12-15-2007

Occurrence of diseases and insect pests in select soybean (Glycine max (L.) Merr.) and sorghum (Sorghum bicolor (L.) Moench) rotations in Mississippi

Sergio Tomas Pichardo

OCCURRENCE OF DISEASES AND INSECT PESTS IN SELECT SOYBEAN (*GLYCINE MAX* (L.) MERR.) AND SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH) ROTATIONS IN MISSISSIPPI

By

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A Dissertation
Submitted to the Faculty of Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in Entomology and Plant Pathology
in the Department of Entomology and Plant Pathology

Mississippi State, Mississippi

December 2007

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Title of Study: OCCURRENCE OF DISEASES AND INSECT PESTS IN SELECT SOYBEAN (*GLYCINE MAX* (L.) MERR.) AND SORGHUM (*SORGHUM BICOLOR* (L.) MOENCH) ROTATIONS IN MISSISSIPPI

Pages in study: 107

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Field and greenhouse studies were conducted during 2004 through 2006 at the Rodney R. Foil Plant Science Research Center, Starkville, MS. Six sorghum and soybean rotation treatments were tested to determine their effect on plant pathogen, insect, and nematode diversity and density levels. Treatments included 1) continuous sorghum, 2) continuous soybean, 3) sorghum-soybean-sorghum rotation, 4) soybean-sorghum-soybean rotation, 5) sorghum-soybean-soybean rotation, and 6) soybean-sorghum-sorghum rotation. Several nematode and insect species were identified during the study, but were always below economic thresholds. Six insect species were identified on soybean during each growing season and used as the indicator species for this study. The most prevalent were threecornered alfalfa hopper (*Spissistilus festinus* (Say) and bean

leaf beetle (*Cerotoma trifurcata* (Forester). Sorghum webworm (*Nola sorghiella* Riley) and corn earworm (*Helicoverpa zea* (Boddie) were the most common insects on sorghum panicles. Rotations did not affect the diversity or density levels of the indicator soybean or sorghum insects during the three year study. Plant disease levels during the investigation showed variable results. Three foliar fungal pathogens including *Diaporthe phaseolorum* (Cooke & Ellis) Sacc. var. *meridionalis*, *Septoria glycines* Hemmi, and *Cercospora sojina* Hara on soybean, and *Gloeocercospora sorghi* D. Brain & Edgerton ex Deighton on sorghum were observed. The only virus disease on soybean was bean pod mottle, but levels were not affected by the rotations during the study. Zonate spot caused by *G. sorghi* was the most prevalent foliar sorghum disease, but was not affected by the rotations. Six frequently isolated fungal pathogens from either soybean or sorghum roots included *Macrophomina phaseolina* (Tassi) G. Goidanich, *Rhizoctonia solani* Kühn, *D. phaseolorum, Aspergillus* spp., *Trichoderma* spp and *Fusarium* spp. Aflatoxin contamination of sorghum seed was low (<20 ppb) the first two years of the study, but was high (790 ppb) in 2006. Significantly greater soybean and sorghum yields were obtained from rotated systems compared to monoculture systems in 2005. In a greenhouse test, *M. phaseolina* infection of soybean led to significantly greater root disease ratings, lower plant height and dry weight than the untreated control. Sorghum plant growth was not affected by *M. phaseolina* and *R. solani*.

DEDICATION

I would like to dedicate this research to my parents Rosendo Pichardo and Mercedes Guido de Pichardo, my wife María Argentina Loáisiga, my daughter Laura Belén, my two sons Sergio Josué and Alvaro Antonio, my brothers Peter Radell, Jose Manuel, Aurelio, Gregorio, Juan Carlos, María Elena, and Rosendo Pichardo. I appreciate the support of my family through this stressful time.

ACKNOWLEDGEMENTS

I would like to express my sincere thanks to my major professors, Dr. Richard E. Baird and Dr. Henry N. Pitre, for their support and guidance in this project. I would also like to thank my committee members: Dr. Gary Windham and Dr. Clarence Collison for their support during the course of this program. I also wish to thank the Department of Entomology and Plant Pathology for giving me the opportunity to pursue doctoral studies, and INTSORMIL (International Sorghum and Millet project) for providing the financial support for this research. My special thanks are extended to Dr. Mario E. Parada, Sandra Woolfolk, Sharon Vaughn, Mona Goodin, and Barbara Perrigin for their support. Finally, I would like to thank Dr. Johnson Zeledon, Dr. Frank Davis, Dr. William Moore, Dr. Peter Radell, Dr. Alan Henn, Dorgelis Villarroel and Dr. Dennis Salazar for their support and encouragement in making this personal goal a reality.

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Soybean

Soybean is a highly efficient producer of protein and oil, both of which are utilized for nourishment of animals and humans (Aldrich and Scott, 1970; Heatherly and Hodges, 1999). Soybeans were rated fifth economically in relation to other agricultural commodities in the United States (U.S), with an estimated value of $15.2 billion in 1998 (Wrather, 1999). According to Hymowitz (1989), the U.S. is the top producer of soybean in the world at 47%, followed by Brazil (19 %), China (11%) and Argentina (10%). Mississippi produced 1.3 billion kg of seed in 1999 (Anonymous, 1999). Soybean production for the 16 southern states in the U.S in 2001 was 6.4 million ha, with an average yield of 2,162 kg/ha. In 2001, a total of 514,350 ha were harvested in Mississippi with an average yield of 2,222 kg/ha.

Soybean is affected by a number of diseases with estimated losses at 26.9 percent in Mississippi (Koenning, 2001). Diseases of soybean are common in mid-southern states of the U.S., such as Mississippi, and can cause serious yield loss or seed quality problems. Major diseases include charcoal rot (*Macrophomina phaseolina* (Tassi) G. Goidanich), soybean pod and stem blight (*Diaporthe phaseolorum* (Cooke and Ellis) Sacc. var. *sojae* (S.G. Lehman) Whemeyer), soybean stem canker **(***Diaporthe phaseolorum* (Cooke and Ellis) Sacc. var. *caulivora* K. L. Athow and R. M. Caldwell), sudden death syndrome (*Fusarium solani* f.sp. *glycine* (Mart.) Sacc.), Phytophthora root rot (*Phytophthora megasperma* var. *sojae* (M. J.

Kaufmann and J. W. Gerdemann), seedling disease complex, and frogeye leaf spot (*Cercospora sojina* Hara). More than 100 species of plant-parasitic nematodes have been reported to feed on or be associated in some way with the roots of soybean plants, but only a few are of economic importance (Noel, 1999). Major nematodes attacking soybean include soybean cyst nematode (*Heterodera glycines* Ichinohe) and root-knot nematodes (*Meloidogyne* spp). Another serious problem in some years, called greenbean syndrome, remains a mystery as to cause, although some researchers believe stink bug feeding is one factor. Minor soybean diseases in the state include bacterial blight (*Pseudomonas syringae* pv. *glycinea* (Coerper) Young *et al.*), bacterial leaf spot (*Xanthomonas campestris* pv. *glycines* (Nakano) Dye), brown spot (*Septoria glycines* (Hemmi), anthracnose (*Colletotrichum dematianum* (Pers.: Fr.) Grove f.sp. *truncatum* (Schwein.) Arx.), target spot (*Corynespora cassiicola* (Berk. & Curt.) Wei), and downy mildew (*Peronospora manshurica* (Naum.) Syd. ex Gaum.) (Baird *et al.,* 2001; Anonymous, 2003; McGee *et al.,* 1980; Roy, 1976; Sinclair, 1992; Kukarek, 2001).

Pathogens that attack soybeans can cause seedling, foliar, pod and seed diseases. The term seedling disease is used to cover seed rot, pre- and postemergence damping-off, and root rot. Most soybean seedling diseases are caused by soilborne fungi, mainly *Pythium* spp. or *Rhizoctonia solani* Kühn, or by seedborne fungi such as those in the *Phomopsis/Diaporthe* complex (Fox *et al.,* 2003). *Pythium* spp. and *R. solani* may cause seedling disease symptoms, while the *Phomopsis/Diaporthe* complex is primarily responsible for seed rot and preemergence damping-off. Poor quality seed resulting from seed coat contamination by *Phomopsis/Diaporthe* and other fungi generally means lower germination and vigor. Cercospora leaf blight (purple seed stain) caused by *Cercospora kikuchii* (Tak. Matsumoto &

Tomoy) M. W. Gardner causes discoloration of the seed from violet to pale or dark purple. Lesions caused by *C. kikuchii* are usually confined to the upper two layers of the seed coat and do not affect bulk density or weight, however, seed quality is reduced. *Alternaria alternata* (Fr.:Fr.) Keissl and *Alternaria tenuissima* (Kunze:Fr.) Weltshire decay seeds and pods after senescence, or following frost damage, insect injury, or wounding. Alternaria seed decay is often associated with feeding by bean leaf beetle, and disease incidence appears to increase with insect damage (Sinclair, 1992). Thus, there is a greater chance of seed rot and a longer period between germination and seedling establishment. Cool (less than 20 °C), wet, poorly drained soils slow germination and the plant-growth processes favor many of the fungi that cause seedling disease (Roy and Abney, 1976; Pathan *et al.,* 1989; Pratt, 1995a; Anonymous, 2003 and Fox *et al.,* 2003).

Some economic insect pests of soybeans migrate from Central and South America into the continental U.S. Species that commonly colonize other crops and/or noncrop vegetation increase populations on these hosts during early season and may damage soybean when the primary host plants become unacceptable (Pitre and Porter, 1990).

Foliage feeding insects are present in practically all soybean fields during the growing season. Most of these pests have chewing mouth parts and cause a characteristic type of defoliation (Dively, 1986). Major insect pests of soybean include bean leaf beetle (*Cerotoma trifurcata* (Forester), beet armyworm (*Spodoptera exigua* (Hubner), green cloverworm (*Plathypena scabra* (Fabricius), cabbage looper (*Trichoplusia ni* (Hubner), soybean looper (*Pseudoplusia includens* Walker), fall armyworm (*Spodoptera frugiperda* (J. E. Smith), corn earworm (*Helicoverpa zea* (Boddie), tobacco budworm (*Heliothis virescens* (Fabricius), Mexican bean beetle (*Epilachna varivestris* Mulsant), yellow stripped armyworm

(*Spodoptera ornithogalli* (Guence), velvetbean caterpillar (*Anticarsia gemmatalis* Hubner), stripped blister beetle (*Epicauta vittata* (Fabricius), saltmarsh caterpillar (*Estigmene acrea* (Drury), southern green stink bug (*Nezara viridula* (Linn.), green stink bug (*Acrosternum hilare* (Say), and threecornered alfalfa hopper (*Spissistilus festinus* (Say) (Hammond, 1996a; Blaine *et al*., 1996).

Most economic losses from soybean arthropod pests in the southern U.S. result from injury to leaf blades and fruit. The major defoliating pests include bean leaf beetle, velvetbean caterpillar, soybean looper, cabbage looper, and green cloverworm. The major pests of fruit are the podworms (*H. zea* and *H virescens*) and the stink bug complex. The southern green and green stink bugs are the predominant stink bug species (Funderburk *et al*., 1989).

Sorghum

Grain sorghum (*Sorghum bicolor* (Linn.) Moench) is one of the most important cereal crops in the world (Zhu *et al.*, 1998). Sorghum is sometimes called "milo" (Nielsen and Johnson, 2003), and was introduced into the U.S. around 1850. In 1966-67, sorghum was grown on 5.6 million ha mainly in the Central and Southern Plains States. Yield for the two years averaged 3,385 kg/ha. Grain sorghum is grown on more than 16.4 million ha in countries such as China, India, and the African continent (Duke, 1983). According to Maunder (2002), the U.S. currently produces approximately 25 % of the world's crop. In the U.S. most of the grain sorghum is used as livestock feed, but in the Orient and Africa it is used primarily as food for humans (Magness *et al.,* 1971; Poehlman *et al*., 1995; Maunder, 2002). The main center of cultivated sorghum is in Africa; having been grown in Ethiopia for

more than 5,000 years. It is also possible that cultivated sorghums were also developed independently in India and China. Today, sorghum is widely distributed throughout the tropics, subtropics, and warm temperate areas of the world. It is the fourth most important cereal grain world-wide, following wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.) and corn (*Zea mays* L.).

In the U.S., grain sorghum is grown as a feed for livestock in areas of the Great Plains that are too hot and dry for growing corn. By growing productive hybrids with high soil fertility and irrigation, the U.S. harvests about one-fourth of the world production of grain sorghum on 10 % of the world's area planted to the crop (Poehlman *et al*., 1995). Sorghum's importance as a feed grain has increased in the U.S. and is very important in the world's human diet, with over 300 million people dependent on it as food (Bukantis; 1980; Vanderlip, 1993).

Grain sorghum plants are coarse annual grasses. Nearly all of the varieties grown in the U.S. are "dwarf" types, with stems under 1.52 m in height and suitable for harvesting with combines (Magness *et al.,* 1971). In other countries, taller-stemmed varieties are grown. Leaves are relatively broad, have numerous but small stomata, and are covered with a waxy bloom. The leaves tend to roll along the midrib under moisture stress. Thus, the plant is more drought resistant than most other grains and requires less water per 0.45 kg of dry matter (Magness *et al.,* 1971; Nielsen and Johnson, 2003). The plants are well suited to drought stressed soils or conditions often considered marginal for corn and is adapted to a wider range of soil types (Kimbrough, 2002). Sorghum can be planted later than corn and still have reasonable yield.

Since yields vary yearly, averages over three or more years are better indicators of variety performance than single year results (Baskin *et al.,* 2003). Sorghum varieties differ in maturity since they have inherited differences in response to light and temperature. Many of the varieties grown in Mississippi are intermediate maturing, usually flowering 60 to 70 days after emergence (Baskin *et al.,* 2003). Varieties are available that will flower in less time, but as a rule, they do not yield as well. Later flowering varieties are also available, but neither early nor late-maturing varieties are as well adapted to most areas in Mississippi as are the intermediate flowering varieties (Baskin *et al.,* 2003).

Sorghum is distinguished among cereals by its broad range of diseases. The diversity of its uses and the range of environments in which it is cultivated ensure that the plant is constantly challenged by pathogens and abiotic diseases (Duke, 1983). In areas where sorghum is traditionally grown, plants may be attacked by as many as five or six foliar pathogens, an array of soilborne organisms, one or more viruses, a phytoplasma, at least two systemic fungal pathogens, and several panicle fungal pathogens. Overlap of disease symptoms is common. Variation in maturity, plant height, pigmentation (both seed and plant), and other morphological characteristics affect disease expression and complicate accurate diagnosis (Dahlberg and Frederiksen, 2000).

Major diseases reported on sorghum include gray leaf spot (*Cercospora sorghi* Ellis & Everth.), anthracnose (*Colletotrichum graminicola* (Ces.) G. W. Wilson), leaf blight (*Exserohilum turcicum* (Pass.) K. J. Leonard & E. G. Suggs), zonate leaf spot (*Gloeocercospora sorghi* D. Brain & Edgerton ex Deighton), bacterial leaf strip (*Burkholderia andropogonis* (Smith) Gillis *et al*.), charcoal rot (*Macrophomina phaseoli* (Tassi) G. Goidanich), milo disease (*Periconia circinata* (L. Mangin) Sacc.), tar spot

(*Phyllachora sorghi* Höhn), rust (*Puccinia purpurea* Cooke), sooty strip (*Ramulispora sorghi* (Ellis & Everth.), downy mildew (*Sclerospora sorghi* W. Weston & Uppal), pokkah boeng (*Fusarium moniliforme* J. Sheld), long smut (*Sorosporium ehrenbergii* Vanky), ergot (*Sphacelia sorghi* McRae, anamorph of *Claviceps africana* Frederiksen), covered kernel smut (*Sphacelotheca sorghi* (Link) G. P. Clinton), loose kernel smut (*S. cruenta* Kühn), and head smut (*S. reiliana* (Kühn) Langdon & Fullerton.

Nematodes causing economic losses to sorghum include *Helicotylenchus cavenessi* Sher, *H. dihystera* (Cobb) Sher, *H. pseudorobustus* (Steiner) Golden, *Hoplolaimus pararobustus* (Schuurmans Stekhoven & Teunissen) Sher, *Meloidogyne incognita* (Kofoid and White) Chitwood, *M. naasi* Franklin, *M. javanica (Treub) Chitwood*, *Peltamigratus nigeriensis* Sher, *Pratylenchus zeae* Graham, *P. hexincisus* Taylor and Jenkins, *P. brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven,, *Quinisulcius acutus* (Allen) Siddiqi, *Rotylenchulus reniformis* Linford & Oliveira, *Scutellonema cavenessi* Sher, *S. clathricaudatum* Whitehead, and *Tylenchorhynchus annulatus* (Cassidy) Golden (Duke, 1983; Dahlberg and Frederiksen, 2000; De Waele and MacDonald, 2000a).

Diseases of sorghum, like those of other crops, vary in severity from year to year and from one field to another, depending upon environment, causal organisms, and the host plant's resistance. Very few foliar diseases are serious problems in Mississippi (Baskin *et al.*, 2003). Stalk and head diseases present the major problems. Anthracnose is a major and commonly occurring disease which can have devastating consequences. Charcoal rot is also a problem following periods of hot, dry weather during plant development. *Fusarium* spp. are associated with sorghum throughout its life cycle from seed to senescence. These fungi also are associated with seedling blight, root and stalk rot, pokkah boeng, grain mold, storage

diseases, and mycotoxicoses. Some species of *Fusarium* associated with these diseases are *F. equiseti* (Corda) Saccardo, *F. graminearum* Schwabe, *F. solani* (Mart.) Saccardo, *F. thapsicum* J. F. Leslie *et al., F. moniliforme* J. Sheld. and *F. equiseti* which is almost always associated with root and stalk rots and is distributed throughout all sorghum growing areas. Fusarium stalk rot and head blight can cause severe yield losses. Stalk rot is usually accompanied by extensive root damage. Fusarium stalk rot is more of a problem when cool, wet weather follows hot, dry conditions. Maximum tillage, high nitrogen fertilization, high plant populations, and continuous cropping to sorghum seem to increase *Fusarium* spp. problems. Fusarium head blight affects the upper stalk and head. Losses caused by *Fusarium* spp. vary from 5-10 % but may approach 100 % in localized areas. Yield reductions are generally attributed directly to poor filling of kernels and to weakened or lodged peduncles, or indirectly to lodging and stalk breakage that hinder harvesting operations. Also, pathogen infections of sorghum kernels often are associated with panicle feeding insects, especially kernel sucking bugs. Often kernels become off-colored, sometimes black, because of pathogen infection associated with insect feeding. Good cultural practices are the best ways to minimize most disease problems (Baskin *et al.,* 2003; Claflin, 2000; Frederiksen, *et al.,* 1991).

Beside diseases, numerous insect pests may attack sorghum in Mississippi. Most important are southern corn rootworm (*Diabrotica undecimpunctuata howardi* (Barber), chinch bug (*Blissus leucopterus leucopterus* (Say), corn leaf aphid (*Rhopalosiphum midis* (Fitch), fall armyworm (*Spodoptera frugiperda* (J. E. Smith), southwestern corn borer (*Diatraea grandiosella* (Dyar), lesser cornstalk borer (*Ellasmopalpus lignosellus* (Zeller), corn earworm (*Helicoverpa zea* (Boddie), sorghum webworm (*Nola sorghiella* (Riley), and

sorghum midge (*Stenodiplosis sorghicola* (Coquillet) (Hamer and Pitre, 2003; Stewart, 2003).

Insect problems in sorghum vary in different areas and growing season (Brooks, 1998). According to Pitre (1985), many of the sorghum pest species that attack the crop occur throughout the U.S. Relatively little attention has been given to the detailed study of many pest species that attack sorghum. However, there are insect pests that attack the crop while it is in the field and cause economical losses.

Crop Rotation

Sorghum and soybeans may be planted in the same cropping system. However, monoculture production of soybeans or grain sorghum generally results in declining grain yields (Roder *et al.,* 1988). Roder *et al*. (1989) reported that soybean root densities were consistently greater when the previous crop was grain sorghum rather than soybean. Grain sorghum as a previous crop, not only resulted in increased root density, but also reduced root diameter. With a reduction in root diameter, the surface area to weight ratio increased. A plant that can produce a larger root surface while reducing root requirements for energy and nutrients could increase above-ground production (Roder *et al.,* 1989).

Soybean is a crop well adapted to many cropping systems throughout most of the U.S. Utilization of soybean in rotations, intercropping, and double-crop systems have increased as farmers investigate ways to reduce chemical and fertilizer inputs (Varvel and Peterson, 1992).

Crop rotation refers to the growing of different crops in a regular sequence and has been shown to increase crop yields. The cause of the higher yields is related to either

increased soil fertility, improved soil physical properties, improved weed control, reduced incidences of diseases and insect pests, or various other factors (Wesley *et al*., 1991). Two Kansas State University studies have documented yield benefits by planting soybeans as part of a sorghum and soybean rotation, rather than keeping a field in continuous sorghum (Peter, 2002).

Cropping systems for grain sorghum vary throughout growing areas, where strategies consist of continuous grain sorghum production and numerous combinations of rotations (Cothren *et al*., 2000). Areas that have adequate rainfall or irrigation may practice continuous sorghum planting as long as yields are maintained at a high level with optimum management practices.

Many reasons exist for using crop rotation, including more effective utilization of resources, risk aversion to weather or prices, reduction of weed, disease, and insect problems, improving soil physical conditions, and utilizing residual nutrients (Jardine, 1998). Results of long-term research in Texas on the benefits of rotating grain sorghum with cotton or soybean indicate sorghum yield enhancements of 26 % with cotton in a 1-year cotton-1-year sorghum rotation, or 2-year cotton rotation scheme. Sorghum rotation with soybean resulted in a 67 % grain yield increase or a 2,318 kg/ha yield advantage over monoculture sorghum when nitrogen fertilizer was withheld (Cothren *et al.,* 2000). The total eradication of diseases in sorghum is not economically feasible, so growers must try to minimize this damage through an integrated pest management system. Planting resistant hybrids, providing optimum growing conditions, rotating with other crops, removing infested debris, planting disease-free seed, proper seedbed preparation, and accurate application of herbicides, insecticides, and fungicides are all methods that can be used to minimize losses from diseases (Jardine, 1998).

Most diseases manageable with rotations are caused by root-or crown-infecting, soilborne pathogens (Baird *et al*., 1996b). These organisms usually spread and increase slowly, so reduction of inoculum levels can have a significant impact on disease development. Crop rotation is an important tool for managing some diseases. If a pathogen does not survive for more than a few years in the absence of a host plant or residue, then rotation to a nonhost crop could be an effective way of reducing disease levels (Zalom and Morse, 1990). Rotations are helpful for controlling some foliage and stem pathogens that survive primarily on host debris in the soil. However, rotations are generally not sufficient by itself to manage diseases that can be wind disseminated or can multiply rapidly when conditions are favorable.

According to Francis *et al*. (1989), conventional wisdom among both researchers and farmers is that crop rotation generally reduces pest problems from insects, plant pathogens, nematodes, and even some weed species. This is generally accomplished by interrupting the reproductive cycle of the crop pest by changing the habitat. Different pests may be found on or with different crops, since they may have specific host ranges. Weeds are somewhat different, since weed seeds of some species can survive for a number of years in the soil, and prevalent weeds will often be associated with a range of crops. Yet the principle applies that crop rotation appears to reduce problems from less mobile pest species. Sumner (1982) and Ware (1996), report that crop rotation is an effective measure in controlling insects that are restrictive in their feeding habits, or have a small and specific host range for reproduction, or that do not move very far when they are feeding. Francis *et al.* (1989) showed that many insect pest problems can be solved or at least managed effectively by crop diversity and rotation. Corn rootworm (*Diabrotica* spp.) can be managed effectively by rotating corn with

soybeans or alfalfa, thus avoiding the need for chemical control. Crops of the same type tend to have similar pests and similar water and heat requirements, and can be considered suitable substitutes when measuring diversity (Beck *et al.,* 1998). Highly dissimilar crops in a rotation will help promote the control of certain crop pests (Brooks, 1998). According to Baird *et al*. (1996b), thorough consideration of crops to be used in a rotation is needed to control target pests.

Farmers around the world practice crop rotation and the benefits are generally known and accepted. Although the benefits of crop rotation have been widely observed, the mechanisms of why these occur are still poorly understood (Francis *et al.,* 1989). Rotation as a management tool is very important for minimizing crop losses from nematodes and other pathogens. Crops such as corn, sorghum, wheat, barley (*Hordeum vulgare* L.), rye (*Secale cereale* L.), oats (*Avena sativa* L.) and alfalfa (*Medicago sativa* L.) suppress different soybean pests. Rotations seem to have the greatest benefit keeping pests in check rather than as a corrective measure (Baird *et al.,* 1996b).

Crop rotation is much more successful for root-infecting, specialized pathogens that can not survive saprophytically in the soil for one or more years (Sumner, 1982). In contrast, root-inhabiting pathogens that can survive saprophytically in the soil on organic matter are not effectively controlled using crop rotation (Sumner, 1982). Baird *et al.* (1996b) and Davis *et al.* (2001) report that mono-cropped fields often develop severe problems with nematodes and/or pathogens. A classic example of a problem from non-rotation was the appearance of the soybean cyst nematode in the 1970s. Soybean was normally planted in a field no more than once every three or four years prior to the 1940's. During the 1970s-1980s soybeans were monocultured in fields not previously planted to peanut (*Arachis hypogaea* L.) or

tobacco (*Nicotiana tabacum* L.). Within three to four years, yields in some fields began to decline due to soybean cyst nematode population increases (Baird *et al.,* 1991). Soybean cyst nematode resistant varieties should never be planted continuously, because new races of the nematode can develop that are capable of reproducing on the resistant varieties (Baird *et al.,* 1991; Wang *et al.,* 2003).

Macrophomina phaseolina is a soilborne pathogen that causes charcoal rot of soybean (Surrette *et al*., 2006). Charcoal rot of soybean and grain sorghum is an important problem in the southern United States, Mexico, and Africa. This fungus can infect over 500 different species of plants, including important agronomic crops such as soybean, corn, grain sorghum and sunflowers. The fungus survives between crop plantings as sclerotia. Viability of sclerotia can be maintained for up to 16 months. Sclerotia are released into the soil as plant debris decays. Sclerotia germinate under conditions of high soil temperature (30^{o}C or higher) and low soil moisture. Infection hyphae produced from sclerotia grow through the soil and infect underground plant parts (Partridge, 1997; Burgess *et al*., 2002; Jardine *et al*., 2003).

Stalk rots, including charcoal rot are considered some of the most serious diseases of corn and sorghum. Charcoal rot is a prevalent disease throughout sorghum growing areas (Frederiksen, 1986; Pratt, 1995b). The causal agent of this disease is extensively distributed in soils worldwide. In the southern states of the U.S, the highest estimated loss in soybean caused by charcoal rot was 3.4 % in 1999. In Mississippi in that year, total yield reduction caused by all diseases was 18.4 %, and charcoal rot accounts for 14 % of this total (Koenning, 2001). Yield losses due to charcoal rot are difficult to quantify because the disease is closely associated with other stress factors like nematode infestations, even at low levels of infestation, since infections interfere with water and nutrients uptake and transport.

Plants with root rot are more severely affected by dry weather than those with a healthy root system (Bain, 1965). In addition, plant pathogenic nematodes can lead to higher disease incidences by providing entrance sites for *M. phaseolin*a (Tu and Chen, 1971; Siddiqui and Husain, 1991; Nischwitz *et al*., 2002).

Aflatoxin

Aflatoxin is a naturally occurring toxin produced by the fungus *Aspergillus flavus* Link (Windham and Williams, 1999). Aflatoxins were first identified as the cause of a severe outbreak of "Turkey X" disease, a toxicosis that killed more than 100,000 turkey poults in England in 1960 (Asplin and Carnaghan, 1961). According to Castegnaro and McGregor (1998), this toxin is the most potent carcinogen found in nature. Aflatoxins produced by *A. flavus* are commonly found in human and animal foods including corn, cottonseed, peanut, and tree nuts. In the southeastern U.S., aflatoxin contamination of corn is a major problem (Payne, 1992; Widstrom, 1996). The Food and Drug Administration's action threshold for aflatoxin is 20 parts per billion (ppb) (Crenshaw, 2002; Nicholson, 2003).

The occurrence of insect pests and plant pathogens in soybean and sorghum rotations in Mississippi has not been reported. Therefore, the objectives of this research were to:

1) determine the effect of soybean-sorghum rotations on insect pest diversity, density, and seasonal incidence,
2) determine the effect of soybean-sorghum rotations on the incidence and severity of root, stem, and foliage diseases,
3) determine the effect of soybean-sorghum rotations on aflatoxin contamination of

sorghum grain, and

4) compare the effect of *M. phaseolina* and *R. solani* separately on soybean and sorghum in the greenhouse.

CHAPTER II

MATERIALS AND METHODS

Field studies to determine the effects of soybean and sorghum crop rotations on plant diseases and insect pests were conducted over a three year period from 2004 through 2006 at the Rodney R. Foil Plant Science Research Center, Mississippi State University, Starkville, MS. The trials were planted in a Leeper silty clay loam, fine smectitic, monoacid, thermic Vertic Epiaquemts soil type (Vaughan *et al*., 2002). The trials were established on May 12 of each year and all land preparation, fertilization, and weed maintenance were based on current recommendations of Mississippi State University Extension Service for growing soybean and sorghum (Funderburg *et al.,* 2003; Baskin, *et al*. 2003; Blaine, 2002a; Blaine, 2002b). The first experiment in 2004 was conducted to collect baseline data.

Plot Design

Six crop planting systems were evaluated to determine their effects on plant disease levels and insect pest diversity and density. The treatments included 1) continuous sorghum, 2) continuous soybean, 3) sorghum-soybean-sorghum rotation, 4) soybean-sorghum-soybean rotation, 5) sorghum-soybean-soybean rotation, and 6) soybean-sorghum-sorghum rotation. Treatments had four replications planted in a completely random design. The hybrid sorghum cultivar Terral TV1050 and soybean variety Pioneer 95B96 (Maturity Group V) were planted each year of the study. Both varieties were reported to be susceptible to *M. phaseolina* (A.

Blaine and E. Larson, pers. comm.). Individual plots were 16 rows wide (15.6 m) by 21.3 m long (15.6m X 21.3 m) with 0.97 m row spacing. Soybean was planted at a rate of 8 seed per 30.5 cm of row using an Almaco® cone planter (Nevada, Iowa). Sorghum was planted at a rate of 5 to 6 seeds per 30.5 cm of row using the same planter.

Data Collection

Field data obtained during the study included plant stand counts, plant heights, root, stem and foliar disease ratings, insect infestations and developmental stages, and nematode species and populations. Stand counts were taken and seedling disease determinations were made at 10, 17, and 24 days after planting (DAP). Ten plants from each row were used for each measurement on each sampling date. Stand count was taken from the two central rows of each plot. Soybean and sorghum foliar disease ratings were taken at first, second, third and fourth months after planting from the same general area within the rows used to sample for incidence of seedling disease. Flags were used to mark sample sites so that the same 10 plants were rated for diseases during the different sampling periods. Insects were collected using different sampling methods which included visual, drop cloth, and sweep net.

Nematode Assay

Soil samples were collected in May and August of each year in all plots to determine nematode levels. Twenty five core samples were randomly taken at a depth of 20 cm in each plot using a "M" pattern. Nematodes were extracted from 100 cm^3 subsample of soil using the semi-automatic elutriator method (Cardenas and Nagler, 2004; Heinz, 2005), and were

identified at the Plant Pathology and Nematology Diagnostic Clinic, Mississippi State University, Starkville, MS.

Soybean Diseases

Root and Hypocotyl Disease Ratings

Rating scales for root and hypocotyl diseases of soybean were based on symptom severity of infected roots and hypocotyl tissues (Baird *et al.*, 1996a). Ten plants were lifted from rows 5 and 12 of each plot 15 and 32 days after emergence, and at R7 (beginning maturity) for soybean and R9 (physiological maturity) for sorghum. Plant roots were washed under running tap water for 5 minutes, dried on paper towels and then assigned a disease rating. Disease severity was estimated using a rating scale of 0 to 5 modified from Baird *et al.*, (1996a) (Table 1). A 1 cm piece of discolored root tissue was removed from each soybean root sample to isolate and identify potential fungal pathogens. If no discolored tissue was available, a piece of apparently healthy tissue was removed at random from the sampled root. The root pieces were surface sterilized in sodium hypochlorite (w/v 0.52 %) for 2 minutes and then placed in 100x10mm Petri dishes to isolate fungi on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI). The PDA was autoclaved for 15 minutes and then allowed to cool at room temperature. After cooling, 0.25 ml of Danitol® solution (fenpropathrin) (miticide), 0.5 ml of chlortetracycline (antibiotic), and 2.5 ml of streptomycin sulfate (antibiotic) were added to the medium. Approximately, 9 ml of PDA were then poured into each Petri dish. The dishes containing root tissue were incubated for 7 days in the laboratory at room temperature. During that time, all fungi growing from the tissue were subcultured onto PDA for later identification.

Table 1. Rating scale [a] for root and hypocotyl diseases of soybean and sorghum, and root disease index formula.

Rating	Scale description
0	No root symptoms (N = Number of asymptomatic plants x 0)
1 =	<2% discoloration and necrosis on roots (VS = Number of plants with very slight symptoms x 1)
2 =	2 to 10 % discoloration and necrosis on roots (SL = Number of plants with slight symptoms x 2)
3 =	11 to 50 % discoloration and necrosis on roots (MO = Number of plants with moderate symptoms x 3)
4 =	>50 % discoloration and necrosis on roots (SE = Number of plants with severe symptoms x 4)
5 =	Plants dead or dying (D/d = Number of dead or dying plants x 5)

$$\text{Root Disease Index} = \frac{(N \times 0) + (VS \times 1) + (SL \times 2) + (MO \times 3) + (SE \times 4) + (D/d \times 5)}{\text{Total number of emerged plants}}$$

[a] Modified from Baird *et al*., 1996a.

Foliar Diseases

Soybean foliage disease ratings were taken during the first week of each month after planting until senescence. Ratings of disease incidence and severity were based on symptom appearance using the rating scales of Fox *et al.* (1996) and W. F Moore. (unpublished) (Tables 2, 3, 4). A subjective rating scale of 0 to 4 was used to rate frogeye leaf spot, Cercospora leaf blight, and Rhizoctonia aerial web blight where 0 represented no disease and 4 the most advanced disease symptoms (W. F. Moore, unpublished). Brown spot, downy mildew, Alternaria leaf spot**,** and bacterial leaf spot symptoms were scored using the rating

scale for Phytophthora severity or sudden death syndrome incidence described in Table 2. Diseases were rated the second week of June and continued monthly until plant senescence. Soybean variety reactions to disease incited by soybean mosaic virus or bean pod mottle virus were rated using the virus rating scale described in Table 5 (See virus section below).

Table 2. Soybean disease rating scales [a] for Phytophthora root and stem rot, and sudden death syndrome on soybean.

Phytophthora rating: Severity as indicated by stand density		Sudden Death Syndrome rating: Incidence and severity			
Rating scale		Incidence		Severity rating	
1 =	0 -10 %	1 =	0 -10 %	0 =	No symptoms
2 =	11-20 %	2 =	11-20 %	1 =	Leaves with yellow spots
3 =	21-30 %	3 =	21-30 %	2 =	Leaves with necrotic lesions
4 =	31-40 %	4 =	31-40 %	3 =	Defoliation occurs
5 =	41-50 %	5 =	41-50 %	4 =	Pods falling
6 =	51-60 %	6 =	51-60 %		
7 =	61-70 %	7 =	61-70 %		
8 =	71-80 %	8 =	71-80 %		
9 =	81-90 %	9 =	81-90 %		
10 =	91-100 %	10 =	91-100 %		

[a] Fox *et al*., 1996.

Table 3. Soybean disease rating scale [a] for stem canker on soybean.

Rating	Scale description
0.0 =	No foliage or stem symptoms
1.0 =	Discrete stem lesions up to 2 mm (about 1/8 inch)
1.2 =	1 to 2 % of plants with foliar symptoms and large stem or crown canker
1.5 =	5% of dead plants with prominent leaf symptoms, usually with additional plants showing stem lesions
2.0 =	25 % of plant showing foliage symptoms and/or dead plants
2.5 =	50 % of plants showing foliage symptoms and/or dead plants
3.0 =	75 % of plants showing foliage symptoms and/or dead plants
4.0 =	100 % of plants showing foliage symptoms and/or dead plants

[a] Modified from W. F. Moore, Mississippi State University, unpublished.

Table 4. Rating scale [a] for soybean diseases frogeye leaf spot, Cercospora leaf blight (purple seed stain) and Rhizoctonia aerial web blight.

Rating	Scale description
0 =	No disease present
1 =	Disease present, but prevalence with low severity, apparently causing little damage
2 =	Intermediate symptoms, estimated leaf area destroyed up to 25 %; disease appears to be of economic importance
3 =	As in 2, but over 25 % of leaf area destroyed
4 =	Most advance symptoms; death of leaves or plants due to disease

[a] Modified from W.F. Moore, Mississippi State University, unpublished.

Confirmation of Soybean Pathogens

Randomly selected plant tissues exhibiting disease symptoms were taken to the laboratory and fungal pathogens were determined using standard macroscopic and microscopic characteristics. Fungi were identified based on conidiogenesis according to standard mycological references (Barnett and Hunter, 1986; Barron, 1972; Domsch *et al.*, 1980; Ellis, 1971; Nelson *et al.*, 1983; Roy *et al.*, 2001). If spores were not available, soybean tissues were placed into moist chambers for 3 to 5 days to induce asexual or sexual reproduction. Leaf and stem tissues were placed in Petri dishes (100 × 10 mm) on sterile filter paper (Whatman's # 4) that was moistened with sterile distilled water. The dishes were sealed with parafilm and held in the laboratory at room temperature. After 3 to 5 days, the plant tissue samples were observed for sporulation and identifications using standard identification keys. Confirmation of the plant pathogen identifications was made by Dr. Richard Baird, plant pathologist, Mississippi State University.

Viruses

Visual ratings for soybean mosaic and bean pod mottle diseases (Table 5) were made on 10 plants from the centered two rows of each plot. The sample sites were located within the center 10 m (the area between 5 m and 15 m from the front of each plot) of the two center rows. The ten plants in each row were flagged to ensure that the same plants were rated monthly.

Enzyme B Linked Immunosorbent Assay (ELISA) test kits developed by AGDIA (AGDIA Inc., Elkhart, IN, 2001) were used to identify soybean mosaic virus and bean pod mottle virus. These methods were used when visible soybean mosaic symptoms and bean

pod mottle virus symptoms were detected in the plots. In August of each of the three years, plants showing virus symptoms were randomly selected from each plot and foliage was collected from the terminals of the plants. The leaves were placed into plastic bags and stored at 4° C until assayed. A minimum of ten replicate plants per plot for all treatments were tested using ELISA. If less than ten plants per plot had visible symptoms, only foliage from plants showing virus symptoms were tested.

Table 5. Soybean virus disease rating scale [a] on soybean.

Rating	Scale description
0 =	No foliage symptoms
1 =	Leaves crinkled
2 =	Leaf shape distorted, with some chlorosis
3 =	Shape distortion and chlorosis severe

[a] Modified from Fox *et al*., 1996.

Soybean Insects

Foliage samples were taken every 14 days after soybean plant emergence until early senescence to determine insect pest diversity, density, and seasonal incidence in treatment plots. Samples were collected using three methods including 1) visual, 2) ground cloth, and 3) sweepnet (Kogan and Pitre, 1980; Blaine *et al*., 1996**)**. Plants in early vegetative stages (V1-V3) in rows 3, 6, 12, and 15 were visually examined at each of four locations in the center of each plot (10 m). Plants in each location were flagged so that the same ten plants in each row could be revisited for sampling on each sampling date. A ground cloth sampling

method was used to sample plants in stages V4-R1 (fourth nodes-beginning bloom). The cloth (a heavy white cloth, 0.97 m^2) was placed on the ground at sample sites between rows 2-3, 5-6, 11-12, and 14-15 for sampling 5 m successive distances inward from the front of each plot. Samples sites were flagged so that no area was sampled more than once. Plants on either side of the row were bent over the cloth and shaken vigorously. Insects on the foliage were dislodged from the plants onto the cloth and soil and were counted and recorded. The third method for sampling soybean foliage employed a sweepnet (0.38 m diameter) for sampling R2-R7 (full bloom-beginning maturity) plant growth stages. A total of 25 sweeps were made in each location within plots (identified above) on rows 4, 6, 11 and 13 for the first samples. Subsequent samples were made on predetermined rows not sampled previously by other methods. All insects not identified in the field and needing further identification were placed into vials with 70% ethanol and taken to the laboratory. Identifications were made using specific taxonomic keys for identification of soybean insects (Curran *et al.*, 1993).

Sorghum Diseases

Root and Hypocotyl Diseases

Methods for evaluating root and hypocotyl diseases of sorghum were the same as for soybean (Table 1). Sorghum foliage disease ratings were taken during the first week of each month until senescence.

Stalk Rot

Data were collected at approximate physiological maturity (stage 9) of sorghum or when the grain was completely mature. Incidence of charcoal stalk rot can be accurately assessed using the stalk crushing evaluation technique (Frederiksen *et al.,* 1991). Near normal sized stalks of standing or lodged plants killed by charcoal rot will crush easily between the forefinger and thumb. Disease confirmation was made by splitting 50 randomized stalks per plot for each treatment (Frederiksen *et al.* 1991). Ten plants from each of rows 4, 6, 11, 13, and 15 were sampled at 5 m successive distances inward from the front of each plot.

Foliar Diseases

Foliar diseases that were observed during this investigation included zonate leaf spot, gray leaf spot, and physiological or genetic spotting. Incidence and severity of foliar diseases was recorded monthly during the growing season using a rating scale of 0 to 5 (Table 6). Twenty plants from each of rows 3, 5, 11, 13, and 15 were sampled for foliar diseases as described for stalk rot.

Confirmation of Sorghum Diseases

Sorghum diseases were confirmed using methods as discussed for soybean diseases. In addition, Dr. Richard Baird, plant pathologist, Mississippi State University confirmed the plant pathogen identifications.

Table 6. Rating scale [a] for foliar diseases of sorghum.

Rating	Scale description
0 =	No foliar disease symptoms
1 =	resistant – disease inconspicuous or present on an occasional plant
2 =	Disease present (over 50 % prevalence with low severity; apparently causing little damage)
3 =	Disease severe (100% prevalent, estimated leaf area destroyed up to 25 %; disease appears to be of economic importance)
4 =	As in 3 but over 25 % of leaf area destroyed
5 =	Death of leaves or plants due to disease

[a] Zummo scale modified by Frederiksen *et al*., 1991.

Sorghum Insects

Sorghum insects were collected weekly from five selected locations within each plot. Insect samples were taken within rows 4, 6, 11, 13 and 15 on successive sample dates at locations as described for soybean samples. Twenty randomly selected sorghum plants within each 5 m row sample were observed visually to determine vegetative plants damaged by cutworms, as well as leaf and other stem feeding insects. Plant defoliation was recorded using the rating scale developed by Frederiksen *et al.* (1991) and damage was related to insect infestation (Table 7). Ten sorghum panicles in each of 5 subsamples from rows 2, 4, 6, 12, and 14 were taken at growth stage 7 (soft-dough). Insects not identified in the field were placed in vials containing 70 % ethanol and taken to the laboratory for positive identification. Photographs and taxonomic keys (Curran *et al*., 1993 and Caballero *et al*., 1994) with assistance from Dr. R. Brown, insect taxonomist, Mississippi State University, were used for

insect determination. Insect pest infestations were recorded to determine their density and seasonal incidence.

Yield

Soybean and sorghum plots were harvested using a Massey Ferguson 8XP Combine (CAGCO Corp., Duluth, GA). Seed yield from each plot was taken from rows 7 to 10. Bags of harvested seed were allowed to dry at ambient temperature to obtain 12% moisture for soybean and 13% moisture for sorghum before being weighed.

Aflatoxin in Grain

Immediately following harvest, aflatoxin contamination was determined from five-50 g sorghum grain subsamples per treatment plot using the Vicam Aflatest (Watertown, Massachusetts) (Windham and Williams, 1999). Sorghum seed was allowed to dry at ambient temperature to obtain 13% moisture and stored at 4^{o}C for two weeks and then assayed.

Table 7. Rating scale [a] for leaf feeding caterpillars of sorghum.

Rating	Scale description
0 =	No foliar symptoms
1 =	Pin-hole lesions only on whorl leaves
2 =	Pin-hole and shot-hole lesions on whorl leaves
3 =	Pin-hole, shot-hole and several small elongated lesions on whorl and furl leaves
4 =	Many small elongated lesions on the whorl leaves and a few medium lesions on whorl
5 =	Many small elongated lesions and several medium elongated lesions on the whorl and furl leaves
6 =	Many small and medium elongated lesions plus a few medium and large elongated lesions on the furl leaves
7 =	Many small and medium elongated lesions plus several large elongated lesions on the furl leaves and several medium and large lesions on the furl leaves
8 =	Many small, medium, and large elongated lesions on the whorl leaves plus many large elongated lesions on the furl leaves
9 =	Many elongated lesions of all sizes on whorl and furl leaves plus elongated or irregular portions of the furl leaves eaten out including basal membrane

[a] Damage by insect pest that feed on sorghum can be rated by either recording percentage of plants that have leaf feeding damage, or extent (%) defoliation. Leaf feeding caterpillars such as fall armyworm and corn earworm feed within the whorl of sorghum plants and leaf damage is apparent only after leaves have extended from the whorl (Frederiksen *et al*., 1991).

Greenhouse Tests

The pathogenicity of different concentrations of *M. phaseolina* and *R solani* to soybean and sorghum was investigated in pot studies in the greenhouse. This study was conducted during the spring of 2006 in the greenhouse located at the Rodney R. Foil Plant Science Center, Mississippi State University, Starkville, MS. Two isolates each of *M. phaseolina* and *R. solani* were evaluated. *M. phaseolina* (A) and *R. solani* (A) were isolated from soybean and *M. phaseolina* (B) and *R. solani* (B) were isolated from sorghum. Soybean and sorghum cultivars were those used in the field study.

Isolate, storage and preparation

Isolates were maintained on PDA in Petri dishes using routine laboratory culturing procedures and stored at 25^{o}C in an incubator until used. Approximately 9 ml of PDA were poured into each of the 10 x 1.5 cm Petri dishes. One cm diameter disks of agar colonized by the fungal isolates were subcultured, and the plates were incubated as above. Prior to inoculum preparation, all isolates were grown at ambient room temperature (21^{o}C) under normal room light conditions (12-hour day) for 72 hours.

Inoculum preparation

Inoculum for the greenhouse studies was produced by separately growing each isolate at 21^{o}C for 14 days in flasks of sterile sand and corn meal (CMS) (100 g of dry sand, 3 g of cornmeal, 15 ml of distilled water)(Baird *et al*., 1996a). Soil used in the greenhouse studies was obtained from the same field used for the field studies. Soil was autoclaved for four hours, cooled for 24 hours and placed into 20 x 100 cm pots with capacity of 2.25 kg (Baird

et al., 1996a). Inoculations were made by mixing the different concentrations of fungi with the soil in all pots. The pots were then watered to saturation. Soybean or sorghum seeds were planted at a depth of 2.54 cm at the rate of six seeds per pot. Plants were grown at a photoperiod of 14 hours of light per day and temperature from 18 to 30^{o}C. Treatments (Table 8) were arranged in a randomized complete block design with four replications per treatment on greenhouse tables.

Soybean and sorghum plants were rated for stand establishment, plant height, and root diseases. Plant stands were obtained at 14 and 34 days after planting (DAP). To determine plant dry weight, the above ground vegetative plant parts were collected at 34 DAP, dried for 2 days at 60^{o}C and weighed using an analytical balance (A-160). Also, the roots were rated for damage by the pathogens using the rating scale presented by Baird (1996a) (Table 1). Plants showing symptoms of charcoal rot caused by *M. phaseolina* and root rot caused by *R. solani* were used to isolate and confirm identification of the pathogen as described previously.

Statistical Analysis

Data obtained from field experiments were subjected to analysis of variance (ANOVA), and means were separated using Least Significant Difference (LSD) test at the 0.05 significance level. Orthogonal contrasts were conducted when variables were specific for either soybean or sorghum. Greenhouse data were subjected to ANOVA and mean separations using LSD at the 0.05 significance level. Statistical analyses were performed using SAS (Statistical Analysis System,), version 9.1 (SAS Institute Inc., Cary, NC., USA).

Table 8. Treatment concentrations of two fungal pathogens applied to soybean and sorghum in the greenhouse in 2006.

Treatment	Treatment concentration
	Pathogen [a] / 2.25 kg soil
T1	*M. phaseolina* A / 1:50
T2	*M. phaseolina* A / 1:100
T3	*M. phaseolina* A / 1:200
T4	*M. phaseolina* A / 1:300
T5	*M. phaseolina* B / 1:50
T6	*M. phaseolina* B / 1:100
T7	*M. phaseolina* B / 1:200
T8	*M. phaseolina* B / 1:300
T9	*R. solani* A / 1:50
T10	*R. solani* A / 1:100
T11	*R. solani* A / 1:200
T12	*R. solani* A / 1:300
T13	*R. solani* B / 1:50
T14	*R. solani* B / 1:100
T15	*R. solani* B / 1:200
T16	*R. solani* B / 1:300
T17	Uninfested control

[a] A= Isolated from soybean; B= Isolated from sorghum.

CHAPTER III

RESULTS

Crop rotation systems involving soybean and sorghum showed variable results when comparing insect and disease levels during the three years of this study. Insect levels varied each year and were almost always below recommended economic threshold levels. Foliage diseases caused by fungi were identified and generally found to be at low levels each year. However, the incidence of charcoal rot was observed to be present at high levels. Bean pod mottle virus was widely distributed across the treatments during the second and third years.

Soybean Insect Pests

Six insect pest species were selected for comparisons of crop rotation systems during each of the three growing seasons. They included threecornered alfalfa hopper (TCAH), bean leaf beetle (BLB), velvetbean caterpillar (VBC), southern green stink bug (SGSB), green stink bug (GSB), and brown stink bug (BSB).

In general, TCAH infestations on soybean and plants girdled by this pest in crop rotation systems in the third year of this rotation study revealed that the insect numbers (Table 9) and crop damage (Table 10) were not influenced by the crop grown on the same site during the previous two years. These same generally infestation results were obtained when BLB (Table 11), VBC larvae (Table 12), SGSB (Table 13), GSB (Table 14) and BSB (Table 15) sample data were summarized by average number of insects over the eleven day

sampling period. All insects identified during the study were always below the economic threshold (Blaine *et al*. 1996)

Sorghum Insect Pests

During this three year study, sorghum webworm and corn earworm were the most prevalent insect pest on the panicles. However, infestation levels were below economic thresholds (Table 16) (Stewart, 2006).

Table 9. Mean number of threecornered alfalfa hopper adults and nymphs per 25 sweeps on soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Sampling dates											
	6/4	6/16	6/27	7/10	7/22	8/6	8/17	8/28	9/10	9/20	9/30	Acc [b]
2004												
SY (1)	0.0	0.0	11.9	17.5	12.2	8.0	7.0	5.1	4.0	3.7	2.5	72.0
2005												
SR (1) – SY (2)	0.0	0.0	7.1	10.0	12.0	17.5	5.3	11.2	16.8	14.0	18.2	121.0
SY (1) – SY (2)	0.0	0.0	7.2	9.5	11.2	17.0	8.5	10.0	17.2	13.0	19.2	112.0
Orthogonal contrasts	NS [c]	NS	NS	NS	NS	NS	A [d]	NS	NS	NS	NS	NS
2006												
SY (1) – **SR (2) – SY (3)**	0	16.0	8.0	9.0	9.5	9.0	7.0	5.0	4.0	4.0	4.5	76.0
SR (1) – **SY (2) – SY (3)**	0	13.1	7.0	8.0	10.3	9.7	5.9	4.9	5.0	4.3	4.0	72.0
SY (1) – SY (2) – SY (3)	0	13.6	8.0	8.0	8.0	10.0	10.0	5.8	5.0	4.6	3.6	77.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006); SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006).

[b] Acc = Accumulated mean number of threecornered alfalfa hoppers across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$.

[d] Letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SY (2) vs. SY (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) - SY (3); C = SY (2) – SY (3) vs. SY (1) - SY (2) – SY (3).

Table 10. Mean number of threecornered alfalfa hopper girdled soybean plants per row meter in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Girdled plants		Acc [b]
	6/16	6/27	
2004			
SY (1)	1.2	1.0	2.0
2005			
SY (1) – SR (2)	1.7	1.3	3.0
SR (1) – SY (2)	1.9	1.1	3.0
2006			
SY (1) – **SR (2) – SY (3)**	1.8	1.8	3.6
SR (1) – **SY (2) – SY (3)**	2.0	1.7	3.7
SY (1) – SY (2) – SY (3)	2.0	1.7	3.7
Orthogonal contrasts	NS [c]	NS	NS

[a] SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006); SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006).

[b] Acc = Accumulated mean number of girdled plants by threecornered alfalfa hoppers across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$; letters are used to designate differences as determined by orthogonal contrasts: A = SY (1) – SR (2) vs. SR (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) – SY (3); C = SY (2) – SY (3) vs. SY (1) – SY (2) – SY (3).

Table 11. Mean number of bean leaf beetle adults and nymphs per 25 sweeps on soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Sampling dates											
	6/4	6/16	6/27	7/10	7/22	8/6	8/17	8/28	9/10	9/20	9/30	Acc [b]
2004												
SY (1)	8.0	9.5	13.1	11.0	12.4	7.5	15.0	5.8	3.6	3.8	3.0	93.0
2005												
SR (1) – SY (2)	0.0	0.0	2.3	13.8	13.8	14.5	5.1	16.2	14.5	23.7	22.1	126.0
SY (1) – SY (2)	0.0	0.0	2.2	16.0	14.0	13.7	9.2	14.7	13.5	23.5	21.5	128.0
Orthogonal contrasts	NS [c]	NS	NS	NS	NS	NS	A [d]	NS	NS	NS	NS	NS
2006												
SY (1) – **SR (2) – SY (3)**	19.0	7.0	9.0	13.0	17.0	11.0	9.0	10.0	7.0	5.0	4.5	116.0
SR (1) – **SY (2) – SY (3)**	15.7	6.2	8.0	10.9	15.8	11.1	7.5	9.9	7.4	5.6	4.0	102.0
SY (1) – SY (2) – SY (3)	15.0	6.3	8.0	11.0	14.6	11.3	7.3	10.3	8.00	5.6	4.8	102.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006); SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006).

[b] Acc = Accumulated mean number of bean leaf beetles across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$.

[d] Letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SY (2) vs. SY (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) - SY (3); C = SY (2) – SY (3) vs. SY (1) - SY (2) – SY (3).

Table 12. Mean number of velvetbean caterpillar larvae per 25 sweeps on soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Sampling dates											
	6/4	6/16	6/27	7/10	7/22	8/6	8/17	8/28	9/10	9/20	9/30	Acc [b]
2004												
SY (1)	0.0	0.0	0.0	0.0	5.9	10.3	9.7	15.4	16.9	12.3	11.8	82.0
2005												
SR (1) – SY (2)	0.0	0.0	0.0	1.5	1.8	9.1	9.7	10.5	7.8	8.5	14.5	63.0
SY (1) – SY (2)	0.0	0.0	0.0	1.5	1.5	8.2	8.7	9.7	8.2	8.7	13.5	60.0
Orthogonal contrasts	NS [c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2006												
SY (1) – **SR (2) – SY (3)**	0.0	0.0	3.0	2.0	1.0	3.0	8.0	10.0	12.0	4.0	15.3	58.0
SR (1) – **SY (2) – SY (3)**	0.0	0.0	2.6	1.2	1.2	2.6	7.1	8.4	10.3	4.2	13.6	51.0
SY (1) – SY (2) – SY (3)	0.0	0.0	2.6	1.3	1.2	2.6	7.3	8.0	10.0	4.6	15.0	53.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006); SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006).

[b] Acc = Accumulated mean number of velvetbean caterpillars across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$; letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SY (2) vs. SY (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) – SY (3); C = SY (2) – SY (3) vs. SY (1) – SY (2) – SY (3).

Table 13. Mean number of southern green stink bug adults and nymphs per 25 sweeps on soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Sampling dates											
	6/4	6/16	6/27	7/10	7/22	8/6	8/17	8/28	9/10	9/20	9/30	Acc [b]
2004												
SY (1)	0.0	0.0	0.0	0.0	0.0	0.9	3.4	3.0	4.0	5.0	6.0	22.0
2005												
SR (1) – SY (2)	0.0	0.0	0.0	1.7	0.8	0.8	0.6	0.6	1.3	1.3	4.0	12.0
SY (1) – SY (2)	0.0	0.0	0.0	1.5	1.2	0.6	0.4	0.7	1.5	1.4	4.2	11.0
Orthogonal contrasts	NS [c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2006												
SY (1) – **SR (2) – SY (3)**	0.0	0.0	0.0	2.0	1.0	2.0	4.0	6.0	4.0	6.0	8.0	33.0
SR (1) – **SY (2) – SY (3)**	0.0	0.0	0.0	1.0	1.2	1.5	3.4	5.2	3.1	4.7	7.0	27.0
SY (1) – SY (2) – SY (3)	0.0	0.0	0.0	1.0	1.0	1.0	2.5	1.3	0.2	2.3	0.7	10.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006); SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006).

[b] Acc = Accumulated mean number of southern green stink bugs across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$; letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SY (2) vs. SY (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) – SY (3); C = SY (2) – SY (3) vs. SY (1) – SY (2) – SY (3).

Table 14. Mean number of green stink bug adults and nymphs per 25 sweeps on soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Sampling dates											
	6/4	6/16	6/27	7/10	7/22	8/6	8/17	8/28	9/10	9/20	9/30	Acc [b]
2004												
SY (1)	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.7	0.7	0.8	1.0	4.0
2005												
SR(1) - SY(2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.8	0.4	2.0
SY(1)– SY(2)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	1.0	0.3	2.0
Orthogonal contrasts	NS [c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2006												
SY (1) – **SR (2) – SY (3)**	0.0	0.0	0.0	1.0	1.0	1.0	3.0	2.0	1.0	3.0	1.0	13.0
SR (1) – **SY (2) – SY (3)**	0.0	0.0	0.0	1.0	1.0	1.0	2.4	1.3	0.4	2.4	0.7	10.0
SY (1) – SY (2) – SY (3)	0.0	0.0	0.0	1.0	1.0	1.0	2.5	1.3	0.4	2.3	0.7	10.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006); SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006).

[b] Acc = Accumulated mean number of green stink bugs across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$; letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SY (2) vs. SY (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) – SY (3); C = SY (2) – SY (3) vs. SY (1) – SY (2) – SY (3).

Table 15. Mean number of brown stink bug adults and nymphs per 25 sweeps on soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Sampling dates											
	6/4	6/16	6/27	7/10	7/22	8/6	8/17	8/28	9/10	9/20	9/30	Acc [b]
2004												
SY (1)	0.0	0.0	0.0	0.0	0.0	0.5	0.5	1.3	1.0	0.6	0.0	4.0
2005												
SR(1) - SY(2)	0.0	0.0	0.0	0.3	0.3	1.0	0.5	1.2	0.8	0.0	0.0	4.0
SY(1)– SY(2)	0.0	0.0	0.0	0.5	0.3	1.2	1.0	0.7	0.5	0.0	0.0	4.0
Orthogonal contrasts	NS [c]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
2006												
SY (1) – **SR (2) – SY (3)**	0.0	0.0	0.0	0.3	1.0	2.0	1.0	0.4	1.0	1.0	0.0	7.0
SR (1) – **SY (2) – SY (3)**	0.0	0.0	0.0	0.5	0.9	0.4	0.6	0.6	1.0	0.7	0.0	5.0
SY (1) – SY (2) – SY (3)	0.0	0.0	0.0	0.5	0.7	0.3	0.6	0.3	1.0	0.7	0.0	4.0
Orthogonal contrasts	NS	NS	NS	NS	NS	A [d]	NS	NS	NS	NS	NS	NS

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006); SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006).

[b] Acc = Accumulated mean number of brown stink bugs across 11 sampling dates.

[c] NS = Not significantly different at $P \leq 0.05$.

[d] Letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SY (2) vs. SY (1) – SY (2); B = SR (2) – SY (3) vs. SY (2) – SY (3); C = SY (2) – SY (3) vs. SY (1) – SY (2) – SY (3).

Table 16. Mean number of sorghum webworm and corn earworm larvae per sorghum panicle in crop systems during 2006. Starkville, MS.

Rotations [a] (Study year)	Insect/Sampling date	
	Sorghum webworm	Corn earworm
	7/24	7/24
2006		
SR (1) - **SR (2) - SR (3)**	0.5	0.4
SR (1) - **SY (2) - SR (3)**	0.5	0.5
SR (1) - SR (2) - SR (3)	0.8	0.7
Orthogonal contrasts	NS [b]	NS

[a] SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006); SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006).

[b] NS = Not significantly different at $P \leq 0.05$; letters are used to designate differences as determined by orthogonal contrasts: A = SR (2) – SR (3) vs. SY (2) - SR (3); B = SY (2) – SR (3) vs. SR (1) - SR (2) – SR (3).

Soybean and Sorghum Diseases

Rotations using soybean and sorghum had varied effects when comparing disease levels during the three years of this study. Disease organisms encountered included both fungi and virus. Three foliar fungal pathogens, including *Diaporthe phaseolorum* var. *meridionalis*, *Septoria glycines*, and *Cercospora sojina,* were observed on soybean, and two on sorghum, including *Gloeocercospora sorghi* and *M. phaseolina*. Bean pod mottle virus was identified on soybean. Five fungal pathogens, including *M. phaseolina, R. solani,* and three identified *Fusarium* spp., including *Fusarium* sp. (A), *Fusarium* sp. (B), and *Fusarium* sp. (C), were isolated from either sorghum or soybean roots. Other fungi isolated from soybean and sorghum roots were *Aspergillus* spp. and *Trichoderma* spp.

Soybean Diseases

Charcoal rot, caused by *M. phaseolina,* was rated at plant growth stages R5 (beginning seed) and R7 each year (Table 17). The disease was observed at high incidence levels late in the crop season each of the three years of this investigation; however, results were similar among treatments.

Stem canker, caused by *D. phaseolorum var. meridionalis,* was observed and rated for disease incidence and severity at R7 in 2004 and 2005, but not in 2006 (Table 17). Stem canker increased in soybean from 2004 to 2005. In 2004, it had an incidence at 9.8%, and severity rating at 1.0. In 2005, continuous soybean had significantly greater severity of stem canker with a rating at 2.5 compared with the sorghum-soybean rotation rated at 1.2. Furthermore, continuous soybean had numerically greater incidence of stem canker at 46.0% compared with the sorghum-soybean rotation at 32.0%.

Brown spot, caused by *Septoria glycines,* was observed in soybean during the first month after planting in 2005 (Table 17). Continuous soybean had significantly greater incidence of brown spot at 81.0% than the sorghum-soybean rotation at 18.0%. The disease was not observed on soybean during 2004 or 2006.

Frogeye leaf spot, caused by *Cercospora sojina,* was observed late in the season on soybean and increased in severity from 2004 to 2005. The disease was not observed in 2006 (Table 17).

Bean pod mottle disease, caused by bean pod mottle virus, was not observed in 2004, but in 2005 and 2006 the disease was present in all plots (Table 17). All plots had about equal levels of disease severity. Some plots showed green stem symptoms and could not be harvested.

Sorghum Diseases

Zonate spot, caused by *Gloeocercospora sorghi,* was the most prevalent sorghum disease observed during 2004 and 2005, but was not observed in 2006 (Table 18). The disease increased in severity during the growing season and continuous sorghum and soybean-sorghum rotations had similar severity ratings.

Charcoal rot symptoms on above ground parts of the sorghum plants were not observed in 2004 and 2005 (Table 18), but in 2006, continuous sorghum had a somewhat lower incidence of disease at 25.0% than soybean-sorghum rotations at 40.0%.

Root Diseases

Root disease ratings for soybean and sorghum in field plots in 2004 were similar during the three sampling dates (Table 19), but in 2005 results were similar among treatments only during the first date. On the second date continuous sorghum had significantly greater root disease ratings at 3.6 than soybean - sorghum rotation rated at 2.5 by causal organisms including *M. phaseolina, R. solani* and *Fusarium* spp. Similar results were observed on the second date for continuous soybean which had significantly greater root disease ratings at 4.0 than sorghum – soybean at 2.6. During the third date, continuous soybean had a significantly greater mean root disease rating at 3.3 than in the sorghum–soybean rotation at 2.4. During the third year of this study, continuous sorghum and soybean-sorghum-sorghum rotations had a greater root disease rating than the other rotation treatments.

Dry plant weight was collected from soybean at V3 (third nodes), V6 (sixth nodes, and R7 (beginning maturity), and sorghum at stages 2 (five-leaf), 3 (growing point differentiation), and 9 (physiological maturity) during each year of the study (Table 19). In 2004, 2005 and 2006 soybean and sorghum plant dry weights in crop systems were not significantly different at the three sampling dates.

Plant stand, plant heights, and aflatoxin data were similar for both crops in the treatment rotations during the second and third years of this study (Table 20).

The rotation treatments had an effect on yields in 2005 (Table 20). In 2005 soybean – sorghum rotations had significantly greater sorghum yield (3343.0 kg/ha) than continuous sorghum (3079.0 kg/ha), and sorghum-soybean rotations had significantly greater soybean yield (2021.0 kg/ha) than continuous soybean (1614.0 kg/ha). In 2006, soybean and sorghum yields were not significantly different for individual crops among the crop rotation treatments.

Table 17. Incidence and severity ratings for soilborne and foliage diseases of soybean in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [b] (Study year)	Charcoal rot		Stem canker		Brown spot	Frogeye leaf spot		Bean pod mottle [a]			
	Incidence		Inc [c]	Sev [d]	Inc [c]	Severity [e]		1	2	3	4
2004											
SY (1)	5.0	90.0	9.8	1.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0
2005											
SR(1) – SY(2	10.0	90.0	32.2	1.2	18.0	2.0	2.0	0.0	1.3	0.3	0.0
SY(1) – SY(2)	15.0	90.0	46.0	2.5	81.0	2.0	2.0	0.0	1.2	0.2	0.0
Orthogonal contrasts	NS [f]	NS	NS	A [g]	A	NS	NS	NS	NS	NS	NS
2006											
SY(1) – **SR(2) – SY(3)**	20.0	98.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.2	0.0
SR(1) – **SY(2) – SY(3)**	20.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.8	0.0
SY(1) – SY(2) – SY(3)	18.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	1.3	0.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Bean pod mottle sampled at 1 = first month, 2 = second month, 3 = third month, and 4 = four month after planting.
[b] SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006); SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006).
[c] Inc = Percentage of soybean plants showing symptoms of either stem canker or brown spot.
[d] Sev = Severity of damage to stem tissue rated using a scale from 0 - 4 (0 being no symptoms, 4 being 100% of plants showing foliage symptoms and/or dead plants.
[e] Amount of soybean foliage affected by the frogeye leaf spot disease rated three and four months after planting using a scale from 0-4 (0 no symptoms, 4 most advance symptoms; death of leaves or plants due to disease.
[f] NS = Not significantly different at $P \leq 0.05$.
[g] Letters are used to designate differences as determined by orthogonal contrasts: A = SR(1) - SY(2) vs. SY(1) - SY(2); B = SR (2) –SY(3) vs. SY(2) - SY(3); C = SY(2) - SY(3) vs. SY(1) - SY(2) - SY(3).

Table 18. Severity ratings for zonate spot on sorghum foliage and incidence of charcoal rot on sorghum stalk in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Zonate spot [b]			Charcoal rot [c]
	30 DAP	60 DAP	90 DAP	Stage 9
2004				
SR (1)	0.0	1.3	1.5	0.0
2005				
SR (1) - SR (2)	0.0	2.0	3.0	0.0
SY (1) - SR (2)	0.0	2.0	3.0	0.0
Orthogonal contrasts		NS	NS	NS
2006				
SR(1) - **SY (2) - SR (3)**	0.0	0.0	0.0	40.0
SY(1) - **SR (2) - SR (3)**	0.0	0.0	0.0	25.0
SR(1) - SR (2) - SR (3)	0.0	0.0	0.0	20.0
Orthogonal contrasts		NS [d]	NS	NS

[a] SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006); SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY (3) = Soybean (2006).

[b] Amount of zonate spot on sorghum foliage rated at three different DAP (days after planting) using a scale from 0-5 (0 being no foliar disease symptoms, 5 being death of leaves or plants due to disease).

[c] Percentage of soft sorghum plant stalks at growth stage 9 (physiological maturity).

[d] NS = Not significantly different at $P \leq 0.05$; letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) - SR (2) vs. SY (1) - SR (2); B = SY (2) - SR (3) vs. SR (2) - SR (3); C = SR (2) - SR (3) vs. (SR (1) - SR (2) – SR (3).

Table 19. Root disease ratings and dry weight (kg) for soybean and sorghum rotations in crop systems during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Root disease [b]			Dry weight		
	15 DAP [c]	32 DAP	R7/R9	17 DAP	34 DAP	R7 /Stage 9 [c]
2004						
SY (1)	2.3a	1.6a	3.0a	0.03	0.06	1.92
SR (1)	2.4a	1.5a	3.0a	0.02	0.03	0.80
2005						
SR (1) - SR (2)	3.1	3.3	3.2	0.02	0.07	2.6
SY (1) - SR (2)	3.6	2.5	3.0	0.03	0.09	3.0
SR (1) - SY (2)	1.5	2.6	2.4	0.04	0.07	1.1
SY (1) - SY (2)	1.7	4.0	3.6	0.03	0.07	0.8
Orthogonal contrasts	NS [d]	A, B [e]	B	NS	NS	NS
2006						
SR (1) - **SY (2) - SR (3)**	1.1	1.2	3.0	0.03	0.02	1.7
SY (1) - **SR (2) - SR (3)**	1.1	1.1	4.0	0.02	0.02	1.9
SR (1) - SR (2) - SR (3)	1.1	1.0	4.0	0.03	0.02	1.8
SY (1) - **SR (2) - SY (3)**	2.1	1.5	1.0	0.01	0.07	0.9
SR (1) - **SY (2) - SY (3)**	1.3	1.6	1.6	0.01	0.07	0.8
SY (1) - SY (2) - SY (3)	1.3	1.5	1.6	0.01	0.05	0.8
Orthogonal contrasts	NS	NS	C	NS	NS	NS

[a] SR (1) = Sorghum (2004); SR (2) = Sorghum (2005); SR (3) = Sorghum (2006); SY (1) = Soybean (2004); SY (2) = Soybean (2005); SY(3) = Soybean (2006).

[b] Root disease rated for the two crops using a scale from 0 - 5 (0 being no root symptoms, 5 being plants dead). Root disease index = (Number of asymptomatic plants x 0) + (Plants with very slight symptoms x 1) + (Plants with slight symptoms x 2) + (Plants with moderate symptoms x 3) + (Plants with severe symptoms x 4) + (Death/dying plants x 5) / Divided by the total number of plants.

[c] DAP = Days after planting; R7 for soybean (physiological maturity), and Stage 9 for sorghum (physiological maturity)

[d] NS = Not significantly different at $P \leq 0.05$.

[e] Letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SR (2) vs. SY (1) - SR (2); B = SR (1) - SY (2) vs. SY (1) - SY (2); C = SY (2) – SR (3) vs. SR (2) – SR (3); D = SR (2) – SR (3) vs. SR (1) – SR (2) – SR (3); E = SR (2) - SY (3) vs. SY (2) - SY (3); F = SY (2) - SY (3) vs. SY (1) - SY (2) - SY (3).

Table 20. Effect of soybean and sorghum rotations on select parameters during 2004-2006. Starkville, MS.

Crop or crop rotations [a] (Study year)	Plants stand [b]			Plant height (cm)	Aflatoxin (ppb) [c]	Yield (kg/ha)
	10 DAP [d]	17 DAP	24 DAP			
2004						
SY (1)	510	541	543	30.0	-	2774.0
SR (1)	470	472	459	46.5	0.5	4041.1
2005						
SR (1) - SR (2)	429.0	418.0	413.0	52.0	0.6	3079.0
SY (1) - SR (2)	418.0	446.0	437.0	47.0	0.3	3543.0
SR (1) - SY (2)	435.0	425.0	413.0	35.0	-	2021.0
SY (1) - SY (2)	430.0	427.0	422.0	31.0	-	1614.0
Orthogonal contrasts	NS [e]	NS	NS	NS	NS	A,B [f]
2006						
SR (1) - **SY (2) - SR (3)**	322.0	334.0	316.0	40.7	660.0	4711.0
SY (1) - **SR (2) - SR (3)**	291.0	298.0	287.0	40.5	532.0	4439.0
SR (1) - SR (2) - SR (3)	291.0	308.0	297.0	40.1	790.0	4166.0
SY (1) - **SR (2) - SY (3)**	322.0	442.0	515.0	32.4	-	1250.0
SR (1) - **SY (2) - SY (3)**	326.0	354.0	347.0	24.6	-	1236.0
SY (1) - SY (2) - SY (3)	340.0	377.0	363.0	22.2	-	1005.0
Orthogonal contrasts	NS	NS	NS	NS	NS	NS

[a] SR (1) = Sorghum (2004); SR(2) = Sorghum (2005); SR(3) = Sorghum (2006); SY(1) = Soybean (2004); SY(2) = Soybean (2005); SY(3) = Soybean (2006).
[b] Mean number of plants stand (cm) per 21.3 m row collected from the two central rows.
[c] Parts per billion (ppb) of aflatoxin determined in sorghum seed.
[d] DAP = Days after planting.
[e] Not significantly different at $P \leq 0.05$.
[f] Letters are used to designate differences as determined by orthogonal contrasts: A = SR (1) – SR (2) vs. SY (1) - SR (2); B = SR (1) - SY (2) vs. SY (1) - SY (2); C = SY (2) – SR (3) vs. SR (2) – SR (3); D = SR (2) – SR (3) vs. SR (1) – SR (2) – SR (3); E = SR (2) - SY (3) vs. SY (2) - SY (3); F = SY (2) - SY (3) vs. SY (1) - SY (2) - SY (3).

Pathogens Isolated from Root Tissue

Six common fungal genera were frequently isolated from soybean and sorghum root tissues collected in the field during this three year study (Tables 21, 22, 23). They included *M. phaseolina, R. solani, D. phaseolorum, Aspergillus* spp., and *Trichoderma* spp. In addition, three species of *Fusarium* were isolated and separated based on color morphologies and included *Fusarium* sp. (A) which was pink, *Fusarium* sp. (B) which was brown, and *Fusarium* sp. (C) which was white with a bluish area around the inoculation point. This area was found to contain sporodochia with macro and micro conidia. The three were separated since species within *Fusarium* are often important pathogens on soybean and sorghum.

In 2004, *M. phaseolina* had similar isolation frequencies from soybean and sorghum across all treatments, but in 2005 this pathogen was isolated from the sorghum-soybean rotation with significantly greater frequency at 81.9% than from continuous sorghum at 35.0% (Table 22). Continuous soybean, sorghum-soybean, and soybean-sorghum rotations had similar *M. phaseolina* isolation frequencies regardless of the rotation system. In 2006, *M. phaseolina* was isolated at greater frequencies than all other pathogens from soybean and sorghum roots (Table 23). Pathogen isolation frequencies showed an increasing trend from the first to third collection dates. By the third date, continuous soybean, continuous sorghum, soybean-sorghum-soybean, and sorghum-sorghum-soybean had 99 to 100% isolation frequencies compared with 63 to 81% for the other treatments.

Isolation frequencies of *R. solani* from soybean and sorghum root tissues were similar between the two crops in 2004 (Table 21). In 2005, continuous sorghum and sorghum-soybean-sorghum rotation had significantly greater isolation frequencies at 23.7% and

21.8%, respectively, than continuous soybean at 5.0%. On the second date, similar isolation frequencies among treatments occurred, however, continuous sorghum and the sorghum-soybean rotation had trend of numerically greater isolation frequencies of *R. solani* than continuous soybean, and the soybean-sorghum rotation. In 2006, *R. solani* was not observed during the three sampling dates.

Based on cultural morphology of isolates from soybean roots, *Diaporthe phaseolorum* var *meridionalis* was the only *Diaporthe* sp. isolated and was observed on stem and foliar plant tissues. In 2004 and 2006, this fungus did not occur in the soybean plots (Tables 21, 22, 23). In 2005 continuous soybean had significantly greater isolation frequencies of *D. phaseolorum* var *meridionalis* at 15.0% than sorghum-soybean at 9.0% on the first date, but was not observed on the last two dates.

Isolation frequencies of *Fusarium* sp. (A) from soybean and sorghum root samples were similar during the first and second sampling dates in 2004 and 2005 (Tables 21, 22, 23). On the third date in 2005, continuous sorghum and the soybean-sorghum rotation had significantly greater isolation frequencies of *Fusarium* sp. (A) at 21.3% and 53.7%, respectively, than continuous soybean or the sorghum-soybean rotation at 5.0% and 4.2%, respectively. In 2006, the isolation frequencies of *Fusarium* sp. (A) were similar among treatments for the first and second dates, but on the third date, continuous sorghum had significantly greater isolation frequencies than continuous soybean, soybean-sorghum-soybean and sorghum-soybean-soybean rotations where no isolatings of *Fusarium* sp. (A) were found.

The brown colored isolates of *Fusarium* sp. (*Fusarium* sp. B) were not observed in 2004. But on the first sampling date in 2005, sorghum-soybean rotation had a significantly

greater isolation frequency of this isolate at 6.2% than continuous sorghum, or continuous soybean at 1.2% and 1.0%, respectively (Tables 21, 22, 23). During the second date, the isolation frequencies of *Fusarium* sp. (B) were similar among treatments. In 2006, sorghum-soybean-soybean and soybean-sorghum-sorghum rotations had significantly greater isolation frequencies of *Fusarium* sp. (B) at 12.5% than continuous sorghum, continuous soybean, or sorghum-soybean-sorghum rotations at 2.5%, 1.2% and 2.5%, respectively. On the second and third dates, isolation frequencies were similar among treatments.

The white *Fusarium* isolates (*Fusarium* sp. C) were not observed in 2004, but in 2005 isolation frequencies of this isolate from soybean and sorghum root samples were similar on first and second dates, but it was not observed on the third sample date (Tables 21, 22, 23). In 2006, results were similar among treatments on first and second sample dates with levels ranging from 10.0% to 20.0% and 18.7% to 27.5%, respectively, but on the third sample date, soybean-sorghum-sorghum rotations had significantly greater isolation frequencies of *Fusarium* sp. (C) at 10.0% than in continuous soybean, sorghum-soybean-sorghum, soybean-sorghum-soybean, or sorghum-soybean-soybean rotations at 0%, 3.7%, 0% and 0%, respectively.

In 2004 soybean and sorghum had similar isolation frequencies of *Aspergillus* spp. during the first and second sampling dates, but on the third sample date, this fungus was not observed (Table 21). In 2005, *Aspergillus* spp. were not observed, but in 2006 the fungus was recorded in continuous soybean and in the sorghum-soybean-sorghum rotation with significantly greater isolation frequencies at 10.0% and 12.5%, respectively, than in the soybean-sorghum-soybean rotation at 0.0% (Tables 22, 23). On the second sampling date,

similar results were observed among the treatments for the isolation frequencies of *Aspergillus* spp.; on the third date it was not observed.

In 2004, soybean and sorghum had similar isolation frequencies of *Trichoderma* spp. during the three sampling dates (Tables 21, 22, 23), but in 2005 the soybean-sorghum planting had significantly greater isolation frequencies of *Trichoderma* spp. at 5.0% than in continuous soybean at 0%, but on second and third sample dates this fungus was not observed. In 2006, continuous sorghum, sorghum-soybean-sorghum, and soybean-sorghum-sorghum had significantly greater *Trichoderma* spp. isolation frequencies at 32.5%, 25.0% and 27.5%, respectively, than continuous soybean, soybean-sorghum-soybean, or sorghum-soybean-soybean during the first date. On the second and third dates the isolation frequencies of *Trichoderma* spp. were similar among treatments.

Additional fungi isolated from soybean and sorghum plots in 2005 included *Nigrospora* spp. and *Rhizoctonia zeae.* However their occurrence was very rare during this investigation.

Nematodes Extracted from Soil Samples

Four nematode species including *Meloidogyn*e spp., *Helicotylenchus* spp, *Rotylenchulus reniformis,* and *Pratylenchus* spp. were isolated from soil samples collected from soybean and sorghum plots during this three year study.

Meloidogyne spp.

In 2004, the root-knot nematode populations were similar in soybean and sorghum plots (Table 24), but in 2005, this nematode was not isolated. In 2006, root-knot nematode

populations were similar among treatments during both sampling dates, and population levels ranged from 0 to 28.0 and 13.0 to 45.0 nematodes per 475 ml of soil, respectively.

Helicotylenchus spp. (spiral nematode).

Spiral nematodes were the most prevalent nematodes isolated from soil samples during this three year study. However, populations were similar among treatments throughout the study period (Table 24).

Rotylenchulus reniformis (reniform nematode).

The reniform nematode was not observed on sampling dates in 2004 and 2005 (Table 24). In 2006, the populations of reniform nematodes were similar among treatments on the two sampling dates. Levels during 2006 ranged from 2.0 to 14.0 nematodes and 16.0 to 183.0 nematodes, respectively.

Pratylenchus spp. (lesion nematode).

In 2004, the lesion nematode was not isolated from soil samples. Only six individuals were isolated in 2005, but in 2006 this nematode was observed in very low and similar infestation levels among treatments (Table 24).

Table 21. Mean percentage of fungi isolated from soybean and sorghum root tissues collected on three dates after planting (DAP) from plants in field plots in 2004. Starkville, MS.

Crop [a]	*Macrophomina phaseolina*			*Rhizoctonia solani*			*Diaporthe* complex		
	17 DAP [b]	34 DAP	R7 [c]/ Stage 9 [d]	17 DAP	34 DAP	R7 /Stage 9	17 DAP	34 DAP	R7 /Stage 9
SY (1)	22.1a [e]	32.9a	48.3a	10.8a	11.0a	0.0	0.0	0.0	0.0
SR (1)	21.3a	29.0a	45.4a	16.7a	17.9a	0.0	0.0	0.0	0.0
	Fusarium sp. (A)			*Aspergillus* spp.			*Trichoderma* spp.		
	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9
SY (1)	5.4a	19.2a	0.0	8.0a	6.0a	0.0	15.0a	8.0a	5.0a
SR (1)	5.5a	13.3a	0.0	10.0a	7.0a	0.0	13.0a	10.0a	3.0a

[a] SY (1) = Soybean (2004); SR (1) = Sorghum (2004).
[b] DAP = Days after planting.
[c] R7 (beginning maturity of soybean).
[d] Stage 9 (Physiological maturity of sorghum).
[e] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.

Table 22. Mean percentage of fungi isolated from soybean and sorghum root tissues collected on three dates after planting (DAP) from plants in field plots in 2005. Starkville, MS.

Rotations [a]	*Macrophomina phaseolina*			*Rhizoctonia solani*			*Diaporthe phaseolorum*		
	17 DAP [b]	34 DAP	R7 [c]/ Stage 9 [d]	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9
SR (1) - SR (2)	25.0a [e]	30.0a	35.0c	23.7a	11.2a	0.0	-	-	-
SY (1) - SY (2)	33.7a	43.7a	61.2ab	5.0b	5.0a	0.0	15.0a	0.0	0.0
SR (1) - SY (2)	28.1a	31.2a	81.9a	21.8a	13.2a	0.0	9.0b	0.0	0.0
SY (1) - SR (2)	28.1a	29.3a	60.0ab	11.2ab	5.3a	0.0	-	-	-
Rotations	*Fusarium* sp. (A)			*Fusarium* sp. (B)			*Fusarium* sp. (C)		
	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9
SR (1) - SR (2)	1.2a	23.7a	21.6a	1.2b	13.0a	0.0	18.7a	27.5a	0.0
SY (1) - SY (2)	11.2a	21.2a	5.0b	1.0b	16.2a	0.0	20.0a	21.2a	0.0
SR (1) - SY (2)	1.8a	22.5a	4.2b	6.2a	14.3a	0.0	13.7a	18.7a	0.0
SY (1) - SR (2)	8.7a	19.3a	53.7a	1.8ab	15.0a	0.0	10.0a	22.5a	0.0
Rotations	*Trichoderma* spp.								
	17 DAP	34 DAP	R7 / Stage 9						
SR (1) - SR (2)	1.2ab	0.0	0.0						
SY (1) - SY (2)	0.0b	0.0	0.0						
SR (1) - SY (2)	5.0a	0.0	0.0						
SY (1) - SR (2)	3.1ab	0.0	0.0						

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SR (1) = Sorghum (2004); Sorghum (2005).
[b] DAP = Days after planting.
[c] R7 (beginning maturity of soybean).
[d] Stage 9 (Physiological maturity of sorghum).
[e] Means followed by the same letter in the same column are not significantly different using LSD with $P = 0.05$.

Table 23. Mean percentage of fungi isolated from soybean and sorghum root tissues collected on three dates after planting (DAP) from plants in field plots in 2006. Starkville, MS.

Rotations [a]	*Macrophomina phaseolina*			*Fusarium* sp. (A)			*Fusarium* sp. (B)		
	17 DAP [b]	34 DAP	R7 [c] / Stage 9 [d]	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9
SR (1) - SR (2) - **SR (3)**	32.5c [e]	17.5b	80.5b	27.5a	33.7a	5.0a	2.5b	1.2a	1.2a
SY (1) - SY (2) - **SY (3)**	65.0ab	48.7a	100.0a	10.0ab	27.5a	0.0b	1.2b	1.5a	0.0a
SR (1) - SY (2) - **SR (3)**	31.2c	18.7b	62.5c	16.2ab	28.7a	1.2ab	2.5b	1.2a	2.5a
SY (1) - SR (2) - **SY (3)**	63.7ab	52.5a	100.0a	6.2ab	30.0a	0.0b	6.2ab	2.5a	0.0a
SR (1) - SY (2) - **SY (3)**	80.0a	45.0a	98.7a	12.5ab	21.2a	0.0b	12.5a	5.0a	0.0a
SY (1) - SR (2) - **SR (3)**	48.7bc	33.7ab	75.0bc	28.7a	22.5a	3.7ab	12.5a	2.5a	5.0a
Rotations	*Fusarium* sp. (C)			*Aspergillus* spp.			*Trichoderma* spp.		
	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9	17 DAP	34 DAP	R7 / Stage 9
SR (1) - SR (2) - **SR (3)**	8.7ab	12.5a	7.5ab	10.0a [d]	7.5a	0.0	32.5a	20.0a	5.0a
SY (1) - SY (2) - **SY (3)**	6.2ab	20.0a	0.0 b	6.2ab	6.5a	0.0	11.2b	7.50a	0.0a
SR (1) - SY (2) - **SR (3)**	5.0ab	8.7a	3.7 b	12.5a	5.0a	0.0	25.0a	13.75a	3.7a
SY (1) - SR (2) - **SY (3)**	4.5ab	26.2a	0.0 b	0.0 b	0.0a	0.0	7.5b	12.5a	0.0a
SR (1) - SY (2) - **SY (3)**	22.5a	26.2a	0.0 b	3.7ab	0.0a	0.0	7.5b	12.5a	1.2a
SY (1) - SR (2) - **SR (3)**	6.2ab	10.0a	10.0a	8.7ab	11.2a	0.0	27.5a	7.5a	2.5a

[a] SY (1) = Soybean (2004); SY (2) = Soybean (2005); SR (1) = Sorghum (2004); Sorghum (2005).
[b] DAP = Days after planting.
[c] R7 (beginning maturity of soybean).
[d] Stage 9 (Physiological maturity of sorghum).
[e] Means followed by the same letter in the same column are not significantly different using LSD with $P = 0.05$

Table 24. Effect of soybean and sorghum rotation on the mean number of nematodes recovered per treatment during 2004-2006. Starkville, MS.

Crop or crop rotations [b] (Study year)	Nematodes samples [a]							
	Meloidogyne spp.		*Helicotylenchus* spp.		*R. reniformis*		*Pratylenchus* spp.	
	5/12	10/05	5/12	10/05	5/12	10/05	5/12	10/05
2004								
SR (1)	1a [c]	1a	18a	400a	0	0	0	0
SY (1)	0a	0a	15a	51a	0	0	0	0
2005								
SR (1) - **SR (2)** - SR (3)	0	0	31a	53a	0	0	6	0
SY (1) - **SY (2)** - SY (3)	0	0	53a	108a	0	0	0	0
SR (1) - **SY (2)** - SR (3)	0	0	21a	398a	0	0	0	0
SY (1) - **SR (2)** - SY (3)	0	0	10a	20a	0	0	0	0
2006								
SR (1) - SR (2) - **SR (3)**	0a	21a	67a	175a	11a	19a	2a	0a
SY (1) - SY (2) - **SY (3)**	19a	13a	262a	444a	8a	31a	2a	0a
SR (1) - SY (2) - **SR (3)**	13a	39a	81a	449a	4a	71a	2a	0a
SY (1) - SR (2) - **SY (3)**	28a	16a	63a	158a	2a	32a	6a	0a
SR (1) - SY (2) - **SY (3)**	12a	16a	153a	1019a	14a	183a	0a	0a
SY (1) - SR (2) - **SR (3)**	23a	45a	155a	134a	2a	16a	2a	6a

[a] From each plot 25 cores were mixed thoroughly and a sample of 475 ml of soil was used to determine mean numbers of nematodes. Samples were collected on 05/12 and 10/05 during the three year study.

[b] SR (1) = Sorghum (2004), SR (2) = Sorghum (2005), SR (3) = Sorghum (2006), SY (1) = Soybean (2004), SY (2) = Soybean (2005), and SY (3) = Soybean (2006).

[c] Means followed by the same letter in the same column are not significantly different using LSD with $P = 0.05$.

Greenhouse Research

Inoculum concentrations of two isolates each of *M. phaseolina* (1 and 2) and *R. solani* (1 and 2) had varied results in pathogenicity to soybean and sorghum during this study in 2006. Results showed statistical differences among treatments on soybean and sorghum, and are discussed below.

Soybean

Plant stands

Treatments of *M. phaseolina* isolate 1 significantly reduced plant stands (Table 25). The uninfested control had statistically greater mean numbers of plants at 5.5 per pot during the first sampling date than the inoculated treatments. *Macrophomina phaseolina* treatment at 1:50 had a significantly lower mean number of plants per pot at 1.3 than the 1:200 treatment at 3.5. Similar results were observed at 34 DAP for plant stands per pot than those at 14 DAP. The uninfested control had a significantly greater plant stand per pot at 5.3 than the 1:50, 1:100, 1:200 or 1:300 treatments of *M. phaseolina* isolate 1 having 1.0, 2.8, 2.5, and 2.8 plants per pot, respectively.

Plant stand per pot was affected by the different treatments of *M. phaseolina* isolate 2 at 14 DAP. The uninfested control had a significantly greater mean number of plants per pot at 5.5 than the 1:50, 1:100, or 1:200 treatment of *M. phaseolina* isolate 2 having 2.0, 3.5, and 3.5 plants per pot, respectively. Treatment 1:50 of *M. phaseolina* isolate 2 had a significantly lower plant stand at 2.0 than the 1:300 having a plant stand of 4.0. At 34 DAP the uninfested control had a significantly greater plant stand per pot at 5.3 than the 1:50 or 1:200 treatments

of *M. phaseolina* isolate 2 at 2.0 and 3.3 plants per pot, respectively. The different concentrations for *R. solani* isolates 1 and 2 had similar plant stands per pot at 14 and 34 DAP.

Plant heights

Macrophomina phaseolina isolate 1 and the uninfested control had similar plants heights across treatments. The 1:50 treatment of *M. phaseolina* isolate 2 had a significantly lower mean plant height at 8.3 cm than the 1:100, 1:200, or 1:300 treatments or the uninfested control having 11.0, 10.0, 10.6, and 11.2 cm, respectively (Table 26). Pots containing the different inoculum treatments of *R. solani* isolates 1 and 2 had similar plant heights.

Root disease ratings

The uninfested control had a significantly lower mean root disease rating at 1.4 than the *M. phaseolina* isolate 1 treatments 1:50, 1:100, 1:200 or 1:300 having 3.4, 2.8, 2.8, and 2.6 ratings, respectively. Pots containing 1:300 had a significantly lower root disease rating at 2.6 than the treatment at 1:50 having a 3.4 rating (Table 27). The uninfested control had a significantly lower mean root disease rating at 1.4 than treatments 1:50, 1:100, 1:200 or 1:300 of *R. solani* isolate 1 at 2.7, 2.8, 2.5, and 2.9, respectively. Also, the *R. solani* isolate 2 root disease ratings were similar among all treatments and the uninfested control.

Plant dry weights

Plants grown in the uninfested control pots had significantly greater dry weights than those grown in inoculated soil among the different treatments of *M. phaseolina* isolates 1 and 2 (Table 28). All treatments of *R. solani* isolate 1 and the uninfested control had similar plant dry weights, but plants grown in pots inoculated with *R. solani* isolate 2 had significantly lower dry weights than the uninfested control.

Root isolation

The uninfested control had significantly lower isolation frequencies of *M. phaseolina* isolate 1 at 0% than the treatments at 1:50, 1:100, 1:200 or 1:300 having 52.5, 47.5, 43.0, and 27.3 %, respectively, (Table 29). Results with *M. phaseolina* isolate 1 were similar to results with isolate 2. The uninfested control had significantly lower *M. phaseolina* isolate 2 isolation frequencies at 0% than treatments 1:50, 1:100 or 1:200 having 49.8, 45.5 and 49.8%, respectively. Isolation frequencies of *R. solani* isolates 1 and 2 and the uninfested control were similar among treatments.

Sorghum

Plant stands

Sorghum plant stand at 14 and 34 DAP were similar among treatments. However, variability in plant stand was high within treatments (Appendix A2).

Plant heights

Sorghum plants in uninfested pots had significantly greater mean height at 6.8 cm than those in pots inoculated with the *M. phaseolina* isolate 1 treatments at 1:50 and 1:100 at 4.9 and 5.0 cm, respectively (Table 30). Similar plant heights were observed among of *M. phaseolina* isolate 2 treatments and the uninfested control, but *R. solani* isolate 1 treatment 1:300 had a significantly greater sorghum mean plant height at 7.2 cm than the 1:50, 1:100 or the uninfested control at 4.5, 5.4, and 6.0 cm, respectively. *Rhizoctonia solani* isolate 2 treatments 1:50 and 1:100 had significantly lower mean plant heights at 4.8 and 4.3 cm than the 1:200, or 1:300 treatments or the uninfested control at 6.9, 6.3, and 6.0 cm, respectively.

Root diseases ratings

Sorghum root disease ratings were similar across *M. phaseolina* isolates 1 and 2, and *R. solani* isolate 2 treatments. The 1:50 treatment of *R. solani* isolate 1 had a significantly greater root diseases rating at 2.1 than the *R. solani* treatments 1:100, 1:200, 1:300 or the control having 1.5, 1.3, 1.3, and 1.2 ratings, respectively (Table 31).

Plant dry weights

The uninfested control had a significantly greater mean weight at 2.4 g than treatments 1:50 or 1:100 treatments with *M. phaseolina* isolate 1 at 0.4 and 1.5 g, respectively. All *M. phaseolina* isolate 2 treatments and the uninfested control were similar except for the 1:50 treatment which had a significantly lower mean plant dry weight at 0.7 g. Dry weights for the uninfested control and *R. solani* isolate 1 treatments were similar except for the 1:50 treatment at 0.6 g. The uninfested control and *R. solani* isolate 2 treatments

1:300 and 1:200 had significantly greater mean plant dry weights at 2.4, 2.3, and 2.1 g, respectively, than treatments 1:50 or 1:100 at 0.9 and 1.4 g, respectively.

Root isolation

Pots containing *M. phaseolina* isolate 1 treatments 1:50, 1:200, and 1:300 had significantly greater isolation frequencies at 54.0, 58.2, and 54.2% than the uninfested control at 0%. Pots containing *M. phaseolina* isolate 2 treatments 1:50, 1:100, 1:200 and 1:300 had significantly greater isolation frequencies at 58.2, 70.8, 50.0, and 58.1% than the uninfested control at 0% (Table 33). Treatments with *R. solani* isolates 1 and 2 were statistically different across treatments. The uninfested control had significantly lower mean isolation frequencies at 0% than *R. solani* isolate 1 treatments at 1:50, 1:100, 1:200, or 1:300 at 65.0, 53.2, 46.0, and 63.0%, respectively. *Rhizoctonia solani* isolate 2 had similar results as isolate 1 with the uninfested control having significantly lower mean isolation frequencies at 0% than pots containing isolate 1 for treatments 1:50, 1:100, 1:200 or 1:300 at 38.0, 46.0, 42.0, and 54.2%, respectively.

Table 25. Effect of different concentrations of fungal pathogens on soybean plant stands in the greenhouse. Starkville, MS., 2006.

Pathogen concentration [a]	Mean plant stand per pot at 14 and 34 days after planting (DAP)							
	Macrophomina phaseolina				*Rhizoctonia solani*			
	Soybean isolate 1 [b]		Sorghum isolate 2 [c]		Soybean isolate 1 [b]		Sorghum isolate 2 [c]	
	14 DAP	34 DAP	14 DAP	34 DAP	14 DAP	34 DAP	14 DAP	34 DAP
1:50	1.3c	1.0c	2.0c	2.0b	4.3	3.3	2.5	2.5
1:100	2.8bc	2.8b	3.5bc	3.3b	3.5	3.3	4.3	4.3
1:200	3.5b	2.5bc	3.5bc	3.5ab	2.5	3.8	4.5	4.3
1:300	3.0bc	2.8b	4.0ab	3.8ab	4.8	5.3	4.0	3.5
Control [e]	5.5a	5.3a	5.5a	5.3a	5.5	5.3	5.5	5.3
LSD ($P \leq 0.05$)	0.0036	0.0010	0.0326	0.0390	NS	NS	NS	NS

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

Table 26. Effect of different concentrations of fungal pathogens to soybean plant heights in the greenhouse. Starkville, MS., 2006.

Mean plant heights per pot at 34 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [a]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]
1:50	8.3	8.3b [d]	9.0	10.2
1:100	8.8	11.0a	10.3	11.3
1:200	10.9	10.0a	10.1	10.4
1:300	9.0	10.6a	9.4	9.8
Control [e]	11.2	11.2a	11.2	11.2
LSD ($P \leq 0.05$)	NS	0.0472	NS	NS

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

Table 27. Effect of different concentrations of fungal pathogens on soybean root diseases in the greenhouse. Starkville, MS., 2006.

	[a] Root disease index at 34 days after planting (DAP)			
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [b]	Soybean isolate 1 [c]	Sorghum isolate 2 [d]	Soybean isolate 1 [c]	Sorghum isolate 2 [d]
1:50	3.4a [e]	3.4a	2.7a	2.7a
1:100	2.8ab	3.4a	2.8a	2.7a
1:200	2.8ab	3.1a	2.5a	2.9a
1:300	2.6 b	3.1a	2.9a	2.4ab
Control [f]	1.4c	1.4b	1.4b	1.4b
LSD ($P \leq 0.05$)	0.0013	0.0003	0.0013	0.005

[a] Root disease rated for soybean using a scale from 0 - 5 (0 being no root symptoms, 5 being plants dead). Root disease index = (Number of asymptomatic plants x 0) + (Plants with very slight symptoms x 1) + (Plants with slight symptoms x 2) + (Plants with moderate symptoms x 3) + (Plants with severe symptoms x 4) + (Death/dying plants x 5) / Divided by the total number of emerged plants.

[b] Ratio of cornmeal sand inoculum pathogen to sterile soil.

[c] *M. phaseolina* and *R solani* isolated from soybean.

[d] *M. phaseolina* and *R solani* isolated from sorghum.

[e] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.

[f] Pots not inoculated with pathogens.

Table 28. Effect of different concentrations of fungal pathogens to soybean plants dry weight in the greenhouse. Starkville, MS., 2006.

Mean dry weight (g) at 34 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [a]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]
1:50	0.4b [d]	0.7b	1.2	0.8
1:100	0.7b	0.9b	1.2	1.9
1:200	0.8b	1.2ab	0.9	1.9
1:300	1.0b	0.9b	1.4	0.9
Control [e]	2.3a	2.3a	2.3	2.3
LSD ($P \leq 0.05$)	0.0014	0.0500	NS	NS

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

Table 29. Isolation frequencies of fungal pathogens from soybean root samples in the greenhouse. Starkville, MS., 2006.

Mean isolation frequencies (%) collected at 44 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [a]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]
1:50	52.5a [d]	49.8a	50.0	45.8
1:100	47.5a	45.5a	45.8	25.0
1:200	43.0a	49.8a	37.2	20.8
1:300	27.3ab	29.3ab	25.0	16.8
Control [e]	0.0b	0.0b	0.0	0.0
LSD ($P \leq 0.05$)	0.0312	0.0318	NS	NS

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

Table 30. Effect of different concentrations of fungal pathogens to sorghum plant heights in the greenhouse. Starkville, MS., 2006.

Mean plant heights per pot at 34 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [a]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]
1:50	4.9b [d]	4.7	4.5 d	4.8b
1:100	5.0b	6.7	5.4cd	4.3b
1:200	6.6a	6.3	7.1ab	6.9a
1:300	6.0ab	5.1	7.2a	6.3a
Control [e]	6.8a	6.0	6.0bc	6.0a
LSD ($P \leq 0.05$)	0.0138	NS	0.0013	0.0009

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

Table 31. Effect of different concentrations of fungal pathogens on sorghum root disease in the greenhouse. Starkville, MS., 2006.

Root disease [a] index at 34 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [b]	Soybean isolate 1 [c]	Sorghum isolate 2 [d]	Soybean isolate 1 [c]	Sorghum isolate 2 [d]
1:50	1.9	1.2	2.1a	2.0
1:100	1.5	1.2	1.5b	1.3
1:200	1.6	1.4	1.3b	1.4
1:300	1.4	1.4	1.3b	1.1
Control [f]	1.2	1.2	1.2b	1.2
LSD ($P \leq 0.05$)	NS	NS	0.0174	NS

[a] Root disease rated for soybean using a scale from 0 - 5 (0 being no root symptoms, 5 being plants dead). Root disease index = (Number of asymptomatic plants x 0) + (Very slight damaged plants x 1) + (Slight damaged plants x 2) + (Moderate damaged plants x 3) + (Severe damaged plants x 4) + (Death/dying plants x 5) / Divided by the total number of emerged plants.
[b] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[c] *M. phaseolina* and *R solani* isolated from soybean.
[d] *M. phaseolina* and *R solani* isolated from sorghum.
[e] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[f] Pots not inoculated with pathogens.

Table 32. Effect of different concentrations of fungal pathogens to sorghum plants dry weight in the greenhouse. Starkville, MS., 2006.

Mean dry weight (g) at 34 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [a]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]
1:50	0.4 c [d]	0.7b	0.6b	0.9c
1:100	1.5 b	1.7a	1.8a	1.4bc
1:200	1.7ab	2.3a	2.3a	2.1ab
1:300	2.1ab	1.6a	1.9a	2.3ab
Control [e]	2.4a	2.4a	2.4a	2.4a
LSD ($P \leq 0.05$)	0.0008	0.0046	0.0213	0.0271

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

Table 33. Isolation frequencies of fungal pathogens from sorghum root samples in the greenhouse. Starkville, MS., 2006.

Mean isolation frequencies (%) collected at 44 days after planting (DAP)				
	Macrophomina phaseolina		*Rhizoctonia solani*	
Pathogen concentration [a]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]	Soybean isolate 1 [b]	Sorghum isolate 2 [c]
1:50	54.0a [d]	58.2a	65.0a	38.0a
1:100	37.1ab	70.8a	53.2a	46.0a
1:200	58.2a	50.0a	46.0a	42.0a
1:300	54.2a	58.1a	63.0a	54.2a
Control [e]	0.0b	0.0b	0.0b	0.0b
LSD ($P \leq 0.05$)	0.0388	0.0036	0.0010	0.0456

[a] Ratio of cornmeal sand inoculum pathogen to sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean.
[c] *M. phaseolina* and *R solani* isolated from sorghum.
[d] Means followed by the same letter in the same column are not significantly different using LSD at $P = 0.05$.
[e] Pots not inoculated with pathogens.

CHAPTER IV

DISCUSSION

Recommendations using soybean and sorghum rotations for insect and disease control have been unavailable to Mississippi producers and for the Southeastern United States. Therefore, a three year study was established in an effort to determine if selected rotation schemes would have an effect on insect pests and disease levels. Results showed that insects and disease pests did not occur at levels that would consistently influence crop yields during the three year study. *Diaporthe phaseolorum* var *meridionalis* was the only foliar pathogen of soybean observed to reduce yields. Only one major soilborne pathogen (*Macrophomina phaseolina*) was observed on soybean during this investigation and was also found to routinely occur on the roots of both soybean and sorghum at high levels, but yield impacts were minimal. *Gloeocercospora sorghi* was the most prevalent sorghum foliar pathogen during the study, but yields were not affected.

Insect Pests

Six soybean and two sorghum insect pests were selected for comparisons of crop systems in this study. These insects are reported to be commonly associated with the respective soybean and sorghum crops throughout the southern United States (Hammond, 1996b; Duyn, 2000), including Mississippi (Blaine *et al*., 1996; Stewart, 2003; Catchot, 2007). Even though these insects are reported to cause significant yield losses (Duyn, 2000)

their levels during this investigation were always below economic thresholds (Blaine *et al*. 1996; Stewart 2003; Catchot, 2007). Furthermore, visible damage, such as girdling by threecornered alfalfa hopper, was low and similar among treatments.

Each year soybean plants outside the study area were planted for other projects approximately one month prior to initiation of this study. Insect populations were present in greater numbers on the adjacent more mature soybean plants than in the study area. Insects are reported to disperse from overwintering areas to early planted soybeans (Jensen *et al*., 1974). Because of the differences in maturity of the plants outside the plots, the selected insects were attracted to the early planted soybean, thus the pest populations were reduced in the study area. Additionally, the low pest levels and similar distribution of all insects collected in the test area and across the different treatments may be attributed to the hot, dry weather (Stewart, 2003), and small plot size (Henry Pitre, pers. comm.). For example, adult moths looking for hosts for oviposition sites landed anywhere throughout the test sites regardless of the type of rotation. The adult insect pests recorded in this investigation are good fliers and can fly easily to nearby areas. Crop rotation studies usually have greatest success against pests with limited dispersal capabilities (Herzog and Funderburk, 1985). The insects that were collected in this study have great mobility, resulting in similar populations within treatment plots.

Pathogens on Soybean

The charcoal rot pathogen, *M. phaseolina,* is considered the most important fungal pathogen that attacks soybeans and causes yield losses in Mississippi and other southern states in the United States (Wrather *et al*., 2001; Koenning, 2006). This pathogen was

isolated from both soybean and sorghum root tissue each of the three years of this study. Previously, it was reported that soybean seedlings may be infected by *M. phaseolina* and show symptoms very early in the growing season with up to 80-100% seedlings being infected 2 to 3 weeks after planting. These infections remain latent until optimal conditions occur for the pathogen, such as low soil moisture and high ambient temperatures, resulting in above ground symptoms and death of the plants. However, if wet, cool weather persists, infected seedlings can survive, but carry a latent infection (Sinclair and Shurtleff, 1975; Seem, 2003; Shaner *et al*., 1999). Above-ground disease symptoms may appear later, between 1 to 4 weeks before normal maturity (R7), and usually during hot, dry weather. The disease is not evident at low temperatures, but pathogen growth commences and symptoms appear between 28 and 35^{O}C (Smith and Wyllie, 1999; Sinclair and Shurtleff, 1975; Yang and Navi, 2003; Meyer *et al*., 1974; Pedersen, 2006b).

In the present study, charcoal rot incidence across treatments was similar from early season through harvest regardless of the type of crop rotation. In 2006, pathogen isolation frequencies were similar to the previous year. Previous research showed that shortened crop sequences, frequently limited to maize and soybean rotated with susceptible crops such as sunflower (*Helianthus annuus* L.), canola (*Brassica rapa* L.) or dry bean (*Phaseolus vulgaris* L.), increased soil plant pathogen inoculum density (Schwartz and Steadman, 1978). In the tropics, where the charcoal rot pathogen causes blight of emerging seedlings, plant losses of up to 77% have been reported (Schwartz and Steadman, 1978). Charcoal rot symptoms were observed at high levels late in the season during the present three year investigation. When severe, the disease reduces yield and seed quality (Smith and Wyllie, 1999; Yang and Shriver, 2004). Results of these studies indicate that length of the rotations did not affect

survival of the charcoal rot pathogen. These results suggest that to obtain reduction in plant pathogen survival, rotations of soybean with maize or sorghum must be for at least three years in heavily infested fields. However, because of the wide host range of *M. phaseolina* and the long survival times of the microsclerotia, crop rotation would probably have little benefit in reducing charcoal rot (Almeida *et al*., 2003).

Levels of stem canker, caused by *D. phaseolorum* var *meridionalis,* varied across treatments during this investigation. Infection rates were greater in 2005 than in 2004. The disease did not occur in 2006. The severity of disease was greater in continuous soybean than in the crop rotations. Continuous soybean may account for greater infection rates, since the stem canker pathogen can increase its inoculum concentration from one year to the next in continuous crop systems (Wrather and Sweets, 1998; Kucharek, 2001; Tingle *et al*., 2003). The pathogen is considered endemic throughout the south, where it can cause losses up to 100% (Fernandez *et al*., 1999). In the present investigation, disease incidence was lower when soybean was rotated with sorghum. Previous studies reported that the fungus overwinters on diseased stems and infected seed, and that crop rotation will reduce overwintering inoculum (Kucharek, 2001; Tingle *et al*., 2003).

Many diseases can be avoided or controlled by rotating crops (Wrather and Sweets, 1998). Most of the organisms that cause soybean diseases need soybean plants as a host in order to thrive and will die over time without this plant. Sorghum is a nonhost to the pathogen, thus, in the present study the inoculum concentrations decreased the following crop season and the incidence and severity of stem canker was lower. Sinclair and Hartman (1999) reported that economic importance of any single disease may vary from one geographic area to another in any one season. A pathogen may be very destructive one season and difficult or

impossible to find the next season. In 2004, stem canker caused the greatest yield reduction to the soybean crop in the southern United States (Wrather and Koenning, 2006).

Symptoms of brown spot, caused by *Septoria glycines,* were observed in soybean only in 2005 during the first month after planting. Disease levels were low, except when wet weather favored the development of the disease. The primary effect of the disease on soybean was defoliation of the lower leaves. Incidence of brown spot was greater in continuous soybean than in rotated systems with sorghum. Brown spot is primarily a cosmetic leaf disease (William Moore, pers. comm.). Koenning (2001) estimated soybean losses of 0.2% caused by *S. glycines* to Mississippi soybean production in 1997, and Sinclair and Hartman (1999) and Grau and Cullen (2007) reported that yield loss estimates were 8 to 15% when 25 to 50% of the soybean canopy was prematurely defoliated. According to Sinclair and Hartman (1999), Anonymous (2001), Dorrance *et al*. (2001), Pedersen (2006a), and Grau and Cullen (2007), Septoria leaf spot is more severe in continuously cropped soybean fields. To control this disease it is recommended that soybean should be rotated with a non-legume crop for at least one year, since this pathogen overwinters on infected plant debris (Sinclair and Hartman, 1999; Grau and Cullen, 2007). Brown spot was not observed in the present investigation in 2006 possibly because of the hot, dry weather. Previous studies by Pratt (1995a) reported that hot, dry weather stops the development of this disease, while Sinclair and Hartman (1999) found that infection of soybean with *S. glycines* and disease development is favored by warm, moist weather, which promotes sporulation of the pathogen in the primary lesions.

Frogeye leaf spot, caused by *Cercospora sojina,* occurred at low levels in soybean plots during 2004 and 2005, but not in 2006. Crop rotations did not have an effect on the

disease during this investigation. The weather was wet at the beginning of the season in 2004 and throughout the crop growing season in 2005 favoring occurrence of the disease (Wrather and Sweets, 1998). Also, the low disease levels during the study period may have been influenced by the characteristic genetic reaction of the soybean variety to this disease. The soybean variety Pioneer 95B96 used in the study was reported to have intermediate resistance to the frogeye leaf spot pathogen (Newman, *et al*., 2002). In 2006, weather was extremely hot and dry, factors that may have accounted for the absence of disease during the last year of this investigation. Wrather and Sweets (1998) reported that leaves that expand during dry weather remain relatively free of disease. During a wet year, symptoms of frogeye leaf spot may appear uniformly over the foliage. Symptoms may appear on young leaflets since they are susceptible to infection (Koenning, 2000; Westphal *et al*., 2006). Since the fungus survives in crop residue, fields should be rotated out of soybean for at least 1 to 2 years (Wrather and Sweets, 1998; Phillips, 1999; Yang and Lundeen, 1999; Koenning, 2000; Sweets, 2003; Pedersen, 2006c; Westphal *et al*., 2006).

Bean pod mottle disease was prevalent throughout the present investigation. In 2004, bean pod mottle symptoms were not observed, but in 2005 and 2006 levels were high in all soybean plots. The disease is wide-spread in the major soybean-growing areas in the southern and southeastern United States (Giesler *et al*., 2002). All treatments containing soybean in the rotation had similar infection levels. Symptoms on the young leaves were very conspicuous in the upper canopy at plant stages R1 to R2 (beginning bloom to full bloom). As the plants matured and temperatures increased**,** symptoms were masked. Previous studies reported that symptoms are masked during periods of high temperature and are not observed on plants after pod set (Gergerich, 1999). During cooler weather during the growing season disease

symptoms will appear (William Moore, pers. comm.). Plants in some of the plots also had green stem (delayed maturity) after the pods had matured, and the leaf petioles were still attached. This condition may have been the result of this virus disease. Plant stem moisture levels were too high preventing harvest of these plants in 2006. The results obtained indicate that the crop system treatments did not have an effect on bean pod mottle disease pattern distribution throughout treatment plots. This situation was possibly due to small plot size and high mobility of the bean leaf beetle adults which could fly from one plot to the next and spread the virus throughout the different treatments. Previous studies report that the bean leaf beetle, which transmits the bean pod mottle virus from infected plants to healthy soybean plants, is the most important vector in the field (Gergerich, 1999).

Pathogens on Sorghum

Zonate spot, caused by *Gloeocercospora sorghi,* was the most prevalent foliar sorghum disease during this investigation, but did not appear to cause economic losses. Rotations did not seem to affect levels of this disease since it was spread uniformly throughout the treatment plots, usually occurring from the second month after planting until senescence. The fact that the disease was spread uniformly in sorghum plots may be attributed to how the conidia are disseminated by wind and rain (Stack, 2003). In 2006 the disease did not occur due to dry weather and hot temperature. This disease is common throughout sorghum producing areas of the United States, occurring during periods of high rainfall (Stack, 2003). Crop rotation, deep tillage, and clean cultivation are recommended to destroy residues of susceptible weed hosts and reduce the losses from this disease (Franklin, 2000; Stack, 2003). Crop rotation is the most feasible disease management option for control

of zonate leaf spot (Franklin, 2000; Stack, 2003). However in the present study, crop rotations did not influence incidence of zonate spot. Diseases of sorghum, like those of other crops, vary in severity yearly and from one locality to another, depending upon environment, causal organisms, and the host plant's resistance (Jardine, 1998).

Symptoms of gray leaf spot, caused by *Cercospora sorghi,* were observed on sorghum plants at low levels and occurred randomly across the rotation treatments. A warm, wet environment is conducive to development and spread of this disease. Conidia are air-dispersed and spread from host debris (Holliday and Mulder, 1974; Odvody, 1999). Crop rotations can reduce surface residue and initial inoculum, thereby delaying the onset of gray leaf spot (Odvody, 1999).

Charcoal rot, caused by *M. phaseolina,* was not observed during the first two years of this investigation. However, in 2006 a high incidence of this disease occurred on sorghum, and was similar across treatments. It is the most common and probably the most important root and stalk rot disease of sorghum (Mughogho and Pande, 1984; Pande, 2000). Occurrence of the disease in 2006 was primarily attributed to unusually dry weather. As in soybean, charcoal rot is particularly destructive on crops during hot, dry weather if the vigorously growing crop is subjected to moisture stress during the postflowering period (Pande, 2000). This would suggest that sorghum with resistance to the pathogen and or moisture stress would be best for charcoal rot control.

Soybean and Sorghum Root Diseases

Periodic ratings of soybean and sorghum for root diseases had variable results during the three year study. Root disease levels were affected by rotation systems. The levels of root

diseases caused by *M. phaseolina*, *R. solani* and *Fusarium* spp. were significantly greater in continuous plantings of soybean and continuous plantings of sorghum than in the rotation treatments consisting of sorghum-soybean, and soybean-sorghum. In 2006, the treatments involving sorghum had greater root disease levels only on the third sampling date. This was unusual, since plants were subjected to prolonged drought stress during most of the growing season.

Aflatoxins

Aflatoxin concentrations were very low in 2004 and 2005, but in 2006 levels were greater than the acceptable 20 ppb tolerance levels for human food (Krausz, 2003). Previous studies have shown that aflatoxin can occur in sorghum grain. Working with four naturally contaminated samples of sorghum from Illinois, Nasir and Jolley (2002) found average aflatoxin levels between 2.7 and 26.5 ppb. Phillips (1996) reported that 400 ppb of aflatoxin occurred in one sorghum sample in Texas. Aflatoxin production appears to be higher at grain moisture levels of 22 to 26% and temperatures of 26 to 38^{o}C (Phillips, 1996; Cassel *et al*., 2001). Aflatoxin increases during crop-water deficits because the growth of *Aspergillus flavus*, which produces the fungus in the weakened crop, is favored by drought (Rosenzweig, 2001). Dry weather (2.0 to 18.0 ml rainfall) and high temperature levels ranging from 29 to 36 ^{o}C in 2006 favored high levels of aflatoxin production by *A. flavus*.

Crop Yields

Yields collected from soybean and sorghum plots varied across crop system treatments during the three years of this investigation. In 2005, rotation treatments had

significantly greater sorghum yield than continuous sorghum, and soybean rotation systems had significantly greater yield than continuous soybean. Sorghum plant dry weights were generally greater in rotation systems with soybean. The higher sorghum yield in 2005 may have occurred because of the increased nitrogen provided by the previous soybean crop. Peter (2002) reported that specific yield benefits are gained by planting soybeans as part of a sorghum-soybean rotation rather than keeping a field in continuous sorghum in Kansas. Soybean had previously been reported to increase sorghum yield following soybean and sorghum rotations (Varvel and Peterson, 1992). Even though root disease levels may be greater in continuous crop systems, previous studies report that sorghum root disease pathogens, normally harmless, only affect yield when the plant stand is reduced (Wrather *et al*., 1999). However, in the present investigation plant stand was not reduced by root diseases.

As indicated above, soybean yields in continuous soybean rotations were significantly reduced in 2005, with the lower yields coinciding with high levels of stem canker. Stem canker can cause losses up to 100% (Fernandez *et al*., 1999). In 2004, stem canker was reported to cause significant yield reduction in the soybean crop in the southern United States (Wrather and Koenning, 2006). In 2006, soybean yields as well as sorghum yields were similar across treatments. Even though the weather was very hot and dry in 2006, sorghum yields were numerically greater in 2006 than in 2004 or 2005. Previous studies report that sorghum, like maize and sugarcane, carries out C4 photosynthesis, a specialization that makes these grasses well adapted to environments with high temperature and water limitation (Edwards *et al*., 2004). The lower soybean yield in 2006 than in 2004 or 2005 may be due to the prolonged drought during 2006. These results are in agreement with Blaine

(2002b) who reported that drought tolerance in soybeans is not available, thus moisture stressed plants will have reduced soybean yield.

Fungi Isolated from Root Tissue

Even though *R. solani* was rarely isolated from both crops in 2005, it was not a predominant pathogen. The fungus causes pre- and post-emergence damping-off and root rot of young and adult plants including soybean and sorghum (Bauske and Kirby, 1992; Yang, and Uphoff, 1999; Nelson, 2003a). Anastomosis group 4 (AG-4) was the primary group isolated from root tissue. Weather during 2004 and 2006 crop growing seasons was unusually hot and dry explaining why in these years *R. solani* AG-4 isolation levels were very low since the pathogen rarely infects plants at temperatures greater than 24 to 26^{o}C (Yang, 1999).

Cool, wet soils favor the development of seedling diseases, since cool temperatures slow seed germination and seedling growth and favor the growth of many fungi that cause seedling diseases (Wrather and Sweets, 1998; Wrather *et al*., 1999). Previous studies report that *R. solani* has a wide host range, and is a very common pathogen with a great diversity of host plants including soybean and sorghum (Ceresini, 1999). In the present study, *R. solani* was isolated with high frequency during early plant growth stages. No visible symptoms were observed during later plant growth stages and the pathogen could not be isolated from older plants. This is in agreement with Paxton and Chamberlain (1968) who reported that young hypocotyl tissue is highly susceptible to *R. solani*, but plants become progressively more resistant to pathogen invasion, attaining complete resistance by the fourth week of plant growth. They also reported that the change in resistance was associated with a change in pectic substances and calcium content of the hypocotyl.

Three species of *Fusarium* were frequently isolated from root tissue during this research. Even though there were statistical differences among crop system treatments, no obvious trend in disease incidence reduction was observed among sorghum-soybean rotations. Previous studies report that *F. solani* and *F. oxysporum* can cause damping-off of seedlings and root rot on older plants (Datnoff and Sinclair, 1988; Yang, and Uphoff, 1999; Nelson, 2003b). Several different, normally harmless, soil inhabitanting fungi, including *Fusarium* spp., *Pythium* spp., *R. solani*, *Aspergillus* spp., and *Phoma* spp., can attack grain sorghum seed and seedlings (Wrather *et al*., 1999).

Isolation frequencies of *Aspergillus* spp. indicated greater frequency occurrence from sorghum root tissues than soybean root tissues. *Aspergillus* spp. are reported to be more prevalent during drought conditions in the later half of the crop growing season (Agrios, 1997). Previous studies have reported *Aspergillus* spp. to be associated with seedling diseases of sorghum (Forbes and Odvody, 2000).

In general, *Trichoderma* spp. isolation frequencies were very low in 2004 and 2005. In 2006, treatments with sorghum had the highest *Trichoderma* spp. isolation frequencies. *Trichoderma* spp. are fungi that are present in nearly all soils and other diverse habitats. In the soil, they frequently are the most prevalent culturable fungi. They are favored by the presence of high levels of plant roots, which they colonize readily. Some strains are highly rhizosphere competent, i.e., able to colonize and grow on roots as they develop. *Trichoderma* spp. are primarily tissue degrading fungi (Hartman, 2000). There are no reports that *Trichoderma* spp. are pathogenic on soybean or sorghum. Species of this genus occur in soil worldwide and are reported to be very efficient mycoparasites and aggressive competitors with plant pathogens (Cook and Baker, 1983). They are used as a biological control agent

against other fungi (Agrios, 1997; Trigiano *et al*., 2004), such as *M. phaseolina,* which has been successfully controlled by *Trichoderma* spp. (Mohamed *et al*., 2004).

Nematodes

Levels of nematodes collected during this three year study were very low in all crop system treatments. A total of four genera were identified across treatments. All four species including *Helicotylenchus* sp., *Pratylenchus* sp., *Rotylenchulus reniformis*, and *Meloidogyne* sp. have been reported to be parasites on both soybean and sorghum (De Waele and Jordan, 1988; Noel and Acosta, 1999; McGawley and Overstreet, 1999; Kinloch and Rodríguez-Kábana, 1999; De Waele and McDonald, 2000b; Buchanan, 2002). In 2005, spiral nematode (*Helicotylenchus* sp) was the most common species identified from the soil samples. The species was found across all treatments during this research**,** but populations levels were below the economic threshold (Balbalian, 2004; Balbalian, 2005; Balbalian, 2006**)**. In 2006, root-knot nematode (*Meloidogyne* sp.) was isolated from all treatments except continuous sorghum. However, since this species was below threshold levels no differences in treatments occurred.

According to Noel (1999), more than 100 species of plant-parasitic nematodes have been reported to feed on or be associated in some way with the roots of soybean plants, but only a few, such as root-knot nematode, are of economic importance. Quantitative surveys of sorghum fields frequently indicate the presence of plant-parasitic nematodes, including root-lesion and root-knot nematodes. Specific information regarding their pathogenicity and economic importance on sorghum is lacking (De Waele and McDonald, 2000a).

Greenhouse Research

Soybean and Sorghum

Macrophomina phaseolina (isolates 1 and 2) and *R. solani* (isolates 1 and 2), inoculated to pots containing soybean or sorghum, infected the plants with variable results in this greenhouse study. Soybean plant stand was reduced by the two isolates of *M. phaseolina*. Previous studies reported that *M. phaseolina* infected seed may not germinate (Smith and Wyllie, 1999). Low vigor and wilted plants with yellowish foliage were observed in pots treated with the highest concentration of *M. phaseolina* (inoculum:soil=1:50). Studies have demonstrated that *M. phaseolina* produces a toxin (botryodiploidin) which inhibits germination and causes seeding wilt (Smith and Wyllie, 1999; Ramezani *et al*., 2007). The treatment level of 1:50 for the two pathogens is a very high level in soil. Earlier studies reported pathogenicity tests conducted in the greenhouse with *M. phaseolina* at concentrations of 1:200 (inoculum:soil) and *R. solani* at concentrations of 1:90, 1:167 and 1:200 (Baird *et al*., 1996a; Baird and Brock, 1999; Baird and Batson, 2000; Carling *et al*., 2002; Baird *et al*., 2003).

During this greenhouse trial the highest concentration of *M. phaseolina* had greater root disease levels and dry weights than the uninfested control. The results of this study are similar to the field study in that soybean roots were routinely infected by *M. phaseolina*, but rates were similar among treatments. Su *et al*. (2001) did not find greater pathogenicity of isolates from soybean, sorghum or corn, and in addition they reported that based on DNA tests, *M. phaseolina* constitutes a single species. Studies conducted under controlled conditions by Jardine *et al*. (2003) indicated that *M. phaseolina* can affect plant growth.

However, previous studies indicated that *M. phaseolina* isolates did not affect dry plant weights of soybean or sorghum (Su *et al*., 2001). In the present study, plants with the *R. solani* isolate treatments had greater disease levels than the plants in the uninfested control pots, but dry weights were similar among treatments. Even though significant differences were observed, the root disease levels caused by *R. solani* were low. Disease levels attributed to *R. solani* were also low during the field study and no plant stand or growth reductions were observed. Previous studies reported that the pathogen disappeared in plants with time and was not isolated from older plants. As indicated previously, Paxton and Chamberlain (1968) reported that young hypocotyl tissue is highly susceptible, to *R. solani*, but becomes progressively more resistant to invasion by the fourth week after planting, at which time it attains complete resistance. This change in resistance was associated with a change in pectic substances and calcium content of the hypocotyl. Temperature played an important role in this greenhouse study. *Rhizoctonia solani* does not survive well at high temperature, and the soil temperature in the greenhouse during this trial averaged 30^{o}C. Optimum soil temperatures for root rot-causing isolates of *R. solani* AG-4 are 24-26^{o}C (Yang, 1999).

Both *M. phaseolina* and *R. solani* infected sorghum plants, had significantly greater root disease ratings, lower plant height and dry weight compared with plants that were not infested. *Rhizoctonia solani* is included in a group of fungi, which in the soil, can attack grain sorghum seed and seedlings, but normally are harmless (Wrather *et al*., 1999).

CHAPTER V

GENERAL CONCLUSIONS

Six identified insect species on soybean, two on sorghum, and several common plant diseases on the respective crops were used as indicators to determine the effects of crop rotation systems on pest occurrence and density levels on these crops over a three year period. Insect pest numbers and disease levels remained below economic thresholds during each year. However, the most prevalent soybean insects were the threecornered alfalfa hopper and bean leaf beetle. Sorghum webworm and corn earworm were the most common insects on sorghum panicles. Crop system rotations did not affect occurrence and density levels of either soybean or sorghum insect pest species or disease incidence during the three year study.

Three foliar fungal pathogens occurred in the soybean rotation systems including *Diaporthe phaseolorum* var. *meridionalis* (stem canker), *Septoria glycines* (brown spot), and *Cercospora sojina* (frogeye leaf spot) on soybean. Bean pod mottle disease was present in soybean, but levels were not affected by rotations during the study. On sorghum, *Gloeocercospora sorghi* (zonate spot), was identified from field collected crop materials. Zonate spot was the most prevalent foliar sorghum disease, but was not affected by the rotations.

Five potential fungal pathogens were isolated from either soybean or sorghum roots. They included *Macrophomina phaseolina* (charcoal rot), *Rhizoctonia solani* (Rhizoctonia rot

root) and three *Fusarium* spp. *Macrophomina phaseolina,* which occurred on both soybean and sorghum crops, was the most frequently isolated pathogen from the roots, but this fungus was not affected by the different rotation systems.

The reniform nematode (*Rotylenchulus reniformis*), spiral nematode (*Helicotylenchus* sp.), lesion nematode (*Pratylenchus* sp.), and root-knot nematode (*Meloidogyne* sp.) were extracted from soil under either soybean or sorghum in the test field. All species identified were below threshold levels during the study. Therefore, low nematode population levels prevented comparison of the rotation treatments.

Aflatoxin contamination of sorghum seed was low (<20 ppb) the first two years of the study, but was high (790 ppb) in 2006. However, the aflatoxin concentration in sorghum seeds was not affected by the rotation systems.

Soybean and sorghum yields were significantly increased in crop rotations in 2005 compared with the respective monocrop systems. However, it is still uncertain why yields increased that year and no other trends were observed the other years of this study.

In a greenhouse investigation, both *M. phaseolina* and *R. solani* infected soybean and sorghum plants and caused significantly higher levels of root disease, lower plant height and dry weight compared with plants that were not infested. Since the levels of the pathogens were low during the field study, no comparisons could be done with the greenhouse tests.

These studies did not demonstrate significant benefits of a soybean/sorghum rotation in a field with low to moderate levels of insects, diseases, or nematodes. Higher levels of these pests and pathogens may be needed to demonstrate the effectiveness of this pest control method.

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APPENDIX

Table A1. Monthly air and soil temperatures and rainfall during three year crop growing seasons (2004-2006). Starkville, MS.

	Average monthly air and soil temperature (°C)									
	May		June		July		August		September	
Year	Air[a]	Soil[b]	Air[a]	Soil[b]	Air[a]	Soil[b]	Air[a]	Soil[b]	Air[a]	Soil[b]
2004	22	23	25	26	27	27	24	28	23	25
2005	21	25	25	27	27	27	30	30	25	27
2006	22	23	25	31	28	32	29	31	21	25

	Average monthly rainfall (ml)				
2004	11.0	27.0	11.0	8.0	13.0
2005	38.0	36.0	41.0	30.0	20.0
2006	2.0	4.0	7.0	11.0	18.0

[a] Air temperature, [b] Soil temperature.

Table A2. Effect of different concentrations of fungal pathogens to sorghum plant stand in the greenhouse in 2006. Starkville, MS.

Pathogen concentration [a]	Mean plant stand per pot at 14 and 34 DAP							
	Macrophomina phaseolina				*Rhizoctonia solani*			
	Soybean isolate [b]		Sorghum isolate [c]		Soybean isolate [b]		Sorghum isolate [c]	
	14 DAP	34 DAP	14 DAP	34 DAP	14 DAP	34 DAP	14 DAP	34 DAP
1:50	1.8b [d]	2.8b	3.7a	5.5a	3.8b	4.3b	5.0a	6.0a
1:100	3.0ab	4.8ab	3.8a	6.3a	4.0ab	5.0ab	5.3a	5.8a
1:200	3.3ab	4.5ab	4.8a	5.8a	5.8a	4.3b	4.3a	4.3b
1:300	4.3ab	5.0ab	4.8a	5.5a	4.8ab	6.3a	5.0a	6.3a
Control [e]	4.8a	5.5a	4.8a	5.5a	4.8a	5.5ab	4.8a	5.5ab
LSD at P = 0.05	0.1500	0.2575	0.7782	0.8688	0.2675	0.1435	0.8843	0.0802

[a] Fungal concentration inoculated into sterile soil.
[b] *M. phaseolina* and *R solani* isolated from soybean
[c] *M. phaseolina* and *R solani* isolated from sorghum
[d] Means followed by the same letter in the same column are not significantly different using LSD at P ≤ 0.05
[e] Pots not inoculated with pathogens.