Host Status of Alfalfa Cultivars and Germ Plasms to *Meloidogyne chitwoodi* Race 2 and Reactions of Selected Cultivars to *M. chitwoodi* and *M. hapla* Infection

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ABSTRACT

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The reproductive efficiency of *Meloidogyne chitwoodi* race 2 was evaluated on 50 alfalfa cultivars and germ plasms. The reproductive factor (final egg density at 55 days ÷ 5,000 [initial egg density]) ranged from 1.1 to 40. An experimental line, W12SR2W1, consistently exhibited a high degree of resistance to *M. chitwoodi* race 2. Reproduction of *M. chitwoodi* race 2 and *M. hapla* on W12SR2W1 was between alfalfa cultivars Lahontan and Nevada Synthetic XX, susceptible and resistant to *M. hapla*, respectively. The pathogenicity of *M. chitwoodi* race 2 and *M. hapla* on selected commercially grown alfalfa cultivars was less clear. They reduced the number of plants per pot of cv. WL-312 alfalfa without affecting its shoot or root growth and temporarily reduced the shoot dry weight of cv. Vernal alfalfa. *M. chitwoodi* race 2 increased cv. Thor alfalfa shoot and root growth and had no adverse effect except to decrease leaf area. *M. hapla* did not alter the shoot or root growth of Thor alfalfa.

The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al) is an important pest of potato (*Solanum tuberosum* L.) and seriously reduces tuber quality (17). Presently, two races of *M. chitwoodi* are identified (7,18). The two races are differentiated by their reproduction on cv. Thor alfalfa (*Medicago sativa* L.) and cv. Red Cored Chantenay carrot (*Daucus carota* L.). Race 1 reproduces on carrot, but reproduces very poorly or not at all on alfalfa. However, alfalfa is a suitable host for race 2, but carrot is not (11). Race 2 has been found in all of the major potato-growing regions of the Pacific Northwest (15). Alfalfa-potato rotations were originally recommended to suppress the *M. chitwoodi* population (17). However, in the presence of race 2, this practice can no longer be recommended. Also, it has been reported that *M. chitwoodi* race 2 may be pathogenic to alfalfa (6). Thus, an alfalfa cultivar with a high degree of resistance to *M. chitwoodi* race 2 would be ideal to reduce damage and suppress *M. chitwoodi* populations in the field.

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The objectives of these studies were to evaluate 1) the host status of 50 alfalfa cultivars and germ plasms to *M. chitwoodi* race 2, and 2) the reaction of three alfalfa cultivars, grown commercially in the state of Washington, to *M. chitwoodi* races. *M. hapla* Chitwood, a known parasite of alfalfa, was included for comparison.

MATERIALS AND METHODS

The nematode populations in these experiments were maintained in the Irrigated Agriculture Research and Extension Center collection (15). Those used were WAMCI (*M. chitwoodi* race 1), ORMC8 (*M. chitwoodi* race 2), and *M. hapla*. Single egg masses of *M. chitwoodi* from cv. Nugaines wheat (*Triticum aestivum* L.) and *M. hapla* from cv. California Wonder pepper (*Capsicum annuum* L.) were increased on cv. Columbian tomato (*Lycopersicon esculentum* Mill.). The *M. chitwoodi* races 1 and 2 were further tested for purity on alfalfa and carrot, respectively. The inocula consisted of eggs collected after shaking tomato roots in 0.5% NaOCl (9).

The alfalfa seeds were water-soaked and incubated at 24 C for 2 days to germinate. The germinated seeds were planted in either 10-cm-diameter plastic pots (holding 625 cm^3 of soil) or Cone-tainers (a plastic cone tapering from 3.75 cm at the top to a blunt point at the bottom) (Ray Leach Cone-tainer Nursery, Canby, OR) containing methyl bromide-fumigated greenhouse-mix soil (84% sand, 10% silt, and 6% clay). At harvest, the eggs were extracted from roots by washing them free of soil and shaking in 0.5% NaOCl. Columbian tomato and California Wonder pepper were included in all tests. Tomato, an excellent host for both *M. chitwoodi* and *M. hapla*, was used as a standard. Pepper, a nonhost for *M. chitwoodi*, was included as a check against impure inocula and contamination with *M. hapla* during the experiment. Nugaines wheat, a nonhost to *M. hapla*, was used for the same reason whenever *M. hapla* was tested. These tests were conducted over a 2-yr period in a greenhouse where temperatures ranged from 20 to 26 C.

Host status of alfalfa cultivars. The host status of 50 alfalfa cultivars and germ plasms to *M. chitwoodi* race 2 was determined in four sets of experiments over a 1-yr period. The plant entries included representatives of eight fall dormancy groups (1).

Ten seedlings per pot were grown for 3 wk before the soil was infested with an initial population (P_i) of 5,000 eggs in 5 ml of water. The egg inoculum was delivered to 5-mm-deep grooves made in the soil surface. It was covered with soil and the pots were watered. Test plants in each set were arranged in randomized complete blocks with five replications. After 55 days, the nematode eggs were extracted to determine the final population (P_f) and reproductive factor ($R = P_f/P_i$) (14). When the R value for any alfalfa was lower than 2, the experiment was repeated.

An experimental line of alfalfa (W12SR2W1) that repeatedly had an R value of less than 2 was tested further. One hundred individual seedlings in pots were inoculated with 5,000 eggs and maintained for 55 days before the roots were washed free of soil and stained with phloxine B (4). The number of egg masses was counted under a dissecting microscope and an egg mass index (EI) was determined (8). Eggs were then extracted and the R factor was calculated. The individual plant was rated a nonhost (immune) when EI and R were zero; a poor host (resistant) when EI = 1–3 and $R < 1$; and a suitable host (susceptible) when EI = 4–5 and $R > 1$.

In addition, W12SR2W1 alfalfa was further tested with *M. chitwoodi* races 1 and 2 and *M. hapla* to ascertain its host status to all common root-knot nematode species and races in the region. Nevada Synthetic XX (Nev Syn XX), a resistant germ plasm, and Lahontan, a cultivar

susceptible to *M. hapla*, were included for comparison (5). One hundred twenty pregerminated seeds of each alfalfa cultivar were individually planted in Cone-tainers and inoculated with 5,000 eggs. The three alfalfa entries per nematode population were arranged in four randomized complete blocks with 30 plants per block. Egg mass index and R values were determined as previously described.

Table 1. Reproductive factor ($R = P_f/P_i$)[u] of *Meloidogyne chitwoodi* race 2 on different alfalfa cultivars 55 days after inoculation with 5,000 eggs (P_i)[v]

Cultivar or germ plasm	Dormancy class[w]	Mean R ± SE[x]
Apollo II	4	40.5 ± 6.2
CW 223	4	39.4 ± 9.1
Pacer	3	33.2 ± 2.5
Vertus	4	32.6 ± 6.9
W38[y]	4	32.0 ± 4.9
Trumpetor	4	31.5 ± 4.4
Oneida	3	31.0 ± 6.5
Lahontan	6	29.7 ± 2.9
Anchor	3	28.7 ± 2.7
Dupuit	4	28.5 ± 2.7
Washoe	5	28.6 ± 3.9
Maxim	4	28.4 ± 5.5
Cimarron	4	28.3 ± 5.3
Eagle	4	26.8 ± 2.3
Maris Kabul	4	26.5 ± 3.3
Washoe (certified)	5	26.7 ± 6.2
Vernema (certified)	4	26.3 ± 7.6
Phytor	3	25.9 ± 2.3
Thor	5	24.8 ± 2.6
Excalibur	4	23.5 ± 4.2
Syn VV[y]	5	22.9 ± 3.0
Vernema (foundation)	4	22.7 ± 6.6
Vancor	2	21.0 ± 6.3
Blazer	3	20.8 ± 4.6
Vernal	2	20.4 ± 3.2
Epic	4	19.5 ± 3.9
Granada	8	19.5 ± 4.3
Action brand	4	18.7 ± 2.4
Moapa 69	7	17.7 ± 3.8
Ranger	3	16.8 ± 6.9
Vernema (breeder)	4	14.8 ± 6.2
Agate	2	11.1 ± 3.4
Arc	4	10.6 ± 2.9
Atra 55	4	10.5 ± 1.5
W-39[y]	4	9.9 ± 2.5
W-37[y]	5	9.4 ± 2.0
DK 135	4	8.5 ± 2.0
WL-312	4	8.5 ± 2.2
W-47[y]	4	8.4 ± 2.8
W-42S$_1$[y]	4	8.0 ± 0.6
Ramsey	2	7.8 ± 3.0
W9SR2W1[y]	4	7.3 ± 1.1
WL-316	4	7.2 ± 1.2
Iroquois	2	6.7 ± 2.7
W45[y]	4	6.9 ± 0.8
Spredor II	1	6.6 ± 3.2
UC Salton	8	5.6 ± 0.8
CUF 101	8	5.2 ± 1.3
Nev Syn XX[y]	5	4.7 ± 1.6
W12SR2W1[y]	4	1.1 ± 0.3[z] 1.6 + 0.4[z]

[u] P_i = initial population; P_f = final population.
[v] Values are means of five replicates. Each replicate consisted of 10 3-wk-old seedlings at the time of inoculation.
[w] 1 = Most dormant, 8 = nondormant type (1).
[x] SE = standard error.
[y] Experimental lines.
[z] Two repeated experiments on W12SR2W1.

Pathogenicity tests. The pathogenicity of *M. chitwoodi* races 1 and 2 and *M. hapla* to Thor and that of race 2 and *M. hapla* to cultivars Vernal and WL-312 was evaluated. Nine to 10 pregerminated alfalfa seeds were planted in plastic pots. The soil was infested by delivering 10^4 eggs in 5 ml of water to the soil surface of pots as previously described. The experiments were arranged in 10 randomized complete blocks on a greenhouse bench. The shoots were periodically harvested and stand counts were determined before the roots were destructively sampled to extract the eggs. The shoots and roots were dried at 60 C for 48 hr and were weighed. The treatment variances were calculated and means were separated according to Duncan's multiple range test.

In the first and second experiments, eggs of all nematode species and races were introduced around roots of Thor alfalfa seedlings and 3-wk-old plants. Pots were maintained in the greenhouse for 100 days after inoculation. The shoots were harvested 75 and 100 days after inoculation. Before the first harvest, 10 trifoliolate leaves per replicate were randomly selected and their area was measured with a Li-Cor LI-3100 leaf area meter.

In the third experiment, Vernal and WL-312 alfalfa were inoculated at seeding time with *M. chitwoodi* race 2 and *M. hapla*. Shoots were harvested, dried, and weighed at 75, 100, and 125 days after planting.

RESULTS

Host status of alfalfa. All 50 alfalfa cultivars and germ plasms supported *M. chitwoodi* race 2 with R values ranging from 1.1 to 40 (Table 1). An experimental line, W12SR2W1, had the lowest R value of 1.1. When the experiment was repeated with this alfalfa, the R value was again low at 1.6 (Table 1).

When 100 seedlings of W12SR2W1 were individually inoculated with *M. chitwoodi* race 2, the average EI and R values were 2.4 and 1.4, respectively. The three host categories of nonhost, poor host, and suitable host consisted of 19, 44, and 37 plants, respectively. Finally, *M. chitwoodi* race 1 failed to reproduce on the three alfalfa cultivars (W12SR2W1, Nev Syn XX, and Lahontan) (R = 0–0.05). *M. chitwoodi* race 2 and *M. hapla* both reproduced on Lahontan but not on resistant Nev Syn XX (Table 2). Reproduction of these two nematodes on W12SR2W1 was between susceptible Lahontan and resistant Nev Syn XX. *M. chitwoodi* races and *M. hapla* reproduced very well on tomato (R = 30–119), but their reproduction on pepper (R = 0–0.01) and wheat (R = 0–0.3) was negligible.

Pathogenicity. *M. chitwoodi* race 1 failed to increase on Thor alfalfa and did not alter the dried shoot and root weight of plants inoculated at seeding. However, it increased ($P = 0.05$) both shoot and root weights of 3-wk-old plants (Table 3). Area per trifoliolate leaf of plants inoculated with race 1 at seeding was smaller ($P = 0.05$) than that of the noninoculated plants. *M. chitwoodi* race 2, however, increased on Thor alfalfa (R = 9–39) and stimulated shoot and root growth of plants inoculated at seeding (Table 3). The area per trifoliolate leaf of these plants was also smaller than that of the control. In this experiment, *M. hapla* reproduced on Thor alfalfa (R = 55–65) without influencing plant growth.

In the third experiment, *M. hapla* reproduced (R = 37–55) on Vernal and WL-312 and reduced ($P = 0.05$) the number of plants per pot of both cultivars. Shoot growth of Vernal was also reduced ($P = 0.05$) 100 days after inoculation, but the differences at 125 days were not significant (Table 4).

Similarly, *M. chitwoodi* race 2 increased on both cultivars (R = 3–9) and temporarily reduced the shoot dry weight of Vernal alfalfa. The nematode reduced ($P = 0.05$) the stand counts of WL-312, but differences in root and shoot growth were not significant (Table 4). Unlike *M. chitwoodi* race 2, *M. hapla* induced galls, lateral root proliferation, and browning of root systems.

DISCUSSION

Alfalfa is a polyploid and is naturally cross-pollinated, which results in a heterozygous population of plants. Therefore, the disease resistance in

Table 2. Egg mass index (EI) and reproductive factor ($R = P_f/P_i$)[x] of *Meloidogyne chitwoodi* race 1 and race 2 and *M. hapla* on three alfalfa cultivars 55 days after inoculation with 5,000 eggs (P_i)[y]

Host cultivar or germ plasm	*M. chitwoodi* race 1		*M. chitwoodi* race 2		*M. hapla*	
	EI[z]	R	EI[z]	R	EI[z]	R
Lahontan	0.78 a	0.05 a	3.45 a	5.45 a	4.40 a	33.77 a
W12SR2W1	0.48 ab	0.01 a	1.95 a	1.80 b	2.58 b	12.43 b
Nevada Syn XX	0.17 b	0.00 a	0.78 c	0.21 c	0.13 c	0.00 c

[x] P_i = initial population; P_f = final population.
[y] Values are means of four replicates (30 individual plants/replicate). Values in each column followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] EI: 0 = 0, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 > 100 egg masses per seedling.

alfalfa is based on the percentage of the plant population that responds to a given pathogen. Because we were interested in determining if *M. chitwoodi* race 2 increases on a given alfalfa cultivar, we tested individual plants in only three cultivars. Therefore, it is possible that some of the cultivars carried the gene(s) for resistance to *M. chitwoodi* race 2 in spite of supporting high R values. For example, in a previous experiment consisting of 10 Nev Syn XX seedlings per pot (five replicates), the R value for *M. chitwoodi* race 2 was 4.7 (Table 1). However, when 120 individual Nev Syn XX seedlings were exposed to this nematode, 109 exhibited a resistant reaction (EI = 0–3 and R < 1) and 11 supported *M. chitwoodi* race 2 reproduction with two seedlings sustaining R > 3.

Our experimental procedure to evaluate the host suitability of alfalfa cultivars was different from the guidelines set by the Alfalfa Improvement Conference Committee on "Standard tests for characterizing disease and insect resistance of alfalfa cultivars" (5). One of the guidelines to determine host suitability of root-knot nematodes on alfalfa is the number of galls produced on roots. *M. chitwoodi* seldom induces galls on alfalfa roots (15). Therefore, the number of egg masses and number of eggs produced were used to measure host suitability (19).

Nev Syn XX and W12SR2W1 alfalfa were ranked first and second, respectively, in resistance to *M. chitwoodi* race 2. Fifty to 66% of W12SR2W1 seedlings failed to support the reproduction of both *M. hapla* and *M. chitwoodi* race 2. W12SR2W1 was screened in the Pacific Northwest for freedom from galling by *M. hapla* before the recognition of *M. chitwoodi* as an independent species. Nev Syn XX parent clones were screened in Nevada, and only those that yielded resistant progeny in tests at Prosser, WA, Corvallis, OR, and Reno, NV, were included in the first synthetic. Therefore, it is possible that W12SR2W1 was exposed to *M. chitwoodi* populations in the process of selection, and that Nev Syn XX parent selection was influenced by tests that may have contained *M. chitwoodi*. Alternatively, the gene(s) for resistance to *M. hapla* may be common (or linked) with that for *M. chitwoodi* resistance. Consistent low reproduction (R = 1.1–1.8) of *M. chitwoodi* race 2 on W12SR2W1 may be a useful characteristic and should be exploited if the cultivar is developed commercially.

W12SR2W1, derived from Beltsville 72 (Saranac AN4W2 Syn 1), was screened for, and is highly resistant (>50%) to, stem nematode (*Ditylenchus dipsaci* (Kühn) Filipjev) and bacterial wilt (*Clavibacter michiganensis* subsp. *insidiosus* Davis et al). It is resistant (31–50%) to anthracnose race 1 (*Colletotrichum trifolii* Bain & Essary), moderately resistant (14–30%) to anthracnose race 2, and has low resistance (6–13%) to Phytophthora root rot (*Phytophthora megasperma* f. sp. *medicaginis* Kwan & Erwin) and Verticillium wilt (*Verticillium albo-atrum* Reinke et Berth.) (R. N. Peaden, *unpublished data*). Verticillium wilt is a serious disease of susceptible alfalfa in the Pacific Northwest (3). Examination of western and central Alfalfa Improvement Conference and Western Canada reports of alfalfa forage yield tests suggests that Verticillium wilt in general does not seriously affect forage yield for up to 3 yr. Individual tests, location, or year may provide specific instances of earlier reduction (2). Thus, W12SR2W1 could be used in 2- to 3-yr rotations.

In these studies, *M. chitwoodi* races 1 and 2 increased Thor alfalfa shoot and root dry weight and had no adverse effect except to decrease area per leaf. *M. hapla*, a known parasite of Thor alfalfa (16), also failed to reduce root or shoot dry weights, despite good reproduction. Although *M. hapla* and *M. chitwoodi* race 2 reduced the number of WL-312 plants, and *M. hapla* reduced plant numbers of Vernal, shoot dry weights were not affected by the end of the experiment. It has been demonstrated that as stand densities decrease over time, mean plant weights increase (12).

M. hapla is generally considered a minor pest of alfalfa. It appears to be most important in early stand establishment (10) and in winter hardiness (13). Presently, we are examining the responses of nonhost and suitable host cultivars of alfalfa to *M. chitwoodi* and *M. hapla* in longer-term experiments in field microplots.

Table 3. Number of plants per pot, shoot dry weight, root dry weight, and leaf area of cultivar Thor alfalfa inoculated with 10^4 eggs of *Meloidogyne chitwoodi* race 1 and race 2 and *M. hapla* at seeding or 3 wk after seeding, and nematode reproductive factor (R) 100 days after inoculation[x]

Treatments	No. of plants	Shoot wt.[y] (g)	Root wt.[y] (g)	Area of 10 trifoliolate leaves (cm^2)	R (P_f/P_i)[z]
		Inoculated at seeding			
Control	9.0 a	3.15 b	1.06 b	16.9 a	0.0
M. chitwoodi race 1	8.8 a	3.27 ab	1.06 b	13.9 b	<0.1
M. chitwoodi race 2	8.5 a	3.78 a	1.69 a	11.4 c	9.1
M. hapla	8.9 a	3.38 ab	1.00 b	···	55.0
		Inoculated 3 wk after seeding			
Control	9.0 a	6.97 b	2.84 b	···	0.0
M. chitwoodi race 1	8.8 a	8.58 a	3.66 a	···	0.2
M. chitwoodi race 2	8.5 a	7.29 b	2.47 b	···	39.8
M. hapla	8.9 a	7.57 ab	2.68 b	···	65.0

[x] Values are means of 10 replicates. Each replicate initially consisted of nine seedlings in 625 cm^3 of soil per pot. Values in each column per inoculation date followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[y] Sum of shoot and root dry weights from harvests at 75 and 100 days after inoculation.
[z] P_i = initial population; P_f = final population.

Table 4. Number of plants per pot, shoot dry weight, and root dry weight of cultivars Vernal and WL-312 alfalfa inoculated with 10^4 eggs of *Meloidogyne chitwoodi* race 2 and *M. hapla* at seeding, and nematode reproductive factor (R) 125 days after inoculation[y]

Treatments	No. of plants	Shoot wt. (g) by days after inoculation			Root wt. (g)	R (P_f/P_i)[z]
		75	100	125		
			Vernal			
Control	9.1 a	4.23 a	2.48 a	2.30 a	2.3 a	0
M. chitwoodi race 2	8.6 ab	4.37 a	2.03 b	1.84 a	1.8 a	9.0
M. hapla	7.9 b	4.36 a	1.92 b	2.02 a	1.8 a	37.7
			WL-312			
Control	9.2 a	5.05 a	2.48 a	1.82 a	2.1 a	0
M. chitwoodi race 2	8.1 b	4.18 a	2.14 a	1.77 a	1.7 a	3.2
M. hapla	8.0 b	4.84 a	2.36 a	1.75 a	1.9 a	55.0

[y] Values are means of 10 replicates. Each replicate initially consisted of 10 seedlings in 625 cm^3 of soil per pot. Values in each column per cultivar followed by the same letter are not significantly different ($P = 0.05$) according to Duncan's multiple range test.
[z] P_i = initial population; P_f = final population.

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