Evaluation of *Meloidogyne incognita* and *Rotylenchulus reniformis* nematode resistant cotton cultivars with supplemental Corteva Agriscience nematicides

by

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Abstract

Meloidogyne incognita (root-knot nematode) and *Rotylenchulus reniformis* (reniform nematode) accounted for an estimated 7% of the cotton yield lost in Alabama in 2020 and 7.5% lost in 2021. New nematode resistant cotton cultivars and new nematicides are becoming available to help manage nematode induced yield reductions. The objectives of this study were: 1) to determine the yield potential of the new *M. incognita* resistance variety PHY 360 W3FE and the *R. reniformis* resistant variety PHY 332 W3FE in nematode infested fields and 2) to evaluate the effects of combining the new nematicide Reklemel™ (fluazaindolizine), with Vydate® C-LV (oxamyl), and the seed treatment BIOST Nematicide 100 with resistant cotton varieties on nematode population levels and cotton lint yield. In 2020 and 2021, four field trials were established in nematode infested fields. Two resistant cultivars, PHY 360 W3FE or PHY 332 W3FE, and a susceptible cultivar, PHY 340 W3FE were evaluated with and without the addition of nematicides BIOST seed treatment and Reklemel plus Vydate® C-LV in-furrow sprays applied at planting. Field trials in 2020 indicated *M. incognita* population levels near 45 days after planting were 63% lower on PHY 360 W3FE and 73% lower for *R. reniformis* on the PHY 332 W3FE compared to the susceptible PHY 340 W3FE. Nematode eggs per gram of root were further reduced with the addition of Reklemel™ and Vydate® C-LV to both susceptible and resistant varieties. In the *M. incognita* tests, BIOST Nematicide 100 (0.026 mg ai/seed) + the mid-rate rate of Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) supported the greatest lint yield (1720 kg/ha), which was increased by 357 kg/ha over the lowest yielding treatment, Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) (1152 kg/ha). In the *R. reniformis* tests, with the mid-rate of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) supported the greatest yields (1777 kg/ha) over the lowest yielding treatment, untreated control by 488 kg/ha. Field trials in 2021 indicated that population levels near 40 days after planting for *M. incognita* were 82% lower on PHY 360 W3FE and 87% lower for *R. reniformis* on the PHY 332 W3FE compared to PHY 340 W3FE. Additionally, in 2021, nematode eggs per gram of root were further reduced 35%, 59%, and 31% after addition of Reklemel and Vydate® C-LV to PHY 340

W3FE, to PHY 360 W3FE, and PHY 332 W3FE, respectively. In the *M. incognita* tests, PHY 360 W3FE with BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) at a medium rate supported the greatest lint yield (14021 kg/ha), which was increased by 310 kg/ha over the lowest yielding treatment, PHY 340 W3FE + BIOST Nematicide 100 (1092 kg/ha). In the *R. reniformis* tests, PHY 332 W3FE with BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha), the medium rate, supported the greatest yields (1591 kg/ha) over the lowest yielding treatment, PHY 340 W3FE by 583 kg/ha. Overall, planting these resistant varieties PHY 360 W3FE and PHY 332 W3FE improved yields an average of 364 kg/ha which is equal to approximately $899/ha while limiting nematode population increases; the addition of the nematicides also further increased yields 152 kg/ha of the nematode resistant varieties equaling approximately $376/ha.

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List of Abbreviations

DAP	Days After Planting
PHY	PhytoGen
RKN	Root-knot nematode
RN	Reniform nematode
SAT	Seed Applied Treatment

Chapter 1: Introduction and Review of Literature

Introduction and Problem Statement

The objective of this research was to improve nematode management in cotton (*Gossypium hirsutum* L.) related to *Meloidogyne incognita* ((Kofoid & White) Chitwood) (root-knot nematode, RKN) and *Rotylenchulus reniformis* (Linford & Oliveira) (reniform nematode, RN) in Alabama. Two main objectives for this project were to evaluate a *M. incognita* resistant cotton cultivar PHY 360 W3FE and a *R. reniformis* resistant cotton cultivar PHY 332 W3FE in nematode infested fields and to determine if there was an additional benefit to adding nematicides. An estimated 659,000 bales of cotton were lost due to plant-parasitic nematodes totaling a 4% loss in U.S cotton production in the 2020 growing season (Lawrence et al. 2021). Plant-parasitic nematodes often damage cotton roots by feeding on the root and providing a pathway for secondary infection of bacteria or fungal pathogens (Lambert and Bekal, 2002). Nematodes can significantly reduce cotton yields because their population numbers can increase dramatically in a growing season. Under the right environmental conditions, nematodes can produce hundreds of eggs in one generation with several generations in a growing season (Wrona et al., 1996).

Plant-parasitic nematodes come from the diverse phylum of molting animals known as Nematoda (Blaxter and Koutsovoulos, 2014). Within Nematoda, there are three orders of plant parasites: Triplonchida (Cobb 1920), Dorylaimida (Pearse 1942), and Tylenchida (Thorne 1949) (Sultana et al, 2013). Most plant-parasitic nematodes belong in the order Tylenchida, but it is hypothesized that the ability of these nematodes to parasitize plants evolved from fungal feeding ancestors who feed on mosses, algae, root hair, and epidermal cells (Holterman et al, 2008). Plant parasitic nematodes are microscopic animals that are worm-shaped and transparent with spear-like mouth parts called stylets which puncture plant cells to feed on the cellular contents (Lee and Atkinson, 1976). Plant-parasitic nematodes can feed on all parts of a plant including roots, stems, leaves, flowers, and even the seeds (Lambert and Bekal, 2002). There are three main types of feeding styles for these nematodes: endoparasitic, semi-endoparasitic, and ectoparasitc (Sato et al, 2019). Endoparastic nematodes completely enter the roots and feed on internal tissues of the plant; semi-endoparasitic nematodes partially enter the plant roots to feed while the posterior part remains in the soil (Perrine-Walker, 2019; Sato et al, 2019). Ectoparasitic nematodes live on the outside of the plant in the soil and don't enter the plant root, but they do use their stylet to puncture plant cells to feed (Decraemer and Geraert, 2006). *Meloidogyne incognita* is an endoparasitic nematode and *R. reniformis* is a semi-endoparastic nematode. *Meloidogyne incognita* was first discovered parasitizing cotton in 1889 and *R. reniformis* was first identified as a pathogen of cotton in 1940 (Atkinson, 1892; Smith, 1940; Sasser, 1954). Recent studies show that *M. incognita* and *R. reniformis* can damage upland cotton production by decreasing yields by nearly 50% in some fields because both pathogens reduce the cotton plants' ability to produce lint (Khanal et al., 2018; Dyer et al., 2019; Lawrence et al, 2021).

Cotton

Cotton is an important cash crop grown worldwide (USDA, 2022). There are four independently domesticated species of cotton: *Gossypium arboreum* L., *G. herbaceum* L., *G. barbadense* L., and *G. hirsutum* L. (Coppens d'Eeckenbrugge and Lacape, 2014; Fang and Percy, 2015). *Gossypium arboreum* L. is a tree cotton native to southern Asia, and *G. herbaceum* L. is levant cotton native to Africa (Lee and Fang, 2015). *Gossypium barbadense* L. referred to as creole cotton is native to South America. *Gossypium hirsutum,* also known as upland cotton, is native to Central America (Lee and Fang, 2015) and accounts for 90% of the world's cotton production, and 97% of the United States cotton production (Cotton Incorporated, 2020; USDA, 2020). More than 12 million acres of upland cotton were planted in the U.S. for the 2020 growing season (National Cotton Council, 2021). Upland cotton is used for a variety of products; however, its main end use is for apparel (USDA, 2020). Cotton is one of the most important cash crops in U.S production and is the leading natural fiber cash crop exported in the United States (Ma et al, 2018; USDA, 2020). An estimated 532,000 acres of upland cotton were harvested in Alabama in the 2019 growing season with a value of production at $331,776,000 at $0.603/lb (USDA, 2020). In

Alabama, 90% of the cotton grown is grown as a monoculture or in a rotation with crops that are also hosts to nematodes; this creates a favorable environment for nematode population levels to continuously increase (Gazaway and McLean, 2003). *Rotylenchulus reniformis* is estimated to reduce cotton yields by 3% across Alabama; however, yield losses of 50% have been reported for individual fields infested with this pathogen. Similarly, M. *incognita* reduces cotton yields in infested fields by an estimated 4% across Alabama, but losses can be much higher in individually infested fields (Lawrence et al, 2021).

Meloidogyne incognita

Meloidogyne spp. nematodes were first reported causing damage on cucumbers in 1855 by Reverend Miles Joseph Berkeley (Mitkowski, and Abawi, 2003). In the late 1800s and early 1900s, numerous names were given to root-knot nematode, including *Heterodera radicicola* (Greeff, 1872), *Anguillula marioni* (Cornu, 1879), *Meloidogyne exiqua* (Goeldi, 1887), *Anguillula arnaria* (Lavergne, 1901), *Hederodera vialac* (Kofoid and White, 1919), *Oxyuris incognita* (Kofoid & White, 1919) (Hunt and Handoo, 2009). In 1949, Chitwood significantly revised the taxonomy of the root-knot nematodes. He distinguished root-knot nematodes from the cyst nematodes, *Heterodera*, into a separate genus, *Meloidogyne*, with five distinct species *M. incognita*, *M. arenaria*, *M. javanica*, *M. hapla*, *M. exiqua* (Hunt and Handoo, 2009).

The root-knot nematode genus, *Meloidogyne* spp., consists of approximately one hundred species found in warm temperate and tropical regions worldwide (Álvarez-Ortega et al, 2019). *Meloidogyne* species destroy approximately 5% of the world crop production (Ralmi et al, 2016). Root-knot nematodes (*Meloidogyn*e spp.) are important common pathogens of several agricultural crops in the U.S. (Ye et al, 2019). More than 3,000 plant species are characterized as host to root-knot nematodes with numerous agricultural crops being attacked by a minimum of at least one root-knot nematode species (Ralmi et al, 2016). *Meloidogyne incognita* is a sedentary endoparasitic nematode that reproduces and feeds on altered living plant cells within the plant roots (Castagnone-Sereno et al, 2013). The altered plant cells are feeding structures described as giant cells (Siddiqui et al, 2014). This feeding habit is symptomized by the knots or galling that form throughout the host plant's root system as the nematode feeds (Siddiqui et al, 2014). The life cycle of *M. incognita* contains an egg stage, four juvenile stages, and an adult stage (Castagnone-Sereno et al, 2013). Castagnone-Sereno et al. (2013) summarized the life cycle of *M. incognita* nematode as follows: the nematode molts inside the egg and hatches as a second-stage juvenile. The second-stage juvenile is the infective stage of the nematode life cycle. Once the second-stage juvenile hatches, it begins searching for a new host. After the nematode finds its host, it invades the root at the zone of elongation behind the root cap and migrates to the vascular cylinder (Castagnone-Sereno et al, 2013; Simon, 2012). The nematode will pierce the plant's vascular cells with its stylet in the zone of elongation (Taylor and Sasser, 1978; Bartlem et al, 2014). The juvenile nematode begins to feed on the cellular contents, causing the cells to differentiate undergoing intense hyperplasia multiplication forming specialized nurse cells or giant cells (Hoth et al, 2008; Taylor and Sasser, 1978). The roots around the feeding nematode begin to develop galls (Mitkowski and Abawi, 2003) which reduce the cotton plant's ability to absorb nutrients leading to wilting, stunting, chlorotic leaves, and yield loss (Overstreet et al., 2016). The *M. incognita* nematode completes its life cycle, and the female nematode produces a gelatinous medium that contains the eggs outside of the root surface (Simon, 2012).

The major *Meloidogyn*e species studied are *M. arenaria*, *M. hapla*, *M. incognita*, *M. javanica,* and *M. enterolobii* with *M. incognita* being the most studied so far of all *Meloidogyne* species (Elling, 2013; Ye et al, 2013; Mitiku, 2018). *Meloidogyne incognita* is just one of the three *Meloidogyne spp*. species in the United States that causes cotton yield losses for farmers each year (Thiessen and Rivera, 2019). *Meloidogyne incognita* was first identified in the southern United States on cotton in 1889 (Sasser, 1954).

Rotylenchulus reniformis

Rotylenchulus reniformis is a sedentary semi-endoparasitic nematode that feeds on plant roots in tropical and subtropical regions (Robinson et al, 1997). *Rotylenchulus reniformis* was first reported in upland cotton in Georgia in 1940 and then in Louisiana in 1941 (Smith, 1940; Smith and Taylor, 1941).

Rotylenchulus reniformis can be found in 11 of the 17 U.S. cotton growing states with the most severe cases of yield loss observed randomly in Alabama, Florida, South Carolina, Louisiana, Arkansas, Mississippi, and Tennessee (Lawrence et al, 2021). *Rotylenchulus reniformis* has a host range of more than 310 plants species, including economically important crops such as cotton, peanuts, peas, soybean, pineapples, tea, and many vegetable crops (Marwoto, 2010). It is the second most important nematode species in cotton (Faske and Starr, 2006) with an estimated average yield loss of 1.48% in the U.S. However, depending on the level of infection, cultivars grown in the field, and environmental conditions, yield loss can be as high as 40% (Bhandari et al, 2015; Dyer et al., 2020). *Rotylenchulus reniformis* population levels are usually highest when the silt and clay portion is nearly 28% and the levels decline as the texture becomes either coarser or finer (Davis et al, 2013; Moore and Lawrence, 2013). In addition, *R. reniformis* have been observed to favor soils with less than 40% sand content (Starr et al, 1993; Moore and Lawrence, 2013).

The life cycle of *R. reniformis* starts with an adult female nematode laying one-celled eggs inside a secreted gelatinous matrix where the embryo develops into a first stage juvenile (Leach et al, 2009). The first cuticle molt will transpire while the nematode is inside of the egg, and the second-stage juvenile will use its stylet to emerge from the eggshell (Leach et al, 2009). After the second-stage juvenile emerges from the shell, the third- and fourth-stage juveniles grow and shed their cuticle (Khanal et al, 2018). Fourth-stage juveniles will mature into adult males and adult vermiform females (Khanal et al, 2018). Unlike *M. incognita* nematodes, where the second-stage juvenile is the infective stage, the adult vermiform female of *R. reniformis* is the infective stage (Khanal et al, 2018). Adult female *R. reniformis* nematodes penetrate cells of the host plant's cortex as it moves to get to an outer endodermal cell that is perpendicular to the root axis (Robinson, 2007). This endodermal cell and an arched sheet of contiguous cells of the pericycle undergo adnate cell wall disbanding and minor hypertrophy without hyperplasia, generating a syncytium that functions as a nutrient source for the developing female (Robinson, 2007). Once the syncytium is formed, the infective vermiform adult female becomes sedentary and ultimately forms into a kidney shape (Ganji et al, 2013). When the adult female nematode is fertilized by the male, the female lays an estimated 60 eggs within a protective gelatinous matrix that is secreted by the vaginal glands (Ganji et al, 2013). Adult female *R. reniformis* nematodes can be identified on a cotton root thorough eggs masses and a single white female with soil particles attached to the egg mass (Overstreet et al., 2016). Only the anterior end of the female *R. reniformis* nematode is inside of the plant root while the posterior end protrudes out of the root. Adult male *R. reniformis* nematodes do not feed (Ganji et al, 2013). Root systems infected with *R. reniformis* nematodes can appear relatively normal unless viewed through a microscope, even though aboveground symptoms are observed in the field (Weaver et al, 2007). This makes the evaluation of *R. reniformis* nematode resistance in cotton challenging. Symptoms of *R. reniformis* nematode on cotton are stunting, wilting, appearance of nutrient deficiencies, and yield loss (Robinson, 2007). Specifically, *R. reniformis* nematode damages cotton production through reduction in yield, boll size, and lint percentage (Weaver et al, 2007).

Management practices for *Meloidogyne incognita* and *Rotylenchulus reniformis* in the southeastern United States.

Before nematode management practices can be utilized, an initial evaluation of the field is necessary. Soil sampling is the main way to know if nematodes are present in a field. Soil sample assays will help identify which nematode genera are present in the soil as well as level of infestation that exists. Nematodes are not usually uniformly distributed throughout a field, so taking several samples across an entire field to see how far infestation is spreading throughout the field is important. Fields can be quite large, so sub-dividing a field based on soil types could provide a better understanding of what the true infestation is. When sampling, use a soil sampling probe or device to obtain soil cores from the row to a depth of 20-25 cm (Gazaway et al, 2019). Approximately 20 or more random samples of soil should be taken from each sub-divided section (Gazaway et al, 2019). Time of sampling and soil moisture are also important when sampling. Ideally, the best time to sample cotton is between August and October because the nematode populations are at their highest (Gazaway et al, 2019). Nematodes are not easily detected in late winter through early spring.

Nematode management strategies consist of crop rotation, weed management, host resistance, sanitation, and nematicides (Westphal, 2011). Crop rotation is a widely used nematode management practice; however, a farmer would need sufficient land for the alternative crop to be economically viable to justify removing the cotton crop (Starr et al., 2007). *Meloidogyne incognita* nematode has a very wide host range of more than 3,000 plant species that includes essential agronomic crops, horticultural crops, and various weeds species (Abad et al., 2003). *Rotylenchulus reniformis* nematode also has a wide host range that includes some agronomic field crops, vegetables, fruits, and ornamentals (Davis et al, 2003; Ayala and Ramírez, 1969). Wheat, corn, and peanuts are not considered hosts for *R. reniformis* nematode; however, corn is a host for the *M. incognita* nematode (Anguelov et al, 2020). *Meloidogyne incognita*’s wide host range makes using an effective crop rotation system very challenging. Peanuts are the only major field crop grown in the southeastern United States that is a non-host of *M. incognita* (Taylor and Sasser, 1978). Cotton-corn and soybean-corn rotations are common in Alabama; however, these rotations will most likely increase *M. incognita* nematode population density unless resistant varieties are used (Davis and Kemerait, 2009). For *R. reniformis* nematode suppression, *R. reniformis* nematode-resistant soybean cultivars, corn and winter grain crops can be included in a rotation system, and this system could improve cotton yields for one growing season (Davis et al, 2003). Since peanuts are a non-host for *M. incognita* and *R. reniformis* nematodes, they provide a possible rotational crop for management of these nematodes in areas of the US where peanuts are grown (Koenning et al., 2004). Selection of a crop for a rotation system and evaluating which nematodes are in a field are important because both will help establish an effective crop rotation system for maximum cotton production (Starr et al, 2007).

Weeds are important when trying to manage nematode infested fields. “Agricultural fields will inevitably have weeds” appear throughout a growing season (Davis and Webster, 2005). Weeds ultimately are competing for the same resources as agricultural crops are in a field; however, weeds are part of the ecology of a field and can have long-lasting effects such as serving as a reservoir for insects, diseases, and nematodes (Davis and Webster, 2005). *Meloidogyne incognita* and *R. reniformis* nematodes can survive on a wide host range of plants that includes weeds found commonly in agricultural fields (Rich et al, 2008; Lawrence et al, 2008). Weeds provide these nematodes the ability to survive on an alternative host which allows for higher nematode levels at planting for the next season or the current season (Thomas et al., 2005; Rich et al, 2008). Therefore, weed management is very important for maintaining an effective nematode control system.

Prevention of nematode infections is the first line of defense against *M. incognita* and *R. reniformis* nematode (McSorley, 1998). Sanitation may include thoroughly cleaning farm equipment used for planting, tillage, and harvesting to remove any soil residue containing nematodes when traveling from field to field. *Meloidogyne incognita* nematode and *R. reniformis* nematode can be difficult to control through sanitation methods but keeping equipment meticulously clean is key (Perry et al, 2009).

Host plant resistance is the most ecologically and cost-effective strategy to provide crop protection against *M. incognita* and *R. reniformis* nematodes (He et al, 2014). Plant tolerance of injury is independent of resistance and pertains to the capability of a host genotype to endure or recover from the damaging effects of nematode infection and produce quality yield (Trudgill, 1991). Nematode resistance is defined as “the ability of a plant to limit a nematode's reproduction when compared with reproduction on a susceptible host” (Robinson et al, 1999; Davis and May, 2003). Cotton cultivars that support fewer than 10% of the nematode reproduction on the susceptible cultivar are normally considered to be highly resistant, and cotton cultivars supporting more than 10% but fewer than the susceptible cultivar are moderately resistant (Davis and Kemerait, 2009). *Rotylenchulus reniformis* nematode resistance had not been commercially licensed in cotton cultivars until 2021 (Koenning et al, 2007; PhytoGen Cottonseed, 2020). Nematode resistant cotton cultivars have numerous advantages over other strategies such as more reliable, less toxic to the environment, and reduces the amount of time a field needs a crop rotation plan (Trudgill, 1991). Crop rotations and nematicides can reduce losses during a growing season; however, nematode population levels can still increase rapidly during the season, so treatments must be repeated annually (Bell et al, 2015). Preferably, nematode resistant cultivars would suppress the nematodes so that additional treatments would not be required (Bell et al, 2015).

With practical and economic limitations to crop rotation and a limited number of high yielding nematode-resistant cultivars available, nematicides continue to be the main strategy of nematode management in cotton in the U.S. (Moore and Lawrence, 2012; Khanal et al., 2018; Lawrence, 2022). There are four common application approaches for nematicides: seed treatment, injection of soil fumigants before planting, in-furrow application of granular or liquid formulations, and foliar spray applications of systemic nematicides post-planting (Greer et al, 2009). Seed treatments show potential in protecting emerging roots from nematode infection early in the season, but they have limitation because their effectiveness only occurs when the nematode populations are low to moderate (Khanal et al, 2018; Starr et al, 2007). Fumigant nematicides are extremely effective; however, they are difficult and expensive to apply because of the equipment required for application, and safety concerns connected with their use has limited their availability in cotton (Faske and Hurd, 2015). Non-fumigant nematicides are the most used nematicides in agricultural production even though the most effective non-fumigants like oxamyl and aldicarb are extremely toxic (Faske and Hurd, 2015). Nematicide effects on nematodes have a relatively short duration of 1 to 6 weeks, and nematode population density might recover during the last half of the growing season (Giannakou et al, 2005; Greer et al, 2009). This can lead to population density being as high, or higher, than non-treated areas by harvest time (Greer et al, 2009).

History of Nematode Resistant Cotton Cultivars

The first report in the world of *Meloidogyne* spp. infesting cotton was in 1892 by Dr. George F. Atkinson, then a professor of Biology at Alabama Polytechnic Institute (now Auburn University) (Atkinson, 1892). Atkinson concluded there was an association between *Meloidogyne* spp. nematode and a fungal pathogen he described as *Fusarium vasinfectum* (Atkinson, 1892). This disease complex led to the start of breeding for *Meloidogyne* spp. resistance in cotton due to nematode infection increasing plant susceptibility to fusarium wilt [*Fusarium oxysporum* Schleft. f. sp. *vasinfectum* (Atk.) Snyd. and Hans] (Shepherd and Huck, 1989). Breeding for *Meloidogyne* spp. nematode resistance in cotton began in the early 1900s, but it was not until 1953 when the first cotton cultivar, Auburn 56, was developed with moderate *M. incognita* resistance (Zhang et al, 2006; Smith, 1964). The first highly *M.incognita* resistant cotton germplasm was Auburn 623 RNR released in March of 1970 by Auburn University Agricultural Experiment Station and the Agricultural Research Service (ARS) of the USDA (Shepherd, 1974). The Auburn 623 RNR line was a cross between Clevewilt 6-3-5 x Mexico Wild (Shepherd, 1974). Clevewilt 6-3-5 was a breeding line that was developed in 1930 by Coker Pedigreed Seed company, and Mexico Wild specific origins are unknown (Shepherd, 1974). *Meloidogyne incognita* resistance genes are broken down into two categories: moderately resistant or highly resistant (Gutierrez et al, 2010). For a cotton cultivar to be highly resistant to *M. incognita* nematode, the cotton cultivar must inherit the resistance gene as a quantitative trait that is controlled by at least two genes (Gutierrez et al, 2010). Moderate *M. incognita* resistance is transmitted as a recessive gene (Gutierrez et al, 2010). It is believed that these resistance genes are associated with chromosomes 11 and 14 (Gutierrez et al, 2010). Chromosome 11 is indicated to suppress the formation of root galls while chromosome 14 is indicated to suppress egg production (He et al, 2014). Breeding efforts have been continuous since the 1970s to produce highly *M. incognita* resistant cotton cultivars with better agronomic characteristics than Auburn 623 RNR (McPherson et al, 2004).

Rotylenchulus reniformis was first observed in Hawaii parasitizing cowpeas in the 1940s (Linford and Oliveira, 1940). The first report of *R. reniformis* nematode infecting cotton was in 1941 in Louisiana (Bhandari et al, 2015). The screening for *R. reniformis* resistant cotton cultivars began in the early 1960s with greenhouse tests done by Birchfield and Brister (Khanal et al, 2018). All upland cotton (*G. hirsutum*) cultivars that have been evaluated are susceptible to *R. reniformis* nematode; however, *R. reniformis* resistance has been detected in other species of cotton (Bhandari et al, 2015). *Rotylenchulus reniformis* resistance has been identified in at least 10 of 50 cotton species, including G. *anomalum*, G. *arboretum,*

G. *aridum*, G. *herbaceum*, G. *raimondii*, G. *somalense*, G. *stocksii*, G. *thurberi*, G. *longicalyx*, and G. *barbadense* (Li et al, 2018). Among these, only G. *longicalyx* displayed total resistance to *R. reniformis* nematode (Li et al, 2018). In other species of cotton, six *R. reniformis* nematode resistance genes have been identified ranging from partial dominant, dominant, and recessive in action (Khanal et al, 2018). Ren^{lon} and Ren^{ari} genes are dominant and identified in *G. longicalyx* on chromosome 11, *G. aridum* on chromosome 21, and $\text{Ren}^{\text{barb1}}$, $\text{Ren}^{\text{barb2}}$, and $\text{Ren}^{\text{barb3}}$ are partially resistant and identified in *G. barbadense* located on chromosomes 21 and 18, respectively (Khanal et al, 2018). Multiple upland cotton cultivars such as Stoneville 4793 (BASF, Ludwigshafen, Germany), Suregrow 521 R (Bayer, Leverkusen, Germany) Suregrow 215 BR (Bayer, Leverkusen, Germany), Paymaster 1218 BR (Bayer, Leverkusen, Germany), and Deltapine 449 BR (Bayer, Leverkusen, Germany) showed potential tolerance to *R. reniformis* nematode infection, but some of the cultivars were inconsistent in their ability to tolerate *R. reniformis* nematode infection or not widely available (Blessitt et al, 2012). PhytoGen seed company allowed network growers to plant the first *R. reniformis* resistant cotton seed for on-farm trials in the 2020 growing season (Boyd, 2020). The *R. reniformis* resistant cotton variety, PHY 332 W3FE, will become available to farmers for the 2022 growing season (Boyd, 2020).

Research Hypothesis

We hypothesized that combining the use of nematode resistant cotton cultivars with additional nematicides would provide for an integrated nematode management system of *M. incognita* and *R. reniformis* nematodes.

The purpose of this study was to compare susceptible and resistant cotton cultivars PHY 340 W3FE (S), and PHY 360 W3FE (*M. incognita* R) and PHY 332 W3FE (*R. reniformis* R), and the effects of additional nematicide combinations on *M. incognita* and *R. reniformis* population development and yield in upland cotton. The objectives of this study were 1) to determine the yield potential of the *M. incognita* resistance variety PHY 360 W3FE and the *R. reniformis* resistant variety PHY 332 W3FE in nematode infested fields and the cultivar effect on the nematode populations development; and 2) to evaluate the effects of combining the new nematicide Reklemel™ (fluazaindolizine), with Vydate® C-LV (oxamyl), and the seed treatment BIOST Nematicide 100 with the cotton genetic resistance on nematode population levels and subsequent cotton lint yield.

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Chapter 2: Evaluation of *Meloidogyne incognita* and *Rotylenchulus reniformis* nematode resistant cotton cultivars in greenhouse and microplot and with supplemental Corteva Agriscience nematicides in field studies

INTRODUCTION

Meloidogyne incognita and *Rotylenchulus reniformis* are major, yield limiting pests of upland cotton, *Gossypium hirsutum*. *Meloidogyne incognita* (Kofoid and White) Chitwood (root-knot nematode, RKN) is one of the most important plant parasitic nematodes globally because of its ability to damage agricultural crops as well as many other cultivated plants worldwide (Wram and Zasada, 2019). *Meloidogyne incognita* accounts for an estimated annual yield loss of over $100 billion worldwide, and $283 million for cotton in the United States (Forghani and Hajihassani, 2020; Lawrence et al, 2022). In Alabama, *M. incognita* is estimated to reduce cotton yield 4.5% which is approximately equivalent to 34,000 bales of cotton (Lawrence et al, 2022). Management of *M. incognita* in cotton production relies heavily on nematicides due to its wide host range and limited resistant varieties (Starr, 2007). The first *M. incognita* resistant cotton cultivar became available in 1953 with moderate *M. incognita* resistance (Zhang et al, 2006; Smith, 1964). There are two genes located on cotton chromosomes 11 and 14 that are responsible for this resistance (Gaudin and Wubben, 2021). Currently there are at least ten cotton cultivars with one or two genes for resistance to *M. incognita* available through Corteva Agriscience, Bayer, and BASF (Kichler, 2021).

Rotylenchulus reniformis Linford and Oliveira (reniform nematode, RN) is one of the most economically important plant parasitic nematodes on upland cotton (Gordon et al, 2022). *Rotylenchulus reniformis* reportedly caused an estimated annual loss of approximately $33 million dollars in the United States in 2020 (Wilson et al, 2020). In Alabama, *R. reniformis* accounts for an estimated 3% of cotton yield loss which is approximately 22,700 bales of cotton lost (Lawrence et al, 2022). Management of *R. reniformis* consists of integrated practices of crop rotation, cover cropping, and nematicides (Davis et al, 2003; Wilson et al, 2020). The first *R. reniformis* resistant cotton cultivars are just becoming available due to challenges of integrating nematode resistance into commercial upland cotton cultivars (Davis et al, 2003). Two *R. reniformis* nematode resistant cultivars with three genes for resistance will be available from PhytoGen in 2022. Now nematode resistant cotton cultivars are another tool in an integrated nematode management system. Cotton cultivars that have some form of resistance to *M. incognita,* whether partially or highly resistant, are listed in Table 1 (Wheeler et al, 2020; Weaver, 2015; Corteva Agriscience, 2020). The four cotton cultivars with partial *R. reniformis* resistance that are just becoming available to growers are listed in Table 2 (Wheeler et al, 2020; Weaver, 2015; Corteva Agriscience, 2020).

New nematode control measures are becoming available with new nematicides and nematicide combinations. Several of the most commonly used nematicides for *M. incognita* and *R. reniformis* control in cotton are listed in Table 3 (Muller et al, 2012; Lawrence, 2021). The seed treatment BIOST Nematicide 100 (Corteva Agriscience; Wilmington, DE) is labeled to protect cotton against nematodes. BIOST Nematicide 100 is a biological nematicide derived from heat killed *Burkholderia rinojenses* and its non-living spent fermentation media with multiple modes of action via enzymes and toxins (Albaugh, 2016). Vydate® C-LV, oxamyl, by comparison, is an older nematicide that has been shown to be effective against *M. incognita* and *R. reniformis* through suppression of nematode population and feeding (Lawrence and McLean, 2000; Mueller, et al, 2012). Reklemel™, fluazaindolizine, is a new nematicide against *M. incognita* and *R. reniformis* with a safer mammalian ectotoxicity profile when compared with currently used fumigants like 1, 3-dichloropropene (Lahm et al, 2017; Talavera et al, 2021). Reklemel™ has been shown to significantly reduce motility and activity of *M. incognita* and *R. reniformis in vitro* (Lahm et al, 2017; Groover and Lawrence, 2021). Seed treatment and in-furrow spray nematicides have been employed for nematode management in cotton over the last two decades. The combination of these selected nematicides stated above and nematode resistant cotton cultivars could impact upland cotton production.

Integrated nematode management practices may now be possible to manage *M. incognita* and *R. reniformis* utilizing host plant resistant cultivars combined with nematicides to reduce nematode population density and plant damage and enhance cotton yields. The integration of nematicides with resistant cultivars could potentially extend the seasons a resistant cultivar could be produced without requiring a crop rotation to manage the nematode pests (Davis and Kemerait, 2009). Repeated planting of the resistant cultivar alone could eventually lead to natural selection and resistance in the nematode population, overwhelming or negating nematode resistance in the cotton cultivar. (Young, 1992; Davis and Kemerait, 2009).

The objectives of this study were 1) to determine the cultivar effect on the nematode population development and yield potential of the *M. incognita* resistance variety PHY 360 W3FE and the *R. reniformis* resistant variety PHY 332 W3FE in nematode infested fields compared to a nematode susceptible cultivar PHY 340 W3FE; and 2) to evaluate the effects of combining the new nematicide Reklemel™ (fluazaindolizine), with Vydate® C-LV (oxamyl), and the seed treatment BIOST Nematicide 100 with the cotton genetic resistance on nematode population levels and subsequent cotton lint yield.

MATERIALS AND METHODS

Greenhouse tests were established to evaluate the population density of *M. incognita* and *R. reniformis* on the susceptible cotton cultivar PHY 340 W3FE and the *M. incognita* resistant cotton cultivar PHY 360 W3FE and the *R. reniformis* resistant cotton cultivar PHY 332 WFE. Microplots were established to evaluate the population density of *M. incognita* and yield potential of cotton cultivars PHY 340 W3FE and PHY 360 W3FE under a natural outdoor environment but within a container to control the nematode population level. Field trials were established in separate *M. incognita* and *R. reniformis* infested cotton fields to determine nematode population development and cotton yield. Nematicide applications were applied at planting to be evaluated alongside the genetics of the nematode resistant cultivars PHY 360 W3FE or PHY 332 W3FE.

Nematode Inoculum and Extraction

Meloidogyne incognita was maintained on corn “Pioneer 1197 YHR” (Corteva Agriscience; Wilmington, DE) in 2020 and “Pioneer 1506 YHR” in 2021 and *R. reniformis* was maintained on cotton “Deltapine 1646 B2XF” (Bayer AG, Leverkusen, Germany) in 2020 and “PhytoGen 340 W3FE” (Corteva Agriscience; Wilmington, DE) in 2021 in 500-cm^3 polystyrene cups (Dart Container

Corporation; Mason, Michigan) at the Plant Science Research Center (PSRC) in Auburn, AL for inoculum. Eggs of *M. incognita* and *R. reniformis* were extracted by a modified root extraction method of Hussey and Barker (1973). The tops of the infected plant were removed, and the roots were dipped in water to remove excess soil. Roots were then placed in a 0.625% NaOCl solution and shaken for four minutes at one g-force on a Barnsted Lab Line Max Q 5000E Class shaker (Thermo Fisher Scientific: Waltham, MA). The eggs were collected on a 25-µm pore sieve, rinsed with water, and washed into 50 mL centrifuge tubes. The contents were mixed with a 1.14 specific gravity sucrose solution and centrifuged at 1400 g-forces for 1 minute (Jenkins, 1964). After centrifugation, the eggs in the supernatant of the sucrose solution were collected on a 25-µm pore sieve and rinsed with water to remove the sucrose solution from the eggs. *Meloidogyne incognita* and *R. reniformis* egg numbers were enumerated using a Nikon TSX 100 inverted microscope at 40x magnification and adjusted to 5,000 eggs/ml.

Greenhouse Trials

Greenhouse tests were conducted at PSRC to determine the effect of *M. incognita* population density on PHY 340 W3FE and PHY 360 W3FE and *R. reniformis* on PHY 340 W3FE and PHY 332 W3FE (Corteva Agriscience; Wilmington, DE) susceptible and resistant cotton, respectively. All seeds were pretreated with the insecticide/fungicide seed treatments metalaxyl 4.0 ST, fludioxonil 4L ST, myclobutanil 240 ST, and imidacloprid. A Kalmia loamy sand texture soil (80% sand, 10% silt, and 10% clay; 1.2% organic matter, pH 6.9) from the Plant Breeding Unit (PBU) near Tallassee, AL was pasteurized at 88°C for 12 hours then allowed to cool for 24 hours and the process was repeated. The pasteurized soil was mixed with sand at a rate of 60:40 soil to sand, and fertilizer and lime were added to the soil at rates recommended by the Auburn University Soil Lab. Seeds were planted in 500-cm^3 polystyrene cups. Four cotton seeds were planted per pot at a depth of 2.5 cm. Nematode inoculum (previously described) was added at planting. Each pot received 5,000 nematode eggs, pipetted 2.5 cm deep in the soil. All plots were arranged in a RCBD with five replications and each test was repeated each year. Plants were watered as needed to maintain soil moisture, and lighting was supplied via 1000-watt halide bulbs producing 110,000 lumens for 14-hour day length. Temperature in the greenhouse ranged from 25°C to 29°C.

Data was collected near 30 DAP for all greenhouse trials. Plant parameters included plant height (PH), shoot fresh weight (SFW), root fresh weight (RFW), and biomass (SFW + RFW). Nematode parameters of *M. incognita* and *R. reniformis* population density included the total number of eggs extracted from the roots and reported on a per gram of root basis.

Microplot Trials

The cotton varieties PHY 340 WFE and PHY 360 WFE tested in the greenhouse experiments were repeated for trials conducted in a microplot setting with *M. incognita*. Microplot pots consisted of a pot within a pot design with one 26.5-liter plastic tree pot placed inside an identical pot with a brick between the two pots to act as a root barrier. The top pot was filled with Kalmia loamy sand (24% sand, 49% silt and 28% clay) from the PBU. Microplots representing 0.3 m of a linear row were arranged in a RCBD with five replications per treatment and the trial was repeated. Each microplot was inoculated with 250 cm^3 of soil containing approximately 50,000 eggs and J2 of *M. incognita* and mixed into the soil evenly. Tests were planted June 2, 2020, and May 14, 2021. Ten cotton seeds were sown in a linear row at a depth of 2.5 cm in each microplot. Shortly after emergence plants were thinned to 6 seedlings per microplot. The microplots received water at 30 ml/min by an automated drip irrigation system that was adjusted throughout the season to run for 15-45 minutes twice a day, for a total of 450 – 4,620 ml of water per microplot per day as needed.

Data were collected near 40 DAP and at plant maturity (154 DAP to 174 DAP). Two cotton plants were excavated from each microplot for plant and nematode analysis at each of the sample data collection times. Plant parameters included PH, SFW, RFW, biomass, and seed cotton yield. Nematode parameters included *M. incognita* root gall ratings and population density measured as number of total eggs extracted from the roots and eggs per g of root. At plant maturity, cotton was hand harvested for all microplot trials on October 15, 2020 and November 5, 2021.

Field Trials

Field trials were conducted at two locations: PBU and Tennessee Valley Research Extension Center (TVREC) near Belle Mina, AL. PBU is naturally infested with *M. incognita* race 3 and initial at plant populations were 222 vermiform life stages per 100cm^3 of soil in 2020 and 508 vermiform life stages per 100cm^3 of soil in 2021. PBU has a soil type of Kalmia loamy sand, which was used in the greenhouse and microplot tests. TVREC was artificially infested in 2007 with *R. reniformis.* For TVREC, the population density at planting in 2020 was 5000 vermiform life stages per 100cm^3 of soil and 1158 vermiform life stages per 100cm^3 of soil in 2021. TVREC soil type is a Decatur silt loam, which consist of 23% sand, 49% silt, and 28% clay, 1% OM pH 6.0 CEC 9 ncmol. Test plots consisted of two rows, 7.6 meters long, with a 1-meter row spacing and a 4.6-meter alley between replications. All trials were placed in a factorial arrangement of a RCBD with ten replications, and each plot was planted with 13 seeds per meter of row at a depth of 2.5 cm using a John Deere MaxEmerge (John Deere; Moline, IL) planter with Almaco cone planters (Almaco; Nevada, IA). At planting, an in-furrow spray application of Reklemel™ and Vydate® C-LV was applied in the furrow directly behind the seed (Table 4 and 5). Planting dates were May 7, 2020 at PBU and May 5, 2020 at TVREC. In-furrow applications were made at rates of 140 g/ha, 280 g/ha, and 560 g/ha for Reklemel™, and 560 g/ha, 1120 g/ha, and 2240 g/ha for Vydate® C-LV at 30 PSI using 8002 flat fan nozzles for PBU and a 30-PSI orifice for TVREC in 2020. All plots were maintained throughout the season with standard herbicide, insecticide, and fertility production practices. Plots were irrigated with a center pivot sprinkler system at PBU and a lateral irrigation system at TVREC as needed throughout the growing season. Plots were harvested on October 7, 2020 and October 21, 2020 for PBU and TVREC, respectively. In 2021, the same cultivars were tested but the nematicide rates were reduced. In-furrow spray applications were applied at 110 g/ha, 140 g/ha, and 280 g/ha for Reklemel™, and 392 g/ha, 560 g/ha, and 1120 g/ha for Vydate® C-LV at 30 PSI using 8002 flat fan nozzles for PBU and a 30-PSI orifice for TVREC in 2021. PBU and TVREC sites were planted on April 27, 2021 and May 7, 2021, respectively and harvested on 20 Oct and 8 Nov.

Field data collection

Plant parameters included plant height (PH), shoot fresh weight (SFW), root fresh weight (RFW), biomass, and seed cotton yield. Additional measurements included stand counts per length of row per plot, visual vigor ratings on a scale of 1-10 (1= dead plants and 10= maximum vigor) and for *M. incognita* trials root-gall ratings on a scale of 0-10 (0 having no knots on roots and 10 all roots severely galled) (Bridge and Page, 1980). Data were obtained for measurements by digging four random representative cotton plants per plot near 40 DAP. Nematode population density was determined following the procedure for *M. incognita* and *R. reniformis* extractions from the roots were used as described in the nematode inoculum after transport from the field to PSRC.

In both years, plots were assessed near 40 DAP for PH, SFW, RFW, plant biomass, and eggs per gram of root. Twenty-five mature bolls were hand harvested per treatment for one rep of each test, and samples were ginned using a 10-saw table-top gin at the PSRC. The seeds and lint collected from the gin were weighed individually and these data were used to calculate the lint ratio for each treatment. Plots were machine-harvested for yield with an Almaco SPC40 plot combine (Nevada, Iowa).

Statistical analysis

Data collected from greenhouse and microplot trials were analyzed in SAS 9.4 (SAS Institute; Cary, NC) using the PROC GLIMMIX procedure. Tukey Kramer LS-means were compared using a standard ANOVA at a significance level of $P \leq 0.05$. Dependent variables were PH, SFW, RFW, biomass, gall ratings, number of *M. incognita* and *R. reniformis* total egg numbers (eggs/root), *M. incognita* and *R. reniformis* eggs per gram of root (eggs/g of root) and seed cotton yield. Random effects included replication and greenhouse and microplot location. Nematode eggs/root and eggs/g of root were log-transformed to fulfill the normal assumption and are presented as LS-means of all replications in the test. Pearson’s correlation coefficient (PROC CORR) was used to determine relationships between nematode eggs/g of root and yield. There were no significant interactions in the greenhouse and microplots tests between 2020 and 2021 thus the data from both years were pooled into a single dataset.

Data collected from field trials were analyzed in SAS 9.4 (SAS Institute; Cary, NC) using the PROC GLIMMIX procedure. A two-way factorial analysis was conducted with variety, nematicides, and variety x nematicides for each year of the trials. Means were separated using Tukey Kramer LS-means test at the $P \leq 0.05$ level. Student panels were produced to determine the normality of the residuals. In the case of nematode eggs/g of root, the data were log-transformed to satisfy the ANOVA assumptions of normally distributed residuals. Repeated tests were combined for ten repetitions for each year.

RESULTS

Meloidogyne incognita **Greenhouse 2020 & 2021**

In the greenhouse setting with *M. incognita*, the nematode resistant cultivar PHY 360 W3FE grew similarly to PHY 340 W3FE in PH, SFW, and total plant biomass (Table 6; Figure 1). However, RFW was greater ($P < 0.0024$) on the PHY 340 W3FE cotton. *Meloidogyne incognita* total egg numbers and eggs per gram of root density were significantly lower ($P < 0.0009$) on the resistant PHY 360 W3FE cultivar compared to the susceptible PHY 340 W3FE. PHY 360 W3FE supported 90% fewer *M. incognita* total eggs and J2 as compared to PHY 340 W3FE. When placed on the per gram of root basis, the pattern was similar with 88% fewer *M. incognita* eggs per gram of root supported on PHY 360 W3FE compared to PHY 340 W3FE.

Rotylenchulus reniformis **Greenhouse 2020 & 2021**

In the greenhouse setting with *R. reniformis*, the nematode resistant cultivar PHY 332 W3FE grew similarly to PHY 340 W3FE in PH, SFW, RFW, and total plant biomass (Table 7). *Rotylenchulus reniformis* total eggs and eggs per gram of root were significantly lower ($P < 0.003$ and $P < 0.0810$) on the resistant PHY 332 W3FE compared to the susceptible PHY 340 W3FE. PHY 322 W3FE supported 77% fewer *R. reniformis* total eggs as compared to PHY 340 W3FE. When placed on the per gram of root basis, the pattern was similar with 60% fewer *R. reniformis* eggs per gram of root supported on PHY 322 W3FE.

Meloidogyne incognita **Microplot 2020 & 2021**

In the microplot setting with *M. incognita*, similar plant grow parameters were observed for PHY 340 W3FE and PHY 360 W3FE (Figure 2). Plant height, SFW, RFW, total plant biomass, and seed cotton yield were similar between the resistant and susceptible cultivars (Table 8). *Meloidogyne incognita* population density as measured by total eggs and eggs per gram of root was significantly lower ($P < 0.0079$ and $P < 0.0087$) with resistant PHY 360 W3FE as the host plant as compared to the susceptible PHY 340 W3FE. Similarly, to the greenhouse tests, the microplot setting also indicated a 90% lower population of *M. incognita* total eggs and J2 growing on PHY 360 W3FE. The population density of eggs per gram of root was also 82% lower. Significant negative correlations between *M. incognita* eggs per gram root and PH ($r= -0.45724$; $P < 0.0030$), SFW ($r= -0.32765$; $P < 0.0390$), and total plant biomass ($r= -0.33309$; $P < 0.0357$) indicated the increase of *M. incognita* eggs reduced overall plant growth.

Meloidogyne incognita **Field 2020**

For field trials nematicides were added to the susceptible and resistant cotton cultivars PHY 340 W3FE and PHY 360 W3FE to determine if there was an additional benefit of combining the two. The factorial analysis in PROC GLIMMIX indicated no significant interactions between the cotton cultivars and nematicides for the plant parameters. Plant stand was greater for the PHY 340 W3FE with 9.4 plants per meter of row surviving compared to 5.8 plants per meter of row for PHY 360 W3FE (Table 9). Plant height and root fresh weight were similar between cotton varieties and nematicide applications. Plant biomass near 40 DAP was greater with PHY 340 W3FE weighing 0.5 g more than PHY 360 W3FE. Significant positive correlations between biomass and lint yield ($r= 0.23025$; $P < 0.0034$) indicated the increase of biomass increased plant growth overall and increased lint yield. The resistant cultivar PHY 360 W3FE supported a significantly higher lint yield than the susceptible cultivar PHY 340 W3FE. Lint yield was increased by 92 kg/ha or 6% when growing PHY 360 W3FE compared to PHY 340 W3FE.

Nematicide combinations at the medium and high rates of Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) and Reklemel^{TM} (1.13 L/ha) + Vydate® C-LV (5.0 L/ha) significantly lowered plant stand compared to all other nematicide combinations. The nematicide combinations did not significantly affect PH, RFW, or total plant biomass. The addition of the nematicide BIOST Nematicide 100 (0.026 mg ai/seed) alone or with all rates of + Reklemel^{TM} (0.28, or 0.56, or 1.13 L/ha) + Vydate® C-LV (1.24, or 2.5, or 5.0 L/ha) supported the highest lint yield compared to the medium rate of Reklemel^{TM} (0.56 L/ha)

+ Vydate® C-LV (2.5 L/ha) alone. Lint yield was increased by an average of 132 kg/ha or 8% with the addition of the BIO^{ST} plus Reklemel™ and Vydate® C-LV nematicide combinations.

The factorial analysis indicated gall ratings were significant for cotton variety response only. Gall ratings conducted with nematode extractions or 40 DAP indicated significantly less galling ($P < 0.001$) on PHY 360 W3FE with at 3.4 gall rating compared to 4.7 rating for PHY 340 W3FE. Final gall ratings at plant harvest had increased over the season; however, the severity of the galling remained lower ($P < 0.001$) on PHY 360 W3FE at 6.1 compared to the 7.1 galling for PHY 340 W3FE. No differences in galling were observed between the nematicide applications. A significant interaction between variety x nematicide was observed for *M. incognita* total eggs and eggs per gram of root. The *M. incognita* population density was higher ($P< 0.05$) for the susceptible PHY 340 W3FE compared to the resistant PHY 360 W3FE (Figure 7). The susceptible PHY 340 W3FE without a nematicide supported the highest *M. incognita* total population density and eggs per gram of root followed by PHY 340 W3FE + the mid-rate of Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha). The addition of the nematicide combinations significantly reduced *M. incognita* total eggs and eggs per gram of root on the susceptible PHY 340 W3FE variety. The addition of the nematicide combinations on PHY 360 W3FE did not further reduce *M. incognita* populations.

Meloidogyne incognita **Field 2021**

In 2021, the tests were repeated with altered nematicide rates, removing the high rate of Reklemel™ plus Vydate® C-LV and adding a lower rate of Reklemel™ (0.21 L/ha) + Vydate® C-LV (0.88 L/ha). The factorial analysis indicated no significant interactions between cotton cultivars and nematicides for plant parameters like the observations in 2020. PHY 340 W3FE supported similar plant stands to PHY 360 W3FE (Table 10). All plant stands ranged from 10.7 to 10.9 plants per meter of row, which is within the optimal range for cotton plant stand (Alabama Cooperative Extension System, 2021). Plant height and RFW were comparable between the two cultivars. The resistant cultivar PHY 360 W3FE plants had a larger total plant biomass than the susceptible PHY 360 W3FE (Figure 3). Significant positive correlations between biomass and lint yield ($r= 0.14483$; $P < 0.0677$) indicated the increase of biomass increased plant growth overall which increased lint yield. Lint yield was increased by 322 kg/ha ($P \geq 0.05$) or 23% when growing PHY 360 W3FE compared to PHY 340 W3FE.

Plant stand was similar between the nematicide combinations (Figure 4). The nematicide combination BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) significantly supported the leading plant height and root fresh weight over no nematicide control and the two lowest rates of Reklemel^{TM} (0.21 and 0.28 L/ha) + Vydate® C-LV (0.88 and 1.24 L/ha) without BIOST Nematicide 100. Plant biomass was also larger in all three rates of the nematicide combinations of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.21, 0.28, and 0.56 L/ha) + Vydate® C-LV (0.88, 1.24 and 2.5 L/ha) compared to the untreated control. The nematicide combinations of BIOST Nematicide 100 (0.026 mg ai/seed) and the mid and high rates of Reklemel^{TM} (0.28 and 0.56 L/ha) + Vydate® C-LV (1.24 and 2.5 L/ha) supported the largest lint yields averaging 352 kg/ha more lint yield compared to the untreated control. The addition of the combination of Reklemel^{TM} + Vydate® C-LV or BIOST Nematicide 100 + Reklemel^{TM} + Vydate® C-LV increased lint yield by 18% over the untreated control while the addition of BIOST Nematicide 100 alone increased lint yield by 9% over the untreated control.

The factorial analysis indicated nematode gall ratings near 40 DAP were significant for cotton variety but not nematicide. Gall ratings conducted with nematode extractions indicated significantly less galling ($P < 0.001$) on PHY 360 W3FE with at 5.3 gall rating compared to 5.9 rating for PHY 340 W3FE. Gall ratings at harvest were significant for cotton variety response and nematicide combinations. Although final gall ratings at plant harvest had increased over the season, the severity of the galling remained lower ($P < 0.001$) on PHY 360 W3FE at 5.5 compared to the 6.8 galling for PHY 340 W3FE. The addition of the seed treatment BIOST Nematicide 100 (0.026 mg ai/seed) supported the highest gall rating of 7.1 at harvest. The combination of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) sustained significantly lower final gall rating ($P < 0.05$) at 5.7.

A significant interaction was observed between cotton cultivar x nematicides for the nematode parameters *Meloidogyne incognita* total eggs and *Meloidogyne incognita* eggs per g of root (Figure 8).

The resistant cultivar PHY 360 W3FE supported 61 % and 77 % fewer *Meloidogyne incognita* total eggs and eggs per gram of root than the susceptible PHY 340 W3FE with no nematicide treatment. The addition of BIOST Nematicide 100 (0.026 mg ai/seed) supported similar population levels to the cultivar without any additional nematicide. *Meloidogyne incognita* total eggs and eggs per gram of root were significantly lower when growing PHY 360 W3FE and the mid-rate nematicide combination of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.28 L/ha) + Vydate® C-LV (1.24 L/ha). PHY 340 W3FE supported the highest *M. incognita* total population density and eggs per gram of root followed by PHY 340 W3FE + BIOST. The addition of the nematicide combinations of Reklemel^{TM} + Vydate® C-LV at all rates further reduced *M. incognita* total eggs and eggs per gram of root with PHY 360 W3FE.

Rotylenchulus reniformis **Field 2020**

The *R. reniformis* field trials added nematicides to the susceptible PHY 340 W3FE and resistant PHY 332 W3FE cotton cultivars to determine if there was an additional benefit of combining the resistant PHY 332 W3FE variety with nematicides (Figure 5). Data analysis indicated no significant interactions between the cotton cultivars and nematicide for the plant parameters. Plant stand was greater for the PHY 332 W3FE with 6.7 plants per meter of row surviving compared to 5.4 plants per meter of row for PHY 340 W3FE (Table 11; Figure 6). Plant stand, plant height, root fresh weight, and biomass were significantly improved on PHY 332 W3FE compared to PHY 340 W3FE. PHY 332 W3FE plants were larger than the susceptible PHY 340 W3FE plants in plant height, root fresh weight, and the total plant biomass. The cotton cultivar significantly affected cotton yield. Lint yield was 714 kg/ha or 38% greater when growing PHY 332 W3FE compared to PHY 340 W3FE.

The addition of the nematicide BIOST Nematicide 100 (0.026 mg ai/seed) alone or with all rates of Reklemel^{TM} (0.28, or 0.56, or 1.13 L/ha) + Vydate® C-LV (1.24, or 2.5, or 5.0 L/ha) did not have a significant effect on plant stand. The nematicide combination of the mid-rate of Reklemel^{TM} (0.28 L/ha) + Vydate® C-LV (1.24 L/ha) with and without BIOST Nematicide 100 (0.026 mg ai/seed) produced the tallest cotton plants ($P \leq 0.05$) as compared to the untreated control. Root fresh weight and total plant biomass were significantly increased with the use of the mid-rate of the nematicide combination

Reklemel^{TM} (0.28 L/ha) + Vydate® C-LV (1.24 L/ha) with or without BIOST Nematicide 100 (0.026 mg ai/seed) as compared to the untreated control. All nematicide combinations of Reklemel^{TM} + Vydate® C-LV at all rates tested significantly supported more cotton lint yield than the untreated control. Lint yield was increased by 488 kg/ha or 27% with the addition of the nematicide combination BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.28 L/ha) + Vydate® C-LV (1.24 L/ha) compared to no nematicide.

A significant interaction was observed between cotton cultivar x nematicides for the nematode parameters for *R. reniformis* total populations as well as eggs per gram of root. (Figure 9). The susceptible variety PHY 340 W3FE alone and PHY 340 W3FE + BIOST Nematicide 100 supported high *R. reniformis* total population density and eggs per gram of root. The *R. reniformis* total egg density and eggs per gram of root numbers for the resistant PHY 332 W3FE were 57% and 73% lower, respectively than on PHY 340 W3FE. The addition of the nematicide combinations significantly reduced *R. reniformis* total eggs and eggs per gram of root with PHY 340 W3FE.

Rotylenchulus reniformis **Field 2021**

In 2021 the tests were repeated with lower nematicide rates removing the high rate of Reklemel plus Vydate and adding a very low rate of Reklemel^{TM} (0.21 L/ha) + Vydate® C-LV (0.88 L/ha). The factorial analysis indicated no significant interactions between the cotton cultivars and nematicide for the plant parameters like the observations in 2020. Plant stand was greater for the PHY 332 W3FE with 5.3 plants per meter of row surviving compared to 3.2 plants per meter of row for PHY 340 W3FE (Table 12). Cultivar did not impact plant height or root fresh weight. Total plant biomass was greater ($P \leq 0.05$) with the resistant PHY 332 W3FE compared to PHY 340 W3FE. Lint yield was increased ($P < 0.001$) by 703 kg/ha or 42% when growing the resistant PHY 332 W3FE compared to PHY 340 W3FE in 2021.

The lowest rate nematicide combination of Reklemel^{TM} (0.21 L/ha) + Vydate® C-LV (0.88 L/ha) supported the best plant stand of 5.2 plants per meter of row as compared to BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) which supported 3.7 plants. Plant height was significantly increased with the nematicide combination BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.21 L/ha) + Vydate® C-LV (0.88 L/ha) as compared to the BIOST Nematicide 100 (0.026 mg ai/seed) alone and the untreated control. The nematicide combinations at the low and mid rates of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.21 or 0.28 L/ha) + Vydate® C-LV (0.88 or 1.24 L/ha) and the mid and high rates of Reklemel^{TM} (0.28 or 0.56 L/ha) + Vydate® C-LV (1.24 or 2.5 L/ha) significantly increased root fresh weight and biomass. The nematicides combinations at the low to mid rates of Reklemel^{TM} (0.28 and 0.56 L/ha) + Vydate® C-LV (1.24 and 2.5 L/ha) alone or in combination with BIOST Nematicide 100 (0.026 mg ai/seed) significantly supported more yield than the BIOST Nematicide alone and the untreated control. Lint yield was also increased ($P < 0.001$) by 583 kg/ha or 37% when using nematicide combination BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) compared to no nematicide.

A significant interaction was observed between the cultivar and nematicides in the presence of *R. reniformis* for total populations density and eggs per gram of root (Figure 10). The susceptible PHY 340 W3FE + BIOST Nematicide 100 supported the highest *R. reniformis* total population density and eggs per gram of root. The addition of the nematicide combinations significantly reduced *R. reniformis* total eggs and eggs per gram of root with both the susceptible PHY 340 W3FE and resistant PHY 332 W3FE. Cultivar PHY 332 W3FE supported the fewest ($P < 0.0384$ and $P < 0.0357$) *R. reniformis* total eggs and eggs per gram of root, and the mid-rate nematicide combination BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) numerically reduced *R. reniformis* compared to no nematicide. The resistant cultivar PHY 332 W3FE supported 86% less *R. reniformis* eggs per gram of root than PHY 340 W3FE.

DISCUSSION

The primary goal of this study was to determine if growing the nematode resistant cultivars PHY 360 W3FE and PHY 332 W3FE would reduce nematode population density and produce a higher cotton yield compared to the nematode susceptible cultivar PHY 340 W3FE. Plant resistance to nematodes is defined as the plant’s capacity to inhibit nematode reproduction (Roberts, 2002; Schrimsher et al. 2014).

Results of our greenhouse, microplot, and field trials confirm with previous studies that PHY 340 W3FE is susceptible to *M. incognita* and *R. reniformis* nematodes while indicating that the new resistant varieties PHY 360 W3FE and PHY 332 W3FE are effective at keeping nematode populations low. Significant progress has been made since the initial introduction of the first commercial cotton cultivar LA887 which was registered with partial resistance to *M. incognita* (Jones et al., 1991; Wheeler et al., 2020). Our results show similar findings of reduction of *M. incognita* and *R. reniformis* populations observed by Shepard and Huck (1947), Schrimsher et al. (2014), and Weaver et al. (2011). In our nematode resistance and yield performance trials, PHY 360 W3FE and PHY 332 W3FE suppressed *M. incognita* and *R. reniformis* populations while yielding more cotton compared to the susceptible PHY 340 W3FE (Zhou and Starr, 2003). These resistant cultivars supported fewer nematodes while producing optimum cotton yields. Additionally, as seen in previous studies, selecting, and growing nematode resistant cotton lines has been shown to reduce nematode reproductive potential for *M. incognita* and *R. reniformis* (Koenning et al., 1996; Schrimsher et al., 2014). We also observed the reduction in nematode populations on these newly released cotton cultivars. These new cotton cultivars have improved from the first PHY 417 WR cotton cultivar, which had high resistance to *M. incognita* but poor yield potential and thus had limited commercial adaptation (Fuchs et al., 2015; Wheeler et al., 2020).

The secondary goal was to evaluate the potential of the addition of BIOST Nematicide 100 seed treatment nematicide and Reklemel^{TM} and Vydate$^{®}$ C-LV in furrow spray nematicides to PHY 360 W3FE and PHY 332 W3FE to further manage *M. incognita* and *R. reniformis* nematodes in cotton. Of these nematicides, BIOST Nematicide 100 and Vydate$^{®}$ C-LV (Oxamyl) are registered for use on cotton; however, Reklemel^{TM} (Corteva Agriscience; Indianapolis, IN) is currently registered for use on fruits and vegetables (Desaeger et al., 2020). Results of our field trials indicate applying BIOST Nematicide 100 as a seed treatment or BIOST Nematicide 100 + Reklemel^{TM} + Vydate® C-LV in-furrow nematicides to PHY 360 W3FE and PHY 332 W3FE further lowered nematode population density. These nematicides also protected the resistant cultivars from natural selection, and the development of potentially nematode resistance genotypes thus allowing for a longer production life of these cultivars. This research confirms that applying nematicides to manage *M. incognita* and *R. reniformis* nematodes reduces populations and often enhances yields (Lawrence and McLean, 2000; Wheeler et al, 2013; Schrimsher et al. 2014).

PBU 2020 & 2021

Meloidogyne incognita

At PBU overall, the resistant PHY 360 W3FE variety produced 21% more lint cotton than the susceptible variety. The combination of the low and mid-rate of BIOST Nematicide 100 + Reklemel^{TM} (0.28 and 0.56 L/ha) + Vydate® C-LV (1.24 and 2.5 L/ha) supported the lowest total eggs and eggs per gram of root. These findings are consistent with Desaeger et al (2020), where applications of Reklemel^{TM} and Vydate® C-LV alone significantly reduced *M. incognita*. The use of the nematicide seed treatment BIOST Nematicide 100 overall produced a growth benefit to the cotton plants with increased plant height, root fresh weight and plant biomass. Similar results were reported by Monfort et al. (2006) and Wilkerson and Allen (2020). PHY 340 W3FE supported the largest root fresh weight potentially due to the galling caused by the large *M. incognita* population density. Chitwood et al. (1952) reported an increase in root fresh weight with larger populations of *M. incognita* in peach rootstocks. The nematicide combination that supported the largest lint yield was the mid-rate of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha). A similar study done by Koenning et al. (2004) indicated that increasing nematicide rate led to increased lint yields. We observed that nematicide applied as a seed treatment and an in-furrow treatment increased lint yield an average of 16% when compared to no nematicide, while adding Reklemel^{TM} + Vydate® alone increased lint yield 17% compared to no nematicide. An application of BIOST Nematicide 100 increased yield by 8% alone. A trial conducted by Dyer et al. (2016) supports this conclusion and found that nematicide seed treatment BIOST Nematicide 100 increased lint yield ranging from 1% to 12% depending on the rate of the seed treatment.

TVREC 2020 & 2021

Rotylenchulus reniformis

At TVREC overall, the resistant PHY 332 W3FE variety produced 39% more lint cotton than the susceptible variety when the data were analyzed as a factorial with all sixteen treatments. The use of the nematicide seed treatment BIOST Nematicide 100 overall produced a growth benefit to the cotton plants with increased plant height, root fresh weight and plant biomass. Xiang et al. (2018) on cotton confirmed the utilization of a biological agent such as *Bacillus* to reduce *M. incognita* population density in cotton. The resistant cultivar PHY 332 W3FE and the nematicide combination of BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel (0.28 L/ha) + Vydate® C-LV (1.24 L/ha) at the mid-rate was the most effective in lowering *R. reniformis* total eggs and eggs per gram of root. This combination also produced the highest lint yield. A previous study done by Khalilian et al. (2003) on cotton confirms similar results with applying variable rates of 1,3-dichloropropene and aldicarb to manage Columbia lance nematode. When using the mid-rate of the nematicide combination BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel^{TM} (0.56 L/ha) + Vydate® C-LV (2.5 L/ha) lint yield was increased 32%. Research supports the use of Reklemel^{TM} and Vydate® C-LV on reducing *R. reniformis* population levels (Lawrence et al, 2007; Lahm et al, 2017). A nematicide applied as a seed treatment or in-furrow increased lint cotton yield an average of 21% when compared to no nematicide; BIOST Nematicide 100 + Reklemel^{TM} + Vydate® C-LV lint yield increased 28%. An application of BIOST Nematicide 100 increased yield by 12% alone. A trial conducted by Dyer et al. (2016) supports this conclusion. A study done by Zimet et al (2002) confirms that there are economic benefits to applying nematicides such as 1,3-D and aldicarb to increase cotton lint yields in the presence of plant-parasitic nematodes. This research also confirms previous studies that have shown a similar result of using nematode resistant cultivars with an addition of a nematicide in cotton (Dyer et al, 2020; Crow et al, 2021). Due to the limited number of nematode resistant cultivars available and the restrictions on certain effective nematicides, more studies should be conducted to evaluate nematode resistant cotton cultivar lines. High yielding nematode resistant cotton cultivars are the most desirable form of nematode management. Continued integrated nematode management research is needed to determine the durability of resistance in these long-awaited *M. incognita* and *R. reniformis* resistant cotton cultivars (Young, 1992).

CONCLUSION

Overall, both resistant cultivars PHY 360 W3FE and PHY 332 W3FE supported higher yield potential than the susceptible PHY 340 W3FE in nematode infested fields. It can be concluded from this two-year study that combining the nematicides Reklemel™, Vydate® C-LV, and BIOST Nematicide 100 with genetic resistance under nematode pressure can increase lint yield in the presence of *M. incognita* and *R. reniformis*. To our knowledge, this is the first published study on Reklemel^{TM} and Vydate® C-LV efficacy as a cotton nematicide combination. These findings indicate that while each rate evaluated was successful at lowering both *M. incognita* and *R. reniformis* population density, the application of the medium rates of Vydate® C-LV and Reklemel^{TM} were more consistent at lowering nematode population density and improving lint yield. In conclusion, the growing of a *M. incognita* and *R. reniformis* nematode resistant cotton cultivar in fields infested with *M. incognita* and *R.* reniformis will produce higher yields than the susceptible varieties available at this time. In addition, resistant cotton genotypes suppress *M. incognita* and *R. reniformis* populations for future growing seasons. The addition of the nematicides further increased yields and lowered *M. incognita* and *R. reniformis* populations. The combination of the two integrated nematode management options of resistant varieties with nematicides appears to have strong potential for sustaining high yields while reducing nematode populations.

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Table 1. Cultivars grouped into *Meloidogyne incognita* (Mi) resistance categories.

Cultivar	Mi category	Company
Fibermax 1621 GL	PR[z]	BASF; Ludwigshafen, Germany
Fibermax 1911 GLT	PR	BASF; Ludwigshafen, Germany
Fibermax 2011 GT	PR	BASF; Ludwigshafen, Germany
Fibermax 1730 GLTP	PR	BASF; Ludwigshafen, Germany
Stoneville 4946 GLB2	PR	BASF; Ludwigshafen, Germany
Stoneville 5600 B2XF	PR	BASF; Ludwigshafen, Germany
Deltapine 2141 NR B3XF	PR	Bayer; Leverkusen, Germany
Deltapine 2143 NR B3XF	PR	Bayer; Leverkusen, Germany
Deltapine 1454NR B2RF	NR[y]	Bayer; Leverkusen, Germany
Deltapine 1558NR B2RF	NR	Bayer; Leverkusen, Germany
Deltapine 1747NR B2RF	NR	Bayer; Leverkusen, Germany
Deltapine 1823NR B2XF	NR	Bayer; Leverkusen, Germany
PhytoGen 250 W3FE	PR	Corteva AgriScience; Indianapolis, IN
PhytoGen 332 W3FE	R[w]	Corteva AgriScience; Indianapolis, IN
PhytoGen 350 W3FE	PR	Corteva AgriScience; Indianapolis, IN
PhytoGen 390 W3FE	R	Corteva AgriScience; Indianapolis, IN
PhytoGen 400 W3FE	PR	Corteva AgriScience; Indianapolis, IN
PhytoGen 430 W3FE	PR	Corteva AgriScience; Indianapolis, IN
PhytoGen 440 W3FE	PR	Corteva AgriScience; Indianapolis, IN
PhytoGen 443 W3FE	R	Corteva AgriScience; Indianapolis, IN
PhytoGen 545 W3FE	R	Corteva AgriScience; Indianapolis, IN
PhytoGen 320 W3FE	PR	Corteva AgriScience; Indianapolis, IN
PhytoGen 360 W3FE	R	Corteva AgriScience; Indianapolis, IN
PhytoGen 417 WRF	HR[x]	Corteva AgriScience; Indianapolis, IN
PhytoGen 427 W3FE	R	Corteva AgriScience; Indianapolis, IN
PhytoGen 480 W3FE	HR	Corteva AgriScience; Indianapolis, IN
PhytoGen 530 W3FE	R	Corteva AgriScience; Indianapolis, IN
PhytoGen 580 W3FE	HR	Corteva AgriScience; Indianapolis, IN

[z] PR are cultivars partial resistant to *M. incognita*.

[y] NR are cultivars that are susceptible to *M. incognita*.

[w] R are cultivars that have some form of resistance but it isn’t fully evaluated yet.

[x] HR are cultivars that have high resistance with two homologous resistant genes.

Table 2. Recommended nematicides to control *Meloidogyne incognita* and *Rotylenchulus reniformis* in Alabama (Alabama Cooperative Extension System, 2021).

Nematicides	**Company**
AgLogic 15G ®	AgLogic; Chapel Hill, NC
Aeris	Bayer CropScience; Monheim am Rhein, Germany
Avicta Duo Cotton ST[z]	Syngenta; Basel, Switzerland
Poncho/Votivo ST	BASF; Ludwigshafen, Germany
Telone II®	Corteva AgriScience; Indianapolis, IN
Vydate® C-LV	Corteva AgriScience; Indianapolis, IN
Velum®	Bayer CropScience; Monheim am Rhein, Germany
Velum Prime®	Bayer CropScience; Monheim am Rhein, Germany

[z] ST is a seed treatment.

Table 3. Cultivars grouped into *Rotylenchulus reniformis* (Rr) resistance categories.

Cultivar	Rr category	Company
Deltapine 2141 NR B3XF	PR[z]	Bayer; Leverkusen, Germany
Deltapine 2143 NR B3XF	PR	Bayer; Leverkusen, Germany
PhytoGen 332 W3FE	R[y]	Corteva AgriScience; Indianapolis, IN
PhytoGen 443 W3FE	R	Corteva AgriScience; Indianapolis, IN

[z] PR are cultivars partial resistant to *R. reniformis*.

[y] R are cultivars that have some form of resistance to *R. reniformis* but it isn't fully evaluated yet.

Table 4. Nematicide rates used in Plant Science Research Center, Auburn, AL microplots and greenhouse test and Tennessee Valley Research and Extension Center, Belle Mina, AL, and Plant Breeding Unit, Shorter, AL field trials in 2020 and 2021.

2020	
Chemical	**Field rate**
Reklemel™ (Fluazaindolizine)	0.28 L/ha
Reklemel™	0.56 L/ha
Reklemel™	1.13 L/ha
Vydate® C-LV (Oxamyl)	1.24 L/ha
Vydate® C-LV	2.5 L/ha
Vydate® C-LV	5.0 L/ha
BIOST Nematicide 100[z]	0.026 mg ai/seed
2021	
Chemical	**Field rate**
Reklemel™ (Fluazaindolizine)	0.21 L/ha
Reklemel™	0.28 L/ha
Reklemel™	0.56 L/ha
Vydate® C-LV (Oxamyl)	0.88 L/ha
Vydate® C-LV	1.24 L/ha
Vydate® C-LV	2.5 L/ha
BIOST Nematicide 100	0.026 mg ai/seed

[z] Heat-killed *Burkholderia rinojenses* and its non-living spent fermentation media.

Table 5. Nematicide combinations applied on greenhouse and microplots at Plant Science Research Center, Auburn, AL, and field trials conducted at the Tennessee Valley Research and Extension Center, Belle Mina, AL and the Plant Breeding Unit, Shorter, AL in 2020 and 2021 with cultivars PHY 340 W3FE, PHY 360 W3FE and PHY 332 W3FE.

No	Treatments		Nematicides
1	PHY 340 W3FE (S)[x]		No nematicide
2	PHY 340 W3FE (S)		Reklemel^{TM} + Vydate® C-LV
3	PHY 340 W3FE (S)		Reklemel^{TM} + Vydate® C-LV
4	PHY 340 W3FE (S)		Reklemel^{TM} + Vydate® C-LV
5	PHY 340 W3FE (S)		BIOST Nematicide 100
6	PHY 340 W3FE (S)		Reklemel^{TM} + Vydate® C-LV + BIOST Nematicide 100
7	PHY 340 W3FE (S)		Reklemel^{TM} + Vydate® C-LV + BIOST Nematicide 100
8	PHY 340 W3FE (S)		Reklemel^{TM} + Vydate® C-LV + BIOST Nematicide 100
	PBU (*M. incognita*)	TVREC (*R. reniformis*)	
9	PHY 360 W3FE (R)[y]	PHY 332 W3FE (R)	No nematicide
10	PHY 360 W3FE (R)	PHY 332 W3FE (R)	Reklemel^{TM} + Vydate® C-LV
11	PHY 360 W3FE (R)	PHY 332 W3FE (R)	Reklemel^{TM} + Vydate® C-LV
12	PHY 360 W3FE (R)	PHY 332 W3FE (R)	Reklemel^{TM} + Vydate® C-LV
13	PHY 360 W3FE (R)	PHY 332 W3FE (R)	BIOST Nematicide 100
14	PHY 360 W3FE (R)	PHY 332 W3FE (R)	Reklemel^{TM} + Vydate® C-LV + BIOST Nematicide 100
15	PHY 360 W3FE (R)	PHY 332 W3FE (R)	Reklemel^{TM} + Vydate® C-LV + BIOST Nematicide 100
16	PHY 360 W3FE (R)	PHY 332 W3FE (R)	Reklemel^{TM} + Vydate® C-LV + BIOST Nematicide 100

[X] Treatments with (S) are the susceptible cultivar.

[Y] Treatments with (R) are the resistant cultivar.

Table 6: Greenhouse trial LS means for cotton cultivar and nematicide combination effects on plant height, shoot fresh weight, root fresh weight, biomass, total *Meloidogyne incognita* eggs, and *M. incognita* eggs per gram of root near 30 DAP at the Plant Science Research Center, Auburn, AL in 2020 and 2021.

Cotton Variety LS-means	**Plant height**	**Shoot fresh weight (g)**	**Root fresh weight (g)**	**Biomass (g)**	**Total *M. incognita* eggs**	***M. incognita* eggs/g of root**[x]
PHY 340 W3FE[z]	11.95 a[y]	6.21 a	6.68 a	12.89 a	1922 a	353 a
PHY 360 W3FE	12.63 a	6.55 a	4.31 b	10.85 a	194 b	42 b

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] values present are LS-means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly. Nematode egg data were log-transformed to satisfy the ANOVA assumption of normally distributed residuals values in the column followed by different letters differ significantly.

[x] Data for *M. incognita* eggs/gram of root was collected from 4 root systems.

Table 7: Greenhouse trial LS means for cotton cultivar and nematicide combination effects on plant height, shoot fresh weight, root fresh weight, biomass, total *Rotylenchulus reniformis* eggs, and *R. reniformis* eggs per gram of root near 30 DAP at the Plant Science Research Center, Auburn, AL in 2020 and 2021.

Cotton Variety LS-means	**Plant height**	**Shoot fresh weight (g)**	**Root fresh weight (g)**	**Biomass (g)**	**Total *R. reniformis* eggs**	***R. reniformis* eggs/g of root**[x]
PHY 340 W3FE[z]	11.97 a[y]	6.24 a	6.55 a	12.79 a	1935 a	358 a
PHY 332 W3FE	11.02 a	5.50 a	4.57 a	10.06 a	447 b	146 a

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] values present are LS-means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly. Nematode egg data were log-transformed to satisfy the ANOVA assumption of normally distributed residuals values in the column followed by different letters differ significantly.

[x] Data for *R. reniformis* eggs/gram of root was collected from 4 root systems.

Table 8: Microplot trial LS means for cotton cultivar and nematicide combination effects on plant height, shoot fresh weight, root fresh weight, biomass, *Meloidogyne incognita* total eggs, *M. incognita* eggs per gram of root near 35 DAP and lint cotton yield at the Plant Science Research Center, Auburn, AL in 2020 and 2021.

Cotton Variety LS-means	**Plant height**	**Shoot fresh weight (g)**	**Root fresh weight (g)**	**Biomass (g)**	**Total *M. incognita* eggs**	***M. incognita* eggs/g of root**[x]	**Seed Cotton Yield (g)**
PHY 340 W3FE[z]	22.59 a[y]	13.86 a	2.30 a	16.15 a	992 a	729 a	40 a
PHY 360 W3FE	23.08 a	12.69 a	1.85 a	14.54 a	103 b	128 b	45 a

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] values present are LS-means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly. Nematode egg data were log-transformed to satisfy the ANOVA assumption of normally distributed residuals values in the column followed by different letters differ significantly.

[x] Data for *M. incognita* eggs/gram of root was collected from 4 root systems.

Table 9: Field trial LS means for cotton cultivar and nematicide combination effects on plant stand, plant height, root fresh weight, plant biomass, and lint cotton yield at the Plant Breeding Unit in 2020.

Source of Variation (F-value)	Stand	Plant height	Root fresh weight (g)	Biomass (g)	Lint cotton yield (kg/ha)
Cotton Variety[z]	279.33****[w]	0.37	2.28	5.48*	4.29**
Nematicide[y]	9.65****	1.62	1.14	1.57	4.54***
Variety x Nematicide	1.07	0.36	0.37	0.31	1.09
Cotton Variety LS-means					
PHY 360 W3FE	44.58[v] b	13.91 a	0.49 a	4.57 b	1621 a
PHY 340 W3FE	71.58 a	13.69 a	0.52 a	5.06 a	1529 b
Nematicide LS-means					
Untreated	63.25 a	14.13 a	0.50 a	4.74 a	1527 ab
Reklemel™ (0.28 L/ha) + Vydate® C-LV (1.24 L/ha)	60.65 a	14.79 a	0.52 a	5.17 a	1489 ab
Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	45.65 b	13.43 a	0.49 a	4.22 a	1363 b
Reklemel™ (1.13 L/ha) + Vydate® C-LV (5.0 L/ha)	48.00 b	13.20 a	0.54 a	4.77 a	1458 ab
BIOST Nematicide 100[x] (0.026 mg ai/seed)	61.35 a	12.74 a	0.47 a	4.41 a	1657 a
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.28 L/ha) + Vydate® CLV (1.24 L/ha)	63.45 a	14.15 a	0.51 a	5.33 a	1716 a
BIOST Nematicide 100(0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	62.70 a	13.74 a	0.50 a	4.81 a	1720 a
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (1.13 L/ha) + Vydate® C-LV (5.0 L/ha)	59.55 a	14.25 a	0.53 a	5.08 a	1671 a

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] Reklemel™ and Vydate® C-LV was applied at the time of planting as an in-furrow spray.

[x] Heat-killed *Burkholderia rinojenses* and its non-living spent fermentation media.

[w] Significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

[v] Values present are LS-means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly.

Table 10: Field trial LS means for cotton cultivar and nematicide combination effects on plant stand, plant height, root fresh weight, plant biomass, and lint cotton yield at the Plant Breeding Unit in 2021.

Source of Variation (F-value)	Stand	Plant height	Root fresh weight (g)	Biomass (g)	Lint cotton yield (kg/ha)
Cotton Variety[z]	4.62^{*}[w]	0.26	0.13	4.17^{**}	95.04^{****}
Nematicide[y]	1.41	9.59^{****}	9.35^{****}	10.42^{***}	3.09^{***}
Variety x Nematicide	0.40	0.58	0.88	0.62	0.60
Cotton Variety LS-means					
PHY 360 W3FE	81.33[v] a	16.88 a	3.26 a	42.44 a	1411 a
PHY 340 W3FE	83.69 a	16.73 a	3.22 a	39.31 b	887 b
Nematicide LS-means					
Untreated	82.25 a	15.09 c	2.34 d	30.82 d	964 b
Reklemel™ (0.21 L/ha) + Vydate® C-LV (0.88 L/ha)	82.35 a	16.53 bc	2.96 bcd	37.03 cd	1221 ab
Reklemel™ (0.28 L/ha) + Vydate® C-LV (1.24 L/ha)	84.00 a	16.41 bc	3.18 bcd	40.07 bcd	1002 ab
Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	81.50 a	17.36 ab	3.41abc	43.25 abc	1171 ab
BIOST Nematicide 100[x] (0.026 mg ai/seed)	84.85 a	14.94 c	2.75 cd	33.15 d	1060 ab
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.21 L/ha) + Vydate® CLV (0.88 L/ha)	82.30 a	17.84 ab	3.71 ab	47.79 ab	1143 ab
BIOST Nematicide 100(0.026 mg ai/seed) + Reklemel™ (0.28 L/ha) + Vydate® C-LV (1.24 L/ha)	83.90 a	17.55 ab	3.41 abc	43.78 abc	1316 a
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	78.90 a	18.70 a	4.09 a	51.11 a	1315 a

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] Reklemel™ and Vydate® C-LV was applied at the time of planting as an in-furrow spray.

[x] Heat-killed *Burkholderia rinojenses* and its non-living spent fermentation media.

[w] Significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

[v] Values present are LS-means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly.

Table 11: Field trial LS means for cotton cultivar and nematicide combination effects on plant stand, plant height, root fresh weight, plant biomass, and lint cotton yield at the Tennessee Valley Research Extension Center in 2020.

Source of Variation (F-value)	Stand	Plant height	Root fresh weight (g)	Biomass (g)	Lint cotton yield (kg/ha)
Cotton Variety[z]	27.99****[w]	14.89***	27.31****	28.12****	295.26****
Nematicide[y]	1.87*	4.05***	5.60****	4.28***	7.86****
Variety x Nematicide	2.48	1.30	0.60	0.52	4.48
Cotton Variety LS-means					
PHY 332 W3FE	51.20[v] a	13.42 a	2.52 a	21.82 a	1998 b
PHY 340 W3FE	41.34 b	12.42 b	1.97 b	16.62 b	1284 a
Nematicide LS-means					
Untreated	51.20 a	11.73 c	1.72 c	14.32 b	1289 c
Reklemel™ (0.28 L/ha) + Vydate® C-LV (1.24 L/ha)	46.85 a	13.33 abc	2.14 abc	19.29 ab	1545 ab
Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	50.35 a	14.10 a	2.64 a	23.70 a	1732 ab
Reklemel™ (1.13 L/ha) + Vydate® C-LV (5.0 L/ha)	46.70 a	12.63 abc	1.98 bc	18.13 ab	1668 ab
BIOST Nematicide 100[x] (0.026 mg ai/seed)	46.80 a	12.36 bc	2.1 abc	18.08 ab	1504 bc
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.28 L/ha) + Vydate® CLV (1.24 L/ha)	40.20 a	12.59 abc	2.12 abc	17.67 ab	1687 ab
BIOST Nematicide 100(0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	45.10 a	13.45 ab	2.71 a	21.95 a	1777 a
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (1.13 L/ha) + Vydate® C-LV (5.0 L/ha)	42.95 a	13.16 abc	2.57 ab	20.60 a	1731 ab

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] Reklemel™ and Vydate® C-LV was applied at the time of planting as an in-furrow spray.

[x] Heat-killed *Burkholderia rinojenses* and its non-living spent fermentation media.

[w] Significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

[v] Values present are LS-means separated using the Tukey-Kramer method at *P*≤0.05. Values in the same column followed by the same letter do not differ significantly.

Table 12: Field trial LS means for cotton cultivar and nematicide combination effects on plant stand, plant height, root fresh weight, plant biomass, and lint cotton yield at the Tennessee Valley Research Extension Center in 2021.

Source of Variation (F-value)	Stand	Plant height	Root fresh weight (g)	Biomass (g)	Lint cotton yield (kg/ha)
Cotton Variety[z]	103.43****[w]	0.04	1.82	5.48**	168.50****
Nematicide[y]	2.46**	4.52***	5.48****	6.32****	7.38****
Variety x Nematicide	1.71	0.32	0.60	0.56	0.51
Cotton Variety LS-means					
PHY 332 W3FE	40.85[v] a	11.22 a	1.20 a	12.46 a	1692 a
PHY 340 W3FE	24.81 b	12.42 a	1.10 a	11.07 b	989 b
Nematicide LS-means					
Untreated	30.07 ab	9.68 c	0.74 c	8.07 c	1008 c
Reklemel (0.21 L/ha) + Vydate® C-LV (0.88 L/ha)	39.62 a	10.61 abc	1.10 abc	10.90 abc	1253 bc
Reklemel™ (0.28 L/ha) + Vydate® C-LV (1.24 L/ha)	34.26 ab	11.64 ab	1.30 a	12.96 ab	1456 ab
Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	35.60 ab	11.48 abc	1.32 a	12.49 ab	1445 ab
BIOST Nematicide 100[x] (0.026 mg ai/seed)	32.43 ab	10.28 bc	0.87 bc	9.30 bc	1099 c
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.21 L/ha) + Vydate® CLV (0.88 L/ha)	32.37 ab	12.15 a	1.33 a	13.55 a	1334 abc
BIOST Nematicide 100(0.026 mg ai/seed) + Reklemel™ (0.28 L/ha) + Vydate® C-LV (1.24 L/ha)	30.19 ab	12.00 ab	1.35 a	14.23 a	1538 ab
BIOST Nematicide 100 (0.026 mg ai/seed) + Reklemel™ (0.56 L/ha) + Vydate® C-LV (2.5 L/ha)	28.10 b	11.63 ab	1.20 ab	12.62 ab	1591 a

[z] All seeds were treated with Metalaxyl 4.0 ST, Fludioxonil 4L ST, Myclobutanil 240 ST, Resonate 600.

[y] Reklemel™ and Vydate® C-LV was applied at the time of planting as an in-furrow spray.

[x] Heat-killed *Burkholderia rinojenses* and its non-living spent fermentation media.

[w] Significance at the 0.1, 0.05, 0.01, and 0.001 level is indicated by *, **, ***, and **** respectively.

[v] Values present are LS-means separated using the Tukey-Kramer method at $P \leq 0.05$. Values in the same column followed by the same letter do not differ significantly.

Figure 1. Greenhouse grown plants at Auburn University's Plant Science Research Center showing the nematode susceptible PHY 340 W3FE on the left and the *Meloidogyne incognita* nematode resistant PHY 360 W3FE on the right.

Figure 2. Microplot test at Auburn University's Plant Science Research Center showing the nematode susceptible PHY 340 W3FE on the left and the *Meloidogyne incognita* nematode resistant PHY 360 W3FE on the right.

Figure 3. Field plot at Auburn University's Plant Breeding Unit (PBU) showing the nematode susceptible PHY 340 W3FE on the left and the *Meloidogyne incognita* nematode resistant PHY 360 W3FE on the right around 106 DAP.

Figure 4. Aerial image of the *Meloidogyne incognita* field plots at the Plant Breeding Unit (PBU) of Auburn University near Tallassee, Alabama. PHY 340 outlined in yellow and PHY 360 outlined in black.

Figure 5. Auburn University’s Tennessee Valley Research Extension Center showing the nematode susceptible PHY 340 W3FE on the left and the *Rotylenchulus reniformis* resistant PHY 332 W3FE on the right 102 DAP.

Figure 6. Aerial image of the *Rotylenchulus reniformis* field plots at the Tennessee Valley Research Extension Center (TVREC) of Auburn University near Bella Mina, Alabama. PHY 340 outlined in orange and PHY 360 outlined in black.

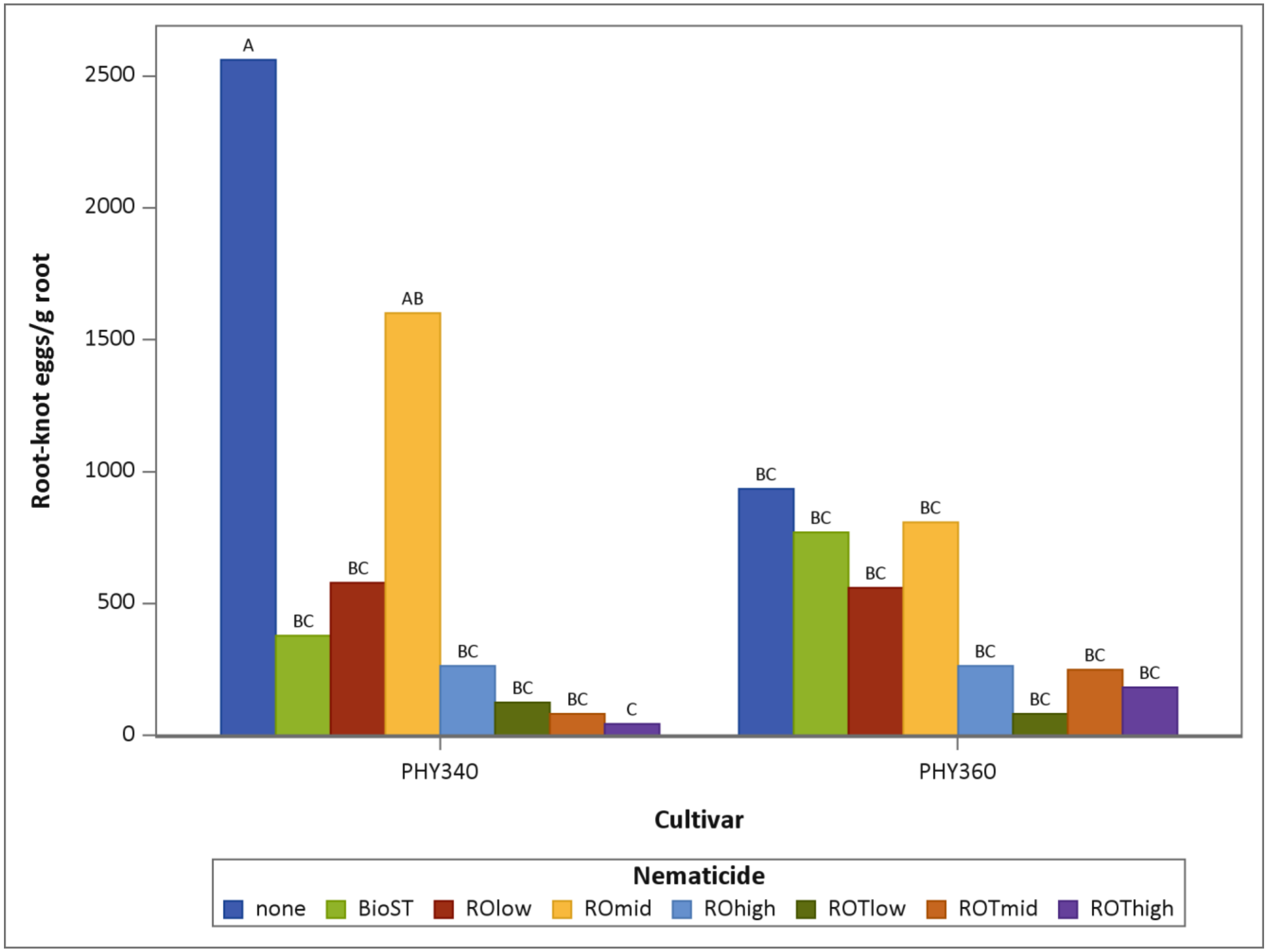

Figure 7. Field trial LS means for cotton cultivar and nematicide combination effects on *Meloidogyne incognita* egg population density expressed as number of eggs per gram of root near 40 DAP at the Plant Breeding Unit in 2020.

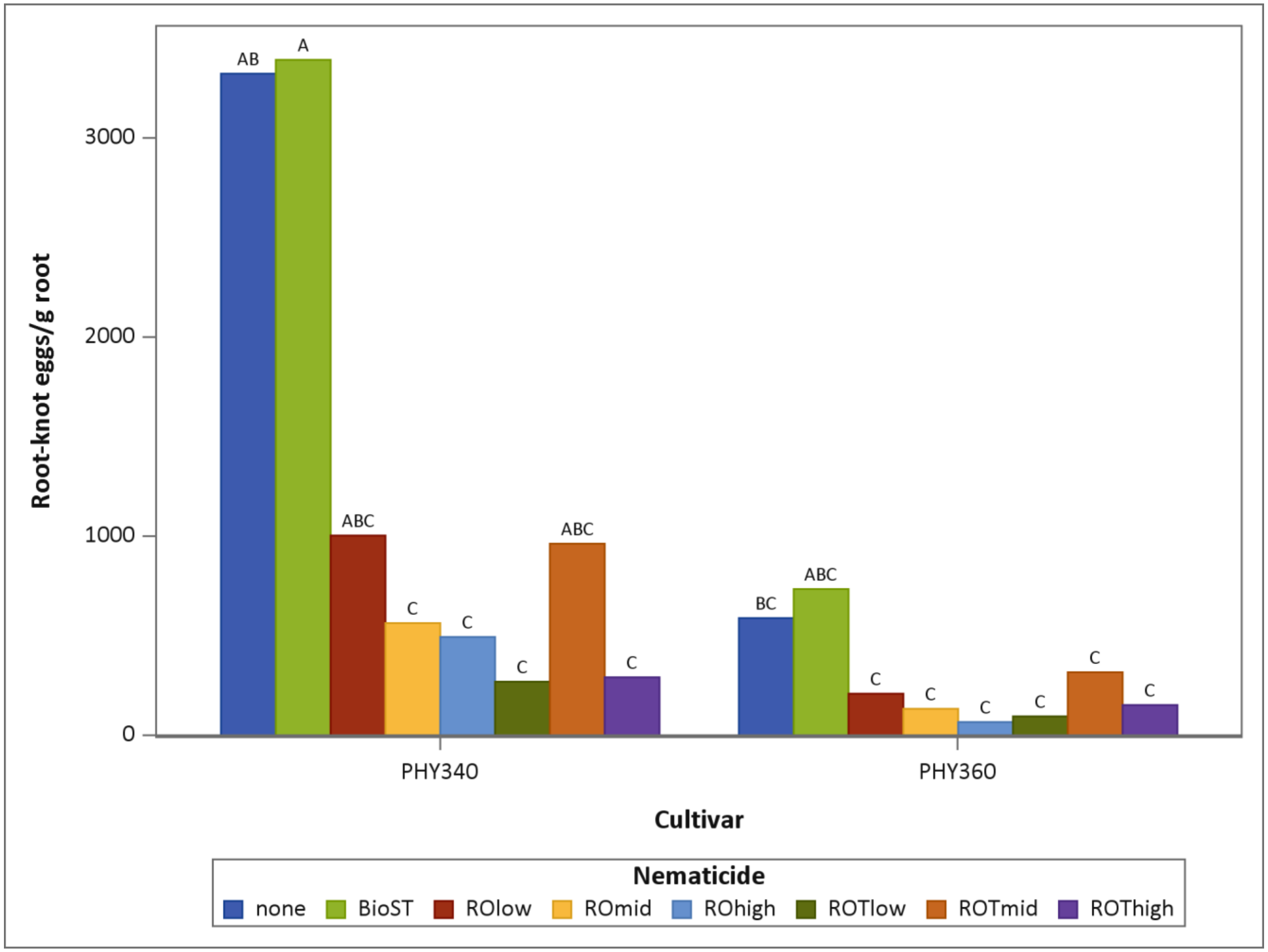

Figure 8. Field trial LS means for cotton cultivar and nematicide combination effects on *Meloidogyne incognita* egg population density expressed as number of eggs per gram of root near 40 DAP at the Plant Breeding Unit in 2021.

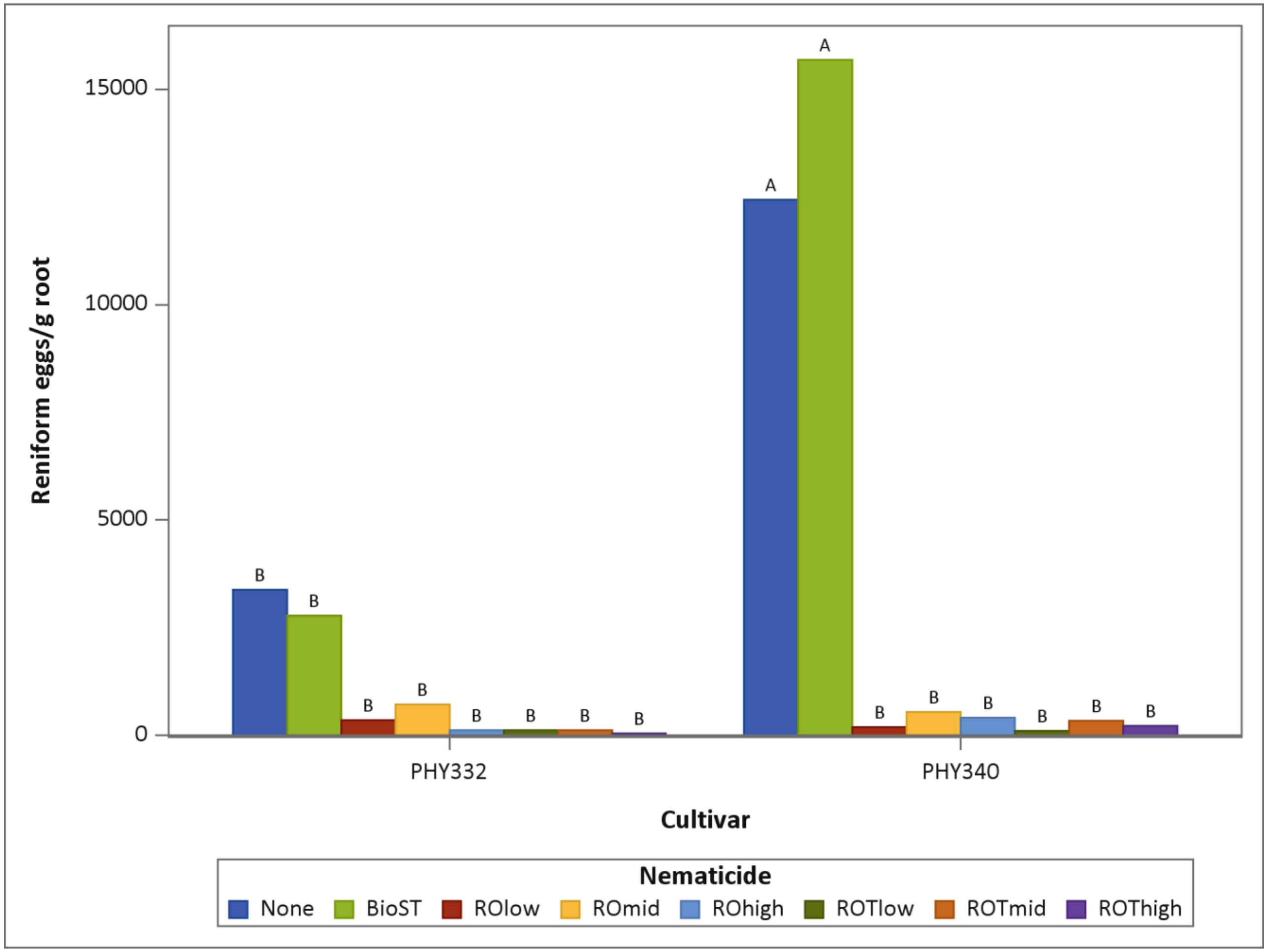

Figure 9. Field trial LS means for cotton cultivar and nematicide combination effects on *Rotylenchulus reniformis* egg population density expressed as number of eggs per gram of root near 40 DAP at the Tennessee Valley Research Extension Center in 2020.

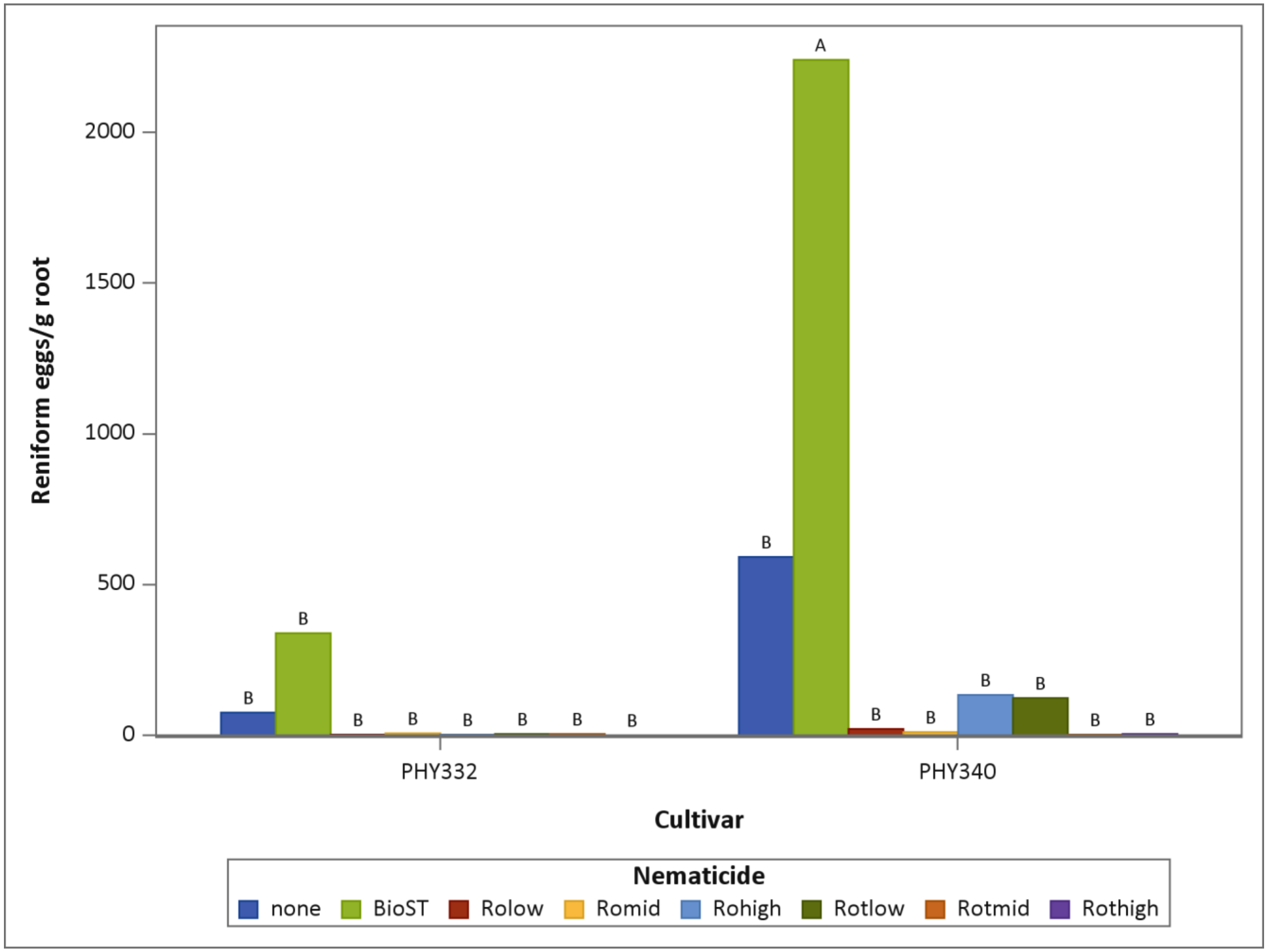

Figure 10. Field trial LS means for cotton cultivar and nematicide combination effects on *Rotylenchulus reniformis* egg population density expressed as number eggs per gram of root near 40 DAP at the Tennessee Valley Research Extension Center in 2021.

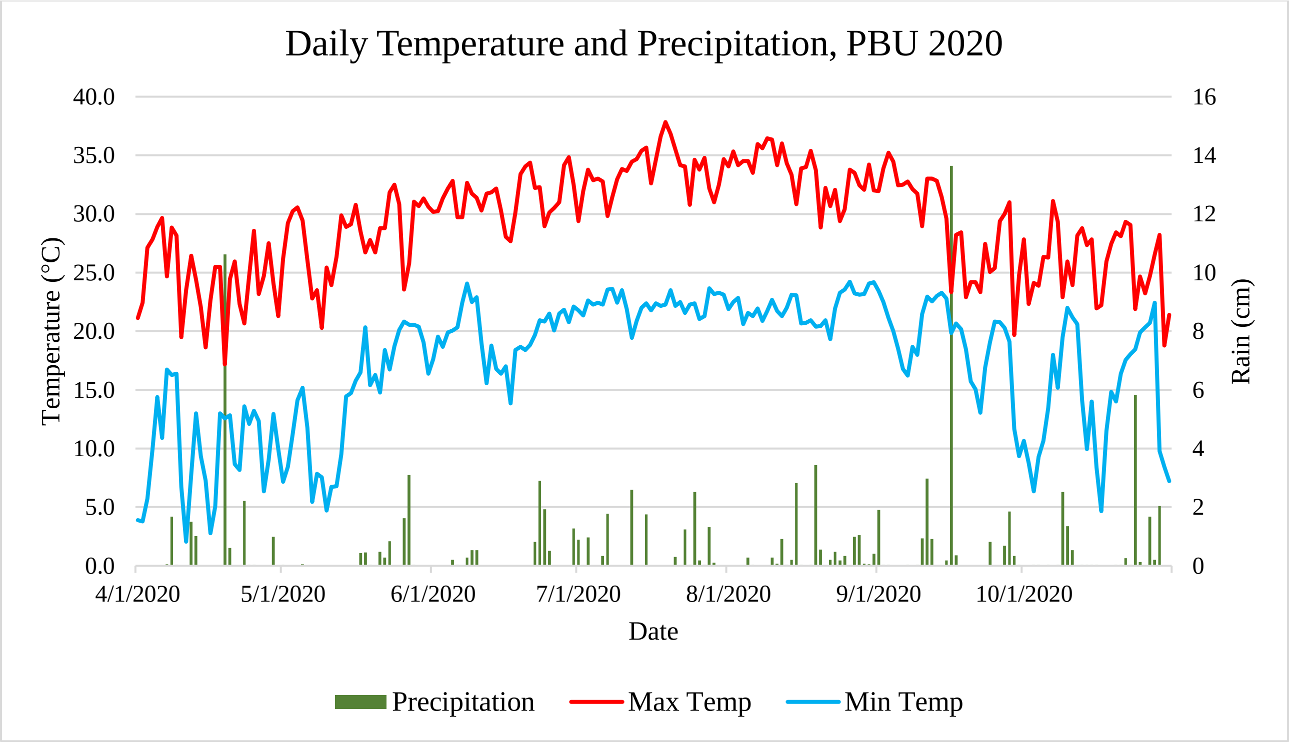

Figure 11. Daily temperatures and precipitation from time of planting until harvest at the Plant Breeding Unit, 2020 near Tallassee, AL.

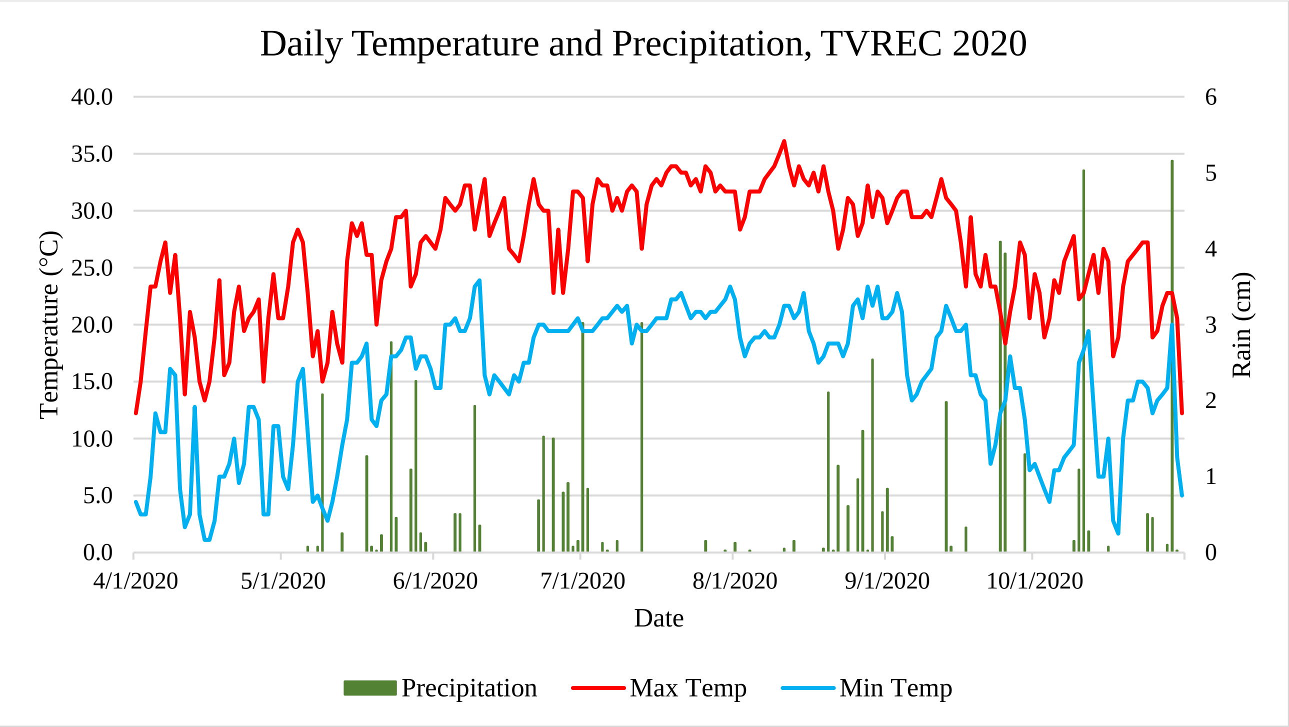

Figure 12. Daily temperatures and precipitation from time of planting until harvest at the Tennessee Valley Research and Extension Center, 2020 near Belle Mina, AL.

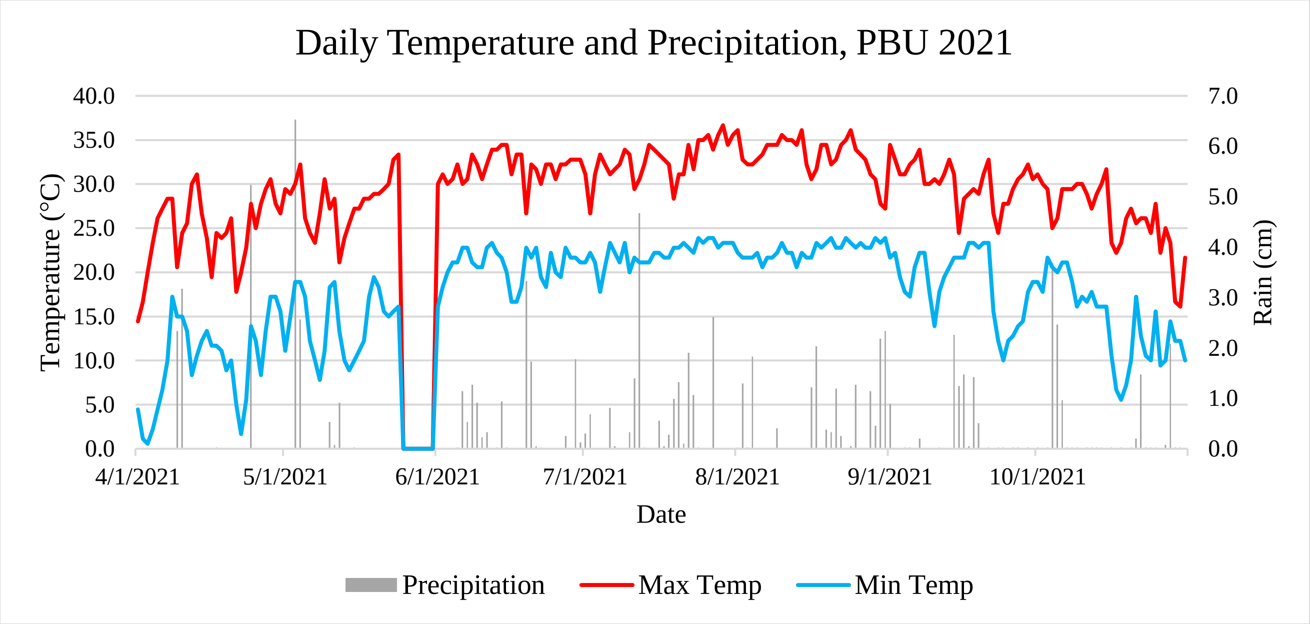

Figure 13. Daily temperatures and precipitation from time of planting until harvest at the Plant Breeding Unit, 2021 near Tallassee, AL.

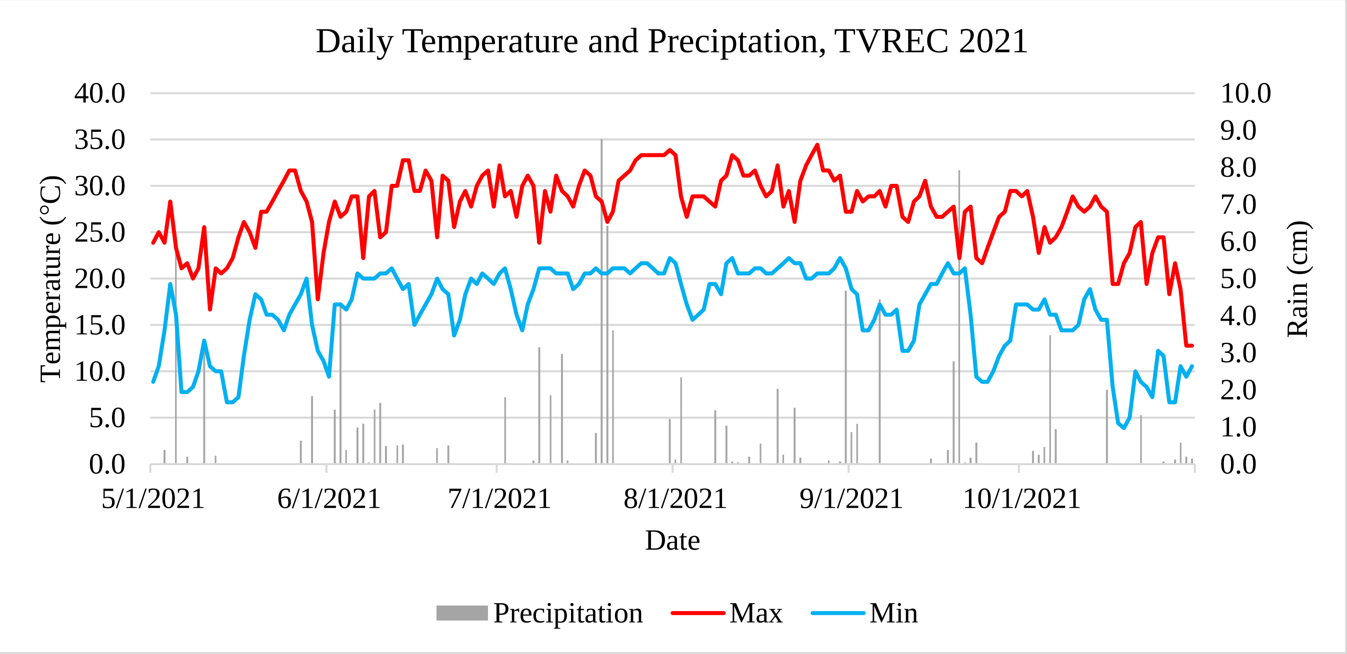

Figure 14. Daily temperatures and precipitation from time of planting until harvest at the Tennessee Valley Research and Extension Center, 2021 near Belle Mina, AL.