THE INFLUENCE OF LEACHATES FROM ROOTS OF MORNINGGLORY (*IPOMOEA LACUNOSA*), HEMP SESBANIA (*SESBANIA EXALTATA*), AND JOHNSONGRASS (*SORGHUM HALEPENSE*) ON REPRODUCTION OF *ROTYLENCHULUS RENIFORMIS* WITH EMPHASIS ON THE ECLOSION AND HATCHING OF EGGS

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ABSTRACT

Pontif, M. J. and E. C. McGawley. 2008. The influence of leachates from roots of Morningglory (*Ipomoea lacunosa*), Hemp Sesbania (*Sesbania exaltata*) and Johnsongrass (*Sorghum halepense*) on eclosion and hatching of eggs of *Rotylenchulus reniformis*. Nematropica 38: 23-35.

In greenhouse experiments, leachates from roots of morningglory and johnsongrass, but not hemp sesbania, caused significant reductions in reproduction of *Rotylenchulus reniformis* on soybean. Laboratory experiments evaluated the influence of nonfiltered and 0.45 and 0.80-µm cellulose membrane filtrates of leachates from the roots of the three weeds on the development and hatch of eggs of *R. reniformis*. Embryonic development and hatch events were divided into four categories: 1—undifferentiated, granular eggs; 2—eggs at the 4 to 8 cell stage; 3—vermiform juveniles within the egg, and 4—hatched juveniles. One ml suspensions of eggs in leachates from each weed, along with appropriate controls, were placed into sterile 3-ml capacity wells. Eggs were enumerated and assigned to the appropriate developmental category over 10 days. Reductions in the development and hatch of eggs occurred with nonfiltered portions of leachates from all three weeds. Over four experiments, 91% of eggs in distilled water hatched by day 10. Significantly reduced hatch occurred with eggs incubated in leachates; averaging 59% for morningglory, 57% for hemp sesbania and 53% for johnsongrass. There were no differences in egg development and hatch in control and leachate samples that passed through a 0.45-µm filter. Control and leachate samples that passed through a 0.80-µm filter retained inhibitory activity similar to that observed for the nonfiltered portion.

Key words: Allelopathy, cotton, eclosion, *Glycine max*, *Gossypium hirsutum*, hatch, hemp sesbania, *Ipomoea lacunosa*, johnsongrass, juvenile, leachates, morningglory, reniform nematode, reproduction, *Rotylenchulus reniformis*, *Sesbania exaltata*, *Sorghum halepense*, soybean, weed.

RESUMEN

Pontif, M. J. and E. C. McGawley. 2008. La influencia de lixiviados de raíces de *Ipomoea lacunosa*, *Sesbania exaltata* y *Sorghum halepense* sobre el desarrollo y la eclosión de *Rotylenchulus reniformis*. Nematropica 38:23-35.

En experimentos de invernadero, se observó una reducción significativa en la reproducción de *Rotylenchulus reniformis* en soya por efecto de lixiviados de *Ipomoea lacunosa* y de *Sorghum halepense*, pero no de *Sesbania exaltata*. En experimentos de laboratorio, se evaluó el efecto de lixiviados de las tres malezas, sin filtrar y filtrados a través de membranas de celulosa de 0.45 y 0.80 µm, sobre la eclosión de *R. reniformis*. Se dividieron los eventos de desarrollo embrionario y eclosión en cuatro categorías: 1—huevos granulares, indiferenciados; 2—huevos en la etapa de desarrollo de 4 a 8 células; 3—juveniles vermiformes de primer estadio, y 4—juveniles eclosionados. Se incubaron las suspensiones de huevos con los lixiviados de cada maleza, y los respectivos controles, en pozos de 3 ml. Se contaron los huevos y se les asignó una categoría de desarrollo durante 10 días. Se observaron reducciones signi-

ficativas en el desarrollo y eclosión de los huevos con los tratamientos no filtrados de las tres malezas. En cuatro experimentos, el 91% de los huevos en agua destilada eclosionó durante los 10 días. Se observó reducción de la eclosión en los huevos incubados con lixiviados; en promedio 59% con *I. lacunosa,* 57% con *S. exaltata* y 53% con *S. halepense*. No se observaron diferencias en el desarrollo y eclosión entre el control y los tratamientos filtrados a través de la membrana de 0.45 µm. Los lixiviados filtrados a través de 0.80 µm retuvieron la actividad inhibitoria observada en los lixiviados no filtrados.
Palabras clave: alelopatía, algodón, eclosión, *Glycine max*, *Gossypium hirsutum*, *Ipomoea lacunosa*, juveniles, lixiviados, malezas, nematodo reniforme, reproducción, *Rotylenchulus reniformis*, *Sesbania exaltata*, *Sorghum halepense*, soya.

INTRODUCTION

Egg development and hatch are important aspects of the life cycle of the reniform nematode. Physical factors such as temperature, moisture and pH and chemical factors such as secretions by other rhizosphere associated microorganisms or plant roots influence nematode egg biology and have a significant effect on the survival of the nematode population. Cucumber root extracts contain compounds that act as attractants and repellents to juveniles of *Meloidogyne incognita* (Castro *et al.*, 1990), and high concentrations of certain salts, including Hoagland's solution salts, may be repellent to juveniles of *Meloidogyne javanica* (Prot, 1978). Certain inorganic ions are attractive to reniform nematode. Riddle and Bird (1985), and Khan, (1985), found that certain concentrations of tomato root leachates may stimulate or suppress hatch of reniform nematode.

The research detailed herein is the continuation of a previous report (Pontif and McGawley, 2007) that documents reduced reniform nematode reproduction on cotton and soybean in microplots in the presence of johnsongrass, and on cotton in the presence of morningglory and hemp sesbania. Subsequent greenhouse experiments with cotton tested and produced data to support the hypothesis that the reduced reproduction observed in microplots resulted from compounds leachable from the roots of the three weed species.

The objective of these greenhouse and laboratory experiments was to evaluate the effect of leachates from roots of morningglory, hemp sesbania and johnsongrass on reproduction of *R. reniformis* as impacted by the development and hatch of eggs.

MATERIALS AND METHODS

General Procedures

Cotton cultivar LA 887 and soybean cultivar Pioneer 96B21 were used in these experiments. Monoxenic cultures of the reniform nematode were isolated from cotton in Alexandria, Louisiana and maintained in the greenhouse on 'Rutgers' tomato. This population was the source of all reniform life stages used in this research. Inoculum for greenhouse experiments was juveniles and immature adults extracted from greenhouse cultures by wet-sieving through nested 250-µm-pore and 38-µm-pore sieves followed by sugar flotation and centrifugation (Jenkins, 1964). Eggs were extracted from greenhouse cultures using the sodium hypochlorite method (Hussey and Barker, 1973), and utilized in laboratory experiments within 2 hours of harvest.

Greenhouse

Forty-eight clay pots having top diameters of 15-cm, each containing 2 kg of steam-pasteurized soil, and representing four replicates of 12 treatments were

arranged in a randomized complete block design on a greenhouse bench. The forty-eight pots of soil were each infested with 300 reniform juveniles, which by soil volume duplicates the infestation level used in previous microplot trials (Pontif and McGawley, 2007). On an adjacent bench, five 30-cm-diam. coco fiber hanging baskets, one each for morningglory, hemp sesbania, johnsongrass, soybean and perlite only, containing 375 g of sterile perlite were suspended 50-cm above the surface of the bench. Two hundred seed of each weed species were planted in each basket. A 30-cm-diam. plastic funnel was affixed to the bottom of each basket. A 25-cm length of tubing connected the bottom of the funnel to the mouth of a foil-wrapped, sterile 1 L plastic bottle positioned on the bench below. The foil wrapped collecting bottles were autoclaved after each use.

Each morning for 45 days, beginning 72 hours after planting, two liters of water was added to each of the hanging baskets, providing approximately two liters of leachate per weed species. These five leachate sources or regular greenhouse tap water, 120 ml per pot, were added immediately to the clay pots on the adjacent bench. Twenty-eight of these pots duplicated the seven plant or plant-weed combinations used in previous microplot research (Pontif and McGawley, 2007). Treatments 1-4 involved each of the four plant species alone, treatments 5-7 were soybean co-cultured with one of the three weeds. The twenty remaining pots contained a single Pioneer B96B21 soybean seedling, infested with reniform nematodes. Of these, four received leachates from morningglory, four from hemp sesbania, four from johnsongrass, four from soybean and four from the basket containing only perlite growing medium. On another greenhouse bench, 24 pots containing a single soybean seedling not infested with reniform nematode, received leachates from the five leachate sources and regular greenhouse tap water. These pots were established to evaluate the effects of the leachates on soybean growth in the absence of the nematode. Over the course of the experiment, temperature, pH of soil, water and leachates were monitored daily. At the conclusion of the experiment, plant tops were removed, placed into a paper bag and dried at 35°C for 10 days. Root systems and soil were separated, and roots were dried as described for tops. Nematodes were extracted from a 150 g composite subsample with the wet-sieving and centrifugal/sugar flotation technique (Jenkins, 1964). Nematodes were counted at 40× using an Olympus CK-2 inverted microscope. Total soil population density per pot (Pf) and the reproductive values (R, where R = Pf/Pi and Pf = the final population level and Pi = infestation level (Oostenbrink, 1966)) were determined. The experiment was repeated once using the identical experimental design and methodology.

Laboratory

In order to assess the effect of weed leachate and control treatments on egg development and hatch, nematodes were placed into four categories: Category 1—undifferentiated, granular eggs; Category 2—eggs at the 4 to 8 cell stage of development; Category 3—vermiform juveniles within the egg, and Category 4—hatched juveniles. Sources and collection of controls and weed leachates were the same as those described for greenhouse experiments. In laboratory experiments distilled water was included as an additional control, bringing the number of treatments in Experiment 1 to six. In Experiment 2, leachate from soybean was included as a seventh treatment.

One-half liter of liquid was collected from each leachate source and transported

to the laboratory. These samples were used to establish nonfiltered and vacuum-filtered (500 ml capacity Nalgene filtration unit with a 0.45-µm cellulose acetate membrane in Experiment 1 and a 0.80-µm membrane in Experiment 2) subsamples for each of the leachate sources. Aqueous suspensions containing known numbers of eggs were then decanted through an autoclaved 500 mesh (25-µm-pore) sieve and immediately washed with nonfiltered or filtered leachate from sample cups. Sterile wash bottles containing the appropriate nonfiltered or filtered leachate samples were then used to backwash eggs into a second set of sterile sample cups. At this point one ml of each egg-leachate suspension was pipetted into each of four cell wells for each treatment (Falcon sterile, polystyrene, nonpyrogenic 24 well, 3-ml capacity tissue culture plates). Eggs in each well were observed and assigned to the appropriate developmental category daily over the next 10 days. The 10-day period was chosen as the duration for these experiments on the basis of work by others who have studied nematode egg biology and on the basis of our preliminary observations with eggs of this isolate of reniform nematode. Experiments 1 and 2 were each repeated once.

Statistical Analysis

Data were analyzed using the "fit Y by X" module of JMP (Version 5.0, The SAS Institute, Cary, NC) to test for main treatment effects. Unless otherwise stated, all differences noted were significant at the 5% level.

RESULTS

Greenhouse

Data from the two greenhouse experiments with soybean were combined and presented in Table 1. Reniform nematode reproduction, both in the presence of the intact johnsongrass weed or leachates from the roots of morningglory or johnsongrass, was reduced significantly. Nematode populations and reproductive rates were reduced to a greater degree by leachates collected from multiple morningglory or johnsongrass seedling roots than by those originating from single, intact plants. Reniform nematode population increases on soybean plants irrigated with leachates from the roots of hemp sesbania was also reduced compared to the soybean control.

Table 1. The influence of plant root leachates on soil populations of *Rotylenchulus reniformis* after 45 days in a greenhouse environment.

	Reniform/2 kg	
Plant species/treatment	Pf[w]	R[x]
Soybean	7739 a	25.8 a
Morningglory	6249 c	20.8 b
Hemp sesbania	5210 d	17.4 c
Johnsongrass	2953 f	9.8 e
Soybean + Morningglory	7657 a	25.5 a
Soybean + Hemp sesbania	7001 abc	23.3 ab
Soybean + Johnsongrass	4102 e	13.7 d
Soybean/MG Leachate[y,z]	6864 bc	22.8 bc
Soybean/HS Leachate	6837 bc	22.7 bc
Soybean/JG Leachate	3295 f	10.9 e
Soybean/Soybean Leachate	7493 ab	24.9 a
Soybean/Perlite Leachate	7575 a	25.3 a

Data are means of 8 replications combined over two experiments. For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ($P \leq 0.05$).

[w]Pf = final population density per 15-cm-diam. clay pot containing 2 kg of soil.

[x]R (reproductive value) = Pf/Pi where Pf = the final population density and Pi = infestation level of 300 vermiform individuals.

[y]MG = morningglory, HS = hemp sesbania, JG = johnsongrass.

[z]Indicates soybean plants to which leachate from morningglory plants was added.

The experiment evaluating leachate effect on soybean growth in the absence of reniform nematode showed no phytotoxic effects. Root, top or plant dry weights of noninoculated soybean irrigated with root leachates from the three weeds were not different when compared to the control after 45 days (Table 2). The average air and soil temperatures ranged from 25-35°C and 20-30°C, respectively. Water and leachate temperatures both ranged from 25 to 30°C. The pH of the soil ranged from 6.8 to 7.2 across treatments. The pH for each of the three weed leachates used was comparable to each other (averaging 6.6 for morningglory, 6.5 for hemp sesbania, 6.8 for johnsongrass) and to the controls that averaged 6.8, 7.1 and 6.8 for soybean, perlite and tap water, respectively.

Laboratory

Experiment 1: There were no differences in egg and juvenile numbers among the nonfiltered and filtered portions of the three controls (Fig. 1). The only exception to this was the nonfiltered cotton leachate control at Day 8 (Fig. 1D). Therefore, all references to the control treatment from this point refer to the "distilled water control". Over both runs of Experiment 1 the greatest amount of developmental inhibition was associated with the nonfiltered portion of leachate from the roots of each of the three weed species. Additionally, over both sets of experiments, differences in eclosion and hatch among the numbers of eggs associated with weed root leachates rarely occurred. Beginning on Day 6 (Fig. 1A) the number of Category 1 eggs associated with the three weed species was approximately equal and averaged 58% more than the number in the control. With the exception of the counts for the morningglory leachate treatment on Day 8, the number of Category 1 eggs in leachates from the weeds remained greater than those of the control through Day 10. With Category 2, which included eggs in the 4-8 cell stages, differences were first apparent on Day 2 when the numbers of eggs were reduced by the leachates from morningglory, hemp sesbania and johnsongrass (Fig. 1B). At the following two intervals, 4 and 6 days, there were no differences between numbers of eggs in weed leachates and controls. On Day 8, all weed leachate treatments had a greater number of Category 2 eggs than did the control. On Day 10 morningglory and hemp sesbania, but not johnsongrass treatments, had a greater number of Category 2 eggs than did the control.

Table 2. Effects of leachates from morningglory, hemp sesbania and johnsongrass on dry weight of noninfested soybean after 45 days in a greenhouse environment.

	Dry weights (g)[z]		
Soybean irrigated with leachates from:	Root	Top	Plant
Control (tap water)	5.8 a	11.4 a	17.2 a
Morningglory	5.3 a	11.1 a	16.4 a
Hemp sesbania	5.4 a	11.1 a	16.5 a
Johnsongrass	5.1 a	10.7 a	15.8 a

Data are means of 8 replications combined over two experiments. For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ($P \leq 0.05$).
[z]Dry weights were determined after 1 week at 35°C.

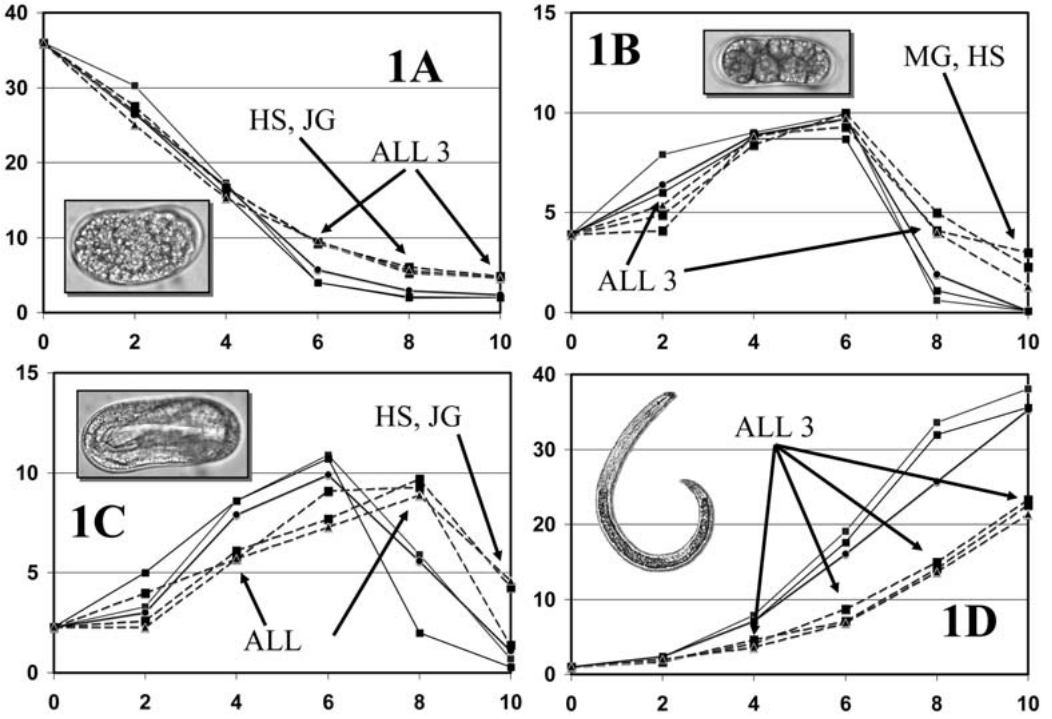

Figure 1. Influence of non-filtered leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in experiment 1. Data are means of 8 replications averaged over two trials. Panel 1A is the numbers of eggs in the undifferentiated, granular stage of development; panel 1B is the numbers of eggs in the 4-8 cell stage of development; panel 1C is the numbers of eggs containing differentiated juveniles and panel 1D is the numbers of hatched juveniles. Solid lines are control treatments: ■ = distilled water, ▲ = perlite and ● = cotton. Dashed lines are weed root leachate treatments: ■ = MG, ● = HS and ▲ = JG. Arrows indicate intervals at which data for weed leachates were significantly different than those of the distilled water control.

No differences in numbers of Category 3 eggs were apparent until Day 4, at which time the numbers of eggs subjected to each of the three weed leachate treatments were less than numbers in the control. Category 3 eggs peaked at six days in the distilled water control but not until Day 8 in weed leachate treatments. On Day 6, only johnsongrass leachate values were less than the control. All leachate treatments had greater numbers of developed juveniles in eggs at Day 8. Except for the morningglory treatment, this trend continued through Day 10 and resulted in reduced numbers of Category 3 eggs present in the control at Day 10.

Beginning at 4 days (Fig. 1D) and continuing throughout the duration of the test, the greatest number of hatched juveniles occurred in distilled water control. At the end of Experiment 1, 91% of the eggs in distilled water had hatched (Table 3). By comparison, only 55, 54 and 51%, had hatched in leachates from morningglory, hemp sesbania and johnsongrass, respectively.

Data for leachates filtered at 0.45 µm indicated that filtering reduced hatch inhibition of leachates (Fig. 2. A-D). For each of the four egg development categories across the ten-day duration of the experiment, there were no differences in the

Table 3. Percentage hatch and mortality of eggs of *Rotylenchulus reniformis* 10 days after exposure to nonfiltered leachates and a distilled water control.

	% Eggs hatched			% Mortality[z]		
Treatment[y]	EXPT.1	EXPT. 2	Combined	EXPT.1	EXPT. 2	Combined
Distilled Water	91 a	91 a	91 a	9 b	9 b	9 b
Cotton	84 a	89 a	87 a	16 b	11 b	13 b
Perlite	85 a	89 a	87 a	15 b	11 b	13 b
Morningglory	55 b	63 b	59 b	45 a	38 a	41 a
Hemp Sesbania	54 b	61 b	57 b	46 a	39 a	43 a
Johnsongrass	51 b	55 b	53 b	49 a	45 a	47 a

Data for experiments 1 and 2 are each means of 8 replications and combined data are means of 16 replications. For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ($P \leq 0.05$).

[y] Root leachate treatments were established by pouring a 1 L of water through coco fiber baskets of perlite growing medium containing seedlings of the respective weed. Control leachate treatments were distilled water, leached growing medium and leachate from cotton seedling roots.

[z] % Mortality indicates #'s of eggs that failed to hatch.

numbers of eggs and juveniles associated with any of the control and weed leachate treatments. After 10 days, 89% of the eggs in distilled water developed into juveniles and hatched. Percentages of eggs that developed into juveniles and hatched were numerically less but not significantly different for weed leachate treatments and averaged 66% for morningglory, 78% for hemp sesbania and 63% for johnsongrass.

Experiment 2: In both trials of Experiment 2 an additional control treatment, leachate from soybean roots, was included since it was a component of this research. There were no differences in egg and juvenile numbers among the nonfiltered portions of the four controls (Fig. 3). As was the case in Experiment 1, the only exception was that the nonfiltered cotton leachate control and it was different only from the distilled water control and only on Day 6 (Fig. 3D). As before, all references to the control from this point refer to the distilled water. On days 4 and 6, numbers of Category 1 eggs in the control were less than those subjected to the three weed leachate treatments (Fig. 3A). On Day 8, more Category 1 eggs were present only in leachates from johnsongrass. The numbers of eggs in Category 1on Day 10 for all treatments were statistically equal.

There were no differences in the number of Category 2 eggs among any of the treatments during the first 6 days (Fig. 3B). Over the course of the next 96 hours, days 8 through 10, numbers of eggs subjected to the leachates from each of the three weeds were greater than those of the control.

During the first 2 days, there were no differences in the numbers of eggs in Category 3 among the treatments (Fig. 3C). All three leachate treatments produced eggs with numbers that were less than those of the control on day 4; only the numbers of eggs in the morningglory and johnsongrass treatments were less on day 6. The opposite occurred on Day 8 in that the numbers of Category 3 eggs were greater in leachates from hemp sesbania and johnsongrass than in the control. The numbers of eggs in this

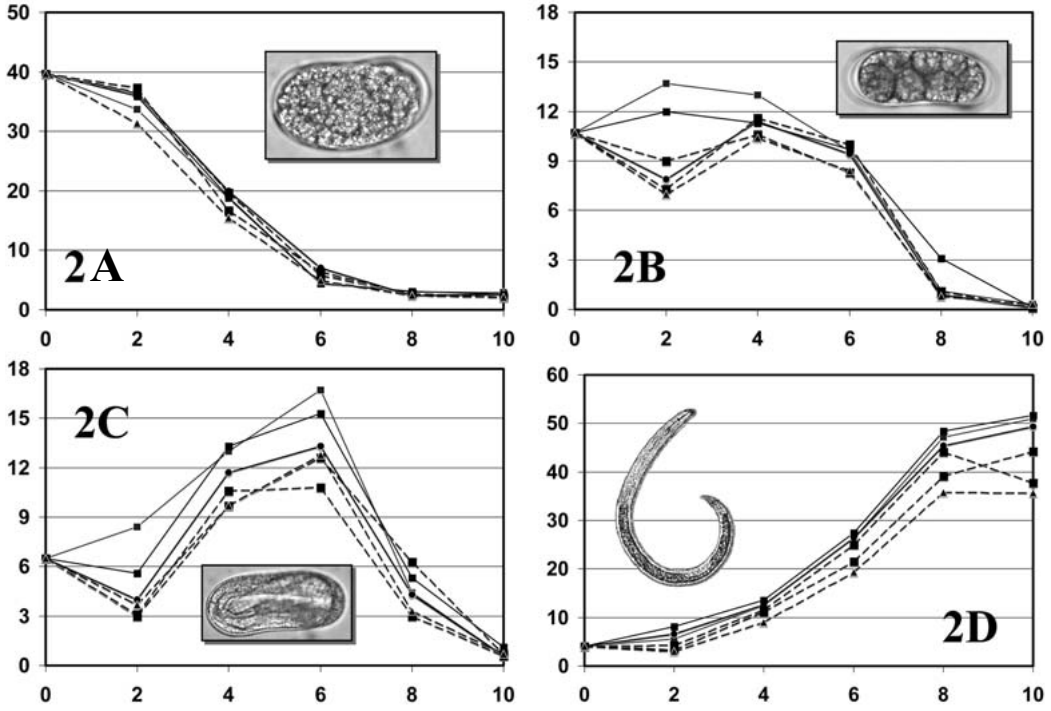

Figure 2. Influence of leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) that passed thru a 0.45-µm filter on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in experiment 1. Data are means of 8 replications averaged over two trials. Panel 1A is the numbers of eggs in the undifferentiated, granular stage of development; panel 1B is the numbers of eggs in the 4-8 cell stage of development; panel 1C is the numbers of eggs containing differentiated juveniles and panel 1D is the numbers of hatched juveniles. Solid lines are control treatments: ■ = distilled water, ▲ = perlite and ● = cotton. Dashed lines are weed root leachate treatments: ■ = MG, ● = HS and ▲ = JG.

category in morningglory leachate were not different from the control at Day 8. On the tenth day, there were no differences among the weed leachate treatments and the control except that numbers of eggs in leachates from hemp sesbania were greater than those of the control. At Day 10 of this experiment differences in the degree of inhibition among the weed leachate treatments was apparent for the first time. The numbers of eggs from morningglory and hemp sesbania were equal but significantly fewer were found with johnsongrass.

The numbers of hatched juveniles, Category 4, were equivalent among treatments for the first 2 days. Thereafter through Day 10, a greater number occurred in the control treatment (Fig. 3D). Over the 10-day period of Experiment 2, 91% of the eggs in distilled water developed and hatched, the exact same percentage as was found over both trials of Experiment 1 (Table 3). The percentages of eggs that developed and hatched in leachates of morningglory averaged 63%, those which developed and hatched in leachates from hemp sesbania and johnsongrass averaged 61 and 55%, respectively.

At only one interval, 6 days, were there significant differences in the numbers of Category 1 eggs associated with control and weed root leachates that passed through

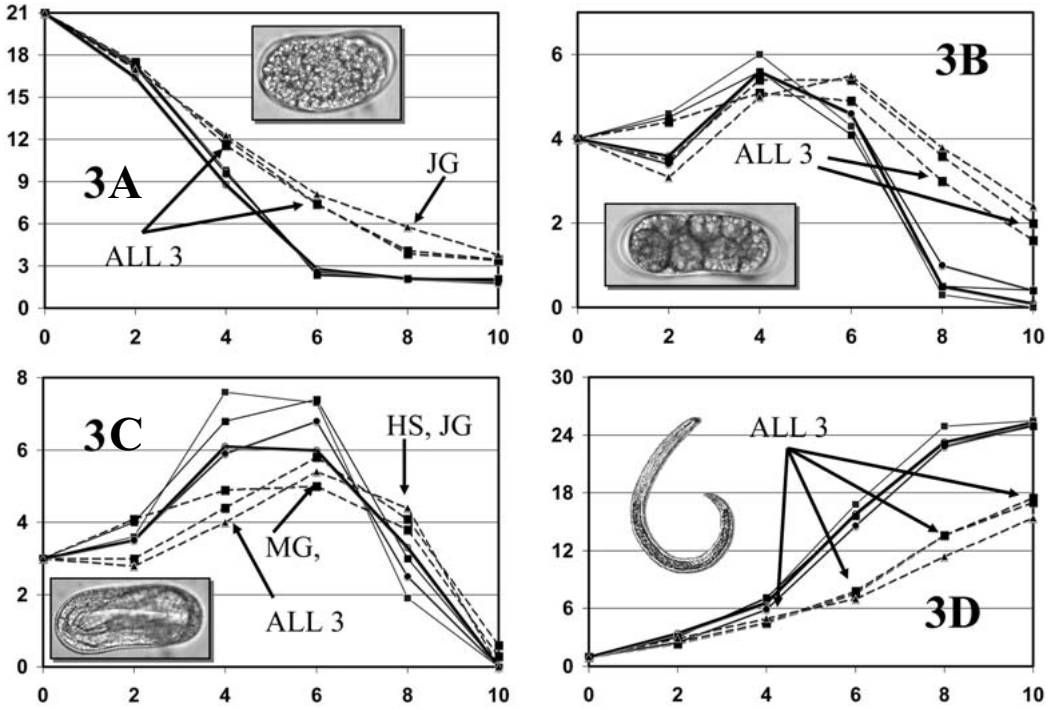

Figure 3. Influence of non-filtered leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in experiment 2. Data are means of 8 replications averaged over two trials. Panel 1A is the numbers of eggs in the undifferentiated, granular stage of development; panel 1B is the numbers of eggs in the 4-8 cell stage of development; panel 1C is the numbers of eggs containing differentiated juveniles and panel 1D is the numbers of hatched juveniles. Solid lines are control treatments: ■ = distilled water, ▲ = perlite and ● = cotton. Dashed lines are weed root leachate treatments: ■ = MG, ● = HS and ▲ = JG. An additional control, leachate from soybean roots (O), was included in this experiment. Arrows indicate intervals at which data for weed leachates were significantly different than those of the distilled water control.

the 0.80-µm filter (Fig. 4A). Egg counts at this interval were significantly greater for the weed leachate treatments.

Numbers of Category 2 eggs present in suspensions representing the control and weed leachate treatments did not differ on Day 2 (Fig. 4B). On Day 4 only the leachate from morningglory produced egg counts that were less than those of the control. At the 6-day interval, there was a difference in the numbers of Category 2 eggs counted for distilled water, soybean and perlite controls. These differences reflected results of the first but not the second run this experiment. There were no differences between other leachate treatments and the control at this interval. Except for morningglory on Day 10 the numbers of eggs from the weed leachates were greater than those of the control on Days 8 and 10.

Category 3 egg counts for control and weed leachates did not differ during the first 48 hours (Fig. 4C). On Day 4 eggs present in leachates from morningglory and johnsongrass were less than those of the control. Relative to the control, egg counts for all three weed leachates were less on Day 6. At Days 8 and 10 no differences among the treatments were observed.

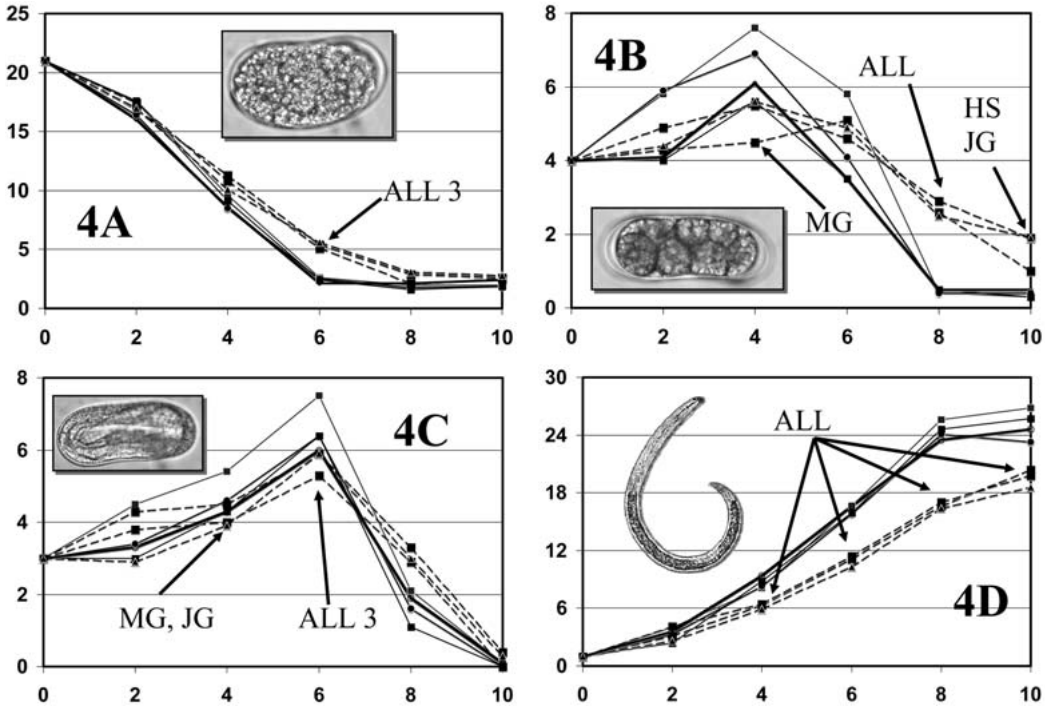

Figure 4. Influence of leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) that passed thru a 0.80-µm filter on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in experiment 2. Data are means of 8 replications averaged over two trials. Panel 1A is the numbers of eggs in the undifferentiated, granular stage of development; panel 1B is the numbers of eggs in the 4-8 cell stage of development; panel 1C is the numbers of eggs containing differentiated juveniles and panel 1D is the numbers of hatched juveniles. Solid lines are control treatments: ■ = distilled water, ▲ = perlite and ● = cotton. Dashed lines are weed root leachate treatments: ■ = MG, ● = HS and ▲ = JG. An additional control, leachate from soybean roots (O), was included in this experiment. Arrows indicate intervals at which data for weed leachates were significantly different than those of the distilled water control.

There were no differences among hatched juveniles, Category 4, on Day 2 but thereafter through Day 10 the numbers of juveniles were reduced significantly in weed leachates (Fig. 4D). At the conclusion of the experiment, 95% of the eggs in the distilled water had developed into juveniles and hatched. By comparison, only 73, 71 and 66% of eggs hatched when exposed to leachates from morningglory, hemp sesbania and johnsongrass, respectively. The pH for each of the three weed leachates was 6.6, 6.5 and 6.8 for morningglory, hemp sesbania and johnsongrass, respectively. The pH for the controls averaged 6.8, 6.9, 6.7 and 7.0 for cotton, soybean, perlite and distilled water, respectively. The pH and temperature (22-25°C) remained the same throughout the duration of the experiments.

DISCUSSION

Over the course of this research six experiments were conducted: two with soybean and weed root leachates in the greenhouse and four with eggs of reniform nematode and weed root leachates in the laboratory. These experiments represent a continuation of the microplot experiments with cotton and soybean and the green-

house leachate tests with cotton reported previously (Pontif and McGawley, 2007).

Overall, the results of these greenhouse experiments with soybean were in agreement with the microplot experiments in that the population increases of *R. reniformis* were reduced in the presence of johnsongrass but not morningglory or hemp sesbania. In these trials, as well as those reported previously for cotton, leachates from weed seedling roots rather than the co-culture with a single weed plant was more inhibitory to the nematode. Since weed plants in all treatments were the same age and because the pH of soil and leachates was equivalent throughout all greenhouse experiments, it is logical to assume that this increased inhibition resulted primarily from the vastly greater number of seedlings associated with the source of weed leachate. This augmented inhibition was consistent in each of the two greenhouse experiments with soybean and each of the two with cotton.

Similar research was conducted by Caswell (1991) in which he collected root exudates from marigold, rhodes grass and tomato plants and evaluated their influence, under greenhouse conditions, on soil populations and egg hatch of reniform nematode. At the conclusion of a 35-day experiment in which exudates from roots of rhodes grass were added to soil containing tomato plants, there was a reduction in populations of reniform nematode that averaged 27%. Additionally, root exudates from rhodes grass significantly reduced the amount of egg hatch that occurred in soil. This reduced egg hatch with rhodes grass was not observed in his *in vitro* egg studies. However, Caswell states that the single *in vitro* experiment did not eliminate the possibility that at different exudate concentrations different results would have been obtained.

The primary reason for the filtration of the root leachates in our experiments was to reduce the opacity of leachate suspension and provide a medium in which egg categories could be accurately counted. The absence of inhibitory activity associated with the 0.45-µm filtered portion of the leachate was probably related to the liner and perlite growth medium in which the weeds were grown. Known allelochemicals such as polythienyls, isothiocyanates, glucosinolates, cyanogenic glycosides, polyacetylenes, alkaloids, terpenoids, sesquiterpenoids and phenolics would not be directly restricted by this size filter but the liner and growth medium components likely congested the 0.45-µm filter pores and impeded their passage. The 0.8-µm filter, however, would permit the passage of all of these leachate components (M. E. Newcomer, Professor, LSU Dept. of Chemistry, personal communication).

Although there is a substantial body of literature that reports the effects of plant extracts and exudates on nematode egg hatching, relatively few (Widmer and Abawi, 2002; Vrain and Barker, 1978) have focused on both the eclosion and hatching processes. This research documents significant influences of leachates from roots of all three weed species on reniform egg development within 48 hours of exposure (Fig. 1B).

With the few exceptions noted earlier the inhibitory effects of the leachates from the three weeds were roughly equivalent. However, the lowest numbers of hatched juveniles occurred with the leachate from johnsongrass in both experiments. Root hairs of *sorghum* species, which includes johnsongrass, are known to exude the phenolic compound sorgoleone, a known allelochemical (Chang *et al.*, 1986) which has been shown to be suppressive to plant parasitic nematodes (Kinloch and Dunavin, 1993; Mojtahedi *et al.*, 1993a). Most studies of nematode-weed interactions have documented the role of the weeds as a biological reservoir for the nematodes during

winter or periods of fallow. A few reports (Ismail and Hasabo, 1995; Wang *et al.*, 2001) document the fact that some weed species do inhibit nematode reproduction, including that of *R. reniformis*.

Investigators have associated exudates, diffusates and leachates from roots with host finding activities of plant-parasitic nematodes and/or the host status of a plant. In general, poor or nonhost produce materials that repel or suppress the nematode (Wang *et al.*, 2001; Siddiqi and Alam, 1987). Good host produce materials which stimulate/enhance host-finding or reproduction by the nematode (Khan, 1985). Our work documents elements of both of these situations. All three of these weeds, morningglory, hemp sesbania and johnsongrass are good hosts of reniform nematode with reproductive values ranging from a low of 9.8 for johnsongrass to a high of 20.8 for morningglory after 45 days in a greenhouse environment and 23.5 for johnsongrass and 49.8 for morningglory after 60 days in microplots (Pontif and McGawley, 2007). In spite of the fact that these three weeds are good hosts *R. reniformis*, leachates from their roots contain materials that inhibit both the development and hatch of eggs, the latter more so than the former. It would be very interesting to study other species of *Ipomoea*, *Sesbania* and *Sorghum* to determine if species that support higher levels of reproduction of reniform nematode lack the ability to produce these inhibitory, leachable materials. Preliminary inoculation studies (data not presented) showed that the nematode did not cause significant damage to any of these three weeds either in the greenhouse or the microplot.

This research demonstrates that morningglory, hemp sesbania and johnsongrass, three weed species endemic in soybean fields in Louisiana and much of the southern U.S., may have a suppressive effect on reproduction of reniform and possibly other major nematode species. These three weeds could have potential use in reniform nematode management programs. Some level of weed presence in the field, especially when involving plant species which are producers of allelochemicals, may benefit growers. The challenge is to select or breed a plant the produces nematicidal agents, but does not have phytotoxic or competitive effect on crops (Ferris *et al.*, 1992). If successful, this would reduce both the monetary and environmental costs associated with herbicide use based on the premise that fields should be maintained 100% weed-free and reduce nematode populations.

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