Resistance to *Meloidogyne incognita* in *Trifolium* Interspecific Hybrids and Species Related to White Clover

G. A. PEDERSON, Research Geneticist, and G. L. WINDHAM, Research Plant Pathologist, USDA-ARS-CSRL, Forage Research Unit, Mississippi State, MS 39762

ABSTRACT

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Eight species of *Trifolium* and seven interspecific hybrids of *Trifolium* related to white clover (*T. repens* L.) were evaluated for resistance to the southern root-knot nematode, *Meloidogyne incognita*. The highest percentages of resistant plants (gall index <2 on a scale of 0–5) were found in MS-2*x* of *T. ambiguum* (38%) and *T. nigrescens* (32%). No resistant plants were observed for *T. argutum*, *T. hybridum*, PI 368173 of *T. occidentale*, *T. repens*, or PI 341939 of *T. uniflorum*. Fertile interspecific hybrids with mean gall indices <2 were (*T. repens* × *T. nigrescens*) × *T. repens*, *T. isthmocarpum* × *T. repens*, and *T. repens* × *T. uniflorum*. Due to its cross-fertility and high level of resistance, *T. nigrescens* may be a valuable source of resistance to *M. incognita* for white clover.

White clover (*Trifolium repens* L.) is the major legume grown in permanent pastures in the southern United States. In this region, root-knot nematodes (*Meloidogyne* spp.) may be a limiting factor in clover production. *M. incognita* (Kofoid & White) Chitwood has been shown to limit the persistence of white clover (3) and restrict the forage yields of arrowleaf (*T. vesiculosum* Savi) and berseem (*T. alexandrinum* L.) clovers (20). Previous investigations have shown relatively little resistance to *M. incognita* in white clover (1,13,16), although a white clover germ plasm with root-knot nematode tolerance has been developed (6).

Interspecific hybridization of *Trifolium* spp. was first suggested as a method to improve white clover in the 1950s (4,14). Potential benefits of interspecific hybridization of *Trifolium* include disease and insect resistance, increased persistence, improved root systems, cold and drought tolerance, and seedling vigor (7,15,18). White clover has been successfully hybridized with six other *Trifolium* spp.: *T. nigrescens* Viv. (4), *T. uniflorum* L. (8,14,15), *T. argutum* Sol. (syn. *T. xerocephalum* Frenzl) (10), *T. occidentale* Coombe (syn. *T. repens* var. *biasolettii* (Steud. & Hochst.) Aschers. & Graebn.) (7), *T. isthmocarpum* Brot. (11), and *T. ambiguum* M. Bieb. (18,21).

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Few of the species compatible with white clover have been evaluated for resistance to *M. incognita*. Some 25 *Trifolium* spp., including *T. repens*, *T. isthmocarpum*, and *T. nigrescens*, have been found to be susceptible to *M. incognita* var. *acrita* (13). Interspecific hybrids of *Trifolium* have rarely been evaluated for characters other than agronomic growth and fertility.

The objective of this study was to determine the susceptibility of eight *Trifolium* spp. and seven interspecific hybrids of *Trifolium* to *M. incognita*.

MATERIALS AND METHODS

Eight species of *Trifolium*, including *T. repens*, *T. hybridum* L., and the six species with which interspecific hybrids with *T. repens* have been reported, were evaluated in this study. Seed of plant introductions of six species was obtained from the Regional Plant Introduction Stations at Experiment, GA, and Geneva, NY. Three populations of *T. ambiguum* produced at Mississippi State from parents with 16, 32, or 48 chromosomes were included. These populations were polycross seed from 35 diploid plants of 14 plant introductions (MS-2*x*), 75 tetraploid plants of 31 plant introductions (MS-4*x*), or 48 hexaploid plants of 23 plant introductions (MS-6*x*). Cuttings of six interspecific hybrids were obtained from E. Rupert, Clemson University, Clemson, SC (5). F_2 plants from the self-fertile hybrid 435 of *T. ambiguum* × *T. repens* (18) were obtained from N. L. Taylor, University of Kentucky, Lexington. Cuttings were made from the parent interspecific hybrids by stolon tips or by dividing the parent plants. Vigor of many of the interspecific hybrids was poor and the number of viable cuttings obtained from each hybrid varied.

Seedlings of the *Trifolium* spp. and cuttings of the interspecific hybrids were transplanted into Super Cell Cone-tainers containing a potting mixture of heat-sterilized sandy loam soil and river sand (80% sand, 6% clay, 14% silt). The planting was made on 18 September 1987. All plants were placed in a greenhouse maintained at a temperature of 26 ± 3 C.

A race 4 population of *M. incognita*, obtained from the Department of Plant Pathology, North Carolina State University, Raleigh, was increased on tomato (*Lycopersicon esculentum* Mill. 'Floradel') in the greenhouse. After 8–10 wk, eggs were collected from tomato roots using NaOCl (9). Four weeks after transplanting, plants of *Trifolium* spp. and interspecific hybrids were inoculated by pipetting a water suspension containing approximately 1,500 nematode eggs into each Cone-tainer. Approximately 60 days after inoculation, the root systems were carefully washed free of soil. Root systems were rated for galling using a gall index and for determining the percent of the root system galled. The gall index consisted of a 0–5 scale, with 0 = no galls, 1 = 1 or 2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 >100. The rating scale used to measure percent of the root system galled (PRSG) consisted of a 0–5 scale, with 0 = no galls, 1 = 1–10% of the root system galled, 2 = 11–25%, 3 = 26–75%, 4 = 76–90%, and 5 = 91–100%.

Fertility of the interspecific hybrids was based on seed production by the hybrid as a female parent. Hand crosses were attempted with white clover in the greenhouse, and open-pollinated crosses were produced in a space-planted study in the field.

The experiment was conducted using a completely randomized design with unequal replication. The data were ranked, and analyses of variance on the ranked data were performed. Means were separated by the Tukey-Kramer test for unequal replication when appropriate (17).

RESULTS AND DISCUSSION

The eight *Trifolium* spp. evaluated varied greatly in their response to *M. incognita*. MS-2*x* of *T. ambiguum* and

Table 1. Root gall ratings of *Trifolium* spp. following inoculation with *Meloidogyne incognita*

Trifolium spp.	Population	Plants inoculated (no.)	Gall index[a] 0	1	2	3	4	5	Mean	PRSG score[b]
T. argutum	PI 353407	23	0	0	7	15	1	0	2.7	2.5
T. ambiguum	MS-2*x*	104	14	26	51	13	0	0	1.6*[c]	1.0*
	MS-4*x*	104	3	17	63	21	0	0	2.0*	1.2*
	MS-6*x*	97	6	9	48	32	2	0	2.2*	1.6*
T. hybridum	PI 206763	107	0	0	0	25	47	35	4.1*	3.8*
T. isthmocarpum	PI 244679	41	0	2	11	28	0	0	2.6*	2.0*
T. nigrescens	PI 206769	40	3	10	15	11	1	0	2.0*	1.4*
T. occidentale	PI 368173	20	0	0	3	10	7	0	3.2	2.6
	PI 368174	8	0	1	3	3	0	1	2.6	1.2*
T. repens	Regal	109	0	0	10	71	24	4	3.2	2.8
T. uniflorum	PI 341938	10	2	0	8	0	0	0	1.4*	0.9*
	PI 341939	10	0	0	6	4	0	0	2.4*	2.5

[a] Gall index: 0 = no galls, 1 = 1 or 2 galls per root system, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 > 100.
[b] Percent of the root system galled: 0 = no galls, 1 = 1–10% of the root system galled, 2 = 11–25%, 3 = 26–75%, 4 = 76–90%, 5 = 91–100%.
[c] Significantly different from *T. repens* at $P < 0.05$ by the Tukey-Kramer test, with unequal replication.

Table 2. Reaction of interspecific hybrids of *Trifolium* to *Meloidogyne incognita*

Interspecific hybrid	Plant number	Cuttings inoculated (no.)	Gall index[a]	PRSG score[b]	Fertile
T. ambiguum × *T. hybridum*	1-2-1	2	2.0	1.0	No
	1-3-1	1	0.0	0.0	No
	1-3-4	5	5.0	5.0	No
T. ambiguum × *T. occidentale*	4-2-2	1	3.0	3.0	No
	4-2-3	2	5.0	5.0	No
T. ambiguum × *T. repens*	···	20[c]	2.5	2.5	Yes
T. isthmocarpum × *T. repens*	5-2-6	3	2.3	2.0	Yes
	5-4-5	10	1.6	1.2	Yes
(*T. repens* × *T. nigrescens*) × *T. repens*	2-3-1	5	0.8	0.6	Yes
	2-3-2	7	2.1	1.3	Yes
	2-3-3	1	0.0	0.0	Yes
T. repens × *T. occidentale*	5-2-2	10	2.6	2.0	Yes
	5-2-3	2	2.5	2.5	Yes
	5-4-1	10	2.5	2.1	Yes
	6-2-1	10	2.3	1.5	Yes
	6-2-2	7	3.0	3.0	Yes
	6-2-3	7	2.3	1.9	No
	6-2-6	10	3.0	2.2	No
T. repens × *T. uniflorum*	2-3-5	10	2.2	1.0	Yes
	2-3-6	4	0.2	0.2	No
	3-4-1	7	3.0	2.7	Yes
	3-4-2	7	2.7	2.1	No
	3-4-3	8	4.6	4.4	No
	3-4-4	9	1.3	1.0	Yes

[a] Gall index: 0 = no galls, 1 = 1 or 2 galls per root system, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 > 100.
[b] Percent of the root system galled: 0 = no galls, 1 = 1–10% of the root system galled, 2 = 11–25%, 3 = 26–75%, 4 = 76–90%, 5 = 91–100%.
[c] F_2 plants produced from seed (not cuttings).

PI 341938 of *T. uniflorum* had the most resistance to *M. incognita* with mean gall indices of 1.6 and 1.4, respectively (Table 1). Four of the species had mean gall indices less ($P < 0.05$) than *T. repens*. *T. hybridum* was the only species with a mean gall index greater ($P < 0.05$) than *T. repens*. With a gall index of 0 or 1 classified as resistant, 38% of MS-2*x* of *T. ambiguum* and 32% of the plants of *T. nigrescens* were resistant to *M. incognita*. No resistant plants were observed for *T. argutum*, *T. hybridum*, PI 368173 of *T. occidentale*, *T. repens*, or PI 341939 of *T. uniflorum*.

Our results disagree with those of McGlohon and Baxter (13), who reported that *T. repens*, *T. isthmocarpum*, and *T. nigrescens* were uniformly susceptible to *M. incognita* var. *acrita*. These differences are probably associated with variability of pathogenicity of geographical isolates of *M. incognita*, common in *Meloidogyne* spp. (12,19). Although *T. repens* appears to be uniformly susceptible to host races of *M. incognita* (Windham, *unpublished*), the response of *T. isthmocarpum* and *T. nigrescens* to host races of *M. incognita* is not known. Variation in pathogenicity of isolates of *M. incognita* within the same host race has also been reported (19).

PRSG scores followed a similar trend, with MS-2*x* of *T. ambiguum* and PI 341938 of *T. uniflorum* having the least galling and *T. hybridum* having the most (Table 1). There was a large difference between mean gall index and PRSG score (2.6 and 1.2, respectively) for PI 368174 of *T. occidentale*. The high gall index and low PRSG score indicated that, although the plant was a good host for the nematode, most of the root system remained fibrous (i.e., was not galled).

Gall indices of the three populations of *T. ambiguum* suggested a relationship between ploidy level and susceptibility to *M. incognita* (Table 1). Hexaploid (MS-6*x*) *T. ambiguum* had a higher ($P < 0.05$, Tukey-Kramer) gall index than the diploid MS-2*x*. No significant difference was found between the tetraploid (MS-4*x*) *T. ambiguum* and the other two populations. MS-2*x* had 38% resistant plants compared with 19 and 15% for MS-4*x* and MS-6*x*, respectively. As the three populations of *T. ambiguum* are synthetics of different parents, the relationship may be merely a measure of the variability within the species. Ploidy level did not appear to be related to nematode susceptibility in the other five diploid (*T. argutum*, *T. hybridum*, *T. isthmocarpum*, *T. nigrescens*, and *T. occidentale*) and two tetraploid (*T. repens* and *T. uniflorum*) species.

Considerable variability was observed for mean gall index among plants within interspecific hybrids of *Trifolium*. The hybrids of *T. repens* × *T. occidentale* showed the most consistent levels of susceptibility, with gall indices ranging from 2.3 to 3.0 (Table 2). Hybrids of *T. ambiguum* × *T. hybridum* and *T. repens* × *T. uniflorum* were the most variable, with mean gall indices ranging from 0.0 to 5.0 and 0.2 to 4.6, respectively. Of the interspecific hybrids with the least galling due to *M. incognita*, only four ([*T. repens* × *T. nigrescens*] × *T. repens* [plant numbers 2-3-1 and 2-3-3], *T. repens* × *T. uniflorum* [3-4-4], and *T. isthmocarpum* × *T. repens* [5-4-5]) were fertile and would be of use in transferring resistance to *M. incognita* to *T. repens*.

The hybrids of (*T. repens* × *T. nigrescens*) × *T. repens* (2-3-1 and 2-3-3) appeared to have the greatest potential, with mean gall indices of 0.8 and 0.0, respectively. Further backcrosses with *T. repens* will be evaluated to determine the feasibility of transferring this resistance.

A comparison of the mean gall index with the PRSG score demonstrated differences in the ability of hybrid root systems to maintain a high proportion of fibrous roots, relative to galled roots, under parasitism of *M. incognita*. The hybrids with high gall indices and low PRSG scores were *T. ambiguum* × *T. hybridum* (1-2-1), (*T. repens* × *T. nigrescens*) × *T. repens* (2-3-2), *T. repens* × *T. occidentale* (6-2-1), and *T. repens* × *T. uniflorum* (2-3-5) (Table 2). The ability of these hybrids to maintain a high proportion of fibrous roots while remaining a good host for the nematode may be an expression of tolerance. Selections for tolerance to *M. incognita* have already been made in white clover (6). Additional progress for nematode tolerance should be feasible in white clover without the use of interspecific hybrids.

Due to the multiple virus resistance and rhizomatous root system of *T. ambiguum*, the hybrid of *T. ambiguum* × *T. repens* has been of great interest recently (2,18,21). F_2 plants from the self-fertile hybrid 435 of *T. ambiguum* × *T. repens* (18) expressed a wide range of galling due to *M. incognita*, with gall indices ranging from 0 to 5. Though four resistant plants were observed, the very low fertility of these plants will make backcrossing to *T. repens* difficult. The resistance observed in the populations of *T. ambiguum* (Table 1) suggests that future hybrids developed from this source should also have good nematode resistance. The difficulty in obtaining hybrids of *T. ambiguum* × *T. repens*, however, may limit the usefulness of *T. ambiguum* as a source of resistance to *M. incognita*.

T. nigrescens appears to be the most valuable species to hybridize with *T. repens* to improve the resistance of white clover to *M. incognita*. Advantages for using *T. nigrescens* include the ease of producing the interspecific hybrid (relative to other interspecific hybrids of *Trifolium*), fertility of the hybrid and backcrosses, the level of resistance to *M. incognita* in *T. nigrescens*, and resistance to *M. incognita* of the present hybrids of (*T. repens* × *T. nigrescens*) × *T. repens*. *T. isthmocarpum* and *T. uniflorum* do not appear to be as useful due to their lower levels of nematode resistance, greater difficulty in producing hybrids with white clover, and greater variability in the fertility and nematode resistance of the present hybrids. Other researchers (8) have concluded that the hybrid of *T. repens* × *T. nigrescens* was of little value for white clover improvement due to poor agronomic characters and lack of virus resistance. Our results suggest that *T. nigrescens* may be a valuable source of resistance to *M. incognita* for white clover. Further selection and backcrossing to white clover would be necessary for the development of a commercially acceptable cultivar.

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