

# RESEARCH/INVESTIGACIÓN

## INFLUENCE OF SOIL NUTRIENTS ON REPRODUCTION AND PATHOGENICITY OF *ROTYLENCHULUS RENIFORMIS* ON COTTON

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### ABSTRACT

Kularathna, M. T., C. Overstreet, E. C. McGawley, D. M. Xavier, and C. M. Martin. 2014. Influence of soil nutrients on reproduction and pathogenicity of *Rotylenchulus reniformis* on cotton. *Nematropica* 44:15-24.

Greenhouse and field studies were conducted to evaluate the effect of soil nutrients on reniform nematode (*Rotylenchulus reniformis*) reproduction and pathogenicity on cotton (*Gossypium hirsutum*). Initial greenhouse studies examined phosphorus (P) and potassium (K) at very low (10 or 44 mg kg<sup>-1</sup>) and high (50 or 123 mg kg<sup>-1</sup>) levels, respectively. Phosphorus produced significant increases in plant height and shoot and root dry weights as well as significant reductions in numbers of nematodes in soil and eggs from roots. Subsequent greenhouse studies evaluated increasing levels of P (10, 20, 35, 60, and 73 mg kg<sup>-1</sup>), K (44, 70, 106, 123, and 153 mg kg<sup>-1</sup>), and sulfur (S) at 3, 12, 20, 40, and 50 mg kg<sup>-1</sup> on cotton growth and nematode reproduction. Phosphorus significantly increased plant height at 15 and 30 d and shoot and root weights at 60 d. Potassium and S had no effect on plant growth with the exception of the highest level of S, which significantly reduced plant height and shoot dry weights. Overall, as P level increased, reproduction of the reniform nematode decreased. Potassium and S, irrespective of level, had no effect on densities of eggs or soil stages of the nematode. Field trials with cotton included combinations of P at 44.8 or 112 kg ha<sup>-1</sup> and S at 5.6 or 22.4 kg ha<sup>-1</sup> with or without 1, 3-dichloropropene at 28.1 L ha<sup>-1</sup>. Nematicide application significantly reduced nematode population density at mid-season and harvest in 2011 and at planting in 2012. In both 2011 and 2012, management of soil nutrients did not significantly influence nematode reproduction. In both years, seed cotton yield was significantly increased with nematicide, but not with supplemental nutrients.

*Key words:* cotton, *Gossypium hirsutum*, pathogenicity, phosphorus, potassium, reniform nematode, reproduction, *Rotylenchulus reniformis*, sulfur.

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### RESUMEN

Kularathna, M. T., C. Overstreet, E. C. McGawley, D. M. Xavier, and C. M. Martin. 2014. Influencia de los nutrientes del suelo sobre la reproducción y patogenicidad de *Rotylenchulus reniformis* en algodón. *Nematropica* 44:15-24.

Se llevaron a cabo estudios en invernadero y campo con el fin de evaluar el efecto de los nutrientes del suelo sobre la reproducción del nemátodo reniforme (*Rotylenchulus reniformis*) y su patogenicidad en algodón (*Gossypium hirsutum*). Los estudios iniciales en invernadero examinaron fósforo (P) y potasio (K) a niveles muy bajos (10 o 44 mg kg<sup>-1</sup>) y altos (50 o 123 mg kg<sup>-1</sup>), respectivamente. El fósforo causó incrementos significativos en la altura de la planta y en los pesos secos de la parte aérea y raíces, así como reducciones significativas en el número de nematodos en suelo y de huevos en las raíces. Estudios posteriores en invernadero evaluaron el efecto de niveles crecientes de P (10, 20, 35, 60, y 73 mg kg<sup>-1</sup>), K (44, 70, 106, 123, y 153 mg kg<sup>-1</sup>), y azufre (S) a 3, 12, 20, 40, y 50 mg kg<sup>-1</sup> sobre el crecimiento del algodón y la reproducción del nemátodo. El fósforo incrementó significativamente la altura de la planta a los 15 y 30 días y el peso de las raíces a los 60 días. El potasio y el azufre no tuvieron efecto sobre el crecimiento vegetal, con la excepción del nivel más alto de S, el cual redujo significativamente la altura de la planta y el peso seco de la parte aérea. En general, la reproducción del nemátodo reniforme se redujo a medida que el nivel de P se incrementaba. Potasio y S, no mostraron efecto sobre las densidades de huevos o estadios en suelo del nemátodo, a ninguno de los niveles. Los ensayos en campo incluyeron combinaciones de P a 44.8 o 112 kg ha<sup>-1</sup> y S a 5.6 o 22.4 kg ha<sup>-1</sup> con o sin 1, 3-dicloropropeno a 28.1 L ha<sup>-1</sup>. La aplicación del nematicida redujo significativamente la densidad de población a mitad del ciclo de cultivo y en la cosecha en 2011 y en la plantación en 2012. En 2011 y 2012, el manejo de los nutrientes del suelo no influyó significativamente en la reproducción del nemátodo. En ambos años, la producción de semillas de algodón se incrementó significativamente con el nematicida, pero no con los nutrientes suplementarios.

*Palabras clave:* Algodón, azufre, fósforo, *Gossypium hirsutum*, nemátodo, patogenicidad, potasio, reniforme, reproducción, *Rotylenchulus reniformis*.

## INTRODUCTION

Cotton (*Gossypium hirsutum*) is known as nature's wonder fiber. It is grown in 17 southern states of the U.S. from Virginia to California (Anonymous, 2013). In 2012, acreage planted to cotton in the U.S. was estimated to be 5 million hectares. Louisiana supplies about 2.5% of the total cotton produced and ranks eleventh among the U.S. cotton-producing states.

Nematodes play a major role in reducing cotton yield, fiber quality, and earliness. Across the Cotton Belt, annual yield losses due to nematodes exceed \$400 million USD (Bagwell *et al.*, 2006). Root-knot and reniform nematodes are responsible for the highest percentages of damage in the mid-South and southeastern U.S. (Overstreet *et al.*, 2010).

At present, reniform nematode has a wide distribution in the cotton-producing area of the southern U.S. and can be found as far west as Texas (Bagwell *et al.*, 2006). During the past 15 to 20 years, reniform nematode has become the dominant nematode species in a number of states, including Louisiana (Overstreet and McGawley, 1998; 2000; Gazaway, 2005; Overstreet, 2006). Damage to cotton can be severe, resulting in dramatic yield reductions. Estimates from 2001-2005 showed that reniform alone caused about \$839.2 million in yield loss (Bagwell *et al.*, 2006). Reniform nematodes cause yield reductions due to induced nutritional deficiencies, fruit abortion, and abnormal crop maturation (Koenning *et al.*, 2004). According to Birchfield (1962), poor stands in some cotton fields in Louisiana were due to high population densities of *R. reniformis*. Over the past several decades, the reniform nematode has become much more widely distributed and losses have increased dramatically in Louisiana. A survey during 1994-1995 showed that reniform nematodes have spread widely through the state and estimated acreage infested was about 510,000 (Overstreet and McGawley, 1996). In 2012, there was a 4% loss in cotton yield in Louisiana due to reniform nematode (Blasingame *et al.*, 2013).

The two most commonly employed tactics for management of reniform and other cotton-associated nematodes are crop rotation and nematicide usage. Other methods of management include the use of resistant varieties, various tillage practices, and in recent times, the use of biological control agents and precision agriculture (Koenning *et al.*, 2004).

The maintenance of adequate soil fertility is essential for an economical yield of cotton. The amount of nutrients available to the plant also plays a major role in overall plant health. Nutrient deficiencies or excesses increase plant vulnerability to diseases and insects and ultimately result in reduced yields (Albers *et al.*, 1993; Knowles *et al.*, 1999).

Adjustment of soil fertility is an aspect of nematode management that scientists are currently investigating. According to the results of Berankova and Saly (1980) in their 3-yr study, mineral fertilizer

has a considerable negative impact on nematode populations. Gruzdeva *et al.* (2007) found that there was a strong correlation between specific nutrients including nitrogen (N), phosphorus (P), and potassium (K) or their combinations with nematode population decline. Rodriguez-Kabana and King (1980) found that adding urea together with molasses was effective for reducing *Meloidogyne arenaria* population density in squash (*Cucurbita* spp). A similar study conducted by Melakeberhan (1999) found that soybeans perform better against cyst nematode (*Heterodera glycines*) when there was a balanced supply of nutrients.

Behm *et al.* (1995) found that the micronutrient zinc had an effect on the hatching of eggs of *H. glycines* in corn. Similarly, macronutrients, such as P, were associated with reduced penetration of roots of sugar beets by juveniles of *H. schachtii* (Bell, 1996). A study conducted on the nutrient status of guava showed that shoot symptoms induced by *M. mayaguensis* were associated with a decrease in soil fertility (Gomes *et al.*, 2008). Ahmad and Siddiqui (2009) also found that N-P-K fertilizer was related to the suppression of *M. incognita* populations associated with tomato. Wolcott *et al.* (2008) showed that high levels of P and Zn nutrients through the soil profile were as effective as 1,3-dichloropropene (Telone II®) in managing both reniform and root-knot nematodes and increasing yields of cotton lint.

The effects of fertilizers on nematode pathology are not fully understood. Some reports suggest that fertilizer incorporation into soil makes crops more susceptible to nematode damage (Agu, 2003; Mahmood *et al.*, 2011). Conversely, there are reports that fertility has no effect on nematode damage to plants (Luc *et al.*, 2007; Ebelhar *et al.*, 2011). Investigations of the influence of soil nutrients on nematode biology and pathology will be an additional step toward the development of effective site-specific nematode management.

The objectives of this research were: i) to evaluate the effects of P, K, and sulfur (S) nutrients on reniform nematode reproduction and pathogenicity on cotton and ii) to evaluate the interactions of P and S nutrients with 1,3-dichloropropene under field conditions on cotton.

## MATERIALS AND METHODS

### *Isolates of Reniform Nematode*

For the first greenhouse study, reniform nematodes were extracted from soil samples obtained from the Northeast Research Station in St. Joseph, LA, and propagated on tomato (cv. Rutgers PS; Seedway, Hall, NY 14463). Hereafter, this population is referred to as the St. Joseph isolate. After 30 d, egg masses were removed from tomato root systems to establish axenic cultures that were maintained in the LSU nematology greenhouse on tomato. For the second, third, and

fourth greenhouse studies, an isolate of reniform nematodes from Rapides Parish was provided by E.C. McGawley from axenic cultures maintained in the greenhouse on tomato. Both isolates have been confirmed as *R. reniformis* by McGawley *et al.* (2010) and Robinson *et al.* (1997).

### General Information

Four greenhouse experiments and one field trial, each repeated once, were conducted during the course of this research. Terracotta pots with an inside top diameter of 10.2 cm were used in greenhouse experiment one, and pots with a top diameter of 15.0 cm were used in greenhouse experiments two, three, and four. Pots in experiment one held 0.5 kg of soil, and those in experiments two, three, and four held 1.6 kg of soil. All soils used in these experiments were steam sterilized for 8 hr at 116°C. Soil mixtures used in greenhouse studies were three parts sterilized Commerce silt loam (fine-silty, mixed, superactive, nonacid, thermic Fluvaqueptic Endoaquepts) collected from the LSU Agricultural Center, Northeast Research Station in St. Joseph, LA, and one part steam-sterilized sand, yielding a final mixture that was 70.1% sand, 25.4% silt, and 2.5% clay, referred to hereafter as “native soil.” Treatments in greenhouse experiments were replicated five times, and those in field experiments were replicated six times.

Inoculum for greenhouse experiments consisted of juveniles, pre-adult females, and males extracted from greenhouse cultures by wet sieving through nested 850- $\mu$ m-pore and 38- $\mu$ m-pore sieves, followed by sugar flotation and centrifugation (Jenkins, 1964). In greenhouse studies, soil was infested with nematodes by pipetting aqueous suspensions of vermiform individuals of *R. reniformis* into a series of depressions arranged into a triangular pattern in soil, 0.5-cm diam.  $\times$  5- to 7.5-cm deep, surrounding a single 10-d-old Stoneville LA887 cotton seedling. Texture of soils was analyzed according to the hydrometer method modified by Day (1965) and the American Society for Testing and Materials (1985). Prior to onset of each greenhouse experiment, soil nutrient levels were determined by the LSU AgCenter Soil Testing and Plant Analysis Laboratory (STPAL) and adjusted according to individual treatment objectives of each experiment. Soil nutrient amendments established for these studies involved multiple levels of P, K, and S. Milligram or kilogram nutrient rates referred to in all trials are levels per kg or ha, respectively. Water-soluble ammonium nitrate (33% N, 45 mg of soil) was used as the N source for greenhouse studies and was applied at 12-d intervals during the study periods. Tensiometers (Irrometer moisture indicator, Irrometer Company, Riverside, CA) were placed into pots to measure the water potential, and plants were watered when the potential fell between 40-50 kPa. Plant heights were measured at 15-d intervals in all

greenhouse studies. Greenhouse experiments were terminated after 60 d, and plant height and dry shoot and root weights were determined after drying at 45°C for 2 d.

Samples of soil for analysis of nematode population densities were collected from greenhouse trials at 60 d. Those for field trials were collected at planting, at mid-season, and at harvest. For both types of trials, 500-g soil samples were processed using the semi-automatic elutriation (Byrd *et al.*, 1976) and sugar flotation and centrifugation (Jenkins, 1964) techniques. Reniform life stages were counted at 40 $\times$  using an inverted microscope and total population densities per pot (Pf) and rate of reproduction (R, where R equals final population divided by inoculum level) were determined. For greenhouse experiments, nematode eggs were extracted from the entire root system by stirring in 0.6% NaOCl for 10 min (Hussey and Barker, 1973) and staining with 1.5 ml of acid fuchsin stain prior to counting.

### Preliminary Phosphorus and Potassium Greenhouse Study

This preliminary experiment was initiated to evaluate the influence of four combinations of P and K on reniform nematode pathogenicity and reproduction on Stoneville LA 887 cotton. Low (10 or 44 mg) or high (50 or 123 mg) levels of the nutrients P and K, respectively, were used. Nutrient combinations evaluated included: low P and high K, low P and low K, high P and low K, and high P and high K. Nematode levels were either 0 or 3,000 vermiform life stages per pot. The trial was a 2 $\times$ 2 $\times$ 2 factorial arranged as a randomized complete block.

### Greenhouse Experiments with P, K, and S

Experiments two, three, and four involved reniform nematode infestation levels of 0 or 10,000 vermiform life stages per pot and five levels of P, K, or S. Analysis of the soil prior to establishment of these experiments showed that it was deficient in P, K, and S with levels averaging 10, 44, and 3 mg, respectively. This nutritional deficiency was desirable so that the individual nutrients could be added back in a stepwise manner in subsequent experiments to evaluate their influence on the interaction of *R. reniformis* with cotton. Commercial triple super phosphate (46% P<sub>2</sub>O<sub>5</sub>), muriate of potash (60% K), and ammonium sulfate (22% S) were used as the sources for P, K, and S. These experiments had a 5  $\times$  2 factorial treatment structure and were arranged as a randomized complete block.

Soil P levels of 10, 20, 35, 60, and 73 mg (considered by the soil testing laboratory to indicate very low, low, medium, high, and very high levels, respectively) were established as main soil amendment treatments. These levels of P in soil were obtained by

adding either 0.015, 0.1, 0.25, or 0.32 g of triple super phosphate to the native soil in each pot. Potassium, S, and N were maintained at levels of 106, 20, and 45 mg, respectively, in all pots. Plants and nematodes were established as described previously.

Soil K levels of 44, 70, 106, 123, and 153 mg were established as described above for P. These levels of K in soil were obtained by adding 0.12, 0.23, 0.33, and 0.41 g of muriate of potash, respectively, to native soil in each pot. Phosphorus was maintained at a level of 35 mg and S and N at levels of 20 and 45 mg, respectively. Plants and nematodes were established as described previously.

Soil S levels of 3, 12, 20, 40, and 50 mg were established as described above for P and K. These levels of S in soil were obtained by adding 0.03, 0.08, 0.2, and 0.3 g of ammonium sulfate, respectively, to native soil in each pot. Phosphorus, K, and N were maintained at levels of 35, 106, and 45 mg, respectively, in all pots. Plants and nematodes were established as described previously.

#### *Field Experiments with Nutrients and Telone II*

To evaluate the interactions of P and S with 1,3-dichloropropene under field conditions, trials were conducted in 2011 and 2012 with the cotton cultivar Phytogen 565 WRF. The field, located at the LSU Agricultural Center, Northeast Research Station at St. Joseph, LA, was severely infested with the reniform nematode and deficient in P and S (8 and <12 mg, respectively). Planting and harvest dates in 2011 and 2012 were 23 May and 3 October and 16 May and 29 October, respectively.

The field was divided into plots 13.7-m long  $\times$  four rows spaced 1 m on center. Four nutrient combinations: P low and S high (44.8 and 22.4 kg), P and S low (44.8 and 5.6 kg), P high and S low (112 and 5.6 kg), and P and S high (112 and 22.4 kg) with and without 1,3-dichloropropene (Telone II<sup>®</sup>, Dow AgroSciences LLC, Indianapolis, IN) at 28.1 L per ha were established as main treatments. The fumigant was applied 2 to 3 wk prior to planting to a depth of 30 cm beneath the row using a four-row applicator with 76.2-cm diameter coulters and a precision application system. Plots received 100.8 kg of N and 89.6 kg of K to ensure availability of these nutrients throughout the duration of the trials. Cotton was harvested using a modified John Deere cotton picker. Both trials were 2 $\times$ 2 $\times$ 2 factorials arranged as randomized complete blocks.

#### *Analysis of Data*

Data obtained from all studies were analyzed using SAS JMP version 10.0 (SAS Institute, Cary, NC) analysis of variance (ANOVA) and Tukey's HSD mean separation technique ( $P < 0.05$ ). Because of a significant year  $\times$  yield interaction in the field study, data were analyzed separately for each year. Analysis

was conducted using the "Fit Model" module of SAS JMP, version 10.0.

## RESULTS

#### *Preliminary P and K Greenhouse Study*

Phosphorus impacted all plant and nematode parameters (Table 1). Shoot height and both shoot and root dry weights were increased, whereas the numbers of eggs per g of dry root and vermiform life stages of the nematode were reduced. There was a significant P  $\times$  Nematode interaction, which influenced vermiform life stage density in soil (Fig. 1). Low P levels tended to promote higher vermiform densities, while significantly lower densities were seen at high P levels. Reniform averaged 39,817 per 500 cm<sup>3</sup> in the high level of P and 165,356, a significantly greater number, at the low level of P. There was also a significant P  $\times$  K  $\times$  Nematode interaction that influenced numbers of eggs per g of dry root (Fig. 2). When levels of K in soil were very low, there were no differences in eggs per g of dry root in soils regardless of their level of P. The greatest number of eggs (22,246) per g of dry weight was recovered from roots growing in soil with very low P and high K, while the lowest number of eggs (2,191) per g of dry weight was recovered from soil with high levels of both P and K.

#### *Greenhouse Experiments with P, K, and S*

There were significant main effects due to both P and nematodes, but no significant P  $\times$  Nematode interactions that influenced plant parameters (Table 2). Phosphorus significantly increased plant height at 15 and 30 d and shoot and root dry weights at 60 d. The only main effect due to the nematodes was a decrease in shoot dry weight. In order to evaluate the effect of levels of P on nematode population density, individual treatment means were compared using Tukey's HSD test (Table 3). Generally, the overall impact of P nutrition on nematode reproduction was inhibitory. The three highest levels of P resulted in significant reductions in the number of eggs recovered from roots. Compared with the 20-mg level of P, increasing levels from 35 to 73 mg resulted in reductions of 72 to 85% in numbers of eggs per g of dry root. The impact of P on vermiform life stages from soil was variable. Compared to the 20-mg level, the 60-mg level had 52% fewer vermiform stages in soil. There were no significant main or interactive effects due to K influencing plant or nematode parameters (Table 4).

Both S and Nematodes influenced plant parameters independently, but there was not a S  $\times$  Nematode interaction (Tables 5). Sulfur decreased both plant height and shoot dry weights at 60 d, while the nematode suppressed plant height at both 30 and 60 d. There were no differences in nematode eggs or vermiform life stages or reproductive index among S levels (Table 6).

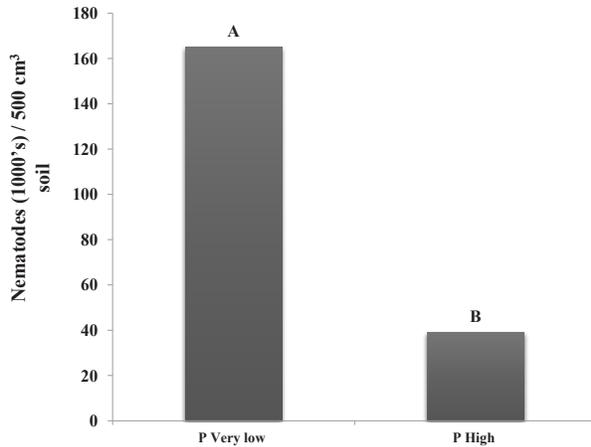


Fig. 1. Vermiform life stages of *Rotylenchulus reniformis* recovered after 60 d in a greenhouse environment from Stoneville LA 887 cotton in soils with very low (10 or 44 mg kg<sup>-1</sup>) and high (50 or 123 mg kg<sup>-1</sup>) levels of phosphorus. Data are means of 10 replications averaged over two trials. Designations as “very low” and “high” are according to the LSU AgCenter Soil Testing and Plant Analysis Laboratory. Bars with different letters are significantly different based on Tukey’s HSD ( $P < 0.05$ ).

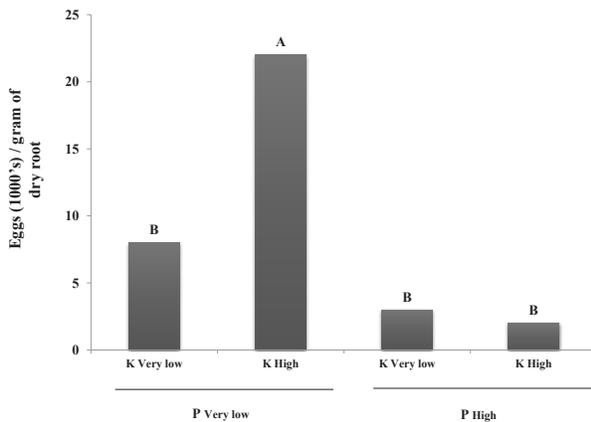


Fig. 2. Eggs of *Rotylenchulus reniformis* per gram of Stoneville LA 887 cotton dry root with very low (10 or 44 mg kg<sup>-1</sup>) and high (50 or 123 mg kg<sup>-1</sup>) levels of phosphorus and potassium, respectively, after 60 d in a greenhouse environment. Data are means of 10 replications averaged over two trials. Designations as “very low” and “high” are according to the LSU AgCenter Soil Testing and Plant Analysis Laboratory. Bars with the same letter are not significantly different based on Tukey’s HSD ( $P < 0.05$ ).

#### Field Experiments with Nutrients and Telone II

There were no significant main effects of P or S on plant or nematode parameters in either 2011 or

2012, with the single exception that S significantly decreased seed cotton yield in 2011 (Table 7). Nematicide application increased yield of cotton and lowered nematode population densities at mid-season and harvest in 2011 and at planting in 2012 (data not shown). No significant interactions among main effects were observed.

## DISCUSSION

Limiting P availability can suppress shoot and root development (Anonymous, 2009), so an appropriate P supply is essential for healthy plant growth. Our greenhouse data provides further support for the observation where P levels of 50 and 60 mg resulted in improved shoot and root dry weights in two different experiments. It is interesting to note that in both of these studies, enhanced plant growth was accompanied by reductions in population of *R. reniformis*. Across both tests, egg densities were 85% lower, and vermiform life stages were 52 to 75% lower at higher levels of P. Smith and Kaplan (1988), in their work with P and the burrowing nematode, *Radopholus citrophilus*, obtained similar results where augmentation of soil P increased shoot and root weights and resulted in concomitant reductions in population densities of the nematode. Similarly, Waceke *et al.* (2002) found that the addition of 150 and 300 kg of P, as either triple super phosphate or single super phosphate, improved the shoot and root weights of plants infected with the root-knot nematode, *M. hapla*. Nematode disease severity and the density of eggs, juveniles, and females were also reduced significantly in their studies.

Unlike P, K level produced no detectable effects on either plant growth or reniform nematode reproduction. Greenhouse studies by Gazaway *et al.* (1996), which evaluated increasing levels of K on cotton plant growth in the presence of reniform nematode, strongly support the conclusions of this research. However, in field trials, Pettigrew *et al.* (2005) found increased cotton growth and a 12% increase in reniform nematode population density in response to increased K levels. Their trials, however, were conducted under field conditions where there was likely a much greater variation in soil type, pH, moisture content, and possibly other key agronomic factors.

Sulfur had no detectable effects on nematode reproduction in either the greenhouse or field at relatively comparable rates. A single report from India indicates that S negatively impacted populations of stunt and reniform nematodes on garlic under field conditions (Kassab and Hafez, 1990). In both the greenhouse and field environments, our data suggest that S nutrition negatively impacted plant growth where plant height and shoot weight were reduced 10% and 15%, respectively, in the greenhouse, and cotton yield was reduced in the field significantly

Table 1. Main and interaction effects (*P* values) of phosphorus, potassium, and *Rotylenchulus reniformis* on Stoneville LA 887 cotton growth parameters and egg and vermiform nematode life stages in a greenhouse environment<sup>v</sup>.

Treatment	DF	Shoot height (cm)	Shoot dry weight (g) <sup>w</sup>	Root dry weight (g)	Eggs per gram of dry root	Vermiform life stages
Phosphorus (P) <sup>x</sup>	1	<0.001**	<0.001**	<0.001**	0.002**	0.010**
Potassium (K) <sup>y</sup>	1	0.651	0.276	0.272	0.054	0.469
Nematode (N) <sup>z</sup>	1	0.741	0.591	0.344	<0.001**	<0.001**
P × K	1	0.652	0.757	0.912	0.036*	0.074
P × N	1	0.626	0.932	0.175	0.002**	0.010**
K × N	1	0.345	0.175	0.470	0.054	0.469
P × K × N	1	0.110	0.776	0.803	0.036*	0.074

<sup>v</sup>Data are combined over two 60-d duration trials with five replications per treatment.

<sup>w</sup>Data are dry weights obtained after two days at 45°C.

<sup>x</sup>Phosphorus levels were 10 or 50 mg kg<sup>-1</sup>.

<sup>y</sup>Potassium levels were 44 or 123 mg kg<sup>-1</sup>.

<sup>z</sup>Reniform nematode levels of 0 or 3,000 vermiform life stages were used as inoculum.

Data analyzed as a 2 × 2 × 2 factorial; \*and\*\* indicate *P* values significant at 0.05 and 0.01% level, respectively.

Table 2. Main and interaction effects (*P* values) of phosphorus and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 d in a greenhouse environment<sup>w</sup>.

Treatment	DF	Plant height (cm)			Shoot dry weight(g) <sup>y</sup>	Root dry weight(g)
		15 <sup>x</sup>	30	60		
Phosphorus (P) <sup>z</sup>	4	<0.001**	0.029*	0.131	<0.001**	<0.001**
Nematode (N)	1	0.408	0.206	0.154	0.004**	0.307
P × N	4	0.624	0.843	0.647	0.565	0.131

<sup>w</sup>Data combined over two 60-d duration trials with five replications per trial. Reniform nematode levels of 0 or 10,000 vermiform life stages were used as inoculum.

<sup>x</sup>Days after inoculation.

<sup>y</sup>Data are dry weights at 60 d and were obtained by drying for 2 d at 45°C.

<sup>z</sup>Phosphorus levels were 10, 20, 35, 60, and 73 mg kg<sup>-1</sup>.

\*and\*\* indicate *P* values significant at 0.05 and 0.01% levels, respectively.

Table 3. Effects of phosphorus on eggs and vermiform life stages of *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 d in a greenhouse environment<sup>w</sup>.

Levels of Phosphorus (mg kg <sup>-1</sup> ) <sup>x</sup>	Eggs (1000's) per gram of dry root <sup>y</sup>	Vermiform life stages (1000's) per 500 cm <sup>2</sup> soil	R value <sup>z</sup>
10	149 ab	337 ab	107.9
20	162 a	359 a	115.1
35	35 b	251 ab	80.5
60	24 b	171 b	54.9
73	45 b	226 ab	72.6

<sup>w</sup>Data combined over two 60-d duration trials with five replications per trial.

<sup>x</sup>Phosphorus levels were 10, 20, 35, 60, and 73 mg kg<sup>-1</sup> considered by LSU AgCenter Soil Testing and Plant Analysis Laboratory to be very low, low, medium, high, and very high respectively.

<sup>y</sup>Within each column, means followed by the same letter are not significantly different based on Tukey's HSD (*P*<0.05).

<sup>z</sup>Reproductive value (R), where  $R = P_f / P_i$  ( $P_f$  is the final population density and  $P_i$  is the infestation level).

Table 4. Main and interaction effects (*P* values) of potassium and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 d in a greenhouse environment<sup>w</sup>.

Treatment	DF	Plant height (cm)			Shoot dry weight(g) <sup>y</sup>	Root dry weight(g)
		15 <sup>x</sup>	30	60		
Potassium (K) <sup>z</sup>	4	0.674	0.476	0.719	0.098	0.964
Nematode (N)	1	0.281	0.558	0.643	0.206	0.634
K × N	4	0.973	0.920	0.680	0.701	0.927

<sup>w</sup>Data combined over two 60-d duration trials with five replications per trial. Reniform nematode levels of 0 or 10,000 vermiform life stages were used as inoculum.

<sup>x</sup>Days after inoculation.

<sup>y</sup>Data are dry weights at 60 d and were obtained by drying for 2 d at 45°C.

<sup>z</sup>Potassium levels were 44, 70, 106, 123, and 153 mg kg<sup>-1</sup>.

Table 5. Main and interaction effects (*P* values) of sulfur and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 d in a greenhouse environment<sup>w</sup>.

Treatment	DF	Plant height (cm)			Shoot dry weight(g) <sup>y</sup>	Root dry weight(g)
		15 <sup>x</sup>	30	60		
Sulfur (S) <sup>z</sup>	4	0.090	0.213	0.020*	0.011**	0.245
Nematode (N)	1	0.335	0.003**	0.004**	0.097	0.204
S × N	4	0.353	0.783	0.474	0.845	0.116

<sup>w</sup>Data combined over two 60-d duration trials with five replications per trial. Reniform nematode levels of 0 or 10,000 vermiform life stages were used as inoculum.

<sup>x</sup>Days after inoculation.

<sup>y</sup>Data are dry weights at 60 d and were obtained by drying for 2 d at 45°C.

<sup>z</sup>Sulfur levels were 3, 12, 20, 40, and 50 mg kg<sup>-1</sup>.

\*and\*\* indicate *P* values significant at 0.05 and 0.01% levels, respectively.

Table 6. Effects of sulfur on eggs and vermiform life stages of *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 d in a greenhouse environment<sup>w</sup>.

Levels of Sulfur (mg kg <sup>-1</sup> ) <sup>x</sup>	Eggs (1000's) per gram of dry root <sup>y</sup>	Vermiform life stages (1000's) per 500 cm <sup>3</sup> soil	R value <sup>z</sup>
3	55 a	180 a	57.8
12	63 a	188 a	60.2
20	61 a	181 a	58.2
40	56 a	148 a	47.4
50	66 a	193 a	62.0

<sup>w</sup>Data combined over two 60-d duration trials with five replications per trial.

<sup>x</sup>Sulfur levels were 10, 20, 35, 60, and 73 mg kg<sup>-1</sup> considered by LSU AgCenter Soil Testing and Plant Analysis Laboratory to be very low, low, medium, high, and very high respectively.

<sup>y</sup>Within each column, means followed by the same letter are not significantly different based on Tukey's HSD (*P*<0.05).

<sup>z</sup>Reproductive value (R), where  $R = P_f / P_i$  ( $P_f$  is the final population density and  $P_i$  is the infestation level).

Table 7. Main and interaction effects (*P* values) of phosphorus, sulfur, and 1,3-D on seed cotton yield and population density of *Rotylenchulus reniformis* at three sampling periods in 2011 and 2012<sup>w</sup>.

Treatment	DF	<i>R. reniformis</i> population density							
		Seed cotton (kg/ha)		Planting		Mid season		Harvest	
		2011	2012	2011	2012	2011	2012	2011	2012
Phosphorus (P) <sup>x</sup>	1	0.170	0.630	0.391	0.705	0.195	0.503	0.674	0.072
Sulfur (S) <sup>y</sup>	1	0.047*	0.324	0.831	0.810	0.438	0.918	0.847	0.720
Nematicide (1-3,D) <sup>z</sup>	1	0.001**	0.128	0.094	0.014**	0.004**	0.173	0.008**	0.151
P x S	1	0.408	0.660	0.273	0.548	0.800	0.630	0.863	0.880
P x 1-3,D	1	0.900	0.475	0.555	0.091	0.981	0.319	0.167	0.572
S x 1-3,D	1	0.878	0.710	0.334	0.260	0.840	0.912	0.119	0.102
P x S x 1-3,D	1	0.214	0.988	0.899	0.583	0.681	0.166	0.707	0.088

<sup>w</sup>Data combined over 2 yr with six replications per trial and analyzed as a 2 x 2 x 2 factorial.

<sup>x</sup>Phosphorus levels were 44.8 or 112 kg ha<sup>-1</sup>.

<sup>y</sup>Sulfur levels were 5.6 or 22.4 kg ha<sup>-1</sup>.

<sup>z</sup>1,3-dichloropropene (1-3,D) was applied as Telone II® at 28.1L ha<sup>-1</sup>.

\*and\*\* indicate *P* values significant at 0.05 and 0.01% level, respectively.

in 2011 and numerically in 2012 at higher S levels. Other field studies evaluating the effects of S nutrition on cotton yields have shown positive results (Júnior *et al.*, 2012; Yin *et al.*, 2011). None of these reports, however, included data for reniform or any other nematode, implying that they were conducted in the absence of a significant nematode infestation.

According to Behm *et al.* (1995), Zn compounds may cause a significant reduction in hatching of eggs of *H. glycines*. Bell (1996) also found that P nutrition negatively influenced hatching of eggs of *H. schachtii* and speculated that the effects on nematode reproduction could be due to a change in root exudates as well as a change in root penetration. Pettigrew *et al.* (2005) suggested that changes in reniform nematode reproduction could also be due to the development of a more robust and extensive cotton root system, which could have been the case in our studies. In our work, reniform reproduction could have been related either directly or indirectly to nutrient status of the soil and may have been exhibited as effects on egg hatching, root penetration, root exudate production, root system development, or, more likely, a combination of these factors.

This research provides impetus for further investigation of the role of mineral nutrition in pathogenesis by nematodes and other soil-borne pathogens. Notable conclusions from this research are: i) nutrient status can affect reproduction and pathogenicity of *R. reniformis* on cotton; ii) of the three nutrients studied, P had the most pronounced effect on both nematode reproduction and cotton growth; and iii) the effect of P on nematode reproduction was negative (averaging a 74% suppression), while the effects on cotton growth were positive, averaging a

68% improvement in shoot and root weights.

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