Journal of Nematology 29(1):55–64. 1997.

Host-Parasite Relationships of *Meloidogyne trifoliophila* Isolates from New Zealand

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Abstract: Root-infecting nematodes are commonly found on white clover in New Zealand pasture where they reduce yield, nitrogen fixation, and persistence. The dominant root-knot nematode on white clover in New Zealand is confirmed in this study as *Meloidogyne trifoliophila* by isozyme phenotype comparison with the type population from Tennessee. Results from a host differential test differed in the host ranges of *M. trifoliophila* and *M. hapla* from New Zealand locations, with *M. trifoliophila* failing to reproduce on the standard host plants of the test. The size and character of white clover root galls differ between species as *M. trifoliophila* galls are large, elongate, and smooth compared to the *M. hapla* galls, which are small, round, inconspicuous, and generally have adventitious, lateral roots. Culture and identification of root-knot nematode populations from sites in the North Island of New Zealand showed that *M. trifoliophila* is more widespread and abundant than *M. hapla.* Similar differential resistant and susceptible galling responses among half-sib families of white clover from a breeding program indicated that all *M. trifoliophila* populations tested were of the same pathotype. This resistant material was not effective in reducing reproduction of *M. hapla. Meloidogyne trifoliophila* did not develop to maturity on six grasses tested, but galls were formed on some species.

Key words: breeding, detection, diagnosis, *Meloidogyne hapla, Meloidogyne trifoliophila,* nematode, New Zealand, pasture, resistance, root-knot nematode, *Trifolium repens,* white clover.

Root-knot nematodes were first recorded from New Zealand (NZ) in 1903 (Clark, 1963) and have been studied since then in horticultural crops (Clark, 1963; Dale, 1972; Mercer, 1994) and particularly in pasture (Yeates, 1977; Watson and Barker, 1993; Mercer and Watson, 1996), where the characteristic galling of roots is commonly found on clovers but rarely on grasses. Until recently, the dominant root-knot nematode on clovers in NZ pastures was thought to be *Meloidogyne hapla* Chitwood. However, in December 1993, distinct contrasts were found in female size and in isozyme phenotype between an isolate collected from white clover (*Trifolium repens* L.) in NZ and a Texas isolate of *M. hapla* (J. L. Starr, unpubl. data). The NZ isolate has since been identified as *Meloidogyne trifoliophila* (E. C. Bernard, pers. comm.), originally described from a population on white clover in Tennessee (Bernard and Eisenback, 1997).

Most NZ pastures rely on the symbiotic fixation of atmospheric nitrogen in rhizobial root nodules on clover roots. Clovers also make important contributions to feed quality and supply as their digestibility and protein levels are superior to that of companion grasses. Greenhouse and field trials (Mercer and Watson, 1996) have consistently demonstrated the benefit of nematode control in white clover. For example, in trials at three sites, nematicides reduced nematode numbers, increased total dry matter yields and N fixation, and improved the ability of white clover to recover from drought (Watson et al., 1994). Control of nematode pathogens in pasture by nematicides, however, is untenable, either economically or ecologically. The alleviation of clover nematode problems is being addressed by: (i) improving resistance and tolerance of white clover to nematodes, (ii) designing pasture management systems that reduce the impact of nematodes, and (iii) improving biological control of nematodes (Mercer and Watson, 1996).

The white clover breeding program for improving resistance to *M. trifoliophila* in NZ has developed a number of half-sib families with partial nematode resistance (van den Bosch and Mercer, 1996a,b), but the response of these half-sib families to *M. hapla* was not known. If the resistance developed

Received for publication 30 July 1996.

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The authors thank J. Grant and G. Vickers for technical help; the Ricegrowers Cooperative, NSW, for supplying rice seed; and R. Cook for supply of *M. hapla* from Wales.

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to *M. trifoliophila* is not effective against *M. hapla* and if the latter is widespread and abundant, then the white clover resistance screening program would need to be expanded to include selecting for resistance to *M. hapla.* Cultures of root-knot nematode were established from sites around NZ to check on the distribution of *M. trifoliophila* and *M. hapla* and verify earlier work (Mercer and Grant, 1993), which reported that white clover from the breeding program was resistant to different populations of *M. trifoliophila* from various parts of NZ.

The objectives of this work were to: (i) further characterize an isolate of *M. trifoliophila* from white clover in NZ by comparing its host-pathogen relationships and isozyme phenotypes with the Tennessee type population of *M. trifoliophila* and with other *Meloidogyne* spp., (ii) survey NZ pastures for *M. trifoliophila* and *M. hapla* and verify that only one pathotype of *M. trifoliophila* is present, (iii) determine the response of white clover germplasm resistant or susceptible to *M. trifoliophila* to a Tennessee isolate of *M. trifoliophila* and determine if this resistance also is effective against *M. hapla* from NZ, and (iv) determine the reaction of a number of grasses to a NZ isolate of *M. trifoliophila.*

Materials and Methods

Host differential tests: One test was conducted at Texas A&M University using a population of *M. trifoliophila* that originated from egg masses collected from a clover pasture at Fitzherbert West, Palmerston North, NZ. This isolate was identified previously as *M. hapla* (Mercer, 1990). Roots of white clover, used to maintain the culture, were crushed under a hand-held roller before extraction with 0.5% NaOCl (Hussey and Barker, 1973). In February 1994, a suspension of ca. 10,000 *M. trifoliophila* eggs was applied to each plant in a host differential test (Hartman and Sasser, 1985) (Table 1) and to white clover. Plants were grown in a coarse sand-peat mix (6:1 v/v) in 100-mm-diam. pots. After 8 weeks, the roots were washed free of soil and the extent of galling assessed on a 0-5 scale. The roots were then

Table 1. Total egg production per plant by six New Zealand isolates of *Meloidogyne* spp. on host differential test plants and on white clover in two tests.

Host species[c]	Test one (Texas)[a]			Test two (Palmerston North): New Zealand populations[b]											
				1		2		3		4		5		6	
	Eggs	*N*[d]	Gall index[e]	Eggs	*N*	Eggs	*N*	Eggs	*N*	Eggs	*N*	Eggs	*N*	Eggs	*N*
Cotton	23	3	0	0		0		670	1	500	1	1,900	1	0	
Tobacco	15	3	0	111,600	3	130,000	3	558,000	3	207,000	3	29,000	2	0	
Pepper	0	3	0	9,000	3	1,400	1	12,000	3	11,000	2	11,000	3	0	
Watermelon	10	2	0	0		0		0		0		0		0	
Peanut	3	3	0	10,000	3	14,000	3	17,000	3	23,000	3	31,000	3	0	
Tomato	6	3	0	269,000	3	436,000	3	341,000	3	542,000	3	75,000	3	0	
White clover	59,000	3	5	303,000	3	97,000	3	346,000	3	312,000	3	166,000	3	249,000	3

[a] Eggs were counted 8 weeks after each plant was inoculated with ca. 10,000 eggs.

[b] Sources of isolates; 1 = roots of kiwifruit from Te Puke, New Zealand, (NZ); 2 = carrot from Pukekohe, NZ; 3 = boronia from Motueka, NZ; 4 = white clover among kiwifruit vines at Te Puke, NZ; 5 = *Scutellaria baicalensis* from Riwaka, NZ; 6 = white clover from Fitzherbert West Palmerston North, NZ. Eggs were counted 10 weeks after each plant was inoculated with ca. 2,000 eggs.

[c] Cotton, 'Deltapine' 61; tobacco, 'NC95'; pepper, 'CalWonder'; watermelon, 'Charleston Grey'; peanut, 'Florunner'; tomato, 'Rutgers'; white clover, 'Regal'.

[d] *N* = number of infected plants.

[e] 0 = no galls; 5 = 91% to 100% of the root system galled.

crushed, and the eggs were extracted as above and counted. A second host differential test was conducted in NZ in August 1994 using five cultures of root-knot nematodes initiated as single egg masses collected from known hosts of *M. hapla* (Table 1) and maintained on 'Rutgers' tomato. The sixth isolate in the second test was the *M. trifoliophila* isolate from the Fitzherbert West pasture site described above. Test plants were grown in a pasteurized sand-soil mix (50:50 v/v; soil was Manawatu silt loam pH 6.1) in 125-mm-diam. pots in a greenhouse (soil temperature range of 18–25 °C). A suspension of ca. 2,000 eggs was applied to each plant. Plants were watered as required and supplied with complete nutrients every 2 weeks. After 10 weeks, eggs were extracted from roots, as above, and counted. From the white clover plants inoculated with isolates 4 (*M. hapla*) and 6 (*M. trifoliophila*) (Table 1), representative galls were selected, measured, and dissected.

Esterase and malate dehydrogenase (MDH) isozyme phenotypes: Isozyme phenotypes were determined at Texas A&M University for mature females of each nematode isolate. *Meloidogyne hapla, M. javanica,* and *M. incognita* were cultured on tomato (*Lycopersicon esculentum* 'Rutgers'), and *M. trifoliophila* was cultured on white clover 'Regal'. Females, a minimum of eight for each species, with well-developed egg masses were dissected from host roots and macerated individually in 0.1 M phosphate extraction buffer (pH 7.4) with 20% sucrose, 2% Triton X-100, and 0.1% bromophenol blue. Electrophoretic separation of esterase and MDH isozymes was accomplished with an automated Phastsystem (Pharmacia, Uppsala, Sweden). Esterase and MDH isozymes were detected by staining for enzymatic activity according to established protocols (Esbenshade and Triantaphyllou, 1985, 1990). MDH phenotypes were used to identify *Meloidogyne* species collected from white clover roots during the survey of NZ pastures because MDH assays gave more consistent results than did esterase assays. Esterase activity deteriorated more rapidly than did MDH during shipment of samples from NZ to Texas.

Pathogenicity comparisons: Four white clover half-sib families were chosen from the breeding program selecting for resistance to *M. trifoliophila;* two half-sib families were partially resistant, and two were susceptible. In Palmerston North, pre-germinated seeds were sown singly in 60-mm-diam. pots containing the Manawatu sand-soil mix. In a galling response test, inoculum was prepared from either the Fitzherbert West culture of *M. trifoliophila* or from a culture established from a Tennessee isolate. In September 1995, inoculum was added as a suspension of ca. 2,000 eggs in 3 ml water around the roots of 2-week-old seedlings. To determine inoculum viability, eggs were added to small plastic dishes and hatched juveniles counted. After 6 days, 98% (N = 100) of the eggs of the Tennessee isolate and 50% (N = 100) of those from the Fitzherbert West isolate had hatched. Pots were arranged randomly in a single block, and trays of pots were rotated weekly. There were 10 plants (replicates) in each half-sib family. Plants were maintained as above, and, after 4 weeks, roots were washed free of soil and galls counted. In a reproduction test, two different pairs of half-sib families were inoculated with eggs of *M. trifoliophila* from the Fitzherbert West culture and from an *M. hapla* culture originating from kiwifruit roots in a Te Puke, NZ orchard. Inoculation protocol and maintenance conditions were as above. Eight weeks after inoculation, roots were washed free of soil, and eggs extracted and counted from all plants as described above.

Grasses experiment: Four seeds of seven grass species and of white clover were sown directly into each 60-mm-diam. pot of the Manawatu sand-soil mix. Ten pots per plant species were used. In December 1995, after 3 weeks, seedlings were thinned to two per pot and inoculated with ca. 2,000 eggs of *M. trifoliophila* from the Fitzherbert West culture. After 5 weeks, soil was washed from the roots, galls counted, and up to four galls per plant, when available, were examined microscopically. Portions of roots from each grass and from white clover were stained with Cotton Blue after the method of Byrd et al.

(1983) and examined under a dissecting microscope for the presence of nematodes and egg masses.

Pathotype test of populations: In October 1995, approximately 2 liters of soil and roots was collected from under white clover plants in permanent pasture at 25 sites around NZ. The sites represented a variety of climates and soils. At each site, cores were taken under 10 white clover plants and bulked in an effort to incorporate the inherent genetic diversity of each nematode population. Half the soil from each site was mixed with pasteurized sand (50:50 v/v), put into 60-mm-diam. pots, and sown with white clover seed. However, populations from all of the sites sampled in the South Island, and some of those from the North Island, failed to establish. After about 6 weeks the cultures were increased by washing soil from the white clover roots, extracting eggs in 0.5% NaOCl, and inoculating new white clover roots in pasteurized sand-soil mix. After about 18 weeks (ca. three generations of root-knot nematode [Mercer, 1990]), egg suspensions were prepared from each culture (Table 2) and inoculated onto two resistant and two susceptible half-sib families of white clover. Germinated seeds were sown singly into 60-mm-diam. pots of pasteurized sand-soil mix in March 1996 and inoculated 2 weeks later. There were 10 plants (replicates) per half-sib family. Plants were maintained as described previously and kept in one block, but those from each site were isolated from each other to prevent contamination. After 4 weeks, roots were washed free of soil and galls of *M. trifoliophila* or *M. hapla* counted.

Results

Root-knot nematode inoculum, prepared from an isolate from white clover in a pasture located in Fitzherbert West, NZ, did not cause galls on any plants in the host differential test conducted at Texas A&M University (Table 1), but white clover was severely galled. Egg production occurred in trace amounts on some plants in the host differential test, whereas large numbers were recovered from white clover. Of the six root-knot nematode populations compared in the test conducted in NZ, five showed the host response typical of *M. hapla* (Hartman and Sasser, 1985) (Table 1). The sixth population, obtained from the same Fitzherbert West culture used in the Texas A&M University test, reproduced only on white clover and not on the plants of the host differential test.

The several isolates of *M. trifoliophila* from NZ and the Tennessee isolate were characterized by a single esterase isozyme with an electrophoretic mobility of 0.32 (Fig. 1A). This esterase phenotype was distinct from the characteristic phenotypes of *M. hapla* and *M. javanica,* and from the characteristic esterase phenotypes of *M. arenaria* and *M. incognita* (data not shown). Some variation in esterase phenotype for *M. hapla* was observed in the form of an additional band of enzyme activity in ca. 10% of the individuals tested (Fig. 1B).

The MDH phenotypes for *M. hapla* and *M. trifoliophila* were each characterized by two bands of activity in 20 of 32 assays. These isozymes had electrophoretic mobilities of 0.56 and 0.64 for *M. hapla* and 0.45 and 0.53 for *M. trifoliophila* (Fig. 1C). No consistent variation in MDH phenotype was observed among the isolates of either species. For both *M. hapla* and *M. trifoliophila,* the occasional absence of the MDH isozyme with the greater mobility appeared to be related to electrophoretic conditions rather than biological variation because, when these isozymes were absent, they were absent from all samples for a given assay (Fig. 1D).

In the survey of root-knot nematode species associated with white clover in NZ pastures, the MDH phenotype was used to distinguish between *M. hapla* and *M. trifoliophila.* Ninety-two percent of the total of 268 individuals tested were identified as *M. trifoliophila,* and 4.5% were identified as *M. hapla* (Table 2). Only 3% of the individuals could not be identified based on their MDH phenotypes.

Root galls on white clover caused by *M. hapla* (isolate 4, Table 1) were round, 0.5 to 1 mm across ($N = 20$), colored the same as the rest of the root (beige), and frequently

TABLE 2. Identification of *Meloidogyne* species parasitizing white clover in New Zealand pasture using malate dehydrogenase (MDH) phenotype and number of galls on white clover half-sib families resistant and susceptible to *M. trifoliophila*.

Location of populations	Map reference[c]		MDH phenotype				Mean number of *M. trifoliophila* galls[a]				Mean number of *M. hapla* galls[b]	
							Resistant half-sib families		Susceptible half-sib families		Resistant half-sib families	Susceptible half-sib families
			Total	*M. trifoliophila*	*M. hapla*	Unknown	c17522	c17534	c17504	c17507	c17522 and c17534	c17504 and c17507
Carterton	S26	143 201	16	13	1	2	19 a	38 b	85 c	84 c	0.1	0
Dairy Flat	R10	029 572	15	14	1	0	20 a	24 a	83 b	86 b	0.3	0
Gisborne	Y18	496 659	13	13	0	0	8 a	22 b	68 c	60 c	0.1	0
Inglewood	Q19	276 109	—	—	—	—	22 a	47 b	147 c	115 c	0	0
Kai Iwi	R22	758 483	19	19	0	0	—	—	—	—	—	—
Keri Keri	P05	960 683	9	9	0	0	—	—	—	—	—	—
Levin	S25	613 051	14	12	0	2	22 a	23 a	77 b	66 b	0.2	0
Manaia	P21	080 829	20	20	0	0	33 a	39 a	106 b	70 c	0.1	0
Normanby	Q21	199 856	16	16	0	0	20 a	32 a	93 b	83 b	0	0
Opotiki	W15	873 342	17	12	5	0	31 a	54 b	124 c	111 c	0.6	0.3
Otaki	S25	445 925	16	16	0	0	19 a	29 a	97 b	86 b	0	0
Palmerston North	T24	877 318	—	—	—	—	39 a	55 a	145 b	128 b	0	0
Patea	Q22	377 603	30	27	3	0	17 a	23 a	83 b	81 b	0.5	0.1
Te Puke	V15	148 666	16	16	0	0	25 a	39 a	100 b	102 b	0.2	0
Tokanui	S15	143 448	16	14	1	1	—	—	—	—	—	—
Tutira	V20	455 122	25	23	1	1	17 a	13 a	69 b	56 b	0.1	0
Woodville	T24	928 508	25	23	0	2	20 a	31 a	74 b	78 b	0	0
				Mean			22 a	34 b	96 c	86 c	0.125	0.029
				Standard error			2	3	7	6	0.049	0.022

[a] Within a location, means without a letter in common differ significantly at $P = 0.05$ (ANOVA). Galls were counted 4 weeks after each plant was inoculated with ca. 2,000 eggs.
[b] Values are means for two half-sib families. Galls were counted 4 weeks after each plant was inoculated with ca. 2,000 eggs.
[c] Map references from New Zealand Map Series 260.

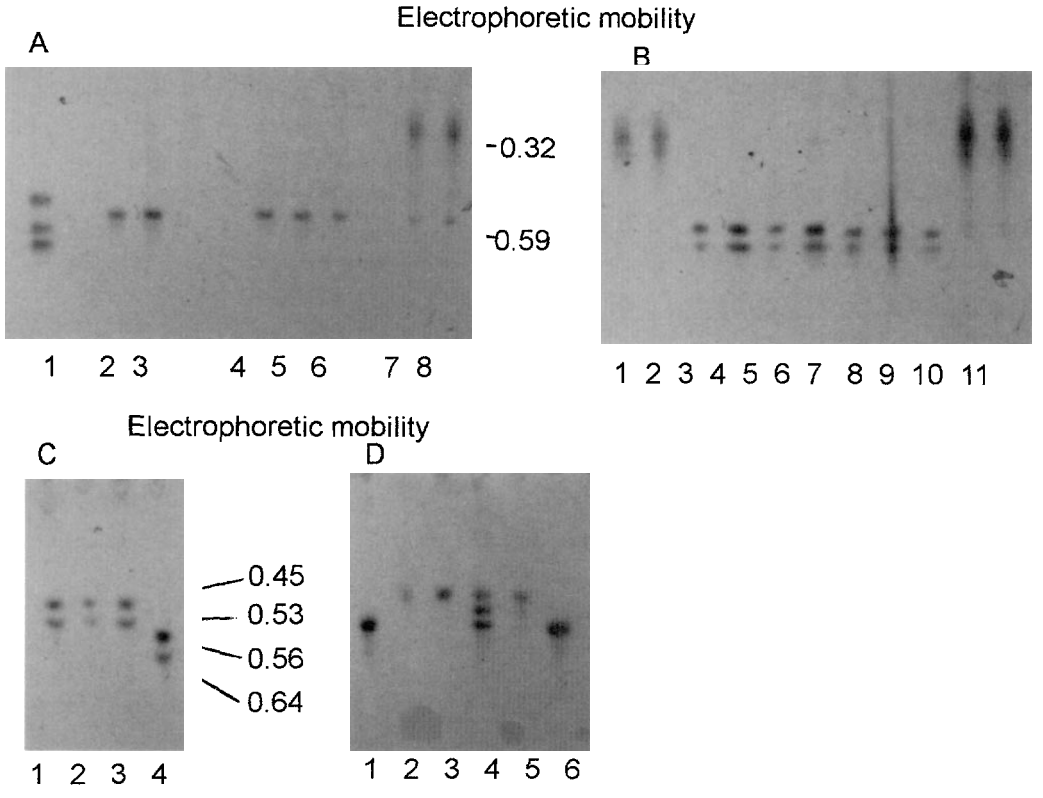

FIG. 1. Esterase and malate dehydrogenase (MDH) phenotypes for *Meloidogyne hapla, M. javanica,* and *M. trifoliophila.* A) Esterase phenotypes—lane 1 is *M. javanica* from Texas (TX); lanes 2,3 are *M. hapla* from New Zealand (NZ); lanes 4,5 are *M. hapla* from TX; lane 6 is *M. hapla* from the United Kingdom; lanes 7 and 8 are *M. trifoliophila* from NZ. B) Esterase phenotypes—lanes 1,2 are *M. trifoliophila* from Tennessee; lanes 3,4,5 are *M. hapla* from NZ; lanes 6,7 are *M. hapla* from TX; lanes 8,9 are *M. hapla* from the United Kingdom; lanes 10,11 are *M. trifoliophila* from NZ. Note in B the second esterase isozyme for all *M. hapla* individuals that was observed in ca. 10% of the assays. C) MDH phenotypes of *Meloidogyne* females collected from pastures in NZ lanes 1,2,3 are *M. trifoliophila;* lane 4 is *M. hapla.* D) MDH phenotypes of *Meloidogyne* females from pastures in NZ—lanes 1,6 are *M. hapla;* lanes 2,3, and 5 are *M. trifoliophila;* lane 4 is an unknown individual. Note in D the absence of the faster migrating MDH isozyme for both *M. hapla* and *M. trifoliophila.* This phenotype was observed in ca. 20% of the assays.

had a yellow egg mass protruding through the root gall epidermis (Fig. 2A). Inside the galls was one or occasionally two females. All but two of 40 *M. hapla* galls examined had adventitious, lateral roots. Galls caused by *M. trifoliophila* were wider and more elongate (mean length = 4 mm, mean width = 1.5 mm, N = 20) (Fig. 2B) than those of *M. hapla.* The *M. trifoliophila* galls were a cream color and lighter than the rest of the root, and less than 1% (N = 200) had a lateral root. Each gall contained 1 to 9 females and egg masses. Egg masses of *M. trifoliophila* were typically embedded within the galls rather than protruding from the gall surface.

For each isolate tested, the mean number of *M. trifoliophila* galls was lower on the resistant lines than on the susceptible ones (Table 2). Overall, the counts of *M. trifoliophila* on resistant half-sib families were about 31% of those on the susceptible ones. Galls typical of *M. hapla* developed from nine populations and were at lower numbers than *M. trifoliophila* galls. Counts of *M. hapla* galls were not compared statistically because of low numbers. However, the overall mean figure was not different between resistant and susceptible half-sib families (Table 2). Seven populations produced galls on half-sib families resistant to *M. trifoliophila* but not on susceptible half-sib families.

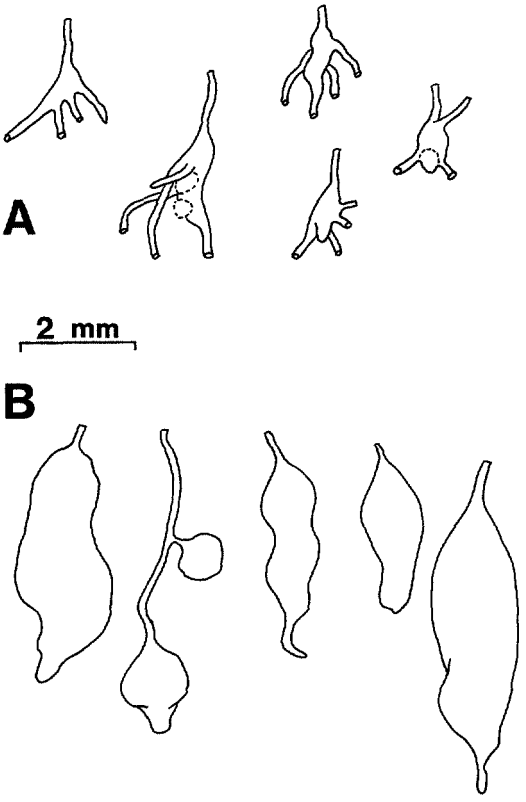

FIG. 2. Root galls from white clover inoculated 6 weeks earlier with A) *Meloidogyne hapla* or B) *Meloidogyne trifoliophila*. White clover root galls differ between nematode species as *M. trifoliophila* galls are large, elongate, and smooth compared to the *M. hapla* galls, which are small, round, inconspicuous, and generally have adventitious, lateral roots. Egg masses are indicated by dashed lines.

The resistance in two half-sib families from a white clover breeding program was effective in reducing galling by isolates of the Fitzherbert West and Tennessee populations of *M. trifoliophila* (Table 3). The resistance in another pair of half-sib families reduced egg production by *M. trifoliophila* but not by *M. hapla* (Table 4).

Few galls from *M. trifoliophila* infection were found on grass roots compared to on white clover roots (Table 5). *Poa annua* was the most heavily infected grass species with a mean of 12 galls per pair of plants. No development beyond swollen J2 was found in galls from *P. annua, P. dilatatum,* and *Eleusine indica.* In the single gall from a rice plant and in galls from *Alopecurus pratensis* and *Echinochloa crus-galli,* low numbers of females were found, but in every case no eggs were seen in the small gelatinous extrusion; males were not found in any galls from grasses. In the stained roots, no further galls, females, or egg masses were seen. In the galls on white clover roots, many males as well as egg masses with eggs were found.

TABLE 3. Galling response of resistant and susceptible half-sib families of white clover to isolates of *Meloidogyne trifoliophila* from Fitzherbert West, Palmerston North, New Zealand, and Tennessee, USA.

White clover seedline		Fitzherbert West		Tennessee	
		Number of galls	*N*	Number of galls	*N*
C1022	Resistant	7 a	10	5 a	10
C1019	Resistant	7 a	8	10 a	10
C1015	Susceptible	29 b	10	72 b	9
C1014	Susceptible	44 b	10	110 b	10

In columns, means without a letter in common differ significantly ($P = 0.001$). ANOVA was conducted on data transformed with $\log_{10}(x + 1)$ before analysis; geometric means are presented. *N* = number of plants.

DISCUSSION

The dominant root-knot nematode on white clover in NZ pasture has been determined as *M. trifoliophila.* The esterase and MDH phenotypes confirm the morphological comparison of NZ isolates with the paratypes of *M. trifoliophila* from Tennessee (E. C. Bernard, pers. comm.), and the survey shows that *M. trifoliophila* is probably the most numerous root-knot nematode species in the pastures examined.

The host differential tests gave an unam-

TABLE 4. Reproduction of *Meloidogyne trifoliophila* and *Meloidogyne hapla* on white clover half-sib families resistant or susceptible to *Meloidogyne trifoliophila.*

White clover seedline		*M. trifoliophila*		*M. hapla*	
		Number of eggs	*N*	Number of eggs	*N*
C17522	Resistant	68,000 a	6	69,000 a	7
C17534	Resistant	60,000 a	6	36,000 a	6
C17504	Susceptible	238,000 b	8	38,000 a	6
C17510	Susceptible	164,000 b	6	25,000 a	5

In columns, means without a letter in common differ significantly. ANOVA was conducted on data transformed to square roots before analysis; geometric means are presented. *N* = number of plants. Eggs were counted 8 weeks after each plant was inoculated with ca. 2,000 eggs.

TABLE 5. Gall numbers on six grasses and white clover inoculated with *Meloidogyne trifoliophila* eggs.

Plant species	Accession number or cultivar	Mean gall number[a]	Numbers of infected plant pairs and total number of plant pairs at harvest
Poa annua L.	Bp 1271	12.0	10, 10
Oryza sativa L.	Amaroo	0	0, 10
Oryza sativa L.	Pelde	0.1	1, 10
Paspalum dilatatum Poir.	Bo 224	0.1	1, 10
Alopecurus pratensis L.	Bz 2554	1.1	4, 9
Eleusine indica (L.) Gaertn.	Bz 2653	0.5	2, 6
Echinochloa cruss-galli (L.) Beauv.	Bz 2643	3.1	5, 10
Trifolium repens L.	Grasslands Huia	147	10, 10

[a] Counts are for two root systems. Galls were counted 4 weeks after each plant was inoculated with ca. 2,000 eggs.

biguous result for distinguishing *M. hapla* from *M. trifoliophila*. This is the first published result of a host differential test on NZ root-knot nematodes and confirms Dale's (1972) listing of carrots, kiwifruit, and white clover as hosts of *M. hapla*. Clark (1963) recorded white clover as a host of *Meloidogyne* sp. but did not record it as a host of *M. hapla* and so may have seen differences between *M. trifoliophila* and *M. hapla* 34 years ago. Further confirmation that some NZ isolates are *M. hapla* comes from MDH phenotypes, which were the same as for *M. hapla* from Texas and the United Kingdom. A host differential test takes too long for routine diagnostic purposes, but the characters of gall size and presence-absence of adventitious, lateral roots are sufficient to allow *M. trifoliophila* galls to be distinguished from those caused by *M. hapla*.

The esterase and MDH phenotypes of *M. trifoliophila* were clearly distinct from those of the other major *Meloidogyne* spp., including *M. hapla* (Esbenshade and Triantaphyllou, 1990). Although some variation was observed for both esterases and MDH, this appeared to be due to experimental error, as the same variation (an additional esterase isozyme for *M. hapla* or loss of an MDH isozyme for *M. trifoliophila* and *M. hapla*) could be observed for all individuals of a species for a single assay and was not consistent across repeated assays of any isolate. These variations were observed in less than 20% of the electrophoresis assays. No apparent difference in esterase or MDH phenotype was observed among the three isolates of *M. hapla* tested or between the NZ and Tennessee isolates of *M. trifoliophila*. The reported esterase and MDH phenotypes for *M. hapla* have only a single isozyme each (Esbenshade and Triantaphyllou, 1985, 1990), with relative electrophoretic mobilities corresponding to that of the slower migrating isozyme of each enzyme activity (0.59 for esterase and 0.56 for MDH in this study). These isozymes were always present in the individuals of the control isolates and were used for identification purposes. The variable detection of the second isozyme for each enzyme activity may be due to variability in extraction of individual females and the greater sensitivity of the Phastsystem relative to other electrophoretic systems.

In the survey of the *Meloidogyne* species associated with white clover in NZ pastures, 3% of the individuals had MDH phenotypes that were not characteristic of *M. hapla* or *M. trifoliophila*. The identity of these individuals is not known.

The reduced numbers of *M. trifoliophila* galls on resistant white clover from all NZ populations tested confirms an earlier finding (Mercer and Grant, 1993) that only one *M. trifoliophila* pathotype is likely to exist on white clover in NZ and that continued use of the local (Fitzherbert West) *M. trifoliophila* population is justified in the resistance breeding program. This pathotype test, together with the earlier one (Mercer and Grant, 1993), brings the number of populations tested to 18. The earlier study reported the root-knot nematode tested as *M. hapla*, but based on data from the current study it was undoubtedly *M. trifoliophila*. The reaction of resistant and susceptible half-sib

families of white clover to the NZ and Tennessee isolates suggests that both isolates are the same pathotype.

The incidence of *M. hapla* was low at the sites where it occurred, and so the species does not appear to present an immediate threat to the use of white clover varieties with resistance to *M. trifoliophila.* However, the possibility that a shift in species composition of the populations occurred in culture must be considered (Eisenback, 1993). In the field, the future use of white clover varieties with resistance to *M. trifoliophila* may increase selection of other nematode species (Young, 1992).

Early investigations showed *M. trifoliophila* to be morphologically similar to *M. graminicola,* which is a parasite of grasses and common in the rice-producing areas of Asia (Bridge et al., 1990). However, we suspected that the dominant root-knot nematode in NZ pastures was not *M. graminicola* because galls were not seen on grasses growing in pastures with heavily galled white clover. The inability of NZ isolates of *M. trifoliophila* to parasitize grasses was demonstrated in our results and confirms the report of Bernard and Jennings (1997) on host range. Windham and Pederson (1992) reported a number of clovers as hosts of a Mississippi isolate of *M. graminicola* but did not test their inoculum on any of the known grass hosts of *M. graminicola.*

The identification (Bernard and Eisenback, 1997) and further characterization of *M. trifoliophila* have allowed more accurate focus of NZ nematode research programs. The isozyme phenotypes and gall characters described above give convenient methods for distinguishing *M. trifoliophila* and *M. hapla.* Gall characteristics alone should be sufficient for determining the incidence of *M. trifoliophila* and *M. hapla* in routine surveys.

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