Reproductive Efficiency of Pacific Northwest Populations of *Meloidogyne chitwoodi* on Alfalfa

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ABSTRACT

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Thirty-two geographic isolates of *Meloidogyne chitwoodi* were evaluated for their ability to reproduce on Thor alfalfa. Plant reactions to these populations ranged from nonhost to a suitable host based on egg mass index (EI) and reproduction factor (R). Thirteen populations that had EI >2 and R >1 were classified as the alfalfa race (race 2). *M. chitwoodi* race 2 was found in major potato production areas in the Pacific Northwest. Therefore, alfalfa rotation can no longer be generally recommended to reduce *M. chitwoodi* population densities.

The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al) and northern root-knot nematode (*M. hapla* Chitwood) are serious pests of potato (*Solanum tuberosum* L.) in the U.S. Pacific Northwest (15). *M. chitwoodi* is more widely distributed than *M. hapla* in this region, and concomitant populations are reported to be rare (10). Economic loss in potatoes is predominantly due to reduced tuber quality and is more severe with *M. chitwoodi* (13). Both species can be controlled by preplant soil fumigation or fumigant-nonfumigant nematicide combination treatments (19), and crop rotation has also been recommended to reduce root-knot nematode population densities before planting potatoes (15). Host ranges are extensive for these two species; however, their reactions differ on two major potato rotation crops, cereals and alfalfa (*Medicago sativa* L.) (3,11). *M. hapla* reproduces well on alfalfa but not cereals, whereas cereals and alfalfa are reported as good hosts and very poor to nonhost for *M. chitwoodi*, respectively. Recent research indicated that two *M. chitwoodi* populations, collected from fields previously cropped to alfalfa in the Pasco, WA, area, reproduced well on several alfalfa cultivars (17). Because alfalfa was recommended as a rotation crop when *M. chitwoodi* was present (15), a study to determine whether this *M. chitwoodi* alfalfa race was localized or widely distributed was conducted and is described herein.

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MATERIALS AND METHODS

M. chitwoodi populations were collected from potato production areas of the western United States. Two types of samples were received: soil samples from infested fields and symptomatic tubers from shipping and processing inspectors. Soil samples were processed by sieving centrifugation (8). Presence of *M. chitwoodi* was determined by tail morphology of second-stage juveniles (10). A suspension of eggs and juveniles from these samples was then poured around the root systems of wheat (*Triticum aestivum* L. 'Nugaines') plants (16) to increase the population. The tuber samples were sliced to expose root-knot females with egg masses. Perineal patterns of females were cut and examined to confirm the presence of *M. chitwoodi*. Eggs were collected by comminuting the tuber slices that contained egg masses in a blender for 30 sec, and the puree was poured around wheat roots. Plants were maintained in a greenhouse at 20–24 C for at least 4 mo to increase nematode populations. The two *M. hapla* isolates used in the study were maintained on pepper (*Capsicum annuum* L. 'California Wonder') (5).

Four plant species were used to differentiate root-knot nematode species and evaluate the reproductive potential of *M. chitwoodi* field populations on alfalfa. Thor alfalfa, which supported two *M. chitwoodi* populations in a preliminary study (17) and is a suitable host for *M. hapla*, was used. California Wonder pepper, a good *M. hapla* host and a nonhost for *M. chitwoodi*, and Nugaines wheat, a good *M. chitwoodi* host and a nonhost for *M. hapla*, were included to ascertain mixed or contaminated cultures. Tomato (*Lycopersicon esculentum* Mill. 'Columbia') is an excellent host for both species (14) and was included as a standard.

Three 3- to 4-wk-old alfalfa seedlings or one seedling of other differential host genera were planted in 10-cm-diameter clay pots containing methyl bromide-fumigated sandy loam soil (3.7% gravel, 51.6% coarse sand, 28.7% fine sand, 10% silt, and 6% clay). Eggs for differential tests were collected by washing wheat or pepper roots free of soil and shaking roots in 0.5% NaOCl (6). Five replicates of each differential host were inoculated by pipetting 5,000 eggs around the root systems. The experiment was conducted over a 7-mo period from January through July in a greenhouse where temperatures ranged from 22 to 26 C. Plants were harvested after 55 days, the root systems were carefully washed free of soil and stained with Phloxine B (1), and egg masses were counted under a dissecting microscope. Females were collected from alfalfa roots that contained egg masses, and at least 15 perineal patterns were examined to determine the *Meloidogyne* species. Eggs were extracted by shaking roots in 1.0% NaOCl (6) and counted.

The compatibility of each field population to the four plant species was evaluated by two criteria: 1) the egg mass index (EI), where a mean EI of 0 = 0, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = $>$100 egg masses per root sample; and 2) the reproduction factor (R), where R = number of eggs recovered per number of eggs inoculated after 55 days (12). A plant species was judged a host if the mean EI >2 (5) and the R >1.

RESULTS

M. chitwoodi isolates capable of reproducing on Thor alfalfa were common in the collection (Table 1). On the basis of EI and R values, 13 of the 32 populations evaluated were the alfalfa host race. This group had representatives from most potato production areas in the Pacific Northwest and one other location in the western United States (Fig. 1, Table 1). Thor alfalfa was rated an extremely poor host, EI and R between 0 and 1, or a nonhost, EI and R = 0, for 10 and nine *M. chitwoodi* populations, respectively (Table 1). WAMc1, the type culture of *M. chitwoodi* used in all

Table 1. Reactions of 32 geographic isolates of *Meloidogyne chitwoodi* and two *M. hapla* populations on Thor alfalfa and Columbia tomato

Meloidogyne sp.	Geographical region	Population code	Host: Alfalfa EI[a]	Alfalfa SE[b]	Alfalfa R[c]	Alfalfa SE	Tomato EI	Tomato SE	Tomato R	Tomato SE
M. chitwoodi	Columbia Basin, WA	WAMc1	0.9	0.4	0.4	0.3	4.9	0.1	48.3	11.3
		WAMc2	1.9	0.5	1.3	1.2	4.6	0.2	21.9	10.1
		WAMc3	0.0	0.0	0.0	0.0	4.4	0.6	11.4	3.3
		WAMc4	1.2	0.7	<0.05	···	5.0	0.0	68.8	0.0
		WAMc5	1.8	1.1	3.5	2.5	4.8	0.2	58.5	18.0
	Pasco area, WA	WAMc6	4.6	0.4	6.6	3.7	5.0	0.0	106.6	40.0
		WAMc7	3.8	0.3	3.2	1.1	4.9	0.1	47.7	9.0
		WAMc8	0.8	0.4	<0.05	···	5.0	0.0	53.4	13.0
		WAMc9	0.6	0.4	<0.05	···	5.0	0.0	149.0	26.7
		WAMc10	4.5	0.5	6.3	2.5	5.0	0.0	47.3	11.3
	Western Washington	WAMc11	0.0	0.0	0.0	0.0	4.5	0.5	24.3	11.6
		WAMc16	0.0	0.0	0.0	0.0	4.4	0.6	10.7	2.3
	Columbia Gorge, WA	WAMc12	0.4	0.4	<0.05	···	5.0	0.0	86.7	16.3
	Yakima Valley, WA	WAMc13	0.0	0.0	0.0	0.0	5.0	0.0	35.7	15.4
	Columbia River, WA	WAMc14	4.4	0.6	11.5	4.6	5.0	0.0	94.1	9.1
		WAMc15	5.0	0.0	21.7	6.1	5.0	0.0	81.7	6.3
	Columbia River, OR	ORMc1	0.0	0.0	0.0	0.0	4.6	0.4	13.6	3.7
		ORMc2	0.6	0.6	0.1	0.1	5.0	0.0	128.2	18.7
		ORMc3	4.2	0.5	13.5	5.9	5.0	0.0	117.2	9.6
		ORMc4	4.8	0.2	12.1	2.4	5.0	0.0	92.7	7.8
	Central Oregon	ORMc5	4.0	0.3	8.1	5.4	5.0	0.0	40.2	12.2
		ORMc6	0.0	0.0	0.0	0.0	5.0	0.0	62.7	19.1
		ORMc7	2.0	0.8	0.8	0.4	5.0	0.0	97.9	4.0
	Klamath Basin, OR	ORMc8	4.8	0.2	8.3	2.0	5.0	0.0	37.3	10.5
		ORMc9	4.2	0.4	2.9	0.8	5.0	0.0	75.6	12.3
		ORMc10	4.8	0.2	12.5	3.0	5.0	0.0	55.7	10.2
	Klamath Basin, CA	CAMc1	0.0	0.0	0.0	0.0	5.0	0.0	44.6	31.6
	Snake River Basin, ID	IDMc1	0.0	0.0	0.0	0.0	5.0	0.0	126.1	4.6
		IDMc2	0.0	0.0	0.0	0.0	5.0	0.0	112.4	5.3
		IDMc3	2.4	1.0	5.8	5.2	5.0	0.0	57.8	11.3
	Utah	UTMc1	1.0	0.6	0.1	0.6	5.0	0.0	127.6	33.9
	San Luis Valley, CO	COMc1	3.2	1.0	7.3	5.9	5.0	0.0	90.0	12.0
M. hapla	Utah	UTMh1	5.0	0.0	34.8	8.4	5.0	0.0	131.6	23.9
	Washington	WAMh1	5.0	0.0	9.0	3.0	5.0	0.0	68.7	22.5

[a] EI = egg mass index, where 0 = 0, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 > 100 egg masses per replicate; mean of five replicates. Geographic isolates producing a mean EI > 2 and R > 1 are considered reproductively efficient on the host.
[b] SE = standard error of the mean.
[c] R = reproduction factor: final population/initial population; mean of five replicates.

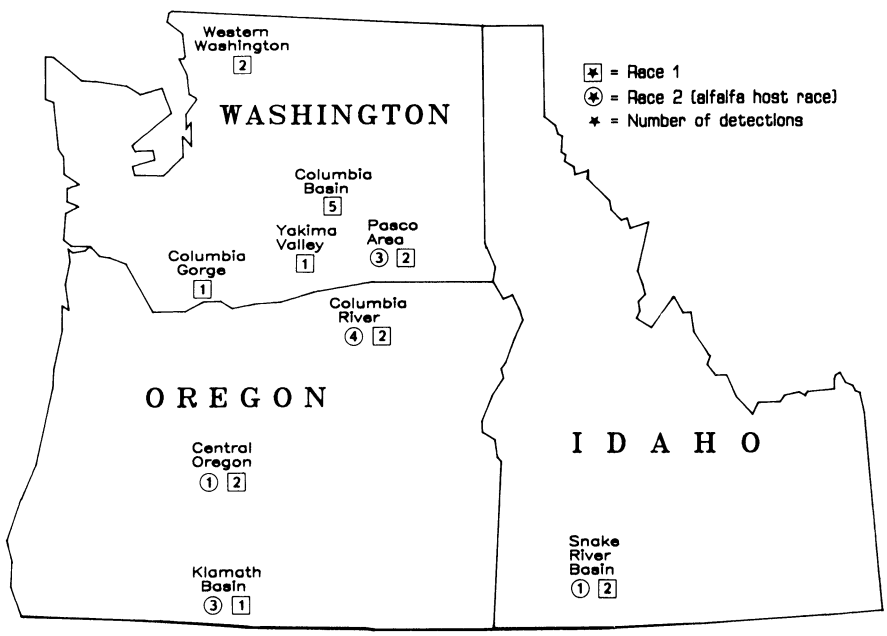

Fig. 1. Distribution of *Meloidogyne chitwoodi* races 1 and 2 in the Pacific Northwest.

previous host range tests, was used in three tests. It reproduced poorly in all trials, with a mean EI = 0.9 and R = 0.4. Similarly, isolates for which Thor alfalfa was rated a nonhost were retested with consistent results. All *M. chitwoodi* populations reproduced well on Columbia tomato (Table 1) and Nugaines wheat. No reproduction was detected with any of these populations on California Wonder pepper. *M. hapla* reproduction on Columbia tomato was comparable to the *M. chitwoodi* populations (Table 1). Nugaines wheat did not support increase of *M. hapla* and reproduction was less on pepper than on Thor alfalfa, with R values on pepper of 3.5 and 7.7 for UTMh1 and WAMh1, respectively. In this experiment, seven *M. chitwoodi* alfalfa race isolates had reproduction rates similar to *M. hapla* on Thor alfalfa (Table 1). The R range for these seven isolates was 8.1–21.7, whereas the two *M. hapla* populations had values of 9.0 (WAMh1) and 34.8 (UTMh1).

Perineal patterns of females excised from alfalfa roots were typically *M. chitwoodi*, with coarse annulation and deeply sunken vulvas. *M. chitwoodi* produced swelling but no galling along the alfalfa root axis or at junctions of lateral roots (Fig. 2), whereas *M. hapla* caused galls and pronounced root proliferation.

DISCUSSION

Major *Meloidogyne* species display intraspecific pathogenic variability. This variability provided the basis for developing a host differential test that separates races within these species (5). However, field populations have not always conformed to host race reaction as defined in these differential tests, and isolates that reproduce on resistant hosts have been reported (9). Goplen et al (4), while testing 20 California populations of *M. incognita*, *M. javanica*, and *M. hapla* against five alfalfa differentials, found variability in host reaction within each species. Triantaphyllou and Sasser (20) reported that an *M. incognita* population reproduced slightly on a resistant tomato cultivar and that its reproductive rate was increased by sequential reinoculation of the progeny on the resistant cultivar. However, Netscher and Taylor (9) inoculated different *M. javanica* and *M. incognita* field populations on a resistant tomato cultivar and reported that three populations that developed egg masses in the first generation failed to reproduce when progeny were inoculated back on the resistant host, but other populations were selected for high reproduction rates.

In our study, the physiological variability in reproduction among the 32 *M. chitwoodi* populations on Thor alfalfa was great. Reproduction rates did not indicate a differential response that clearly separated two host races but rather a continuum from nonhost to suitable host. We have observed both no reproduction and good reproduction (R = 30) with different populations when alfalfa was inoculated with inoculum collected from alfalfa (*unpublished*). Experiments are currently in progress to ascertain whether populations with only trace reproduction on alfalfa will shift to an alfalfa host race when cultured continuously on alfalfa or if the host-parasite relationship is stable. If the range of reproduction observed among the field populations is a product of the duration to which each population was exposed to alfalfa, then all *M. chitwoodi* populations may potentially become an alfalfa host race during the crop cycle. The stability of this trait must be known before the development of a host differential will be practical and rotation recommendations can be developed.

Alfalfa is cross-pollinated and extremely heterogeneous for many traits, including susceptibility to root-knot nematodes (4). Elgin et al (2) reported that although Thor alfalfa was considered susceptible to *M. hapla*, 5% of the plants tested were rated resistant. In preliminary studies with individual Thor alfalfa plants inoculated with 5,000 eggs, egg mass production ranged from 0 to 88 and 0 to 137 per plant after 55 days for two *M. chitwoodi* isolates (17). The number of plants was increased to three per pot in our study; however, the variability, as expressed by the standard error, remained high (Table 1).

Presence of a *M. chitwoodi* alfalfa host race in the Pacific Northwest may have several ramifications in addition to alfalfa yield reduction. Potato is an important irrigated crop in this region, and *M. chitwoodi*, one of its dominant pests, has an economic threshold density of <1 egg/250 cm^3 of soil (18). Of the major rotation crops, wheat, corn (*Zea mays* L.), and alfalfa, only alfalfa could be recommended to reduce populations of this species. Two factors may act to maintain or increase *M. chitwoodi* alfalfa race frequency within a field population during alfalfa cropping. First, alfalfa stands are in production four or more years. Second, *M. chitwoodi* reproduces in cool soils (7), which allows development to commence in early spring and extend into late fall. Thus, there is a prolonged period of uninterrupted monoculture during which those *M. chitwoodi* individuals that develop on alfalfa may reproduce. In this case, fumigation may be necessary following alfalfa, which significantly increases the production cost of a subsequent potato crop. Moreover, if nematodes develop in deep alfalfa root systems, these populations will be difficult to control in lower soil profiles with conventional fumigation methods.

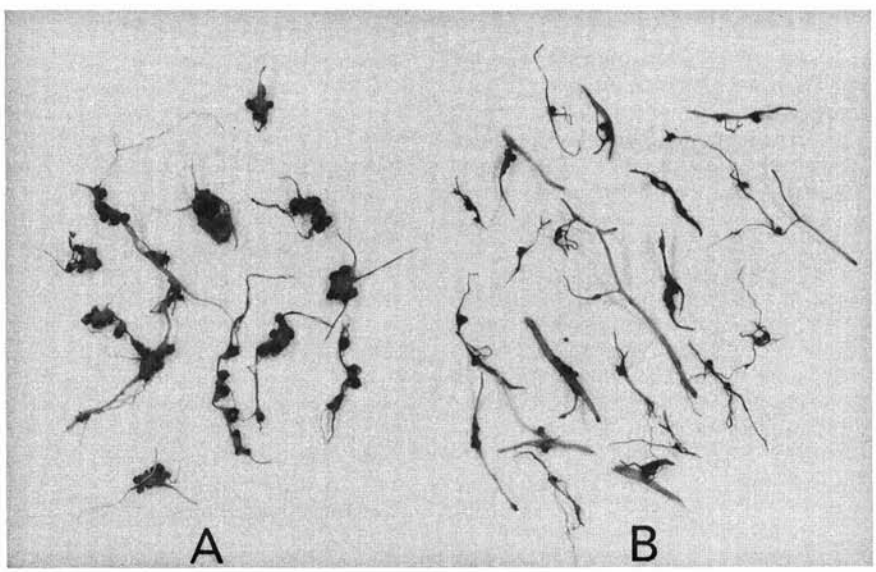

Fig. 2. Thor alfalfa roots infected with **(A)** *Meloidogyne hapla* and **(B)** *M. chitwoodi*.

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