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Evaluation of 15 *Trifolium* spp. and of *Medicago sativa* as Hosts of Four *Meloidogyne* spp. Found in New Zealand

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Abstract: The predominant root-knot nematode in New Zealand pastures is *Meloidogyne trifoliophila,* identified until recently as *M. hapla.* Clarification was needed on the host range of these two species on legumes found in New Zealand pastures and on clover species closely related to *Trifolium repens.* In a greenhouse test, 15 *Trifolium* spp. and *Medicago sativa* were inoculated with eggs of *M. trifoliophila, M. hapla, M. incognita,* or *M. javanica.* All legumes tested were hosts to some degree to each of the root-knot nematodes used, except for *T. striatum* and *M. sativa* whose status as hosts to *M. trifoliophila* was doubtful. Low galling rates occurred on *T. glomeratum* infected by *M. hapla* (mean of 3% of the root system galled), on *T. semipilosum* infected by *M. javanica* (2%), on *T. striatum* infected by *M. trifoliophila* (2%), and on *T. micranthum* (4%) and *M. sativa* (6%) infected by *M. incognita.* The most heavily parasitized clovers were *T. repens* infected by *M. trifoliophila* (92%), *T. pratense* infected by *M. incognita* (91%), and *T. argutum* infected by *M. incognita* (88%).

Key words: alfalfa, breeding, clover, detection, diagnosis, lucerne, *Meloidogyne hapla, Meloidogyne incognita, Meloidogyne javanica, Meloidogyne trifoliophila,* nematode, New Zealand, pasture, resistance, root-knot nematode, *Trifolium* spp., white clover.

The predominant root-knot nematode infecting white clover (*Trifolium repens* L.) in New Zealand pastures has been called into question because of a misidentification of many populations originally identified as *Meloidogyne hapla* Chitwood but now recognized as a recently described species, *M. trifoliophila* Bernard and Eisenback (Bernard and Eisenback, 1997). New Zealand studies of pasture root-knot nematodes on host range (Mercer, 1989; Mercer and Woodfield, 1986), distribution (Mercer and Woodfield, 1986; Skipp and Christensen, 1983), resistance screening (Grandison, 1976; Yeates et al., 1973), and interactions with VAM fungi (Cooper and Grandison, 1986) referred to *M. hapla* but not to *M. trifoliophila.*

A resistance screening project has identified resistance in white clover to *M. trifoliophila* (van den Bosch and Mercer, 1996) but not to *M. hapla* (Mercer et al., 1997). The use of this resistance in New Zealand pastures may select for parasitism by *M. hapla* and any other species of root-knot nematodes that may be found in the legumes that exist locally. The following study was conducted to clarify the status of common pasture legumes as hosts of four root-knot nematodes. Some species of *Trifolium* that can be hybridized with *T. repens* were included in case they could be used to introduce resistance into white clover. *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood were included in the test as these have been identified from New Zealand (C. J. Barber, pers. comm.).

MATERIALS AND METHODS

The *M. trifoliophila* population used in this study originated from egg masses collected from infected white clover in a pasture at Fitzherbert West, Palmerston North, New Zealand. The *M. hapla* population originated from egg masses from roots of kiwifruit at Te Puke, New Zealand. The *M. incognita* population (isolate 85-3) and the *M. javanica* population (isolate 93-9) were supplied by J. L. Starr, Texas A&M University, as New Zealand isolates were not available. Nematode cultures were identified by isozyme phenotype, host range and morphology as described by Mercer et al. (1997).

Pre-germinated seeds were sown, one per 70-cm^3 compartment, in methyl bromide-sterilized 50:50 sand-soil (Manawatu silt loam, pH 6.1) mix held in Rootrainers (Carran Industries, New Zealand). The layout was blocked by root-knot nematode species

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(one block each) separated from each other to prevent contamination. The arrangement of the legume species was randomized within each block. Ten seeds of each of the legume species in Table 1 were sown on 28 March 1995. Seven days later, inoculum prepared by an NaOCl method (Hussey and Barker, 1973) was injected around the roots at 1,000 eggs per plant. Rootrainers were kept in a greenhouse at 18 °C to 24 °C, watered from above as required, and plants were supplied with full nutrients fortnightly (half-strength "Thrive," Yates, New Zealand). However, since unseasonably low temperatures delayed development, plants were moved to a controlled temperature room (25 °C, 10 hours of light) 6 weeks after inoculation. Roots were washed free of soil on 13 June 1995 and visible egg masses and galls counted. An estimate on the percentage of the root system galled was determined. Data were analyzed with analysis of variance, and means were compared at $P = 0.05$ with LSD. Data on percentage of root system galled were transformed with arcsine ($\sqrt{x}$) before analysis.

Results and Discussion

Growth and survival of plants was generally good except in *T. arvense, T. glomeratum,* and *T. occidentale,* where fewer than half of the plants survived (Table 1). *Trifolium argutum* plants were stunted. Least galling occurred on *T. glomeratum* infected by *M. hapla, T. striatum* infected by *M. trifoliophila, T. semipilosum* infected by *M. javanica,* and *T. micranthum* and *M. sativa* infected by *M. incognita* (Table 1). The galls on *T. striatum* and on *M. sativa* were smaller than those on other plants. The most heavily parasitized on a percentage-of-the-root-system-galled basis were *T. repens* parasitized by *M. trifoliophila* (92%), *T. pratense* by *M. incognita* (91%), and *T. argutum* by *M. incognita* (88%). The egg masses of *M. trifoliophila* remain deeply embedded within a spongy root gall; thus, fewer egg masses were seen on roots infected by this species.

The low gall numbers, small gall size, and absence of visible egg masses suggest that *T. striatum* and *M. sativa* are not good hosts of *M. trifoliophila.* All other legumes tested were hosts to some degree to all of the root-knot nematodes tested. More definitive determination of host status could be ascertained by counting eggs (Windham and Pederson, 1992), but these data were not collected in this study. Mercer (1989) reported *T. striatum* as a nonhost of *M. hapla,* but this nematode was, in fact, more recently identified as *M. trifoliophila.* The response of alfalfa to *M. trifoliophila* was similar to the results of Bernard and Jennings (1997), where four of nine entries were not galled and the other five had low mean galling indices. Alfalfa was a good host for the other three root-knot nematodes in this test, confirming earlier reports (Griffin et al., 1996).

The host status of various legumes for root-knot nematodes reported by Yeates et al. (1973), Grandison (1976), and Mercer and Woodfield (1986) are confusing. However, regardless of the root-knot nematode species these researchers used, the legumes in their studies are now confirmed in this study as hosts of *M. hapla* and *M. trifoliophila* (except for *T. striatum* and *M. sativa*). This report adds 10 *Trifolium* spp. to the list of hosts of *M. trifoliophila* published by Bernard and Jennings (1997): *T. ambiguum, T. arvense, T. dubium, T. glomeratum, T. hybridum, T. medium, T. micranthum, T. occidentale, T. semipilosum,* and *T. argutum.*

Windham and Pederson (1992) compared reproduction by *M. graminicola* and *M. incognita* on 23 *Trifolium* spp., including seven of the species used in this test. However, the identification of the *M. graminicola* isolate used by Windham and Pederson (1992) has been questioned by Bernard and Jennings (1997), who reported that morphologically it more closely resembled *M. trifoliophila* than *M. graminicola.* If the *M. graminicola* isolate used by Windham and Pederson (1992) is found to be *M. trifoliophila,* then the results of this test confirm the host status of the seven species common to both studies.

The *Trifolium* spp. in this test that have been hybridized with *T. repens* (*T. nigrescens, T. occidentale,* and *T. argutum*) did not ex-

TABLE 1. Numbers of plants surviving, mean number of galls per plant, mean percentage of root system galled, and mean number of egg massess on roots of 16 legumes (*Trifolium* spp., *Medicago sativa*) infected with *Meloidogyne hapla* (MH), *M. trifoliophila* (MT), *M. javanica* (MJ), or *M. incognita* (MI).

		Surviving plant number				Number of galls[a]				Percent galling				Number of egg masses[b]			
Host plant	Accession	MH	MT	MJ	MI	MH	MT	MJ	MI	MH	MT	MJ	MI	MH	MT	MJ	MI
T. ambiguum	Az 1134	9	8	9	8	59	41	28	29	54	40	48	84	49	2	14	17
T. argutum	Az 1618	4	6	10	9	43	11	28	39	76	80	67	88	30	2	5	21
T. arvense	Az 3124	2	2	1	4	28	15	38	19	50	55	30	30	11	1	0	9
T. dubium	Az 3079	9	8	9	9	42	29	11	22	34	22	3	21	26	0	7	17
T. glomeratum	Az 3025	3	3	1	3	5	13	17	24	3	60	17	33	2	1	16	13
T. hybridum	Ab 273	8	8	9	9	85	40	36	49	71	64	60	47	65	4	18	24
T. medium	Z 150	9	9	7	8	10	13	36	28	2	15	53	19	2	0	7	3
T. micranthum	Az 2026	7	9	7	8	14	63	22	12	2	56	6	4	4	2	9	5
T. nigrescens	Az 2225	5	6	6	8	39	16	34	44	24	69	54	70	29	2	14	18
T. occidentale	—	3	3	2	4	18	12	15	18	8	47	25	46	13	0	15	5
T. pratense	F 2657	8	9	8	9	81	22	75	55	55	20	78	91	51	0	51	30
T. repens	G. 'Huia'	8	10	9	9	32	21	50	43	40	92	45	52	19	8	15	18
T. semipilosum	Az 1922	5	6	6	8	22	18	4	27	28	48	2	54	10	1	1	8
T. striatum	Az 1805	7	6	9	7	56	7	54	62	41	2	64	53	25	0	36	19
T. subterraneum	Ak 711	9	7	6	7	93	49	62	101	54	7	68	79	72	1	31	72
M. sativa	Af 2401	9	9	9	9	19	9	17	10	9	2	22	6	8	0	7	3
Mean						45	26	34	37	35	41	44	49	29	2	16	18
LSD ($P < 0.05$)						5.9	4.8	4.5	4.8	4.4	5.1	5.6	4.3	5.1	0.9	2.8	3.3

[a] Galls were counted 10 weeks after inoculation with ca. 1,000 eggs.
[b] Most MT egg masses were completely embedded in root tissue and therefore were not counted.

hibit resistance to any of the *Meloidogyne* spp. in our study. Resistant genotypes may be identified in screenings of greater numbers of genotypes than used here. For example, Mercer (1989) reported only one *T. semipilosum* genotype highly resistant to *M. hapla* (now *M. trifoliophila*) among 10 tested but in later screenings found 41 highly resistant genotypes out of a total of 245 tested (Mercer and Grant, 1993).

This study has clarified the host range among common New Zealand pasture legumes for root-knot nematodes and shows that nearly all may support populations of the *Meloidogyne* spp. found locally. This should be taken into account in ecological studies and when designing procedures for the introduction and field testing of resistant cultivars.

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