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The influence of morningglory (*Ipomoea lacunosa*), hemp sesbania (*Sesbania exaltata*), and johnsongrass (*Sorghum halepense*) on reproduction of *Rotylenchulus reniformis* on cotton *Gossypium hirsutum* L. and soybean *Glycine max.* (L.) merrill

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**THE INFLUENCE OF MORNINGGLORY (*IPOMOEA LACUNOSA*), HEMP SESBANIA  
(*SESBANIA EXALTATA*), AND JOHNSONGRASS (*SORGHUM HALEPENSE*) ON  
REPRODUCTION OF *ROTYLENCHULUS RENIFORMIS* ON COTTON *GOSSYPIUM*  
*HIRSUTUM* L. AND SOYBEAN *GLYCINE MAX.* (L.) MERRILL**

A Dissertation

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
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requirements for the degree of  
Doctor of Philosophy

in

The Department of Plant Pathology and Crop Physiology

by  
Michael J. Pontif  
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## ABSTRACT

Microplot studies were conducted to evaluate the effects of cotton (LA. 887), soybean (Pioneer 96B21), and three endemic weed species, pitted morningglory (*Ipomoea lacunosa*), hemp sesbania (*Sesbania exaltata*), and johnsongrass (*Sorghum halepense*), on reproduction of the reniform nematode, (*Rotylenchulus reniformis*). Over two microplot trials the co-culture of cotton with any of the three weeds suppressed numbers of reniform nematode juveniles in soil. When grown singly, reniform nematode reproductive values after 60 days on cotton averaged 69.0, while those for morningglory, hemp sesbania, and johnsongrass averaged 42.0, 23.5, and 18.0, respectively. Reproductive values for cotton co-cultured with morningglory averaged 38.7. Those for the cotton-hemp sesbania and cotton-johnsongrass combinations averaged 23.5 and 26.2, respectively. Reniform reproduction data for soybean cultured alone or with the three weeds in two trials showed reduced reproduction of reniform nematode only in the presence of johnsongrass. Suppression of reniform nematode reproduction likely resulted from the secretion of allelopathic compounds by weed roots and from crowding due to the increased amount of biomass present in microplots containing two plant species. Data from subsequent greenhouse experiments conducted with cotton and soybean and leachates from each of the three weed species supported the allelopathy hypothesis. Reniform reproduction on cotton and soybean plants irrigated with leachates from the roots of morningglory, hemp sesbania and johnsongrass was significantly reduced compared to soybean irrigated with water. Laboratory experiments conducted in which reniform nematode eggs were exposed to leachates from roots of morningglory, hemp sesbania and johnsongrass, nonfiltered and filtered through a .45  $\mu\text{m}$  and a



.80 $\mu$ m filter unit resulted in suppression of hatch and delayed development of reniform eggs in the nonfiltered portions of both filter units and the filtered portion of the .80  $\mu$ m filter.

# CHAPTER 1

## INTRODUCTION

### THE RENIFORM NEMATODE

The nematode genus *Rotylenchulus* was first described in 1940. It was given its name as a result of its morphological affinity with nematodes in the genus *Rotylenchus* and its biological similarity to *Tylenchulus* (Linford and Oliveira, 1940). The genus is in the zoological family Hoplolaimidae and the subfamily Rotylenchulinae. It contains 10 species, with *Rotylenchulus reniformis* being the type species and the only species known to be of major economic importance and the species with the widest geographical distribution and host range (Robinson *et al.*, 1997; Mai and Mullin, 1996). Species in *Rotylenchulus* are separated into five groups based on juvenile lip and tail morphology, with *R. reniformis* as the only species in group III. It is characterized by a high, conoid, rounded, annulated lip region and by the hyaline portion of the tail (h) being less than 13µm (Robinson *et al.*, 1997).

The reniform nematode, *R. reniformis*, is a sedentary, semi-endoparasite of plants found in tropical and sub-tropical regions. It was first described in 1940 by Linford and Oliveira when it was found in a pineapple field in Hawaii. It was the only species in the genus until 1961 when a second species, *Rotylenchulus parvus* (Williams, 1960), was transferred from the genus *Helicotylenchulus* into the genus *Rotylenchulus* (Robinson, *et al.*, 1997). Between 1961 and 1990, eight more species of *Rotylenchulus* were described, with *R. brevitubulus* (Van den Berg, 1990) being the most recent. Despite the nine species described since *R. reniformis*, it remains the only species of known economical importance, and it is the most intensely investigated (Robinson *et al.*, 1997). Of the 10 species of *Rotylenchulus*, *R. reniformis* and *R. parvus* are the only ones known to be in the United States (Lehman and Inserra, 1990). The known host range

of *R. reniformis* includes 314 different plant species (of 364 studied) in 77 families (Robinson *et al.*, 1997), 56 of which are of agricultural importance.

The term “reniform” refers to the kidney-shape of the body of the mature female. Mature females of *Rotylenchulus* are reniform in shape; males are vermiform. The primary morphological characteristics used to speciate *R. reniformis* are the presence of males, the length of the stylet (16-21 $\mu$ m), and the position of the vulva ( $v > 63\%$ ). Secondary morphological characteristics of importance include the distance from the dorsal esophageal gland orifice (DEGO) to the stylet knobs ( $> 1/2$  stylet length for *R. reniformis*), the shape and annulation of the lip region, and the length of h (usually  $h > 2x$  anal body diameter) (Mai and Mullin, 1996; Robinson *et al.*, 1997). The reniform life cycle begins with the egg stage. The first stage juvenile (J1) molts to the second stage (J2) in the egg, and, under optimum conditions, the J2 hatches 1 to 2 weeks after eggs are laid (Robinson *et al.*, 1997). The third and fourth stage juveniles, J3 and J4, often have superimposed cuticles (Linford and Oliveira, 1940) and the reniform body becomes shorter and smaller after each molt (Robinson *et al.*, 1997). When eggs are hatched in water, there is usually a 1:1 ratio of males and females after the last molt (reached 1 to 2 weeks after hatch). Reniform nematodes can remain in a state of anhydrobiosis for as long as 20 years without the presence of host plants (Birchfield, 1961). If there is a suitable host nearby, immature females (J4) infect roots intercellularly and induce syncytia, permanent hypertrophic feeding sites in the stele of the root. Entirely dependent on the syncytia for nutrients, the females become reniform or kidney shaped (Robinson *et al.*, 1997). The anterior end of the female is buried in the root while the posterior end usually protrudes. Reproductive maturity is reached 1 to 2 weeks after root penetration (Robinson *et al.*, 1997). The mature female deposits eggs in a gelatinous matrix produced by the vaginal glands. Reniform

nematodes produce an average of 54 eggs per egg mass, and usually there are less than 74 eggs per mass (Linford and Oliveira, 1940). Fecundity of females of *R. reniformis* is much lower than that of root-knot (*Meloidogne* spp.) and soybean cyst (*Heterodera glycines*) nematodes, which average 100 eggs per female (Taylor and Sasser, 1978; Young, 1992).

The entire life cycle of *R. reniformis* can be completed in less than three weeks at optimal temperatures (29.5 °C) but may require more than two years if the nematodes are anhydrobiotic (Linford and Oliveira, 1940; Rebois, 1973a; Robinson *et al.*, 1997). Males of *R. reniformis* do not feed, and their life cycle is about eight days shorter than that of females (Gaur and Perry, 1991; Sivakumar and Seshadri, 1971). Reniform nematodes have the unique ability to develop from egg hatch through the fourth stage juvenile in water or soil without the presence of a host (Linford and Oliveira, 1940; Sivakumar and Seshadri, 1971).

The documented geographical range and economic importance of *R. reniformis* in the United States, especially the southeast United States, has increased each year over the last 15 years (Birchfield and Jones, 1961; Overstreet and McGawley, 1994 and 1998). The explanation for this phenomenon may rely primarily in the advances in developing resistance to root-knot and soybean cyst nematodes. As resistance to these two important nematodes has become widely available in many crop species, reniform nematode has flourished as the result of reduced competition (Stetina *et al.*, 1997). The threat from reniform nematodes will continue to escalate until adequate resistance to the nematode is identified. Contradictions in cultivar responses, host range, and reproductive rates reported by nematologists have suggested the existence of biotypes/races of *R. reniformis* as has been shown for root-knot and soybean cyst nematodes (Golden *et al.*, 1970; Hartman and Sasser, 1985; Niblack *et al.* 2002; Sasser, 1972; Swanson and Guidry, 1984; Taylor and Sasser, 1978). Breeding efforts are hindered by the lack of knowledge

of races or biotypes of *R. reniformis*. This awareness must precede successful breeding activity, as was the case for soybean cyst nematode (Golden *et al.*, 1970).

There is a growing body of evidence to suggest the existence of races of *R. reniformis*, especially in Louisiana (Birchfield and Brister, 1962; Birchfield, 1962; McGawley and Overstreet, 1995). As early as the 1960's, Birchfield and Brister (1962) and Birchfield (1962) indicated that the Louisiana populations of *R. reniformis* were physiologically different from other reniform populations and suggested the existence of "different strains of the organism." This has been further confirmed by McGawley and Overstreet (1995), who have reported differences in pathogenicity and reproduction of *R. reniformis* populations on cotton and soybean in Louisiana.

Populations of *R. reniformis* have been reported with different host ranges (Dasgupta and Seshadri, 1971a and b; McGawley and Overstreet, 1995; Mehta and Sundara, 1989; Routaray *et al.*, 1988; Srivastava and Sethi, 1968). *Zea mays* is usually considered resistant to *R. reniformis* (Robinson *et al.*, 1997), but Srivastava and Sethi (1986) reported steady population levels in a corn field in India. Chilli, *Capsicum annuum*, is also considered resistant to *R. reniformis*, but some varieties are susceptible to populations in India (Routaray *et al.*, 1988). Dasgupta and Seshadri (1971a and b) inoculated 10 populations of *R. reniformis* onto seedlings of castor bean (*Ricinus communis*), cowpea (*Vigna unguiculata*) and cotton (*Gossypium hirsutum*). They found that nine of the populations reproduced on all three plant species, although the levels of reproduction and ratio of males to females varied. These nine populations were designated as Race A. One population only colonized cowpea, and it was termed Race B. Reports of sugarcane as a host for *R. reniformis* have varied over the years, but it is generally not considered

a host in most parts of the world. However, Mehta and Sundara (1989) detected reproduction of *R. reniformis* on sugarcane under controlled conditions in India.

Another source of population variation is the ratio of males to females. Some populations of *R. reniformis* have very different percentages of males. These populations are broken into three groups, based on whether males were common, rare, or absent. These populations reproduced either parthenogenically or amphimictically (Robinson *et al.*, 1997). Sivakumar and Seshadri (1971) found that females were able to reproduce parthenogenically in the absence of males, even if they originated from a population where males naturally occurred.

Populations of *R. reniformis* are known to react differently to temperature and moisture (Heald and Inserra, 1988; Rebois, 1973a and b). This difference may reflect adaptation based on the geographical area from which they originated (Heald and Inserra, 1988), or it may be evidence of discrete races. Rebois (1973a and b) and Heald and Inserra (1988) did a series of studies to determine the effect of temperature and moisture on infectivity and reproduction of several reniform populations. Moisture content was not a limiting factor, since the optimum moisture level for reniform growth and infection is generally the same as conditions that are best for the growth of the host plant (Rebois, 1973b). Temperature was found to be an important factor for *R. reniformis* (Heald and Inserra, 1988; Rebois, 1973a), with the optimum temperature for infectivity and reproduction being 29.5 °C (Rebois, 1973a). Heald and Inserra (1988) reported that populations varied in reproductive rates at a sub-optimal temperature (15°C). None of the populations were able to reproduce at 10°C.

Since the original description of *R. reniformis* did not indicate the number of specimens examined to define the species, Lehman and Inserra (1990) looked at nine populations of *R. reniformis* to determine acceptable variation in morphology within the species. Unfortunately,

seven of the nine populations they examined were from Florida, hence they did not adequately represent the geographical diversity of the nematode. Single egg mass cultures were not established, so populations used may actually have been a mixture of populations. It is therefore not surprising that they found very little variation in mean stylet length, vulva position, and male and juvenile stylet length. Body length varied from 302-470  $\mu\text{m}$ , with a mean of 354 to 415  $\mu\text{m}$ , depending on the population. Tail characteristics were also variable, with length ranging from 19.6-30.3  $\mu\text{m}$ , and population means of 22-25.9  $\mu\text{m}$ . The hyaline portion of the tail (h) ranged from 3.4-9.3  $\mu\text{m}$ , with population means of 5.4-7.3  $\mu\text{m}$ . Linford and Oliveira (1940) noted variances in egg length from 70-118  $\mu\text{m}$  and width from 34-49  $\mu\text{m}$  (mean 94  $\mu\text{m}$  by 42  $\mu\text{m}$ ). Diameter of the egg masses also varied, from 0.5 to 0.8 mm (Linford and Oliveira, 1940).

#### **RENIFORM ON COTTON AND SOYBEANS**

Cotton is a major crop grown around the world. Only wheat (*Triticum* spp.), rice (*Oryza sativa*), soybean (*Glycine max*) and corn (*Zea mays*) surpass cotton (*Gossypium hirsutum*) returns (Robinson, 2007). In the past year world cotton production totaled 117 million bale equivalents. The United States crop farm gate value was 6.5 billion dollars in 2006. In the last NCCA (National Cotton Council of America) annual report, half the losses attributed to nematodes were due to *R. reniformis*. In Louisiana, over 3000 fields are infested with *R. reniformis*, which represents every cotton-producing parish in the state. Infestation levels are as much as 100% in four of the most productive parishes in Louisiana. The reniform nematode has become one of the most economically important pathogens on cotton. The first report of reniform nematode on cotton was in 1940, since then *R. reniformis* has been detected in every cotton producing state in the southeastern United States (Robinson *et al.*, 1997). There are no commercially available cotton cultivars that provide resistance to the reniform nematode (Starr,

1998; Usery *et al.* 2005). Nematicides, such as 1,3 dichloropropene, oxamyl and aldicarb, are used to manage reniform nematode problems. Even with the use of nematicides, nematode population densities increase by the end of the growing season thereby requiring the application of more nematicides each year which becomes a risk to sustainable agriculture. Reniform nematode damage is difficult to identify in the field. Infected plants exhibit various degrees of stunting, signs of potassium deficiency, reduced cotton production, and early maturity. Flowering and fruit set is consistently delayed one or two fruiting branches up the main stem. Symptoms usually appear in localized areas or "pockets" in newly infested fields. In fields where reniform nematodes have become well established, stunting and other signs of reniform damage are fairly uniform throughout the field. Cotton roots damaged by reniform nematodes are generally smaller and more sparse than healthy roots, but otherwise, they appear normal. After rinsing roots, soil particles can be seen sticking to the gelatinous egg masses embedding the kidney-shaped females protruding from the root surface. A soil nematode analysis is the only means for identifying reniform nematode infestations.

What makes *R. reniformis* so damaging to cotton production? Robinson (2007) listed six biological attributes of *R. reniformis* that make it a successful parasite of cotton: 1) Cotton is an excellent host of *R. reniformis*; 2) *R. reniformis* has a short life cycle, as little as 17 days from egg to egg at 27 °C to 32 °C, and has the ability to survive in the soil in its vermiform stage for long periods of time in the absence of a suitable host; 3) *R. reniformis* damages taproot penetration and root colonization of the soil far less than does *M. incognita*; 4) *R. reniformis* establishes feeding sites all along primary, secondary, and tertiary roots which results in more reproductive females on a root system. This translates into a high potential rate of population increase compared to other nematodes that parasitize cotton; 5) the cuticle (outer-covering) of *R.*



*reniformis* is retained during the three juvenile molts which may provide protection from antagonists like *Pasteuria penetrans*, which is devastating to *Meloidogyne* spp.; 6) *R. reniformis* can build up high population densities in a wide range of soils in contrast to root-knot, lance and sting nematodes which favor or are limited to sandy soils.

Unmanaged weed populations lend to the problems with reniform nematodes in cotton fields. Some weeds are excellent host of *R. reniformis*, such as several species of morningglory, which can support even greater populations than cotton.

In the United States, the 2007 soybean planted area was estimated at 67.1 million acres, down 11% from the record high of almost 75 million acres in 2006. Area for harvest, at 63.3 million acres, was also down 15% from 2006 (National Agricultural Statistics Service, USDA). This was the lowest planted and harvested area for soybean since 1995. Many farmers across the country shifted to planting more corn in 2007, at the expense of soybean. However, increases in soybean area occurred across the southeast, where some farmers shifted from cotton to corn and soybean (National Agricultural Statistics Service, USDA). In Louisiana, there were 585,000 acres of soybeans planted in 2007, which represents the largest planted commodity in the state. Although soybean cyst nematode is the primary pathogen attacking soybean, reniform and root-knot nematodes are being detected more than ever in field surveys (Palmer, 2001). The best methods for management of reniform nematode in soybean are: 1) variety selection, 2) crop rotation with a nonhost or poor host, and 3) nematicides (McGawley *et al.*, 2006). There are reniform nematode resistant soybean cultivars available, although none are highly resistant and some population increase can be expected during the season on these cultivars. The effectiveness of resistant cultivars decreases over time if they are continually grown in nematode infested fields. Growers who have fields with a history of nematode problems need to develop a strategy that includes crop rotation and rotation of nematode resistant and susceptible varieties. Rotation

with corn and grain sorghum is an excellent, but not widely practiced, management tactic. Nematicides, such as Telone and Temik, are efficacious against reniform nematode, but monetary and environmental costs are usually prohibitive.

Over the last 20 years, there has been a growing concern over the use of environmentally harmful nematicides, not only from the public but also from governmental agencies. With current management practices of reniform nematode in cotton being limited to nematicides and crop rotation, which many cotton farmers cannot do, and the recent spread of *R. reniformis* in the United States, the search for new alternative management practices to control reniform nematodes is the top priority of many nematologists.

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## CHAPTER 2

### MICROPLOT AND GREENHOUSE STUDIES WITH PITTED MORNINGGLORY, HEMP SESBANIA AND JOHNSONGRASS ON REPRODUCTION OF *ROTYLENCHULUS RENIFORMIS* ON COTTON AND SOYBEAN

#### INTRODUCTION

The reniform nematode (*Rotylenchulus reniformis*) has become the most economically important pest species associated with upland cotton (*Gossypium hirsutum*) production in the southeast United States (Lawrence, 2004). It has been found in all 11 states that make up the Cotton Belt. Of the 6.2 million acres of cotton produced in the southeast, 19 percent is infested with reniform nematode. Infestations are estimated from 1.4 to 55 percent in each state, with the highest in Alabama, Louisiana and Mississippi. Losses to reniform nematode from 2000 through 2003 averaged 5.0%, 6.9%, and 6.0% in Louisiana, Mississippi, and Alabama, respectively. Cotton loss due to reniform nematode in these three states during this period was estimated at 1.14 million bales (Blasingame and Patel, 2001, 2002, 2003, 2004).

Nematodes have been a problem in cotton fields in Louisiana for as long as the crop has been produced. It was not until the late nineteenth century, however, that nematodes were recognized as being casually related too much of this loss (Overstreet and McGawley, 1997). *Rotylenchulus reniformis* was first described in Hawaii in 1940 by Linford and Oliveira. Shortly thereafter, it was reported in the continental United States as a parasite of cotton in Georgia (Smith, 1940) and Louisiana (Smith and Taylor, 1941). It was not until 1965 that reniform was shown to be an important parasite of soybean (Fassuliotis and Rau, 1967). Only the pre-adult females of reniform nematodes infect cotton and soybean roots. Females produce 75-80 eggs per egg mass within three weeks of infection. With a relatively short life cycle of only three weeks, soil populations increase rapidly during a single growing season (Lawrence and McLean, 2001).

In the past decade, there has been an increase in research effort and awareness of the pathogenicity of this nematode (Koenning *et al.* 2004). Over 620,000 acres of cotton were planted in Louisiana in 2006 (National Agricultural Statistics Service, USDA), and reniform nematode now occurs in every cotton-producing parish (Overstreet and McGawley, 1997). During the past 10 years, 26% of cotton fields in which the reniform nematode has been detected have population densities over 10,000 per 500 cm<sup>3</sup> of soil and 10% over 20,000 per 500 cm<sup>3</sup> of soil. The most commonly employed methods for management of reniform nematode are: 1) nematicides, 2) crop rotation with a nonhost or poor host, and 3) variety selection improvements (McGawley *et al.*, 2006). Currently there are no commercially available cotton varieties resistant to reniform nematode (Lawrence and McLean, 2001; Koenning *et al.*, 2004). Rotation with corn and grain sorghum is an excellent management tactic. Nematicides, such as Telone and Temik, are efficacious against reniform nematode, but monetary and environmental costs are usually prohibitive.

Almost 75 million acres of soybean were planted in the United States in 2006, a 2.8 million acre increase from 2005. Soybean growers are encouraged by high prices with the largest increase in acreage being in Louisiana, Mississippi and Minnesota (National Agricultural Statistics Service, USDA). Although soybean cyst nematode is the primary pathogen attacking soybean, reniform and root-knot nematodes are being detected more than ever in field surveys (Palmer, 2001). In many fields in the southeast United States, cotton is planted year after year, encouraging reniform populations to build up to highly damaging levels.

Cotton and soybean roots survive for months after harvest. In years when there is a delay in the onset of cool temperatures (<15°C), nematodes can feed and reproduce on stubble and associated weed roots, thus maintaining high population densities through to the next planting

season (Kinloch and Rich, 2001). When soils warm in the spring, weeds that are hosts of pathogenic nematodes may provide sustenance such that nematode soil population densities become elevated prior to planting. Weeds allow plant-parasitic nematodes to survive in the absence or presence of the crop, providing a source of nematode inoculum for the following season (Myers *et al.*, 2004). There are many weeds, particularly broad-leaved ones, which are good hosts for reniform nematode (Hollis, 2003). Numerous studies (McSorley and Campbell, 1980; Inserra, *et al.*, 1989; Schroeder, J.S. *et al.*, 1993; Thomas, S.H. *et al.*, 1996; Schroeder, J., 2004) have documented the interaction of nematodes and weeds (Queneherve *et al.*, 1995; Noling and Gilreath, 2002). Moreover, weeds that are good hosts for nematodes can diminish the nematode-suppressive effect of a rotation crop (Davis, 2004).

Although most weeds are hosts for nematodes, others are known which produce allelopathic substances that suppress reproduction and thereby reduce populations in the soil. Allelochemicals are plant metabolites or their products that are released into the microenvironment or rhizosphere. Allelopathic compounds are released through volatilization, exudation from roots, leaching from plants or residues, and decomposition of residues (Halbrendt, 1996). The possibility of using naturally occurring allelochemicals for nematode control has advantages over the current use of toxic chemicals. Many crop and weed species have been evaluated for chemical activity against nematodes. Results of these investigations revealed that numerous plant species produce nematicidal compounds (Halbrendt, 1996). Growing nematode suppressive crops is a management tactic that can be effective but which has received much less attention by plant scientists. Suppressives combat infestations of plant parasitic nematodes and other soil pathogens naturally without fumigants or non-host crop rotations. Plants, such as marigolds (*Tagetes patula*), chrysanthemum (*Chrysanthemum spp.*),



velvet bean (*Mucuna pruriens*), and rapeseed (*Brassica napus*), produce nematicidal and nematostatic (suppressive) organic compounds. These compounds are toxic to nematodes and are released from the roots of living plants. For example, toxic thiophenes have been recovered from marigold root extracts and from undisturbed rhizospheres (Caswell *et al.*, 1991; McGawley *et al.*, 1991).

Failure to observe differences in population density and/or life stage distribution in fields known to be infested with reniform nematode in spite of rotation of cotton and soybeans with non-hosts or fallow prompted an evaluation of the impact of indigenous weed species on reproduction of *R. reniformis*. The objective of this research were to evaluate reniform nematode reproduction on cotton and soybean in the presence and absence of morningglory, hemp sesbania and johnsongrass, three weed species endemic on both crops in Louisiana.

## **MATERIALS AND METHODS**

### **General Procedures**

Cultivars of cotton and soybean used in all microplot and greenhouse experiments were LA 887 and Pioneer 96B21, respectively. Monoxenic cultures of reniform nematode were isolated from cotton in Alexandria, Louisiana and maintained in the greenhouse on Rutgers tomato. This population was the source of all inoculum. Seedlings of cotton, soybean and all three weed species were produced in seedling trays in the greenhouse and then transplanted into microplots. Microplots were clay pots having top diameters of 30.5-cm with soil capacities of 15 kg. Each pot contained 15 kg of methyl bromide-treated Commerce silt loam soil (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts). All microplot experiments were established in May or June and harvested 60 days after inoculation. Standard fertilization

and insect management practices were used in all microplots. At harvest, plant material was dried at 35 °C for 10 days and then weighed.

### **Microplots**

This project was initiated with the establishment of a microplot trial with cotton, pitted morningglory (henceforth referred to as morningglory), hemp sesbania and johnsongrass. Each microplot was placed into a preformed depression in the soil with only the rim of the pot exposed. The 49 microplots were spaced 1 m apart in a six-by-eight pattern. The entire area was covered with a 14-m-long by 6.5-m-wide aluminum quonset hut frame that was open at both ends and covered with 4 ml polyethylene plastic. Each microplot area was equipped with overhead fans and an automated micro-mist irrigation system in which there was no water splashing. Misters delivered 5 L / nozzle twice daily and pots received approximately 250 ml at each interval. Reflective shade cloth was placed over the plastic cover so that soil and air temperatures in microplots were within 2-3 °C of those in the field. Light intensity under the reflective cloth was measured as  $512\mu\text{E} \cdot \text{s}^{-1} \cdot \text{m}^{-2}$ , which is approximately 78% of full sunlight. The pH of the soil in all microplot experiments ranged from 6.7-7.2.

Planting and harvest dates for cotton experiments in year 1 and year 2 were 01 June and 09 August, respectively. Soybean planting and harvest dates in year 1 were 10 June and 18 August and 07 June and 14 August in year 2. Treatments were arranged in a randomized complete block design. Each microplot was infested with approximately 2,000 reniform juveniles. These infestation levels mimic preplant levels of reniform nematodes commonly found in cotton and soybean fields in Louisiana. Inoculum for all tests consisted of juveniles and pre-adults extracted from greenhouse cultures by wet-sieving through nested 250- $\mu\text{m}$ -pore and

38- $\mu$ m-pore sieves followed by sugar flotation and centrifugation (Jenkins, 1964). Ten days after transplanting, soil was infested by pipetting reniform nematode suspensions into depressions (1.5-cm-diam. by 3- and 6-cm deep) surrounding the bases of the plants. Seven treatments were employed: treatments 1- 4 involved each of the four plant species alone and the final three treatments included cotton or soybean co-cultured with one of the three weeds. Each treatment was replicated seven times for a total of 49 microplots. Each microplot trial was run for 60 days after infestation, allowing for at least two generations of reniform nematode. Trials were terminated at this time because of concern that root growth and subsequent effects on reniform reproduction, would be restricted by microplot size. When microplot trials were terminated, six soil cores (2.5-cm diam. by 30-cm deep) were collected from each microplot, bulked and mixed thoroughly. Nematodes were extracted from a 150 g composite subsample with wet-sieving and centrifugal/sugar flotation technique (Jenkins, 1964). Immature life-stages of the reniform nematode were enumerated at 40X using an Olympus CK-2 inverted microscope. Total 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. Plant tops were removed and placed into a paper bag. Bags were then placed in a drying oven at 35 °C for 10 days and then weighed. Root systems were removed from the microplots by carefully washing away soil over a 3-cm mesh screen allowing soil to pass through and preserving the intact root system. The intact root systems were visually inspected for signs and symptoms of nematode damage. Following inspection, the roots were then placed into paper bags and transferred to a heated environment for drying.

Two soybean experiments were conducted over the same two-year period and the identical experimental design and methodology was employed.

## **Greenhouse**

The hypothesis that the suppression of reniform reproduction observed in microplots was due to allelopathic compounds was tested in the greenhouse. Fifty clay pots having top diameters of 15-cm, each containing 2 kg of steam-sterilized soil, and representing five replicates of 10 treatments were arranged in a randomized complete block design on a greenhouse bench. Each of the pots was inoculated with 300 reniform juveniles, which by soil volume duplicates the infestation level used in the microplot trials. On an adjacent bench, six 30-cm-diam. coco fiber hanging baskets, two each for morningglory, hemp sesbania and johnsongrass, containing 375 g of sterile perlite were suspended 50-cm above the surface of the bench. One 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 500 ml plastic bottle positioned on the bench below.

Each morning for 45 days, beginning 72 hours after planting, 500 ml of water was added to each of the hanging baskets; providing approximately 1 liter of leachate per weed species. These three leachate sources or regular tap water, 120 ml per pot, were added immediately to the clay pots on the adjacent bench. Thirty-five of these pots duplicated the original seven plant or plant-weed combinations used in the microplots. The remaining 15 pots contained a single LA 887 cotton seedling and five received leachates from morningglory, five from hemp sesbania and five from johnsongrass. Over the course of this greenhouse trial, temperature and pH of soil, water and leachates was monitored daily. The foil wrapped collecting bottles were autoclaved after each use. The experiment was repeated once and two additional controls, leachate from cotton seedlings and leachate from baskets containing only perlite were included. Planting and

harvesting dates were 16 November and 03 January and 03 March and 29 April for the first and second experiments, respectively.

To evaluate effects of leachates on plant growth, a preliminary 45-day duration experiment was conducted in which leachates from each of the three weeds plus a tap water control were added to 15-cm-diam clay pots with 2 kg of steam sterilized soil containing single LA 887 cotton seedlings. As with above treatments, 120 mls of leachate from weeds or tap water was added to pots representing appropriate treatments each morning for 45 days.

### **Statistical Analysis**

Analysis of variance and Tukey's HSD means separation procedures were performed on plant and nematode numbers using the "Fit Model" module of SAS JMP, version 5.0 (SAS Institute, Cary, NC). Differences noted were significant at the 5% level. Since there were year by treatment interactions with the soybean trials, data for each year is presented separately.

## **RESULTS**

### **Cotton**

The absence of year by treatment interactions allowed data for the cotton microplot trials to be combined for analysis and presentation. Over both microplot trials, reniform population density at 60 days on cotton averaged approximately 138 thousand individuals per microplot, representing a reproductive factor of 69.0 (Table 2.1). Numbers of reniform individuals per microplot and reproductive values for morningglory, hemp sesbania and johnsongrass, when alone were 84, 47 and 36 thousand and 42.0, 23.5 and 18, respectively. These values represented a reduction from the cotton alone treatment of 39%, 66% and 74%, respectively. When alone, both population density and reproductive values for morningglory were statistically equal to

those for cotton. For hemp sesbania and johnsongrass, however, these values were both significantly less than those for cotton.

Table 2.1. Influence of cotton and three cotton-weed combinations on reproduction of *Rotylenchulus reniformis* after 60 days in a microplot environment.

Plant species	Pf <sup>a</sup>	R <sup>b</sup>	Root dry weight (g) <sup>c</sup>	
			Cotton	Weed
Cotton	138 a	69.0 a	26.1 a	--
Morningglory	84 ab	42.0 ab	--	26.7 b
Hemp sesbania	47 b	23.5 b	--	41.7 b
Johnsongrass	36 b	18.0 b	--	291.2 a
C <sup>d</sup> + MG	77 b	38.7 b	4.0 b	26.7 b
C + HS	47 b	23.5 b	5.1 b	34.0 b
C + JG	52 b	26.2 b	4.9 b	304.5 a

Data are means of 14 replications over two trials.

For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ( $P \leq 0.05$ ).

<sup>a</sup>Pf = final population density in 1000s per 30-cm-diam. clay pot containing 15 kg of soil.

R (reproductive value) = Pf/Pi where Pf = the final population density and Pi = infestation level of 2000 vermiform nematodes.

<sup>c</sup>Root weights were determined by drying roots for one week at 35 °C.

<sup>d</sup>C = cotton, MG = morningglory, HS = hemp sesbania, JG = johnsongrass, C + MG, C + HS, C + JG represent combined plantings.

Relative to cotton alone, the co-culture of cotton with any of the three weeds resulted in a significant decline in reniform population density. Numbers of reniform individuals per microplot and reproductive values for morningglory, hemp sesbania and johnsongrass, when co-cultured with cotton were 77, 47 and 52 thousand, respectively. Those values represented 44%, 66%, and 62% reductions from the cotton alone treatment. Reproductive index data followed the same trend. Cotton root weights, at 60 days after inoculation, were reduced significantly in the

presence of each of the three weed species. Weights of weed root systems, however, were not reduced when they were co-cultured with cotton.

### **Soybean**

Due to treatment by year interactions the soybean data is presented separately. On soybean, reniform population densities at 60 days in year one ranged from a high of almost 300 thousand individuals per microplot for the soybean alone treatment to a low of just over 72 thousand for johnsongrass alone (Table 2.2). These levels represented a range in reproductive rate of 146.1 to 36.2. Singly, soybean was a significantly better host for *R. reniformis* in both years than was either hemp sesbania or johnsongrass. Soybean was a significantly better host for *R. reniformis* than morningglory in year one. However, in year two, reproduction by *R. reniformis* on morningglory was statistically indistinguishable to that on soybean. In year one, morningglory was a significantly better host of *R. reniformis* than johnsongrass and in year two, morningglory was a significantly better host than both hemp sesbania and johnsongrass. In both years of the microplot trial, only the co-culture of johnsongrass with soybean resulted in populations of reniform nematode that were reduced significantly below those for soybean alone.

### **Greenhouse**

The preliminary experiment evaluating weed leachate effect on cotton growth in the absence of reniform nematode showed no phytotoxic effects (Table 2.3). Root dry weights at 45 days were not significantly different among treatments. Top weight of cotton plants irrigated with leachates from johnsongrass was reduced significantly but this did not alter final plant weights that were statistically indistinguishable among all treatments. Data from both greenhouse experiments with cotton supported the allelopathy hypothesis (Table 2.4). Reniform nematode reproduction, both in the presence of the intact weed or leachates from their roots, was reduced significantly.

Table 2.2. Influence of soybean and three soybean–weed combinations on reproduction of *Rotylenchulus reniformis* after 60 days in a microplot environment.

Plant species	Year 1				Year 2			
	Pf <sup>a</sup>	R <sup>b</sup>	Root		Pf	R	Root	
			dry weight (g) <sup>c</sup>				dry weight (g)	
			<u>Soybean</u>	<u>Weed</u>			<u>Soybean</u>	<u>Weed</u>
Soybean	292 ab	146.1 ab	26.2 a	--	71 ab	35.9 ab	22.9 bc	--
MG	192 c	96.3 c	--	9.1 b	57 b	28.4 b	--	7.8 c
HS	141 cd	70.6 cd	--	10.1 b	34 c	17.2 c	--	25.0 bc
JG	72 d	36.2 d	--	361.2 a	21 c	10.4 c	--	62.5 a
S <sup>d</sup> + MG	374 a	187.1 a	26.5 a	7.8 b	89 a	44.4 a	37.9 ab	5.8 c
S + HS	221 bc	110.6 bc	24.8 a	5.5 b	56 b	27.9 b	42.6 a	20.8 c
S + JG	162 c	81.0 c	35.7 a	374.8 a	32 c	16.0 c	21.9 c	54.8 ab

Data are means of five replications.

For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ( $P \leq 0.05$ ).

<sup>a</sup>Pf = final population density in 1000s per 30-cm-diam. clay pot containing 15 kg of soil.

R (reproductive value) = Pf/Pi where Pf = the final population density and Pi = infestation level of 2000 vermiform nematodes.

<sup>c</sup>Root weights were determined by drying roots for one week at 35 °C.

<sup>d</sup>S = soybean, MG = morningglory, HS = hemp sesbania, JG = johnsongrass, S + MG, S + HS, S + JG represent combined plantings.



Table 2.3. Effects of leachates from morningglory, hemp sesbania and johnsongrass on dry weight of noninfested cotton after 45 days in a greenhouse environment.

Cotton irrigated with leachates from:	<u>Dry weights (g)<sup>a</sup></u>		
	Root	Top	Plant
Control (tap water)	2.3 a	11.1 a	13.4 a
Morningglory	2.0 a	10.7 ab	12.7 a
Hemp sesbania	2.1 a	10.5 ab	12.6 a
Johnsongrass	2.1 a	10.2 b	12.3 a

Data are means of 14 replications, averaged over two experiments.

For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ( $P \leq 0.05$ ).

<sup>a</sup>Dry weights were determined after 1 week at 35 °C.

Moreover, with the single exception of the cotton/johnsongrass leachate treatment in experiment one, nematode populations and reproductive rates were reduced to a significantly greater degree by leachates collected from multiple seedling roots than by those theoretically originating from single, intact plants. In experiment one, the average air and soil temperatures ranged from 12-21 °C and 14-19 °C, respectively. Water and leachate temperatures both ranged from 17-22 °C, respectively. The pH of the soil in experiment one ranged from 6.9-7.2 across treatments. The pH values for each of the three leachates used in experiment one were comparable to each other (averaging 6.6 for morningglory, 6.5 for hemp sesbania and 6.8 for johnsongrass) and to the water control, which averaged 6.8. In experiment two, the average air and soil temperatures ranged from 25-35 °C and 20-30 °C, respectively. Water and leachate temperatures both ranged from 25-30 °C, respectively. The pH data for experiment two was identical to that for experiment one. Values for the two additional controls in experiment two were within these same temperature and pH ranges.

Table 2.4. The influence of plant root leachates on soil populations of *Rotylenchulus reniformis* after 45 days in a greenhouse environment.

Plant species/Treatment	Experiment 1		Experiment 2	
	Pf <sup>a</sup>	R <sup>b</sup>	Pf	R
Cotton	4,756 a	15.8 a	8,899 a	29.6 a
Morningglory	4,537a	15.1 a	7,828 abc	26.0 ab
Hemp sesbania	3,207 b	10.6 b	5,379 d	17.9 cd
Johnsongrass	3,025 b	10.0 b	5,182 d	17.2 cde
Cotton + Morningglory	1,421 cd	4.7 cd	6,778 c	22.5 bc
Cotton + Hemp sesbania	1,731 c	5.7 c	3,476 e	11.5 e
Cotton + Johnsongrass	1,276 cd	4.2 cd	3,717 e	12.3 de
Cotton / MG leachate <sup>c</sup>	109 e	0.4 e	1,224 f	4.0 f
Cotton / HS leachate	638 de	2.1 de	1,443 f	4.8 f
Cotton / JG leachate	619 de	2.0 de	1,312 f	4.3 f
Cotton / Cotton leachate	--	--	7,587 bc	25.2 ab
Cotton / Perlite leachate	--	--	8,637 ab	28.7 a

Data are means of five replications for each experiment (experiment one ran from 16 November through 03 January and experiment two from 03 March through 29 April).

For each parameter, means followed by the same letter are not significantly different based on ANOVA and Tukey's HSD Tests ( $P \leq 0.05$ ).

<sup>a</sup>Pf = final population density per 15-cm-diam. clay pot containing 2 kg of soil.

<sup>b</sup>R (reproductive value) = Pf/Pi where Pf = the final population density and Pi = infestation level of 300 vermiform nematodes.

<sup>c</sup>MG = morningglory, HS = hemp sesbania, JG = johnsongrass.

## DISCUSSION

Over the course of this research, six individual experiments, four in microplots and two in the greenhouse, were conducted to evaluate the influence of three weed hosts on reproduction of *R. reniformis*. Among the weeds, averaged across all four of the microplot trials, morningglory was the best host with an average reproductive value at 60 days of 55.6. Hemp sesbania and johnsongrass followed with reproductive values at 60 days averaging 37.1 and 21.5, respectively. Microplot data from Carter (1995), who worked with the same three weeds, rated the suitability of these three weeds to *R. reniformis* in the same order at the conclusion of a 76-day duration greenhouse experiment with soybean. A recent report by Lawrence *et al.* (2006) in Mississippi reports that morningglory and hemp sesbania, but not johnsongrass, are hosts of the reniform nematode.

Modes of nematode suppression by cover crops or weeds can be categorized as providing a nonhost or poor host environment for nematodes (Rodriguez-Kabana *et al.*, 1988), producing allelochemicals (Halbrendt, 1996) or acting as trap crops to the nematode (Gardner and Caswell-Chen, 1994).

Singly, all three of the weeds used in this investigation were hosts of reniform nematode. The co-culture of johnsongrass with either cotton or soybean significantly reduced reproduction of the nematode, and the co-culture of either morningglory or hemp sesbania reduced reproduction on cotton but not soybean. The two greenhouse experiments, conducted subsequent to the cotton microplot trials, suggested that the reproductive inhibition observed with cotton resulted from an allelopathic, leachable product(s) produced by the three weeds. Subsequent greenhouse experiments with soybean and leachates from the three weeds, shows that inhibition of reniform nematode reproduction on soybean is also suppressed by leachates from

morningglory, hemp sesbania and johnsongrass. Subsequent research results of *in vitro* tests of the effects of leachates from the roots of each of the three weeds on eggs of *R. reniformis* nematode follows this chapter.

This research demonstrates that the suppression in reproduction of reniform in greenhouse trials resulted largely as the result of allelopathic compounds produced by the weeds. The results of this research suggest that allelopathy is a major factor that limited reproduction of reniform nematodes in our microplot trials. The reduction in cotton root weights at the end of the trial in microplots where they were co-cultured with any of the weeds were the result of reniform nematode pathology on cotton plants already contending with the presence of a concomitant weed species requiring space, water and nutrients. Data for dry top weights and that obtained by adding root and top weights together to determine plant weights, (data not shown), follows the same trend as that of root weights.

Caswell (1991) conducted research to assess the influence of several accompanying plant species on the reproduction of *R. reniformis* on tomato. In these experiments, tomato was planted alone or was co-cultured with either rhodes grass or marigold. At 102 days after infestation, reproductive values for reniform nematode, when co-cultured, were significantly reduced relative to those for tomato alone. Inhibition of reproduction by reniform nematode was attributed to allelopathy in the case of marigold and was unexplained for rhodes grass although the inhibition was greater than that of a fallow treatment.

Most plant species that produce allelochemicals, for example *Crotalaria juncea*, *B. napus* and *T. patula* (Caswell, 1991; Wang *et al.*, 2001), are poor or non-hosts of the target nematode. This research with morningglory, hemp sesbania and johnsongrass along with that documented

for African marigold, *T. erecta*, (Wang, 2001) constitute some cases in which plants that are hosts of the nematode are also producers of allelochemicals.

This research suggests that morningglory, hemp sesbania and johnsongrass, three weed species endemic in cotton and 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. This does not suggest that producers should abandon current weed control practices. However, some level of weed presence in the field, especially that which involves species which are producers of allelochemicals, would reduce both the monetary and environmental costs associated with herbicide use based on the premise that fields should be maintained 100% weed-free.

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## CHAPTER 3

### INFLUENCE OF LEACHATES FROM ROOTS OF PITTED MORNINGGLORY, HEMP SESBANIA AND JOHNSONGRASS ON REPRODUCTION AND ECLOSION AND HATCHING OF EGGS OF *ROTYLENCHULUS RENIFORMIS*

#### INTRODUCTION

The reniform nematode, *Rotylenchulus reniformis*, is the most important pathogen on cotton and one of the most important pathogens of soybean in the United States. The estimated annual loss to the U.S. cotton crop is over 525,000 bales, which represents a value of 130 million dollars. Economic losses caused by reniform nematodes occur due to infected cotton plants producing fewer and smaller bolls, which results in lower harvestable yield (Jones *et al.*, 1959; Lawrence and McLean, 2001). The damage threshold for *R. reniformis* in cotton in Louisiana is 1500 nematodes per 500 cm<sup>3</sup> of soil (Overstreet, *pers. com*). There are many factors that apply when considering the population density threshold at which damage from reniform nematode can be expected. Those factors include, but are not limited to, soil type and texture, temperature and water availability (Robinson, 2007). Reniform nematode soil populations can increase quite rapidly during a single growing season. The nematode is able to build up to such high populations in the soil because of a relatively short life cycle, a wide host range and the ability to survive adverse conditions in a quiescent or anhydrobiotic state. It has also been reported that reniform nematodes can exist at soil depths between 60 and 120 cm, which is well below the zone affected by tillage or nematicide applications (Westphal and Smart, 2003; Robinson *et al.*, 2005). Typical reniform nematode damage symptomology includes light green or chlorotic foliage, stunting, reduced number of secondary roots, nutritional deficiencies and abnormal maturation of the crop. Current practices employed for the management of *R. reniformis* are the use of nematicides, crop rotation with a nonhost or poor host and variety selection. Rotation crops include corn, peanut, grain sorghum and resistant soybean, but crop rotation is not a widely

practiced management technique in some areas. In order for rotational crops to be practical, alternative crops must provide an adequate return to the grower and production of the crop must result in sufficient cotton yield increases to justify moving land from cotton production (Koenning *et al.*, 2004). Currently there are no resistant cotton varieties commercially available (Lawrence and McLean, 2001; Koenning *et al.*, 2004). There are reniform nematode resistant soybean cultivars available, although none are highly resistant and some population increase can be expected during the season when these cultivars are used.

Nematode management in cotton is largely dependent upon nematicides, such as aldicarb and 1, 3-dichloropropene, and it is the most frequently utilized method for controlling *R. reniformis* (Kinloch and Rich, 2001; Lawrence and McLean, 2001; Koenning *et al.*, 2004). The use of nematicides has increasingly come under scrutiny by the public and government agencies because of toxicological and environmental concerns. With the increased production costs and health risks associated with the use of nematicides and the possibility of eventual nematode resistance, a shift to alternative controls of reniform nematode is eminent.

To manage *R. reniformis* below the damage threshold, one approach is to select cover crops possessing multiple suppressive mechanisms. The nonhost, rhodes grass (*Chloris gayana*), and the poor hosts sunn hemp (*Crotalaria juncea*), marigold (*Tagetes patula*), and panola grass (*Digitaria eriantha*) all reduced reniform nematode populations in Hawaiian pineapple (*Ananas comosus*) soils as well as or better than allowing the soil to remain fallow (Caswell *et al.*, 1991a). Root exudates of *T. minuta* L. have been found to have nematicidal activity against *R. reniformis* (Siddiqui and Alam, 1987). Although most weeds are hosts for nematodes, others are known which produce allelopathic substances that suppress nematode reproduction and thereby reduce populations in the soil. Allelochemicals are plant metabolites or their products that are released into the microenvironment or rhizosphere. Allelopathic compounds are released through

volatilization, exudation from roots, leaching from plants or residues, and decomposition of residues (Halbrendt, 1996). The possibility of using naturally occurring allelochemicals for nematode control has advantages over the current use of toxic nematicides. Many crop and weed species have been evaluated for chemical activity against nematodes. Results of these investigations revealed that numerous plant species produce nematocidal compounds (Halbrendt, 1996). Plants, such as marigolds (*Tagetes patula*), chrysanthemum (*Chrysanthemum spp.*), velvet bean (*Mucuna pruriens*), and rapeseed (*Brassica napus*), produce nematocidal and nematostatic (suppressible) organic compounds. These compounds are toxic to nematodes and are released from the roots of living plants.

Nematode egg hatch is an important part of the life cycle of the reniform nematode (Hamlen and Bloom, 1968). Factors that influence this process may have a highly significant effect on the survival of the nematode. These factors may also influence the nematode's generation time and its ability to ward off competitors or predators. Root leachates and seasonal changes in environmental, physical and chemical factors also affect the eclosion and hatching of eggs of plant parasitic nematodes. Root leachates and rhizosphere chemicals may also stimulate egg hatch and act as the stimulus for juvenile orientation to roots (Caswell *et al.*, 1991b).

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 *M. 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; Nematropica, submitted) that documents significantly reduced reniform nematode reproduction on cotton and soybean in microplots in the presence of johnsongrass (*sorghum halepense*) and on cotton in the presence of morningglory (*ipomoea lacunosa*) and hemp sesbania (*sesbania exaltata*). 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.

This report details results of greenhouse experiments testing the allelopathy hypothesis with soybean and laboratory experiments with cotton and soybean evaluating the effect of leachates from roots of morningglory, hemp sesbania and johnsongrass on the eclosion and hatch of eggs of *R. reniformis*.

## **MATERIALS AND METHODS**

### **General Procedures**

Cultivars of cotton and soybean used in experiments were LA 887 and Pioneer 96B21, respectively. Monoxenic cultures of reniform nematode were isolated from cotton in Alexandria, Louisiana and maintained in the greenhouse on Rutgers (*Lycopersicon esculentum*) tomato. This population was the source of all reniform life stages used in greenhouse and laboratory experiments. Inoculum for greenhouse experiments consisted of juveniles and preadults extracted from greenhouse cultures by wet-sieving through nested 250- $\mu\text{m}$ -pore and 38- $\mu\text{m}$ -pore sieves followed by sugar flotation and centrifugation (Jenkins, 1964). Eggs of reniform nematode from greenhouse cultures maintained on Rutgers tomato were extracted using the sodium hypochlorite method (Hussey and Barker, 1973), and utilized in laboratory experiments within two hours of harvest.

## **Greenhouse**

Forty-eight clay pots having top diameters of 15-cm, each containing 2 kg of steam-sterilized soil, and representing four replicates of 12 treatments were arranged in a randomized complete block design on a greenhouse bench. The forty-eight pots were infested with 300 reniform juveniles, which by soil volume duplicates the infestation level used in previous microplot trials (Pontif and McGawley, 2007; Nematropica, submitted). 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, 2 liters of water was added to each of the hanging baskets, providing approximately 2 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; Nematropica, submitted). 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 leachate from the five leachates sources or 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, air and soil temperature and the pH of soil, water and leachates was 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 enumerated at 40X 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, the process was arbitrarily divided 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 an additional control, distilled water, was included bringing the number of treatments in Experiment one to six. In Experiment 2 a seventh treatment, leachates from the roots of soybean, were included.

One-half liter of liquid was collected from each leachate source and transported to the nematology laboratory. These samples were used to establish nonfiltered and vacuum-filtered (500 ml capacity Nalgene filtration unit with a 0.45  $\mu\text{m}$  cellulose acetate membrane in Experiment 1 and a 0.80  $\mu\text{m}$  membrane in Experiment 2) subsamples for each of the leachate sources. Aqueous suspensions containing known numbers of eggs were then decanted over an autoclaved 500 mesh (25  $\mu\text{m}$ -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 1 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). Numbers of eggs/juveniles in each of the four categories in each well were determined daily over a period of 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 for a total of 4 experiments.

### **Statistical Analysis**

Analysis of variance and Tukey's HSD means separation procedures were performed on plant and nematode numbers using the "Fit Model" module of SAS JMP, version 5.0 (SAS Institute, Cary, NC). Differences noted were significant at the 5% level.

## **RESULTS**

### **Greenhouse**

Data from the two greenhouse experiments with soybean were combined and presented in Table 3.1. 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 significantly greater degree by leachates collected from multiple morningglory or johnsongrass seedling roots than by those originating from single, intact plants. Reniform nematode reproduction on soybean plants irrigated with leachates from the roots of hemp sesbania was reduced compared to the soybean control.

The experiment evaluating leachate effect on soybean growth in the absence of reniform nematode showed no phytotoxic effects (Table 3.2). Root, top or plant dry weights of noninfested soybean irrigated with leachates from the roots of the three weeds were not significantly different when compared to the control after 45 days. The average air and soil temperatures ranged from 25-35 °C and 20-30 °C, respectively. Water and leachate temperatures both ranged from 25-30 °C. The pH of the soil ranged from 6.8-7.2 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 and 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 (Figure 3.1). The only exception to this was the nonfiltered cotton leachate control at Day 8 (Figure 3.1D). Therefore all references to the control treatment from this point refer to the “distilled water control”. Over both trials 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,



Table 3.1. The influence of plant root leachates on soil populations of *Rotylenchulus reniformis* after 45 days in a greenhouse environment.

Plant Species/Treatment	Reniform / 2 kg	
	Pf <sup>a</sup>	R <sup>b</sup>
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 <sup>c,d</sup>	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 eight 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 ( $P \leq 0.05$ ).

<sup>a</sup>Pf = final population density per 15-cm-diam. clay pot containing 2 kg of soil.

<sup>b</sup>R (reproductive value) = Pf/Pi where Pf = the final population density and Pi = infestation level of 300 vermiform individuals.

<sup>c</sup>MG = morningglory, HS = hemp sesbania, JG = johnsongrass.

<sup>d</sup>Indicates soybean plants to which leachate from morningglory plants were added.

Table 3.2. Effects of leachates from morningglory, hemp sesbania and johnsongrass on dry weight of noninfested soybean after 45 days in a greenhouse environment.

Soybean irrigated with leachates from:	<u>Dry Weights (g)<sup>a</sup></u>		
	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 eight 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 ( $P \leq 0.05$ ).

<sup>a</sup>Dry weights were determined after 1 week at 35 °C.

over both sets of experiments, there were rarely statistically significant differences among the numbers of eggs associated with weed root leachates. Where differences did occur among weed leachates they will be indicated. Beginning on Day 6 (Figure 3.1A) 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. Concerning eggs for which exposure to leachates were in the Category 2 stage of development, 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 all three weed species (Figure 3.1B). At the following two intervals, four and six 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. 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 (Figure 3C). The numbers of eggs containing developed juveniles, Category 3, peaked at six days in the distilled water control and but not until Day 8 in weed leachate treatments. On Day 6, only the data for johnsongrass were less than the control. All leachate treatments had greater numbers of fully 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 juveniles present in the control at Day 10.

Beginning at four days (Figure 3.1D) and continuing throughout the duration of the test, a greater number of hatched juveniles occurred in distilled water control. At the conclusion of Experiment 1, 91% of the eggs in the distilled water had developed into juveniles and hatched (Table 3.3). By comparison, only 55, 54 and 51%, each a reduction in egg development and hatch, occurred with leachates from morningglory, hemp sesbania and johnsongrass, respectively.

Results for treatments established following passage of the leachates through a 0.45  $\mu\text{m}$  filter indicated that the inhibitory effect of weed leachates on reniform egg development and hatch when the leachate was passed thru the filter of this size opening were lost. (Figure 3.2 A-D). For each of the four egg development categories across the 10-day duration of the experiment, there were no differences in the 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. There were no differences in egg and juvenile numbers among the nonfiltered portions of the four controls (Figure 3.3). As was the case in Experiment 1, the only exception was the nonfiltered cotton leachate control, and it was different only from the distilled water control on Day 6 (Figure 3.3D). As before, all references to the control from this point refer to distilled water. On days four and six, numbers of Category 1 eggs in the control were less than those subjected to the three weed leachate treatments (Figure 3.3A). On Day 8, more Category 1 eggs were present only in leachates from johnsongrass. The numbers of eggs in Category 1 on 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 six days (Figure 3.3B). Over the course of the next 96 hours, days eight 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 two days, there were no differences in the numbers of eggs in Category 3 among the treatments (Figure 3.3C). Treatments with all three weed leachates resulted in numbers of eggs that were less than those of the control on day four. On Day 6 the numbers of eggs in morningglory and johnsongrass were less. The opposite occurred on Day 8, in that the numbers of eggs remaining in Category 3 were greater in leachates from hemp sesbania and johnsongrass than in the control. The numbers of eggs in this category in morningglory leachate were not different from the control at Day 8. On the 10th, there were no differences among the weed leachate treatments and the control except that numbers of Category 3 eggs in leachates from hemp sesbania were greater than those of the control.

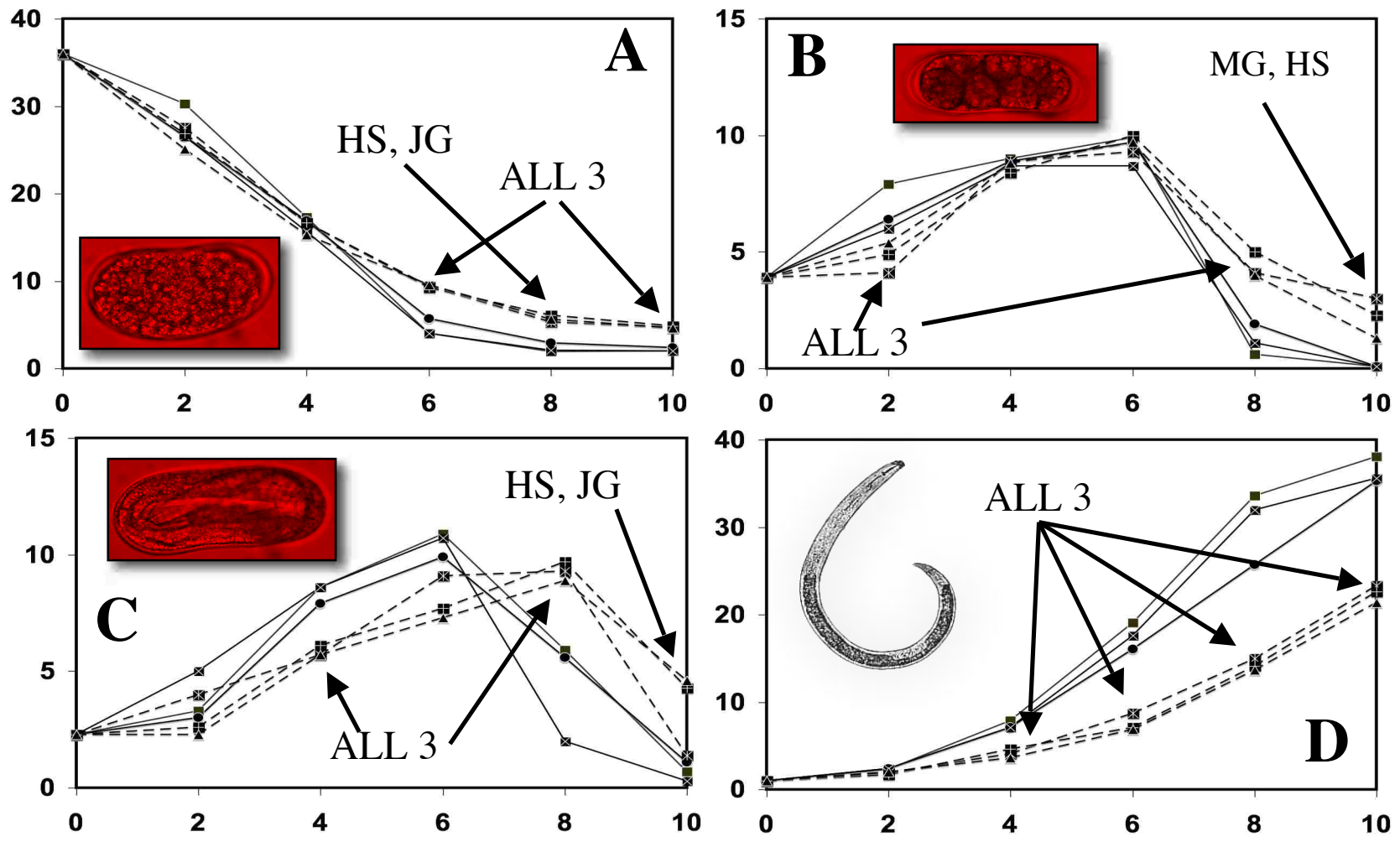


Figure 3.1 Influence of time and non-filtered leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in Experiment 1. Data are means of eight replications averaged over two trials. Panel A is the numbers of eggs in the undifferentiated, granular stage of development; panel B is the numbers of eggs in the 4-8 cell stage of development; panel C is the numbers of eggs containing differentiated juveniles and panel D 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.

Table 3.3. Percentages for hatch and mortality of eggs of *Rotylenchulus reniformis* 10 days after exposure to nonfiltered weed leachates or distilled water, cotton or perlite controls.

Treatment <sup>a</sup>	% of Eggs that Hatched			% Mortality		
	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 eight 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 ( $P \leq 0.05$ ).

<sup>a</sup>Root leachate treatments were established by pouring 1 L of water thru 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.

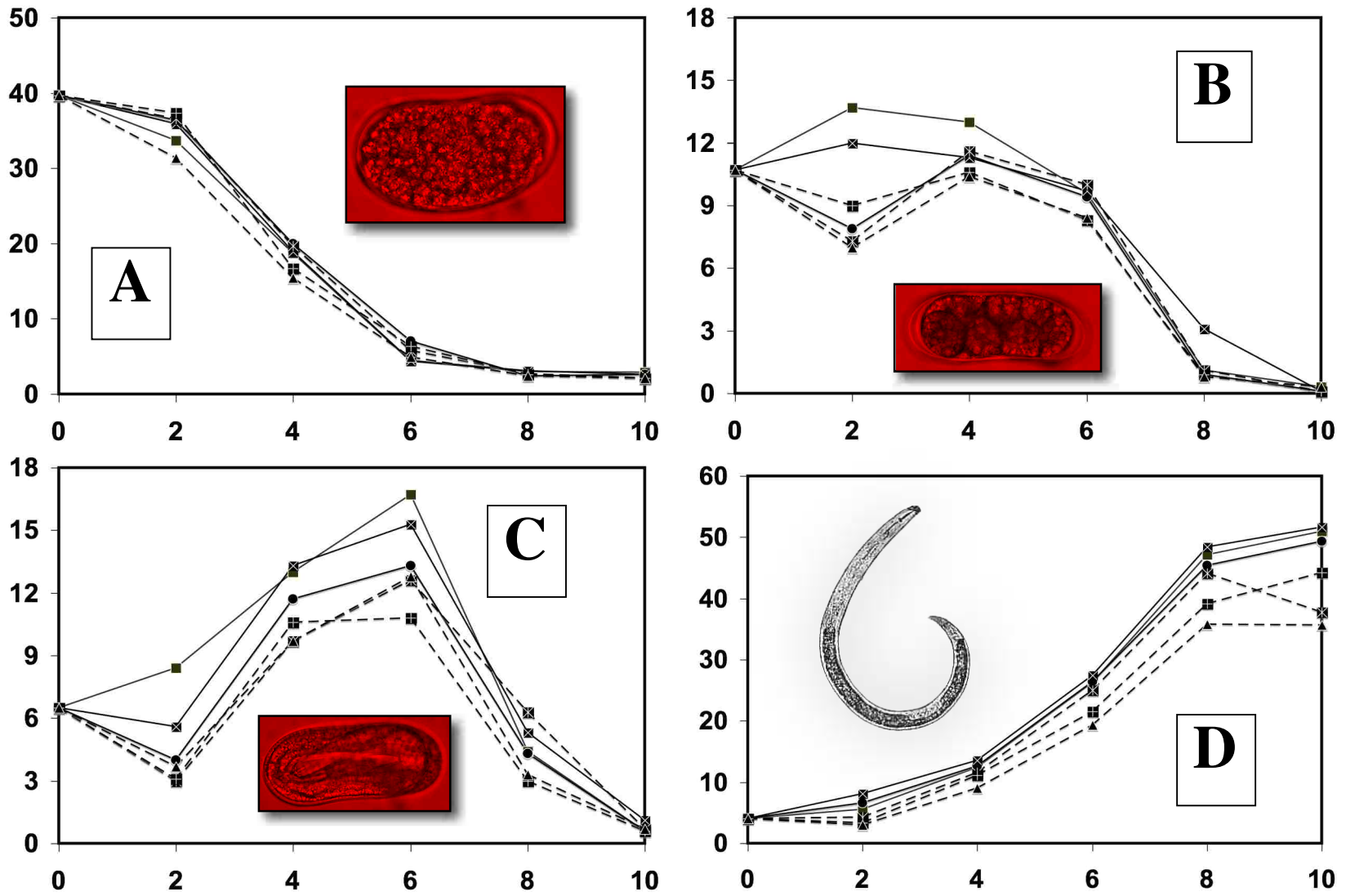


Figure 3.2 Influence of time and leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) that passed thru a  $0.45\mu\text{m}$  filter on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in Experiment 1. Data are means of eight replications averaged over two trials. Panel A is the numbers of eggs in the undifferentiated, granular stage of development; panel B is the numbers of eggs in the 4-8 cell stage of development; panel C is the numbers of eggs containing differentiated juveniles and panel D 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.

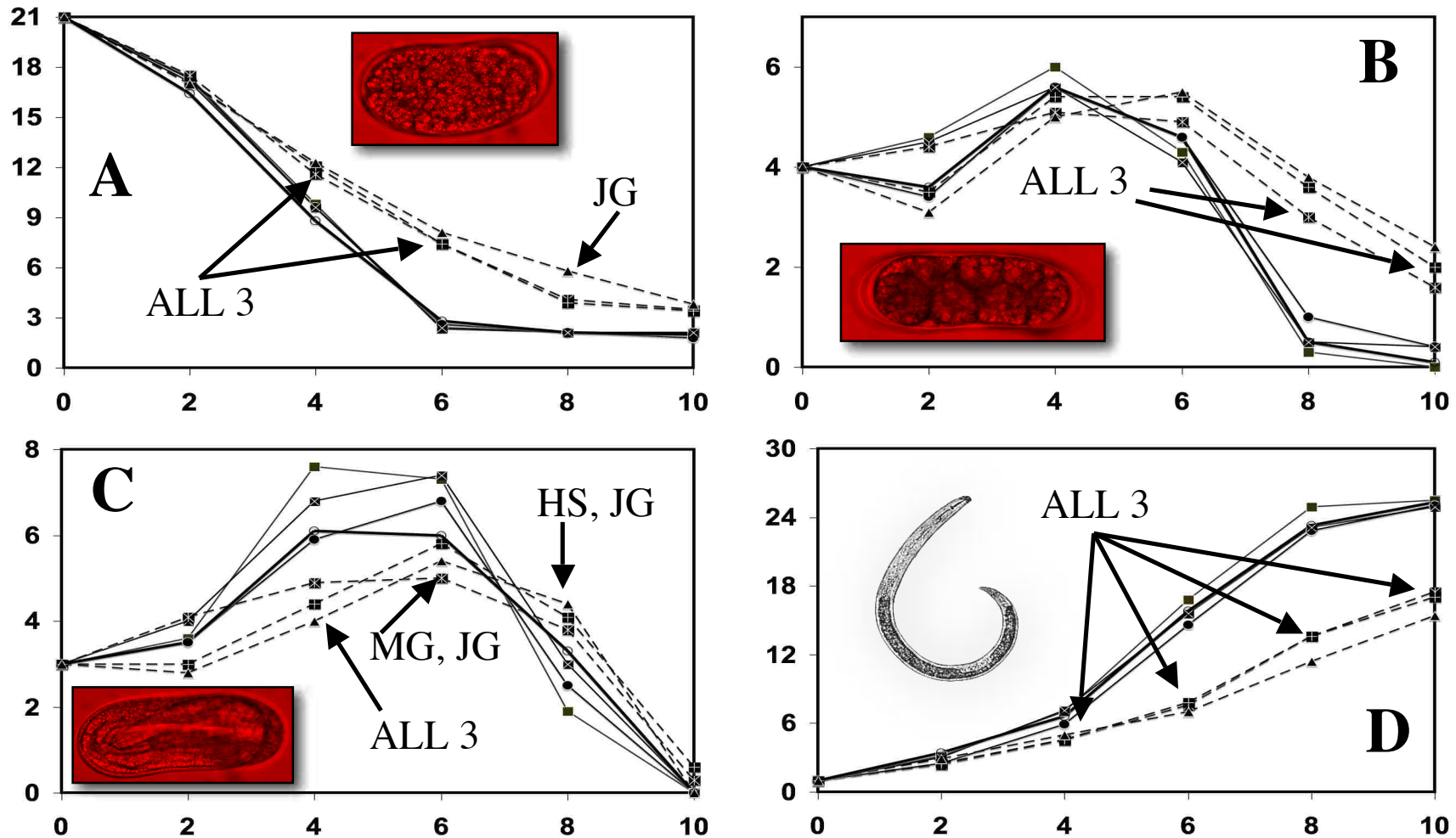


Figure 3.3 Influence of time and non-filtered leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in Experiment 2. Data are means of eight replications averaged over two trials. Panel A is the numbers of eggs in the undifferentiated, granular stage of development; panel B is the numbers of eggs in the 4-8 cell stage of development; panel C is the numbers of eggs containing differentiated juveniles and panel D 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 (○), was included in this experiment. Arrows indicate intervals at which data for weed leachates were significantly different than those of the distilled water 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 two days. Thereafter through Day 10, a greater number occurred in the control treatment (Figure 3.3D). Over the ten-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 3.3). The percentages of eggs that developed and hatched over the course of Experiment 2 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, six days, were there significant differences in the numbers of Category 1 eggs associated with control and weed root leachates that passed through the 0.80 $\mu$ m filter (Figure 3.4A). 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 (Figure 3.4B). On Day 4, only the leachate from morningglory resulted in egg counts that were less than those of the control. At the six-day interval, there was a difference in the numbers of Category 2 eggs counted for distilled water and soybean and perlite leachate controls. These differences reflected results of the first but not the second run of 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 all weed leachates remaining in Category 2 were greater than those of the control on Days 8 and 10.

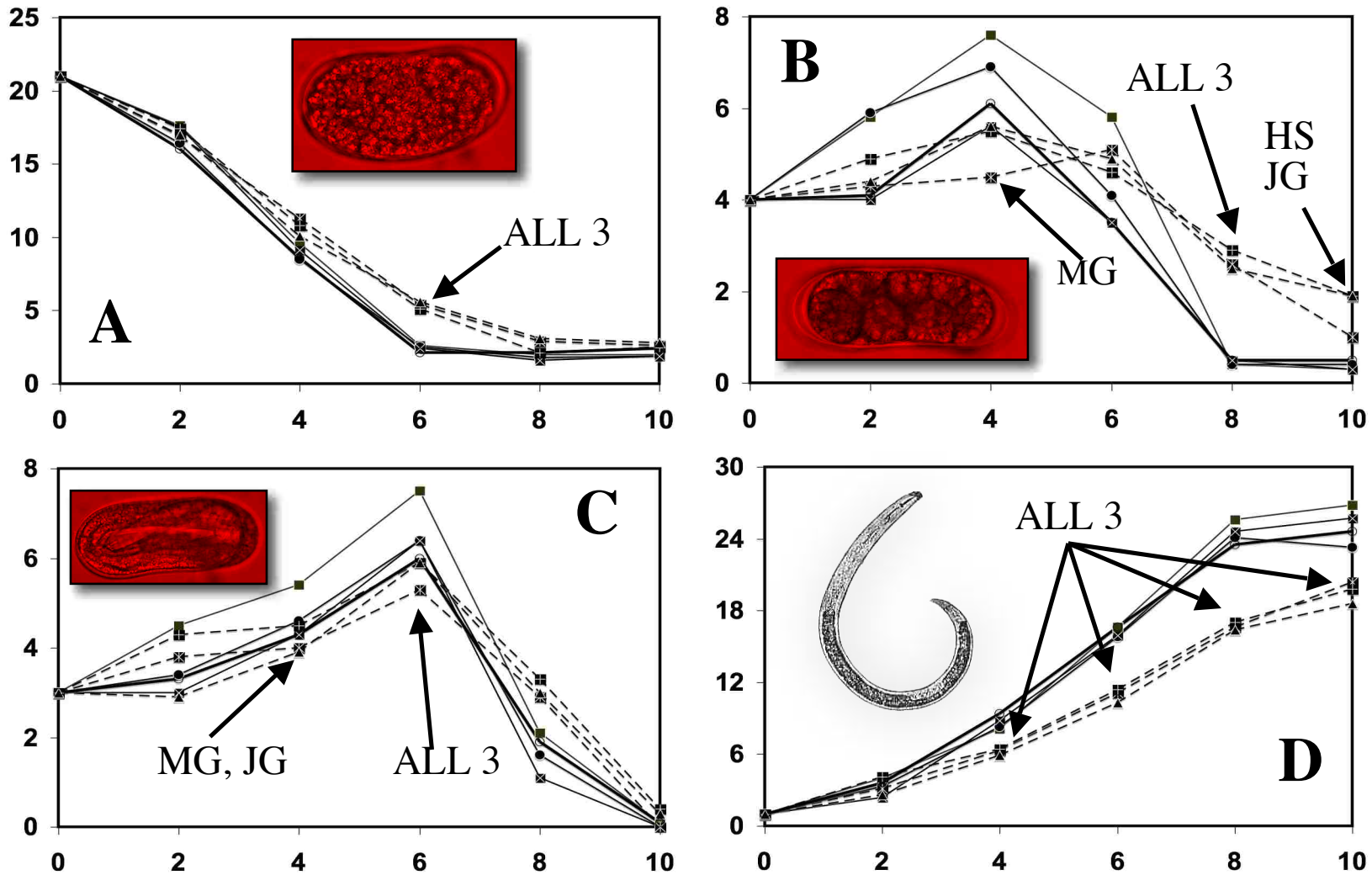


Figure 3.4 Influence of time and leachates from roots of morningglory (MG), hemp sesbania (HS) and johnsongrass (JG) that passed thru a  $0.80\mu\text{m}$  filter on eclosion and hatch of eggs of *Rotylenchulus reniformis* over 10 days in Experiment 2 Data are means of eight replications averaged over two trials. Panel A is the numbers of eggs in the undifferentiated, granular stage of development; panel B is the numbers of eggs in the 4-8 cell stage of development; panel C is the numbers of eggs containing differentiated juveniles and panel D 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.

Category 3 egg counts for control and weed leachates did not differ during the first 48 hours (Figure 3.4C). 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 eight and 10 no differences among the treatments were observed.

There were no differences among hatched juveniles, Category 4, on Day 2 but thereafter through Day 10, the numbers of juveniles were significantly less in weed leachates (Figure 3.4D). At the conclusion of Experiment 2, 95% of the eggs in the distilled water had developed into juveniles and hatched. By comparison, only 73, 71 and 66%, each a significant reduction in egg development and hatch, occurred with 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 all 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 greenhouse leachate tests with cotton reported previously (Pontif and McGawley, 2007; Nematropica, submitted).

Overall, the results of the greenhouse experiments with soybean were in agreement with the microplot experiments, in that reproduction of *R. reniformis* was 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 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 with different exudate concentrations different results would have been obtained.

The primary reason for the filtration of the root leachates in these 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 $\mu$ m 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 $\mu$ m filter pores and impeded their passage. The 0.80 $\mu$ m 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.

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* sp., 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 document the fact that some weed species do inhibit nematode reproduction, including that of *R. reniformis* (Ismail and Hasabo, 1995; Wang *et al.*, 2001).

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 nonhosts produce materials that repel or suppress the nematode. Good hosts produce materials which stimulate/enhance host-finding or reproduction by the nematode (Khan, 1985; Castro *et al.*, 1990). 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. 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 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 and are damaged by the nematode. 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 United States, 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. Results of experiments conducted in controlled greenhouse and laboratory environments do not always translate to success in large field production. Some population level of weed presence in the field, especially that which involves 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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## CHAPTER 4

### SUMMARY AND CONCLUSIONS

Although there are currently no commercial cotton cultivars available with resistance to reniform nematode, some genes that may confer resistance have been identified (Robinson, 2007). However, incorporation of these genes into commercial cultivars is proving to be a difficult task. Until a resistant cultivar is successfully produced, alternate control strategies will have to be employed. Although allelochemicals offer some management potential (Halbrendt, 1996; Ferris, *et al.*, 1992; Chitwood, 2002; Wang 2002; Dufour 2003; Kokalis-Burelle and Rodriguez-Kabana, 2006) they are short-lived in the soil, easily metabolized or hydrolyzed, and require that plants producing them remain actively growing and hence secreting them into the rhizosphere (Cheng, 1992).

The retention, transformation and transport of allelochemicals are influenced by soil physical and chemical conditions, microbial populations and environmental conditions (Cheng, 1992). Physical, microbiological and environmental factors contribute to the inconsistency of nematode control observed in research trials on crop rotation and cover crop systems, biofumigation and biochemical pesticides (Kokalis-Burelle and Rodriguez-Kabana, 2006). Greenhouse and microplot studies demonstrate that allelopathic rotation crops can suppress populations of plant –parasitic nematodes, but there are very few reports of successful application in commercial agriculture. In order for allelopathy to be a commercially viable option, the technique must be both economical and compatible with farming practices. At this time most known allelopathic plants do not fulfill these requirements. Common problems associated with the broad use of a rotation crop containing allelopathic properties include: plants

that are not adapted to the climate or soil, seeds that are too expensive or unavailable and the benefits of the allelopathic rotation crop is not cost effective compared to the use of nematicides. The use of allelopathic crop rotations will need to be adapted to meet the requirements for different cropping systems and nematode problems (Halbrendt, 1996). A good example of an allelopathic cover crop is *Crotalaria* spp., which produces alkaloids and monocrotaline that are both toxic to nematodes. *Crotalaria* spp. can be used as preplant cover crops, intercrops, or soil amendments. When used as cover crops, *Croatalria* spp. reduces plant-parasitic nematode populations by acting as a nonhost or poor host, producing allelochemicals that are toxic or inhibitory, providing a niche for antagonistic flora and fauna and trapping the nematode. *Crotalaria* spp. has the potential to be used to manage *R. reniformis*, but the residual effects are short term and the number of nematodes will resurge on subsequent host crops. Integrating other management strategies with *Crotalaria* could offer promising new management approaches (Anaya, 2006). While research continues to improve nonchemical, alternative approaches that will eventually become the management strategies of choice, short-term nematode control needs will continue to depend on synthetic chemical nematicides.

*Rotylenchulus reniformis* has several traits that serve it well as a plant parasitic nematode. The ability to thrive in many types of soil, to survive under adverse conditions, to produce extremely high populations, and to reproduce on a wide variety of crop and weed hosts makes *R. reniformis* a formidable pest of cotton and soybean. Weeds affect nematodes in a myriad of ways; they serve as alternate sources of sustenance, they protect from pesticides and the environment by supplying a biological “shelter in the storm,” they suppress reproduction through the production of allelopathic compounds and exert indirect effects through competition with crops. With the growing concern over environmentally incompatible nematicides, the need for

new and innovative management tactics to control nematodes and other serious agricultural pests has become a global priority.

Crop producers with nematode-related problems need to develop management strategies that include the responsible use of agrichemicals and the employment and exploitation of natural antagonisms such as predators, competition and allelochemicals, both complemented with physical measures, such as crop rotation and resistant varieties. The weed-nematode data developed in these studies shows clearly that some weeds and their metabolic products have nematostatic/nematicidal properties. These data are some of the first to focus attention on the “ever-present and much maligned” weed as a possible source of crop protection chemistry.

This research was conceptualized on the basis of field observations and included experiments conducted in microplot, greenhouse and laboratory environments. Morningglory, hemp sesbania and johnsongrass, three weeds endemic in cotton and soybean fields in the southern United States, have been shown to inhibit reproduction of *R. reniformis*, currently one of the most serious nematodes affecting plant agriculture. Over five years of microplot experiments with cotton and soybean, the co-culture of each of these weeds with either cotton or soybean resulted in reduced soil populations of *R. reniformis*. Determination of whether or not this reduced nematode reproduction resulted from allelochemicals produced by the weeds, competition between the crop and the weed or from a combination of these factors required advancement of the studies to a greenhouse environment. A series of preliminary and four major greenhouse-based experiments were conducted to test the hypothesis that the reductions in reniform populations observed in microplot studies resulted from root products leachable from the root systems of the three weeds.

Data from leachate trials in the greenhouse again demonstrated that reproduction of reniform nematode was suppressed by all three weeds with cotton and by johnsongrass with soybean. Additionally, these studies showed clearly that leachates from the weeds, in the absence of the weeds themselves, would suppress nematode reproduction. The next step in these investigations was to advance the study to a laboratory environment to determine which life stage or stages were affected by the leachates.

Studies with eggs of *R. reniformis* in the lab showed that the mechanism by which the leachates from the three weeds inhibit reproduction is by suppressing the hatch of juveniles from the eggs.

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## VITA

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