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Effect of Soil Temperature on Reproduction of *Meloidogyne chitwoodi* and *M. hapla* Alone and in Combination on Potato and *M. chitwoodi* on Rotation Plants[1]

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Abstract: *Meloidogyne chitwoodi* developed and reproduced more rapidly than *M. hapla* in potato roots at 15, 20, or 25 C when both species of nematodes were inoculated simultaneously at 250 or 1,000 juveniles of each. At 30 C significantly more *M. hapla* than *M. chitwoodi* females were found at the lower inoculum level after 41 days. More *M. chitwoodi* than *M. hapla* juveniles were extracted from soil at 15, 20, and 25 C, but only at the lower inoculum level at 30 C. Potato was considered a more suitable host for *M. chitwoodi* than *M. hapla* because of *M. chitwoodi*'s greater reproduction at 15, 20, and 25 C. Corn and wheat cultivars tested supported *M. chitwoodi* reproduction at temperatures of 10, 15, 20, and 25 C, but fewest eggs were produced on these plants at 20 C. Temperatures of 10 to 25 C had little influence on the low reproduction of *M. chitwoodi* on four alfalfa cultivars. *M. chitwoodi* reproduced on the alfalfa entry Mn PL9HF.

Key words: Columbia root-knot nematode, northern root-knot nematode, alfalfa, wheat, corn.

Meloidogyne chitwoodi Golden, O'Bannon, Santo, and Finley, and *M. hapla* Chitwood are serious pests of potato (*Solanum tuberosum* L.) in the Pacific northwest (8). *Meloidogyne chitwoodi* readily reproduces on corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) but reproduces poorly on alfalfa (*Medicago sativa* L.) (7). These crops are commonly used in rotations with potato.

A survey in the Pacific northwest (6) revealed that *M. chitwoodi* occurred alone in 83% of potato tubers examined and *M. hapla* in 11%. While only 6% of the tuber samples contained both species, this mixture of species was evenly distributed in all the areas surveyed. The higher incidence of *M. chitwoodi* on potato (6) suggests that it is the dominant species, and this dominance is attributed to its ability to develop and reproduce over a wider temperature range than *M. hapla* (9). Also, grain crops often rotated with potato are good hosts of *M. chitwoodi* but not *M. hapla* (10). Alfalfa, a major rotation crop with potato, is susceptible to *M. hapla* and is not usually grown when this species is present. Small grains used in rotation to reduce *M. hapla*, were not previously known to be good hosts of *M. chitwoodi* (8).

Low soil temperatures inhibit reproduction of *M. chitwoodi* less than *M. hapla* (9). *M. chitwoodi* can invade host roots and develop at temperatures as low as 7–10 C (3,5). Soil temperatures in the Pacific northwest are variable but generally range throughout the year within the infective

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TABLE 1. Effect of four soil temperatures on development of combined *Meloidogyne chitwoodi* and *M. hapla* on Russet Burbank potato.†

Initial population (Pi)		% Mature ♀ and J2/root system							
		15 C		20 C		25 C		30 C	
		♀	J2	♀	J2	♀	J2	♀	J2
250	*M. chitwoodi*	94*	99*	89*	94*	79*	91*	29	68*
+									
250	*M. hapla*	6	1	11	6	21	9	71*	32
1,000	*M. chitwoodi*	97*	93*	83*	97*	77*	90*	43	58
+									
1,000	*M. hapla*	3	7	17	3	23	10	57	42

* Differences comparing percentages of females or juveniles at either Pi are significant at $P < 0.05$.
† Treatments 20, 25, and 30 C harvested 41 days and 15 C 48 days after inoculation.

and developmental limits of *M. chitwoodi*, but not always within the range of *M. hapla*.

One experiment was conducted to determine how soil temperature influenced reproduction of individual and combined *M. chitwoodi* and *M. hapla* on potato. A second experiment examined development and reproduction of *M. chitwoodi* on alfalfa, corn, and wheat at different temperatures.

MATERIALS AND METHODS

Meloidogyne chitwoodi and *M. hapla* were isolated from field populations on potato and alfalfa, respectively, and increased on tomato (*Lycopersicon esculentum* Mill. cv. Roza) in the greenhouse. Nematode eggs for inoculum were extracted from galled tomato roots by the NaOCl method (4).

Experiment 1: Single-eye seed pieces of Russet Burbank potato were planted in methyl bromide-fumigated sand in metal flats. After 5 weeks, plants with seed pieces removed were transplanted into 1-liter plastic beakers, one plant per beaker, containing 800 cm^3 of methyl bromide-fumigated sandy loam soil. Plants were held in the greenhouse for 1 week and then the pots were transferred to temperature tanks in a growth room. After an additional week, 500 and 2,000 *M. chitwoodi* or *M. hapla* separately, or 250 and 1,000 eggs of each species combined, were pipetted in 10 ml of water around the roots of each plant. Noninoculated plants served as controls. Plants were positioned randomly in seven replicates and grown at constant soil temperatures of 15, 20, 25, or 30 C. Ambient growth room temperature was 24 C. Fourteen-hour day length was provided by cool white fluorescent bulbs at an intensity of 4.8×10^3 lux, situated 91 cm above the temperature tanks.

Plants grown at 20, 25, or 30 C were harvested after 41 days; plants grown at 15

TABLE 2. Eggs (per gram dry root basis) recovered from Russet Burbank potato inoculated with *Meloidogyne chitwoodi* and *M. hapla* alone and in combination at two inoculum levels and four soil temperatures.†

Species	Initial population (Pi)	Soil temperature (C)			
		15	20	25	30
M. chitwoodi	500	35 bc‡	270 a	2,390 b	420 c
	2,000	290 a	830 a	10,720 a	1,540 ab
M. hapla	500	1 d	10 b	680 c	310 c
	2,000	22 c	33 b	2,330 b	650 bc
Combined§	500	36 bc	290 a	2,440 b	430 c
	2,000	160 b	330 a	8,260 a	1,610 a

† Treatments at 20, 25, and 30 C harvested 41 days and at 15 C 48 days after inoculation.
‡ Numbers followed by the same letter in columns are not significantly different according to Duncan's multiple-range test ($P < 0.05$).
§ Equal numbers, 250 and 1,000 of *M. chitwoodi* and *M. hapla*.

TABLE 3. Egg masses (EM) and eggs (E) (10^3) recovered from alfalfa, corn, and wheat inoculated with *Meloidogyne chitwoodi* and grown at four soil temperatures for 75 days (per gram dry root basis).

	10 C		15 C		20 C		25 C	
Cultivars	EM	E	EM	E	EM	E	EM	E
Alfalfa								
Saranac	0 c†	0 c	0 d	0 e	0 d	0 d	1.5 d	1 d
Thor	0 c	0 c	0 d	0 e	0 d	0 d	0 3	0 d
Washoe	0 c	0 c	0.5 d	0.8 e	0 d	0 d	0 e	0 d
DuPuits	0 c	0 c	0 d	0 e	0 d	0 d	1 de	1.4 d
Mn PL9HF	0 c	0 c	1.6 c	3.1 d	0 d	0 d	2.6 c	4.7 c
Corn								
KT-626	2.3 b	3.5 b	4.2 a‡	7.6 bc	3.9 b	7.3 b	4.1 b	9.2 b
KE-497	1.4 b	8.8 bc	2.7 b	6.2 c	2.3 c	5.4 c	3.6 bc	8.1 b
Jubilee	2.2 b	2.5 b	4.4 a	8.2 ab	3.8 b	7.6 b	4.4 b	9.5 b
Wheat								
Fielder (spring)	4.0 a	6.1 a	4.9 a	9.8 a	5.3 a	9.8 a	6.3 a	11.9 a
Nugaines (winter)	3.9 a	6.2 a	4.3 a	9.2 ab	4.8 ab	9.7 a	5.8 a	12.4 a

† Numbers followed by the same letter in columns are not significantly different according to Duncan's multiple-range test ($P < 0.05$) based on log-transformed data.

‡ Underscored egg mass (EM) numbers on corn and wheat in rows are not significantly different according to Duncan's multiple-range test ($P < 0.05$).

C were harvested at 48 days when egg masses were first observed on roots. Roots were carefully washed free of soil and weighed after being blotted dry. Roots of seedlings from each harvest date were cut into 1-cm lengths, mixed, and separated into two equal parts by weight. Nematode reproduction was measured by extracting eggs from half of the roots with NaOCl (4). The other half of the roots were stained with hot acid fuchsin-lactoglycerol and cleared in lactoglycerol (1). About 30–35 mature females were dissected from each root sample for species determination by perineal pattern. Juveniles were extracted from 250 cm^3 soil from each pot by a combination of elutriation and centrifugal flotation. One hundred juveniles, extracted from soil from each pot, were differentiated by tail morphology (6).

Experiment 2: The influence of temperature on reproduction of *M. chitwoodi* on alfalfa entries 'Saranac,' 'Thor,' 'Washoe,' 'DuPuit,' and 'Mn PL9HF'; corn entries 'KT-626,' 'KE-497,' and 'Jubilee'; and wheat entries 'Fielder' (spring) and 'Nugaines' (winter) was determined. Seeds were germinated on moist filter paper in petri dishes for 3 days and then planted in plastic beakers containing 400 cm^3 of fumigated sandy loam soil. Beakers with seedlings were completely randomized in controlled air temperature tanks in seven replicates and maintained at constant soil temperatures of 10, 15, 20, or 25 C with an ambient temperature of 24 C. Roots of all seedlings were each inoculated with 5,000 *M. chitwoodi* eggs in 10 ml water 7 days after placing in temperature tanks. Other growing conditions were as in Experiment 1. Roots of extra inoculated seedlings were checked at intervals for presence of egg masses; after 75 days inoculated seedlings at all temperatures had egg masses. Roots were carefully washed free of soil and placed in phloxine B solution (2) to stain egg masses. Egg masses on the entire root system were counted, after which eggs were extracted by NaOCl. Roots were then oven dried and weighed.

RESULTS

Experiment 1: Examination of perineal patterns of nematodes removed from roots growing in soil inoculated with both *M. chitwoodi* and *M. hapla* revealed that more *M. chitwoodi* than *M. hapla* developed and reproduced in potato roots at 15, 20 or 25 C (Table 1). Also, more *M. chitwoodi* than *M. hapla* juveniles were recovered from soil

samples. At 30 C more *M. hapla* than *M. chitwoodi* females developed at the low Pi, but not at the high Pi. In fact, there was a lower proportion of *M. hapla* females at the higher than at the lower Pi. A higher proportion of *M. chitwoodi* than *M. hapla* juveniles were extracted from the 30 C soil at the low Pi, but not at the high Pi.

Numbers of *M. chitwoodi* eggs recovered per gram of dry potato root were higher at 15, 20, and 25 C, but not at 30 C, than *M. hapla* eggs at the same inoculum level (Table 2). When the two species were combined, the number of eggs recovered were similar to the numbers recovered with *M. chitwoodi* alone at all temperatures and inoculum levels.

Experiment 2: The *M. chitwoodi* population used in this study did not readily establish on alfalfa at any temperature, nor did temperature affect the reaction of any alfalfa cultivar to this nematode (Table 3). Only Mn PL9HF was susceptible to *M. chitwoodi* in this test just as it was in a previous test (7). All corn and wheat cultivars tested supported nematode reproduction at all temperatures, although fewer eggs ($P < 0.05$) were produced on these plants at 10 C than at higher temperatures.

Discussion

Previous investigations in the Pacific northwest demonstrated that *M. chitwoodi* is dominant to *M. hapla* on potato (7,8). This study confirms that *M. chitwoodi,* either singly or with *M. hapla,* colonizes potato more successfully than does *M. hapla.* Similar observations have been made on other commercial potato cultivars (Santo, unpubl.).

Low soil temperatures, such as occur in the Pacific northwest, favor early invasion of potato roots by *M. chitwoodi. M. chitwoodi* reproduction in some rotation crops was sufficiently great, even at 10 C, that high inoculum levels would be left in the soil ready to attack the new potato roots. Earlier invasion and subsequent reproduction may then result in more *M. chitwoodi* generations per year than *M. hapla* under these conditions.

Several alfalfa cultivars were poor hosts or failed to support reproduction of *M. chitwoodi* in our greenhouse (7) and controlled temperature studies reported here. Recently potatoes in two fields in Washington were severely infected by *M. chitwoodi* where potatoes followed alfalfa; this situation is being investigated. Small grains, extensively used in rotation with potato, are a principal source of *M. chitwoodi* inoculum. Since these crops are in the field year around, several generations of the nematode can develop annually, necessitating application of control measures every time potatoes are planted.

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