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# Reaction of *Trifolium repens* Cultivars and Germplasms to *Meloidogyne incognita*[1]

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*Abstract:* Ten cultivars and 13 germplasms of white clover (*Trifolium repens*) were evaluated in the greenhouse for resistance to the southern root-knot nematode, *Meloidogyne incognita* race 4. One hundred plants of each cultivar or germplasm were rated for percentage of the root system galled (PRSG) at 60 days after inoculation with root-knot nematode eggs. Tillman (9%) and SRVR (19%) had the highest percentage of resistant plants (PRSG = 0 or 1 on a scale of 0–5) for the cultivars and germplasms, respectively. No resistant plants were selected from the cultivars California Ladino or Sacramento, or from the germplasms Brown Loam population or Brown Loam Synthetic #6. Resistant plants identified in this study were used to initiate a recurrent selection program for resistance to *M. incognita*.

*Key words:* clover, *Meloidogyne incognita*, nematode, resistance, southern root-knot nematode, *Trifolium repens*.

White clover (*Trifolium repens* L.) is one of the most widely grown pasture legumes in the southern United States. *Trifolium repens*, an excellent forage crop, provides nitrogen to companion plants by fixing atmospheric nitrogen; it can also be used as a cover crop (8). Although white clover is a perennial, stands often begin declining by the second or third year after planting. Root-knot nematodes (*Meloidogyne* spp.) are considered one of the major biotic factors contributing to this lack of persistence (1,2,4). Clover roots infected with root-knot nematodes are stunted, may be heavily galled, and can deteriorate rapidly, which leads to plant death. *Meloidogyne* spp. are particularly damaging to white clover following the hot, dry summer months of July and August and can severely limit forage production in the fall (4,17).

Control of root-knot nematodes in forage production systems is difficult. Use of nematicides is not desirable because of the expense, the low crop value, and the associated environmental hazards. Tolerance has been considered as a strategy for managing *Meloidogyne* spp. on white clover. SC-1 was released in 1973 as a "root-knot nematode tolerant" germplasm (6). The usefulness of this germplasm appears to be limited. In a recent study (16), SC-1 was moderately tolerant to only two or eight populations of *M. incognita* (Kofoid & White) Chitwood. The eight *M. incognita* populations reproduced as well or better on SC-1 as on the other germplasms or cultivars tested.

Selection of white clover with root-knot nematode resistance has had limited success in the past. Bain (3) selected seedlings that had "partial" resistance to *M. incognita acrita* Chitwood & Oteifa. Several white clover introductions were also identified with resistance to root-knot nematodes (3). In Alabama, 10,000 white clover plants were evaluated to identify genotypes resistant to *M. incognita acrita* (15), and plants with moderate root-knot nematode resistance were identified. Interspecific hybrids related to white clover also have been screened for root-knot nematode resistance (13). A backcross hybrid of *T. repens* × *T. nigrescens* Viv. exhibited a high level of resistance to *M. incognita*. *Trifolium nigrescens* is partially cross fertile with white clover and may be a valuable source of resistance.

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Even though resistant white clover genotypes have been identified, no germplasm or cultivar resistant to root-knot nematodes has been released. Research has not been conducted to evaluate the resistance of recently developed or released white clover cultivars and germplasms. The objectives of our study were to determine the reaction of white clover cultivars and germplasms to *M. incognita* and to select plants resistant to root-knot nematodes to incorporate into a white clover breeding program.

## Materials and Methods

The white clover cultivars evaluated were Arcadia, California Ladino, Dusi, Louisiana S-1, Nolin's Improved, Osceola, Regal, Sacramento, Tamar, and Tillman. Germplasms tested included Brown Loam population, Brown Loam Synthetic #6, C/W 600, C/W L-8-100, C/W L-8-101, Florida XP-2, Florida XP-3, K-6-8, KO 176 (Arcadia), NC-7, SC-1, SC Medium Flowering, and Southern Regional Virus Resistant (SRVR). Seeds were germinated on water agar at 22 C. Germinated seed were transplanted into Super Cell Cone-tainers (Ray Leach Cone-tainer Nursery, Canby, OR) containing a methyl bromide-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 watered into the soil.

A race 4 population of *M. incognita* was obtained from Dr. K. R. Barker of North Carolina State University. Inoculum was increased on *Lycopersicon esculentum* Mill. cv. Floradel in greenhouse pot cultures. After 8 to 10 weeks, eggs were collected from tomato roots with NaOCl (10). Approximately 4 weeks after transplanting, seedlings were inoculated by pipetting 1,500 eggs over the root system. The cultivar and germplasm evaluations were conducted separately. Cultivars were grown in a greenhouse at 28 ± 4 C, and germplasms were grown at 29 ± 5 C. Cultivars were transplanted on 30 July 1986, they were inoculated with *M. incognita* on 4 September 1986, and their root systems were rated on 4 November 1986. Germplasms were transplanted on 4 March 1987, they were inoculated with *M. incognita* on 6 April 1987, and their root systems were rated on 6 June 1987.

To evaluate root galling, roots were carefully washed free of soil and stained with Phloxine B (5,9). Root galling was assessed by determining the percentage of the root system galled (PRSG). The PRSG rating scale was as follows: 0 = no galling, 1 = 1–10%; 2 = 11–25%; 3 = 26–75%; 4 = 76–90%; and 5 = 91–100% of the root system galled. Dead plants were given a rating of 5. Plants with a 0 or 1 PRSG score were considered resistant to *M. incognita* and were repotted for crossing and evaluation of the progeny.

The experimental design was a randomized complete block with four replicates of 25 plants from each entry. The data for the two evaluations were analyzed separately by analysis of variance to determine differences among white clover cultivars and germplasms. Cultivar or germplasm means were compared by Fisher's protected least significant difference test (FLSD), $P = 0.05$.

## Results

All of the white clover cultivars were very susceptible to the *M. incognita* race 4 population (Table 1). Although differences ($P = 0.01$) were found among cultivars, all were excellent hosts for the southern root-knot nematode. Numerically, Nolin's Improved had the highest PRSG score (4.5), and Louisiana S-1, Osceola, and Tillman had the lowest (3.8). Tamar had the greatest number of highly susceptible plants (scores of 4 or 5), with 87, whereas Louisiana S-1 had the least, with 58. The cultivars with the highest and lowest mortality rates were Tamar (29%) and Louisiana S-1 (3%). Of the 1,000 plants representing 10 cultivars, only 29 plants were classified as resistant (scores of 0 or 1). The greatest number of resistant plants were selected out of Tillman (9 plants). No resistant plants

TABLE 1. Root gall ratings and percentages of mortality of 10 *Trifolium repens* cultivars following inoculation with *Meloidogyne incognita.*

| Cultivar | Number of plants with PRSG† score | | | | | | PRSG Mean | Percentage of mortality |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | | |
| Arcadia | 1 | 0 | 5 | 21 | 17 | 56 | 4.2 | 8 |
| California Ladino | 0 | 0 | 3 | 18 | 41 | 38 | 4.1 | 5 |
| Dusi | 2 | 1 | 4 | 11 | 10 | 72 | 4.4 | 19 |
| Louisiana S-1 | 0 | 6 | 1 | 35 | 25 | 33 | 3.8 | 3 |
| Nolin's Improved | 3 | 0 | 3 | 9 | 8 | 77 | 4.5 | 6 |
| Osceola | 4 | 0 | 8 | 18 | 36 | 34 | 3.8 | 11 |
| Regal | 1 | 1 | 3 | 12 | 21 | 62 | 4.4 | 9 |
| Sacramento | 0 | 0 | 4 | 18 | 33 | 45 | 4.2 | 14 |
| Tamar | 1 | 0 | 2 | 10 | 27 | 60 | 4.4 | 29 |
| Tillman | 6 | 3 | 8 | 20 | 13 | 50 | 3.8 | 4 |
| FLSD ($P = 0.05$) | | | | | | | 0.3 | 8 |

† Percentage of the root system galled: 0 = no galls, 1 = 1–10%, 2 = 11–25%, 3 = 26–75%, 4 = 76–90%, 5 = 91–100%. Dead plants were given a 5 rating.

were found in the cultivars California Ladino or Sacramento.

The white clover germplasms were also very susceptible to *M. incognita* (Table 2). Numerically, the Brown Loam population had the highest PRSG score (4.4), and SRVR had the lowest score (2.8). The Brown Loam population had the highest mortality rate (15%) and also had the highest number of highly susceptible plants (84). SRVR had the lowest mortality rate (2%) and the least number of highly susceptible plants (23). Of 1,300 plants evaluated from the 13 germplasms, 54 were classified as resistant. SRVR and C/W L-8-100 had the most resistant plants, with 19 and 10, respectively. No resistant plants were found in the Brown Loam population or in Brown Loam Synthetic #6. Arcadia (KO 176), the only entry included in both tests, had PRSG scores of 4.2 in the cultivar evaluation and 3.9 in the germplasm evaluation. Three cultivars (Osceola, Tillman, and Louisiana S-1) and five germplasms (NC-7, C/W L-8-

TABLE 2. Galling reaction and percentage of mortality of 13 *Trifolium repens* germplasms to *Meloidogyne incognita.*

| Germplasm | Number of plants with PRSG† score | | | | | | PRSG mean | Percentage of mortality |
|---|---|---|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | 4 | 5 | | |
| Brown Loam population | 0 | 0 | 0 | 16 | 26 | 58 | 4.4 | 15 |
| Brown Loam Synthetic #6 | 0 | 0 | 15 | 35 | 9 | 41 | 3.8 | 6 |
| C/W 600 | 0 | 5 | 8 | 35 | 19 | 33 | 3.7 | 10 |
| C/W L-8-100 | 2 | 8 | 20 | 31 | 13 | 26 | 3.2 | 7 |
| C/W L-8-101 | 0 | 4 | 26 | 44 | 13 | 13 | 3.0 | 6 |
| Florida XP-2 | 1 | 5 | 28 | 39 | 9 | 18 | 3.0 | 8 |
| Florida XP-3 | 0 | 1 | 14 | 31 | 13 | 41 | 3.8 | 12 |
| K-6-8 | 0 | 3 | 6 | 26 | 10 | 55 | 4.1 | 14 |
| KO 176 (Arcadia) | 0 | 1 | 10 | 31 | 12 | 46 | 3.9 | 13 |
| NC-7 | 0 | 1 | 11 | 52 | 11 | 25 | 3.5 | 6 |
| SC-1 | 0 | 2 | 11 | 37 | 24 | 26 | 3.6 | 7 |
| SC Medium Flowering | 0 | 2 | 16 | 25 | 23 | 34 | 3.7 | 7 |
| SRVR | 1 | 18 | 21 | 37 | 4 | 19 | 2.8 | 2 |
| FLSD ($P = 0.05$) | | | | | | | 0.3 | 8 |

† Percentage of the root system galled: 0 = no galls, 1 = 1–10%, 2 = 11–25%, 3 = 26–75%, 4 = 76–90%, 5 = 91–100%. Dead plants were given a rating of 5.

100, C/W L-8-101, Florida XP-2, and SRVR) had significantly lower ($P = 0.05$) PRSG scores than Arcadia (Tables 1 and 2). Tamar and Dusi had a higher ($P = 0.05$) mortality rate than Arcadia, and only SRVR had a lower ($P = 0.05$) mortality rate than Arcadia.

## Discussion

This study confirms results found in other studies (12,14,16) that white clover is highly susceptible to the southern root-knot nematode. The mean PRSG ratings for all of the cultivars and germplasms would indicate a susceptible host (mean PRSG score $\geq 2.0$). Also, the high mortality rate of several of the cultivars and germplasms indicate the pathogenicity of *M. incognita* on white clover. Out of 2,300 plants evaluated in this study, only 83 plants were considered resistant using the PRSG system. This scoring system has been used to separate plants with a high proportion of fibrous roots relative to galled roots (13). This allowed detection of plants with moderate resistance to *M. incognita* that may have been discarded using a traditional gall index scoring system.

The *M. incognita* race 4 population used in this study may also have facilitated the detection of moderate levels of resistance. This population, although pathogenic, was the least aggressive of eight *M. incognita* populations toward white clover cultivars and germplasms in an earlier greenhouse study (16). Plant reaction to highly aggressive populations could prevent the identification of moderate levels of resistance.

The Brown Loam population and Brown Loam Synthetic #6 were the only germplasms with no plants resistant to root-knot nematodes. These two populations were derived from material originally selected for drought tolerance in a field at the Brown Loam Branch Experiment Station, Raymond, Mississippi (11). Apparently, the ability of these white clover selections to withstand droughty conditions is not related to root-knot nematode resistance.

SC-1 was released in 1973 as a "nematode tolerant germplasm" (6), and its reaction in this study was similar to that in another recent study (16). SC-1 had a mean PRSG score of 3.6 in the present study and a PRSG score of 3.5 in the previous study comparing aggressiveness of *M. incognita* populations (16). The usefulness of the SC-1 germplasm in a screening program for nematode resistance appears to be limited because only 2 of 100 SC-1 plants showed resistance in this study. Also, root-knot nematode populations reproduced as well or better on SC-1 than on other white clover germplasms and cultivars (16). Tillman, Regal, and SC-1 are related in that five of six parent clones of Tillman and two of five parent clones of Regal are also parents of SC-1 (145 parent clones). Tillman may be useful in a selection program because it had the most resistant plants (nine plants) of the 10 cultivars in our study.

SRVR shows the most promise as a source of resistance to root-knot nematodes. This germplasm had the lowest PRSG score, the lowest mortality rate, and the highest number of resistant plants of all the germplasms and cultivars tested. In addition to being a source of nematode resistance, SRVR was selected for resistance to peanut stunt virus, clover yellow vein virus, and alfalfa mosaic virus, which are the three main viruses affecting white clover in the southeastern United States (7). Incorporating root-knot nematode resistance into SRVR would yield a multiple-pest-resistant white clover. This could result in increased yields and persistence of white clover in forage productions systems where plants are under virus and nematode pressure.

The resistant plants selected in this study have been used to initiate a recurrent selection program for *M. incognita* resistance in white clover. The range of cultivars and germplasms evaluated should provide a genetically broad-based resistant plant population. Additional selections from SRVR are being used in a second recurrent selection program to incorporate root-knot nematode resistance into this virus-resistant germplasm. Development of nematode resistant white clover would greatly

contribute to control of *M. incognita* in forage production systems in the southeastern United States.

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