Variation in Virulence

Within *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla* on *Solanum* spp.

J. G. van der Beek, L. M. Poleij, C. Zijlstra, R. Janssen, and G. J. W. Janssen

First, second, and third authors: Research Institute for Plant Protection (IPO-DLO), P.O. Box 9060, NL-6700 GW Wageningen, the Netherlands; fourth and fifth authors: Centre for Plant Breeding and Reproduction Research (CPRO-DLO), P.O. Box 16, NL-6700 AA Wageningen, the Netherlands.

Accepted for publication 19 March 1998.

ABSTRACT

Van der Beek, J. G., Poleij, L. M., Zijlstra, C., Janssen, R., and Janssen, G. J. W. 1998. Variation in virulence within *Meloidogyne chitwoodi*, *M. fallax*, and *M. hapla* on *Solanum* spp. *Phytopathology* 88:658-665.

The virulence of *Meloidogyne hapla*, *M. chitwoodi*, and *M. fallax* was studied on genotypes of *Solanum* spp. in a greenhouse. Juveniles of 11 *M. hapla* race A isolates, 3 *M. hapla* race B isolates, and 5 mono-female lines of a *M. hapla* race A isolate were inoculated on *S. chacoense*, *S. hougasii*, and *S. sparsipilum*. Juveniles of eight *M. chitwoodi* isolates, five *M. fallax* isolates, and six mono-female lines of a *M. chitwoodi* isolate were inoculated on *S. bulbocastanum*, *S. chacoense*, *S. hougasii*, *S. stoloniferum*, and *S. tuberosum*. Virulence was expressed as nematode reproduction 8 weeks after inoculation. Nematode reproduction was estimated by the number of egg masses and, in one experiment, by the number of hatched second-stage juveniles per inoculated juvenile. Considerable variation in virulence and resistance was observed among *M.*

hapla isolates and plant genotypes, respectively. The *M. hapla* isolate-plant species interaction was highly significant. The response to *M. chitwoodi* ranged from susceptible (*S. tuberosum* and *S. chacoense*) to highly resistant (*S. bulbocastanum* and *S. hougasii*). *S. tuberosum* was susceptible to *M. fallax*, whereas all four wild species were resistant. In contrast to *M. hapla*, no significant isolate-plant genotype interaction was obtained for *M. chitwoodi* or *M. fallax*, indicating no or little intraspecific variation in virulence. *M. chitwoodi* juveniles in species mixtures with *M. fallax* isolates appeared to be able to break the resistance of *S. bulbocastanum* and *S. hougasii*. Significant differences among mono-female lines of *M. hapla* and *M. chitwoodi* were observed, indicating heterogeneity of pathogenicity within meiotic parthenogenic *Meloidogyne* populations.

Additional keywords: genetic variation, intraspecific specialization, potato, root-knot nematode.

The root-knot nematode species *Meloidogyne chitwoodi* Golden et al. and *M. hapla* Chitwood can cause growth and yield reductions and quality loss in a large number of crops. In potato (*Solanum tuberosum* L.), these root-knot nematodes are able to reproduce on roots, causing yield reduction (21). *M. fallax* Karssen, which was described recently (22), is allopatric with *M. chitwoodi* in the Netherlands, and its host range is similar to that of *M. chitwoodi*. *M. hapla* causes little quality damage due to gall formation on the outside of tubers, in contrast to *M. chitwoodi* (7). Recent observations indicate that *M. chitwoodi* and *M. fallax* reproduce similarly on tubers, and both result in quality loss (J. G. van der Beek, unpublished data). In addition to yield reduction and quality loss, an increase in the nematode population due to cultivation of a susceptible host makes these pathogens a potential threat to agriculture in Western Europe, because many crops in the current rotation schemes are good hosts (33). However, *M. hapla* does not reproduce on monocotyledonous crops, which may be used in rotation with dicotyledons as an effective control measure.

The introduction of resistant potato cultivars also would improve the efficiency of crop rotation in reducing this threat. However, in current potato cultivars, no desirable level of resistance to *M. chitwoodi* and *M. hapla* has been identified (7,17).

Thus far, intraspecific variation in *M. chitwoodi* has been described by the occurrence of three host races that can be distinguished on alfalfa cv. Thor (nonhost for race 1 and host for races 2 and 3), carrot cv. Red Cored Chantenay (host for race 1 and non-

host for races 2 and 3), and *S. bulbocastanum* Dun. SB22 (nonhost for races 1 and 2 and host for race 3) (26,27,36).

Resistance to *M. chitwoodi* and *M. fallax* has been identified in accessions of wild tuber-bearing *Solanum* species, originating mainly from Central America, such as *S. bulbocastanum* and *S. hougasii* Corr. (5,6,18). Resistance to *M. chitwoodi* has been transferred from a resistant genotype of *S. bulbocastanum* to the gene pool of the cultivated potato by somatic hybridization (1,7), and a single gene has been localized on chromosome 11 that appears to control this resistance (8). Also, various levels of resistance to *M. hapla* have been reported (9,15,18). Recent investigations indicated the presence of variation in host responses to different *M. hapla* isolates on some genotypes of *S. bulbocastanum*, *S. hougasii*, *S. chacoense* Bitt., *S. gourlayi*, *S. sparsipilum*, and *S. spegazzinii* that were selected for resistance (19).

In general, the efficacy of resistance depends largely on genetic variation in the virulence of the pathogen and factors involved in the durability of resistance. Often the presence of resistance is concluded from experiments with one or a few isolates. The current study focused on the variation in virulence within *M. chitwoodi*, *M. fallax*, and *M. hapla* by testing several isolates and mono-female lines on some completely resistant and incompletely resistant genotypes of *Solanum* spp. In this paper, virulence is defined as the ability of an isolate of a *Meloidogyne* species to reproduce significantly on resistant host plants that prevent or suppress reproduction of other isolates of the *Meloidogyne* species (adapted from Roberts [34]).

MATERIALS AND METHODS

Nematode isolates and plant genotypes. A total of 8 *M. chitwoodi*, 5 *M. fallax*, and 14 *M. hapla* isolates were used in this

Corresponding author: J. G. van der Beek
E-mail address: j.g.vanderbeek@IPO.DLO.NL

study (Table 1). All isolates, except three, were from geographically different sites in the Netherlands, and originated from various hosts. *M. chitwoodi* isolate Cba was obtained from the United States (Oregon) and represents race 1; *M. hapla* isolates Haw and Han originated from France and South Korea, respectively. Of the *M. hapla* isolates, 11 were of the facultative meiotic parthenogenetic race A, and 3 were of the obligate mitotic parthenogenetic race B. Races A and B were distinguished by examining oocytes for the presence (race A) or absence (race B) of a second maturation division, using Hoechst 33258 (Polysciences Inc., Warrington, PA) for chromosome staining (42). All isolates were maintained and propagated on *Lycopersicon esculentum* L. 'Moneymaker' in glass-house compartments at 20 ± 2°C and 70 to 80% relative humidity (RH). During maintenance, the isolates were tested regularly for possible contamination with other species by observing isozyme phenotypes of esterase and malate dehydrogenase (12,23) and analysis of internal transcribed spacer (ITS) regions of ribosomal DNA (46,47). Additionally, during the experiment, samples of the inocula of the isolates were collected and verified for absence of contamination by analysis of ITS regions. The same DNA test was performed with samples of nematode progenies of the isolates extracted from test plants at harvest.

Six mono-female lines (Cb1 to Cb6) from *M. chitwoodi* isolate Cb and five mono-female lines (Ha1, Ha2, Ha4, Ha5, and Ha6) from *M. hapla* race A isolate Ha were developed by inoculating root tips of *S. tuberosum* cv. Eigenheimer grown in petri dishes containing 2.0 technical agar no. 3 (Oxoid, London) with second-stage juveniles (J2). To ensure parthenogenetic offspring, only one J2 was inoculated per petri dish. Consequently, the progeny of such a juvenile are termed a mono-female line. This procedure was repeated for seven consecutive cycles in petri dishes. The mono-female lines obtained each originated from a different egg mass of the original isolates, with one exception: Cb1 and Cb2 were derived from two J2 from the same egg mass after one generation of parthenogenesis. The resulting 11 single egg masses were propagated separately on plants of cv. Moneymaker prior to preservation in liquid nitrogen (43). After thawing, two generations followed on tomato plants to obtain adequate amounts of J2 for the experiment.

The nematode isolates and mono-female lines of *M. chitwoodi* and *M. fallax* were tested on susceptible *S. tuberosum* cv. Nicola and genotypes of four wild *Solanum* spp.: *S. bulbocastanum* 93-60-2, *S. chacoense* 93-68-2, *S. hougasii* 93-71-6, and *S. stoloniferum* 93-STOL-2. The nematode isolates and mono-female lines of *M. hapla* were tested on genotypes of three wild tuber-bearing *Solanum* spp.: *S. chacoense* 93-68-2, *S. hougasii* 93-71-6, and *S. sparsipilum* 93-107-1. Genotypes of the wild species were selected from previous experiments because of their observed variation in response to one, two, or three isolates of *M. chitwoodi*, *M. fallax*, or *M. hapla* (19). In those experiments, the *S. bulbocastanum* and *S. hougasii* genotypes were completely resistant to *M. chitwoodi* and *M. fallax*, *S. stoloniferum* was incompletely resistant to *M. chitwoodi* and completely resistant to *M. fallax*, and *S. chacoense* was susceptible to *M. chitwoodi* and completely resistant to *M. fallax*. Except for *S. tuberosum* cv. Nicola, these genotypes were maintained and propagated in vitro, ensuring rapid multiplication. To obtain the proper number of plants per genotype, shoots were cut every 2 to 3 weeks and transferred to new tubes with Murashige and Skoog medium containing 30 g of sucrose per liter (28). Three weeks after the last cut, plantlets were large enough for in vivo transplantation.

Test for virulence. In vitro plantlets of the wild *Solanum* spp. and tuber pieces with top sprouts of cv. Nicola were transplanted to 350-ml clay pots, which were filled with moist silver sand containing 0.2% slow-release fertilizer (Osmocote; Sierra Chemical Company, Milpitas, CA) and 0.08% N-P-K (12-10-18) and placed in a greenhouse compartment at 20 ± 2°C at 70 to 80% RH with additional light for 16 h per day. During the first 4 days after

transplantation, RH was kept at ≈100% by covering the plants originating from in vitro culture with transparent plastic.

To prepare the nematode inoculum, heavily infested tomato roots were placed on trays in a mist cabinet at 20°C with a water temperature of 25°C (37). One- to two-day-old hatched J2, which settled on the bottom of the tray, were collected over approximately three weeks and kept at 4°C. Prior to inoculation, the J2 were poured over a paper filter. Inoculum was collected after 1 day, and inoculation was done on plantlets randomly assigned to treatment by twice applying a quantity of 4.5 ml of inoculum in a groove ≈1 cm deep around the plant while continuously mixing the inoculum. An average inoculation density of 1.6 J2 per ml of sand was used, resulting in a total of ≈560 J2 per pot. Noninoculated control plants were included in the experiment.

For practical reasons, tests with *M. chitwoodi* and *M. fallax* isolates were carried out at a different time than tests with *M. hapla*. Also, tests with the mono-female lines were carried out at a different time than those with the isolates. This resulted in four experiments arranged in a complete randomized block design, each with five replicates. Eight weeks after inoculation, the roots were rinsed free of sand and stained with phloxine B (10). Egg masses were counted visually.

For the experiments with *M. chitwoodi* and *M. fallax* isolates and lines, roots were ground in a table blender, and eggs were extracted by shaking the roots for 4 min in 0.5% NaOCl (extraction method adapted from Hussey and Barker [16]). Eggs were

TABLE 1. *Meloidogyne hapla*, *M. chitwoodi*, and *M. fallax* isolates used in the experiments

Isolate	Origin*	Host of origin	Year of sampling	Obtained ^y
<i>M. hapla</i> ^z				
Ha (A)	Lisse	Peony	1990	PD
Hb (A)	Zwaanshoek	<i>Astilbe</i> sp.	1990	PD
Hc (A)	Bavel	<i>Aconitum</i> sp.	1990	PD
He (A)	Drouwenerveen	Potato	1992	PAV
Hf (A)	Borgers	Carrot	1992	PAV
Hi (A)	Smilde	Immortelle	1992	PAV
Hj (A)	Smilde	Carrot	1992	PAV
Hk (A)	Slochteren	Chicory	1992	PAV
Hak (A)	Mussel	Carrot	1993	HLB
Haw (A)	Antibes (Fr.)	Grape	Pre-1977	INRA
Hbz (A)	Amerzoden	Rose	1990	PD
Hh (B)	Wachtum	Fallow (weed)	1992	PAV
Han (B)	S. Korea	<i>Hosta</i> sp.	1990	PD
Hbr (B)	Hoorn	<i>Veronica</i> sp.	1992	PD
<i>M. chitwoodi</i>				
Ca	NL	Maize	1989	PD
Cb	NL	Wheat	1990	PD
Ck	NL	Tomato	1989	PD
Co	NL	Black salsify	1993	PAV
Cx	NL	Potato	1993	PD
Cy	NL	Potato	1993	PD
Caq	NL	Potato	1993	CPRO
Cba	OR	Potato	Unknown	WSU
<i>M. fallax</i>				
Fa	NL	Early primrose	1992	PAGV
Fb	NL	Beet	1991	PD
Fc	NL	Potato	1993	PD
Fd	NL	Potato	1993	PD
Fe	NL	Unknown	1994	BLGG

* Fr. = France; NL = Netherlands; Oregon = Oregon.

^y PD = Plant Protection Service, Wageningen, the Netherlands; PAV = Research Station for Arable Farming and Field Production of Vegetables, Lelystad, the Netherlands; HLB = Hilbrands Laboratory for Soilborne Diseases and Pests, Assen, the Netherlands; INRA = Institut National de Recherche Agronomique, Montpellier, France; CPRO = DLO-Centre for Plant Breeding and Reproduction Research, Wageningen, the Netherlands; WSU = Washington State University, Department of Plant Pathology, Prosser; BLGG = Laboratory for Soil and Crop Testing, Oosterbeek, the Netherlands.

^z Letters in parentheses indicate cytological race.

separated from root particles with a series of sieves. Subsequently, eggs were collected and hatched on 20-µm mesh sieves, and J2 were counted after 3 weeks of incubation at 20°C. Total numbers of hatched J2 were estimated by counting three samples of 1 ml of J2 suspension with a Contextvision MicroGOP2000 image analysis package (Struers Vision AB, Linköping, Sweden) (3). The reproduction rate, Pf/Pi (final population divided by initial population), was estimated by the number of hatched J2 per inoculated J2. The root volume was estimated according to a scale with four response categories ranging from small (= 1) to large (= 4) root volume.

Statistical analysis. Linear logistic regression was employed to explain the number of egg masses observed (Y) in response to

TABLE 2. Mean number of egg masses for combinations of *Meloidogyne hapla* isolates and *Solanum chacoense*, *S. hougasii*, and *S. sparsipilum*

Isolate ^y	Host ^z			Isolate mean
	<i>S. chacoense</i>	<i>S. hougasii</i>	<i>S. sparsipilum</i>	
Ha	0.5 a	8.8 b	0.0 a	3.1
Hb	0.0 a	2.0 a	0.0 a	0.6
Hc	0.0 a	3.8 b	1.2 ab	1.6
He	1.8 a	16.4 b	4.5 a	7.6
Hf	28.5 b	0.0 a	48.5 c	25.6
Hi	8.3 a	35.3 b	41.0 b	28.2
Hj	6.0 b	0.0 a	41.8 c	15.9
Hk	0.3 a	0.0 a	3.0 a	1.0
Hak	10.4 b	0.0 a	43.8 c	18.1
Haw	0.8	0.0	15.8	5.5
Hbz	14.5 b	0.4 a	2.8 a	5.9
Hh	3.3 a	2.2 a	1.5 a	2.3
Han	11.4	0.0	3.8	5.1
Hbr	0.6 a	2.2 a	4.0 a	2.3
Host mean	6.0	4.6	14.7	8.5

^y Isolates Haw and Han were contaminated with *M. chitwoodi* and excluded from the statistical analysis.

^z Means with common letters in a row are not significantly different at $P < 0.05$, using the F distribution for the deviance statistic on the logit scale.

TABLE 3. Mean number of egg masses for combinations of *Meloidogyne hapla* isolate Ha and mono-female lines of isolate Ha with *Solanum chacoense*, *S. hougasii*, and *S. sparsipilum*

Line	Host			Isolate mean ^z
	<i>S. chacoense</i>	<i>S. hougasii</i>	<i>S. sparsipilum</i>	
Ha	0.2	13.8	0.4	4.8 a
Ha1	0.8	38.7	0.6	13.4 b
Ha2	0.0	13.6	0.6	4.7 a
Ha4	0.0	3.0	0.2	1.1 a
Ha5	0.0	12.0	0.0	4.0 a
Ha6	0.8	41.0	1.2	14.3 b
Host mean ^z	0.3 p	22.0 q	0.5 p	

^z Means followed by the same letter are not significantly different at $P < 0.05$, using the F distribution for the deviance statistic on the logit scale.

TABLE 4. Analysis of deviance for linear logistic regression of egg-mass counts of *Meloidogyne hapla* isolates inoculated on *Solanum chacoense*, *S. hougasii*, and *S. sparsipilum*

Source	df	Deviance	Mean deviance	Deviance ratio ^z
Replication	4	47.4	11.84	2.55*
Isolates	11	1,451.5	131.95	28.39***
<i>Solanum</i> genotype	2	386.6	193.30	41.59***
Isolates- <i>Solanum</i> genotype	22	1,016.3	46.20	9.94***
Residual	122	567.1	4.65	
Total	161	3,468.9	21.55	

^z * and *** indicate deviance ratios are significant at $P < 0.05$ and $P < 0.001$, respectively, using the F distribution.

several classifying variables: replicate, *Solanum* genotype, *Meloidogyne* isolate, and interaction between *Solanum* genotype and *Meloidogyne* isolate. The model assumes that the variance in the number of egg masses observed is due to a distribution more variable than the binomial and that the variance can be adequately described by variance proportional to binomial variance (24). The linear logistic model is represented as

$$\log[\mu_{ijk}/(n - \mu_{ijk})] = \text{constant} + \text{replicate}_i + \text{isolate}_j + \text{genotype}_k + \text{isolate} \cdot \text{genotype}_{jk}$$

$$\text{var}(Y_{ijk}) = \sigma^2 \mu_{ijk}(n - \mu_{ijk})/n$$

where Y_{ijk} and μ_{ijk} are the observed and expected numbers of egg masses for isolate j and genotype k in replicate i , respectively, n is the number of inoculated J2 and denotes the dispersion parameter. If $\sigma^2 > 1$, there is overdispersion compared to binomial variance.

Loglinear regression models with variance proportional to Poisson variance were employed to describe the response of the ratios of the number of produced J2 and number of inoculated J2 to the same classifying variables. The loglinear model is represented as

$$\log(\mu_{ijk}) = \text{constant} + \text{replicate}_i + \text{isolate}_j + \text{genotype}_k + \text{isolate} \cdot \text{genotype}_{jk}$$

$$\text{var}(R_{ijk}) = \phi \mu_{ijk}$$

where R_{ijk} and μ_{ijk} are the observed and expected values of the ratio for isolate j and genotype k in replicate i , respectively, and ϕ denotes the dispersion parameter. If $\phi > 1$, there is overdispersion compared to Poisson variance.

The logistic and loglinear models were fitted to the data by the quasi-likelihood method (45). For the logistic and loglinear models, analysis of deviance was used as a generalization of the analysis of variance (ANOVA) for data with normal errors. In an analysis of deviance table, each deviance represents the variation accounted for by its corresponding term, having eliminated the effects of terms above it but ignoring any effects of those terms below it. The criterion for inclusion of a term in the model was an F test for the ratio of the mean deviance for the particular term to a current estimate for the overdispersion (the mean deviance of the rest).

TABLE 5. Analysis of deviance for linear logistic regression of egg-mass counts of *Meloidogyne hapla* isolate Ha and mono-female lines from isolate Ha inoculated on *Solanum chacoense*, *S. hougasii*, and *S. sparsipilum*

Source	df	Deviance	Mean deviance	Deviance ratio ^z
Replication	4	123.7	30.92	4.56***
Lines	5	219.1	43.83	6.46***
<i>Solanum</i> genotype	2	954.9	477.45	70.40***
Lines- <i>Solanum</i> genotype	10	9.6	0.96	0.14
Residual	61	413.9	6.79	
Total	82	1,721.1	20.99	

^z *** indicates deviance ratio is significant at $P < 0.001$, using the F distribution.

TABLE 6. Mean number of egg masses for two *Meloidogyne hapla* isolates (Hi and Hk) compared with two related isolates on three *Solanum* spp. in separate experiments

Isolate ^z	Host		
	<i>S. chacoense</i>	<i>S. hougasii</i>	<i>S. sparsipilum</i>
Hi	8.3	35.3	41.0
HSMB	32.8	6.0	21.3
Hk	0.3	0.0	3.0
HSL	0.8	15.3	3.0

^z The pairs of isolates Hi and HSMB and Hk and HSL originated as pairs from the same soil samples in 1992 but were propagated separate from each other for 4 years.

Pairwise differences between treatment means on the logit scale were assessed by *t* tests. However, in cases in which zero treatment means were obtained, *t* tests for assessing pairwise differences between treatment means on the logistic or logarithmic scales were no longer valid. Fitting logistic and loglinear models in the case of zero means results in underflow (2), as the result of an inability to represent $-\infty$ (the estimated treatment mean on the logistic or logarithmic scale) on a computer.

Zero means were considered random, assuming that positive counts were possible. Pairwise testing was performed by an *F* test for the ratio of the difference between residual deviances of the model, considering the groups compared as one common group, the model as different, and the residual mean deviance from the latter model. All statistical analyses were performed by Genstat 5 (release 3, Clarendon Press, Oxford).

RESULTS

***M. hapla* on *Solanum* spp.** Analysis of rDNA of *M. hapla* juveniles hatched from eggs collected from roots 8 weeks after inoculation revealed no contamination of the isolates with other *Meloidogyne* spp., except for Han and Haw, which each contained $\approx 5\%$ *M. chitwoodi*. Virulence data for Han and Haw, therefore, should be interpreted cautiously. These two isolates were omitted from the statistical analysis.

None of the three *Solanum* genotypes showed absolute resistance to all *M. hapla* isolates, and none of the nematode isolates showed absolute avirulence to all three *Solanum* genotypes (Tables 2 and 3). However, 25% of the isolate-*Solanum* genotype combinations were incompatible. The plots of deviance residuals against fitted values showed no severe anomalies (data not shown). Based on calculated *P* values for the *F* tests of the mean deviance ratios, significant effects for the interactions between *Solanum* genotypes and *M. hapla* isolates were detected (Table 4). For each isolate, pairwise differences between the means were analyzed (Table 2). Large differences among paired means were found, for example, between isolates He and Hf for combinations with all three *Solanum* species (Table 2).

Significant effects of *Solanum* genotype and mono-female lines were detected, but the line-*Solanum* genotype interaction was not

statistically significant (Table 5) in contrast to the isolate-*Solanum* genotype interaction (Table 4). Mean numbers of egg masses of the mono-female lines were higher than or equal to those of isolate Ha (Table 3).

The host responses of isolates Hi and Hk were compared to those of isolates from a previous study (19), HSMB and HSL, respectively. Isolates Hi and HSMB were collected in 1992 from the same soil sample from Smilde, and isolates Hk and HSL were collected from Slochteren (both sites in the Netherlands) but maintained and propagated separately. Isolates HSMB and HSL were tested by Janssen et al. (19) on the same *Solanum* genotypes used in this study. Large differences in virulence were found between these paired isolates (Table 6). Isolate HSL could reproduce moderately on the *S. hougasii* genotype, whereas isolate Hk was completely avirulent. Isolates HSMB and Hi varied on all genotypes with regard to virulence.

***M. chitwoodi* and *M. fallax* on *Solanum* spp.** The tested genotypes of *S. bulbocastanum* and *S. hougasii* demonstrated extremely high levels of resistance to all *M. chitwoodi* and *M. fallax* isolates and mono-female lines (Tables 7 and 8). Only three isolates were able to reproduce on one or both of these two *Solanum* spp. Isolate Fc was able to reproduce on the *S. bulbocastanum* and *S. hougasii* genotypes (Table 7). Isolates Fd and Ck produced only one egg mass each on *S. bulbocastanum* and *S. hougasii*, respectively (Table 7).

Restriction analysis of ITS regions of rDNA, generated from J2 from inocula and nematode progenies extracted from cv. Nicola, indicated that these isolates (T, Fc, Fd, and Ck) were contaminated with one other species. *M. chitwoodi* isolate Ck was contaminated with 10% juveniles of *M. hapla*, which could explain the single egg mass found on *S. hougasii*. In two *M. fallax* isolates, low levels of contamination with *M. chitwoodi* were found: Fc and Fd both contained $\approx 2\%$ *M. chitwoodi* juveniles in the inocula, which resulted in $\approx 10\%$ contamination in their progenies on cv. Nicola at 8 weeks after inoculation. The resistance-breaking ability of *M. fallax* isolate Fc on *S. bulbocastanum* appeared to be totally attributable to *M. chitwoodi*, which was present as a contaminant in Fc, because DNA analysis of the extracted J2 from *S. bulbocastanum* revealed only the *M. chitwoodi* pattern. This result

TABLE 7. Mean number of egg masses (EM) and final population divided by initial population (*Pf/Pi*) for combinations of *Meloidogyne chitwoodi* and *M. fallax* isolates and *Solanum bulbocastanum*, *S. chacoense*, *S. hougasii*, *S. stoloniferum*, and *S. tuberosum*, arranged according to increasing overall mean number of egg masses

Isolate ^w	Host										Mean ^x	
	<i>S. bulbocastanum</i> ^y		<i>S. chacoense</i>		<i>S. hougasii</i>		<i>S. stoloniferum</i>		<i>S. tuberosum</i>			
	EM	<i>Pf/Pi</i>	EM	<i>Pf/Pi</i>	EM	<i>Pf/Pi</i>	EM	<i>Pf/Pi</i>	EM	<i>Pf/Pi</i>	EM	<i>Pf/Pi</i>
Co	0	0	23.8	13.6	0	0	2.4	2.2	76.4	24.3	21.4 a	8.6 a
Ck	0	0	33.6	12.3	0.3	0.0	13.6	6.1	67.0	31.5	23.8	11.0
Cba	0	0	37.0	22.3	0	0	2.0	0.7	90.4	38.9	27.0 ab	11.8 ab
Ca	0	0	41.6	26.7	0	0	3.8	2.6	87.6	81.5	28.9 ab	24.1 c
Caq	0	0	53.2	39.7	0	0	5.0	7.9	84.8	57.7	29.8 ab	22.0 bc
Cb	0	0	57.6	61.2	0	0	3.2	6.8	95.0	67.1	32.5 bc	27.6 c
Cy	0	0	60.0	9.3	0	0	1.2	0.0	121.2	49.5	38.0 bc	12.3 a
Cx	0	0	58.6	43.9	0	0	1.6	6.8	144.2	79.8	42.6 c	27.2 c
Mean ^z	0	0	45.7 b	27.1 b	0.0	0.0	4.1 a	3.2 a	95.8 c	52.8 c	29.3	18.1
Fe	0	0	1.6	0.0	0	0	0.2	0.6	79.2	10.8	16.9	2.5 a
Fa	0	0	1.8	0.2	0	0	0.4	0.0	81.8	28.2	17.5	5.9 b
Fd	0.2	0.0	0.4	0.0	0	0	0.8	0.0	92.0	42.0	19.5	8.8
Fb	0	0	0.2	0.0	0	0	0.2	0.0	107.6	26.3	22.5	5.7 b
Fc	10.2	1.3	0.4	0.3	3.8	0.4	6.0	0.7	131.6	23.0	31.5	4.7
Mean ^z	0	0	1.0 a	0.1 a	0	0	1.5 a	0.2 a	90.2 b	26.8 b	19.1	5.7

^w Contaminated isolates Ck, Fc, and Fd were excluded from statistical analyses.

^x For each response variable, number of egg masses (EM) and *Pf/Pi*, means followed by the same letter are not significantly different at *P* < 0.05, using the *F* distribution for the deviance statistic on the logit scale for EM and log scale for *Pf/Pi*.

^y Nonzero values are means for egg masses (EM) and *Pf/Pi*, entirely attributable to *M. chitwoodi* contamination in *M. fallax* isolates. These means were excluded from the marginal means.

^z For each response variable, number of egg masses (EM) and *Pf/Pi*, means, specified per *Meloidogyne* species, followed by the same letter are not significantly different at *P* < 0.05, using the *F* distribution for the deviance statistic on the logit scale for EM and log scale for *Pf/Pi*.

was confirmed by morphological identification of juveniles of Fc, which had reproduced on *S. bulbocastanum* (G. Karssen, *personal communication*).

Similarly, morphological identification of the extracted progenies of Fc from *S. hougasii* and Fd from *S. bulbocastanum* after harvest indicated that only the contaminating *M. chitwoodi* J2 in these *M. fallax* isolates were able to reproduce on their respective hosts. Morphological identification also confirmed that the single egg mass produced by *M. chitwoodi* mono-female line Cb1 on *S. hougasii* (Table 8) was true to species. Species identification by analysis of ITS regions of rDNA revealed the absence of contamination of the isolates with *M. incognita* or other species, which might occur as contaminants under greenhouse conditions. Absolute levels of resistance were demonstrated in *S. bulbocastanum* and *S. hougasii* genotypes to all tested *M. fallax* and *M. chitwoodi* isolates and mono-female lines, except for contaminated *M. fallax* isolates Fc and Fd and mono-female line Cb1. Because the genotypes of *S. bulbocastanum* and *S. hougasii* showed no differentiation for the two parameters, they did not contribute to the variation and were omitted from the statistical analyses. Consequently, results and discussion of regression analyses are based solely on the genotypes of *S. chacoense*, *S. stoloniferum*, and *S. tuberosum*.

The interaction terms for isolate-*Solanum* spp. (Tables 9 and 10) and lines-*Solanum* spp. (Table 11) were not significant. Plots of deviance residuals against fitted values revealed no anomalies (data not shown). Differences between *Solanum* spp. were highly significant for both parameters (Tables 9 through 11). Differences between isolates and between mono-female lines of Cb were significant for both parameters for *M. chitwoodi* (Tables 9 through 11). For *M. fallax*, differences between isolates were significant for Pf/Pi but not for number of egg masses. Further, analysis showed a clear *Meloidogyne* spp.-*Solanum* spp. interaction effect (data not shown), mainly caused by differences in the responses of *S. chacoense* and *S. stoloniferum* to the two *Meloidogyne* species (Table 7).

The *M. chitwoodi* isolates showed different levels of reproduction on the genotype of *S. stoloniferum*, in contrast to the *M. fallax* isolates, which were avirulent, except for one (Table 7). The differentiating *M. fallax* isolate was the contaminated isolate Fc. The *S. chacoense* genotype varied in susceptibility to *M. chitwoodi* isolates but showed incomplete resistance to *M. fallax*. Cv. Nicola was susceptible to both *M. fallax* and *M. chitwoodi*. On this commercial cultivar, the level of susceptibility for the mean number of egg masses was similar for *M. chitwoodi* and *M. fallax*, but the mean Pf/Pi value was ≈50% lower for *M. fallax* compared to *M. chitwoodi*.

ANOVA of data for root volume revealed no significant differences between *S. tuberosum*, *S. stoloniferum*, and *S. chacoense* (mean root volume values were 2.8, 2.7, and 3.1, respectively; data not shown), suggesting the coparameter root volume had no notable influence on the significant *Solanum* spp. effect for the parameters.

Using the mean deviance of the residual as an estimate of the dispersion parameter in the logistic and loglinear models resulted in estimates much larger than one (Tables 9 through 11), indicating overdispersion (i.e., the data are from distributions more variable than binomial and Poisson distributions).

DISCUSSION

Variation in virulence. Considerable variation in virulence was demonstrated among the 14 isolates of *M. hapla* when tested on the *Solanum* genotypes. This result agrees with the variation found by Janssen et al. (19). The observed variation was accompanied by a highly significant interaction effect for mean number of egg masses, indicating genetic differences for virulence in *M. hapla* and for resistance versus susceptibility in *Solanum* genotypes. *M. hapla* is a cytologically complex species, with varying chromosome numbers, ploidy levels, and reproduction systems (39,40). Despite the variability in this species, pathogenic races, as have been described in *M. chitwoodi*, *M. arenaria*, and *M. incognita*, have not yet been reported for *M. hapla*. Intraspecific variation in *M. hapla* has been recorded twice (13,14), with two *M. hapla* isolates tested on two genotypes of *Medicago sativa*. In contrast, the absence of a significant cultivar-isolate interaction effect in white clover was demonstrated when eight *M. hapla* isolates from New Zealand were tested against two resistant and two susceptible cultivars (25). This discrepancy may be due to a possibly narrower genetic basis for *M. hapla* in New Zealand compared to Western Europe and the United States or to differences in host plants.

The 14 *M. hapla* isolates also were dissimilar. No association was found between virulence and cytological race. Under the assumption that interactions are based on genetic differences, this surprising result indicates the presence of a range of comparable virulence factors in both the meiotic parthenogenetic race A isolates as well as in the mitotic parthenogenetic race B isolates. Based on the current results and given the random choice of *M. hapla* isolates, there are strong indications that many virulence factors are involved. At least five patterns could be distinguished among the *M. hapla* isolates based on differences between the numbers of egg masses for each isolate on the three host genotypes (Table 2), implying the presence of approximately five virulence factors in the *M. hapla* isolates. Apparently, these genes are preserved in *M. hapla* without selection pressure from resistance genes, because these genes have never been introduced before in the Netherlands. We postulate that these virulence factors are maintained in the gene pool by a continuously changing selection pressure, assured by the polyphagous nature and large cytological variation of the nematode species. *M. hapla* is able to reproduce on many dicotyledons, including weeds. This genetically broad supply of host genotypes may ensure the preservation of many virulence factors.

The absence of significant plant genotype-nematode isolate interactions within *M. chitwoodi* and *M. fallax* leads us to conclude

TABLE 8. Mean number of egg masses for combinations of *Meloidogyne chitwoodi* isolate Cb and mono-female lines of isolate Cb with *Solanum bulbocastanum*, *S. chacoense*, *S. hougasii*, *S. stoloniferum*, and *S. tuberosum*

Isolate	Host					Isolate mean ^z
	<i>S. bulbocastanum</i>	<i>S. chacoense</i>	<i>S. hougasii</i>	<i>S. stoloniferum</i>	<i>S. tuberosum</i>	
Cb	0	57.8	0	2.4	92.4	31.8 bc
Cb1	0	41.5	0.2	4.6	91.0	26.8 a–c
Cb2	0	28.8	0	3.3	97.8	25.8 ab
Cb3	0	31.0	0	3.2	79.4	22.7 a
Cb4	0	25.0	0	2.4	87.6	23.0 a
Cb5	0	69.2	0	3.0	98.6	35.6 c
Cb6	0	28.3	0	3.8	70.8	20.3 a
Host mean ^z	0	40.9 p	0	3.2 q	87.9 r	26.5

^z Means followed by the same letter are not significantly different at *P* < 0.05, using the *F* distribution for the deviance statistic on the logit scale.

that little variation in virulence exists among the tested isolates of *M. chitwoodi* and *M. fallax* toward the three *Solanum* genotypes in this study. These differences in variability in host response between *M. hapla* on one side and *M. chitwoodi* and *M. fallax* on the other may be explained by an introduction of *M. chitwoodi* into the Netherlands that is more recent than that of *M. hapla*. The similarity of the American *M. chitwoodi* isolate Cba to the Dutch isolates may indicate the same origin or similarity in virulence patterns in *M. chitwoodi* in a wide geographic area. Further studies are needed to provide decisive answers.

Despite the low variation in virulence in relation to *Solanum* spp., the current study indicates the presence of intraspecific specialization for virulence in *M. chitwoodi* and its absence in the five *M. fallax* isolates, because the reproduction of isolate Fc on *S. bulbocastanum* and *S. hougasii* and of Fd on *S. bulbocastanum* was caused entirely by the presence of *M. chitwoodi*. It is remarkable that the contaminating *M. chitwoodi* in isolate Fc in the current study did not reproduce significantly on *S. chacoense*, as did the nine *M. chitwoodi* isolates (Table 7).

In addition to possible intraspecific specialization, a synergistic effect of the two incompatible *Meloidogyne* spp. could explain the breaking of *S. bulbocastanum* and *S. hougasii* resistance to *M. chitwoodi*. Synergistic effects inducing susceptibility have been reported in interspecific studies in *Meloidogyne* but involved inoculation of a compatible *Meloidogyne* species prior to the incompatible species, such as inoculation with *M. arenaria* or *M. hapla* prior to *M. incognita* race 1 on tobacco cv. NC95 (11) or *M. hapla* prior to *M. incognita* race 3 on *Mi*-resistant tomato cv. Celebrity (31), using the split-root technique. In an experiment supplementary to the current study, no indication of such a synergistic effect was obtained, because no reproduction was observed with mixtures of *M. fallax* and *M. chitwoodi* isolates as inoculum on *S. bulbocastanum* 93-60-2 (data not shown). To test whether the *M. chitwoodi* contamination in Fc originated from a host race 3 isolate, this contamination was isolated and tested on *S. bulbocastanum* 93-60-2 and SB22. No reproduction was observed (data not shown), indicating the *M. chitwoodi* contamination does not belong to host race 3. Moreover, the observations for Fc on *S. bulbocastanum* 93-60-2 in the current paper were not repeatable. Despite the fact that *M. chitwoodi* contaminants did reproduce on *S. bulbocastanum* and *S. hougasii*, we conclude that there is little or no variation in the *M. chitwoodi* isolates.

Differences in variation in virulence can be due either to different rates of virulent and avirulent nematodes within a population, to dosage effects of avirulence genes, or to both. Based on experiments with the mono-female lines, we concluded that genetic variation in pathogenicity is present in the original isolates

TABLE 9. Analyses of deviance for linear regression of egg-mass counts for *Meloidogyne chitwoodi* and *M. fallax* isolates inoculated on *Solanum chacoense*, *S. stoloniferum*, and *S. tuberosum*

Source	df	Deviance	Mean deviance	Deviance ratio ^z
<i>M. chitwoodi</i>				
Replication	4	157	39	2.71*
Isolates	6	257	43	2.96*
<i>Solanum</i> spp.	2	4,459	2,230	154.05***
Residual	92	1,331	14	
Total	104	6,204	60	
<i>M. fallax</i>				
Replication	4	91	23	3.11*
Isolates	2	25	12	1.70 ns
<i>Solanum</i> spp.	2	2,919	1,460	199.53***
Residual	36	263	7	
Total	44	3,298	75	

^z * and *** indicate deviance ratios are significant at $P < 0.05$ and $P < 0.001$, respectively, and ns indicates not significant, using the *F* distribution.

(e.g., Cb), because significant differences exist between these lines. These differences show that a meiotic parthenogenetic *Meloidogyne* population consists of genotypes with different pathogenicity. For breeding purposes, the most pathogenic lines should be chosen, which possibly could be homozygous for virulence gene(s).

Selection for virulence. Several authors have reported selection for virulence under laboratory conditions (4,20,29,32,38,41). In all of these studies, virulent isolates were obtained after several successive generations of selection for virulence originating from avirulent isolates. This laboratory-selected virulence is sometimes confusingly referred to as "B-race" (32). Despite the laboratory-selected virulence of the pathogen, the *Mi*-gene in tomato was effective against *M. incognita*, *M. javanica*, and *M. arenaria* from the time of its introgression from *Lycopersicon peruvianum* to cultivated tomato, until recently (35).

Selection for virulence under natural conditions has been demonstrated, for example, in *M. incognita*, *M. arenaria*, and *M. javanica* on cultivars of groundnut (*Arachis hypogaea*), cotton (*Gossypium hirsutum*), strawberry (*Fragaria × ananassa*), and sweet pepper (*Capsicum frutescens*), as discussed by Netscher (30). In the current study, the striking differences between isolates from identical field populations indicates that large genetic variation in virulence can be expected within one field population. Divergence between two isolates from the same field population may be explained by selection of different genotypes during multiplication, beginning with a mixture of genotypes for virulence. Also, contamination of *M. hapla* isolates with other isolates of *M. hapla* cannot be excluded, because no routine detection is currently available to discriminate between isolates of the same species. In contrast, no variation in virulence was found in isolate Ha when studying five mono-female lines, because no mono-female line-

TABLE 10. Analyses of deviance for loglinear regression of *Pf/Pi* (final population divided by initial population) of *Meloidogyne chitwoodi* and *M. fallax* isolates inoculated on genotypes of *Solanum chacoense*, *S. stoloniferum*, and *S. tuberosum*

Source	df	Deviance	Mean deviance	Deviance ratio ^z
<i>M. chitwoodi</i>				
Replication	4	269	67	6.06***
Isolates	6	497	83	7.45***
<i>Solanum</i> spp.	2	1,859	930	83.67***
Residual	88	978	11	
Total	100	3,603	36	
<i>M. fallax</i>				
Replication	4	106	26	7.04***
Isolates	2	47	23	6.21***
<i>Solanum</i> spp.	2	631	315	84.02***
Residual	34	128	4	
Total	44	911	22	

^z *** indicates deviance ratio is significant at $P < 0.001$, using the *F* distribution.

TABLE 11. Analysis of deviance for linear logistic regression of egg-mass counts of *Meloidogyne chitwoodi* isolate Cb and mono-female lines from isolate Cb inoculated on *Solanum chacoense*, *S. stoloniferum*, and *S. tuberosum*

Source	df	Deviance	Mean deviance	Deviance ratio ^z
Replication	4	59	15	2.66**
Lines	6	123	20	3.73**
<i>Solanum</i> spp.	2	3,266	1,633	296.95***
Residual	86	4,73	6	
Total	98	3,921	40	

^z ** and *** indicate deviance ratios are significant at $P < 0.01$ and $P < 0.001$, respectively, using the *F* distribution.

Solanum sp. interaction was observed, which indicates the presence of only one common virulence factor. Apparently field populations of *M. hapla* race A may reflect a large diversity in virulence, but once in captivity, a tendency toward homogeneity in virulence can occur due to uniformity in host and environmental conditions. Despite the lack of variation in virulence within isolate Ha, significant differences in mean numbers of egg masses between the mono-female lines were observed. The virulence pattern of Ha was comparable in the two experiments with *M. hapla* isolates, indicating the differences between the lines were caused by genetic differences and were not the result of large environmental effects. The occurrence of more than one virulence factor in a field population should discourage the use of such heterogeneous populations in resistance tests and argues in favor of the use of more homogenous isolates, such as mono-female isolates.

Contamination. For proper testing of isolates, their purity should be guaranteed. One of the factors that influences isolate purity is contamination with nematodes from other *Meloidogyne* species. Because we used precise methods to test for this kind of contamination, we could identify the few isolates that were contaminated with small numbers of other nematodes. Deviations from consistency in resistance level seemed to be due to contamination, as for isolates Ck and Fc on *S. stoloniferum* and isolate Fc on *S. bulbocastanum*. There are several possible reasons for the contamination of *M. fallax*, varying from mixed field populations to cross-infection during the maintenance program or inoculum preparation. We concluded that despite serious preventive precautions a constant risk for contamination is present in a program using a range of isolates for resistance testing. These mixed-species isolates hinder the interpretation of the results. Only identification of reproduced juveniles, as for isolates Fc and Fd on *S. bulbocastanum* and isolate Fc on *S. hougasii*, can limit misinterpretations. These results stress the importance of making every effort to avoid working with contaminated isolates and emphasize the importance of testing for possible contamination. Until now, only contamination with different species could be detected. The degree of genetic homogeneity within a given isolate is unknown.

Resistance. In this paper, *M. hapla* response varied from total absence to high mean numbers of egg masses on a host genotype. On average, *S. sparsipilum* was significantly more susceptible than the other two *Solanum* genotypes tested, showing more highly compatible combinations with isolates. The three *Solanum* genotypes were dissimilar, suggesting differences in genetic control of resistance to the tested isolates.

The significant difference in reproductive ability between *M. chitwoodi* and *M. fallax* supports the species status of *M. fallax*, which only recently has been recognized as distinctly different from the related *M. chitwoodi* (22). *M. fallax* appeared to be less virulent than *M. chitwoodi* on the tested genotypes of *S. chacoense* and *S. stoloniferum*, but both nematode species were equally virulent on *S. bulbocastanum*, *S. hougasii*, and *S. tuberosum* cv. Nicola. In general, resistance of *Solanum* spp. is effective against both *M. chitwoodi* and *M. fallax*, as was shown by Janssen et al. (18) in resistance trials testing 64 *Solanum* spp.: only a few genotypes of *S. chacoense* and *S. stoloniferum* (the same genotypes used in this study) were differentiated between these *Meloidogyne* spp. The current study specified the consistency of this differentiation. For practical breeding purposes, however, it is important to use sources for resistance that are effective for both nematode species, because both species are allopatric.

On susceptible *S. tuberosum* cv. Nicola, mean numbers of egg masses were similar for *M. chitwoodi* and *M. fallax*, showing high levels of virulence. However, the mean value of Pf/Pi for *M. fallax* was 50% lower than for *M. chitwoodi*. This phenomenon was observed in other experiments as well (44) and was presumed to be due to a shorter life cycle for *M. fallax*, for which at the time of egg extraction many J2 had already hatched and left the egg

mass. This difference in life cycle was confirmed by M. M. A. Khan (*personal communication*), who studied the development of these two species in potato and maize. The current results emphasize that when measuring reproduction at the interspecific level, Pf/Pi values estimated on egg or J2 counts from extraction with NaOCl might not be representative when life cycles differ between tested species. Observations of numbers of egg masses, therefore, would be much more reliable than numbers of Pf/Pi in such situations.

In conclusion, when testing resistance with isolates of *M. hapla*, attention should be paid to the variation in virulence to different *Solanum* genotypes. Screening for resistance should include testing against a range of isolates that represent the variation in virulence in the species. Furthermore, the efficacy of resistance breeding depends on the durability of the resistance genes. The presence of various genetic factors for virulence amongst *M. hapla* isolates and for resistance in the tested *Solanum* genotypes stresses the need for genetic analyses. The studied genotypes of *S. bulbocastanum* and *S. hougasii* showed high levels of broad-spectrum resistance to the root-knot nematode species *M. chitwoodi* and *M. fallax*. Additionally, high levels of resistance were demonstrated toward *M. fallax* by the genotypes of *S. chacoense* and *S. stoloniferum*. These results provide promising breeding perspectives. Intraspecific specialization in *M. chitwoodi* and *M. fallax* seems lower and less pronounced and, thus, much easier to handle in breeding programs compared to *M. hapla*. However, the virulence of *M. chitwoodi* juveniles in isolate Fc on *S. bulbocastanum* and *S. hougasii* raised overly optimistic expectations because the spread of this and possibly other virulent isolates or races in the Netherlands, as well as the effect of mixtures of species, are not yet known. Because ample knowledge of the durability of resistance in *S. bulbocastanum* and *S. hougasii* is still lacking, incomplete resistance like that in *S. stoloniferum* to *M. chitwoodi* should not be discarded.

ACKNOWLEDGMENTS

This research was supported by EC project FAIR1-CT95-0896. We thank the Plant Protection Service, Wageningen, the Netherlands; the Research Station for Arable Farming and Field Production of Vegetables, Lelystad, the Netherlands; the Hilbrands Laboratory for Soilborne Diseases and Pests, Assen, the Netherlands; the Laboratory for Soil and Crop Testing, Oosterbeek, the Netherlands; Washington State University, Department of Plant Pathology, Prosser; and the Institut National de Recherche Agronomique, Montpellier, France, for providing nematode isolates. We also thank A. van Norel for technical assistance, P. F. G. Vereijken for statistical advice and analyses, and R. F. Hoekstra, J. Hoogendoorn, P. W. Th. Maas, and C. H. van Silfhout for critical reading of the manuscript.

LITERATURE CITED

1. Austin, S., Pohlman, J. D., Brown, C. R., Mojtahedi, H., Santo, G. S., Douches, D. S., and Helgeson, J. P. 1993. Interspecific somatic hybridization between *Solanum tuberosum* L. and *S. bulbocastanum* Dun. as a means of transferring nematode resistance. *Am. Potato J.* 70:485-495.
2. Baker, R. J., Clarke, M. R. B., and Lane, P. W. 1985. Zero entries in contingency tables. *Comput. Stat. Data Anal.* 3:33-45.
3. Been, T. H., Meijer, E. M. J., Benier, J. E., and Knol, J. B. 1996. Using image analysis for counting larvae of potato cyst nematodes (*Globodera* spec.). *Fundam. Appl. Nematol.* 19:297-304.
4. Bost, S. C., and Triantaphyllou, A. C. 1982. Genetic basis of the epidemiologic effects of resistance to *Meloidogyne incognita* in the tomato cultivar Small Fry. *J. Nematol.* 14:540-544.
5. Brown, C. R., Mojtahedi, H., and Santo, G. S. 1989. Comparison of reproductive efficiency of *Meloidogyne chitwoodi* on *Solanum bulbocastanum* in soil and in vitro tests. *Plant Dis.* 73:957-959.
6. Brown, C. R., Mojtahedi, H., and Santo, G. S. 1991. Resistance to Columbia root-knot nematode in *Solanum* spp. and in hybrids of *S. hougasii* with tetraploid cultivated potato. *Am. Potato J.* 68:445-452.
7. Brown, C. R., Mojtahedi, H., Santo, G. S., and Austin-Phillips, S. 1994. Enhancing resistance to root-knot nematodes derived from wild *Solanum*

- species in potato germplasm. Pages 426-438 in: *Advances in Potato Pest Biology and Management*. G. W. Zehnder, M. L. Powelson, R. K. Jansson, and K. V. Raman, eds. The American Phytopathological Society, St. Paul, MN.
8. Brown, C. R., Yang, C. P., Mojtahedi, H., Santo, G. S., and Masuelli, R. 1996. RFLP analysis of resistance of Columbia root-knot nematode derived from *Solanum bulbocastanum* in a BC₂ population. *Theor. Appl. Genet.* 92:572-576.
 9. Brücher, H. 1967. Root-knot eelworm resistance in some South American tuber-forming *Solanum* species. *Am. Potato J.* 44:370-375.
 10. Dickson, D. W., and Struble, F. B. 1965. A sieving-staining technique for extraction of egg-masses of *Meloidogyne incognita* from soil. (Abstr.) *Phytopathology* 55:497.
 11. Eisenback, J. D. 1983. Loss of resistance in tobacco cultivar 'NC95' by infection of *M. arenaria* or *M. hapla*. (Abstr.) *J. Nematol.* 15:478.
 12. Esbenshade, P. R., and Triantaphyllou, A. C. 1990. Isozyme phenotypes for the identification of *Meloidogyne* species. *J. Nematol.* 22:10-15.
 13. Goplen, B. P., Stanford, E. H., and Allen, M. W. 1959. Demonstration of physiological races within three root-knot nematode species attacking alfalfa. *Phytopathology* 49:653-656.
 14. Griffin, G. D., and McKenry, M. V. 1989. Susceptibility of Nevada Synthetic XX germplasm to a California race of *Meloidogyne hapla*. *J. Nematol.* 21:292-293.
 15. Hoyman, W. G. 1974. Reaction of *Solanum tuberosum* and *Solanum* species to *Meloidogyne hapla*. *Am. Potato J.* 51:281-286.
 16. Hussey, R. S., and Barker, K. R. 1973. A comparison of methods of collecting inocula of *Meloidogyne* spp. including a new technique. *Plant Dis. Rep.* 57:1025-1028.
 17. Janssen, G. J. W., Van Norel, A., Verkerk-Bakker, B., and Janssen, R. 1995. Detecting resistance to the root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi* in potato and wild *Solanum* spp. *Potato Res.* 38:353-362.
 18. Janssen, G. J. W., Van Norel, A., Verkerk-Bakker, B., and Janssen, R. 1996. Resistance to *Meloidogyne chitwoodi*, *M. fallax* and *M. hapla* in wild tuber-bearing *Solanum* spp. *Euphytica* 92:287-294.
 19. Janssen, G. J. W., Van Norel, A., Verkerk-Bakker, B., and Janssen, R. 1997. Intra- and interspecific variation in root-knot nematodes, *Meloidogyne* spp., for resistance in wild tuber-bearing *Solanum* species. *Fund. Appl. Nematol.* 20:449-457.
 20. Jarquin-Barberena, H., Dalmasso, A., de Guiran, G., and Cardin, M. C. 1991. Acquired virulence in the plant-parasitic nematode *Meloidogyne incognita*. 1. Biological analysis of the phenomenon. *Rev. Nematol.* 14:214-219.
 21. Jatala, P., and Bridge, J. 1990. Nematode parasites of root and tuber crops. Pages 137-180 in: *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*. M. Luc, R. A. Sikora, and J. Bridge, eds. CAB International, Wallingford, England, U.K.
 22. Karssen, G. 1996. Description of *Meloidogyne fallax* n. sp. (Nematoda: Heteroderidae), a root-knot nematode from the Netherlands. *Fundam. Appl. Nematol.* 19:593-599.
 23. Karssen, G., van Hoenselaar, T., Verkerk-Bakker, B., and Janssen, R. 1995. Species identification of cyst and root-knot nematodes from potato by electrophoresis of individual females. *Electrophoresis* 16:105-109.
 24. McCullagh, P., and Nelder, J. A. 1989. *Generalized Linear Models*. 2nd ed. Chapman & Hall, London.
 25. Mercer, C. F., and Grant, J. L. 1993. Reproduction of *Meloidogyne hapla* and *Heterodera trifolii* from several sites in New Zealand on resistant and susceptible lines of white clover. *Nematologica* 39:312-321.
 26. Mojtahedi, H., Santo, G. S., Brown, C. R., Ferris, H., and Williamson, V. 1994. A new host race of *Meloidogyne chitwoodi* from California. *Plant Dis.* 78:1010.
 27. Mojtahedi, H., Santo, G. S., and Wilson, J. H. 1988. Host tests to differentiate *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla*. *J. Nematol.* 20:468-473.
 28. Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.
 29. Netscher, C. 1977. Observations and preliminary studies on the occurrence of resistance breaking biotypes of *Meloidogyne* spp. on tomato. *Cah. ORSTOM Ser. Biol.* 11:173-176.
 30. Netscher, C. 1978. Morphological and Physiological Variability of Species of *Meloidogyne* in West Africa and Implications of Their Control. Veenman, Wageningen, Netherlands.
 31. Ogalllo, J. L., and McClure, M. A. 1996. Systemic acquired resistance and susceptibility to root-knot nematodes in tomato. *Phytopathology* 86:498-501.
 32. Riggs, R. D., and Winstead, N. N. 1959. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. *Phytopathology* 49:716-724.
 33. Roberts, P. A. 1992. Current status of the availability, development and use of plant resistance to nematodes. *J. Nematol.* 24:213-227.
 34. Roberts, P. A. 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host plant resistance. *Annu. Rev. Phytopathol.* 33:199-221.
 35. Roberts, P. A., Dalmasso, A., Cap, G. B., and Castagnone-Sereno, P. 1990. Resistance in *Lycopersicon peruvianum* to isolates of *Mi* gene-compatible *Meloidogyne* populations. *J. Nematol.* 22:585-589.
 36. Santo, G. S., and Pinkerton, J. N. 1985. A second race of *Meloidogyne chitwoodi* discovered in Washington State. *Plant Dis.* 69:361.
 37. Seinhorst, J. W. 1988. The Estimation of Densities of Nematode Populations in Soil and Plants. *Sveriges Lantbruksuniversitet, Uppsala, Sweden*.
 38. Stephan, Z. A. 1982. The influence of temperature and storage time on eggs of four species of *Meloidogyne*. *Nematol. Mediter.* 10:167-173.
 39. Triantaphyllou, A. C. 1966. Polyploidy and reproductive patterns in the root-knot nematode *Meloidogyne hapla*. *J. Morphol.* 118:403-414.
 40. Triantaphyllou, A. C. 1984. Polyploidy in meiotic parthenogenetic populations of *Meloidogyne hapla* and a mechanism of conversion to diploidy. *Rev. Nematol.* 7:65-72.
 41. Triantaphyllou, A. C., and Sasser, J. N. 1960. Variation in perineal patterns and host specificity of *Meloidogyne incognita*. *Phytopathology* 50:724-735.
 42. Van der Beek, J. G. 1997. Interaction between root-knot nematodes and *Solanum* spp. Variation in pathogenicity, cytology, proteins and DNA. Ph.D. thesis. Agricultural University Wageningen, Wageningen, Netherlands.
 43. Van der Beek, J. G., Veldhuis, W. B. J., Zijlstra, C., and Van Silfhout, C. H. 1996. Preservation of *Meloidogyne hapla* and *M. chitwoodi* in liquid nitrogen: Differences in response between populations. *Fundam. Appl. Nematol.* 19:227-234.
 44. Van der Beek, J. G., Vereijken, P. F. G., Poleij, L. M., and Van Silfhout, C. M. 1998. Isolate-by-cultivar interaction in root-knot nematodes *Meloidogyne hapla*, *M. chitwoodi* and *M. fallax* on potato. *Can. J. Bot.* 76:75-82.
 45. Welham, S. J., and Thompson, R. 1997. Likelihood ratio tests for fixed model terms using residual maximum likelihood. *J. R. Stat. Soc. B* 59:701-714.
 46. Zijlstra, C., Lever, A. E. M., Uenk, B. J., and Van Silfhout, C. H. 1995. Differences between ITS regions of isolates of the root-knot nematodes *Meloidogyne hapla* and *M. chitwoodi*. *Phytopathology* 85:1231-1237.
 47. Zijlstra, C., Uenk, B. J., and Van Silfhout, C. H. 1997. A reliable, precise method to differentiate species of root-knot nematodes in mixtures on the basis of ITS-RFLPs. *Fundam. Appl. Nematol.* 20:59-63.