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Influence of soil nutrients on reproduction and pathogenicity of *Rotylenchulus reniformis* on cotton

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

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Plant Pathology and Crop Physiology

by

Herath Mudiyanseelage Kularathna
B.Sc. (Honors) University of Colombo, Sri Lanka, 2006
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ABSTRACT

Among the variety of pathogens of cotton (*Gossypium hirsutum*), nematodes play a major role in reducing yield. Across the U.S. cotton belt, millions of dollars are lost annually due to nematode infestation. In the Mid-South and Southeast United States, root-knot nematode (*Meloidogyne incognita*) and reniform nematode (*Rotylenchulus reniformis*) are responsible for the highest percentage of damage. Crop rotation and nematicides are currently the most commonly used management strategies for nematode management. Soil fertility, which has a direct effect on plant growth, is also known to influence disease severity. Therefore, soil fertility would be an additional factor to consider for management of nematodes. The objectives of these studies were to evaluate the effects of soil nutrients on reniform nematode reproduction and pathogenicity on cotton. Four 60-day-duration greenhouse studies were conducted to evaluate the effect of different soil nutrients on reniform nematode pathogenicity and reproduction. Nutrients used in greenhouse studies were phosphorus (P), potassium (K) and sulfur (S). For the first study, P and K were used in high (112 kg ha⁻¹) and low (0 kg ha⁻¹) levels with a soil mixture of 70.1% sand, 25.4% silt, and 2.5% clay. Treatments for the second, third and fourth studies were five increasing levels of P (10, 20, 35, 60, and 73 mg kg⁻¹), K (44, 70, 106, 123, and 153 mg kg⁻¹), and S (3, 12, 20, 40, and 50 mg kg⁻¹) mixed with soil comprised of 68% sand, 30% silt and 2% clay. Application of P produced a significant increase in plant shoot and root dry weights in studies one and two. Similarly, reproduction of reniform nematodes in these two studies were significantly influenced by levels of P. Studies three and four focused on K and S and did not show any effect on reproduction of reniform nematodes. Treatment with S had a significant negative influence on shoot height and dry weights. Under field conditions, nematicide application significantly reduced nematode population density at mid-season and at 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 nutrients.

INTRODUCTION

Cotton is known as nature's wonder fiber. It holds the first place as a food and fiber crop in the U.S. by producing fiber and cotton seed products (Anonymous, 2011a,b). Cotton is grown around the world, and the U.S. takes third place by producing 14% of the world's total. The cotton industry generates about 200,000 jobs in the United States and has an annual revenue of 2 billion U.S. dollars. Cotton is grown in the southern states of the U.S. from Virginia to California and includes 17 states (Anonymous, 2009a). In 2011, acreage planted to cotton in the U.S. was estimated to be 5.5 million hectares, an increase of 25% from 2010 (Anonymous, 2011c). Louisiana supplies about 2.5% of the total cotton produced and ranked tenth among the U.S. cotton producing states (Anonymous, 2011d).

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 U.S. dollars (Bagwell *et al.*, 2006). According to Koenning *et al.* (2004), yield losses caused by nematodes have increased significantly. This is probably due to a lack of resistant cultivars, limited use of crop rotation, spread of reniform nematodes within cotton producing areas, and the lack of low cost and efficient nematicides (Koenning *et al.*, 2004). Several species of plant parasitic nematodes are damaging to cotton in the United States. These include root-knot (*Meloidogyne incognita*), reniform (*Rotylenchulus reniformis*), lance (*Hoplolaimus spp.*) and sting (*Belonolaimus spp.*) nematodes (Koenning *et al.*, 2004, 1999).

Root-knot (RKN) and reniform nematodes are responsible for the highest percentage of damage in mid-South and Southeastern U.S. (C. Overstreet, pers. comm.; Overstreet *et al.*, 2010). Root-knot nematode infests almost all United States cotton producing areas (Bagwell *et al.*, 2006). Southern root-knot nematode races 3 and 4 are well adapted to reproduce on cotton

(Koenning *et al.*, 2004). The climate and soil types in the U.S. cotton growing area are ideal for survival and reproduction of RKN. In 2004, the estimated production loss due to RKN was approximately \$214 million (Gazaway, 2005).

Reniform nematode was first described as a parasite of cotton in Hawaii by Linford and Oliveira in 1940 (Linford and Oliveira, 1940). At present, the nematode has a wide distribution in the cotton producing area of the southern U.S. and can be found even as far west as Texas (Bagwell *et al.*, 2006). During the past 15 -20 years, reniform nematode has become the dominant nematode species in a number of states, including Louisiana (Gazaway, 2005; Overstreet and McGawley, 1998; 2000; Overstreet, 2006). Damage can be severe, resulting in dramatic yield reductions. Estimates from 2001-2005 showed that reniform alone caused about 839.2 million dollars yield loss (Bagwell *et al.*, 2006). Reniform nematode causes yield losses due to induced nutritional deficiencies, fruit abortion, and abnormal crop maturation (Koenning *et al.*, 2004). Symptoms caused by *Rotylenchulus reniformis* are similar to that of other nematodes. That is , plants become stunted, develop poorly with low yields, and lack vigor (Overstreet and Wolcott, 2007). Detection of reniform nematode damage is very difficult to diagnose because they do not produce distinctive galling symptoms like root-knot nematode (Overstreet and Wolcott, 2007). This nematode is considered a tropical/sub-tropical pest and has a wide host range (Koenning *et al.*, 2004; Robinson *et al.*, 1997). Reniform nematode can survive in soil for long periods without the presence of the host due to its ability to enter an anhydrobiotic state. This state of reduced metabolism provides for higher survival of the nematode. Additionally, high reproductive rate and ability to migrate deep in soil allows the nematode to survive and repopulate the “cultivation layer” of soil when conditions are favorable (Koenning *et al.*, 2004).

In 1941, Smith and Taylor identified reniform nematode as a pest of cotton in Louisiana. 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, 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 infected was about 510,000 (Overstreet and McGawley, 1996). In 2007, there was a 4% loss of cotton yield due to reniform nematodes and a 3% loss due to RKN in Louisiana (Blasingame *et al.*, 2008). Research reported by McGawley *et al.* (2010; 2011) on cotton and soybeans has shown that there were significant differences in reproduction and pathogenicity among different geographical isolates of reniform nematodes from several U.S. states, including Louisiana, Texas, Hawaii, Arkansas, Alabama, and Mississippi.

There are a number of different techniques that have been employed for the control of reniform nematodes. Some common strategies practiced for management include crop rotation, biological control, nematicide application, and the use of precision agriculture (Koenning *et al.*, 2004).

Crop rotation is a widely used cultural practice for nematode management. A crop that is selected as a rotational crop should provide a reasonable economical income for the grower and also decrease the nematode populations to a level favorable for cotton production the next season. Davis *et al.* (2003) suggested that rotation with corn or highly resistant soybeans could reduce reniform nematode levels. Cotton followed by two seasons of corn produced a significant reduction in the population density of reniform nematode (Erwin *et al.*, 2007; Stetina *et al.*, 2007). In most production fields, two or more species of nematodes occur concomitantly. This makes the selection of rotational crop more difficult. Rotational crops such as corn may reduce

populations of reniform nematodes, but have only minimal effectiveness against root-knot nematode (Overstreet *et al.*, 2010).

Currently, there are no cultivars of commercial cotton resistant to reniform nematode. Resistance has been documented in other *Gossypium* species (Koenning *et al.*, 2004; Robinson and Percival, 1997; Yik and Birchfield, 1984), but has not yet been successfully transferred to commercial cultivars. Developing resistant breeding lines that can be used as parental material will take considerable time and effort. Scientists have also identified tolerant cotton varieties that produce acceptable yields in the presence of nematode (Koenning *et al.*, 2004). Romano *et al.* (2009) identified molecular markers that can be useful in developing reniform resistant cultivars. Some resistant breeding lines were released in 2007, but they showed early stunting in some sites in the presence of high populations of reniform nematodes (Nichols *et al.*, 2010).

Use of biological agents is another management tool for nematode parasites of cotton. Studies have shown that the nematophagous Arkansas fungus (ARF), is capable of suppressing reniform populations (Wang *et al.*, 2004). *Pasteuria* spp. are also known to parasitize reniform nematodes and reduce their population density (Hewlett *et al.*, 2010). These bacteria attack juveniles of the nematodes, as well as mature males and females (Schmidt *et al.*, 2010).

Among the different management options, nematicides are the primary and most widely used tactic (Koenning *et al.*, 2004; Starr *et al.*, 2007). Historically, aldicarb has been the dominant nematicide used in cotton, but it is no longer available. Also, most fumigant nematicides may soon be unavailable. Avicta Complete Cotton, Aeris seed-applied insecticide/nematicide, and Poncho/Votivo are currently the most widely used nematicides. Previous studies showed that the commonly used nematicide Telone II can significantly reduce reniform populations and increased yields of cotton (Overstreet *et al.*, 2001; 2007). Seed

treatment nematicides are also available when the nematode populations are present in low to moderate population levels (Erwin *et al.*, 2010). Also, nematicides can be combined with another management strategy. According to data of Royal and Hammes (2005), crop rotation plus nematicide application resulted in significant reduction in reniform nematode populations. Lawrence and Mclean (2002) showed that foliar application of oxymyl with aldicarb significantly decreased *M. incognita* populations on cotton. Nematicides provide protection against some nematodes, but they also increase production cost and have many negative environmental impacts (Starr *et al.*, 2007).

Precision farming is a new and novel approach for management of agricultural pests. Modern technologies like geographic information systems (GIS) and global positioning systems (GPS) are incorporated into the development of precision farming strategies (Koenning *et al.*, 2004; Nutter *et al.*, 2002). Over the years, producers have usually applied a single rate of nematicide across an entire field (Khalilian *et al.*, 2001; Wrather *et al.*, 2002). However, it is well known that nematode distribution in a field is not uniform (Khalilian *et al.*, 2001; Perry *et al.*, 2006). Therefore, single rate application of nematicides is inefficient, costly and irresponsible (Erwin *et al.*, 2007; Khalilian *et al.*, 2001; Wrather *et al.*, 2002). Wrather *et al.* (2002) showed that responses obtained for the site specific application of aldicarb are similar to those for single rate application when combating *M. incognita*.

According to Khalilian *et al.* (2001), both soil type and texture have a direct impact on nematode population density. Overstreet *et al.* (2007) observed that soil texture has a direct impact on Telone II fumigant response against nematodes. A study conducted from 1991-1993 showed that reproduction of *M. incognita* was greater in coarse textured soils whereas *R. reniformis* reproduced better in sandy loam soil (Koenning *et al.*, 1996). Development of novel

machinery like the Veris 3100 Soil EC Mapping System could be used to identify nematode “hot spots” in cotton fields by determining soil texture in different locations (Perry *et al.*, 2006).

Today, considerable research is underway to augment the effectiveness of site specific nematode management technology.

For an economical cotton yield, maintaining an adequate soil fertility level is essential. The amount of fertilizer available to the plant also plays a major role in overall plant health. Excessive fertilizer availability or deficiencies make plants vulnerable to diseases and insects and ultimately result in low yields (Albers *et al.*, 1993; Knowels *et al.*, 1999).

Nematode management via soil fertility adjustment is another aspect that scientists are investigating. According to the results of Berankova and Saly (1980) in their 3-year study, mineral fertilizer had a considerable negative impact on nematode populations. Gruzdeva *et al.* (2007) also found that there was a strong correlation of particular nutrients including nitrogen (N), phosphorus (P), potassium (K), or their combinations with nematode population decline. Rodriguez 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*) with a balanced nutrient supply.

Behm *et al.* (1995), found that the micronutrient zinc (Zn) had an effect on the hatching of eggs of *Heterodera glycines* in corn. Similarly, macronutrients, such as P, were associated with reduced penetration by juveniles of *Heterodera schachtii* on sugar beet (Bell, 1996). A study conducted on nutrient status of guava showed that shoot symptoms induced by *Meloidogyne 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 suppression of *M. incognita* populations in tomato.

Plants absorb fertilizer in ionic form from the soil solution. According to Ronan and Queneherve (2002), ionic compounds such as ammonium and nitrates have exhibited repellent activity against *M. incognita*. The application of fertilizer can interfere with the mode of parasitism of nematodes. Vestergard (2004) observed that ectoparasitic nematodes were stimulated by nitrogen fertilizer whereas migratory endoparasites are inhibited at high and balanced fertilization.

On the other hand, there are controversial findings regarding the effect of fertilizer on nematode management. There are reports that fertilizer incorporation into soil has made the crops less resistant to nematode damage while others indicate incorporation made them more severe (Agu, 2003; Mahmood *et al.*, 2011). Also, some fertilizer application reports have not shown any measurable effects against nematodes (Luc *et al.*, 2007). Ebelhar *et al.* (2011) observed that there were no marked effects of N and P fertilizer on reniform nematodes in a corn and cotton rotation system. Studies conducted by Wolcott *et al.* (2008) in nematode infested cotton fields showed that high levels of P and Zn nutrients through the soil profile were capable of increasing lint yields, which were similar to the yield response using the nematicide Telone II. Soil nutrients might have the ability to offset nematode damage and increase yields. Indeed, the effects of fertilizers on nematode pathology are not clearly understood. Therefore, looking at the effects of nutrients on nematodes biology will be another approach towards developing site-specific nematode management strategies.

Objectives of the Research

1. Evaluate the effects of phosphorus, potassium, and sulfur (S) fertilizers on reniform nematode reproduction and pathogenicity on cotton.
2. Evaluate the interactions of P and S fertilizers with 1,3-dichloropropene under field conditions on cotton.

MATERIALS AND METHODS

Isolates of Reniform Nematode

For the initial greenhouse study, reniform nematodes were extracted from soil samples obtained from the Northeast Research Station in St. Joseph, Louisiana and propagated on tomato (cultivar Rutgers PS Seedway; Hall, New York 14463), here after referred to as St. Joseph isolate. After 30 days, egg masses were removed from tomato root systems to establish axenic cultures. Cultures were maintained in the LSU nematology greenhouse on tomato for use as inoculum for the first greenhouse study. For the second, third and fourth greenhouse studies, an isolate of reniform nematodes from Rapides Parish was supplied/collected by E. C. McGawley and obtained from axenic cultures maintained in the LSU nematology greenhouse on tomato and use as the inoculum. These isolates had been confirmed *R. reniformis* as described by McGawley *et al.*, (2010) and Robinson *et al.*, (1997).

General Information

A series of greenhouse experiments and two field trials were conducted. Terra-cotta 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 prior to use. Commerce silt soil was collected from the Northeast Research Station. Soil mixtures used in the greenhouse studies were three parts sterilized Commerce silt loam (fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) and one part steam-sterilized sand. All experiments were repeated. Inoculum for all greenhouse experiments consisted of juveniles, pre-adult females and males extracted from greenhouse cultures by wet-sieving through nested 850- μm -pore and 38- μm -pore

sieves, followed by sugar flotation and centrifugation (Jenkins, 1964). In all greenhouse studies described herein, soil was infested with nematodes by pipetting aqueous suspensions of vermiform individuals of *R. reniformis*. Nematodes were pipetted into a series of depressions arranged into a triangular pattern in soil, 0.5 cm diameter X 5-7.5 cm deep, surrounding a single ten day old seedling. Additionally, the cotton cultivar Stoneville LA887 was used because of its susceptibility to reniform nematode (McGawley *et al.*, 2010). Two cotton seeds were planted in each pot and covered with a plastic bag to optimize seed germination. Bags were removed once seeds were established and thinned to one seedling per pot. Water soluble ammonium nitrate (33% N, 45 mg kg⁻¹ of soil) was used as the nitrogen (N) source for all the greenhouse studies and applied at twelve day intervals during the study periods. Soil texture was analyzed both for greenhouse and field soils 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 analyzed by the LSU AgCenter Soil Testing and Plant Analysis Laboratory (STPAL) using Mehlich – 3 extraction method. Soil nutrient treatments used for the studies were phosphorus, potassium, and sulfur. Tensiometers were placed into pots in greenhouse trials to measure the water potential during the study. Water potential is an indicator of soil dryness. Plants were watered when the water potential fell between 40-50 kPa. Plant heights were measured at fifteen day intervals in all four greenhouse studies. Optimum soil pH for cotton falls between pH 5.8 - 8.0 (Anonymous, 2012). Tap water in the greenhouse was above pH 8.4, which is not suitable for optimum cotton growth. This also could affect the nutrient availability for the plants. Therefore, tap water was adjusted to pH 6.0 - 7.0 by mixing 11.6 ml of 2M HCl acid in 18.9 liters of tap water (pH 8.4) and employed for each greenhouse experiment throughout the study period. Soil samples for nematode analysis were processed

using semi-automatic elutriation (Byrd *et al.*, 1976) and centrifugal-flotation (Jenkins, 1964). Vermiform reniform nematodes were counted at a magnification of 40X using an inverted microscope. Total population densities per pot (Pf) were determined. For greenhouse experiments, reniform nematode eggs were extracted from fresh root tissue by stirring in 0.6% NaOCl for 10 minutes (Hussey and Barker, 1973) and stained with acid fusion stain before counting at 100X using an inverted microscope. At the end of each greenhouse study, plant height and dry shoot and root weights were determined. Plant materials were placed in paper bags and oven-dried at 45°C for two days. Dry plant shoot samples from each greenhouse study were collected for nutrient analysis and sent to LSU AgCenter STPAL. The cotton cultivar PhytoGen 565 WRF, susceptible to reniform nematode (Overstreet, pers. comm.), was used in the field study. For both greenhouse and field studies, pre-plant and at harvest soil samples were obtained for soil nutrient analysis. Experimental durations were 60 days for greenhouse studies and full season for field studies.

Greenhouse Experiments

The first greenhouse study evaluated the influence of high and low phosphorus (112 kg ha⁻¹ and 0 kg ha⁻¹ respectively) and potassium (112 kg ha⁻¹ and 0 kg ha⁻¹ respectively) levels on reniform nematode pathogenicity and reproduction. Greenhouse studies two, three, and four investigated the effect of increasing levels of phosphorus, potassium, and sulfur on reniform nematode pathogenicity and reproduction (see individual experiment descriptions for details). Prior to initiation of experiments, soil in all studies was submitted to the soil testing laboratory for analysis. Based on recommendations, soil nutrient levels were adjusted according to individual treatment objectives of each study. Each study was replicated five times.

Experiment 1

This preliminary experiment was initiated to evaluate the influence of four combinations of phosphorus and potassium on reniform nematode pathogenicity and reproduction on Stoneville LA 887 cotton. Treatments in this experiment contained two levels of P (0 or 112 kg ha⁻¹), two levels of K (0 or 112 kg ha⁻¹), and two nematode levels (0 or 3000 vermiform life stages) arranged as a factorial structure. A steam-sterilized soil mixture of 70.1% sand, 25.4% silt, and 2.5% clay was used for the experiment. This experiment had a 2 x 2 x 2 factorial treatment structure and was arranged as a randomized complete block design.

Experiments 2, 3, and 4 (General procedures)

Experiments two, three, and four involved reniform nematode infestation levels of 0 or 10,000 vermiform life stages per pot and five increasing levels of phosphorus (P), potassium (K), or sulfur (S). In these experiments, a Commerce silt loam soil was mixed with sand to produce a mixture containing 68% sand, 30% silt, and 2% clay. After mixing the entire soil lot was steam sterilized. Analysis of soil showed that the mixture was deficient in P, K, and S with levels averaging 7.5 mg kg⁻¹, 40.6 mg kg⁻¹, and 6 mg kg⁻¹ respectively. This soil nutrient deficiency status was desirable so that in subsequent experiments the individual nutrients could be added back in a stepwise manner to evaluate their impact on pathogenicity and reproduction of *R. reniformis*. Commercial triple super phosphate (46% P₂O₅), muriate of potash (60% K), ammonium sulfate (22% S), or ammonium nitrate (33% N) were used as the sources for P, K, S and, N. Appropriate amounts of each of these were hand ground with a mortar and pestle. The powdered form of each of these nutrients were individually dissolved in deionized water, and a 20 ml aliquot mixed with soil to establish each of five nutrient treatments. The experiment had a 5 x 2 factorial treatment structure and was arranged as a randomized complete block design.

Experiment 2

Soil phosphorus (P) levels of 10, 20, 35, 60, and 73 mg kg⁻¹ (considered by soil testing laboratory results as very low, low, medium, high, and very high, respectively) were established as main soil amendment treatments by mixing 0.015, 0.1, 0.25, and 0.32 g of triple super phosphate (TSP) respectively. Fertility levels of K, S, and N of 106, 20, and 45 mg kg⁻¹ respectively were established in soil in all pots. Plants and nematodes were then established as described above.

Experiment 3

Soil potassium (K) levels of 44, 70, 106, 123, and 153 mg kg⁻¹ (considered by soil testing laboratory results as very low, low, medium, high, and very high, respectively) were established as main soil amendment treatments by mixing 0.12, 0.23, 0.33, and 0.41 g of muriate of potash respectively. Fertility levels of P, S, and N of 35, 20, and 45 mg kg⁻¹ respectively were established in soil in all pots. Plants and nematodes were then established as described above.

Experiment 4

Soil sulfur (S) levels of 3, 12, 20, 40, and 50 mg kg⁻¹ (considered by soil testing laboratory results as very low, low, medium, high, and very high, respectively) were established as main soil amendment treatments by mixing 0.03, 0.08, 0.2, and 0.3g of ammonium sulfate respectively. Fertility levels of P, K, and N of 35, 106, and 45 mg kg⁻¹, respectively, were established in soil in all pots. Plants and nematodes were then established as described above.

Field Experiments

This study was originally conducted in 2011 and repeated in 2012 in a field severely infested with reniform nematode located at Northeast Research Station in St. Joseph, Louisiana. Initial soil nutrient analysis indicated that the field was low in phosphorus (P) and sulfur (S) (8 mg kg⁻¹

for P and $<12 \text{ mg kg}^{-1}$ for S). The field was divided into plots with a length of 13.7 m and a width of four rows having a row spacing of 1 m. Four different fertilizer combinations using P and S were applied in the study. Fertilizer levels of 112 kg ha^{-1} or 44.8 kg ha^{-1} (high or low levels of P, respectively) were combined with 22.4 kg ha^{-1} or 5.6 kg ha^{-1} (high or low levels of S, respectively). Fertility levels were compared with the presence or absence of the nematicide 1,3-dichloropropene at 28.1 L ha^{-1} . The fumigant was applied 2-3 weeks prior to planting using a four-row applicator with 76.2 cm yetter coulters and a precision application system to a depth of 30 cm beneath the row. Each treatment was replicated six times across the field. All plots received N and K fertilizers in 100.8 kg ha^{-1} and 89.6 kg ha^{-1} , respectively, (considered by soil testing laboratory as high levels) to ensure an adequate supply of those two nutrients. At the end of the growing season, cotton was harvested using a modified John Deere cotton picker and yields were measured for each treatment and control plots. Experiment had a $2 \times 2 \times 2$ factorial treatment structure and was arranged in a randomized complete block design.

Analysis of Data

Data obtained from the greenhouse studies were analyzed using analysis of variance (ANOVA) and Tukey's HSD mean separation technique ($P \leq 0.05$). Treatments in greenhouse study one were arranged in a $2 \times 2 \times 2$ factorial with main effects being P, K and nematodes. Greenhouse studies two, three and four treatments were arranged in a 5×2 factorial with main effects being treatments and nematodes. Field study treatments were also arranged in $2 \times 2 \times 2$ factorial with main effects being P, S, and nematicide. Because of a significant year x yield interaction, data was not pooled but presented for each year. Analysis was conducted using the "Fit Model" module of SAS JMP, version 10.0 (SAS Institute, Cary, NC).

RESULTS

Greenhouse Experiment 1

Results from the first greenhouse test indicate that phosphorus (P) had a significant effect and impacted shoot height, and shoot and root dry weights. The average height of plants growing in soil with a high level of P was 38.5 cm, and was significantly greater than those of the low P level, which averaged 25.4 cm. The high level of P significantly increased both shoot and root dry weights (4.4 and 1.4 g, respectively) compared to P low levels (1.1 and 0.5 g, shoot and root weights respectively). Other main and interactive affects were not significant (Table 1).

Table 1. Main and interaction effects (P values) of phosphorus, potassium, and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^v.

Treatment	DF	Shoot height (cm) ^z	Shoot dry weight (g) ^z	Root dry weight (g) ^z
Phosphorus (P) ^x	1	<0.001**	<0.001**	<0.001**
Potassium (K) ^y	1	0.651	0.276	0.272
Nematode (Nema) ^w	1	0.741	0.591	0.344
P x K	1	0.652	0.757	0.912
P x Nema	1	0.626	0.932	0.175
K x Nema	1	0.345	0.175	0.470
P x K x Nema	1	0.110	0.776	0.803

^vData combined over two 60 day duration trials with five replications per trial.

^wReniform nematode levels of 0 or 3000 vermiform life stages were used as inoculum.

^xPhosphorus levels were 0 or 112 kg ha⁻¹.

^yPotassium levels were 0 or 112 kg ha⁻¹.

^zData are dry weights obtained after two days at 45°C.

Data analyzed as a 2 x 2 x 2 factorial.

** Indicate a P value significant at 0.01% level.

Reniform nematode eggs and vermiform life stage counts were significantly affected by P and nematode (Table 2). Soil with high P levels resulted in egg and vermiform life stage counts that were significantly lower (2488 eggs per gram of dry root and 19,909 vermiform life stages per 500 cm³ soil, respectively) than soil with low P levels (15,564 eggs per gram of dry root and 82,678 vermiform stages per 500 cm³ soil, respectively). There was a significant interaction between P and potassium (K) that influenced egg numbers (Figure 1). Significantly higher

number of eggs per gram of dry root was observed when K level was high and P level was low. Egg counts were similar with K at both levels when P was low. There were significant P x Nematode interactions which influenced egg production (Figure 2) and vermiform life stage density in soil (Figure 3). Egg production was 4976 per gram of dry root in the presence of the high level of P and 31,128 per gram of dry root at low levels. Vermiform densities averaged 39,817 per 500 cm³ in the high level of P and 165,356 per 500 cm³ at low levels of P. Additionally there was a significant P x Nematode x K interaction which influenced egg production.

Table 2. Main and interaction effects (P values) of phosphorus, potassium, and *Rotylenchulus reniformis* on egg and vermiform life stages on Stoneville LA 887 cotton after 60 days in a greenhouse environment^v.

Treatment	DF	Eggs per gram of dry root ^z	Vermiform life stages per 500 cm ³ soil ^z
Phosphorus (P) ^x	1	0.002**	0.010**
Potassium (K) ^y	1	0.054	0.469
Nematode (Nema) ^w	1	<0.001**	<0.001**
P x K	1	0.036*	0.074
P x Nema	1	0.002**	0.010**
K x Nema	1	0.054	0.469
P x K x Nema	1	0.036*	0.074

^vData combined over two 60 day duration trials with five replications per trial.

^wReniform nematode levels of 0 or 3000 vermiform life stages were used as inoculum.

^xPhosphorus levels were 0 or 112 kg ha⁻¹.

^yPotassium levels were 0 or 112 kg ha⁻¹.

^zData analyzed as a 2 x 2 x 2 factorial.

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

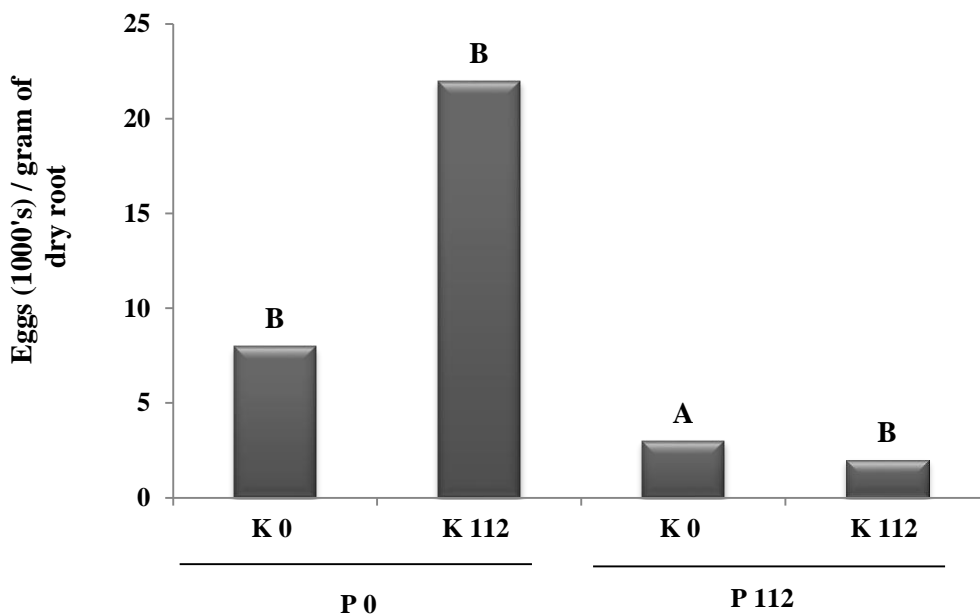


Figure 1. Reniform eggs per gram of Stoneville LA 887 cotton dry root with phosphorus levels at 0 and 112 kg ha⁻¹, respectively, in the absence or presence of potassium (K) at 0 and 112 kg ha⁻¹, respectively. Within each column, means followed by the same letter are not significantly different based on Tukey's HSD ($P \leq 0.05$).

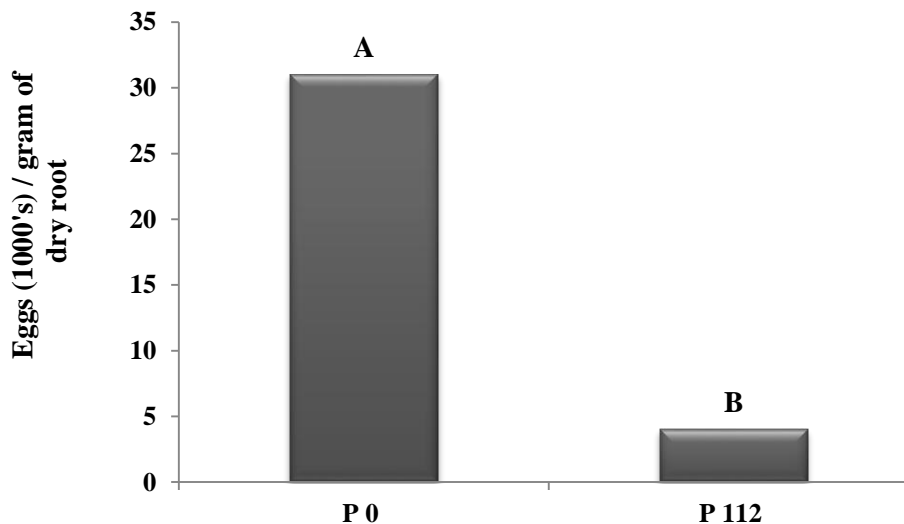


Figure 2. Reniform eggs per gram of Stoneville LA 887 cotton dry root in the absence or presence of phosphorus at 0 and 112 kg ha⁻¹ respectively. Within each column, means followed by the same letter are not significantly different based on Tukey's HSD ($P \leq 0.05$).

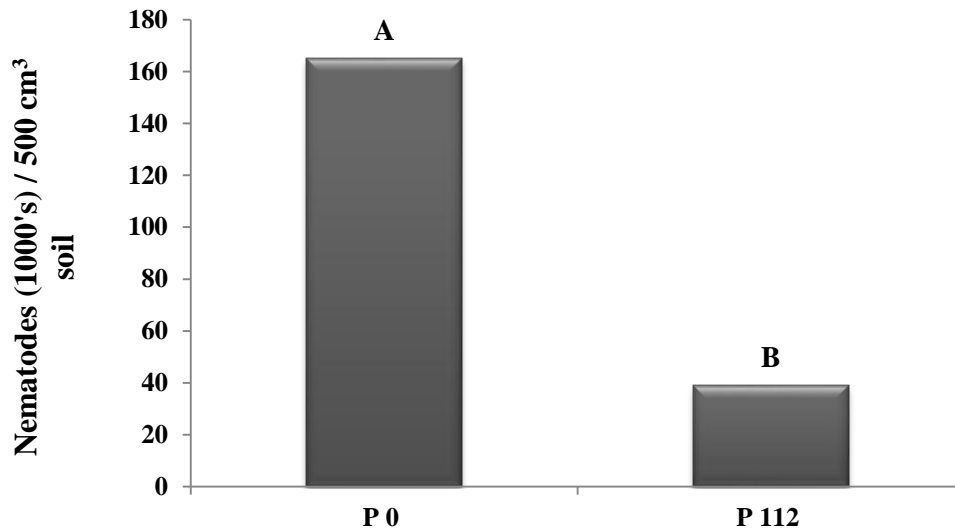


Figure 3. Vermiform nematode counts per 500 cm³ of soil in the absence or presence of phosphorus at 0 and 112 kg ha⁻¹, respectively, on Stoneville LA 887 cotton. Within each column, means followed by the same letter are not significantly different based on Tukey's HSD ($P \leq 0.05$).

Greenhouse Experiment 2

In the second greenhouse study, varying phosphorus (P) levels significantly affected shoot height and shoot and root dry weights (Table 3). At 15 days after inoculation, P treatments with 35, 60, and 73 mg kg⁻¹ showed a significantly higher increase in shoot height compared to P levels at 10 or 20 mg kg⁻¹. A similar trend was seen at 30 days after planting, but was not observed at 60 days. Dry shoot weights for P applied at 35, 60, and 73 mg kg⁻¹ averaged 14.4, 13.9, and 13.6 g, respectively, and were significantly higher than P applied at 10 or 20 mg kg⁻¹ which averaged 9.0 and 11.2 g, respectively. Root weights were significantly higher for P at the 73 mg kg⁻¹ rate (2.5 g) than either of the two lowest P rates (1.6 g for the 10 mg kg⁻¹ rate and 2.1 g for the 20 mg kg⁻¹ rate). Nematode infestation levels of 10,000 vermiform stages significantly affected shoot dry weight, but not root dry weight or shoot height. Nematode inoculation resulted in a difference of shoot weight observed as 13g for the absence of the nematode and 11.8 g when reniform was present. With increasing P levels from 35 to 73 mg kg⁻¹, there was a reduction in

egg counts compared to 20 mg kg⁻¹ P level (Table 4). Vermiform stages of reniform nematode averaged 359,771 for the P rate of 20 mg kg⁻¹ and was significantly higher than P at the 60 mg kg⁻¹ rate, which averaged 171,565 vermiform stages.

Table 3. Main and interaction effects (P values) of phosphorus and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^v.

Source	DF	Plant height 15 DAI ^v	Plant height 30 DAI	Plant height 60 DAI	Shoot dry weight(g) ^z	Root dry weight(g) ^z
Phosphorus (P) ^x	4	<0.001**	0.029*	0.131	<0.001**	<0.001**
Nematode (Nema) ^w	1	0.408	0.206	0.154	0.004**	0.307
P x Nema	4	0.624	0.843	0.647	0.565	0.131

^vData combined over two 60 day duration trials with five replications per trial.

^wReniform nematode levels of 0 or 10,000 vermiform life stages were used as inoculum.

^xPhosphorus levels were 10, 20, 35, 60, and 73 mg kg⁻¹.

^yDAI = days after inoculation.

^zData are dry weights obtained after two days at 45°C.

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

Table 4. Effects of phosphorus on eggs and vermiform life stages of *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^w.

Phosphorus treatment (mg kg ⁻¹) ^x	Eggs (1000's) per gram of dry root ^y	Vermiform life stages (1000's) per 500 cm ³ soil ^y	R value ^z
10	149ab	337ab	107.9
20	162a	359a	115.1
35	35b	251ab	80.5
60	24b	171b	54.9
73	45b	226ab	72.6

^wData combined over two 60 day duration trials with five replications per trial.

^xPhosphorus levels were 10, 20, 35, 60, and 73 mg kg⁻¹ considered by LSU AgCenter Soil Testing and Plant Analysis Laboratory to be very low, low, medium, high, and very high respectively.

^yWithin each column, means followed by the same letter are not significantly different based on Tukey's HSD (P≤0.05).

^zReproductive value (R), where R = P_f/ P_i (P_f is the final population density and P_i is the initial infestation level).

Greenhouse Experiment 3

Potassium (K) treatments in the third greenhouse study did not affect either shoot height or shoot and root dry weights (Table 5). Similarly, there was not a significant main effect of nematodes or the interaction of K x Nematode on any of the plant measurements. There also

were not any significant differences with increasing levels of K on either eggs per gram of dry root or vermiform stages in the soil (Table 6).

Table 5. Main and interaction effects (P values) of potassium and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^v.

Source	DF	Plant height 15 DAI ^v	Plant height 30 DAI	Plant height 60 DAI	Shoot dry weight (g) ^z	Root dry weight (g) ^z
Potassium (K) ^x	4	0.674	0.476	0.719	0.098	0.964
Nematode (Nema) ^w	1	0.281	0.558	0.643	0.206	0.634
K x Nema	4	0.973	0.920	0.680	0.701	0.927

^vData combined over two 60 day duration trials with five replications per trial.

^wReniform nematode levels of 0 or 10,000 vermiform life stages were used as inoculum.

^xPotassium levels were 44, 70, 106, 123, and 153 mg kg⁻¹.

^yDAI = days after inoculation.

^zData are dry weights obtained after two days at 45°C.

** indicate a P value significant at 0.01% level.

Table 6. Effects of potassium on eggs and vermiform life stages of *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^w.

Potassium treatment (mg kg ⁻¹) ^x	Eggs (1000's) per gram of dry root ^y	Vermiform life stages (1000's) per 500 cm ³ soil ^y	R value ^z
44	48a	154a	49.2
70	50a	174a	55.7
106	42a	148a	47.5
123	34a	170a	54.5
153	51a	165a	53.0

^wData combined over two 60 day duration trials with five replications per trial.

^xPotassium levels were 44, 70, 106, 123, and 153 mg kg⁻¹ considered by LSU AgCenter Soil Testing and Plant Analysis Laboratory to be very low, low, medium, high, and very high, respectively.

^yWithin each column, means followed by the same letter are not significantly different based on Tukey's HSD (P≤0.05).

^zReproductive value (R), where R = P_f/ P_i (P_f is the final population density and P_i is the initial infestation level).

Greenhouse Experiment 4

Sulfur (S) was the main nutrient evaluated in the fourth greenhouse study. Shoot height and dry shoot weight was significantly affected by S (Table 7). When S was applied at 50 mg kg⁻¹, plants were significantly shorter than the lowest rate of S (70.5 and 78.2 cm, respectively). Dry shoot weight followed the same trend for increasing rates of S (11.1 g for the very high rate and

13.6 g for the lowest rate). Nematodes had a significant main effect on plant height at 30 days after inoculation. Plant height was 59.4 cm in the absence of the nematode and 54.4 cm when reniform was present. A similar trend was seen 60 days after inoculation. There was no significance in the S X Nematode interaction in this experiment.

Table 7. Main and interaction effects (P values) of sulfur and *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^v.

Source	DF	Plant height 15 DAI ^y	Plant height 30 DAI	Plant height 60 DAI	Shoot dry weight (g) ^z	Root dry weight (g) ^z
Sulfur (S) ^x	4	0.090	0.213	0.020*	0.011**	0.245
Nematode (Nema) ^w	1	0.335	0.003**	0.004**	0.097	0.204
S X Nema	4	0.353	0.783	0.474	0.845	0.116

^vData combined over two 60 day duration trials with five replications per trial.

^wReniform nematode levels of 0 or 10,000 vermiform life stages were used as inoculum.

^xSulfur levels were 3, 12, 20, 40, and 50 mg kg⁻¹.

^yDAI = days after inoculation.

^zData are dry weights obtained after two days at 45°C.

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

Table 8 summarized the effect of S on reniform nematode eggs per gram of dry roots and vermiform life stages per 500 cm³ of soil observed in this experiment.

Table 8. Effects of sulfur on eggs and vermiform life stages of *Rotylenchulus reniformis* on Stoneville LA 887 cotton after 60 days in a greenhouse environment^w.

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

^wData combined over two 60 day duration trials with five replications per trial.

^xSulfur levels were 3, 12, 20, 40, and 50 mg kg⁻¹ considered by LSU AgCenter Soil Testing and Plant Analysis Laboratory to be very low, low, medium, high, and very high, respectively.

^yWithin each column, means followed by the same letter are not significantly different based on Tukey's HSD (P≤0.05).

^zReproductive value (R), where R = P_f/ P_i (P_f is the final population density and P_i is the initial infestation level).

Neither eggs per gram of dry roots nor vermiform life stages for 500 cm³ soil differed significantly among the various levels of S. Reproductive values ranged from 47.43 with S at 40 mg kg⁻¹ to 62.06 with S at 50 mg kg⁻¹.

Field Experiments

There were no significant main effects of phosphorus (P) and Sulfur (S) on nematode reproduction at any of the three sampling times in both years (Table 9). The soil fumigant 1,3-dichloropropene reduced reniform nematode populations at mid-season and harvest in 2011 and at planting in 2012. Mid-season and harvest levels of reniform nematode in 2011 were 16,873 and 108,642 per 500 cm³ of soil, respectively, for the fumigant and 32,730 and 172,000 per 500 cm³ of soil, respectively, in the untreated. Nematode populations at planting for 2012 were 107,700 per 500 cm³ of soil for the fumigant and 166,200 vermiform stages per 500 cm³ of soil for the untreated. There were no significant interactions on nematode population with the soil nutrients and the nematicide.

Table 9. Main and interaction effects (P values) of phosphorus, sulfur, and nematicide on population density of *Rotylenchulus reniformis* per 500 cm³ soil at three sampling intervals^v.

Interaction	DF	At-planting ^z		Mid-season ^z		At-harvest ^z	
		2011	2012	2011	2012	2011	2012
Phosphorus (P) ^w	1	0.391	0.705	0.195	0.503	0.674	0.072
Sulfur (S) ^x	1	0.831	0.810	0.438	0.918	0.847	0.720
Nematicide (1-3,D) ^y	1	0.094	0.014**	0.004**	0.173	0.008**	0.151
P x S	1	0.273	0.548	0.800	0.630	0.863	0.880
P x 1-3,D	1	0.555	0.091	0.981	0.319	0.167	0.572
S x 1-3,D	1	0.334	0.260	0.840	0.912	0.119	0.102
P x S x 1-3,D	1	0.899	0.583	0.681	0.166	0.707	0.088

^vData combined over two 5 month duration studies with six replications per trial.

^wPhosphorus levels were 44.8 or 112 kg ha⁻¹.

^xSulfur levels were 5.6 or 22.4 kg ha⁻¹.

^yNematicide used was 1,3-dichloropropene (1-3,D) at 28.1L ha⁻¹.

^zData analyzed as a 2 x 2 x 2 factorial.

Cotton cultivar was Phytogen 565 WRF.

** indicate a P value significant at 0.01% level.

Seed cotton yield was significantly affected by nematicide and S treatments in 2011 (Table 10). The high level of S actually reduced yield (1811.4 kg ha⁻¹ of seed cotton) compared to the low level (2032 kg ha⁻¹ of seed cotton). The nematicide significantly improved yield with the fumigant averaging 2099.4 kg ha⁻¹ of seed cotton compared to 1744 kg ha⁻¹ of seed cotton for the untreated. There were not any significant interactions of the nematicides and nutrients on yield in either years of the study.

Table 10. Main and interaction effects (P values) of phosphorus, sulfur, and nematicide on yield of Phytogen 565 WRF cotton ^v.

Interaction	DF	Seed cotton ha ⁻¹ ^z	
		2011	2012
Phosphorus (P) ^w	1	0.170	0.630
Sulfur (S) ^x	1	0.047*	0.324
Nematicide (1-3,D) ^y	1	0.001**	0.128
P x S	1	0.408	0.660
P x 1-3,D	1	0.900	0.475
S x 1-3,D	1	0.878	0.710
P x S x 1-3,D	1	0.214	0.988

^vData combined over two 5 month duration studies with six replications per trial.

^wPhosphorus levels were 44.8 or 112 kg ha⁻¹.

^xSulfur levels were 5.6 or 22.4 kg ha⁻¹.

^y Nematicide used was 1,3-dichloropropene (1-3,D) at 28.1L ha⁻¹.

^zData analyzed as a 2 x 2 x 2 factorial.

Cotton cultivar was Phytogen 565 WRF.

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

DISCUSSION

In many instances, cotton fields in Louisiana heavily infested with reniform nematodes also show nutrient deficiency symptoms. Therefore, these studies were conducted to: 1) evaluate the effects of soil nutrients on reniform nematode reproduction and pathogenicity under greenhouse conditions: and, 2) evaluate the interactions of phosphorus and sulfur nutrients with 1,3-dichloropropene under field conditions on cotton.

In the first greenhouse study, a phosphorus level of 112 kg ha⁻¹ increased plant shoot height by 52% and shoot and root dry weights by 75 and 69%, respectively. Related to this, there are reports of reductions in shoot and root development observed under conditions of limiting phosphorus availability (Anonymous, 2009b). In the second study, 60 mg kg⁻¹ of phosphorus resulted in a 55% increase in shoot dry weight and a 75% increase in root weight over that observed with 10 mg kg⁻¹. In both of these studies, the enhanced plant growth was accompanied by reductions in population of *R. reniformis*. Across both tests, egg densities were reduced by 85%, and vermiform life stages by 52 to 75%. Smith and Kaplan (1988), in their work with phosphorus and the burrowing nematode (*Radopholus citrophilus*), found results similar to those reported herein. That is, augmentation of soil phosphorus produced an increase in shoot and root heights of rough lemons, and also resulted in concomitant reductions in population densities of the nematode. This is further supported by the data of Waceke *et al.* (2002). Addition of 150 and 300 kg ha⁻¹ of phosphorus, as either triple super phosphate or single super phosphate, improved the shoot and root weights of pyrethrum plants infected with root-knot nematode (*Meloidogyne hapla*). Also, nematode disease severity and the density of eggs, juveniles, and females were reduced significantly.

Unlike the results for phosphorus discussed above, those for potassium produce no detectable effects on either plant growth or reniform nematode reproduction in these trials. Greenhouse studies by Gazaway *et al.* (1996), which evaluated increasing levels of potassium on cotton plant growth in the presence of reniform nematode, strongly support the conclusions of this research. That is, increasing potassium levels did not ameliorate damage caused by the nematode. Pettigrew *et al.* (2005), evaluated the potassium effects on cotton with identical levels 0 and 112 kg ha⁻¹, and found increased cotton growth and a 12% increase in reniform nematode population density. Their trials, however, were conducted under field conditions where there were likely a much greater variation in soil type, pH, moisture content, and possibly other agronomic factors.

Sulfur produced no detectable effects on nematode reproduction in either the greenhouse or field environment at relatively comparable rates. A single report from India indicates that sulfur negatively impacted populations of stunt and reniform nematodes on garlic under field conditions (Kassab and Hafez, 1990). In both environments of studies reported herein, sulfur nutrition negatively impacted plant growth. Specifically, plant height and shoot weight were reduced 10% and 15%, respectively, in the greenhouse, and cotton yield was reduced in the field trials significantly in 2011 and numerically in 2012. Data from other field studies involved in effects of sulfur nutrition on cotton yields have shown positive results (Júnior *et al.*, 2012; Yin *et al.*, 2011). None of these reports included data for reniform or any other nematode. Therefore, in all likelihood, these trials were conducted in an area where there was not a significant nematode infestation.

SUMMARY AND CONCLUSIONS

This research with *Rotylenchulus reniformis* and the common and important nutrients phosphorus, potassium, and sulfur has been investigated under greenhouse and field conditions over multiple years. The most notable conclusions from this research are:

- 1) Nutrient status does effect reproduction and pathogenicity of *R. reniformis* on cotton.
- 2) Of the three nutrients, phosphorus, potassium, and sulfur, phosphorus has the most pronounced effect on both nematode reproduction and cotton growth.
- 3) The effect of phosphorus on nematode reproduction was negative and averaged 74%, and the effects of cotton growth was positive and averaged 68% for shoot and root weights.

This research provides impetus for further investigation of the role of mineral nutrition in pathogenesis by nematodes and other soil borne pathogens.

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