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# A Pathotype System to Describe Intraspecific Variation in Pathogenicity of *Meloidogyne chitwoodi*[1]

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*Abstract:* Tests of eight Dutch *Meloidogyne chitwoodi* isolates to the differential set for host races 1 and 2 in *M. chitwoodi* provided no evidence for the existence of host race 2 in the Netherlands. The data showed deviations from expected reactions on the differential hosts, which raised doubts of the usefulness of the host race classification in *M. chitwoodi*. The term ''pathotype'' is proposed for groups of isolates of one *Meloidogyne* sp. that exhibit the same level of pathogenicity on genotypes of one host species. We recommend that the pathotype classification be applied in pathogen-host relationships when several genotypes of a *Meloidogyne* sp. are tested on several genotypes of one host species. Three pathotypes of *M. chitwoodi* were identified on *Solanum bulbocastanum,* suggesting at least two different genetic factors for virulence and resistance in the pathogen and the host species, respectively. The occurrence of several virulence factors in *M. chitwoodi* will complicate the successful application of resistance factors from *S. bulbocastanum* for developing resistant potato cultivars.

*Key words:* biotype, *Daucus carota,* host race, intraspecific variation, *Medicago sativa, Meloidogyne chitwoodi,* nematode, pathogen-by-host interaction, pathotype, resistance, root-knot nematode, *Solanum bulbocastanum,* virulence.

For nematode control and risk avoidance, crop rotation with unsuitable or resistant host plants is one of the most effective environmentally safe strategies. Practical application, however, can be complicated by intraspecific variation in pathogenicity as shown in some plant-parasitic nematodes. Pathogenicity refers to the ability of nematodes to infect a plant species, reproduce on it, and cause disease symptoms. Different types of variation are denoted in such terms as forma specialist, host race, race, biotype, and pathotype.

To describe intraspecific variation in pathogenicity in *Meloidogyne* spp., two concepts are used. The best known is the host race concept, which is based on host plant differentials (Hartman and Sasser, 1985; Sasser and Triantaphyllou, 1977; Taylor and Sasser, 1978). Differential host tests are used to detect mixed nematode populations consisting of more than one species and to distinguish host races (Hartman and Sasser, 1985). The test differentiated among *M. incognita* (Kofoid & White) Chitwood host races 1, 2, 3, and 4, *M. javanica* (Treub) Chitwood (without host race specification), *M. hapla* Chitwood (without host race specification), and *M. arenaria* (Neal) Chitwood host races 1 and 2, according to their reproduction on specific cultivars of cotton, tobacco, pepper, watermelon, peanut, and tomato. More recently, a differential set was proposed for *M. chitwoodi* Golden, O'Bannon, Santo & Finley, to distinguish among host races 1, 2, and 3 with specific cultivars of carrot, alfalfa, and *Solanum bulbocastanum* Dun. (Mojtahedi et al., 1988; Mojtahedi and Santo, 1994). In nematology, the term ''host race'' is often related to intraspecific variation in nematodes to a set of plants from different host genera.

The second way of classifying intraspecific variation, by biotypes, is based on responses of populations of a *Meloidogyne* species to more than one genotype of one host plant species. Roberts (1995) applied and elaborated this concept to the *M. incognita*-tomato relationship. This concept is comparable to the physiological race concept in fungi.

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The aim of this research was to study the intraspecific variation in *M. chitwoodi* in The Netherlands. In addition, we sought to determine the utility of host race and biotype testing in describing variation in *M. chitwoodi*.

## Materials and Methods

*Nematode isolates:* A root-knot nematode isolate is a sample of a population, which is maintained in captivity. An isolate is not necessarily genetically homozygous or homogeneous but represents a group of conspecific individuals from a common geographical site. Twelve isolates of *M. chitwoodi* were used in this study (Table 1). Four of the *M. chitwoodi* isolates originated from the United States; all other isolates originated from various sites in The Netherlands. The four U.S. isolates represented the three host races: Cba (race 1), Cbd and Cbf (race 2), and Cbh (race 3), according to their reproduction on specific cultivars of three genera. The isolates were maintained and propagated on *Lycopersicon esculentum* Miller cv. Moneymaker. The specific identities of the isolates were confirmed with isozyme phenotypes for esterase and malate dehydrogenase (Esbenshade and Triantaphyllou, 1990; Karssen, 1995) and by analysis of internally transcribed spacer (ITS) regions of ribosomal DNA (Zijlstra et al., 1995).

*Plant genotypes:* Host races 1 and 2 were distinguished on *Daucus carota* L. cv. Red Cored Chantenay (susceptible to host race 1 isolates and resistant to host race 2) and *Medicago sativa* L. cv. Thor (resistant to host race 1 isolates and susceptible to host race 2) provided by H. Mojtahedi and *L. esculentum* cv. Moneymaker as a susceptible control. In a second experiment, host race 3 and nematode × host genotype interaction were studied on the same cultivars of carrot and alfalfa, and *S. bulbocastanum* Dunnett 'SB22' (provided by C.R. Brown), *S. bulbocastanum* '93-60-2', and *S. tuberosum* L. cv. Nicola as a susceptible control. The host race 3 isolate of *M. chitwoodi* reproduces well on *S. bulbocastanum* 'SB22' and reacts similarly to host race 2 isolates on the differential cultivars of carrot and alfalfa (Mojtahedi and Santo, 1994). *Solanum bulbocastanum* '93-60-2' was used in previous experiments in which it appeared to resist all tested isolates of *M. chitwoodi* and *M. fallax* (Janssen et al., 1997), except to one *M. chitwoodi* isolate (Van der Beek et al., 1998). To obtain the proper number of in vitro plants of *S. bulbocastanum,* shoots were cut every 2–3 weeks and transferred into new tubes with MS medium containing 30 g/liter sucrose (Murashige and Skoog, 1962). Three weeks after the last cut, rooted cuttings were large enough for transplantation into soil.

*Nematode infestation and experimental design:* Carrot, alfalfa, and tomato seedlings, rooted

Table 1. Isolates of *Meloidogyne chitwoodi* used for study.

| Isolate | Geographic origin | Previous host | Year of isolation | Source[a] |
|---|---|---|---|---|
| Ca | The Netherlands | Maize | 1989 | PD |
| Cb | The Netherlands | Wheat | 1990 | PD |
| Ck | The Netherlands | Tomato | 1989 | PD |
| Co | The Netherlands | Black salsify | 1993 | PAV |
| Cx | The Netherlands | Potato | 1993 | PD |
| Cy | The Netherlands | Potato | 1993 | PD |
| Cz | The Netherlands | Potato | 1993 | PD |
| Caq | The Netherlands | Potato | 1993 | PD |
| Cba | Oregon | Potato | Unknown | WSU; ORMc12, race 1 |
| Cbd | Washington | Potato | Unknown | WSU; WAMc30, race 2 |
| Cbf | Oregon | Potato | Unknown | WSU; ORMc8, race 2 |
| Cbh | California | Potato | Unknown | WSU; CAMc2, race 3 |

[a] PD: Plant Protection Service, Wageningen, The Netherlands; PAV: Applied Research for Arable Farming and Field Production of Vegetables, Lelystad, The Netherlands; WSU: Washington State University, Prosser, Washington, USA; ORMc: *M. chitwoodi* isolate from Oregon; WAMc: *M. chitwoodi* isolate from Washington; CAMc: *M. chitwoodi* isolate from California.

cuttings of the two *S. bulbocastanum* genotypes, and germinating potato tuber buds were transplanted to 350-$cm^3$ clay pots, which were filled with moist silver-sand containing 0.2% slow-release fertilizer (Osmocote Plus; NPK 15-11-13 + 2 MgO + micros) and 0.08% NPK 12-10-18, and placed in a greenhouse compartment at 20 ± 2 °C and 70% to 80% relative humidity. The transplanted cuttings were kept at approximately 100% humidity during the first 3 days under a plastic covering.

The nematode inoculum was collected from heavily galled tomato roots on trays in a mist cabinet at 20 °C with water temperature of 25 °C (Seinhorst, 1988). Hatched second-stage juveniles (J2) that settled on the bottom of the tray were collected at least every other day and kept at 4 °C. After 2 weeks, the collected J2 were poured over a cotton wool filter. Continuously stirred inoculum was applied in 9 ml suspension in a notch approximately 1 cm deep around the plant. Approximately 560 J2 (1.6 J2/ml soil) were added to each pot. Eight weeks after soil infestation, the roots were rinsed free from sand. Egg masses were stained red with phloxine-B (Dickson and Struble, 1965) and the number per plant was recorded.

Two experiments were performed. The first experiment, to test for host races 1 and 2, involved 10 *M. chitwoodi* isolates (Table 1) and three host species: carrot, alfalfa, and tomato, in 10 replicates. The second experiment, to test for host race 3 and to study nematode isolate × host genotype interactions on *S. bulbocastanum,* involved three *M. chitwoodi* isolates and the host species carrot, alfalfa, potato, and the two *S. bulbocastanum* genotypes, in 6 replicates. Both experiments were arranged in randomized complete blocks.

*Statistical analysis:* The number of egg masses was used to assess nematode reproduction. Logistic regression models were fitted with replicate, isolate, host, and the isolate × host interaction as independant variables. The dispersion of the egg mass data was assumed to be pseudobinomial, i.e. var $(Y) = n\sigma^2 \mu(1 - \mu)$, where $\mu$ is the expected proportion, n is the number of inoculated J2, and $\sigma^2$ is the dispersion parameter. Treatment effects were tested by means of *F*-statistics. Results were considered significant at $P < 0.05$. The analyses were performed with the Genstat 5 program (Genstat 5, Release 3, Clarendon Press, Oxford, UK).

## Results

Nine of the 10 *M. chitwoodi* isolates were host race 1 in the test on carrot, alfalfa, and tomato (Table 2). All isolates were pure *M. chitwoodi* isolates, and no contamination with *M. hapla* or other species was detected by rDNA analysis. Isolate Cbf, representing host race 2, produced an average of only 3.0 egg masses/plant on alfalfa. In 4 of the 10 replicates of this isolate, no egg masses were produced on alfalfa. Some other isolates also produced a few egg masses on alfalfa. In contrast, on carrot, all isolates reproduced moderately well. Also on carrot, in some replicates no egg masses developed in several isolates. The isolates failed to reproduce in 12% of 78 observations for host race 1 isolates on carrot and in 50% of 16 observations for host race 2 isolates on alfalfa. A significant isolate × host interaction occurred. This effect was mainly due to differences between the isolates on carrot and not on alfalfa. Pairwise comparison did not reveal significant differences between Cbf and any other isolate on alfalfa.

Table 2. Mean numbers of egg masses per plant produced by 10 *Meloidogyne chitwoodi* isolates 8 weeks after soil infestation with approximately 560 juveniles per plant. The two American isolates Cba and Cbf represent race 1 and race 2, respectively.

| Isolate | Carrot 'Red Cored Chantenay' | Alfalfa 'Thor' | Tomato 'Moneymaker' |
|---|---|---|---|
| Ca | 6.9 | 0.0 | 87.0 |
| Cb | 5.0 | 0.1 | 51.3 |
| Ck | 9.4 | 0.0 | 100.3 |
| Co | 7.0 | 0.0 | 38.0 |
| Cx | 5.6 | 0.1 | 77.8 |
| Cy | 6.1 | 0.3 | 54.0 |
| Cz | 15.1 | 0.0 | 67.3 |
| Caq | 9.0 | 0.0 | 64.0 |
| Cba | 19.3 | 0.0 | 24.0 |
| Cbf | 9.2 | 3.0 | 45.5 |

Data are means of 10 replicates.

In experiment 2 (Table 3) only one Dutch isolate (Ca) from experiment 1 was tested for host race 3, as all failed to reproduce on alfalfa and appeared to belong to host race 1. Host race 2 isolate Cbd produced a large number of egg masses on *S. bulbocastanum* '93-60-2' but not on *S. bulbocastanum* 'SB22'. Host race 3 isolate Cbh produced large numbers of egg masses on both *S. bulbocastanum* genotypes. All replicates of isolate Cbh reproduced well on the differential host *S. bulbocastanum* 'SB22'. The isolate X host interaction was significant.

## Discussion

There is no evidence that *M. chitwoodi* host race 2 occurs in The Netherlands, as all eight tested Dutch isolates were host race 1. The situation in The Netherlands seems different from the two other areas in the world examined for *M. chitwoodi* host race type: in the Pacific northwest of the United States, approximately 40% of 32 tested isolates were host race 2 and 60% host race 1 (Pinkerton et al., 1987); and in Tlaxcala State in Mexico, 11 of 12 *M. chitwoodi* isolates were host race 2 and only one isolate was host race 1 (Cuevas, 1995). Differences in origin of and in selection pressure by different host plant genotypes may be involved in this variation in host race type in various places in the world. Possibly the lower reproductive level of the Dutch isolates on carrot compared with the U.S. isolates would also support such a difference.

Classification of *M. chitwoodi* isolates in host races 1 and 2 was not unambiguous. The alfalfa cultivar appeared to be a poor host of the *M. chitwoodi* isolates tested. Variable numbers of egg masses of race 2 isolates on alfalfa have been explained by the cross-pollinating nature of this crop, resulting in heterogeneity for susceptibility to root-knot nematodes and possible gene shifts in the stock (Pinkerton et al., 1987). A similar explanation may be valid for carrot. Also, heterogeneity in meiotic parthenogenetic populations of *Meloidogyne* (Van der Beek, 1997) may be involved. Production of egg masses by host race 2 isolates on carrot, as was observed for isolate Cbf, was also observed by H. Mojtahedi (pers. comm.) and appeared, for as yet unknown reasons, after the isolate had been put into culture in the greenhouse. The low numbers of egg masses of host race 2 isolate Cbf on alfalfa, the recorded compatible reaction of this isolate on carrot, and the frequent failure to produce egg masses on the two hosts hamper a clear distinction between host races 1 and 2. As the differential reactions of host races 1 and 2 are not stable, conclusions on the host race status of *M. chitwoodi* isolates should be drawn cautiously.

The significant nematode isolate × host genotype interaction among the *M. chitwoodi* isolates on *S. bulbocastanum* genotypes confirmed similar observations by Brown et al. (1989). In our study, this interaction points to at least two different virulence factors in the pathogen on *S. bulbocastanum* and may indicate that several different genes for resistance to *M. chitwoodi* are present in *S. bulbocastanum*. These genes could be employed to improve the resistance of *S. tuberosum* to this nematode. However, because the corresponding virulence factors are already present in some *M. chitwoodi* isolates, the durability and usefulness of the resistance must still be assessed. In various studies, the inclusion of more isolates per host race revealed differences in nematode reproduction between isolates of one host race. For example, differences among host race 1 isolates of *M. arenaria* were found in tests on tobacco (Noe, 1992); isolate GA-7 was able to reproduce on resistant cultivars Speight G-70 and Northrup-King K326. The results of Ibrahim

Table 3. Mean numbers of egg masses per plant produced by three *Meloidogyne chitwoodi* isolates 8 weeks after soil infestation with approximately 560 juveniles on *Solanum bulbocastanum* 'SB22', *S. bulbocastanum* '93-60-2', *S. tuberosum* cv. Nicola.

| Isolate | SB22 | 93-60-2 | Nicola |
|---|---|---|---|
| Ca | 0.0 a | 0.0 a | 101.5 c |
| Cbd | 0.7 a | 31.3 b | 33.8 b |
| Cbh | 36.7 b | 40.8 b | 77.5 c |

Nematode isolate-*Solanum* genotype combinations with common letters do not differ significantly for responses on the logit scale ($P < 0.05$). Data are means of six replicates.

et al. (1993) indicate that statistical interaction exists between triticale cultivars Beagle 82 and Florida 201 and *M. arenaria* host race 2 isolates Florence and Pelion, although the authors did not mention this observation themselves.

The classification into host races, in contrast to that of biotypes, relates responses of isolates to single genotypes of different host species. Consequently, the host race concept is independent of the biotype concept and a host race can be composed of different biotypes. This is illustrated in a theoretical example (Table 4), in which host races 1 and 2 are distinguished on cultivars I of hosts X and Y. Isolates A and C, both belonging to host race 1, when tested on cultivars I of hosts X and Y, belong to different biotypes according to their response to different cultivars of hosts X and Y. This example further shows that host race classification is dependent on choice of genotypes. If cultivars II of hosts X and Y had been chosen as differential hosts to distinguish among host races, the host race classification of isolate A would have been different, due to significant isolate × host cultivar interactions, and isolate A would belong to a different host race than isolate C. In conclusion, because host race designation is only applicable when the prescribed host genotypes are used strictly, its validity is relative and classification accordingly is of arbitrary significance as soon as significant nematode isolate × host cultivar interactions occurs. The nematode isolate × host cultivar interactions, which are ignored by the host race concept, are the basis of the biotype concept. The hypothetical isolates A, B, and C would belong to three different biotypes, due to significant interaction of these three isolates with the two cultivars of host X, and the same is true in relation to host Y (Table 4).

The relative significance of the host race concept in the presence of nematode isolate × host cultivar interactions is illustrated by our results on different *S. bulbocastanum* genotypes. The compatible reaction of isolate Cbh (representing host race 3) and the incompatible reaction of isolate Cbd (originally identified as host race 2) on *S. bulbocastanum* 'SB22' confirm the original host race status of these isolates. However, if host race 3 had been defined using *S. bulbocastanum* '93-60-2' as a differential host, isolates Cbd and Cbh would both belong to host race 3. To avoid this confusion, intraspecific variation in pathogenicity with respect to one host species should be described only in terms of nematode isolate X host genotype interactions or aggressiveness.

Pathogenicity comprises aggressiveness and virulence. Aggressiveness, which is the relative degree to which a pathogen is able to reproduce and cause disease symptoms on a genotype of a host species, is quantitative in nature. Virulence, which is the ability to reproduce and cause disease symptoms on a specific host genotype, is characterized qualitatively. For *Meloidogyne,* host race and biotype are defined as follows: ''host race'' reflects the isolate's ability to reproduce on genotypes of different host plant species (Hartman and Sasser, 1985); ''biotype'' describes the isolate's ability to reproduce on different genotypes of a single host plant

TABLE 4. Theoretical example of interaction among three isolates of a hypothetical *Meloidogyne* sp. with genotypes I and II of two hypothetical host species X and Y, showing a host race classification based on cultivars I different from that based on cultivars II.

| *Meloidogyne* species isolate | Host cultivars | | | | *Meloidogyne* sp. | |
|---|---|---|---|---|---|---|
| | Species X | | Species Y | | | |
| | I | II | I | II | Host race[a] based on I | Host race based on II |
| A | + | − | − | + | 1 | 2 |
| B | − | − | + | + | 2 | 2 |
| C | + | + | − | − | 1 | 1 |

[a] Host race classification based on cultivars I and II of species A and B: host race 1 compatible with host A and incompatible with host race B; host race 2 incompatible with host race A and compatible with host race B.

TABLE 5. Pathotype designation of three *Meloidogyne chitwoodi* isolates and resistance factor designation of *Solanum bulbocastanum* '93-60-2' (Rf1) and 'SB22' (Rf2). The index of reproduction is the number of egg masses produced on the *S. bulbocastanum* genotypes as a proportion of the number produced on potato cv. Nicola (S).

| Isolate | Index of reproduction | | | Pathotype |
|---|---|---|---|---|
| | S | Rf1 | Rf2 | |
| Ca | 1.00 | 0.00 | 0.00 | *S.bul.*0/1,2[a] |
| Cbd | 1.00 | 0.93 | 0.02 | *S.bul.*1/2 |
| Cbh | 1.00 | 0.53 | 0.47 | *S.bul.*1,2/ |

[a] Numbers before the slash designate *S. bulbocastanum* resistance factors not effective against the corresponding nematode isolate; numbers following the slash are resistance factors effective against the corresponding nematode isolate.

species (Roberts, 1995; Triantaphyllou, 1987). A biotype is a collective name for isolates with similar virulence patterns. Different biotypes are distinguished by different (a)virulence reactions to host plant resistance factors.

Isolates with equivalent patterns of pathogenic variation have been described as pathotypes in various cyst nematodes: *Globodera* spp. (Kort et al., 1977), *Heterodera avenae* (Andersen and Andersen, 1982), and *H. schachtii* (Müller, 1992; Trudgill, 1986). These pathotypes fit the definition of biotype (Roberts, 1995) for *Meloidogyne* spp. Originally, biotype referred to genetically homogenous individuals (Rieger et al., 1976), and afterwards the term was used to describe various types of biological variation, as in physiology, parasitic ability, and resistance. In plant pathology the term ''pathotype'' would be more appropriate (Dropkin, 1988) and is hereby proposed to describe groups of isolates of a *Meloidogyne* sp. that show equivalent patterns of virulence on each tested genotype of a host species, regardless of their levels of aggressiveness. Andersen and Andersen (1982) and Trudgill (1986) defined pathotypes in cyst nematodes as groups of virulent genotypes in cases of known gene-for-gene relationships. Applying this definition to *Meloidogyne* spp. would require genetic studies in hybrid progenies, which are hampered by the parthenogenetic nature of many species of this genus (Roberts, 1995). In other pathogenic organisms without a known sexual phase (e.g., yellow rust), a gene-for-gene relationship can be assumed (Zadoks, 1961). Recently, however, Triantaphyllou (1993) and Van der Beek and Karssen (1997) demonstrated the potential for hybridization in meiotic parthenogenetic *Meloidogyne* spp., which, combined with molecular characterization of isolates, opens the possibility of genetic studies in intraspecific hybrid progenies.

The three pathotypes of *M. chitwoodi* on *S. bulbocastanum* (Table 5) are coded analogous to Roberts (1995). This notation clearly identifies which resistance factors in *S. bulbocastanum* are effective against the pathotype and which are not. More isolates tested can be grouped in this scheme, which can be subdivided or extended. Consequently, a set of differentiating isolates can be selected, which would contain all known variation in the species, to test for resistance in *S. bulbocastanum.*

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