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Comparison of Reproduction by *Meloidogyne graminicola* and *M. incognita* on *Trifolium* Species[1]

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Abstract: The reproductive potential of *Meloidogyne graminicola* was compared with that of *M. incognita* on *Trifolium* species in greenhouse studies. Twenty-five *Trifolium* plant introductions, cultivars, or populations representing 23 species were evaluated for nematode reproduction and root galling 45 days after inoculation with 3,000 eggs of *M. graminicola* or *M. incognita.* Root galling and egg production by the two root-knot nematode species was similar on most of the *Trifolium* species. In a separate study, the effect of initial population densities (Pi) of *M. graminicola* and *M. incognita* on the growth of white clover (*T. repens*) was determined. Reproductive and pathogenic capabilities of *M. graminicola* and *M. incognita* on *Trifolium* spp. were similar. Pi levels of both root-knot nematode species as low as 125 eggs per 10-cm-d pots severely galled white clover plants after 90 days. *Meloidogyne graminicola* has the potential to be a major pest of *Trifolium* species in the southeastern United States.

Key words: clover, *Meloidogyne graminicola, Meloidogyne incognita,* nematode, pathogenicity, resistance, rice root-knot nematode, southern root-knot nematode, *Trifolium* spp.

The rice root-knot nematode, *Meloidogyne graminicola* Golden & Birchfield, has been isolated in three southeastern states of the United States. The rice root-knot nematode was first isolated from roots of barnyard grass, *Echinochloa colonum* L., collected in Louisiana in 1965 (1,4). In 1984, *M. graminicola* was found on purple nutsedge, *Cyperus rotundus* L., in Georgia (9). Recently, the rice root-knot nematode was isolated from white clover, *Trifolium repens* L., growing in a pasture in Oktibbeha County, Mississippi (14). This was the first report of a *Trifolium* sp. as a host for the rice root-knot nematode.

Additional information on the host status of *Trifolium* spp. for *M. graminicola* and the damage potential of *M. graminicola* on *Trifolium* spp. is needed. The objectives of this study were to compare the ability of *M. graminicola* and *M. incognita* (Kofoid & White) Chitwood (a known pathogen of *Trifolium* species [8,11,12,15]) to induce galls and reproduce on a number of *Trifolium* species and to compare the relationship between shoot growth of *T. repens* and initial inoculum density of *M. graminicola* to that of *M. incognita.*

Materials and Methods

Reproduction of M. graminicola and M. incognita on Trifolium species: A population of *M. graminicola* was isolated from white clover growing in a pasture in Oktibbeha County, Mississippi. A population of *M. incognita* race 4 was obtained from the Department of Plant Pathology, North Carolina State University, Raleigh. *Meloidogyne graminicola* and *M. incognita* were maintained on white clover (cv. Regal) and tomato (*Lycopersicon esculentum* Mill. cv. Floradel) in the greenhouse, respectively. After 8–10 weeks, eggs were collected from white clover and tomato roots with NaOCl (7).

Twenty-three *Trifolium* species, including two subspecies and a variety of *T. subterraneum* L., were evaluated in this study. Seeds were placed on water agar and incubated at 22 C. Germinated seed were transplanted into Super Cell Cone-tainers (Stuewe & Sons, Corvallis, OR) containing a methyl bromide-treated mixture of sandy loam soil and river sand (80% sand, 6% clay, 14% silt). A commercial preparation of *Rhizobium leguminosarum* biovar*tri-*

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minosarum biovar *trifolii* Jordan was broadcast over the seedlings and watered into the soil. Eight weeks after transplanting, seedlings were inoculated by pipetting a water suspension containing approximately 3,000 eggs of either *M. graminicola* or *M. incognita* into each cone-tainer. Plants were grown in a greenhouse maintained at ca. 26 C.

Forty-five days after inoculation, the root systems were carefully washed free of soil and stained with Phloxine B (3,6). Root systems were rated for galling with a gall index. The gall index (GI) consisted of a 0–5 scale, with 0 = 0, 1 = 1 or 2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = >100 galls. *Trifolium* sp. with mean GI ratings <3.0 were designated resistant. After rating for galling, roots were cut into 1-cm segments and comminuted in a blender for 5 seconds. Eggs were then extracted from each root system with NaOCl (7) and counted. Ostenbrink's (10) R factor (RF = final egg number/initial egg number) and the number of eggs per gram of fresh root were determined.

The experiment was conducted twice using a randomized complete block design with three replications per clover–nematode treatment. To compare reproduction and root galling between the two root-knot nematode species, data were subjected to statistical analysis of variance with a general linear models procedure (13). To compare the host status of the *Trifolium* species with *T. repens,* analyses of variance on the gall indices and RF values for *M. graminicola* were performed. Because viable seed of seven *Trifolium* species were available for only one run of the experiment, means were separated by the Tukey-Kramer test for unequal replication (13).

Effect of initial population densities on growth of T. repens: Trifolium repens seed were sown in 10-cm-d clay pots containing the potting medium described in the previous section, and seedlings were inoculated with *Rhizobium* in an identical manner. Inocula for *M. graminicola* and *M. incognita* were increased on Regal white clover and Floradel tomato, respectively. Initial population densities (Pi) were 0, 125, 250, 500, 1,000, 2,000, 4,000, 8,000, and 16,000 nematode eggs per pot. When 8 weeks old, seedlings were thinned to one per pot and each pot was infested with nematode eggs of the appropriate Pi level. Plants were grown in a greenhouse maintained at ca. 26 C.

After 90 days, clover shoots were harvested, dried, and weighed. At harvest, roots were carefully washed free of soil and stained with Phloxine B. Roots were rated for galling using the previously described GI and by determining the percentage of the root system galled (PRSG). The PRSG rating scale consisted of a 0–5 scale, with 0 = no galls, 1 = 1–10%, 2 = 11–25%, 3 = 26–75%, 4 = 76–90%, and 5 = 91–100% of the root system galled.

The experiment was conducted twice using a randomized complete block design with five replicates per treatment. Analysis of variance was performed on all data, and regression analyses compared white clover shoot growth with Pi. For these analyses, Pi were transformed to log $(x + 1)$. The homogeneity of regression coefficients was tested to compare the slopes using the procedure described in Statistical Procedures for Agricultural Research (5).

Results

Ability of M. graminicola and M. incognita to induce galls and reproduce on Trifolium species: Most of the *Trifolium* species evaluated were susceptible (GI $\geq$ 3.0) to *M. graminicola* (Table 1). Of the eight *Trifolium* species that had lower ($P \leq 0.05$) GI ratings than *T. repens,* only *T. carolinianum* Michx. was classified as resistant for *M. graminicola. Meloidogyne graminicola* had higher ($P \leq 0.05$) GI ratings than *M. incognita* on 12 of the 25 *Trifolium* genotypes. None of the *Trifolium* species inoculated with *M. incognita* had significantly higher GI ratings than those inoculated with *M. graminicola. Trifolium ambiguum* M. Bieb., *T. carolinianum,* and *T. tomentosum* L. inoculated

TABLE 1. Reproduction factor (RF), eggs per gram of fresh root (EGR), and galling of selected *Trifolium* species by *Meloidogyne graminicola* (Mg) and *M. incognita* (Mi).

Genotype	RF†			EGR			Gall index‡		
	Mg	Mi	*P* value	Mg	Mi	*P* value	Mg	Mi	*P* value
T. nigrescens PI 120139§	7.6	4.0	0.59	14,223	7,509	0.53	5.0	3.0	0.01
T. michelianum PI 120136§	7.2	2.4	0.22	7,266	1,299	0.23	5.0	4.6	0.42
T. alexandrinum Bigbee	7.1	5.5	0.47	7,817	4,713	0.04	5.0	4.0	0.01
T. repens Regal	6.6	2.9	0.09	11,666	3,342	0.07	5.0	3.6	0.01
T. lappaceum‖	6.3	6.9	0.73	6,833	4,641	0.20	5.0	4.3	0.01
T. subterraneum ssp. *subterraneum* Mt. Barker	5.8	5.2	0.61	4,964	3,975	0.32	5.0	3.5	0.01
T. vesiculosum Yuchi	5.7	1.8	0.01	3,168	935	0.01	5.0	4.1	0.02
T. rueppellianum PI 246354	5.6	3.6	0.15	8,280	5,629	0.39	5.0	3.7	0.02
T. isthomocarpum PI 244679	4.5	2.7	0.30	3,796	4,804	0.54	4.8	3.0	0.01
T. radiosum PI 206771§	4.4	4.0	0.20	7,957	3,161	0.92	5.0	3.5	0.47
T. incarnatum Chief	4.2	3.1	0.46	3,999	2,312	0.13	4.5	3.1	0.01
T. subterraneum var. *yanninicum* Meteora	4.1	3.0	0.23	7,221	2,332	0.07	5.0	3.1	0.01
T. resupinatum PI 110431	3.6	2.6	0.59	4,915	3,080	0.52	4.8	3.2	0.03
T. pratense Kenland	1.9	4.4	0.04	2,298	4,136	0.30	3.8*	3.6	0.74
T. subterraneum spp. *brachycalycinum* Clare	1.8	3.2	0.24	2,780	4,075	0.14	3.6	3.6	0.91
T. africanum PI 369885§	1.6	0.6	0.34	13,648	2,370	0.17	4.5	3.0	0.20
T. glomeratum PI 291788§	1.6	4.9	0.12	4,572	7,505	0.31	4.6	3.5	0.01
T. polymorphum PI 233554§	1.6	0.6	0.60	2,894	6,903	0.47	3.5*	4.0	0.40
T. occidentale PI 368173	1.4	0.4	0.01	9,150	5,390	0.07	3.2*	3.5	0.56
T. burchellianum PI 369916	1.0*	1.2	0.62	14,029	8,263	0.27	3.5*	3.8	0.38
T. fragiferum Palestine§	0.8*	1.8	0.19	2,062	2,863	0.08	5.0	3.5	0.20
T. tomentosum PI 238368	0.5*	0.6	0.74	7,121	7,607	0.88	3.0*	2.5	0.17
T. ambiguum MS-6X germplasm	0.3*	0.7	0.34	1,004*	2,001	0.17	3.1*	2.6	0.12
T. montanum PI 205314	0.2*	0.8	0.38	854*	5,000	0.28	3.5*	3.1	0.19
T. carolinianum¶	0.1*	0.3	0.05	2,015*	3,548	0.38	1.8*	2.6	0.06

* Significantly different from *T. repens* at $P = 0.05$ by the Tukey-Kramer test.
† RF = final number of eggs/initial number of eggs.
‡ Rating scale: 0 = no galls per root system, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, 5 = >100 galls per root system.
§ Viable seed for these species were available for only one run of the experiment.
‖ Collected in Mississippi.
¶ Collected in Texas.

with *M. incognita* were the only species with resistant GI ratings.

Reproduction by *M. graminicola* on 22 of the *Trifolium* species was comparable to reproduction by *M. incognita. Trifolium occidentale* Coombe. and *T. vesiculosum* Savi. had higher ($P \leq 0.05$) RF values for *M. graminicola* than for *M. incognita.* RF values for *M. incognita* were greater ($P \leq 0.05$) than RF values for *M. graminicola* on two clover species; they were less than those for *M. graminicola* on one species. *Trifolium ambiguum, T. carolinianum. T. tomentosum,* and *T. montanum* L. were the only species that maintained both root-knot species below initial population densities (RF values < 1.0). Only three *Trifolium* species had lower ($P \leq 0.05$) RF values than *T. repens* for both nematode species.

Reproduction by *M. graminicola,* as measured by eggs per gram of root (EGR), was also comparable to reproduction of *M. incognita* on the *Trifolium* species. *Trifolium alexandrinum* and *T. vesiculosum* had higher ($P \leq 0.05$) EGR values for *M. graminicola* than for *M. incognita.* None of the *Trifolium* species had significantly higher EGR values for *M. incognita* than for *M. graminicola. Trifolium ambiguum, T. carolinianum,* and *T. montanum* had lower ($P \leq 0.05$) EGR values than *T. repens* for *M. graminicola.*

Effect of initial population densities on growth of T. repens: The relationship between Pi to clover growth was best described by a linear model for *M. graminicola* and *M. incognita* (Fig. 1). As Pi increased, there was a sharp suppression of

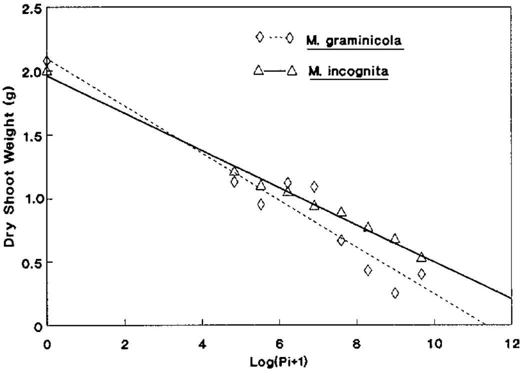

FIG. 1. Relationship between white clover dry shoot weights and initial nematode population density (Pi). $x = \log(x + 1)$ of Pi; *M. graminicola* linear regression equation: $y = 2.097 - 0.185x$, $r^2 = 0.92$; *M. incognita* linear regression equation: $y = 1.964 - 0.146x$, $r^2 = 0.99$.

white clover shoot growth. The test for homogeneity of regression coefficients indicated that the slopes for the linear models for *M. graminicola* and *M. incognita* were not different.

Both root-knot nematode species severely galled *T. repens,* as indicated by number of galls per root system and the amount of the root system galled. Although statistical differences ($P \leq 0.05$) for gall ratings between Pi levels were observed, all nematode-infected roots had highly galled roots. GI ratings ranged from 4.9–5.0 and 4.5–5.0 for *M. graminicola* and *M. incognita,* respectively. PRSG values for *M. graminicola* and *M. incognita* were also extremely high, with a range of 4.6–5.0 and 4.0–5.0, respectively.

DISCUSSION

In this study, *M. graminicola* severely galled the roots and increased in number on most of the *Trifolium* species. This report significantly increases the number of *Trifolium* species documented as hosts for *M. graminicola.* Previously, white clover (*T. repens*) was the only clover reported as a host for *M. graminicola* (14). Arrowleaf (*T. vesiculosum*), berseem (*T. alexandrinum*), and crimson (*T. incarnatum* L.) clovers, which are commonly grown in the Southeast as forage and cover crops, were excellent hosts for *M. graminicola.* Additional leguminous hosts of *M. graminicola* include *Glycine max* (L.) Merr., *Phaseolus vulgaris* L., and *Vicia faba* L. (2).

Meloidogyne graminicola galled roots and reproduced at higher rates than *M. incognita* on most of the *Trifolium* species evaluated. The most striking example was *T. vesiculosum,* which had significantly higher GI levels and reproduction by *M. graminicola* than by *M. incognita.* Although the two root-knot species varied in their ability to gall *Trifolium* species, the level of root galling indicated that most of the *Trifolium* species evaluated were susceptible to both root-knot nematode species.

With GI scores as a measure of resistance, none of the *Trifolium* species was susceptible to one root-knot species and resistant to the other, except for *T. ambiguum* and *T. tomentosum.* Kura clover (*T. ambiguum*) had a susceptible gall rating for *M. graminicola* (3.1) and a resistant gall rating for *M. incognita* (2.6). This germplasm had previously been reported to have moderate resistance to *M. incognita* and suggested as a possible source of nematode resistance for *T. repens* (11).

Two native clover species performed well in our evaluations. Carolina clover (*T. carolinianum*), which grows wild in the southeastern United States, had the lowest RF values for both root-knot species and a resistant GI score for *M. graminicola. Trifolium carolinianum* is also resistant to Florida populations of *M. incognita* (K. H. Quesenberry, pers. comm.). Buffalo clover (*T. reflexum* L.), another Southeastern native with root-knot nematode resistance, showed little root galling and supported low reproduction for both nematode species (Windham, unpubl. data). The use of native clover species as forage crops and for nematode management has not been fully exploited and should be considered in future studies.

Several clovers (i.e., *T. africanum* Ser., *T. burchellianum, T. montanum,* and *T. occidentale*) had relatively low RF values for both or one of the root-knot species, which would indicate these clovers are poor hosts. However, these plants had less vigorous root systems than most of the other clovers, which contributed to the low RF values. By determining nematode reproduction on a per gram of root basis (EGR), these clovers were found to support nematode numbers similar to the other clover species.

The ability of *M. graminicola* to suppress white clover growth in comparison with *M. incognita* emphasizes the potential importance of this nematode species. In addition to reproducing on clovers, a number of plants in the Gramineae family are hosts for *M. graminicola,* which increases the number of potential hosts in forage production systems (2). There is a need for additional investigations to determine the incidence of *M. graminicola* and its damage potential on clovers in the southeastern United States. Depending on the distribution of *M. graminicola,* this nematode could be a major limiting factor in production of *Trifolium* species.

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