EFFECT OF BAHIAGRASS (*PASPALUM NOTATUM* FLUEGGE) ON NEMATODE POPULATIONS IN THE FIELD AND THEIR BEHAVIOR UNDER GREENHOUSE AND LABORATORY CONDITIONS

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ABSTRACT

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Field experiments were established to study the impact of cotton-bahiagrass-bahiagrass-peanut (CBBP) and peanut-cotton-cotton-peanut (PCCP) rotations on plant-parasitic nematode populations during 2003 to 2006 in north Florida, USA. The CBBP rotation reduced soil populations of *Meloidogyne incognita*, *Rotylenchulus reniformis*, and *Helicotylenchus dihystera*, but not *Mesocriconema ornatum* compared to the PCCP rotation in three of the four test years. Greenhouse pot experiments that consisted of amending field soil with bahiagrass root and leaf pieces reduced galling of tomato and reproduction of *Meloidogyne arenaria* when compared to non-amended soils. Under laboratory conditions, juveniles of *M. arenaria* actively moved to root zones of bahiagrass and to root pieces grown in water agar, but no feeding or root penetration was observed.

Key words: Cotton, crop rotation, diseases, management, *Meloidogyne arenaria*, *Meloidogyne incognita*, nematodes, peanut, tomato.

RESUMEN

Tsigbey, F. K., J. R. Rich, J. J. Marois, and D. L. Wright. 2009. Efecto del pasto *Paspalum notatum* Fluegge sobre las poblaciones de nematodos en el campo y su comportamiento en condiciones de invernadero y de laboratorio. Nematropica 39:111-119.

Se efectuaron experimentos de campo para estudiar el efecto de las rotaciones algodón-pasto-pasto-maní (CBBP) y maní-algodón-algodón-maní (PCCP) sobre las poblaciones de nematodos fitoparásitos de 2003 a 2006 en el norte de Florida, EEUU. La rotación CBBP redujo las poblaciones de *Meloidogyne incognita*, *Rotylenchulus reniformis* y *Helicotylenchus dihystera*, pero no las de *Mesocriconema ornatum* en comparación con la rotación PCCP, en tres de los cuatro años del estudio. En los experimentos de invernadero se observó que en suelo enmendado con pedazos de raíces y hojas del pasto se redujo el agallamiento en tomate y la reproducción de *Meloidogyne arenaria* comparados con suelo sin enmendar. En condiciones de laboratorio, los juveniles de *M. arenaria* se movieron activamente hacia las zonas radicales del pasto, pero no se observó alimentación ni penetración de las raíces.

Palabras clave: algodón, enfermedades, manejo, maní, *Meloidogyne arenaria*, *Meloidogyne incognita*, nematodos, rotación de cultivos, tomate.

INTRODUCTION

Peanut (*Arachis hypogaea* L.) and cotton (*Gossypium hirsutum* L.) are important crops in the United States and widely grown by farmers in the southern part of the country (Rodríguez-Kábana *et al.*, 1991). Approximately 30 to 50% of the input costs in peanut production in the southeastern United States are allocated to

managing weeds, insects, and diseases including those caused by nematodes (Garcia-Casellas, 2004). The current trend of escalating production costs exerts tremendous pressure on farmers to cut costs and adopt alternative but sustainable production methods. Crop rotation offers opportunities to reduce pest pressures, and additionally, to improve and sustain soil fertility.

Plant-parasitic nematodes are damaging to peanut and cause an estimated 12% annual loss in crop yield and quality (Sasser and Freckman, 1987; Koenning *et al.*, 1999). Sharma (1985) reported that several nematode species attack peanut but the most prevalent include *Meloidogyne arenaria* (Neal) Chitwood (root-knot nematode), *Pratylenchus brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven (lesion nematode), and *Belonolaimus longicaudatus* Rau (sting nematode). Sasser (1977) listed three root-knot nematode species that are damaging on peanut: *M. arenaria*, *M. javanica*, and *M. hapla*. Among these, *M. arenaria* (the dominant species in the United States) and *M. javanica* occur in warmer regions of the world while *M. hapla* occurs in cooler regions (Dickson and De Waele, 2005). Root-knot nematodes cause galling on peanut roots, pegs, and pods and severely infected plants have stunted growth (Rich and Kinloch, 2007).

Current options for nematode management on peanut and cotton are limited to crop rotation and nematicides (Rich and Kinloch, 2007). Among crops recommended in rotation with peanut and cotton is bahiagrass. This perennial grass is an excellent plant in rotation since it is a nonhost of root-knot nematodes, effectively suppressing populations below economic thresholds for subsequent crops such as peanut (Rodríguez-Kábana, 1994; Johnson *et al.*, 1999). Additionally, bahiagrass reduces other soil-borne diseases and improves nutrient recycling and soil structure (Katsvairo *et al.*, 2006). The mechanism of nematode suppression by bahiagrass is little understood but some authors have suggested release of toxic metabolites or increased presence of biological antagonists (Kloepper *et al.*, 1991; Widmer and Abawi, 2000; Chitwood, 2002; Wang *et al.*, 2003). The objectives of this research were to: 1) monitor soil nematode populations on peanut in bahiagrass and conventional rotations, 2) investigate the effects of soil incorporation of bahiagrass roots and leaves on the reproduction of *M. arenaria*, and 3) observe nematode behavior in response to the presence of agar-grown bahiagrass seedlings.

MATERIALS AND METHODS

Effect of rotation on field populations of nematodes

Field experiments were conducted at the North Florida Research and Education Center in Quincy, Florida from 2003 to 2006 on rotation plots that were first established in 2000 and consisted of a bahiagrass (B) rotation with peanut (P) and a conventional cotton-peanut (CP) rotation. The cropping sequence for the bahiagrass rotation was cotton in the first year followed by bahiagrass for two consecutive years and peanut in the fourth year (CBBP). The conventional rotation consisted of growing peanut in the first year, cotton for two years followed by peanut in the fourth year (PCCP). Management practices for peanut were conducted according to the Florida Cooperative Extension Service recommendations (Wright *et al.*, 2007). Individual plots measured 22.8 m in length by 18.4 m wide (20 peanut rows). The rotations were in a strip block design (Little and Hills, 1978), both within and across years with 4, 4, 6 and 8 replicates in 2003, 2004, 2005, and 2006, respectively, for the PCCP rota-

tion, whereas the CBBP rotation had 8, 10, 6, and 6 replications in the corresponding years.

The bahiagrass was killed in the fall of each year by applying glyphosate (Roundup WeatherMAX; Monsanto, Kansas City, MO). A winter oat (*Avena sativa* L.) cv. Florida 501 cover crop was planted at the seeding rate of 71 kg/ha and was killed 124, 97, 123, and 120 days after planting in 2003, 2004, 2005, and 2006, respectively, by broadcast spraying glyphosate. Seedbeds for peanut plantings were prepared using strip-tilling equipment (Kelly Mfg. Corporation, Tifton, GA). Georgia Green peanut cultivar was planted on 7 May 2003 and 10 May 2004 with a Monosem pneumatic planter (ATI, Inc. Lenexa, KS) at 6 seeds per 31 cm of row and 91-cm single-row spacing. Phorate (Thimet 20-G; Micro Flo Company LLC. Memphis, TN) at 6 kg ha^{-1} was applied in furrow at planting. AP3 peanut variety was planted on 13 May 2005 and 17 May 2006 with a Monosem pneumatic Twin Row Planter (ATI, Inc. Lenexa, KS) at 3 seeds per 31 cm of row on twin rows with simultaneous application of phorate. Plant-parasitic nematode population densities were monitored at peanut harvest in October of each year by randomly collecting 10 soil cores (2.5-cm-diam) to 20 cm deep and in-row from each plot. The 10 soil cores were combined, mixed well, and nematodes were extracted from a 100 cm^3 sub-sample from each plot by centrifugal flotation (Jenkins, 1964). Nematodes were counted under a stereo-microscope using a 2 mm gridded (60 × 15 mm) Petri dish Corning® (Corning, New York). Identification of nematodes to species was done at the Florida Department of Agriculture Division of Plant Industry using identification keys according to Cobb (1893), Raski (1958), Loof and De Grisse (1989), and Robinson *et al.* (1997).

Effect of bahiagrass on populations of Meloidogyne arenaria race 1 inter-planted with tomato

The effect of bahiagrass root and leaf cuttings on survival of *M. arenaria* race 1 was investigated in the greenhouse using tomato (*Solanum lycopersicum* L.) cv. Rutgers. Field soil (fine loamy siliceous, thermic Plinthic Kandiudult) was collected and air-dried for one month under greenhouse conditions before experiment initiation. The soil was spread thinly (2 cm thick) on white polyethylene with periodic mixing to allow drying by solarization. The dry soil was sieved through a 7-mm-diam mesh screen to remove clods and large organic debris. Pot culture assays consisting of bahiagrass roots and leaves cuttings were conducted in five 16-cm-diam polyethylene replicate pots filled with 1.2 kg of the dry field soil for each treatment. Bahiagrass leaves and roots obtained from growing plants in the field were cut into 2-cm-long pieces and dried for 30 days on a greenhouse bench. These were mixed in different ratios to constitute 2% organic matter content (w:w).

Treatment and amendment ratios for leaves (L) and roots (R) were added to individual pots as follows: L0R0 = Only nematode egg inoculation, no leaves or roots, L12R12 = 1:1 Leaves to roots (12 g of leaves and 12 g of roots), L8R16 = 1:2 Leaves to roots (8 g of leaves and 16 g of roots), L16R8 = 2:1 Leaves to roots (16 g of leaves to 8 g of roots), L0R24 = Roots only (24 g dry roots), and L24R0 = Leaves only (24 g dry leaves). After thoroughly mixing amendments into soil, 500 ml of water was added to the amended soil and allowed to drain for 24 hrs before addition of nematode eggs. Eggs of *M. arenaria* obtained from the University of Florida Nematology Department were extracted from galled roots of tomato cv. Rutgers using NaOCl (Hussey and Barker, 1973). Three holes (1-

cm-diam × 2-cm-deep) were created in the potted soil using a spatula. Egg suspensions of *M. arenaria* (10 000 eggs per pot) were deposited into the holes and then covered with soil. Pots were maintained for 10 days for residue decomposition and egg hatch before three-week-old Rutgers tomato seedlings were transplanted. Pots were placed on a green house bench in a completely randomized design. During the experiments, temperatures in the greenhouse ranged between 17-36°C and average day length was 12-14 hrs. Tomato plants were watered as necessary and fertilized weekly with Peters Special fertilizer (20-20-20) for two months prior to experiment termination. Plant roots were removed from each pot and washed with tap water. The plant roots were rated for root-knot galling using the 0-10 scale where, 0 = no galling, and 10 = 100% root galling (Zeck, 1971). Nematode eggs on the roots were extracted using the NaOCl method described above and counted. Shoot and root weights were determined after being allowed to air-dry at 24 ° C under laboratory conditions to remove excess water. The experiment was conducted three times.

Juvenile nematode movement on agar-grown bahiagrass and tomato seedlings

Seeds of bahiagrass cultivars Pensacola, Paraguay, and Argentine, and the tomato cv. Rutgers were surface-sterilized twice in 100% NaOCl for 10 minutes and rinsed in four changes of sterile distilled water. Fifty seeds were then air-dried in a laminar flow chamber in the laboratory under sterile conditions and later plated on 0.6% water agar (3 g agar in 500 ml de-ionized water) and incubated in Petri dishes (150 × 20 mm) at 24°C for two weeks. The plates were each inoculated with approximately 50 eggs of *M. arenaria* suspended in sterile distilled water by placing them 10 cm from bahiagrass and tomato seedling roots. Unequal number of plates were obtained (7 for Rutgers, 11 for Pensacola, 10 for Paraguay, and 10 for Argentine varieties) as a result of the removal of plates found to be contaminated by other micro-organisms. Similarly, ten 2-cm-long root pieces containing root tips of two-month-old bahiagrass seedlings grown on agar were excised and placed horizontally into holes that were dug in the agar medium in six Petri dishes, and the root pieces covered with agar. Egg suspensions of *M. arenaria* were deposited 10 cm from each root piece as described above and incubated at 24°C in the laboratory. Five days after inoculation, and thereafter weekly, roots pieces of both bahiagrass and tomato were decolorized in NaOCl and later stained in red food dye (Thies *et al.*, 2002) to detect any penetration by root-knot nematode second-stage juveniles (J2). The number of J2 that migrated to living and excised roots was counted after two weeks under a stereoscopic microscope at x40 magnification and their behavior along roots for an additional 4 weeks was observed. The experiment was terminated after three months.

Data analyses

Field data of soil nematode populations were log transformed and analyzed in SAS version 9.1 using PROC GLM (SAS, Inc., Cary, NC) system. Means separation was conducted using Fisher's least significant difference at $P \leq 0.05$. The greenhouse data were transformed to log 10 for numbers greater than 100 before analyses using SAS PROC GLM (SAS, Inc., Cary, NC). Means separation for the greenhouse data were conducted using Tukey's Studentized (HSD) test at $P \leq 0.05$. The laboratory data studying nematode movement was analyzed using PROC GLM (SAS, Inc., Cary,

NC). Means separation was conducted using Fisher's least significant difference at $P \leq 0.05$.

RESULTS

Soil population densities of ring (*Mesocriconema ornatum*), spiral (*Helicotylenchus dihystera*), reniform (*Rotylenchulus reniformis*), and root-knot nematode (*Meloidogyne incognita* race 3) in the rotations varied from year to year. Populations of spiral, reniform, and root-knot nematodes remained consistently greater in the PCCP than in CBBP rotation soils throughout the four years (Table 1). Populations of ring nematodes were significantly ($P \leq 0.05$) greater in the bahiagrass (CBBP) rotation than in the conventional (PCCP) in years 2003 and 2005 but not 2004 and 2006. Across the four years (2003-2006), populations of ring nematodes were greater following bahiagrass than cotton in the conventional rotation. Across the four years, reniform, spiral, and root-knot nematodes were lower in the bahiagrass rotation than in the conventional rotation.

Incorporation of bahiagrass plant parts significantly affected ($P \leq 0.05$) tomato shoot weight, galling index, mean number of nematode eggs per root and per gram of root but not root weight (Table 2). Tomato plants amended with 16 g leaf + 8 g root (L16R8) had the highest shoot weight

Table 1. Effect of rotation on soil nematode populations in peanut during 2003-2006.

		Nematode population/100 cm^3 soil[z]			
Year/ Peanut Variety	Rotation[y]	Ring	Spiral	Reniform	RKN
2003/ Georgia Green	CBBP	239.0	18.0	8.0	3.0
	PCCP	83.0	32.0	122.0	20.0
	LSD ($P \leq 0.05$)	99.8	20.4	66.9	13.0
2004/ Georgia Green	CBBP	85.0	8.0	23.0	23.0
	PCCP	190.0	30.0	343.0	26.0
	LSD ($P \leq 0.05$)	120.3	26.7	252.2	8.5
2005/ AP3	CBBP	180.0	13.0	6.0	5.0
	PCCP	81.0	34.0	97.0	37.0
	LSD ($P \leq 0.05$)	78.7	22.1	38.6	11.8
2006/ AP3	CBBP	81.0	34.0	97.0	37.0
	PCCP	81.0	36.0	538.0	40.0
	LSD ($P \leq 0.05$)	86.6	22.6	248.9	11.5
2003-2006	CBBP	188.0	17.0	15.0	11.0
	PCCP	82.0	33.0	275.0	31.0
	LSD ($P \leq 0.05$)	45.2	10.3	103.9	8.2

[y]Yearly rotation sequences were bahiagrass (B), cotton (C), and peanut (P).
[z]Ring nematode (*Mesocriconema ornatum*), Spiral nematode (*Helicotylenchus dihystera*) Reniform nematode (*Rotylenchulus reniformis*), and RKN (*Meloidogyne incognita*).
Means separation was conducted using Fisher's least significant difference ($P \leq 0.05$)

Table 2. The influence of bahiagrass residues on growth and root galling of tomato plants infected with *Meloidogyne arenaria*[x].

	Fresh weight (g)			Number of *M. arenaria* eggs	
Treatment[y]	Shoot	Root	Galling index[z]	Total per root	Per gram root
L0R0	48.2	28.4	6.7	465 504	16 391
L12R12	59.5	32.0	4.1	208 192	6 505
L8R16	48.1	28.7	4.5	140 744	4 904
L16R8	67.5	27.6	3.9	148 985	5 398
L0R24	54.7	31.4	4.5	216 629	6 899
L24R0	59.0	32.0	4.2	137 696	4 303
HSD (P 0.05)	14.1	8.3	1.6	143 794	5 404

[x]Each value in table represents a mean of 15 observations over three experiments.
[y]Treatment represents the proportion of bahiagrass leaf and root ratios: L0R0 = Nematode egg inoculation with no bahiagrass amendment, L12R12 = Leaves to roots (12 g of leaves and 12 g of roots), L8R16 = 1:2 Leaves to roots (8 g of leaves and 16 g of roots), L16R8 = 2:1 Leaves to roots (16 g of leaves to 8 g of roots), L0R24 = 2% dry cut root (24 g dry root), and L24R0 = 2% organic matter of dry cut bahiagrass leaves (24 g dry cut leaves). Treatments were replicated 5 times and soil in each replicate pots were infested with 10 000 eggs of *M. arenaria*.
[z]Root gall index was scored on a scale of 0-10 where 0 = no galling, 10 = 100% root galling.

(67.5 g) but lowest root weight (27.6 g), whereas those planted into pots amended with 8 g leaf + 16 g root (L8R16) showed lowest shoot weight. Plants grown in soil not amended with bahiagrass cuttings (L0R0) had a significantly greater galling index than those amended with cuttings. Total number of eggs per root system and per gram of root was significantly greater for plants grown in un-amended soil than in amended soil regardless of bahiagrass plant part proportions. No significant differences in egg production on tomato were observed from incorporating differing ratios of bahiagrass leaves and roots.

In the tests utilizing water agar, *M. arenaria* J2 were observed moving on the surface of the agar within three days after adding the egg suspension. The number of J2 that moved to the root zones of tomato, bahiagrass root pieces and seedlings on agar were significantly different ($P \leq 0.05$) (Table 3). Bahiagrass cv. Pensacola had the highest numbers of J2 (16) followed by tomato (12), and cv. Paraguay the least numbers of J2 (4). Juveniles moved on average 3.7 cm towards living roots of tomato, 2.5, 3.1, and 2.6 cm towards bahiagrass cvs. Argentine, Paraguay, and Pensacola, respectively. The maximum distance moved (6.3 cm) by juveniles was recorded on cut bahiagrass root pieces that were embedded in agar and was significantly greater than all other distances travelled in media (Table 3). Observations over 6 weeks showed that the J2-infected tomato roots were galled. No galls were observed on bahiagrass seedlings but live J2 were present and moving in the root zones.

DISCUSSION

Plant-parasitic nematode population densities were lower in the bahiagrass (CBBP) than the conventional (PCCP)

Table 3. Effect of bahiagrass and tomato roots on motility of *Meloidogyne arenaria* juveniles (J2) after two weeks on water agar.

Plant - cultivar	Mean number of juveniles on roots	Ave distance moved by juveniles to roots (cm)
Bahiagrass		
Argentine	9	2.5
Paraguay	4	3.1
Pensacola	16	2.6
Tomato		
Rutgers	12	3.7
Bahiagrass root pieces[y]	7	6.3
LSD ($P \leq 0.05$)	1.3	0.22

[y]Ten bahiagrass root pieces (2 cm) were assayed regardless of the variety.
Means separation was conducted using Fisher's least significant difference ($P \leq 0.05$).

rotation, particularly those of *M. incognita* race 3. Previous studies have demonstrated population reductions of both *M. incognita* and *M. arenaria* after bahiagrass rotation (Rodríguez-Kábana *et al.*, 1994; Johnson et al., 1999; Sumner *et al.*, 1999). Mechanisms of *Meloidogyne* population reduction under a bahiagrass rotation were attributed to the non-host status of bahiagrass and the possible stimulation of nematode antagonists such as *Pasteuria penetrans* (Timper *et al.*, 2001). The bahiagrass rotation did not suppress the ring (*M. ornatum*) nematode populations, although it has previously been used to suppress populations of ring nematode in young peach orchards (Nyczepir and Bertrand, 2000). Similarly, Zehr *et al.* (1990) reported that bahiagrass did not support *M. xenoplax* population under greenhouse conditions when seedlings were inoculated with the nematode. The high population of ring nematode could not be explained from present data; however, this nematode is not known to be a problem in cotton or peanut production in Florida USA (Rich and Kinloch, 2005; 2007).

Incorporation of plant material into soil has been reported to be successful in suppressing nematode populations (Wang, 2000). Soil amendment with either fresh or decomposed plant material alters the soil physical, chemical, and biological equilibrium and increases the diversity of microbial populations which may enhance nematode suppression. Reproduction of *M. incognita* was inhibited in soils planted with digitgrass (*Digitaria eriantha syn. decumbens*) (Haroon and Smart, 1983). Similarly, chopped leaves of brassicas have been reported to successfully lower *M. javanica* numbers when incorporated into soils (Akhtar and Malik, 2000; McLeod and Steel, 1999). The incorporation of bahiagrass residues into soil in this study successfully reduced *M. arenaria* reproduction on tomato. Both roots and leaves of bahiagrass were equally effective in reducing egg production regardless of plant part proportion. However, the mechanism of nematode suppression when using bahiagrass amendments was not investigated in these studies: however other mechanisms impli-

cated in previous studies could have been involved including release of volatile compounds and encouragement of antagonists (Rodríguez-Kábana *et al.*, 1994; Widmer and Abawi, 2000; Wang *et al.*, 2003).

The non-host status of bahiagrass to *M. arenaria* and other nematode species has been well documented (Dickson and Hewlett, 1989; Rodríguez-Kábana *et al.*, 1988). However, the behavior of *M. arenaria* J2 around the root zones of bahiagrass has not been studied. In the present study, J2 of *M. arenaria* actively moved on both living and excised bahiagrass roots in water agar. Since no feeding or root penetration was observed when J2 moved near the roots, these results confirm previous studies of the non-host status of bahiagrass to *M. arenaria*. The movement of *M. arenaria* J2 to cut root pieces in media in large numbers is an indication that exudates from bahiagrass roots may be acting as an attractant to nematodes. Thus, root exudates may act to reduce nematode populations in soils by trapping J2 in root zones where they cannot feed and therefore may die. Our data suggests that nematode stimuli in finding the bahiagrass roots may be different than the response to entering roots and feeding.

Data from these tests indicated that bahiagrass rotations (CBBP) reduced populations of spiral (*H. dihystera*), reniform (*R. reniformis*), and root-knot nematode (*M. incognita*), but not ring (*M. ornatum*) nematodes when compared to the conventional PCCP rotation. These data also suggests that incorporation of bahiagrass plant parts into soil inhibited egg production of *M. arenaria,* and reduced root galling under greenhouse conditions. Bahiagrass root exudates may also actively attract root-knot nematode J2 but in the absence of feeding and root penetration they die.

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