THE EFFECT OF COVER CROPS ON SUPPRESSION OF NEMATODES ON

PEANUTS AND COTTON IN ALABAMA

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THE EFFECT OF COVER CROPS ON SUPPRESSION OF NEMATODES ON PEANUTS AND COTTON IN ALABAMA

Sandeep Reddy Marla

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THE EFFECT OF COVER CROPS ON SUPPRESSION OF NEMATODES ON PEANUTS AND COTTON IN ALABAMA

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VITA

Sandeep Reddy Marla, son of Pulla Reddy Marla and Lalitha Marla was born on October 21, 1981, in Khammam, Andhra Pradesh, India. He has one younger sister Swapna Marla. Mr. Sandeep Marla graduated in Bachelor of Agricultural Sciences from Acharya N G Ranga Agricultural University, Rajendra Nagar, Hyderabad, India, in 2005. He joined the graduate school at Auburn University, Alabama to pursue a Master's program in the Department of Entomology and Plant Pathology in January 2006.

THESIS ABSTRACT

THE EFFECT OF COVER CROPS ON SUPPRESSION OF NEMATODES ON PEANUTS AND COTTON IN ALABAMA

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Cover crops were evaluated in the greenhouse and in field locations to determine their host status and nematode suppressive effect on root-knot nematodes, *Meloidogyne incognita* and *M. arenaria* and the reniform nematode, *Rotylenchulus reniformis*. The winter grain cover crop cultivars included commercially available cultivars of wheat '*Triticum aestivum*' 'Pioneer 26R12', 'AGS 2000', 'Coker 9152', 'Panola'; oats '*Avena sativa*' 'Georgia Mitchell' and 'Bob'; rye '*Secale cereale*' 'Elbon' and 'Abruzzi'. This research also evaluated the host status and nematode suppressive effect of *Crotolaria juncea* populations. The treatments included the *C. juncea* populations; PI 207657, PI 314239, PI 322377, PI 391567 and PI 426626 collected in different countries and the commercially available cultivar 'Tropic Sun'.

Field evaluations of winter grain cover crop cultivars described previously were conducted at WREC in Headland, AL and in a grower's field in Huxford, AL. There were no significant differences ($P \leq 0.05$) between cover crop cultivars on nematodes. This was most probably due to severe drought and uneven rainfall during both the cropping years. However, the greenhouse studies indicated that 'Elbon' rye; oats 'Bob' and 'Georgia Mitchell' supported low populations of *M. incognita*. While 'Bob' oats and the rye 'Elbon' and 'Abruzzi' supported significantly ($P \geq 0.05$) lower *R. reniformis* populations.

C. juncea populations were able to significantly suppress ($P \geq 0.05$) *M. incognita* and *R. reniformis* in the greenhouse tests. *C. juncea* roots stained with McCormick Schilling® red food color were found to contain all juvenile stages, low numbers of mature females of *M. incognita* with egg masses and 1-2 female reniform nematodes per 10 gm of roots, indicating that these nematodes were able to infest and reproduce on *C. juncea* populations. Freeze-dried root exudates tested against both *M. incognita* and *R. reniformis* demonstrated that concentrated exudates could kill both nematodes whereas the water control had no effect. Field trial at EVSRC in Shorter, AL indicated that there were no significant differences observed on *Meloidogyne* spp. suppression among the *C. juncea* populations, might be due to severe drought and extreme high temperatures.

The knowledge obtained from this study suggests that some winter cover crop cultivars and *C. juncea* populations may be poor hosts and suitable for crop rotation in a region with specific nematode histories, thus minimizing usage of synthetic nematicides and yield losses. However, further research studies should focus on extensive long-term field studies under controlled irrigation conditions.

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I. LITERATURE REVIEW

Plant-parasitic nematodes

Nematodes are microscopic, unsegmented invertebrate roundworms with bilateral symmetry (Veech, 1984). Nematodes are ubiquitous and regarded as an important part of every ecosystem. Most of the nematodes are beneficial because of their free-living and saprophytic nature and play a major role in decomposition of organic matter and nutrient recycling. Only 10% of the total known nematodes are considered to be plant-parasites (Schumann and D'Arcy, 2006). These parasitic nematodes are obligate parasites and always require a host plant for their survival. Most of the plant-parasitic nematodes belong to order Tylenchida while a few belong to order Dorylaimida (Agrios, 1997).

Plant-parasitic nematodes are identified by the presence of a stylet which is a specialized feeding structure used for penetrating the plant tissues. They can feed externally as ectoparasites or develop as endoparasites within the roots. Plant-parasitic nematodes life cycle starts with the egg and develops through four juvenile stages with the last molt to an adult (Schumann and D'Arcy, 2006). Plant-parasitic nematodes have a very wide host range and are found to infest a wide variety of agronomic crops.

Plant-parasitic nematodes are identified as one of the major limiting plant parasites for all the cash crops throughout the world. The Society of Nematology and

other organizations estimates worldwide crop losses due to nematodes at $100 billion per annum (Schumann and D'Arcy, 2006). In the United States, plant-parasitic nematodes cause an estimated $10 billion of crop losses each year (Mani et al., 2005). Two major crops infested by nematodes are peanuts and cotton. Severe economic losses in peanuts and cotton results from infestations of plant-parasitic nematodes, such as the root-knot nematode, *Meloidogyne* spp. and the reniform nematode, *Rotylenchulus reniformis* (Rodriguez-Kabana et al., 1991). These nematodes reduce crop productivity by their direct action and association in pathogenic disease complexes. *Meloidogyne* spp. account for yield losses up to 12% in peanuts (Handoo, 1998) and *R. reniformis* causes around 9 % loss in cotton (Blassingame, 2007). These parasitic nematodes cause severe yield losses when infested during early stages of crop growth.

Peanuts and root-knot nematode

Peanuts (*Arachis hypogaea* L.) belong to family Fabaceae and have their origin from Peru (South America). Peanuts are one of the most important oil seed crops in the world. Peanut is a self-pollinated, erect or prostate, sparsely hairy, annual herbaceous legume and geocarpic as it produces underground pods.

Peanuts play a pivotal role in the crop economy of the United States and in the Southeastern United States. The U.S. peanut production accounted to 3.37 billion pounds in the year 2006 (NASS, USDA, 2006), a substantial decrease from the 2005 production of 4.86 billion pounds. In Alabama, the peanut production accounted to 331 million pounds

(NASS, USDA, 2006). The harvested area decreased from 1.62 to 1.21 million acres from the year 2005 to 2006 in the nation (NASS, USDA, 2006). The reason for the gradual decrease in peanut yields is not clear but may be due to adverse climatic conditions, severe pest problems and a commodity market shift. Approximately 60% of the peanut production in the United States occurs in Georgia, Florida and Alabama (Fletcher, 2002). Damage caused by the *Meloidogyne* spp. is one of the most serious constraints for peanut production in these states.

Meloidogyne spp. nematodes are present throughout the world and found to attack more than 2000 plant species (Agrios, 1997). *Meloidogyne* spp. are sedentary endoparasites. They establish feeding sites inside the roots known as giant cells and continue their life cycle within the roots. The life cycle of a *Meloidogyne* spp. nematode is completed in 3-4 weeks under favorable conditions. In the Southeastern United States, the peanut root-knot nematode, *Meloidogyne arenaria*, is one of the major *Meloidogyne* spp. that causes severe yield losses in peanuts (Rodriguez-Kabana et al., 1991). *Meloidogyne* spp. can occasionally damage the entire crop when the infestation is wide spread. The symptoms produced by *Meloidogyne* spp. include formation of galls on roots, pegs and pods (Porter et al., 1984). In case of severe infestations, plants are stunted in growth and light green in color, resembling nutrient deficiencies. Nematode damage can be identified by the presence of small spots with a dark center on peanut pods (Porter et al., 1984). Above ground symptoms includes yellowing of foliage, midday wilting and stunted growth in patches over the entire field. The losses incurred by these nematodes

can be effectively reduced by use of synthetic nematicides, fallowing and crop rotation with non-host crops.

Cotton and reniform nematode

Cotton (*Gossypium* spp.) is the most important fiber crop of the world. Cotton is a perennial plant with indeterminate growth habitat but is cultivated as an annual crop. There are four domesticated species of cotton: *Gossypium arboretum* L., *Gossypium herbaceum* L., *Gossypium barbadense* L., and *Gossypium hirsutum* L. (Lee, 1984). Cotton can be grown effectively around the world between the latitudes of 47^0 north and 32^0 south (Lee, 1984). The growing period for cotton is from six to eight months depending on climatical conditions and the weather patterns. Cotton is generally planted between April and early June and generally harvested from September to October.

Cotton is primarily grown for fiber and the seeds are an important source of oil. The United States is the second-largest producer and the largest exporter of cotton in the world (Fry, 2001). The U.S. cotton production in the year 2006 accounted to 20.5 million bales (NASS, USDA, 2006) while cotton production in 2005 is 23.2 million bales (NASS, USDA, 2005).

Cotton production in the Southeastern United States is hindered by insects and plant-parasitic nematodes (Koenning et al., 2004). The major nematode infesting cotton in the Southeastern states is the reniform nematode, *Rotylenchulus reniformis*, accounts

for around 9% of the yield losses on this crop (Blassingame, 2007). In surveys of Alabama cotton fields conducted by Gazaway and McLean (2003), 47% of the fields contained *R. reniformis*. The symptoms produced by *R. reniformis* infestation are not conspicuous, and require root and soil analysis to determine the nematode's presence (Gaur and Perry, 1991). Symptoms produced by *R. reniformis* include uneven growth of plants, severe stunting, yellowing of foliage and premature death of the plants (Lawrence and McLean, 2001).

Rotylenchulus reniformis is a sedentary, semi-endoparasitic nematode that infests a wide range of agronomic crops. The life cycle consists of an egg, four juveniles and an adult stage, and is completed within 24-29 days from egg to egg (Guar and Perry, 1991). Adult males do not feed on the roots. The vermiform females penetrate roots to establish a feeding site in the stele (Gaur and Perry, 1991).

Winter cover crops

Cover crops are planted between cycles of the main cash crop or intercropped with cash crops to improve soil fertility, soil structure, water infiltration, and reduce soil erosion (Hooks et al., 1998). They have the potential to suppress pathogens, weeds and nematode pests**.** According to Barker and Koenning (1998) crop rotation with cover crops provided diversity in time and space and is often considered as a preferred means to manage plant-parasitic nematodes. A significant reduction of *R. reniformis* nematodes has been observed when cotton is rotated with winter grain crops (Jones et al., 2006). In

addition to controlling parasitic nematodes, specific cereal crops have also decreased weeds more efficiently at early stages of crop growth (Wang et al., 2004a). Taking into consideration of all the above mentioned beneficial qualities, winter cover crops may serve as an alternative to chemical nematicides.

Meloidogyne spp. and *R. reniformis* are major plant nematode pathogens causing severe yield losses in the Southeastern United States. The present method of controlling these nematodes is use of synthetic nematicides; however, it is not cost-effective and can be harmful to environment and humans. Crop rotation with cover crops can offer a supplement to this current nematode management strategy (McSorley, 1999). Crittenden (1961) has reported many commercial wheat cultivars to be hosts of *M. incognita* and *M. javanica*. Recent studies with winter cover crops demonstrated some winter grain crops decreased numbers of *M. incognita* better than the leguminous cover crops (Wang et al., 2004a). Field evaluations described that winter grain cover crops maintained low populations of *M. incognita* throughout the winter season but nematode densities were found to increase after planting a susceptible host (Wang et al., 2004a). Greenhouse studies for evaluating winter cover crop cultivar host status has observed reduction of *R. reniformis* populations on 'Gulf' ryegrass (*Lolium multiflorum*), 'Wren's Abruzzi' rye (*Secale cereale*), 'Soil Saver' blackoats (*Avena strigosa*), ' AU Homer' lupin (*Lupinus albus*), and 'Coker 9663' wheat (*Triticum aestivum*), suggesting that these crops were non-host crops (Jones et al., 2006).

In field experiments, Wang et al., (2004a) observed that rye and oats were poor hosts of *M. incognita* and reduced nematode populations more effectively than fallow.

Crop rotation of cotton with rye supported the least number of *M. incognita* eggs and there was very low root-galling in cotton during the next cropping season (Timper et al., 2006). McSorley (1994) described rye to be partially suppressive to *M. arenaria*; however, there was no decrease in the nematode populations. A regression model of *M. arenaria* indicated slight decline of populations over the initial populations (Pi), indicating that rye can be used as a winter cover crop or rotation crop (McSorley, 1994). Planting rye as cover crop can also lower the risk of increasing populations of *M. incognita* when compared to use of clovers and vetches (Timper et al., 2006). According to Zasada et al., (2005) rye tissue degradation products contained chemicals DIBOA (2, 4-dihydroxy-(2H)-1,4-benzoxazin-3(4H)-one) and DIMBOA (2,4-dihydroxy-7-methoxy-(2H)-1,4-benzoxazin-3(4H)-one). These chemicals resulted in a mortality rate of 73% and 71% for *M. incognita*. However, the nematode suppressive effect lasted through a single cropping season and nematode densities increased again after planting susceptible crops. Specific cover crops are found to suppress plant-parasitic nematodes but the mechanism of suppression is not clearly known. Reduced numbers of plant-parasitic nematode abundance in cover crops may be due to poor host status, production of allelochemicals or enhancement of nematode-antagonistic flora and fauna (Wang et al., 2002).

Sunn hemp

Crotalaria juncea L. (sunn hemp) used as a cover crop in crop rotation has been demonstrated to have many beneficial qualities. It is an effective legume cover crop that

adds nitrogen and organic matter to the soil and enhances soil fertility (Wang et al., 2004b). *Crotalaria juncea* has the potential to grow and cover the soil surface rapidly while protecting the soil surface from erosion (Mansoer et al., 1997). In addition to controlling weeds and soil erosion, *C. juncea* also produced a biomass of 7.6 Mg/ha after 14WAP with an average N content of 144 kg/ha (Balkcom and Reeves, 2005). *Crotalaria juncea* can be grown as a green manure crop or intercropped with the cash crops.

Crotalaria juncea has shown high degree of resistance to several *Meloidogyne* spp. (Wang et al., 2004b) and increase free-living nematode populations in pineapple cultivation (Wang et al., 2003). Besides increasing beneficial nematode populations, *C. juncea* can increase nitrogen especially in organic production systems (McSorley, 1999). This combination of nematode and nitrogen management could be especially useful in sustainable and organic production systems where neither nematicides nor synthetic nitrogen fertilizers could be used (McSorley, 1999). In West Africa, *Crotalaria* spp. such as *C. podocarpa, C. senegalensis* and *C. sphaericarpa* are used as nematostatic green manure crops (Jourand et al., 2004). *Crotalaria juncea* used for green manure production can also be used in crop rotation to decrease nematode population levels and propagate arbuscular mychorrhizal (AM) fungi for subsequent crops (Germani and Plenchette, 2004). *Crotalaria juncea* can also be cultivated as green manure crop and ploughed into the soil in organic farming systems. Several research studies have found that this leguminous cover crop has considerable potential for use by farmers in developing countries to control nematode populations in low-value cropping systems (Jourand et al., 2004).

Crotalaria juncea plant residues incorporated into soil have been described to contain antagonistic activity against some plant-parasitic nematodes (Rodriguez-Kabana and Kloepper, 1998). Previous studies describe *C. juncea* as a poor host to many important plant-parasitic nematodes, including *M. incognita, M. javanica, M. arenaria*, *R. reniformis* and *Pratylenchus brachyurus* (Wang et al., 2004b). Germani and Plenchette (2004) indicated that plant extracts of *C. juncea* apparently inhibited egg hatching and were found to be lethal to second-stage juveniles (J2) of *M. incognita*. The aqueous crude extracts from *C. juncea* shoot and root paralyzed J2 of *M. incognita*, *M. javanica* and *M. mayaguensis* (Jourand et al., 2004). McSorley (1999) indicated *C. juncea* are highly resistant to nematodes but not immune. Significant differences are observed in invasion and developmental rates of *M. incognita* and *M. javanica* on *C. juncea* and on susceptible control. McSorley et al., (1994) described *C. juncea* as a trap crop that reduced population densities of several *Meloidogyne* spp., while enhancing the yield of subsequent nematode susceptible vegetable crop. However, these favorable effects last only through a single cropping season.

Crotalaria juncea is found to be a poor host to *R. reniformis*, allowing the nematode to penetrate the roots but restricting their development and reproduction (Wang et al., 2003). Nematode invasion rates on *C. juncea* are very low when compared to the susceptible control (tomato). The juvenile J2 which invaded the tomato plants developed into adults, while those on *C. juncea* rarely developed beyond the third-stage juvenile J3, thus confirming *C. juncea* to be nonhosts or poor hosts (Germani and Plenchette, 2004). Previous studies have reported the ability of *C. juncea* to enhance the activity of

nematode-antagonistic microorganisms (Rodriguez-Kabana and Kloepper, 1998) and population densities of free-living nematodes (Wang et al., 2002). *Crotalaria juncea* amendments when incorporated into soils with low organic matter increased numbers of beneficial nematodes. Research studies of Wang et al., (2004b) with *C. juncea* hay as organic fertilizer is found to increase shoot and root weight of squash and were able to enhance the activity of some nematode-antagonistic fungi in soils. Moreover, *C. juncea* also enhanced free-living nematodes which play a major role in nutrient cycling (Wang et al., 2004b). Plant extracts of *C. juncea* apparently inhibited hatching of eggs and are lethal to second-stage juveniles of *M. incognita and R. reniformis* (Rich and Rahi, 1995). Chemical analysis of root and leaf exudates from *Crotalaria* spp. described the presence of allelopathic compounds such as monocrotaline and pyrrolizidine alkaloids (Rich and Rahi, 1995). These compounds produced are toxic to plant-parasitic nematodes and inhibited the formation of *Meloidogyne* spp. galls (Araya and Caswell-Chen, 1994). The mechanism by which *C. juncea* limits the plant-parasitic nematodes is not clearly known but this may due to the production of nematostatic or nematicidal compounds contained in the root system or in the aerial vegetative parts (Germani and Plenchette, 2004).

II. EFFECT OF WINTER GRAIN CROP CULTIVARS ON *MELOIDOGYNE* SPP. AND *ROTYLENCHULUS RENIFORMIS* SUPPRESSION

INTRODUCTION

In Southeastern United States, most of the peanuts (*Arachis hypogaea* L.) and cotton (*Gossypium hirsutum*) are monocultured, creating a favorable environment for enhancing plant-parasitic nematode densities (Rodriguez-Kabana et al., 1991). These pests cause severe yield losses when they infest plants at early stages of the crop growth. The most effective strategy for controlling plant-parasitic nematodes is chemical-based management. However, these synthetic nematicides may potentially have adverse affects on human health and environment.

Crop rotation with cover crops is often considered as the most practical means of controlling plant-parasitic nematodes (Barker and Koenning, 1998). Knowledge of the host status of cover crops plays a vital role in successful usage of these crops since many crops are susceptible to plant-parasitic nematodes. There are contradictory reports in the literature on susceptibility of cover crops. Some literatures suggest that winter cover crops decrease plant-parasitic nematodes while other suggests that they may be non-hosts (Jones et al., 2006). For instance, many commercial wheat (*Triticum aestivum*) cultivars were hosts of *Meloidogyne incognita* and *Meloidogyne javanica* (Crittenden, 1961;

Opperman et al., 1988) and resulted severe infestations in the next cropping season. Wang et al., (2004a) described rye (*Secale cereale*) and oats (*Avena sativa*) to be poor-hosts of *M. incognita*. Previous studies showed that lower plant-parasitic nematode densities in cover crops may be due to poor-host status, production of allelochemicals or enhancement of nematode-antagonistic flora and fauna (Wang et al., 2002). This research was conducted to determine the effect of commercially available winter grain cover crop cultivars on root-knot nematode, *Meloidogyne* spp. and the reniform nematode, *Rotylenchulus reniformis* suppression.

MATERIALS AND METHODS

Field experiments were conducted at two locations, Wiregrass Research and Extension Center (WREC), Headland, AL, and in a grower's field near Huxford, AL. The field evaluations were conducted for two cropping cycles (2005-06 and 2006-07). The following winter grain cover crops, wheat (*Triticum aestivum*) cultivars 'AGS 2000', 'Coker 9152', 'Pioneer 26R24'; oats (*Avena sativa*) cultivars 'Bob' and 'Georgia Mitchell'; rye (*Secale cereale*) cultivars 'Elbon' and 'Abruzzi' were used for evaluations. During the second cropping year (2006-07), in addition to the above cultivars, wheat (*Triticum aestivum*) cultivars 'Pioneer 26R61', 'Pioneer 26R12' and 'Panola' were also evaluated for nematode suppression. Fallow was used as control for all the field studies.

These research plots were planted in November 2005 with the winter grain cover crops and sampled monthly. In April, the cover crops were harvested and yield data was

collected. After harvesting of cover crops at WREC, peanuts (*Arachis hypogaea*) were planted. In Huxford, AL, cotton (*Gossypium hirsutum* cv. DP 555 BG/RR) was planted in the same plot.

Nematode extraction and quantification

Each plot was sampled monthly in a zigzag pattern using a soil probe. Five samples of soil per treatment were taken per plot. Soil samples were stored in a one-gallon plastic bag, at 10 ^{o}C until the nematodes were extracted. Each soil sample was mixed thoroughly and a 100 cm^3 aliquot of soil was taken from each sample to extract the nematodes.

Nematodes were extracted using gravity screening and centrifugal flotation method (Jenkins, 1964). Aliquots of soil (100 cm^3) taken from each sample was mixed with water in a container. The sediments were allowed to settle to the bottom. The solution was passed through a series of nested sieves (60-µm on top, 350-µm in middle and 500-µm at the base). The material on the 500-µm sieve was washed into a 50-ml plastic tube and centrifuged for 4 min at 2400 rpm. The supernatant was decanted and 1M sucrose solution was added to the plastic tubes and centrifuged for 2 min at 1200 rpm. The sucrose supernatant containing the nematodes was decanted into a 500-µm sieve, rinsed with water and collected into separate test tubes. Nematodes were identified to genus level and counted on grid plates on a Nikon T-100® inverted microscope at 10x magnification.

Field evaluations

Experiment I was conducted at WREC, Headland, AL to evaluate the effect of winter grain cover crops on peanut root-knot nematode, *Meloidogyne arenaria* suppression. Each plot was 10 m in length and 4 m in width. The soil type was Dothan sandy loam. This experiment was conducted for two years from November 2005 to October 2007. The treatments included winter grain cover crop cultivars of wheat, oats and rye described previously and one fallow (control). This experiment was arranged in a completely randomized block design, replicated four times. The winter grain cover crop treatments were row planted in November 2005 and 2006 using a John Deere® tractor mounted plot planter with 15 cm spacing between the rows in individual plots, harvested after maturity, and replanted with peanuts in June 2006 and 2007. Peanuts were harvested at maturity in October 2006 and November 2007 and the yield was recorded separately for each plot. The yield of winter grain crop cultivars and peanuts was recorded for both these years. Soil sampling was done at monthly intervals during the cover crop life cycle and at pre-season (June), mid-season (August) and harvesting (November) during the peanut cropping cycle. Nematodes were extracted and quantified as described above.

Experiment II was conducted in a grower's field near Huxford, AL to evaluate the effect of winter grain cover crops on reniform nematode, *Rotylenchulus reniformis*. The soil type was Ruston very fine sandy loam. Each plot size measured 10 m in length and 4 m in width. The experiment arrangement was similar to the above experiment. This experiment was repeated for two years. The same winter grain cover crops used in experiment I were planted in November 2005 and 2006, harvested and shoot weights of

winter grain crops was recorded. Cotton was planted in the same plots with 90 cm spacing between the rows during June 2006 and 2007, and was harvested in October 2006 and November 2007. After harvesting, yield of cotton was recorded. Soil samples were collected as described previously and nematodes were extracted from soil samples. Soil sampling was done at monthly intervals during the winter cover crop life cycle, at pre-season (June), mid-season (August), and at the time of harvesting (November) during the main cash crop cycle. Nematodes were extracted, and quantified to genus level.

Greenhouse evaluations

Greenhouse evaluations were conducted at the Plant Science Research Center, located on the campus of Auburn University, Auburn, AL. Two experiments were conducted to evaluate the host status of winter grain crops for *M. incognita* and *R. reniformis*. Isolates of *M. incognita* and *R. reniformis* nematodes were maintained in the greenhouse on tomato (*Lycopersicon esculentum* cv. Rutgers) and cotton (*Gossypium hirsutum* cv. DP 555 BG/RR), respectively. The winter grain crop treatments used were wheat (*Triticum aestivum*) cultivars 'AGS 2000', 'Pioneer 26R12'; oats (*Avena sativa*) cultivars 'Bob' and 'Georgia Mitchell'; rye (*Secale cereale*) cultivars 'Elbon' and 'Abruzzi'. Tomato and cotton were the controls for *M. incognita* and *R. reniformis* evaluations respectively. The soil used was a mixture of autoclaved loamy sand soil field soil (72.5%, 25%, 2.5%, S-S-C, pH 6.4) and sand in the ratio of 3:1 in 500 cm^3 polystyrene cups. Both the experiments were arranged in a completely randomized block design on raised benches, replicated ten times. Both the *M. incognita* and *R. reniformis* experiments were repeated for the second time**.** Winter grain crop seeds were hand-sown

into the cups, allowed to germinate and grow. One week after germination of winter grain crops nematode eggs were infested as described below.

On the day of nematode infestation, nematode eggs from the host plants were extracted using hypochlorite method by agitating roots in 0.6% sodium hypochlorite solution (Hussey and Barker, 1973). Eggs were collected by washing through a 350-µm and 500-µm sieve. Nematode eggs were rinsed with water and the solution containing nematode eggs was collected. The number of eggs for innoculum was standardized by adding water to the solution. Every individual cup was infested with ca. 4000 *M. incognita* eggs or ca. 2000 *R. reniformis* eggs near the root zone by making small holes at the base of the plant. After 50 days, the roots were washed gently with water; the shoot weight and root weight of the winter grain crops was recorded. Nematode eggs present on the winter grain crop roots were extracted as described above and counted. Nematode eggs present per gram of root were determined using the formula (Number of eggs/gm of root = Total number of eggs present on plant / Total root weight of the plant) and the data were analyzed as described in data analysis.

Data analysis

The field data and the greenhouse data of nematode populations were log transformed and analyzed separately for each experiment. The transformed values were submitted to analysis of variance using Generalized Linear Model in Statistical Analytical System software (SAS institute, Inc., Cary, NC). Probability of F-value was used to determine the significant effects of winter grain cover crop treatments ($P \geq 0.05$).

Treatment means were separated by Fisher's protected Least Significant Difference (LSD) and the effect of winter grain cover crop treatments on *M. incognita* and *R. reniformis* nematodes was compared.

RESULTS

Field evaluations

In the field studies during 2005-06 at WREC, there was no significant difference ($P \leq 0.05$) observed in the nematode suppression among the different winter grain crop cultivars evaluated (Table 1). Even though there were no significant differences on nematode populations among winter grain cover crops, the numerically decreasing order of winter grain crop susceptibility to *M. arenaria* nematode demonstrated was 'Bob' oats, 'AGS 2000' wheat, 'Elbon' rye, 'Georgia Mitchell' rye, 'Pioneer 26R24' wheat, Fallow, 'Coker 9152' wheat and 'Abruzzi' rye.

During this cropping cycle, *M. arenaria* counts remained low throughout the winter cropping season and were lowest at harvest of winter grain cover crops. Nematode densities were found to gradually decrease from sowing to harvesting of winter grain cover crops (Table 1). Nematode populations were found to resurge after planting of peanuts. Highest numbers of *M. arenaria* were present after harvesting of peanuts.

During the second cropping season of 2006-07 at WREC, the winter grain cover crops had no significant effect on nematode suppression ($P \leq 0.05$) (Table 2). The

decreasing order of numerically relative host susceptibility was 'Coker 9152' wheat, 'Elbon' rye, 'Pioneer 26R61' wheat, Fallow, 'AGS 2000' wheat, 'Georgia Mitchell' oats, 'Bob' rye, 'Abruzzi' rye, and 'Panola' wheat. The overall nematode populations were highest during the harvesting of winter grain cover crops (April), followed by nematode counts during harvesting of peanuts in October (Table 2). There was no significant difference between the cover crop yields and the peanut yields during the two cropping seasons.

In Experiment II during 2005-06 Huxford, AL, the winter grain cover crop cultivars supported low *R. reniformis* nematode populations. No significant difference ($P \leq 0.05$) was observed among the winter grain cover crop cultivars on *R. reniformis* populations (Table 3). Nematode counts were varied between different sampling months. Highest nematode counts were recorded at mid-season sampling of cotton followed by pre-plant sampling of cover crops (Table 3). Nematode counts were comparatively low throughout the winter grain cover cropping cycle.

In the cropping cycle of 2006-07 at Huxford, AL, there were no significant differences ($P \leq 0.05$) on nematode susceptibility between the different winter grain cover crop cultivars evaluated (Table 4). The numerically decreasing order of winter grain cover crop susceptibility for *R. reniformis* nematode was 'Georgia Mitchell' oats, 'Bob' oats, 'Abruzzi' rye, 'Panola' wheat, 'Pioneer 26R61' wheat, 'Coker 9152' wheat, 'Elbon' rye, Fallow, and 'AGS 2000' wheat. The nematode densities were highest during initial stages of cover crop growth 'January', and remained high throughout the rest of the winter grain cover crop cycle. Nematode densities were very low at pre-planting of

cover crop 'November'. There was no significant relationship between the winter cover crop yields and the cotton yields during both cropping years.

Greenhouse evaluations

Winter grain crop cultivars had a significant effect ($P \geq 0.05$) on the rate of *M. incognita* nematode reproduction (Table 5). The nematode reproduction among the treatments ranged from 3.516 [Log (x+1) of number of *M. incognita* per gram of root weight) on 'Rutgers' tomato to 1.854 'Bob' oats. Among the winter grain crops evaluated, wheat cultivars supported highest number of nematodes, followed by rye, and oats supported the lowest number of *M. incognita* nematodes (Table 5). Wheat supported numerically high amounts of *M. incognita* nematodes than oats and rye cultivars; however, the reproduction of *M. incognita* nematodes on wheat was less than the tomato control. The nematode susceptibility of winter grain crop cultivars in decreasing order was Tomato, 'AGS 2000' wheat, 'Pioneer 26R12' wheat, 'Abruzzi' rye, 'Elbon' rye, 'Georgia Mitchell' oats, and 'Bob' oats (Table 5).

The greenhouse evaluations of *R. reniformis* nematodes, there was a significant relationship ($P \geq 0.05$) on *R. reniformis* nematode reproduction among the winter grain crop cultivars and the control (Table 6). Cotton supported numerically highest number of *R. reniformis* nematodes. Among the winter grain crops, 'Pioneer 26R12' wheat supported numerically highest number of *R. reniformis* nematodes and 'Abruzzi' rye supported the lowest number of *R. reniformis* nematodes (Table 6). The decreasing order of relative host status of winter grain crops to *R. reniformis* nematode demonstrated from

this experiment was Cotton, 'Pioneer 26R12' wheat, 'Georgia Mitchell' oats, 'AGS 2000' wheat, 'Bob' oats, 'Elbon' rye, and 'Abruzzi' rye (Table 6).

DISCUSSION

Field evaluations

In experiment I at WREC, the reason for gradual decrease in nematode populations among different sampling intervals was due to adverse climatic conditions. There was a severe drought during the winter grain cover crop cycle, resulted in poor crop growth and no significant differences between the winter grain crop yields and nematode suppression, as observed in the greenhouse evaluations. Highest numbers of *M. arenaria* nematode populations were present at time of harvesting of peanuts, which indicated that under field conditions the winter grain cover crops had no residual effects on *M. arenaria* populations. However, this was previously observed by Wang et al., (2004a).

During the first cropping year 2005-06 at WREC, Wheat cultivar 'Pioneer 26R24' had no effect on nematode suppressive effect, therefore this cultivar was removed from the trials and other wheat cultivars described above were included during the second cropping year.

In 2006-07 at WREC, 'Panola' wheat and 'Abruzzi' rye supported very low *M. arenaria* reproduction, this may be due to uneven rainfall during the winter grain cover

crop cycle. *Meloidogyne arenaria* nematode densities were high during winter grain cover crop harvesting; which may have been the result of increased rainfall before harvesting of winter cover crops.

Experiment II conducted at Huxford, AL, during 2006-07 demonstrated contrasting results from that of the first cropping year. In 2005-06 cover crop cycle, *R. reniformis* nematode densities were low on winter grain cover crop cultivars, whereas *R. reniformis* densities were high during the 2006-07 winter grain cover crop cycle. The reason for this contrasting result was due to uneven rainfall received during cover crop cycle. The greenhouse results suggest that rye cultivars and oats may be used as winter cover crops in crop rotation to manage *R. reniformis* nematodes. However, due to adverse climatic conditions these field studies were inconclusive.

Greenhouse evaluations

Among the different winter grain crop cultivars evaluated in the greenhouse, wheat cultivars 'AGS 2000' and 'Pioneer 26R12' served as hosts for *M. incognita*. 'Abruzzi' rye also supported higher number of *M. incognita* nematodes, similar to wheat cultivars. While cultivars 'Elbon', 'Bob' and 'Georgia Mitchell' were found to be poor hosts, supporting very low populations of *M. incognita*. This experiment demonstrated that wheat cultivars of 'Pioneer 26R12', 'AGS 2000', and rye 'Abruzzi' were more susceptible to *M. incognita* nematode, suggesting that these crops should not be used as cover crop in crop rotation sequences to manage *M. incognita* nematodes. Based on the difference in the levels of reproduction of *M. incognita* nematodes on winter grain crops,

oats cultivars ‘Georgia Mitchell’ and ‘Bob’, and ‘Elbon’ rye gave the better results and could be recommended in crop rotation with peanuts to control *M. incognita* nematodes. These greenhouse results were similar to those reported by Wang et al., (2004a).

Winter grain crop cultivars supported low reproduction of *R. reniformis* nematode. There was a significant difference between the winter grain crop cultivars and the control. Among the winter grain crop cultivars, wheat cultivars ‘Pioneer 26R12’ and ‘AGS 2000’ were most susceptible to *R. reniformis*, indicating these cultivars were good hosts for *R. reniformis*. Oats cultivar ‘Georgia Mitchell’ supported high *R. reniformis* populations, suggesting this cultivar should not be used in crop rotation. However, the oats cultivar ‘Bob’ supported low nematode reproduction between the different winter grain crops evaluated. Therefore, cultivars selection is important in crop rotation. *Rotylenchulus reniformis* reproductions were very low on rye cultivars ‘Abruzzi’ and ‘Elbon’, suggesting these cultivars to be poor-hosts and can be used in a crop rotation sequence with cotton for managing *R. reniformis* nematodes.

Table 1. Effect of winter grain cover crop cultivars on populations of peanut root-knot nematode, *Meloidogyne arenaria* at Wiregrass Research and Extension Center, Headland, AL, during the cropping cycle of year 2005-06.

Cultivar	Winter grain cover crop sampling					Peanut sampling		
	November[a]	January[a]	February[a]	March[a]	April[a]	June[a]	August[a]	October[a]
Abruzzi	39.3	13.5	11.5	5.5	13.8	10	22	148.3
Fallow	42.5	20.6	11.9	12.3	7.5	15.9	38.1	296.8
Pioneer 26R24	49.9	7.4	11.1	20.6	6.3	8.8	45	202.5
AGS 2000	17.3	17	15.4	19.9	11.6	11.8	49.9	126.9
Elbon	12.5	17.3	5	14.3	18.8	10	88.3	68
Bob	32.8	9.3	19.3	24.3	21.3	7	34.8	128
Coker 9152	12.4	18.5	9.6	13	10.4	14.8	44.4	148.1
Georgia Mitchell	23.8	17.3	9.8	13	15	10.3	33.3	137
LSD ($P \leq 0.05$)	48.1	18.2	13.4	17.5	15.7	16	37.7	216.4

[a] Number of nematodes present in a 100 cm^3 sample.
Cover crop was planted in the middle of November 2005 and was harvested in April 2006. Peanuts were planted in June and harvested in October.
Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 2. Effect of winter grain cover crop cultivars on populations of peanut root-knot nematode, *Meloidogyne arenaria* at Wiregrass Research and Extension Center, Headland, AL, during the cropping cycle of year 2006-07.

Cultivar	Winter grain cover crop sampling					Peanut sampling		
	November[a]	January[a]	February[a]	March[a]	April[a]	June[a]	August[a]	October[a]
Coker 9152	148.2	12.6	11.8	78.8	527.8	89.5	109.3	154.3
Georgia Mitchell	137	19.5	35.5	62	353.8	77	51.3	206
Pioneer 26R12	202.5	41.2	26	41	315	121.7	51	102.8
AGS 2000	126.8	15.5	23.3	23.8	167.3	109.2	134.8	238
Pioneer 26R61	44	44	9.5	43	225	83.5	44.8	199.3
Elbon	68	12.6	10.8	47.8	360.3	96	102.8	250.8
Panola	11	11	22	29.3	212.3	70.5	57.5	57.5
Fallow	296.7	21.2	12.5	46.5	630.8	96	31.8	154.5
Bob	128	20.7	16.5	38.3	379.5	83.5	83.5	218.8
Abruzzi	148.2	25	9.5	60.3	147.8	51.3	64.3	96.3
LSD ($P \leq 0.05$)	216.4	29	25	39.5	315	136	76.5	151.3

[a] Number of nematodes present in a 100 cm^3 sample.

Cover crop was planted in the middle of November 2006 and was harvested in April 2007. Peanuts were planted in June and harvested in October.

Means within columns followed by different letters are significantly different according to Fischer's protected LSD ($P \leq 0.05$).

Table 3. Effect of winter grain cover crop cultivars on populations of reniform nematode, *Rotylenchulus reniformis* at Huxford, AL, during the cropping cycle of year 2005-06.

Cultivar	Winter grain cover crop sampling					Cotton sampling		
	November[a]	January[a]	February[a]	March[a]	April[a]	June[a]	August[a]	October[a]
AGS 2000	653.5	157	99.3	108.8	115.3	115.3	2085.5	367.75
Pioneer 26R24	651.2	150	93.8	85.5	105.3	105.3	1981.5	357.25
Fallow	534.7	230	121.2	35	117.8	117.8	1370.7	594.5
Elbon	564.5	256	120.5	106	82.5	82.5	3894.2	369.25
Bob	527.5	136	115	120	135.3	135.3	1660.5	366.5
Abruzzi	616	165	75.3	87	129.5	129.5	2703.5	281
Georgia Mitchell	508.7	83.5	77	66	106.3	106.3	2420.2	538.25
Coker 9152	484	149.5	145.2	55.5	130.8	130.8	2511.5	193.5
Panola	627	119	69.8	61	96	96	1344.5	419.25
LSD ($P \leq 0.05$)	345.9	180.9	67.9	81.7	60.7	60.7	1164.9	303.7

[a] Number of nematodes present in a 100 cm^3 sample.

Cover crop was planted in the middle of November 2006 and was harvested in April 2007. Cotton was planted in June and harvested in October.

Means within columns followed by different letters are significantly different according to Fischer's protected LSD test ($P \leq 0.05$).

Table 4. Effect of winter grain cover crop cultivars on populations of reniform nematode, *Rotylenchulus reniformis* at Huxford, AL, during the cropping cycle of year 2006-07.

Cultivar	Winter grain cover crop sampling					Cotton sampling		
	November[a]	January[a]	February[a]	March[a]	April[a]	June[a]	August[a]	October[a]
AGS 2000	107.5	334.3	263.5	444	289.3	289.3	386.3	377
Pioneer 26R24	210	373	265.7	405.3	482.5	482.5	534	454.3
Fallow	145.3	701.5	328	334.5	379.3	379.3	341	467.5
Elbon	174.8	379.5	334.5	533.8	373	373	437.7	403.3
Bob	352	389.8	424.3	431	733.8	733.8	585.5	476
Abruzzi	238.5	862.3	547	386	540.5	540.5	450.3	365.3
Georgia Mitchell	300.8	798	579	637	546.8	546.8	302.3	420.3
Coker 9152	280.3	669	456.8	450.5	624	624	180	186.3
Panola	282	430.8	353.8	508.3	630.5	630.5	405.3	411.8
LSD ($P \leq 0.05$)	161.3	531.3	531.4	361.5	417.8	417.8	317.4	236.3

[a] Number of nematodes present in a 100 cm^3 sample.

Cover crop was planted in the middle of November 2006 and was harvested in April 2007. Cotton was planted in June and harvested in October.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 5. Effect of winter grain crop cultivars on the populations of root-knot nematode, *Meloidogyne incognita* in the greenhouse at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

Cultivar	Shoot weight	Root weight	Eggs/gm of root	Nematode eggs[a]	
Georgia Mitchell	10.82	10.04	930	2.2857	bc
Bob	13.46	12.73	673	1.8548	c
Abruzzi	9.06	18.26	1365	2.6643	b
Elbon	9.70	23.65	1063	2.4005	bc
Pioneer26R12	10.11	21.94	1612	2.8570	b
AGS2000	11.15	19.57	1695	2.9014	b
Tomato	9.77	8.71	3951	3.5162	a
LSD ($P \leq 0.05$)				0.864	

[a] Log (x+1) of the number of nematode eggs present per gram of root weight.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 6. Effect of winter grain crop cultivars on populations of reniform nematode, *Rotylenchulus reniformis* in the greenhouse at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

Cultivar	Shoot weight	Root weight	Eggs/gm of root	Nematode eggs[a]	
Georgia Mitchell	13.85	9.90	5082.9	1.5813	bc
Bob	16.68	11.64	520.3	1.0533	cde
Abruzzi	16.35	28.81	58.5	0.5724	e
Elbon	20.22	30.07	156.5	0.9655	de
Pioneer 26R12	19.12	27.19	1063.4	1.6126	b
AGS 2000	19.93	29.56	1417.4	1.4565	bcd
Cotton	7.20	6.32	15137.62	3.1334	a
LSD ($P \leq 0.05$)				0.543	

[a] Log (x+1) of the number of nematode eggs present per gram of root weight.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

III. EVALUATION OF *CROTALARIA JUNCEA* POPULATIONS TO MANAGE PLANT-PARASITIC NEMATODES

INTRODUCTION

Crotalaria juncea L. (sunn hemp) is a legume crop that has received attention due to its green manure properties, the potential to fix nitrogen and its ability to suppress plant-parasitic nematodes (McSorley et al., 1999). It is also reported to increase number of free-living nematodes and improve nutrient levels in soils with low organic matter content. Considering all these beneficial qualities, *C. juncea* may be used effectively in organic and sustainable agricultural systems to suppress plant-parasitic nematodes. The major constraint affecting the extensive use of *C. juncea* in the continental United States is its limited reproduction and non-availability of seeds on large scale for cultivation. *Crotalaria juncea* cannot reproduce under these climatic conditions. In United States, most of the *C. juncea* evaluations and breeding programs were limited to Hawaii (Rotar and Joy, 1983).

To overcome the problem of seed production, *C. juncea* populations collected from different countries were evaluated for their ability to produce seeds under the Southeastern U.S. climatic conditions. Along with the seed production evaluations, the nematode suppressive effect of these populations was evaluated. This research was conducted to evaluate the efficiency of different *C. juncea* populations to suppress the

southern root-knot nematode, *Meloidogyne incognita* and the reniform nematode, *Rotylenchulus reniformis.*

MATERIALS AND METHODS

Crotalaria juncea populations collected in different countries were obtained from the National Plant Germplasm System. Seed of these populations were increased under the same environmental conditions and under isolation in 2003 at Tallassee, AL. The populations were: PI 207657 from Srilanka, PI 314239 from Russia, PI 322377 from Brazil, PI 391567 from South Africa and PI 426626 from Pakistan. These *C. juncea* populations were evaluated for their ability to suppress southern root-knot nematode, *M. incognita* and reniform nematode, *R. reniformis* in the greenhouse and in the field. The *C. juncea* host status was evaluated in the greenhouse at the Plant Science Research Center, Auburn University, AL and in one field experiment at E V Smith Research Center (EVSRC), Shorter, AL during the summer of 2006. Tropic Sun (*C. juncea* cv. Tropic Sun) was used as the control in the field experiment. Additional populations of Selection FCU-2005 and Selection PBU-2005 were also used in mixed planting experiments and in a field evaluation.

Nematode extraction from roots

Meloidogyne incognita and *R. reniformis* populations were maintained on the host plants of tomato (*Lycopersicon esculentum* cv. Rutgers) and cotton (*Gossypium hirsutum*

cv. DP 555 BG/RR), respectively. Nematode eggs on the roots were extracted using 0.6% sodium hypochlorite solution (Hussey and Barker, 1973). The hypochlorite solution containing nematode eggs was allowed to pass through a series of sieves with 350-µm sieve on top and 500-µm at the base. Nematode eggs were collected on 500-µm sieve and were gently washed with water. The solution containing nematode eggs was standardized and quantified under an (Nikon-T 100®) inverted microscope.

Greenhouse evaluations of *C. juncea* populations

Experiments were conducted to evaluate the host status of *C. juncea* populations for plant-parasitic nematodes. *Crotalaria juncea* populations were grown in 500 cm^3 polystyrene cups filled with autoclaved loamy sand soil field soil (72.5%, 25%, 2.5%, S-S-C, pH 6.4) and fine sand in 3:1 ratio. One *C. juncea* seed of each treatment was hand-sown in each cup. Tomato (*Lycopersicon esculentum* cv. Rutgers) and cotton (*Gossypium hirsutum* cv. DP 555 BG/RR) were used as the controls for *M. incognita* and *R. reniformis* nematodes, respectively. The experiment was arranged in a completely randomized block design, replicated eight times and repeated twice. *Crotalaria juncea* seeds were allowed to germinate and grow for one week. On the day of nematode infestation, nematode eggs were extracted from host plants as described previously.

One week after the *C. juncea* germination, the seedlings were inoculated with ca. 4000 eggs of *M. incognita* or ca. 2000 eggs of *R. reniformis* by making small holes near the root zone of the plants by using a Repipett® Jr. Dispenser. After 50 days the *C. juncea* roots were gently washed, fresh weight of the aerial parts and root weight was

recorded. Nematodes present on the roots of *C. juncea* populations were extracted as described above and the numbers of eggs present were counted. The number of eggs present per gram of root was calculated, and the data were analyzed.

Staining of *C. juncea* roots

10 grams of *C. juncea* roots of all the populations infested with *M. incognita* or *R. reniformis* were stained using McCormick Schilling® red food color (Thies et al., 2002). *Crotalaria juncea* roots were washed gently with water and blotted dry by using a paper towel. The roots were cut in to 2-cm-pieces and were suspended for 15 sec in a 500-ml beaker containing 10% (v/v) solution of McCormick Schilling® red food color (Thies et al., 2002). The stained roots were rinsed in tap water and blotted dry. *Meloidogyne incognita* and *R. reniformis* egg masses present in the roots were observed and counted under a Nikon T-100® inverted microscope (10x).

To identify the nematode presence within the root tissues, the stained roots were suspended into acidified glycerin (40 ml glycerin and 5 drops of 5N HCL). The roots were mounted between glass microscopic slides to observe the juvenile stages present in roots and photographs were taken using a Nikon® Coolpix4500 camera (4 mega pixels). For counting the number of *M. incognita* juveniles present within the roots, the roots were chopped in an Oster® blender for 20 sec by adding water; the chopped suspension was observed under a Nikon® inverted microscope and juvenile stages were counted.

Nematicidal activity of freeze-dried *C. juncea* root exudates

To evaluate the nematicidal activity of *C. juncea* root exudates, the roots of *C. juncea* plants were submerged in a beaker containing 250 ml distilled water. The beakers were wrapped with an aluminum foil to prevent photolysis of the exudates. The beakers were agitated using an Environ® automatic shaker @ 150 rpm for 12 hours. *Crotalaria juncea* root exudates collected in the beakers were decanted into brown plastic bottles and stored at 0 ^{o}C. These exudates were freeze-dried using a freeze drier.

Freeze-dried root exudates were reconstituted by adding 15 ml of distilled water. Nematode isolates maintained on susceptible hosts of tomato and cotton was extracted using 0.6% NaOCl (Hussey and Barker, 1973). Nematode eggs were allowed to hatch by placing in an incubator at 25 ^{o}C for one day. Approximately, 25 second-stage juveniles (J2) were handpicked and added to the glass vials containing 5 grams of sand (Halbrendt et al., 2007). The treatments included three different concentrations of root exudates, 50-µl, 100-µl, 250-µl and the water control. Root exudates were added using a 1000-µl Eppendorf® micropipette, and water was added to make up the volumes in the vials to one ml. This entire experiment was arranged in a complete randomized block design in five replications. After 24 hours, the nematodes were washed into a series of sieves with 350-µm on top and 500-µm at the base. The suspended solution on 500-µm sieve was transferred in to Petri plates and the number of alive J2 stages was counted under Nikon® inverted microscope. The nematodes that were moving and burst open when squished using a needle was considered alive. In contrast, nematodes that were straight in shape, paralyzed and didn't burst open when squished were considered dead.

Mixed planting of *C. juncea*

Crotalaria juncea populations described previously were planted with tomato or cotton in the greenhouse to evaluate their potential to suppress *M. incognita* or *R. reniformis*. Autoclaved loamy sand soil field soil (72.5%, 25%, 2.5%, S-S-C, pH 6.4) and sand were mixed in 3:1 ratio and placed in 2000 cm^3 plastic pots. Plastic squares cut from a large plastic screen were placed vertically in the center of each pot, dividing it in to equal portions. One seed of a *C. juncea* population was hand-sown in one half of the pot and one tomato or cotton seed was sown on the other half of the pot. The control pots were planted with tomato or cotton plants on both sides of the screen. The entire experiment was arranged in a completely randomized block design on raised benches in 8 replications and both the experiments were repeated twice.

Two weeks after germination of *C. juncea* plants, ca. 6000 *M. incognita* or *R. reniformis* nematodes were infested at the center of the pot near the base of the screen by making small holes. Shoot weight and root weight of *C. juncea* and tomato or cotton controls was recorded after 50 days. Nematode present on the roots were extracted using hypochlorite method and number of eggs present per gram of root was determined.

Field evaluation

Field evaluation of *C. juncea* populations was conducted to evaluate their effect on *Meloidogyne* spp. at E V Smith Research Center, Shorter, AL. This experiment was conducted during the summer months from June to August 2007. The treatments included the *C. juncea* populations described above, one fallow and tomato control, replicated four

times. *Crotalaria juncea* populations were planted as a summer crop. Each plot (1 m^2) was sampled in a zigzag pattern before planting *C. juncea*. Five probes of soil was taken, composited into one sample per plot and stored in plastic bags. These samples were maintained at a temperature of 10 ^oC until nematode extraction. A subsample of soil (100 cm^3) was used and nematodes were extracted using gravity screening and centrifugal flotation method and quantified (Jenkins, 1964).

Each plot was hand sown with five *C. juncea* seeds per population by marking with a wooden marker. Tomato was planted as a control in one plot. Weeding was done manually every week for the entire experimental period. All plants and root systems were harvested after 50 days by digging with a shovel. Plants from each plot were placed in separate plastic bags. Fresh weight of leaves and stem and roots was recorded. Soil samples were collected at time of harvesting and nematodes were extracted. Nematodes present on the roots were extracted as described above in nematode extraction from roots.

After harvest of *C. juncea*, all the plots were replanted with two-wk-old tomato seedlings at the same place where the *C. juncea* plants were planted previously. Tomato plants were harvested manually 50 days after planting and soil samples were collected. Fresh weight and root weights were recorded, and nematodes were extracted from the soil samples and tomato roots.

Data analysis

The greenhouse data represented the number of eggs present per gram of root weight and the field data reflected the number of nematodes present in each soil sample.

Data from both field and greenhouse were log transformed and the transformed data was analyzed using Proc GLM in Statistical Analysis Systems software (SAS institute, Inc., Cary, NC). The significance of effects of *C. juncea* treatments was determined by the probability of F-value (P less than or equal to 0.05). Treatment means was separated by Fisher's protected Least Significant Differences, and the suppressive effect of *C. juncea* populations on *M. incognita* and *R. reniformis* nematodes was compared.

RESULTS

Greenhouse evaluations of *C. juncea* populations

Crotalaria juncea populations supported very low populations of *M. incognita* nematodes. There was a significant difference in *M. incognita* populations ($P \geq 0.05$) between all the *C. juncea* populations and the control. In *M. incognita* experiment, all the *C. juncea* populations supported low reproduction and tomato supported the highest reproduction (Table 1). Among the different *C. juncea* populations evaluated, there were no significant differences between the populations. The numerically relative decreasing order of *M. incognita* reproduction on *C. juncea* populations was tomato, PI 207657, PI 314239, PI 322377, PI 391567, and PI 426626 (Table 1).

Crotalaria juncea populations also demonstrated a significant difference ($P \geq 0.05$) on suppression of *R. reniformis* reproduction (Table 2). All the *C. juncea* populations supported low *R. reniformis* reproduction while the control cotton supported

the highest *R. reniformis* populations (Table 2). Between the *C. juncea* populations, PI 322377 supported numerically the lowest *R. reniformis* reproduction and population PI 314239 supported the highest *R. reniformis* reproduction. However, there were no significant differences between *C. juncea* populations on *R. reniformis* reproduction. The numerically relative decreasing susceptibility of *C. juncea* for *R. reniformis* was cotton, PI 314239, PI 391567, PI 426626, PI 207657, and PI 322377.

Staining of *C. juncea* roots

All the *C. juncea* populations were found to contain few *M. incognita* nematode juveniles (Fig. 1) and adults (Fig. 2) within the root tissues. All the juvenile stages J2, J3 of *M. incognita* were observed within the roots. In case of *R. reniformis* nematodes, only 1-2 adult females (Fig. 3 and 4) were present semi-endoparasitically inside the *C. juncea* root tissues.

Nematicidal activity of freeze-dried *C. juncea* root exudates

Crotalaria juncea freeze-dried root exudates were found to kill most of the *M. incognita* (Table 3) and *R. reniformis* nematodes (Table 4). There was a significant difference ($P \leq 0.05$) in the nematode mortality rates between the *C. juncea* root exudates and the water control. The nematode mortality rate was highest at 250-µl concentration. Higher concentrations resulted in higher mortality rates.

Mixed planting of *C. juncea*

Mixed planting of *C. juncea* populations had no significant effect ($P \leq 0.05$) on *M. incognita* populations (Table 5). However, population's PI 391567, PI 314239, Selection FCU-05, PI 207657, PI 322377, and Tropic Sun supported numerically lower *M. incognita* reproduction than the control.

Crotalaria juncea populations had no significant effect ($P \leq 0.05$) on *R. reniformis* suppression (Table 6). Planting of cotton with *C. juncea* population's PI 322377, PI 426626, Selection PBU-2005, Tropic Sun and Selection FCU-2005 supported numerically lower *R. reniformis* nematode densities than the control.

Field evaluation

Field evaluations provided no significant difference ($P \leq 0.05$) between the *C. juncea* populations, follow and the tomato control (Table 7). However, the continuous cultivation of tomato followed by tomato supported numerically highest *Meloidogyne spp.* counts whereas tomato grown in the plots after harvesting of *C. juncea* was found to support lower nematode populations (Table 7). *C. juncea* populations PI 322377, Tropic Sun and PI 207657 were found to reduce *Meloidogyne spp.* counts when compared to the fallow treatment.

DISCUSSION

Greenhouse evaluations of *C. juncea* populations

All *C. juncea* populations tested suppressed *M. incognita* populations more effectively when compared to tomato control. Greenhouse studies revealed that *C. juncea* populations were effective in reducing the *M. incognita* nematode densities. The difference in *M. incognita* reproduction between *C. juncea* populations may be due to the different genetic constitution of the plants.

Crotalaria juncea populations were found to significantly decrease *R. reniformis* nematodes when compared to cotton control. Among the *C. juncea* populations, PI 322377 supported numerically the lowest *R. reniformis* nematode reproduction. In contrast, population PI 314239 supported the highest *R. reniformis* reproduction. The reason for varying nematode reproduction levels on *C. juncea* populations may be due to the different genetic constitution of the plants. However, all the *C. juncea* populations were able to significantly decrease *R. reniformis* populations.

Staining of *C. juncea* roots

Different *M. incognita* juvenile stages and few adults were present within the root system, indicating there was penetration of *M. incognita*. This experiment demonstrated that *M. incognita* juveniles were able to pierce the *C. juncea* roots, but in limited numbers.

Adult females of the *R. reniformis* nematodes were present semi-endoparasitically inside the roots, indicating that *R. reniformis* can infest and reproduce on the *C. juncea* roots. However, the *R. reniformis* reproduction was very low when compared to the cotton control.

Nematicidal activity of freeze-dried *C. juncea* root exudates

Crotalaria juncea freeze-dried root exudates were able to kill the *M. incognita* and *R. reniformis* nematodes. The mechanism responsible for nematode mortality is not clearly known but might be due to some nematotoxic compounds released from the roots in to the water. Root exudates may serve as an alternative to chemical nematicides in organic production systems. Further biochemical studies have to be conducted to determine the chemical nature of the compounds present in the *C. juncea* root exudates. The chemical nature of *C. juncea* root exudates is not known but these exudates were found to kill the nematodes.

Mixed planting of *C. juncea*

In the mixed planting of *C. juncea* with tomato or cotton, *C. juncea* populations were able to suppress the nematodes when compared to control. The reason for numerically low nematode densities on tomato or cotton mixed planted with *C. juncea* might be the result of nematode suppression resulted from root exudates produced by the *C. juncea* populations. However, there was no significant difference ($P \leq 0.05$) on nematode populations between the control and *C. juncea* planted with tomato or cotton. Nematodes may have been more affected if the *C. juncea* was planted earlier than tomato

or cotton. Further studies should focus on mixed cropping *C. juncea* populations with agronomic crops to control plant-parasitic nematodes.

Field evaluation

Field studies demonstrated that there were no significant differences between the continuous cultivation of tomato and the tomato grown in rotation after *C. juncea*. However, *C. juncea* populations were found to decrease small populations of *Meloidogyne spp.* densities on the tomato grown after *C. juncea* cultivation. In contrast, continuous cultivation of tomato increased *Meloidogyne spp.* densities during the summer months and tomato grown in the next cropping season. Extreme temperatures that prevailed during the summer months under field conditions, accompanied by lack of rainfall might be reason for no significant differences as was observed in the greenhouse evaluations. Even though the plots were hand-irrigated, the lack of moisture and high temperatures may have played a role. Therefore, further field studies with *C. juncea* populations under controlled irrigation conditions should be carried out to determine the potential of *C. juncea* populations as summer cover crop to manage plant-parasitic nematodes in organic farming and sustainable agriculture.

Table 1. Effect of *Crotalaria juncea* populations on southern root-knot nematode, *Meloidogyne incognita* under controlled conditions at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

Cultivar	Shoot weight	Root weight	Nematode eggs[a]	Nematode eggs[b]	
PI 207657	11.4	8.7	220	1.3070	b
PI 314239	15.2	12.4	151	1.1659	b
PI 322377	13.5	14	76	1.0272	b
PI 391567	17.1	13.6	101	0.7319	b
PI 426626	16.0	11.8	37	0.5170	b
Tomato	10.5	12.3	11171	3.7292	a
LSD ($P \leq 0.05$)				0.8636	

[a] Number of nematodes present per gram of root weight.

[b] Log (x+1) of the number of nematode eggs present per gram of root weight.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 2. Effect of *Crotalaria juncea* populations on reniform nematode, *Rotylenchulus reniformis* under controlled conditions at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

Cultivar	Shoot weight	Root weight	Nematode eggs[a]	Nematode eggs[b]	
PI 207657	16.1	16.5	26.1	0.4853	cd
PI 314239	16.1	15.4	282.4	1.1440	b
PI 322377	16.5	20.2	13.9	0.1637	d
PI 391567	16.6	16.9	135.2	0.8913	bc
PI426626	17.0	17.4	80.1	0.6817	bcd
Cotton	9.5	9.5	2122.8	3.2416	a
LSD ($P \leq 0.05$)				0.7539	

[a] Number of nematodes present per gram of root weight.

[b] Log (x+1) of the number of nematode eggs present per gram of root weight.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 3. Evaluation of the nematicidal activity of *Crotalaria juncea* populations root exudates on root-knot nematode, *Meloidogyne incognita*.

Population	Root weight	Number of nematodes alive in different concentration of *C. juncea* root exudates		
		50-µl[a]	100-µl[a]	250-µl[a]
PI 207657	38.1	0.18 de	0.06 c	0 c
PI 314239	23.9	0.64 b	0.48 b	0.4 b
PI 322377	33.3	0.48 bc	0.3 bc	0.25 b
PI 391567	28.6	0.33 cd	0.15 c	0.06 c
PI 426626	33.7	0.14 e	0.12 c	0 c
Control	--	1.24 a	1.24 a	1.25 a
LSD ($P \leq 0.05$)		0.17	0.2	0.16

[a] Log (x+1) of number of nematodes alive in different concentrations of *Crotalaria juncea* root-exudates.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 4. Evaluation of the nematicidal activity of *Crotalaria juncea* populations root exudates on reniform nematode, *Rotylenchulus reniformis*.

Population	Root weight	Number of nematodes alive in different concentration of *C. juncea* root exudates		
		50µl	100µl	250µl
PI 207657	38.1	0.37 c	0.18 c	0 d
PI 314239	23.9	0.68 b	0.48 b	0.27 bc
PI 322377	33.3	0.62 b	0.39 bc	0.37 b
PI 391567	28.6	0.4 c	0.27 bc	0.15 cd
PI 426626	33.7	0.27 c	0.18 c	0 d
Control	--	1.25 a	1.27 a	1.26 a
LSD ($P \leq 0.05$)		0.19	0.28	0.15

[a] Log (x+1) of number of nematodes alive in different concentrations of *Crotalaria juncea* root-exudates.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 5. Effect of *Crotalaria juncea* populations planted with tomato on root-knot nematode, *Meloidogyne* incognita under controlled conditions at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

C. juncea populations'	Tomato plants mixed planted with *C. juncea*			
	Shoot weight	Root weight	Nematode eggs[a]	Nematode eggs[b]
PI 207657	30.1	5.9	8992	3.66
PI 314239	34.7	8.7	9963	3.77
PI 322377	44.8	7.4	6211	3.63
PI 391567	28.35	6.0	10953	3.88
PI 426626	42.3	8.1	13675	4.09
Tropic Sun	20.4	4.9	7961	3.4
Selection FCU-05	30.7	7.7	11939	3.71
Selection PBU-05	23.8	5.5	15070	3.95
Tomato	38.8	10.8	9622	3.89
LSD ($P \leq 0.05$)				0.59

[a] Number of nematodes present per gram of root weight.

[b] Log (x+1) of the number of nematode eggs present per gram of root weight on tomato plants mixed planted with *C. juncea.*

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 6. Effect of *Crotalaria juncea* populations planted with cotton on reniform nematode, *Rotylenchulus reniformis* suppression under controlled conditions at the Plant Science Research Center, located on campus of Auburn University, Auburn, AL.

Cultivar	Cotton plants mixed planted with *C. juncea*			
	Shoot weight	Root weight	Nematode eggs[a]	Nematode eggs[b]
PI 207657	29.4	5	1977	3.19
PI 314239	28.3	4.3	2774	3.22
PI 322377	33.3	6.4	1853	3.12
PI 391567	32.2	7.7	2738	3.39
PI 426626	30.22	7.7	1375	2.99
Tropic Sun	42	9.7	1552	2.89
Selection FCU-05	47.7	8.3	634	2.08
Selection PBU-05	29.5	4.2	2658	2.92
Cotton	41.8	12.1	2054	3.15
LSD ($P \leq 0.05$)				0.7628

[a] Number of nematodes present per gram of root weight.

[b] Log (x+1) of the number of nematode eggs present per gram of root weight on cotton mixed planted with *Crotalaria juncea* populations.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

Table 7. Effect of *Crotalaria juncea* populations on root-knot nematode, *Meloidogyne* spp. under field conditions at E V Smith Research Center, Shorter, AL.

Population	Tomato planted after harvesting of *C. juncea*			
	Shoot weight	Root weight	Nematode eggs[a]	Nematode eggs[b]
PI 207657	336.5	38.35	115.2	2.1585
PI 314239	447	47.975	341.2	2.5447
PI 322377	307.25	40.425	505.2	2.3267
PI 391567	357	47.3	496	2.6646
PI 426626	297.25	39.75	463.5	2.6425
Tropic Sun	356.5	42.725	450.7	2.2481
FCU-05	418.25	51.575	508.6	2.6646
PBU-05	195	25.15	437.2	2.6318
Fallow	263.33	37.03	543	2.5304
Tomato	193.75	29.55	3565.9	3.2087
LSD ($P \leq 0.05$)				0.6963

[a] Number of nematodes present per gram of root weight.

[b] Log (x+1) of the number of nematode eggs present per gram of root weight.

Means within columns followed by different letters are significantly different according to Fischer's protected Least Significant Difference test ($P \leq 0.05$).

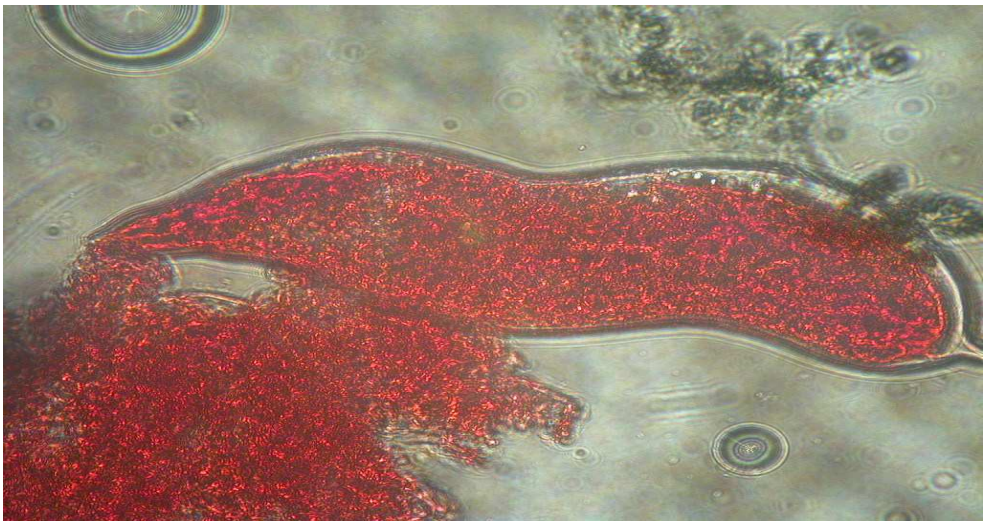

Fig 1. Third-stage juvenile (J3) of root-knot nematode, *Meloidogyne* incognita present inside the *Crotalaria juncea* roots, stained using a McCormick Schilling® red food color, observed under a Nikon® eclipse 80*i* microscope at 40x magnification, photographed using a Nikon® Coolpix4500 camera (4 mega pixels).

Fig 2. Adult female of root-knot nematode, *Meloidogyne incognita* present inside the *Crotalaria juncea* roots, stained using a McCormick Schilling® red food color, observed under a Nikon® eclipse 80*i* microscope at 40x magnification, photographed using a Nikon® Coolpix4500 camera (4 mega pixels).

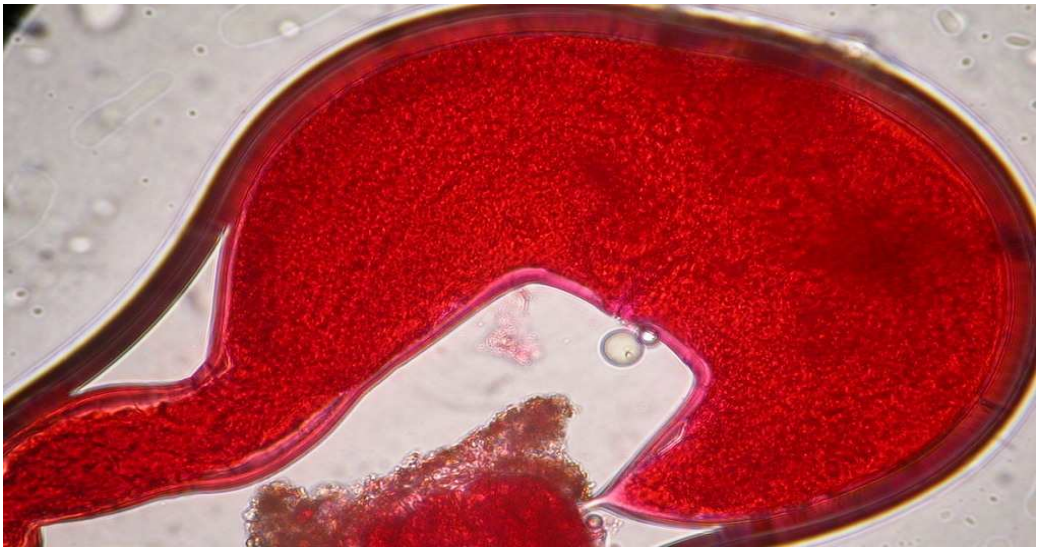

Fig 3. Female reniform nematode, *Rotylenchulus reniformis* present semi-endoparasitically on *Crotalaria juncea* roots, stained using a McCormick Schilling® red food color, observed under a Nikon® Eclipse 80*i* microscope at 40x magnification, photographed using a Nikon® Coolpix 4500 camera (4 mega pixels).

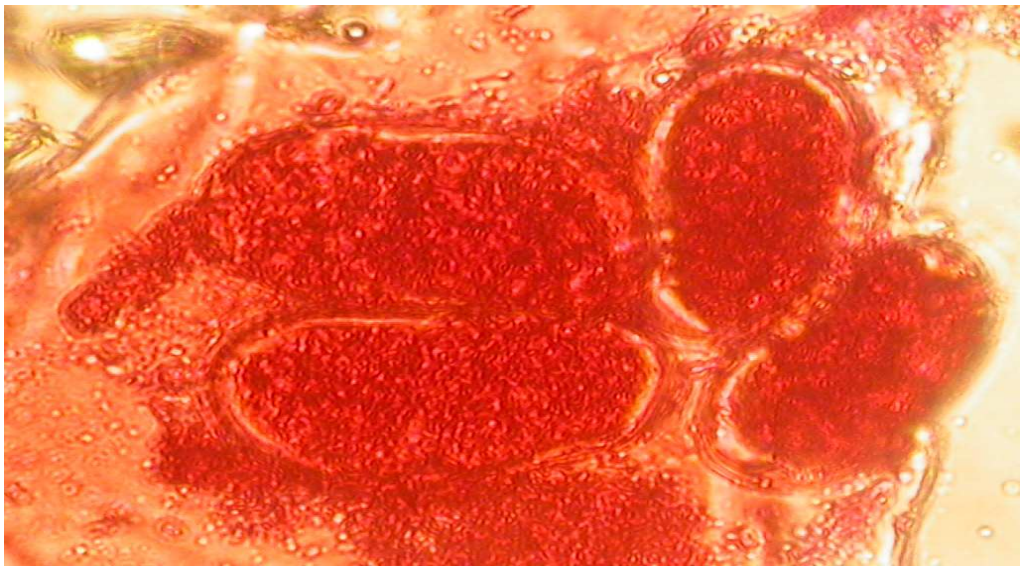

Fig 4. Nematode eggs inside an adult female of reniform nematode, *Rotylenchulus reniformis* present semi-endoparasitically on *Crotalaria juncea* roots, stained using a McCormick Schilling® red food color, observed under a Nikon® eclipse 80*i* microscope at 40x magnification, photographed using a Nikon® Coolpix4500 camera (4 mega pixels).

IV. SUMMARY

Cover crops were evaluated in the greenhouse and in field locations to determine their host status and nematode suppressive effect on root-knot nematodes, *Meloidogyne incognita* and *M. arenaria* and the reniform nematode, *Rotylenchulus reniformis*. The winter grain cover crop cultivars included four commercially available cultivars of wheat (*Triticum aestivum*) 'Pioneer 26R12', 'AGS 2000', 'Coker 9152', 'Panola'; two cultivars of oats (*Avena sativa*) 'Georgia Mitchell' and 'Bob'; and two cultivars of rye (*Secale cereale*) 'Elbon' and 'Abruzzi'. This research also evaluated the host status and nematode suppressive effect of *Crotolaria juncea* (sunn hemp) populations. The treatments included the *C. juncea* populations; PI 207657 from Srilanka, PI 314239 from Russia, PI 322377 from Brazil, PI 391567 from South Africa and PI 426626 from Pakistan collected in different countries and increased under the same environmental conditions and under isolation in 2003 at Tallassee, AL. *Crotalaria juncea* commercially available cultivar 'Tropic Sun' was also used for evaluations.

Winter grain cover crop cultivars described above were evaluated to determine *M. arenaria* and *R. reniformis* suppressiveness and their subsequent effects on peanuts and cotton. Field evaluations were conducted at Wiregrass Research and Extension Center, Headland, AL and in a grower's field in Huxford, AL to determine the effect of winter grain cover crops on *M. arenaria* and *R. reniformis*, respectively. The treatments included

commercially available cultivars of wheat, oats and rye described above at both the test locations. There were no significant differences ($P \leq 0.05$) between winter grain cover crop cultivars on nematode suppression. This was most probably due to severe drought and uneven rainfall during both the cropping years at the two test locations. However, the greenhouse studies indicated that the wheat cultivars 'Pioneer 26R12' and 'AGS 2000'and rye cultivar 'Abruzzi' were hosts to *M. incognita* nematodes, whereas 'Elbon' rye, oats cultivars 'Bob' and 'Georgia Mitchell' supported low populations of *M. incognita*. The greenhouse studies of winter cover crop cultivars with *R. reniformis* nematodes demonstrated that the wheat cultivars 'Pioneer 26R12' and 'AGS 2000' and oats cultivar 'Georgia Mitchell' were good hosts, while 'Bob' oats and the rye cultivars 'Elbon' and 'Abruzzi' supported significantly ($P \geq 0.05$) lower *R. reniformis* nematode populations. This suggests that these oats and rye cultivars are poor hosts and might be used in a crop rotation sequence with agronomic crops to manage these nematodes.

Crotalaria juncea populations were able to suppress *M. incognita* and reniform nematodes in the greenhouse tests. Significant difference ($P \geq 0.05$) was observed in nematode reproduction between *C. juncea* populations and the control. This indicated that the *C. juncea* populations could be an efficient summer cover crop to manage *M. incognita* and *R. reniformis* nematodes. Roots of *C.* juncea populations infested with *M. incognita* and *R. reniformis* were stained using a McCormick Schilling® red food color (Thies et al., 2002) to determine if either genus could complete its life cycle on the roots. All juvenile stages of *M. incognita* were found as well as low numbers of mature females with egg masses and 1-2 adult female reniform nematodes were present per 10 gm of

roots, indicating that these nematodes were able to infest and reproduce on the roots of *C. juncea* populations. However, the reproduction on *C. juncea* was very low when compared to the controls. In the evaluation of the nematicidal activity of *C. juncea* root exudates, root exudates were collected from each population and freeze-dried. The exudates were reconstituted and tested against both *M. incognita* and *R. reniformis* nematodes at several concentrations. All concentrations could kill both nematodes whereas the water control had no effect. The field trial conducted in the summer (2007) at E V Smith Research Center, Shorter, AL indicated that continuous cultivation of tomato followed by tomato increased the nematode densities whereas tomato planted after *C. juncea* was found to support low *Meloidogyne* spp. nematode densities. However, there were no significant differences observed on *Meloidogyne* spp. suppression between the different *C. juncea* populations evaluated.

The knowledge obtained from this study suggests that some winter cover crop cultivars and *C. juncea* populations may be suitable for crop rotation in a region with specific nematode histories, thus minimizing usage of synthetic nematicides and yield losses. However, further research studies should focus on extensive long-term field studies on cover crops nematode suppressive effect under controlled irrigation conditions.

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