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Crop Rotation Studies with Velvetbean (*Mucuna deeringiana*) for the Management of *Meloidogyne* spp.

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Abstract: Results from a greenhouse experiment at Cabrils, Spain, with two velvetbean (*Mucuna deeringiana*) accessions (Florida and Mozambique) growing in sterilized sandy loam and inoculated with *Meloidogyne arenaria* race 2, *M. incognita* race 1, and *M. javanica* revealed that the legume was not a host for these nematodes. In contrast, roots of 'Clemson Spineless' okra (*Hibiscus esculentum*), 'Summer Crookneck' squash (*Cucurbita pepo*), and 'Davis' soybean (*Glycine max*) were galled by all three root-knot nematodes. Greenhouse experiments at Auburn, Alabama, using soils infested with *Heterodera glycines* (race 14) + *M. incognita* or with *H. glycines* + *M. arenaria* (race 2) showed that, in contrast to Davis soybean, a Mexican and the Florida velvetbean accessions were not hosts for the nematodes. An experiment with 'Florunner' peanut (*Arachis hypogaea*) and the Florida velvetbean in a field infested with *M. arenaria* (race 1), near Headland, Alabama, showed that significant juvenile populations of the nematode at peanut harvest time were present only in plots with peanut. A microplot rotation experiment demonstrated that 'Black Beauty' eggplant (*Solanum melongena*) following the Florida velvetbean had heavier shoots and lower numbers of *M. arenaria* juveniles in the roots and in the soil than eggplant after Summer Crookneck squash or Davis soybean.

Key words: cropping system, *Heterodera glycines, Meloidogyne arenaria, Meloidogyne incognita, Meloidogyne javanica, Mucuna deeringiana,* nematode, nematode control, pest management, root-knot nematode, rotation, soybean cyst nematode, velvetbean.

Currently, nematode control in commercial production of many vegetable crops is based on the use of a few restricted nonfumigant nematicides or on preplant applications of limited number of fumigants (methyl bromide, metham sodium, or compositions containing 1,3-D) (1). These materials may not be available to producers in the future because of environmental concerns. It is therefore important to find alternative methods of control. Crop rotation can be an effective method for the management of nematode problems (6,7,18). There are many examples where crops that are nonhosts of *Meloidogyne* spp. have been used in rotation with susceptible crops to manage problems caused by these nematodes (18). Corn (*Zea mays*), cotton (*Gossypium hirsutum*), sorghum (*Sorghum bicolor*), and a number of other crops can be used to manage root-knot problems in peanut (*Arachis hypogaea*) and soybean (*Glycine max*) (7,10,11,16). A number of less common or exotic crops are also promising for the development of new rotation systems to control root-knot nematodes (12,13). Velvetbean (*Mucuna deeringiana*) is an African legume that has been used in the southern United States as a forage and cover crop. Root exudates of this plant are suppressive to *Meloidogyne* spp. (19), and there is evidence that velvetbean may be an effective rotation crop for the management of root-knot and other soilborne problems (5,19). This study presents information on host response of velvetbean to three *Meloidogyne* spp. and on the potential of this crop for use in rotations to manage root-knot nematodes.

Materials and Methods

The response of velvetbean to three *Meloidogyne* spp. was compared to those of five other plant species in a greenhouse experiment (Experiment 1) at the Institut de Recerca i Tecnologia Agroalimentàries (IRTA), Cabrils, Barcelona, Spain. *Meloidogyne* spp. cultures were from single egg masses originally isolated from infested plants in Barcelona province. These were: *M. arenaria* (race 2) from tomato (*Lycopersicon esculentum*) in Cabrera de Mar; *M. in-*

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ognita (race 1) from kiwi (*Actinidia deliciosa*) in Tordera; and *M. javanica* from fig (*Ficus carica*) in Cabrils. Cultures were maintained on 'Garrigues' almond (*Prunus amygdalus*). Eggs and juveniles were extracted from roots after maceration in NaOCl solution (2). An aqueous suspension of the inoculum was prepared to deliver 2,500 juveniles and eggs in 5 ml of suspension. Seeds of velvetbean, 'Clemson Spineless' okra (*Hibiscus esculentum*), 'Stoneville' cotton (*Gossypium hirsutum*), 'Davis' soybean (*Glycine max*), and 'Summer Crookneck' squash (*Cucurbita pepo*) were planted in pots (5 seeds/pot) containing 500 g of fine (≤1 mm mesh), sterilized loamy sand (pH = 8.3, organic matter content ≤1.0% [w/w], and cation exchange capacity [C.E.C.] ≤10 meq/100 g soil). Two types of velvetbeans were evaluated: a Florida accession obtained from a commercial source in Alabama and a Mozambique accession obtained from the Regional Plant Introduction Station (USDA, ARS, SAA; Griffin, GA 30223). Plants were allowed to grow for 2 weeks before inoculation. Inoculum was divided and delivered into each pot through five holes 4 to 5 cm deep placed equidistantly in a circle midway from the pot wall and its center. There were five pots per plant and nematode species in the experiment. Inoculated pots were placed in a greenhouse where the plants were maintained under optimal growing conditions, with temperatures fluctuating between 20 and 27 C. Five weeks after inoculation, the plants were removed from the pots, the roots were carefully washed to remove the sand, the number of nematode galls were counted, and fresh root weights were recorded.

Two greenhouse experiments were conducted at the Auburn University campus to compare the response of velvetbean with those of Davis soybean and Summer Crookneck squash in soil infested with the soybean cyst nematode (*Heterodera glycines* race 14) together with *M. incognita* (Experiment 2), and in soil infested with *H. glycines* and *M. arenaria* race 2 (Experiment 3). The soils were from soybean fields near Elberta, Baldwin County, in southwest Alabama. They were sandy loams with pH = 6.2, organic matter content <1.0% (w/w), and C.E.C. <10 meq/100 g soil. For each experiment, the field soil was mixed 50:50 (v:v) with fine (<1 mm mesh) sand, and the moist (60% field capacity) mixture was then apportioned in 1-kg amounts and placed in one-liter-capacity, 10-cm-d cylindrical PVC pots. Soil in the pots was planted with five seeds per pot; in each experiment there were eight pots per plant species. In Experiment 2 (*H. glycines* + *M. arenaria*), two velvetbean accessions were used, the Florida velvetbean used in the IRTA experiment and a Mexican accession from Tabasco provided by Dr. Roberto García (Departamento de Fitopatología, Escuela de Postgraduados, Montecillos, México). In Experiment 3, only the Florida velvetbean was used.

The seeded pots were placed in a greenhouse where the resulting plants were maintained in optimal conditions for growth. After 6 weeks, soil and plants were removed from the pots. A subsample of 100 cm^3 soil was taken from each pot. Plants were washed free of soil, the roots were weighed, and the numbers of galls caused by *Meloidogyne* spp. and numbers of cysts and mature females of *H. glycines* were counted. The general degree of galling in the roots was assessed using Zeck's root-knot index on a 0–10 scale, in which 0 represents no galls and 10 maximal galling (20). The roots were incubated in water for 72 hours to extract nematodes according to the "salad bowl" incubation technique (14). Nematodes in soil samples were also extracted using the same technique.

The effectiveness of velvetbean as a rotation crop for the management of *M. arenaria* was studied in a microplot experiment at the Old Agronomy Farm on the Auburn University campus. Microplots (30.5 cm × 30.5 cm) were delimited by terra-cotta chimney liners with walls 2.5 cm thick. The liners had been placed into holes dug into the ground so that the top 10 cm of the liners were above the soil line.

The liners were filled with soil infested with *M. arenaria* (30 juveniles/100 cm^3 soil). The soil had the same texture and properties as that used for the Auburn greenhouse experiments. Microplots were planted (five seeds/microplot) with Davis soybean, Summer Crookneck squash, and Florida velvetbean. There were 16 microplots for each plant species: eight were treated at-plant with aldicarb and the other eight were left untreated. Aldicarb was applied at a rate of 9 kg a.i./ha on a broadcast basis. The granules were spread evenly over the surface of the microplot and were then worked into the soil to a depth of 3–5 cm using a trowel. There were six treatments in the experiment arranged in a randomized complete block design. The microplots were watered as needed by means of a drip irrigation system. Weed control was by hand, and each plot was fertilized soon after emergence of plants with 20 g of 14-14-14 Osmocote (Grace/Sierra, Milpitas, CA) slow-release fertilizer. The plants were allowed to grow for 3 months when they were cut leaving the roots in the soil. Each microplot was then planted with a single 3-week-old 'Black Beauty' eggplant (*Solanum melongena*). The eggplants were fertilized as described with Osmocote and grew for 2 months, when an early frost killed them. At this time, four 2.5-cm-diam soil cores were taken from each microplot to a depth of 20–25 cm using a standard soil probe. The cores were pooled and a 100-cm^3 subsample was used for nematological analysis with the "salad bowl" incubation technique (14). The eggplants were then removed, the soil was washed from the roots, and the fresh weights of roots and shoots were recorded. Galls caused by *M. arenaria* in the roots were counted, and the root system from each plot was incubated to determine nematode numbers using the salad bowl incubation technique.

The effect of velvetbean on populations of *M. arenaria* (race 1) was studied at the Wiregrass substation, near Headland, Alabama, in a peanut (*Arachis hypogaea*) field. The field had been in peanut with winter fallow for the preceding 15 years. The soil was a sandy loam with pH = 6.0, organic matter content <1.0% (w/w), and C.E.C. <10 meq/100 g soil. Plots in the experiment were eight rows wide and 6 m long; the row width was 0.9 m. The three treatments were 'Florunner' peanut planted with at-plant application of aldicarb, peanut without aldicarb, and Florida velvetbean planted in rows at 11 kg seeds/ha. Aldicarb was applied in a 20-cm-wide band with the seed furrow in the middle of the band at a rate of 31 g a.i./100 m row; the granules were incorporated 2–3 cm into the soil by the action of spring-activated tines attached to the planting equipment. Cultural practices and control of foliar diseases, insects, and weeds in peanut were according to recommendations for the area (3,4). No pesticides were used in plots with velvetbean. The field was watered as needed using a center pivot irrigation system. There were eight replications (plots) per treatment arranged in a randomized complete block design. Peanut and velvetbean were planted on 9 May 1989, and soil samples for nematode analysis were taken on 29 August just before peanut harvest to coincide with the period of maximal *M. arenaria* juvenile numbers in soil (15). Soil samples were collected from each plot and consisted of 18–20 2.5-cm-diam cores taken with a standard probe. The cores were taken from the plots at approximately 0.3-cm spacings and from the root zone to a depth of 20–25 cm. Cores from a plot were pooled and a 100-cm^3 subsample was used to determine nematode numbers as described for the other experiments.

All data were subjected to standard procedures for analysis of variance (9,17). Fisher's least significant differences (F.L.S.D.) were calculated when F values were significant and are included in the tables of results. Unless otherwise stated, all differences referred to in the text were significant at $P \leq 0.05$.

Results and Discussion

Experiment 1: All three *Meloidogyne* spp. failed to develop significant numbers of

TABLE 1. Host response of two velvetbeans and five field crop plant species to *Meloidogyne arenaria* (race 2), *M. incognita* (race 1), and *M. javanica* in a greenhouse experiment (Experiment 1).

Crop	*M. arenaria* race 2		*M. incognita* race 1		*M. javanica*	
	Fresh root weight (g)	No. galls per pot	Fresh root weight (g)	No. galls per pot	Fresh root weight (g)	No. galls per pot
'Stoneville' cotton	2.49	0	2.26	0	3.02	0
'Clemson Spineless' okra	2.46	58	1.51	65	3.65	221
'Florunner' peanut	4.53	0	3.83	0	3.84	0
'Davis' soybean	11.13	160	9.40	19	10.45	152
'Summer Crookneck' squash	3.17	81	2.38	91	3.79	93
Florida velvetbean	4.15	0	3.27	0	4.38	0
Mozambique velvetbean	5.02	2	3.32	0	5.38	0
F.L.S.D. ($P = 0.05$)	1.92	25	1.52	18	1.53	24

Data are means of five replications.

galls in roots of velvetbean, peanut, and cotton. This contrasted with okra, soybean, and squash, in which significant numbers of galls were detected in the roots (Table 1). For each plant species in the experiment, root development was comparable in pots with different *Meloidogyne* spp.

Experiment 2: No galls from *M. incognita* were found in roots of the Florida velvetbean and no juveniles of the nematode were extracted from roots of either the Florida or the Mexican accessions (Table 2). Soybean and squash roots were moderately galled (root-knot index values = 5–6) and contained significant juvenile populations of *M. incognita*. Cysts, mature females, and juveniles of *H. glycines* were found only in soybean roots. Roots of both types of velvetbean contained only males of *H. glycines*. Juvenile populations of *M. incognita* in soil were significant only in soil with squash or soybean, whereas *H. glycines* juveniles were high in numbers only in soil with soybean.

Experiment 3: In contrast with squash and soybean, roots of velvetbean had no galls from *M. arenaria*, and no juveniles of the nematode were extracted from them (Table 3). Significant numbers of cysts, females, and juveniles of *H. glycines* were associated only with soybean roots. Numbers of juveniles of *M. arenaria* were highest in soil with squash and were few or none in soils with soybean or velvetbean.

Microplot experiment: Eggplants following velvetbean had heavier shoots than those grown after squash or soybean (Table 4). Application of aldicarb did not increase eggplant shoot weight (Table 4) but had an adverse effect on root weights of eggplants

TABLE 2. Development of *Meloidogyne incognita* and *Heterodera glycines* on soybean, squash, and velvetbean planted in a greenhouse in soil infested with the two nematode species (Experiment 2).

Crop	No. galls per g root	Galling index†	Nematodes per pot				
			H. glycines cysts and females	Juveniles in roots		Juveniles per 100 cm^3 soil	
				M. incognita	*H. glycines*	*M. incognita*	*H. glycines*
'Davis' soybean	22	4.9	12	518	84	31	188
'Summer Crookneck' squash	30	5.9	0	557	0	66	11
Florida velvetbean	0	0.0	0	0	36‡	7	7
Mexican velvetbean	13	0.0	0	0	33‡	13	9
F.L.S.D. ($P = 0.05$)	3	2.0	2	133	16	17	33

Data are means of eight replications.
† Zeck's (20) galling index: 0–10.
‡ All males.

TABLE 3. Development of *Meloidogyne arenaria* race 2 and *Heterodera glycines* race 14 in soybean, squash, and Florida velvetbean planted in a greenhouse in soil infested with the two nematode species (Experiment 3).

Crop	Fresh root weight (g)	No. galls per g root	Galling index†	Nematodes per pot				
				H. glycines cysts and females	Juveniles in roots		Juveniles per 100 cm^3 soil	
					M. incognita	*H. glycines*	*M. incognita*	*H. glycines*
'Davis' soybean	0.92	30	5.0	4	175	97	3	220
'Summer Crookneck' squash	0.44	33	5.5	0	53	0	15	24
Florida velvetbean	0.94	0	0.0	0	0	12	0	10
F.L.S.D. ($P = 0.05$)		3	0.4	1.5	39	15	4	50

Data are means of eight replications.
† Zeck's (20) galling index: 0–10.

TABLE 4. Effect of the previous crop on growth of 'Black Beauty' eggplant and development of *Meloidogyne arenaria* race 2 in a microplot experiment at the Old Agronomy Farm on the Auburn Campus.

Previous crop	Nematicide treatment†	Fresh top weight (g)	Galling index‡	Galls per g of root	Juveniles per g of root	Juveniles per 100 cm^3 soil
Squash	(−)	44.1	7.1	13.6	114	209
Squash	(+)	38.8	5.1	10.5	12	29
Soybean	(−)	50.3	6.4	17.0	77	166
Soybean	(+)	56.0	5.6	10.5	41	175
Velvetbean	(−)	80.5	5.1	7.9	27	90
Velvetbean	(+)	70.3	3.9	4.5	13	44
F.L.S.D. (P = 0.05)		24.4	2.0	5.6	37	135

Data are means of eight replications.
† (−) = without nematicide treatment; (+) = with an at-plant application to eggplant of aldicarb at 9 kg a.i./ha.
‡ Zeck's (20) galling index: 0–10.

following squash (data not shown). The lowest number of galls per gram of root was for eggplants following velvetbean. Aldicarb reduced numbers of galls in eggplants following soybean. The numbers of *M. arenaria* juveniles per gram of fresh root were lowest in eggplant after velvetbean and highest in eggplant after squash. Aldicarb reduced juvenile populations in eggplant roots following squash. *Meloidogyne arenaria* juvenile soil populations were lowest in soil from microplots with eggplants after velvetbean with aldicarb and highest in those following squash without aldicarb. Aldicarb reduced juvenile populations in eggplants after squash.

Field experiment: Highest numbers of *M. arenaria* juveniles were found in plots with Florunner peanut and the lowest in those with velvetbean (Table 5). Aldicarb reduced juvenile numbers in peanut plots; however, populations in plots with nematicide were higher than in those with velvetbean.

TABLE 5. Numbers of *Meloidogyne arenaria* race 1 juveniles immediately before harvest from a field experiment with 'Florunner' peanut and Florida velvetbean at the Wiregrass substation, near Headland, in southeast Alabama.

Crop	Juveniles per 100 cm^3 soil
'Florunner' peanut	188
'Florunner' peanut with aldicarb†	74
Florida velvetbean	23
F.L.S.D. (P = 0.05)	31

Data are means of eight replications.
† Aldicarb was applied at-plant at 31 g a.i./100 m row in a 20-cm-wide band, with the seed furrow in the middle of the band.

Results of this study show that velvetbean is a poor host for the common *Meloidogyne* spp. in Alabama. This property was true of the three different types of velvetbean tested in our experiments. Velvetbean can be used in combination with corn or other crops to improve soil fertility and to suppress a number of soilborne pathogens (5). There is evidence that root exudates from this plant may exert a suppressive effect on root-knot nematodes (19). Recently, Kloepper et al. (8) showed that rhizosphere bacteria of velvetbean were markedly different from those of soybean and other crops. It is possible that the peculiar rhizosphere microflora of this legume may exert a suppressive effect on *Meloidogyne* spp. Our results also indicate that velvetbean may be suppressive to *H. glycines,* suggesting that the effect of the legume on nematodes may be more generalized. There is need to determine the spectrum of activity of velvetbean on other nematodes.

Results from the microplot and field experiments indicate that velvetbean may be an excellent rotation crop for the management of root-knot nematode problems in the production of vegetables and other crops. In these experiments, we did not incorporate velvetbean green matter into the soil. The amount of green manure produced by this legume can be in excess of 10 tons dry matter/ha. Incorporation

into the soil of such material could enhance soil fertility and possibly result in significant destruction of *Meloidogyne* inoculum through stimulation of microbial activity. Velvetbean grows quickly when soil temperatures are >20–25 C and is ideally suited for the humid subtropical and tropical regions of the world where root-knot nematode problems are common in crop production.

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