EVALUATION OF *LEUCAENA* SPECIES GERMPLASM FOR GENETIC RESISTANCE TO *MELOIDOGYNE INCOGNITA* AND *M. JAVANICA*[1]

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ABSTRACT

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There has been increasing interest in adapting tropical legumes to parts of the Southern United States and worldwide. Fast-growing trees, such as *Leucaena* (Benth.) offer promise as forage and biomass crops, and easily adapt to subtropical environments. Few disease and pest problems have been linked to *Leucaena* spp., but a low level of root-knot nematode galling was observed in a forage evaluation planting in Florida. Our objectives for this study were to evaluate diverse *Leucaena* spp. for genetic resistance to two root-knot nematode species prevalent in Florida soils. Ninety-seven accessions of *Leucaena* spp. were screened in a shadehouse for reaction to Florida populations of race 3 of *Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood. The results from the nematode study, using three levels of nematode inoculum (0, 3, and 9 eggs/cm^3 of soil) on 11-week-old plants grown in 150-cm^3 plastic containers, showed a general absence of root galling of the plant by either nematode species, with few exceptions. During 1983, a split-plot experimental design was used to evaluate *M. incognita*-inoculated versus non-inoculated *Leucaena* spp. grown under field conditions on Myakka fine sand. The root reactions of the 17 accessions grown in the field supported the findings of the *M. incognita*-inoculated container study.

Additional key words: Alysicarpus vaginalis, root-knot nematodes.

RESUMEN

Zimet, A. R., K. H. Quesenberry, and R. A. Dunn. 1986. Evaluación del germoplasma de especies de *Leucaena* en relación con su resistencia genetica a *Meloidogyne incognita* y a *M. javanica*. Nematropica 16:99-108.

Existe un interés creciente en adaptar las legumbres tropicales a las regiones del Sur de los Estados Unidos de America y del mundo entero. Arboles de crecimiento rápido como *Leucaena* (Benth.) son prometedores como productores de forrajes y materia vegetal viva, ya que son fácilmente adaptables al ambiente subtropical. Pocas enfermedades y plagas han sido relacionadas con *Leucaena* spp., pero en una evaluación de forrajes efectuada en Florida, se observó un nivel bajo del nematodo nodulador. El objetivo de éste estudio fue evaluar diversas especies de *Leucaena* para determinar su resistencia genética a dos especies del nematodo nodulador que son comunes en los suelos de Florida. Noventa y siete introducciones de *Leucaena* spp. fueron evaluadas en un umbraculo para determinar su reacción a la raza 3 de *Meloidogyne incognita* (Kofoid and White) Chitwood y a *M. javanica* (Treub) Chitwood. Se utilizaron tres niveles de inocolo del nematodo (0, 3 y 9 huevecillos por cm^3 de suelo) en plantas de 11 semanas de edad, mantenidas en macetas plásticas de 150 cm^3 de capacidad. En general, con pocas excepciones, los resultados del estudio mostraron una ausencia de nódulos de nematodos en las raices. Durante 1983 se uso un diseño experimental de parcelas divididas para evaluar, bajo condiciones de campo, las introducciones de *Leucaena* spp. inoculadas con *M. incognita.* La reacción de las raices de las introducciones probadas en el campo estuvieron de acuerdo con los resultados de los estudios conducidos en el umbraculo.

Palabras claves adicionales: Alysicarpus vaginalis, nematodos noduladores.

INTRODUCTION

Nematode susceptibility of cultivated crops is of widespread importance. Two species of root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood and *M. javanica* (Treub) Chitwood] are prevalent in warmer soils and affect a wide range of crops. The current international interest in *Leucaena* (Benth.) as a forage and biomass crop suggested a need to evaluate the response of these tree legumes to root-knot nematodes. Although once described as a virtually disease and insect free crop (8), several observations of disease and insect occurrence on *Leucaena* spp. have been recently cited (2,5,7,9).

Nematological references to *Leucaena* spp. are available, although information on root-knot nematode (*Meloidogyne* spp.) infection of *Leucaena* spp. has been cited by Bessey (1) and Thrower (12). Severe infection of the roots of *L. glauca,* synonymous with *L. leucocephala* (Lam.) de Wit, by *M. javanica* was reported in Papua, New Guinea (12). Nematode-inoculated plants were killed outright or severely galled and had lower yields than uninoculated control plants. Mature females and egg-masses were recovered, demonstrating successful nematode reproduction in *L. glauca.* More recently, resistance to *M. incognita* in *L. leucocephala* was reported by Hasan and co-workers (3). Limited information on nematode parasitism and the low incidence of root-knot nematode galling observed on a forage *L. leucocephala* at Gainesville, Florida warranted further study in this area.

The identification of root-knot nematode resistance in plants requires working with rather large plant populations. Recently, Quesenberry *et al.* (10) reported the use of small plastic containers as a technique for screening forage legumes for response to root-knot nematodes. Results from 150-cm^3 containers correlated better than those from 50-cm^3 containers with galling observed in field plots. The following experiments were used to evaluate: 1) part of the USDA *Leucaena* plant introduction collection for response to *M. javanica* and race 3 of *M. incognita,* and 2) the use of 150-cm^3 containers in screening woody legumes for nematode resistance.

MATERIALS AND METHODS

Experiment 1

Ninety-seven *Leucaena* accessions (Table 1) and a Florida common alyceclover, *Alysicarpus vaginalis* (L.) DC., were germinated under sterile conditions (seeds were surface-sterilized in 95% EtOH for 30 sec., immersed in 0.2% acidified $HgCl_2$ for 3 min and rinsed 6 times in sterile, deionized water). Two-day-old seedlings were transplanted into 150-cm^3 Conetainers® (Ray Leach Conetainer Nursery, Canby, Oregon) containing a steam-sterilized 1:1 mixture of Myakka fine sand (sandy, siliceous, hyperthermic Aeric Haplaquod) and coarse sand. The soil mixture was fertilized with a commercial micronutrient mixture (FTE® 503) prior to autoclaving. Plants were watered once a week with full strength Hoagland's solution (4) or tap water as needed. At 21 days, seedlings were infested with 0, 3, or 9 eggs of race 3 of *M. incognita*/cm^3 soil, supplied in a concentrated 1-ml aliquot applied with an automatic pipet to a depth of approximately 6 cm. Nematode inoculum was produced on 'Rutgers' tomato and isolated by the method of Hussey and Barker (6). Conetainers were arranged in a randomized complete-block design with 5 replications. Seedlings were grown under 30% shade in a shadehouse. The seedlings were evaluated 60 days after inoculation and roots were scored on a visual rating scale (11) of 0 to 5 (0 = no galls, 1 = 1 or 2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = > 100 galls per root system) as described by Quesenberry *et al.* (10).

Experiment 2

This experiment was conducted at the same time and under the same conditions as Experiment 1, except that *M. javanica* eggs were substituted as the inoculum.

Experiment 3

Surface-sterilized seeds (as described previously) of 17 *Leucaena* accessions and Florida common alyceclover (Table 1) were germinated asceptically. Two-day-old seedlings were planted into Todd® Planter Flats (Speedling Manufacturing, Inc., Sun City, Florida) containing steam-sterilized commercial potting soil. After 30 days, half of the seedlings were inoculated with approximately 9 eggs of race 3 of *M. incognita* per cm^3 of soil directly into the Todd Planter Flats while the other half

Table 1. List of *Alysicarpus* and *Leucaena* accessions used in experiments

U.S.D.A. plant introductions and Univ. of Florida accessions[z]	Species	Origin
*Alyceclover	*A. vaginalis*	Florida
UF-1	*L. leucocephala*	Florida
*UF-2	*L. leucocephala*	Florida
VI-2	*L. leucocephala*	U.S. Virgin Islands
VI-3	*L. leucocephala*	U.S. Virgin Islands
164061	*L. leucocephala*	Brazil
188810	*L. leucocephala*	Philippines
247682	*L. leucocephala*	Belgian Congo
263695	*L. esculenta*	Mexico
274470	*L. leucocephala*	South Africa
279180	*L. leucocephala*	India
279577	*L. leucocephala*	Taiwan
281605	*L. leucocephala*	U.S. Virgin Islands
281606	*L. leucocephala*	Colombia
281627	*L. leucocephala*	Australia
281636	*L. leucocephala*	Tanganyika
281766	*L. leucocephala* x *L. pulverulenta*	New Guinea
281767	*L. leucocephala* x *L. glabrata*	New Guinea
*281771	*L. leucocephala*	New Guinea
281772	*L. leucocephala*	New Guinea
281775	*L. leucocephala*	New Guinea
281777	*L. leucocephala*	New Caledonia
281778	*L. leucocephala*	New Caledonia
281779	*L. leucocephala*	New Caledonia
281780	*L. leucocephala*	Puerto Rico
281781	*L. leucocephala*	Ceylon
281782	*L. leucocephala*	Ghana
281783	*L. leucocephala*	Sierra Leone
*281784	*L. leucocephala*	Senegal
282396	*L. leucocephala*	Philippines
282404	*L. leucocephala*	Colombia
*282405	*L.* sp.	Honduras
*282458	*L. leucocephala*	Colombia
*282460	*L. insularum*	Australia
282462	*L. leucocephala*	Australia

Table 1. List of *Alysicarpus* and *Leucaena* accessions used in experiments (continued).

U.S.D.A. plant introductions and Univ. of Florida accessions[z]	Species	Origin
282463	*L. leucocephala*	Australia
282464	*L. leucocephala*	Australia
*282465	*L. leucocephala*	Australia
282466	*L. leucocephala*	Australia
282467	*L. leucocephala*	Australia
*282468	*L. leucocephala*	Australia
282469	*L. leucocephala*	Australia
282470	*L. leucocephala*	Australia
*282471	*L. leucocephala*	Australia
282472	*L. leucocephala*	Australia
282473	*L. leucocephala*	Australia
*282474	*L.* sp.	Australia
*282692	*L. leucocephala*	Mexico
282817	*L. leucocephala*	Taiwan
283697	*L. leucocephala*	New Caledonia
286248	*L. lanceolata*	Mexico
286295	*L. leucocephala*	Ivory Coast
288001	*L. leucocephala*	U.S.A.
288003	*L. leucocephala*	U.S.A.
288004	*L. leucocephala*	U.S.A.
288005	*L. leucocephala*	U.S.A.
288007	*L. leucocephala*	U.S.A.
288008	*L. leucocephala*	U.S.A.
288009	*L. pulverulenta*	U.S.A.
288011	*L. leucocephala*	U.S.A.
292345	*L. leucocephala*	Bolivia
295360	*L. leucocephala*	Taiwan
295362	*L. leucocephala*	Taiwan
295363	*L. leucocephala*	Taiwan
*295364	*L. leucocephala*	Taiwan
300010	*L. leucocephala*	South Africa
300011	*L. leucocephala*	South Africa
*304650	*L. leucocephala*	Argentina
305453	*L. leucocephala*	Sierra Leone
308544	*L. leucocephala*	Colombia
308568	*L. leucocephala*	Venezuela

Table 1. List of *Alysicarpus* and *Leucaena* accessions used in experiments (continued).

U.S.D.A. plant introductions and Univ. of Florida accessions[z]	Species	Origin
311128	*L. leucocephala*	Nicaragua
311513	*L. leucocephala*	Brazil
317908	*L. leucocephala*	U.S.A.
317909	*L. leucocephala*	U.S.A.
317910	*L. leucocephala*	U.S.A.
317911	*L. leucocephala*	U.S.A.
317912	*L. leucocephala*	U.S.A.
317916	*L. pulverulenta* x *L. leucocephala*	U.S.A.
*317918	*L.* sp.	U.S.A.
322553	*L. leucocephala*	Brazil
331798	*L. leucocephala*	Australia
364373	*L. leucocephala*	Nigeria
367835	*L. leucocephala*	Argentina
370749	*L. leucocephala*	U.S.A.
*384516	*L. leucocephala*	Bahamas
384517	*L. leucocephala*	Bahamas
415703	*L. leucocephala*	Taiwan
435930	*L. leucocephala*	U.S.A.
443479	*L. leucocephala*	El Salvador
443559	*L. leucocephala*	Mexico
*443599	*L. leucocephala*	Mexico
443604	*L. leucocephala*	Mexico
443608	*L. leucocephala*	Mexico
443700	*L. leucocephala*	Mexico

[z]Asterisk (*) indicates U.S.D.A. plant introductions and Univ. of Florida accessions also included in field experiment.

received no eggs and served as control plants. The infested and non-infested plants with attached root balls were then immediately transplanted to a fumigated field treated with 37 L/ha of ethylene dibromide (Soilbrom-90 EC®, Great Lakes Chemical Corp.). The field was located on Myakka fine sand (sandy, siliceous, hyperthermic, Aeric Haplaquod) at the Beef Research Unit, University of Florida, Gainesville, Florida. The experimental design was a split-plot with nematode infestation versus no infestation as the main plots, and *Leucaena* and alyceclover accessions as the subplots. There were 8 replications. Whole plants were removed after 120 days of growth in the field, and root gall counts (as previously described) and whole plant dry-matter yields were recorded. Plant dry matter yields of alyceclover were not included in the comparisons.

RESULTS

Experiments 1 and 2

In the shadehouse, an assessment of root-knot nematode resistance to *Leucaena* spp. and a known root-knot susceptible control plant, alyceclover, grown in Conetainers, provided a relatively rapid and inexpensive method for screening large quantities of plants in a limited space. Several *Leucaena* accessions grown in containers showed only a low incidence of root galling in response to either *M. incognita* or *M. javanica.* Although variation in gall ratings for those accessions were statistically significant ($P = 0.05$), gall numbers were so low that they appeared of little biological importance. In the *M. incognita* inoculation experiment, *Leucaena* PI 286295 had a 1.6 gall rating at the 9 egg/cm^3 inoculation level but no more than 2 or 3 galls per root system were observed on any plant. No apparent galling at the 3 egg/cm^3 soil inoculum level was noted on PI 286295. One other genotype reacted mildly to *M. incognita,* a hybrid of *L. leucocephala* x *L. pulverulenta,* PI 281767, which had a gall score of 1.0 at the 3 egg/cm^3 inoculum level, but only a 0.2 score at the 9 egg/cm^3 level. The remainder of the accessions in both experiment had gall scores of less than 1.0, and most accessions had no galls.

The Florida common alyceclover accession, a highly susceptible genotype to root-knot, provided an excellent example of plant susceptibility to both nematode species. The alyceclover accession had gall ratings of 4.0 for the 3 egg/cm^3 level and 4.8 for the 9 egg/cm^3 the level of the *M. incognita*-inoculated experiment. In the *M. javanica*-inoculated study, alyceclover had gall ratings of 3.4 and 5.0 for the 3 and 9 egg/cm^3 levels, respectively. The less than 5.0 gall rating on the nematode-inoculated alyceclover was attributed to decomposition of highly-galled roots, which actually lowered the final root galling score. Uninoculated control alyceclover plants, in both studies, were free of any galling and received gall rating scores of 0.

Experiment 3

Field data correlated well with the shadehouse results obtained from *M. incognita* in Conetainers. Nematode-inoculated *Leucaena* accessions had no root galling while the infested alyceclover accession was heavily galled (Table 2). In one replication, an uninoculated alyceclover entry showed appreciable galling (rated at 3), although planted in a fumigated soil. This was possibly due to an increase in nematode populations after fumigation, faulty fumigation, or nematode contamination from inoculated plants. Fumigation had no significant effect on dry matter yields of *Leucaena* accessions (Table 2). The accession UF-2 exhibited greater early seedling vigor than other *Leucaena* accessions, although the magnitude of the yield difference was small.

Table 2. Comparison of *M. incognita* infested and non-infested *Leucaena* and *Alysicarpus* accessions for root galling response and dry matter yield under field conditions.

	Root gall rating[y,z]		Pland dry matter yield, g/plant[z]	
Accession	Non-infested	Infested	Non-infested	Infested
Alyceclover	0.4 a	5.0 a	3530	2810
UF-2	0 b	0 b	320 a	325 a
281771	0 b	0 b	89 bc	111 b
281784	0 b	0 b	83 bc	124 b
282405	0 b	0 b	244 ab	230 ab
282458	0 b	0 b	131 bc	144 b
282460	0 b	0 b	224 abc	154 b
282465	0 b	0 b	154 bc	136 b
282468	0 b	0 b	203 abc	135 b
282471	0 b	0 b	146 bc	149 b
282474	0 b	0 b	93 bc	88 b
282692	0 b	0 b	111 bc	201 ab
288009	0 b	0 b	119 bc	86 b
295364	0 b	0 b	106 bc	70 b
304650	0 b	0 b	70 c	144 b
317918	0 b	0 b	91 bc	137 b
384516	0 b	0 b	79 c	127 b
443599	0 b	0 b	83 bc	143 b

[y]Gall rating: 0 = no galls, 1 = 1 or 2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls, 5 = > 100 galls per root system.

[z]Means in the same column not followed by the same letter are significantly different at the 0.05 level. No significant overall treatment or treatment x genotype interaction effect.

DISCUSSION

Data from these tests indicate a high degree of root-knot nematode resistance in *Leucaena* spp. to the races of *M. incognita* and *M. javanica* used in these experiments. Races or biotypes of *Meloidogyne* species are common and our data do not preclude different responses when using other nematode populations. The North Carolina State University Differential Host Test (11) divides *M. incognita* into four races, although no distinct races of *M. javanica* have been found.

The highly root-knot susceptible alyceclover accession provided an excellent positive check of the viability of nematode inocula. Although 3 eggs/cm^3 soil rates were suitable for an inoculum load in Conetainers, instituting a "light" and a "heavy" inoculum load level as well as an uninoculated check insured against gall counting error due to "root-pruning" by the nematode. Root pruning refers to a phenomenon whereby nematode feeding has stunted growth of lateral roots and/or caused such severe injury that they have sloughed from the tap root, so little or no galling of lateral roots can be observed. Hence low gall scores would be recorded when the plant actually has marked response to the nematode. Little or no root pruning was observed in *Leucaena.*

From a practical point of view, cultivation of a highly root-knot nematode resistant *Leucaena* could conceivably decrease the *Meloidogyne* spp. population in field soil. Ideally, root-knot nematode populations could be reduced below the economic damage threshold for interplanted or subsequent crops after a period of time.

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