VIRULENCE OF FOUR FLORIDA ISOLATES OF *MELOIDOGYNE MAYAGUENSIS* TO SELECTED SOYBEAN GENOTYPES

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ABSTRACT

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The reproduction and virulence of four Florida isolates of *Meloidogyne mayaguensis* to the root-knot nematode resistant ('Benning', 'Boggs', 'Bragg', 'Forrest', 'Haskell', 'Lee 74'and G-93-9009) and susceptible ('Bossier', 'GoSoy 17', 'Pickett') soybean genotypes were evaluated in a growth chamber. Two levels of each nematode isolate (low = 2,500 eggs or J2/ plant; high = 5,000 eggs or J2/ plant) were used. None of the genotypes were immune to the isolates of *M. mayaguensis*. Differences in reproduction and virulence were detected among the nematode isolates at both inoculum levels. All four isolates overcame the root-knot nematode resistance genes, including the *Mir1* gene. The root-knot nematode resistant soybean 'Bragg' was resistant (egg mass index values (EMI) ranging from 0.1-2.0) to isolates 2 and 3, but susceptible (EMI > 2) to isolate 1 at both inoculum levels, whereas 'Forest', which possess the *Mir1* gene was resistant to only isolate 3. The root-knot nematode resistant soybean 'Boggs' and line G93-9009 were resistant to isolate 4. All soybean genotypes were susceptible to isolate 1 at both inoculum levels, except that soybean 'Lee 74' sustained low egg mass (EMI = 2.00) at the low inoculum level ($P \leq 0.05$). The results of this study showed for the first time that gene(s) that confer resistance to at least one of the major root-knot nematode species in soybean may also confer resistance to some isolates of *M. mayaguensis*.

Key words: *Glycine max*, host response, *Meloidogyne mayaguensis*, resistance, root-knot nematode, soybean, virulence.

RESUMEN

Cetintas, R., J. A. Brito, and D. W. Dickson. 2008. Virulencia de cuatro aislamientos de *Meloidogyne mayaguensis* de Florida a algunos genotipos de soya. Nematropica 38:127-135.

Se evaluó la reproducción y la virulencia de cuatro aislamientos de *Meloidogyne mayaguensis* de Florida en genotipos de soya resistentes al nematodo agallador ('Benning', 'Boggs', 'Bragg', 'Forrest', 'Haskell', 'Lee 74'y G-93-9009) y susceptibles al nematodo agallador ('Bossier', 'GoSoy 17', 'Pickett'), en cámaras de crecimiento. Se utilizaron dos niveles de inócuo de cada aislamiento (bajo = 2,500 J2 o huevos/ planta; alto = 5,000 huevos o J2/ planta). Ninguno de los genotipos evaluados fue inmune a los aislamientos de *M. mayaguensis*. Se detectaron diferencias en la reproducción y la virulencia entre los aislamientos con ambos niveles de inóculo. Los cuatro aislamientos vencieron los genes de resistencia, incluyendo al gen *Mir1*. El genotipo de soya resistente 'Bragg' fue resistente (valores de índice de masa de huevos (EMI) entre 0.1 y 2.0) a los aislamientos 2 y 3, pero susceptible (EMI > 2) al aislamiento 1 con ambos niveles de inóculo, mientras que 'Forest', que posee el gen *Mir1,* fue resistente a sólo el aislamiento 3. La variedad resistente 'Boggs' y la línea G93-9009 fueron resistentes al aislamiento 4. Todos los genotipos de soya evaluados fueron susceptibles al aislamiento 1 con ambos niveles de inóculo. La excepción fue 'Lee 74' que sostuvo un índice de masa de huevos bajo (EMI = 2.00) con el nivel de inóculo bajo ($P \leq 0.05$). Los resultados de este estudio muestran por primera vez que los genes que confieren resistencia a por lo menos una de las principales especies de nematodo agallador en soya también pueden brindar resistencia a algunos aislamientos de *M. mayaguensis.*

Palabras clave: *Glycine max*, *Meloidogyne mayaguensis*, nematodo agallador, resistencia, respuesta de hospedante, soya, virulencia.

INTRODUCTION

Meloidogyne mayaguensis is a highly virulent root-knot nematode originally described from egg plant (*Solanum melongena* L.) collected in Puerto Rico (Rammah and Hirschmann, 1988). This nematode has been found in Brazil (Carneiro *et al.*, 2006), Cuba (Rodriguez *et al.*, 2003), France (Blok *et al.,* 2002), South Africa (Willers, 1997), and West Africa (Fargette *et al.*, 1996; Fargette, 1987).

Meloidogyne mayaguensis has been detected in Florida (USA) since 2002 on cover crops, fruit trees, weeds and several ornamental and agronomic plants (Brito *et al.*, 2004; Brito *et al*, 2008; Cetintas *et al.*, 2007; Kaur *et al.*, 2007; Mendes *et al.*, 2007).

Populations of *M. mayaguensis* from outside the U.S. have been reported to overcome root-knot nematode resistance genes in several economically important crops, including the *Mi-1* gene in tomato (*Solanum lycopersicum* Mill), unidentified root-knot nematode resistance gene(s) in pepper (*Capsicum annuum* L.) and sweet potato (*Ipomoea batatas* [L.] Lam.) (Fargette, 1987; Fargette *et al.*, 1996; Carneiro *et al.*, 2006; Brito *et al.*, 2007a). Similarly, isolates of *M. mayaguensis* from Florida also overcame the resistance of other root-knot nematode resistance genes, including *N* and *Tabasco* genes in bell pepper (*Capsicum annuum* L.) 'Charleston Belle' and thee lines of sweet pepper, respectively (Brito *et al.*, 2007a), and also the *Rk* gene in cowpea (*Vigna unguiculata* (L.) Walp.) (Brito *et al.*, 2007b).

In the southeastern United States, soybean (*Glicine max* [L.] Merril) is an economically important crop that is seriously damaged by root-knot nematodes (Ha *et al.*, 2004; Harris *et al.*, 2003). Soybean cultivars with resistance to the major root-knot nematodes (*M. arenaria, M. incognita* and *M. javanica*) have been developed and are available in the United States (Baker and Harris, 1979; Boerma *et al.*, 1994, 1997, 2000; Ha *et al.*, 2004). The use of these root-knot nematode-resistant soybean cultivars has been included in management programs to suppress soil populations and improve crop yield (Boerma and Hussey, 1992).

Sources of genetic resistance to *M. incognita* (Luzzi *et al.*, 1987; Luzzi *et al.*, 1994), *M. arenaria* (Luzzi *et al.*, 1987; Tamulonis *et al.*, 1997; Harris *et al.*, 2003); and to *M. javanica* (Luzzi *et al.*, 1987) are known in soybean. For instance, soybean cultivars such as 'Bragg' (Ha *et al.*, 2004), 'Forrest' (Luzzi, 1994), Benning (Boerma *et al.*, 1997), Boggs (Boerma *et al.*, 2000), G93-9009 (Luzzi et al., 1996), Haskell (Boerma *et al.*, 1994), and Lee 74 (Ha *et al.*, 2004) are found to have resistance to one or more of these *Meloidogyne* spp.

Currently very little is known about the capability of *M. mayaguensis* to reproduce on soybean, including cultivars that are resistant to the three major root-knot nematode species. The objectives of this study were to determine the reproduction and virulence of four isolates of *M. mayaguensis* from Florida on selected root-knot nematode resistant and susceptible soybean genotypes, and to determine if there is any variation in the virulence among these nematode isolates.

MATERIALS AND METHODS

Nematode origin

The designations, host plants, and origins of the four isolates of *M. mayaguensis* used in this study from Florida were: isolate 1 (N01-283-14B) and isolate 2 (N01-304-15B) from unidentified ornamental plants in Broward and Palm Beach Counties, respectively; isolate 3 (N01-341-4B) obtained from an unidentified weed plant in Hendry County; and isolate 4 (N01-514-3B) from unidentified tropical fruit trees in Dade County. Single egg mass isolates were established from each field populations and reared on 'Rutgers' tomato in separate greenhouses. Species identification was confirmed by subjecting at least 26 females of each isolate to polyacrylamide gel electrophoresis (Cetintas *et al.*, 2007).

Experimental Design

The experiment was designed as a 4 × 2 × 12 factorial treatment arrangement in a randomized complete block with five replications. The trial was conducted twice. Factors and the levels for each factors consisted of two trials, four *M. mayaguensis* isolates: isolate 1, 2, 3, and 4., two inoculum levels: low (2,500 eggs or J2s/plant) and high (5,000 eggs or J2s/plant), and 12 plant genotypes: 10 root-knot nematode resistant and susceptible soybean genotypes (Table 1) with 'Poinsett 64' cucumber (*Cucumis sativus* L.) and 'Rutgers' tomato used as controls, to verify the viability of the inoculum.

Four-week-old seedlings were transplanted into 15-cm-diam clay pots containing pasteurized soil. Five days after transplanting, each seedling was inoculated with the appropriate inoculum level from the same extraction for each particular nematode isolate. Eggs were extracted from tomato roots using 0.6% NaOCl (Hussey and Barker, 1973). Tests were carried out in a growth room for 60 days, with temperature averaging 28 ± 8°C. Plants were watered daily and fertilized bi-weekly with Peter's fertilizer (20-20-20) (United Industries Corp., St. Louis, MO) using 3 g/L.

Table 1. Host suitability of the ten soybean genotypes to *Meloidogyne* spp. used in this study.

Soybean Genotypes	*Meloidogyne* spp.	Host status	Source
Bragg	*M. incognita, and M. javanica*	R[y]	Lehman *et al.*, 1979; Ha *et al.*, 2004
Forrest	*M. incognita*	R	Luzzi *et al.*, 1994; Ha *et al.*, 2004
Benning	*M. arenaria*, *M. incognita*, and *M. javanica*	R	Boerma *et al.*, 1997; Ha *et al.*, 2004
Boggs	*M. incognita*, and *M. javanica*	R	Boerma *et al.*, 2000
	M. arenaria	S[z]	
GaSoy 17	*M. arenaria*, and *M. incognita*	S	Baker and Harris, 1979; Ha *et al.*, 2004
Bossier	*M. incognita,* and *M. javanica*	S	Lehman *et al.*, 1979; Ha *et al.*, 2004
G93-9009	*M. incognita*	R	Luzzi et al., 1996
Haskell	*M. arenaria*, *M. incognita* and *M. javanica*	R	Boerma *et al.*, 1994; Ha *et al.*, 2004
Lee 74	M. incognita	R	Ha *et al.*, 2004
Pickett	M. incognita	S	Kinloch, 1974

[y]R = resistant.
[z]S = susceptible.

At harvest, plants were removed from pots, and root systems were washed and stained with McCormick red food color (McCormick Company, Inc., Sparks, MD) (Thies, *et al.*, 2002). Root galling and egg mass indices were assessed using a 0 to 5 scale, where 0 = no egg mass, 1 = 1-2 egg masses, 2 = 3-10 egg masses, 3 = 11-30 egg masses, 4 = 31-100 egg masses and 5 = >100 egg masses (Taylor and Sasser, 1978). The values of these indices were used to rate the host response of the soybean cultivars to *M. mayaguensis* isolates as described by Taylor and Sasser (1978). According to this rating system, an immune response does not allow any egg production, a resistant response allows egg mass index values ranging from 0.1-2.0, and a susceptible response allows egg mass index values > 2.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) with SAS software (SAS Institute, Cary, NC) and mean separation was conducted using Tukey's studentized range (HSD) test ($P \leq 0.05$). The experiment was conducted twice; no differences were detected ($P > 0.05$) between the trials (Table 2), therefore pooled data was analyzed and presented (Table 3 and 4).

RESULTS AND DISCUSSION

Nematode isolates, plant genotypes and inoculum levels influenced root galling and egg mass indices ($P \leq 0.01$) (Table 2). Nematode isolates × plant genotypes and inoculum level × plant genotypes interactions ($P \leq 0.01$) were detected for both gall and egg mass indices ($P \leq 0.01$) (Table 2). Additionally, interactions of nematode isolates × inoculum levels and nematode isolate × inoculum level × plant genotypes were observed for the number of egg masses ($P \leq 0.01$) but not for the number of galls (Table 2).

All nematode isolates reproduced well and induced root galling on both controls 'Rutgers' tomato and 'Poinsett 64' cucumber (egg mass and galling indices = 5, data not shown) at both levels. This showed that the same extraction of an isolate used for both high and low inoculum levels sustained a very good viability. Therefore, having differences in egg mass and galling indices between nematode inoculums was not a consequence of nematode viabilities. None of the soybean genotypes was immune to *M. mayaguensis* (Tables 3 and 4). However, differences in reproduction and virulence were observed among the isolates.

The soybean 'Bragg' had the smallest egg mass (≤1.4) and galling (≤1.8) indices when inoculated with isolates 2, 3 and 4 at the low inoculum level and only with isolate 2 at high inoculum level ($P \leq 0.05$) (Tables 3 and 4). However, 'Bragg' was susceptible (EMI > 2) to isolate 1. This cultivar is very promising because it is also resistant to other root-knot nematode species (Table 1).

The soybean 'Forrest', which is resistant to *M. incognita* (Table 1), maintained a resistant response (0.1< EMI < 2) only to isolate 3 with egg mass indices ranging from 1.6-1.8 (Table 4). A similar response was reported for an isolate of *M. mayaguensis* from Cuba, which reproduced poorly on this cultivar (Rodriquez *et al.*, 2003). The other isolates reproduced well on 'Forrest' and caused high root galling and egg mass indices ($P \leq 0.05$) (Tables 3 and 4). This result shows that two out of four *M. mayaguensis* isolates evaluated overcame the *Rmi1* resistance gene in 'Forrest' soybean as reported in West Africa (Fargette, 1987); which confirms the variable response of 'Forrest' to *M. mayaguensis* isolates from different geographical areas.

For the root-knot nematode resistant 'Boggs', only isolate 4 had low number of

Table 2. Analysis of variance for the effects of two trials, four isolates of *Meloidogyne mayaguensis*, twelve plant genotypes, and two inoculum levels, and their interactions on the root galling and egg mass indices.

Source of variation	df	Galling index[v]	*F* value	Egg mass index[w]	*F* value
Trials (T)	1	ns[x]	1.38	ns	0.20
Nematode isolates (N)	3	**[y]	36.51	**	58.99
Inoculum level (I)	1	**	169.24	**	127.79
Plant genotypes (P)	11	**	46.93	**	59.13
T × N	3	ns	0.94	**	9.08
T × I	1	ns	0.30	*[z]	4.48
T × P	11	**	15.03	**	15.48
N × I	3	ns	2.47	**	3.82
N × P	33	**	9.19	**	14.08
I × P	11	**	5.36	**	4.50
T × N × I	3	**	5.83	**	8.12
T × N × P	33	**	9.27	**	6.17
T × I × P	11	ns	1.38	ns	1.58
N × I ×P	33	ns	1.26	**	2.25
T × N × I × P	33	ns	0.87	*	1.45

[v]Root galling indices: 0-5 scale, where 0 = no galls, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = >100 galls (Taylor and Sasser, 1978).
[w]Egg mass indices: 0-5 scale, where 0 = no egg mass, 1 = 1-2 egg masses, 2 = 3-10 egg masses, 3 = 11-30 egg masses, 4 = 31-100 egg masses and 5 = >100 egg masses (Taylor and Sasser, 1978).
[x]ns = not significant.
[y]** = significant at $P \leq 0.01$ level.
[z]* = significant at $P \leq 0.05$ level.

Table 3. Effect of two inoculum levels on root galling of four isolates of *Meloidogyne mayaguensis* on ten selected soybean genotypes.

		Galling index Low (2, 500 eggs or J2/plant)[z]				Galling index High (5,000 eggs or J2/plant)			
		M. mayaguensis isolates				*M. mayaguensis* isolates			
Soybean genotypes	*Meloidogyne* spp. to which genotypes have resistance	1	2	3	4	1	2	3	4
Bragg	Mi and Mj	3.6 cdA	1.6 dB	1.6 dB	1.8 eB	3.6 bA	1.8 cB	2.4 cAB	3.2 cA
Forrest	Mi	4.4 bA	2.8 cB	1.8 dC	4.0 abcA	4.8 aA	3.2 bB	2.6 cB	5.0 aA
Benning	Ma, Mi, and Mj	2.8 fB	5.0 aA	3.6 abB	3.6 bcB	3.6 bB	5.0 aA	4.8 aA	4.6 abA
Boggs	Mi, and Mj	4.7 aA	5.0 aA	3.6 abB	2.8 deB	4.6 aA	5.0 aA	4.6 abA	2.8 cB
GaSoy 17		4.0 bcAB	5.0 aA	3.8 abBC	3.0 cdC	5.0 aA	4.8 aA	4.8 aA	4.2 bB
Bossier		3.8 bcdB	5.0 aA	3.0 bcB	3.6 bcB	4.8 aA	5.0 aA	4.4 abAB	4.8 aA
G93-9009	Mi	3.6 cdB	4.8 abA	2.4 cdC	3.8 bcB	4.6 aA	5.0 aA	3.8 bB	4.4 abAB
Haskell	Ma, Mi, and Mj	4.8 aA	4.8 abA	3.0 bcB	4.4 abA	5.0 aA	5.0 aA	3.8 bB	5.0 aA
Lee 74	Mi	2.6 eB	4.4 bA	4.2 aA	4.0 abcA	4.4 aA	4.8 aA	4.8 aA	4.6 abA
Pickett		4.6 aA	4.6 abA	3.4 bB	4.8 aA	4.8 aA	4.8 aA	4.4 abA	5.0 aA

[z]Root galling index: 0-5 scale, where 0 = no root galls, 1 = 1-2 root galls, 2 = 3-10 root galls, 3 = 11-30 root galls, 4 = 31-100 root galls and 5 = >100 root galls (Taylor and Sasser, 1978).

Data are means of ten replications (pooled data of two trials). Means followed by the same lower case letter within a column or by the same upper case letter within a row in the same inoculum level are not significantly different according to Tukey's studentized range (HSD) test ($P \leq 0.05$).

Table 4. Effect of two inoculum levels on reproduction of four isolates of *Meloidogyne mayaguensis* on ten selected soybean genotypes.

Soybean Genotypes	*Meloidogyne* spp. to which genotypes have resistance	Egg mass index Low (2, 500 eggs or J2/plant)[z]				Egg mass index High (5,000 eggs or J2/plant)			
		M. mayaguensis isolates				*M. mayaguensis* isolates			
		1	2	3	4	1	2	3	4
Bragg	Mi and Mj	2.6 cA	0.8 eB	1.4 deB	1.0 eB	3.2 cA	1.2 dC	2.0 dBC	2.4 cAB
Forrest	Mi	3.4 bcA	2.2 dB	1.6 dC	3.2 bA	4.6 abA	2.0 cB	1.8 dB	4.2 abA
Benning	Ma, Mi, and Mj	2.6 cB	5.0 aA	3.0 bB	2.2 cdB	3.8 bB	5.0 aA	4.4 abAB	3.2 bC
Boggs	Mi, and Mj	3.8 bAB	4.6 abA	2.8 bcB	2.0 dC	4.4 abA	4.8 aA	4.6 aA	1.6 dB
GaSoy 17		4.0 abB	5.0 aA	2.8 bcC	2.6 cC	3.2 cB	4.8 aA	4.8 aA	2.2 cdC
Bossier		3.0 bcB	4.4 abA	2.2 cB	2.4 cdB	3.2 cB	5.0 aA	3.6 cB	3.2 bB
G93-9009	Mi	2.8 cB	4.0 bcA	1.4 deC	1.8 dC	3.2 cB	5.0 aA	3.6 cB	1.0 eC
Haskell	Ma, Mi, and Mj	4.6 aA	4.6 abA	1.2 eB	1.8 dB	5.0 aA	5.0 aA	4.0 bB	3.2 bBC
Lee74	Mi	2.0 dB	3.8 bcA	3.6 aA	3.4 abA	3.6 bcB	4.8 aA	4.6 aA	4.0 abAB
Pickett		4.0 abA	3.6 cA	3.2 bA	3.8 aA	3.4 bcB	3.2 bB	3.6 cB	4.8 aA

[z]Egg mass index: 0-5 scale, where 0 = no egg mass, 1 = 1-2 egg masses, 2 = 3-10 egg masses, 3 = 11-30 egg masses, 4 = 31-100 egg masses and 5 = >100 egg masses (Taylor and Sasser, 1978).

Data are means of ten replications (pooled data of two trials). Means followed by the same lower case letter within a column or by the same upper case letter within a row in the same inoculum level are not significantly different according to Tukey's studentized range (HSD) test ($P \leq 0.05$).

egg masses, but was susceptible to isolates 1, 2, and 3 (Table 4). Similarly, the soybean line G93-9009, which is highly resistant to *M. incognita* (Table 1), was also resistant to isolate 4, sustaining low egg mass indices, with values of 1.8 and 1.0 for low and high inoculation levels, respectively ($P \leq 0.05$) (Table 4).

The soybean 'Haskell', which is resistant to the three major root-knot nematode species (Table 1), sustained low egg mass indices of isolates 3 and 4 at low inoculum levels, but it was susceptible to the other isolates (Table 4).

Understanding the variability in reproduction and virulence among isolates of *M. mayaguensis* is important for selecting and implementing appropriate management strategies. The use of resistant cultivars is environmentally safe and one of the oldest and most effective methods to manage root-knot nematodes; however, the emergence of virulent root-knot nematode populations (Fargette *et al.*, 1996; Brito *et al.*, 2007a) limits its usefulness as a control strategy. Previous studies have shown that the genes that confer resistance to the major root-knot nematode species in bell pepper, cowpea, tomato, and sweet pepper cultivars did not confer resistance by *M. mayaguensis* (Brito *et a*l., 2007a; 2007b; Carneiro *et al.*, 2006; Fargette, 1987). Our results indicated that resistance gene(s), including the *Mir1* and other unidentified genes which confer resistance in soybean to the major root-knot nematode species, may also confers resistance to some isolates of *M. mayaguensis.* Although none of the genotypes of soybean was immune to the isolates of *M. mayaguensis* evaluated, the root-knot nematode resistant soybean 'Bragg', 'Boggs', 'Forest' and G93-9009 were resistant to at least one of the isolates of *M. mayaguensis.* These results provide information on possible sources of *M. mayaguensis* resistance for soybean breeding programs as well as management of this nematode species utilizing the resistant soybean cultivars identified.

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