |
| 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 | + | - | - | + | - | - |
| <i>G. pallida</i> | | | | | | |
| 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 $P < 0.001$.

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

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 $P < 0.001$.

+ 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

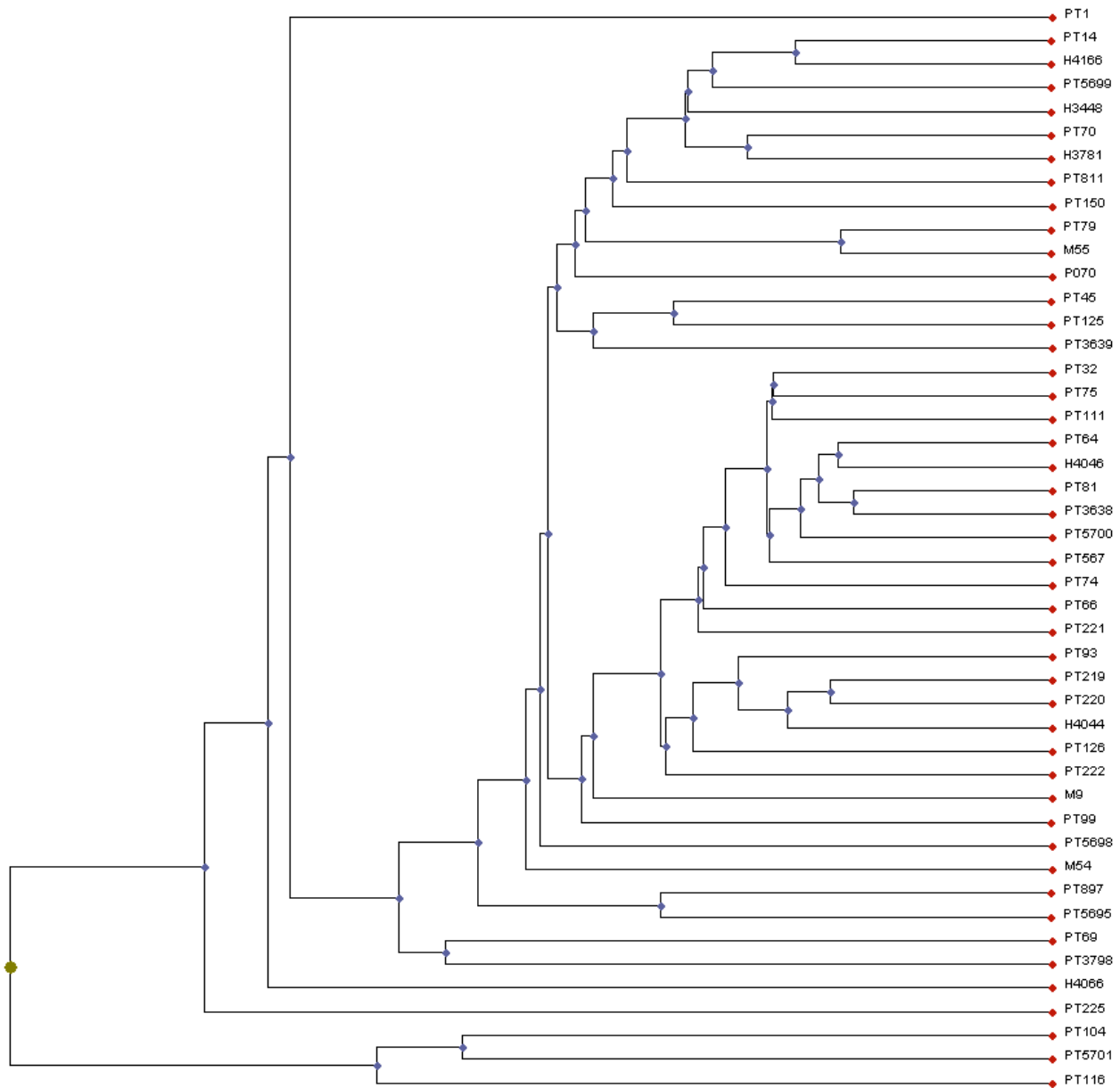


Figure 1. Relationships of 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on Hierarchical Cluster Analyses of the cyst numbers produced on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles / pot. For isolate codes see Table 1.

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

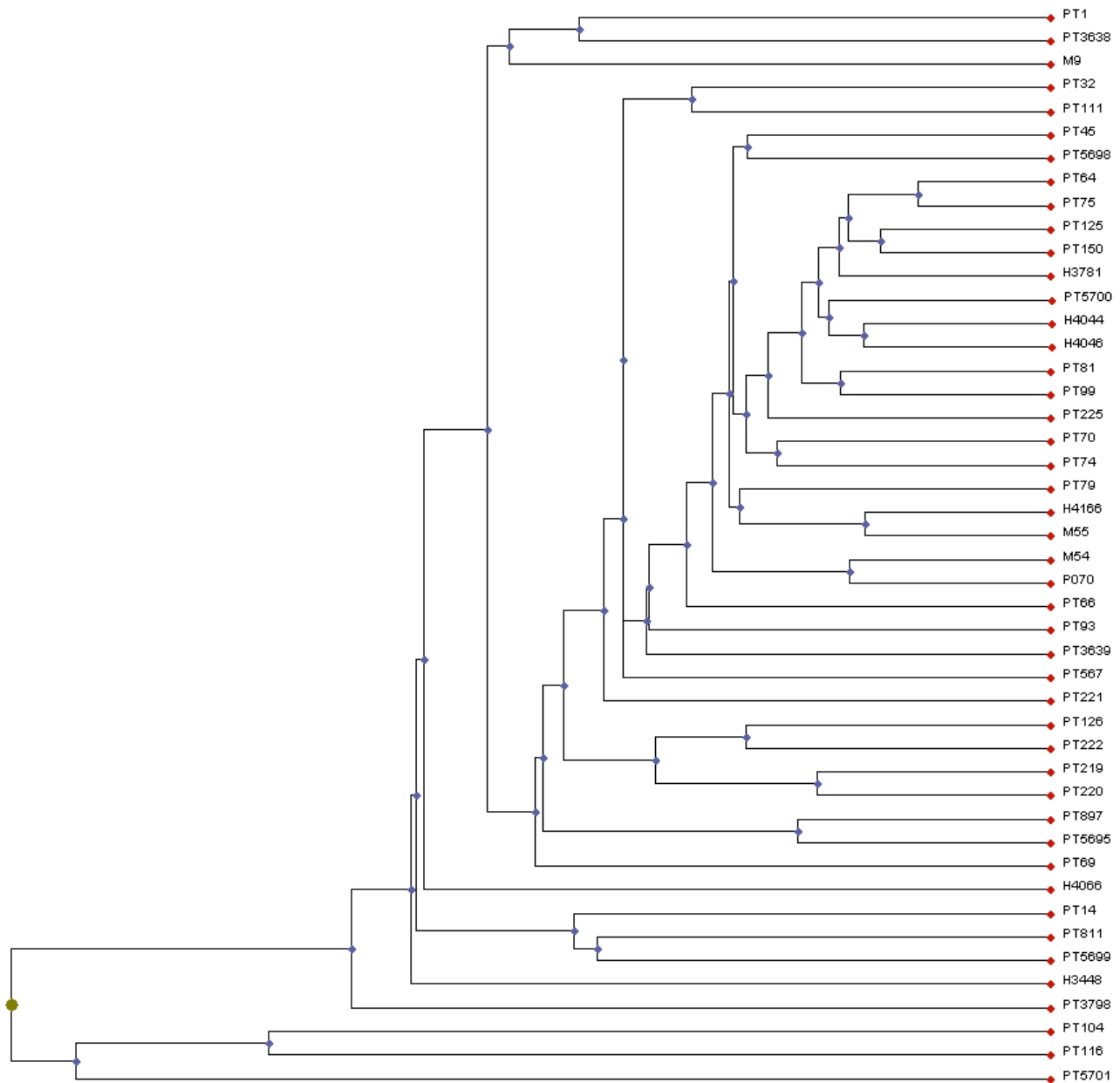


Figure 2. Relationships of 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on Hierarchical Cluster Analyses of the egg + second-stage juveniles numbers produced on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot. For isolate codes see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

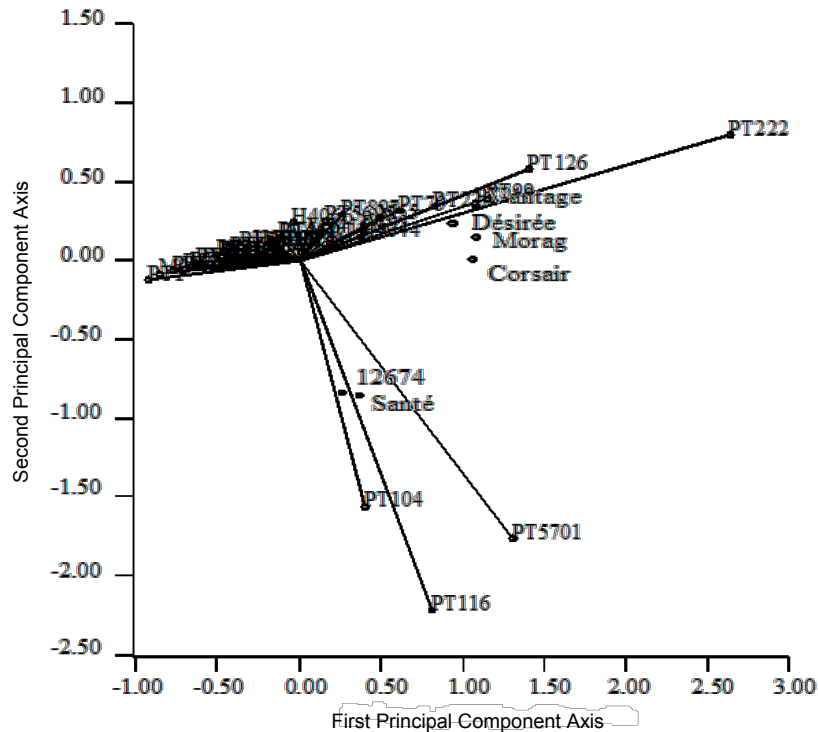


Figure 3. Principal Component Analysis of the host genotypes \times nematode isolates interaction effects for 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on the cyst numbers produced on six differential potato genotypes inoculated with 4000 eggs + second-stage juveniles/pot.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

ance of partially resistant potato genotypes and virulence of the nematode isolates generally were ranked in similar order (Mugniery *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

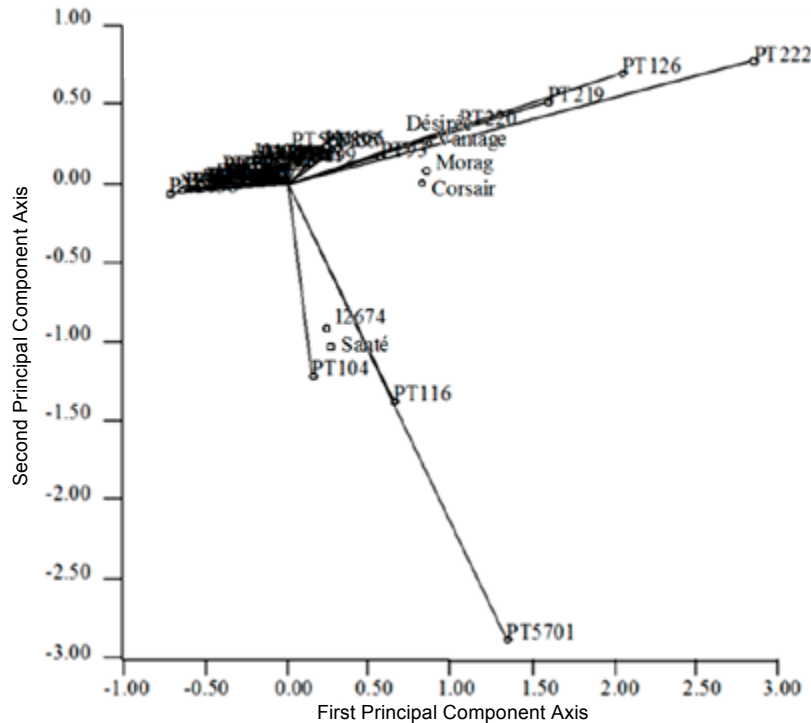


Figure 4. Principal Component Analysis of the host genotypes \times nematode isolates interaction effects for 43 isolates of *Globodera rostochiensis* and three of *G. pallida* based on the egg + second-stage juveniles numbers produced on six differential potato genotypes inoculated with 4000 eggs+ second-stage 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 \times 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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