Management of *Rotylenchulus reniformis* and *Meloidogyne incognita* on *Gossypium hirsutum* in Alabama and the economic impact of additional fertilizer and nematicide applications

by

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Abstract

The reniform nematode, *Rotylenchulus reniformis* and the southern root-knot nematode *Meloidogyne incognita* are some of the most damaging plant parasitic nematodes on upland cotton in the United States. Management strategies often reduce nematode populations without increasing yield. The overall objective of this research was to integrate additional fertilizer and nematicide combinations into current practices to establish economical and sustainable nematode management strategies. Microplot and field trials were run to evaluate fertilizer and nematicide combinations applied at the pinhead square (PHS) and first bloom (FB) plant growth stages to reduce nematode population density and promote plant growth and yield. Cost efficiency was evaluated based on profit from lint yields and chemical input costs.

Microplot and field trials were conducted throughout two growing seasons (2019 – 2020). Data combined from 2019 and 2020 suggested a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur was the most effective in increasing seed cotton yields in the *R. reniformis* microplot trials. In *R. reniformis* field trials, a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS supported the largest lint yield and profit per hectare at $1175.87. This combination increased profit by $137 per hectare when compared to treatments with no nematicides. In *M. incognita* microplot trials, an application of 28-0-0-5 at PHS resulted in the lowest *M. incognita* eggs per gram of root and the combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB resulted in the highest seed cotton yields. In *M. incognita* field trials, a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB supported the largest lint yields and profit per hectare at $784.00, resulting in a $54 per hectare increase over treatments with no nematicides. These results suggest that combinations utilizing fertilizers and nematicides in addition to current fertility management show potential to promote yield and profit in *R. reniformis* and *M. incognita* infested cotton fields.

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List of Abbreviations

FB	First Bloom
PHS	Pinhead Square
ST	Seed Treatment

Chapter 1: Introduction and Review of Literature

Introduction and Problem Statement

The reniform nematode (*Rotylenchulus reniformis*) (Linford and Oliveira) and the southern root-knot nematode (*Meloidogyne incognita*) (Kofoid and White) are the most economically important nematodes on upland cotton (*Gossypium hirsutum* L.) (Robinson, 2007). Nearly 182 thousand hectares of upland cotton were planted in Alabama in 2020 (USDA, 2021). In the same year, *M. incognita* and *R. reniformis* caused a combined estimated yield loss of approximately 22 thousand hectares, representing a 12% yield loss in Alabama (Lawrence et al., 2021). Ideal management strategies use a combination of the most effective and cost-efficient practices based on nematode species and levels, equipment accessibility, financial resources and environmental conditions (Grabau, 2017). For most growers, the economics of cotton production are the driving forces behind management (Starr et al., 2007). Integrating nematode management into current agricultural practices would allow cotton growers to mitigate nematode damage and promote yield. Common nematode management strategies include crop rotation, nematicides and host resistance (Starr et al., 2007). Nematicides are commercially available to growers in a variety of applications. Seed treatments and foliar sprays can adequately reduce *M. incognita* and *R. reniformis* populations (Lawrence and McLean, 2002; Faske and Hurd, 2015), and tend to be the most common treatments because of cost, efficacy and equipment convenience. High rates of fertilizer have been documented to limit nematode induced crop damage (Chawla and Prasad, 1973), reduce plant stress and promote plant growth (Whitaker, 2018). However, there is very little research done on the effect of fertilizer on cotton production systems that are infested with nematodes. Most research is conducted either on the efficacy of nematicides (Lawrence et al., 1990) or the importance of nitrogen fertilizer in cotton (Duncan and Raper, 2019). Utilizing a combination of additional nematicides and fertilizers would allow growers to integrate a nematode management strategy into current agricultural practices.

The primary objective of this study was to analyze the impact of additional fertilizer and nematicide combinations on *R. reniformis* and *M. incognita* populations and cotton yield. The two main objectives of this research were: 1) to evaluate the effects of additional fertilizer and nematicide applications at two critical plant growth stages; 2) to determine the financial impact of additional fertilizer and nematicide combinations using input costs and revenue.

Gossypium hirsutum

Cotton (*Gossypium*) is one of the most important fibers produced worldwide and is a staple in the United States and global economies. Nearly 14 million acres of cotton are harvested in the United States equating to $38 billion dollars each year (Wilkins et al., 2000). Upland cotton is the most widely grown cotton species worldwide consisting of nearly 90% of global cotton production (Glade et al., 1996; Wakelyn et al., 2006). Cotton is restricted to tropical and subtropical regions because of elevated temperatures and humidity that are ideal for production (Luttrell et al., 1994). Upland cotton production is localized primarily to the southern United States because of the subtropical environment (Figure 1). The upland cotton cultivar ‘DP 1646 B2XF’ is the most widely planted across the southeast and in Alabama (USDA 2021).

Rotylenchulus reniformis

The reniform nematode (*R. reniformis*) was first reported in Hawaii by Lindford and Oliveira in 1940. The nematode is a soil-borne semi endoparasitic nematode that is known to cause damage to a broad range of hosts, including vegetables, fruits, row crops, and ornamentals (Leach et al., 2012, Robinson et al., 1997). *Rotylenchulus reniformis* can be found in tropical and subtropical regions and prefer soil with high silt and clay concentrations (Dropkin, 1980). In 2020, an estimated 145 thousand bales of cotton were lost in the United States cotton belt due to *R. reniformis* making this nematode economically important in the southeast (Lawrence et al., 2021).

Damage from *R. reniformis* can be severe for cotton growers, making a proactive management approach important in a cotton cropping system. Soil samples are often taken late summer or early spring to determine nematode levels after a growing season is concluded or before it begins (Barker and Campbell, 1981). Sampling will determine nematode genera, levels and field distribution (Wrona et al., 1996). *Rotylenchulus reniformis* damage threshold is minimal, at 2 – 20 nematodes/500cm^3 of soil, although thresholds vary widely across locations, soil types and crops (Khanal et al., 2018, Blasingame et al., 2002). Above ground symptoms of *R. reniformis* damage are stunted plants due to limited root development that produce a wave-like pattern across the canopy (Lawrence and Lawrence, 2020), reduced boll size and number of bolls that result in reduced lint yield (Jones et al., 1959). As nematode levels increase, nematodes and inflicted damage will become more evenly distributed across the field (Robinson, 2007). Management of this nematode can be difficult due to a short lifecycle and survival at multiple soil depths (Koenning et al., 2007, Robinson et al., 1997).

Infection starts as the young female penetrates the plant root to establish a permanent feeding site (Wang 2013). The posterior portion of the nematode will remain on the exterior of the plant root and begin to swell as the nematode matures (Robinson et al., 1997). A gelatinous matrix forms around the posterior of the female where she lays her eggs after seven or eight days (Sivakumar and Seshadri, 1971). Typically, females lay around 60 eggs in an egg mass. When conditions are favorable, embryogenesis results in a first-stage juvenile (J1) within the egg

(Linford and Oliveira, 1940). The J1 molts into a second-stage juvenile (J2) which emerges from the egg, into the soil. The third and fourth stage molts (J3 and J4) occur in the soil; the vermiform nematodes are not parasitic during these stages (Robinson et al., 1997). A young female reaches an infective stage between 1 -2 weeks after hatching (Lawrence and McLean, 2001). Males have less developed feeding structures and are not parasitic (Koenning et al., 2004). Under favorable conditions, the lifecycle can be completed in 25-30 days (Birchfield, 1962).

Meloidogyne incognita

The southern root-knot nematode (*M. incognita*) is a sedentary endoparastic nematode found across the world and widely throughout the United States (Koenning et al., 2004). They have a broad host range of over 2000 species including agricultural crops and weeds (Thomas and Kirkpatrick, 2001). In 2020, nearly 473 thousand bales of cotton were estimated lost in the United States cotton belt due to *M. incognita* (Lawrence et al., 2021). The extensive host range and economic impact makes them one of the most economically important nematodes throughout the world (Sasser, 1977).

Visual aboveground symptoms include stunted plant growth and wilting due to reduced water and nutrient uptake (Lawrence and Lawrence, 2020, Davis and May, 2003). When the root system is penetrated, secondary soil-borne pathogens can enter the plant system (Jenkins et al., 1995). However, the most identifiable symptoms are the massive galls formed on host root system (Chitwood, 1949). Galls are formed through the formation of giant cells. Giant cells contain enlarged nuclei and cytoplasm which supply nutrients for the nematode throughout its lifecycle (Jones et al., 2013).

Implementing a management strategy becomes crucial to mitigate yield losses. Soil samples will give growers accurate identification of species and levels of nematodes in a field (Neher and Campbell, 1996). Sampling is suggested for late spring, summer or early fall when environmental conditions including temperature and moisture will provide the most accurate nematode populations (Olsen, 2000). Analysis of soil samples for *M. incognita* allows growers to make economically responsible and accurate pest management strategies. The damage threshold for *M. incognita* is low at 10 juveniles/ 500 cm^3 soil (Starr et al., 1989).

Embroyogenesis results in the development of the first-stage juvenile (J1) within the egg (Moens et al., 2009). The second-stage juvenile (J2) emerges from the egg and begins to search for an appropriate host (Taylor and Sasser, 1978). The J2 searches for a suitable host and penetrates the root meristem, migrating to an area of cell elongation before they establish a feeding site (Taylor and Sasser, 1978). Effectors, secreted through the salivary glands of the nematode, are inserted into the root cells through the stylet (Favery et al., 2015). The J2 nematode goes through the third and fourth stage molt (J3 and J4) within the plant root before reaching maturity. Once a mature adult, the female deposits eggs in a gelatinous matrix on her posterior region (Taylor and Sasser, 1978). Under favorable environmental conditions, a female can lay around 500 – 2000 eggs (Tyler, 1933). Males are not necessary for reproduction, as reproduction primarily occurs via parthenogenesis (Moens et al., 2009). After 10 days, a new generation of juveniles will begin to hatch (Blasingame et al., 2002). Population growth is rapid due to multiple generations during one growing season (Calderon-Urrea et al., 2016).

Management Strategies

Cultural management strategies for *R. reniformis* and *M. incognita* may include crop rotation, weed removal, cover crops and field fallowing (Creech et al., 1995). Crop rotation is one of the most effective management strategies by reducing overall nematode populations for future growing seasons (Bridge, 1996). *Rotylenchulus reniformis* and *M. incognita* are obligate parasites meaning they need a host to survive. Alternating a host with a non-host can reduce overall nematode populations in a field (Duncan, 1991). Popular alternative hosts for *M. incognita* include grain sorghum (*Sorghum bicolor*), and soybean (*Glycine max*) cultivars (Kirkpatrick and Thomas, 2012). *Meloidogyne incognita* has a broad host range making it difficult to find suitable alternative hosts (Trivedi and Barker, 1986). Corn (*Zea mays*), peanut (*Arachis hypogaea*) and wheat (*Triticum*) are ideal alternative hosts for *R. reniformis* in a crop rotation system (Robinson, 2007). In a single growing season, crop rotation cycles of either cotton-corn or cotton-peanut for *R. reniformis* and *M. incognita* resulted in nematode population reductions (Lawrence et al., 2008, Kirkpatrick and Sasser, 1984). However, crop rotation is not a sustainable practice in locations where monoculture is predominant. Monoculture has become an increasingly popular practice in cotton systems in the southeast due to environment, and equipment accessibility (Shaw et al., 2002).

Resistant cultivars provide an economical form of management for specific nematode species (Barker and Koenning, 1998). Nematode resistant cultivars limit reproduction when compared to the reproduction on a susceptible cultivar (Cook and Evans, 1987). Until recently, there were no commercially available cotton cultivars that provide resistance for *R. reniformis* (Koenning et al., 2000). The cultivars PhytoGen 443 W3FE and 332 W3FE were released for the 2021 growing season and are resistant to *R. reniformis* and *M. incognita*. A study conducted by

Turner et al. (2021) found the cultivar PHY 332 increased cotton lint yields by 51% in a reniform infested field when compared to a non-resistant cotton variety. Tolerant and resistant cotton varieties provide practical management approaches by integrating *M. incognita* resistant genes into commercially available cultivars (Davis and May, 2003, McClure et al., 1973). Many *M. incognita* resistant cultivars focus on reducing nematode reproduction rather than yield potential (Faske and Starr, 2009). Breeding efforts concentrate on *M. incognita* resistant cultivars and strives to produce higher yields and lower nematode reproduction (Koenning et al., 2001). Growers who experience *R. reniformis* and *M. incognita* damage must assess input costs of potential nematicide management strategies to determine which would provide the greatest return. Alternative management strategies must be implemented to negate *R. reniformis* levels due to the scarcity of resistant cultivars. Tolerant *R. reniformis* cotton varieties are available but show minimal reductions in nematode reproduction and indicate poor yield performance (Koenning et al., 2000, Usery et al., 2005).

Nematicides are the primary management strategy for plant parasitic nematodes due to a lack to high yielding resistant cultivars and limitations on crop rotation (Starr et al., 2007). Nematicides are an effective management strategy for reducing *R. reniformis* and *M. incognita* populations and increasing cotton yields (Lawrence et al., 1990). Nematicides applied to the soil are often broad-spectrum, encompassing insecticide or fungicide capabilities (Chitwood, 2003). The host root system will be more readily protected during the first 4 to 6 weeks after a nematicide application (Greer et al., 2009). Non-fumigant nematicides are the most widely used treatment because they do not require specialized equipment making seed treatments, foliar sprays and in-furrow applications popular (Faske and Hurd, 2015). A study conducted by Lawrence et al. (1990) found that aldicarb (AgLogic™ 15G, Chapel Hill, NC) and fenamiphos

(Nemacur®, Bayer CropScience, Research Triangle Park, NC) applied as a granular or in-furrow sprays were able to reduce *R. reniformis* levels and provide increased profit in a cotton cropping system. In another study, the seed treatment abamectin (Syngenta, Crop Protection, Greensboro, NC) resulted in minimal root-galling and suppressed infection from *M. incognita* (Monfort et al., 2006).

Nematicide seed treatments provide easy application before planting, and early protection against nematode populations. Additional benefits include reduced chemical applications and limited exposure to harmful chemicals (Wilson et al., 2020). Seed treatments apply a nematicide directly to the soil and are highly effective against plant parasitic nematodes prior to feeding site establishment (Hawk and Faske, 2020). Seed treatment applications can be effective for up to 2 weeks after planting, allowing the cotton to germinate and establish a root system (Monfort et al., 2006). Nematodes must come in direct contact with the seed treatment for it to be effective. Thiodicarb (Aeris®, Bayer CropSciences, Research Triangle Park, NC) and fluopyram (COPeO™ Prime, BASF Agricultural, Florham Park, NJ) are common nematicide seed treatments used on cotton. A study conducted by Groover et al., (2020) showed that an application of fluopyram significantly reduced *R. reniformis* population density on cotton. A similar study conducted by Spinks et al., (2020) demonstrated *M. incognita* population density ranging from low to severe was mitigated with the application of a fluopyram seed treatment.

Foliar nematicides allow growers the option to apply a nematicide after planting and germination have occurred. This is ideal for growers who do not realize their fields are nematode infested prior to planting. Oxamyl (Vydate® C-LV, Corteva, Wilmington, DE) is a systemic insecticide/nematicide that moves primarily downward through the plant to inhibit nematode feeding (Wheeler et al., 2013). Systemic nematicides work rapidly once applied to the soil or plant tissue (Noling, 2000). Multiple studies conducted using oxamyl applied at pinhead square mitigated *R. reniformis* and *M. incognita* populations and resulted in increased seed cotton yields (Radewald et al., 1970; Rich and Bird, 1973; Hammes et al., 1996). Foliar nematicide applications are recommended for use in combination with at-planting nematicide treatments (Faske and Starr, 2009). Studies conducted by Lawrence and McLean (2000, 2002) with a foliar application of oxamyl in combination with an in-furrow application of aldicarb produced a positive effect on cotton plant growth and boll production and reduced *R. reniformis* and *M. incognita* levels.

Nematicides are crucial in a pest management system to reduce *R. reniformis* and *M. incognita* levels. There are many formulations and application techniques to best fit grower production practices and maximize nematicidal efficacy (Chitwood, 2003). Seed treatments and in-furrow applications are applied before or during planting for early season protection. Post-emergent nematicides such as foliar sprays can be applied in combination with early season treatments for further control of moderate or advanced nematode populations (Mueller, 2017). A combination of a seed treatment nematicide Aeris® (thiodicarb) with two foliar applications of Vydate® C-LV (oxamyl) applied after germination and the 2- 8 leaf stage reduced *R. reniformis* and *M. incognita* populations. Multiple nematicide applications throughout the growing season are necessary when nematode populations are exceptionally high (Minton et al., 1980). A study conducted by Farr (1984) found that an application of Vydate® C-LV (oxamyl) applied at planting, 30 day and 60 days after planting generated a higher lint yield than the control treatment.

Plant parasitic nematodes are a major problem on upland cotton in the southeastern United States. Management of *R. reniformis* and *M. incognita* primarily rely on the use of nematicides. Nematicides reduce nematode populations and protect cotton plants from nematode induced damage and yield losses. Employing nematicides in conjunction with additional fertility practices could promote growth and yield while reducing nematode populations. Growers often oppose extensive new management strategies due to limited resources and increased economic risks (Schomberg et al., 2003). Utilizing current grower fertility practices eliminates the purchase of extraneous fertilizers and promotes a financially practical management system

Fertilizers

Cotton growth and yield production are directly correlated with nutrient uptake (Bange et al., 2004). Nutrient uptake in cotton is slowest during the first 40 days and begins to increase once pinhead square (PHS) is reached (Wright et al., 2003). A study conducted by Bassett et al. (1970) estimated that first bloom (FB) occurred in mid-July, indicating a transfer of nitrogen accumulation from leaves to fruit. Multiple applications of fertilizer are common in cotton cropping systems due to potential leaching, denitrification or volatilization (Gerik et al., 1998). Sidedress applications between first square and first bloom are suggested for growers in the Southeast (Whitaker et al., 2018). A study conducted by Mullins et al. (2003) suggested that fertilizer applied during peak uptake, considered to be during early bloom and peak bloom, has the potential to increase lint yields between 8.4 and 13.7%.

Commercially available fertilizers can be applied as a broadcast spray, dribbled, knifed-in or banded during planting (Oldham, 2017). Broadcast applications increase flexibility and improve application time, allowing for more efficient fertility management (Bazen et al., 2007). Banded applications of liquid fertilizers increased overall lint yields by 9%. (Guthrie, 1991). A study conducted by Roberts et al., (1999) showed no significant differences in yield between broadcast and injected applications of fertilizer. Fertilizer application methods are often chosen by growers based off equipment accessibility and fertilizer input costs. There is a trade off when deciding between liquid and granular formulations of fertilizer. Liquid fertilizers are popular due to easy application and absence of specialized equipment or labor. Granular fertilizers including ammonium sulfate can be more cost efficient when purchased in bulk when compared to liquid fertilizers (Isleib, 2016).

Nitrogen management in a cotton cropping system affects yield and lint quality more than any other plant nutrient (Khan et al., 2017). Nitrogen deficiencies, often seen as chlorosis of the mature leaves, and over application, have adverse effects on plant maturity and lint yields (McConnell et al., 1995; Weir et al., 1996). Nitrogen is most commonly applied to the soil before or during planting (Gerik et al., 1998). Common inorganic fertilizers include ammonium sulfate, urea, anhydrous ammonia, and ammonium nitrate (Jones, 1982). A study conducted by Ferrari et al. (2015) found that ammonium sulfate increased seed cotton yields when applied as a broadcast fertilizer.

Sulfur is another influential compound that promotes cotton growth and yield through the formation of proteins and amino acids (Dunn et al., 2008). Inadequate levels of sulfur, as seen in pale green or yellow colors on younger leaves, can reduce cotton yield and quality (Scherer, 2001; Yin et al., 2012). Deficiencies are often a result of the use of fertilizers and pesticides containing little or no sulfur (Chen et al., 2005). Sulfur applications are often applied pre-plant, as a side dress or foliar spray (Ensminger, 1958). Foliar applications are often applied mid-season and supplement a soil fertility program (Oosterhuis et al., 1991). Common sulfur fertilizers are ammonium sulfate $(NH_4)_2SO_4$, gypsum $CaSO_4$-H_2O and ammonium thiosulfate $(NH_4)_2S_2O_3$. Sulfur applied at 30lbs/A on sandy soils has resulted in as large as a bale increase in lint (Wright et al., 2003). Other studies conducted by Mullins (2013) on coastal plain soil with ammonium sulfate, elemental sulfur, potassium sulfate and potassium thiosulfate resulted in positive yield responses over a 3-year trial.

Rotylenchulus reniformis and *M. incognita* cause greater yield losses on cotton plants under nutrient and moisture stress than non-stressed plants (Mueller, 1994). Cotton yield losses due to *R. reniformis* and *M. incognita* are often dependent on environmental factors and stressors (Crow et al., 2020). Fertilizer amendments have shown nematicidal effects while simultaneously promoting yield (Muller and Gooch, 1982). Managing fertility in nematode infested fields could help to alleviate stressful environmental conditions and avoid yield losses (NCC, 2007). Maintaining soil fertility and promoting plant health may improve plant tolerance to nematode infections despite not reducing nematode populations (Grabau, 2017). A study conducted by Dyer (2019) found that an application of AgraLi®, liquid fertilizer (Evonik Industries, Essen Germany) used in combination with a nematicide reduced *R. reniformis* levels and increased seed cotton yield by 417 kg/ha. Nutrient inconsistencies and fertilizer application costs have prompted research for cost-efficient strategies (Overstreet et al., 2014).

Maximizing profit is the most important factor in any cotton production system. Growers must take into consideration input costs, financial risks and potential revenue. Management strategies for pests and fertility programs play a huge role in the economic viability of the cotton-cropping program. However, there is little research evaluating nematicide treatments in combination with additional fertilizer applications as a management strategy for *R. reniformis* and *M. incognita*. Increasing fertilizer applications have the potential to reduce plant stress and promote growth and yield. Integrating increased fertility practices into nematode management could help growers mitigate nematode induced yield losses.

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Figure 1: *Gossypium hirsutum* distribution map of the United States by county (USDA Upland Cotton Production)

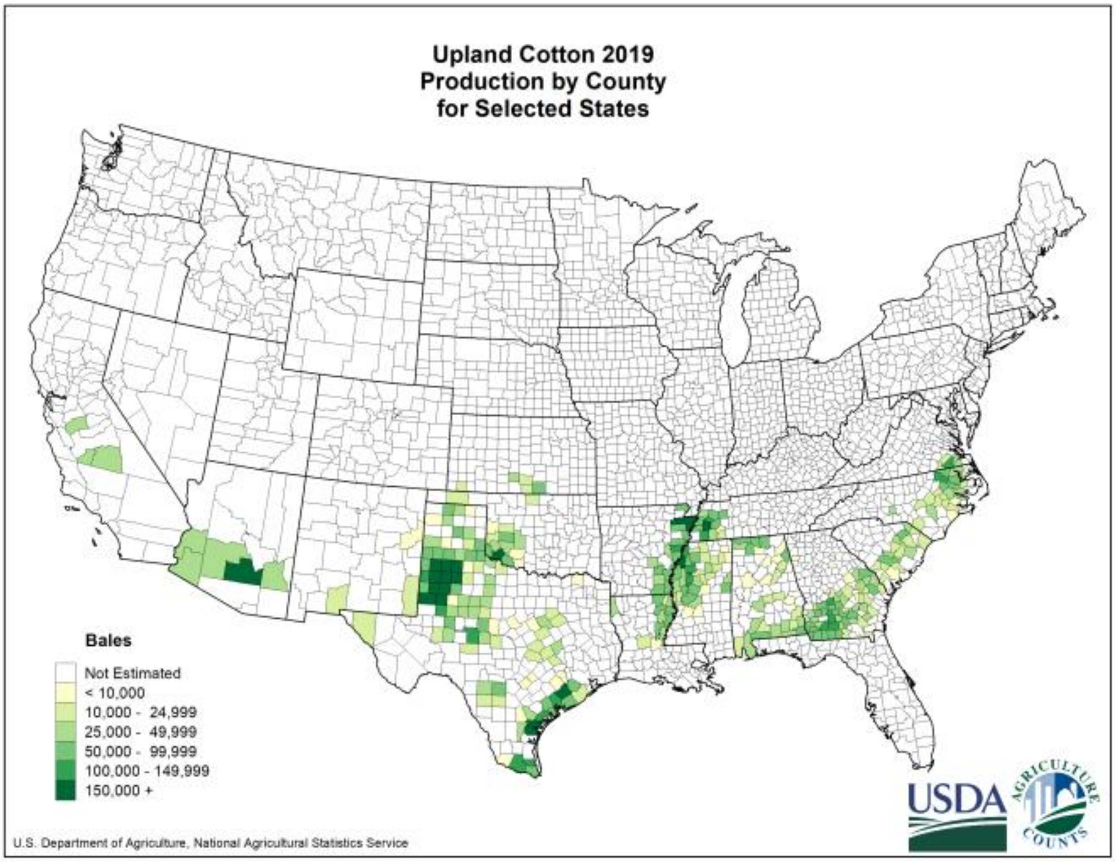
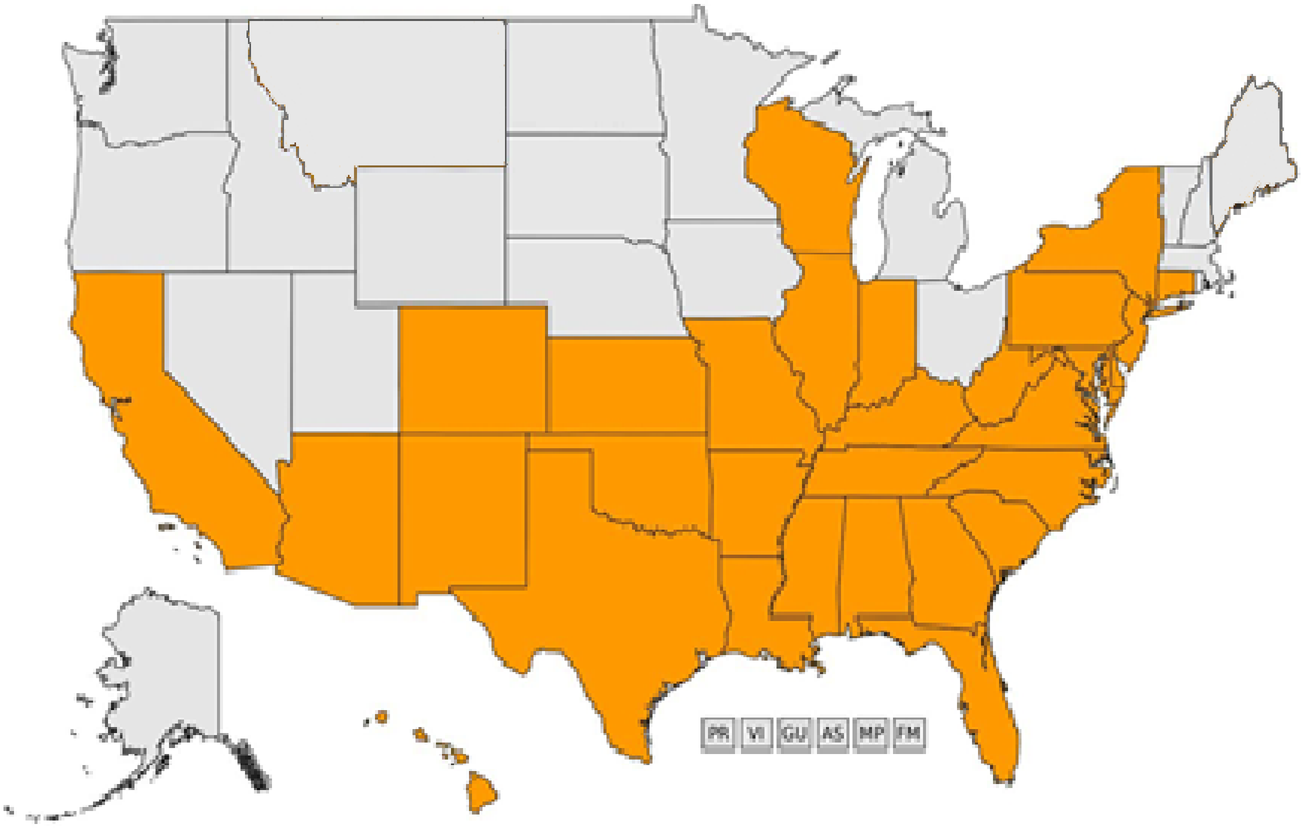


Figure 2: *Rotylenchulus reniformis* distribution map of the United States by state (Dr. D. R. Dyer, Personal Communication)

Figure 3: *Meloidogyne incognita* distribution map of the United States by state (Dr. D.R. Dyer, Personal Communication)



Chapter 2: Economic impact of additional fertilizer and nematicide applications on management of *Rotylenchulus reniformis* and *Meloidogyne incognita* on *Gossypium hirsutum* in Alabama.

Introduction

There is a need for enhanced management strategies for *R. reniformis* and *M. incognita* in cotton cropping systems that focus on reducing nematode populations and promoting plant growth and yield. Fertilizer soil amendments have been suggested as having nematicidal effects while increasing plant yield (Muller and Gooch, 1982). A study conducted using a synthetic nitrogen based soil treatment on olives decreased *R. reniformis* levels by 31% (Badra and Khattab, 1980). High rates of nitrogen fertilizer in cotton cropping systems have reduced plant parasitic nematode induced plant stress and increased overall yield (Whitaker, 2018). Integrating additional fertilizer applications with nematicide management strategies has the potential to mitigate plant stress and promote yield.

The purpose of this study was to evaluate selected fertilizer and nematicide combinations for management potential of *R. reniformis* and *M. incognita* and economic impacts on upland cotton cropping systems. The objectives of this study were 1) to assess selected fertilizer and nematicide combinations applied at pinhead square and first bloom to reduce nematode populations and promote yield; and 2) determine the economic practicality of additional fertilizer and nematicide applications as a management strategy on upland cotton.

Rotylenchulus reniformis (Linford and Oliveira) is a semi endoparasitic nematode that partially enters the plant root while the posterior extrudes from the root surface (Robinson et al., 1997). *Rotylenchulus reniformis* has a broad host range and can cause extensive cotton yield losses making it an economically important nematode (Greer et al., 2009; Dropkin, 1980). Common symptoms include stunted plant growth, limited root development, reduced boll size and yield (Jones et al., 1959; Lawrence and McLean, 2001). In 2020, *R. reniformis* caused an estimated yield loss of 23,700 bales in Alabama, equating to an estimated 8 million dollars. (Lawrence et al., 2021; USDA, 2021). Management strategies currently focus on chemical nematicides and crop rotation, but there is need for improvement. These management strategies may not be ideal in the southeast where cropping systems are heavily based on monoculture (Shaw et al., 2002). Utilizing chemical nematicides at significant plant growth stages has the potential to promote chemical uptake and increase efficacy. This modified management strategy focuses on reducing nematode induced plant stress during critical plant development, improving current strategies.

Meloidogyne incognita (Kofoid and White) Chitwood is an economically important pest on field crops across the United States and worldwide (Koenning et al., 2004). It is sedentary endoparasitic nematode that enters the plant roots where it reproduces and feeds, generating galls on the root system (Lawrence and McLean, 2001; Jones et al., 2013). The galls or knots produced on the root system give the nematode its common name, the root-knot nematode (Moens, et al., 2009). *Meloidogyne incognita* favor tropical and subtropical environments and reproduce rapidly in soils with high concentrations of sand (Robinson et al., 1987). Common symptoms include stunted plant growth, wilting, and yield loss (Lawrence and Lawrence, 2020). Current management strategies rely heavily on the use of chemical nematicides to reduce *M. incognita* population levels. There is a need for the additional integration of strategies focusing on yield that are financially practical for growers.

Fertility management in a cotton cropping system is crucial in plant growth promotion and yield. Nitrogen significantly impacts plant growth, boll development and lint yield more than any other fertilizer compound (Chen et al., 2019). Studies have shown that nitrogen applied as a liquid or granular increased overall seed cotton and lint yield (Guthrie, 1991; Ferrari et al., 2015). Sulfur is commonly applied in combination with nitrogen fertilizers to improve cotton yield and quality (Scherer, 2001). Additional fertilizer applications in the southeast are suggested between first square and first bloom, during peak nutrition uptake (Whitaker et al., 2018). Studies have shown that nitrogen and sulfur applied between early bloom and peak bloom increased lint yield on average by 11% (Mullins et al., 2003).

Materials and Methods

Microplot and field tests were established to test the impact of additional fertilizer and nematicide applications timed at pinhead square (PHS) and first bloom (FB) to enhance management of *R. reniformis* and *M. incognita*, increase cotton lint yield and determine the economics of these practices.

Nematicides and fertilizers

Additional nematicides and fertilizers were evaluated in various combinations for their ability to reduce *R. reniformis* and *M. incognita* population density and promote cotton yield. A nematicide seed treatment was applied before planting to the cotton seed (Table 1). Aeris® (Bayer CropScience, Research Triangle Park, NC) (Imidacloprid and Thiodicarb) was the seed treatment in 2019 and COPeO™ Prime (BASF, Florham Park, NJ) (Fluopyram) was applied in 2020. The additional nematicide Vydate® C-LV (DuPont, Wilmington, DE) (Oxamyl) was applied at pinhead square (PHS) and/or first bloom (FB)(Figure 4). Supplementary fertilizers

$(NH_4)_2SO_4$, 28-0-0-5 and Max-In® Sulfur (WinField United, Arden Hills, MN) were applied once at PHS or twice at PHS and FB. Trials were conducted with 12 fertilizer and nematicide combinations utilizing a base fertilizer of $(NH_4)_2SO_4$ or 28-0-0-5 applied at PHS or PHS and FB. A nematicide seed treatment was applied to the cotton seeds in 10 treatments, before planting. Applications of Vydate® C-LV or Vydate® C-LV and Max-In® Sulfur were applied at PHS to 4 treatments. Sequential applications of Vydate® C-LV and Max-In® Sulfur were applied at FB to 2 treatments (Table 2).

Nematode Extraction

To obtain nematode population levels, eggs were extracted from the cotton roots using a modified method of Hussey and Barker (1973). Eggs were collected for *R. reniformis* and *M. incognita* by placing roots in a 0.625% NaOCl solution and shaken for four minutes on a Barnstead Lab Line Max Q 5000 E Class shaker (Conquer Scientific: San Diego, CA). Roots were rinsed with water and scrubbed by hand; eggs were collected on a 25-µm sieve and poured into a 50mL centrifuge tube. The product was further processed by sucrose centrifugation following the modified methodology of Jenkins (1964). Contents were centrifuged at 220 g-forces for 1 minute and then rinsed with water; eggs were collected on a 25-µm sieve. Eggs were enumerated via a Nikon TSX 100 inverted microscope at a 40x magnification.

Microplot Tests

Microplot trials were conducted in 2019 and 2020 at the Plant Science Research Center (PSRC) in Auburn, AL. Four tests were conducted, one each year for *R. reniformis* and *M. incognita*. Microplots were 26.5 L plastic pots filled with Kalmia loamy sand (24% sand, 49% silt and 28% clay) from the Plant Breeding Unit (PBU) or Decatur silt loam (24% sand, 49% silt and 28% clay) from the Tennessee Valley Research and Extension Center (TVREC) and represent 0.3 m of row in the field. Each microplot was inoculated with 250 cm^3 of soil containing approximately 50,000 eggs and vermiform life stages of either *R. reniformis* or *M. incognita* and placed in the base of the planting furrow. Nematode inoculum was obtained from Auburn University which isolated *R. reniformis* and *M. incognita* from the Tennessee Valley Research and Extension Center near Belle Mina, AL and the Plant Breeding Unit near Tallassee, AL, respectively *Rotylenchulus reniformis* populations were maintained on cotton "Phytogen 340 W3FE" (Corteva Agriscience, Wilmington, DE) and *M. incognita* populations were maintained on corn "Mycogen 2H723" (Dow AgroScience, Indianapolis, IN). Nematode inoculum was prepared in 500 cm^3 polystyrene pots at the Plant Science Research Center. A pre-plant application of 13-13-13 was applied at 0.13kg/m and was broadcast and hand tilled into the soil. DP 1646 B2XF (Bayer CropScience, Research Triangle, NC) were pretreated with an insecticide/fungicide seed treatment by Bayer CropScience (metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, fluxapyroxa). Seed receiving an additional nematicide seed treatment were treated with Aeris® (Imidacloprid and Thiodicarb) at 0.375mg ai/seed applied in 2019 and COPeO™ Prime (Fluopyram) at 0.30mg ai/seed applied in 2020 before planting using a Gustafson laboratory table top seed treater (Pinckard, 1971). Seeds were air dried for 24 hours before planting. Tests were planted on May 14, 2019, and June 1, 2020. Ten cotton seeds were planted 2.5 cm deep into a furrow in each microplot and thinned to five seedlings after germination. Irrigation was administered through a drip irrigation system at 30 ml/min and was adjusted throughout the season to run for 15 to 45 minutes twice a day.

All tests were arranged in a randomized complete block design (RCBD), with 5 replications. The first additional application of fertilizers and nematicides were applied at (PHS)

44 days DAP in 2019 and 39 DAP in 2020. At PHS, ammonium sulfate, $(NH_4)_2SO_4$ was applied by hand to the base of the plant at a rate of 0.52g/m. At PHS, a narrow indention 5 cm beside and 5 cm below the base of treated plants was created with a hand spade and 28-0-0-5 was pipetted at a rate 0.289 ml/m. Max-In® Sulfur and Vydate® C-LV were applied as foliar sprays via a handheld spray bottle at rates of .007ml/m and .004ml/m. The second application of additional fertilizers was applied at (FB) 75 DAP in 2019 and 62 DAP in 2020. All fertilizers and nematicides were applied identically at PHS and FB. In 2020, microplots received the same management practices as in 2019 with the exception that the nematicide seed treatment Aeris® was replaced with Copeo™ Prime.

Microplot Data Collection

Data were collected at (PHS) 44 DAP and (FB) 75 DAP in 2019 and at (PHS) 39 DAP and (FB) 62 DAP in 2020. One cotton plant was excavated from each microplot for plant and nematode data at each of the sample data collection times. Plant parameters included plant height (PH), and root fresh weight (RFW) and seed cotton yield. Nematode parameters of *R. reniformis* and *M. incognita* population density included number of eggs per g of root. At plant maturity, cotton was hand harvested for all microplot trials on (162 DAP) October 22, 2019, and (154 DAP) October 15, 2020.

Field Tests

Field trials were conducted at Tennessee Valley Research and Extension Center (TVREC) near Belle Mina, AL, and the Plant Breeding Unit (PBU) near Tallassee, AL. Both research stations-maintained plots throughout the growing season with standard herbicide, insecticide, and fertility practices. Fertility practices at both locations included a pre-plant application of 28-0-0-5 at 112 kg/ha in late April followed by a sidedress application of 28-0-0-5 at 34 kg/ha applied in mid-July. TVREC was artificially infested with *R. reniformis* in 2007, the initial population density at planting averaged 5000 vermiform life stages per $100cm^3$ of soil in 2019 and 2020. The soil type in this field is a Decatur silt loam. PBU is naturally infested with *M. incognita* and the initial population at planting was 77 J2 per $100cm^3$ of soil in a Kalmia loamy sand soil type. The trials were arranged in a RCBD with 5 replications and the entire test was repeated within each year. Both sites were planted using a John Deere MaxEmerge planter (Moline, Illinois) equipped with Almaco cone planters (Nevada, Iowa). Trials were planted with DP 1646 B2XF at a rate of 100 seeds per 7.6 m. Plots at TVREC consisted of 2 rows that were 7.6 m long with 1.01 m row spacing and a 6 m wide alley. Plots at PBU consisted of 2 rows that were 7.6 m long with 0.91m row spacing and a 6 m wide alley.

Additional fertilizer, nematicides, and seed treatment applications were identical to the microplot trials (Table 1 & 2). TVREC was planted on April 30, 2019. PBU was planted on April 25, 2019, and replanted May 15, 2019, due to poor plant stand because of a 22cm rainfall four days after planting. TVREC was planted on May 4, 2020, and PBU was planted on May 7, 2020. The additional fertilizer and nematicide combinations were applied at PHS (49 DAP) and FB (75 DAP), in 2019 and at PHS (40 DAP) and FB (69 DAP) in 2020. Ammonium sulfate was applied by hand to the base of the plant at a rate of 108 kg/ha. 28-0-0-5 was knifed into the soil at a rate of 89 L/ha 5cm beside and 5 cm below the plant with a liquid fertilizer applicator and fertilizer disc. WinField Max-In® Sulfur and Vydate® C-LV (Oxamyl) were applied at rates of 1.5 L/ha and 0.8 L/ha at 25 PSI with a Case IH 265 tractor equipped with a 4 boom sprayer at PHS and FB. Entire plots were machine harvested with a Case International Harvester 2555 cotton picker with Harvest Weigh Mobile by System Scales at TVREC (157 DAP) and PBU (160 DAP) in 2019 and at TVREC (168 DAP) and PBU (147 DAP) in 2020.

Field Data Collection

Four representative cotton plants from each plot were randomly removed from each plot to collect plant and nematode data at PHS and FB. The plant growth parameters included plant stand, plant height, root fresh weight and cotton yield. Nematode population density was collected as in the microplot trials after transport from the field to PSRC. Fifty mature bolls were hand harvested from the first rep of each test. Samples were ginned using a 10-saw table-top gin at the PSRC. The lint and seeds collected from the gin were weighed individually and these data were used to calculate the lint ratio for each treatment. All plots were machine harvested to determine seed cotton yields.

Data Analysis

Data collected from microplot, and field trials were analyzed with SAS 9.4 (SAS Institute, Cary, NC) using the PROC GLIMMIX procedure. LS-means were compared using ANOVA, and Tukey-Kramer multiple pair wise comparison at a significance level of $P \leq 0.10$. Dependent variables included plant stand, plant height, root fresh weight, *R. reniformis* and *M. incognita* eggs per gram of root (eggs/g of root), seed cotton yield (kg/ha) and mean ($/kg). Fixed effects comprised of nematicide and fertilizer treatments at PHS and/or FB. Random effects comprised of replication, test repetition and location.

There were no significant interactions between 2019 and 2020 thus the data from both years were combined into a single dataset. Different nematicide seed treatments were used in both years; therefore, we analyzed the effects of a general nematicide seed treatment and not a specific chemical.

Profit Calculation

Revenue was calculated using the most current price from the USDA upland cotton announcement (https://www.fsa.usda.gov/Internet/FSA_EPAS_Reports.pdf) of \$1.32/kg in 2019 and \$1.54/kg in 2020 and the lint ratio from each treatment. Fertilizer and nematicide input costs were acquired through a local sales agricultural (Stephen Till, personal communications) representative in 2019 and 2020 (Table 3). Input costs were subtracted from revenue to determine profit for individual treatment combinations. Upper, lower, and mean profit for each combination was determined using a confidence interval from an ANOVA test at $P \leq 0.10$.

Results

Microplots 2019 & 2020

Rotylenchulus reniformis

In the microplot setting with *R. reniformis*, the additional fertilizer and nematicide applications did not significantly increase plant height or root fresh weight at either PHS or FB (Table 4: Figure 5). *Rotylenchulus reniformis* eggs per gram of root were significantly reduced when data collected at PHS and FB were combined for the nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS application compared to the control application of $(NH_4)_2SO_4$ at PHS with no ST nematicide. A significant increase in seed cotton yield was measured with the maximum input combination of the nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur applied at PHS and FB when compared to the combination of the nematicide ST + $(NH_4)_2SO_4$ applied at PHS ($P \leq 0.10$).

Meloidogyne incognita

The *M. incognita* infested microplot trials found the additional fertilizer and nematicide combinations did not significantly increase plant height or root fresh weight sampled at PHS or FB (Table 5: Figure 6). *Meloidogyne incognita* eggs per gram of root were lowest in the application of 28-0-0-5 at PHS, and in the combinations of the nematicide ST + 28-0-0-5 + Vydate® C-LV at PHS and a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur at PHS and FB. Seed cotton yields were similar across all fertilizer and nematicide combinations in the microplot tests.

Field Tests 2019 & 2020

Rotylenchulus reniformis

Plant stand was not affected by the additional fertilizer or Vydate® C-LV combinations (Table 6: Figure 7). The additional fertilizer and nematicide combinations did not significantly increase root fresh weight at PHS or FB. *Rotylenchulus reniformis* eggs per gram of root were not significantly decreased at PHS or FB or the combination of PHS plus FB. The combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur at PHS and FB had the lowest *R. reniformis* eggs per gram of root at the PHS and PHS + FB sampling dates. The combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS supported a significantly larger lint yield than the treatments with no nematicide ST, which applied $(NH_4)_2SO_4$ at PHS, 28-0-0-5 at PHS and the combination of a nematicide ST + 28-0-0-5 at PHS and FB ($P \leq 0.10$). Lint yield was increased by 546 kg/ha, 603 kg/ha, 580 kg/ha or 31%, 34% and 33% with the combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS when compared to the three lowest yielding combinations, $(NH_4)_2SO_4$ at PHS, 28-0-0-5 at PHS and a nematicide ST + 28-0-0-5 at PHS and FB.

Mean profits for fertilizer applications of $(NH_4)_2SO_4$ and 28-0-0-5 at PHS with no nematicides averaged $853.62 (Table 7: Figure 8). A nematicide ST with an additional fertilizer application at PHS increased mean profit by $46/ha from an additional fertilizer alone. A nematicide ST with an additional application of fertilizer at PHS and FB increased profit by $32/ha from an additional fertilizer alone. Combinations with Vydate® C-LV applied at PHS had an increased profit of $137/ha from an additional fertilizer alone. Fertilizer and nematicide combinations that included Vydate® C-LV at PHS and FB had an increased profit of $48/ha. All combinations were compared to the mean profit of treatments of an additional fertilizer with no nematicides to determine increased profit per hectare. The combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS had the greatest overall mean profit of $1175.87 which was significantly greater than the mean profit of $(NH_4)_2SO_4$ at PHS, 28-0-0-5 at PHS and a nematicide ST + 28-0-0-5 at PHS and FB.

Meloidogyne incognita Field 2019 & 2020

Plant stand was not affected by the additional fertilizer or Vydate® C-LV combinations (Table 8: Figure 9). The additional fertilizer and nematicide combinations did not significantly increase root fresh weight at PHS or FB. *Meloidogyne incognita* eggs per gram of root were not significantly reduced in samples taken at PHS, FB or PHS + FB. A nematicide ST + $(NH_4)_2SO_4$ at PHS and FB had the lowest *M. incognita* eggs per gram of root, represented by the combined sample. The combination of a nematicide ST + 28-0-0-5 + Vydate® C- LV + Max-In® at PHS and FB had a significantly higher lint yield when compared to application of $(NH_4)_2SO_4$ at PHS or 28-0-0-5 at PHS ($P \leq 0.10$). Lint yield was increased by 433 kg/ha and 447 kg/ha or 35% and

38% with the combination of a nematicide ST + 28-0-0-5 + Vydate® C- LV + Max-In® at PHS and FB when compared to the lowest yielding treatments, $(NH_4)_2SO_4$ at PHS and 28-0-0-5 at PHS.

Mean profits for fertilizer applications of $(NH_4)_2SO_4$ and 28-0-0-5 at PHS with no nematicides averaged $548.53 (Table 9: Figure 10). A nematicide ST with an additional fertilizer application at PHS increased mean profit by $54/ha from an additional fertilizer application alone. A nematicide ST with an additional application of fertilizer at PHS and FB increased profit by $103/ha from an additional fertilizer application alone. Fertilizer combinations with Vydate® C-LV applied at PHS had an increased profit of $93/ha from an additional fertilizer application alone. Fertilizer and nematicide combinations that included Vydate® C-LV at PHS and FB had an increased profit of $131/ha. All combinations were compared to the mean profit of treatments of an additional fertilizer with no nematicides to determine increased profit per hectare. The combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB had the greatest overall mean profit of $784.00 which was significantly greater than the mean profit of treatments of $(NH_4)_2SO_4$ or 28-0-0-5 at PHS.

Discussion

Rotylenchulus reniformis Microplot 2019 & 2020

A nematicide ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur at PHS was the most effective combination in reducing *R. reniformis* eggs per gram of root at PHS and FB. The combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur at PHS and FB was the most effective treatment in increasing seed cotton yield. The combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur at PHS and FB increased seed cotton yields by 50% when compared to the combination of a nematicide ST + $(NH_4)_2SO_4$ at PHS. Research supports the use of Vydate® C- LV on cotton during PHS and sequential treatments reducing *R. reniformis* population levels (Hammes et al., 1999). Applications of nematicides when analyzed individually increased lint yields by 8% with a nematicide ST, 19% with a single Vydate® C-LV application and 29% with a double application of Vydate®C-LV.

Meloidogyne incognita Microplot 2019 & 2020

The combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB was the most effective treatment in increasing root fresh weight at PHS and FB. The application of 28-0-0-5 at PHS had the lowest *M. incognita* eggs per gram of root, followed by a nematicide ST + $(NH_4)_2SO_4$ + Vydate® + Max-In® Sulfur at PHS and FB. Studies found that plants which had increased nitrogen available had lower nematode population levels (Miller and Wihrheim, 1966; Rodriguez-Kabana, 1986). Seed cotton yield was greatest with the combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB. The combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB also supported the largest root fresh weight among treatments at PHS and FB. Similarly, a study conducted by Bednarz et al., (2000) found that cotton yields were greatest using 28-0-0-5 in a loamy sand soil type. Analyzing nematicide applications individually indicated overall seed cotton yield was increased by 7% with a nematicide ST and 6% with two foliar applications of Vydate® C-LV.

Rotylenchulus reniformis Field 2019 & 2020

At TVREC, the combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur at PHS was the most effective at increasing root fresh weight at FB. A nematicide ST

+ (NH4)2SO4 + Vydate® C-LV + Max-In® Sulfur at PHS and FB had the lowest *R. reniformis* eggs per gram of root, closely followed by the combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS. These findings are supported by Badra and Elgindi (1979), where foliar applications of Vydate® C-LV significantly reduced *R. reniformis* population levels. Similarly, the use of a nematicide ST COPeO™ Prime (Fluopyram) inhibited *R. reniformis* from increasing on cotton root systems (Faske and Hurd, 2015). The combination that supported the largest lint yield was a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS. Nematicide applications were analyzed individually in response to lint yield. An application of a nematicide ST increased overall yield by 11%. A trial conducted by Groover et al. (2020) supports this conclusion and found that a nematicide ST (COPeO™ Prime) increased lint yield by 14%. A single foliar application of Vydate® C-LV increased overall lint yield by 13%, while two applications of Vydate® C-LV increased overall lint yield by 2%. Increases in yield with single or multiple Vydate® C-LV applications on cotton were also found in a study conducted by Hammes et al. (1999).

The combination with the largest mean profit in dollars/ha was a nematicide ST+ $(NH_4)_2SO_4$ + Vydate® C-LV at PHS. This combination also supported the largest lint yield. Based on the lower and upper profits, there is a 90% chance when using this fertilizer and nematicide combination in a *R. reniformis* infested field, the mean profit will fall between \$991.15/ha and \$1360.59/ha. The increased mean profit of this combination could be contingent on the moderate fertilizer and nematicide input costs. The overall input cost of this combination was \$55.96/ha which was \$46.17 cheaper than the most expensive $(NH_4)_2SO_4$ based combination. This evaluation is supported by Zimet el al. (2002) who reported financial returns in *R. reniformis* fields with lower chemical rates equating to reduced chemical input costs.

Similarly, a study conducted by Koenning et al., (2007) saw an increase in yield with the use of nematicides in *R. reniformis* infested fields but when conducting an economic analysis determined the profit from the additional yield did not cover the increased chemical costs.

Meloidogyne incognita Field 2019 & 2020

At PBU, the combination that supported the largest root fresh weight at FB was a nematicide ST + $(NH_4)_2SO_4$ at PHS. *Meloidogyne incognita* eggs per gram of root combined were lowest in the treatment combination of a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-In® Sulfur at PHS. This combination decreased *M. incognita* eggs per gram of root by 15% when compared to the treatment of $(NH_4)_2SO_4$ at PHS, with no nematicide. A study conducted by Faske et al. (2015) also found that a nematicide ST reduced *M. incognita* population levels when compared to treatments with only a base fungicide. The combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV + Max-In® Sulfur at PHS and FB was the most effective at increasing lint yield. This combination also supported the largest seed cotton yield in the *M. incognita* microplot trials in 2019 and 2020. The application of a nematicide ST (COPeO™ Prime) provided the greatest yield protection against *M. incognita* in trials conducted by Faske et al. (2021). This finding contrasts with Anderson et al. (2012) who found applications of Vydate® C-LV did not have an impact on cotton lint yield. Nematicide applications were analyzed individually to evaluate lint yield responses. The application of a nematicide ST increased overall lint by 20%, a single Vydate® C-LV application increased overall lint by 11%, and sequential Vydate® C-LV applications increased overall lint by 5%.

The largest mean profit was obtained with the combination of a nematicide ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur at PHS and FB. This combination also supported the largest lint yields, correlating increased lint yields with additional profit. Established on lower and upper profit, there is a 90% chance that if this combination is used in a *M. incognita* populated field, profit will fall between \$692.42/ha and \$875.58/ha. The input cost of this combination was \$83.67/ha, making it one of the most expensive treatment combinations. A study conducted by Zimet et al. (2004) found that nematicide treatments in *M. incognita* fields with fewer input costs had reduced lint yields resulting in partial net returns.

There was a decrease in *R. reniformis* eggs per gram of root and an increase in seed cotton and lint yield with the combination of a nematicide and fertilizer at either PHS or PHS + FB. Greater profit per hectare was obtained in *R. reniformis* infested soil with combinations that included at least one nematicide in combination with an additional fertilizer at either PHS or PHS + FB. The three highest profiting treatments from field trials had an additional application of $(NH_4)_2SO_4$ at PHS or PHS + FB. The same trend was seen with *M. incognita*; eggs per gram of root were reduced and seed cotton and lint yield increased in combinations with a nematicide ST + an additional fertilizer combination at PHS or PHS + FB. Profit increased in combinations with a nematicide in combination with an additional application of fertilizer at PHS or PHS + FB. The three highest profiting treatments from field trials had an additional application of 28-0-0-5 at PHS or PHS+ FB.

Conclusion

Our finding suggests that utilizing a nematicide with a fertilizer will increase yield and profit for growers with *R. reniformis* or *M. incognita* infested cotton fields. In *R. reniformis* field trials, 10 out of 10 fertilizer and nematicide combinations increased lint yields and 9 out of 10 combinations increased profits when compared to treatments with no nematicides. The three highest yielding combinations, a nematicide ST + $(NH_4)_2SO_4$ at PHS and FB, a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV at PHS and a nematicide ST + $(NH_4)_2SO_4$ + Vydate® C-LV + Max-

In® Sulfur at PHS also had the largest profits. Combinations with applications of $(NH_4)_2SO_4$ were the most economical in *R. reniformis* infested fields. These field trials suggested that the application of a nematicide ST increased lint yield by 8% and a single application of Vydate® C-LV increased lint yield by 19%. The addition of a nematicide ST increased profit by $32/ha and an additional application of Vydate® C-LV at PHS increased profit by $137/ha when compared to an additional fertilizer alone. In *M. incognita* field trials, 10 out of 10 fertilizer nematicide combinations increased lint yields and profit when compared to treatments with no nematicides. The three highest yielding combinations were a nematicide ST + Vydate® C-LV + Max-In® Sulfur at PHS and FB, a nematicide ST + 28-0-0-5 at PHS and FB, and a nematicide ST + 28-0-0-5 + Vydate® C-LV at PHS also had the largest profits. Combinations with applications of 28-0-0-5 were the most economical in *M. incognita* infested fields. These trails suggested that the application of a nematicide ST increased lint yield by 20%, a single application of Vydate® C-LV increased lint yield by 11% and sequential Vydate® C-LV applications increased overall lint by 5%. The addition of a nematicide ST increased profit by $54/ha when compared to an additional fertilizer alone. The addition of a single application of Vydate® C-LV increased profit by $93/ha and sequential applications of Vydate® C-LV at PHS and FB increased profit by $131/ha. In conclusion, our hypothesis that combining nematicides with fertilizers at PHS and FB plant growth stages can provide a management system for *R. reniformis* or *M. incognita* infested cotton fields with potential for economic gains.

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Table 1. Additional fertilizer and nematicide rates and application method used in Plant Science Research Center, Auburn, AL microplots and Tennessee Valley Research and Extension Center, Belle Mina, AL, and Plant Breeding Unit, Shorter, AL field trials in 2019 and 2020. Chemicals were applied on the upland cotton cultivar DP 1646 B2XF[z].

Chemical[z]	**Microplot rate**	**Field rate**	**Application**
$(NH_4)_2SO_4$ (21-0-0-24)	.52 g/m	108 kg/ha	Broadcast
28-0-0-5	0.289 ml/m	89 L/ha	Knifed
Vydate® C-LV (Oxamyl)	.004 ml/m	0.8 L/ha	Foliar spray
Max-In® Sulfur (0-0-19-13)	.007 ml/m	1.5 L/ha	Foliar spray
Aeris® (Imidacloprid & Thiodicarb)	0.375 mg ai/seed	0.375mg ai/seed	Seed treatment
COPeO™ Prime (Fluopyram	0.30 mg ai/seed	0.30mg ai/seed	Seed treatment

[Z] All DP 1646 B2XF seeds were pretreated with a BASF fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

Table 2. Additional fertilizer and nematicide combinations and application to upland cotton (DP 1646 B2XF) applied on Plant Science Research Center, Auburn, AL, microplots and field trials conducted at the Tennessee Valley Research and Extension Center, Belle Mina, AL and the Plant Breeding Unit, Shorter, AL in 2019 and 2020. Applications were at pinhead square (PHS) and/or first bloom (FB) cotton growth stage.

2019			
Treatment	**Fertilizer**	**Nematicides**	**Application**
1	$(NH_4)_2SO_4$	Untreated[z]	PHS
2	28-0-0-5	Untreated	PHS
3	$(NH_4)_2SO_4$	Aeris®	PHS
4	28-0-0-5	Aeris®	PHS
5	$(NH_4)_2SO_4$	Aeris®	PHS + FB
6	28-0-0-5	Aeris®	PHS + FB
7	$(NH_4)_2SO_4$	Aeris® + Vydate® C-LV	PHS
8	28-0-0-5	Aeris® + Vydate® C-LV	PHS
9	$(NH_4)_2SO_4$ + Max-In® Sulfur	Aeris® + Vydate® C-LV	PHS
10	28-0-0-5 + Max-In® Sulfur	Aeris® + Vydate® C-LV	PHS
11	$(NH_4)_2SO_4$ + Max-In® Sulfur	Aeris® + Vydate® C-LV	PHS + FB
12	28-0-0-5 + Max-In® Sulfur	Aeris® + Vydate® C-LV	PHS + FB

2020			
Treatment	**Fertilizer**	**Nematicides**	**Application**
1	$(NH_4)_2SO_4$	Untreated	PHS
2	28-0-0-5	Untreated	PHS
3	$(NH_4)_2SO_4$	COPeO™	PHS
4	28-0-0-5	COPeO™	PHS
5	$(NH_4)_2SO_4$	COPeO™	PHS + FB
6	28-0-0-5	COPeO™	PHS + FB
7	$(NH_4)_2SO_4$	COPeO™ + Vydate® C- LV	PHS
8	28-0-0-5	COPeO™ + Vydate® C-LV	PHS
9	$(NH_4)_2SO_4$ + Max-In® Sulfur	COPeO™ + Vydate® C-LV	PHS
10	28-0-0-5 + Max-In® Sulfur	COPeO™ + Vydate® C-LV	PHS
11	$(NH_4)_2SO_4$ + Max-In® Sulfur	COPeO™ + Vydate® C-LV	PHS + FB
12	28-0-0-5 + Max-In® Sulfur	COPeO™ + Vydate® C-LV	PHS + FB

[z] All DP 1646 B2XF seeds were pretreated by with a BASF fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

Table 3. Additional fertilizer and nematicide input costs for 2019 and 2020. Cost estimates were based off 2019 and 2020 market prices determine by a local agriculture sales representative.

Additional Input	2019 Additional Input Costs	2020 Additional Input Costs
$(NH_4)_2SO_4$	$54.50/ha	$51.63/ha
28-0-0-5	$30.50/ha	$29.90/ha
Seed Treatment [z]	$45.65/ha	$48.50/ha
Vydate C-LV	$38.30/ha	$38.94/ha
Max-In Sulfur	$11.35/ha	$11.20/ha

[z] The seed treatment Aeris® was used in 2019 and switched to COPeO™ Prime in 2020. The seed treatment input cost represents the combined average cost of Aeris® and COPeO™ Prime.

Table 4. Microplot LS means[z] from 2019 and 2020 of the effect of additional nematicide and fertilizer combinations on DP 1646 B2XF[y] plant height, cotton root fresh weight at pinhead square (PHS) (44 DAP in 2019 and 39 in 2020) and first bloom (FB) (75 DAP in 2019 and 62 DAP in 2020), *Rotylenchulus reniformis* eggs per gram of root at PHS, FB, PHS and FB sample data summed, and seed cotton yield at the Plant Science Research Center.

No	**Treatments**	**Plant height (cm)**	**Root fresh weight (g) PHS[x]**	***R. reniformis* eggs/g root PHS**	**Root fresh weight (g) FB[w]**	***R. reniformis* eggs/g root FB**	***R. reniformis* eggs/g root- PHS + FB**		**Seed cotton yield (g)**	
1	$(NH_4)_2SO_4$ - PHS	68	4.00	178	8.49	130	308	a	36	ab
2	28-0-0-5 – PHS	67	2.76	107	8.28	76	183	ab	33	ab
3	ST[v] + $(NH_4)_2SO_4$ – PHS	71	4.51	137	11.22	71	208	ab	28	b
4	ST + 28-0-0-5 – PHS	66	2.83	137	12.29	134	271	ab	36	ab
5	ST + $(NH_4)_2SO_4$ – PHS + FB	68	4.02	71	16.47	50	121	ab	45	ab
6	ST + 28-0-0-5 – PHS + FB	67	3.81	116	15.54	136	252	ab	41	ab
7	ST + $(NH_4)_2SO_4$ + Vydate® C-LV- PHS	71	3.42	85	9.47	67	152	ab	41	ab
8	ST + 28-0-0-5 + Vydate® C-LV- PHS	73	3.44	78	12.58	32	110	ab	47	ab
9	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur - PHS	71	3.78	224	11.66	48	272	ab	47	ab
10	ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur – PHS	73	2.88	43	11.89	27	70	b	44	ab
11	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max- In® Sulfur – PHS + FB	68	2.62	121	10.31	99	220	ab	55	a
12	ST + 28-0-0-5 + Vydate® C-LV+ Max-In®Sulfur – PHS + FB	73	3.75	90	12.88	57	147	ab	47	ab

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[z] LS-means followed by the same letter are not significantly different at $P \leq 0.10$ as determined by the Tukey Kramer method.

[y] All DP 1646 B2XF seeds were pretreated with a company fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

[x] PHS refers to the pinhead square plant growth stage when the first additional combination of fertilizers and nematicides were applied

[w] FB refers to the first bloom plant growth stage when the second additional combination of fertilizers and nematicides were applied

[v] ST refers to nematicide seed treatment, Aeris® (Thiodicarb) applied in 2019 and COPeO™ Prime (Fluopyram) applied in 2020

Table 5. Microplot LS means[z] from 2019 and 2020 of the effect of additional nematicide and fertilizer combinations on DP 1646 B2XF[y] plant height, cotton root fresh weight at pinhead square (PHS) (44 DAP in 2019 and 39 in 2020) and first bloom (FB) (75 DAP in 2019 and 62 DAP in 2020) *Meloidogyne incognita* eggs per gram of root at PHS, FB, PHS and FB sample data summed, and seed cotton yield at the Plant Science Research Center.

No	**Treatments**	**Plant height(cm)**	**Root fresh weight (g) PHS[x]**	***M. incognita* eggs/g root PHS**	**Root fresh weight (g) FB[w]**	***M. incognita* eggs/g root FB**	***M. incognita* eggs/g root PHS + FB**	**Seed cotton yield (g)**
1	$(NH_4)_2SO_4$ - PHS	51	3.53	165	7.28	389 ab	554 ab	34
2	28-0-0-5 – PHS	52	3.24	50	6.46	80 b	130 b	34
3	ST[v] + $(NH_4)_2SO_4$ – PHS	55	3.39	163	4.97	242 ab	405 ab	33
4	ST + 28-0-0-5 – PHS	61	4.14	65	6.21	159 ab	224 ab	32
5	ST + $(NH_4)_2SO_4$ – PHS + FB	42	2.56	257	6.84	420 a	677 a	33
6	ST + 28-0-0-5 – PHS + FB	45	2.78	124	6.40	185 ab	309 ab	47
7	ST + $(NH_4)_2SO_4$ + Vydate® C-LV- PHS	52	4.31	82	5.52	159 ab	241 ab	24
8	ST + 28-0-0-5 + Vydate® C-LV- PHS	55	4.41	54	5.30	92 b	146 b	34
9	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur - PHS	57	4.73	61	5.56	130 ab	191 ab	22
10	ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur – PHS	52	3.32	143	5.17	265 ab	408 ab	29
11	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur – PHS + FB	54	4.41	52	6.69	81 b	133 b	23
12	ST + 28-0-0-5 + Vydate® C-LV+ Max-In®Sulfur – PHS + FB	59	4.84	150	7.45	314 ab	464 ab	49

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[z] LS-means followed by the same letter are not significantly different at $P \leq 0.10$ as determined by the Tukey Kramer method

[y] All DP 1646 B2XF seeds were pretreated with a company fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

[x] PHS refers to the pinhead square plant growth stage when the first additional combination of fertilizers and nematicides were applied

[w] FB refers to the first bloom plant growth stage when the second additional combination of fertilizers and nematicides were applied

[v] ST referees to nematicide seed treatment, Aeris® (Thiodicarb) applied in 2019 and COPeO™ Prime(Fluopyram) applied in 2020

Table 6. Field trial LS Means[z] from 2019 and 2020 of the effect of nematicide and fertilizer combinations on DP 1646 B2XF[y] stand, cotton root fresh weight at pinhead square (PHS) (49 DAP in 2019 and 40 in 2020) and first bloom (FB) (75 DAP in 2019 and 69 DAP in 2020) *Rotylenchulus reniformis* eggs per gram of root at PHS, FB, PHS and FB sample data summed, and yield at the Tennessee Valley Research and Extension Center.

No	Treatments	Stand[x]	Root fresh weight (g) PHS[w]	*R. reniformis* eggs/g root PHS	Root fresh weight (g) FB[v]	*R. reniformis* eggs/g root FB	*R. reniformis* eggs/g root PHS + FB	Lint (kg/ha)
1	$(NH_4)_2SO_4$ - PHS	59	2.69	5570	12.09	153	5723	1257 b
2	28-0-0-5 – PHS	56	2.67	6580	11.59	241	6821	1200 b
3	ST[u] + $(NH_4)_2SO_4$ - PHS	57	2.97	4553	13.14	182	4735	1345 ab[z]
4	ST + 28-0-0-5 - PHS	56	3.10	4209	12.89	145	4354	1368 ab
5	ST + $(NH_4)_2SO_4$ – PHS + FB	56	3.12	2416	12.51	142	3126	1566 ab
6	ST + 28-0-0-5 – PHS + FB	55	2.29	4980	11.22	160	5140	1223 b
7	ST + $(NH_4)_2SO_4$ + Vydate® C-LV- PHS	57	3.36	2972	13.66	154	2558	1803 a
8	ST + 28-0-0-5 + Vydate® C-LV- PHS	57	2.91	5244	13.60	149	5393	1411 ab
9	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur - PHS	58	2.70	5398	14.21	121	5519	1542 ab
10	ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur - PHS	55	2.81	4051	12.08	105	4156	1412 ab
11	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max- In® Sulfur – PHS + FB	58	3.01	2120	13.01	108	2228	1432 ab
12	ST + 28-0-0-5 + Vydate® C-LV+ Max-In®Sulfur – PHS + FB	59	2.99	5529	11.98	141	5670	1455 ab

[z] LS-means followed by the same letter are not significantly different at $P \leq 0.10$ as determined by the Tukey Kramer method

[y] All DP 1646 B2XF seeds were pretreated with a BASF fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

[x] Stand count is the number of seedlings per 7.6 m of row collected 14 DAP

[w] PHS refers to the pinhead square plant growth stage when the first additional combination of fertilizers and nematicides were applied

[v] FB refers to the first bloom plant growth stage when the second additional combination of fertilizers and nematicides were applied

[u] ST refers to nematicide seed treatment, Aeris® (Thiodicarb) applied in 2019 and COPeO™ Prime(Fluopyram) applied in 2020

Table 7. Field trial LS Means[z] from 2019 and 2020 representing profit mean (\$/ha), and lower and upper profit determined by ANOVA ($P \leq 0.10$) for fertilizer and nematicide combinations on DP 1646 B2XF[y] at the Tennessee Valley Research and Extension Center. Revenue was calculated using prices determine by the United States Department of Agriculture upland cotton announcement of \$1.32/ha in 2019 and \$1.54/ha in 2020. Profit was calculated by subtracting additional input costs (\$/ha) from revenue.

No	Treatments	Mean profit	Lower profit	Upper profit	Additional fertilizer and nematicide input cost[x]
1	$(NH_4)_2SO_4$ - PHS[w]	\$862.69 b	\$677.97	\$1047.41	\$56.06
2	28-0-0-5 – PHS	\$844.55 b	\$659.83	\$1029.27	\$30.20
3	ST[v] + $(NH_4)_2SO_4$ – PHS	\$880.33 ab	\$695.61	\$1065.05	\$100.13
4	ST + 28-0-0-5 – PHS	\$920.39 ab	\$735.67	\$1105.11	\$77.27
5	ST + $(NH_4)_2SO_4$ – PHS + FB[u]	\$988.46 ab	\$803.74	\$1173.18	\$153.19
6	ST + 28-0-0-5 – PHS + FB	\$784.57 b	\$599.84	\$962.29	\$107.47
7	ST + $(NH_4)_2SO_4$ + Vydate® C-LV- PHS	\$1175.87 a	\$991.15	\$1360.59	\$138.73
8	ST + 28-0-0-5 + Vydate® C-LV- PHS	\$913.44 ab	\$728.72	\$1098.16	\$115.87
9	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur – PHS	\$974.36 ab	\$789.64	\$1159.08	\$150.00
10	ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur – PHS	\$902.49 ab	\$717.77	\$1087.21	\$127.14
11	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max- In® Sulfur – PHS + FB	\$882.36 ab	\$697.64	\$1067.08	\$252.93
12	ST + 28-0-0-5 + Vydate® C-LV+ Max-In®Sulfur – PHS + FB	\$922.73 ab	\$738.01	\$1107.45	\$207.21

[z] LS-means followed by the same letter are not significantly different at $P \leq 0.10$ as determined by ANOVA

[y] All DP 1646 B2XF seeds were pretreated with a BASF fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

[x] Additional input costs from 2019 and 2020 were averaged to determine a single input cost for treatment analysis

[w] PHS refers to the pinhead square plant growth stage, when the first combination of additional fertilizers and nematicides were applied

[v] ST refers to nematicide seed treatment, Aeris® (Thiodicarb) applied in 2019 and COPeO™ Prime(Fluopyram) applied in 2020

[u] FB refers to the first bloom plant growth stage, when the second combination of fertilizers and nematicides were applied

Table 8. Field trial LS Means^z from 2019 and 2020 of the effect of nematicide and fertilizer combinations on DP 1646 B2XF^y cotton root fresh weight at pinhead square (PHS) (49 DAP in 2019 and 40 in 2020) and first bloom (FB) (75 DAP in 2019 and 69 DAP in 2020) *Meloidogyne incognita* eggs per gram of root at PHS, FB, PHS and FB sample data summed, and yield at the Plant Breeding Unit.

No	Treatments	Stand^x	Root fresh weight (g) PHS^w	*M. incognita* eggs/g root PHS	Root fresh weight (g) FB^v	*M. incognita* eggs/g root FB	*M. incognita* eggs/g root PHS + FB	Lint (kg/ha)
1	$(NH_4)_2SO_4$ - PHS	44	7.47	563	14.89	133	696	832 b
2	28-0-0-5 – PHS	41	8.90	1397	13.32	264	1661	788 b
3	ST^u + $(NH_4)_2SO_4$ – PHS	46	8.84	613	15.50	163	776	918 ab
4	ST + 28-0-0-5 – PHS	52	9.85	664	14.35	160	824	978 ab
5	ST + $(NH_4)_2SO_4$ – PHS + FB	51	10.61	389	11.73	176	590	1044 ab
6	ST + 28-0-0-5 – PHS + FB	53	7.83	937	13.85	269	1206	1101 ab
7	ST + $(NH_4)_2SO_4$ + Vydate® C-LV-PHS	52	8.89	678	12.94	194	872	1050 ab
8	ST + 28-0-0-5 + Vydate® C-LV-PHS	54	8.75	833	13.35	271	1104	1096 ab
9	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur - PHS	55	8.86	647	13.51	63	710	1051 ab
10	ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur - PHS	56	7.95	490	12.47	100	565	1052 ab
11	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max- In® Sulfur – PHS + FB	48	9.27	742	13.86	351	1093	1010 ab
12	ST + 28-0-0-5 + Vydate® C-LV+ Max-In®Sulfur – PHS + FB	57	10.04	1025	13.01	190	1215	1265 a

[z] LS-means followed by the same letter are not significantly different at $P \leq 0.10$ as determined by the Tukey Kramer method

[y] All DP 1646 B2XF seeds were pretreated with a BASF fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

[x] Stand count is the number of seedlings per 7.6 m of row collected 14 DAP

[w] PHS refers to the pinhead square plant growth stage at 49 DAP, when the first combination of fertilizers and nematicides were applied

[v] FB refers to the first bloom plant growth stage at 85 DAP, when the second combination of fertilizers and nematicides were applied

[u] ST refers to nematicide seed treatment, Aeris® (Thiodicarb) applied in 2019 and COPeO™ Prime(Fluopyram) applied in 2020

Table 9. Field trial LS Means[z] from 2019 and 2020 representing profit mean ($/ha), and lower and upper profit determined by ANOVA ($P \leq 0.10$) for fertilizer and nematicide combinations on DP 1646 B2XF[y] at the Plant Breeding Unit. Revenue was calculated using prices determine by the United States Department of Agriculture upland cotton announcement of $1.32/ha in 2019 and $1.54/ha in 2020. Profit was calculated by subtracting additional input costs ($/ha) from revenue.

No	Treatments	Mean profit		Lower profit	Upper profit	Additional fertilizer and nematicide input cost[x]
1	$(NH_4)_2SO_4$ – PHS[w]	$553.01	b	$461.43	$644.59	$56.06
2	28-0-0-5 - PHS	$544.05	b	$452.47	$635.63	$30.20
3	ST[v] + $(NH_4)_2SO_4$ - PHS	$569.21	ab	$477.63	$660.79	$100.13
4	ST + 28-0-0-5 - PHS	$635.85	ab	$544.27	$727.43	$77.27
5	ST + $(NH_4)_2SO_4$ – PHS + FB[u]	$608.01	ab	$516.43	$699.59	$153.19
6	ST + 28-0-0-5 – PHS + FB	$695.76	ab	$604.18	$787.34	$107.47
7	ST + $(NH_4)_2SO_4$ + Vydate® C-LV- PHS	$627.25	ab	$535.67	$718.83	$138.73
8	ST + 28-0-0-5 + Vydate® C-LV- PHS	$683.50	ab	$591.92	$775.08	$115.87
9	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max-In® Sulfur - PHS	$616.22	ab	$524.64	$707.80	$150.00
10	ST + 28-0-0-5 + Vydate® C-LV+ Max-In® Sulfur - PHS	$640.59	ab	$549.01	$732.17	$127.14
11	ST + $(NH_4)_2SO_4$ + Vydate® C-LV+ Max- In® Sulfur – PHS + FB	$575.53	ab	$483.95	$667.11	$252.93
12	ST + 28-0-0-5 + Vydate® C-LV+ Max-In®Sulfur – PHS + FB	$784.00	a	$692.42	$875.58	$207.21

[z] LS-means followed by the same letter are not significantly different at $P \leq 0.10$ as determined by ANOVA

[y] All DP 1646 B2XF seeds were pretreated with a BASF fungicide and insecticide metalaxyl, pyraclostrobin, myclobutanil, imidacloprid, and fluxapyroxa

[x] Additional input costs from 2019 and 2020 were averaged to determine a single input cost for treatment analysis

[w] PHS refers to the pinhead square plant growth stage, when the first additional combination of fertilizers and nematicides were applied

[v] ST refers to nematicide seed treatment, Aeris® (Thiodicarb) applied in 2019 and COPeO™ Prime(Fluopyram) applied in 2020

[u] FB refers to the first bloom plant growth stage, when the second combination of additional fertilizers and nematicides were applied

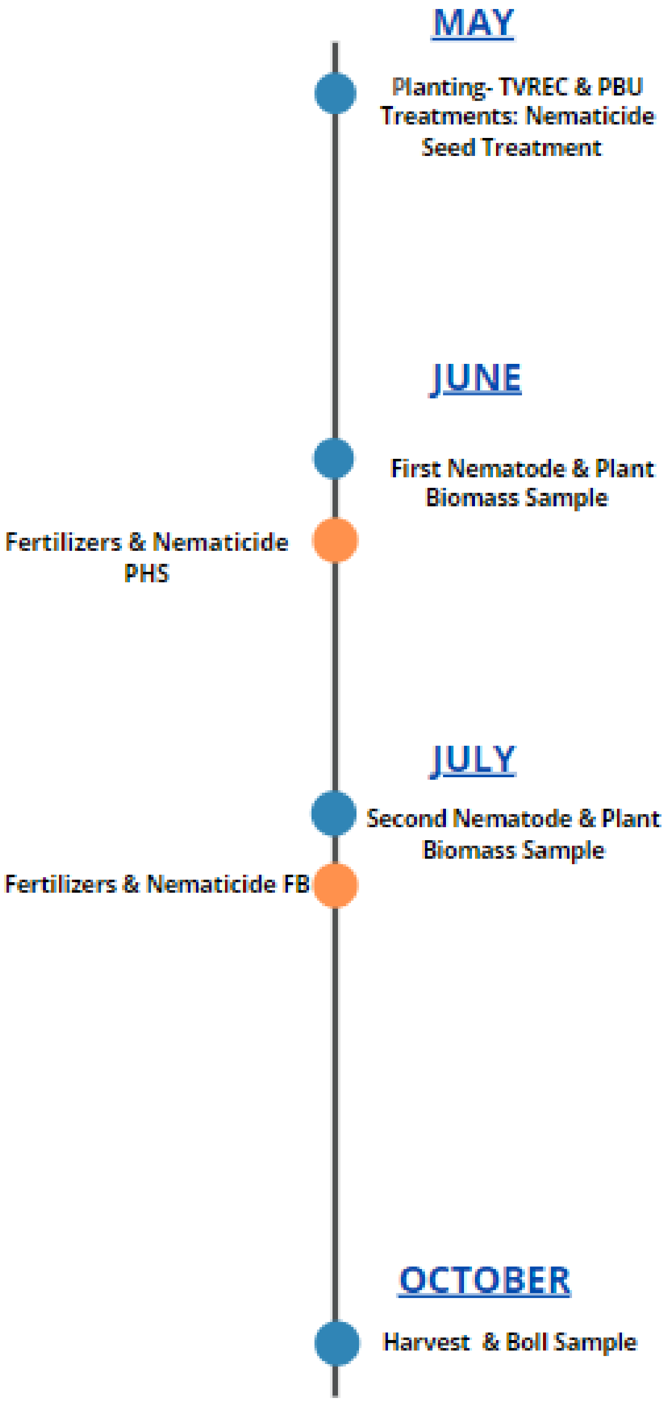

Figure 4. Timeline of planting, sampling, fertilizer and nematicide applications and harvest on microplot and field trials in 2019 and 2020.

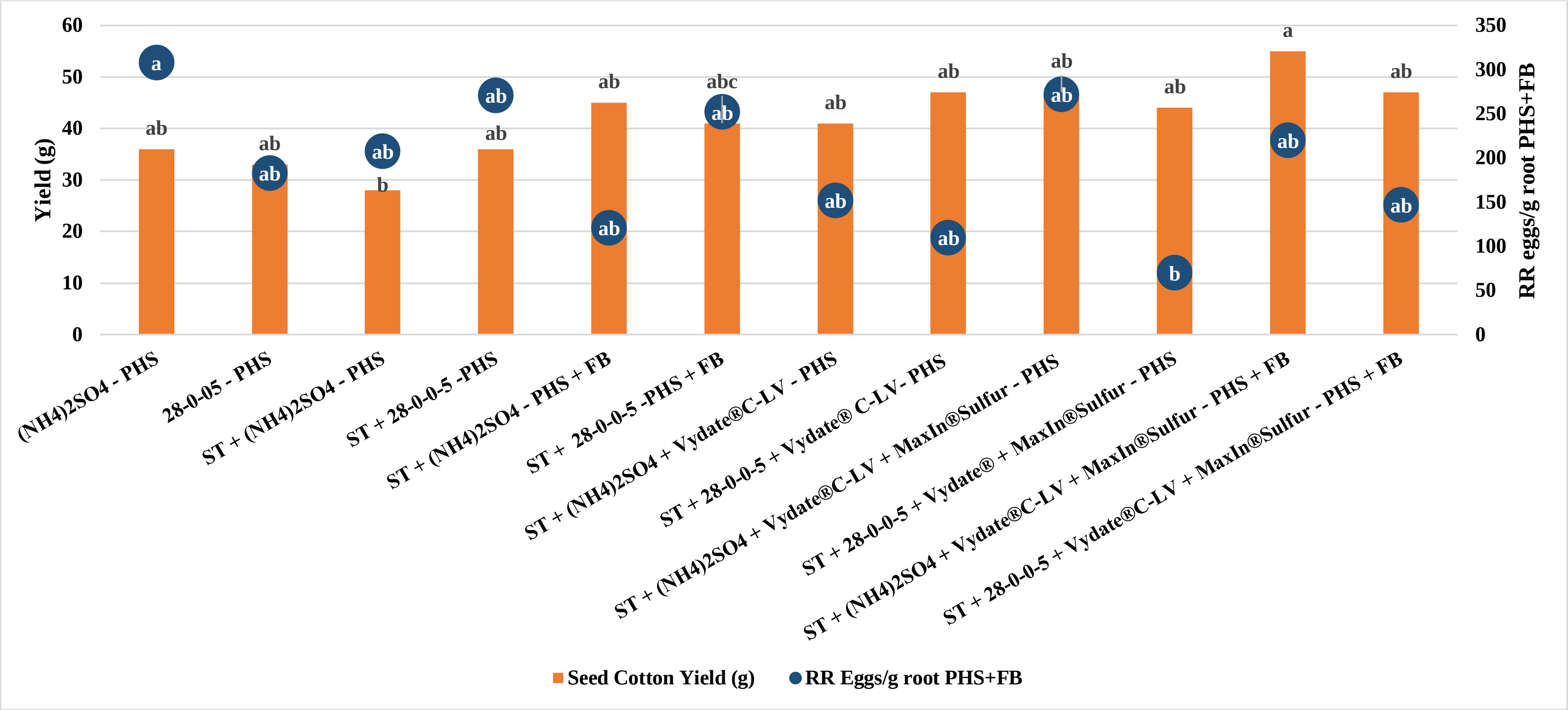

Figure 5. Microplot LS means from 2019 and 2020 of the effect of additional fertilizer and nematicide combinations on DP 1646 B2XF seed cotton yield and *Rotylenchulus reniformis* eggs per gram of root at PHS and FB at the Plant Science Research Center in Auburn, AL. Means of bars with the same letter above are not significantly different (Tukey Kramer, P ≤ 0.10).

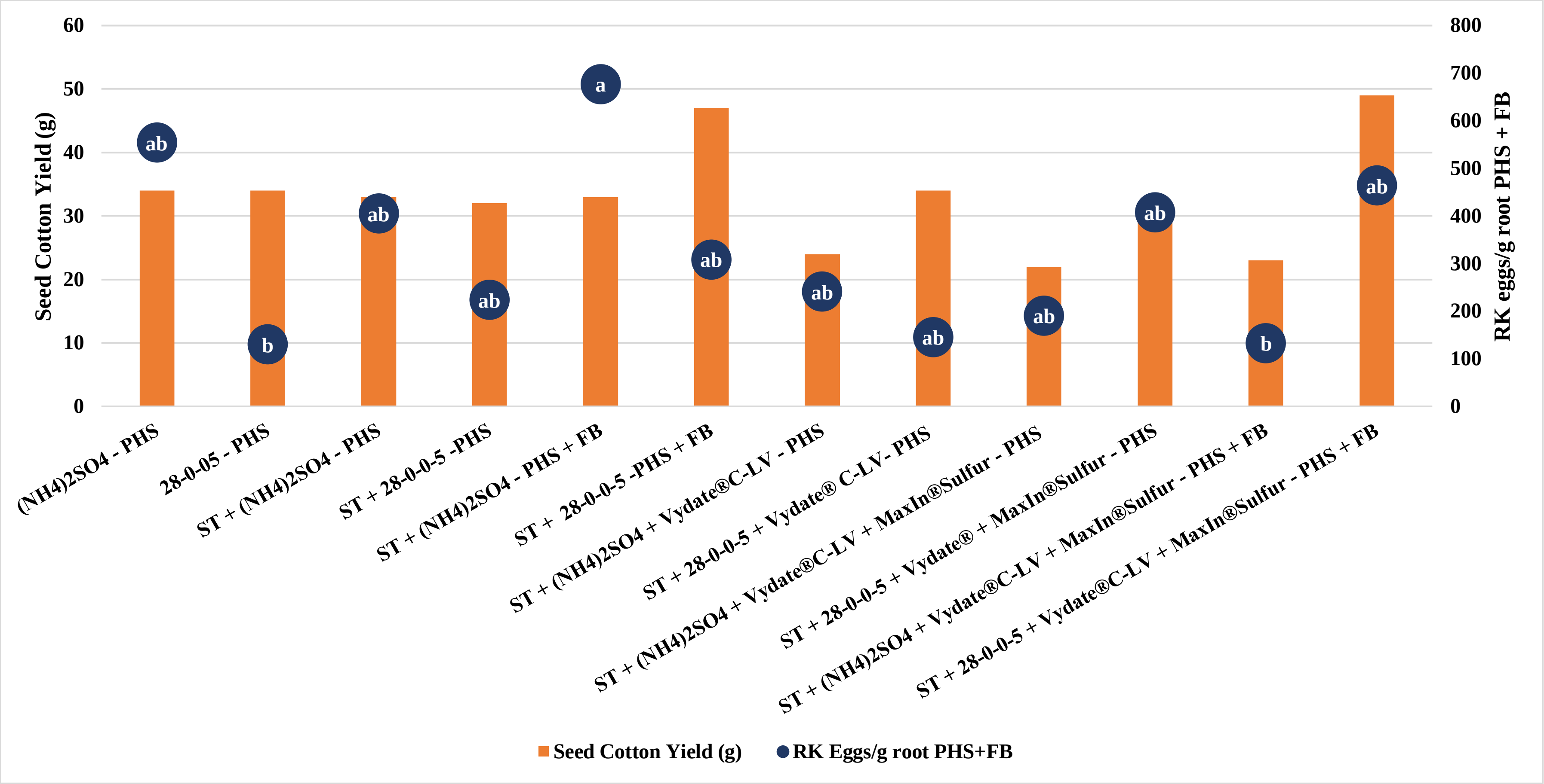

Figure 6. Microplot LS means from 2019 and 2020 of the effect of additional fertilizer and nematicide combinations on DP 1646 B2XF seed cotton yield and *Meloidogyne incognita* eggs per gram of root at PHS and FB at the Plant Science Research Center in Auburn, AL. Means of bars with the same letter above are not significantly different (Tukey Kramer, $P \leq 0.10$).

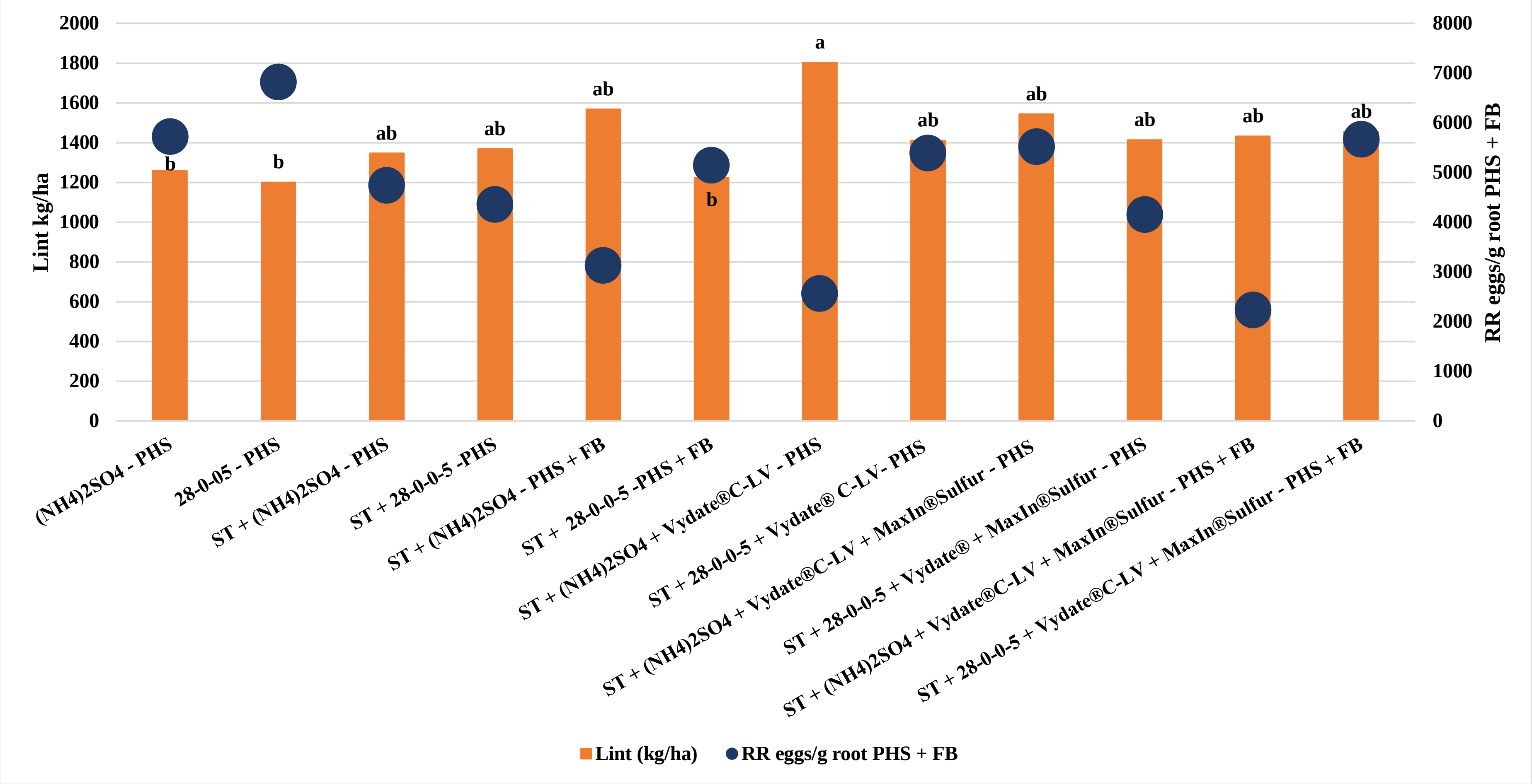

Figure 7. Field trial LS means from 2019 and 2020 of the effect of additional fertilizer and nematicide combinations on DP 1646 B2XF seed cotton yield and *Rotylenchulus reniformis* eggs per gram of root at PHS and FB at the Tennessee Valley Research and Extension Center in Belle Mina, AL. Means of bars with the same letter above are not significantly different (Tukey Kramer, $P \leq 0.10$).

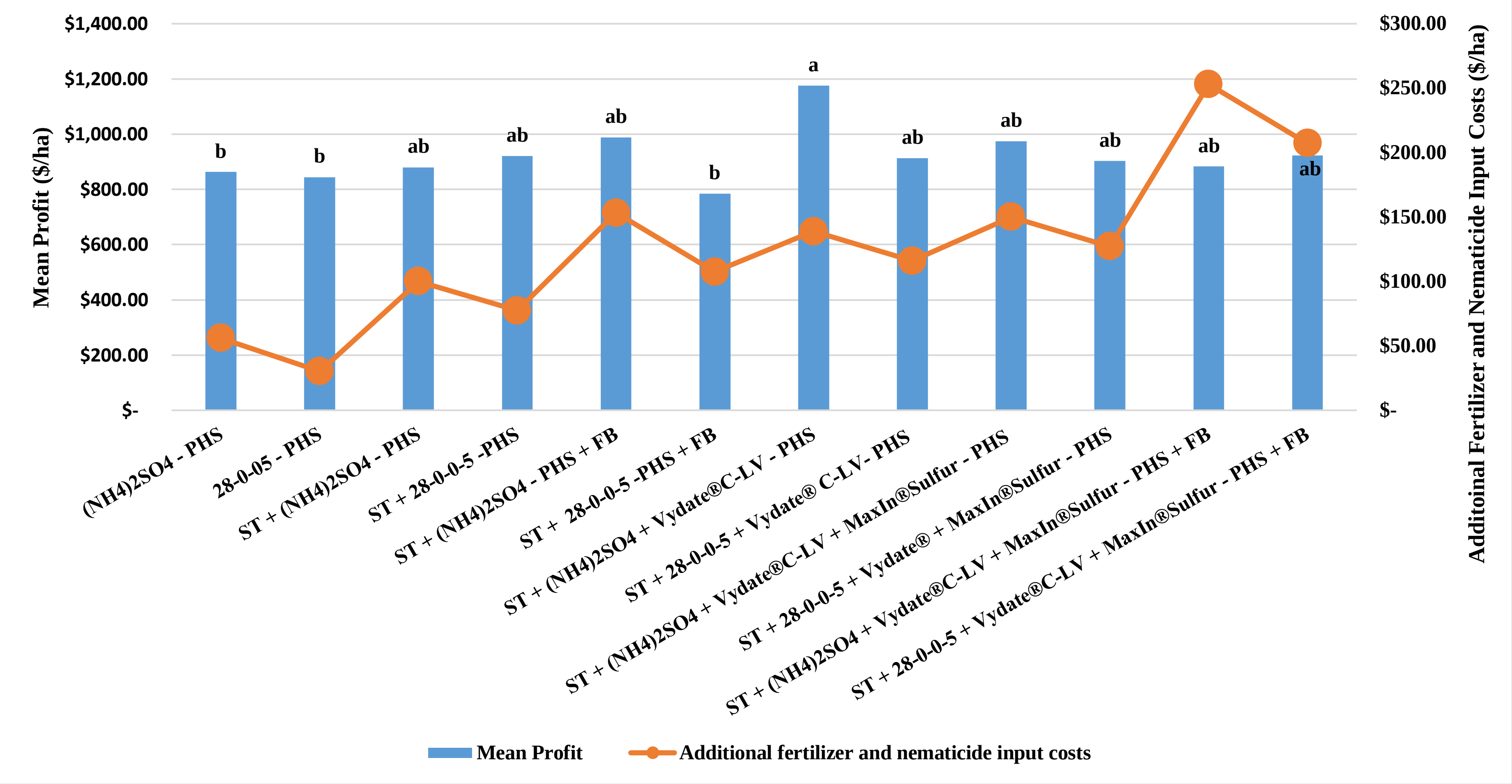

Figure 8. Field trial LS means from 2019 and 2020 representing profit mean ($/ha), and lower and upper profit determined by ANOVA ($P \leq 0.10$) for fertilizer and nematicide combinations on DP 1646 B2XF at the Tennessee Valley Research and Extension Center. Revenue was calculated using prices determine by the United States Department of Agriculture upland cotton announcement of $1.32/ha in 2019 and $1.54/ha in 2020. Profit was calculated by subtracting additional input costs ($/ha) from revenue. Input costs were determined from a local sales representative in Alabama. Additional input costs from 2019 and 2020 were averaged to determine a single input cost for treatment analysis.

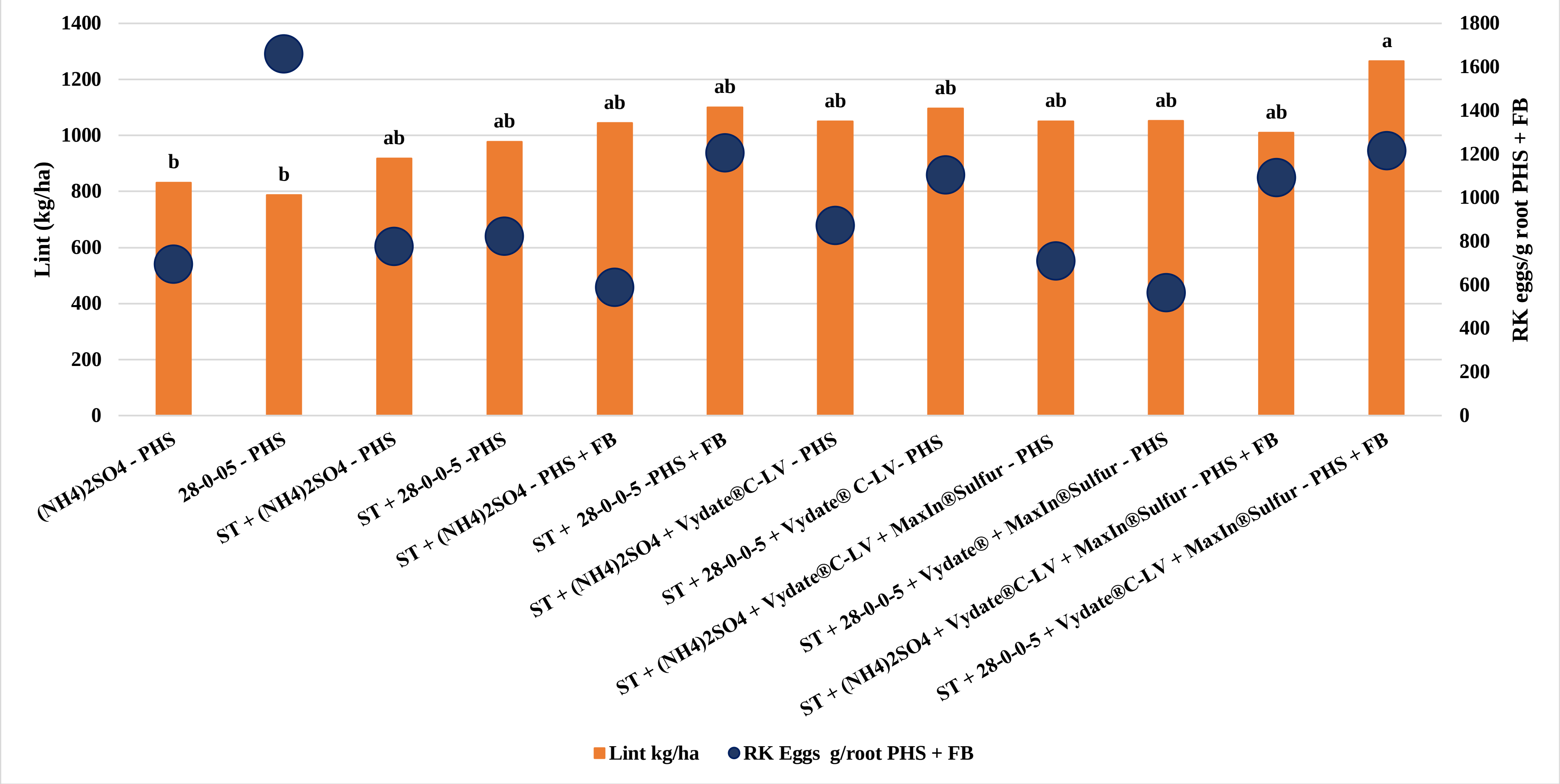

Figure 9. Field trial LS means from 2019 and 2020 of the effect of additional fertilizer and nematicide combinations on DP 1646 B2XF seed cotton yield and *Meloidogyne incognita* eggs per gram of root at PHS and FB at the Plant Breeding Unit in Tallassee, AL. Means of bars with the same letter above are not significantly different (Tukey Kramer, $P \leq 0.10$).

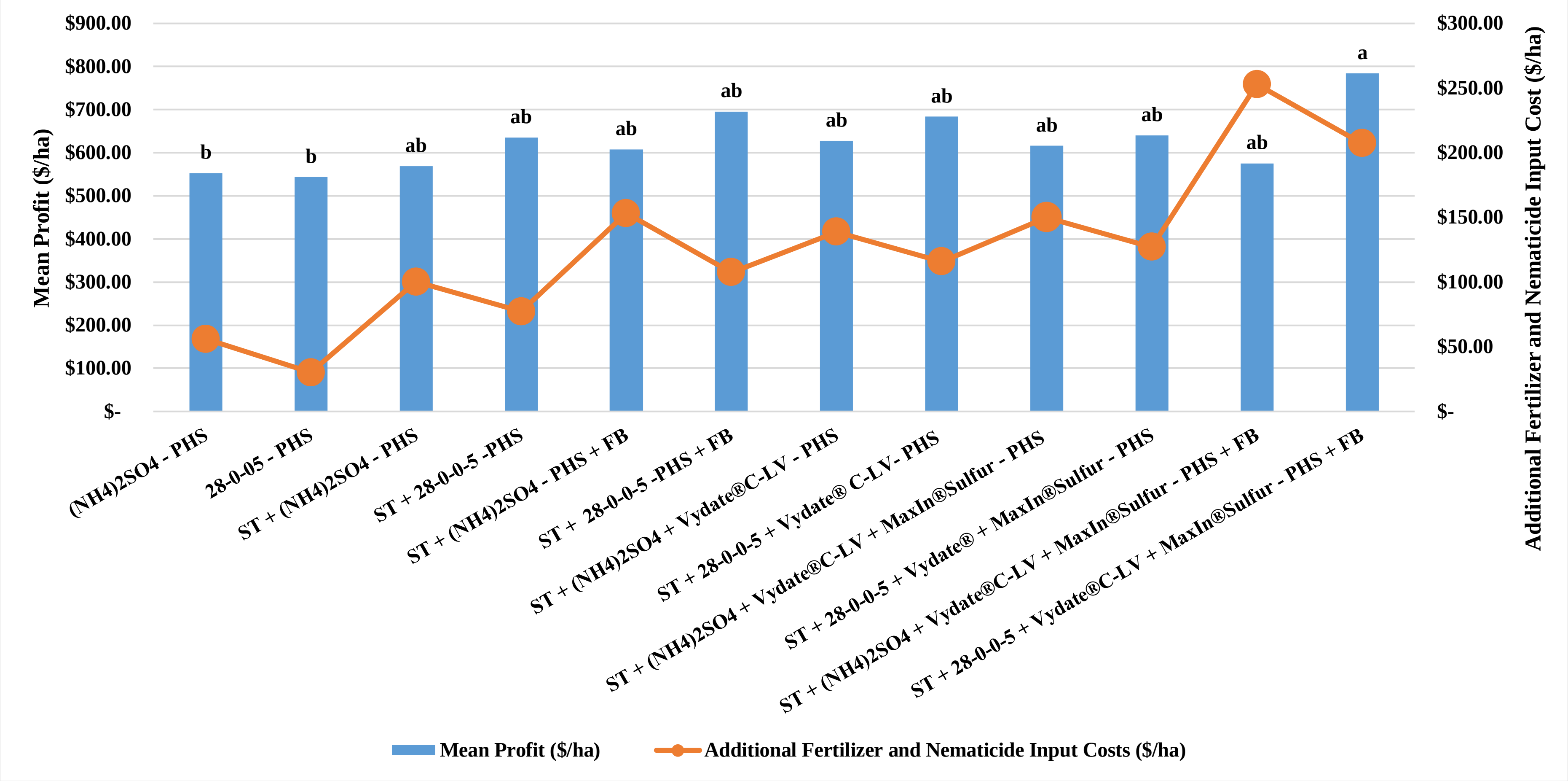

Figure 10. Field trial LS means from 2019 and 2020 representing profit mean ($/ha), and lower and upper profit determined by ANOVA ($P \leq 0.10$) for fertilizer and nematicide combinations on DP 1646 B2XF at the Plant Breeding Unit. Revenue was calculated using prices determine by the United States Department of Agriculture upland cotton announcement of $1.32/ha in 2019 and $1.54/ha in 2020. Profit was calculated by subtracting additional input costs ($/ha) from revenue. Input costs were determined from a local sales representative in Alabama. Additional input costs from 2019 and 2020 were averaged to determine a single input cost for treatment analysis.