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Responses of *Meloidogyne arenaria* and *M. incognita* to Green Manures and Supplemental Urea in Glasshouse Culture

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Abstract: The recent loss of many effective nematicides has led to renewed interest in alternative methods of nematode management. Greenhouse experiments were conducted to determine the effects of rapeseed and velvetbean green manures, and supplemental urea, on the root-knot nematodes *Meloidogyne arenaria* and *M. incognita*. Green manures were incorporated with *M. arenaria*-infested soil using rates totaling 200, 300, and 400 mg N/kg soil. Squash plants grown in this soil were evaluated using a gall index and plant dry weight. A second experiment tested ratios of rapeseed green manure to urea resulting in rates of 50, 100, and 150 mg N/kg soil on viability of *M. incognita* eggs and degree of galling on squash test plants. A third experiment examined combinations of velvetbean green manure and urea resulting in rates of 100, 200, and 300 mg N/kg soil on viability of *M. incognita* eggs. When applied at rates of 200, 300, and 400 mg N/kg soil, rapeseed green manure was more effective than velvetbean green manure at reducing galling of squash roots caused by *M. arenaria*. Decreased viability of *M. incognita* eggs was observed from treatments that received rates ≥200 mg N/kg soil with higher percentages of N from urea.

Key words: alginate, ammonia, *Brassica napus, Cucurbita pepo,* green manure, *Meloidoyne arenaria, Meloidogyne incognita, Mucuna deeringiana,* nitrogen, organic amendment, rapeseed, root-knot nematode, squash, velvetbean.

The recent removal of many effective nematicides and the pending loss of methyl bromide as a soil fumigant will greatly affect available methods of nematode control (3). Although it is widely known that crop rotations can aid nematode management, many producers do not view rotations as economically feasible production options (11). Use of green manure crops might increase the feasibility of rotations by decreasing the time between plantings of the main crop. Some compounds released from decaying plant material have shown nematode-suppressive properties such as ammoniacal nitrogen (10) and isothiocyanates (8,9).

The first objective of our research was to ascertain at what N rates rapeseed (*Brassica napus*) and velvetbean (*Mucuna deeringiana*) green manures are effective in reducing damage caused by *Meloidogyne arenaria* and *M. incognita* on vegetables. The second objective was to determine if N from green manure sources might be supplemented by N from an industrial source to enhance nematode effects. The third objective was to determine the effect of green manures and urea on viability of *M. incognita* eggs using a new technique for the study of nematode eggs (12).

Materials and Methods

A Dothan fine sandy loam soil (fine-loamy, siliceous, thermic Plinthic Paleudalt) naturally infested with *M. arenaria* race 1 was used in this study. Soil was collected from around peanut plants during mid-summer, and was sieved through a 2-mm screen and mixed 1:1 in a media mixer with washed river sand. The resulting experimental soil was 91.3% sand, 6.3% silt, and 2.4% clay; pH 6.3.

Rapeseed green manure was obtained from greenhouse-grown plants that were harvested 4 months after planting. Velvetbean green manure came from greenhouse-grown plants harvested 2 to 4 months after planting for experiment 1 and field-grown plants harvested 2 to 4 months after planting for experiment 3.

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Green manure consisted of all aboveground parts. The plant material was freeze dried and stored in sealed freezer storage bags at −2°C to preserve volatile compounds in plant tissues. Plant material was ground mechanically to pass through a 5-mm screen.

Differences in N added will result when green manures are incorporated on a dry matter basis. Because N has been shown to impact nematode populations (10), green manures incorporated on an N basis might provide a more balanced view of their effectiveness for nematode control. Therefore, all green manures used in these experiments were added based on total N. Total N and carbon (C) content of green manures was determined via dry combustion techniques (Leco, St. Joseph, MI) and are reported as the average of five subsamples.

Experiments were performed in the greenhouse using a completely randomized design with eight replications. Lighting was provided by ambient sunlight, and temperatures were maintained at 21 °C night and 30°C day. Data were analyzed by SAS using ANOVA with means separated by LSD (SAS User's Guide: version 6.04, Cary, NC).

Experiment 1: Rapeseed and velvetbean green manures were incorporated with 4 kg of soil at rates of 200, 300, and 400 mg N/kg soil. Percentage N contents were 3.2% and 2.5% for rapeseed and velvetbean green manure, respectively. These N rates correspond to field dry matter rates of 14, 21, and 28 Mg plant material/ha for rapeseed and 18, 27, and 36 Mg/ha for velvetbean. An additional treatment was amended with rates of 40 mg N/kg soil, 30 mg P_2O_5/kg soil, and 60 mg K_2O/kg soil as recommended by the Auburn University soil testing laboratory (1). Fertilizer sources were urea (46-0-0), triple superphosphate (0-46-0), and muriate of potash (0-0-60). After incorporation of treatments, soil was placed into 11-liter pots. One squash cv. Super Sett seedling was transplanted into each pot immediately following incorporation of treatments.

Plants were watered as needed. After 8 weeks, plant roots were evaluated for galls caused by *M. arenaria* using a gall index (0-10) (15). Plants were dried at 65 °C and their weights recorded.

Experiment 2: This experiment examined the effects of combinations of urea and rapeseed green manure on *M. incognita* at three N rates (50, 100, 150 mg N/kg soil) using five ratios of rapeseed green manure-N:urea-N to make total N rates. Ratios were 0:4, 1:3, 1:1, 3:1, and 4:0. Green manure was determined to be 3.4% total N. The control in this experiment was unamended soil.

Green manure and urea were mixed with 2 kg of soil and placed into PVC cylinders measuring 10 cm diam. and 20 cm high. An additional 2.5-cm section of pipe was cut, and the two sections were glued together with a section of 2-mm wire mesh. A no. 2 filter paper was placed on top of the mesh to keep soil from washing.

Alginate films containing *M. incognita* eggs were used to examine effects of treatments (12). Eggs of *M. incognita* were obtained from infected tomato cv. Rutgers using 1% NaOCl (5).

One alginate film was buried vertically in each pot, placed just below the soil surface. The film was removed after 4 days and replaced with a second film. The process was repeated with a third film. After removal, the films were washed gently to remove soil and cut to 2.5 cm^2. Each film was placed into a small petri dish filled with distilled water and allowed to incubate for 8 days. As *M. incognita* juveniles (J2) hatched, they moved into the water. At the end of incubation, petri dishes were stored at 10 °C until numbers of J2 were counted over a period of 14 days.

Fourteen days following incorporation of amendments, two additional films were buried in each pot to provide nematode inoculum for gall index determination. A squash seedling also was transplanted into each pot at this time and allowed to grow for 53 days. To maintain control plants, all treatments were fertilized three times. Fertilizer supplied 30 mg N/kg soil, 32 mg

P_2O_5/kg soil, and 34 mg K_2O/kg soil at each application along with micronutrients. Plants were fertilized 20, 32, and 47 days after planting.

Fifty-three days after planting, plant tops were harvested, dried, and weighed. Plant roots were rated for galling. Soil samples were collected for NH_3 analysis.

During the ammonification process, NH_3 is converted to NH_4 by soil microorganisms. Because NH_3 is difficult to measure, soil NH_4 is used to compare NH_3 levels between treatments. Soil samples were dried and 2.5-g subsamples extracted with 2M KCl (7) for determination of soil NH_4-N. Determinations were made via colorimetric analysis using a microplate reader (14).

Experiment 3: Similar to experiment 2, this experiment tested effects of combinations of urea and velvetbean green manure on *M. incognita* at three N rates. Nitrogen rates used in this study were 100, 200, 300 mg N/kg soil, and ratios of velvetbean green manure-N:urea-N were the same as in experiment 2. Velvetbean green manure was 3.4% N. The control was unamended soil.

Because plants in experiment 2 developed secondary infections from fungal root pathogens, soil in this experiment was autoclaved before use. Treatments were mixed with 2 kg of soil and placed into the PVC cylinders described in experiment 2.

Procedures involving treatment effects on hatching of *M. incognita* eggs were identical to those used in experiment 2. Fourteen days after incorporation of amendments, two alginate films were added and squash transplanted as before. All plants received fertilizer at the same rates as in experiment 2. Plants were fertilized on days 7 and 14 after transplanting.

After 2 weeks of growth, plants in some treatments began to experience symptoms of NH_3 toxicity including severe wilting and poor root development. Consequently, to prevent loss of the experiment, plants were harvested after 22 days of growth. Aboveground dry weights of each plant were recorded, and root systems were evaluated for galling. Soil NH_4 levels were determined as in experiment 2.

Results

Experiment 1: Squash plants growing in soil amended with rapeseed green manure had less galling than control plants ($P \leq 0.05$) (Fig. 1). At 300 and 400 mg N/kg soil, no galling was observed on squash roots from rapeseed treatments. Velvetbean green manure did not reduce galling ($P \leq 0.05$), and galling was actually higher at 400 mg N/kg soil than on fertilized control plants and plants receiving 200 and 300 mg N/kg soil as velvetbean green manure.

Phytotoxic effects were observed on rapeseed treatments at 300 and 400 mg N/kg soil rates, resulting in decreased plant dry weight. Rapeseed green manure, when applied at 200 mg N/kg soil, and all velvetbean treatments did not exhibit phytotoxic effects. There were no differences in dry weights of squash plants grown in these treatments and those of fertilized control plants.

Experiment 2: Nitrogen rate had no effect on number of *M. incognita* J2 hatching from alginate films. There were differences ($P \leq 0.05$) among ratios of N source, but these differences were not consistent with increases in the ratio of either rapeseed N or urea N.

Because of significant interaction of N rate and N source ratio for galling, means could not be compared across N rates. Therefore, gall index comparisons were made within the same rate of N. Differences ($P \leq 0.05$) in galling of roots were observed only for treatments amended with 150 mg N/kg soil (Table 1). There were no treatments with less galling than the control.

Shoot dry weight increased ($P \leq 0.001$) with increasing rates of N. Differences ($P \leq 0.05$) were observed among ratios of N source, but could not be attributed to increases in ratio of either rapeseed N or urea N. Data from soil NH_4 levels were inconclusive and therefore not shown.

Experiment 3: Because of interactions (*P*

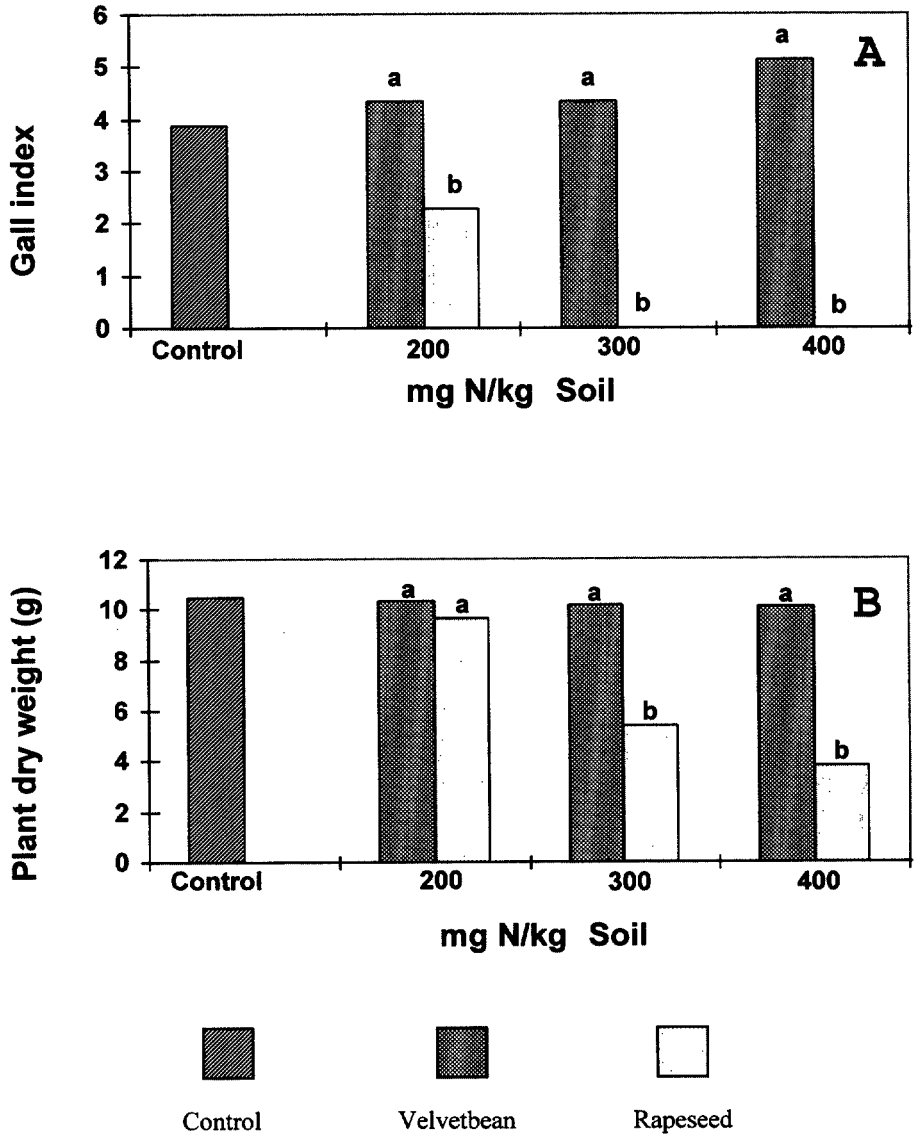

FIG. 1. Effects of velvetbean and rapeseed green manures when applied at rates of 200, 300, and 400 mg N/kg soil on A) gall index (0–10) of squash roots caused by *M. arenaria* and B) squash plant dry weight. Means with common letters are not significantly different at the same N rate.

≤ 0.05) between N rate and N source ratio, means could not be compared across N rates. Therefore, all comparisons are made within the same rate of N.

Treatment effects on hatching of *M. incognita* eggs were difficult to interpret due to variability among N rates and among films. Results from film 1 (1 to 4 days following incorporation) were inconclusive. When 75% on N was supplied by urea at rates of 200 mg N/kg soil, all alginate decomposed from film 2 (5 to 8 days after incorporation). Only data from film 3 (9 to 12 days following incorporation) are reported (Table 2). Numbers of hatched juveniles were lowest from film 3 when 75% of N was supplied by urea and 25% by velvetbean green manure at 200 and 300 mg N/kg.

Ratio of N source had significant effects on gall index, but these effects were not consistent with increases in velvetbean or

TABLE 1. Gall-index ratings (0–10) from squash roots as influenced by N rate and ratio of rapeseed green manure-N:urea-N.

	Rate of N added (mg N/kg soil)			
Ratio	0	50	100	150
Control	4.0			
1:3[a]		6.1 a	6.1 a	5.4 bc
1:1		6.3 a	6.6 a	6.1 abc
3:1		6.8 a	5.9 a	6.3 ab
4:0		6.1 a	6.6 a	4.8 c
0:4		6.0 a	5.9 a	6.3 a

Due to interaction between ratios across N rates, mean comparisons are made within the same rate of N. Means within columns with common letters are not significantly different within N rate according to LSD mean separation ($P \leq 0.05$).

[a]Ratio of total N supplied by rapeseed green manure:urea.

urea N source. Ratio of N source was not consistent with increases in shoot dry weights. Highest shoot dry weights were obtained at 200 and 300 mg N/kg soil when 100% of N was supplied by velvetbean.

Soil NH_4 levels were influenced by both rate of N and ratio of N supplied by urea. With increasing rates of N added, levels of NH_4 in soil increased. As ratio of N supplied by urea increased, soil NH_4 levels increased as well. Maximum NH_4 levels were obtained at 200 and 300 mg N/kg soil when 75% and 100% of N was supplied by urea.

DISCUSSION

The rapeseed cultivar used in these studies, 'Humus,' is high in glucosinolates (2). As glucosinolates break down in the soil, isothiocyanates are released and could explain both reduced galling and phytotoxic effects on squash plants in experiment 1 (8,9). Velvetbean does not contain glucosinolates and did not exhibit these effects.

Phytotoxic effects from rapeseed green manure might be alleviated by allowing a sufficient time interval before planting. In experiment 1, squash was transplanted immediately following incorporation of amendments and phytotoxicity was observed. Squash plants were transplanted 14 days after incorporation in experiment 2 and showed no phytotoxicity.

TABLE 2. Number of *Meloidogyne incognita* second-stage juveniles (J2) hatched from alginate film 3 after being in soil during days 9–12 following incorporation of amendments, gall index ratings (0–10) of squash roots, squash shoot dry weight (g), and soil NH_4/kg soil as influenced by N rate and ratio of velvetbean green manure-N:urea-N, experiment 3.

	Treatments (mg N/kg soil)														
	0	100				200				300					
Ratio	0:0[a]	1:3	1:1	3:1	4:0	0:4	1:3	1:1	3:1	4:0	0:4	1:3	1:1	3:1	4:0	0:4
Flm 3	24	49 a	45 a	35 a	34 a	36 a	4 d	39 abc	57 ab	38 bc	38 abc	9 cd	17 cd	42 abc	50 ab	23 bcd
Galls	2.9	1.9 ab	1.5 b	2.1 ab	1.6 ab	0.6 c	0.0 b	0.0 b	0.3 b	0.9 a	0.0 b	0.0 a	0.1 a	0.1 a	0.5 a	0.0 a
Shoot	2.1	3.7 a	3.7 a	4.1 a	3.4 a	3.4 a	2.4 b	4.2 a	3.9 b	4.6 a	2.9 b	2.6 b	2.4 b	1.8 b	5.1 a	2.4 b
NH_4	15	32 a	19 b	14 b	11 b	44 a	58 a	34 b	11 c	11 c	52 a	106 a	56 b	31 c	13 c	91 a

Due to interaction between ratios across N rates, mean comparisons are made within the same rate of N. Means across columns with common letters are not significantly different using LSD mean separation ($P \leq 0.05$).

[a] Ratio of total N supplied by velvetbean green manure:urea.

Hatching of *M. incognita* eggs was unaffected by rapeseed green manure and urea. This is consistent with the research of Mojtahedi et al. (9), who found hatching of *M. chitwoodi* eggs unaffected by similar rates of the rapeseed cv. Jupiter. Johnson and Shamiyeh (6) found that urea, when added at rates near 1,000 mg N/kg soil, had less effect on hatching of *M. javanica* and *M. arenaria* than did alfalfa plant material added at the same rate of N. Rates of urea used in experiment 2 (50–150 mg N/kg soil) were much lower.

Lack of correlation between total N added and level of NH_4 in soil might be explained by the time period that elapsed between incorporation of amendments and soil NH_4 readings. Over time, NH_4 is converted to NO_3 by soil bacteria. Both NO_3 and NH_4 are removed from soil due to plant uptake and leaching. Because NH_4 readings were taken 53 days following incorporation, any initial differences in soil NH_4 might be undetected by this late date.

In experiment 3, NH_3 toxicity developed only in those treatments receiving a high percentage (75% to 100%) of N from urea. Highest shoot dry weights were found in the treatment receiving 100% of N from velvetbean green manure at the two highest levels of N. This is consistent with experiment 1, where no toxicity developed in velvetbean treatments of up to 400 mg N/kg soil.

No galling of roots was observed in treatments where levels of soil NH_4 were above 60 ppm at 38 days following incorporation. Soil NH_3 levels might explain effects on hatching of *M. incognita* eggs from film 3. Previous research has shown that a carbon source supplied along with urea increases urease levels in soil. Higher levels of urease led to urea being broken down more rapidly, thereby increasing rate of NH_3 released (4,13).

Soil in experiment 3 was autoclaved before use. This might impact soil microbial populations. Lower initial numbers of nitrifiers in soil could affect soil NH_4 and NH_3 levels.

Alginate decomposing from film 2 could be related to soil NH_4. Alginate decomposes in the presence of monovalent cations such as NH_4^+. Stimulated microbial activity in these treatments is another possible explanation. By-products of microorganisms might cause breakdown of the alginate.

In conclusion, incorporation of rapeseed green manure at rates $\geq$200 mg N/kg soil was effective at reducing galling caused by *M. arenaria*. Ammonia released when velvetbean green manure was combined with supplemental urea at rates $\geq$200 mg N/kg soil, when a higher percentage of N came from the urea source, reduced viability of *M. incognita* eggs in autoclaved soil. Green manures and supplemental N sources, particularly those releasing NH_3 or other compounds having nematode effects, might be useful tools in nematode management in the future.

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