Fundam. appl. Nematol., 1997, **20** (5), 449-457

Intra- and interspecific variation of root-knot nematodes, *Meloidogyne* spp., with regard to resistance in wild tuber-bearing *Solanum* species

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Accepted for publication 15 November 1996.

Summary - Genotypes of wild *Solanum* species were tested to determine the level of resistance to root-knot nematodes and to detect the presence of virulent populations within *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla*. High resistance to all tested populations of *M. chitwoodi* and *M. fallax* was observed in genotypes of *Solanum bulbocastanum*, *S. hougasii*, *S. cardiophyllum* and *S. fendleri*. Some genotypes of *S. chacoense* and *S. stoloniferum* showed moderate resistance to *M. fallax*, but no or lesser resistance to *M. chitwoodi*. There was little variation in virulence in populations of *M. chitwoodi* and *M. fallax* found on resistant plants. In contrast, large differences in virulence toward resistant genotypes of *S. bulbocastanum*, *S. hougasii*, *S. chacoense*, *S. gourlayi*, *S. sparsipilum* and *S. spegazzinii* were observed between four populations of *M. hapla*. It was found that resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla* is not linked to resistance to the nematode species adapted to high temperature, *i.e.*, *M. incognita*, *M. arenaria* and *M. javanica*.

Résumé - ***Variabilité intra- et interspécifique chez les nématodes* Meloidogyne *spp. au regard de la résistance présente chez les espèces tubéreuses sauvages de* Solanum** - Des génotypes appartenant à des espèces sauvages de *Solanum* ont été testés pour déterminer leur niveau de résistance aux nématodes *Meloidogyne* et pour détecter la présence de populations virulentes chez *Meloidogyne chitwoodi*, *M. fallax* et *M. hapla*. Une résistance élevée de toutes les populations testées appartenant à *M. chitwoodi* et *M. fallax* est observée chez des génotypes de *Solanum bulbocastanum*, *S. hougasii*, *S. cardiophyllum* et *S. fendleri*. Quelques génotypes de *S. chacoense* et *S. stoloniferum* font montre d'une résistance modérée envers *M. fallax*, mais non, ou à un moindre degré, envers *M. chitwoodi*. Il n'y a que peu de différence dans la virulence observée chez les plantes résistantes entre les populations de *M. chitwoodi* et *M. fallax*. Par contre, des différences notables sont observées entre populations de *M. hapla* pour leur virulence envers des génotypes résistants de *S. bulbocastanum*, *S. hougasii*, *S. chacoense*, *S. gourlayi*, *S. sparsipilum* et *S. spegazzinii*. Il a été observé que la résistance à *M. chitwoodi*, *M. fallax* et/ou *M. hapla* ne correspond pas à la résistance des espèces adaptées aux températures élevées *M. arenaria*, *M. incognita* et *M. javanica*.

Key-words : evolution, genetic variation, *Meloidogyne arenaria*, *M. chitwoodi*, *M. fallax*, *M. hapla*, *M. incognita*, *M. javanica*, potato, selection, virulence.

In North-Western Europe, root-knot nematodes, *Meloidogyne* spp., are expected to become serious pests for potato crops due to the reduced use of nematicides and a change in crop rotation in favour of highly profitable host crops like vegetables. The predominant *Meloidogyne* species attacking potato are *M. hapla* Chitwood, *M. chitwoodi* Golden, O'Bannon, Santo & Finley, and *M. fallax* Karssen. These nematodes can cause serious economic losses due to reduction in yield and lower quality of the tubers. In particular, *M. chitwoodi* and *M. fallax* cause severe external malformations of tubers and internal necrotic spotting, which renders tubers unsuitable for consumption or processing. Resistance would be very effective in controlling root-knot nematodes, but is not available in currently used potato cultivars (Brown *et al.*, 1994; Janssen *et al.*, 1995).

Resistance screening trials in glasshouses revealed genotypes from various wild tuber-bearing *Solanum* spp. with resistance to *M. chitwoodi* (Brown *et al.*, 1989, 1991; Janssen *et al.*, 1996), *M. fallax*, and/or *M. hapla* (Janssen *et al.*, 1996). However, these screening trials have been made with only one or a few populations of a *Meloidogyne* species and virulence toward supposedly resistant *Solanum* species might already exist. So far, one population of *M. chitwoodi* virulent toward resistant genotypes of *S. bulbocastanum* has been reported (Mojtahedi & Santo, 1994). By definition, virulent populations are those that can reproduce significantly on resistant host plants that prevent

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ISSN 1164-5571/97/05 $ 7.00 / © *Gauthier-Villars - ORSTOM*

or suppress reproduction of avirulent populations of the same parasite species (Roberts, 1995).

Conversely, it has been shown that resistance in some wild *Solanum* spp. can be effective to a broader spectrum of *Meloidogyne* spp. Preliminary results indicated that resistance to both *M. chitwoodi* and *M. fallax* occurs in genotypes of several North-American *Solanum* spp. (Janssen *et al.*, 1996). In subtropical and tropical climates, *M. incognita* (Kofoid & White) Chitwood, *M. arenaria* (Neal) Chitwood, and *M. javanica* (Treub) Chitwood, cause serious economic problems for potato growers (Iwanaga *et al.*, 1989). Resistance to these *Meloidogyne* spp. has been identified in various wild *Solanum* species (Brücher, 1967; Jatala & Mendoza, 1978; Nirula *et al.*, 1967, 1969) and some of these resistant species have also been selected for resistance against *M. chitwoodi*, *M. fallax*, and/or *M. hapla* (Janssen *et al.*, 1996).

The occurrence in the field of virulent individuals or related (virulent) nematode species can result in a rapid decrease of the effectiveness of a resistant cultivar. In some areas of the Netherlands, the extensive use of cultivars with resistance to *Globodera rostochiensis* has led to a great increase in the proportion of fields infected with *G. pallida*. Therefore, the evaluation of the resistance of wild *Solanum* spp. against an array of *Meloidogyne* populations, before its introduction into new potato cultivars, will allow for a better prediction of the durability of resistance and will help defining better management strategies for resistance genes.

This study describes the results of glasshouse experiments carried out to determine the level of resistance of wild *Solanum* genotypes and the variation in virulence of several *Meloidogyne* populations against the resistant genotypes. The occurrence of intra- and interspecific variation for resistance and the implications this may have for the development and use of new resistant cultivars are discussed.

Materials and methods

Plant material

Resistant and susceptible genotypes of wild tuber-bearing *Solanum* species, *S. bulbocastanum*, *S. hougasii*, *S. iopetalum*, *S. fendleri*, *S. cardiophyllum*, *S. stoloniferum*, *S. microdontum*, *S. gourlayi*, *S. sparsipilum*, *S. spegazzinii*, *S. brachistotrichum* and *S. arnezii*, had been identified in resistance screening trials (Janssen *et al.*, 1996). Some resistant genotypes were crossed with susceptible ones to provide progenies in order to investigate the inheritance of resistance (Janssen *et al.*, unpubl.). The genotypes were multiplied under short day conditions in a glasshouse to induce tubers, and the tubers were stored at 5°C until use.

When possible, *in vitro* multiplication was used for rapid multiplication of genotypes. Young growing shoots were sterilized for 10 min in 4 % NaOCl-solution and then transferred into sterile tubes containing MS 30 medium (Murashige & Skoog, 1962) with Cefotaxime sodium. Shoots were cut every 2 to 3 weeks and transferred into new tubes with MS 30 medium, until the required number of plants per genotype was obtained. An experiment was carried out with several resistant and susceptible genotypes to compare the relative level of infection between plants originating from tissue culture and those obtained from tubers. No significant difference was found in number of egg masses between the two types of plant material used.

Inoculum

Field populations of *M. hapla*, *M. chitwoodi* and *M. fallax* were obtained from infested soil and infected plant material, collected from several areas in the Netherlands from 1991 to 1993 (Table 1). To obtain species specific populations the infested soil or infected roots were added to young growing tomato plants and after 10 weeks 30 to 40 females with egg masses were individually identified to species level by isozyme pattern of esterase and malate dehydrogenase (Esbenshade & Triantaphyllou, 1990; van Meggelen *et al.*, 1994). The egg masses were used to inoculate young tomato plants cv. Nematex and, from 12 weeks onwards, these plants were used for obtaining inoculum. To maintain the purity of populations, the procedure of identification of females and inoculation with egg masses was repeated approximately every 3 to 4 months. Contamination with other nematode populations during propagation was prevented by using sterilized sand and pots and by spatial isolation. All populations were maintained on tomato plants in a temperature-controlled glasshouse (22 ± 2°C).

Isolates of *M. incognita*, *M. javanica* and *M. arenaria*, originating from single egg masses, were kindly provided by Dr. M. Fargette (Montpellier, France) (Table 1) and were multiplied for one generation on potato cv. Première. Subsequently, ten to twenty females per isolate were used for isozyme analysis and no contamination with other species was found.

To obtain juveniles for inoculation, eggs were harvested from roots of tomato and potato plants approximately three months after inoculation by dissolving egg masses with 0.5 % NaOCl solution (Hussey & Barker, 1973). Second stage juveniles were hatched in water and stored at 4°C for up to one month until use.

Resistance tests

– *Resistance to* M. chitwoodi, M. fallax *and/or* M. hapla

Table 1. *Origin of* Meloidogyne *populations and isolates used in this study.*

Area	Country	*Meloidogyne* sp.	Last field-grown crop (year)	Designation
Smilde	The Netherlands	*M. hapla*	carrot (1992)	HSMA
Smilde	The Netherlands	*M. hapla*	everlasting flowers (1992)	HSMB
Roggel	The Netherlands	*M. hapla*	hemp (1992)	HRO
Slochteren	The Netherlands	*M. hapla*	chicory (1992)	HSL
Rips	The Netherlands	*M. chitwoodi*	maize (1991)	CHR
Heide	The Netherlands	*M. chitwoodi*	scorzonera (1993)	CHE
Middenmeer	The Netherlands	*M. chitwoodi*	potato (1992)	CHW
Baexem	The Netherlands	*M. fallax*	potato (1992)	FB
Vreedepeel	The Netherlands	*M. fallax*	scorzonera (1992)	FVR
Kempen	The Netherlands	*M. fallax*	scorzonera (1992)	FK
Unknown	Burkina Faso	*M. incognita*	unknown	Mi67*
Unknown	Russia	*M. incognita*	unknown	Mi82*
Unknown	Russia	*M. arenaria*	unknown	Ma84*
Ste Anne	French W. Indies	*M. arenaria*	unknown	Ma29*
Queensland	Australia	*M. javanica*	unknown	Mj44*
Unknown	Marocco	*M. javanica*	unknown	Mj57*

* Isolates kindly provided by Dr. M. Fargette, Montpellier, France.

Genotypes of a *Solanum* species were tested with two to four populations of each *Meloidogyne* species to which resistance had been observed, and with one population of the *Meloidogyne* species to which the genotypes were originally found susceptible.

Plantlets from tissue culture or tubers were grown in clay pots of 350 cm^3, filled with moist silver sand and additional NPK nutrients, in a temperature-controlled glasshouse (22 ± 2°C). Then ten *Meloidogyne* populations were represented as main plots in four replicates, physically separated by splashing boards to avoid cross contamination. Genotypes were randomly assigned as subplots of three to ten *Meloidogyne* populations, depending on the observed resistance of the *Solanum* species (Janssen *et al.*, 1996). When the plants showed vigorous growth, *i.e.*, 2 to 4 weeks after planting, they were inoculated with 400 juveniles of *M. chitwoodi*, *M. fallax*, or *M. hapla* per pot, using an automatic syringe. Plants were individually harvested 8 weeks after inoculation. Roots were washed clean of sand, stained with a phloxine-B solution (Dickson & Strubble, 1965) and egg masses were counted. The potato cvs Darwina and Nicola and the tomato cv. Moneymaker were included as controls.

Due to the large differences in levels of resistance of *Solanum* genotypes and the unbalanced design of the experiment no analysis of variance could be made. Instead, for each genotype, standard errors were calculated over the means and compared using a Student's t-test ($P \leq 0.05$).

– *Resistance to* M. incognita, M. arenaria *and* M. javanica

A second experiment was set up to investigate whether resistance to the temperate *Meloidogyne* spp. in various *Solanum* spp. was also effective against some *Meloidogyne* spp. adapted to high temperature. Besides populations of *M. chitwoodi*, *M. fallax* and *M. hapla*, two isolates each of *M. incognita*, *M. arenaria* and *M. javanica* were used. The experimental method described above was used again for this second experiment. Genotypes were represented by four randomly placed replicates for each nematode population/isolate. Nematode populations/isolates were analyzed separately with ANOVA using Genstat (Payne *et al.*, 1987).

Results

In the first experiment, large differences in the resistance to *M. chitwoodi*, *M. fallax* and *M. hapla*, and to different populations of each of these *Meloidogyne* species, were observed in the genotypes of the various *Solanum* species. Mean numbers of egg masses of all genotype/*Meloidogyne* population combinations tested are shown in Table 2. The results of the earlier screening experiments are also presented as reference.

Table 2. *Mean numbers of egg masses of populations of* Meloidogyne fallax *(Mf),* M. chitwoodi *(Mc) and* M. hapla *(Mh) on roots of genotypes of various* Solanum *spp. The resistance ratings of earlier screening experiments are indicated as reference.*

	Resistance rating*			*M. fallax*			*M. chitwoodi*			*M. hapla*			
	Mf	Mc	Mh	FB**	FVR	FK	CHW	CHE	CHR	HRO	HSL	HSMB	HSMA
S. bulbocastanum													
93-57-5***	sus	sus	res	48.0 *bc*	59.7 *bc*	60.2 *bc*	77.2 *c*	50.7 *bc*	4.7 *a*	37.7 *b*	0.5 *a*	0.8 *a*	--****
93-60-2	res	res	res	0.0 *a*	0.0 *a*	--	0.3 *a*	0.0 *a*	0.0 *a*	35.0 *b*	1.0 *a*	0.3 *a*	--
93-D26-3	res	res	res	0.0 *a*	0.0 *a*	--	--	0.5 *a*	0.0 *a*	17.0 *b*	--	0.3 *a*	--
M94-98-1	--	res	--	25.3 *c*	13.5 *b*	17.8 *bc*	28.3 *c*	17.3 *bc*	0.0 *a*	17.5 *bc*	0.5 *a*	0.8 *a*	--
M94-106-2	--	res	--	0.0	0.0	0.0	0.0	0.3	0.3	--	0.0	0.0	--
M94-106-4	--	res	--	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	28.3 *b*	0.0 *a*	0.0 *a*	--
S. hougasii													
93-71-6	res	res	res	0.0 *a*	0.0 *a*	--	0.0 *a*	0.0 *a*	0.0 *a*	41.5 *d*	15.3 *c*	5.0 *b*	1.0 *a*
93-71-28	res	res	res	0.0 *a*	0.0 *a*	--	0.0 *a*	0.3 *a*	0.0 *a*	36.7 *c*	12.0 *b*	5.7 *b*	--
S. iopetalum													
93-108-1	sus	sus	sus	72.5 *d*	52.3 *abc*	--	63.5 *cd*	63.7 *cd*	54.0 *bc*	--	49.7 *ab*	56.0 *bcd*	38.3 *a*
93-108-11	sus	sus	sus	71.5 *d*	52.0 *a*	--	53.3 *ab*	48.4 *a*	72.5 *bcd*	54.7 *abc*	46.0 *a*	71.2 *cd*	46.2 *a*
S. cardiophyllum													
93-CAR-1	res	res	sus	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	--	--	17.3 *b*	--
93-79-10	res	res	sus	0.0 *a*	0.5 *a*	0.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	--	--	12.3 *b*	--
93-79-13	res	res	sus	0.3 *a*	0.0 *a*	0.0 *a*	0.3 *a*	0.0 *a*	0.0 *a*	--	--	25.3 *b*	--
S. fendleri													
93-114-5	res	res	sus	0.0 *a*	0.0 *a*	0.3 *a*	1.0 *a*	0.0 *a*	0.3 *a*	--	--	14.0 *b*	--
93-114-11	res	res	sus	0.3 *a*	0.3 *a*	0.8 *a*	0.0 *a*	0.0 *a*	0.0 *a*	--	--	25.0 *b*	--
93-114-12	res	res	sus	0.0 *a*	0.0 *a*	0.5 *a*	0.0 *a*	0.0 *a*	0.0 *a*	--	--	21.7 *b*	--
93-115-7	sus	sus	sus	--	62.0 *abc*	73.7 *bc*	51.0 *ab*	87.3 *c*	80.0 *c*	--	--	46.0 *a*	--
93-115-14	sus	sus	sus	99.0 *b*	--	84.3 *ab*	74.7 *ab*	72.5 *ab*	70.2 *ab*	--	--	61.2 *a*	--
93-115-18	sus	sus	sus	83.3 *ab*	93.0 *bc*	66.0 *a*	110.0 *c*	74.2 *ab*	59.7 *a*	--	--	76.7 *ab*	--
M94-33-3	--	res	--	0.0 *a*	0.0 *a*	1.0 *a*	0.0 *a*	0.0 *a*	0.0 *a*	--	--	27.8 *b*	--
M94-33-5	--	res	--	0.0 *a*	0.0 *a*	0.3 *a*	0.3 *a*	0.0 *a*	0.0 *a*	--	--	42.0 *b*	--
M94-51-1	--	res	--	0.0	0.3	0.0	0.0	0.3	0.0	--	--	--	--
S. stoloniferum													
93-STOL-2	res	res	sus	1.3 *a*	1.3 *a*	0.8 *a*	12.0 *b*	6.7 *ab*	5.7 *ab*	--	--	56.2 *c*	--
93-STOL-3	res	res	sus	1.5 *a*	2.5 *a*	1.5 *a*	3.3 *a*	4.0 *a*	2.2 *a*	--	--	53.5 *b*	--
93-STOL-4	res	res	sus	0.3 *ab*	0.0 *a*	1.5 *abc*	10.0 *d*	3.7 *c*	2.5 *bc*	--	--	42.5 *d*	--
93-STOZ-1	sus	sus	sus	100.2 *b*	104.0 *b*	74.0 *a*	118.7 *b*	111.2 *b*	--	--	--	68.0 *a*	--
M94-23-1	res	--	--	1.0 *a*	0.5 *a*	1.0 *a*	29.0 *b*	--	32.2 *b*	--	--	35.0 *b*	--
M94-131-5	res	--	--	3.7 *a*	6.7 *ab*	15.8 *abc*	20.0 *bcd*	34.3 *cd*	40.0 *cd*	--	--	46.0 *d*	--
S. chacoense													
93-24-3	sus	sus	res	24.8 *b*	51.0 *c*	--	--	84.7 *d*	--	0.3 *a*	0.5 *a*	0.3 *a*	0.8 *a*
93-24-27	sus	sus	res	40.7 *c*	27.0 *b*	--	--	51.0 *c*	--	0.5 *a*	2.0 *a*	0.8 *a*	0.8 *a*
93-68-1	res	sus	res	0.5 *a*	0.0 *a*	--	--	--	--	--	1.0 *a*	2.0 *b*	5.0 *b*
93-68-2	res	sus	res	0.0 *a*	0.3 *a*	--	--	45.7 *b*	--	0.8 *a*	0.8 *a*	32.7 *b*	--

End of Table 2 next page

Table 2. *(cont.)*

	Resistance rating*			*M. fallax*			*M. chitwoodi*			*M. hapla*			
	Mf	Mc	Mh	FB**	FVR	FK	CHW	CHE	CHR	HRO	HSL	HSMB	HSMA
S. chacoense (cont.)													
93-68-4	res	sus	res	5.2 *a*	1.5 *a*	--	--	78.2 *b*	--	2.2 *a*	2.2 *a*	3.2 *a*	1.0 *a*
93-68-6	sus	sus	sus	80.0 *c*	57.0 *c*	--	--	68.7 *c*	--	4.2 *a*	2.7 *a*	36.2 *b*	29.0 *b*
93-68-11	sus	sus	res	25.3 *c*	8.3 *b*	--	--	78.5 *d*	--	0.8 *a*	0.7 *a*	2.7 *a*	2.7 *ab*
93-113-1	res	sus	res	2.0 *a*	0.5 *a*	--	--	28.0 *b*	--	0.3 *a*	0.3 *a*	0.0 *a*	0.0 *a*
93-113-2	res	sus	res	1.0 *a*	0.3 *a*	--	--	70.7 *b*	--	7.3 *a*	1.0 *a*	5.3 *a*	--
M94-28-1	--	--	res	8.0 *a*	6.5 *a*	--	--	54.2 *b*	--	2.5 *a*	4.0 *a*	34.7 *b*	33.0 *b*
M94-28-4	--	--	res	24.0 *b*	17.7 *b*	--	--	65.7 *c*	--	0.8 *a*	3.5 *a*	3.7 *a*	5.7 *a*
M94-115-2	--	--	res	37.5 *c*	52.2 *d*	--	--	72.5 *e*	--	0.5 *a*	3.7 *ab*	0.3 *a*	4.0 *b*
M94-93-5	--	--	res	61.2 *c*	50.7 *bc*	--	--	37.7 *b*	--	0.3 *a*	0.8 *a*	0.3 *a*	0.0 *a*
S. microdontum													
93-MCD-4	sus	sus	res	66.0 *c*	--	--	--	40.5 *b*	--	1.5 *a*	1.3 *a*	0.3 *a*	--
93-MCD-17	sus	sus	res	--	--	--	--	39.0 *b*	--	1.0 *a*	3.0 *a*	1.5 *a*	--
S. gourlayi													
93-94-7	sus	sus	res	44.0 *b*	--	--	--	32.0 *b*	--	4.5 *a*	2.7 *a*	23.7 *b*	--
93-94-8	sus	sus	res	50.2 *d*	--	--	--	30.8 *c*	--	1.0 *a*	1.5 *a*	13.7 *b*	--
S. sparsipilum													
93-107-1	sus	sus	res	10.3 *ab*	--	--	--	45.2 *c*	--	3.0 *a*	3.0 *a*	21.3 *b*	32.0 *bc*
93-107-5	sus	sus	sus	--	--	--	--	--	--	2.3 *a*	1.3 *a*	17.5 *b*	--
S. spegazzinii													
93-58-1	sus	sus	res	30.5 *b*	--	--	--	--	--	1.5 *a*	2.0 *a*	20.8 *b*	--
S. tuberosum													
Darwina	sus	sus	sus	101.7 *c*	103.2 *c*	72.2 *b*	79.5 *bc*	73.7 *b*	58.3 *ab*	50.0 *a*	43.7 *a*	47.0 *a*	48.7 *a*
Nicola	sus	sus	sus	110.2 *c*	101.2 *bc*	94.0 *bc*	108.7 *c*	97.5 *bc*	71.2 *a*	63.5 *a*	72.2 *a*	82.7 *ab*	--
L. esculentum													
MoneyMak.	sus	sus	sus	52.0 *ab*	67.7 *b*	50.2 *ab*	53.7 *ab*	33.0 *a*	31.3 *a*	56.5 *ab*	65.7 *ab*	78.2 *b*	86.2 *b*

* The genotypes were considered to be resistant if roots have less than five egg masses, and to be susceptible if they have more than five egg masses (Janssen *et al.*, 1996).
** See Table 1 for population codes.
*** For each genotype, means not followed by the same letter are significantly different according to Student's t-test ($P \leq 0.05$).
**** --= not tested.

High resistance to all populations of both *M. chitwoodi* and *M. fallax* was present in most genotypes of *S. bulbocastanum*, *S. hougasii*, *S. fendleri* and *S. cardiophyllum*. Only the genotypes 93-57-5 and M94-98-1 of *S. bulbocastanum* showed significant differences between populations, *i.e.*, they were susceptible to all populations of *M. chitwoodi* and *M. fallax* except CHR. Genotypes of *S. stoloniferum* showed moderate resistance to *M. fallax*, but lesser or no resistance to *M. chitwoodi*. The difference between *M. fallax* and *M. chitwoodi* was most clearly expressed by some genotypes of *S. chacoense*, which were resistant to *M. fallax*, but susceptible to *M. chitwoodi*.

The effectiveness of the plant resistance to *M. hapla* varied greatly, depending on the nematode populations used. All genotypes of *S. bulbocastanum* were resistant to the *M. hapla* populations HSL and HSMB, but susceptible to HRO. The level of resistance of genotypes of *S. hougasii* varied depending on nematode populations from resistant (HSMA) via intermediate (HSMB, HSL) to susceptible levels (HRO). Plants of *S. chacoense* expressed resistance to

Table 3. *Mean numbers of egg masses of isolates of* Meloidogyne incognita, M. arenaria *and* M. javanica *on roots of* Solanum *genotypes with resistance to* M. hapla, M. chitwoodi *and/or* M. fallax.

Genotype	*M. fallax*	*M. chitwoodi*	*M. hapla*		*M. incognita*		*M. arenaria*		*M. javanica*	
	FVR*	CHW	HSL	HSMB	Mi67	Mi82	Ma84	Ma29	Mj44	Mj57
S. bulbocastanum										
93-60-2	0.0 *a***	0.0 *a*	0.0 *a*	0.0 *a*	83.5 *c*	33.0 *bc*	32.2 *cde*	56.0 *de*	25.7 *bc*	12.3 *a*
S. brachistotrichum										
93-7986	0.0 *a*	0.0 *a*	17.0 *c*	2.0 *ab*	12.5 *ab*	--***	59.3 *f*	49.2 *de*	11.8 *ab*	35.0 *a*
S. cardiophyllum										
93-CAR-1	0.0 *a*	0.0 *a*	0.5 *a*	3.2 *ab*	13.0 *ab*	21.3 *ab*	20.0 *bcd*	13.0 *ab*	14.3 *ab*	--
S. hougasii										
93-71-6	0.0 *a*	0.0 *a*	15.3 *c*	1.2 *ab*	9.0 *a*	14.0 *ab*	33.5 *cdef*	17.0 *abc*	13.5 *ab*	12.8 *a*
S. fendleri										
93-114-11	0.3 *a*	0.0 *a*	25.5 *c*	44.0 *c*	27.3 *b*	44.3 *c*	41.7 *def*	47.5 *cde*	42.2 *cd*	33.5 *a*
S. stoloniferum										
93-STOL-2	0.8 *a*	13.0 *b*	5.5 *b*	49.0 *c*	13.5 *ab*	9.5 *a*	1.0 *a*	2.7 *a*	3.2 *a*	11.8 *a*
S. arnezii										
93-138-1	70.5 *c*	102.5 *d*	0.0 *a*	6.5 *b*	17.7 *ab*	18.5 *ab*	9.3 *b*	32.7 *bcd*	61.5 *d*	25.5 *a*
S. chacoense										
93-24-3	18.7 *b*	63.0 *c*	0.0 *a*	0.0 *a*	7.3 *a*	10.8 *a*	19.7 *bc*	43.0 *cde*	23.2 *bc*	17.7 *a*
S. tuberosum										
Nicola	84.5 *c*	98.3 *d*	45.7 *d*	--	66.7 *c*	125.5 *d*	48.3 *ef*	72.7 *e*	72.5 *d*	73.5 *b*

* See Table 1 for population codes.
** Within each column, means not followed by the same letter are significantly different (L.S.D., $P \leq 0.05$).
*** = not tested.

M. hapla, but some genotypes were resistant to only some populations of *M. hapla* (*e.g.*, 93-68-6), others to all populations of *M. hapla* (*e.g.*, 93-24-3). Some genotypes were resistant to both *M. hapla* and *M. fallax* (*e.g.*, 93-113-1). Genotypes of *S. microdontum* were resistant to all three tested populations of *M. hapla*, whereas genotypes of *S. gourlayi*, *S. sparsipilum* and *S. spegazzinii* were only resistant to HRO and HSL, and not resistant to HSMB. Genotypes of *S. tuberosum* and tomato exhibited little or no differences in susceptibility.

In the second experiment, genotypes of the wild *Solanum* sources with resistance to *M. hapla*, *M. chitwoodi* and/or *M. fallax* were tested against isolates of *M. incognita*, *M. javanica* and *M. arenaria* to investigate whether the resistance was also effective against these species. As a control, populations of *M. chitwoodi*, *M. fallax* and *M. hapla* were included. The results (Table 3) with *M. chitwoodi*, *M. fallax* and *M. hapla* confirmed the results from the first experiment (Table 2). No absolute resistance to any of the subtropical and tropical nematode species was observed, and most genotypes showed moderate to high levels of nematode reproduction. In screening trials with *M. chitwoodi*, *M. fallax* and *M. hapla*, an arbitrary level of five egg masses per plant was set as the upper limit for the definition of resistance (Janssen *et al.*, 1996). Using this criterion, only *S. stoloniferum* 93-STOL-2 would have been selected for resistance to *M. arenaria* and to one isolate of *M. javanica*.

Discussion

The presence of resistance to root-knot nematodes in various wild tuber-bearing *Solanum* spp. as previously reported (Janssen *et al.*, 1996), has been confirmed in this study. A high level of resistance to *M. chitwoodi* and *M. fallax* was observed in genotypes of *S. bulbocastanum*, *S. hougasii*, *S. brachistotrichum*, *S. cardiophyllum* and *S. fendleri*. Since no genotypes of these *Solanum* species had an infection level significantly different for *M. chitwoodi* and for *M. fallax*, it is likely that the resistance to both nematode species is based on the same resistance gene(s) occurring in these North-American *Solanum* species. This will be

further investigated with crossing experiments. With *S. chacoense* and *S. stoloniferum*, significant differences between *M. chitwoodi* and *M. fallax* were observed for some genotypes, but resistance was not as effective as that observed in genotypes of the *Solanum* species mentioned above. *M. fallax* has only recently been differentiated from *M. chitwoodi* (Karssen, 1996) and the two species are closely related in their morphology. Few differences are known regarding host range (van Meggelen *et al.*, 1994; Karssen, 1996), and the likely distribution of *M. fallax* seems comparable to that of *M. chitwoodi* (Karssen, 1996). The similar behaviour of both nematode species on various North-American *Solanum* spp. is a further indication that *M. chitwoodi* and *M. fallax* should be considered as genetically closely related species, as compared to other *Meloidogyne* species like *M. hapla* and *M. incognita*.

Variability in virulence within *Meloidogyne* species was most noticeable with *M. hapla*. This species occurs worldwide as one of the four most common species of the genus (Sasser & Carter, 1985) and large genetic variation within the species is expected. However, it is surprising that large differences in virulence toward *Solanum* genotypes, which are native to North and South-America (Hawkes, 1990), were found between *M. hapla* populations from the Netherlands. The presence of virulent populations is not likely to be the result of a selection of virulent forms by these resistant *Solanum* spp., since resistance is not yet present in the currently used potato cultivars (Brown *et al.*, 1994; Janssen *et al.*, 1995). A similar situation is found in tomato cultivars bearing the *Mi*-gene from *Lycopersicon peruvianum*. Resistance breaking field populations of root-knot nematodes have been found that had not been previously exposed to resistant cultivars (Netscher, 1977; Prot, 1984; Fargette, 1987; Roberts & Thomason, 1989). Roberts and Thomason (1989) suggested that this virulence is genetically closely related to genes controlling other traits that improve the fitness of the population. Nevertheless the possibility of a recent introduction, in the last decades, of *Meloidogyne* populations from other areas and continents could also explain the occurrence of virulent *M. hapla* populations.

Little variation in virulence was noticed within *M. chitwoodi*. The population CHR of *M. chitwoodi* did not reproduce on *S. bulbocastanum* M94-98-1 and had a low level of reproduction on 93-57-5, two genotypes that were susceptible to the other populations of *M. chitwoodi* and *M. fallax*. None of the tested populations was virulent toward the genotypes of *S. bulbocastanum*, but the existence of *M. chitwoodi* populations virulent toward this *Solanum* species has been reported in the U.S.A. (Mojtahedi & Santo, 1994).

Most *Solanum* genotypes with resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla* were susceptible to *M. incognita*, *M. arenaria* or *M. javanica*. Only *S. stoloniferum* 93-STOL-2 was moderately resistant to some isolates of *M. arenaria* and *M. javanica*. Resistance to these subtropical and tropical nematode species has been observed in several *Solanum* species tested (Brücher, 1967; Nirula *et al.*, 1967, 1969; Jatala & Mendoza, 1978) and attempts have been made to transfer resistance into cultivated potatoes (Gomez *et al.*, 1983; Iwanaga *et al.*, 1989). Although the nematode isolates used in this study do not represent the complete genetic variability of these *Meloidogyne* species adapted to high temperature, it is not expected that the resistance to *M. chitwoodi*, *M. fallax* or *M. hapla* investigated will be effective against *M. incognita*, *M. javanica* and *M. arenaria*.

Within *Meloidogyne* spp., genetic variation resulting in differences in morphological characters, biochemical and molecular markers, host range, and (a)virulence factors has often been reported, but such differences could not be used for the differentiation of well-defined groups within *Meloidogyne* spp., and this may prove to be impossible. Variation in chromosome number, in, *e.g.*, the A- and B-race of *M. hapla* and aneuploid populations in various *Meloidogyne* spp., has been described (Triantaphyllou, 1985), but this merely complicates the characterization of groups based on host ranges and (a)virulence factors (Roberts, 1995). A differential host test has been developed to differentiate (races of) *M. arenaria*, *M. incognita*, *M. javanica* and *M. hapla* (Sasser, 1979; Sasser & Carter, 1985), but the use of this test is restricted to practical purposes and meant for preliminary identification. Host range differences within the species have also been described for *M. hapla* (Sasser, 1972) and *M. chitwoodi* (Santo & Pinkerton, 1985), but only for the latter species did it lead to a differentiation into races. Race deviations have also been used to differentiate (a)virulent populations on resistant host plants. The terms "B population" or, later, "B-race" have been introduced for populations of *M. incognita*, *M. javanica* and *M. arenaria* reproducing on resistant tomatoes carrying the *Mi*-gene (Riggs & Whitehead, 1959; Netscher, 1977) and a population of *M. chitwoodi* virulent toward resistant *S. bulbocastanum* has been designated as race 3 (Mojtahedi & Santo, 1994).

This study has shown that virulence toward several resistant *Solanum* spp. exists within *M. hapla* and *M. chitwoodi*. The four populations of *M. hapla* tested behaved differently toward the various genotypes of the different *Solanum* species, which shows that more than one (a)virulence factors are involved, and present in different combinations. Assuming that (a)virulence is genetically based, many combinations

are possible depending on the number of resistance sources. Moreover, the (a)virulence factors can inherit independently of other characters used for race differentiations. For example, on root-knot nematode resistant cowpea, both virulent and avirulent populations have been identified within the races 1 and 3 of *M. incognita* (Roberts *et al.*, 1995). Therefore, we would prefer to base the identification of new races on characters such as host ranges and ploidy level, but not on the (a)virulent behaviour of populations. For example, population HRO of *M. hapla*, virulent toward *M. hapla*-resistant *S. hougasii*, should not be described as a new "race." According to this guideline, race 3 of *M. chitwoodi* (Mojtahedi & Santo, 1994) should be included in race 2 of *M. chitwoodi*, but designated as a population virulent toward resistant *S. bulbocastanum*. When resistance from wild *Solanum* species is incorporated into new cultivars and used in cropping systems, a biotype scheme as proposed by Roberts (1995) may be developed for a more convenient designation of populations.

To investigate virulence groups, new procedures for multiplication, identification, and preservation of nematode populations/isolates are necessary. The current method of rearing populations on tomato plants with accurate species identification of females using isozyme patterns (Janssen *et al.*, 1995) cannot exclude the possibility of contamination by populations of the same *Meloidogyne* species differing in virulence factors, nor preserve the genetic variation of a population. To solve the second problem, the storage of nematode populations/isolates in liquid nitrogen is an effective and reliable method for long-term preservation of *Meloidogyne* germplasm (van der Beek *et al.*, 1996). The multiplication on differential plants, *i.e.*, resistant genotypes, and the use of molecular markers should give an insight into the genetic background of virulence and other characters.

Resistance sources from various *Solanum* species are now available for introduction of resistance into cultivated potato. However, the existence of variation in virulence and the interactions observed with different sources of resistance warn us that introgression of resistance into cultivated potato should proceed with caution. Moreover, no resistant genotype to all tested populations of *M. hapla*, *M. chitwoodi* and *M. fallax* has been identified. To avoid selection of virulent populations or competitive nematode species, the introduction of resistance from more than one *Solanum* species into new potato cultivars is highly recommended. From our results, it can be anticipated that this will be time-consuming but possible for resistance to *M. chitwoodi*, *M. fallax* and/or *M. hapla*. Additionally, resistance, and specially resistance to *M. hapla*, needs to be evaluated against a large number of populations that would be representative of the pathogenicity of the species to ensure the effectiveness of the resistance before laborious efforts are undertaken to introduce this trait into new potato cultivars.

Acknowledgements

The authors wish to thank Mireille Fargette and Evert Brommer for providing initial nematode populations, Hans van der Beek, Leontine Colon, Mireille Fargette, Coosje Hoogendoorn and Evert Jacobsen for their critical comments on the manuscript and Paul Keizer for his statistical support. This work was financially supported by the S.S.A. (Stichting Stimulering Aardappelonderzoek).

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