PATHOTYPES AND HETEROGENEITY OF ITALIAN POPULATIONS OF *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA*

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Summary. Investigations were undertaken on the pathotypes and heterogeneity of Italian populations of the potato cyst nematodes, *Globodera rostochiensis* and *G. pallida*. All nematode populations were collected from the field and reproduced on a susceptible potato cultivar. To identify pathotypes, the differential *Solanum* spp. clones were grown in pots containing 1000 cm^3 of soil infested with 5 eggs/cm^3 of either nematode species, in a glasshouse at 20 ± 2 °C. Seventy days after potato emergence, the nematode population density in each pot was determined and the pathotype identified according to nematode reproduction rate. There were sixteen populations of *G. rostochiensis* and ten of *G. pallida*. All populations of *G. rostochiensis* reproduced more or less exclusively on both *Solanum tuberosum* cv. Spunta (susceptible) and cv. Saturna (possessing the H1resistant gene), so were considered pathotype Ro2. Of the populations of *G. pallida*, six were pathotype Pa3 as they reproduced on all of the differential clones, three did not reproduce on the clone $(Vt^n)^2$62.33.3 of *S. vernei* and were classified as Pa2, and one population could not be clearly classified. The heterogeneity of seven populations of *G. rostochiensis* was investigated by examining the reproduction of ten single cyst derived populations for each of the original populations on potato cvs Spunta and Saturna, and on clone KTT/60.21.19 of *S. kurtzianum*. All of the single cyst populations derived from five of the parent populations were identical to their parent populations as they reproduced only on the first two cultivars, while one from a population from Puglia and five from a population from Calabria also reproduced on clone KTT/60.21.19.

Key words: Potato, potato cyst nematodes.

The potato cyst nematodes, *Globodera rostochiensis* (Woll.) Skarbilovich and *G. pallida* (Stone) Behrens, are found in most of the major potato growing areas of the world (Turner and Evans, 1998). They are also widespread and cause severe yield losses to potato (*Solanum tuberosum* L.) in Italy (Greco *et al*., 1993). The control of these nematodes with crop rotation, chemicals and soil solarization is feasible but these control methods may cause pollution, and may be costly or difficult to implement. The use of resistant cultivars, however, is safe and easy and therefore attractive to farmers. Unfortunately, in Europe, five pathotypes of *G. rostochiensis* and three of *G. pallida* have been identified (Kort *et al*., 1977; Fleming and Powers, 1998; Scurrah *et al*., 2005). Moreover, many of the available resistant potato cultivars are resistant only to pathotype Ro1 of *G. rostochiensis*, and only a few are reported resistant to all five pathotypes of this species. Therefore, the choice of a resistant cultivar requires precise information on the nematode species and pathotypes present in a given area or, better, in a given field. Such information was limited in Italy (Di Vito, 1981). Therefore, investigations have been undertaken since 1997 to identify pathotypes of populations of potato cyst nematodes present in the different regions of the country and obtain insights on their heterogeneity. When an electrophoretic analysis of isozyme patterns from cysts of standard *Globodera* pathotypes was attempted, it was not possible to obtain specific markers for each pathotypes (Fox and Atkinson, 1984; Molinari, 2006). Moreover, grouping of potato cyst nematode populations by molecular techniques (Bakker *et al*., 1992; Blok and Phillips, 1995; Cunha *et al*., 2006)) has sometimes proved not to coincide with nematode pathotypes as classified according to Kort *et al*. (1977). Therefore, we chose to use a method based on the differential *Solanum* spp. clones of Kort *et al*. (1977).

MATERIALS AND METHODS

Identification of the pathotypes. The sixteen populations of *G. rostochiensis* (Table I) were from the regions Calabria, Campania, Abruzzi, Emilia-Romagna, Puglia and Trentino Alto Adige, while the ten populations of *G. pallida* (Table II) were from Campania, Puglia and Sicilia. They were collected from a single position in each field or from the rhizosphere of a few potato plants, close to each other, when present. These populations were reared on a susceptible potato cultivar (Spunta or Sieglinde) to produce sufficient new cysts for use in the tests. The host differentials used were the cvs Spunta and Saturna of *S. tuberosum* and the clones of *S. kurtzianum* Bitt. *et* Witt. (KTT/60.21.19), *S. multidissectum* Hawkes (P55/7) and *S. vernei* Bitt. *et* Witt. [GLKS-58-1642.4, $(Vt^n)^2$62.33.3] suggested by Kort *et al*. (1977) and Canto-Saenz and Mayer de Scurrah (1977). The clone GP 6 AD (CIP code 2800090.10), which differentiates between the Andean pathotypes PA5 and PA6 (Llontop *et al*., 1987), was also included in the test of the four populations of *G. rostochiensis* from Trentino Alto Adige and for two populations of *G. pallida* from Puglia (Polignano 3) and Sicilia (Siracusa). All differen-

tial clones (Tables I and II), except the *S. tuberosum* cv. Spunta used as susceptible check, were supplied by the International Potato Center, Lima, Peru, as *in vitro* cultures. These were multiplied to obtain tubers or cuttings, although sprouting tubers were used in most of the tests. They were planted in 14-cm-diameter clay pots containing about 1000 cm^3 of sandy soil (89% sand) infested with 5 eggs/g of either nematode population. The pots were arranged randomly on a bench in a glasshouse at 20 ± 2 °C. During the growing period the pots were irrigated as required and fertilised every other week with a liquid fertiliser. Insecticides were used to control whiteflies and only rarely other insects. About 70-80 days after emergence, the plants were cut off at ground level and the soil in the pots left to dry. The soil of each pot was then separately mixed and nematode cysts were extracted from a 200 g sub-sample with a Fenwick can. The cysts were further separated from the soil debris with the ethanol flotation method (Seinhorst, 1974), separated from soil particles, counted, crushed according to Bijloo's modified method (Seinhorst and Ouden, 1966), and their egg content determined in order to calculate the reproduction rate of the nematodes on the differential clones. The nematode was assumed to have failed to reproduce on a differential clone when the reproduction rate on that clone was ≤ 1.

Investigation of the heterogeneity of virulence of the nematode populations. To assess the heterogeneity of Italian populations of *G. rostochiensis*, the populations from Ronzo, Bellaria, Fucino Valley 1, Barletta, Molfetta, Polignano 2, and Celino, were used to obtain populations from single cysts. Twenty cysts from each of these populations were introduced singly into the rhizosphere of 10-day-old plants of cvs Spunta or Sieglinde reared in 14-cm-diameter clay pots filled with the same soil as above. After 60-70 days the old plants were removed and new tubers transplanted to increase the nematode population. Forty to fifty days later the root ball of these plants was checked for the presence of nematode females on the roots. The pots that showed root infestation were left for a further twenty days to allow females to turn into cysts, which could then be used to further increase the nematode populations. For this, the soil of these small pots was mixed with 3.2 dm^3 of steam sterilised soil of the same texture, and the mixture was transferred to 21-cm-diameter clay pots in which tubers of the susceptible potato cultivar were planted. These pots were maintained in the same glasshouse or outdoors in the shade during autumn-spring. At the end of the growth cycle, the nematode population density in each pot was determined by extracting the cysts from a 200 g sample of dried soil and counting the eggs as described above. The nematode populations derived from single cysts from each of the seven mentioned localities were then used for further investigation. Each was used to infest soil lots (texture as mentioned) with 10 eggs of the nematode/g and fifteen 14-cm-diameter clay pots were filled with infested soil of each population. As the parent nematode populations had all been found to be Ro2, five pots were sown with the cv. Spunta (susceptible to all pathotypes), five with cv. Saturna (possessing the H1 gene conferring resistance to Ro1 but susceptible to Ro2), and five with the clone KTT 60.21.19, which is resistant to both of the pathotypes Ro1 and Ro2. Potato plantlets used in these tests were rooted cuttings obtained from mother plants by an adaptation of the method suggested by Bryan *et al.* (1981).

About 40 days after transplanting, the root ball of each potato plant was inspected for the presence of nematode females. The soil of the pots in which the plants of the clone KTT 60.21.19 were free of nematode females was discarded, but that of the pots with roots of this clone showing even a few females was saved and transplanted with tubers or rooted cuttings of potato cv. Spunta to increase the nematode population. The cv. Spunta was used because preliminary attempts to increase nematode populations on the clone KTT 60.21.19 failed. These populations were used to infest the soil of five 14-cm-diameter pots to which were added single rooted cuttings of the clone KTT 60.21.19. Forty-fifty days after transplanting, these pots were again checked visually for the presence of nematode females on the root ball, and the plants in the pots found infested were left to grow for about twenty days more to allow the females to turn into cysts. The nematode soil population density was determined as described above.

RESULTS

Identification of the pathotypes. Although variation occurred in the reproduction of the *G. rostochiensis* populations on the different *Solanum* spp. clones, the data clearly demonstrated that the nematode reproduced well only on the susceptible potato cvs Spunta or Sieglinde and on the cv. Saturna carrying the resistant gene H1 (Table I). Moreover, when the clone GP 70 was also included among the differential clones (for populations from Trentino Alto Adige), the nematode reproduction was similar to that on the previously mentioned cultivars. Therefore all sixteen populations of *G. rostochiensis* were identified as pathotype Ro2 according to Kort *et al.* (1977). However, the populations from Bevacqua and Cardamone (Calabria region) were not tested on clone KTT/60.21.10 and that from Cardamone did not reproduce well even on the susceptible cv. Spunta. Therefore, there are some doubts about the pathotype designation of these two populations. Population Polignano 2 (Puglia region) also showed some reproduction on the clones KTT/60.21.19 and GLKS-58-1642.4 but results from the second test suggested that at least most of the cysts of this population were of pathotype Ro2.

Six populations of *G. pallida* reproduced well on all of the differential clones, although differences could be observed among the populations (Table II). These popula-

tions, all from the Puglia region, were classified as pathotype Pa3. The population from Campania (Cimitile), one from Puglia (Polignano 5) and that from Sicilia (Siracusa) did not reproduce on the clone $(Vt^n)^2$62.33.3 and were classified as Pa2. A population from Puglia (Polignano 4) clearly reproduced only on the cv. Spunta and clone KTT/60.21.19, and not on the clones P55/7 and $(Vt^n)^2$62.33.3, but the reproduction of this population on

Table I. Reproduction rates on the differential hosts and pathotype designation according to Kort *et al.* (1977) of Italian populations of *G. rostochiensis*.

Nematode population by **Region** and locality	*Solanum* species, cultivar or clone					Pathotype designation
	S. tuberosum cv. Spunta	*S. tuberosum* cv. Saturna	*S. kurtzianum* KTT/60.21.19	*S. vernei* GLKS-58-1642.4	*S. vernei* $(Vt^n)^2$62.33.3	
Trentino Alto Adige						
Creino	9.1	2.2	0.4	0.2	0.3	Ro2
Lomaso	3.9	3.2	0.3	0.7	0.9	Ro2
Ramgo	16.6	6.7	0.2	0.2	0.2?	Ro2
Ronzo	3.8	1.5	0.8	0.8	0.9	Ro2
Emilia Romagna						
Bellaria	20	8.4	0.4	0.4	0.5	Ro2
Abruzzo						
Fucino valley 1	21	15	0.2	0.3	0.2	Ro2
Fucino valley 2	11	28	1	1	1	Ro2
Fucino valley 3	9	10	1.2	0.7	0.2	Ro2
Puglia						
Barletta	4	1.5	0.1	0.3	0.1	Ro2
Molfetta	7,6	17	0.5	0.9	0.5	Ro2
Polignano 1	158	78	0.3	1.0	0.8	Ro2
Polignano 2	9	3	2.4	1.7	0.6	Ro2/Ro3
Campania						
Acerra	8.6	5.7	0.9	1.5	0.4	Ro2
Calabria						
Bevacqua	16.9	45,9	NT[1]	1.1	1.4	Ro2?
Cardamone	1,5	1,9	NT	1.2	0.9	Ro2?
Celino	3	3	0.7	0.6	0.6	Ro2

[1]NT = Not tested.

Table II. Reproduction rates on the differential hosts and pathotype designation according to Kort *et al.* (1977) of Italian populations of *G. pallida*.

Nematode population by **Region** and locality	*Solanum* species, cultivar or clone					Pathotype designation
	S. tuberosum cv. Spunta	*S. multidissectum* P55/7	*S. kurtzianum* KTT/60.21.19	*S. vernei* GLKS-58-1642.4	*S. vernei* $(Vt^n)^2$62.33.3	
Campania						
Cimitile (NA)	20	2.2	2.2	1.9	0.5	Pa2
Puglia						
Polignano 3	26	2.5	4.7	2.8	3.9	Pa3
Polignano 4	6.6	0.9	3.9	NT[1]	0.5	?
Polignano 5	17.9	9.5	2.7	2.2	1	Pa2
Polignano 6	88	8	23	51	4.7	Pa3
Monopoli	48	6	13	12	3	Pa3
Zapponeta	63.7	11.6	24.9	6.6	10.2	Pa3
Margherita di Savoia 1	87.7	11.1	11.2	7.4	2.8	Pa3
Margherita di Savoia 2	79.5	14.2	22.3	8.0	3.8	Pa3
Sicilia						
Siracusa	7.3	2.4	1.0	7.8	0.4	Pa2

[1]NT = Not tested.

clone GLKS-58-1642.4 was not tested and, therefore, it could not be classified. A population from Puglia (Polignano 1) and that from Sicilia (Siracusa), tested also on clone GP 70, reproduced as well on this clone as on the susceptible cv. Spunta.

Investigation of the heterogeneity of virulence of the nematode populations. Of the populations of *G. rostochiensis* derived from a single cyst (ten from each original population), all those from Ronzo (Trentino region), Fucino Valley 1 (Abruzzo region) and Molfetta (Puglia region) showed females only on the root balls of the cvs Spunta and Saturna and not on those of clone KTT 60.21.19. Only four single cyst populations derived from the population from Bellaria (Emilia Romagna region) each developed a few females on one or two plants of the clone KTT 60.21.19. Planting this clone in soil in which the nematode populations had been increased on the susceptible cv. Spunta did not allow the development of any female on the roots. The cysts extracted from the soil infested with these sub-populations contained an average of only 28 eggs. Of the single cyst populations derived from the population collected at Barletta (Puglia), only one developed a few females on a plant of KTT 60.21.19. However, further increase of the nematode population on cv. Spunta and re-planting of the clone KTT 60.21.19 failed to reveal any female on the root ball. Of the population Polignano 2 (Puglia region), four single cyst derived populations each produced a few females on one or two plants of clone KTT 60.21.19, but the development of females on the roots of the four plants of clone KTT 60.21.19 was confirmed in only one after its increases on the cv. Spunta. Soil analysis of the pots with these infected plants revealed an increase (twofold) of the nematode populations and of the eggs per cyst (177 *vs* 136 at planting).

Finally, of the single cyst populations from Celino (Calabria), all developed yellow females on the roots of one to four plants of clone KTT 60.21.19. However, after increasing the nematode population on the cv. Spunta and re-testing, only five populations confirmed their development on KTT 60.21.19. Analysis of the soil samples in which these infested plants were grown, despite the very large nematode populations at planting (294-537 eggs/g soil), revealed that the nematode population remained at the same level (two single cyst derived populations) or increased slightly (1.1-1.3×). Moreover the number of eggs per cyst was similar (155 or 174) or slightly greater (1.2×) or smaller (0.9×) than that (155-220) of the cysts inoculated before planting the clone KTT 60.21.19.

DISCUSSION

In Italy, Di Vito (1981) investigated three populations of *G. pallida* and five of *G. rostochiensis* and identified pathotypes Pa2 and Pa3 of *G. pallida* and only pathotype Ro1 of *G. rostochiensis*. Our study confirmed these findings for *G. pallida* but demonstrated that, for *G. rostochiensis*, Ro2 must perhaps be considered the prevalent pathotype.

The investigation has highlighted difficulties in designating pathotypes according to Kort *et al.* (1977). The scheme proposed by these authors considers a reproduction rate of ×1 as the borderline to designate pathotypes of potato cyst nematode populations on their proposed differential *Solanum* clones. Therefore, when the reproduction rate of a population on a clone is close to ×1, the designation of the pathotype is problematic, confirming doubts and criticisms expressed by others (Trudgill, 1985; Fleming and Powers, 1998).

Nijboer and Parlevliet (1990) discussed the international pathotype scheme proposed by Kort *et al.* (1977) and Canto-Saenz and Mayer de Scurrah (1977) and considered only three valid pathotypes of *G. rostochiensis* for Europe, Ro1 (= ex Ro1 and Ro4), Ro3 (= ex Ro2 and Ro3) and Ro5 (= ex Ro5), while for *G. pallida* they considered the identification of the three European pathotypes not reliable. Therefore, according to these authors, the Italian populations of *G. rostochiensis* should be considered as pathotype Ro3.

At first sight, the Italian populations of *G. rostochiensis* and, to a lesser extent, of *G. pallida* would appear rather homogeneous, with only one pathotype of *G. rostochiensis* and two of *G. pallida*. This would indicate that potato cultivars resistant to pathotype Ro2 (according to Kort *et al.*, 1977) of *G. rostochiensis* and Pa2/3 of *G. pallida* would be useful to control most populations of potato cyst nematodes all over the country. However, the analysis of the single cyst derived populations of *G. rostochiensis* demonstrates that this is not always true and that some of them contain a few cysts that develop on the clone KTT 60.21.19 of *S. kunurtzianum*, and these could become prevalent under the genetic pressure following the use of cultivars resistant to pathotypes Ro1 and/or Ro2. Moreover, in the Puglia, Campania and Sicilia regions, both potato cyst nematode species occur in mixed populations. Therefore, as most potato cultivars are resistant to only pathotype(s) of one particular species, a shift in the relative prevalence of the species would occur following the use of a cultivar resistant to only one of the species.

Because of the difficulties and doubts over identification of pathotypes of potato cyst nematodes with the differential host test method, the use of methods that avoid uncertainty is desirable. Several authors have been able to differentiate nematode populations by molecular and biochemical methods (Fox and Atkinson, 1984; Bakker *et al.*, 1992; Blok and Phillips, 1995; Cunha *et al.*, 2006; Molinari 2006). Most of such methods are able to distinguish *G. rostochiensis* from *G. pallida* and, in some cases, allow the discrimination of nematode populations of different geographic origin (Molinari *et al.,* 2005; Cunha *et al.*, 2006). Hinch *et al.* (1998), working with Australian and European populations of potato cyst nematodes, were able to characterize all the

European pathotypes using high performance capillary electrophoresis. Although some problems were encountered, the authors found this method to have potential to discriminate pathotypes of potato cyst nematodes, although validation with a larger number of nematode populations was suggested. Recently, Cunha *et al.* (2006) used high performance capillary gel electrophoresis to differentiate 49 Portuguese populations of these nematodes and one population for each of the eight European pathotypes. They obtained some protein profiles specific for each pathotype but, in general, their grouping of populations did not fit with the pathotypes/virulence groups differentiated in Europe by the host reaction test. On the other hand, superoxide dismutase (SOD) patterns of cyst extracts from *Globodera* populations consisted of up to 17 bands and allowed the discrimination of Pa2 from Pa3, within *G. pallida* pathotypes, although it was not possible to discriminate Ro1, Ro2, Ro3, and Ro4 from Ro5, within *G. rostochiensis* pathotypes (Molinari *et al.,* 2005; Molinari, 2006).

All this clearly indicates that there is still much work to do to standardise a molecular or biochemical method for a full identification of potato cyst nematode pathotypes, grouped according to their different virulence on potato germplasms. Molecular techniques are becoming more and more sophisticated and there is a strong demand for an alternative identification method to the current bioassays that is likely to be more precise, easier, faster and cheaper to perform than the tests used so far. Such a test might lead to a classification of potato cyst nematode populations different from that obtained from the current pathotype schemes.

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LITERATURE CITED

Bakker J., Bouwman-Smits L. and Gommers F.J., 1992. Genetic relationship between *Globodera pallida* pathotypes in Europe assessed by using two dimensional gel electrophoresis of proteins. *Fundamental and Applied Nematology, 15*: 481-490.

Blok V.C. and Phillips M.S., 1995. The use of repeat sequence primers for investigating genetic diversity between populations of potato cyst nematodes with differing virulence. *Fundamental and Applied Nematology, 18*: 575-582.

Bryan J.E., Jackson M.T. and Meléndez N., 1981. *Técnica de multiplicación rápida de papa.* Centro Internacional de la Papa (CIP), Lima, Peru, 20 pp.

Canto Saenz M. and Mayer de Scurrah M., 1977. Races of the potato cyst nematode in the Andean region and a new system of classification. *Nematologica, 23*: 340-349.

Cunha M.J.M. da, Conceição I.L.P.M. da, Abrantes I.M. de O. and Santos M.S.N. de A., 2006. Assessment of the use of high-performance capillary gel electrophoresis to differentiate isolates of *Globodera* spp. *Nematology, 8*: 139-146.

Di Vito M., 1981. Contributo alla conoscenza dei patotipi di nematode cisticoli della patata presenti in Italia. *Informatore Fitopatologico, 31(10)*: 21-23.

Fleming C.C. and Powers T.O., 1998. Potato cyst nematodes: species, pathotypes and virulence concepts. Pp. 51-57. *In*: Potato Cyst Nematodes – Biology, Distribution and Control (Marks R.J. and Brodie B.B., eds). CAB International, Wallingford, UK.

Fox P.C. and Atkinson H.J., 1984. Isoelectric focusing of general protein and specific enzymes from pathotypes of *Globodera rostochiensis* and *G. pallida*. *Parasitology, 88*: 131-139.

Greco N., D'Addabbo T., Brandonisio A. and Elia F., 1993. Damage to Italian crops caused by cyst-forming nematodes. *Journal of Nematology, 25(4S)*: 836-842.

Hinch J.M., Alberdi F., Smith S.C. Woodward J.R. and Evans K., 1998. Discrimination of European and Australian *Globodera rostochiensis* and *G. pallida* pathotypes by high performance capillary electrophoresis. *Fundamental and Applied Nematology, 21*: 123-128.

Kort J., Ross H., Rumpenhorst H.J. and Stone A.R., 1977. An international scheme for identifying and classifying pathotypes of potato cyst-nematodes *Globodera rostochiensis* and *G. pallida*. *Nematologica, 23*: 333-339.

Llontop J.A., Franco J. and Scurrah M., 1987. Identificación de especies y razas del nematodo quiste de la papa *Globodera* spp. en la zona papera de La Libertad, Peru. *Abstracts XIX Annual Meeting of the Organization of Tropical American Nematologists, October 18-23, 1987, Santiago, Chile*, p. 9.

Molinari S., 2006. Isozyme phenotypes of plant-parasitic nematodes as markers of their genetic variability. XXVIII International Symposium of the European Society of Nematologists, 5-9 June, Blagoevgrad, Bulgaria, p. 47 (Abstract).

Molinari S., Lugo Z., Greco N., Crozzoli R., Perichi G. and Castellano G., 2005. Identification of root-knot nematode (*Meloidogyne* spp.) populations from Venezuela by isozyme electrophoresis patterns. *Nematropica, 35*: 87 (Abstract).

Nijboer H. and Parlevliet J.E., 1990. Pathotype-specificity in potato cyst nematodes, a reconsideration. *Euphytica, 49*: 39-47.

Scurrah M.I., Björn N. and Bridge J., 2005. Nematode Parasites of Solanum and sweet potatoes. Pp. 193-219. *In*: Plant Parasitic Nematodes in Subtropical

and Tropical Agriculture - Second Edition (Luc M., Sikora R.A. and Bridge J., eds). CABI Publishing, Wallingford, UK.

Seinhorst J.W., 1974. Separation of *Heterodera* cysts from organic debris using ethanol. *Nematologica*, *20*: 367-369.

Seinhorst J.W. and Ouden H. den, 1966. An improvement of Bijloo's method for determining the egg content of *Heterodera* cysts. *Nematologica*, *12*: 170-171.

Trudgill D.L., 1985. Potato cyst nematodes: a critical review of the current pathotyping scheme. *EPPO Bulletin*, *15*: 273-279.

Turner S.J. and Evans K., 1998. The origin, global distribution and biology of potato cyst nematodes (*Globodera rostochiensis* Woll. and *Globodera pallida* Stone). Pp. 7-26. *In*: Potato Cyst Nematodes - Biology, Distribution and Control (Marks R.J. and Brodie B.B., eds). CAB International, Wallingford, UK.

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