Journal of Nematology 20(3):468–473. 1988.
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Host Tests to Differentiate *Meloidogyne chitwoodi* Races 1 and 2 and *M. hapla*[1]

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Abstract: The reproductive factor (R = final egg density at 55 days ÷ 5,000, initial egg density) of *Meloidogyne chitwoodi* race 2 (alfalfa race) on 46 crop cultivars ranged from 0 to 130. The reproductive efficiency of *M. chitwoodi* race 1 (non-alfalfa race) and *M. chitwoodi* race 2 was compared on selected crop cultivars. The basic difference between the two races lay in their differential reproduction on Thor alfalfa and Red Cored Chantenay carrot. *M. chitwoodi* race 2 reproduced on alfalfa but not on carrot. Conversely, alfalfa was a poor host and carrots were suitable for *M. chitwoodi* race 1. Based on host responses to *M. chitwoodi* races and *M. hapla,* a new differential host test was proposed to distinguish the common root-knot nematode species of the Pacific Northwest.

Key words: Columbia root-knot nematode, differential host test, *Meloidogyne chitwoodi, M. hapla,* northern root-knot nematode.

The Columbia root-knot nematode, *Meloidogyne chitwoodi* Golden et al., 1980, is a serious pest of potato (*Solanum tuberosum* L.) in the U.S. Pacific Northwest (10). Earlier research indicated that alfalfa (*Medicago sativa* L.) was not a suitable host to the type culture of *M. chitwoodi* (10). Later research, however, showed that certain populations of *M. chitwoodi* were cabable of reproducing on alfalfa; these were designated as the *M. chitwoodi* alfalfa race (race 2) (11). A subsequent study showed that race 2 reproduced on 54 alfalfa cultivars (5). Thus alfalfa can no longer be generally recommended as a rotation crop with potato to suppress *M. chitwoodi* populations. Studies on the distribution of this new race indicate that it is present in all the major potato growing regions of the Pacific Northwest (9); however, no differential host test was available to separate concomitant populations of *M. chitwoodi* races.

The objectives of this study were 1) to evaluate the host status of different crop cultivars to *M. chitwoodi* race 2 and 2) to develop a differential host test that can distinguish the two races of *M. chitwoodi* from each other and from *M. hapla* Chitwood, 1949, another root-knot nematode species common in the Pacific Northwest (2).

MATERIALS AND METHODS

Experiments were conducted with *Meloidogyne chitwoodi* and *M. hapla* isolates from the Irrigated Agriculture Research and Extension Center, Prosser, Washington, collection (9). The inoculum consisted of 5,000 eggs in 5 ml water pipetted around the root systems of test plants. Egg inocula were obtained from infected tomato (*Lycopersicon esculentum* Mill. cv. Columbian) roots by the NaOCl method (4).

Seeds were planted in methyl bromide-fumigated soil in cavity trays for 3 weeks before transplanting to 10-cm-d plastic pots containing methyl-bromide fumigated loamy sand (84% sand, 10% silt, 6% clay). Five replications of each plant were inoculated at the time of transplanting. Columbian tomato and pepper (*Capsicum annuum* L. cv. California Wonder) plants were included with each experiment. Columbian tomato, an excellent host for both races of *M. chitwoodi,* was used as a standard. California Wonder pepper, a nonhost for *M. chitwoodi,* was included as a check for possible *M. hapla* contamination.

Host suitability was assessed after 55 days by washing the roots free of soil, extracting the eggs (4), and calculating the reproduction factor (R = final egg density ÷ initial egg density) (8).

Host range studies: Forty-six plant culti-

Received for publication 8 October 1987.

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vars were tested with *M. chitwoodi* race 2 (ORMC8) over a 1-year period. Alfalfa (*Medicago sativa* L. cv. Thor) was included with each experiment to ascertain ability of the nematode to reproduce on alfalfa throughout the studies. When new crop cultivars were tested or the status of a plant as a host to *M. chitwoodi* race 2 was substantially different from the reported data for *M. chitwoodi* race 1 (7), the experiment was repeated and an isolate of type culture (WAMC1) was included. The experiments were conducted in a greenhouse where the temperature was 22–28 C. Based on the R values, the test plants were grouped in four host categories: R = 0–0.09, nonhost; R = 0.1–0.9, poor host; R = 1–2, moderate host; R > 2, suitable host.

Differential host tests: Ten race 2 and two race 1 populations of *M. chitwoodi* were reared on wheat (*Triticum aestivum* L. cv. Nugaines) and an isolate of *M. hapla* was reared on pepper. The populations were subsequently increased on Columbian tomato. Egg inocula obtained from tomato were introduced around carrot (*Daucus carota* L. cv. Red Cored Chantenay) roots, and reproductive factors of all isolates were determined. In a second experiment, single egg masses of selected populations of *M. chitwoodi* were obtained from wheat and reared on tomato. The egg inocula from tomato were introduced to Red Cored Chantenay carrot and a clonal Thor alfalfa, selected as susceptible to *M. chitwoodi* race 2. After 55 days, the root systems of test plants were stained with Phloxin B (1). Egg masses were counted and indexed (3), then eggs were extracted and reproductive factors were calculated.

Results and Discussion

Host range studies: M. chitwoodi race 2 successfully reproduced on tomato and alfalfa roots; the R values on these hosts in five sets of experiments were 12–134 on tomato and 1.5–11 on alfalfa. The R values for 28 of the 46 plant entries tested were 0–0.9, indicating they were nonhosts or poor hosts of *M. chitwoodi* race 2 (Table 1).

Among the nonhosts and poor hosts for race 2 were *Beta vulgaris* L. cv. U&I Hybrid No. 9 sugarbeet and Gold Pak carrot. These two plants were moderate hosts for *M. chitwoodi* race 1 in an earlier study (7). In a subsequent test with both races of *M. chitwoodi*, sugarbeet remained a poor host for *M. chitwoodi* race 2 and was also a poor host to *M. chitwoodi* race 1, contrary to an earlier report (7). The discrepancy between the two studies was due to different methods used to express nematode reproduction. In the previous report (7), the host indexing was based on the number of eggs per gram of dried root. Therefore, on a very small root system, like sugarbeet feeder roots, the actual number of eggs produced may have been inflated and the host status of sugarbeet to *M. chitwoodi* overestimated. Conversely, nematode reproduction on a large root system may be underestimated. Thus a standardized method of reporting nematode reproduction and evaluating the host status of plants is important (12).

Three carrot cultivars including Gold Pak (Table 1) again failed to support *M. chitwoodi* race 2 populations but were suitable hosts for *M. chitwoodi* race 1 (Table 2). In differential host tests, Red Cored Chantenay carrot was selected as an additional differential host to separate the two races.

Nine onion (*Alium cepa* L.) cultivars tested were nonhosts or poor hosts (R = 0–0.2) for *M. chitwoodi* race 2 (Table 1). In another test, these onion cultivars were also shown to be nonhosts or poor hosts for *M. chitwoodi* race 1 (Table 2). A similar reaction of these onions to *M. hapla* was also observed (unpubl.).

Two field corn (Idahybrid 303, Northrup King 497) and one sweet corn (Jubilee) cultivars were suitable hosts for *M. chitwoodi* race 2 (Table 1). Previously these corn varieties were reported to be poor hosts for *M. chitwoodi* race 1 (7). All three corn cultivars were retested with *M. chitwoodi* races 1 and 2. Several other sweet corn cultivars were also tested. In the second test, Jubilee sweet corn was a moderate host (R = 1.9) for *M. chitwoodi* race 1 and the two field corn cultivars were suitable hosts (Table 2) to this race, contrary to a pre-

TABLE 1. Reproductive factor (R) of *Meloidogyne chitwoodi* race 2 (alfalfa race) on several plant species.

Scientific name	Common name	Horticultural variety	R (Pf/Pi)	Host status†
Chenopodiaceae				
Beta vulgaris	Sugar beet	U&I Hybrid No. 9	0.4	PH
Cruciferae				
Raphanus sativus	Fodder radish	Nerus	0.8	PH
Brassica rapa	Turnip (Hyb.)	Forage Star	0.0	NH
Cucurbitaceae				
Citrullus vulgaris	Watermelon	Charleston Grey	< 0.1	NH
Graminae				
Avena sativa	Oat	Park	24.5	SH
Hordeum vulgare	Barley	Boyer	13.4	SH
Sorghum vulgare	Sudangrass		0.4	PH
Triticum aestivum	Wheat (spring)	Fielder	36.8	SH
	Wheat (winter)	Nugaines	17.3	SH
Zea mays	Field corn	Idahybrid 303	12.1	SH
		Northrup King 497	6.9	SH
		Pioneer 3232	8.6	SH
	Sweet corn	Candy Bar	1.7	MH
		Jubilee	5.8	SH
		Kandy Kiss	0.9	PH
		Style Pak	1.4	MH
		Style Sweet	2.1	SH
		Sweet Tooth	0.8	PH
		Sweet Treat	9.0	SH
Labiatae				
Mentha cardiaca	Spearmint	Scotch	0.0	NH
Leguminosae				
Arachis hypogaea	Peanut	Florrunner	0.0	NH
Phaseolus limensis	Lima bean	Henderson Baby Bush	0.0	NH
Phaseolus vulgaris	Snap bean	Apollo	94.4	SH
	Bean	Blue Mountain	130.8	SH
Pisum sativum	Garden pea	Dark Skin Perfection	39.5	SH
		Alaska	6.8	SH
Vigna unguiculata	Cowpea	California Black Eye No. 5	0.0	NH
Trifolium pratense	Red clover		17.8	SH
Medicago sativa	Alfalfa	Thor	6.6‡	SH
Liliaceae				
Alium cepa	Onion	Carmen	< 0.1	NH
		Cima	0.0	NH
		Granada	0.0	NH
		Magnum	< 0.1	NH
		Rocket	< 0.1	NH
		Snow White	0.2	PH
		Vega	< 0.1	NH
		Walla Walla Sweet	0.2	PH
		Yula	< 0.1	NH
Asparagus officinalis	Asparagus	Mary Washington	0.0	NH
Malvaceae				
Gossypium hirsutum	Cotton	Delta Pine 16	< 0.1	NH
Rosaceae				
Fragaria chiloensis	Strawberry	Quinault	0.0	NH
Solanaceae				
Capsicum annuum	Pepper	California Wonder	< 0.1‡	NH
Lycopersicon esculentum	Tomato	Columbian	47.3‡	SH
Solanum melongena	Eggplant	Ichiban	0.2	PH

TABLE 1. Continued.

Scientific name	Common name	Horticultural variety	R (Pf/Pi)	Host status†
Umbelliferae				
Daucus carota	Carrot	Red Cored Chantenay	< 0.1	NH
		Gold Pak	0.0	NH
		Imperator 58	< 0.1	NH

Average of five replicates.
† R = 0–0.09, nonhost (NH); R = 0.1–0.9, poor host (PH); R = 1–2, moderate host (MH); R > 2, suitable host (SH).
‡ Average of five experiments.

vious report (7). Again, the discrepancy may be due to different methods used.

Of the remaining sweet corn cultivars, three (Candy Bar, Style Pak, and Sweet Tooth) consistently supported lower reproduction (R = 0.8–1.7) of both *M. chitwoodi* races than other corn cultivars tested (Tables 1, 2). More than 40,000 acres of sweet corn are grown in Washington annually (13), and a good portion of this acreage is rotated with potato in the Columbia Basin where *M. chitwoodi* infestation is widespread. Our greenhouse observations suggest that some of the sweet corn cultivars may be useful in rotation with potato to reduce *M. chitwoodi* field population. Presently, *M. chitwoodi* races are being monitored on several sweet corn cultivars under field conditions.

Differential host test: All *M. chitwoodi* and *M. hapla* populations successfully reproduced on tomato; R values on this host were 2.6–102.8. Pepper inoculated with *M. chitwoodi* and wheat plants with *M. hapla* remained uninfected. *M. hapla* and the two *M. chitwoodi* race 1 populations (WAMC1, WAMC11) successfully reproduced on Red Cored Chantenay carrot; R values for these three populations were 54.7, 9.0, and 10.7, respectively. Conversely, reproduction of *M. chitwoodi* race 2 populations on carrot was variable. Carrot failed to sustain the

TABLE 2. Reproductive factor (R) of *M. chitwoodi* race 1 on selected crop cultivars 55 days after inoculation with 5,000 eggs.

Common name	Horticultural variety	R (Pf/Pi)	Host status†
Sugarbeet		0.2	PH
Field corn	Idahybrid 303	6.5	SH
	Northrup King 497	2.2	SH
Sweet corn	Candy Bar	1.1	MH
	Jubilee	1.9	MH
	Kandy Kiss	6.4	SH
	Style Pak	1.1	MH
	Style Sweet	2.1	SH
	Sweet Tooth	1.2	MH
	Sweet Treat	16.9	SH
Onion	Carmen	0.0	NH
	Cima	0.0	NH
	Granada	0.0	NH
	Magnum	0.03	NH
	Rocket	0.0	NH
	Snow White	0.0	NH
	Vega	0.07	NH
	Walla Walla Sweet	0.0	NH
	Yula	0.05	NH
Carrot	Red Cored Chantenay	4.4	SH
	Gold Pak	1.3	MH
	Imperator 58	11.6	SH

Average of five replicates
† R = 0–0.09, nonhost (NH); R = 0.1–0.9, poor host (PH); R = 1–2, moderate host (MH); R > 2, suitable host (SH).

TABLE 3. Reproductive factor (R) of several field isolates of *M. chitwoodi* populations and *M. hapla* on Red Cored Chantenay carrot 55 days after inoculating with 5,000 eggs.

Meloidogyne populations	Race designation†	R (Pf/Pi) on carrot‡
M. hapla		54.9
M. chitwoodi		
WAMC1	1	10.7
WAMC11	1	9.0
COMC1	2	7.2
ORMC4	2	1.1
ORMC5	2	0.05
IDMC3	2	0.05
ORMC3	2	0.04
WAMC7	2	0.01
WAMC10	2	0.01
WAMC15	2	0.01
WAMC6	2	< 0.01
ORMC8	2	< 0.01

† From Pinkerton et al. (9).
‡ Average of four replicates.

TABLE 4. Egg mass indices and reproductive factor (R) of single egg mass isolates of selected *Meloidogyne chitwoodi* populations on Thor alfalfa and Red Cored Chantenay carrot 55 days after inoculating with 5,000 eggs.

M. chitwoodi populations	Alfalfa Egg mass index†	Alfalfa Reproductive factor (Pf/Pi)	Carrot Egg mass index†	Carrot Reproductive factor (Pf/Pi)
WAMC1	0.0 b	0.0 b	4.0 a	2.2 a
ORMC4	0.0 b	0.0 b	5.0 a	1.3 a
COMC1	4.6 a	9.5 a	2.0 b	0.1 b
ORMC8	5.0 a	7.0 a	1.8 b	< 0.01 b

Average of five replicates. Averages in each column followed by the same letter are not significantly different at $P = 0.05$ according to Duncan's multiple-range test.

† Egg masses were indexed according to Hartman and Sasser (3).

initial inoculum level of eight populations (R = 0.008–0.05), but COMC1 and ORMC4, previously reported to increase on alfalfa and designated as *M. chitwoodi* race 2 (9), increased on carrots (Table 3). Single egg mass culture of these two populations along with WAMC1 (race 1) and ORMC8 (race 2) were retested on carrot and clonal Thor alfalfa. Single egg mass cultures of WAMC1 and ORMC4 again increased on carrots but failed to increase on alfalfa (Table 4). Conversely, COMC1 and ORMC8 failed to increase on carrots (R = 0–0.2) but reproduced on alfalfa (R = 3–7). These results indicate that COMC1 and ORMC4 contained both *M. chitwoodi* races. Detection of these mixed races of *M. chitwoodi* on alfalfa alone was not possible.

Data from this study and field trials (unpubl.) show that the differential response of Red Cored Chantenay carrot toward race 1 and race 2 of *M. chitwoodi* is consistent. Therefore, to separate *M. hapla* and the *M. chitwoodi* races, we propose to add Red Cored Chantenay carrot and Thor alfalfa to the differential host list reported by Nyczepir et al. (6). These differential hosts—Nugaines wheat, California Wonder pepper, Thor alfalfa, Red Cored Chantenay carrot and Columbian tomato—are derived from seeds and are easy to grow in a greenhouse. Furthermore, reaction of *M. hapla* and *M. chitwoodi* races on these

TABLE 5. Response of differential hosts to *Meloidogyne hapla* and *M. chitwoodi* races.

	Differential host reactions				
	Wheat	Pepper	Alfalfa	Carrot	Tomato
M. hapla	−	+	+	+	+
M. chitwoodi					
Race 1	+	−	−	+	+
Race 2	+	−	+	−	+

Wheat, Nugaines; pepper, California Wonder; alfalfa, Thor; carrot, Red Cored Chantenay; tomato, Columbian. + = the ability of root-knot nematode species to maintain a reproductive factor > 1 by 55 days.

plants (Table 5) is distinct and dependable. Thus this test, along with salient morphological characters of these nematode species (6), will be useful (3) for identification of the different species and races, especially within concomitant populations.

To separate *M. hapla* and *M. chitwoodi* races from the three major root-knot nematode species (*M. incognita, M. javanica,* and *M. arenaria*), *Citrullus vulgaris* Schrad cv. Charleston Grey watermelon, a nonhost to *M. hapla* (2) and *M. chitwoodi* races (7), can be added to the differential host list (6).

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