Relationship Between Levels of Cyanide in Sudangrass Hybrids Incorporated into Soil and Suppression of *Meloidogyne hapla*

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Abstract: Sudangrass cv. Trudan 8 has been demonstrated to suppress infection of vegetables by *Meloidogyne hapla* (Mh). Hydrogen cyanide, released from the degradation of the cyanogenic glucoside (dhurrin) during decomposition of Trudan 8, was the primary factor involved in suppression of Mh on vegetables. The cyanide ion level in leaf tissue of 14 hybrids of sudangrass varied between 0.04 (cv. SX-8) to 1.84 parts per million (cv. 840F). The suppressive activity of the sudangrass hybrids against Mh was assessed in greenhouse tests by incorporating various amounts of leaf tissue into organic soil. After 1 week, eggs of Mh were added to the soil (8 eggs/cm^3 soil), which was then planted with lettuce as a bioassay plant. After 8 weeks, the lettuce roots were washed and rated for root-gall severity (RGS). Incorporation of sudangrass tissue resulted in a reduction of RGS up to 54%. There was a correlation between the amount of free cyanide incorporated into the soil and the reduction in RGS. Other green manures of cyanogenic plants tested were white clover, which resulted in a 45% reduction in RGS, and flax, which resulted in a 53% reduction in Mh penetration of lettuce roots. These results suggest that cyanogenic plants have potential as nematicidal green manures.

Key words: clover, control, cyanide, disease suppression, flax, green manure, *Meloidogyne hapla,* sudangrass.

Addition of organic matter to soil has been demonstrated to have many beneficial attributes to agriculture, including disease suppression. Various cover crops and the incorporation of green manures also suppress nematode infection (Kinloch and Dunavin, 1993; Prot et al., 1992; Viaene and Abawi, 1998). Viaene and Abawi (1998) recently tested various cover crops and their green manures for suppression of the northern root-knot nematode *Meloidogyne hapla,* an emerging pest of vegetables grown in organic soils in New York (Abawi and Laird, 1994). Many of the cover cops tested, including sudangrass hybrid [*Sorghum sudanense* (Piper) Stapf × *S. sudanense*] cv. Trudan 8, were nonhosts for this nematode. However, only sudangrass was effective in reducing the egg production and root-galling severity ratings on the subsequent lettuce crop when incorporated as a green manure.

The mechanisms of nematode and disease suppression include both biological and chemical factors, depending on the type of amendment and environmental conditions (Hoitink et al., 1993; Hoitink and Fahy, 1986; Widmer and Abawi, 2000; Widmer et al., 1998). In previous studies, the addition of organic matter was shown to increase soil microbial activity (Rothwell and Hortenstine, 1969; Widmer and Abawi, 2000) that may have a direct effect on plant-parasitic nematode populations (Kaplan et al., 1992; Kloepper et al., 1991; Riegel and Noe, 2000). Chemical factors also have been shown to be important in nematode suppression and mortality, especially when isothiocyanates are released (Jing and Halbrendt, 1994). The production of volatile and nonvolatile toxic compounds during decomposition has been demonstrated to inhibit plant-parasitic nematodes (Abawi and Thurston, 1994; Patrick et al., 1965).

The primary mechanism of *M. hapla* suppression on vegetables by a green manure of sudangrass hybrid cv. Trudan 8 was shown to be the release of hydrogen cyanide (Widmer and Abawi, 2000). Other sudangrass hybrids also contain varying levels of cyanogenic compounds within their tissues (Harrington, 1966). When different cultivars and different amounts of sudangrass were incorporated into soil, the number of *M. chitwoodi* on tomato roots was reduced compared to unamended or wheat-amended soil (Mojtahedi et al. 1993a). Other plant species such as clover and flax, which contain cyanogenic compounds (Pederson et al., 1996; Schroder, 1977; Seigler, 1976; Trione, 1960), also may suppress nematode infection of vegetables when incorporated as a green manure. Mojtahedi et al. (1993b) showed a direct relationship between the concentration of glucosinolates present in the leaves of rapeseed and the reduction of the population of *M. chitwoodi* in soil. Establishing a relationship between the amount of cyanide in leaf tissue and nematode suppression would be useful for selecting a potential cover crop and the rate of incorporation necessary for effective nematode suppression. This study was conducted to test different hybrids of sudangrass and other cyanogenic plants for their ability to suppress *M. hapla* infection and to determine a relationship between the cyanide levels within leaf tissue and nematode suppression. A summary of the results was reported previously (Widmer and Abawi, 1999).

Materials and Methods

Nematode inoculum: Populations of *Meloidogyne hapla* were maintained on tomato, *Lycopersicon esculentum* Mill. cv. Rutgers, in the greenhouse. Eggs were extracted from tomato roots with 0.5% sodium hypochlorite (Hussey and Barker, 1973).

Plant tissue: Seeds of sudangrass hybrid Trudan 8 were obtained from Northrup King Company (Minne-

apolis, MN). Seeds of sudangrass hybrids 811F, 841F, 849F, 877F, 819F, 839F, 840F, and 855F were obtained from Pioneer Brand Seed (Johnston, IA). Seeds of sudangrass hybrids ST-6E, SX-8, SX-15, and SX-17 were obtained from DeKalb Genetics Corporation (Lubbock, TX). Seeds of flax (*Linum usitatissimum* L.) accessions PI289121 and PI522532, and white clover (*Trifolium repens* L.) accessions PI287998, PI217444, PI287990, PI214207, PI214208, and P1516411 were obtained from the USDA seed repositories at the North Central Regional PI Station (Ames, IA) and the Western Region PI Station (Pullman, WA), respectively. Plants were grown for 6 to 8 weeks in the greenhouse to obtain leaf tissue.

Preparation of plant extracts: Tissue-extract solutions were prepared by homogenizing 10 g of leaf tissue in a blender for 30 seconds with 100 ml of water. The suspension was filtered through two layers of cheesecloth and centrifuged twice for 30 minutes at 11,950 *g*. The supernatant was carefully decanted after the second centrifugation and filtered through a 0.2-μm filter. The solutions were sterilized by filtering through a sterile 0.2-μm 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 (1987). A test tube (2.2-cm i.d., 7-cm height) was placed in a glass jar (4-cm i.d.) with a screwcap lid. Ten milliliters of the tissue extract, prepared as described above, or standard cyanide solutions were pipeted into the glass jars along with 2.5 ml of 5.0 N HCl. The extract and acid solutions were mixed, and 1 ml of 1.0 N NaOH was pipeted into the inner test tube well. The jars were sealed and placed on an orbital shaker for 6 hours at 45 °C agitating at 100 revolutions per minute. The flasks were then cooled to 4 to 10 °C by placing them in a freezer for approximately 15 minutes.

The inner tubes were removed from the jars, and the solutions were diluted with 2 ml of 0.1 N NaOH. A 1-ml aliquot was removed, acidified with 0.5 ml of 1.0 N acetic acid, and combined with 5 ml of N-chlorosuccinimide-succinimide reagent and 1 ml of barbituric acid-pyridine reagent. After 10 minutes, the absorbance at 580 nm was recorded on a UVIKON 860 spectrophotometer (Kontron Instruments, Zurich, Switzerland). The absorbance readings were converted to cyanide concentrations in parts per million by comparison to the standard curve.

Incorporation of cyanogenic plant material: The leaf tissue of sudangrass hybrids Trudan 8, 'Piper', SX-17, SX-15, SX-8, SX-6E, 811F, 841F, 849F, 877F, 819F, 839F, 840F, and 855F, the flax accessions (PI289121 and PI522532), and white clover accessions (PI287998, PI217444, PI287990, PI214207, PI214208, and PI516411) were cut into approximately 1-cm segments. Each leaf sample was weighed and incorporated at the rate of 37.5 g tissue per 1,000 cm^3 of natural organic

soil (unsterilized, Carlisle muck with up to 80% organic matter). The amended soils were transferred to clay pots and maintained at 21 °C and 75% relative humidity. An unamended soil was included as a control.

After 1 week, the soil treatments were infested with *M. hapla* eggs (8 eggs/cm^3 soil) and mixed thoroughly in plastic bags. The soil was then divided into five 400-cm^3 pots for each of the sudangrass hybrids and white clover accessions or five 100-cm^3 cups for each of the flax accessions. Two lettuce seeds (*Lactuca sativa* L. cv. Montello) were planted in each 400-cm^3 pot, and a single 1-week-old lettuce seedling was transplanted into each 100-cm^3 cup. The pots and cups were placed in the growth chamber at 21 °C and 75% relative humidity. Plants were watered daily and fertilized weekly with 20,20,20 NPK.

The lettuce seedlings in the 100-cm^3 cups were removed after 12 days. The roots were washed, blotted dry, and stained with acid fuchsin stain (Byrd et al., 1983). The number of nematodes within the roots was counted using a dissecting microscope at 40×.

The lettuce plants in the 400-cm^3 pots were removed from the soil after 8 weeks. The roots were washed free of soil. Root-gall severity (RGS) caused by *M. hapla* was rated 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. The experiment was repeated once. The data were subjected to analysis of variance (ANOVA) and the least significance difference (LSD) multiple-range test to separate the means of the RGS of the different sudangrass hybrids and white clover accessions.

Correlation of tissue cyanide levels and nematode suppression: The leaf tissue of the sudangrass hybrids Trudan 8, 840F, 877F, 849F, SX-8, and SX-15 were cut into approximately 1-cm segments. Leaf samples were weighed and incorporated as either 37.5 (100%), 7.5 (20%), 3.75 (10%), or 1.875 g (5%) of tissue per 1,000 cm^3 of natural organic soil (unsterilized, Carlisle muck with up to 80% organic matter). The amended soils were transferred to clay pots and maintained at 21 °C and 75% relative humidity. An unamended soil was included as a control.

After 1 week, the soil treatments were infested with *M. hapla* eggs (8 eggs/cm^3 soil) and mixed thoroughly in plastic bags. The soil was then divided into five 400-cm^3 pots for each of the sudangrass hybrids. Different incorporation rates of tissue of white clover and flax were not possible due to limited availability of plant material. Two lettuce seeds (*Lactuca sativa* L. cv. Montello) were planted in each 400-cm^3 pot. The pots were placed in the growth chamber at 21 °C and 75% relative humidity. Plants were watered daily and fertilized weekly with 20,20,20 NPK.

The lettuce plants in the 400-cm^3 pots were removed from the soil after 8 weeks. The roots were washed free

of soil. Root-gall severity caused by *M. hapla* was rated on a scale from 1 (no galling) to 9, described previously. The experiment was repeated once. The data were subjected to ANOVA and the LSD multiple-range test to separate the means of the RGS rating of the different sudangrass hybrids and incorporation rates of leaf tissue. To establish a correlation between the amount of cyanide incorporated into the soil and reduction of RGS, the results from all sudangrass hybrids tested were combined. The concentration of cyanide in the leaf tissue was first converted to cyanide concentration incorporated into the soil. The effect of increasing the concentration of cyanide in the soil on the RGS rating on lettuce was analyzed by regression analysis.

Effect of plant extract concentration on egg development: The effect of various dilutions of sudangrass extract on *M. hapla* egg development was evaluated based on the procedure by Widmer and Abawi (2000). Sterile extract of hybrid Trudan 8 was prepared as described previously. The sudangrass extract was diluted with sterile deionized water to final concentrations of 50, 25, 10, 1, and 0.5% of the original prepared extract. *Meloidogyne hapla* eggs were separated at the undeveloped early embryonic stage of development by observing the eggs under a dissecting microscope and individually removing them with a pipet. Fifty eggs were transferred to microcentrifuge tubes. Enough sterile water was added to the tubes to bring the volume up to approximately 0.5 ml, and then 0.5 ml of 1% NaOCl solution was added to surface-sterilize the eggs. The egg suspension was centrifuged for 5 minutes at 16,000g in an Eppendorf 5415C (Brinkman Instruments, Inc., Westbury,

NY). The supernatant was carefully decanted and the eggs washed twice with sterile deionized water. After the last washing, the supernatant was decanted and the eggs were resuspended in the various dilutions of sudangrass extract. The same procedure was followed for controls using 100% of the original prepared extract and sterile deionized water. The egg suspensions were poured into sterile plastic petri plates (20-mm-diam.) and placed in the dark at 25 °C. The percentage of eggs that contained first-stage juveniles was calculated over 12 days every 24 hours. After 12 days, a curve was calculated to show egg development exposed to the various treatments. The impact of the sudangrass extract was determined by calculating the corrected percentage of undeveloped eggs using Abbott's formula (Abbott, 1925): $U = (D - T)/D$, where U is the corrected percentage of undeveloped eggs, D is the percentage of developed eggs in the control, and T is the percentage of developed eggs exposed to the sudangrass extracts. The effect of increasing the concentration of sudangrass extract on egg development was analyzed by regression analysis. The experiment was repeated once.

Results

The cyanide ion level in the leaf tissue of the sudangrass hybrids varied between 0.04 to 1.84 parts per million (ppm) (Fig. 1). The amount of cyanide in the white clover accessions ranged from 1.00 to 7.13 ppm (Fig. 2). One accession of white clover (PI516411) was acyanogenic, with a concentration of 0.01 ppm. The values for the two flax accessions were 17.0 and 29.0 ppm for PI289121 and PI522532, respectively.
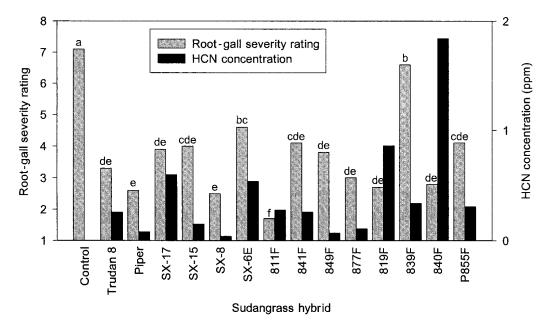

FIG. 1. Cyanide content (parts per million; μg/g) of leaf tissue of sudangrass hybrids and their effect on root galling on lettuce (*Lactuca sativa* L. cv. Montello) when incorporated into *Meloidogyne hapla*-infested soil as a green manure. Cut leaves were mixed into soil at 37.5 g/1,000 cm³. The root-gall severity (RGS) rating is based on a scale of 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. The columns representing the RGS rating with similar letters are not different according to LSD analysis (*P* > 0.05).

Fig. 2. Cyanide content (parts per million; µg/g) of leaf tissue of white clover accessions and their effect on root galling on lettuce (*Lactuca sativa* L. cv. Montello) when incorporated into *Meloidogyne hapla*-infested soil as a green manure. Cut leaves were mixed into soil at 37.5 g/1,000 cm³. The root-gall severity (RGS) rating is based on a scale of 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. The columns representing the RGS rating with similar lette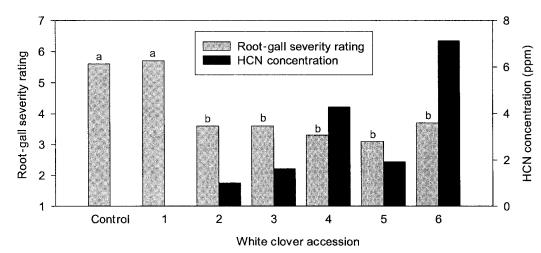rs are not different according to LSD analysis (*P* > 0.05). White clover accessions 1, 2, 3, 4, 5, and 6 were PI516411, PI217444, PI214208, PI214207, PI494747, and PI287988, respectively.

Results from the two trials within the single and variable-rate experiments were similar; therefore, data were combined. In all of the sudangrass cultivars tested, significant reduction in RGS was achieved in comparison to the control when 37.5 g of tissue was incorporated per liter of soil (Figs. 1, 3). When less tissue was incorporated into the soil, the RGS rating increased, regardless of the sudangrass hybrid tested (Fig. 3). At the rate of 37.5 g/liter of soil, no correlation between the CN⁻ content of the leaf tissues and suppression of *M. hapla* was observed (*P* = 0.45). However, when the leaf tissue from the variable-rate experiment was converted to CN-

concentration in soil, there was a negative relationship between the level of cyanide incorporated into the soil and the amount of root galling (*P* = 0.01). This relationship is described by the equation: $\log_{10}y = 1.8 - 0.4x$ ($r^2 = 0.82$), where the variable y is the amount of cyanide incorporated per liter of soil and x is the RGS rating (Fig. 4).

The green manure of white clover also suppressed galling of *M. hapla* when incorporated into the soil, except for the cultivar that was acyanogenic (Fig. 2). When 37.5 g of flax was incorporated into 1 liter of soil, both PI522532 and PI289121 reduced the number of
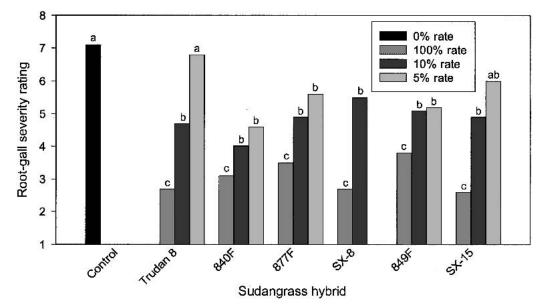

Fig. 3. The effect of different amounts of incorporated leaf tissue from sudangrass hybrids on root galling on lettuce (*Lactuca sativa* L. cv. Montello) caused by *Meloidogyne hapla*. The 100%, 10%, and 5% rates equal 37.5, 3.75, and 1.875 g of tissue per 1,000 cm³ of soil. The root-gall severity (RGS) rating is based on a scale of 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. Columns within a specific hybrid with the same letter are not significantly different (*P* > 0.05).
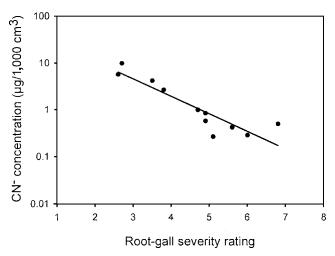
FIG. 4.   The relationship between cyanide concentration within the leaf tissue of sudangrass hybrids incorporated into soil and root galling on lettuce (*Lactuca sativa* L. cv. Montello) caused by *Meloidogyne hapla*. The root-gall severity (RGS) rating is based on a scale of 1 (no galling) to 9 (>80% 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. The equation of the line is: $\log_{10}y = 1.8 - 0.4x$, where the variable y is the amount of cyanide incorporated per liter of soil and x is the RGS rating ($P = 0.01$; $r^2 = 0.82$).

nematodes that penetrated lettuce roots (7.8 and 7.9 nematodes per root system, respectively) compared to unamended soil (16.5 nematodes per root system; $P = 0.004$).

When undeveloped eggs were exposed to an increasing percentage of sudangrass extract, there was a decrease in the development of these eggs [$y = 67 - 28.7(\log_{10}x)$; $P = 0.01$; $r^2 = 0.77$], where the variable y is the percentage of developed eggs and x is the percentage of extract concentration (Fig. 5). When the results
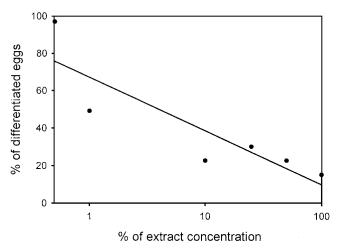

FIG. 5.   The percentage of *Meloidogyne hapla* eggs that developed from a nondifferentiated embryo into a first-stage juvenile after 12 days' exposure to various dilutions of leaf extract from sudangrass hybrid cv. Trudan 8. The line is represented by the equation: $y = 67 - 28.7(\log_{10}x)$, where the variable y is the percentage of differentiated eggs and x is the percentage of extract concentration ($P = 0.01$; $r^2 = 0.77$).

are corrected for the undeveloped eggs exposed to the water control, a dilution resulting in less than 4% sudangrass extract reduced the number of eggs developed to J1 stage by more than 50%.

DISCUSSION

We confirmed that incorporating green manure of sudangrass hybrids suppressed *M. hapla* infection of host crops (Viaene and Abawi, 1998; Widmer and Abawi, 1998). All of the sudangrass hybrids tested suppressed infection of the host, but to varying degrees. Mojtahedi et al. (1993a) also showed that different sudangrass hybrids differed in their suppression of *Meloidogyne* spp. We had predicted that this variation in nematode suppression among the hybrids would be related to the amount of CN^- within the leaf tissue because cyanide was previously determined to be the primary mechanism involved in this system (Widmer and Abawi, 2000). When the hybrids were incorporated at a single rate, no correlation was found; however, when the concentration of CN^- incorporated into soil was determined, there was a negative relationship between soil concentration and root galling. Thus, it is important to know the CN^- concentration within the leaf tissue and the rate of incorporation when selecting an appropriate green manure. Other cyanogenic crops, such as flax and white clover, also reduced root galling and penetration of *M. hapla,* whereas an acyanogenic white clover had no effect on root galling. Despite higher concentrations of CN^- in the tissue of flax and white clover than in the sudangrass hybrids, they were no more suppressive to *M. hapla.*

The lack of correlation between concentrations of CN^- in the leaf tissue and nematode suppression may be due to other chemical components of the leaves that affect the release of cyanide in the soil. The cell composition of different plants varies in their ratios of lignin, polyphenols, and nitrogen (Albersheim, 1976), which affect the decomposition rate of tissues in soil (Carreiro et al., 1999; Senesviratne et al., 1998; Tian et al., 1992; Vanlauwe et al., 1997). Although the cell wall constituents of the selected sudangrass hybrids in this study were not compared, differences have been documented among sorghum hybrids (Gerhardt et al., 1994). The level of glucosidase, which is involved in the release of free cyanide, also may vary among sudangrass hybrids. This would affect the rate of cyanide release and the concentration within the soil. Because HCN is volatile, the slow release of HCN may not yield high enough concentrations to affect the eggs. Also, there is evidence that the concentration of cyanide does not increase suppression above a certain level (Widmer and Abawi, 2000).

To understand the impact of cyanide on the nematode, a more detailed study was done on the impact of egg development because we have previously shown

that cyanide affects eggs more than juveniles (Widmer and Abawi, 2000). Differences in the development of *M. hapla* eggs, corresponding to various levels of CN⁻ exposure, were observed when eggs were incubated in dilutions of sudangrass extract. Decreased egg development may contribute to the reduction in root galling observed with increasing concentrations of CN⁻ in soil.

In New York, growing and incorporating a sudangrass cover crop may not be the most appropriate system for the management of *M. hapla* in vegetables. Ideally, the nematode-antagonistic crop should be planted after the main vegetable crop is harvested and then grown for 6 to 8 weeks before incorporation as a green manure in autumn to achieve sufficient nematicidal activity (Viaene and Abawi, 1998). Thus, the field would not have to be taken out of production for the main vegetable crop. However, environmental conditions in New York do not usually provide a long enough period to grow sudangrass after the harvest of major vegetable crops and before the first frost. After a frost, the amount of cyanide in sudangrass tissue increases but then decreases over time (Harrington, 1966). This decrease would diminish the effectiveness of the sudangrass green manure for nematode suppression. Therefore, it is important to test other cover crops, such as flax and white clover, that are suppressive to nematodes when incorporated as a green manure. These cover crops are better adapted to northern climates because of their cold hardiness (Dillman, 1941; Klebesadel, 1986) and, therefore, may be a more compatible double crop for vegetables in New York.

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