

Effect of Castor and Velvetbean Organic Amendments on *Meloidogyne arenaria* in Greenhouse Experiments¹

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Abstract: Effectiveness of castor (*Ricinus communis*) and velvetbean (*Mucuna deeringiana*) amendments was tested for suppression of the root-knot nematode (*Meloidogyne arenaria*) and growth of okra (*Hibiscus esculentus*) in three greenhouse experiments. Regression analysis was used to relate nematode population data or plant growth responses to various rates (0, 1, 2, 4, or 8 g/560 cm³ soil pot) of each amendment in separate experiments. In general, plant growth parameters responded positively to the amendment rate until a level of about 4 g to 5 g of velvetbean or castor amendment/pot. Similar trends were observed for nematode galls, egg masses, and second-stage juveniles extracted from root systems. In most circumstances, quadratic equations best expressed the relationships between plant or nematode parameters and rates of velvetbean or castor amendment, leading to the assumption that a best rate of the amendment for plant growth or nematode suppression can be predicted. In a third experiment, in which both amendments were compared directly, velvetbean amendment was more efficient than castor in suppressing nematodes as well as in improving plant growth.

Key words: *Hibiscus esculentus*, *Meloidogyne arenaria*, *Mucuna deeringiana*, nematode, nematode management, okra, *Ricinus communis*, root-knot nematode.

A variety of organic amendments has been used for management of nematodes. These amendments are associated either with reduced infection or survival of nematodes or with increases in microbial and animal species antagonistic to nematodes (Ichinohe, 1985; Linford et al., 1938; Mankau, 1968; Mankau and Minteer, 1962; Muller and Gooch, 1982; Rodríguez-Kábana, 1986; Sitaramaiah and Singh, 1978; Trivedi and Barker, 1986; Watson, 1945). Of the amendments tested by Ritzinger (1997), castor (*Ricinus communis* L.) and velvetbean (*Mucuna deeringiana* [Bort.] Merr., *M. pruriens* DC) were the most effective in improving plant growth and reducing nematode population levels. There was insufficient evidence in these studies to conclude if nematode suppression was due to the release of toxic substances, to improved plant nutrition, or to the enhanced growth of antagonistic organisms. Effectiveness of nematode suppression by organic amendments generally depends on the amount of the amendment used, C/N ratio, and time of decomposition (McSorley and Gallaher, 1995a, 1995b).

Singh and Sitaramaiah (1994) reported that the decomposition rate of an amendment depends on the soil type and the climate. Since the rate of organic amendment decomposition is also related to the amount applied, nematode suppression is related to both the quantity and quality of the decomposition products (Singh and Sitaramaiah, 1994). Although many studies have shown that the amount of the organic amendment is important in nematode suppression (Holtz and Vandecaveye, 1938; Linford et al., 1938; Mankau, 1968; Mankau and Minteer, 1962; McSorley and Gallaher, 1995b; Rodríguez-Kábana, 1986; Sitaramaiah and Singh, 1978; Watson, 1945), further research on the quality and quantity of amendments still is needed to stimulate their widespread use (McSorley and Gallaher, 1995a, 1995b).

Castor and velvetbean were chosen for greenhouse studies because they had been used in earlier tests as organic amendments, had suppressed *Meloidogyne arenaria* (Neal) Chitwood race 1, and had provided the best plant growth response among several amendments tested (Ritzinger, 1997). The objectives of these greenhouse experiments were to determine which amendment type was more effective in improving plant growth and suppressing *M. arenaria* race 1 on okra (*Hibiscus esculentus* L.) and to determine the effects of various application rates

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of these amendments on nematode levels and plant growth.

MATERIALS AND METHODS

Three experiments were carried out in a greenhouse during summer and fall 1996 on the University of Florida campus in Gainesville, Florida. Castor and velvetbean plants to be used as dry organic amendments for all three experiments were grown in 18-cm-diam. clay pots. Above-ground parts of both types of plants were harvested before the reproductive stage. Leaves and stems of each were chopped separately, mixed well, and then air-dried until constant weight was reached. It required 6.9 g and 4.4 g of fresh castor and velvetbean, respectively, to obtain 1.0 g of dry amendment. A mineral analysis of each amendment, determined according to methodology described elsewhere (Ritzinger, 1997), is presented in Table 1.

For all experiments, 2-week-old seedlings of 'Clemson Spineless' okra (*Hibiscus esculentus* L.) were used as test plants. Seedlings were transplanted, one per pot, into plastic pots filled with 560 cm³ of steam-sterilized field soil mixed with sand in a ratio of 1:1 by volume. The composition of the soil mixture for all experiments was 95% sand, 4% clay, and 1% silt.

One day after transplanting, the pots received nematode inoculum and amendment treatments according to the requirements of each experiment. The inoculum treatment consisted of 1,000 fresh second-stage juveniles (J2) of *Meloidogyne arenaria* race 1, which had been increased on 'Rutgers' tomato (*Lycopersicon esculentum* Mill.). Pots with no J2 constituted the no-inoculum treatment. Amendment treatments are

specified below for each experiment. Plants were maintained in a greenhouse and sprayed twice per week with a dilute soap solution to reduce infestations of whiteflies. No other pesticides or fertilizers were applied.

At harvest, okra plants were removed from the soil, and stem diameter, plant height, and fresh top weight were recorded. Root galls and egg masses were rated according to the root-knot index of Taylor and Sasser (1978), where 0 = 0 galls or egg masses per root system, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 galls or egg masses per root system. In the first and second experiments, eggs were extracted from egg masses in the okra root systems with 1.05% NaOCl (Hussey and Barker, 1973). The extracted eggs were then incubated on Baermann trays (Rodríguez-Kábana and Pope, 1981) for 7 days, and the hatched J2 were counted. In the third experiment, the same technique was used but with only 10 egg masses from each root system. After those 10 egg masses were removed from the root system, the remaining root system was immersed in a phloxine B solution (0.15 g/l tap water) (Southey, 1982) for rating all the remaining egg masses and galls. At harvest, a soil sample was taken from each pot to determine the final population of J2 in 100 cm³ soil from the infested treatments by means of the centrifugal flotation technique (Jenkins, 1964).

The experimental design differed somewhat for each experiment; however, data were statistically analyzed as a completely randomized factorial design in all cases. In the first two experiments, which involved different rates of amendments, regression analysis was used to determine the pattern of response of each measured variable to the

TABLE 1. Mineral analysis and C/N ratio of the organic amendment treatments.

Amendment	C/N ratio	Macronutrients (percent)					Micronutrients (ppm)			
		N	P	K	Ca	Mg	Mn	Zn	Cu	Fe
Castor	7.91	2.26	0.34	3.20	1.64	0.53	280	105	34.25	61.50
Velvetbean	8.68	2.20	0.23	1.39	1.40	0.39	460	1,070	12.00	60.75

Data are means of five replications from the aerial plant parts harvested before the reproductive stage.

amendment rate at each inoculum level. Since a quadratic regression resulted in a better fit (higher r^2) for all cases, it was chosen to represent the trend rather than a linear regression. Analysis of variance was applied to the factorial designs and, when a main effect was significant with no interactions, a separate analysis was carried out and means were separated with Tukey's test (SAS Institute, Cary, NC).

Experiment 1 – velvetbean amendment: On 27 May 1996, 2-week-old okra seedlings were transplanted individually into each pot receiving inoculum and amendment rate treatments. The inoculum treatment consisted of 0 or 1,000 J2/pot. The amendment was applied as a mulch at the top of the pot at rates of 0, 1, 2, 4, or 8 g of dry velvetbean per pot. The experimental design was a 5×2 factorial with 10 replications (5 for each harvest date). On 1 July, 35 days after inoculation, half of the experiment was harvested and plant parameters and nematode data were recorded. On 23 July, 57 days after inoculation, the remainder of the experiment was harvested and the same plant and nematode parameters were recorded. At this time, J2 were extracted from 100 cm³ soil and counted.

Experiment 2 – castor amendment: Two-week-old okra seedlings were transplanted and inoculated on 18 June 1996. The inoculum consisted of 0 or 1,000 J2/pot. The amendment consisted of dry castor applied as a mulch at rates of 0, 1, 2, 4, or 8 g/pot. The experiment was a 5×2 factorial with 10 rep-

lications. On 25 July, 37 days after inoculation, half of the experiment was harvested following the same procedure as in the first experiment. On 17 August, 60 days after inoculation, the remaining pots of the experiment were harvested.

Experiment 3 – combined amendments, velvetbean, and castor: On 24 September 1996, 2-week-old okra seedlings were inoculated with 0 or 1,000 J2/pot. The amendment treatments were castor or velvetbean, both applied at rates of 2 or 8 g/pot. The experimental design was a $2 \times 2 \times 2$ factorial with 12 replications, including two inoculum densities, two amendments, and two amendment rates. Thirty days after inoculation, on 24 October, half of the experiment was harvested and all the plant and nematode data were recorded, except J2 were not extracted from soil. The remainder of the experiment was harvested on 7 November, 45 days after inoculation.

RESULTS AND DISCUSSION

Experiment 1 – velvetbean amendment: There were significant ($P \leq 0.01$) interactions between inoculum and amendment rate for stem diameter at 35 and 57 days and for plant height at 35 days after inoculation (Table 2). The relationships between these plant parameters and amendment rate were expressed with quadratic regression equations (Figs. 1, 2). In general, these plant parameters increased to a certain point as amendment rate increased and tended to

TABLE 2. Effect of inoculum level (0 or 1,000) of *Meloidogyne arenaria* and soil amendment rate (0, 1, 2, 4, or 8 g) on growth of okra in greenhouse experiments. Numbers are F-values from analysis of variance.

Treatment effect	Stem diameter		Plant height		Fresh top weight	
	Mid ^a	Final ^a	Mid	Final	Mid	Final
Experiment 1 (Velvetbean amendment)						
Inoculum level	1.4	6.9**	8.7**	14.7**	3.1	2.1
Amendment rate	15.7**	34.7**	2.9*	9.4**	24.1**	44.4**
Inoculum \times rate	4.9**	5.2**	4.8**	2.5	1.5	2.1
Experiment 2 (Castor amendment)						
Inoculum level	11.2**	24.7**	2.9	100.6**	17.8**	85.3**
Amendment rate	74.7**	129.3**	10.6**	46.7**	149.3**	143.4**
Inoculum \times rate	0.7	1.3	0.8	1.3	0.7	2.1

^a Midseason harvest at 35 (Experiment 1) or 37 (Experiment 2) days after inoculation; final harvest at 57 (Experiment 1) or 60 (Experiment 2) days after inoculation.

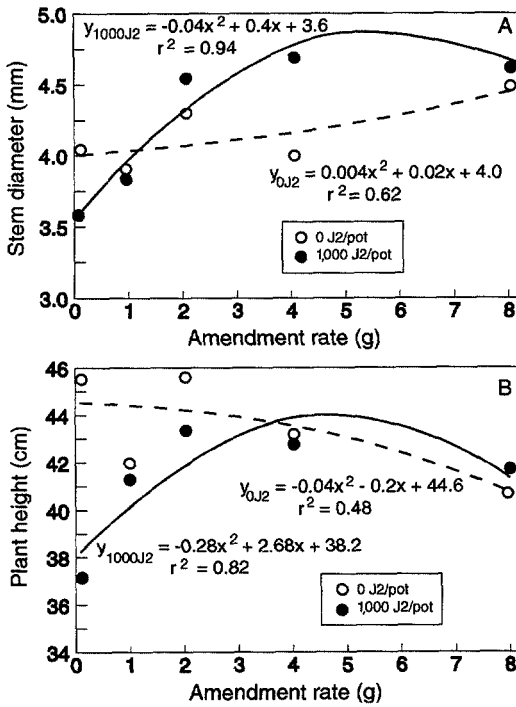


FIG. 1. Relationships between okra plant growth at 35 days and application rate of velvetbean amendment in pots inoculated or not inoculated with second-stage juveniles (J2) of *Meloidogyne arenaria*. A) Stem diameter. B) Plant height. All r^2 values significant at $P \leq 0.01$ (Fig. 1A) or $P \leq 0.05$ (Fig. 1B).

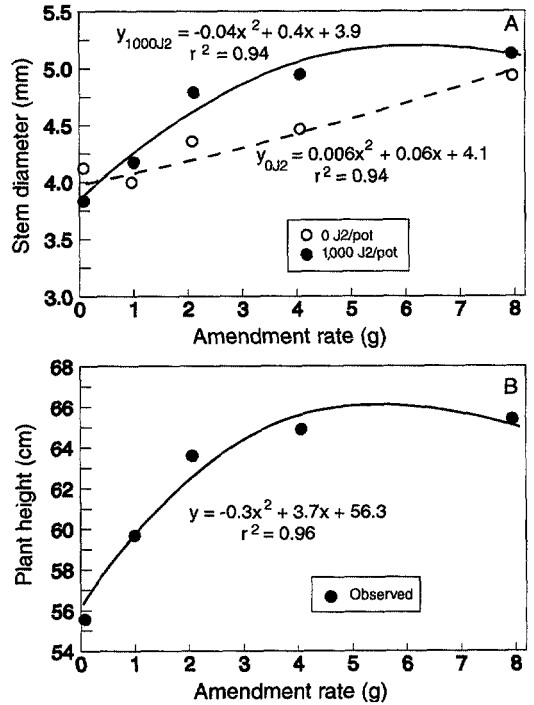


FIG. 2. Relationships between okra plant growth at 57 days and application rate of velvetbean amendment in pots inoculated or not inoculated with second-stage juveniles (J2) of *Meloidogyne arenaria*. A) Stem diameter. B) Plant height pooled across inoculation treatments since no interactions occurred. Both r^2 values significant at $P \leq 0.01$.

level off at the highest rate (Figs. 1,2). The quadratic shape was more evident for the inoculated pots and approached a more linear shape with the uninoculated pots. An exception was observed for plant height at 35 days with the no-inoculum treatment (Fig. 2A), although the r^2 value was relatively low (0.48).

Amendment rates were significantly different ($P \leq 0.05$), but no amendment rate \times inoculum interaction occurred for plant height at 57 days after inoculation or for top fresh weight at 35 and 57 days after inoculation. Plant height at 57 days exhibited a quadratic response to amendment rate for data pooled across inoculum rate (data were pooled since no interaction occurred) (Fig. 2B). Data for fresh top weight at 35 days showed a similar trend, but the relationship between top fresh weight at 57 days and amendment rate was better explained by a linear regression ($r^2 = 0.98$), at least within

the rates tested in this experiment (Table 3). Most plant parameters responded positively to amendment rates up to 4 g, but did not improve further with rates up to 8 g (Figs. 1,2). The highest response to the amendment under most of these quadratic regressions was predicted to occur between rates of 5 g and 6 g of the amendment (Figs. 1,2). Above these levels, other factors, such as toxicity of the amendment, may have inhibited plant response.

Root galling at 35 days after inoculation and J2 extracted from egg masses and soil at 57 days after inoculation decreased ($P \leq 0.01$) with amendment rate (Table 4). Responses of these parameters were best fit by quadratic regression equations. Egg mass ratings at 35 days (mean = 4.08) and 57 days (mean = 4.01), gall ratings at 57 days (mean = 4.96), and J2 extracted from egg masses at 35 days (mean = 47.8) were not affected by amendment rate.

TABLE 3. Effect of rates of velvetbean or castor amendments on growth of okra in greenhouse experiments.

Parameter measured	Amendment rate (g)					Regression equation ^a	r ²
	0	1	2	4	8		
	Experiment 1 (Velvetbean amendment)						
Top fresh weight (g), 35 days	9.0 ^b	11.8	11.6	13.2	16.2	$y = -0.04x^2 + 1.14x + 9.7$	0.94
Top fresh weight (g), 57 days	9.8	11.3	13.8	15.3	20.6	$y = 1.3x + 10.2$	0.98
	Experiment 2 (Castor amendment)						
Stem diameter (mm), 37 days	3.8	4.2	4.6	5.0	5.7	$y = -0.02x^2 + 0.4x + 3.8$	0.99
Stem diameter (mm), 60 days	3.9	4.6	5.0	5.7	6.4	$y = 0.03x^2 + 0.6x + 4.0$	0.99
Plant height (cm), 37 days	36.9	41.7	44.4	43.7	44.4	$y = -0.03x^2 + 2.8x + 38.1$	0.80
Plant height (cm), 60 days	47.9	55.9	57.0	64.9	67.5	$y = 0.4x^2 + 5.7x + 48.7$	0.97
Top fresh weight (g), 37 days	6.3	10.4	13.6	16.2	19.8	$y = -0.23x^2 + 3.4x + 6.8$	0.98
Top fresh weight (g), 60 days	7.2	11.6	14.0	20.9	28.6	$y = -0.16x^2 + 4.0x + 7.3$	0.99

^a Best-fit regression equation (linear or quadratic); y = parameter measured, x = amendment rate in g. All r² values are significant at $P \leq 0.01$.

^b Data are means of 10 replications, pooled across inoculum levels.

In general, plant parameters responded positively to amendment rate up to a level of about 4 g to 5 g of velvetbean per pot, then levelled off or decreased at higher rates. There was an optimum rate of the amendment that promoted the best plant response; beyond that rate the amendment could become toxic to the plant or at least non-beneficial. A similar trend was noted for the nematode evaluations. The best plant growth responses and nematode suppression under the velvetbean amendment were recorded at rates of 4 g to 6 g of the amendment/pot.

Experiment 2 – castor amendment: Relationships between plant parameters and rate of castor amendment were similar to results obtained with velvetbean, except that no in-

oculum × amendment rate interactions were observed (Tables 2, 3). The relationships between these plant parameters and amendment rate were better fitted by quadratic regression equations, which had higher r² values than corresponding linear models (Table 3). The quadratic regression is consistent with the hypothesis that at higher rates of the organic amendment, the decomposition products of the amendment might be phytotoxic. Much of the research that has investigated the use of organic amendments for biological control has indicated that efficacy is dependent upon the amendment rate as well as the C/N ratio. Other workers have concluded that the decomposition of some amendments might be toxic to the plant, even before suppression of nema-

TABLE 4. Effect of rates of velvetbean or castor amendments on egg mass or gall ratings and on numbers of second-stage juveniles (J2) of *Meloidogyne arenaria* on okra in greenhouse experiments.

Parameter measured	Amendment rate (g)					Regression equation ^a	r ²
	0	1	2	4	8		
	Experiment 1 (Velvetbean amendment)						
Gall rating, 35 days	4.8	4.6	4.0	4.0	4.0	$y = 0.03x^2 - 0.3x + 4.8$	0.88
J2 from egg masses, 57 days	1,199	812	753	769	643	$y = 13.4x^2 - 160x + 1088$	0.75
J2/100 cm ³ soil, 57 days	362	200	104	109	50	$y = 8.4x^2 - 100x + 323$	0.87
	Experiment 2 (Castor amendment)						
Egg mass rating, 37 days	4.6	4.8	4.2	4.0	4.0	$y = 0.02x^2 - 0.3x + 5.0$	0.77
J2 from egg masses, 37 days	653	820	729	562	424	$y = -3.8x^2 - 10x + 732$	0.76

Data are means of five replications. All r² values significant at $P \leq 0.01$.

^a Galls or egg masses rated on 0-to-5 scale: 0 = 0; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls or egg masses per root system.

^b Best-fit regression equation (linear or quadratic); y = parameter measured, x = amendment rate in g. All r² values are significant at $P \leq 0.01$.

todes (Brown, 1987; Muller and Gooch, 1982; Rodríguez-Kábana, 1986; Stirling, 1991; Trivedi and Barker, 1986). In our studies, significant toxicity symptoms from amendment application were not observed, only a levelling off in plant performance at the highest amendment rates used.

Egg mass index and J2 extracted from the egg masses at 37 days were affected by amendment rate ($P \leq 0.01$) (Table 4). Egg mass index at 60 days (mean 4.72), gall ratings at 37 days (mean = 4.04) and 60 days (mean = 5.0), and J2 from the egg masses at 60 days (mean = 971) were not affected.

In general, plant parameters increased progressively in response to the amendment rate up to 4 g to 6 g/pot, and then either levelled off or decreased. This trend was represented by the quadratic regression. For both experiments, effects on plant parameters were observed during both harvest periods.

Experiment 3 – combined amendments, velvetbean and castor: There was a significant interaction ($P \leq 0.05$) between amendment type and amendment rate for all plant parameters, and a triple interaction ($P \leq 0.05$) among inoculum level, amendment type, and amendment rate only for stem diameter at 45 days after inoculation (Table 5). For most plant parameters, there was no significant difference from the castor amendment at rates of 2 g or 8 g/pot, but plant growth was greater at 8 g/pot than at 2 g/pot of velvetbean amendment (Table 6). Effects of

TABLE 6. Effect of amendment and amendment rates on okra in a greenhouse experiment (Experiment 3—combined experiment).

Amendrate rate (g)	Amendment		Mean
	Velvetbean	Castor	
	Stem diameter (mm) 30 days		
2	3.3 b ^a	3.5 a	3.4 B ^b
8	3.8 a	3.4 a	3.6 A
Mean	3.5 A ^b	3.4 A	3.5
	Stem diameter (mm) 45 days		
2	3.4 b	3.6 a	3.5 B
8	4.0 a	3.5 a	3.8 A
Mean	3.7 A	3.6 A	3.6
	Plant height (cm) 30 days		
2	33.6 b	33.7 a	33.6 A
8	36.1 a	32.7 a	34.4 A
Mean	34.8 A	33.2 B	34.0
	Plant height (cm) 45 days		
2	37.4 b	38.7 a	38.0 B
8	44.9 a	38.4 a	41.7 A
Mean	41.2 A	38.5 B	39.8
	Top fresh weight (g) 45 days		
2	7.4 b	8.4 a	7.9 B
8	12.6 a	8.8 a	10.7 A
Mean	10.0 A	8.6 B	9.3

^a Data followed by small letters are means of six replications. Means followed by the same small letters in columns are not significantly different ($P \leq 0.05$), according to Tukey's test.

^b Capital letters refer to comparisons between main effect means in columns or in rows.

amendment on *M. arenaria* varied in this experiment. There was a significant interaction ($P \leq 0.05$) between amendment and amendment rate for galls per gram dry root, with the highest number of galls recorded at 8 g of the castor amendment (Table 7). Egg mass index was affected by the amendment

TABLE 5. Effect of organic amendment type, amendment rate, and inoculum level of second-stage juveniles of *Meloidogyne arenaria* on okra in a greenhouse experiment (Experiment 3). Numbers are F-values from the analysis of variance.

Treatment effect	Stem diameter		Plant height		Fresh top weight
	30 days	45 days	30 days	45 days	
Amendment (A) ^a	2.15	2.57	5.94*	5.95*	9.65**
Amendment rate (B) ^b	8.99**	15.13**	1.11	11.34**	40.43**
Inoculum level (C) ^c	8.24**	0.05	4.83*	1.84	0.12
Interaction (AB)	16.15**	25.34**	6.24*	12.96**	28.50**
Interaction (AC)	0.20	2.57	0.15	0.66	0.05
Interaction (BC)	2.54	0.47	0.15	0.04	0.10
Interaction (ABC)	0.04	5.24*	0.04	0.00	0.32

^a Velvetbean or castor

^b Rates of 2 g or 8 g/pot.

^c Levels of 0 or 1,000 J2/pot.

TABLE 7. Effect of amendment type and amendment rate (2 g or 8 g) on egg masses, galls, and second-stage juveniles (J2) of *Meloidogyne arenaria*, experiment 3 (combined experiment).

Amendment rate	Amendment		Mean
	Velvetbean	Castor	
	Egg mass index ^a		
2	4.8 a ^b	4.8 a	4.8 A ^c
8	4.2 a	4.5 a	4.3 B
Mean	4.5 A ^c	4.7 A	4.6
	Eggs/g dry root		
2	14.8 a	13.6 a	14.2 A
8	12.1 a	17.3 a	16.2 A
Mean	13.4 A	15.4 A	14.4
	Gall index ^a		
2	4.5 a	4.8 a	4.7 A
8	4.7 a	5.0 a	4.8 A
Mean	4.6 A	4.9 A	4.7
	Galls/g dry root		
2	13.5 a	13.6 b	13.6 A
8	13.5 a	19.0 a	16.2 A
Mean	13.5 B	16.3 A	14.9
	J2/10 egg masses		
2	1,433 a	1,220 a	1,326 A
8	1,262 a	1,216 a	1,239 A
Mean	1,348 A	1,218 A	1,283
	J2/100 cm ³ soil		
2	7.5 a	17.3 a	12.4 A
8	7.7 a	13.0 a	10.3 A
Mean	7.6 B	15.2 A	11.4

^a Egg masses or galls rated on 0-to-5 scale: 0 = 0; 1 = 1-2; 2 = 3-10; 3 = 11-30; 4 = 31-100; 5 = more than 100 galls or egg masses root system.

^b Data are means of six replications: Means followed by the same small letters in columns for each parameter are not significantly different at $P \leq 0.05$, according to Tukey's test.

^c Capital letters refer to comparisons between main effect means in columns or in rows.

rate ($P \leq 0.05$), while the number of J2 extracted from soil was affected ($P \leq 0.05$) by the amendment type (Table 7). Where differences occurred, velvetbean amendment was more efficient than castor in suppressing *M. arenaria*.

In the first and second experiments, there were significant responses of plant parameters and nematodes to velvetbean and castor amendments applied at low rates, but at the highest rate (8 g/pot), there were small differences or no additional effects on plant parameters or nematode suppression. Thus, the differences recorded between 2 g and 8 g of velvetbean amendment in the third experiment are puzzling. Under the conditions of the third experiment, in which both

amendments were compared directly, the velvetbean amendment appeared to be more efficient in suppressing nematodes and in improving plant growth. It is possible that during amendment decomposition, velvetbean may release substances that improve plant growth and suppress nematodes better than the castor amendment, as all the other parameters were constant. In addition, only 4.4 g of fresh velvetbean was needed to obtain 1 g of dry velvetbean amendment, compared to 6.9 g of fresh castor. The greater density and efficacy of the velvetbean amendment compared to the castor amendment suggests that less velvetbean amendment would be necessary to promote plant growth and nematode suppression. Thus, velvetbean was a more effective amendment than castor under the conditions of these tests. The optimum rate for plant growth and nematode suppression was 4 g to 6 g/pot.

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