

A cost-effective approach for combining nematicides, starter fertilizers, and plant growth regulators in order to create a sustainable management system for the southern root-knot nematode, *Meloidogyne incognita*, in corn

by

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Abstract

The southern root-knot nematode, *Meloidogyne incognita*, is one of the most problematic plant-parasitic nematodes in the southeastern United States. Significant yield reductions can occur for some of the most economically important crops in this area such as cotton, corn, and soybean. Literature suggests that a 30% reduction in yield can occur on corn due to this nematode. No resistant corn hybrids are available today, and cultural management techniques can be difficult to implement. We propose an integrated system combining nematicides with direct yield and growth promoting inputs such as pop-up and starter fertilizers and plant growth regulators in order to create a sustainable, profitable system. We evaluated this by first screening each individual product in a greenhouse setting to select the best input from each category. These inputs were integrated into field trials to assess their ability to reduce nematode population density, increase early growth, and thus increase yield.

In greenhouse trials, Counter® 20G was the most effective nematicide reducing root-knot population density by 85%, which led to an 18% increase in plant biomass. Most in-furrow starter fertilizers (pop-ups) increased plant biomass at 45 days after planting (DAP) without increasing root-knot population density. The plant growth regulator, Ascend®, in-furrow application (IFS) was the only treatment that significantly increased plant biomass (23%) at 45 DAP. Field trials were planted over two growing seasons (2016-2017) at two different locations: a field with a very high root-knot population density, Plant Breeding Unit (PBU) in Tallahassee, AL,

and a field with a moderate population density, Brewton Agricultural Research Unit (BARU) in Brewton, AL. In the second season a 5x5 (5 cm below; 5 cm beside the planting furrow) application of starter fertilizer was evaluated along with the previous pop-up fertilizers. For the 2016 growing season, the nematicide, growth regulator, and pop-up fertilizer combination significantly increased plant height and plant biomass at both locations; however only at BARU did a significant increase in yield occur, proving that the integrated system has a greater effect on yield at a lower population density. At BARU, the net return on yield for the three input system was \$113/ha, whereas at PBU the cost of application did not compensate for the gain in yield. Counter[®]20G + Ascend[®] IFS without pop-up fertilizers was the only combination to provide a positive return on yield at both locations (\$2.61/ha at PBU and \$54.98/ha at BARU). For the 2017 growing season multiple inputs increased early plant growth, but because of unforeseen environmental conditions, their effect on yield could not be accurately portrayed. All input combinations including the 5x5 starter fertilizer increased plant biomass by an average of 77% compared to the control at PBU, which was a 47% increase compared to pop-up fertilizers. Counter[®]20G + starter fertilizer provided the greatest increase (112%) at that location. Many input combinations increased biomass at BARU; however, pop-up fertilizer combinations averaged a 4% higher increase in biomass than the starter fertilizer compared to the control. Our system of combining a nematicide with pop-up or starter fertilizers and/or plant growth regulators shows promise in efficiently managing plant-parasitic nematodes in corn; however, the economic benefits were best under lower root-knot nematode population density.

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Chapter 1: Introduction and Review of Literature

Introduction and Problem Statement

The objective of this research is to measure the impact of the southern root-knot nematode (RKN) (*Meloidogyne incognita* (Kofoid and White) Chitwood) on corn (*Zea mays* L.) grain production. Two main objectives of this research are to provide more information on potential yield loss caused by the southern RKN in Alabama as well as developing an alternative economical management strategy that combines nematicides with commercial agricultural inputs marketed as plant health and growth enhancement products. In 2016, an estimated 1.48 million tons of corn were lost due to plant-parasitic nematodes in the United States, and 1.03 million tons were lost just in the southernmost states where nematodes were the second most important plant pathogen (Mueller et al., 2016). This is similar to Koenning et al. (1999) findings where plant-parasitic nematodes caused a 20% loss in southern corn-producing states as opposed to only a 5% loss in northern states. They valued the total losses in corn from all nematode genera to be worth 22.99 billion dollars for the year of 1994. Nematodes can damage corn roots in two ways: direct injury by mechanically or chemically injuring the roots or indirect damage by favoring entry of fungal or bacterial pathogens resulting in disease complexes (Windham, 1998). Diagnosing yield losses due to nematodes may be difficult due to corn's extensive fibrous root system. The plant may mask the symptoms until higher population densities develop or until unfavorable environmental conditions occur (Windham, 1998)

More than 60 nematode species have been documented as corn parasites in North America, and because of environmental differences in these species, only three to seven species are found in a field at one time (Norton, 1983). Within these species, only a few can cause significant damage within their own localized populations (Windham, 1998). Among these are the root-lesion nematodes (*Pratylenchus* spp.) Filipjev, sting nematodes (*Belonolaimus* spp) Steiner, needle nematodes (*Longidorus breviannulatus*) Norton and Hoffman, stubby-root nematodes (*Paratrichodorus minor* (Colbran)) Siddiqi, and root-knot nematodes (*Meloidogyne* spp.) Goldei. Other nematodes usually occur in mixtures and yield losses can be difficult to assess (Windham, 1998). The root-lesion nematode is classified as a migratory endoparasitic nematode that causes necrotic lesions on the roots. Common species in southeastern U.S. include *P. zae* Graham and *P. brachyurus* (Godfrey) Filipjev and Shururmans-Stekhoven. *Pratylenchus zae* has been shown to reduce corn yields by 20% (Tarte, 1971). Sting and needle nematodes are relatively large ectoparasites that feed on the outside of the root near the tip, and they can cause severe yield loss in soils containing 80% or more sand content (Windham, 1998). Almost no yield is predicted in fields containing 30 sting nematodes per 100 cm³ of soil (McSorley and Dickson, 1989), and a 62% reduction can occur when the needle nematode reaches 10 nematodes per 100 cm³ of soil (Malek et al., 1980). Stubby-root nematodes are a major nematode pest of the coastal plain region of the southeastern U.S. (Christie, 1953). These ectoparasitic nematodes feed on almost exclusively on the root tips causing a halt in root growth, which in turn gives the root system a “stubby” appearance (Windham, 1998). Initial populations of this nematode exceeding 20 nematodes per 100 cm³ of

soil can suppress yields (Barker and Olthof, 1976). Root-knot nematodes are commonly associated with corn in the southeastern U.S. (Barker, 1974; Gazaway et al., 1991).

Meloidogyne incognita, *M. arenaria* (peanut RKN (Neal)) Chitwood, and *M. javanica* (javanese RKN (Treub)) are the major root-knot species that serve as hosts to corn in North America. In Alabama, a survey indicated that 7% of the fields sampled (totaling 603 ha) were infested with *M. incognita* ranging from light to high infestations (Gazaway and McLean, 2003).

Due to its prevalence in Alabama and the potential yield loss that exists with major agronomic crops grown in the state, we focused on *M. incognita*. This species thrives in tropical and sub-tropical climates and can survive in a wide variety of soil types, but the most severe damage occurs in coarse-textured sandy soils (Van Gundy, 1985). *Meloidogyne incognita* has a very wide host range of up to 3,000 plant species (Jepson, 1987). This includes major agronomic crops such as corn, soybean, alfalfa, and cotton, as well as a wide variety of weeds, making cultural management such as crop rotation very difficult. Added plant stress including unfavorable environmental conditions, dramatically increase the severity of *M. incognita* infestations. A corn root system infested with southern RKN can exhibit moisture stress, reduced nutrient uptake, and subsequent pathogenic infections due to the galling and suppressed root growth induced by the pest's feeding (Mitkowski and Abawi, 2003). O'bannon and Reynolds (1965) found that cotton without RKN used 184% more water than cotton infected with RKN when soil moisture fluctuated between 50% and 100% field capacity. Wheeler et al. (1991) found that as water or nutrient stress increased, more relative yield was lost per RKN. This agrees with Wheeler et al. (2013) where they evaluated the relationship

between environmental variables and the response to nematicides in cotton in Texas and Alabama, and found that as moisture increased during the month of planting, nematicide treatments caused a reduction in galls, and vice versa for non-nematicide treatments. For these reasons, unfavorable environmental conditions can create a “doubling effect” on yield loss due to RKN. In heavily infested fields, yield losses of up to 30% can occur in corn (Gazaway et al., 1991).

In the past three years, the price of corn has stayed under \$157.47/t (\$4.00/bu) for the typical harvest months of August through October (Figure 1). The price on Oct 3, 2017 was \$138.18/t (\$3.51/bu) (Prices). In Alabama, 94% of corn was harvested by Oct 1, 2017 (USDA,



Figure 1. Trend in corn prices from 2015 to 2017

2017b). This puts an added pressure on producers to efficiently maximize their yields while still being able to produce a profitable net return. Producers must carefully manage inputs such as

water, fertilizer, and pesticides to maximize profit. Producers may not be willing to spend the money necessary for adequate management of plant-parasitic nematodes. This seems to be the case for a variety of reasons. It is a common myth that the corn root system is extensive enough to overcome RKN feeding, and that the damage that does occur is not enough to warrant a nematicide application (Kemerait, 2016). However, damage caused by nematodes, in general, is usually grossly underestimated due to the symptoms resembling nutrient deficiencies, drought, or other abiotic factors (Windham, 1998). Misdiagnosing nematode symptoms occurs because a producer is often unaware of effects of nematode feeding, and how their feeding makes the host crop more susceptible to the aforementioned environmental stresses. As mentioned previously, cultural management of RKN in corn is often very difficult, and few management options exist besides the application of nematicides. The goal of this research is to create an economical management system that ties in fertility and plant health management with nematicidal applications for an efficient management system of southern RKN-infested corn. We also seek to provide more information on potential yield loss when RKN is present in a field.

Corn

More than 90 million acres in the U.S. are planted to corn, with its primary use as the main ingredient in livestock feed. Other uses include food and industrial products such as starch, corn oil, industrial alcohol, and ethanol fuel (USDA ERS, 2017). Ethanol production is the main reason for the recent surge in corn acreage due to government offered incentives, but the role that improved agricultural technology plays should not be overlooked (USDA ERS, 2017).

The 2016 growing season for corn in Alabama began as a promising year with a 31% increase in hectares planted (dryland and irrigated) from 2015 (105,218 to 137,593 ha) (USDA, 2017a). This increase was due to growers assessing the low prices of other commodity crops, and deciding on corn being the least risky option for making a profit. However, due to several regions of the state experiencing drought in 2016, corn yield was down to 7,532 kg/ha (120 bu/A) (dryland and irrigated), the lowest yield in Alabama since 2012 where the average yield was only 6,151 kg/ha (98 bu/A) (USDA, 2014). Value of production in corn for grain in 2016 was \$132,300,000 at \$137.79/t (\$3.50/bu) making it the third most valuable commodity crop in Alabama behind cotton and hay (USDA, 2016). Despite the relatively high production of corn, Alabama is still known as a corn-deficit state due to the enormous amounts of grain needed to feed the large poultry and livestock industries. In the United States, record highs in both average corn yield (10,922 kg/ha or 174.6 bu/A) and production (384.81 million tons or 15.15 trillion bushels) occurred (USDA, 2016). The U.S. is the world's largest producer of corn and exports between 10 and 20 percent of its annual production (USDA ERS, 2017).

Meloidogyne incognita

The root-knot nematode genus, *Meloidogyne spp.*, comprises of over 100 species and are one of the most damaging plant-parasitic nematodes (Elling, 2013). As a whole, plant-parasitic nematodes account for 14% of the worldwide losses, which translates into an astounding \$100 billion annually. *Meloidogyne spp.* accounts for nearly one-third of those losses (Mitkowski and Abawi, 2003). Within the genera exists the species *M. incognita*, which is known as one of the most widespread and economically important nematode pathogens. It

survives in a wide array of soil textures, but it is most severe in coarse-textured soil (Van Gundy, 1985). In Alabama, southern RKN suppresses yield in three of the state's most economically important crops: cotton, corn, and soybean. In 2016, an estimated 14,200 cotton bales (2% of total yield) were lost costing producers 5.2 million dollars (Lawrence et al. 2016). In corn, yield loss has been observed to be anywhere between 2.2-11.4% when the population density is 100 juveniles per 100 cm³ of soil (Bowen et al., 2008). In 2016, 2,722 tons of soybeans were lost due to southern RKN in Alabama compared to 31,056 total tons lost across the southern states (Allen et al., 2016). Because significant yield losses can occur in these commonly grown crops in Alabama, different management strategies such as resistant varieties or chemical and biological control need to be evaluated for their ability to reduce *M. incognita*.

Meloidogyne incognita is a sedentary endoparasitic nematode that reproduces and feeds on modified living plant cells within plant roots, creating variable sized galls or knots giving it its common name, the root-knot nematode (Moens et al., 2009). The root-knot life cycle has three main stages: egg, juvenile, and adult, and its life cycle begins with the egg stage (Taylor and Sasser, 1978). Embryogenesis is followed by the development of the first-stage juvenile within the egg (Moens et al., 2009). The first-stage juvenile molts into the second-stage juvenile (J2) which then emerges from the egg via use of its fully formed stylet, moves into the soil, and seeks a suitable host to begin its feeding (Moens et al., 2009; Taylor and Sasser, 1978). Eggs will only hatch under favorable environmental conditions such as adequate temperature and moisture that are conducive for the J2 to move to its new host (Moens et al., 2009; Tiwari et al., 2009). Once a suitable host is located, the J2 will penetrate just above the root cap in the

apical meristem and move through undifferentiated root cells until it comes to the region of cell elongation (Taylor and Sasser, 1978). The J2 becomes sedentary and removes cell contents with its stylet. Piercing of a cell wall and removal of cell contents with its stylet, along with esophageal gland secretions, induces the cells to enlarge into specialized nurse cells from which the nematode will feed (Moens et al., 2009). Formation of these cells also induces hypertrophy and hyperplasia (Taylor and Sasser, 1978) and creates a metabolic sink, which causes nutrient imbalances in susceptible hosts (McClure, 1977), not to mention limiting proliferation of the branching roots which decreases the capacity of nutrient extraction from the soil. Under favorable conditions, the J2 will molt into its final two juvenile stages (J3 and J4) before reaching the adult stage. The J3 and J4 stages will lack a functional stylet and do not feed; however, the combined duration of these stages is typically only 4-6 days (Moens et al., 2009). After the fourth molt, the stylet is regenerated and the uterus and vagina are formed (Taylor and Sasser, 1978). No males are needed for reproduction in *M. incognita*, for it reproduces via obligatory parthenogenesis (Moens et al., 2009). The adult female will continue to draw nutrients and enlarge inside the feeding site eventually forming the distinctive symptomatic galls that are unique to the root-knot nematode (Taylor and Sasser, 1978). She will then produce eggs both inside and outside of her body in an egg sac contained within a gelatinous matrix (Taylor and Sasser, 1978). A single female can lay about 500-1500 eggs during her life, which can last up to two or three months (Tiwari et al., 2009). The eggs hatch when conditions are favorable, and the cycle begins again.

Cultural management of southern RKN can be difficult due to a variety of reasons. This nematode is an obligate parasite meaning they need a suitable host to live and feed on to survive. Because of this, crop rotation can be an effective management tool; however, due to the species' very wide crop host range, crop rotation with non-hosts can be difficult in Alabama based on the limited variety of crops that are grown. Of the major field crops grown, peanuts are the only non-host of southern RKN (Taylor and Sasser, 1978). Although feasible, a peanut-corn rotation is not a common practice in Alabama. In a three-year microplot experiment with *M. incognita* race 3, Kirkpatrick and Sasser (1984) found that juvenile population density, egg density, and root gall indices all increased in cotton plots following cotton or corn monoculture or cotton followed by corn than in cotton plots following other sequences containing peanut and/or soybean. Soybean-corn and cotton-corn rotations are common in Alabama, and RKN population density will most likely increase unless resistant varieties are used. Soybeans varieties vary from highly susceptible to resistant (Hussey et al., 1991). Commercially available cotton varieties also have resistance to southern RKN but there are limited varieties available (Robinson et al., 1999).

Corn planted for two years followed by cotton can maintain a comparable population density to that of a cotton monoculture (Kirkpatrick and Sasser, 1984). Corn was once considered a non-host of southern RKN, but it is believed that resistance has since been lost due to a lack of screening in corn breeding lines (Lawrence et al. 2006). Aung et al. (1990) evaluated forty-three open-pollinated corn varieties for resistance to southern RKN, and found that two varieties, "Tebeau" and "Old Raccoon" exhibited the highest levels of resistance with

Oostenbrink (1966) reproduction factor (Rf) values of 0.2 and 0.4, respectively. While this is notable, still no commercial hybrids currently have resistance to southern RKN. Windham and Williams (1987) screened 64 commercial corn hybrids in a greenhouse setting and concluded that all of them were good host to southern RKN. Lawrence et al. (2006) screened ninety-two transgenic and non-transgenic commercial corn hybrids in a greenhouse setting for host suitability to southern RKN, and also found that all hybrids were good hosts to southern RKN. These findings are important to growers because they must be able to manage damaging southern RKN population density by different means. Knowing threshold levels for various crops is important when it comes to managing nematodes. Damage thresholds for nematodes are defined as the maximum number of nematodes that the plant may support without damage. In Georgia, fall sampling thresholds are 50 J2's per 100 cm³ of soil for cotton and corn and 60 J2's per 100 cm³ of soil for soybean (Jagdale 2013). If southern RKN exist above these thresholds, actions such as the implementation of chemical and biological control must be taken to reduce yield loss.

Nematicides are considered the most effective way to manage plant-parasitic nematodes. Some are broad-spectrum toxicants with high volatility promoting dispersal throughout the soil (Chitwood, 2003). Typically, they are non-selective and only nematodes are subject to exposure (Haydock et al., 2006). Nematicides only protect roots from nematodes for up to 30 days after application (Somasundaram et al., 1989). There are four different forms of nematicides registered for use in corn: fumigants, granules, seed treatments, and biologicals. Fumigant products like Vapam[®] (metam sodium) from AMVAC Chemical Corporation, Los

Angeles, CA and Telone II® (1,3-Dichloropropene) from Dow AgroScience, Indianapolis, IN are not as popular in corn as in other crops because of the high costs of product application of the nematicides. Based on University of Georgia's corn production guide, Kemeraite (2016) states that fumigation with Telone II® prior to planting corn can result in better root growth, plant growth, nutrient uptake, and less time to reach reproductive growth stages and maturity, which can lead to an economic increase in yield when significant nematode population density is present. The granular nematicide/insecticide, Counter® 20G (Terbufos) from AMVAC Chemical Corporation, is one of the more popular options for growers due to its dual purpose of protecting against corn rootworms and nematodes. In recent studies in Alabama, Counter® 20G has been shown to increase yields in southern RKN-infested fields by up to 1883 kg/ha (Hagen et al., 2012a; Hagen et al. 2012b; and Lawrence et al. 2009;). Lawrence et al. (2009) found that a 51% decrease in *M. incognita* eggs/g of root with the application of Counter 20G allowed for significant increases in seedling fresh weights and grain yield compared to the untreated control. Lawrence et al., (2010a) found that in a southern RKN infested sandy loam field in Mississippi, Counter® 20G increased yield compared to the untreated control by 2,636 kg/ha, while Telone II® only increased yield by 377 kg/ha. Hagan et al. (2015) found that Counter® 20G reduced southern RKN population density, which led to a 45% increase in seedling fresh weights and a 13.5% increase in grain yield compared to the untreated control.

Seed treatments are becoming more popular in managing nematodes due to environmental concerns and restrictions on pesticide use. Two different seed treatments are available with varying formulations for corn nematode management. Avicta® from Syngenta

Seedcare (Greensboro, NC) is currently advertised as Avicta Complete Corn with Vibrance® (Avicta® + Cruiser® + Vibrance®). Avicta® is the nematicide component with the active ingredient, abamectin; Cruiser® is the insecticide component with the active ingredient, thiamethoxam; and Vibrance® is the fungicide component with the active ingredient sedaxane. Hybrids containing this seed treatment, or other variations of it, are only sold on Syngenta's Northrup King corn hybrids. Results of the efficacy of abamectin have been variable. Lawrence et al. (2009, 2010a, and 2010b) found that the seed treatment abamectin significantly increased yield compared to the untreated control, and were similar to Counter® 20G in two of the three trials; however, significant reductions in southern RKN population density did not occur. Hagan et al. (2015) found that abamectin did not increase yield or reduce southern RKN reproduction as compared to insecticide seed treatment controls. This variability can be attributed to environmental conditions such as soil moisture or soil type (Wheeler et al., 2013).

The second seed treatment is the bionematicide, Poncho®/VOTiVO® (Bayer CropScience, Research Triangle Park, NC). Poncho® is the insecticide component with the active ingredient, clothianidin, and VOTiVO® is the bionematicide component with the active ingredient, *Bacillus firmus*. The bacterium's purpose as advertised by Bayer CropScience is to colonize the root system, thus forming a barrier that limits nematode feeding.

Poncho®/VOTiVO® can be included in the seed treatment package in hybrids of Pioneer and Dekalb. Just as seen with abamectin in Hagan et al. (2015), Poncho®/VOTiVO® seed treatment was not as effective as Counter® 20G in decreasing nematode reproduction measured by Rf values or in increasing grain yield.

Management of the southern RKN in corn can be challenging due to the nematode's wide host range and prevalence in Alabama, as well as a lack of resistant corn hybrids. Other approaches need to be evaluated to create an effective management system for managing southern RKN. It is well understood that nematicidal treatments can offer protection that allows for the development of young susceptible plants (Chitwood, 2003). This protection, along with other growth promoting treatments, could potentially allow for superior early growth of corn and an economical yield gain for producers under risk of damaging southern RKN population levels.

Starter fertilizers

Starter fertilizers are defined as small amounts of nutrients that can be applied at planting in close proximity to the seed (Hergert et al., 2012). Applying fertilizer below the soil surface increases the concentrations of relatively immobile nutrients such as P and K in a small volume (Barber and Kovar, 1985). Placing the nutrients in close proximity to the seed will enable a seedling with a limited root system to obtain nutrients that it otherwise could not (Beegle et al., 2007; Vossenkemper and Shanahan, 2012). The added nutrients will aid in establishing a larger root system, which in turn, will allow the roots to more efficiently scavenge for nutrients already present in the soil. Rapid, early plant growth may help the young plant tolerate diseases and insects, as well as gain a competitive advantage against weeds (Beegle et al., 2007). Success of starter fertilizers in managing pathogenic stresses have been documented in soybean and cotton, but not in corn. Miller (2016) found that in-furrow starter fertilizers in soybeans grown under pathogenic stresses (*Rhizoctonia solani* and *Heterodera glycines*)

significantly increased yield. Luangkhot (2016) also found similar results in cotton where he combined starter fertilizers and the nematicide, Velum Total® (imidacloprid + fluopyram) from Bayer CropScience, Research Triangle Park, NC, to increase cotton yield under *Rotylenchulus reniformis* pressure. Starter fertilizers can vary greatly on the composition of nutrients. They may contain essential macronutrients (N, P, K, and S), micronutrients, or a combination of both. The composition of starter fertilizers depends on what the producer needs based upon a soil test from various public and private soil testing services, nutrient requirements for a certain crop, or special soil and environmental conditions that need to be overcome.

Several studies have shown the benefit of starter fertilizers in improving early plant growth in corn; however, most have concluded that increases in early growth often do not correlate with increased yield (Bermudez and Mallarino, 2002; Kaiser et al., 2005; Mascagni and Boquet, 1996; Vetsch and Randall, 2000). They conclude that there may be many variables that affect starter fertilizers' influence on grain yield such as climate, environment, agronomic practices, and methods of applying the fertilizers. For example, starter fertilizers may provide benefits based on climatic variability and the effect that it has on corn production. Studies have documented the effect of ENSO, an oscillation that occurs every 3 to 7 years between warm and cold phases of sea surface temperature in the Equatorial Pacific, on corn production in the southeast (Mourtzinis et al., 2016; Tian et al., 2015). A local extension publication highlights the effect that ENSO can have on corn production (Woli et al., 2013). The El Niño phase of ENSO can result in lower winter temperatures, higher winter-spring rainfall, and drier and hotter summers than normal in northern and southern parts of Alabama. Wet and cool springs can

affect germination, increase nitrogen and potassium leaching and reduce phosphorus uptake. Cold soils can affect the mineralization of organic N to inorganic N (Cassman and Munns, 1980), as well as the release of solid-phase P to liquid-phase P (Arambarri and Talibudeen, 1959). Drier and warmer summers can impact yield due to water stress and excessive heat during pollination and grain-filling, and earlier planting dates are recommended to avoid this in El Niño years, but early planting may create a less favorable environment for corn germination due to the cooler soil temperatures. Ketcheson (1968) reported that the use of starter fertilizers has the highest propensity to increase grain yields when temperature conditions favor low yields. Starter fertilizers have been shown to advance silking date (Mascagni and Boquet, 1996) which may be beneficial in El Niño years. The La Niña phase causes warmer and drier conditions from fall to spring, and wetter and cooler summers in northern and central parts of Alabama. Warmer winters may result in increased density of southern RKN due to them remaining active for longer periods in the late fall/winter and becoming active earlier in the spring. Starter fertilizers may benefit in La Niña years by increasing early plant growth of corn, thus increasing tolerance to heavy southern RKN population density.

Throughout recent years, there has been a shift in tillage practices with many corn producers shifting from conventional to conservation-tillage systems. However, poor early-season growth and germination in conservation tillage systems are a result of early planting in cold soils (Warrington and Kanemasu, 1983). Reasons for this can be explained by poor aeration in reduced tillage systems causing reduced root growth (Bauder et al., 1981), coupled by the residue acting as an insulator, thus prolonging periods of wet, cold soils (Johnson and

Lowery, 1985). Hindrances of nutrient availability (N and P) in wet, cold soils have been previously discussed, as well as the benefits associated with using starter fertilizers. For these reasons, it can be said that N and P are the most important nutrients in starter fertilizers. Touchton and Karim (1986) found that most grain yield improvements with starter fertilizer are due to N as opposed to P; however, this is dependent upon the environment. Randall and Hoelt (1988) attributed grain increases to P due to high-P requirements for early corn growth. Rehm et al. (1988) suggested that increases in yield and growth could be attributed to starter fertilizers when applied in fields with low soil test P (STP). Conventionally tilled and medium to high STP soils rarely show increases in yield with the use of starter fertilizers (Randall and Hoelt, 1988; Rehm et al., 1988).

Many studies have also looked into different placement methods of starter fertilizers. Starter fertilizers can be applied in a band below and/or beside the seed or in the furrow with the seed (Bermudez and Mallarino, 2002). Placement methods of fertilizer will vary based on the available equipment. Typically producers will apply the fertilizer five cm beside and five cm below the seed (5x5) to prevent toxicity and salt injury to the seed, but this requires special equipment (Mortvedt, 1976; Rehm and Lamb, 2009). Liquid ammonium orthophosphate (11-33-0) or ammonium polyphosphate (10-34-0) has been shown to reduce corn yield in sandy soil due to toxicity to the seed regardless of rate (Walker et al., 1984). In this case, it is more applicable to apply the starters in the 2x2 placement. It can still be practical and economical to apply starters in-furrow with the seed. For example, in a corn-cotton production system which is very common in the mid-South and Southeast, cotton producers will already have in-furrow

equipment for insecticide applications (Mascagni and Boquet, 1996). It has been shown that applying lower rates to the seed can advance silking date, increase grain yield, and decrease grain moisture at harvest (Mascagni and Boquet, 1996). Decreasing grain moisture at harvest may reduce grain drying expenses enough to offset the cost of application (Kaiser et al., 2016). Bates et al. (1966) also reported increased nutrient uptake, faster growth, earlier maturity, and higher corn yields in both greenhouse and field studies using seed-placed fertilizers. Kaiser et al. (2005) compared the effect of broadcast P-K fertilization versus in-furrow P-K starter fertilization on increasing corn yield, early growth, and nutrient uptake. They found that the strategically placed starter P-K fertilizer was able to produce statistically similar yields at four sites in Iowa while only using one-eighth of the P and K used by the broadcast fertilizer, as well as increase early corn growth and P and K uptake at most sites. Increases in early corn growth with starters compared to broadcast fertilization has been observed in other research as well (Touchton, 1988; Vetsch and Randall, 2002).

No research has been performed on the value of starter fertilizers to corn under the threat of plant-parasitic nematodes, more specifically *M. incognita*. As discussed earlier, *M. incognita* has the propensity to decrease root branching due to its symptomatic galling (Taylor and Sasser, 1978). Because the physiological processes involved in gall formation creates a metabolic sink, nutrient imbalances within an affected plant can occur (McClure, 1977). Starter fertilizers are valued for supplying readily available nutrients in close proximity to the seed (Hergert et al., 2012). This can benefit corn germination in cold, wet soils that may occur in the El Niño phase of ENSO, or in a conservation tillage system when planting early. Starter fertilizers

can also hasten the time to maturity, which may shield crucial reproductive stages from environmental stresses (Bates et al., 1966; Mascagni and Boquet, 1996). The benefits that starter fertilizers have from an agronomic perspective may translate to pathological benefits. With documented benefits of increasing early plant growth, starter fertilizers may prove to be an effective technique to managing nematodes in corn due to its multifaceted benefits.

Plant Growth Regulators

Plant hormones are used on over one million hectares worldwide and on a wide range of crops (Thomas, 1982). The majority of use is in high-value horticultural crops to improve quality, but they are used in the agronomic spectrum as well. Plant hormones are defined as a group of naturally occurring organic substances that influence physiological processes at low concentrations (Davies, 1995). These processes include growth and differentiation of plant cells and tissues. Plant hormones can either be produced locally in the tissue, or transported from the site of production to the target area. The fact that they can be transported from one site to another is a unique characteristic and was first observed by Darwin in his studies of phototropism. He was able to deduce that a signal derived from the plant apex was transported to the base of the plant where cell elongation occurred and allowed the stem to bend towards the light source (Gray, 2004). This was the discovery of one of the three major plant growth hormones, auxins.

Auxin is the oldest known plant hormone used in agriculture. Besides phototropism, auxins are responsible for apical dominance. Auxin influences stem elongation, and regulates the activity of meristems, therefore, it is known as the hormone that shapes plant architecture

(Gallavotti, 2013). The endogenous form of auxin is indole acetic acid (IAA) and it is not used in agricultural due to its inclination to be rapidly broken down to inactive products by microorganisms and light (Gianfagna, 1995). Instead, synthetic compounds such as indolebutyric acid (IBA) were created. It was found that IBA can increase root development in the propagation of stem cuttings (Gianfagna, 1995). Oosterhuis and Zhao (1994) found that early root growth is promoted by exogenous applications of IBA and gibberellic acid (GA) to cotton. In their study, they simulated in-furrow field applications in a growth chamber by lining up the pots and spraying down the row with a pressurized CO₂ backpack sprayer. The application increased root length by 47%, root dry weight by 29%, and number of lateral roots per plant by 75%, which resulted in an overall increase in nutrient uptake. IBA is still used for its ability to promote root development and has the potential to be used to mitigate damage caused by plant-parasitic nematodes.

The second major plant growth hormone is cytokinin. Cytokinin was first isolated by Miller and his associates and they named it kinetin because of its ability to produce cytokinesis (Miller et al., 1955). Some of the main functions of cytokinin are stimulated cell division, growth of lateral buds, leaf expansion, and chlorophyll synthesis (Davies, 1995). The stimulated growth and added photosynthetic capacity makes cytokinin a valuable plant growth hormone.

Gibberellic acid (GA) is the third major plant growth hormone, and it similar to cytokinins in that they both promote growth and stem elongation. In seedless grape production GA is used to promote stem growth in grape clusters in to create looser clusters that are less susceptible to disease (Taiz and Zeiger, 2010). GA foliar application to corn has shown an increase in plant

height, but no significant effect has been observed on yield (Alder et al., 1959; Cherry et al., 1960). This, along with its growth promoting advantages, can make GA an effective treatment against plant-parasitic nematodes when applied with a suitable ratio of other plant hormones.

The combination of these three types of plant hormones is quite common in commercially available plant growth regulators (PGR). For example, Ascend® (WinField Solutions LLC, Saint Paul, Minnesota) is sold as a ratio of 0.090% cytokinin, 0.030% gibberellic acid, and 0.045% indolebutyric acid per gallon. Winfield (2017) advertises it to increase plant growth at multiple stages of corn growth such as emergence and vegetative growth stages, and it can be applied as a seed treatment, in-furrow spray, or foliar spray (3 to 10 leaf stage (Ritchie et al., 1986)). Fawcett et al. (2016) performed on-farm growth regulator trials in Iowa to evaluate the performance of popular PGRs. In one trial, Ascend was applied in-furrow to corn and in the other it was foliar applied at the V5 stage. The in-furrow application increased yield by 63 kg/ha (1 bu/A) and the foliar application increased yield by 439 kg/ha (7 bu/A); however, both were not significant increases. They concluded that their research agrees with many others in that growth can be increased, but yield increases are less common. This is similar to reports of starter fertilizer treatments as discussed earlier. Both PGRs and starter fertilizers need to be evaluated separately and together for their ability to increase plant tolerance to plant-parasitic nematodes.

We reviewed three potential agricultural inputs for management of southern RKN in corn. We hypothesized that combining the use of nematicides with starter fertilizers and/or plant growth regulators would provide for a complete management system of southern RKN.

Nematicides would protect young corn plants by mitigating nematode feeding on the roots, while starter fertilizers and plant growth regulators provided rapid, early plant growth. We screened products related to each input for effectiveness in a greenhouse setting for increasing early root and shoot growth and overall plant health while under southern RKN pressure, as well as the products' efficacy in reducing nematode population density. The most effective treatments were integrated into microplot and field trials to assess if increases in early plant growth can provide an economical return on yield when southern RKN is present.

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Chapter 2: Nematicides, Starter Fertilizers, and Plant Growth Regulators Implementation into a Corn Production System.

INTRODUCTION

Meloidogyne incognita (Kofoid and White) Chitwood race 3 is a major pest of southeastern field crops in the United States (Koenning et al., 1999). It is a sedentary endoparasitic nematode that reproduces and feeds on modified living plant cells within plant roots, creating variable sized galls or knots giving it its common name, the root-knot nematode (RKN) (Moens et al., 2009). It survives in a wide array of soil textures, but it is most severe in coarse-textured soil (Van Gundy, 1985). In Alabama, a survey indicated that 7% of the fields sampled (totaling 603 ha) were infested with southern RKN ranging from light to high infestations (Gazaway and McLean, 2003). Southern RKN suppresses yield in three of the state's most economically important crops: cotton, corn, and soybean. In 2016, an estimated 14,200 cotton bales (2% of total yield) was lost costing producers 5.2 million dollars (Lawrence et al. 2016). In corn, yield loss has been observed to be anywhere between 2.2-11.4% when the population density is 100 juveniles/100 cm³ of soil (Bowen et al., 2008). In heavily infested fields, yield losses of up to 30% can occur in corn (Gazaway et al., 1991). In 2016, 2,722 tons of soybeans were lost due to southern RKN in Alabama compared to the 31,056 total tons lost across the southern states (Allen et al., 2016). *Meloidogyne incognita* has a very wide host range of up to 3,000 plant species, ranging from important agronomic and horticultural crops to

various weeds species (Jepson, 1987). This makes deploying cultural management strategies very difficult, including an effective crop rotation system. Of the major field crops grown, peanuts are the only non-host of southern RKN (Taylor and Sasser, 1978). Although feasible, a peanut-corn rotation is not a common practice in Alabama. Soybean-corn and cotton-corn rotations are common in Alabama, and RKN population density will most likely increase unless resistant varieties are used. Corn planted for two years followed by cotton can maintain a comparable population density to that of a cotton monoculture (Kirkpatrick and Sasser, 1984). Corn was once considered a non-host of southern RKN, but it is believed that resistance has since been lost due to a lack of screening in corn breeding lines (Lawrence et al. 2006). Aung et al. (1990) evaluated forty-three open-pollinated corn varieties for resistance to southern RKN, and found that two varieties, “Tebeau” and “Old Raccoon” exhibited the highest levels of resistance with Oostenbrink (1966) reproduction factor (Rf) values of 0.2 and 0.4, respectively. While this is notable, still no commercial hybrids currently have resistance to southern RKN. Management of the southern RKN in corn can be challenging due to its wide host range, lack of resistant hybrids, and prevalence in Alabama. Other approaches need to be evaluated to create an effective management system for managing southern RKN.

Nematicides are considered the most effective way to manage plant-parasitic nematodes. Some are broad-spectrum toxicants with high volatility promoting dispersal throughout the soil (Chitwood, 2003). Typically, they are non-selective and all nematodes are subject to exposure (Haydock et al., 2006). Nematicides only protect roots from nematodes for potentially up to 30 days after application (Somasundaram et al., 1989). There are four

different forms of nematicides registered for use in corn: fumigants, granules, seed treatments, and biologicals. Fumigant products like Vapam® (metam sodium) from AMVAC Chemical Corporation, Los Angeles, CA and Telone II® (1,3-Dichloropropene) from Dow AgroScience, Indianapolis, IN are not as popular in corn as in other crops because of the high costs of application of the nematicides.

The granular nematicide/insecticide, Counter® 20G (Terbufos) from AMVAC Chemical Corporation, is one of the more popular options for growers due to its dual efficacy of protecting against corn rootworms and nematodes. In recent studies in Alabama, Counter® 20G was shown to increase yields in southern RKN-infested fields by up to 1883 kg/ha (Hagen et al., 2012a; Hagen et al. 2012b; and Lawrence et al. 2009;). Hagan et al. (2015) found that Counter® 20G reduced southern RKN population density, which led to a 45% increase in seedling fresh weights and a 13.5% increase in grain yield compared to the untreated control. Lawrence et al. (2009) observed similar increases in seedling fresh weights and grain yield with the use of Counter® 20G. Lawrence et al., (2010a) found that in a southern RKN-infested field in Mississippi, Counter® 20G increased yield as compared to the untreated control by 2,636 kg/ha, while Telone II® only increased yield by 377 kg/ha.

Seed treatments are becoming more popular in managing nematodes due to increased regulations on pesticides. They are typically safer and less harmful to the environment. Two different seed treatments are available with varying formulations for corn nematode management. Avicta® from Syngenta Seedcare (Greensboro, NC) is currently advertised as Avicta Complete Corn with Vibrance® (Avicta® + Cruiser® + Vibrance®). Avicta® is the

nematicide component with the active ingredient, abamectin; Cruiser® is the insecticide component with the active ingredient, thiamethoxam; and Vibrance® is the fungicide component with the active ingredient sedaxane. Hybrids containing this seed treatment, or other variations of it, are only sold on Syngenta's Northrup King corn hybrids. Results of the efficacy of abamectin have been variable. Lawrence et al. (2009, 2010a, and 2010b) found that abamectin significantly increased yield compared to the untreated control, and were similar to Counter® 20G in two of the three trials; however, significant reductions in southern RKN population density did not occur. Hagan et al. (2015) found that abamectin did not increase yield nor reduce southern RKN reproduction as compared to insecticide seed treatment controls. The second seed treatment is the bionematicide, Poncho®/VOTiVO® (Bayer CropScience, Research Triangle Park, NC). Poncho® is the insecticide component with the active ingredient, clothianidin, and VOTiVO® is the bionematicide component with the active ingredient, *Bacillus firmus*. The bacterium's purpose as advertised by Bayer CropScience is to colonize the root system, thus forming a barrier that limits nematode feeding.

Poncho®/VOTiVO® is included in the seed treatment package in hybrids of Pioneer and Dekalb. Just as seen with abamectin in Hagan et al. (2015), Poncho®/VOTiVO® seed treatment was not as effective as Counter® 20G in decreasing nematode reproduction measured by Rf values or in increasing grain yield. The benefits that nematicides have in protecting plants at early growth stages and allowing for yield increases have been well documented, but minimal research has been done on using plant health and growth enhancement products such as starter fertilizers and plant growth regulators as a means to minimize losses from plant-parasitic nematodes.

Starter fertilizers are valued for their ability to apply readily available nutrients in close proximity to the seed (Hergert et al., 2012). Placing the nutrients in close proximity to the seed will enable a seedling with a limited root system to obtain nutrients that were otherwise unavailable (Beegle et al., 2007; Vossenkemper and Shanahan, 2012). Rapid, early plant growth may help the young plant tolerate diseases and insects, as well as gain a competitive advantage against weeds (Beegle et al., 2007).

Several studies have shown the benefits of starter fertilizers in increasing early plant growth, but most concluded that early growth increases often do not correlate with increased yield (Bermudez and Mallarino, 2002; Kaiser et al., 2005; Mascagni and Boquet, 1998; Vetsch and Randall, 2000). Significant growth responses from starter fertilizers are more pronounced under unfavorable environmental conditions such as planting early in cold soils, which may limit germination and nutrient availability in the soil, or when certain agronomic practices are used such as conservation tillage. Ketcheson (1968) reported that the use of starter fertilizers has the highest propensity to increase grain yields when temperature conditions favor low yields. Cold soils can affect the mineralization of organic N to inorganic N (Cassman and Munns, 1980), as well as the release of solid-phase P to liquid-phase P (Arambarri and Talibudeen, 1959). It has been suggested that N and P, not K, are the most important nutrients in starter fertilizers. Touchton and Karim (1986) found that most grain yield improvements with starter fertilizer are due to N as opposed to P; however, this is dependent upon the environment. According to Camberato and Nielson (2017), a rule of thumb is that corn needs 1.0 – 1.2 lbs. of actual nitrogen for each bushel of corn produced. Randall and Hoeft (1988) attributed grain increases

to P due to high-P requirements for early corn growth. Rehm et al. (1988) suggested that increases in yield and growth could be attributed to starter fertilizers when applied in fields with low soil test P (STP). Tillage practices also influence the effectiveness of starter fertilizers. Reduced tillage systems can have poor aeration which leads to reduced root growth (Bauder et al., 1981). Also the residue can act as an insulator which will prolong periods of wet, cold soils (Johnson and Lowery, 1985). Abnormally dry and warm summers can impact yield due to water stress and excessive heat during pollination and grain-filling (Woli et al., 2013). Starter fertilizers can also hasten the time to maturity, which may shield crucial reproductive stages from environmental stresses (Bates et al., 1966; Mascagni and Boquet, 1998).

Starter fertilizers can be applied in a band below and/or beside the seed or in the furrow with the seed (Bermudez and Mallarino, 2002). Placement methods of fertilizer will vary based on the available equipment. Typically producers will apply the fertilizer five cm beside and five cm below the seed (5x5) to prevent toxicity and salt injury to the seed, but this requires special equipment (Mortvedt, 1976; Rehm and Lamb, 2009). It can still be practical to apply starters in-furrow with the seed. For example, in a corn-cotton production system which is very common in the mid-South, cotton producers will already have in-furrow equipment for insecticide applications (Mascagni and Boquet, 1998). Applying starter fertilizers in the seed furrow can advance silking date, increase grain yield, and decrease grain moisture at harvest (Mascagni and Boquet, 1998). Decreasing grain moisture at harvest may reduce grain drying expenses enough to offset the cost of application (Kaiser et al., 2016). Bates et al. (1966) also reported increased nutrient uptake, faster growth, earlier maturity, and higher corn yields in both greenhouse and

field studies using seed-placed fertilizers. Liquid ammonium orthophosphate (11-33-0) or ammonium polyphosphate (10-34-0) has been shown to reduce corn yield in sandy soil due to toxicity to the seed regardless of rate (Walker et al., 1984). In this case, the 5x5 application is needed to avoid injury to the seed.

The benefits that starter fertilizers have from an agronomic perspective may translate to pathological benefits. With documented benefits of increasing early plant growth, starter fertilizers may be an effective technique in managing nematodes in corn. *Meloidogyne incognita* has the propensity to decrease lateral roots of a root system due to its symptomatic galling (Taylor and Sasser, 1978). Because the physiological processes involved in gall formation creates a metabolic sink, nutrient imbalances within an affected plant can occur (McClure, 1977). Starter fertilizers have the potential to help plants overcome side effects that come with *M. incognita* parasitism.

Plant growth regulators have the potential to offer the same type of beneficial growth benefits as starter fertilizers. They are mainly used in high-value horticultural crops to improve quality, but they are used in the agronomic spectrum as well. They contain plant hormones which are defined as a group of naturally occurring organic substances that influence physiological processes at low concentrations (Davies, 1995). The primary plant hormones used for growth stimulation are auxin, gibberellic acid (GA), and cytokinin (Davies, 1995). Auxin influences stem elongation, and regulates the activity of meristems; therefore, it is known as the hormone that shapes plant architecture (Gallavotti, 2013). The endogenous form of auxin is indole acetic acid (IAA) and it is not used in agricultural due to its inclination to be rapidly

broken down to inactive products by microorganisms and light (Gianfagna, 1995). GA promotes growth and stem elongation (Taiz and Zeiger, 2010). Oosterhuis and Zhao (1994) found that early root growth is promoted by exogenous applications of IBA and GA to cotton. In their study, they simulated in-furrow field applications in a growth chamber by lining up the pots and spraying down the row with a pressurized CO₂ backpack sprayer. The application increased root length by 47%, root dry weight by 29%, and number of lateral roots per plant by 75%, which resulted in an overall increase in nutrient uptake. In seedless grape production GA is used to promote stem growth in grape clusters to create looser clusters that are less susceptible to disease (Taiz and Zeiger, 2010). GA foliar application to corn showed an increase in plant height, but no significant effect was observed on yield (Alder et al., 1959; Cherry et al., 1960). Cytokinin stimulates cell division, growth of lateral buds, leaf expansion, and chlorophyll synthesis (Davies, 1995). The stimulated growth and added photosynthetic capacity makes cytokinin a valuable plant growth hormone.

There are many commercial products containing these plant hormones for use in agriculture. A popular product is Ascend® (WinField Solutions LLC, Saint Paul, Minnesota) which is sold as a ratio of 0.090% cytokinin, 0.030% gibberellic acid, and 0.045% indolebutyric acid per gallon. Winfield (2017) advertises it to increase plant growth at multiple stages of corn growth such as emergence and vegetative growth stages, and it can be applied as a seed treatment, in-furrow spray, or foliar spray (3 to 10 leaf stage (Ritchie et al., 1986)). Fawcett et al. (2016) compared multiple plant growth regulators in on-farm trials in Iowa. In one trial, Ascend® was applied to corn in-furrow at planting, and in the other it was foliar applied at the 5-leaf stage.

The in-furrow application increased yield by 63 kg/ha (1 bu/A) and the foliar application increased yield by 439 kg/ha (7 bu/A); however, both were not significant increases. They concluded that their research agrees with many others in that growth can be increased, but yield increases are less common. This is similar to reports of starter fertilizer treatments as discussed earlier. Both PGRs and starter fertilizers need to be evaluated separately and together for their ability to increase plant tolerance to plant-parasitic nematodes.

We reviewed three potential agricultural inputs for management of southern RKN in corn. We hypothesized that combining nematicides with starter fertilizers and/or plant growth regulators would provide a complete management system of southern RKN. Nematicides would protect young corn plants by mitigating the effect of nematode feeding on the roots, while starter fertilizers and plant growth regulators provided rapid, early plant growth. Individual inputs will be screened in a greenhouse setting, and the most effective treatments will be integrated into microplot and field trials to assess if increases in early plant growth can provide an economical return on yield when southern RKN is present.

MATERIALS AND METHODS

Greenhouse Tests 2015

All greenhouse testing was performed at the Plant Science Research Center (PSRC) in Auburn, AL. Field soil used for all tests was acquired from the Plant Breeding Unit (PBU) in Tallahassee, AL. The soil is a Kalmia loamy sand (80% sand, 10% silt, and 10% clay) that was mixed with sand for a (60:40 v/v) mix. The soil mixture was steam pasteurized at 180 °C for 90

minutes, cooled for 24 hours, and steam pasteurized again prior to use. Pre-plant fertilizer and lime were added to the pasteurized soil as recommended by the soil analysis conducted by the Soil, Forage and Water Testing Laboratory of Auburn University. Plastic cone-tainers (983cc D60L Deepots; Stuewe & Sons, Tangent, OR) were used and placed in 30.5 x 37.6 x 23.9 cm D20T support trays. Individual tests were wrapped in a reflective foam board to equalize temperature gradients among cone-tainers. Each cone-tainer was planted with two corn seeds of Mycogen 2H723 (Dow AgroScience, Indianapolis, IN) that were pretreated with insecticide/fungicide seed treatment, CruiserMaxx® Corn 1250 (thiamethoxam @ 1.25 mg a.i./seed + azoxystrobin @ 0.0025 mg a.i./seed + fludioxonil @ 0.0065 mg a.i./seed + mefenoxam @ 0.005 mg a.i./seed + thiabendazole @ 0.05 mg a.i./seed). Seedlings were thinned to one seed per cone-tainer after emergence. Plants were watered as needed to maintain soil moisture between 40% and 60% of the field capacity. Lighting was supplied via 1000-watt halide bulbs producing 110,000 lumens at 14 hours per day and temperatures in the greenhouse ranged from 24°C to 35°C.

Nematode Inoculum

Meloidogyne incognita race 3, originally isolated from an infested field at the Plant Breeding Unit of Auburn University and maintained on corn “Mycogen 2H723” (Dow AgroScience, Indianapolis, IN) in 500 cm³ polystyrene pots in a greenhouse at PSRC, was used as inoculum in the experiments (Xiang et al., 2017). Eggs of *M. incognita* were extracted from corn roots by placing the root systems in a 0.625 % NaOCl solution and shaking the roots for 4 min on a rotary shaker at 120 rpm (Castillo et al., 2013; Hussey and Barker, 1973). Eggs were rinsed

with tap water, collected on a 25- μ m-pore sieve, then processed by sucrose centrifugation-flotation at 240 g-forces for 1 minute (Jenkins, 1964). Eggs were enumerated at 40 \times magnification using an inverted TS100 Nikon microscope and standardized to 2,000 eggs/ml, where 5 ml containing 10,000 eggs were pipetted into each cone-tainer.

Data collection

All experiments were arranged in a random complete block design (RCBD) with five replications. Data were collected at 14 and 45 days after planting (DAP), and each experiment was repeated once. Early plant growth responses were measured 14 DAP. Plant parameters measured included plant height, root fresh weight (RFW), shoot fresh weight (SFW), and biomass (SFW + RFW). At 45 DAP, *M. incognita* population density and early growth parameters were measured. *Meloidogyne incognita* population density was measured by extracting eggs from the corn roots as previously described. Nematode eggs were enumerated at 40 \times magnification using an inverted TS100 Nikon microscope and quantified as total eggs per root and as eggs per gram of RFW.

Nematicide trials

Five nematicides were evaluated for their efficacy in reducing *M. incognita* population density and improving early plant growth. Of those five nematicides, all are registered for use on corn except for ILeVO[®] (Bayer CropScience, Research Triangle Park, NC). ILeVO[®] is currently registered for use on soybean and contains the active ingredient, fluopyram. All nematicides were seed treatments except Counter[®] 20G (AMVAC Chemical Corporation, Los Angeles, CA), which was applied at planting as an in-furrow granule. Avicta[®] 500 FS (Syngenta Crop

Protection, Basel, Switzerland), Poncho®/VOTiVO®, and ILeVO® (Bayer CropScience, Research Triangle Park, NC) were all applied to the seeds in a slurry using a Gustafson laboratory tabletop seed-treater. Seeds were allowed to dry at room temperature for 24 hours before planting. The nematicide treatments and rates used for these trials were as follows: (1) untreated control, (2) Counter® 20G (terbufos: 7.3 kg/ha), (3) Avicta® 500 FS (abamectin: 0.15 mg a.i. /seed), (4) Poncho®/VOTiVO® (clothianidin + *Bacillus firmus*: 79.8 ml/ 80,000 seeds), (5) ILeVO® (fluopyram: 0.15 mg a.i./seed), and (6) Poncho®/VOTiVO® + ILeVO®.

Starter fertilizer trials

Low salt index starter fertilizers were evaluated both alone and in combinations for their ability to mitigate *M. incognita* feeding damage and increase plant biomass. Three fertilizers were from Agroliquid (St. Johns, MI) and 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%). An organic fertilizer alternative, Hydrolyzed Fish Fertilizer (2-4-1) from Neptune's Harvest (Ocean Crest Seafoods Inc., Gloucester, MA) was also evaluated. All fertilizers were applied as simulated in-furrow sprays by pipetting the products into the seed furrow. The starter fertilizer treatments were as follows: (1) untreated control, (2) Sure-K® (9.4 L/ha), (3) Pro-Germinator® (28 L/ha), (4) Micro 500® (2.35 L/ha), (5) Hydrolyzed Fish Fertilizer (28 L/ha), (6) Sure-K® + Pro-Germinator®, (7) Sure-K® + Micro 500®, (8) Pro-Germinator® + Micro 500®, (9) Hydrolyzed Fish Fertilizer + Micro 500®, and (10) Sure-K® + Pro-Germinator® + Micro 500®.

Plant growth regulator trials

Ascend® plant growth regulator (cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%) (WinField Solutions LLC, Saint Paul, Minnesota) was evaluated for the efficacy of different application methods for increasing plant biomass and mitigating *M. incognita* feeding damage. Plant growth regulator treatments were as follows: (1) untreated control, (2) Ascend® seed treatment (ST: 88.7 ml/CWT), (3) Ascend® in-furrow spray (IFS: 365 ml/ha), (4) Ascend® foliar spray at 3 leaf stage (FS: 512 ml/ha), (5) Ascend® ST + IFS, (6) Ascend® ST + FS, (7) Ascend® IFS + FS, (8) Ascend® ST + IFS + FS .

Microplot Tests

Treatments that increased early growth parameters and mitigated *M. incognita* feeding damage in greenhouse trials were selected for evaluation in microplot settings. Microplot trials were conducted in two separate years (2016 and 2017) at PSRC in Auburn, AL. All plots were arranged in a RCBD with five replications. Microplots represented 0.3 m of row in the field. Nematode inoculum of 150 cm³ of soil containing 25,000 eggs and J2 stages was added to each microplot in the planting furrow. All plots were maintained throughout the season with standard herbicide, insecticide, and fertility production practices as recommended by the Alabama Cooperative Extension System.

Microplot 2016

Microplots at PSRC consisted of 26.5-L pots filled with naturally infested *M. incognita* Kalmia loamy sand as previously described. The treatments and their respective rates were as follows: (1) untreated control, (2) Counter® 20G (7.3 kg/ha), (3) ILeVO® ST (0.15 mg a.i./seed), (4) Counter® 20G + Ascend® IFS (365 ml/ha), (5) ILeVO® + Ascend® IFS, (6) Counter® 20G + Pro-

Germinator[®] (28 L/ha) + Sure-K[®] (9.4 L/ha) + Micro 500[®] (2.35 L/ha), (7) ILeVO[®] + Pro-Germinator[®] + Sure-K[®] + Micro 500[®], (8) Counter[®] 20G + Ascend[®] IFS + Pro-Germinator[®] + Sure-K[®] + Micro 500[®], (9) ILeVO[®] + Ascend[®] IFS + Pro-Germinator[®] + Sure-K[®] + Micro 500[®], and (10) Counter[®] 20G + ILeVO[®] + Ascend[®] IFS + Pro-Germinator[®] + Sure-K[®] + Micro 500[®].

Four Mycogen 2C797 (Dow AgroScience, Indianapolis, IN) seeds were planted 2.5 cm deep in the row furrow on 6 Apr. Nematode inoculum was added in the planting furrow as previously described. The nematicide seed treatment, ILeVO[®], was applied to the seeds as previously described. In-furrow liquid preparations were pipetted in the open furrow over top of the seeds at simulated spray output rate of 187 L/ha. Counter[®] 20G granule application was applied over the top of the seeds in the open furrow. Each microplot received water at 30 ml/min by an automated drip irrigation system adjusted throughout the season to run for 15 to 45 min twice a day, for a total of 450 to 1,350 ml of water per microplot per day.

Microplot 2017

Modifications to the treatment list we made in 2017. We eliminated ILeVO[®] to focus on one nematicide, Counter[®] 20G, and added a 5x5 (2 in x 2 in) application of starter fertilizer that allowed for a higher nutrient concentration due to the distance it was placed from the seed. In addition, we wanted to look at the performance of Ascend[®] IFS and all fertilizer treatments both with and without a nematicide. To further assess the treatments' effect on plant health, leaf chlorophyll content was measured with a SPAD 502 chlorophyll meter (Spectrum Technologies, Inc., Aurora, IL).

A new location at PSRC was used for microplot evaluations in 2017. Microplots consisted of clay flue liners (30 cm x 30 cm x 61 cm) in a Marvyn loamy sand (77% sand, 9% silt, 14% clay; 1.1% OM, 6.1 pH). Natural populations of *M. incognita* at planting were 927 J2s per 100 cm³ of soil. Ammonium polyphosphate (APP) (10-34-0) and Urea Ammonium Nitrate Sulfate (UANs) (25-0-0-3) were used for the 5x5 application, and were referred to as “starter mix”. The in-furrow fertilizers from 2016 were used again and were referred to as “pop-ups”. Only Counter[®] 20G was used for the nematicide component. The treatments were as follows: (1) untreated control, (2) Counter[®] 20G (3) pop-ups, (4) starter mix, (5) Ascend[®] IFS, (6) Counter[®] 20G + pop-ups, (7) Counter[®] 20G + starter mix, (8) Counter[®] 20G + Ascend[®] IFS, (9) pop-ups + Ascend[®] IFS, (10) starter mix + Ascend[®] IFS, (11) Counter[®] 20G + pop-ups + Ascend[®] IFS, and (12) Counter[®] 20G + starter mix + Ascend[®] IFS.

Two Mycogen 2C786 seeds per 0.3 meters of row were planted on 21 Apr. Procedures for applying Counter[®] 20G and in-furrow liquid fertilizers were as described from the 2016 microplot experiment. The starter mix was applied 5 cm beside and 5 cm below the seeds by first measuring the appropriate horizontal length away from the seed furrow. Next, a slit in the soil was made by hand with a thin makeshift metal object 7.5 cm deep (2.5 cm extra to account for planting depth) extending the length of the adjacent seed furrow. APP was pipetted in the slit at rate of 72 L/ha (7.7 gal/A) and UANs was applied at 176 L/ha (18.8 gal/A) to simulate a field application of 60 units (lbs/A) of N, 30 units of P₂O₅, and 6 units of S. Each microplot received 0.18 ml of water per day by an automated drip irrigation system adjusted throughout the season to run twice a day for 15 minutes, for a total of 1,893 ml of water per day.

Microplot data collection

One representative corn plant was harvested per microplot at 35 DAP to evaluate early plant growth parameters and *M. incognita* population density as described in the greenhouse trials. *Meloidogyne incognita* eggs were extracted from the root system as previously described. One additional measurement was taken in 2017 (SPAD meter readings). The SPAD meter was calibrated for consistent readings, and the sensor was placed halfway up the uppermost leaf with a fully formed leaf collar between the midrib and the edge of the leaf to standardize measurements and minimize natural variation in greenness that occurs across leaves and plants. Data were an average of two readings per plant on each side of the appropriate leaf's midrib.

Field Tests

Field trials were conducted at two locations: Plant Breeding Unit (PBU) in Tallassee, AL and Brewton Agricultural Research Unit (BARU) in Brewton, AL. Both fields were cropped using a conventional tillage system via disk harrow. Standard fertilizer and herbicide applications were applied to each field as recommended by the Alabama Cooperative Extension System. PBU is naturally infested with *M. incognita* race 3 at a high population density, and the soil at this farm is a Kalmia loamy sand. BARU is naturally infested with *M. incognita* race 3 at a low population density, and the soil is a Benndale fine sandy loam (73% sand, 20% silt, and 7% clay). A John Deere MaxEmerge planter (Moline, Illinois) equipped with Almaco cone planters (Nevada, Iowa) was used for planting at both field sites. Counter[®] 20G granular applications

were made via chemical hoppers attached to the planter. PBU was irrigated with a center pivot, and BARU was irrigated with an overhead sprinkler system as needed.

Field 2016

The same treatment list, seed (Mycogen 2C797), and procedures for treating seed from the 2016 microplot trial were used. PBU and BARU sites were planted to a depth of 2.5 cm at a rate of 8.3 seeds per meter of row (89,660 seeds/ha) on 21 Apr and 7 Apr, respectively. Plots at PBU consisted of two rows; 7.6 m long with 0.9 m row spacing. Plots at BARU consisted of two rows; 5.5 m long with 0.9 m row spacing. In-furrow spray applications were applied at 102.8 L/ha at 40 PSI using 8003 flat fan nozzles at both locations. Plots were harvested on 7 Sep and 26 Aug for PBU and BARU, respectively.

Field 2017

The same treatment list and seed (Mycogen 2C786) from the 2017 microplots were used. PBU and BARU sites were planted at a rate of 6.6 seeds per meter of row (71,729 seeds/ha) on 19 May and 11 Apr, respectively. Planting at PBU was late because the trial was replanted due to poor plant stands. The plot dimensions for each location remained the same as in 2016. Application of starter fertilizer 5 cm below and 5 cm beside (2x2) the seed was made possible with the purchasing of G2 fertilizer discs (Schaffert Manufacturing & Sales, Indianola, NE). A 20 cm single disc opener was mounted on the press wheel brackets of the John Deere planter with Zipper closing wheels. APP was applied at rate of 122 L/ha (13 gal/A) and UANS was applied at 87 L/ha (9.3 gal/A). Forty units of N, 50 units of P₂O₅, and 3 units of S were applied in this mix. In-furrow spray applications were applied at an output rate of 102.8

L/ha and 46.8 L/ha at 40 PSI using 8003 flat fan nozzles for PBU and BARU, respectively. Starter mix (2x2) applications were applied at an output rate of 46.8 L/ha at both locations. Plots were harvested on 29 Aug and 23 Aug for PBU and BARU, respectively.

Field data collection

The same plant growth parameters and procedure for *M. incognita* extractions from the roots were used as described from the microplots trials. Additional measurements included stand counts per length of row per plot and visual vigor ratings on a scale of 1-10 (1 being extremely poor and 10 being extremely good). Data were obtained for measurements by digging two random representative corn plants per plot. The entire plot was machine-harvested for yield and grain moisture content with an Almaco SPC40 plot combine (Nevada, Iowa). Grain yields were adjusted to 15.0% moisture and converted to yield in kg/ha.

In 2016, plots were assessed at 40 DAP and 140 DAP (yield) at PBU and at 45 DAP and 142 DAP (yield) at BARU. In 2017, plots were assessed at 35 DAP and 103 DAP (yield) at PBU and at 31 DAP and 135 DAP (yield) at BARU, with additional measurements included as previously mentioned for the microplots in 2017. Procedures for SPAD meter readings were conducted as previously mentioned, except five representative plants per plot were used in the field.

Data analysis

Data collected from greenhouse, microplot, and field trials were analyzed in SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. Dependent variables included stand count (Stand), plant vigor (Vigor), SPAD 502 Chlorophyll Meter readings (SPAD), plant

height, root fresh weight (RFW), shoot fresh weight (SFW), biomass (RFW + SFW), number of *M. incognita* eggs per total root (eggs/root), *M. incognita* eggs per gram of root (eggs/g of root), moisture content of corn per plot at harvest, and grain yield (kg/ha). Fixed effects were nematicide, starter fertilizer, and PGR treatments, and the random effects included replication and repeat in time. Student panels were generated to determine the normality of the residuals. A log transformation was required for eggs/root and eggs/g of root to satisfy the normal assumptions. LS-means were compared between the treatments and the untreated control by Dunnett's method at significance levels of $P \leq 0.05$ (**) and $P \leq 0.10$ (*). LS-means presented in the tables with different symbols indicate a significant difference.

RESULTS

Greenhouse Testing

Nematicides

Nematicide treatments did not increase early plant growth (plant height, SFW, RFW, biomass) compared to the control at 14 DAP (Table 1). The untreated control had a greater plant height than Poncho®/VOTIVO® ($P \leq 0.05$) as well as a higher RFW and biomass compared to Counter® 20G and Poncho®/VOTIVO® + ILeVO® ($P \leq 0.05$). The later date (45 DAP) allowed for enough time for plant growth benefits due to the nematicides' ability to reduce *M. incognita* population density (Table 1). It is evident that the top half of the roots of the untreated control (Figure 2A) exhibited more visual thinning than the five nematicide treatments (Figure 2B-F) and this was confirmed by a 58% increase in eggs/g of root compared to all nematicide treatments. Plant height was significantly increased compared to the control

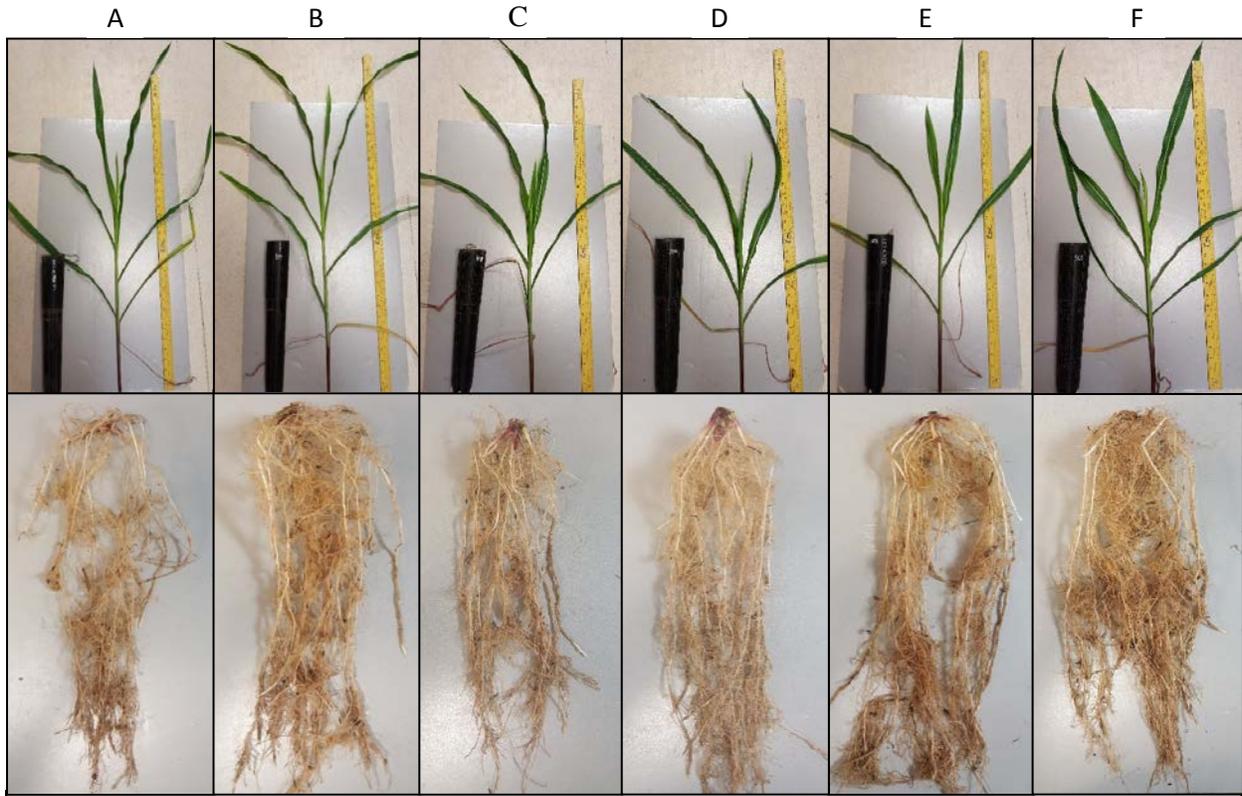


Figure 2: Nematicide greenhouse trial at 45 DAP depicting shoot growth (top) and corresponding root growth (bottom) of corn with 10,000 *Meloidogyne incognita* eggs inoculated at planting. Column A = untreated control; Column B = Counter® 20G; Column C = Avicta® 500FS; Column D = Poncho®/VOTiVO®; Column E = ILeVO®; Column F = Poncho®/VOTiVO® + ILeVO®

with the nematicide treatments, Poncho®/VOTiVO® and Poncho®/VOTiVO® + ILeVO® ($P \leq 0.05$) and with Avicta® 500 FS and ILeVO® ($P \leq 0.10$). Counter® 20G significantly increased plant biomass and decreased eggs/root and eggs/g of root compared to the control ($P \leq 0.05$). Poncho®/VOTiVO® + ILeVO® also decreased eggs/root ($P \leq 0.10$) and eggs/g of root ($P \leq 0.05$), but had no significant effect on plant biomass.

Starter fertilizers

Starter fertilizers and different combinations of those starter fertilizers had no significant effect on early plant growth parameters (plant height, SFW, RFW, biomass)

compared to the untreated control at 14 DAP (Table 2). The micronutrient encompassing fertilizer (Micro 500[®]) decreased plant height ($P \leq 0.05$) and plant biomass ($P \leq 0.10$) compared to the control. Benefits of starter fertilizers to plant growth parameters were seen at 45 DAP (Table 2). All treatments except two, Sure-K[®] + Micro 500[®] and Neptune's Harvest + Micro 500[®], increased plant height compared to the control ($P \leq 0.05$). Plant biomass was significantly increased with the following treatments: Sure-K[®], Pro-Germ[®], Sure-K[®] + Pro-Germ[®], Sure-K[®] + Micro 500[®], Pro-Germ[®] + Micro 500[®], and Sure-K[®] + Pro-Germ[®] + Micro 500[®] ($P \leq 0.05$). No significant increases or decreases in eggs/root were observed; however, Sure-K[®] + Micro 500[®] decreased eggs/g of root compared to the control ($P \leq 0.10$).

Plant growth regulator

Ascend[®] plant growth regulator applications did not increase plant height, RFW, or plant biomass compared to the control at 14 DAP (Table 3). Ascend[®] IFS + FS was the only treatment that increased SFW compared to the control at 14 DAP ($P \leq 0.10$). The triple application (Ascend[®] ST + IFS + FS) decreased RFW at 14 DAP ($P \leq 0.05$). At 45 DAP, only one treatment, Ascend[®] IFS, increased plant biomass compared to the control (Table 3: Figure 3B) ($P \leq 0.05$). A significant increase in eggs/root was observed with Ascend[®] ST + IFS + FS (Figure 3C) ($P \leq 0.10$), but there was no statistical differences in eggs/g of root. Figure 3 shows the difference between the root systems of the untreated control (A), Ascend[®] IFS (B), and Ascend[®] ST + IFS + FS (C), and galled root system of Ascend[®] ST + IFS + FS which supported significantly higher eggs/root than the control.



Figure 3: Ascend® application effect on corn root biomass inoculated with 10,000 *Meloidogyne incognita* eggs per cone in the greenhouse 45 DAP. Picture A = untreated control; Picture B = Ascend®IFS; Picture C = Ascend®ST + IFS + FS; Picture D = magnified root of "Picture C" showing excessive root galling.

The following treatments were chosen to be integrated in to microplot and field trials. For nematicides, Counter® 20G was selected based on its efficacy in decreasing *M. incognita* population density, which allowed for a significant increase in plant biomass. ILeVO® is not registered for use on corn, but it was also selected due to its effectiveness in decreasing nematode population density. Because of the broad spectrum of nutrients encompassed within them and no single fertilizer standing out, the starter fertilizer combination, Sure-K® + Pro-Germ® + Micro 500® was selected for further evaluations. Ascend® IFS was chosen due to its ability to increase plant biomass without increasing nematode egg production, as well as its mixing compatibility with in-furrow starter fertilizers.

Microplot 2016

Treatments were evaluated for increased plant growth and *M. incognita* egg production at 35 DAP (Table 4). No differences in plant height among treatments were observed, but one treatment (Counter[®] 20G + Ascend[®] IFS) significantly increased plant biomass compared to the untreated control ($P \leq 0.10$). ILeVO[®], alone, significantly decreased eggs/root at $P \leq 0.05$, while ILeVO[®] + Ascend[®] IFS, and ILeVO[®] + SF decreased eggs/root at $P \leq 0.10$. *Meloidogyne incognita* eggs/g of root were decreased with ILeVO[®] and ILeVO[®] + SF treatments ($P \leq 0.05$) as well as with Counter[®] 20G and ILeVO[®] + Ascend[®] IFS ($P \leq 0.10$).

Field 2016

Nematicides, growth hormones, and pop-up fertilizer combination effects on early plant growth parameters, harvest moisture content and yield were evaluated at two locations. Plant stand and vigor had no significant differences compared to the control at either location (data were not presented). At PBU, *M. incognita* eggs/g of root ranged from a low of 191 with Counter[®] + Ascend[®] IFS + SF to a high of 2769 with ILeVO[®] + SF; however, there were no significant differences compared to the untreated control (Table 5). Counter[®] + Ascend[®] IFS + SF was the only treatment to significantly increase both plant height and plant biomass compared to the untreated control at 40 DAP ($P \leq 0.05$). No significant increases in grain yield were observed compared to the control, however; numerical increases in grain yield occurred with all treatments containing Counter[®] 20G. Harvest moisture content was significantly decreased compared to the control with ILeVO[®] + Ascend[®] IFS + SF ($P \leq 0.10$).

At BARU, Counter[®] 20G, Counter[®] + SF, and Counter[®] + Ascend[®] IFS + SF all significantly increased plant height compared to the untreated control, with the latter also increasing plant

biomass at 45 DAP ($P \leq 0.05$) (Table 6). Counter[®] + SF also increased plant biomass at ($P \leq 0.10$). ILeVO[®] and ILeVO[®] + SF were the only two treatments that did not decrease *M. incognita* eggs/g of root. Counter[®] + SF and Counter[®] + Ascend[®] + SF significantly increased grain yield compared to the control ($P \leq 0.05$). ILeVO[®] + SF significantly decreased harvest moisture content compared to the control ($P \leq 0.10$).

Microplot 2017

Modifications to the treatment list were made in 2017 and evaluated for early plant growth parameters and *M. incognita* egg production at 35 DAP (Table 7). Plant chlorophyll content increased with Counter[®] + SF, SF + Ascend[®] IFS, and Counter[®] + SF + Ascend[®] IFS treatments compared to untreated control ($P \leq 0.05$). There were no significant differences in plant height or plant biomass compared to the control. Counter[®] + Pop-up + Ascend[®] IFS decreased eggs/root and eggs/g of root compared to control ($P \leq 0.05$). Eggs/g of root also decreased with Counter[®] 20G, alone ($P \leq 0.10$).

Field 2017

Plant stand and vigor had no significant differences in 2017. At PBU, only significant differences in early plant growth at 35 DAP were observed (Table 8). SPAD readings ranged from 38.58 – 42.86 and there were no significant differences in treatments compared to the untreated control. All treatments including the 5x5 fertilizer application (SF) averaged slightly higher SPAD readings than treatments including the in-furrow fertilizer application (Pop-up) (41.55 vs 40.18). Plant height was significantly increased compared to the untreated control at

$P \leq 0.05$ with SF, Counter[®] + SF, SF + Ascend[®] IFS, Counter[®] + Pop-up + Ascend[®] IFS, and Counter[®] + SF + Ascend[®] IFS, and at $P \leq 0.10$ with Counter[®] + Pop-up. Plant biomass was significantly increased compared to the control at $P \leq 0.05$ with SF, Counter[®] + SF, and Counter[®] + SF + Ascend[®] IFS, and at $P \leq 0.10$ with SF + Ascend[®] IFS. Plant height and plant biomass were significantly increased over the untreated control with all treatments containing SF ($P \leq 0.05$; $P \leq 0.10$). Only Counter[®] + SF + Ascend[®] IFS significantly decreased moisture content at harvest ($P \leq 0.05$). There were no significant differences in eggs/g of root or yield compared to the control. The control had the lowest numeric yield, but no significant differences compared to other treatments due to low yields at this location.

At BARU, the starter fertilizer mix (SF) significantly increased plant height compared to the untreated control at 31 DAP ($P \leq 0.10$) (Table 9). Pop-up, SF, Counter[®] + Pop-up, and Counter[®] + SF + Ascend[®] IFS all significantly increased plant biomass compared to the untreated control ($P \leq 0.05$), as well as Counter[®] + SF, Counter[®] + Ascend[®] IFS, Pop-up + Ascend[®] IFS, and Counter[®] + Pop-up + Ascend[®] IFS ($P \leq 0.10$). Pop-up + Ascend[®] IFS significantly decreased eggs/g of root ($P \leq 0.05$) even though no nematicide was used. The only other treatment to decrease eggs/g of root was Counter[®] + Pop-up + Ascend[®] IFS ($P \leq 0.10$). There were no significant differences for SPAD meter readings, harvest moisture content, or yield compared to the untreated control at BARU.

DISCUSSION

Greenhouse

Nematicide

At 14 DAP, corn plants were shorter compared to the untreated control. The later date (45 DAP) allowed for enough time for plant growth benefits due to the nematicides' ability to reduce *M. incognita* population density. Counter® 20G and Poncho®/VOTiVO® + ILeVO® effectively decreased eggs/g of root by 88% and 66%, respectively. Counter® 20G's ability to increase plant biomass and decrease eggs/g of root is supported by Lawrence et al. (2009). They found that Counter® 15G significantly increased root fresh weights, and decreased *M. incognita* eggs/g of root in field studies in Alabama. Evidence of superior performance of Counter® 20G over seed treatment nematicides is supported by Hagan et al. (2015). They found that Counter® 20G was the only nematicide treatment that consistently reduced *M. incognita* juvenile population density, and increased seedling growth.

Starter fertilizer

Just as seen with the nematicide trial, differences in growth were not observed until 45 DAP in starter fertilizer greenhouse trials. Micro 500® and Neptune's Harvest were the only treatments that did not increase plant biomass significantly, and all treatments containing either Pro-Germ® or Sure-K® increased plant biomass compared to the control. Micro 500® was not effective as a stand-alone fertilizer because it does not contain any essential macronutrients. Touchton and Karim (1986) found that increases in early season growth rates in corn were attributed to N-containing fertilizers as opposed to fertilizers not containing N. They found that average plant height was 51 cm when N was not included and 68 cm when it was included. Micro 500® may have more benefits in early season plant growth when combined

with N-containing fertilizers. Prior research supports the ability of starter fertilizers to increase early plant growth (Bermudez and Mallarino, 2002; Kaiser et al., 2005; Touchton and Karim, 1986; Vetsch and Randall, 2000; Vetsch and Randall, 2002); however, these experiments were in conservation tillage and low soil test P soils. Combining starter fertilizers with nematicides has the potential to act as a dual system that both enhances plant health due to stimulated plant growth, and decreases nematode population density, thus giving corn an even greater advantage in a situation with damaging population levels and potentially increasing yield. Luangkhot (2016) found that yield increases occurred in cotton when using Sure-K[®] + Micro 500[®] + Velum Total[®] (imidacloprid + fluopyram) in a heavily infested *Rotylenchulus reniformis* field. Dodge (2017) found that Sure-K[®] + Micro 500[®] + Avicta[®] increased yield over the control by 225 kg/ha in a *M. incognita*-infested soybean field.

Plant Growth Regulator

Once again, just as with nematicides and starter fertilizers, the majority of plant growth regulator treatments did not have a significant effect on early plant growth at 14 DAP; however, at 45 DAP, Ascend[®] IFS significantly increased biomass by 23% over the control. This supports findings by Dodge (2017), where the Ascend[®] IFS application significantly increased soybean biomass by 24% in a high population density of *M. incognita*. Three of the four Ascend[®] application combinations increased *M. incognita* egg production, and the triple combination, Ascend[®] ST + IFS + FS, significantly increased eggs/root. This may be evidence that the oversupply of plant hormones may create ideal feeding sites and rapid reproduction of *M. incognita*. Balasubramaniam and Rangaswami (1962) reported the first evidence in the role

that auxin plays in feeding cell development. They showed that there was an increase in indole compounds in galls induced by *M. incognita*, *M. hapla*, and *M. javanica*. Lohar et al. (2004) transgenically expressed cytokinin oxidase genes to directly regulate cytokinin levels *in planta* and revealed a correlation between the number of feeding sites induced by *Meloidogyne* spp. and the level of cytokinin.

Microplot 2016

The most effective treatment in decreasing eggs/root (98%) and eggs/g of root (97%) was ILeVO®; however, this reduction in nematode population density did not translate to an increase in plant biomass or plant height at 35 DAP. ILeVO® + Ascend® IFS and ILeVO® + SF significantly decreased eggs/root by 83% and 90%, respectively. Research supports ILeVO®'s ability to reduce *M. incognita* population density; however, it was performed in soybean (Jackson et al., 2014). Plant growth was not reduced compared to the control with these treatments due to the addition of Ascend® IFS and SF. A reduction in eggs/root by 62% allowed Counter® + Ascend® IFS to significantly increase plant biomass. Counter® 20G significantly decreased eggs/g of root (83%) but not biomass due to no other inputs being included.

Field 2016

At both locations, Counter® + Ascend® IFS + SF was the most effective treatment at increasing early plant growth; however, only at BARU did a significant decrease in eggs/g of root translate to a significant increase in early plant growth, although a 84% decrease in eggs/g of root was observed at PBU. These findings are supported by Hagan et al. (2015), where a significant reduction in *M. incognita* reproduction with the application of Counter® 20G

translated to a 45% increase in corn seedling fresh weights. At BARU, an increase in early plant growth translated to a significant yield increase that provided a positive net return which is also supported by Hagan et al. (2015). They observed that Counter® 20G significantly increased seedling fresh weights, which resulted in a 13.5% increase in corn yield.

At BARU, *M. incognita* population density was much lower than PBU, and the lower density provided a more dramatic response. All but two treatments, ILeVO® and ILeVO® + SF, significantly decreased eggs/g of root, which contradicts results from the 2016 microplot test where the two treatments significantly decreased eggs/g of root. Ascend® provided contradicting results for increases in early plant growth. At PBU, adding Ascend® to Counter® 20G numerically increased plant height and plant biomass compared to Counter, alone, whereas at BARU the same did not numerically increase plant height or biomass compared to Counter® 20G without Ascend®. This contradicts Alder et al. (1959); Cherry et al. (1960) findings that application of GA to corn increased plant height. They found no relationship between plant height and significant increases in corn yield, which agrees with our findings of Counter® + Ascend® IFS not significantly increasing yield at either location. This is similar to Fawcett et al. (2016), where they observed only a 63 kg/ha increase in yield with the in-furrow application of Ascend®. Adding the in-furrow starter fertilizer to Counter® 20G and Ascend® IFS, significantly increased plant height and biomass over the control at both locations and yield at BARU. This may be explained by Oosterhuis and Zhao (1994) findings that exogenous applications of GA and IBA to cotton in a growth chamber increased root length by 47%, root dry weight by 29%, and number of lateral roots by 75%, which resulted in an overall increase in nutrient uptake.

The addition of starter fertilizers to Ascend[®] enhanced the plants' ability to uptake nutrients, which explained the significant increases in early plant growth. The ability of nematicides, starter fertilizers, and Ascend[®], together, to increase yield under nematode pressure is supported by Dodge (2017) who observed a 14% increase in soybean yield with a similar application, and Luangkhot (2016) who observed a 17% increase in cotton yield with a similar application. Our findings support Dodge (2017) and Luangkhot (2016) findings in that Counter[®] + Ascend[®] IFS + SF increased yield. At PBU, yield was increased by 659 kg/ha, but this was not enough to offset the cost of application with a break-even yield of 1327 kg/ha (Table 13). At BARU, yield was increased by 2,148 kg/ha which provided the highest net return among all treatments (\$113.13/ha). Counter[®] + Ascend[®] IFS was the only treatment that provided a net increase in dollars per hectare at both PBU and BARU (\$2.61/ha and \$54.98/ha, respectively). At BARU, all treatments containing Counter[®] 20G provided a net gain in dollars per hectare. In recent studies in Alabama, Counter[®] 20G increased yields in root-knot-infested fields by up to 1883 kg/ha (Hagen et al., 2012a; Hagen et al. 2012b; and Lawrence et al. 2009;). Only two treatments, ILeVO[®] + Ascend[®] IFS + SF, and ILeVO[®] + SF significantly decreased harvest moisture at either location. Reasons for this may be due to the inclusion of in-furrow starter fertilizer. Various research supports the ability of in-furrow starter fertilizers to decrease grain moisture at harvest (Bullock et al., 1993; Kaiser et al., 2016; Mascagni and Boquet, 1998). ILeVO[®] was not an effective nematicide treatment for corn and was not included in the following growing season.

Microplot 2017

The SPAD 502 meter is a convenient tool to measure relative leaf chlorophyll concentration and subsequent plant N status, and it is commonly used to provide data for timely N-fertilization (Blackmer and Schepers, 1995; Piekielek et al., 1995). It provides rapid assessment without destruction of plant tissue, which is required by tissue nutrient analysis (Blackmer and Schepers, 1995). Three out of four treatments containing the 5x5 starter fertilizer (SF) significantly increased SPAD meter readings compared to the control ($P < 0.05$) at 35 DAP. This is not surprising due to SF containing the highest amount of N (60 units). Schepers et al. (1992) found that SPAD meter readings correlated well with leaf N concentrations. The increase in chlorophyll content did not provide for any significant increases in plant height or plant biomass even though Counter[®] + SF + Ascend[®] IFS increased plant height 11% and plant biomass by 33%. All treatments containing Counter[®] 20G decreased eggs/root by 76% compared to treatments without the nematicide, not including the untreated control. Counter[®] 20G, without any additional inputs, significantly decreased eggs/g of root in both years of microplot experiments (by 83% in 2016 and by 84% in 2017). This is similar to Lawrence et al. (2009), where Counter[®] 15G significantly decreased eggs/g of root by 51% compared to the control.

Field 2017

SPAD readings were not significant and the 5x5 fertilizer application averaged slightly higher than the pop-ups at one location, but not at the other. This does not support Bullock and Anderson (1998) findings that there was a significant correlation with N-fertilizer rate and SPAD

meter readings. At both locations, plant height and plant biomass were significantly increased with the 5x5 fertilizer application, alone. The 5x5 application increased biomass at PBU 47% more than the pop-up fertilizers did, but there were no increases in grain yield. This supports other findings that 5x5 placement of starter fertilizers often increased early plant growth to a greater extent than yield (Bullock et al., 1993; Randall and Hoeft, 1988). Bullock et al. (1993) found that early season dry weight was increased by 15-20% with a 5x5 starter fertilizer application, but there was no effect on final dry weight. They also found lower harvest moisture, but grain yield was not affected. This supports our research in that Counter® + SF + Ascend® IFS significantly decreased moisture content at harvest at PBU. Although pop-up fertilizers can result in early plant growth, they are often not used due to toxicity to the seed, which causes poor seedling emergence and decreased stand (Mortvedt, 1976). Results with pop-up fertilizers are often variable due to differences in soil moisture and soil texture (Randall and Hoeft, 1988). This supports our findings where at BARU, pop-up fertilizers increased plant biomass by an average 4% more than treatments containing 5x5 fertilizers, and they increased biomass over the control by 28%. Counter® 20G did not significantly increase early growth parameters, but the addition of the 5x5 application and Ascend® IFS to Counter®20G provided significant increases in early plant growth. No treatments including Counter® 20G significantly decreased eggs/g of root at either location so differences in early plant growth cannot be attributed to the nematicide's reduction in root-knot population density which contradicts findings by Hagen et al., (2015); Lawrence et al., (2009). Significant increases in early plant growth were attributed to the application of starter fertilizers and Ascend®. No treatment

containing Counter® 20G, alone, increased plant height or plant biomass at either location, whereas the 5x5 application, alone, increased both growth parameters at both locations and the pop-up application, alone, increased both growth parameters at BARU. This contradicts findings by Dodge (2017) and Luangkhot (2016) who both saw significant increases in early plant growth in treatments containing just the nematicide without additional inputs. Reasons for no significant effects on yield can be attributed to environmental conditions. At PBU, the late planting due to poor stands from the first planting (Figure 4) resulted in poor yields. Usual planting dates for corn in Alabama are from Mar 25 – Apr 25 (USDA, 2010). Planting corn this late with a medium maturity hybrid (Mycogen 2C786) increased risk of high temperatures during crucial reproductive stages (Woli et al., 2013). According to Neild and Newman (2017), corn usually begins to stress when temperatures exceed 32 °C (90 °F) during tasseling-silking and grainfill stages. At 6 weeks after plant emergence, daily maximum temperatures exceed 32 °C from Jul 2 – Aug 1 (Figure 8). Six weeks after plant emergence is when the 12th leaf is fully emerged and when the number of ovules are determined on the major (top) ear (Ritchie et al., 1986). In the following weeks, crucial reproductive stages were initiated, and the high temperatures during this time affected final yield. At BARU, Only one treatment, Counter® 20G, had a higher yield (1 kg/ha) than the control. This is due to variations within the field and the favorable location of the control (Figure 5). These variations can be attributed to poor drainage leading to excess moisture on the bottom side of the field.



Figure 4: Picture of the first planting at PBU in 2017, 29 DAP. This shows evidence of poor germination believed to be caused by excess moisture following planting. This extends from the first replication to the fifth, causing the trial to be replanted in a different location on the farm.



Figure 5: Drone picture at BARU 67 DAP showing field variation and poor corn growth on the bottom side of the field relative to the rest of the field. The right side of the red arrow represents the 2-row, 4-rep, 12-treatment test (6 plots long, 8 plots wide) with the first rep planted in the first 4 rows. Reps are stacked vertically instead of horizontally to fit the trial in the field. Plots were randomized on a 12 plot long, 4 plot wide basis, and the actual layout of the trial was not known until planting occurred making the randomization off. The untreated control (stars) were primarily in the back half of the field where plants appear the healthiest, which may explain the high value of yield in the control.

CONCLUSION

Results from field trials were greatly influenced by environmental conditions at the two locations over two years (Figure 5-8). It can be concluded from this two-year study that adding starter fertilizers regardless of application type and/or Ascend® PGR to Counter® 20G can greatly increase early plant growth in *M. incognita*-infested fields; however, yield increases did not always occur. Only in one trial, BARU 2016, did combinations of a nematicide, plant growth regulator, and starter fertilizer significantly increase yield. In 2016, the increases in early corn growth translated to a significant yield increase (2,148 kg/ha more than the control) with Counter® + Ascend® IFS + SF, and this occurred at the location with a lower root-knot

population density. Here, a net gain of \$113.13/ha occurred. At the higher root-knot population density, this treatment did not provide a positive net return. Counter[®] + Ascend[®] IFS was the only treatment that provided a positive net return at both locations (\$2.61/ha and \$54.98/ha, respectively), although only numeric increases in early plant growth occurred. Additionally, Counter[®] 20G proved to be an effective nematicide option. In microplot trials, all treatments containing Counter[®] 20G decreased root-knot eggs/g of root by an average of 84%. At BARU in 2016, all treatments containing Counter[®] 20G decreased eggs/g of root by 91% and provided a net gain in dollars per hectare. Unforeseen circumstances in 2017 trials made the benefits of treatments on yield difficult to assess; however, early plant growth benefits were observed with some of the treatments. The 5x5 starter fertilizer performed dramatically better than the pop-up application at PBU, increasing biomass by an average of 76% over the control as opposed to 20% by the pop-ups; however, pop-ups increased biomass by an average of 4% more than the 2x2 application at BARU. There are not many options for corn growers when it comes to managing nematodes, but our integrated system shows the potential for economic gains during times where corn prices are low by utilizing corn's high nutrient requirements and effective nematicides to minimize poor early season growth. In conclusion, our hypothesis that combining nematicides with starter fertilizers and/or plant growth regulators would provide a complete management system for root-knot nematodes in corn by increasing early plant growth and ultimately yield, was not consistently observed; however, our findings suggest that there is potential for these inputs to provide a cost-effective management strategy.

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Table 1. Nematicide effect on corn plant height, shoot and root fresh weight, plant biomass, and *Meloidogyne incognita* eggs per root, and eggs per gram of root in greenhouse trials

Treatment	Rate	14 Days after planting				45 Days after planting			
		Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Plant biomass ^z (g)	Plant height (cm)	Plant biomass (g)	<i>Meloidogyne incognita</i> ^y Eggs/root	Eggs/g of root
Control	---	28.8 ^x	2.01	1.61	3.63	46.1	25.86	22230	2065
Counter [®] 20G (terbufos)	7.3 kg/ha	25.4	1.71*	1.30**	3.01 **	50.0	30.54**	3370**	251 **
Avicta [®] 500 FS (abamectin)	0.15 mg a.i./seed	25.8	1.96	1.48	3.44	51.7*	28.53	13331	1340
Poncho [®] /VOTIVO [®] (clothianidin + Bacillus firmus)	79.8 ml/80,000 seeds	25.4**	1.84	1.42	3.26	54.8**	28.01	9928	875
ILeVO [®] (fluopyram)	0.15 mg a.i./seed	27.9	2.05	1.40 *	3.46	52.0*	28.02	4054	489
Poncho [®] /VOTIVO [®] + ILeVO [®] (clothianidin + Bacillus firmus + fluopyram)	79.8 ml/80,000 seeds + 0.15 mg a.i./seed	26.0	1.76*	1.29 **	3.05 **	54.0**	29.03	8090 *	692 **

^zPlant biomass is calculated by the sum of shoot and root fresh weights

^y*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

*Means in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 2. Starter fertilizer effect on corn plant height, shoot and root fresh weight, plant biomass, and *Meloidogyne incognita* eggs per root, and eggs per gram of root in greenhouse trials

Treatment	Rate	14 Days after planting				45 Days after planting			
		Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Plant biomass ² (g)	Plant height (cm)	Plant biomass (g)	<i>Meloidogyne incognita</i> ^y	
								Eggs/root	Eggs/g of root
Control	---	32.6 ^x	2.28	1.81	4.09	76.8	66.23	174392	7917
Sure-K [®]	9.4 L/ha	29.8	2.19	1.88	4.08	87.1 ^{**}	80.45 ^{**}	204249	9178
Pro-Germ [®]	28 L/ha	32.9	2.64	1.97	4.60	88.0 ^{**}	81.37 ^{**}	133614	5183
Micro 500 [®]	2.35 L/ha	25.7 ^{**}	1.83	1.49	3.32 [*]	88.9 ^{**}	72.23	105524	4313
Neptune's Harvest	28 L/ha	29.9	2.46	1.76	4.22	88.2 ^{**}	74.30	130707	5211
Sure-K [®] + Pro-Germ [®]	9.4 L/ha + 28 L/ha	31.3	2.14	1.61	3.75	87.0 ^{**}	80.44 ^{**}	140962	5234
Sure-K [®] + Micro 500 [®]	9.4 L/ha + 2.35 L/ha	31.2	2.09	1.68	3.77	80.3	80.15 ^{**}	79027	2866 [*]
Pro-Germ [®] + Micro 500 [®]	28 L/ha + 2.35 L/ha	35.2	2.63	1.76	4.38	87.1 ^{**}	77.44 ^{**}	295984	13593
Neptune's Harvest + Micro 500 [®]	28 L/ha + 2.35 L/ha	29.4	2.11	1.61	3.71	81.8	75.59 [*]	162244	7860
Sure-K [®] + Pro-Germ [®] + Micro 500 [®]	9.4 L/ha + 28 L/ha + 2.35 L/ha	33.1	2.50	1.62	4.12	86.4 ^{**}	77.97 ^{**}	89552	3715

²Plant biomass is calculated by the sum of shoot and root fresh weights

^y*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^{*}Means in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 3. Plant growth regulator effects on corn plant height, shoot and root fresh weight, plant biomass, and *Meloidogyne incognita* eggs per root, and eggs per gram of root in greenhouse trials

Treatment	Rate	14 Days after planting				45 Days after planting			
		Plant height (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Plant biomass ^z (g)	Plant height (cm)	Plant biomass (g)	<i>Meloidogyne incognita</i> ^x	
								Eggs/root	Eggs/g of root
Control	---	29.9 ^w	2.15	2.08	4.23	75.9	51.96	96138	5829
Ascend® ST ^y	88.7 ml/cwt	31.3	2.34	2.16	4.50	79.9	57.80	81344	4470
Ascend® IFS	365 ml/ha	30.2	2.36	2.09	4.46	77.9	63.86 ^{**}	85099	4683
Ascend® FS	512 ml/ha	31.5	2.45	1.91	4.36	75.5	54.78	174508	10887
Ascend® ST + IFS	88.7 ml/cwt + 365 ml/ha	31.0	2.20	1.90	4.10	71.7	50.18	68037	5656
Ascend® ST + FS	88.7 ml/cwt + 512 ml/ha	29.1	2.33	1.73 [*]	4.06	75.5	57.05	125400	9622
Ascend® IFS + FS	365 ml/ha + 512 ml/ha	31.1	2.52 [*]	1.98	4.50	79.9	55.78	153641	12865
Ascend® ST + IFS + FS	88.7 ml/cwt + 365 ml/ha + 512 ml/ha	30.0	2.34	1.68 ^{**}	4.02	80.4	58.80	194446 [*]	13277

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yAscend® contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%. Ascend® ST was applied as a seed treatment; IFS was an in-furrow spray; and FS was a foliar spray applied at the 3-leaf stage.

^x*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^wData represents LS-means, and means in the same column followed by * P < 0.10; ** P < 0.05 are significantly different compared to the control according to Dunnett's P values

Table 4. The effect of nematicide, starter fertilizer, and plant growth regulator combinations on corn plant height, plant biomass, and *Meloidogyne incognita* eggs per root and eggs per gram of root at 35 days after planting (DAP) in the microplot at PSRC 2016

Treatment	Rate	Plant height (cm)	Plant biomass ^z (g)	<i>Meloidogyne incognita</i> ^w	
				Eggs/root	Eggs/g of root
Control	---	74.2 ^y	53.06	3433	286
Counter [®] 20G (terbufos)	7.3 kg/ha	72.8	65.60	816	50*
ILeVO [®] (fluopyram)	0.15 mg ai/seed	72.2	45.86	61**	9**
Counter [®] + Ascend [®] IFS ^y	7.3 kg/ha + 365 ml/ha	74.9	70.24*	1288	71
ILeVO [®] + Ascend [®] IFS	0.15 mg ai/seed + 365 ml/ha	71.0	61.22	574*	23*
Counter [®] + SF ^x	7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	69.7	56.48	1486	141
ILeVO [®] + SF	0.15 mg ai/seed + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	72.2	57.38	332*	21**
Counter [®] + Ascend [®] IFS + SF	7.3 kg/ha + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	73.8	63.58	1900	124
ILeVO [®] + Ascend [®] IFS + SF	0.15 mg ai/seed + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	71.0	51.72	757	59
Counter [®] + ILeVO [®] + Ascend [®] IFS + SF	7.3 kg/ha + 0.15 mg ai/seed + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	72.9	59.08	6718	563

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yAscend[®] contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%; IFS was an in-furrow spray

^xSF was a starter fertilizer mix containing 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%)

^w*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^yData represents LS-means, and means in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 5. The effect of nematicide, starter fertilizer, and plant growth regulator combinations on corn plant height, plant biomass, *Meloidogyne incognita* eggs per gram of root, harvest moisture, and yield at PBU 2016

Treatment	Rate	40 Days after planting			140 Days after planting	
		Plant height (cm)	Plant biomass ^z (g)	<i>M. incognita</i> ^w eggs/g of root	Moisture content (%)	Yield (kg/ha)
Control	---	85.9	150.64	1188	14.52	10673
Counter [®] 20G (terbufos)	7.3 kg/ha	83.5	153.65	1062	14.39	10793
ILeVO [®] (fluopyram)	0.15 mg ai/seed	84.3	127.90	2436	14.31	9791*
Counter [®] + Ascend [®] IFS ^y	7.3 kg/ha + 365 ml/ha	88.1	181.61	481	14.16	11212
ILeVO [®] + Ascend [®] IFS	0.15 mg ai/seed + 365 ml/ha	80.7	127.12	226	14.33	9892
Counter [®] + SF ^x	7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	90.9	179.39	895	14.40	10746
ILeVO [®] + SF	0.15 mg ai/seed + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	84.3	156.61	2769	14.46	10961
Counter [®] + Ascend [®] IFS + SF	7.3 kg/ha + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	97.8**	220.02**	191	14.29	11332
ILeVO [®] + Ascend [®] IFS + SF	0.15 mg ai/seed + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	89.0	182.23	2061	14.15*	10467
Counter [®] + ILeVO [®] + Ascend [®] IFS + SF	0.15 mg ai/seed + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	89.9	180.36	402	14.26	10613

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yAscend[®] contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%; IFS was an in-furrow spray

^xSF was a starter fertilizer mix containing 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%)

^w*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^yMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 6. The effect of nematicide, starter fertilizer, and plant growth regulator combinations on corn plant height, plant biomass, *Meloidogyne incognita* eggs per gram of root, harvest moisture, and yield at BARU 2016

Treatment	Rate	45 Days after planting			142 Days after planting	
		Plant height (cm)	Plant biomass ^z (g)	<i>M. incognita</i> ^w eggs/g of root	Moisture content (%)	Yield (kg/ha)
Control	---	90.0	222.88	25	15.65	7159
Counter® 20G (terbufos)	7.3 kg/ha	99.0**	249.45	2**	15.67	8009
ILeVO® (fluopyram)	0.15 mg ai/seed	86.1	165.14	12	14.91	6830
Counter® + Ascend® IFS ^y	7.3 kg/ha + 365 ml/ha	93.4	243.33	3*	16.59	8078
ILeVO® + Ascend® IFS	0.15 mg ai/seed + 365 ml/ha	89.8	203.11	3*	16.44	7252
Counter® + SF ^x	7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	102.4**	297.57*	1**	15.20	8883**
ILeVO® + SF	0.15 mg ai/seed + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	85.3	217.04	8	14.58*	6932
Counter® + Ascend® IFS + SF	7.3 kg/ha + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	104.2**	376.12**	3**	15.86	9307**
ILeVO® + Ascend® IFS + SF	0.15 mg ai/seed + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	88.7	236.19	5*	15.32	6941
Counter® + ILeVO® + Ascend® IFS + SF	7.3 kg/ha + 0.15 mg ai/seed + 365 ml/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	87.4	185.14	2**	15.12	6938

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yAscend® contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%; IFS was an in-furrow spray

^xSF was a starter fertilizer mix containing 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%)

^w*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^vMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 7. The effect of nematicide, starter fertilizer, and plant growth regulator combinations on corn leaf chlorophyll content, plant height, plant biomass, *Meloidogyne incognita* total eggs per root, and eggs per gram of root at 35 DAP in the microplot at PSRC 2017

Treatment	Rate	SPAD ^u	Plant height (cm)	Plant biomass ^z (g)	<i>Meloidogyne incognita</i> ^v	
					Eggs/root	Eggs/g of root
Control	---	40.60 ^t	93.2	141.38	3162	158
Counter [®] 20G (terbufos)	7.3 kg/ha 9.4 L/ha + 28 L/ha + 2.35 L/ha	45.02	94.2	149.77	582	25*
Pop-up ^y	72 L/ha + 176 L/ha	44.28	99.4	170.01	2060	107
SF ^x	L/ha	44.80	96.6	167.86	3986	216
Ascend [®] IFS ^w	365 ml/ha 7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	43.22	95.2	137.24	2027	84
Counter [®] + Pop-up	7.3 kg/ha + (72 L/ha + 176 L/ha)	40.18	98.1	165.85	171	13
Counter [®] + SF	7.3 kg/ha + 365 ml/ha	46.94**	94.4	161.05	626	43
Counter [®] + Ascend [®] IFS	365 ml/ha (9.4 L/ha + 28 L/ha + 2.35 L/ha) + 365 ml/ha	43.58	89.2	121.14	1542	106
Pop-up + Ascend [®] IFS	72 L/ha + 176 L/ha + 365 ml/ha	42.38	97.8	187.69	3046	323
SF + Ascend [®] IFS	176 L/ha + 365 ml/ha 7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha) + 365 ml/ha	48.04**	93.2	140.19	533	27
Counter [®] + Pop-up + Ascend [®] IFS	7.3 kg/ha + (72 L/ha + 176 L/ha) + 365 ml/ha	39.94	97.4	186.32	61**	5**
Counter [®] + SF + Ascend [®] IFS	176 L/ha + 365 ml/ha	48.08**	103.0	187.64	404	37

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yPop-up was a fertilizer mix applied in the seed furrow containing 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%)

^xSF was a fertilizer mix applied "5x5" from the seed furrow containing APP (10-34-0) and UANS (25-0-0-3)

^wAscend[®] contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%; IFS was an in-furrow spray

^v*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^uSPAD were readings of chlorophyll content measure by the SPAD 502 chlorophyll meter (Spectrum Technologies)

^tMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 8. The effect of nematicide, starter fertilizer, and plant growth regulator combinations on corn leaf chlorophyll content, plant height, plant biomass, *Meloidogyne incognita* eggs per gram of root, harvest moisture, and yield at PBU 2017

Treatment	Rate	SPAD ^u	35 Days after planting		103 Days after planting		
			Plant height (cm)	Plant biomass ^z (g)	<i>M. incognita</i> eggs/g of root ^v	Moisture content (%)	Yield (kg/ha)
Control	---	40.86 ^t	75.20	94.97	991	20.38	1794
Counter [®] 20G (terbufos)	7.3 kg/ha 9.4 L/ha + 28 L/ha + 2.35 L/ha	42.86	77.80	105.15	1222	19.97	2134
Pop-up ^y	72 L/ha + 176 L/ha	40.02	75.20	108.10	282	19.42	2067
SF ^x	L/ha	42.36	90.00**	168.31**	411	18.51	2128
Ascend [®] IFS ^w	365 ml/ha	39.98	81.70	115.49	400	19.84	1942
Counter [®] + Pop-up	7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha)	39.80	83.40*	119.54	787	19.30	1868
Counter [®] + SF	7.3 kg/ha + (72 L/ha + 176 L/ha)	42.00	92.50**	201.03**	492	19.28	2001
Counter [®] + Ascend [®] IFS	7.3 kg/ha + 365 ml/ha	40.82	81.40	108.15	441	19.18	1862
Pop-up + Ascend [®] IFS	(9.4 L/ha + 28 L/ha + 2.35 L/ha) + 365 ml/ha	38.58	78.90	97.12	555	19.77	1797
SF + Ascend [®] IFS	(72 L/ha + 176 L/ha) + 365 ml/ha	40.12	85.30**	135.56*	550	19.55	1960
Counter [®] + Pop-up + Ascend [®] IFS	7.3 kg/ha + (9.4 L/ha + 28 L/ha + 2.35 L/ha) + 365 ml/ha	42.30	85.80**	130.83	906	19.24	1855
Counter [®] + SF + Ascend [®] IFS	7.3 kg/ha + (72 L/ha + 176 L/ha) + 365 ml/ha	41.72	90.90**	164.24**	1111	17.89**	1862

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yPop-up was a fertilizer mix applied in the seed furrow containing 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%)

^xSF was a fertilizer mix applied "5x5" from the seed furrow containing APP (10-34-0) and UANS (25-0-0-3)

^wAscend[®] contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%; IFS was an in-furrow spray

^v*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^uSPAD were readings of chlorophyll content measure by the SPAD 502 chlorophyll meter (Spectrum Technologies)

^tMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 9. The effect of nematicide, starter fertilizer, and plant growth regulator combinations on corn leaf chlorophyll content, plant height, plant biomass, *Meloidogyne incognita* eggs per gram of root, harvest moisture, and yield at BARU 2017

Treatment	Rate	SPAD ^u	31 Days after planting		135 Days after planting		
			Plant height (cm)	Plant biomass ^z (g)	<i>M. incognita</i> eggs/g of root ^v	Moisture content (%)	Yield (kg/ha)
Control	---	34.72 ^t	66.88	99.81	63	20.00	8223
Counter [®] 20G (terbufos)	7.3 kg/ha + 9.4 L/ha + 28 L/ha +	36.40	66.50	113.92	10	19.18	8224
Pop-up ^y	2.35 L/ha + 72 L/ha +	33.55	70.38	125.97**	41	19.46	6792
SF ^x	176 L/ha	34.30	71.13*	134.08**	58	18.89	8219
Ascend [®] IFS ^w	365 ml/ha + 7.3 kg/ha + (9.4 L/ha +	35.38	69.00	116.96	169	19.34	7709
Counter [®] + Pop-up	28 L/ha + 2.35 L/ha)	36.43	67.13	137.90**	82	20.09	7086
Counter [®] + SF	7.3 kg/ha + 176 L/ha)	37.85	68.38	121.72*	43	18.75	8093
Counter [®] + Ascend [®] IFS	365 ml/ha + (9.4 L/ha +	35.75	69.13	122.84*	9	19.22	7825
Pop-up + Ascend [®] IFS	28 L/ha + 2.35 L/ha) +	35.08	68.38	123.70*	1**	19.46	7256
SF + Ascend [®] IFS	365 ml/ha + (72 L/ha +	33.00	68.25	111.63	84	19.43	7476
Counter [®] + Pop-up + Ascend [®] IFS	176 L/ha) + 365 ml/ha +	38.00	67.00	123.90*	3*	18.82	7026
Counter [®] + SF + Ascend [®] IFS	7.3 kg/ha + (9.4 L/ha +	34.38	68.25	125.26**	16	18.62	6525
	28 L/ha + 2.35 L/ha) +						
	365 ml/ha + (72 L/ha +						
	176 L/ha) +						
	365 ml/ha						

^zPlant biomass was calculated by the sum of shoot and root fresh weights

^yPop-up was a fertilizer mix applied in the seed furrow containing 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%)

^xSF was a fertilizer mix applied "5x5" from the seed furrow containing APP (10-34-0) and UANS (25-0-0-3)

^wAscend[®] contains cytokinin 0.090%, gibberellic acid 0.03% and indole butyric acid 0.045%; IFS was an in-furrow spray

^v*Meloidogyne incognita* eggs were log transformed to satisfy model assumptions

^uSPAD were readings of chlorophyll content measure by the SPAD 502 chlorophyll meter (Spectrum Technologies)

^tMeans in the same column followed by * $P < 0.10$; ** $P < 0.05$ are significantly different compared to the control according to Dunnett's P values

Table 10. The economic return on corn yield compared to the untreated control based on cost of application of treatments containing Counter® 20G

Treatment	Cost of Application ^z (\$/ha)	Break even Yield ^y (kg/ha)	PBU 2016		BARU 2016	
			Yield above control (kg/ha)	Net gain/loss per hectare ^x (\$/ha)	Yield above control (kg/ha)	Net gain/loss per hectare (\$/ha)
Counter® 20G	53.35	387	120	(36.79)	850	63.80
Counter® + Ascend® IFS	71.68	520	539	2.61	919	54.98
Counter® + SF	164.55	1194	73	(154.46)	1724	73.03
Counter® + Ascend® IFS + SF	182.88	1327	659	(92.04)	2148	113.13

^zCost of application was based on corresponding rates applied in-furrow to 0.91 m row widths

^yBreak even yield was calculated at a corn price of \$137.79/t

^xNet gain/loss per hectare was calculated by the following: (Yield above control – Break even yield) * corn price

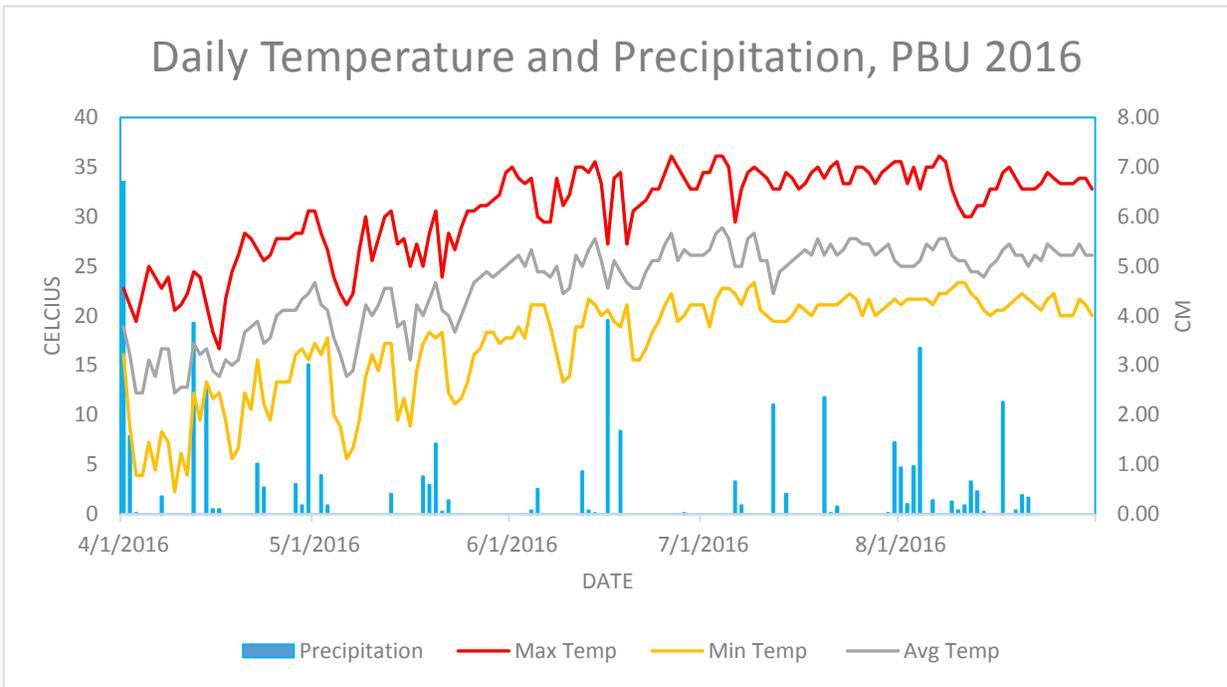


Figure 6: Daily temperature and precipitation at PBU, 2016. Data were retrieved from AWIS Weather Services, reported by Alabama Cooperative Extension System, Auburn University.

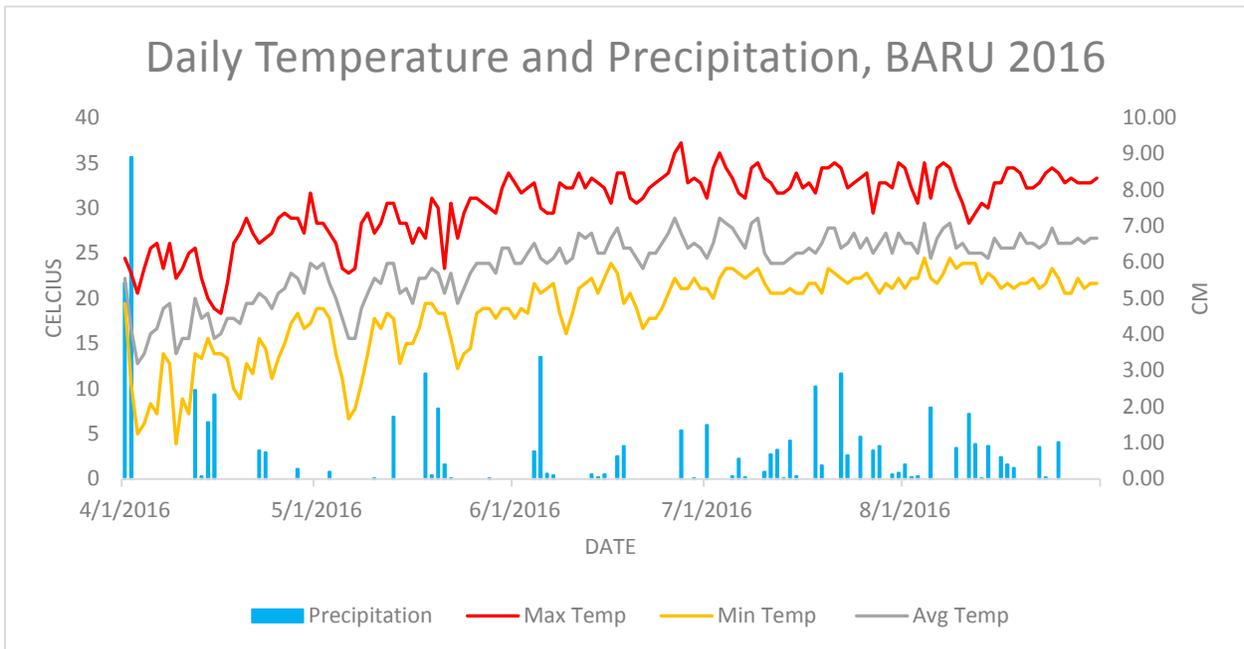


Figure 7: Daily temperature and precipitation at BARU, 2016. Data were retrieved from AWIS Weather Services, reported by Alabama Cooperative Extension System, Auburn University.

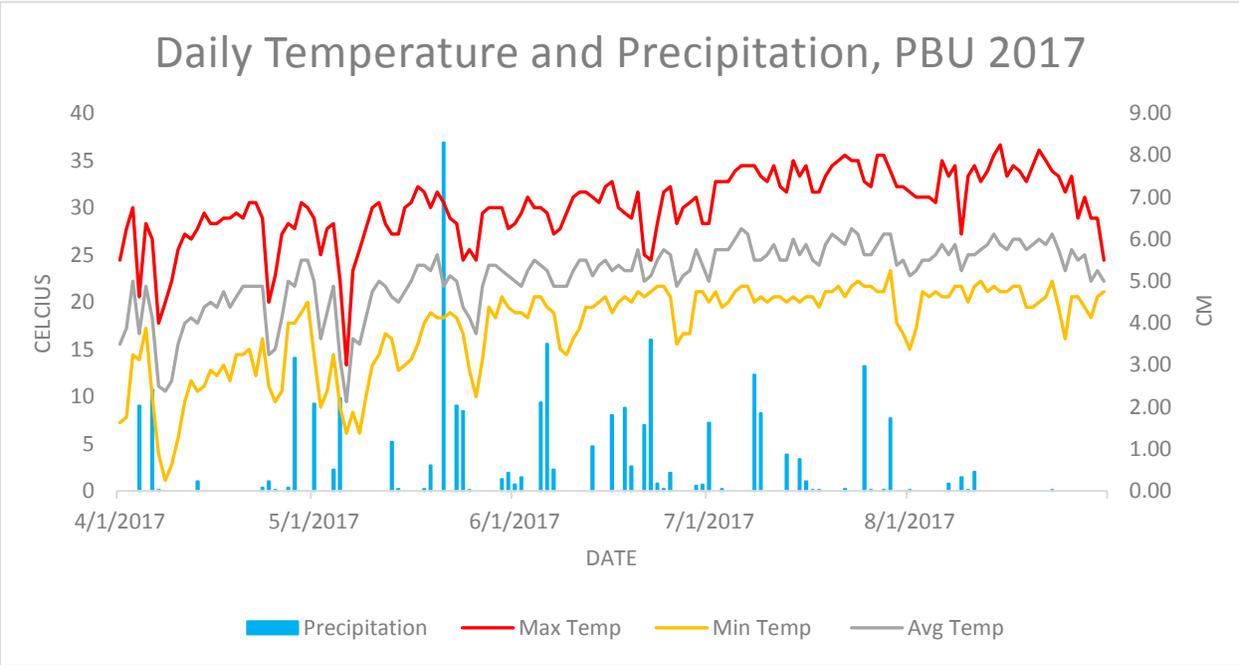


Figure 8: Daily temperature and precipitation at PBU, 2017. Data were retrieved from AWIS Weather Services, reported by Alabama Cooperative Extension System, Auburn University.

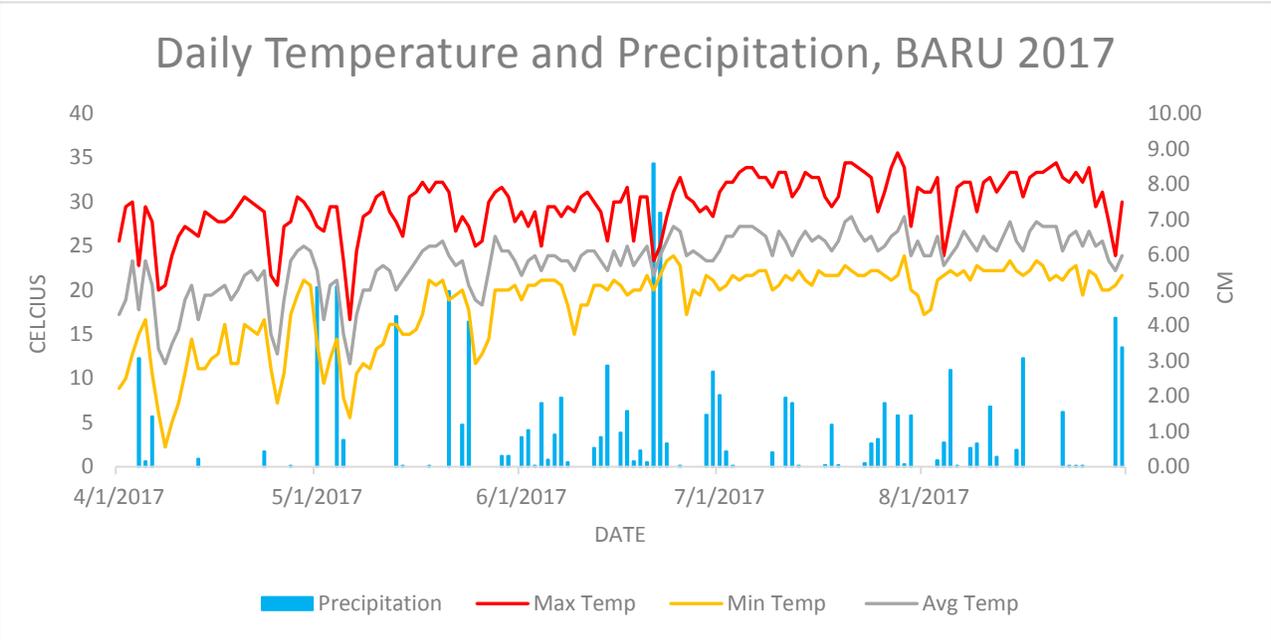


Figure 9: Daily temperature and precipitation at BARU, 2017. Data were retrieved from AWIS Weather Services, reported by Alabama Cooperative Extension System, Auburn University.