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Suppression of Root-knot Nematode Populations with Selected Rapeseed Cultivars as Green Manure[1]

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Abstract: *Meloidogyne chitwoodi* races 1 and 2 and *M. hapla* reproduced on 12 cultivars of *Brassica napus* and two cultivars of *B. campestris*. The mean reproductive factors (Rf), Rf = Pf at 55 days ÷ 5,000, for the three nematodes were 8.3, 2.2, and 14.3, respectively. All three nematodes reproduced more efficiently ($P < 0.05$) on *B. campestris* than on *B. napus*. Amending *M. chitwoodi*-infested soil in plastic bags with chopped shoots of Jupiter rapeseed reduced the nematode population more ($P < 0.05$) than amendment with wheat shoots. Incorporating Jupiter shoots to soil heavily infested with *M. chitwoodi* in microplots reduced the nematode population more ($P < 0.05$) than fallow or corn shoot treatments. The greatest reduction in nematode population density was attained by cropping rapeseed for 2 months and incorporating it into the soil as a green manure.

Key words: *Brassica* spp., canola, Columbia root-knot nematode, glucosinolate, host suitability test, *Meloidogyne chitwoodi, M. hapla,* nematicide, Northern root-knot nematode, organic amendment, reproductive factor.

Rapeseed (*Brassica napus* L. and *Brassica campestris* L.), grown in many countries for producing edible canola oil, industrial oil, and protein, ranks fifth among oil-seed crops in the world (2). Because of undesirable erucic acid in the oil and glucosinolates in the oil-free meal, this crop has been slow in gaining popularity in the United States. After the release of high-yielding cultivars containing low levels of erucic acid and glucosinolates, and approval of the Federal Drug Administration of canola oil consumption, growers in the western United States began to try rapeseed as an alternate crop. Approximately 5,000 ha are grown in eastern Washington (20).

Erucic acid, a long-chain fatty acid, is undesirable in edible rapeseed oil because it is not digested readily; high-quality industrial lubricants are extracted from rapeseed cultivars with high erucic acid (> 45% of the fatty acid composition) (18). Glucosinolates are sulfur-containing compounds in all parts of the plant (15). The glucosinolates alone are not harmful; however, in broken tissue, they are hydrolyzed by enzymatic action of plant myrosinase to bitter tasting, toxic, and goitrogenic compounds (10). Oil-free rapeseed meal containing > 30 μmole glucosinolate/g is unpalatable for animal feed. The breakdown products of glucosinolates include various forms of fungicidal and bactericidal isothiocyanates (5). Isothiocyanates include methyl isothiocyanate, a breakdown product of metham sodium, which is an effective nematicide (8).

Control of root-knot nematodes, *Meloidogyne chitwoodi* Golden et al. and *M. hapla* Chitwood, on potatoes in the northwestern United States is heavily dependent on soil fumigation with 1,3-dichloropropene and metham sodium (12,16). The continued availability of these nematicides is of major concern to potato growers. Thus, the search for alternative measures to manage root-knot nematode on potato and other vegetable crops has become increasingly important. The objectives of this study were to determine the host suitability of rapeseed cultivars to *M. chitwoodi* races 1 and 2 and *M. hapla* and to evaluate the use of rapeseed as a soil amendment in managing *M. chitwoodi.*

Materials and Methods

Host test studies: Isolates of *M. chitwoodi* races 1 and 2 and *M. hapla* were obtained from the Washington State University Ir-

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TABLE 1. Reproductive factor (Pf/Pi) of *Meloidogyne chitwoodi* races 1 (MC1) and 2 (MC2) and *M. hapla* (MH) on selected rapeseed (*Brassica* spp.) cultivars 55 days after inoculation with 5,000 eggs.

		Reproductive factor		
	Cultivar†	MC1	MC2	MH
B. campestris	Candle (S)	36.3 b	4.7 fgh	82.2 a
	Tobin (S)	25.0 cd	4.1 fgh	30.2 bc
B. napus	Tribute (S)	11.4 efgh	3.8 fgh	3.9 fgh
	Westar (S)	6.9 fgh	1.2 h	19.0 de
	Altex (S)	4.4 fgh	2.9 fgh	10.9 efgh
	Arabella (W)	6.1 fgh	1.8 gh	2.6 fgh
	Jupiter (W)	5.7 fgh	1.1 h	12.5 ef
	Lindora (W)	5.3 fgh	1.8 gh	2.8 fgh
	Rubin (W)	4.1 fgh	1.1 h	8.7 efgh
	Santana (W)	2.9 fgh	2.5 fgh	5.1 fgh
	Bridger (W)	2.4 fgh	1.5 h	12.1 efg
	Liradonna (W)	2.2 fgh	1.0 h	3.1 fgh
	Ceres (W)	1.9 gh	1.5 h	3.7 fgh
	Cascade (W)	1.8 gh	2.1 fgh	3.6 fgh
Mean		8.3	2.2	14.3

Values are means of five replicates. Means in each row and column followed by the same letter do not differ ($P < 0.05$) according to least significant difference (LSD = 10.6).
† S = spring cultivar, W = winter cultivar.

rigated Agriculture Research and Extension Center, Prosser, Washington, root-knot nematode collection (14). Egg inocula were obtained from infected tomato (*Lycopersicon esculentum* Mill. cv. Columbian) plants by the NaOCl method (7). For each nematode isolate, 5,000 eggs (Pi) in 5 ml water were applied to the soil surface in 10-cm-d plastic pots containing 10 3-week-old rapeseed plants and covered with a thin layer of soil. The potting medium (850 g/pot) was a loamy sand soil (84% sand, 10% silt, 6% clay; 0.5% organic matter, pH 6.9) previously treated with methyl bromide. The test plant entries included two cultivars of *Brassica campestris* and 12 cultivars of *B. napus* (Table 1). Columbian tomato, an excellent host for both species of nematodes, was used as a standard. Pepper (*Capsicum anuum* L. cv. California Wonder), a nonhost for *M. chitwoodi* and a host for *M. hapla,* and wheat (*Triticum aestivum* L. cv. Nugaines), a nonhost for *M. hapla* and a host for *M. chitwoodi,* were also included to detect any contamination of nematode inocula. Treatments were replicated five times and arranged in a randomized complete block design on a greenhouse bench at 22–26 C during the experiment. Host suitability was assessed after 55 days by washing the roots free of soil, extracting the eggs (7), and calculating the reproductive factor (Rf) as follows: Rf = final egg density (Pf) ÷ initial egg density (Pi) (11). The Rf for all nematodes was subjected to factorial analysis (nematodes, factor 1 × rapeseed cultivars, factor 2), and the means were separated by least significant difference (LSD).

Soil amendment–greenhouse study: Two separate tests were conducted to determine the effect of soil-incorporated rapeseed shoots on the survival of *M. chitwoodi* race 1. Potting soil was infested by incorporating chopped tomato roots infected with *M. chitwoodi* and incubated for 10 days to obtain a mixture of eggs and second-stage juveniles. Forty grams of shoots from Jupiter or Bridger rapeseed or Nugaines wheat were chopped (2–3 cm long) and mixed with 500 g *M. chitwoodi*-infested soil. The amended soils were placed in plastic bags (15 × 38 cm) and 15 g nonfumigated field soil was added to facilitate decomposition of plant tissue during the incubation period. The sealed bags were incubated at 15–18 C for 1 month before the content of each bag was placed in a 7.5-cm-d clay pot and bioassayed with a 3-week-old tomato seedling. The seedlings were

removed after 3 weeks, the root systems were washed free of soil and stained with acid fuchsin (3), and the nematodes within the roots were counted.

In the first test, Jupiter rapeseed was compared to treatment with no plant parts. In the second test, Jupiter and Bridger rapeseeds were compared to Nugaines wheat shoots or no plant parts. The treatments were replicated five times in a randomized complete block design. Data were subjected to analysis of variance and means were separated by Duncan's multiple-range test.

Soil amendment–microplot study: Loamy sand field soil (81% sand, 17% silt, 2% clay; 0.9% organic matter, pH 6.9) naturally infested with *M. chitwoodi* was placed in 19-liter bucket microplots (13) on 17 May 1989. An additional 500 g soil infested with *M. chitwoodi* was added to each bucket and thoroughly mixed in the top 15 cm. A single Columbian tomato seedling, five field corn (*Zea mays* L. cv. Pioneer 3732) or 10 Jupiter rapeseed seeds were planted in each bucket. Treatments were arranged in a complete randomized block design with five replicates. The buckets were irrigated and weeded by hand as needed and fertilized with Osmocote (14-14-14, Sierra Chemical Co., Milpitas, CA) slow release fertilizer. After 2 months, the nematode population in the soil was assessed by removing five 2.5-cm-d soil cores from each bucket and bioassaying 500 g soil with a tomato seedling as described for the greenhouse study.

The tops of all plants were removed at the soil line, and 1,000 g chopped (ca. 5 cm length) rapeseed or corn tops were incorporated into the top 15 cm of soil, either of buckets from which they were harvested or of tomato buckets. An additional set of buckets planted with tomato were processed the same as the other buckets, but no plant material was added. These buckets served as a fallow treatment to measure the natural decline of nematodes. The buckets were kept moist for 1 month, and nematode population was determined by bioassay as before.

Results and Discussion

Host test studies: Meloidogyne chitwoodi races 1 and 2 and *M. hapla* successfully reproduced on tomato; the Rf values on this host in four sets of experiments ranged from 4 to 100. Reproduction of *M. chitwoodi* races 1 and 2 on pepper and *M. hapla* on wheat was negligible (Rf < 0.1).

Meloidogyne hapla and *M. chitwoodi* races 1 and 2 reproduced on all 14 rapeseed cultivars with mean Rf values of 14.3, 8.3, and 2.2, respectively. *Meloidogyne hapla* was more ($P < 0.05$) reproductive than *M. chitwoodi* races 1 and 2 on two and five of the rapeseed cultivars, respectively (Table 1). Similarly, *M. chitwoodi* race 1 reproduced better ($P < 0.05$) than race 2 on two of the rapeseed cultivars. *Brassica campestris* was generally a more suitable host to root-knot nematodes than *B. napus.* All three nematode populations increased on Jupiter, which contained higher glucosinolates than the other cultivars.

Soil amendment–greenhouse study: At the end of the incubation period, the chopped shoots were soft, water soaked, and decomposing. The amended soils were wetter than the control due to the leakage of plant sap from disintegrating tissue. Also, the bags containing the rapeseed shoots gave off a pungent odor. In both tests, some of the bioassay tomato seedlings planted in the rapeseed amended soil died and had to be replanted several times. In addition, the tomato root systems harvested from these soils exhibited various degrees of discoloration and browning, which indicated that a substance released from the decomposition of rapeseed was phytotoxic to tomato seedlings. The tomato seedlings planted in unamended soil or soil amended with wheat shoots grew normally.

In the first test, the mean number of nematodes surviving in the rapeseed amended soil was significantly ($P < 0.05$) less than in the control (Table 2). In the second test, Jupiter killed more ($P < 0.05$) nematodes than did the control or wheat treatments (Table 2). Results suggested that the effects of Jupiter rapeseed on nema-

TABLE 2. Number of infective *Meloidogyne chitwoodi* detected in 500 g soil amended with shoots of different crops and incubated in sealed plastic bags at 15–18 C for 1 month.

Crop	Cultivar	Test 1	Test 2
None		1,280 a	2,246 a
Rapeseed	Jupiter	4 b	2 c
	Bridger		33 b
Wheat	Nugaines		85 b

Values are means of five replicates. Means in each column followed by the same letter do not differ ($P < 0.05$) according to Duncan's multiple-range test.

TABLE 3. Number of infective *Meloidogyne chitwoodi* detected in 500 g soil collected from microplots before and 1 month after incorporating the shoots of different crops into the soil.

Crop†	Amendment	Before	After
Tomato	None (fallow)	1,893 a	138 a
Tomato	Corn	1,317 a	331 a
Corn	Corn	0 b	185 a
Tomato	Rapeseed	2,578 a	19 b
Rapeseed	Rapeseed	1 b	2 c

Values are means of five replicates. Means in each column followed by the same letter do not differ ($P < 0.05$) according to Duncan's multiple-range test.

† Crops were grown for 2 months in *M. chitwoodi*-infested soil.

todes were beyond the normal effects attributed to the green manure amendment; however, there was no difference between the wheat or Bridger rapeseed treatments. The differing effects of Jupiter and Bridger on *M. chitwoodi* could be due to the higher levels of glucosinolates in Jupiter. Jupiter, a winter rapeseed, was released by Svalof (Swedish Seed Association) and contained > 30 μmoles of glucosinolates/g of defatted meal. In contrast, Bridger is an industrial lubricant quality cultivar with < 30 μmoles of glucosinolates/g of defatted meal and 52% erucic acid in the seeds (1). The direct evidence to support this hypothesis could be derived from monitoring the gas(es) evolved during the incubation period.

Soil amendment–microplot study: Meloidogyne chitwoodi reproduced on tomato in greater ($P < 0.05$) numbers than on corn or rapeseed before amending the soil (Table 3). Despite the fact that Pioneer 3732 field corn (9) and Jupiter rapeseed (Table 1) are reported suitable hosts for *M. chitwoodi* under greenhouse conditions, few, if any, nematodes were recovered from around their roots 2 months after planting. A similar differential rate of reproduction of *M. chitwoodi* on carrot in the greenhouse and field was previously reported (17). It is possible that the nematodes in the corn or rapeseed roots had not yet reached the state of maturity to contribute to nematode population in the soil.

After a month of fallow (no amendment), the population of *M. chitwoodi* declined by 93% (Table 3). Amending the soil with rapeseed shoots caused a further ($P < 0.05$) decline of the population. The number of nematodes in the buckets amended with corn shoots was not different from the fallow treatment. Davis et al. (4) reported that amending the soil with rapeseed meal significantly reduced the population of *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans Stekhoven, but amendment with rapeseed shoots did not. The failure to reduce *P. neglectus*, relative to *M. chitwoodi*, in this study may be due to the ability of the nematode to tolerate certain chemicals. We have consistently observed in commercially fumigated fields that *P. neglectus* appears to survive soil fumigation better than *M. chitwoodi* or *M. hapla* (Santo, unpubl.). More important, however, the concentration of glucosinolates in leaves and shoots of rapeseed decreases as the plant matures (19). Hence, if the breakdown products of glucosinolates are responsible for nematicidal activities in rapeseed shoots, then the age of plant at incorporation may significantly influence the suppression of nematode populations. Our results showed that growing Jupiter rapeseed in infested soil for 2 months and incorporating the shoots with the soil resulted in the lowest ($P < 0.05$) final population of *M. chitwoodi*. Thus, rapeseed as a cover crop may play an important role in reducing the impact of root-knot nematode populations on potato and vegetable crops. Spring incorporated winter rapeseed, as green manure following

wheat, increased potato yields (6). In the present rotational scheme for potatoes, wheat and sweet corn, commonly grown before potatoes, are harvested before August 1. Used as a cover crop, rapeseed would be planted in early August and incorporated as green manure in the field. Growing rapeseed as a green-manure cover crop is more acceptable to vegetable and potato growers than allowing it to produce seed and possibly become a noxious weed.

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