Mechanism of Suppression of *Meloidogyne hapla* and Its Damage by a Green Manure of Sudan Grass

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ABSTRACT

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Meloidogyne hapla is a continuing problem on vegetables in the state of New York. Sudan grass has been demonstrated to suppress infection and damage to susceptible vegetables caused by this nematode when incorporated as a green manure. Some Sudan grass cultivars contain the cyanogenic glucoside dhurrin that is degraded through an intermediate step to *p*-hydroxybenzaldehyde (*p*-HBA) and HCN. Incubating *M. hapla* eggs in Sudan grass extract resulted in a 55% reduction in the number of juveniles (J2) penetrating lettuce roots. Juveniles exposed to the extracts were not affected. Sudan grass extract affected egg maturation by delaying development, but did not affect hatching. Exposing eggs to a 0.1 ppm CN⁻ solution reduced the number of nematodes that penetrated the roots by 48%, while exposing J2 to the same concentration reduced nematode infection by only 4%. However, exposure of eggs to *p*-HBA did not reduce J2 penetration into roots. After purifying Sudan grass extract through size-exclusion resin, only the fractions collected that contained cyanide suppressed infection of lettuce roots by *M. hapla* J2 when eggs were exposed to these fractions. These results suggest that CN⁻ is the primary factor involved in the suppression of *M. hapla* by a green manure of Sudan grass.

Additional keywords: northern root-knot nematode

The northern root-knot nematode, *Meloidogyne hapla* Chitwood, has a wide host range consisting of more than 550 diverse crop and weed species (17). Recent work in New York has documented the increasing occurrence and damage of this nematode on vegetables grown on organic soils (1,28). Control of root-knot nematodes has been accomplished primarily through the use of chemical nematicides, crop rotations, and resistant cultivars on a few selected crops (10,23). Vydate was found to be highly effective and economical in controlling *M. hapla* on carrots and other vegetables in New York State (2; G. S. Abawi, unpublished). However, many nematicides are highly toxic chemicals and have raised concerns regarding environmental contamination and human health. As a result, the use of a number of nematicides has been cancelled, voluntarily withdrawn, scheduled to be phased-out in the near future, or is currently under review. Thus, alternative measures to control nematodes are urgently needed.

The use of rotational crops that are nonhosts, poor hosts, or antagonistic to nema-

todes is one of the oldest and most effective cultural practices for controlling nematodes (10,22,23,27). For example, monoculture of carrots has been shown to increase root-knot population and damage, whereas rotations, including oats and other crops, decreased the damage and increased marketable yield (9). However, designing an effective rotation is difficult because of the relatively long time required, limited availability of land, economics of production, and the wide host range of the root-knot nematode. Therefore, there is interest in using cover crops and their green manures as a control option against the root-knot nematode, specifically the northern root-knot nematode in organic soils in New York State.

Cover crops that are nonhosts or poor hosts are known to reduce populations of plant-parasitic nematodes. In addition, extensive information exists in the literature documenting the fact that the incorporation of cover crops with antagonistic properties as green manures usually results in the suppression of populations and damage from parasitic nematodes, as well as other root pathogens (3,5,10,22,27). Addition of green manures also stimulates soil microbial activities and increases accumulations of plant decomposition products and microbial metabolites that can be deleterious to nematodes.

Recently, several studies have shown that Sudan grass (*Sorghum sudanense* (Piper) Stapf), sorghum-Sudan grass hy-

brids (*S. bicolor* (L.) Moench × *S. sudanense*), and Sudan grass hybrids (*S. sudanense* × *S. sudanense*) are effective in suppressing infections and damage of vegetables by *Meloidogyne* spp. when incorporated into the soil as green manures (19,28,29). Viaene and Abawi (28) tested various cover crops and their green manures for effectiveness in suppressing the northern root-knot nematode, *M. hapla*. Although many of the cover crops tested, including Sudan grass hybrid cv. Trudan 8, were nonhosts to this nematode, only a green manure of Sudan grass was highly effective in reducing the egg production and root-gall severity ratings on the subsequent lettuce crop.

In general, pathogen suppression related to the addition of organic matter has been attributed to an increase in microbial activity or release of toxic compounds (14,15,32). A greater diversity of microorganisms occurs in soil as organic matter is increased, thus increasing the chances of microbial competition and antagonisms against soilborne pathogens (6). However, under most natural conditions, these antagonists are very poor competitors and their populations may rapidly fall below the level necessary to have an impact on the pathogen, once the organic food base is consumed (6,14). Also, suppression relying on this mechanism is likely to be more site specific because the antagonistic organism would have to be indigenous or specifically introduced. Environmental conditions such as soil moisture or availability of antagonistic-specific nutrients, which promote the growth of the antagonist, would be required to maintain or increase populations in order to be effective in suppression. Suppression affected by a chemical mechanism would be less site specific because the toxic compound is present within the amendment and it is released upon incorporation and decomposition in soil. The major inconvenience in a system using this mechanism is the short duration of time it is effective; therefore, repeated applications may be necessary. For example, the *in vivo* production of the toxic compound ceases shortly after incorporation and is released into the soil where it can be quickly volatilized or degraded microbially or chemically.

Sudan grass contains a cyanoglucoside compound, called dhurrin, present primarily in the epidermal cells of the leaf tissue (4,18). The cellular contents of the epider-

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mal and the mesophyll cells, which contain the enzyme β -glucosidase, become mixed when the tissue is damaged or begins to decompose. The enzyme catalyzes the hydrolysis of dhurrin to yield *p*-hydroxymandelonitrile, which then dissociates to produce free HCN and *p*-hydroxybenzaldehyde (p-HBA; Fig. 1). Hydrogen cyanide is known to be toxic to many different organisms, including nematodes (21). The objective of this study was to examine the relationship between dhurrin and its decomposition by-products, including HCN specifically, from a green manure of Sudan grass, and the suppression of *M. hapla*. A summary of these results has been previously presented (30).

MATERIALS AND METHODS

Nematode inoculum. Populations of *M. hapla* were maintained on tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, in the greenhouse. Eggs were extracted from the tomato roots with 0.5% sodium hypochlorite as described by Hussey and Barker (16).

Test tissues (green manure). Seed of Sudan grass hybrid cv. Trudan 8, referred to hereafter as Sudan grass, were obtained from Northrup King Company (Minneapolis, MN). Barley (*Hordeum vulgare* L. cv. Ledger) seed was obtained from a local source. Plants were grown for 6 to 8 weeks in the greenhouse.

Suppression efficacy of Sudan grass green manure over time. Aboveground tissues of Sudan grass were cut into segments approximately 3 cm in length and thoroughly incorporated at 37.5 g/1,000 cm³ soil in a composite sample of organic (Carlisle muck with up to 80% organic matter) soils obtained from commercial onion, lettuce, and carrot fields in New York. This correlates to approximately 20 tons/ha in the field. The amended soil was divided into batches of 2,500 cm³. A nonamended soil treatment was also included as a control. The soils were transferred to clay pots and incubated at 21°C and 75% relative humidity (RH) for 1, 2, 4, 6, or 8 weeks.

After each specific incubation period, the soil lots were removed and infested with 20,000 *M. hapla* eggs (8 eggs/cm³ soil). The infested soils were thoroughly mixed and placed into five 400-cm³ pots and five 100-cm³ cups. Two 1-week-old lettuce (*Lactuca sativa* L., cv. Montello) seedlings were planted in each of the 400-cm³ pots and one in each of the 100-cm³ cups. The pots and cups were maintained in a greenhouse with an average temperature range of 21 to 24°C. Plants were watered daily, fertilized weekly with a recommended solution of complete fertilizer (N-P-K, 20-20-20), and sprayed for the control of foliar insects as needed.

The lettuce seedlings in the 100-cm³ cups were removed after 12 days. The roots from each time period were washed free of soil, excised from the shoot, blotted

dry, and weighed together. The roots were cleared and stained using a modified procedure by Byrd et al. (11). The roots were cleared for 4 min in a solution of 1% NaOCl. The roots were collected on a 60-mesh screen and rinsed thoroughly with tap water. The roots were transferred to a 100-ml beaker containing 49 ml of water and 1 ml of stain (3.5 g of acid fuchsin, 250 ml of acetic acid, and 750 ml of deionized water). The root suspension was brought to a boil for 5 s in a microwave oven and then allowed to cool for about 5 min. The roots were collected on a 60-mesh screen and rinsed with tap water. The roots were stored in a beaker containing glycerin at room temperature for no longer than 2 days before examination. The roots were carefully pressed between two microscope slides and the number of nematodes that penetrated the root system was counted using a dissecting microscope.

The lettuce plants in the 400-cm³ pots were removed after 8 weeks. The roots were washed free of soil and evaluated for root-galling severity caused by *M. hapla* on a scale from 1 (no galling) to 9 (>80% of the root system galled). Ratings of 2 to 8 indicated that 1 to 3, 4 to 10, 11 to 25, 26 to 35, 36 to 55, 56 to 65, and 66 to 80% of the root system was galled, respectively.

Data were subjected to analysis of variance using the general linear models procedure (GLM; SAS Institute, Cary, NC). The means were compared statistically using least significant differences. The experiment was repeated once.

Preparation of sterile plant extracts. Tissue-extract solutions were prepared by homogenizing 10 g of Sudan grass or barley leaves and stems in a blender for 30 s with 100 ml of water. Sudan grass tissue was also homogenized, as described above, in 100 ml of 50 mM McIlvaine's buffer, pH 7.0, or 100 ml of 50 mM McIlvaine's buffer, pH 7.0, containing 1 mM silver nitrate. Each suspension was filtered through two layers of cheesecloth, centrifuged for 30 min at 11,950 $\times$ g, carefully decanted, and the supernatant centrifuged again. The supernatant was carefully decanted after the second centrifugation and filtered through a 0.2- μ m filter. The solutions were sterilized by filtering through a sterile 0.2- μ m syringe-type filter.

Cyanide concentration assay. The amount of cyanide in the leaf tissue was measured using the modified procedure described by Dartnall and Burns (12). The bottom of a cut test tube (2.2-cm i.d.) was placed in a glass jar (4-cm i.d.) with a screwcap lid. A total of 10 ml of the tissue extract, prepared as described above, or standard cyanide solutions was pipetted into the glass jars. Then, 1 ml of 1.0 N NaOH was pipetted into the inner test-tube well and 2.5 ml of 5 N HCl was pipetted into the jar with the solution to be tested. The jars were sealed and placed on an orbital shaker for 6 h at 45°C, agitating at 100 rpm. The flasks were cooled to 4 to 10°C by placing them in a freezer for approximately 15 min.

The inner tubes were removed and 2 ml of 0.1 N NaOH were added. A 1.0-ml aliquot was removed and acidified with 0.5 ml of 1.0 N acetic acid. To this was added 5.0 ml of succinimide and 1.0 ml of barbituric acid reagents. After 10 min, the absorbance at 580 nm was recorded on a UVIKON 860 spectrophotometer (Kontron Instruments, Zurich, Switzerland). The absorbance readings were converted to cyanide concentrations (ppm) by comparing to the standard curve.

Effect of plant extracts and cyanide on nematode infection. The Sudan grass extract, prepared as described above, was diluted with water to final concentrations of 50, 25, 10, 1, and 0.5% of the original extract solution. Cyanide solutions of 5, 1, 0.1, 0.05, and 0.01 ppm were prepared by diluting a 10-ppm stock solution. Solutions of 5, 1, 0.1, and 0.01 mM *p*-hydroxymandelonitrile (p-HMN), corresponding to 125, 25, 2.5, and 0.25 ppm CN⁻, respectively, were also prepared. In addition, a solution of 1 mM p-HBA was prepared. In all, 4,000 *M. hapla* eggs or juveniles (J2) were collected on a 30- μ m-mesh screen. The eggs or J2 were washed off the screen into a sterile beaker with sterile plant extract solutions, water, or cyanide solutions. The suspensions were covered and incubated at 8°C for 3 days. Pasteurized organic soil (1,000 cm³ of soil microwaved for 4 min; 13) was infested with the suspensions, mixed thoroughly, and divided into five 100-cm³ cups. A 1-week-old lettuce seedling was planted in each cup and

Fig. 1. Breakdown of dhurrin to the intermediate *p*-hydroxymandelonitrile and the final products of *p*-hydroxybenzaldehyde and hydrogen cyanide.

placed in a growth chamber with 12-h light at 21°C and 75% RH. After 12 days, the roots were removed, processed, and stained with the acid fuchsin stain as described above, and the number of nematodes that had penetrated the roots was determined. The effect of increasing the concentrations of Sudan grass extract and cyanide on infection was analyzed by regression analysis. The concentrations of cyanide and p-HMN and the extract dilutions were transformed to $\log(x + 1)$ values before regression analysis was performed. All experiments were repeated at least twice.

Effect of Sudan grass extract on egg development. Freshly extracted *M. hapla* eggs were separated by individually removing the eggs from a suspension in water with a Pasteur pipette, based upon whether the eggs contained undifferentiated nematode embryos or vermiform juveniles. The separated eggs were placed in a microcentrifuge tube and centrifuged for 4 min at $16,000 \times g$ in an Eppendorf 5415C centrifuge (Brinkman Instruments, Inc., Westbury, NY). The supernatant was carefully removed and the eggs were resuspended in either sterile deionized water or sterile Sudan grass extract, prepared as described above. The egg suspensions were poured into sterile plastic Petri plates (60 mm in diameter), covered, and incubated in the dark at 25°C. The changes in egg development and hatching were noted over time. Data were subjected to analysis of variance using GLM (SAS Institute). The treatments were compared over time using repeated measures analysis. The experiment was repeated once.

Sudan grass-extract purification. Enough Sephadex G-50-150 size exclusion resin was suspended in water to swell the resin to a volume of 25 ml. After rinsing several times with deionized water passed through an ion-exchange water purifier, the slurry was poured into a plastic syringe (18 mm in diameter) with a layer of cotton on the bottom, bringing the total volume of the resin to 24 ml. The column was placed

in a cold room at 8°C and the flow rate was adjusted to 7.8 ml/h. After the column was equilibrated with water, 10 ml of Sudan grass extract, prepared as described above, was carefully pipetted onto the column. After the extract was loaded onto the column, the column was washed with water. In all, 12 fractions (5 ml each) were collected. The column was washed thoroughly with water and another 10 ml of Sudan grass extract was loaded onto the column. The corresponding fractions from each run were pooled. The fractions were assayed for their cyanide content using the assay described above.

Suppressive activity of the fractions was tested by exposing *M. hapla* eggs (4,000) to the fractions for 3 days at 4°C and setting up the bioassay test, as described above. Data were subjected to analysis of variance using GLM (SAS Institute). The means were compared statistically using least significant differences. The relationship between suppressive activity and cyanide presence in the fractions was determined by calculating the correlation coefficient. The experiment was repeated once.

Effect of green manure on microbial activity. The amount of carbon dioxide produced by the microorganisms in soil as affected by the various treatments was measured by the modified procedure of Bartha and Pramer (8). Sudan grass leaf and stem tissue, grown for 6 to 8 weeks, was incorporated into organic soil (37.5 g/1,000 cm³ of soil) infested with *M. hapla* eggs (8 eggs/cm³ of soil). Sufficient water was added to the soil to increase the moisture level to 50% and a 100-g sample (50 g dry weight) was added to the bottom of a glass jar. A glass tube containing 10 ml of 1 N NaOH was placed on a metal stand above the soil in the jar. The jar was sealed with a rubber stopper that was mounted by an Ascarite filter with a stopper, stopcock, and a syringe needle. The needle was capped with a rubber policeman and its tip covered with a short polyethylene tubing

that contacted the very bottom of the tube containing the alkali. The jars were covered with aluminum foil and placed in a growth chamber at 21°C. Every 7 days, the NaOH solution was collected by first removing the ascarite filter stopper and opening the stopcock. The rubber policeman was replaced with a syringe, and the alkali was then removed and added to a flask containing 1 ml of a 50% BaCl₂ solution and 1 ml of phenolphthalein solution. Fresh NaOH was added to the tube through the syringe. The rubber policeman was placed on top of the needle, the filter stopper was returned on the ascarite filter, and the stopcock closed. The solution was titrated to equilibrium against 1.044 N HCl until the solution remained a light pink color. The amount of CO₂ produced (milligrams per gram of dry soil) was calculated by the following formula: $[(B - V)NE]/\text{soil dry weight (g)} = \text{mg CO}_2$, where B = volume of HCl titrated against control, V = volume of HCl titrated against samples, N = normality of HCl (1.044 N), and E = equivalent weight of CO₂ (22).

A soil core was removed from each repetition and combined into one sample for the treatment. The nematodes were extracted from each soil sample by the modified Baermann funnel technique (7). A soil sample (20 cm³) was distributed evenly on tissue paper which was placed on top of a 6-mesh screen containing a milk filter (KenAG, Ashland, OH). This was placed in a metal pan containing enough water to barely cover the soil. After 4 days, the water was removed by pouring into a beaker and fresh water was added. After an additional 4 days, the water from the pan was collected and pooled with the previous water sample. Both free-living and plant-parasitic nematodes were counted under a dissecting microscope (40×) and the total calculated for number per cubic centimeter of soil.

The effect of the amendment on bacterial densities was calculated by mixing 3.33 g of soil with 100 ml of sterile water and then making several dilutions, giving a final dilution of $1:3 \times 10^6$ (wet weight). From this final dilution, 1 ml was pipetted into each of 10 petri plates (150 mm in diameter), and cooled, 10% tryptic soy broth medium, containing 100 ppm of cycloheximide, was poured into each plate, swirled gently, and allowed to cool. The plates were incubated in the dark at 25°C and the number of colonies was recorded after 3 days.

The effect of the amendment on soil fungi was estimated by mixing 3.33 g of soil with 100 ml of sterile water and removing 1 ml of the suspension and adding it to 99 ml of sterile water, giving a final dilution of 1:3000 (wet weight). From this final dilution, 1 ml was pipetted into each of 10 Petri plates (150 mm in diameter) and cooled potato dextrose agar, containing 100 ppm of streptomycin sulfate, 50 ppm

Table 1. Influence of incubation time on suppression of *Meloidogyne hapla* infection of lettuce roots grown in Sudan grass-amended soils^w

Incubation period ^z	J2/root system ^x		Root-gall severity index ^y	
	No Sudan	Sudan	No Sudan	Sudan
1 week	21.6 a	8.6 b	7.0 a	3.6 b
2 weeks	32.6 a	4.8 b	7.0 a	4.6 b
4 weeks	29.0 a	7.8 b	6.0 a	3.4 b
6 weeks	26.8 a	11.8 a	8.5 a	8.0 a
8 weeks	48.4 a	21.0 a	8.0 a	7.1 a

^w Values in the same row followed by the same letter are not significantly different according to least significant difference analysis ($P \leq 0.05$).

^x The number of *M. hapla* juveniles (J2) per root system penetrating 1-week-old lettuce seedlings recorded after 12 days. Organic soil was infested with *M. hapla* eggs either nonamended (No Sudan) or amended (Sudan) with Sudan grass cv. Trudan 8 (37.5 g/1,000 cm³ of soil).

^y Root-gall severity index of lettuce roots recorded at 8 weeks after planting, based upon the percentage of roots with galls, where 1 = no galls present, 2 = 1 to 3%, 3 = 4 to 10%, 4 = 11 to 25%, 5 = 26 to 35%, 6 = 36 to 50%, 7 = 51 to 65%, 8 = 66 to 80%, and 9 = >80% infected. All noninfested controls had a root-gall severity index of 1 (data not shown).

^z Time period between the incorporation of Sudan grass and soil infestation with *M. hapla* eggs.

of chlortetracycline, and 0.1% tergitol NP-10, was poured into each plate, swirled gently, and allowed to cool. The plates were incubated at room temperature under a fluorescent light.

A sample of the soil was dried overnight in an oven to calculate the percentage of moisture for each treatment. The experiment was repeated and the effects of the treatments were compared over time using an SAS procedure for repeated measures analysis.

RESULTS

Suppression efficacy of green manure over time. Results from both tests were similar; therefore, the data were combined for statistical analysis. Significantly fewer *M. hapla* J2 penetrated roots of lettuce growing in the Sudan grass-amended soils (up to 4 weeks after the incorporation of Sudan grass tissue) as compared to those growing in the nonamended soil (Table 1). Similarly, root-galling severity ratings were significantly lower on roots of lettuce growing in the Sudan grass-amended soils. For example, root-galling severity ratings averaged 3.4 and 6.0 on roots of lettuce seedlings in Sudan grass-amended and nonamended soils, respectively, in which eggs had been added at 4 weeks after incorporation. However, the number of J2 in roots and root-galling severity ratings were not significantly different on roots of lettuce in which eggs had been added 6 or 8 weeks after incorporation of Sudan grass.

Cyanide concentration in leaf tissue. The equation for the CN^- standard curve estimation was: $CN^- \text{ (ppm)} = (ABS_{580} - 0.003)/1.5477$. Based upon an absorbance reading at 580 nm of 0.510 and the above-stated equation, the calculated CN^- content of the leaf tissue of Sudan grass cv. Trudan 8 was 0.33 ppm.

Effect of plant extracts and cyanide on nematode infection. Exposing eggs of *M. hapla* to the extract of Sudan grass resulted in a significantly lower number of J2 in lettuce roots as compared to eggs exposed to water or an extract of barley (Table 2). However, no significant differences in numbers of J2 in lettuce roots were observed when J2 were exposed to water or extracts of Sudan grass and barley. In addition, when eggs were exposed to Sudan grass extract prepared from tissue that was homogenized in buffer containing silver ions, which inhibits the enzyme β -glucosidase, there were 31 J2 per lettuce root, which was not significantly different from the 21 J2 per lettuce root from eggs exposed to water. The number of J2 that penetrated the root systems of lettuce was significantly affected by the CN^- concentration (Fig. 2). However, the effective dose against eggs was lower (0.01 ppm) than that affecting the J2 (1.0 ppm). The equations $y = 658 - 8,231 \times \log(x + 1) + 23,411 \times [\log(x + 1)]^2$ and $y = 3,366 - 5,349 \times \log(x + 1) + 3,893 \times [\log(x + 1)]^2$

best fit the curves generated to describe the effect of CN^- on eggs ($r^2 = 0.81$) and juveniles ($r^2 = 0.998$), respectively. Extract of Sudan grass diluted to as low as 1% of the

original Sudan grass extract prepared was still effective in suppressing infection of roots by *M. hapla*, but the maximum activity of Sudan grass extract occurred at a

Table 2. Penetration of lettuce roots by *Meloidogyne hapla* as affected by exposing eggs or juveniles (J2) to water or extracts of Sudan grass or barley

Treatment ^x	<i>M. hapla</i> J2/root system ^w	
	Egg inoculum ^y	J2 inoculum ^z
Water	25.8 a	10.2 a
Barley extract	21.8 a	20.4 a
Sudan grass extract	3.0 b	14.6 a

^w The number of *M. hapla* J2 per root system penetrating 1-week-old lettuce seedlings recorded after 12 days. Values in the same column followed by the same letter are not significantly different according to least significant difference analysis ($P \leq 0.05$).

^x Eggs or juveniles of *M. hapla* were exposed to either sterile water, sterile barley extract (10 g/100 ml of water), or sterile Sudan grass extract (10 g/100 ml of water) for 3 days at 4°C.

^y Eggs extracted from tomato roots infected with *M. hapla*.

^z Juveniles hatched from *M. hapla* eggs.

Fig. 2. Effect of exposing eggs or juveniles of *Meloidogyne hapla* to various concentrations of cyanide and the infection of 1-week-old lettuce roots grown in infested soil for 12 days.

Fig. 3. Effect of exposing eggs of *Meloidogyne hapla* to various concentrations of Sudan grass extract (10 g of tissue/100 ml of water) on the infection of 1-week-old lettuce seedlings grown in infested soil for 12 days.

concentration greater than 10% (Fig. 3). The equation $y = 342 - 1756 \times \log(x + 1) + 4200 \times [\log(x + 1)]^2$ best fit the curve generated in this study ($r^2 = 0.59$). Infection of lettuce seedlings by *M. hapla* was decreased as the concentration of p-HMN was increased from 0.01 to 5.0 mM (Table 3). The equation $y = 55.9 - 178 \times \log(x + 1) + 152 \times [\log(x + 1)]^2$ best fit the curve generated to describe the affect of p-HMN on eggs ($r^2 = 0.99$). In contrast, there was no effect on infection of lettuce roots by *M. hapla* by exposing eggs to the noncyanogenic compound p-HBA at 1.0 mM.

Effect of Sudan grass extract on egg development. Repeated measures analysis showed a significantly higher rate of egg development change for eggs exposed to water compared to eggs exposed to Sudan grass extract ($P < 0.01$). Only 17% of the eggs counted after 5 days developed into juveniles when eggs in the nondifferentiated stage were exposed to Sudan grass extract, compared to 35% of the eggs exposed to water. However, there was not a

significant difference over time between the treatments in the percentage of J2 that hatched from eggs originally selected at the prejuvenile stage. After 5 days, 33% of the eggs exposed to water hatched, while 28% of eggs exposed to Sudan grass extract hatched.

Purified column fractions of Sudan grass extract. There was a significant correlation between the suppressive activity against *M. hapla* and the cyanide content of the fractions of Sudan grass extract collected after passing through the size-exclusion resin ($r = -0.804$). Fractions which showed no suppressive activity against *M. hapla* did not contain cyanide, as indicated by the colorimetric assay results (Table 4). In contrast, fractions that exhibited a suppressive activity against *M. hapla* did contain cyanide.

Effect of green manure on microbial activity. Total respiration in the Sudan grass-amended treatment, as measured by the amount of CO_2 produced, was significantly higher than the nonamended control as measured by repeated measures analysis (Fig. 4). This was supported by the microbial enumeration results, which showed higher soil populations of nematodes, fungi, and bacteria in the Sudan grass-amended treatment (Fig. 5). In the Sudan grass-amended soil, bacterial populations increased dramatically after the first week of incorporation, declined quickly, and then leveled off. Fungal populations also increased after the first week, but maintained high populations through the duration of the study. Nematode populations increased sharply after 3 weeks and then slowly declined over time.

DISCUSSION

In this and previous studies (19,28,29), Sudan grass was demonstrated to suppress

infections by *Meloidogyne* spp. to host crops when incorporated as a green manure. Suppression of plant pathogens by green manures has been primarily attributed to two mechanisms of action: chemical or microbial (6). This study focused on determining the chemical mechanism involved in the suppression of the root-knot nematode and the identification of a chemical compound or compounds, because exposure of *M. hapla* eggs to sterile Sudan grass extracts inhibited root infection by the subsequent J2 of *M. hapla*. Barley extract was used as a control because incorporation of barley into the soil as a green manure did not exhibit suppressive activity (G. S. Abawi, unpublished data). The hypothesis that cyanide was the compound responsible for suppression was initially based upon what is known biochemically about Sudan grass and earlier observations and speculations made in this and other studies. Cyanide is known to be toxic to different organisms and is present within Sudan grass tissue initially as a cyanogenic glucoside. This compound is enzymatically degraded to an intermediate compound, p-HMN, which freely dissociates into p-HBA and HCN. Exposing *M. hapla* eggs to various concentrations of cyanide demonstrated an increase in suppression as the concentration was increased. Exposure of eggs to a concentration as low as 0.01 ppm of CN^- was enough to affect potential infection by J2. Calculations determined an ED_{50} value of approximately 0.1 ppm. More direct evidence that the cyanide within Sudan grass extract was having the most impact was demonstrated by exposing eggs to different fractions collected after passing the extract through a size-exclusion resin. Those fractions which did not have any cyanide present, as determined by the colorimetric

Table 3. Effect of exposing *Meloidogyne hapla* eggs to different concentrations of p-hydroxy-mandelonitrile (p-HMN) and to p-hydroxybenzaldehyde (p-HBA) on the number of juveniles (J2) that penetrated lettuce roots

Solution	<i>M. hapla</i> J2/root system ^y
Water	56.2 ^z
0.01 mM p-HMN	55.0
0.1 mM p-HMN	48.6
1.0 mM p-HMN	16.2
5.0 mM p-HMN	9.6
1.0 mM p-HBA	32.6

^y The number of *M. hapla* J2 per root system penetrating 1-week-old lettuce seedlings recorded after 12 days.

^z Regression analysis determined a significant effect of p-HMN concentration on the number of nematodes penetrating the root system ($P \leq 0.01$).

Table 4. The relationship between the cyanide content of purified Sudan grass-extract fractions passed through size-exclusion resin, Sephadex G-50-150, and the suppressive activity against *Meloidogyne hapla*

Fraction ^x	CN^- level (ppm) ^y	<i>M. hapla</i> J2/root system ^z
Water	0.00	29.4 ± 22.2
III	0.04	36.2 ± 11.7
IV	0.27	7.8 ± 3.0
V	0.28	21.4 ± 14.6
VI	0.24	26.8 ± 21.7
VII	0.08	33.0 ± 20.3
VIII	0.01	48.6 ± 10.4

^x Refers to the fraction number (12 total) of the extract that was collected after passing 10 ml of Sudan grass extract through the size-exclusion resin (5 ml/fraction). Water = control.

^y Cyanide level, as determined by the colorimetric assay and then converted to ppm based upon the standard curve generated.

^z The number of *M. hapla* juveniles (J2) per root system penetrating 1-week-old lettuce seedlings was recorded after 12 days.

Fig. 4. Amount of carbon dioxide evolved in Sudan-grass-amended (37.5 g/1,000 cm³ of soil) and nonamended organic soil over time.

assay, did not suppress infection. Cyanide still bonded to the phenolic compound, as in the cyanogenic glucoside, does not exhibit suppressive activity because no reduction of infection occurs when the enzyme β -glucosidase is inactivated by silver ions. Activity of the cyanide molecule in p-HMN is more difficult to determine, because the dissociation of cyanide from p-HMN occurs freely at a pH above 3.5 (25). This made it difficult to determine if p-HMN has suppressive activity because a pH below 3.5 would, in itself, directly affect the eggs.

The conclusion that cyanide is responsible for suppression would likely explain why activity was lost when the amended soil was incubated for longer than 6 weeks. Hydrogen cyanide is very volatile and, after 6 weeks, most of the cyanide could have been released from the tissue after decomposition in the soil and microbially degraded or volatilized into the soil atmosphere and eventually out of the soil.

The HCN compound is toxic to many different organisms; therefore, there is concern that it may also inhibit beneficial organisms. Although the affects of cyanide on specific organisms, besides *M. hapla*, were not tested in this study, there was an overall increase in soil respiration in the Sudan grass-amended soils, as was evident by higher carbon dioxide evolution. The latter also included increases in the total populations of nematodes, bacteria, and fungi, as indicated by direct counts of live nematodes or bacterial and fungal CFU growing on general media. Selected species of these organisms may have been impacted, however. A study is currently being conducted to examine the effects of a Sudan grass green manure and extract on several nematode species.

The only stages in the life cycle of *M. hapla* vulnerable to chemicals or the effects of cultural practices would be at the egg and J2 stages before root penetration. Once the J2 migrate and penetrate the root, they complete their life cycle in the root host until the females lay their eggs outside the roots in a gelatinous matrix. These nematodes are protected from chemical compounds unless those compounds are systemic. The eggs also undergo several stages of development, from a single-celled embryo to a fully formed juvenile. Although the exact effect of Sudan grass extracts on eggs is not known at this time, cyanide appears to have more of an effect on egg development rather than on hatching, at least in the concentrations of cyanide within the leaf tissue tested in this study. This effect on early egg development might explain some variability observed among different experiments. When nematode eggs are extracted from tomato roots for inoculum, they are not all at the same stages of development. If the ratio of eggs that are at later developmental stages

or contain J2 is higher, the cyanide (within the Sudan grass extract) may not have as much of an impact as if the batch of eggs utilized have a higher ratio of undifferentiated or earlier developmental egg stages.

The concentration of cyanide found in the leaves of cv. Trudan 8 was 0.33 ppm. This might explain why there was no inhibitory effect when J2 were exposed to the Sudan grass extract. This concentration

is significantly lower than the 1.0 ppm demonstrated to have an exposure effect against J2 of *M. hapla*. Other varieties and hybrids of Sudan grass have varying amounts of cyanide in the leaf tissue, showing a correlation between the amount of tissue incorporated and suppression of *M. hapla* (31).

There is great potential for this green-manure application system to be used as an

Fig. 5. Population changes over time of general (A) bacteria, (B) fungi, and (C) nematodes in Sudan-grass-amended (37.5 g/1,000 cm³ of soil) and nonamended organic soil.

effective and sustainable management option to control *M. hapla*. Synthetic pesticides are constantly under review for human health and environmental impact reasons; therefore, farmers are looking for alternative sustainable practices that they can safely apply if these pesticides are removed. The disadvantage of this specific system is that it may not be the most practical under New York conditions. In order to be most effective, the Sudan grass cover crop should be grown for only 6 to 8 weeks and then incorporated into the soil before the first frost (19,28). However, the last frost-free date for vegetable production in this region of New York is about 14 October, which does not allow enough time after harvesting the main crop to grow Sudan grass and incorporate it before the first frost. Other plants, including clover and flax, that also contain cyanogenic compounds may have some potential for use as a green manure (20,24,26). Currently, another study is underway to test other Sudan grass cultivars and various other cyanogenic plants, which may be more suitable for local conditions, for suppressive activity (31).

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