SUSCEPTIBILITY OF PIGEON PEA (*CAJANUS CAJAN*) CULTIVARS AND LINES TO *MELOIDOGYNE JAVANICA*

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ABSTRACT

Acosta, N., N. Vicente, and J. Toro. 1985. Susceptibility of pigeon pea (*Cajanus cajan*) cultivars and lines to *Meloidogyne javanica*. Nematropica 16:1-10.

Greenhouse and field tests were conducted to determine the susceptibility of various pigeon pea cultivars and lines developed in Puerto Rico to a population of *Meloidogyne javanica* from Coamo, Puerto Rico. In the first greenhouse test, shoot dry weights and number of bacterial nodules fron inoculated plants were significantly higher than from noninoculated plants. Gall indices of 5 on a 0-5 rating scale were found in all cultivars and lines evaluated. The cultivar Blanco was the tallest entry, had the highest shoot and root dry weights, and the second highest number of *M. javanica* eggs and second-stage juveniles recovered from the roots. The same cultivar showed the highest number of bacterial nodules in inoculated plants. The 8 pigeon pea cultivars and lines tested in a second greenhouse experiment were also susceptible to the *M. javanica* population. Dry weight in noninoculated plants was significantly higher than in inoculated. Highest nematode populations were recorded from line 98, which also showed the highest dry weights. Similar results were obtained in the field. Root gall indices and values of a reproduction factor demonstrated that all cultivars and lines are susceptible to the *M. javanica* population from Coamo. This was evidenced by the many giant cells and different stages of development of the nematode found in root sections. This is the first report on the histopathology of *M. javanica* on pigeon pea.
Additional key words: resistance, root-knot nematodes, histopathology.

RESUMEN

Acosta, N., N. Vicente y J. Toro. 1985. Susceptibilidad de cultivares y líneas de gandul (*Cajanus cajan*)a *Meloidogyne javanica*. Nematropica 16:1-10.

Se establecieron experimentos en invernadero y en el campo para determinar la susceptibilidad de varios cultivares y líneas de gandul desarrolladas en Puerto Rico a una población de *Meloidogyne javanica* de Coamo, Puerto Rico. En el primer ensayo en invernadero, los valores de peso seco de las partes aéreas y el número de nódulos bacterianos en plantas inoculadas fueron significativamente más altos que los de las no inoculadas. Los valores del índice de nodulación fueron iguales a 5, en un rango de 0-5, en todos los cultivares y líneas evaluadas. El cultivar Blanco fue el más alto, tuvo los valores más altos de peso seco de las partes aéreas y de las raíces y fue el segundo más alto en la cantidad

de huevos y segundos estadios juveniles recobrados de las raíces. El mismo cultivar mostró los valores más altos de nódulos bacterianos en las plantas inoculadas. En un segundo experimento en invernadero, 8 cultivares y líneas de gandul resultaron ser susceptibles a la población de *M. javanica.* Los valores de peso seco de las plantas sin inocular fueron significativamente más altos que en las inoculadas. A pesar de que la reproducción del nematodo fue alta en todos los cultivares y líneas, los valores más altos se obtuvieron en la línea 98, la que mostró, además, los valores más altos de peso seco. Resultados similares se obtuvieron en el campo. Los valores del indice de nodulación y del factor de reproducción demostraron que todos los cultivares y líneas son susceptibles a la población de *M. javanica* de Coamo. Esto fue evidenciado por el gran número de células gigantes en diferentes etapas de desarrollo del nematodo encontradas en las secciones de raíces. Este es el primer informe de la histopatología de *M. javanica* en gandul.
Palabras claves adicionales: resistencia, nematodo nodulador, histopatología.

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.) Huth] is a tropical bush legume of Indian or African origin used for grain, fresh peas, or as forage (9). It is an important cash crop and protein source in the tropical and subtropical regions on the world (7). In addition to its high nutritional value, pigeon pea can tolerate low soil fertility and drought without alterations in yield and also has only minor disease problems (1).

During 1983-84, pigeon pea production in Puerto Rico was more than 105,000 cwt, the highest producer among the edible legumes. As in most tropical countries, plant-parasitic nematodes constitute one of the major problems affecting Puerto Rican agriculture (3). Even though severe damage caused by nematodes has been observed recently in commercial plantings of pigeon pea, little or no information concerning economic losses due to nematode damage in the crop is available.

Pigeon pea is an excellent host of the reniform nematode *Rotylenchulus reniformis* (3,10), the root-knot nematodes *Meloidogyne javanica* (Treub) Chitwood (2) and *M. incognita* (Kofoid and White) Chitwood (8), and other nematode species (12). The damage caused by the reniform nematode consists of marked necrosis, trimming of root tips, growth retardation (3), chlorosis, and reduction in the number of bacterial nodules (10). Personal observations on private farms have shown that massive attack of root-knot nematodes could lead to a total loss of the crop.

Pigeon pea production in Puerto Rico satisfies only 60% of the demand, and hence, considerable amounts of fresh peas are imported. In order to reduce importations, production must be improved and increased. This could be achieved by utilizing nematode-resistant cultivars with good horticultural characteristics complemented with adequate control practices. The study reported herein was conducted to deter-

mine the suitability of various pigeon pea cultivars and lines to a population of *M. javanica* from Coamo, Puerto Rico.

MATERIALS AND METHODS

Two greenhouse and one field test were conducted to determine the suitability of pigeon pea cultivars and breeding lines to a population of *M. javanica* from the pigeon pea cultivar Pinto from Coamo, Puerto Rico.

In the first greenhouse test, 6-week-old pigeon pea seedlings were planted into 15-cm-diam plastic pots containing 1300 cm^3 of a steam-sterilized soil (65% sand, 15% clay, and 20% silt) with pH 6.5. Five groups of 10 pots each were transplanted with 2 plants of one of the following cultivars: Kaki, Pinto, Blanco, 2B-Bushy, or line 147, a breeding line developed in Puerto Rico by selection using the pedigree method after crossing 'Kaki' with PI 5690, an introduction from Trinidad, W.I. All pots received 11,808 *M. javanica* eggs and second-stage juveniles added with an oxford pipette. A second set of 5 groups of 10 pots, each planted with 2 plants from each of the above mentioned cultivars or lines, received supernatant (suspension of organisms, nematode-free).

Nematode eggs and larvae were extracted from the roots of the pigeon pea cultivar Pinto by the method of Hussey and Barker (5). Ten replicates per cultivar, 5 inoculated and 5 noninoculated, were arranged in a split-plot design on a greenhouse bench for 90 days. The initial height of the two plants per pot was measured before nematode inoculation and at the end of the test. Plants were watered as needed and a 20-20-20 fertilizer was applied monthly.

At harvest, 90 days after treatment, mean values of shoot height, dry shoot and root weights, gall indeex (0=0, 1=1-2, 2=3-10, 3=11-30, 4=31-100, 5= over 100 galls), number of eggs and bacterial nodules were recorded. Second stage juveniles from the entire content of soil in each pot were extracted utilizing the method of Christie and Perry (4). Data were statistically analyzed using ANOVA. Means between treated and untreated plants within a cultivar were compared using a t-test and those among cultivars were compared using Duncan's multiple range test.

In a second greenhouse experiment cultivars 2B-Bushy, Pinto, Blanco, and Kaki, and breeding lines 149, 147, 98, and 59 were evaluated. Lines 59 and 149 were selected from a population obtained by the irradiation with Co 60 of seeds from plants 21-B4 of unknown origin. Line 98 was obtained from seeds of plants 8AB-4 of unknown origin irradiated with Co 60. Eight groups of 12 pots each were planted with one plant per 10-cm-diameter pot, containing a methyl bromide-

sterilized soil. A week later 30,500 eggs and second-stage juveniles of *M. javanica* were added to half of the pots per cultivar or line. Treatments were replicated 6 times and arranged in a split-plot design. Inoculation methods, fertilizer, watering, and other procedures were as in the first test. Plants were harvested 45 days after inoculation. Data on shoot and root dry weight, root-knot index, and nematode reproduction (number of eggs and second-stage juveniles/root system) were recorded. Additionally, nematode-infected roots were examined histologically. Root segments (1-cm long) were fixed in formalin-aceto-alcohol (FAA) solution, dehydrated in tertiary butyl alcohol, and thereafter embedded in 49-56C paraplast. Longitudinal and transverse sections 20 μm thick were made with a rotary microtome. Sections mounted on glass slides were stained with safranin 0 and fast green (6) and examined under the microscope.

A field test was established in Coamo, the main pigeon pea producing area in Puerto Rico. Cultivars Blanco, Kaki, Pinto, and 2B-Bushy, and lines 149, 147, 98, and 59 were planted in a field heavily infested with *M. javanica* and previously planted to 'Pinto'. The 3-row plots were 3.05 m long × 0.305 m apart. Five plants were placed 0.61 m apart in each row. Treatments were arranged in a partially-balanced incomplete-block design with 4 replicates per treatment. No chemical products, nodulating bacteria, fertilizer, or irrigation were applied throughout the experiment. Five months after planting, data on weight of pods and root gall index (0-5) per plot were recorded. Data recorded were evaluated for significance according to the Duncan's multiple range test.

RESULTS AND DISCUSSION

In the first greenhouse test, noninoculated cultivars and lines, except 'Blanco', were taller than inoculated plants, although significant differences were not obtained among treatments (Table 1). Shoot dry weights of inoculated plants were significantly higher than those of noninoculated with all cultivars and lines, perhaps indicating an increase in plant foliage due to a compensation for root damage by the nematodes. Noninoculated plants of line 147 had significantly higher root dry weights than inoculated plants of this line. Root deterioration due to nematode damage could have been the cause of lower root weights in inoculated plants. All cultivars were good hosts for *M. javanica* based on root gall indices and on nematode reproduction. Numbers of bacterial nodules in roots of inoculated plants were significantly higher than in noninoculated plants (Table 2).

Comparisons among inoculated cultivars showed cultivar Blanco had significantly higher values in height, shoot and root dry weights, and number of bacterial nodules (Table 3). The highest numbers of

Table 1. Comparisons between values from pigeon pea plants inoculated with *Meloidogyne javanica* and those noninoculated within the same cultivar.

Cultivar	Height (cm)[z]		Dry weight (g)			
			Shoot		Root	
	I	NI	I	NI	I	NI
Blanco	31.1	27.2	22.4	11.5*	19.3	19.8
Kaki	17.9	23.1	18.9	9.7*	17.4	17.6
Pinto	21.9	25.3	20.1	10.0*	17.8	18.4
2B-Bushy	25.8	26.3	19.2	9.6*	17.8	18.6
147	26.3	30.0	19.4	10.3*	17.6	19.2*

[z]Values from inoculated (I) and noninoculated (NI) plants within the same cultivar are compared statistically. An asterisk (*) indicates a significant (P = 0.05) difference between I and NI within each pair, according to a t-test.

Table 2. Comparisons between numbers of eggs and larvae of *M. javanica* and bacterial nodules from inoculated and noninoculated plants within the same cultivar.

Cultivar	Eggs and 2nd stage juveniles[z]		Gall index		Bacterial nodules	
	I	NI	I	NI	I	NI
Blanco	61,600	0*	5	0*	67.7	16.1*
Kaki	22,700	0*	5	0*	41.8	10.5*
Pinto	39,100	0*	5	0*	23.9	9.2*
2B-Bushy	32,300	0*	5	0*	34.2	11.8*
147	105,200	0*	5	0*	32.5	20.0*

[z]Values from inoculated (I) and noninoculated (NI) plants within the same cultivar are compared statistically. An asterisk (*) indicates a significant (P = 0.05) difference between I and NI within each pair, according to a t-test.

nematode eggs and second-stage juveniles were obtained from line 147 and the cultivar Blanco. Based on the number of bacterial nodules and growth and development of plants, these data indicate that 'Blanco' may have some tolerance, whereas other cultivars are highly susceptible to *M. javanica* (11). The cultivar Pinto is apparently highly susceptible, having the lowest number of bacterial nodules.

Table 3. Effects of *Meloidogyne javanica* inoculations on pigeon pea cultivars and lines.

Cultivar	Height[z] (cm)	Dry weight (g)		Number/pot	
		Shoots	Roots	Eggs and 2nd-stage juveniles	Bacterial nodules
Blanco	31.1 c	22.4 b	19.3 b	61,600 ab	58 c
Kaki	17.9 a	18.9 a	17.4 a	22,700 b	42 a
Pinto	21.9 ab	20.1 a	17.8 a	39,100 b	24 b
2B-Bushy	25.8 b	19.2 a	17.9 a	32,300 b	34 ab
147	26.3 b	19.4 a	17.7 a	105,200 a	35 ab

[z]Values in vertical columns followed by the same letters do not differ (P = 0.05), according to Duncan's multiple range test.

Table 4. Comparisons between pigeon pea cultivars and lines inoculated with *Meloidogyne javanica* and those noninoculated within the same cultivar.

Cultivar	Dry weight (g)[z]			
	Shoot		Root	
	I	NI	I	NI
98	0.63	1.36*	0.36	0.72*
Pinto	0.55	1.04*	0.35	0.53*
149	0.54	1.08*	0.33	0.57*
Blanco	0.44	0.97*	0.33	0.62*
147	0.50	0.97*	0.32	0.67*
Kaki	0.43	1.16*	0.24	0.58*
2B-Bushy	0.50	1.15*	0.26	0.52*
59	0.31	1.08*	0.27	0.53*

[z]Values from inoculated (I) and noninoculated (NI) plants within the same cultivars are compared statistically. An asterisk (*) indicates a significant (P = 0.05) difference between I and NI within each pair, according to a t-test.

In the second greenhouse test, shoot and root dry weights of all inoculated cultivars and lines were significantly lower than those in noninoculated controls (Table 4). Comparisons between inoculated cultivars and lines showed significant differences only in shoot dry weights and reproduction (Table 5). Line 98 showed the highest shoot dry weight and line 59 showed the lowest value. Results from comparisons

Table 5. Comparisons between the suitability of pigeon pea cultivars and lines to *M. javanica.*

Cultivar	Dry weight (g)[z]		Gall index[y]	Reproduction (eggs and 2nd-stage juveniles)
	Shoot	Root		
98	0.63 a	0.36 a	5 a	469,000 a
Pinto	0.55 ab	0.35 a	5 a	245,000 d
149	0.54 ab	0.33 a	5 a	238,000 d
Blanco	0.44 ab	0.33 a	5 a	368,000 b
147	0.50 ab	0.32 a	5 a	310,000 c
Kaki	0.43 ab	0.24 a	5 a	222,000 de
2B-Bushy	0.50 ab	0.26 a	5 a	194,000 e
59	0.31 b	0.27 a	5 a	141,000 f

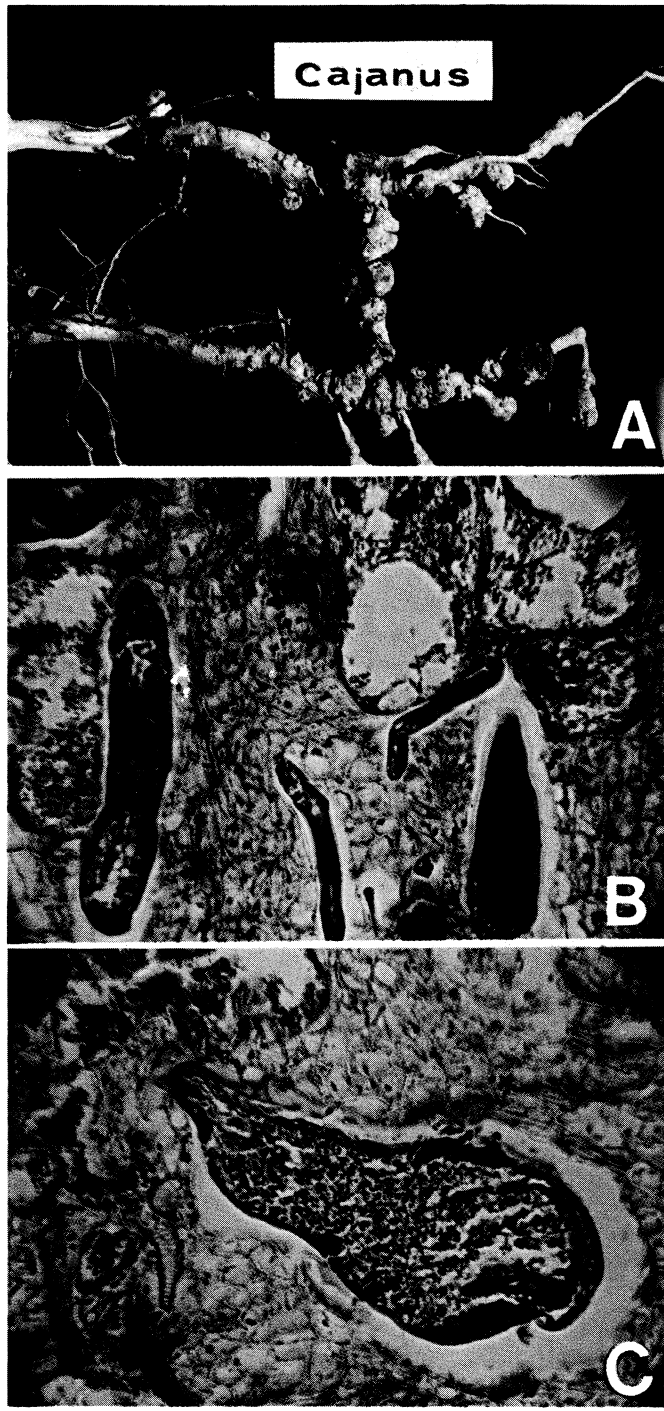
[y]Root-knot index based on the following scale: 0 = 0, 1 = 1-2, 2 = 3-10, 3 = 11-30, 4 = 31-100, 5 = greater than 100 galls.
[z]All values in vertical columns followed by the same latter do not differ (P = 0.05), according to Duncan's multiple range test.

Table 6. Values of fresh weight of pigeon pea pods and root gall indices in pigeon pea cultivars and lines from a field infested with *M. javanica,* Coamo, P. R.

Cultivar	Pod fresh weight/plot[z] (g)	Root gall index (0-5)
98	138.8 ab	4.4 a
Pinto	152.3 ab	4.7 a
149	261.5 a	4.9 a
Blanco	276.7 a	5.0 a
147	83.8 b	4.6 a
Kaki	208.5 ab	4.9 a
2B-Bushy	129.1 ab	4.3 a
59	169.7 ab	5.0 a

[z]Values in vertical columns followed by the same letters do not differ (P = 0.05), according to Duncan's multiple range test.

of root dry weights and root gall indices among cultivars and lines were not significantly different. Inoculated plants had many smaller bacterial nodules, whereas noninoculated had larger nodules but in fewer numbers. Nematode reproduction was high and significantly different among cultivars and lines. The highest values were recorded from line


Cajanus
A
B
C

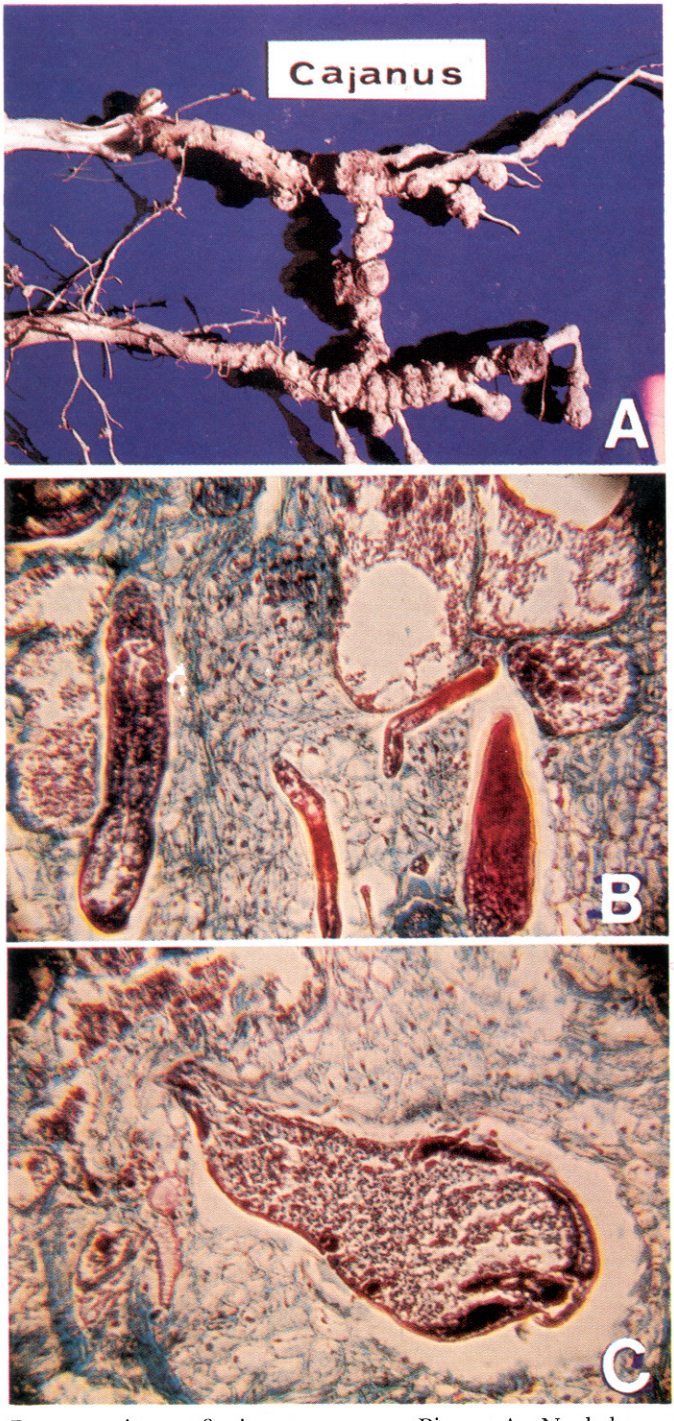


Fig. 1. Root sections of pigeon pea cv. Pinto. A. Nodules caused by *Meloidogyne javanica* in pigeon pea roots (100X); B. root-knot larvae at different stages of development feeding on giant cells (200X); C. a young female nematode feeding on giant cells around the nematode head (200X).

98, which also had the highest dry weights. The lowest values were from line 59, which had very low dry weights.

In the field experiment, the highest yield was obtained from the cultivar Blanco with a root gall index of 5.0, whereas the lowest was recorded from line 147 with a 4.6 gall index value (Table 6). These findings confirmed greenhouse results.

Histological examination of nematode infected roots, regardless of the cultivar or line, demonstrated the presence of different stages of development of the nematode and immature females feeding on giant cells (Fig. 1). This is the first report on the histopathology of *M. javanica* on pigeon pea.

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