An Evaluation of Plant Growth Regulator, Starter Fertilizer, and Nematicide Inputs to Promote Soybean Growth and Support Greater Yield in *Meloidogyne incognita* Infested Fields in Alabama

by

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Abstract

The Southern Root-knot nematode, *Meloidogyne incognita*, is a significant root parasite that poses a threat to sustainable production of many crops, including soybean. Management strategies for this nematode include: crop rotation, fumigation, and utilizing a resistant variety. An alternative management strategy implementing plant growth promoting inputs was proposed herein in order to provide a profitable and sustainable solution to diminished soybean yield due to root-knot nematode. The direct inputs of a nematicide, plant growth regulator, and starter fertilizer at planting will effectively reduce nematode population density and increase yield by supporting greater plant biomass in the vegetative stages of growth. This hypothesis was evaluated by:

1) greenhouse trials of starter fertilizer, plant growth regulator and nematicide inputs to determine their effect on soybean plant biomass and root-knot nematode population density; 2) inputs based on greenhouse performance were evaluated in field trials in two locations infested with *M. incognita*; 3) the effect and efficacy of nematicide treatment was further characterized on five soybean varieties planted in four root-knot nematode infested locations in Alabama.

In greenhouse trials, starter fertilizer treatments increased plant fresh shoot weight in the first 45 days of soybean development and had no impact on *M. incognita* population density. Plant growth regulator treatments stimulated root and shoot growth, increasing total plant biomass within the first 45 days of soybean growth. The nematicide treatments significantly reduced nematode population density by as much as 93% at 30 days after planting. Combining nematicide seed treatment and starter fertilizer plus plant growth regulator treatments applied as in-furrow sprays did not significantly increase yield or reduce nematode population at either field location. However the combination of Avicta nematicide and the starter fertilizer treatment significantly reduced nematode population density at one location, and significantly increased yield by 20% over the control at the other site. In variety trials across four locations, the effect of the Avicta nematicide treatment varied by variety; a root-knot susceptible variety, Progeny 5333RY, responded significantly better to the nematicide than all other varieties tested. Avicta increased yield of Progeny 5333RY at two locations by 39% and 48%, respectively. The Avicta seed treatment did not significantly increase yield of a root-knot resistant variety (Mycogen 5N522R2) at any location, however the root-knot resistant variety produced the numerically highest yields at the location with the greatest nematode population density. Ultimately, the system of nematode management using inputs must be evaluated based on soybean variety, location, and nematode population density.

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Table of Contents

List of Tables

Chapter 2:

Chapter 3:

List of Figures

Chapter 2:

Chapter 3:

Chapter 1: Introduction and Review of Literature

The objective of the research presented herein is to provide an alternative method to manage root-knot nematode in infested soybean fields. This will be accomplished through the use of selected inputs used to maximize plant growth and reduce root-knot population density in the first 40 days of growth. The effects of the inputs will, ideally, translate into increased yields.

Soybean

Soybean (*Glycine Max* L. Merr) has become an increasingly popular crop because of its widespread use in food products, animal feed, and seed oil. The U.S. soybean planted acreage has risen by 8 million acres from 2001 to 2016 (NASS, 2016).The U.S. planted 83.3 million acres of soybean in the 2014 season (USDA, 2014). U.S. soybean exports reached a record high in 2014-2015 and demand is expected to increase in the future (O'Brien, 2015). China is a major importer of soybean; their imports are projected to increase from 83 million tons in 2016/17 to 109.5 million tons in 2025/26 (Tani Lee *et al.*, 2016). China imports approximately 1 billion bushels of soybeans annually from the U.S. (Lloyd, 2016). Masuda and Goldsmith (2009) suggested that food production and security will be a problem in the future because of limited acreage for growing edible crops such as soybean, therefore, it is important to increase yields to meet national and global demands.

Soybeans in Alabama

Alabama production of soybeans totaled 420,000 acres planted for 2016 (up from 140,000 in 2001), with an average yield of 41 bushels an acre and total yield of 20,090,000 bushels (NASS, 2016). Alabama's soybean production equates to approximately 1% of U.S. soybean production, ranking 23rd in in the U.S. (NASS, 2016). The greatest production of soybeans in Alabama occurs in North Alabama (Fig 1).

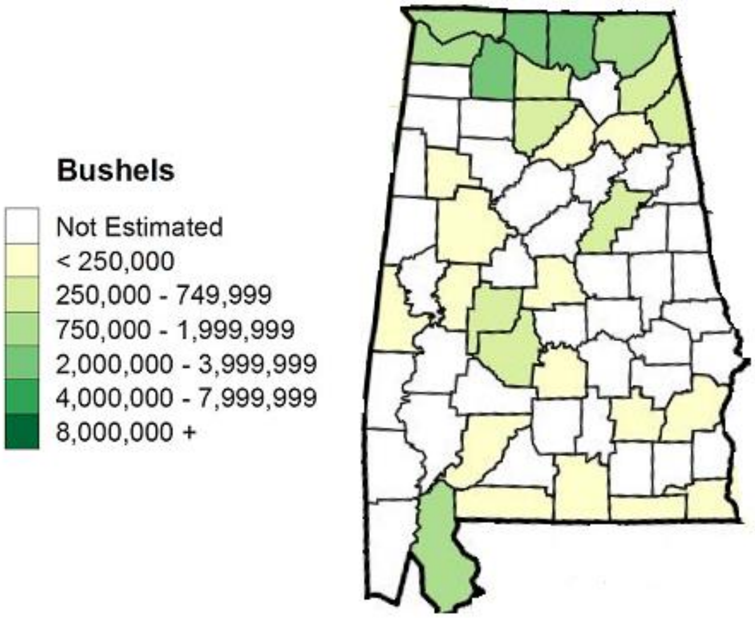

Figure 1. 2012 Alabama soybean production by county in total bushels of soybean produced (data from NASS, 2016).

Over the last 15 years soybean production in Alabama has increased approximately 20% per year (NASS, 2016). Increasing soybean demand must be met by increased planted and harvested acreage as well as increases in yield per acre. The input costs of dryland soybean, which include fertilizer, lime, herbicide, insecticide, fungicides, insurance, machinery, labor, and general overhead costs, total approximately $377.81 per acre (Runge, 2016). Commodity prices as well as yield per acre determine if growers can profit from planting soybean. For example: production above 40 bushels per acre at a commodity price of $10 per bushel nets a return of $84.33 per acre (Runge, 2016). As soybean prices rise with increased demand, many states, including Alabama, may increase acreage of planted soybean to meet these demands.

Soybean Yield Potential and Limitations

World food supply demands require maximizing soybean yield potential. A new world record of soybean yield was set by Randy Dowdy of Georgia. Dowdy's soybean yield reached an average of 171 bushels per acre (Begemann, 2016). This contrasts with the current average soybean yield in Georgia and Alabama: 43 and 41 bushels per acre, respectively (NASS, 2016). Maximizing yield of soybean is dependent upon many factors. Limiting factors of soybean yield include: available nutrients, agronomic practices (tillage system, and row spacing) (Pederson and Lauer, 2004), season length, water, atmospheric CO_2, pathogens, pests, weed competition, and improvements in genetic potential (Koester *et al*., 2014).

Meloidogyne incognita

Southern root-knot nematode (*Meloidogyne incognita*) is a plant-parasite of many important agronomic crops such as soybean, cotton, and corn. Serious yield reductions of soybeans occur in locations with sandy-loam soil and high initial population density of *M. incognita* (Windham and Barker, 1986). In 2010, *M. incognita* reduced soybean yields in the United States by approximately 7,556,000 bushels (Koenning and Wrather, 2010). Fourie *et* al. (2010) and Antonio (1988) reported that the nematode can cause approximately 55-60% crop loss on soybeans. Kinloch (1974) reported that *M. incognita* caused 53-90% yield loss on susceptible varieties and 32-40% yield loss on resistant varieties in the coastal plain of Florida.

Fields infested with Southern root-knot nematode require appropriate attention and planning to prevent crop losses. Crop rotation for *M. incognita* is important because this nematode causes significant crop losses on many crops including corn (loss: 2.2-11.4% per 100 juveniles/ 100cc soil) (Bowen *et al.*, 2008). This is a problem because corn is commonly used in rotation with soybean and because corn profit margins may not allow economic use of nematicides to reduce nematode populations. An integrative pest management approach to root-knot nematode infestations focuses on reducing nematode population density and increasing plant biomass during early crop development stages to reduce loss in yield from the pathogen. Luangkhot, 2016). *Meloidogyne incognita* is endemic to much of the southeastern United States and is distributed in many of the counties of Alabama (Fig. 2). Kratochvil *et al.* (2004) determined that the most accurate times to sample *M. incognita* infested fields to determine if an economic threshold has been reached are midsummer or early fall. Kinloch (1982) defined a relationship between overwintering *M. incognita* population density and soybean yield in the Coastal Plains as a 5.31 kg/ha loss for each *M. incognita* second stage juvenile found in a 10cm^3 preseason soil sample. A 10cm^3 pre-season shoil sample containing 100 juveniles would then cause approximately 8 bushels per acre loss equating to an approximate 20% yield loss on a 41 bushel/acre soybean crop. Clemson University nematode threshold recommendations specifies that soil samples be taken within three weeks of soybean harvest; a 100 cm^3 soil sample at harvest containing more than 75 vermiform stage *M. incognita* warrants control measures for soybean growers in the Coastal Plains for the next crop year (Mueller, 2009). The University of Georgia economic threshold for soybean post-harvest samples is stated as greater than 60 second stage juveniles per 100 cm^3 of soil (J2's), however, anywhere from 1-59 J2's per 100 cm^3 of soil may affect the crop (Jagdale *et al*., 2013). Soil samples taken in the spring prior to planting to estimate nematode populations are more variable than and not as accurate as a post-harvest sampling for predicting yield losses from root-knot nematode

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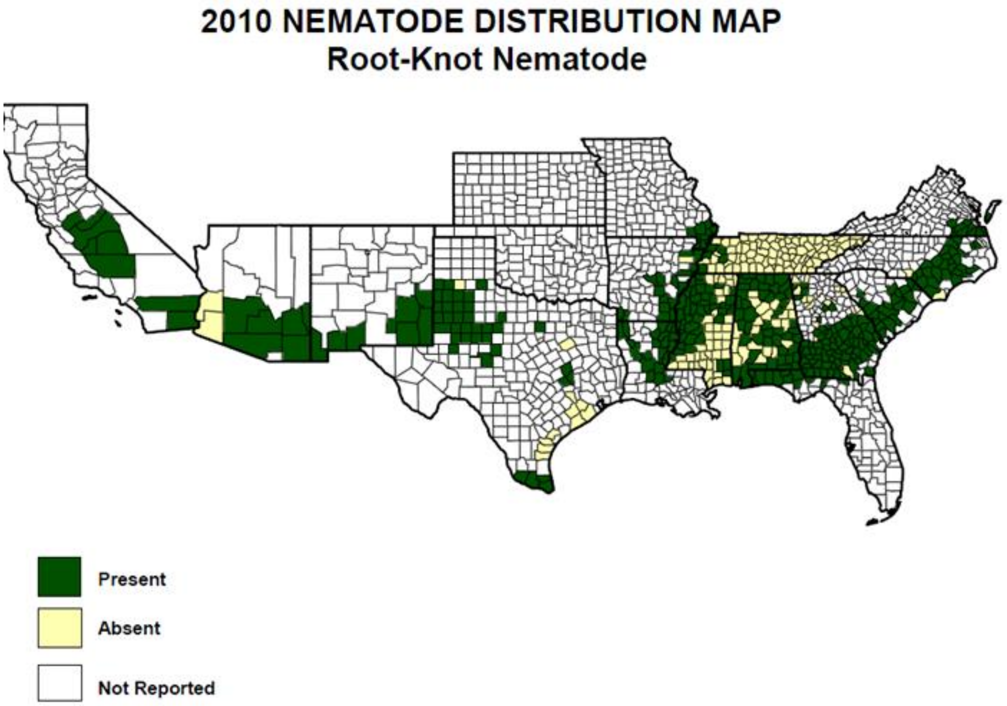

Figure 2. 2010 United States distribution of Root-knot Nematode by county (National Cotton Council, 2010).

Life Cycle & Symptoms

Meloidogyne incognita is an obligate plant-parasite, requiring approximately 28-30 days to complete one life cycle under ideal environmental conditions (Perry *et al.*, 2009). The life cycle begins when a mature female lays eggs. Inside the egg, a single molt of the nematode occurs, and after hatching the second stage juvenile will migrate to a root, where it penetrates root tips by mechanically piercing apical cells with its stylet. The stylet is much like a hypodermic needle, allowing plant parasitic nematodes to cause cellular damage and uptake of plant cellular nutrients. After its migration, the second stage juvenile initiates a feeding site in the vascular cylinder and molts two more times (to J3 and J4).Throughout its development, the nematode excretes proteins from esophageal glands which cause hypertrophy and hyperplasia of the vascular cylinder of the root, initiating a feeding site (McClure, 1977). The cells inside this feeding site are referred to as giant cells because of nematode-induced hypertrophy. The female nematode may initiate formation of 3-6 giant cells in a localized area of the vascular cylinder. The giant cell formation with the surrounding hypertrophy and hyperplasia of root cells, causes the root galling that is the main symptom of this genus of plant parasitic nematodes. The nematodes effectively create a metabolic sink in galled roots, which causes a nutrient imbalance in affected plants (McClure, 1977). As the female reaches maturity, she enters into a reproductive phase (Perry *et al*, 2009). *Meloidogyne incognita* reproduces by mitotic parthenogenesis (asexual reproduction of eggs by mitosis) (Eisenback *et al.*, 1981); an individual adult female is estimated to lay 200-500 eggs (Perry *et al.*, 2009). The adult female nematode continues to draw nutrients from the feeding site. The galling also occludes xylem and phloem elements necessary for nutrient transport; causing the foliar chlorosis and yellowing symptoms associated with root-knot infection (Allen *et al.,* 2005). Carneiro *et al.* (2002) observed that *M. incognita* interferes with nutrient uptake and translocation in soybean; total nutrients were more prevalent in roots than shoots at 50 days after planting. Fortnum *et al.* (1991) reported shoot weight loss and increased root weights of tomato plants infested with an initial *M. incognita* population density of 100,000 eggs per plant at 40 days.

Interaction with Other Pathogens

Meloidogyne incognita interacts with several fungal plant pathogens causing severe disease on many crops. Pathogenic fungi and *M. incognita* may form a disease complex where the nematode enables fungal entry into plant roots, causing wilt, root necrosis, leaf chlorosis and defoliation. Akinsanmi and Adekunle (2003) reported that fungal pathogens: *Fusarium oxysporum* (Schlecht.) f sp. g*lycines* and *Sclerotium rolfsii* (Sacc.) form disease complexes with

M. incognita on soybean. Together, the pathogens caused stunted shoot and root development. Additionally, high field populations of *M. incognita* cause increased incidence and severity of root-rot and seedling diseases which can be caused by *Pythium* spp., *Rhizoctonia solani* (Kuhn), *or Thielaviopsis basicola* (Berk and Broome) (Perry *et al.*, 2009).

Fusarium wilt is a significant disease of soybean. The disease was estimated to cause a 10,627,000 bushel yield reduction in soybeans in the U.S. in 2010 (Wrather and Koenning, 2010). *Meloidogyne incognita* entry and development of galling of plant roots facilitates *Fusarium* infection, which subsequently kills the developing female nematodes inside the plant roots (El-Shawadfy *et al*., 1988; Moussa and Hague, 1988). Mai and Abawi (1987) determined that wilt symptoms developed earlier in the presence of *M. incognita* suggesting that the presence of root-knot nematodes increased incidence of wilt on multiple plant hosts. *Fusarium oxysporum* has a wide distribution in the U.S., occupying much of the Cotton Belt including a wide distribution in Alabama (Bennett *et al*., 201; Kappelman, 1983).

Management of Meloidogyne incognita

There are several options for soybean growers attempting control of *M. incognita* infestations. While some options such as fumigation, biological control, soil solarizaton, and fallow practice (Lawrence and McLean, 1999) have efficacy, the simplest and most frequently implemented control methods are the use of crop rotation, resistant varieties, and nematicide treatment (Kirkpatrick *et al.,*2014). Crop rotation is very important to prevent population density from increasing to economic threshold levels. Soybean rotations that include peanut, rice, or grain sorghum are recommended to reduce population density of *M. incognita* (Kirkpatrick and Sasser, 1984; Kirkpatrick *et al.*, 2014). Data from Kirkpatrick *et al*. (2014) demonstrate that planting a variety resistant to *M. incognita* can result in a yield improvement of 10 to 25%. Seed companies provide soybean resistant varieties that are available in maturity groups grown in Alabama (IV, V, and VI). Information on available soybean varieties in these maturity groups may be obtained through the Alabama Cooperative Extension System, which publishes soybean variety performance reports every year (available from aces.edu), and or a seed company representative. Lee *et al.* (2015) recently published a list of 24 genetic accessions or sources of resistance to soybean cyst nematode and their levels of resistance to root-knot nematode and reniform nematode.

The recent history of nematicides, includes the use of soil fumigants such as methyl bromide, vapam, and chloropicrin, as well as aldicarb. Aldicarb is an insecticide/ nematicide that was very effective but due to toxicity concerns was voluntarily removed from production (Cone, 2010). Recently developed nematicides for soybeans have been implemented as seed treatments (See Table 1 in Chapter 2 for a list of nematicides labeled for soybean). Avicta (Abamectin) from Syngenta Crop Protection and ILeVO (Fluypram) from Bayer Crop Science are two seed treatments currently available for control of plant parasitic nematodes, including *M. incognita*. These seed treatments are effective at reducing nematode population density but are often economically challenging to implement (Allen *et al*., 2005). Avicta costs about $10 per acre in addition to fungicide/insecticide seed treatments (Heatherly, 2015). ILeVO costs approximately $13 per acre (or $2 per oz.) in addition to fungicides and insecticides (Heatherly, 2016). Soybean prices, as well as seed costs for a resistant variety, will ultimately dictate whether a grower will apply nematicides to a soybean crop; higher soybean prices will allow nematicides to become a viable option. The application of a nematicide may be economically beneficial if nematode population density has reached an action threshold. Action threshold is described as “the minimum pest population to which a fixed dosage of a pesticide is applied" (Osteen *et al.*, 1988). Economic threshold for nematode pests can be found for many crops through U.S. University Extension publications and web pages; a post-harvest 500cc soil sample containing approximately 200-300 second stage juveniles constitutes a threshold level for Alabama (Lawrence and McLean, 1999). Applying a nematicide at an action threshold can recoup yields that would have otherwise been lost; however, the application may not be profitable (Heatherly, 2015).

Proposed Management Strategy

A composite solution for pest management of *M. incognita* on soybean could be achieved through the application of multiple commercial product inputs. The proposed inputs are hypothesized to stimulate plant health and soybean growth in order to prevent *M. incognita* induced plant stress and or offset any nutrient deficiencies that can be caused by root-knot nematode infection. Several studies have demonstrated the efficacy of this strategy. Maw *et al*. (2011) indicated that starter nitrogen fertilizer significantly increased soybean biomass in vegetative stages (V5) and increased yield under drought stress. The study found a significant correlation between soybean biomass at V5 and yield. Miller (2016) implemented starter fertilizer to successfully reduce the impact of *Rhizoctonia solani* and *Heterdoera glycines* (Ichinohe, 1952) while increasing soybean yield compared to the control treatment.

The management strategy herein is focused on increasing soybean biomass and reducing *M. incognita* (a source of plant stress) population density through the appropriate selection of growth promoting inputs and nematicide treatments that will result in greater yield. Plant growth regulators and starter fertilizers are excellent growth promoting inputs that may be paired with a nematicide to achieve this goal. Ascend® is a popular plant growth promoter product which contains the plant growth regulators cytokinin, indolebutryic acid, and gibberellic acid. This plant growth promoter is applied in order to accelerate shoot and root growth.

Starter fertilizers are sources of nitrogen, potassium and phosphorous. These products applied at planting have been shown to increase soybean yield by 5% (Osborne and Riedell, 2006). Melakeberhan (1999) demonstrated that the application of synthetic fertilizer benefited soybean photosynthetic rate under soybean cyst nematode stress and also suggests that the fertilizer reduces the effect of nutrient deficiency caused by the pathogen. Nematicide treatment is an essential component of this strategy. In 2013, 70% of soybean seeds were treated with a fungicide, nematicide or insecticide (Gaspar *et al*., 2014). Today's available fungicides, insecticides and nematicides have the potential to significantly increase plant stands and subsequent yields when applied as a seed treatment (Gaspar *et al.*, 2014). A review of the applications of these inputs and their effects on *M. incognita* and other nematodes is necessary in order to adequately understand their application.

Plant Growth Regulators

Indolebutryic acid, cytokinin, and gibberellic acid are classic plant hormones or growth regulators. They are chemicals that drive plant cell division, flowering, fruiting, and elongation (Zhang *et al.,* 1997). Cytokinin and gibberellic acid are also involved in prevention of plant senescence, delaying the loss of chlorophyll that is essential to plant growth and yield production (Zhang *et al.,* 1997).

Indolebutryic acid, also known as IBA, is a type of auxin known for its effect on root proliferation *(*Ludwig-Müller, 2000). It is primarily used commercially as a rooting substance for transplants. While there is little literature pertaining to its effect on soybeans, IBA has been suggested to stimulate fruit setting by regulating transcription and cell signaling at reproductive stages (Davies, 1995; de Jong, 2009). IBA application to hypocotyls of soybean induced significantly higher numbers of lateral roots than control plants (Chao *et al.*, 2001). This is important as an increase in root surface area allows for additional water and nutrient absorption for plant growth.

Cytokinins as well as auxins have been used to induce and increase pod set in soybeans (Cho *et al*., 2002). Cho *et al*. (2002) used synthetic plant growth regulators to increase seed weight, seed yield, and pod number when applied as a foliar spray to soybean at reproductive growth stages R1 (one flower at any node) and R3 (pod is ½ cm long at one of the four uppermost nodes with completely unrolled leaf). Additionally, Nonokawa *et al.* (2007) confirmed that exogenous cytokinin application can increase pod set when applied 7 days after flowing. Overall, it is posited that exogenous cytokinin application to soybean may increase yield by approximately 3% (Nagel *et al.*, 2001).

Gibberellic acid (GA) is a phytohormone involved in the control of plant height and seed germination (Taiz and Zeiger, 2010). Application of GA stimulates shoot elongation of soybean but in some instances can cause reduced pod set, and thus, yield. Bostrack and Struckmeyer (1964) found that GA application results in taller plants as well as a longer vegetative stage of production resulting in a later and less productive reproduction phase.

Ghorbanli *et al.* (2000) conducted studies on soybean seedlings with GA application to offset toxicity effects of plants inoculated with cadmium. In these experiments, GA increased plant leaf area and stem length. GA seed treatment application resulted in increased height of soybeans; however, resulting yields were significantly lower than control plots (Howell *et al.*, 1960). Mislevy *et al.* (1989) confirmed the results of Howell *et al.* (1960) demonstrating 8-10% yield loss of soybean when GA was applied to seedlings via foliar spray. However, internode length of soybean was increased and taller plants allowed harvesting of pods typically unreached by the harvester which ultimately resulted in no loss of harvestable yield.

Dual application of cytokinin and GA has been shown to offset the observed yield losses caused by GA (Leite *et al*., 2003). Foliar application of combinations of plant growth regulators used on soybean have resulted in increased biomass but not significant increases in yield (Fawcett *et al.*, 2015); however, more treatment application types are necessary for confirmation.

Nematode-Interaction with Plant Growth Regulators

Plant growth regulators appear to have significant function in relation to *M. incognita* and the formation of giant cells in infected plant roots (Goverse and Bird, 2011). *Meloidogyne incognita* second stage juveniles and adult females have been shown to secrete cytokinins from pharyngeal glands via the stylet (Dimalla and van Staden, 1977; De Meutter *et al.*, 2003). Cytokinins produced by *Meloidogyne* nematodes have been implicated to have key and specific roles in the development of root galls (Jones and Goto, 2011).

Bird and Loveys (1980) confirmed the findings of Dropkin *et al.*, (1969) that the exogenous addition of cytokinin to tomato increased its susceptibility to *M. incognita*. . Auxins (indole based compounds) have also been found to contribute to formation of giant cells (Giebel, 1974). Yu and Viglierchio (1964) detected indole-butyric- acid (IBA, an auxin) in egg masses of *M. incognita*. De Meutter *et al.* (2003) demonstrated through mass spectrometric analysis that *M. incognita* produces pharyngeal excretions of auxins. Kyndt, *et al*. (2016) revealed that *M. incognita* may manipulate auxin regulation of *Arabidopsis thaliana* in root gall formation; plant mutants without specific auxin regulatory genes produced fewer and smaller galls. Hutangura *et al.* (1999) suggests that auxin is required to trigger gall development of root-knot nematodes in white clover. Scientific literature has revealed that exogenous application of plant growth regulators may induce susceptibility in resistant plants to *M. incognita* infection (Jones and Goto, 2011). Use of plant growth regulators to increase soybean biomass may positively affect *M. incognita* population density by enhancing gall development or inducing susceptibility.

Starter Fertilizers

Starter or" pop up" fertilizers are liquid sources of macronutrients (nitrogen, phosphorous and potassium) and micronutrients (zinc, copper, iron, manganese, and boron). Starter fertilizers are routinely applied to soils that have low macro and/or micro-nutrients at planting (Tisdale *et al.*, 1985). Starter fertilizers are used to increase initial plant growth in terms of total plant biomass which will be partitioned into increased yield at harvest.

Starter fertilizers have typically been used in corn production due the crop's high nitrogen and other macronutrient demands (Tisdale *et al.*, 1985). Soybeans naturally develop nitrogen fixing nodules that produce in symbiosis with nitrogen fixing Rhizobacteria such as *Rhizobium* (Zahran 1999). However utilizing a starter fertilizer for soybeans may directly increase yield under certain conditions.

Starter Fertilizer Use in Multi-Crop Seasons

Growers with a long growing season (122 days for corn and 70-100 days for soybean) can maximize their profit by planting two crops in the same season. Starter fertilizers can greatly benefit the second crop in the cycle, which will likely have fewer nutrients available than the first crop planted (Tisdale *et al*, 1985). Corn planted in March and harvested in mid-July allows a short season soybean crop to be planted immediately after the corn is harvested. This would be a situation where a starter fertilizer could benefit a soybean crop. Starling *et* al. (1998) demonstrated that when planting a late soybean crop following corn, an initial broadcast supply of nitrogen at a rate of 50 kilograms of nitrogen per hectare immediately after planting increased soybean height at R1 stage (first reproductive stage; beginning of bloom) (Pedersen, 2004) as well as yield at harvest across seven field locations in Alabama. Starter fertilizer application may be of interest to growers in the Southeastern U.S., where growing two crops in the same season is possible, especially in rotation with corn or small grains. For example, growers with a corn-soybean rotation may benefit from starter fertilizer application. In the Northern Great Plains of the U.S., Osborne and Riedell (2006) demonstrated that soybean yields were improved when supplied with a starter fertilizer in a corn-soybean rotation. In a three year experiment, the data revealed that 16 kg/ha nitrogen increased soybean yield by 6% while increasing plant biomass in V3-V4 stages (vegetative growth stages corresponding to the first 21 days of plant growth, Pedersen, 2004).

Starter Fertilizer Use in Nutrient Limiting Soil Conditions

Starter fertilizers may also increase yield under conditions of cool soil temperature during planting. Planting early in the season allows a crop to grow longer and possibly increase yield. Applying a starter fertilizer at an early planting date may be of benefit because of the nature of nutrient cycling and availability under cool conditions. Nutrients are less available to plants in cold soil temperatures, especially under low soil moisture conditions. Warmer temperatures are pivotal in supporting chemical reactions that demineralize nutrients, making them available to plants (Pregitzer and King, 2005). Early planting dates will likely have cooler soil temperatures and less available nutrients. Yield increases from application of starter fertilizers may be observed in conditions of cold soils, locations of nutrient immobilization, and in compacted soils that are adverse to root penetration (Touchton and Rickerl, 1986). The results of Staton (2012) demonstrated that starter fertilizer increased soybean yield in soils with low test levels of potassium and phosphorous.

Starter Fertilizer Implementation with Adequate Nutrient Levels

Several researchers have discussed the efficacy of starter fertilizer use with soybeans under standard planting conditions and planting date. Rehm and Lamb (2010) used liquid fertilizers (10-15-0, 4-4-8 and 3-8-15) at rates of 32 or 56 L/ha and found that fertilizer use negatively affected emergence and stand counts. However, fertilizer application did not affect yield or stand counts in the silty-clay loam soil. Clapp and Small (1967) utilized liquid (54.25 L/ha 5-8-4) and granular (4.5 kg/A 10-15-5) fertilizer in the seed zone of soybeans which resulted in a reduced stand and yield proposedly due to seed injury. Gordon (1999) successfully used starter fertilizer in a ridge till system, applying it by the 2x2 system (2” to the right and 2” below seed depth). A rate of 34 kg/a muriate of potash (7-21-7) achieved greatest soybean yield with 2x2 application. However, in-furrow application of the same liquid fertilizer: salt of potash or muriate of potash (7-21-7) at a rate of 45.35 kg/a reduced plant stand and yield.

Additionally, the role of micronutrients is important for soybean production. Starter fertilizer may be effective as well if liquid micronutrients are included in soil or foliar application. Randall *et al.* (1975) utilized soil and foliar manganese treatments to increase soybean yields. Boron (Ross *et al.*, 2006) and zinc (Sutradhar *et al.*, 2016) are also important for soybean photosynthesis and yield potential.

Application with Soil Borne Disease

The appropriate selection and application rate of a liquid starter fertilizer is key to producing high yielding crops. The objective of utilizing starter fertilizer in this study was to increase early development of the plant, resulting in a healthier plant that is able to survive and reproduce in the presence of the soil born pathogen *M. incognita*. The application of starter fertilizer to reduce plant stress from soil borne disease and increase yield has been implemented by Luangkhot (2016) and Miller (2016) in cotton and soybean respectively. Luangkhot (2016) successfully demonstrated that a combination of starter fertilizer (2-1-6 with micronutrient package) and nematicide treatment (Velum Total®) significantly increased plant biomass and reduced *Rotylenchulus reniformis* population density at 42 days after planting. The nematicide-fertilizer combination also resulted in a significant increase in yield. Miller (2016) experimented with using two starter fertilizers to increase soybean yields under *Rhizoctonia solani* (causal agent of root-rot and damping off) and *Heterodera glycines* (soybean cyst nematode or SCN) stress. The results indicated that liquid fertilizers (3-10-13 and 7-12-11) applied in-furrow at planting significantly increased yield while numerically reducing SCN population densities at midseason. A low salt index starter fertilizer incorporating macro as well as micronutrients to increase early soybean development may provide yield increases in fields infested with root-knot nematodes.

Interaction with M. incognita

It has been reported that high populations of *M. incognita* can cause yield loss through nutrient deficiencies on many crops, including soybeans. Root-knot nematodes form root galls that act as “metabolic sinks” characterized by the accumulation of photosynthates and carbon

(McClure, 1977). A study on common bean (*Phaseolus vulgaris*) by Melakeberhan *et al.* (1985) concluded that *M. incognita* caused physiological changes in plants, altering nutrient concentration and location, which appeared to be the cause of yield loss in infected plants. The plant nutrient concentration after one week of growth was reported to be at reduced levels for K, Zn and Mn and an increased level of Ca in shoots (Melakeberhan *et al* 1985). In a subsequent greenhouse study, Melakeberhan (2006) found that soybeans inoculated with *M. incognita* (15,000 eggs/800cc soil) had reduced nematode population density and higher rates of photosynthesis when fertilizer was applied early in plant development. This experiment suggests that starter fertilizer application may lead to increased yield in soybeans parasitized by *M. incognita*. Farahat *et al*. (2015) discovered that application of organic and inorganic fertilizers reduced *M. incognita* population density on eggplant. Starter fertilizer was not as effective as the nematicide treatment used (Vydate), however the data demonstrated significant differences in *M. incognita* population density from inorganic and organic fertilizer application compared with control plants. The literature suggests that starter fertilizer application to soybean may have significant impacts on increasing plant biomass and reducing *M. incognita* population density. Use of an appropriate starter fertilizer may prevent or offset yield loss caused by this nematode.

Nematicide Treatments and Efficacy

Nematicide application is an effective way to decrease *M. incognita* population densities in many crops, including soybeans. Nematicides such as Aldicarb, dibromide chloropicrin and soil fumigants have been effective in reducing nematode population density and increasing yield (Minton *et al.*, 1978; Minton *et al.*, 1980. The scheduled removal of Aldicarb (Temik from Bayer Crop Science, Raleigh, NC) from the market, has led to the development of alternative nematicides such as abamectin (Avicta from Syngenta, Greensboro, NC) and fluopyram (ILeVO from Bayer Crop Science, Raleigh, NC) (Gowen, 1992; O'Brien *et al.*, 2012).

Seed treatment is currently being popularized as a convenient and economical method of nematicide application. Several commercial seed treatable nematicides are currently available for soybeans, including: Avicta (abamectin), PONCHO/VOTiVO (*Bacillus firmus*) and ILeVO (fluopyram). ILeVO may also be applied in-furrow at planting and has been proven to effectively suppress *M. incognita* population density on soybean at the beginning of the growing season (Jackson *et al.*, 2015). Abamectin as a seed treatment in combination with a fungicide and insecticide have to be effective in increasing soybean plant stand and yield (Gaspar *et al.*, 2014,).

Abamectin is a mixture of avermectins, a compound isolated from an actinomycete in the 1970's that had nematicidal properties. It was later developed into a plant parasitic nematicide and offered as the product Avicta by Syngenta (Wislocki et al., 1989; Burg *et al.*, 1979). Faske and Starr (2006) conducted a toxicity study of abamectin on juveniles of *M. incognita*, concluding that exposure to the nematicide caused "irreversible paralysis and mortality". Sub lethal rates of abamectin reduced the number of nematodes in infected plants. Cabrera *et al.* (2009) confirmed these results stating that seed treatment with abamectin was effective in reducing *M. incognita* population density early in plant development. Field trial results on soybean demonstrated that abamectin significantly reduced *M. incognita* population density and produced higher yields compared with untreated controls (Fourie *et al*., 2015). Monfort *et al.* (2006) conducted field trials with abamectin treated cotton seed, where *M. incognita* population density was reduced 14 days after planting (DAP). However, at 21 DAP and at harvest, there was no significant difference in population density between abamectin treated and an untreated control.

Bayer Crop Science (Raleigh, NC) produces two nematicides labeled for soybean. ILeVO (fluopyram) is a succinate dehydrogenase inhibitor that has fungicide and nematicidal activity. PONCHO/VOTiVO (*Bacillus firmus* and clothianidin), which is labeled for a range of crops and plant parasitic nematodes (Wilson, 2013). Faske and Hurd (2015) determined that exposure to low concentrations of fluopyram caused nematode immobility and reduced infection rate of tomato roots. Dodge and Lawrence (2015b) conducted field trials using fluopyram treated soybean seed. In this experiment Velum Total (Bayer Crop Science, Raleigh, NC) (Velum Total is a combination of an insecticide and nematicide: imidacloprid and fluopyram): a combination of fungicide and nematicide was also shown to increase yields when applied as a seed treatment to several soybean varieties. The yields of two UniSouth Genetic varieties were improved 34% and 40% by nematicide application (Dodge and Lawrence, 2015a). Yield increased by 2% on average across all 10 varieties (Dodge and Lawrence, 2015a). Applying a nematicide as a seed treatment in fields infested with *M. incognita* increased yield over control treatments and is important for protecting soybeans during early development to prevent yield losses.

The reviewed literature outlines the effectiveness of inputs that stimulate soybean growth as well as nematicides that reduce nematode population density. The purpose of this thesis is to evaluate the application of these inputs to stimulate early plant growth of soybean and decrease yield loss due to *M. incognita*.

Chapter 2: The Effects of Plant Growth Regulator, Starter Fertilizer, and Nematicide on Soybean Plant Biomass, *Meloidogyne incognita* Population Density, and Yield in Greenhouse and Field Trials

Introduction

Meloidogyne incognita race 3 (Kofoid and White, 1919; Chitwood 1949), also known as the Southern Root-knot nematode, is a significant plant pathogen of soybeans in the Southeastern United States. This endoparasite of plants establish feeding sites within the vascular cylinder of the roots, resulting in the formation of root-galls which reduce the diameter and efficiency of vascular tissue and prevent nutrient translocation (Carneiro *et al.*, 2002; Dorhout t *et al.,* 1991; Melakerberhan, *et al.*, 1985). Plants infected with root-knot nematode typically have nutrient deficiencies as the root galls act as a metabolic sink (McClure, 1977; Melakerberhan *et al.*, 1985). This phenomenon has been observed in soybean infected with *M. incognita.* Carneiro *et al*. (2002) found that *M. incognita* affected translocation of nutrients, causing roots to have greater nutrient concentration than shoots 50 days after planting. In 2010, this nematode was responsible for an estimated yield loss of 7.5 million bushels of soybean in the U.S. (Koenning and Wrather, 2010). Soybean yield is reduced by 53-90% on *M. incognita* susceptible varieties and 32-40% on resistant soybean varieties in the coastal plains of the U.S (Kinloch, 1974). This nematode is a constant threat to soybean production in the Southeastern U.S. Among the states in the southeast region, Alabama has increased its production of soybean by 280,000 planted acres since 2001 (NASS, 2016). In total, the U.S. has increased its soybean production by 8 million planted acres since 2001 (NASS, 2016). The expanding planted acreage represents a response to the soybean import demands of China and the increased use of soybean in human food products (Lloyd, 2016; Masuda and Goldsmith, 2009). *Meloidogyne incognita* limits the economic yield potential of soybean in the mid-south, posing a challenge to expanding production in this region of the U.S. (Lawrence and McLean, 1999).

Current management strategies for *M. incognita* include using root-knot nematode resistant cultivars, crop rotation with a non-host, and nematicide applications (Kirkpatrick *et al*., 2014). Resistant varieties limit reproduction of root-knot nematodes while preventing yield loss. Glass *et al*. (2015) recorded that the root-knot resistant variety Mycogen 5N550R2 (Mycogen Seeds Indianapolis, IN) outperformed the susceptible variety UA 5141 (University of Arkansas System Division of Agriculture, Fayetteville, AR) across multiple locations in Alabama. Yield differences between the varieties at two nematode infested locations (Brewton Agricultural Research Unit and Gulf Coast Research and Extension Center) were 5 and 8 bushels/acre(bu/a), respectively. Crop rotation is an important component of nematode management. Planting a non-host such as peanut will prevent the *M. incognita* population density from increasing. New nematicide treatments such as ILeVO (Bayer CropScience, Raleigh, NC) have shown efficacy for reducing root-knot nematode population density on soybean in Alabama (Dodge and Lawrence, 2015). In addition to these strategies, a new method of management of *M. incognita* as well as reniform nematode was recently explored by Luangkhot (2016). This method, as modeled on cotton in Alabama, implemented the combination of plant growth regulator and starter fertilizer treatments, paired with an in-furrow nematicide. The hypothesis behind this method is that increasing plant biomass in vegetative stages of soybean development as well as reducing the nematode population density will translate to greater yield. Maw *et al.* (2011) established a correlation between increases in soybean biomass in vegetative stages and increased yield which supports the objective of evaluating these inputs as tools to improve root-knot nematode management. The results of the Luangkhot (2016) study demonstrated that when applied together, the inputs significantly increased cotton plant biomass and reduced *R. reniformis* population density while increasing yield.

The application of plant growth regulator and starter fertilizer has shown interesting effects on *M. incognita* population density and soybean growth. The primary plant growth regulators are cytokinin, gibberellic acid (GA), and auxin (e.g. indole butyric acid: IBA; Davies, 1995). These compounds stimulate plant cell division and differentiation, flowering, fruiting, and stem elongation and root growth respectively (Zhang et al., 1997; Leite *et al*, 2003; Ludwig-Muller, 2000). The foliar application of cytokinin to soybean during reproductive stages, R1 (beginning flowering) and R3 (beginning pod set; Pedersen, 2004), induces greater pod set which results in increased yield (Cho *et al.*, 2002). However, the individual application of gibberellic acid has been reported to have negative effects on soybean yield (Howell *et al.* 1960; Mislevy *et al.* 1989). Indole butyric acid has been proven to stimulate increased root growth in the number of lateral roots (Chao *et al.*2001). The application of commercial plant growth regulators, such as Ascend **(**WinField Solutions L.L.C. Apopka, FL; containing cytokinin, GA, and IBA)**,** to soybean may have negatively affect soybean yield. Endres *et al* (2013) and Hartschub and Prochaska (2013) reported that soybean treated with Ascend produced slightly lower yield than control plots when applied via foliar spray at reproductive stages R3-R4 (Pedersen, 2004). Plant growth regulators also have significant interactions with root-knot nematodes. The application of cytokinin to tomato increased susceptibility of the plant to root-knot nematode (Dropkin and Hegelson, 1969). Cytokinin, which is secreted from the pharyngeal glands of *M. incognita* (Dimalla and van Staden, 1977), plays a significant role in the development of the feeding sites of *M. incognita* (Abelenda and Prat, 2013). Additionally, *M. incognita* secretes auxins from its pharyngeal glands (De Meutter *et al*., 2003) which may alter the plant’s development and regulation of auxin in order to establish feeding sites and form root galls (Kyndt *et al*. 2016). Further study of the effects of plant growth regulators is necessary to determine if they have applications in nematode management.

Starter fertilizers, which are sources of initial macronutrients (nitrogen, phosphorous, and potassium), are applied at planting through an in-furrow spray, banded application, or 2x2 (applied 2 inches to the side and 2 inches in soil depth). Osborne and Ridell (2006) demonstrated that in a corn-soybean rotation, banded starter fertilizer application of 16 kg/ha nitrogen at planting increased soybean biomass at V3-V4 (third and fourth node on the main stem, respectively; Pederson, 2004) and increased yield by an average of 5% across a three year study. Starter fertilizer application by 2x2 at a rate of 34kg/A of (7-21-7) successfully increased soybean yield (Gordon, 1999). Clapp and Small (1967) observed reduced emergence of soybean after application of liquid 5-8-4 at a rate of 54.25 L/ha. Rehm and Lamb (2010) also observed that application of liquid starter fertilizers (10-15-0, 4-4-8 and 3-8-15 at 32 or 56 L/ha) in-furrow reduced soybean stand counts.

Starter fertilizer may affect population density of *M. incognita* and have use in reducing the impact of other soil-borne diseases. Starter fertilizer application reduced nematode population density of soybeans inoculated with a high density of *M. incognita* (15,000 eggs/800cc soil); soybeans with fertilizer in this experiment had greater rates of photosynthesis than control plants (Melakeberhan, 2006). Similarly, Miller (2016) effectively used in-furrow starter fertilizers (3-10-13; 7-12-11) to reduce the impact of soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) and *Rhizoctonia solani* (Kuhn) on soybean. Fertilizer treatments in Miller's study produced significantly greater yields and had numerically lower soybean cyst nematode (SCN) population density than control plots.

Nematicides have been proven to reduce *M. incognita* population densities and prevent yield losses. Of particular interest are two nematicides applied as seed treatments: Avicta (0.15mg abamectin /seed; Syngenta Crop Protection, Greensboro, NC) and ILeVO (0.15mg fluopyram/seed; Bayer CropScience, Raleigh, NC). Experimental trials demonstrated that abamectin reduced *M. incognita* population density during early plant development (Cabrera *et al*. 2009; Monfort *et al*. 2006). Fourie et al. (2015) demonstrated the efficacy of abamectin in soybean field trials in reducing *M. incognita* population density, and increasing yield. Bayer Crop Science has recently released the fluopyram product as a nematicide seed treatment for soybean. Jackson *et al* (2014) observed that soybeans treated with fluopyram reduced *M. incognita* population density at 30 days after planting, equal in performance to abamectin (0.15 mg/seed); fluopyram treatments had numerically greater yield than the control. The observations of Dodge and Lawrence (2015) concur with these results: fluopyram application significantly increased soybean yield by 21% in field trials in Alabama. Pairing a nematicide seed treatment with starter fertilizer and plant growth regulator treatments may be an effective method of managing root-knot nematode in soybeans.

The overall hypothesis of this research is that an alternative *M. incognita* nematode management strategy utilizing multiple growth promoting inputs combined with a nematicide will reduce nematode population density and support greater plant biomass in vegetative stages, which will result in an increase in yield at harvest. The objectives necessary to support this hypothesis include: 1) greenhouse evaluations of starter fertilizer, plant growth regulator and nematicide inputs separately to determine their effect on soybean plant biomass and *M. incognita* nematode population density; 2) selected inputs based on greenhouse performance to be evaluated in combinations in field trials in two locations. The information from these studies will potentially provide an alternative and economic method of managing *M. incognita* nematode on soybean.

Materials and Methods

Greenhouse studies

The greenhouse evaluations of starter fertilizer, plant growth regulator and nematicide experiments were conducted at the Plant Science Research Center (PSRC) greenhouse located at Auburn University, Auburn, AL. All experiments were arranged in a randomized complete block design (RCBD) with five replications and each experiment was repeated at least twice. The field soil used in the greenhouse experiments was a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) collected from the Plant Breeding Unit (PBU) located at the E.V. Smith Research Center of Auburn University, near Tallassee, AL. Soil was steam pasteurized at 180 °C for 90 minutes, cooled for 24 hours, and then the steam pasteurizing process was repeated prior to use. Soil was mixed with 14.5 mg/kg N as an equivalent application of 32.6 kg of N per hectare recommended for 60-80 bushel/acre soybean (Schmidt, 2014). All experiments were performed in 1000 cm^3 plastic cone-tainers (Stuewe & Sons Inc., Tangent, Oregon) filled with a soil sand mix (60:40 v/v). Pots were insulated in foam boards to standardize temperatures. Two soybean seeds were sown per pot, and thinned to one plant per pot five days after planting (DAP). *Meloidogyne incognita* inoculum (described below) was added at planting.

Plants were watered as needed. Supplemental light of 1000 watt halide bulbs producing 110,000 lumens was supplied to maintain day length of 14 hours per day. Greenhouse temperatures ranged from 21°C to 35 °C. Soil moisture was kept between 40 and 60% of the field capacity. Entire plants were harvested at 21 and 45 days after planting in plant growth regulators and starter fertilizer trials. Nematicide trial length was 30 days in order to extract the first generation of eggs produced by the females which developed from the initial inoculation. Plant measurements recorded included plant height, shoot and root fresh weights, and biomass (shoot + root weight). Nematode data recorded were total *M. incognita* eggs and *M. incognita* eggs per gram of root (eggs/g).

Plant material

All trials were conducted using Asgrow 5935 from Monsanto Company © (St. Louis, MO) seed pre-treated with Acceleron (metalaxyl 0.00023 mg ai/seed, fluxapyroxad 0.0082 mg ai/seed, imidacloprid 0.747 mg ai/seed, and pyraclostrobin0.0084 mg/ai seed; Monsanto Company ©, St. Louis, MO) fungicide and insecticide treatment.

Nematode Inoculum

Meloidogyne incognita used as the inoculum for these experiments was originally isolated from an infested field at PBU and maintained on corn plants "Mycogen 2H723" (Dow AgroScience , Indianapolis, IN) in 500 cm^3 polystyrene pots in the greenhouse. Sixty days after inoculation, infested stock corn roots were submerged in a 0.625% NaOCl solution on a Barnstead Lab Line Max Q 5000 E Class shaker table (Conquer Scientific, San Diego, CA) for 4 minutes at 1 g-force. Roots were placed over a 25 µm sieve while being rinsed and brushed abrasively to remove eggs (Hussey and Barker, 1973). Contents of the sieve were decanted into 50 mL centrifuge tubes and suspended in sucrose (sp. gravity 1.14) based on Jenkins (1964) methodology. Centrifuge tubes were spun at 427 g-forces for 1 minute before being decanted over a 25 µm pore sieve and enumerated using the Nikon TSX 100 inverted microscope (Nikon Instruments Inc., Melville, NY) at 40x magnification. The nematode suspension was adjusted to 10,000 eggs and juveniles per milliliter (mL). One mL of suspension was pipetted into each cone via pre-made individual holes in the soil. All treatments, including the water control, received 10,000 eggs and juveniles per replicate.

Plant Growth Regulators

Plant growth regulator (PGR) trials screened multiple applications of Ascend plant growth regulator (WinField Solutions L.L.C., Apopka, FL) for effects on soybean plant biomass and *M. incognita* population density. The components of Ascend are cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%. Applications of Ascend (Table 2) were an in-furrow spray pipetted into a seed furrow to simulate field conditions (IFS) according to the technique outlined by Luangkhot (2016) and Schrimsher *et al.* (2014). (IFS: 292 mL/ha), foliar spray (FS) applied at unifoliate leaf stage or VC (Pedersen, 2004) (FS: 233mL/ha), and seed treatment (ST: 89 mL/CWT).

Plant growth regulator greenhouse treatments consisted of (1) an untreated control, (2) Ascend seed treatment (ST 88.7 mL/CWT), (3) Ascend in-furrow spray (IFS 292 mL/ha), (4) Ascend foliar spray (FS 233 mL/ha) applied at VC stage, (5) Ascend ST + IFS, (6) Ascend ST + FS, (7) Ascend IFS + FS, and (8) Ascend ST + IFS + FS.

Commercial Starter Fertilizer Products

Low salt index starter fertilizer treatments were evaluated in the greenhouse for their efficacy in increasing plant biomass under *M. incognita* infection and their effect on *M. incognita* population density. Three synthetic liquid fertilizers from Agroliquid (St. Johns, MI) included Sure-K (2-1-6), Pro-Germinator (9-24-3), and Micro 500 (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%) which are available in Alabama. One organic fertilizer: Neptune’s Harvest (Ocean Crest Seafoods Inc, Gloucester, MA) was included for an alternative comparison. All treatments were applied as simulated in-furrow sprays (IFS) utilizing the previously described method at planting. The treatments consisted of (1) an untreated control, (2) Sure-K IFS (9.33 L/ha), (3) Pro-Germinator IFS (4.66 L/ha), (4) Micro 500 IFS (2.34L/ha), (5) Neptune's Harvest IFS (7.41 L/ha), (6) Sure-K IFS + Micro 500 IFS, (7) Pro-Germinator IFS + Micro 500 IFS, and (8) Neptune's Harvest IFS + Micro 500 IFS

Nematicide Seed Treatments

Nematicide trials were conducted using commercial products from Syngenta Crop Protection (Basel, Switzerland) and Bayer CropScience (Research Triangle Park, North Carolina USA). These products were applied as seed treatments in slurry at the recommended rates using a Gustafson laboratory tabletop seed treater (Table 4). The Syngenta products utilized in these trials were the nematicide Avicta (abamectin 0.15 mg ai/seed) alone or combined with the insecticide and fungicide CruiserMaxx (fungicides: fludioxonil, mefanoxam plus insecticide: thiamethoxam 88.7mL mL/ CWT) from Syngenta and ILeVO (fungicide/nematicide: fluopyram 0.15 mg/ai seed) and Poncho/VOTiVO (insecticide: clothiandin and nematicide: Bacillus firmus 0.13 mg ai/seed). from Bayer CropScience The seven nematicide seed treatments consisted of (1) an untreated control, (2) CruiserMaxx , (3) Avicta, (4) CruiserMaxx + Avicta, (5) Poncho/VOTiVO, (6) ILeVO, and (7) Poncho/VOTiVO + ILeVO.

Field Trials

Treatments used in field trials included the best performing inputs as determined in greenhouse trials. The Ascend IFS plant growth regulator treatment was selected because it effectively increased plant biomass at 45DAP and was compatible mixing with the in-furrow spray starter fertilizer treatments. Sure-K + Micro-500 was selected over other starter fertilizer treatments due to slightly reduced germination rates observed in Pro-Germinator treatments and because the Neptune's Harvest had a viscosity that was incompatible with the tractor’s in-furrow spray nozzle. These treatments were additively combined with two separate nematicide treatments: Avicta and ILeVO. Treatments evaluated in the field trials were (1) untreated control, (2) ILeVO (3) Avicta, (4) ILeVO + Sure-K + Micro-500 IFS, (5) Avicta+ Sure-K + Micro-500 IFS, (6) ILeVO + Ascend IFS (7) Avicta + Ascend IFS, (8) Avicta + Sure-K + Micro-500 IFS + Ascend IFS (9) ILeVO + Sure-K + Micro-500 IFS + Ascend. Seeds were treated with nematicides before planting. The plant growth regulator and starter fertilizer were applied at rates previously mentioned: 9.33 L/ha (Sure-K) + 2.34 L/ha (Micro-500) and 292 mL/ha (Ascend IFS), through in-furrow application at planting. The in-furrow applications were applied in 93.48 L/ha at 40 PSI using 8003 flat fan nozzles. Trials were planted at the Plant Breeding Unit (PBU) in Tallassee Alabama and at the Brewton Agricultural Research Unit (BARU) in Brewton Alabama. The soil type at PBU (32.487668;-58.882875) and BARU (latitude 31.140404,-87.050274) are Kalmia loamy sand (80% sand, 10% silt, 10% clay) and Benndale fine sandy loam (73% sand, 20% silt, and 7% clay), respectively. Both locations are naturally infested with *M. incognita* race 3. Trials were organized in a RCBD with five replications for a total of 50 experimental units per location. Four row plots were 7.6 m long with 0.9 m row spacing at PBU, and 5.5 m long with 0.9 m row spacing at BARU. The soybean variety Asgrow 5935 (maturity group V) was used for field trials with a seeding rate of 6 seeds per 0.3 m and planted with a John Deere MaxEmerge planter (Moline, Illinois) and Almaco cone planters (Nevada, Iowa). Production practices at PBU and BARU were pre planting inputs of 10-13-27 at a rate of 102 kg/A, sulfur at 5 kg /A, and 0.22kg/A boron. Post planting, pre-emergence herbicides 629 mL/A of Dual Magnum (s-metolachlor; Syngenta, Greensboro, NC) and 946 mL/A Roundup (glyphosate; Monsanto, St. Louis, MO) were applied. Post emergence herbicide: Roundup Power

Max (glyphosate; Monsanto, St. Louis, MO) was applied at the same rate as Roundup near 7, 36, and 60DAP. Planting and harvest dates for PBU and BARU were April 26 and May 9 and Oct 4th and 12th, respectively.

Plant stand counts were taken 14 DAP. Plant stand was determined by counting the number of emerging and standing plants in a 1.5 m section of the inner, left row of each plot. Four row plots were sampled at 32 (PBU) and 38 (BARU) DAP. Two whole plants from each of the two innermost rows were collected from each location and measured for plant height, shoot and root fresh weight, biomass, *M. incognita* eggs and eggs per gram of root (eggs/g). Additionally, post-harvest (7 days after harvest) soil samples were taken to assess general *M. incognita* nematode population density at each location. Planting area from each location was divided into five subsections. Ten 100 cm^3 samples per subsection were taken at a depth of 20 cm with a soil probe in a zig zag pattern (Lawrence and McLean, 1999). Subsection samples were homogenized and four 100 cm^3 soil samples were processed for nematode extraction via sucrose centrifugation as described previously. Average second stage juveniles per 100 cm^3 of soil were calculated for each subsection. Averaging the subsection samples gave a number to represent population density at each location.

Data Analysis

Data from all greenhouse and field trials were analyzed utilizing SAS 9.4 (SAS Institute, Inc. Cary, NC) using the PROC GLIMMIX procedure. LSMEANS for each data parameter (plant height, shoot and root fresh weight, plant biomass, and eggs per gram of root) were compared to the control treatment using Dunnett’s. Model assumptions for ANOVA tests: normally distributed data and homogeneity were checked using studentized residual plots obtained from student panel plots in SAS. All plant parameters were analyzed using a normal distribution. The *M. incognita* nematode populations required a log-normal distribution transformation to satisfy the normality assumption. The LSMEANS estimates for the lognormal distribution function were back transformed to the original vales using PROC MEANS and the original mean values are presented in the tables. The critical *P*-value of 0.10 was used for separating least-squares means. For greenhouse trials, Dunnett's multiple comparison *P* values were used to indicate significant differences between treatments and the control. Response data from the field trials were analyzed separately also utilizing Dunnett's multiple comparison P values.

Results

Plant Growth Regulator Greenhouse Trials

Plant growth regulator applications enhanced soybean plant fresh weight and biomass when compared to the control at 21 DAP. Ascend ST significantly increased root ($P \leq 0.05$), and shoot weights ($P \leq 0.10$) as well as plant biomass ($P \leq 0.05$) at 21 DAP (Table 2). Ascend ST + IFS significantly increased plant biomass ($P \leq 0.05$), shoot fresh weight ($P \leq 0.05$) and plant height ($P \leq 0.10$) over the control at 21 DAP (Table 2). At 45 DAP Ascend ST+IFS retained its advantages in shoot fresh weight ($P \leq 0.05$) and biomass ($P \leq 0.05$) over the control. Ascend IFS increased plant biomass compared to the control ($P \leq 0.10$; Table 3). The Ascend IFS + FS significantly increased *M. incognita* eggs per gram of root by 100% compared to the control ($P \leq 0.10$; Table 3). The Ascend IFS treatment was then selected for field evaluations due to its efficacy in increasing plant biomass as well as its compatibility in mixing with a starter fertilizer IFS; applying inputs as seed treatments and or in-furrow sprays reduces total cost of application.

Starter Fertilizer Greenhouse Trials

Starter fertilizer treatments did not significantly increase plant growth over the control at 21 DAP (Table 4). Pro-Germinator, Sure-K, Micro-500 ($P \leq 0.10$), Neptune's Harvest ($P \leq 0.05$), Pro-Germinator + Micro-500 ($P \leq 0.05$), and Neptune's Harvest + Micro-500 ($P \leq 0.05$) did significantly increased shoot fresh weight over the control at 45 DAP (Table 5). Plant biomass was significantly increased compared to the control by Neptune's Harvest ($P \leq 0.10$), Micro-500 ($P \leq 0.10$), Pro-Germinator ($P \leq 0.05$), and Pro-Germinator + Micro-500 ($P \leq 0.05$). Starter fertilizer had no significant effect on total *M. incognita* eggs at 45DAP; *M. incognita* eggs per gram of root were not significantly different among treatments (Table 5). Pearson's correlation coefficient tests indicated that *M. incognita* eggs increased with plant biomass in a weak, positive relationship ($R^2 = 0.29773$; $P \leq 0.05$).

Nematicide Greenhouse Trials

Avicta, Avicta + Cruisermaxx, ILeVO, and Poncho/VOTiVO + ILeVO significantly reduced total population densities of *M. incognita* in eggs and eggs per gram of root. The ratio of *M incognita* eggs per gram of root was reduced by 91%, 87%, 74%, and 93% on average, respectively ($P \leq 0.05$, Table 6). Plant parameters of root and shoot fresh weights and biomass were also increased by Cruisermaxx alone ($P \leq 0.10$) and Poncho/VOTiVO ($P \leq 0.05$) indicating plant growth stimulation from these products. Cruisermaxx alone also increased root and shoot weight ($P \leq 0.10$). Symptoms of phytotoxicity were observed on soybeans treated with ILeVO in the form of cracked cotyledons and stunted growth of seedlings during the trials. No phytotoxicity was observed with any of the other nematicides tested.

Field Trial Results

Plant Breeding Unit Trial

Field trials are presented separately as conditions between locations warranted. Post-harvest soil samples recorded an average of 25 *M. incognita* juveniles per 100cm^3 of soil. Stand counts, plant height, shoot and root fresh weight, plant biomass, and yield were not statistically different among treatments. Avicta + Sure-K + Micro-500 IFS significantly reduced *M. incognita* eggs per gram of root compared to the control at 35 DAP ($P \leq 0.10$, Table 7). Pearson's correlation coefficients disclosed that shoot fresh weight had a significant, weak negative relationship with *M. incognita* eggs per gram of root ($R^2 = -0.31$, $P \leq 0.05$). Biomass was significantly correlated with eggs per gram of root in a weak negative relationship ($R^2 = -0.27$, $P \leq 0.10$).

Brewton Agricultural Research Unit Trial

Average *M. incognita* population density in post-harvest soil samples was 21 J2 per 100cm^3. Stand counts were not significantly different among treatments at 14 DAP. Avicta increased shoot fresh weight and plant biomass ($P \leq 0.05$), while Avicta + Sure-K + Micro-500 IFS +Ascend IFS significantly increased plant biomass ($P \leq 0.10$, Table 8) ILeVO + Sure-K + Micro-500 IFS at this location, reduced shoot fresh weight and plant biomass when compared to the control ($P \leq 0.05$, Table 8). The combination of Avicta + Sure-K + Micro-500 IFS significantly increased yield ($P \leq 0.0353$, Table 8). Pearson's correlation coefficients demonstrated a significant, weak positive relationship between biomass and yield ($R^2 = 0.35$, $P \leq 0.05$). Plant biomass was also significantly correlated with shoot fresh weight ($R^2 = 0.99$, $P \leq 0.001$) and plant height ($R^2 = 0.47$, $P \leq 0.001$).

Discussion

Plant Growth Regulator Greenhouse Trials

The Ascend plant growth regulator treatments resulted in significant increases in plant root and shoot fresh weight, as well as biomass at 21 DAP in the greenhouse trials. This information supports the findings of Chao *et al.* (2001) that demonstrated increased root development caused by cytokinin application. The combination of seed and in-furrow spray treatments successfully increased plant biomass over both time periods while having no effect on *M. incognita* eggs per gram of root. These results are somewhat comparable to the results of Luangkhot (2016), who found that Ascend treatment significantly increased root weights of cotton seedling grown in an *M. incognita*-infested field. However, the results of this study's greenhouse trials found that Ascend seed treatment combined with an in-furrow spray increased soybean shoot weight under greenhouse conditions. The exogenous application of plant growth regulator (which contains cytokinin) as an in-furrow spray followed by a foliar spray to soybean supported higher numbers of *M. incognita* eggs per gram of root but did not reduce plant growth. These results do not agree with the observations of Dropkin and Hegelson (1969) who demonstrated that exogenous application of cytokinin (0.4 micromolar-0.8 micromolar kinetin) to root-knot resistant tomato converted the observed resistant response to the response of a susceptible plant when infected with *M. javanica*. Dropkin and Hegelson (1969) observed that exogenous cytokinin application increased gall formation from 29% to 65-73% and allowed 55-57% of inoculated juveniles to grow, contrasting with the 4% that survived in the resistant host.

Starter Fertilizer Greenhouse Trials

Starter fertilizer treatments did not significantly increase root and shoot weights in the first 21 days of growth. Significant increases in plant biomass advantages were observed at 45

DAP from Neptune’s Harvest treatments, Pro Germinator treatments, and Micro-500. Additionally, Sure-K significantly increased shoot fresh weight at 45 DAP. These results agree with observations made by Osborne and Riedell (2006) that suggested there was a yield benefit from applying starter fertilizers on soybean. The results from both time periods suggest that starter fertilizers primarily affect shoot fresh weight. Fortnum *et al*. (1991) demonstrated that *M. incognita* affects biomass partitioning in tomato causing a disproportionate amount of nutrients supplied to the roots, while shoot weights were negatively impacted at 40 days. Nutrient assimilation in soybean is associated with plant biomass gain in the vegetative growth stages (Fabre and Planchon, 2000). In greenhouse trials, starter fertilizer increased vegetative growth significantly 45 DAP. This increase in plant biomass may increase yield as nutrients are partitioned to seed. Luangkhot (2016) observed that Sure-K + Micro-500 was beneficial to cotton growth and yield when applied in-furrow on cotton.

Nematicide Greenhouse Trials

The greenhouse nematicide trials showed significant reductions of *M. incognita* population density at 30 DAP. Avicta and ILeVO nematicides effectively reduced the ratio of *M. incognita* eggs per gram of root. These results confirm the findings of Cabrera *et al.* (2009), Faske and Starr (2006), and Faske (2009) which demonstrated that abamectin effectively reduces *M. incognita* population density early in plant development. Trial results indicated that ILeVO effectively reduced nematode population density which agreed with the findings of Faske and Hurd (2015). Nematicide and insecticide treatments did not significantly increase plant growth at 30 DAP compared with the control. The results of these greenhouse screenings confirm the observations made by Zaworski *et al* (2014) that ILeVO causes phytotoxic effects on soybean and may reduce fresh root weight of treated plants.

Field Trials

Plant Breeding Unit

The average *M. incognita* density in post-harvest soil samples at PBU did not reach the economic threshold of 60-70 100cm^3 defined by Mueller (2009) and Jagdale *et al.* (2013). There were no significant differences in plant growth parameters among all treatments and the control at 35 DAP. Avicta + Sure-K + Micro-500 IFS significantly reduced *M. incognita* eggs per gram of root by 67% when compared to the control at 35 DAP. Avicta + Sure-K + Micro-500 reduced nematode population density suggesting that this application is effective for management of root-knot nematodes on soybean. Starter fertilizers did not show significant reductions of *M. incognita* population density in the greenhouse trials, however their combination with the nematicides may have a role in the reduction in *M. incognita* population density observed at PBU. This is supported by the conclusions of Melakeberhan (2006) who reported that starter fertilizer application reduced *M. incognita* eggs per gram of root of soybean during vegetative stages. It is worth noting that Avicta singly supported a 67kg/ha (1 Bu/a) increase over the untreated control which concurs with soybean yield improvements from nematicide application observed by Dodge and Lawrence (2015) and Gaspar *et al.*(2015). The addition of the starter fertilizer Sure-K +Micro-500 IFS to Avicta produced 225 kg/ha (3.4 Bu/a) more than the control. Adding the plant growth regulator Ascend as an in furrow spray increased yield over the control by 532 kg/ha (7.92 Bu/a). The numeric increase in yield observed by the application of Avicta + Sure-K + Micro-500 Ascend IFS, mirrors the observations of Luangkhot (2016) who observed that nematicide combined with starter fertilizer and plant growth regulator significantly increased cotton yield in a reniform nematode infested field in Alabama.

Brewton Agricultural Research Unit Trial

The *M. incognita* post-harvest soil samples at BARU did not cross the population density threshold. The Avicta treatment effectively increased shoot weight and plant biomass over the control. Avicta and Avicta + Sure-K +Micro-500 IFS + Ascend IFS significantly increased plant biomass by 38% and 21%. These treatments increased plant growth at 38 DAP. A significant correlation between plant biomass and yield was observed at this location. This confirms the findings of Maw *et al*. (2011) showing a correlation between biomass in the vegetative growth stages and yield. Treatments with Avicta all numerically increased yield similar to what was observed at PBU. Avicta + Sure-K +Micro-500 IFS increased yield by 450 kg/ha (6.7 Bu/a) or 20% when compared to the control which concurs with the results of Miller (2016) who found that starter fertilizer application under *R.solani* and *H. glycines* increased soybean yield. It also supports the results of Osborne and Ridell (2006) which demonstrated that starter fertilizer application increased soybean yield. The correlation of biomass and yield and significant yield improvement observed by the combined inputs: Avicta + Sure-K +Micro-500 IFS suggest that these treatments have efficacy in increasing early plant growth and yield in *M. incognita*-infested fields.

Treatment performance differed between locations. At PBU, the combination of the plant growth regulator and starter fertilizer combined with Avicta gave the greatest numeric increase in yield while at BARU, Avicta + Sure-K + Micro-500 increased yield significantly. These results are indicative that a program utilizing a nematicide combined with starter fertilizer and plant growth regulator has potential efficacy in increasing soybean yield in fields infested with *M. incognita*.

Chapter 3: Group V Soybean (*Glycine max*) Selection and Nematicide Effects in Root-knot Nematode (*Meloidogyne incognita*) Infested Fields of Alabama

INTRODUCTION

Soybean (*Glycine max* L. Merrill) is one of the most planted and exported crops in the U.S. Soybean acreage in th U.S. has risen by 8 million acres since 2001. China annually imports approximately 1 billion bushels of soybean from the U.S. (NASS, 2016, Lloyd 2016). Alabama planted soybean acreage has increased by 280,000 acres since 2001, totaling 420,000 planted acres in 2016, and accounts for 1% of the U.S. soybean production (NASS, 2016). The Southern root-knot nematode, *Meloidogyne incognita* (Kofoid and White, 1919; Chitwood, 1949), causes significant yield loss of soybean. In 2010, *M. incognita* suppressed U.S. soybean yield by approximately 7.5 million bushels (Koenning and Wrather, 2010). In the coastal plains of the Southeastern U. S., the root-knot nematode can cause yield losses of susceptible soybean varieties of up to 90%, and 32-40% yield loss with resistant varieties have been reported (Kinloch, 1974). Kinloch (1974) observed that resistant varieties combined with a nematicide treatment produced the greatest yields in *M. incognita*-infested fields planted. Similar to Kinloch (1974), Minton *et al.* (1980) demonstrated that dibromide chloropicrin nematicide application increased yield of root-knot nematode resistant, intermediate, and ‘low’ (low resistance) soybean varieties in *M. incognita*-infested fields. Herman *et al*. (1990) also indicated that planting a root-knot resistant soybean variety in a heavily infested field produced significantly greater yield than a susceptible variety. Gaspar *et al*. (2014) observed that seed treatments with the nematicide, abamectin, increased soybean yield in multiple locations; the efficacy of this chemical for management of *M. incognita* was confirmed by Faske and Starr (2006). The purpose of this research was to determine the effect of treatment with abamectin on the performance of *M. incognita* resistant, *Heterodera glycines* (Ichinohe, 1952) resistant, and *M. incognita* susceseeptible soybean cultivars by measuring the ability to reduce nematode population density and increase plant biomass and yield across three locations in Alabama.

MATERIALS & METHODS

Experimental field trials were conducted at the Brewton Agricultural Research Unit (BARU, Brewton, AL, coordinates: 31.140404,-87.050274), Gulf Coast Extension and Research Center (GCREC, Fairhope, AL; coordinates 30.543481,-87.881660), Plant Breeding Unit (PBU, Tallassee, AL ; coordinates: 32.487668;-58.882875), and the Prattville Agricultural Research Unit (PARU, Prattville, AL; coordinates 32.427031,-86.444525). Trials were planted in root-knot nematode infested fields at these locations. Five maturity group V soybean varieties were used in these experiments. Nematode resistance ratings were obtained from the seed companies. Varieties included the root-knot nematode resistant variety Mycogen 5N522R2 (Mycogen Seeds Indianapolis, IN), the soybean cyst nematode (SCN) resistant variety USG 75T40 (UniSouth Genetics, Inc. Dickson, TN), Asgrow 5935 (disease ratings not available), and root-knot susceptible varieties: UA 5414RR (University of Arkansas System Division of Agriculture, Fayetteville, AR) and Progeny 5333RY (Progeny Ag Products, Wynne, AR). The 10 treatments include the varieties alone (5 treatments) and the same varieties treated with the Avicta nematicide (5 treatments) (abamectin 0.15 mg/seed; Syngenta Crop Protection, Greensboro, NC). The nematicide was applied in slurry using a Gustafson laboratory tabletop seed treater. The field trials at each location were arranged in a randomized complete block design with four row plots. A split plot design was used to plant two rows without nematicide and two rows planted to the same variety treated with Avicta. Treatments were replicated five times at each of the four locations for a total of 20 observations per treatment. Main plots consisted of 4 rows that were 6 m long with 0.9 m row spacing Trials were planted 26 Apr. (PBU), 29 Apr (PARU), 9 May (BARU), and 12 May (GCREC), at a seeding rate of 6 seeds per 0.3 m row. Production practices included pre-emergence herbicide application of 629mL/A of Dual Mag and 946mL/A Roundup, post emergence application of Roundup Power Max was sprayed approximately 7, 36, and 60 days after planting (DAP). Four entire plant samples per plot were taken 34, 38, 46 and 48 DAP at each location, respectively. Plant height (in cm), shoot and root fresh weights (in g) and total biomass (sum of root and shoot fresh weights) were recorded from these samples. Nematodes were extracted from roots by 4 min. agitation in a 6% NaOCl solution, captured on a 25-µm sieve, and enumerated to calculate nematode population density in eggs per gram of fresh root weight. Trials were machine harvested at maturity on 3, 5, 12, and 31 Oct. at PBU, PARU, BREW, and GCREC respectively. The data sets were pooled to determine significant interactions between location, variety, and nematicide as well as to compare varieties with and without Avicta by paired T-test to determine the effect of Avicta on treatment variables across all locations and varieties. Individual treatment means were then analyzed by pairwise T-test to compare variety performance after adding Avicta. Data were analyzed utilizing SAS 9.4 using the PROC TTEST procedure to compare means between untreated and nematicide treated plots for each variety. All plant parameters were analyzed assuming normal distribution. *Melodogyne incognita* population density required a log-normal distribution transformation to satisfy the normality assumption; estimates for the lognormal distribution function were back transformed to the original values using PROC MEANS and the original mean values are presented in the tables. Means were compared statistically using a 90% confidence interval ($t \leq 0.10$).

RESULTS

Variety performance measured in yield and plant biomass differed by location. Yield and biomass were significantly higher at GCREC than PBU representing a significant interaction between location and variety performance ($P \leq 0.0001$ and $P \leq 0.0045$ respectively; Table 1). Variety response to Avicta varied with regard to varietal nematode susceptibility. Avicta increased the yields of Progeny 5333RY significantly greater than for the other varieties ($P \leq 0.02$) Treatments at PBU had significantly greater *M. incognita* population density than all other locations, averaging 3498 eggs per gram of root ($P \leq 0.0001$, Table 2). Treatments at GCREC and PARU were not significantly different in *M. incognita* population density (415 and 311 eggs per gram of root, respectively). However the nematode population density of treatments at GCREC and PARU were significantly greater than treatments at BARU (178 eggs per gram of root; $P \leq 0.0001$, Table 2). Because of these significant interactions, data were analyzed and presented by individual variety and by location. Temperature and rainfall data charts from each station depicts higher minimum daily temperatures occurred at GCREC than all other locations (Figures 1-4). Rainfall at all locations diminished in the months of September and October.

Performance of Treatments by Location

Brewton

Variety performance at BARU indicated that the nematicide significantly increased the yield and plant biomass of several varieties when compared to their untreated counterparts. The nematicide treatment significantly increased biomass of the *M. incognita* resistant and susceptible varieties (Table 3). Nematicide treatment also reduced the nematode population density as measured by *M. incognita* eggs per gram of root for UA 5414RR ($P \leq 0.08$), a root-knot nematode susceptible variety (Table 3). The increases in biomass of the susceptible variety UA 5414RR did not correspond to an increase in yield; however, the increased biomass of Progeny 5333RY ($P \leq 0.03$) did correspond to an increase in yield of 39% when treated with Avicta (Table 3). The nematicide application also supported a greater yield in the *H. glycines*- resistant USG 75T40 even though nematode population density was snot suppressed. Pearson's correlation coefficients indicated biomass was negatively correlated with nematode population density at this location (R^2= -0.5798, $P<0.0001$).

Gulf Coast

Variety performance at the GCREC was affected by higher *M. incognita* population densities in this field as well as an overall greater yield. The nematicide application significantly reduced nematode population density ($P \leq 0.09$) and increased plant biomass ($P \leq 0.09$) of the *M. incognita* susceptible UA 5414RR, but did not significantly increase yield (Table 4). The nematicide treatment significantly increased yield of the susceptible variety Progeny 5333RY by 48% ($P \leq 0.06$). Plant biomass negatively correlated with nematode population density at GCREC (R^2=-0.37, $P<0.0085$).

Plant Breeding Unit

The fields at PBU have been infested with *M. incognita* for decades and support the highest nematode population density of all locations (data not shown). The application of the nematicide did not affect the plant biomass for any of the varieties tested. The nematicide did reduce nematode population density of Asgrow 5935 ($P \leq 0.05$) and the susceptible Progeny 5333RY ($P \leq 0.09$) by 48% and 50%, respectively (Table 5). Plant biomass was negatively correlated with nematode population density (R^2-0.3412 $P<0.014$). Yield was negatively correlated with nematode population density (R^2-.2531, $P<0.0763$) and positively correlated with plant biomass ($R^2=0.2436$, 0.0882).

Prattville

The plant biomass at this location was increased by the nematicide treatment for Asgrow 5935, however none of the other soybean varieties responded with an increase in biomass. The nematicide did significantly reduce nematode population density of Mycogen 5N522R2, Asgrow 59335, and Progeny 5333RY by 77% ($P\leq 009$), 84% ($P\leq 0.06$), and 42% ($P\leq 0.05$), respectively (Table 6). However, this reduction in nematode density did not correspond to an increase in yield for any of the varieties. Plant biomass and yield were negatively correlated with nematode population density ($R^2=-0.23$, $P<0.971$; $R^2= -0.35503$, $P<0.0114$); yield was positively correlated with biomass ($R^2=0.534$, $P<0.0001$).

DISCUSSION

This trial successfully demonstrated how a range of soybean varieties respond to treatment with a nematicide when planted in root-knot infested fields. The *M. incognita* resistant variety yielded over 2688 kg/ha (40 bushels per acre) at three locations with or without Avicta. The nematicide seed treatment did not significantly affect the nematode population density of the SCN resistant variety at any location but did increase its yield by 42% at BARU. The *M. incognita* susceptible varieties responded slightly differently to treatment with Avicta. The application of Avicta to UA 5414RR positively affected biomass; however, this did not result in significantly greater yield. Alternatively, applying Avicta to the susceptible Progeny 5333RY variety significantly increased yield while only increasing biomass at one location. The interaction statistics showed that Progeny 5333RY responded better than the other varieties to the nematicide treatment when comparing yields. Avicta significantly increased yield of this variety by of 39% and 48%, respectively. These observations demonstrate that susceptible soybean varieties respond differently to an application of Avicta. At all locations plant biomass was found to be negatively correlated with nematode population density, which concurs with the findings of Fortnum *et al.* (1991) which determined that *M. incognita* can affect biomass partitioning. Yield was positively correlated with plant biomass and negatively correlated with nematode population density at PBU and PARU. These findings support the results of Kinloch *et al.* (1985) who observed a negative correlation between root galling index and soybean cultivar yield. Avicta effectively increased yield of several soybean varieties in multiple locations in this study which is consistent with the observations reported by Gaspar *et al* (2014). However, the nematicide failed to significantly increase yield of the root-knot resistant variety (Mycogen 5N522R2), Asgrow 5935, and one of the susceptible varieties (UA 5414RR). The data from these trials demonstrate that Avicta can significantly increase the yield of *M. incognita* susceptible soybean varieties as well as an *H. glycines* resistant variety while reducing nematode population density. Avicta was of less benefit to the *M. incognita* resistant variety. These results contrast with the findings of Kinloch (1974) and Minton *et al.* (1980) that demonstrated that a nematicide application increased yields of resistant and susceptible soybean varieties in neatode infested fields. Overall, the performance of the root-knot resistant variety in this trial was consistent across locations, and although nematicide treatment did not affect its yield, a *M. incognita* resistant variety should still be one of the major components of any management strategy for growers with *M. incognita* infestations. Ultimately, an economic decision based primarily on nematode population density must be made in order to decide between planting a resistant soybean variety or planting a susceptible/tolerant variety treated with a nematicide.

Overall Conclusion

In conclusion, the research and results of this thesis may have impact on management decisions concerning *M. incognita* on soybean. The efficacy of using plant growth promoting inputs in combination with nematicides to increase plant growth and manage *M. incognita* has not been confirmed by our results. The growth promoting inputs showed efficacy in increasing soybean growth and biomass in vegetative stages, and nematicide treatments effectively reduced population density in greenhouse trials. The results of field trials demonstrated that a combination of inputs can increase plant biomass, however, Avicta combined with starter fertilizer was the only combination that effectively increased yield and reduced nematode population density. Field trials across four locations in Alabama evaluating the response of maturity group V soybean varieties to the Avicta nematicide application revealed interesting results. While location significantly impacted yield, *M. incognita* eggs per gram of root, and plant biomass, the nematicide showed efficacy in increasing the yields of Progeny 5333RY (a *M. incognita* susceptible variety) and USG 75T40 (a *Heterodera glycines* resistant variety). Furthermore, Avicta increased the yield of Progeny 5333RY significantly more than other varieties. Avicta did not affect the yield of the *M. incognita* resistant variety Mycogen 5N522R2, however this variety was consistent in yield production across locations, supporting the fact that using resistant varieties is the primary method to root-knot nematode management. The results of this research indicate that the use of plant growth promoting and nematicide inputs may be an effective method of management of *M. incognita* on soybean with the caveat that decisions regarding their implementation are guided by the nematode population density in the target field.

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Appendix A

Table 1. Commercially available nematicides, active ingredients, manufacturers, rates and applications labeled for soybean.

Nematicide	Active Ingredient	Manufacturer	Rate	Application
Telone II*	1,3,-dichloropropene	Dow Agrosciences	Maximum rate: 187 L/ha	Fumigant
K-Pam*	Potassium N-methyldithiocarbamate	AMVAC Chemical	Maximum rate: 280 L/ha	Soil injection
Vapam*	Sodium methyldithiobarbamate	AMVAC Chemical	Maximum rate: 358kg/ha	Soil injection
PONCHO/VOTiVO	Clothiandin/*Bacillus firmus*	Bayer CropScience	0.13 mg ai/seed	Seed treatment
Clariva pn	*Pasteuria nishizawae*	Syngenta Crop Protection	29.6 to 88.7 mL per 45.4 kg seed	Seed treatment
ILeVO	Fluopyram	Bayer CropScience	0.15mg ai/seed	Seed treatment
Avicta	Abamectin	Syngenta Crop Protection	0.15mg ai/seed	Seed treatment

*Indicates pre-planting nematicide

Table 2. The effect of plant growth regulator treatments on average soybean root and shoot fresh weight, plant biomass and plant height at 21 DAP in greenhouse trials.

Treatment	Rate	Root fresh weight (g)	Shoot fresh weight (g)	Plant height (cm)	Plant Biomass (g[x]
Control	---	1.43 [z]	2.16	20.0	3.60
Ascend ST[y]	88.7 mL/cwt	2.58**	2.76*	22.1	5.32**
Ascend IFS	292 mL/ha	1.71	2.68	21.1	4.39
Ascend FS	233 mL/ha	1.54	2.41	20.6	3.96
Ascend ST + FS	88.7 mL/cwt + 233 mL/ha	1.65	2.46	22.2	4.12
Ascend ST + IFS	88.7 mL/cwt +292 ml/ha	1.94	2.92**	24.7*	4.86**
Ascend IFS + FS	292 mL/ha +233mL/ha	1.50	2.12	20.1	3.62
Ascend ST + IFS + FS	88.7 mL/cwt + 292 + 233 mL/ha	1.84	2.64	22.4	4.49

[x] Plant biomass calculated by the sum of root and shoot fresh weight in grams.

[y] Ascend contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%. Ascend ST is applied as a seed treatment; Ascend IFS is an in-furrow spray; and Ascend FS as a foliar spray applied at 2nd true leaf stage.

[z] Means in the same column followed by * ($P \leq 0.10$) and ** ($P \leq 0.05$) are significantly different than the control according to Dunnett's *P* values.

Table 3. The effect of plant growth regulator treatments on average soybean root and shoot fresh weight, plant biomass, *Meloidogyne incognita* total egg numbers, and eggs per gram of root at 45 DAP in greenhouse trials.

					Meloidogyne incognita	
Treatment	Rate	Root fresh weight (g)	Shoot fresh weight (g)	Plant biomass (g)[x]	Total eggs / root	Eggs/g root
Control	---	7.17[z]	7.31	14.49	35040	4722
Ascend ST[y]	88.7 mL/cwt	8.43	8.55	16.98	67327	7118
Ascend IFS	292 mL/ha	9.05	8.95	18.01*	40231	4166
Ascend FS	233 mL/ha	5.63	6.91	12.54	29200	5598
Ascend ST + FS	88.7 mL/cwt + 233 mL/ha	7.08	8.29	15.37	31332	4291
Ascend ST + IFS	88.7 mL/ha +292.2 mL/ha	9.29	9.29**	18.59**	71276	5702
Ascend IFS + FS	292 mL/ha +233 mL/cwt	6.32	7.24	13.56	55156	9543**
Ascend ST + IFS + FS	88.7 mL/cwt + 292 mL/ha + 233 mL/ha	7.48	7.57	15.06	44774	6630

[x] Plant biomass calculated by the sum of root and shoot fresh weight in grams

[y] Ascend contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%. Ascend ST is applied as a seed treatment; Ascend IFS is an in-furrow spray; and Ascend FS as a foliar spray applied at 2nd true leaf stage.

[z] Means in the same column followed by * ($P \leq 0.10$) and ** ($P \leq 0.05$) are significantly different than the

Table 4. The effect of starter fertilizer treatments on average soybean root and shoot fresh weight, plant height and plant biomass at 21 DAP in greenhouse trials.

Treatment	Rate	Root fresh weight (g)	Shoot fresh weight (g)	Plant height (cm)	Plant biomass (g)[x]
Control	---	1.522 [y]	1.71	16	3.23
Sure-K [z]	9.33 L/ha	1.82	2.48	18.15	4.31
Pro-Germinator	4.64 L/ ha	1.99	1.95	18.4	3.95
Micro 500	2.32 L/ ha	1.78	2.21	18.05	3.98
Neptune's Harvest	7.41 L/ ha	1.72	2.41	19	4.13
Sure-K + Micro 500	9.33 L/ ha + 2.32 L/ha	1.43	1.87	17.25	3.31
Pro-Germinator + Micro 500	4.64 L/ ha + 2.32 L/ha	1.43	2.09	18.55	3.52
Neptune's Harvest + Micro 500	7.41 L/ ha + 2.32 L/ha	1.89	2.57	16.95	4.47

[x] Plant biomass calculated by the sum of root and shoot fresh weight in grams.

[z] Fertilizers selected in these trials were Sure-K (2-1-6) , Pro-Germinator (9-24-3) , Micro 500 (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%) and Neptune's Harvest (2-4-1).

[y] Means of 5 replications per treatment in the same column followed by* $P \leq 0.10$; ** $P \leq 0.05$ are significantly different as compared to the control according to Dunnett's.

Table 5. The effect of starter fertilizer treatments on average soybean root and shoot fresh weight, plant biomass, *Meloidogyne incognita* total eggs per root system, and eggs per gram of root at 45 DAP in greenhouse trials.

					Meloidogyne incognita	
Treatment	Rate	Root fresh weight (g)	Shoot fresh weight (g)	Plant biomass (g)[x]	Total eggs/ root system	Eggs/g root
Control	---	3.29 [z]	4.42	7.71	4637	1404
Sure-K [y]	9.33 L/ha	4.01	5.37*	9.39	5755	1505
Pro-Germinator	4.64 L/ ha	3.91	6.44**	10.36**	5932	1505
Micro 500	2.32 L/ ha	3.58	6.04**	9.63*	3787	1098
Neptune's Harvest	7.41 L/ ha	3.81	5.79**	9.60*	6434	2307
Sure-K + Micro 500	9.33 L/ ha + 2.32 L/ha	3.51	4.92	8.44	6728	1717
Pro-Germinator + Micro 500	4.64 L/ ha + 2.32 L/ha	4.31	6.17**	10.48**	3634	848
Neptune's Harvest + Micro 500	7.41 L/ ha + 2.32 L/ha	3.57	5.53**	9.09	4078	1147

[x] Plant biomass calculated by the sum of root and shoot fresh weight in grams.

[y] Fertilizers selected in these trials were Sure-K (2-1-6), Pro-Germinator (9-24-3), Micro 500 (B 0.02%, Cu 0.25%, Fe 0.37%, Mn 1.2%, and Zn 1.8%) and Neptune's Harvest (2-4-1).

[z] Means of 5 replications per treatment in the same column followed by * $P \leq 0.10$; ** $P \leq 0.05$ are significantly different when compared to the control according to Dunnett's *P* values.

Table 6. The effect of nematicide and insecticide seed treatments on average soybean root and shoot fresh weight, plant biomass, *Meloidogyne incognita* total eggs per root system, and eggs per gram of root at 30 DAP in greenhouse trials.

					Meloidogyne incognita	
Treatment	Rate	Root fresh weight	Shoot fresh weight	Plant biomass (g)[x]	Total eggs/root	Eggs/g root
Control	---	4.67[z]	9.44	14.11	49955	11225
Cruisermaxx[y]	9.28 L/ha	3.18*	7.49*	10.68*	21785**	8457
Avicta	0.15 mg ai/seed	3.90	7.94	11.84	2735**	1323**
Cruisermaxx+ Avicta	88.7mL/CWT +0.15 mg ai/seed	5.10	9.70	14.81	6026**	1323**
PONCHO/ VOTiVO	0.13 mg ai/ seed	2.45**	7.47*	9.92**	36662	20114
ILeVO	0.15 mg ai/seed	3.00**	7.88	10.89*	5258**	1780**
PONCHO/VOTiVO+ ILeVO	0.13 mg ai/seed+ 0.15 mg ai/seed	3.35	8.53	11.89	2433**	664**

[x] Plant biomass calculated by the sum of root and shoot fresh weight in grams.

[y] Nematicides and fungicides selected in these trials were Cruisermaxx (fludioxonil, mefenoxam, and thiamethoxam) Avicta (Abamectin), Poncho/VOTiVO (Clothiandin and *Bacillus firmus*), and ILeVO (fluopyram).

[z] Means, representing 5 replications in the same column followed by * $P \leq 0.10$; ** $P \leq 0.05$ are significantly different than the control according to Dunnett's *P* values.

Table 7. The effect of plant growth regulator, starter fertilizer and nematicide combinations on soybean root fresh weight, plant biomass, *Meloidogyne incognita* eggs per gram of root at 35 DAP, and yield at PBU.

Treatment	Rate	Shoot fresh weight (g)	Biomass (g)[y]	*M. incognita* eggs/g root	Yield kg/ha
Control	---	34.80 [z]	43.89	2408	3798
ILeVO ST[x]	0.15 mg ai/seed	36.63	45.16	921	3439
Avicta ST	0.15 mg ai/seed	38.37	47.81	1064	3867
ILeVO +Sure-K +Micro-500 IFS	0.15 mg ai/seed + 9.33 L/ ha + 2.32 L/ha	39.39	48.9	861	3380
Avicta+ Sure-K +Micro-500 IFS	0.15 mg ai/seed + 9.33 L/ ha + 2.32 L/ha	40.81	50.75	801*	4025
ILeVO+ Ascend IFS	0.15mg ai/seed 292mL/ha	33.79	42.24	1259	3472
Avicta+ Ascend IFS	0.15mg ai/ seed 292mL/ha	46.03	57.03	1115	3701
ILeVO+Sure-K +Micro-500 IFS +Ascend IFS	0.15mg ai/seed + 9.33 L/ ha + 2.32 L/ha + 292m L/ha	35.92	44.63	1133	3314
Avicta+Sure-K +Micro-500 IFS +Ascend IFS	0.15 mg ai/seed + 9.33 L/ ha + 2.32 L/ha + 292 mL/ha	38.72	48.59	1488	4312

[x] ST indicates seed treatment; IFS indicates in-furrow spray treatment.

[y] Biomass calculated by the sum of root and shoot fresh weight in grams.

[z] Means of 5 replicates per treatment in the same column followed by* $P \leq 0.10$; ** $P \leq 0.05$ are significantly different than the control according to Dunnett's P values.

Table 8. The effect of plant growth regulator, starter fertilizer and nematicide combinations on soybean root fresh weight, plant biomass, *Meloidogyne incognita* eggs per gram of root at 38 DAP, and yield at BARU

Treatment	Rate	Shoot fresh weight (g)	Biomass (g)[y]	*M. incognita*	Yield: kg/ha
Control	---	38.47[z]	47.05	3	2209
ILeVO ST[x]	0.15 mg ai/seed	35.26	44.06	2	2214
Avicta ST	0.15 mg ai/seed	52.48**	64.91**	1	2567
ILeVO +Sure-K +Micro-500 IFS	0.15 mg ai/seed +9.28L/ha +2.32 L/ha	28.85**	35.93**	51	2124
Avicta + Sure-K +Micro-500 IFS	0.15 mg ai/seed +9.28L/ha +2.32 L/ha	39.47	47.99	37	2652**
ILeVO + Ascend IFS	0.15mg ai/seed 292mL/ha	39.78	49.1	24	2412
Avicta + Ascend IFS	0.15mg ai/ seed 292m L/ha	36.79	44.76	30	2294
ILeVO +Sure-K +Micro-500 IFS +Ascend IFS	0.15mg ai/seed 9.28 L/ ha + 2.32L/ha + 292m L/ha	35.00	42.76	3	1939
Avicta +Sure-K +Micro-500 IFS +Ascend IFS	0.15 mg ai/seed + 9.33 L/ ha +2.32L/ha + 292 mL/ha	46.14	57.12*	2	2309

[x] ST indicates seed treatment; IFS indicates in-furrow spray treatment.

[y] Biomass calculated by the sum of root and shoot fresh weight in grams.

[z] Means of 5 replicates per treatment in the same column followed by* $P \leq 0.10$; ** $P \leq 0.05$ are significantly different than the control according to Dunnett's P values.

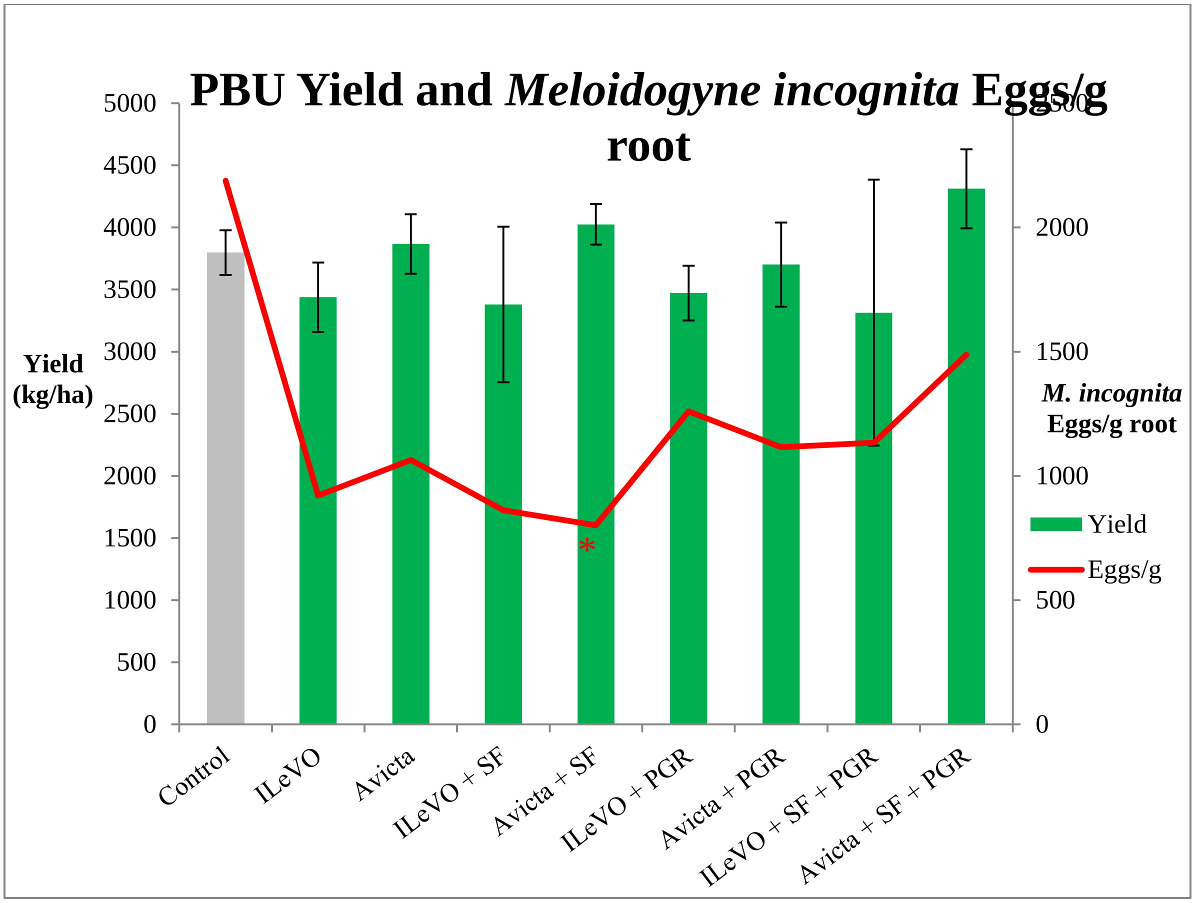

Figure 1. Soybean yield and *Meloidogyne incognita* population density at the Plant Breeding Unit (PBU). Yield presented in kilograms per hectare and nematode population density as the ratio of *Meloidogyne incognita* eggs divided by root weight in grams. Asterisks (black for yields and red for eggs per gram of root;* for $P<0.10$ and ** for $P<0.05$) indicate a treatment is significantly different than the control by Dunnett's.

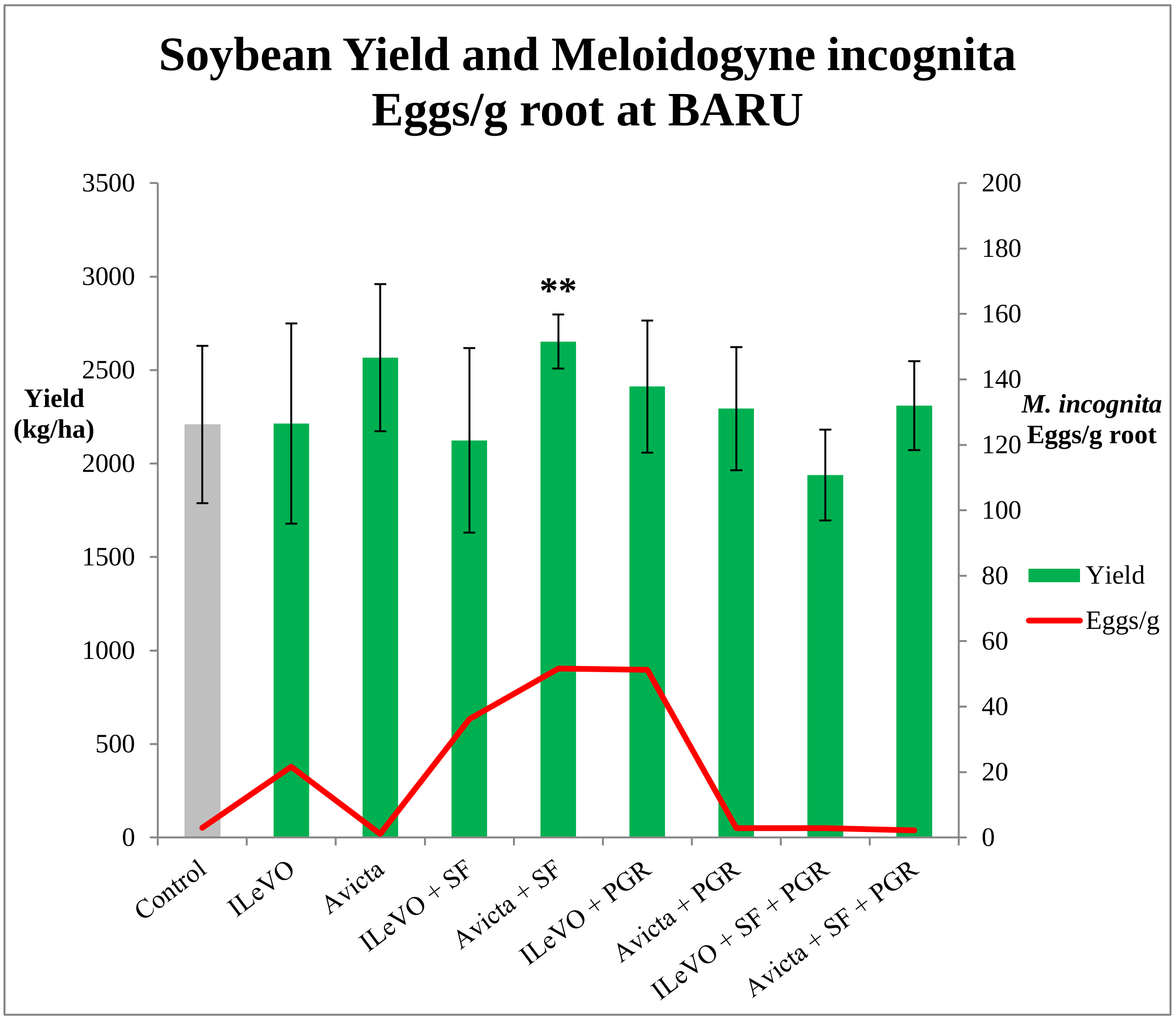

Figure 2. Soybean yield and *Meloidogyne incognita* population density at the Brewton Agricultural Research Unit (BARU). Yield presented in kilograms per hectare and nematode population density as the ratio of *Meloidogyne incognita* eggs divided by root weight in grams. Asterisks (black for yields and red for eggs per gram of root * for *P*<0.10 and ** for *P*<0.05) indicate a treatment is significantly different than the control by Dunnett's.

Appendix B

Table 1. Type 3 interaction effects of variety, Avicta, and location on *Meloidogyne incognita* eggs per gram of root, biomass, and yield of soybean varieties at four locations in Alabama.

			Eggs/g root		Biomass		Yield	
Effect	Num DF	Den DF	F Value	Pr>F	F value	Pr>F	F value	Pr>F
Variety	4	155	5.78	0.0002	4.23	0.0028	18.01	<0.0001
Avicta	1	155	2.45	0.1198	1.45	0.23	4.61	0.0333
Location	3	155	72.02	<0.0001	32.29	<0.0001	23	<0.0001
Vareity x Avicta	4	155	1.26	0.2862	0.87	0.4853	2.85	**0.0259**
Variety x Location	12	155	3.02	**0.0008**	2.53	**0.0045**	6.01	**<0.0001**
Avictax Location	3	155	1.11	0.3472	1.86	0.1388	2.18	0.0921
Variety x Avicta x Location	12	155	0.72	0.7343	0.97	0.4838	0.35	0.9781

Num DF and Dem DF (numerator and denominator degrees of freedom respectively)

Significant interactions determined by P <0.05 are in bold.

Yield calculated in Bu/a; nematode population density presented as a ratio of total eggs divided by root weight in grams; and biomass calculated as the sun of fresh shoot and root weights.

Table 2. Nematode population density by location; data represent log-normal transformed ratio of *Meloidogyne incognita* eggs per gram of root.

Location	Location	Difference of mean	SE	DF	t Value	Pr > |t|	P-value
BARU	GCREC	-3.617	0.3367	170	-10.74	<.0001	<.0001
BARU	PBU	-6.9879	0.3349	170	-20.87	<.0001	<.0001
BARU	PARU	-3.8616	0.3349	170	-11.53	<.0001	<.0001
GCREC	PBU	-3.371	0.3367	170	-10.01	<.0001	<.0001
GCREC	PARU	-0.2446	0.3367	170	-0.73	0.4685	0.8864
PBU	PARU	3.1263	0.3349	170	9.34	<.0001	<.0001

PBU: Plant Breeding Unit, BARU: Brewton Agricultural Research Unit, GCREC: Gulf Coast Research and Extension Center, and PARU: Prattville Agricultural Research Unit.

DF indicates degrees of freedom; SE: standard error.

Significant differences indicated by Tukey by $P < 0.05$ and $P < 0.10$.

Table 3. Soybean variety yield, *Melodogyne incognita* population density and biomass response to nematicide at the Brewton Agricultural Research Unit (BARU).

		Biomass (g)[x]		*M.incognita* eggs/g[y]		Yield (kg/ha)[z]	
		Mean[w]	Pr>|t|	Mean	Pr>|t|	Mean	Pr>|t|
Mycogen 5N522R2[R]	Avicta	77.4	0.06*	8	0.72	2440	0.49
	Untreated	57.4		1		2265	
USG 75T40[R]	Avicta	73.3	0.14	1165	0.69	2198	0.07*
	Untreated	57.9		142		1553	
Asgrow 5935	Avicta	64.1	0.22	38	0.52	2373	0.71
	Untreated	51.3		321		2118	
UA 5414RR[S]	Avicta	72.1	0.03**	40	0.08*	1808	0.15
	Untreated	49.1		1		2326	
Progeny 5333RY[S]	Avicta	63.9	0.02**	8	0.49	2729	0.03**
	Untreated	40.4		55		1963	

Superscripts [R] and [S] indicate nematode resistant and susceptible varieties, respectively.

[w] Means in each column sorted by variety are compared statistically by t-test.

[x] Plant biomass calculated as the sum of fresh shoot and root weights collected between 30-45 DAP.

[y] Nematode population density presented as a ratio of total eggs divided by root weight in grams.

[z] Yield presented in kg/ha.

*Significant at t <0.10; **significant at t <0.05.

Avicta (abamectin 0.15mg ai/seed) from Syngenta Crop Protection, Greensboro, NC

Table 4. Soybean variety yield, *Meloidogyne incognita* population density and biomass response to nematicide at the Gulf Coast Research and Extension Center (GCREC).

		Biomass (g)^x		*M.incognita* eggs/g^y		Yield (kg/ha)^z	
		Mean^w	Pr>|t|	Mean	Pr>|t|	Mean	Pr>|t|
Mycogen 5N522R2^R	Avicta	77.1	0.38	88	0.98	3233	0.77
	Untreated	102		96		3099	
USG 75T40^R	Avicta	59.2	0.82	209	0.78	2837	0.23
	Untreated	65.5		300		2292	
Asgrow 5935	Avicta	120	0.49	81	0.13	4813	0.24
	Untreated	101		838		4275	
UA 5414RR^S	Avicta	156	0.09*	49	0.09*	2608	0.92
	Untreated	105		313		2561	
Progeny 5333RY^S	Avicta	74	0.49	621	0.65	2696	0.06*
	Untreated	93.4		513		1822	

Superscripts ^{R} and ^{S} indicate nematode resistant and susceptible varieties, respectively.

^{W}Means in each column, sorted by variety, are compared statistically by t-test.

^{x}Plant biomass calculated as the sum of fresh shoot and root weights collected between 30-45 DAP.

^{y}Nematode population density presented as a ratio of total eggs divided by root weight in grams.

^{z}Yield presented in kg/ha.

*Significant at t <0.10; **significant at t <0.05.

Avicta (abamectin 0.15mg ai/seed) from Syngenta Crop Protection, Greensboro, NC

Table 5. Soybean variety yield, *Meloidogyne incognita* population density, and biomass response to nematicide at the Plant Breeding Unit (PBU).

		Biomass (g)[x]		*M.incognita* eggs/g[y]		Yield (kg/ha)[z]	
		Mean[w]	Pr>|t|	Mean	Pr>|t|	Mean	Pr>|t|
Mycogen 5N522R2^R	Avicta	46.6	0.72	2399	0.94	2743	0.93
	Untreated	44.3		2183		2709	
USG 75T40^R	Avicta	44.2	0.59	5474	0.93	2057	0.47
	Untreated	40.7		5328		1761	
Asgrow 5935	Avicta	53.8	0.29	1303	0.059*	2548	0.8
	Untreated	46.8		2513		2649	
UA 5414RR^S	Avicta	37.4	0.94	3212	0.78	2292	0.55
	Untreated	36.9		3310		2050	
Progeny 5333RY^S	Avicta	33.6	0.78	3065	0.098*	1627	0.27
	Untreated	31.8		6194		1183	

Superscripts ^{R} and ^{S} indicate nematode resistant and susceptible varieties, respectively.

[W] Means in each column, sorted by variety, are compared statistically by t-test.

[x] Plant biomass calculated as the sum of fresh shoot and root weights collected between 30-45 DAP.

[y] Nematode population density presented as a ratio of total eggs divided by root weight in grams.

[z] Yield presented in kg/ha.

*Significant at t <0.10; **significant at t <0.05.

Avicta (abamectin 0.15mg ai/seed) from Syngenta Crop Protection, Greensboro, NC

Table 6. Soybean variety yield, *Meloidogyne incognita* population density, and biomass response to nematicide at the Prattville Agricultural Research Unit (PARU).

		Biomass (g)[x]		*M.incognita* eggs/g[y]		Yield (kg/ha)[z]	
		Mean[w]	Pr>|t|	Mean	Pr>|t|	Mean	Pr>|t|
Mycogen 5N522R2^{R}	Avicta	76.6	0.22	63	0.096*	2716	0.25
	Untreated	91.1		271		2971	
USG 75T40^{R}	Avicta	56.3	0.93	127	0.184	2225	0.31
	Untreated	57.2		359		2003	
Asgrow 5935	Avicta	64.6	0.031**	37	0.064*	2178	0.18
	Untreated	90.7		238		2474	
UA 5414RR^{S}	Avicta	63.5	0.95	72	0.16	2279	0.0058**
	Untreated	64.1		235		2917	
Progeny 5333RY^{S}	Avicta	60.9	0.52	1007	0.052*	2218	0.43
	Untreated	53.3		1738		2050	

Superscripts ^{R} and ^{S} indicate nematode resistant and susceptible varieties, respectively.

[w] Means in each column, sorted by variety, are compared statistically by t-test.

[x] Plant biomass calculated as the sum of fresh shoot and root weights collected between 30-45 DAP.

[y] Nematode population density presented as a ratio of total eggs divided by root weight in grams.

[z] Yield presented in kg/ha.

*Significant at t <0.10; **significant at t <0.05.

Avicta (abamectin 0.15mg ai/seed) from Syngenta Crop Protection, Greensboro, NC

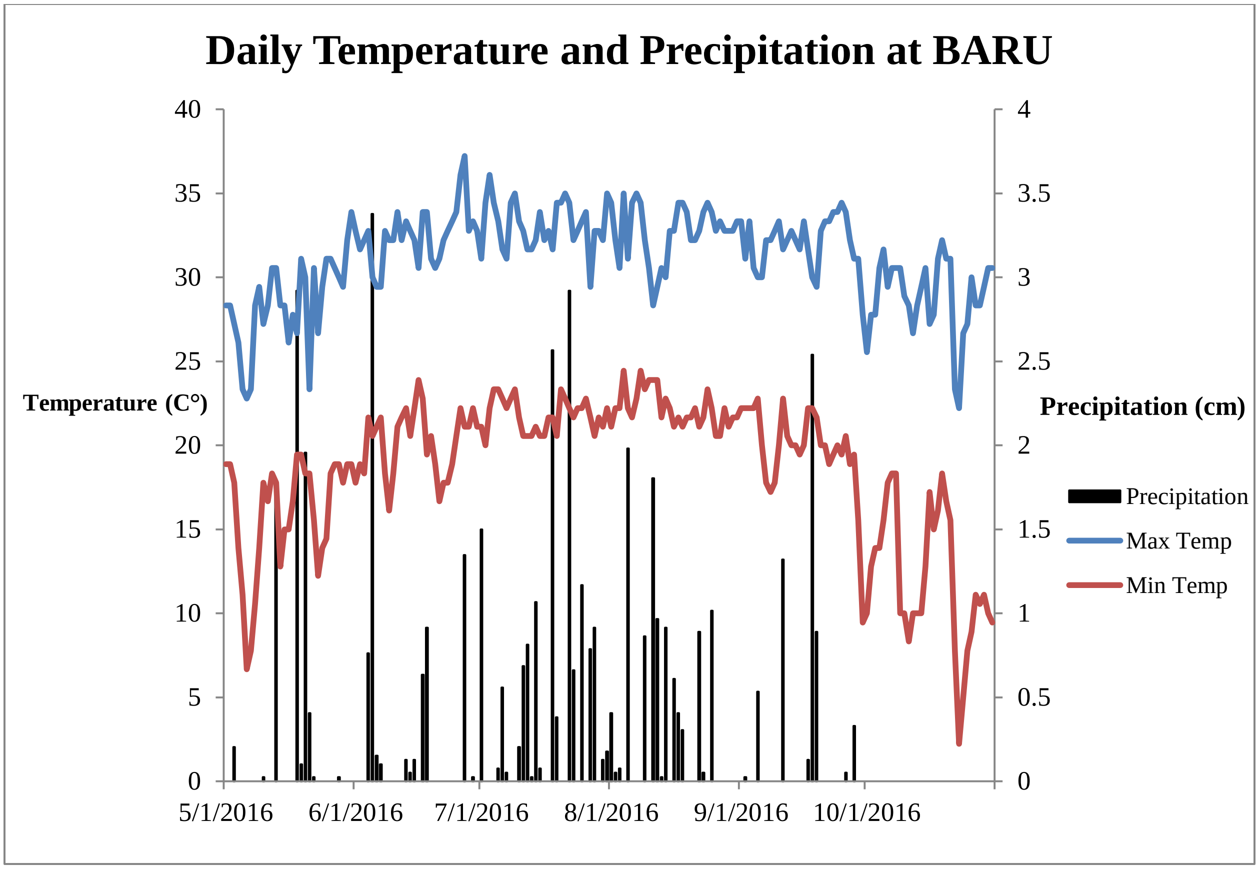

Figure 1. Daily temperature and precipitation recorded at Brewton Agricultural Research Unit (BARU). Daily temperature presented in Celsius and daily rainfall in cm of precipitation

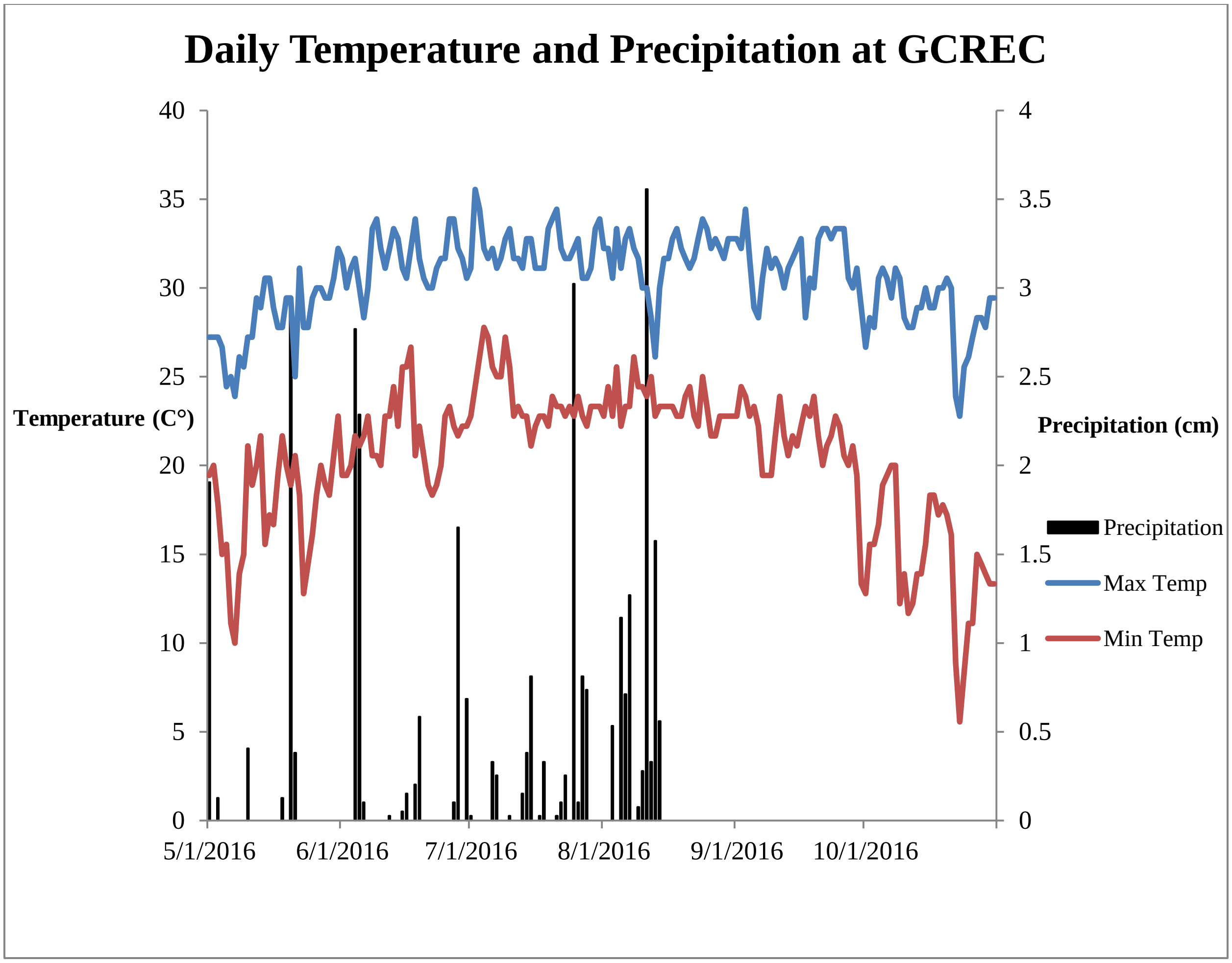

Figure 2. Daily temperature and precipitation recorded at the Gulf Coast Research and Extension Center(GCREC). Daily temperature presented in Celsius and daily rainfall in cm of precipitation.

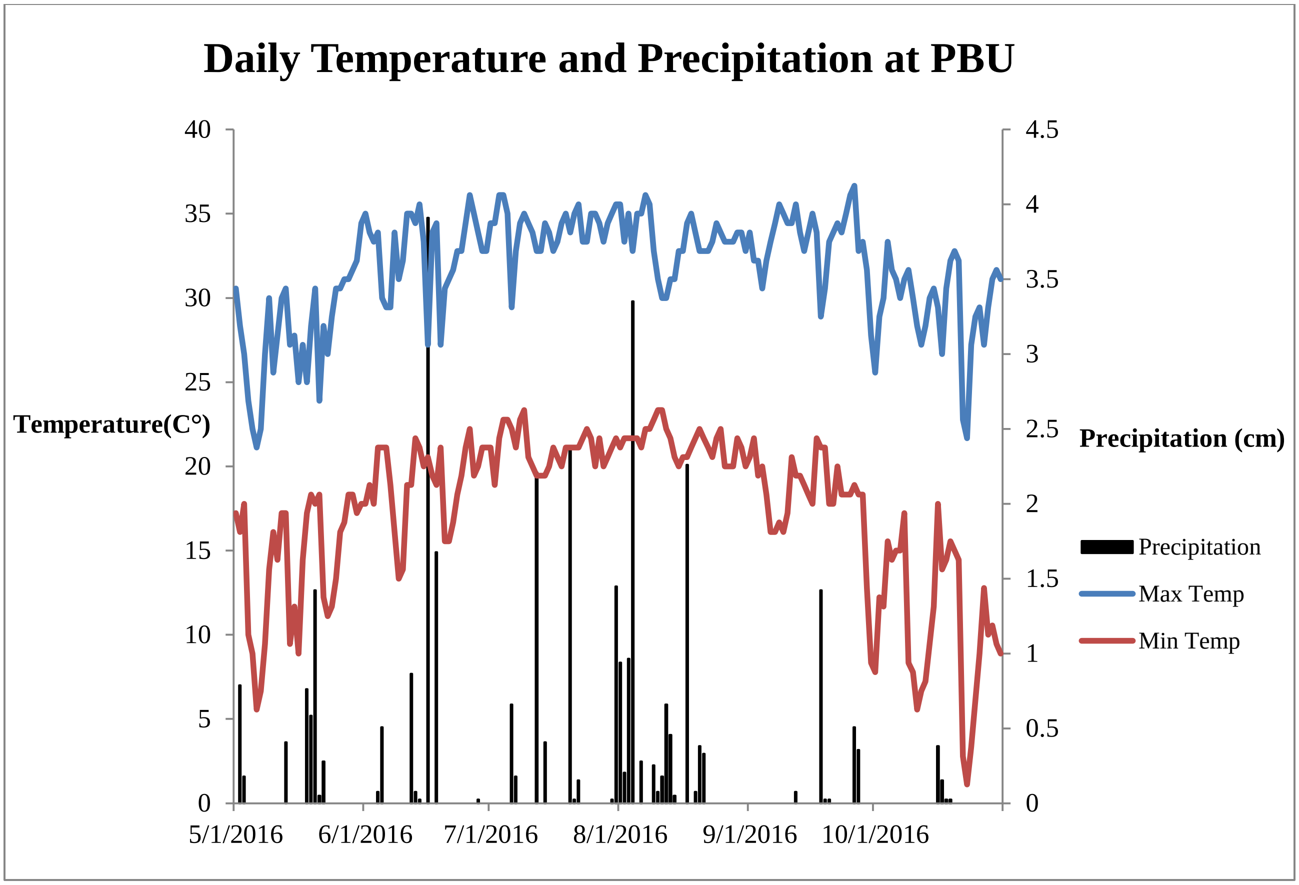

Figure 3. Daily temperature and precipitation recorded at the Plant Breeding Unit (PBU). Daily temperature presented in Celsius and daily rainfall in cm of precipitation.

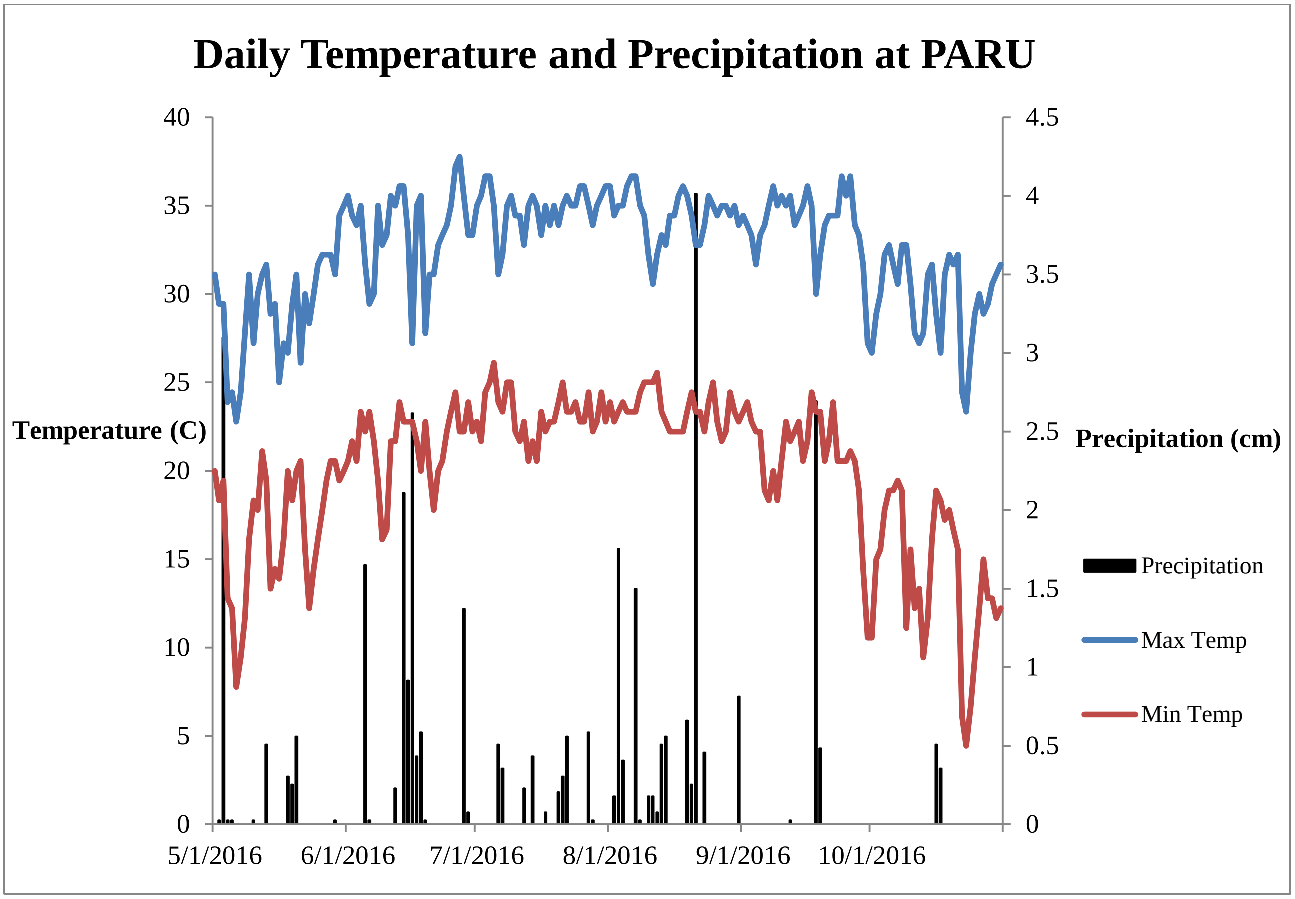

Figure 4. Daily temperature and precipitation recorded at the Prattville Agricultural Research Unit (PARU). Daily temperature presented in Celsius and daily rainfall in cm of precipitation.