SCREENING OF CLONES OF *SOLANUM* SPP. FOR RESISTANCE TO POTATO CYST NEMATODES, *GLOBODERA ROSTOCHIENSIS* AND *G. PALLIDA*

N. Greco[1], D. Carputo[2], L. Frusciante[2], G. Russo[1] and A. Brandonisio[1]

[1] *C.N.R., Istituto per la Protezione delle Piante, Sezione di Bari, Via G. Amendola 165/A, 70126 Bari, Italy*
[2] *Department of Soil, Plant and Environmental Sciences, Universitá di Napoli "Federico II", Via Universitá 100, 80055 Portici (NA), Italy*

Summary. Screenings were undertaken to assess the reactions of 26 clones of various tuber-bearing *Solanum* species to Italian populations of the potato cyst nematodes, *Globodera rostochiensis* Ro2 and *G. pallida* Pa3. The clones were: three of *Solanum acaule*, five of *S. bulbocastanum*, one of *S. canasense*, three of *S. chacoense*, two of *S. commersonii*, three of *S. cardiophyllum*, one of *S. etuberosum*, four of *S. fendleri*, one of *S. phureja* x *S. tuberosum*, one of *S. multidissectum*, and two of *S. tarijense*. Their reactions were compared with that of *S. tuberosum* cv. Spunta. Plants of each of the clones were obtained by *in vitro* culture and were grown in clay pots containing 1000 cm^3 of soil infested with 15-20 eggs/g of either nematode species. Nematode development in the roots was assessed about 40 days after transplanting the clones and the dynamics of nematode soil populations were determined about 70 days after transplanting. In general, development of both nematode species was less in the wild species of *Solanum*. Although no clone was completely resistant, based on the percentage of females and cysts developed on the roots, nematode soil population density, reproduction rate and number of eggs per cyst observed in different tests, clones *acl* 1A and *acl* 1E of *S. acaule*, *cph* 1C and *cph* 2D of *S. cardiophyllum* and *fen* 1D, *fen* 1E, *fen* 2A and *fen* 2D of *S. fendleri* could be considered partially resistant to *G. rostochiensis*. Moreover, clones *acl* 1A, *fen* 1D, *fen* 1E, *fen* 2A, *blb* 1E of *S. bulbocastanum* and *chc* 1B of *S. chacoense* were partially resistant to *G. pallida*.

The cyst nematodes, *Globodera rostochiensis* (Woll.) Behrens and *G. pallida* (Stone) Behrens, are among the most damaging nematodes of potatoes world wide (Turner and Evans, 1998). In Italy, the former species appears all over the country, while the latter prevails in some southern regions (Greco *et al*., 1993). Microplot experiments (Greco *et al*., 1982; Seinhorst, 1982) demonstrated an average tolerance limit of potato to both nematodes of 1.9 eggs/g soil and that yield losses of 50% and 80% would occur when cultivating potatoes in fields infested with 32 or 100 eggs/g soil, respectively. The value of the potato yield lost annually due to cyst nematode attack in Italy was estimated at about 11,000,000 euros (Greco *et al*., 1993).

Although crop rotation, nematocides and soil solarization are effective, the use of resistant potato cultivars is the most promising way of controlling these plant parasitic nematodes. However, most of the available potato cultivars resistant to potato cyst nematodes are resistant only to pathotype Ro1 of *G. rostochiensis*, with few also resistant to other pathotypes (Whitehead and Turner, 1998). Moreover, the resistance to *G. pallida* occurring in the resistant cultivars available in Europe is only partial (Whitehead and Turner, 1998). In Italy, the most widespread pathotypes are Ro2 of *G. rostochiensis* and Pa3 of *G. pallida* (Greco *et al*., 1999). The numerous wild tuber-bearing species of *Solanum* may represent a worthwhile source of genes to develop new varieties with improved resistance and quality traits (Pavek and Corsini, 2001). Potato breeders have a number of strategies to exploit the genetic diversity found in *Solanum* species. These strategies include sexual hybridization, symmetric and asymmetric somatic fusion, gene isolation and genetic transformation. The first step in any of the afore-mentioned strategies is the identification of potentially useful genotypes within available germplasm. In light of this, the aim of the present investigation was to screen new clones of *Solanum* spp. for resistance towards both nematode species in order to identify sources of resistance valuable against Italian populations of these parasites.

MATERIALS AND METHODS

The population of *G. rostochiensis* was from a field at Molfetta and that of *G. pallida* from a field at Polignano a Mare, both in the province of Bari and both planted to potato the previous year. Nematodes used in the first and second tests were from the field, while those of the third test were from glasshouse cultures raised on susceptible potato cultivars (Spunta or Sieglinde). Nematode cysts were extracted from the soil with a Fenwick can, dried in the shade along with soil debris, sieved through a 25-mesh sieve to remove larger pieces of debris, and then mixed with 2 kg of river sand. To estimate the nematode population density of the cyst-sand mixture, five 10-g samples were poured onto a 60-mesh sieve and sprayed with tap water. Cysts were then collected from the sieve, counted and crushed according to Bijloo's modified method (Seinhorst and Den Ouden, 1966) and their egg content determined. An appropriate amount of the cyst-sand mixture was then thoroughly

mixed with the sandy potting soil (89% sand) to give a nematode population density of 20 eggs/g. The infested soil was distributed in 14-cm-diameter clay pots, each receiving 1000 cm^3 of soil. Four pots were planted with each clone/nematode species combination.

The clones of the *Solanum* spp. were from the germplasm collection of the Department of Soil, Plant and Environmental Sciences, University of Naples, Italy, where they are maintained *in vitro*. To produce plant material for screening tests, 4-week-old micro-propagated plants were transferred to styrofoam trays in a growth chamber at 18-20 °C for adaptation. After two weeks, plants were transplanted into 5-cm-diameter plastic pots in a glasshouse at 18-20 °C. The 26 clones tested (Tables I-VIII) were three of *S. acaule* Bitt., five of *S. bubocastanum* Dunal, one of *S. canasense* Hawkes, three of *S. chacoense* Bitter, two of *S. commersonii* Dun., three of *S. cardiophyllum* Lindl., one of *S. etuberosum* Lindl., four of *S. fendleri* A. Gray, one of *S. phureja* Juz. *et* Bukasov x *S. tuberosum* L., one of *S. multidissectum* Hawkes, and two of *S. tarijense* Hawkes. Tubers of *S. tuberosum* cv. Spunta were sown in four more pots to provide plants that served as controls. All pots were arranged in a glasshouse at 18-22 °C and irrigated and fertilized as required.

Table I. First screening. Developmental stages of *Globodera rostochiensis* Ro2 in the roots of new clones of *Solanum* spp. 40 days after transplanting into pots containing nematode-infested soil. Nematode soil population at transplanting was 20 eggs/g.

Solanum sp. and clone	Nematodes/ root	Nematode developmental stages as % of the total per root			
		Second stage juveniles	3° and 4° stage females	Females and cysts	Males
S. acaule					
acl 1A	130 hi	35.4 cdefgh	10.8 jkl	5.4 ghij	48.3 ab
acl 1E	157 hi	34.0 cdefghi	6.8 kl	1.8 ij	57.4 a
acl 2A	272 efghi	14.2 hi	35.7abcdefgh	3.0 hij	47.0 ab
S. bulbocastanum					
blb 1D	885 abcd	15.8 ghi	44.0 abcd	27.8 bc	12.4 defgh
blb 1E	295 efghi	33.0 cdefghi	51.8 ab	9.0 fghij	5.3 gh
blb 2A	790 abcde	10.1 hi	38.9 abcdef	36.0 b	14.8 defgh
blb 2D	472 defghi	22.8 efghi	42.4 abcde	20.7 cdef	14.2 defgh
blb 2E	315 efghi	47.0 cde	33.1 bcdefghi	12.6 efghi	7.0 fgh
S. canasense					
can 1B	707 cdefg	25.8 defghi	12.8 ijkl	14.1 defgh	47.3 ab
S. chacoense					
chc 1A	592 defgh	10.8 hi	55.0 a	19.6 cdef	14.5 defgh
chc 1B	1302 a	16.0 ghi	46.9 abc	14.6 defgh	22.4 cdefg
chc 1C	312 efghi	40.5 cdefg	24.6defghijk	25.8 bcd	9.0 fgh
S. commersonii					
cmm 1T	312 efghi	49.7 cd	15.0 hijkl	8.9 efghi	26.3 cdef
S. cardiophyllum					
cph 1C	107 hi	59.0 bc	25.8 defghijk	0.0 j	15.0 defgh
cph 2D	145 hi	55.2 c	22.2 efghijkl	1.9 ij	20.7 cdefg
cph 2E	222 ghi	41.7 cdef	26.9cdefghijk	14.8 defgh	16.5 defgh
S. etuberosum					
etb 3	1287 ab	24.7 defghi	36.6 abcdefg	15.9 cdefg	22.8 cdefg
S. fendleri					
fen 1D	20 i	83.3 ab	16.7 hijkl	0.0 j	0.0 h
fen 1E	30 i	87.5 a	2.5 l	0.0 j	10.0 efgh
fen 2A	250 fghi	58.0 bc	17.8 ghijkl	0.0 j	24.2 cdefg
fen 2D	217 ghi	47.2 cde	9.0 jkl	5.2 ghij	37.8 abc
S. phureja x *S. tuberosum*					
UP88-P5	765 bcdef	18.7 fghi	33.9 bcdefgh	17.7 cdef	29.6 bcde
S. multidissectum					
mlt A1	515 defghi	19.1 fghi	33.8 bcdefgh	16.0 cdefg	31.0 bcd
S. tarijense					
tar 1C	390 defghi	41.8 cdef	18.1 fghijkl	23.4 cde	17.0 defgh
tar 2B	615 defgh	12.8 hi	36.3 abcdefg	21.3 cde	29.4 bcde
S. tuberosum (control)					
cv. Spunta	1195 abc	8.6 i	28.7 cdefghij	49.4 a	12.4 defgh

Means in the same column flanked by a common letter are not significantly different at $P \leq 0.5$, according to Duncan's Multiple Range Test.

Forty days after transplanting, the plants were uprooted and the roots from each pot were gently washed in water. Excess water was removed and the roots were weighed and cut into 0.5-cm-long pieces. All nematode specimens were extracted from the roots using Coolen's method (Coolen, 1979), counted and classified according to developmental stages.

Clones on the roots of which females were few or did not develop at all were screened again to confirm their reaction to the nematodes. However, in this second screening, clone *acl* 1A of *S. acaule* was not re-tested, while clone *cmm* 1 of *S. commersonii*, previously not screened, was included. Advanced clone S8632 of *S. tuberosum* from the University of Naples breeding programme was used as the control. Eight pots were planted to each clone/nematode species combination; four of them were processed as before, while potatoes in the remaining four plots were left to grow for a further 30 days to give the nematodes that had penetrated the roots time to reach the cyst stage. Plants in these pots were then cut at ground level, the soil was left to dry and a 200-g sample per pot processed with the Fenwick can. Cysts were further separated from soil debris by means of flotation in alcohol (Seinhorst, 1974), counted, crushed according to Bijloo's modified method (Seinhorst and Den Ouden, 1966) and their egg content determined.

A third test was conducted to confirm results of cyst development and nematode reproduction on the most promising clones (Tables IV and VIII). For this, the tubers produced during the second text were cleaned, checked for absence of nematode cysts under a stereomicroscope and, when sprouting, they were planted singly in 14-cm diameter clay pots containing 1000 cm^3 soil infested with either nematode species. Each clone was replicated four times according to a randomised block design. The test was terminated 68 days after planting. Cysts were then extracted and their content determined as described earlier.

Data were statistically analysed and means compared by means of Duncan's Multiple Range Test.

RESULTS

Generally, the number of nematode specimens per root and root weights varied greatly between the different *Solanum* species and sometimes between different clones of the same species.

Globodera rostochiensis. In the first screening (Table I), significantly fewer nematodes per root were observed in all clones of *S. acaule*, *S. commersonii*, *S. cardiophyllum*, *S. fendleri*, *S. multidissectum* and *S. tarijense*, and in clones *blb* 1E, *blb* 2D and *blb* 2E of *S. bulbocastanum* and *chc* 1A and *chc* 1C of *S. chacoense*, compared to cv. Spunta of *S. tuberosum*. The percentages of second stage juveniles found were significantly higher than in the control in the clones of *S. commersonii*, *S. cardiophyllum* and *S. fendleri*, and in one clone each of *S. acaule* (*acl* 1A), *S. bulbocastanum* (*blb* 2E), *S. chacoense* (*chc* 1C) and *S. tarijense* (*tar* 1C). A reduction of the proportion of third and fourth stage specimens was observed only in the roots of clones *acl* 1E of *S. acaule* and *fen* 1E of *S. fendleri*. Female specimens were 49.4 % of the total in the roots of the *S. tuberosum* control and few or absent from the roots of all clones of *S. acaule* and *S. fendleri* and clones *cph* 1C and *cph* 2D of *S. cardiophyl-*

Table II. Second screening. Developmental stages of *G. rostochiensis* Ro2 in the roots of selected new clones of *Solanum* spp. 40 days after transplanting into pots containing nematode-infested soil. Nematode soil population at transplanting was 20 eggs/g.

Solanum sp. and clone	Nematodes/ root	Nematode developmental stages as % of the total per root			
		Second stage juveniles	3° and 4° stage females	Females and cysts	Males
S. acaule					
acl 1E	182 bcd	13.6 ab	23.6 b	9.3 a	53.5 c
acl 2A	202 cd	25.8 abc	26.9 b	10.8 a	36.5 bc
S. commersonii					
cmm 1	310 d	35.7 bc	23.7 b	19.6 ab	20.9 ab
S. cardiophyllum					
cph 1C	135 abc	35.2 bc	27.9 b	14.7 ab	22.2 ab
cph 2D	97 abc	42.1 c	15.6 ab	26.8 ab	15.5 ab
S. fendleri					
fen 1D	45 ab	46.7 cd	20.0 ab	20.0 ab	13.3 ab
fen 1E	27 a	13.2 ab	13.2 ab	40.0 bc	33.2 bc
fen 2A	7 a	75.0 e	0.0 a	25.0 ab	0.0 a
fen 2D	7 a	0.0 a	0.0 a	100.0 d	0.0 a
S. tuberosum					
S8632 (control)	227 cd	22.5 abc	30.8 b	25.2 ab	21.4 ab

Means in the same column flanked by a common letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

Table III. Second screening. Reproduction of *G. rostochiensis* Ro2 on selected new clones of wild *Solanum* spp. Soil population density of the nematode at planting (*Pi*) was 19.2 eggs/g; mean number of eggs/cyst at planting was 50.2.

Solanum species and clone	Eggs and juveniles/ g soil at harvest (*Pf*)	Reproduction rate (*Pf/Pi*)	Eggs/cyst
S. acaule			
acl 1A	45.9 a	2.4 a	121.7 a
acl 1E	53.3 a	2.8 a	124.9 ab
acl 2A	68.6 ab	3.6 ab	143.1 abc
S. commersonii			
cmm 1	187.9 c	9.8 c	192.3 bc
S. cardiophyllum			
cph 1C	70.5 ab	3.7 ab	160.4 abc
cph 2D	94.0 ab	4.9 ab	170.1 abc
S. fendleri			
fen 1D	64.3 ab	3.3 ab	127.5 abc
fen 1E	67.1 ab	3.5 ab	131.8 abc
fen 2A	49.2 a	2.6 a	123.4 a
fen 2D	46.2 a	2.4 a	122.5 a
S. tuberosum			
S8632 (control)	152.3 bc	7.9 bc	194.4 c

Means in the same column flanked by a common letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

Table IV. Third test. Reproduction of *G. rostochiensis* Ro2 on selected new clones of wild *Solanum* spp. Soil population density of the nematode at planting (*Pi*) was 16.7 eggs/g; mean number of eggs/cyst at planting was 298.

Solanum species and clone	Eggs and juveniles/ g soil at harvest (*Pf*)	Reproduction rate (*Pf/Pi*)	Eggs/cyst
S. acaule			
acl 1E	23.0 a	1.6 a	185.7 a
acl 2A	70.0 a	4.8 a	226.0 a
S. cardiophyllum			
cph 1C	43.7 a	3.0 a	237.0 a
cph 2D	102.5 a	7.0 a	321.0 b
S. fendleri			
fen 1D	232.2 b	16.0 b	228.0 a
fen 1E	92.2 a	6.3 a	181.0 a
fen 2A	232.7 b	15.9 b	227.0 a
fen 2D	213.2 b	14.6 b	229.2 a
S. tuberosum			
Cv. Spunta (control)	1199.0 c	82.1 c	460.7 c

Means in the same column flanked by a common letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

lum. In the roots of other clones, females occurred in quite large percentages but were significantly less than in the roots of *S. tuberosum*. Percentages of male stages were significantly larger than in the control only in the roots of all clones of *S. acaule* and *S. canasense* and those of clone *fen* 2D of *S. fendleri*.

In the second screening the differences between the re-tested clones were less (Table II). The total numbers of nematodes in the roots of *S. fendleri* were less than in the roots of the control but data on other developmental stages of the nematode on the clones in this test could not be interpreted clearly. The nematode soil population density (Table III) increased in all pots but, except for pots planted to *S. commersonii*, the reproduction rate was smaller than that in pots planted to *S. tuberosum*. Eggs per cyst were 194 on *S. tuberosum* and were significantly less on clones *acl* 1A and *acl* 1E of *S. acaule* and *fen* 1D, *fen* 2A and *fen* 2D of *S. fendleri* (Table III). However, for the three variables that were measured, the differences from the control were significant only for clones *acl* 1A and *acl* 1E of *S. acaule* and *fen* 2A and *fen* 2D of *S. fendleri*.

Table V. First screening. Developmental stages of *G. pallida* Pa3 in the roots of new clones of *Solanum* spp. 40 days after transplanting into pots containing nematode-infested soil. Nematode soil population density at transplanting was 20 eggs/g.

Solanum sp. and clone	Nematodes/ root	Nematode developmental stages as % of the total per root			
		Second stage Juveniles	3° and 4° stage females	Females and cysts	Males
S. acaule					
acl 1A	27 jkl	57.5 efg	0.0 a	0.0 a	42.5 fg
acl 1E	57 hijkl	16.0 ab	30.9 defghi	11.3 abcd	41.7 efg
acl 2A	50 ijkl	37.1 bcdef	21.0 bcdef	17.1 abcdef	24.6 cde
S. bulbocastanum					
blb 1D	302 bc	26.3 bcd	31.5 defghi	27.5 bcdefg	14.7 abcd
blb 1E	127 defghijkl	27.4 bcd	40.0 ghi	9.4 abcd	23.2 cd
blb 2A	260 bc	15.3 ab	32.9 defghi	45.2 g	6.7 abcd
blb 2D	185 cdefgh	27.0 bcd	21.0 bcdef	25.4 bcdefg	26.5 def
blb 2E	162 defghi	30.0 bcd	43.3 hi	18.3 abcdef	8.3 abcd
S. canasense					
can 1B	227 cdef	31.3 bcde	12.3 ab	4.9 ab	51.4 g
S. chacoense					
chc 1A	117 efghijkl	24.6 abcd	43.0 hi	29.4 cdefg	2.9 ab
chc 1B	155 defghij	34.4 bcdef	41.98 hi	6.7 abc	16.9 abcd
chc 1C	187 cdefgh	44.4 cdef	36.9 efghi	15.6 abcde	3.2 ab
S. commersonii					
cmm 1T	312 bc	31.2 bcde	30.1 cdefghi	33.4 defg	5.3 abc
S. cardiophyllum					
cph 1C	85 ghijkl	35.6 bcdef	13.0 abc	36.7 efg	14.6 abcd
cph 2D	152 defghij	12.7 ab	38.6 fghi	41.0 fg	7.6 abcd
cph 2E	102 fghijkl	17.3 abc	26.9 bcdefgh	45.9 g	9.8 abcd
S. etuberosum					
etb 3	672 a	31.8 bcde	47.4 i	19.2 abcdef	1.6 ab
S. fendleri					
fen 1D	0 l	0.0 a	0.0 a	0.0 a	0.0 a
fen 1E	17 kl	66.7 g	26.7 bcdefgh	0.0 a	6.7 abcd
fen 2A	252 cde	51.78 defg	19.98 bcde	11.8 abcd	16.3 abcd
fen 2D	130 defghijkl	29.45 bcd	36.8 efghi	19.3 abcdef	14.4 abcd
S. phureja x *S. tuberosum*					
UP88-P5	207 cdefg	27.42 bcd	23.9 bcdefg	28.2 bcdefg	20.5 bcd
S. multidissectum					
mlt A1	100 fghijkl	32.42 bcde	22.2 defghi	27.8 bcdefg	17.5 abcd
S. tarijense					
tar 1C	222 cdef	38.45 bcdef	30.7 bcdefg	17.6 abcdef	13.2 abcd
tar 2B	147 defghijk	59.5 fg	11.3 ab	11.2 abcd	18.0 abcd
S. tuberosum (control)					
cv. Spunta	392 b	35.45 bcdef	17.6 bcd	37.3 efg	9.6 abcd

Means in the same column flanked by a common letter are not significantly different at $P \leq 0.05$, according to Duncan's Multiple Range Test.

In the third test (Table IV), the cv. Spunta was used as a control and clone *cmm* 1 of *S. commersonii* was not included as it was found very susceptible in the second screening. The third test confirmed the results of the previous test. However, soil population densities and reproduction rates of *G. rostochiensis* were much less and in the range of 1.9% (clone *acl* 1E) to 19.4% (clone *fen* 2A) of that of the control. Also, egg contents of the cysts were significantly reduced, by from 70% (clone *cph* 2D) to 39% (clone *fen* 1E).

Globodera pallida. The numbers of nematodes found in the roots (Table V) were significantly fewer than in the control in most of the clones, except for two clones of *S. bulbocastanum* and the clones of *S. commersonii*, and they were greater in the clone of *S. etuberosum*. No nematode was found in the roots of clone *fen* 1D of *S. fendleri*. With the exception of the roots of clone *fen* 1D of *S. fendleri*, which were free of nematodes, the percentages of second stage juveniles did not differ significantly from the control in most of the clones. However, they were much less in the clones *acl* 1E of *S.*

Table VI. Second screening. Developmental stages of *G. pallida* Pa3 in the roots of selected new clones of *Solanum* spp. 40 days after transplanting into pots containing nematode-infested soil. Nematode soil population density at transplanting was 16 eggs/g.

Solanum sp. and clone	Nematodes/ root	Nematode developmental stages as % of the total per root			
		Second stage juveniles	3° and 4° stage females	Females and cysts	Males
S. commersonii					
cmm 1	1797 c	5.1 b	36.7 c	29.9 b	28.5 b
S. fendleri					
fen 1D	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
fen 1E	0.0 a	0.0 a	0.0 a	0.0 a	0.0 a
S. tuberosum					
S8632 (control)	937 b	8.5 c	12.1 b	47.0 c	32.2 b

Means in the same column flanked by a common letter are not significantly different at P ≤ 0.05, according to Duncan's Multiple Range Test.

Table VII. Second screening. Reproduction of *G. pallida* Pa3 on selected new clones of wild *Solanum* spp. Soil population density of the nematode at planting (*Pi*) was 16 eggs/g; mean number of eggs/cyst at planting was 37.

Solanum species and clone	Eggs and juveniles/ g soil at harvest (*Pf*)	Reproduction rate (*Pf/Pi*)	Eggs/cyst
S. acaule			
acl 1A	34.8 a	2.2 a	87.1 a
S. commersonii			
cmm 1	340.8 b	21.2 b	221.0 b
S. fendleri			
fen 1D	21.2 a	1.3 a	51.7 a
fen 1E	23.6 a	1.5 a	66.7 a
S. tuberosum			
S8632 (control)	534.0 c	33.4 c	304.1 c

Means in the same column flanked by a common letter are not significantly different at P ≤ 0.05, according to Duncan's Multiple Range Test.

Table VIII. Third screening. Reproduction of *G. pallida* Pa3 on selected new clones of wild *Solanum* spp. Soil population density of the nematode at planting (*Pi*) was 14.6 eggs/g; mean number of eggs/cyst at planting was 220.

Solanum species and clone	Eggs and juveniles/ g soil at harvest (*Pf*)	Reproduction rate (*Pf/Pi*)	Eggs/cyst
S. fendleri			
fen 1D	60.7 a	3.6 a	186 a
fen 1E	79.5 a	4.8 a	187 a
S. tuberosum			
cv. Spunta (control)	709.5 b	42.5 b	286 b

Means in the same column flanked by a common letter are not significantly different at P ≤ 0.05, according to Duncan's Multiple Range Test.

acaule, *blb* 2A of *S. bulbocastanum* and *cph* 2D of *S. cardiophyllum* and significantly more in the root of the clone *fen* 1E of *S. fendleri*. Third and fourth stage juveniles were absent from the roots of clone *acl* 1A of *S. acaule* and clone *fen* 1D of *S. fendleri*, and occurred in larger proportions than in the control in the roots of clones *blb* 1E (*S. bulbocastanum*), all those of *S. chacoense*, *cph* 2D (*S. cardiophyllum*), and *fen* 2D (*S. fendleri*). Females did not develop on clones *acl* 1A of *S. acaule* and *fen* 1D and *fen* 1E of *S. fendleri* and occurred in significantly smaller proportions than in the control in clones *acl* 1E of *S. acaule*, *blb* 1E of *S. bulbocastanum*, *chc* 1b of *S. chacoense* and *fen* 2A of *S. fendleri*. Percentages of male stages were significantly larger than in the control only in the roots of clones *acl* 1A and *acl* 1E of *S. acaule*, and *can* 1D of *S. canasense*.

Of the clones of interest that were re-tested against *S. tuberosum*, only those of *S. fendleri* were found free of nematodes (Table VI). In the second screening, the nematodes invaded and developed very well on clone *cmm* 1 of *S. commersoni*, tested for the first time. In the soil (Table VII), the nematode population density reached in pots planted with the wild clones was significantly smaller than in the control, being 63.4% of the control for clone *cmm* 1 of *S. commersonii* and as low as 4 to 6.5% for clones of *S. acaule* and *S. fendleri*. Thus, the reproduction rate of the nematode was 33x on the control, 2.2x on clone *acl* 1A of *S. acaule* and 1.3x and 1.5x on clones *fen* 1D and *fen* 1E of *S. fendleri*, respectively. The number of eggs per cyst was also less on the wild clones than on *S. tuberosum*, with cysts developed on clones of *S. acaule* and *S. fendleri* containing fewest eggs.

The third test confirmed the results obtained with the clones of *S. fendleri*, although the percentage reductions of nematode population densities, reproduction rate and cyst contents were a little larger than in the previous test (Table VIII).

DISCUSSION

The screenings highlighted some difficulties that arise when using and comparing clones belonging to different *Solanum* species. The species may have different environmental requirements, such as temperature, relative humidity of the air and day length. Therefore, the conditions in our glasshouse may not have been equally suitable for all of the *Solanum* species tested. In fact, development of both aerial parts and roots differed greatly among the *Solanum* species and some, such as *S. fendleri*, produced many more stolons than roots. Stolons are also known to be attacked by cyst nematodes although they may not be as susceptible as roots to the nematodes. This suggests that the inheritance of the resistance observed in *S. fendleri* should be checked in progenies obtained by crossing this species with *S. tuberosum*.

Overall evaluation of the data shows that, although no clone can be considered completely resistant to a species of potato cyst nematode, several of them are very interesting. Conceição *et al.* (2005) found no difference in the number of eggs per cyst of *G. pallida* on susceptible and partially resistant potato genotypes, thus confirming the findings of Turner (1990). In our tests, resistance to both nematodes was expressed both in reduced numbers of new cysts produced (not reported), and in smaller numbers of eggs per cyst, and therefore by lower reproduction rates compared to the susceptible potato, thereby confirming the results of Mullin and Brodie (1988) for *G. rostochiensis*. All clones of *S. acaule* and *S. fendleri* showed a good level of resistance to pathotype Ro2 of *G. rostochiensis*. Moreover, clones *acl 1A* of *S. acaule* and *fen* 1D and *fen* 1E of *S. fendleri* were also partially resistant to *G. pallida* Pa3. Of interest also is the resistance to *G. pallida* shown by clones *blb* 1E of *S. bulbocastanum*, *chc* 1B of *S. chacoense* and *fen* 2A of *S. fendleri*. Castelli *et al.* (2003) also reported resistance to *G. pallida* pathotype Pa 2/3 and *G. rostochiensis* pathotype Ro1 in the same clones of *S. acaule* and *S. fendleri*. Whether this resistance is controlled by major gene(s) or by major and minor genes needs to be investigated, along with its inheritance and its effect on selection of virulent nematode populations. *Solanum bulbocastanum* and *S. chacoense* are diploid, while *S. acaule* and *S. fendleri* are both tetraploid. To use these species in sexual hybridization programmes, different strategies will have to be used. *Solanum fendleri* is the only species that can be directly crossed with tetraploid *S. tuberosum*. For the other three species, post-zygotic incompatibility barriers require genome manipulations through 2n gametes (Carputo and Barone, 2005). In particular, *S. chacoense* can be crossed with *S. tuberosum* haploids (2n = 2x = 24) to produce diploid hybrids. If hybrids produce 2n gametes, they can be used in unilateral sexual polyploidization crossing with *S. tuberosum* schemes (4x × 2x crosses) to synthesize tetraploid hybrids. For the other two species, strategies based on the production of ploidy bridges can be employed (reviewed in Carputo and Barone, 2005).

Although tolerance is also an important criterion to consider in breeding programmes (Dale *et al.*, 1988; Phillips, 1994), it was not assessed in our tests.

Wild *Solanum* species are also known to possess resistance to several biotic and abiotic stresses (Hawkes and Hjerting, 1989). For instance, *S. acaule* is resistant to frost and is of interest for breeding programmes to obtain early cultivars that are usually sown in mid-autumn or as soon as possible in late winter-early spring and, therefore, may suffer from frosts. Cultivation of early potatoes is important in south Italy and most Mediterranean countries. Therefore, testing the clones found to be resistant to *G. rostochiensis* and *G. pallida* in our tests also for their reaction to other stresses would be useful to better address the needs of breeding programmes.

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Contribution no. 97 from Department of Soil, Plant and Environmental Sciences, University of Naples "Federico II".

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