AGGRESSIVENESS OF *MELOIDOGYNE INCOGNITA* HOST RACES ON WHITE CLOVER[1]

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Accepted:
4.X.1989
Aceptado:

ABSTRACT

Windham, G. L., and G. A. Pederson. 1989. Aggressiveness of *Meloidogyne incongnita* host races on white clover. Nematrópica 19:177–183.

The host efficiency and sensitivity of three *Trifolium repens* cultivars (Regal, Louisiana S-1, Osceola) and two germplasms (SC-1, Brown Loam Synthetic No. 6) to eight populations of *Meloidogyne incognita* representing races 1, 2, 3, and 4 were determined in greenhouse experiments. Roots were stained with Phloxine B and rated for nematode egg masses and galling. The three cultivars and two germplasms were excellent hosts for all eight *M. incognita* populations. Populations from all four *M. incognita* host races severely galled the roots of the white clover cultivars and germplasms. A tolerance index was calculated for each white clover-nematode treatment. SC-1, which was released as a root-knot tolerant germplasm, exhibited moderate tolerance to a race 1 and a race 4 population, and Louisiana S-1 was moderately tolerant to a race 2 population.

Key words: aggresssiveness, *Meloidogyne incognita*, reproduction, southern root-knot nematode, tolerance, *Trifolium repens*, white clover.

RESUMEN

Windham, G. L., y G. A. Pederson. 1989. Agresividad de estirpes de hospedantes de *Meloidogyne incognita* en trébol blanco. Nematrópica 19:177–183.

Se determinó con experimentos de invernadero la eficacia y susceptibilidad de tres cultivares de *Trifolium repens* (Regal, Louisiana S-1, Osceola) y de dos germoplasmas (SC-1, Brown Loam Synthetic No. 6) a poblaciones de *Meloidogyne incognita* representantes de las razas 1, 2, 3, y 4. Las evaluaciones se efectuaron tiñendo las raíces con Floxina B y determinando el grado de agallamiento y el número de masas de huevecillos. Los tres cultivares del trébol fueron buenos hospedantes de las ocho poblaciones de *M. incognita* agallando severamente las raíces. Se calculó un indice de tolerancia para cada uno de los tratamientos trébol-nematodo con el cual SC-1 presentado como germoplasma tolerante al agallamiento de la raiz resultó ser moderadamente tolerante a las poblaciones de las razas 1 y 4 y el Louisiana S-1 moderadamente tolerante a la población de la raza 2.

Palabras claves: agresividad, *Meloidogyne incognita*, reproducción, nematodo agallador sureño, tolerancia, *Trifolium repens*, trébol blanco.

[1]Contribution of the USDA Agricultural Research Service in cooperation with the Mississippi Agricultural and Forestry Experiment Station, Mississippi State, MS 39762. Published as Paper J-7191 of the Mississippi Agricultural and Forestry Exeriment Station.

INTRODUCTION

White clover (*Trifolium repens* L.) is one of the most widely grown forage legumes in the southern United States. Although generally classified as a perennial, clover stands usually begin to decline by the second or third year after planting. Root-knot nematodes have been suggested as one of several pathogens that may limit white clover growth (1,2,13). The susceptibility of *T. repens* to *Meloidogyne* spp. has been well documented (11,12). Symptoms include severe galling of roots and stunted shoot growth.

The aggressiveness of several *Meloidogyne* species that occur in the southeastern United States was demonstrated on *T. repens* (4). However, the variation in aggressiveness of *M. incognita* (Kofoid & White) Chitwood host races on white clover has not been reported. Resistance to *M. incognita* in certain crops may be race secific (10), and may be population specific within host races (16).

Control measures for root-knot nematodes in forage production systems are limited. The expense of nematicides and low crop value make chemical control prohibitive. Although some progress has been reported in identifying resistant genotypes (3), no root-knot resistant white clover cultivars are available. A white clover germplasm (SC-1) which was developed for tolerance to *M. incognita* has been released (7). The tolerance of SC-1 to the four host races of *M. incognita* has not been reported.

The objectives of our study were 1) to compare the tolerance of SC-1 with other white clover germplasms and cultivars to the four host races of *M. incognita,* and 2) to determine the variation in aggressiveness of the *M. incognita* host races on *T. repens.*

MATERIALS AND METHODS

Eight populations of *M. incognita* representing the four host races were selected for this study (Table 1). Race 1, 2, and 3 populations were collected in Mississippi by Dr. S. C. Bost, University of Tennessee, and a race 4 population was obtained from Dr. K. R. Barker, North Carolina State University. Inoculum was increased on *Lycopersicon esculentum* Mill. cv. Floradel. After 8–10 weeks, eggs were collected from tomato roots using NaOCl (9).

White clover germplasms used in this study were SC-1 and Brown Loam Synthetic No. 6. The white clover cultivars included 'Regal', 'Osceola', and 'Louisiana S-1'. Seeds were germinated on water agar and incubated at 22 C. Germinated seed were transplanted into Super Cell Cone-tainers® (Ray Leach Cone-tainer Nursery, Canby, Oregon, U.S.A.) containing a heat sterilized mixture of sandy loam soil and river sand (80% sand, 6% clay, 14% silt). A commercial preparation of *Rhizobium leguminosarum* biovar *trifolii* Jordan was broadcast over the seedlings and

Table 1. Designation of *Meloidogyne incognita* populations by number, state of origin, and host race.

Population no.	State of origin	Host race
MS-4	Mississippi	1
MS-11	Mississippi	1
MS-13	Mississippi	1
MS-8	Mississippi	2
MS-3	Mississippi	3
MS-5	Mississippi	3
MS-6	Mississippi	3
NC-1	North Carolina	4

watered into the soil. Four weeks after transplanting, seedlings were inoculated with the appropriate nematode population by pipetting 1 500 eggs over the root system. Noninoculated controls for each white clover line were included to determine the effect of the nematode populations on plant growth. Plants were grown in a greenhouse at 29 ± 5 C. When the experiment was repeated, the average temperature was 29 ± 6 C.

After 60 days, shoots were harvested, dried, and weighed. Roots were carefully washed free of soil and stained with Phloxine B (6,8). Nematode reproduction was determined by counting egg masses and using the following scale: 0 = 0; 1 = 1–2; 2 = 3–10; 3 = 11–30; 4 = 31–100; and 5 = > 100 egg masses per root system. Root galling was assessed by determining the percent of the root system galled (PRSG). The PRSG rating scale was: 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 experimental design was a 5 × 8 factorial in a randomized complete block with ten replications. The factors were five white clover lines and eight *M. incognita* populations. Data from both experiments were combined for analysis. Analysis of variance was performed on all data to determine clover line effects, nematode population effects, and interactions. Nematode reproduction and effects on each line were compared by least significant differences (LSD) $P = 0.05$. A tolerance index [(shoot dry weight of nematode-infected clover/shoot dry weight of non-infected clover) × 100] was calculated for each white clover-nematode treatment. Levels of tolerance were defined as high (index values of 96 to 100), moderate (index values of 68 to 95), or intolerant (index values of 0–67) (5).

RESULTS

Roots of the five white clover lines were galled severely by all eight root-knot nematode populations from the four host races (Table 2). Although there were significant ($P = 0.05$) differences in the amount of galling caused by the different nematode populations, all of the pop-

Table 2. Root gall ratings for five lines of white clover following inoculation with eight *Meloidogyne incognita* populations.

M. incognita		PRSG Score/line[z]					
Race	Population no.	SC-1	Brown Loam Synthetic No. 6	Regal	Louisiana S-1	Osceola	Mean
1	MS-4	4.1	4.6	4.6	4.9	4.3	4.5
1	MS-11	4.3	4.7	4.0	4.3	4.3	4.3
1	MS-13	4.3	4.3	4.1	4.1	4.1	4.1
2	MS-8	4.3	4.2	4.1	4.3	4.6	4.3
3	MS-3	4.0	4.3	4.3	4.0	4.3	4.1
3	MS-5	4.0	4.3	4.3	4.2	3.9	4.1
3	MS-6	3.9	4.2	4.2	4.1	4.4	4.1
4	NC-1	3.5	3.9	3.7	3.5	3.6	3.6
LSD (0.05)		0.6	0.6	0.5	0.5	0.5	

[z]PRSG (percent root system galled) rating scale: 0 = no galls; 1 = 1–10%; 2 = 11–25%; 3 = 26–75%; 4 = 76–90%; and 5 = 91–100% of the root system galled.

ulations were pathogenic to white clover. The NC-1 population tended to be less aggressive, having the lowest mean PRSG score (3.6) on the white clovers tested. The most aggressive nematode population was MS-4 which had a mean PRSG score of 4.5. No differences ($P = 0.05$) were observed among the five white clover lines for PRSG score. Although the nematode-tolerant germplasm (SC-1) had the lowest mean PRSG score, the roots of SC-1 were galled severely by all *M. incognita* populations. No interactions among white clover lines and nematode populations were observed for PRSG score.

All of the white clover lines were excellent hosts for the eight populations of the four *M. incognita* host races (Table 3). Nematode reproduction was high on SC-1 as well as the other white clovers. Egg mass

Table 3. Reproduction of eight populations of *Meloidogyne incognita* on five lines of white clover.

M. incognita		Egg mass index/line[z]					
Race	Population no.	SC-1	Brown Loam Synthetic No. 6	Regal	Louisiana S-1	Osceola	Mean
1	MS-4	4.4	4.7	4.7	4.9	4.5	4.6
1	MS-11	4.6	4.9	4.4	4.6	4.4	4.5
1	MS-13	4.7	4.7	4.8	4.8	4.5	4.7
2	MS-8	4.9	4.9	4.7	4.7	4.9	4.8
3	MS-3	4.4	4.5	4.7	4.6	4.5	4.5
3	MS-5	4.7	4.4	4.7	4.6	4.3	4.5
3	MS-6	4.8	4.7	4.9	4.7	4.9	4.8
4	NC-1	4.2	4.7	4.6	4.5	4.4	4.4
LSD (0.05)		0.2	0.4	0.3	0.3	0.4	

[z]Rating scale: 0 = 0; 1 = 1–2; 2 = 3–10; 3 = 11–30; 4 = 31–100; and 5 = > 100 egg masses/root system.

indices for SC-1 ranged from 4.2 for race 4 to 4.9 for race 2. Mean egg mass indices ranged from 4.4 to 4.8 for the NC-1 and MS-8 nematode populations, respectively. Although statistical differences between nematode populations for reproduction were observed, all of the five white clover lines were very efficient hosts for *M. incognita.*

The white clover lines were tolerant to few of the *M. incognita* populations (Table 4). SC-1, which was released as a nematode tolerant germplasm (6), showed only moderate tolerance to two nematode populations, MS-11 and NC-1. The tolerance index values for SC-1 ranged from 38 to 85. Louisiana S-1 exhibited moderate tolerance to a race 2 population (MS-8). Brown Loam Synthetic No. 6, Regal, and Osceola were intolerant to all eight root-knot nematode populations. No interactions between white clover lines and *M. incognita* populations were observed for tolerance.

DISCUSSION

Resistance to *M. incognita* in white clover is a desirable method of controlling this pathogen in pasture and forage systems. Use of resistant cultivars is a more economical method of nematode control than use of nematicides, and presents no hazards to the environment. Limited progress has been made in identifying white clover genotypes with root-knot nematode resistance (3,13). Interspecific hybrids related to white clover also have been evaluated for nematode resistance (12). A *T. repens* × *T. nigrescens* Viv. backcross hybrid exhibited a high level of resisance to *M. incognitá. T. nigrescens* is partially cross fertile with white clover and may be a valuable source of resistance. At present, no *M. incognita*-resistant cultivars of white clover are available.

Table 4. Tolerance index for five lines of white clover to eight populations of *Meloidogyne incognita.*

M. incognita		Tolerance index/line[z]					
Race	Population no.	SC-1	Brown Loam Synthetic No. 6	Regal	Louisiana S-1	Osceola	Mean
1	MS-4	38	38	42	27	32	35
1	MS-11	69	33	47	39	35	44
1	MS-13	55	39	41	47	45	45
2	MS-8	54	43	48	75	52	54
3	MS-3	65	43	39	59	42	49
3	MS-5	50	64	56	45	48	52
3	MS-6	39	50	44	46	37	43
4	NC-1	85	63	52	62	51	62
LSD (0.05)		25	24	NS	21	NS	

[z]Tolerance index = (dry shoot weight of nematode-infected plants/dry shoot weight of check plants) × 100.

Tolerance is an alternative form of control. However, SC-1, the only germplasm having root-knot tolerance (7), exhibited moderate tolerance to only two of the *M. incognita* populations in this study. In addition, SC-1 was an excellent host for all populations of *M. incognita*. High nematode reproduction is a major problem associated with cultivars having tolerance to nematodes. Populations increase during the growing season and thus high numbers of nematodes are present to infect, in the case of clovers, companion grasses or subsequent crops. Also, tolerant crops infected with large numbers of nematodes are more likely to be predisposed to root-rot disease complexes (14).

The white clover genotypes evaluated in this study were uniformly susceptible to all of the *M. incognita* populations. No differences in pathogenicity among the *M. incognita* host races were observed. These results agree with the findings of Windham and Barker (16). Differences in host efficiency of plants not included in the North Carolina Differential Host Test (15) are found among geographically different root-knot nematode populations within the same race, so resistance may not necessarily be race specific.

Since all populations were pathogenic to white clover, any of the root-knot nematode populations in our study could be used to screen for resistance. The NC-1 population would be the most preferred population to use to identify moderate levels of resistance because it had the lowest mean PRSG score and the highest mean tolerance index. The Mississippi populations were so aggressive that only very high levels of resistance could be identified. Use of highly aggressive populations for initial evaluations would prevent identification of white clover with moderate levels of resistance. MS-4 which had the highest PRSG score and the lowest mean tolerance index could be used in subsequent testing for high levels of resistance once a source of moderate *M. incognita* resistance in white clover was identified.

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Received for publication: *4.V.1989*
Recibido para publicar:

ACKNOWLEDGEMENTS

We thank Luther Brister, Mary Hardy, and James Manuel for technical assistance in this study.