MELOIDOGYNE INCOGNITA RESISTANCE QTLS IN UPLAND COTTON HAVE DIFFERENT EFFECTS ON NEMATODE DEVELOPMENT, BUT DO NOT AFFECT FUSARIUM WILT SEVERITY

by

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(Under the Direction of Richard Davis and Peng Chee)

ABSTRACT

The germplasm sources highly resistant to *Meloidogyne incognita* (Southern root-knot nematode) in Upland cotton (*Gossypium hirsutum* L) contain the resistance QTLs *qMI-C11* and *qMi-C14*. Previous research documented resistance expressed at two stages of nematode development, and later research documented an epistatic interaction between the two QTLs, both of which suggest the QTLs have different modes of action. Our experiments demonstrated that *qMi-C11* reduces the number of galls and egg masses, total egg production, and eggs/egg mass whereas *qMi-C14* reduces the number of egg masses, total egg production, and eggs/egg mass without reducing galling. *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) interacts with *M. incognita* resulting in increased Fusarium wilt (FW), therefore, we tested the effect of the nematode resistance QTLs on the severity of FW. A reduction of plant growth and an increase in FW severity was seen on all genotypes regardless of nematode resistance QTLs when coinfected with Fov and *M. incognita.* FW has been reported more frequently in Georgia in recent years, which suggests that something affecting the disease complex may have changed. In 2015 and 2016, a survey of Georgia cotton fields was conducted. The survey showed that Fov race 1 is still the dominant race, but Fov genotypes not previously reported in Georgia were found. Additionally, many instances of FW in Georgia are due to Fov interacting with *Belonolaimus longicaudatus* and not *M. incognita* as previously believed.

INDEX WORDS: *Meloidogyne incognita, Fusarium oxysporum* f.sp. *vasinfectum,* Resistance, QTL, Survey

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DEDICATION

This dissertation is dedicated to God, my family, and friends. I thank God for giving me strength and the wonderful family and friends that supported me through hard times and carried and motivated me to pursue my dreams.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

ROOT-KNOT NEMATODE

Root-knot nematode (RKN) is the common name for plant parasitic nematodes in the genus *Meloidogyne. Meloidogyne spp*. have a wide host range (Alpizar et al., 2007; Castillo et al., 2003, Creech et al., 1995; Miyashita et al., 2014,) and cause visible galls to form on the roots of parasitized plants. *Meloidogyne incognita* is the most important root-knot species and causes agricultural losses worldwide. This species has four races that are separated based on differential host plants (Sasser and Carter, 1985) with races 3 and 4 causing damage to cotton (Galbieri and Asmus, 2016). The United States is the third largest producer of cotton in the world producing 3024 million Kg in 2016 for an average harvest of 958 Kg/ha (The Economic Outlook for U.S. Cotton, 2017). *Meloidogyne incognita* is estimated to cause losses of 3.4% (107 million Kg) valued at $147 million in the U.S.A. cotton belt (Lawrence et al., 2015).

The motile form *M. incognita* is the second stage juvenile (J2) that moves out of the egg and through the soil pores until it finds a plant root that it can infect. The J2 penetrates a susceptible plant root tip, migrates through the root to the cortical tissue, where it establishes a feeding site (Starr and Bridge, 2007). When a feeding site is established, no further migration of the nematode will occur, which is why the nematode is classified as a sedentary endoparasite. Then, the J2 starts to swell (a stage called a swollen J2, or SJ2) and the nematode molts into successive developmental stages: third (J3) and fourth stage juveniles (J4), and finally an adult, which are overwhelmingly female (Taylor and Sasser, 1978). Males can be formed, but they are not common and are not necessary for reproduction of this parthenogenetic species. Females are rounded in shape and typically produce egg masses that protrude onto the surface of the root. The egg mass is protected by a gelatin-like compound and contains around 300-1000 eggs per egg mass (Tang et al., 1994). The egg masses rupture the root tissue and it is exposed on the root surface, which is visible to the naked eye as white dots over the galls (Starr and Bridge, 2007). A significant aspect of feeding site establishment involves the formation of giant cells by repeated mitosis without separation by cytokinesis and hyperplasia. The physical changes in the root around the giant cells result in a visible root gall, which is a diagnostic symptom of *M. incognita* parasitism. The length of the *M. incognita* life cycle is largely driven by temperature and is shorter when the weather is warmer (Babadoost, 2014).

Root-knot nematodes interact with several fungi, including *Fusarium oxysporum* f.sp. *vasinfectum* (Fov, the Fusarium wilt pathogen), *Verticilium dahliae* (the Verticillium wilt pathogen), and *Rhizoctonia solani* (the cause of damping off in cotton) and *Thielaviopsis basicola* (Al-Hazmi and Al-Nadary, 2015; Bell et al., 2017; Katsantonis et al., 2003; Ma et al., 2014). Although Fov can damage cotton plants on its own, Fusarium wilt (FW) is well documented to be much more severe as the result of a disease complex involving the interaction of Fov and RKN (Chawla et al., 2012; Cooper and Brodie, 1963). Therefore, FW is a much greater problem when the two pathogens occur together. The primary method of controlling FW is by controlling RKN through the use of nematicides (Kemerait et al., 2008). The most widely used nematicide in cotton for many years was aldicarb, but it was phased out beginning in 2011 (Cone, 2010). In the last few years, RKN-resistant cotton varieties have become available and are beginning to be used for RKN control, and it is generally assumed that they should also suppress FW. Nematicides are typically applied at or before planting and reduce the population levels of both target and nontarget nematode species.

FUSARIUM WILT OF COTTON

The causal agent of Fusarium wilt (FW) in cotton is the phytopathogenic *Fusarium oxysporum* f. sp. *vasinfectum* (Fov). Disease symptoms typically appear in middle to late spring or early fall (DeVay et al., 1997, Starr et al., 1989). Fov invades root cells and moves upward causing vascular discoloration (Shi et al., 1991). The fungal mycelium and its toxin production and plant defenses such as tyloses and phenolic compounds (Beckman, 2000; Beckman, 1964; VanderMolen et al., 1987) can clog the xylem. Plants severely injured will wilt and die. The first visual symptoms are typically that the leaves start yellowing around the veins and quickly senesce. Plant growth is typically affected and plants are stunted, often forming a patch in the field (Nelson, 1981). Water splash, contaminated soil debris, and agricultural machines help spread the pathogen to non-contaminated areas. Fov can be a soil and seed-borne pathogen (Bell et al., 2017; Kreitlow et al., 1961) and can be spread easily state-to-state or even overseas. Fov has been classified into two pathotypes based on infection mode. The root-rot pathotype causes wilting independent of nematode infection with severe root damage and the vascular competent pathotype is dependent of the nematode to cause severe disease and does not cause severe root damage (Bell et al., 2016). Vascular-competent pathotypes cause disease when inoculated using stem-puncture techniques but not via infested soil assays, whereas root-rot pathotypes cause disease in soil assays but not from stem-punctures (Bell et al., 2017; Bell et al., 2016).

Fov is subdivided into races, genotypes, and biotypes. Genotypes can be distinguished based on small genetic variations among isolates. Isolates with morphological or physiological differences without known genetic differences are labeled biotypes (Downie, 2010). Race is a broader term for isolates where there is usually knowledge of both genotypic and phenotypic variations. Initially, host differentials were used to distinguish races. For example, race 1 was different from race 2 because race 1 does not cause disease in soybean and tobacco, but race 2 was pathogenic in those plants. Because Fov molecular DNA analysis, multigene genealogy, vegetative compatibility groups (VCG), and greenhouse pathogenicity tests started showing similarities among the classified races (Holmes et al., 2009; Kim et al., 2005; Skovgaard et al., 2001; Egamberdiev et al., 2013), host differentials were deemed to be an inadequate form of race classification. Therefore, the previous race classification was replaced by genetic analogy classification, where isolates were identified and classified according to DNA sequencing. Isolates with novel DNA sequences were classified into genotypes (Holmes et al., 2009). The genetic classification is based on Fov nuclear and ribosomal regions including translational elongation factor (EF-1α), intergenic spacer (IGS) region, phosphate permease (PHO), and beta-tubulin (BT) (Cianchetta et al., 2015; Kim et al., 2005). In Georgia, the most common races of Fov are 1, 2, and 8, and the most common genotypes are LA108 and LA110 (Cianchetta et al., 2015; Holmes et al., 2009). Those races are described to interact with root-knot nematode to cause severe wilt disease (Bell et al., 2017). However, other nematode species also have been reported to interact with Fov, including *Pratylenchus penetrans* (lesion nematode), *Belonolaimus longicaudatus* (sting nematode), and *Rotylenchulus reniformis* (reniform nematode) (Cooper and Brodie, 1963; Seinhorst and Kuniyasu, 1971; Neal, 1954).

Fov has no sexual reproduction, and therefore, genetic variability occurs through mutation or a parasexual cycle. The parasexual cycle is initiated when two vegetative compatible haploid hyphae cells fuse to form a heterokaryon. The two nuclei then fuse to form a diploid cell (mitotic recombinant) that later will undergo haploidization. Additionally, hyphae can be formed from three different conidia types: microconidia (unicellular), macroconidia (multicellular), and chlamydospores. The main resistant structure that can persist in the soil is the chlamydospore (Bennett, 2012). Management of Fusarium wilt can be achieved with rotation with non-host crops, non-infested seeds, and moderately resistant cotton cultivars (Scott et al., 2011). In Georgia, cotton is one of the crops most affected by Fusarium wilt. Because there are no highly resistant cotton varieties to Fov, usually management is done by rotating cotton with peanut to decrease *M. incognita* population levels and consequently to control FW in the field.

RESISTANCE IN COTTON

A few cotton cultivars with a high level of resistance to *M. incognita* have been released in the past four years such as Deltapine 1454 and 1558 NR B2RF and Phytogen 487 WRF (Lawrence and Glass, 2015). The studies in cotton on resistance to *M. incognita* began with the transgressive segregant Auburn 623 RNR, which carries genes imparting a high level of resistance to RKN and FW (Shepherd, 1982). Auburn 623 RNR was the progeny of Clevewilt-6 and Wild Mexican Jack Jones, two cotton genotypes with moderate resistance to RKN (Shepherd, 1974). Auburn 623 RNR was crossed with Auburn 56 to develop Auburn 634 RNR, which also is highly resistant to *M. incognita* (Klump and Thomas, 1987). Auburn 634 RNR was then used to develop a number of RKN resistance lines referred to the M-line series (Fig. 1).

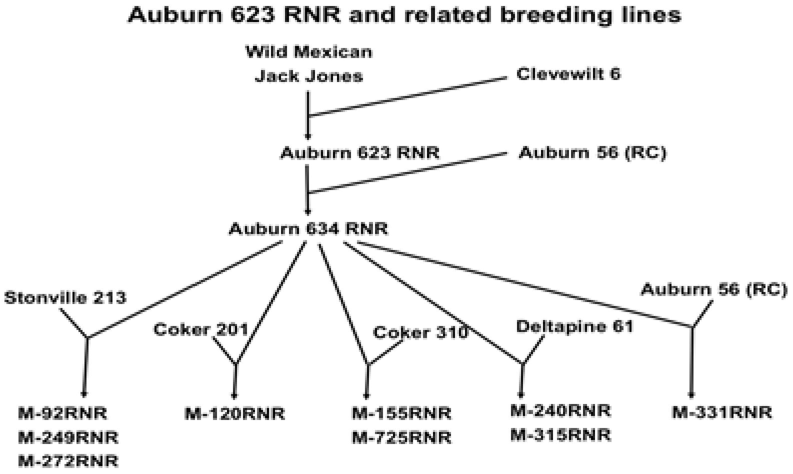

Figure 1.1 Pedigree of RKN resistant M-series lines (Robinson et al., 2001).

McPherson et al. (2004) observed the segregation of the resistance genes from M-315 RNR, one of the M-lines, and they concluded that the resistance is controlled by a dominant (*Mi*1) and an additive gene (*Mi*2). Later, two major QTLs controlling RKN resistance were found on chromosomes 11 and 14 (Shen et al., 2006; Ynturi et al., 2006). The resistance QTLs on chromosome 11 (*qMi-C11*) and 14 (*qMi-C14*) were shown originally to be inherited from Clevewilt-6 and Wild Mexican Jack Jones, respectively (Gutierrez et al., 2010). The major QTL *qMi-C11* was described to have a strong effect on root-galling and egg production whereas the QTL *qMi-C14* has little effect on galling but reduces egg production (Gutiérrez et al., 2010; He et al., 2014). Cotton genotypes with both *qMi-C11* and *qMi-C14* are highly resistant to RKN. All the M-lines developed from Auburn 634 RNR carry the two known genes for resistance to *M. incognita* (Shepherd et al., 1996).

Several isogenic lines were developed from M-120 RNR in the UGA cotton molecular breeding lab to evaluate the individual effects of RKN resistance QTLs *qMi-C11* and *qMi-C14*.

These lines were produced to study the effect of both QTLs together and separately on the root-knot nematode life cycle and on the plant's response to infection by RKN. The RKN resistant line M-120 RNR is homozygous for both QTLs and the line Coker 201 is the RKN susceptible recurrent parent used in creating M-120 RNR (Fig. 1). M-120 RNR and Coker 201 were crossed and selfed twice to obtain a segregating F2 population. Chromosome specific molecular markers were used to identify plants that carried only one of the two QTLs. Plants homozygous for the resistance QTL on either chromosome 11 or 14 were selected to advance in each generation. The resulting isolines carry an RKN resistance QTL either on chromosome 11 or chromosome 14. These isolines provide a valuable research tool for studying the effects of the nematode-resistance QTLs.

QTLs on chromosome 11 and 14 were also reported to impart resistance to FW in cotton (Ulloa et al., 2011; Ulloa et al., 2013), but it is not known if they have any relationship with the QTL regions for RKN resistance. The QTLs controlling resistance to FW differ according to Fov race (Ulloa et al., 2013; Ulloa et al., 2011). Fov races were characterized in the past according to infectivity in different crops. As previously explained, the Fusarium-Root-knot nematode disease complex increases Fusarium wilt severity (Chawla et al., 2012; Garber et al., 1979), and controlling RKN can reduce FW incidence and severity. The mechanism by which RKN infection allows Fov to overcome host resistance to FW is not known, and the Fov-RKN interaction is poorly understood. The increase of Fov growth appears to be associated with giant cell formation in tomato (Fattah and Webster, 1983). Microscopic analyses showed increased hyphal growth as giant cells increased in size in Fusarium-resistant cultivars. In the xylem of Fusarium-resistant cultivars, the mycelium invasion and fungal crystalline inclusions inside the giant cells coincided with the J4 and adult stages of *Meloidogyne javanica* development (Fattah and Webster, 1983). The M-315 RNR cotton germplasm line contains both QTLs *qMi-C11* and *qMi-C14* and has shown resistance to Fusarium wilt (Scott et al., 2011). If gall formation is what precipitates the interaction with the fungus, then resistant plant genotypes that significantly reduce gall formation may reduce Fov infection and FW. The QTL *qMi-C11* is believed to cause gall reduction (Shen et al., 2006), however, both QTLs together have an epistatic effect (He et al., 2014) and it is not known the effect of individual QTLs have on FW.

HYPOTHESIS OF THE STUDIES

Based on previous research, we hypothesize that:

1. The resistance QTLs *qMi*-C11 and *qMi*-C14 have different mechanisms in conferring resistance to *M. incognita* in cotton.
2. The isogenic lines with the *qMi*-C11 locus induces *M. incognita* J2s to leave the root system.
3. The isogenic lines with *qMi*-C11 and/or *qMi*-C14 have an effect on eggs/egg mass, percentage egg hatch, and gall size.
4. The resistance QTLs *qMi*-C11 and *qMi*-C14 have different effects on Fov race 1 in the presence or absence of *M. incognita*.

Additionally, we obtain knowledge of the predominant Fov races in Georgia and determine the predominant plant parasitic nematodes associated with Fov infected fields.

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CHAPTER 2

RESISTANCE QTL *QMI-C11* AND *QMI-C14* IN COTTON HAVE DIFFERENT EFFECTS ON THE DEVELOPMENT OF *MELOIDOGYNE INCOGNITA.*[1]

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[1] M. B. Da Silva, P. W. Chee, P. Kumar, B. Nichols, and R. F. Davis. To be summited to Plant disease.

ABSTRACT

QTLs *qMi-C11* and *qMi-C14* impart resistance to *Meloidogyne incognita* in cotton. Breeders had previously backcrossed both QTLs into Coker 201 (susceptible) to create M-120 RNR (highly resistant), and we crossed Coker 201 and M-120 RNR to create isogenic lines with either *qMi-C11* or *qMi-C14*. Previous work suggests different modes of action for *qMi-C11* and *qMi-C14*. To document individual and combined effects of the QTLs on nematode development and reproduction, Coker 201 (neither QTL), M-120 RNR (both QTLs), CH11 isoline (*qMi-C11*), and CH14 isoline (*qMi-C14*) were inoculated with *M. incognita*. At 4, 8, 12, 16, 20, 25, and 30 days after inoculation (DAI), roots were stained to observe nematode developmental stages (J2, swollen J2, J3, J4, and Female), and the number of galls was counted. At 20, 25, 30 and 40 DAI, *M. incognita* eggs were harvested and counted. At 30 DAI, 80% of the nematodes on Coker 201 were female compared to 50%, 40%, and 33% females on CH14, CH11, and M-120 RNR, respectively, and a greater proportion of nematodes remained in the J2 stage in M-120 RNR (41%), CH11 (58%) and CH14 (27%) than in Coker 201 (9%). More nematodes progressed to the J3 or J4 stage on Coker 201 and CH14 than on CH11 or M-120 RNR. Coker 201 and CH14 had more galls than M-120 RNR. Coker 201 had more eggs than the other genotypes on 30 DAI. This study confirms that *qMi-C11* and *qMi-C14* act at different times and have different effects on the development of *M. incognita* and therefore, have different modes of action.

INTRODUCTION

The United States is the third largest producer of cotton in the world with an average harvest of 958 Kg/ha (3024 million Kg) in 2016 (The Economic Outlook for U.S. Cotton 2017). *Meloidogyne incognita*, the Southern root-knot nematode, is an important pathogen of cotton that is estimated to cause losses of 3.4% (107 million Kg) valued at $147 million in the U.S.A. cotton belt (Lawrence et al., 2015). The most commonly used management options to control *M. incognita* in cotton in the US are based primarily on the use of nematicides, which may be applied as seed treatments, in-furrow applications at planting, or pre-plant fumigation (Burris et al., 2010; Davis and May, 2005; Wheeler et al., 2013). Crop rotation with poor or non-host crops is not a viable option for all growers due to limited hectarage or profitability of effective rotation crops, and nematicides are typically expensive and do not provide season long control. The best option for cost-effective, long-term management is the use of resistant varieties. Cotton cultivars with a high level of resistance to *M. incognita* were first available to farmers in 2014 with the resistant varieties Deltapine 1454 and 1558 NR B2RF and Phytogen 487 WRF (Lawrence and Glass, 2015). However, only 0.16% of the fields in US plant these resistant varieties (cotton varieties planted 2017, USDA).

The primary *M. incognita*-resistant cotton lines used in breeding programs derive their nematode resistance from Auburn 623 RNR (Gutiérrez et al., 2010; Jenkins et al., 1995; Klump and Thomas, 1987; Shen et al., 2006), which carries genes imparting a high level of resistance to *M. incognita* and Fusarium wilt (Shepherd, 1982). Auburn 623 RNR was the progeny from a cross between Clevewilt-6 and Wild Mexican Jack Jones, two cotton genotypes with moderate resistance to *M. incognita* (Shepherd, 1974). Auburn 623 RNR was the resistance source for Auburn 634 RNR, and Auburn 634 RNR was used to develop a number of *M. incognita*-resistant lines referred to as the M-line series. All the M-lines developed from Auburn 634 RNR are highly resistant to *M. incognita* (Shepherd et al., 1996). M-lines such as M-315 RNR and M-120 RNR have been instrumental in studying *M. incognita* resistance in cotton. McPherson et al. (2004) first suggested that there were two resistance genes in the M-lines, and two major QTLs controlling *M. incognita* resistance were subsequently identified on chromosomes 11 and 14 (Shen et al., 2006; Ynturi et al., 2006). The resistance QTLs on chromosome 11 (*qMi-C11*) and 14 (*qMi-C14*) were shown to be inherited from Clevewilt-6 and Wild Mexican Jack Jones, respectively (Gutierrez et al., 2010; Shen et al., 2006). Cotton genotypes with both *qMi-C11* and *qMi-C14* are highly resistant to *M. incognita*.

Resistance to *M. incognita* in cotton in the germplasm line M-315 RNR was shown to be due to post-penetration interference with nematode development (Jenkins et al., 1995). *Meloidogyne incognita* eggs hatch to release second stage juveniles (J2), the infective form of the nematode, which penetrate into roots and establish feeding sites. J2s that successfully establish feeding sites become sedentary, slightly swell (SJ2 stage), and then develop through two additional juvenile stages (J3 and J4) before becoming adults (Gheysen and Jones, 2006; Taylor and Sasser, 1978). Interference with *M. incognita* development in resistant cotton compared to a susceptible genotype has been reported at both 6-8 days after inoculation (DAI) and also at 18-24 DAI (Jenkins et al., 1995; McPherson et al., 2004; McClure et al., 1974; Mota et al., 2013), which approximately coincides with the transition from SJ2 to J3 and with egg production by females, respectively. In cotton populations with both resistance QTLs that were segregating for resistance to *M. incognita*, *qMi-C11* had a significant association with a reduction in root galling and egg production, whereas *qMi-C14* had little effect on galling but had a significant association with a reduction in egg production (Gutiérrez et al., 2010, He et al., 2014).

Isogenic lines were developed from M-120 RNR in the UGA Cotton Molecular Breeding Laboratory to evaluate the individual effects of the resistance QTLs *qMi-C11* and *qMi-C14* on *M. incognita* development and reproduction on the plant’s response to infection by the nematode.

The genotype Coker 201 has neither QTL, but it was the *M. incognita*-susceptible recurrent parent used in creating M-120 RNR, which is homozygous for both QTLs. The isogenic M-120 RNR and Coker 201 were crossed to create F1 seed that were self-pollinated to obtain an F2 population for which *qMi-C11* and *qMi-C14* were segregating. Molecular markers were used to identify plants that carried only one of the two QTLs. Plants homozygous for the resistance QTLs on either chromosome 11 or 14 were selected to advance in each generation resulting in isogenic lines that carry either *qMi-C11* or *qMi-C14*. The objective of this study was to characterize the effects of *qMi-C11* and *qMi-C14* together and individually on *M. incognita* development and reproduction in cotton isogenic lines.

MATERIALS AND METHODS

An experiment was conducted in the greenhouse to evaluate nematode reproduction and development on isogenic cotton lines that differed in QTLs for resistance to *M. incognita*. Four cotton genotypes were evaluated: CH11 (with the resistance QTL *qMi-C11*), CH14 (with *qMi-C14*), the susceptible cultivar Coker 201 (with neither QTL), and the resistant germplasm line M-120 RNR (*qMi-C11* and *qMi-C14*). Cotton seeds were germinated in vermiculite and grown for two weeks. Seedlings of similar size then were transplanted, one per pot, into 10.6 cm x 10.6 cm x 12.4 cm pots filled with approximately 500 ml of steam-pasteurized soil (Tifton loamy sand). At transplant, seedlings were infected with 4,000 *M. incognita* J2 per plant. Nematode inoculum from greenhouse cultures was obtained from eggplant roots which were washed free of soil and placed in a mist chamber for 5 days to obtain J2 from egg hatch. Pots were grouped by sampling date and genotypes within a sampling date were arranged in a completely randomized design with six replications per genotype per sample date. The experiment was conducted twice.

The developmental progression of *M. incognita* was evaluated at 4, 8, 12, 16, 20, 25, and 30 DAI on each of the four genotypes. On each sample date, roots were stained to make it easier to see and count nematodes inside the roots, and the numbers of J2, swollen J2 (SJ2), J3, J4, and mature females were counted. To stain the roots, a slightly modified method of Byrd Jr. et al. (1983) was followed. Roots were carefully cleaned by immersing the soil in water and gently washing them free of soil. Individual root systems were soaked in 30 ml bleach solution (5.25% NaOCl) for 4 min and then rinsed under running tap water before soaking for 15 min in 30 ml of water. Roots were then placed in 30 ml of tap water plus 1 ml of cotton blue solution (0.35g of cotton blue powder to 250 ml of lactic acid and 750 ml of distilled water) and the roots in stain solution were microwaved just until the solution started boiling (approximately 10 sec). Roots were destained so that the nematodes could be seen more easily by rinsing the roots in tap water to remove excess stain and then soaking the roots in non-acidified glycerin. The roots and glycerin were microwaved just until it began to boil. After the glycerin cooled down, roots were observed under an inverted microscope and the number of nematodes at each developmental stage was recorded. Each plant also was assessed for the number of root galls at 25, 30 and 40 DAI and total egg production at 20, 25, 30, and 40 DAI. Eggs were extracted by washing roots free of soil and shaking the root system in a beaker with 1.23% NaOCl for 4 min and eggs were collected on a 500-mesh sieve.

The number of nematodes observed at each developmental stage (J2+SJ2, J3+J4, or female) also was expressed as a percentage of the total number of nematodes observed (J2+SJ2+J3+J4+female). Data for all variables were analyzed for differences among cotton genotypes using the PROC MIXED procedure in SAS 9.3 statistical software (SAS Institute Inc., Cary, NC). No interactions were seen between trial and genotype for the number or percentage of nematodes at each developmental classification (total number, J2 + SJ2, J3+J4, and female), therefore, the data from both trials were combined for analysis. Similarly, data from the two trials for egg counts and gall numbers were combined for analysis. Egg counts were log10 transformed prior to analysis.

RESULTS

The total number of nematodes (sum of all developmental stages) observed in the roots did not differ among the genotypes at 4 and 8 DAI (Fig. 2.1 A). The total number of nematodes observed in the roots did not differ between Coker 201 and CH14 on any sampling date, except on 30 DAI. The total numbers of nematodes in the roots did not differ between M-120 RNR and CH11 on any sampling date (Fig. 2.1 A). However, differences in total number of nematodes between Coker 201 and the genotypes carrying *qMi-C11* (CH11 and M-120 RNR) were observed. Coker 201 had more total nematode numbers in the roots than CH11 at 12, 16, 20, 25, and 30 DAI and more than M-120 RNR 12 DAI. Although M-120 RNR typically had numerically fewer total nematode numbers than other genotypes, statistical differences were lacking between M-120 RNR and CH11.

The number of nematodes in the J2 plus SJ2 stages did not differ among genotypes at either 4 or 8 DAI (Fig. 2.1 B). The number of J2 plus SJ2 in the roots of Coker 201 and CH14 did not differ on any sampling date. Coker 201 and CH14 had greater J2 plus SJ2 counts than both CH11 and M-120 RNR only at 12 DAI. M-120 RNR had lower J2 and SJ2 than Coker 201 and CH14 on all sampling dates with statistical differences at 12, 16, and 20 DAI. The J2 and SJ2 counts in the roots differed between M-120 RNR and CH11 only at 20 DAI when CH11 had higher nematode counts (Fig 2.1 B).

Differences in the combined counts of J3 plus J4 stages were first observed at 8 DAI (Fig. 2.1 C). Coker 201 and CH14 had similar J3 plus J4 levels on all sampling dates. However, Coker 201 had higher J3 plus J4 levels at 8, 16, 20, 25, and 30 DAI than either CH11 or M-120 RNR, and CH14 had greater J3 plus J4 counts than either CH11 or M-120 RNR from 12 to 30 DAI (Fig. 2.1 C). M-120 RNR and CH11 had relatively low levels of J3 plus J4 and they did not differ statistically from each other on any sampling date. J3 plus J4 counts reached a high of 50 nematodes for Coker 201 and CH14 at 20 DAI, the counts did not exceed 20 nematodes for CH11 and M-120 RNR.

Females were first observed 12 DAI, and Coker 201 had numerically more females than the other genotypes on all sampling dates thereafter (Fig. 2.1 D). Beginning 16 DAI, the number of females was statistically greater on Coker 201 than on CH11 or M-120 RNR, whereas the numbers of females on CH14 was similar to Coker 201 on all sampling dates except at 30 DAI when CH14 had fewer females than Coker 201. The numbers of females on M-120 RNR and CH11 did not differ statistically from each other on any sampling date with both maintaining relatively low levels (<40 females). Although CH14 was similar to Coker 201 and CH11 was similar to M-120 RNR, CH14 and CH11 did not differ statistically in the number of females, however, CH14 had more females numerically than CH11 from 16 to 30 DAI.

The number of J2 plus SJ2 in CH14 as a percentage of the total number of all developmental stages was statistically similar to Coker 201 on all sample dates, and the percentage of nematodes in J2 plus SJ2 stages in CH11 was similar to M-120 RNR on all sampling dates. The percentage of nematodes at J2 plus SJ2 stages was smaller for Coker 201 than CH11 and M-120 RNR after 12 DAI. At 30 DAI, the percentage of nematodes in the J2 plus SJ2 stages was the least in Coker 201 at only 8% compared to 45%, 18 %, and 37% for CH11, CH14, and M-120 RNR, respectively (Fig. 2.2 A). The minimum percentage of nematodes in the J2 plus SJ2 stages on any sampling date was 8% for Coker 201, 44% for CH11, 18% for CH14, and 37% for M-120 RNR (Fig. 2.2 A). CH11 had a greater percentage of J2 plus SJ2 than CH14 at 20, 25 and 30 DAI.

The number of J3 plus J4 as a percentage of the total number of nematodes for Coker 201 only differed from the percentage in CH14 at 30 DAI when CH14 had a greater percentage of J3 plus J4 than Coker 201 (Fig. 2.2 B). The percentage of nematodes at J3 plus J4 stages on M-120 RNR was lower than that on Coker 201 at 20 and 25 DAI. CH11 and CH14 did not differ statistically except at 30 DAI when the percentage of nematodes at J3 plus J4 stages on CH14 was greater than on CH11. The percentage of nematodes in the J3 plus J4 stages did not exceed 35%, 35%, 25%, and 28% on any sampling date for Coker 201, CH14, CH11, and M-120 RNR, respectively (Fig. 2.2 B).

Females were a greater percentage of the total number of nematodes for Coker 201 than for CH11 and M-120 RNR beginning at 16 DAI and continuing for later sampling dates. At 30 DAI, 80% of the nematodes on Coker 201 were female whereas CH14, CH11, and M-120 RNR had just 50%, 40%, and 33% females, respectively. The percentage of nematodes that were females did not differ on CH14, CH11, and M-120 RNR except at 25 DAI (Fig. 2.2 C). The 40 DAI evaluation date had the development stages confounded by a second *M. incognita* generation resulting from first-generation eggs that hatched, therefore, that data is not presented.

Total egg production and the total number of galls per root system also were evaluated. Coker 201 and CH14 had similar number of galls per root system at 25, 30, and 40 DAI, and M-120 RNR and CH11 also had similar levels of galling (Fig. 2.3). Both CH11 and M-120 RNR had fewer galls than Coker 201 at 30 and 40 DAI. Coker 201 supported greater egg production than the other genotypes at both 30 and 40 DAI (Fig. 2.4). CH11 and CH14 had statistically similar levels of egg production.

DISCUSSION

The data in our study for J2 plus SJ2 and for J3 plus J4 were combined for analysis because there was a lot of variability in the individual stages and combining the stages made trends more evident and increased our ability to show that numerical differences were statistically significant. Previous research documented that resistance to *M. incognita* in cotton from the Auburn 623 RNR source is expressed at two stages of nematode development and that there is an epistatic interaction between the two QTLs, both of which suggest the QTLs have different modes of action (He et al., 2014, Jenkins et al., 1995). Two QTLs, *qMi-C11* and *qMi-C14*, each confer partial resistance to *M. incognita*. In our study, an increase in total nematode numbers was observed between 4 and 20 DAI on all genotypes, and then nematode levels remained steady or declined. Previous studies also showed a decrease in total nematode numbers after 20 DAI for some resistant cotton lines and resistant melon accessions (Faske, 2013; Faske and Starr, 2009; Jenkins et al., 1995; Stetina, 2015). Our study showed an increase in total nematode numbers at 40 DAI because a second generation of *M. incognita* had been produced and the generations could not be distinguished. Although *M. incognita* stages were easily identifiable inside the roots after staining, the total nematode numbers and J2 plus SJ2 percentage that we observed may have been affected by mechanical handling during our extraction procedure. Nematodes may have been lost on the early sampling dates because root tips and surrounding tissues of young seedlings, which is where nematodes would be soon after penetration, are very delicate and are prone to being broken off when roots are removed from soil. As seedling age, the sections of the root containing nematodes strengthen and do not break off as readily resulting in less loss of infected root tissue and an apparent increase in the total number of nematodes. Another possibility is that new infections may occur many days after inoculation.

The numbers of J2 plus SJ2 were similar for all genotypes at 4 and 8 DAI, which suggests that the resistance QTLs do not affect the entrance of *M. incognita* into the roots. Previous research also found that penetration of *M. incognita* is not affected by the resistance genes from the Auburn 623 RNR source (Creech et al., 1995; Faske and Starr, 2009; McClure et al., 1974). Although the total number of nematodes in the roots in our study increased by a relatively large amount after inoculation until 20 DAI, the number of J2 plus SJ2 increased only modestly after 8 DAI because the increased ability to observe J2s as young roots matured was countered by nematodes maturing into J3s and later stages. The actual counts of J2s plus SJ2s increased slightly through 20 DAI, however, the J2 plus SJ2 as a percentage of the total declined for all genotypes as some nematodes matured. The decline in percentage was more rapid for Coker 201 and CH14 resulting in a lower percentage of J2 plus SJ2 on those genotypes. The greater percentage of J2 plus SJ2 in M-120 RNR and CH11 indicates that fewer nematodes progress to J3 in plants with *qMi-C11*, which confirms the conclusions from previous studies of the Auburn 623 resistance source (Faske and Starr, 2009; Jenkins et al., 1995). It is possible that the lower numbers of J3 plus J4 on M-120 RNR and CH11 may indicate that J2s may leave the roots or die following penetration, not progressing to further stages, whereas they develop normally on CH14 or Coker 201. Emigration of *Meloidogyne* spp. from the roots of resistant plants when J2s fail to establish feeding sites has been reported for melon and soybean accessions (Faske, 2013; Pedrosa et al., 1996). Nematodes that remain in the roots but fail to successfully establish a feeding site will die and will not be observed and counted. Egression and death are the likely causes for the lower total number of nematodes observed on M-120 RNR and CH11.

The J3 plus J4 counts increased through 20 DAI and then decreased for all genotypes, but the changes were greater for Coker 201 and CH14 because more nematodes progressed from SJ2 to J3. Although the numbers of J3 plus J4 were greater for Coker 201 and CH14 than for M-120 RNR and CH11, J3 plus J4 as a percentage of the total did not differ consistently among the genotypes. However, at 30 DAI, both cotton genotypes containing *qMi-C14* had a larger percentage (statistically for CH14 and numerically for M-120 RNR) of J3 plus J4 than the genotypes that did not have that QTL because that QTL prevents some nematodes from developing into females. From the time plants were inoculated until 25 DAI, CH14 was indistinguishable from the susceptible Coker 201 in the total number of nematodes, the number of nematodes at each developmental stage, or the proportion of nematodes at the various developmental stages. However, by 30 DAI, Coker 201 had approximately twice as many females as CH14 resulting in a greater proportion of nematodes on Coker 201 reaching the female stage because *qMi-C14* stopped many nematodes from developing into females on CH14.

In a normal *M. incognita* life cycle, a J2 penetrates a root tip and establishes a feeding site by causing giant cells to form which ultimately results in a gall on the root (Miyashita et al., 2014). Once a feeding site is successfully established, the nematode can progress through the developmental stages and produce eggs. Resistance genes reduce nematode reproduction by interfering with some element of that process. In our study, cotton genotypes containing *qMi-C11* had less galling than genotypes without the QTL, which supports the conclusion that *qMi-C11* somehow interferes with the successful establishment of a feeding site thereby ultimately resulting in fewer nematodes maturing and producing eggs. We also observed on genotypes containing the *qMi-C11* locus a few galls in which we did not observe any nematodes, a phenomenon previously observed and labeled "empty galls" (McClure et al., 1974). It is not known whether the nematodes that initiate the empty galls die and disintegrate, move to establish a feeding site elsewhere, or leave the root.

Both QTLs affected the level of development of *M. incognita*. The *qMi-C11* QTL retards an early stage of nematode development, and *qMi-C14* retards J4 to female development, and both QTLs result in lowered egg production compared to a susceptible line. Jenkins et al. (1995) had also observed weak progression of nematodes at the begging and later stage of the *M. incognita* life cycle. In addition, egg production in the lines containing *qMi-C11* and *qMi-C14* show that these lines have good resistance to *M. incognita*, but they are not immune.

Creech et al. (1995) and Jenkins et al. (1995) documented inhibited nematode development, egg production, and gall production in M-315 RNR, which contains both resistance genes from the Auburn 623 RNR source of resistance, and they speculated that the resistance gene derived from Clevewilt (now known to be associated with *qMi-C11*) affected nematode development at around 6 DAI and that the gene derived from Wild Mexican Jack Jones (now known to be associated with *qMi-C14*) was responsible for affecting development around 24 DAI. Later research documented positive epistatic effects between *qMi-C11* and *qMi-C14* resulting in greater-than-additive effects (He et al., 2014), which means that the individual effects of each resistance QTL are best studied without the influence of the other QTL being present. Our study utilized isolines that separated the QTLs and proved that *qMi-C11* and *qMi-C14* act at different times and have different effects on the development of *M. incognita* in cotton, therefore, we conclude that *qMi-C11* and *qMi-C14* must have different modes of action.

Combining (pyramiding) resistance genes with different modes of action into a single genotype is likely the best method of improving disease control and prolonging the usefulness of the resistance genes by minimizing selection pressure on the pathogen (Djian-Caporalino et al., 2014).

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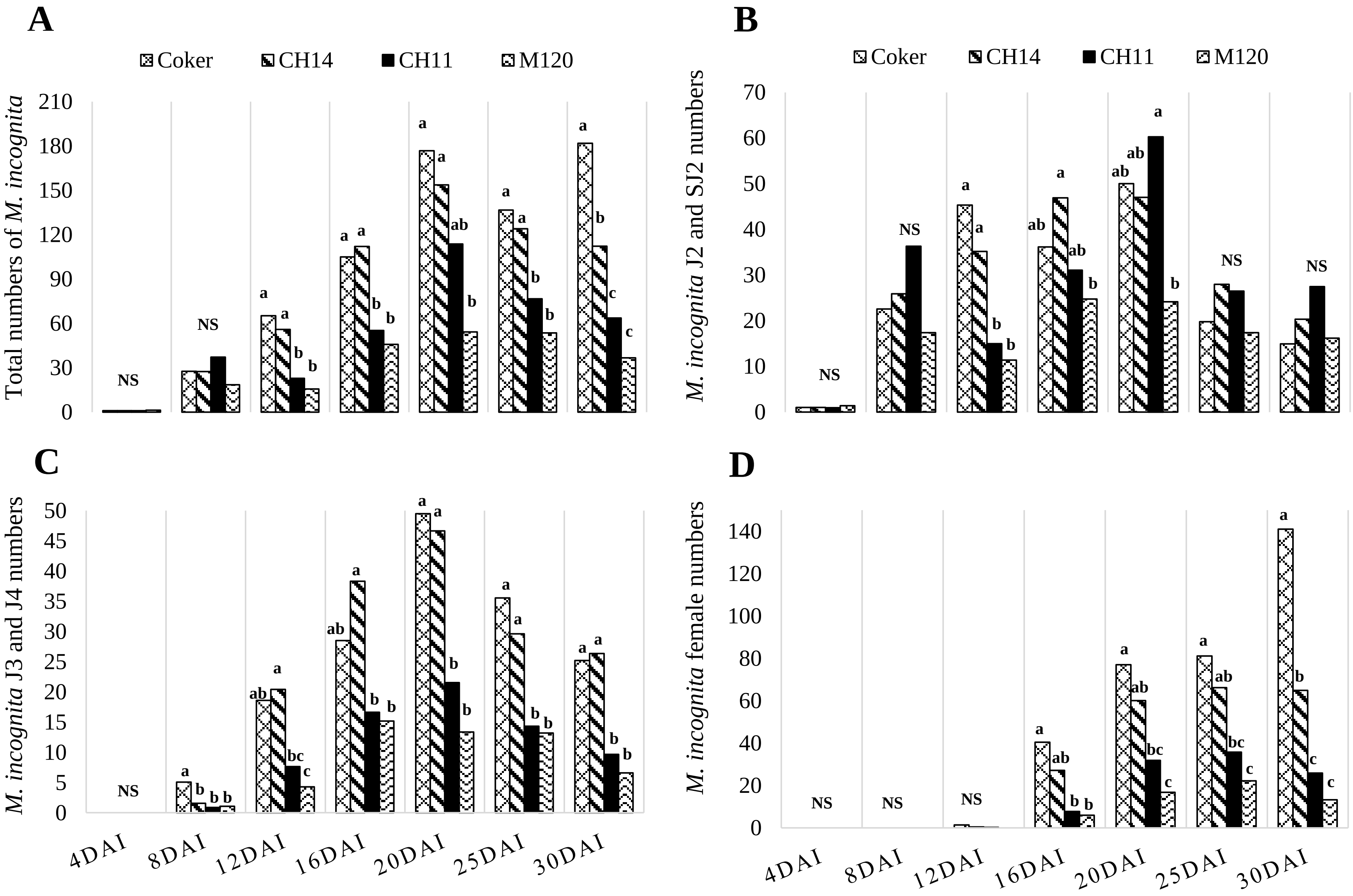
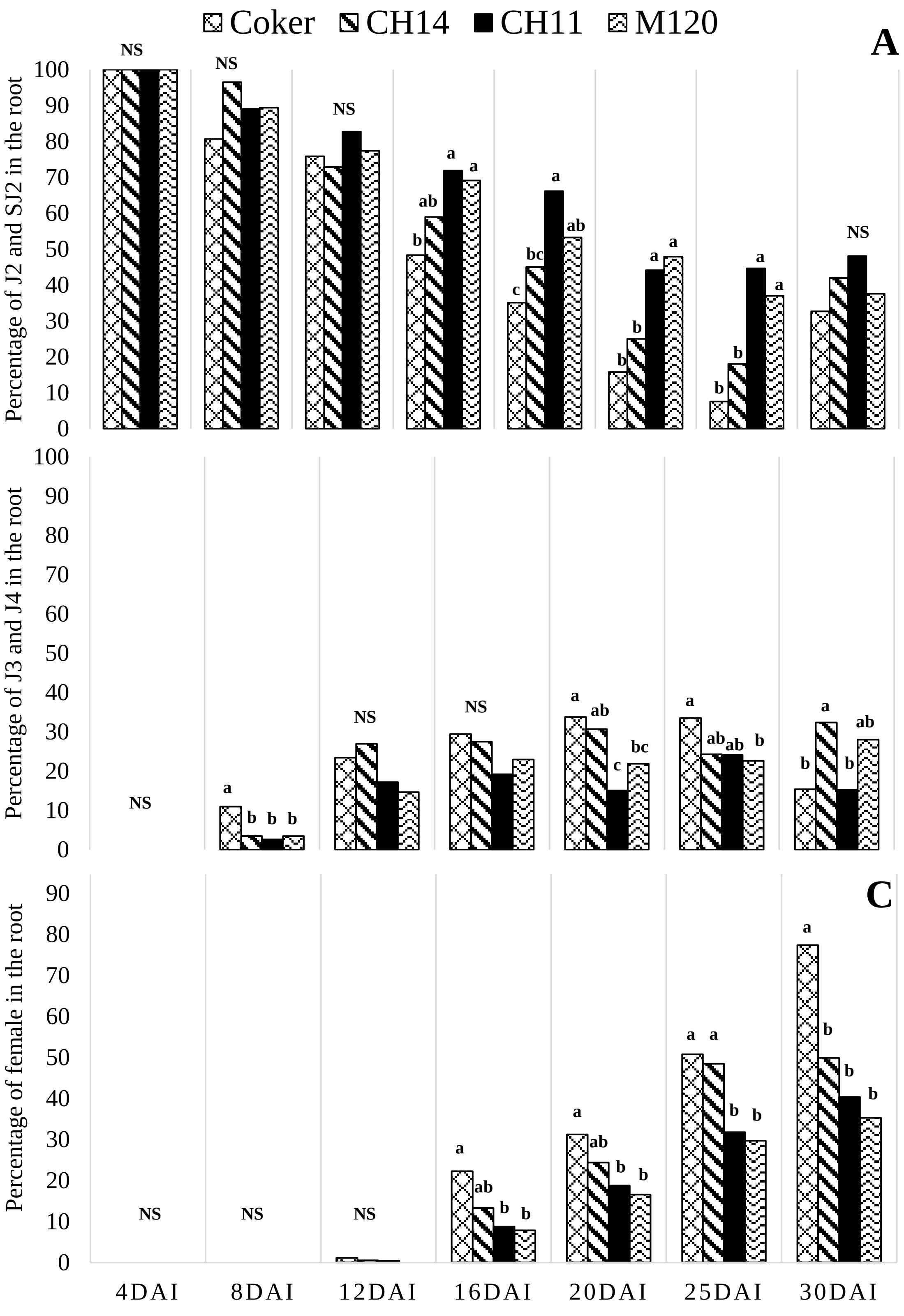

A
Coker
CH14
CH11
M120
Total numbers of M. incognita
0
30
60
90
120
150
180
210
NS
NS
a a b b
a a b b
a a ab b
a a b b
a b c c
B
Coker
CH14
CH11
M120
M. incognita J2 and SJ2 numbers
0
10
20
30
40
50
60
70
NS
NS
a a b b
ab a ab b
ab ab a b
NS
NS
C
M. incognita J3 and J4 numbers
0
5
10
15
20
25
30
35
40
45
50
NS
a b b b
ab a bc c
ab a b b
a a b b
a a b b
a a b b
4DAI
8DAI
12DAI
16DAI
20DAI
25DAI
30DAI
D
M. incognita female numbers
0
20
40
60
80
100
120
140
NS
NS
NS
a ab b b
a ab bc c
a ab bc c
a b c c
4DAI
8DAI
12DAI
16DAI
20DAI
25DAI
30DAI

Fig. 2.1



▨ Coker
▧ CH14
■ CH11
▨ M120
A
C
Percentage of J2 and SJ2 in the root
Percentage of J3 and J4 in the root
Percentage of female in the root
0
10
20
30
40
50
60
70
80
90
100
NS
NS
NS
b
ab
a
a
c
bc
a
ab
b
b
a
a
b
b
a
a
NS
NS
a
b
b
b
NS
NS
a
ab
c
bc
a
ab
ab
b
b
a
b
ab
NS
NS
NS
a
ab
b
b
a
ab
b
b
a
a
b
b
a
b
b
b
4DAI
8DAI
12DAI
16DAI
20DAI
25DAI
30DAI

Figure 2.2

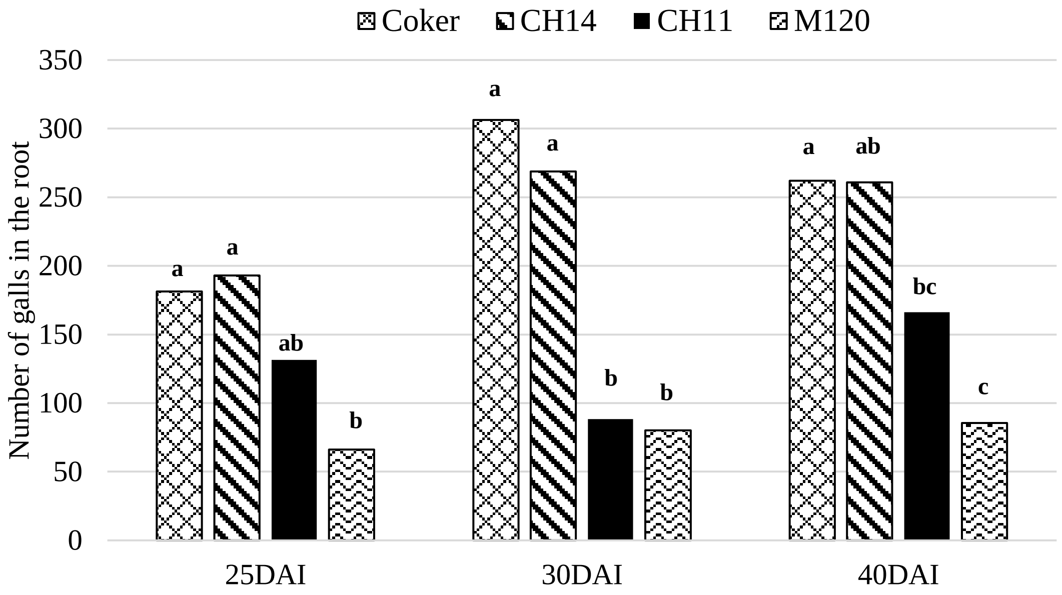

Coker
CH14
CH11
M120
350
300
250
200
150
100
50
0
Number of galls in the root
a
a
ab
b
a
a
b
b
a
ab
bc
c
25DAI
30DAI
40DAI

Figure 2.3

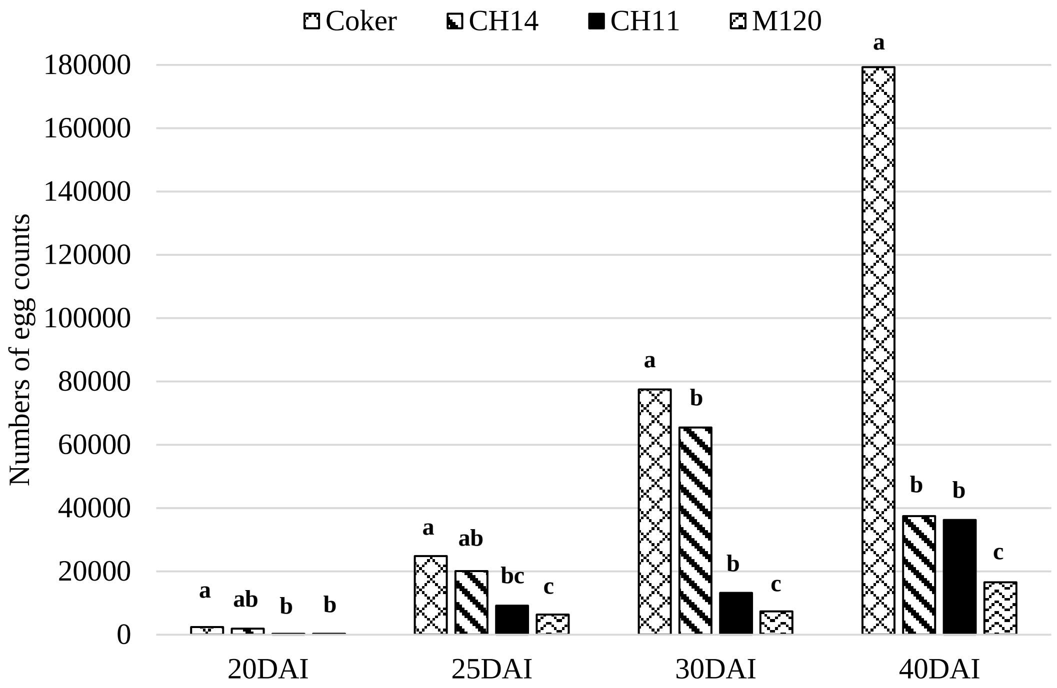

Coker
CH14
CH11
M120
Numbers of egg counts
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
a
ab
b
b
20DAI
a
ab
bc
c
25DAI
a
b
b
c
30DAI
a
b
b
c
40DAI

Fig. 2.4

Figure 2.1. Number of *Meloidogyne incognita* at different developmental stages in the roots of cotton isolines that differ in *M. incognita*-resistance genes. Total numbers are the sum of all development stages (A). Second stage juveniles (J2) plus swollen J2 (SJ2) (B). Third stage juveniles (J3) plus fourth stage juveniles (J4) (C). Females (D). DAI = days after inoculation. Different letter over bars within a sampling date indicate significant differences at α=0.05 according to Tukey's test (NS = not significant).

Figure 2.2. *Meloidogyne incognita* at different developmental stages (expressed as a percentage of the total number of nematodes) in the roots of cotton isolines that differ in *M. incognita*-resistance genes. Second stage juveniles (J2) plus swollen J2 (SJ2) (A). Third stage juveniles (J3) plus fourth stage juveniles (J4) (B). Females (C). DAI = days after inoculation. Different letter over bars within a sampling date indicate significant differences at α=0.05 according to Tukey's test (NS = not significant).

Figure 2.3. Number of galls presented on the roots of cotton isolines that differ in *M. incognita*-resistance genes at 25, 30, and 40 days after inoculation (DAI). Different letter over bars within a sampling date indicate significant differences at α=0.05 according to Tukey's test.

Figure 2.4. Number of eggs harvested from the roots of cotton isolines that differ in *M. incognita*-resistance genes at 20, 25, 30, and 40 days after inoculation (DAI) on the four genotypes. Different letter over bars within a sampling date indicate significant differences at α=0.05 according to Tukey's test.

CHAPTER 3

THE EFFECT OF TWO QTLS FOR RESISTANCE TO *MELOIDOGYNE INCOGNITA* IN COTTON ON NEMATODE EGRESSION FROM ROOTS AND FECUNDITY [2]

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[2] M. B. Da Silva, P. W. Chee, P. Kumar, B. Nichols, and R. F. Davis. To be summited to Journal of Nematology.

ABSTRACT

Cotton is widely grown in the southern US and *Meloidogyne incognita* is the most significant pathogen of cotton in the US. M-120 RNR germplasm is highly resistant to *M. incognita* due to two resistance QTLs, *qMi-C11* and *qMi-C14*. Previous research showed that both QTLs reduce total egg production, the QTLs affect *M. incogni*ta development at different times in the life cycle, and the QTLs do not appear to affect initial penetration of *M. incognita*, however, the genotypes containing *qMi-C11* had fewer nematodes in the roots 8 days after inoculation, which may indicate *M. incognita* egression from roots. Three greenhouse trials were conducted using cotton isolines to determine whether *qMi-C11* and *qMi-C14* affect egression of *M. incognita* juveniles from roots. Two-week-old seedlings were inoculated with *M. incognita*; two days after inoculation (DAI), roots were rinsed and seedlings were transplanted into small cones filled with vermiculite. On each of the five sampling dates (4, 6, 8, 10, and 12 DAI), nematodes that egressed from roots were extracted from the vermiculite and counted and roots were stained to count nematodes that remained in the roots. The effect of the resistance QTLs on *M. incognita* egression from the roots differed among the trials. Beginning 6 DAI, nematode egression was consistently numerically greater, but inconsistently statistically different, from plants with both QTLs than from plants with neither QTL. Plants with only one QTL generally did not differ from plants with both QTLs, and the effects of *qMi-C11* and *qMi-C14* did not differ in any consistent way. At 40 DAI in a separate experiment, plants with neither QTL had more eggs per egg mass than plants with both QTLs, whereas plants with only one QTL were intermediate and did not differ from each other. Root gall size was measured in two trials with seedlings in clear plastic bags. Individual galls were labeled when they first appeared (day 0) and each gall’s size was measured at 0, 7, and 14 days. No consistent differences in gall size

were observed. We conclude that 1) *qMi-C11* and *qMi-C14* do not significantly stimulate nematode egression from cotton roots, 2) both *qMi-C11* and *qMi-C14* reduce *M. incognita* eggs/egg mass, and 3) neither *qMi-C11* nor *qMi-C14* affect gall size.

INTRODUCTION

Meloidogyne incognita, the Southern root-knot nematode, causes greater total damage than any other pathogen of cotton in the United States and is responsible for losses of $147 million/year (Lawrence et al., 2015). The infective second stage juveniles (J2) are mobile and penetrate into the root system to find a feeding site. If a feeding site is successfully established, then the nematode ceases movement and progresses to subsequent developmental stages. Some plants can inhibit the establishment of *Meloidogyne spp*. feeding sites through a hypersensitive response (Davies et al., 2015), which has been suggested as a mechanism of resistance to *M. incognita* in cotton (Mota et al., 2013). Failure to establish a feeding site may lead a nematode to leave the root (Timper et al., 2000). Nematode egression from the roots of resistant plants has been documented for peanut, potato, alfalfa, and tomato (Timper et al., 2000; Mojtahedi et al., 1988; Mullin and Brodie, 1988).

Cotton germplasm with resistance to *M. incognita* is available (Shepherd et al., 1996), and that resistance is due to two major QTLs, *qMi-C11* and *qMi-C14*. One QTL, *qMi-C11*, has a strong effect on root-galling and nematode reproduction whereas the other QTL, *qMi-C14*, has little effect on galling but does reduce reproduction (Gutiérrez et al., 2010; He et al., 2014). Current research utilizing isogenic lines that separate *qMi-C11* and *qMi-C14* documents that *qMi-C11* inhibits successful establishment of a feeding site and subsequent development of the nematode whereas *qMi-C14* allows the successful establishment of a feeding site but then inhibits fourth-stage juveniles (J4) from becoming adults (Silva et al. unpublished data). Inhibition of gall formation was also observed by Jenkins et al. (1995) on the M-315 RNR resistant genotype, which contains *qMi-C11* and *qMi-C14*. Galls increased in size until 20 DAI and then ceased to develop further (Jenkins et al., 1995).

Previous research found that the initial penetration of *M. incognita* into cotton roots was not affected by the *qMi-C11* and *qMi-C14* sources of resistance (Creech et al., 1995). We hypothesized that *qMi-C11*, which inhibits feeding site establishment, gall formation, and nematode development early in the infection process, leads nematodes to leave the root and may also affect gall size. The primary objective of this study was to determine whether the resistance QTLs *qMi-C11* and *qMi-C14* affect egression of *M. incognita* J2 from cotton roots. Additionally, the effect of the resistance QTLs on eggs/egg mass, percentage egg hatch, and gall size was evaluated.

MATERIALS AND METHODS

The isogenic cotton lines Coker 201, M-120 RNR, CH11, and CH14 were used to evaluate the effects of *qMi-C11* and *qMi-C14* on nematode egression from roots. The germplasm line M-120 RNR was derived from backcrossing resistance to *M. incognita* (later attributed to the resistance QTLs *qMi-C11* and *qMi-C14*) into the susceptible cultivar Coker 201 (Shepherd et al., 1996). M-120 RNR contains both *qMi-C11* and *qMi-C14* (He et al., 2014). CH11 (containing *qMi-C11*) and CH14 (containing *qMi-C14*) were created by crossing M-120 RNR and Coker 201, self-pollinating plants for multiple generations, and selecting plants with either *qMi-C11* or *qMi-C14* beginning in the F2 generation.

Egression from roots was evaluated by planting seeds of the four isogenic lines in small tubes (Ray Leach Cone-TainersTM size RLC4; 2.5 cm × 16.1 cm) containing vermiculite and inoculating 2 week-old seedlings with 3,000 *M. incognita* J2s in the greenhouse. Each tube held one seedling. Two days after inoculation, plants were removed from tubes and roots were gently rinsed with water thereby ensuring that only nematodes inside roots remained associated with the seedlings, and then seedlings were replanted into different Cone-TainersTM (size SC10; 3.8cm × 21.0 cm) with fresh vermiculite. At days 4, 6, 8, 10, and 12 after inoculation (DAI), *M. incognita* J2s were collected from vermiculite (Jenkins, 1964) and nematodes inside the roots were stained and counted using a modified version of Byrd Jr et al. (1983). For the staining procedure, roots were carefully removed from cones, gently rinsed clean of vermiculite, soaked in a bleach solution (5.25% NaOCl) for 4 min., and then soaked in tap water for 15 min. Roots were immersed in a solution of one ml of cotton blue solution (0.35%) in 30 ml tap water and then microwaved for approximately 15 s just until the solution started to boil. Cotton blue solution was prepared by adding 0.35 g of cotton blue powder to 250 ml of lactic acid and 750 ml of distilled water. Roots were destained to better see nematodes inside the roots by washing stained roots in tap water and then putting them in a beaker containing glycerin. Each trial consisted of six replicates per genotype in a randomized complete block design and the experiment was conducted three times.

Separate experiments were conducted in the greenhouse to evaluate whether the resistance QTLs influenced the number of *M. incognita* eggs per egg mass on the four cotton isolines. Seeds were sown in trays containing vermiculite and two-week-old seedlings were transferred (one seedling per pot) to 10.6 cm × 10.6 cm × 12.4 cm pots filled with approximately 1175 cm3 sand loamy soil. At the time of transfer, seedlings were inoculated with 5,000 *M.*

incognita eggs. The average number of eggs per egg mass was determined at 30 and 40 DAI by harvesting 10 egg masses per plant (different plants at 30 and 40 DAI), dissolving the gelatinous matrix with 0.82% NaOCl for 30 s, and counting the eggs. The four cotton genotypes were arranged in a randomized complete block design with seven replications. This experiment was conducted three times.

Percentage egg hatch (% of total eggs produced on each of the four cotton isolines) was measured to determine whether the resistance QTLs affected the viability of the eggs. Seeds of the isolines were germinated in vermiculite and two-week-old seedlings were transplanted (one seedling per pot) into 10.6 cm × 10.6 cm × 12.4 cm pots filled with sand loamy soil. Seedlings were inoculated with 7,000 *M. incognita* eggs/pot at transplanting. At 40 DAI, plants were removed from pots, soil was gently rinsed from roots, and roots were soaked in 0.5% NaOCl solution for 2 min to extract eggs. The eggs were then harvested on a 500 mesh sieve, rinsed with water, counted, and transferred to Kimwipes® tissue placed on top of hardware cloth (0.64 cm × 0.64 cm mesh) positioned on small bowls (1.72 L) to allow egg hatch. The bowls were placed in a mist chamber for 5 days. Percentage egg hatch was calculated from the initial egg counts and the number of J2s released from the eggs after 5 days. Each trial had seven replicates per cotton genotype in a randomized complete block design, and the experiment was conducted twice.

The effects of the resistance QTLs on gall size were evaluated by comparing the size of galls produced on the four cotton isolines. Seeds of the isolines were sown (one seed per bag) in 10 cm × 15 cm × 0.004 cm propylene clear bags containing vermiculite. Seven days after planting, bags were inoculated with 2,000 *M. incognita* J2, and then bags were monitored daily to detect the first appearance of root galling on any of the genotypes. Gall size (cm2) started to be measured at 10 DAI using a scanner and WinRHIZO™ software. When galling first appeared in the experiment, individual galls were labeled and those galls were measured again 7 and 14 days after the initial measurement (recorded as days 0, 7, and 14). The total number of galls measured per plant varied and ranged from one to 12. The experiment had ten replications of each cotton isoline in a randomized complete block design. Plants were grown in a growth chamber at 28 °C with 12 hrs daylight per day. The experiment was conducted twice.

Data were analyzed by analysis of variance (ANOVA) using the PROCMIXED procedure in SAS 9.3 statistical software (SAS Institute Inc., Cary, NC). Cotton genotype and trial were identified as fixed effects in the analyses. Statistical differences among means were identified using t-tests. For the nematode egression study and the eggs per egg mass study, mean separation to identify differences among cotton genotypes were performed within a DAI. There was a significant trial × cotton genotype interaction for the nematode egression trials, so data from the trials were not combined for analysis. In contrast, there was no significant trial × cotton genotype interaction for eggs per egg mass, percentage egg hatch, or gall size, so those data were combined for analysis.

RESULTS

In the egression study, the total number of nematodes did not consistently differ among the cotton isolines (Fig. 3.1A-3.1C), although there appeared to be a trend where Coker 201 (the susceptible standard with neither resistance QTL) consistently had numerically more nematodes than the other genotypes at 10 and 12 DAI. The total number of nematodes observed increased as Trial 1 progressed, but an increase was not observed in Trials 2 and 3. CH11 and CH14 generally did not differ from M-120 RNR in the total number of nematodes observed. Results for the number of nematodes that egressed from the roots into the vermiculite were inconsistent among trials with more nematodes egressing from the roots of M-120 RNR than Coker 201 in Trial 1 but not in Trials 2 or 3 (Fig. 3.1D-3.1F). CH11 and CH14 generally did not differ from M-120 RNR in the number of nematodes that egressed from the roots. There were no consistent statistical or numerical patterns among cotton genotypes for differences in the number of nematodes that egressed from the roots. The number of nematodes that remained inside the roots was greater in Coker 201 than in M-120 RNR beginning 10 DAI in Trial 2 and 8 DAI in Trial 3; although differences were not significant in Trial 1, numerical differences were consistent beginning 8 DAI (Fig. 3.1G-3.1I). CH11 and CH14 did not consistently differ from M-120 RNR in the number of nematodes that remained inside the roots.

The number of nematodes that egressed from roots expressed as a percentage of the total number of nematodes differed among the cotton genotypes. Beginning 8 DAI, the percentage of nematodes that had egressed from the roots of M-120 RNR was generally greater than the percentage from the roots of Coker 201 with differences that were always numerically greater in all trials and generally statistically greater in Trials 2 and 3 (Fig. 3.2A-3.2C). CH11 and CH14 did not consistently differ from M-120 RNR in all trials. The percentage of nematodes remaining inside the roots was the inverse of the percentage egressing from roots, with Coker 201 generally retaining a greater percentage of nematodes inside the roots than M-120 RNR and with CH11 and CH14 not consistently differing from M-120 RNR (Fig. 3.2D-3.2F).

The number of eggs per egg mass was two to three times greater at 40 DAI than at 30 DAI on all cotton genotypes. At 30 DAI, Coker 201 had the most eggs/egg mass numerically, but only CH11 was significantly less than Coker 201 (Table 3.1). At 40 DAI, Coker 201 had more eggs per eggs mass than the other genotypes, M-120 RNR had the fewest, and CH11 and

CH14 were intermediate. Percentage egg hatch did not differ among the genotypes and ranged from 15 to 20% (Table 3.1).

The first gall size measurement (day 0) showed differences among the genotypes. M-120 RNR had the smallest gall area of 0.0056 cm^2 and was significantly different from all the other genotypes. Gall size did not differ among genotypes on days 7 and 14. On CH11, galls were statistically larger on day 7 than on day 14 and numerically larger than on day 0. M120-RNR had smaller galls on day 0 than on day 7 and 14. The largest average gall size was on day 7 for all genotypes: Coker 201, CH11, CH14, and M-120 RNR were 0.009, 0.01, 0.009, and 0.007 cm^2, respectively (Table 3.1).

DISCUSSION

One potential effect of plant resistance is the induction of nematode egression caused by the failure to establish a feeding site. Past studies observed significant egression of *M. incognita* from the roots of wild melons resistant to *M. incognita* and of *Globodera rostochiensis* from resistant potato (Faske, 2013; Mullin and Brodie, 1988). Despite some numerical trends in our studies that suggest that the susceptible Coker 201 may have had more total nematodes inside the roots than other genotypes on later sampling dates, statistical differences were not consistent for the total number of nematodes in the roots or the number of nematodes that egressed from the roots. That is consistent with the hypothesis that initial penetration into the roots is not affected by the resistance QTLs (Creech et al., 1995). There was a consistent pattern of Coker 201 having more nematodes that remained inside the roots at later sampling dates with a consistent numerical pattern in all three trials that was statistically significant in two of the three trials. It is possible that the consistent numerical trends reveal true differences that are not supported by the statistics because there was too much variation to detect statistical differences with the number of replications that we used. The percentage data consistently showed that from 8 DAI onward M-120 RNR had a numerically greater percentage outside the roots than Coker 201, but the differences were not always statistically significant. Many *Globodera rostochiensis* J2 egressed from roots of both resistant and susceptible potato cultivars (Mullin and Brodie, 1988), which is similar to our observations on cotton with the resistance QTLs *qMi-C11* and *qMi-C14*.

Based on the lack of consistent statistical differences, we conclude that nematode egression from roots does not appear to be a significant mechanism of resistance imparted by the resistance QTLs. Our conclusion agrees with previous research (McClure et al. 1974) that demonstrated the level of *M. incognita* egression from the moderately resistant Clevewilt-6 was similar to egression from a susceptible line. Clevewilt-6 was the source of the resistance QTL *qMi-C11* in the development of M-120 RNR (Gutierrez et al., 2010; Shepherd et al., 1996). The combination of *qMi-C11* and *qMi-C14* in M-120 RNR was demonstrated to be epistatic for galling development (He et al., 2014). The epistatic effect that reduces galling had the potential to cause greater egression from M-120 RNR, however, little evidence for that was seen in our results. Despite a small difference in percentage egression from M-120 RNR compared to Coker 201 after 8 days, M-120 RNR did not differ in any consistent way from the isolines with only one QTL. Therefore, we conclude that *qMi-C11* and *qMi-C14*, either alone or in combination, have little or no effect on the egression of *M. incognita* from cotton roots. A hypersensitive response is another potential mechanism of host-plant resistance (Alpizar et al., 2007; Freire et al., 2010) that could be occurring in cotton with *qMi-C11* and *qMi-C14,* but we did not attempt to document hypersensitive responses in this study.

Egg masses at 40 DAI contained two to three times as many eggs as egg masses at 30 DAI. Although the difference between the susceptible and the resistant standards was not significant at 30 DAI, the susceptible cultivar had more eggs numerically and that difference increased and was significant at 40 DAI, which may suggest that the difference is a result of the rate of egg production. However, additional research is needed to determine whether differences in the number of eggs per egg mass at 40 DAI were caused by different rates of egg production, a longer period of egg production on the susceptible standard, or perhaps both. Previous research with cotton genotype carrying both *qMi-C11* and *qMi-C14* also found fewer eggs per egg mass (Creech et al., 1995), but that study was not able to test the two QTLs individually in isogenic backgrounds. Another study showed that Clevewilt-6 (the donor of *qMi-C11*) and Wild Mexican Jack Jones (the donor of *qMi-C14*) (Gutierrez et al., 2010) did not differ from a susceptible genotype in the number of eggs per egg mass after 42 DAI (Faske and Starr, 2009); although tested individually, the QTLs were not in isogenic backgrounds, which could have affected the results. Although *qMi-C11* and *qMi-C14* both reduced the number of eggs per egg mass compared to the susceptible genotype, combining *qMi-C11* and *qMi-C14* did not significantly decrease the number of eggs per egg mass compared to *qMi-C14* alone, which suggests that the effects are not additive. However, *qMi-C11* and *qMi-C14* both reduced the number of eggs per egg mass, which likely contributes greatly to the expression of resistance to *M. incognita* in cotton.

The reduction of M-120 RNR gall size may be due to the epistatic effect of *qMi-C11* and *qMi-C14*, however, galls were smaller only on day 0, which suggests that the observed difference may have been due to delayed development. A previous study with resistant coffee plants (Alpizar et al., 2007) found galls with a diameter below 1 mm (small galls) or between 1-3 mm (medium galls) mostly on resistant lines and galls above 3 mm only on susceptible lines. Although cotton is a woody plant as are coffee plants, in our experiments all genotypes presented small and medium galls with none above 3 mm regardless of resistance. M-120 RNR in our study had more small than medium galls whereas the other genotypes presented more medium gall size. Jenkins et al. (1995) observed consistently smaller galls on M-315 RNR starting at 8 DAI than on the susceptible and partially resistant genotypes. Although our results had statistical differences only at 0 day, M-120 RNR maintained small gall size (below 1 mm) throughout the experiment. Our results showed evidence that M-120 RNR reduces gall size, however it is not clear if it is due to epistatic effect of the QTLs.

Percentage egg hatch did not differ among the genotypes in our study. We conclude that the QTLs that impart resistance to *M. incognita* in cotton, *qMi-C11* and *qMi-C14*, do not cause a reduction in egg hatching.

Overall, this study showed that nematode egression is observed in all genotypes and does not seem to be significantly affected by resistance, that the number of eggs per egg mass contributes to the observed levels of resistance, that gall size is reduced compared to susceptible plants when both resistance QTLs are present, and that percentage egg hatch is not affected by the resistance QTLs. These finding increase our knowledge of the specific elements that contribute to *M. incognita* resistance in cotton due to the resistance QTLs *qMi-C11* and *qMi-C14.*

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Table 3.1. Gall size, percentage egg hatch, and eggs/egg mass for cotton isolines that differ in *M. incognita*-resistance genes at 0, 7, and 14 days.

	Gall size (cm^2) [1]			Hatch (%)	Egg/ egg mass	
days	0	7	14	40 DAI[2]	30 DAI	40 DAI
Coker	0.0085 **A a**	0.0091 **A a**	0.0081 **A a**	17.20 **A**	182 **A**	495 **A**
CH14	0.0080 **A a**	0.0090 **A a**	0.0080 **A a**	14.96 **A**	123 **AB**	325 **BC**
CH11	0.0091 **A ab**	0.0100 **A a**	0.0068 **A b**	19.24 **A**	146 **B**	386 **B**
M-120	0.0056 **B b**	0.0074 **A a**	0.0075 **A a**	18.60 **A**	135 **AB**	300 **C**

[1] Days after appearance of first gall.

[2] DAI = days after inoculation.

Upper case letters within a column indicate differences among genotypes. Lower case letters indicate differences among gall ages within a genotype. Different letters indicate significant differences at α=0.05.

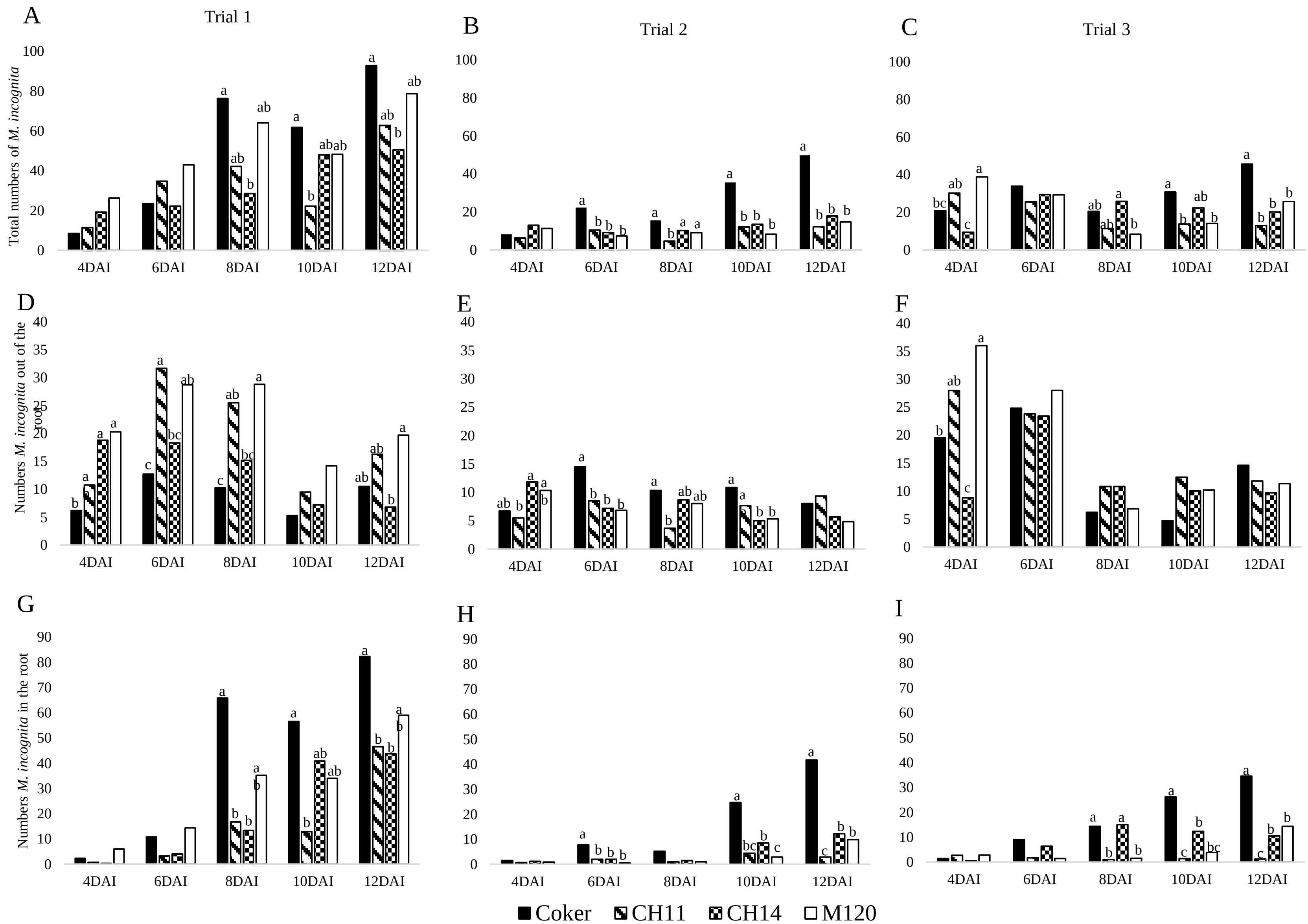
A
Trial 1
B
Trial 2
C
Trial 3
Total numbers of *M. incognita*
0
20
40
60
80
100
D
E
F
Numbers *M. incognita* out of the root
0
5
10
15
20
25
30
35
40
G
H
I
Numbers *M. incognita* in the root
0
10
20
30
40
50
60
70
80
90
4DAI
6DAI
8DAI
10DAI
12DAI
■ Coker
▨ CH11
▩ CH14
□ M120

Figure 3.1

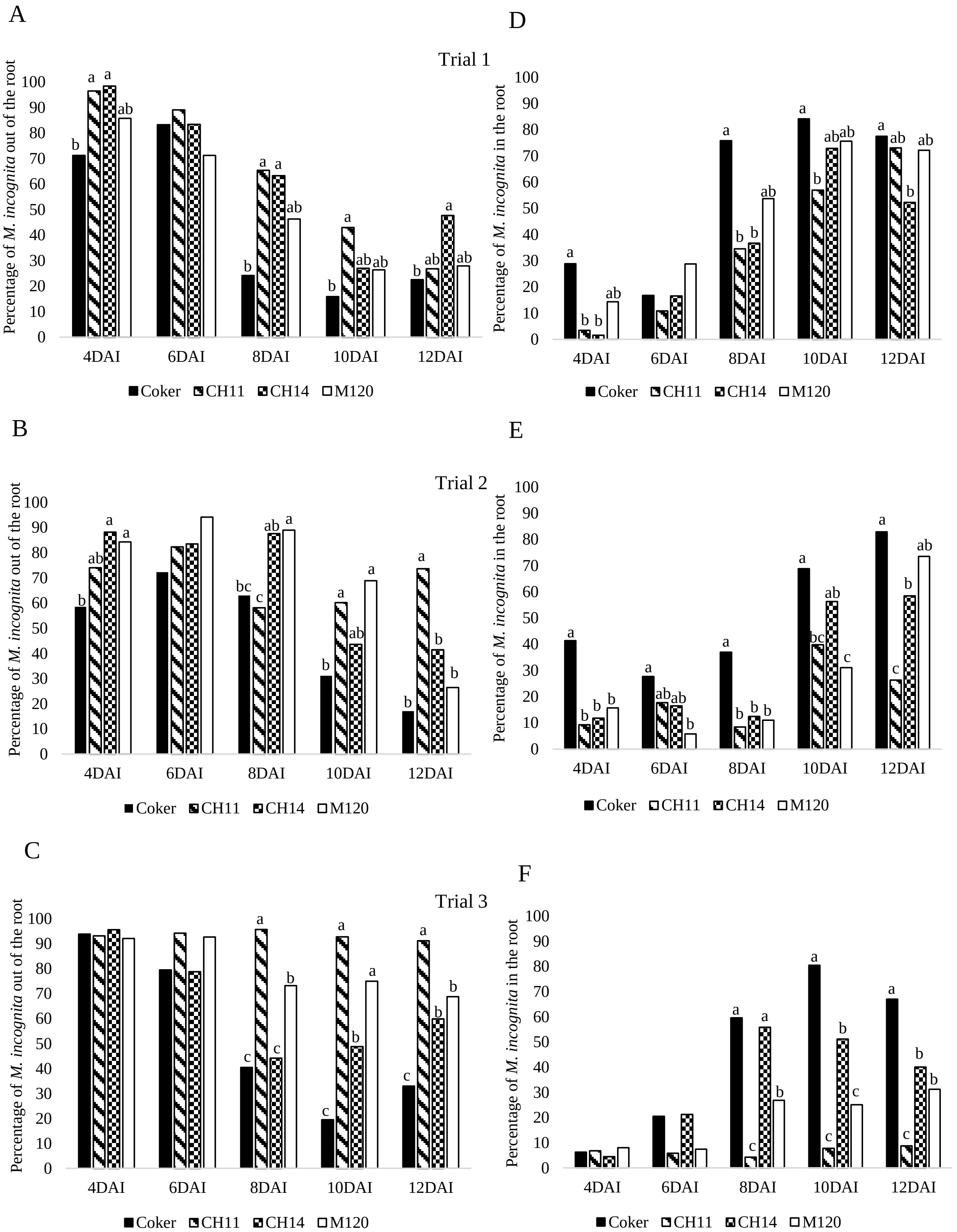
A
Trial 1
Percentage of M. incognita out of the root
b a a ab
a a ab
b a ab ab
b ab a ab
4DAI 6DAI 8DAI 10DAI 12DAI
■Coker ▨CH11 ▩CH14 □M120
D
Percentage of M. incognita in the root
a b b ab
a b b ab
a b ab ab
a ab b ab
B
Trial 2
Percentage of M. incognita out of the root
b ab a a
bc c ab a
b a ab a
b a b b
E
Percentage of M. incognita in the root
a b b b
a ab ab b
a b b b
a bc ab c
a c b ab
C
Trial 3
Percentage of M. incognita out of the root
c a c b
c a b a
c a b b
F
Percentage of M. incognita in the root
a c a b
a c b c
a c b b
0 10 20 30 40 50 60 70 80 90 100

Figure 3.2

Figure 3.1. Numbers of *Meloidogyne incognita* remaining inside roots, egressed from roots, and total counts on four cotton isolines that differ in *M. incognita*-resistance genes in three experimental trials. DAI = days after inoculation. Different letters over bars within a sampling date indicate significant differences at α=0.05. Bars with no letters showed no significant difference within a sampling date.

Figure 3.2. Percentage of *Meloidogyne incognita* inside and outside of the root of four cotton isolines that differ in *M. incognita*-resistance genes in three experimental trials. DAI = days after inoculation. Different letters over bars within a sampling date indicate significant differences at α=0.05. Bars with no letters showed no significant difference within a sampling date.

CHAPTER 4

FUSARIUM WILT OF COTTON MAY COMMONLY RESULT FROM THE INTERACTION OF *FUSARIUM OXYSPORUM* F. SP. *VASINFECTUM* WITH *BELONOLAIMUS LONGICAUDATUS*[3]

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[3] Mychele B. da Silva, Davis, R. F., Doan, H., Nichols, B., Kemerait, R., Brewer, M., Peng W. Chee. To be submitted to Plant Disease.

ABSTRACT

The interaction between *Fusarium oxysporum* f.sp. *vasinfectum* (Fov) and *Meloidogyne incognita* (root-knot nematode) resulting in Fusarium wilt (FW) is well known. Although *Belonolaimus longicaudatus* (sting nematode) can interact with Fov and cause Fusarium Wilt, it has long been believed that virtually all of the FW in Georgia is caused by the interaction of Fov with *M. incognita*. In recent years, Fusarium wilt has been reported more frequently in Georgia, which suggests that something affecting the disease complex may have changed. In 2015 and 2016, a survey of 27 Georgia cotton fields in 10 counties was conducted. At least 10 soil and stem samples per field were collected from plants showing symptoms of FW to quantify plant parasitic nematode levels and identify Fov races. Fov race 1 was identified in all samples in 2015, but one sample also had the LA 110 genotype and another sample also had the LA 108 genotype. In 2016, all Fov races and genotypes found in 2015 were present, however, MDS-12 and LA127/140 also were found. *Meloidogyne incognita* was present in 18% of fields in 2015 and 40% in 2016, and *B. longicaudatus* was present in all fields in 2015 and 75% of fields in 2016. *Meloidogyne incognita* and *B. longicaudatus* were present, respectively, in 18% and 55% of the samples in 2015 and 40% and 51% in 2016. However, *M. incognita* without *B. longicaudatus* was found in 7% of samples in 2015 and 34% in 2016, whereas *B. longicaudatus* without *M. incognita* was found in 45% in 2015 and 44% in 2016. We conclude that 1) Fov race 1 continues to be the dominant race in Georgia, and 2) many instances of Fusarium wilt in Georgia may be due to Fov interacting with *B. longicaudatus* and not *M. incognita* as previously believed.

INTRODUCTION

Fusarium oxysporum is a widespread pathogen causing Fusarium wilt (FW) of numerous plants species, including watermelon, banana, and cotton (Gordon and Martyn, 1997). Typical symptoms of FW are chlorosis of leaves and wilting of plants due to clogging of the xylem, which results in a characteristic vascular discoloration. *Fusarium oxysporum* is divided into formae speciales based on an isolate's ability to reproduce on particular plant species. *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) causes FW in cotton. FW incidence fluctuates greatly from year to year due to different management practices and environmental conditions (Hermanto et al., 2009; Lawrence et al., 2017). *Meloidogyne incognita* causes the greatest losses of any pathogen of cotton in the U.S. (Lawrence et al., 2015), and the synergistic interaction of Fov with *Meloidogyne incognita*, the southern root-knot nematode, that results in greatly increased FW is well documented (Cooper and Brodie, 1963; Garber et al., 1979). Other nematode species also have been reported to interact with Fov, including *Pratylenchus penetrans* (lesion nematode), *Belonolaimus longicaudatus* (sting nematode), and *Rotylenchulus reniformis* (reniform nematode) (Cooper and Brodie, 1963; Neal, 1954; Seinhorst and Kuniyasu, 1971). Although nematodes in several genera have been reported to be capable of increasing FW, it has commonly been believed throughout the cotton-growing areas of the U.S. that the interaction of Fov with *M. incognita* was the most significant cause of FW in cotton and the involvement of other nematodes was rarely considered.

Fov is divided into races, biotypes, and genotypes. Genotypes can be distinguished based on small genetic variations among isolates. Isolates with morphological or physiological differences without known genetic differences are labeled biotypes (Downie, 2010). Race is a broader term for isolates where there is usually knowledge of both genotypic and phenotypic variations. In Georgia, the most common races of Fov are 1, 2, and 8, and the most common genotypes are LA108 and LA110 (Cianchetta et al., 2015; Holmes et al., 2009). However, isolates within a race can have different levels of virulence (Glass et al., 2002). The race classification originally was based on pathogenicity of an isolate to different crop species. For example, race 1 caused disease on tobacco but not soybean, and race 2 was capable of infecting both crops (Armstrong and Armstrong, 1958). The original race classification scheme that used host differentials to distinguish races is no longer acceptable as DNA sequencing indicates that races 3 and 5 are the same, as are races 4 and 7, which also is supported by their being in the same vegetative compatibility group (VCG) (Kim et al., 2005; Skovgaard et al., 2001).

In southern Georgia, awareness of FW among farmers, extension agents and consultants has increased greatly in recent years, as have yield losses attributed to this disease (Bob Kemerait, University of Georgia, personal communication). The phasing out of aldicarb (Cone, 2010; Kemerait et al., 2008), which was a widely used and effective nematicide, from 2011 to 2016 may have allowed plant parasitic nematodes develop and reproduce in the fields more freely than when aldicarb was used. Because of the interaction of the nematode and the fungus, the severity of FW is positively correlated with *M. incognita* population levels (Garber et al., 1979). Fov race 4 and Australian biotypes are unique because they can cause severe FW without interacting with *M. incognita* (Kim et al., 2005), but those genotypes of Fov have not been found in the U.S. outside of California and Texas (Halpern et al., 2017). However, the movement of soil, equipment, plant material, or seed could spread those genotypes to other cotton-producing areas of the country. The reason for the recently increased incidence of FW in Georgia is not known. We conducted a survey of individual plants in cotton fields in southern Georgia showing symptoms of FW to determine which races of Fov were causing FW and which plant parasitic nematode species were associated with the diseased plants.

MATERIAL AND METHODS

A survey was conducted in southern Georgia to identify the Fov races and genotypes causing FW and to document the association of FW with plant parasitic nematodes. Soil and plant samples were collected between June and November in 2015 and 2016 from cotton fields showing symptoms of FW. The survey included six counties and 11 fields in 2015 and seven counties and 17 fields in 2016 (Table 1). The Tifton fields 1, 4, and 5 in 2015 are the same as fields 3, 5, and 4 in 2016, respectively. County extension agents provided general information about soil proprieties such as soil texture, previous crop, and cotton varieties planted.

Eight to fifteen samples were collected from each field. For each individual sample, an individual plant showing symptoms of FW was arbitrarily selected and carefully dug up and observed for presence or absence of galls. The soil associated with that plant’s root system and a piece of the diseased plant’s stem was sealed in a plastic bag. The samples were stored in a cold room at 10 °C until processing. Nematodes were extracted from 150 cm^3 of soil for each sample by the centrifugal flotation method (Jenkins, 1964), and the number of each genus of plant-parasitic nematodes present was recorded.

Fov was isolated by disinfesting infected stem pieces in 0.875% NaOCl for 1 min, and then placing them into Komada selective medium (Komada, 1975) for 5 days. Five stem pieces per plant were cut open longitudinally and placed on a single plate. Individual colonies were then selected and transferred to a new plate containing Komada medium and allowed to grow for 7 days. Then, single spore isolations were made through successive serial dilution of conidial suspensions of 10^1 and plated. DNA was extracted separately from at least three different single-spore isolates per field. DNA extraction was accomplished by inoculating 125 ml flasks of potato dextrose broth with a mycelial plug (0.7 cm diameter) from the single-spore isolates and allowing them to grow for four days on a rotational shaker at 100 rpm. All the mycelium was then dried overnight on a sterile plate prior to using the modified DNA extraction method of Abd-Elsalam et al. (2003). Mycelium was macerated in liquid nitrogen and then put into 1.5 ml Eppendorf tubes prior to adding extraction buffer (200mM Tris pH=8.5; 250 mM NaCl; 25 mM EDTA; and 0.5% SDS). The sample was mixed on a vortex mixer and 4 µl of RNAase was added prior to incubation in a water bath for 65 °C for 10 min. Then, 130 µl of 3M sodium acetate (pH=5.2) was added and the tubes were held at -20 °C for 10 min. After centrifuging the sample for 15 min at 4000 rpm, a 400 µl aliquot of the supernatant was collected and transferred to a 1.5 ml Epperndorf tube. The sample was then mixed in a 1:1 ratio with chloroform for 3 min and then centrifuged for 10 min at 8000 rpm. The supernatant was removed and 650 µl of isopropanol was added to the tube to precipitate the DNA. Isopropanol was removed after centrifuging tubes for 10 min at 4000 rpm. Finally, a 300 µl of 70% ethanol was added into the tubes and centrifuged for 1 min at 4000 rpm. Ethanol was removed from the tube and DNA allowed to dry overnight. Deionized water (100 µl) was added to rehydrate the DNA. The DNA extracted was sequenced based on PCR products of translation elongation factor (EF-1α) and Intergenic Spacer (IGS) region primers as described by Kim et al. (2005) and Cianchetta et al. (2015) and were compared to Fov sequences deposited in the GeneBank database to identify race or genotype.

RESULTS

Fov race 1 was found in all counties in 2015 and 2016. In 2015, no previously unreported races or genotypes were found in Georgia. In addition to race 1, genotype LA108 was found in Lowndes county and LA110 in Tift County. In 2016, two new genotypes, LA127 and LA140, and a race variant MDS-12 were identified in the fields sampled. Fov races 2 and 8 were found in Lowndes County; race 8 and genotypes LA127, LA140, LA110 and LA108 in Tift County; genotypes LA108 and LA110 in Cook County; genotypes LA108 and LA110 and MDS-12 in Colquitt County; race 2 and genotype LA110 in Coffee County; genotype LA108 in Ware County; and genotype LA110 in Worth and Tattnall Counties (Table 4.2 and Fig. 4.1).

The number of samples for each county in our survey (both years combined) with one or more of the three species of nematodes (*M. incognita*, *P. brachyurus*, and *B. longicaudatus*) reported to interact with Fov to cause FW is listed in Table 4.3. A majority of the total survey samples (all counties for both years) contained *B. longicaudatus* either alone (34% of samples) or together with *P. brachyurus* (20%). *Pratylenchus brachyurus* and *M. incognita*, alone and together, were present in 7.3%, 6.6% and 2.4% of the samples, respectively. *Meloidogyne incognita* and *B. longicaudatus* together in the same sample were 2.4% of samples. A greater number of samples were found with *B. longicaudatus* alone in Tattnall County (64% of samples) and in Ware County (65% of samples) than in the other counties (Table 4.3). *Belonolaimus longicaudatus* and *M. incognita* were found together infrequently in only 2.5% of the samples, all of which were in Tift and Worth counties.

For the fields included in our survey in 2015, the predominant cotton cultivar planted was DP 1252 B2RF, which is susceptible to *M. incognita*. The *M. incognita*-resistant varieties planted were DP1454 NR B2RF and PHY 487 WRF, which were planted on 18% of the fields sampled. In 2016, the resistant variety DP1558 NR B2RF was planted in 35% of the fields sampled (Table 4.1). *Belonolaimus longicaudatus* was present in all counties sampled in 2015 and in 70% of the counties in 2016 (Table 4.2). *Meloidogyne incognita* was found in 50% of the counties in 2015 and 70% in 2016. Other plant-parasitic nematodes associated with FW-damaged plants in our survey were *Pratylenchus brachyurus* (lesion), *Helicotylenchus* spp. (spiral), *Hoplolaimus columbus* (lance), *Criconemella* spp. (ring) and *Rotylenchulus reniformis* (reniform) (Table 4.2). Except for *R. reniformis*, these other genera were typically found at low population densities (data not shown).

For comparison to the results in our survey, samples from cotton fields submitted to the Extension Nematology Laboratory at the University of Georgia from 2013 to 2016 were examined. During those four years, *B. longicaudatus* was detected in 1.22% of the samples from all counties throughout Georgia.

DISCUSSION

Belonolaimus longicaudatus can interact with Fov to cause FW in cotton (Cooper and Brodie, 1963), and control of *B. longicaudatus* can reduce FW in the field (Brodie and Hauser, 1970). Aldicarb, which was phased out in the US beginning in 2011 (Cone, 2010), was widely used for nematode control in cotton production. Although aldicarb was applied primarily to manage other nematode species, non-target nematode species including *B. longicaudatus* also would have been suppressed. Aldicarb is effective at reducing *B. longicaudatus* levels in the field (Rhoades, 1981, Weingartner and Shumaker, 1990). In our samples from plants with FW, *B. longicaudatus* was found much more frequently than expected based on the frequency that the nematode is found in samples submitted to the University of Georgia Extension Nematology Laboratory. The removal of aldicarb may have allowed *B. longicaudatus* to thrive resulting in increased incidence and greater population levels of *B. longicaudatus* resulting in more interactions with Fov and greater FW incidence. Better control of *B. longicaudatus* should help reduce the incidence of FW. In fields where *B. longicaudatus* was not found, *M. incognita* was present. FW may have been more severe in fields where both *B. longicaudatus* and *M. incognita* occurred together (Yang et al., 1976). A few individual plant samples had neither nematode found associated with its roots, which could mean that neither nematode was involved in causing FW on that plant or that any nematodes involved were below our detection limit.

Race 1 was the predominant race of Fov in South Georgia in previous surveys (Cianchetta et al., 2015, Holmes et al., 2009). Additionally, races 2 and 8 and genotypes LA108 and LA110 also have been identified in Georgia (Cianchetta et al., 2015, Holmes et al., 2009). However, our survey is the first to report the presence of genotypes MDS-12, LA127, and LA140 in Georgia cotton fields. MDS-12 has been previously found in Alabama, which was the first report of this genotype in the USA (Bennett et al., 2013). Importantly, our survey did not find Fov race 4 or the Australia biotype, which cause severe levels of FW regardless of *M. incognita* infection (Kim et al., 2005). Therefore, all of our isolates are presumed to be able to interact with *M. incognita*.

Although the underlying mechanism of how *M. incognita* infection increases the severity of FW is unknown, it has been shown that *M. incognita* population levels positively correlate with FW incidence severity (Garber et al., 1979). However, some fields in our survey in Berrien, Cook, Lowndes, and Tattnall counties with high levels of FW did not have high levels of *M. incognita*, and the nematode was not detected at all in some fields. Most our fields were infested with *B. longicaudatus*, with which our Fov isolates were likely to be interacting. Although other nematode species may form a disease complex with Fusarium, (Cooper and Brodie, 1963, Seinhorst and Kuniyasu, 1971), our survey showed greater evidence of *B. longicaudatus* being the one causing an increase in FW in Georgia cotton fields.

Another possible explanation for the recent increase in the incidence of FW in Georgia could be a genetic change in Fov allowing it to become more virulent. Fov race 4 is highly virulent, and phylogenic trees have shown that even though LA110 and LA108 still appear to interact with nematodes to increase FW severity, their genetic similarity to race 4 may suggest that they share the high virulence of race 4 (Holmes et al., 2009). However, race 4 is a root-rot pathotype that is not considered a vascular-competent pathogen, whereas LA110 and LA108 are vascular competent pathotypes that require nematode infection to increase disease severity in the field (Bell et al., 2017). The difference in amino acid pattern between race 4 and LA108 and LA110 could be related to virulence or the need to interact with nematodes to cause severe disease under field conditions.

The interaction of Fov with nematodes other than *M. incognita* is not well documented, has been the subject of limited research, and is seldom mentioned as a possible factor in FW incidence. The relatively common interaction of Fov with *B. longicaudatus* identified in our study requires additional research to be more fully understood. Better recognition that *B. longicaudatus* can interact with Fov to cause FW in cotton may lead to better control of FW by allowing growers to identify at-risk fields and minimize FW through better control of the nematode.

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Table 4.1. Description of the fields in Georgia where Fov was collected.

	County	Field	Cotton Variety[1]	Soil description[2]	Planting date	Sampling date
2015	Benhill	1	DP1252	Sandy loam	May 15	August 13
	Berrien	1	DP1252	Leefield loamy sand	April 24	August 28
	Cook	1	DP1252	Filk	May 1	July 24
	Lowndes	1	DP1050	Sandy	May 8	August 28
	Tattnall	1	DP1050	Sandy	May 20	Mid August
		2	DP1050/DP1137	Sandy	May 15	Mid August
	Tift	1	DP1252/ PHY 487/ DP 1454	Stilson and Dothan loamy sand	May 4	June 24
		2	PHY333	Ocilla loamy sand	June 2	August 28
		3	DP1252	Ocilla loamy sand	unknown	July 10
		4	DP1252/ DP1555	Tifton and Dothan loamy sand	unknown	September 30
		5	DP1454	Dothan loamy sand	May 7	June 19
2016	Coffee	1	ST6182	Sandy loamy	May15	August 30
	Cook	1	DP1252	Sandy loamy	May 5	August 26
	Colquitt	1	DP1538	Leefield loamy sand	April 20	August 31
		2	DP1252	Dothan	1st week of May	August 31
		3	DP1553	Ocilla fine	Mid May	August 31
		4	unknown	unknown	unknown	December 1
	Tattnall	1	DP1558	Fuquay, Osier, and Tift loamy sand	May 13	September 22
		2	DP1553/DP1558	Pelham loamy sand	April 27	September 22
		3	DP 1553/DP1558	Pelham loamy sand	April 29	September 22
	Tift	1	DP1252	Ocilla and Stilton loamy sand	April 24	August 24
		2	DP1252	Dothan, Fuquay, and Ocilla loamy sand	April 26	August 24
		3	DP1252	Ocilla and Dothan loamy sand	April 23	August 24
		4	ST5115/ST6182	unknown	June 1	August 26
		5	DP1558	Sandy	May 15	August 26
	Ware	1	PHY444	Sandy	May 18-20	September 7
		2	DP1558	Sandy	May 16	September 7
	Worth	1	DP1558	Sand loamy and Tift	May 7	September 21

[1] DP 1252 B2RF, DP 1050 B2RF, DP 1137 B2RF, DP 1555 B2RF, DP 1454 NR B2RF, DP 1558 NR B2RF, DP 1538 B2XF, DP 1553 B2XF, PHY 487 WRF, PHY 333 WRF, PHY 444 WRF, ST 6182 GLT, and ST 5115 GLT.

[2] Information provided by county agents.

Table 4.2. Fov races and plant parasitic nematodes found in South Georgia field in 2015 and 2016.

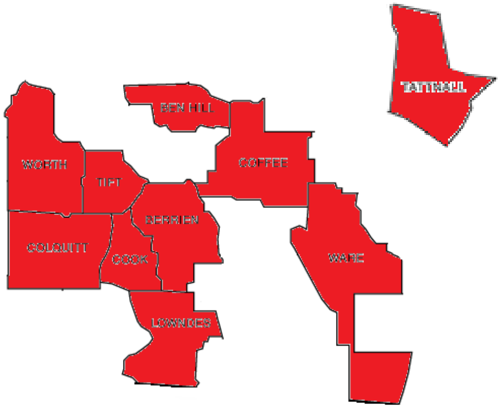
	Fov		Nematode species [1]													
County	Race description		*Meloidogyne incognita*		*Belonolaimus longicaudatus*		*Criconemella* spp.		*Pratylenchus brachyurus*		*Helicotylenchus* spp.		*Hoplolaimus columbus*		*Rotylenchulus reniformis*	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Benhill	1	ns[2]	Y[3]	ns	Y	ns	Y	ns	Y	ns	N	ns	N	ns	N	ns
Berrien	1	ns	Y	ns	Y	ns	Y	ns	Y	ns	Y	ns	N	ns	N	ns
Coffee	ns	1, 2, LA110	ns	Y	ns	N	ns	Y	ns	Y	ns	N	ns	N	ns	N
Cook	1, LA108, LA110	1, LA110	N	N	Y	Y	N	Y	Y	Y	N	Y	N	N	N	N
Colquitt	ns	1, 4-like, LA110, LA108	ns	Y	ns	Y	ns	Y	ns	Y	ns	Y	ns	N	ns	Y
Lowndes	1, 2, 8, LA108, LA110	NS	N	ns	Y	ns	Y	ns	Y	ns	Y	ns	N	ns	N	ns
Tattnall	1	1, LA110	N	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	N
Tift	1,8, LA110, LA108	1,8, LA108, LA110, LA127/140	Y	Y	Y	Y	Y	Y	Y	Y	Y	N	N	N	N	N
Ware	ns	1, 8	ns	Y	ns	N	ns	Y	ns	Y	ns	N	ns	N	ns	N
Worth	ns	1, LA110	ns	Y	ns	Y	ns	Y	ns	Y	ns	N	ns	N	ns	N

[1] *Melodoigyne incognita*, *Belonolaimus* spp., *Pratylenchus brachyurus*, *Hoplolaimus columbus*, and *Rotylenchus* spp. have been reported to interact with *Fusarium oxysporum* f. sp. *vasinfectum* to increase Fusarium wilt.

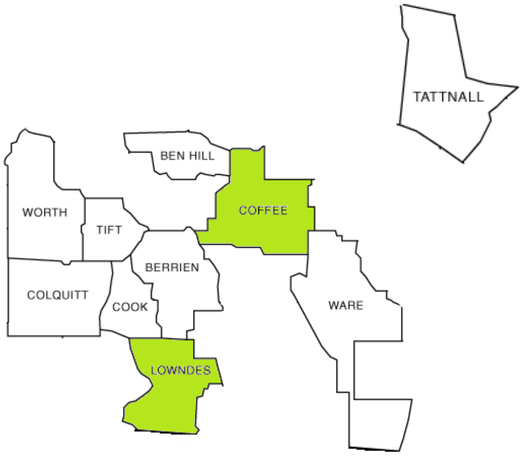
[2] not sampled

Table 4.3. Total samples collected infected with Fov all over Georgia counties and respective occurrence of *M. incognita*, *B. longicaudatus*, and *Pratylenchus brachyurus* in all combinations together or separately in the individual samples.

			Samples with concomitant occurrence of nematodes			
County	Total samples		*Meloidogyne incognita*	*Belonolaimus* spp.	*Pratylenchus* spp.	All three
		M. incognita	1			
Ben Hill	10	*Belonolaimus*	0	6		0
		Pratylenchus	0	1	0	
		M, incognita	0			
Berrien	10	*Belonolaimus*	0	6		0
		Pratylenchus	0	1	4	
		M. incognita	4			
Coffee	8	*Belonolaimus*	0	0		0
		Pratylenchus	4	0	0	
		M. incognita	0			
Cook	23	*Belonolaimus*	0	3		0
		Pratylenchus	0	11	5	
		M. incognita	9			
Colquitt	40	*Belonolaimus.*	0	1		0
		Pratylenchus	16	8	2	
		M. incognita	0			
Lowndes	10	*Belonolaimus*	0	6		0
		Pratylenchus	0	4	0	
		M. incognita	0			
Tattnall	50	*Belonolaimus*	0	32		0
		Pratylenchus	0	6	0	
		M. incognita	5			
Tift	108	*Belonolaimus*	6	28		11
		Pratylenchus	4	9	8	
		M. incognita	0			
Ware	20	*Belonolaimus*	0	13		0
		Pratylenchus	0	7	0	
		M. incognita	0			
Worth	10	*Belonolaimus*	1	3		0
		Pratylenchus	0	4	2	



🟥 Race 1



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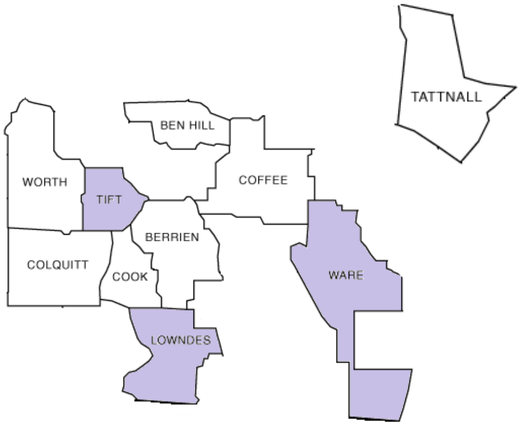
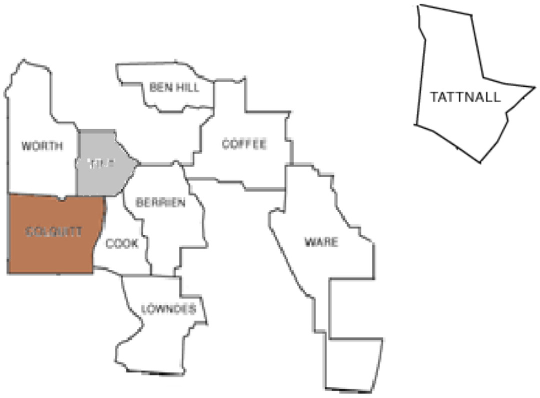
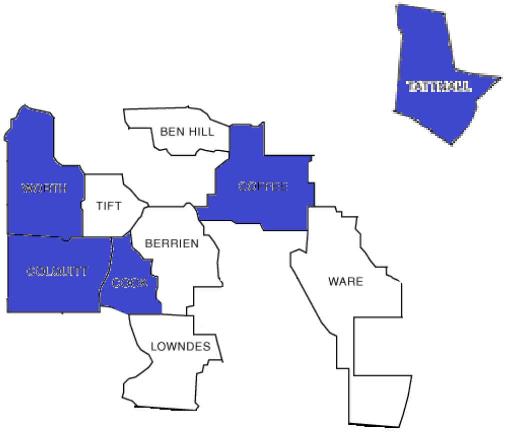


🟪 Race 8

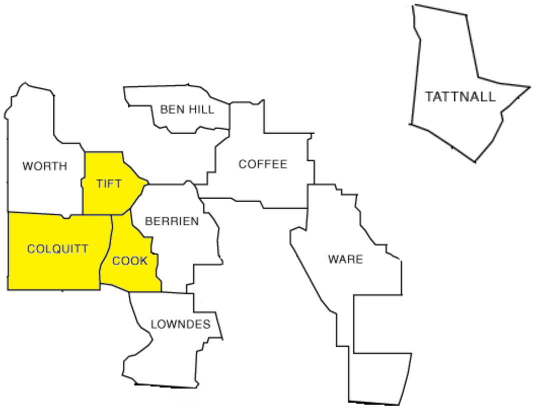


🟪 MDS-12

🟫 LA127/140



🟦 LA110



🟨 LA108

Figure 4.1.

Figure 4.1. Map of the counties in Georgia that Fov races and genotypes were found. Race and genotypes are distinguished by color.

CHAPTER 5

RESISTANCE OF COTTON TO *MELOIDOGYNE INCOGNITA* DID NOT DECREASE PLANT MORTALITY FROM FUSARIUM WILT[4]

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[4] Mychele B. da Silva, Davis, R. F., Doan, H., Nichols, B., Kemerait, R., Brewer, M., Peng W. Chee. To be submitted to Journal of Nematology.

ABSTRACT

Fusarium oxysporum f. sp. *vasinfectum* (Fov) is the causal agent of Fusarium wilt of cotton. Fov interacts with *Meloidogyne incognita* (RKN) and increase Fusarium wilt severity. Both pathogens together can cause devastating losses in cotton production. Early studies have revealed that gall formation primes this interaction. To verify that the Fov-RKN interaction is related to gall production, Rowden (susceptible to RKN and Fov), CH11 (RKN resistance QTL *qMi-C11* - reduces gall formation), CH14 (RKN resistance QTL *qMi-C14* – no gall effect), M-120 RNR (containing *qMi-C11* and *qMi-C14*), Clevewilt-6 (containing *qMi-C11*), and Coker 201 (susceptible to RKN) were sown and inoculated with RKN alone, Fov alone, combined RKN and Fov, and neither pathogen. The leaf and vascular scoring scale varied between 1 (healthy plant) and 5 (vascular discoloration >75% or >90% damaged leaves to dead). All plant evaluations (plant scoring, galling count, plant growth, and plant mortality) were done at 28 days after inoculation (DAI). At 14 DAI, only nematode galling counts were done. There were seven plants replicated per genotype per treatment. Two trials were set up for 14 DAI and three at 28 DAI in a randomized complete block design. Plant growth was reduced on all genotypes in combined Fov and RKN treatments. Gall numbers were not affected by the presence of Fov on any genotype except for Coker 201 in trial A and Rowden in trials A and B at 14 DAI. However, at 28 DAI, galling was reduced for Coker 201 in trial A and for Rowden in trial B. Percentage of dead plants was higher with combined Fov and RKN on all genotypes, but CH11 had overall lower mortality at 28 DAI than other genotypes. Foliar damage and vascular discoloration scoring were higher for all genotypes in combined Fov and RKN treatment. In conclusion, combined Fov and RKN reduced plant growth and increased plant mortality, but the QTLs *qMi-C11* and *qMi-C14* did not

affect the interaction of Fov and *M. incognita*. Thus, there is not enough evidence from our work to conclude that low gall forming resistant genotypes reduce Fov severity.

INTRODUCTION

Fusarium wilt (FW) of cotton is caused by *Fusarium oxysporum* f. sp. *vasinfectum* (Fov). When Fov causes FW, symptoms typically include interveinal chlorosis and wilting of the leaves, stunting of the plant, and darkening of the vascular system. FW can cause annual yield reduction of 10 million Kg with economic loss of $7 million in USA (Lawrence et al., 2017). *Melodoigyne incognita* (RKN) is the most economically damaging pathogen of cotton in the U.S.A. (Lawrence et al., 2017) and coinfection of cotton with Fov and *M. incognita* can result in a disease complex that greatly increases the severity of FW (Chawla et al., 2012; Cooper and Brodie, 1963). In Georgia, the damage of Fov and *M. incognita* in the same field may cause estimated yield losses of $26 million (Lawrence et al., 2017). Fov race 4 and the Australia biotype cause severe FW regardless of *M. incognita*, but they have not been found in the southeastern USA.

Management of FW is primarily through the control of *M. incognita* (Scott et al., 2011). Although control of *M. incognita* can reduce FW incidence, virulence variability of Fov isolates belonging to the same race may still cause significant FW disease (Chawla et al., 2012). Fov is persistent in the soil because it can produce chlamydospores that can survive for years (Bennett, 2012), making eradication of the fungus difficult or impossible. No cotton cultivars are highly resistant to Fov, and moderately resistant genotypes are more susceptible to FW in the presence of the nematode (Wang and Roberts, 2006).

Fov is subdivided into races, genotypes, and biotypes. Initially, races were identified based on an isolate's pathogenicity to different plant cultivars (Ridgway et al., 1984). However, the original race classification system is now deemed inadequate. For example, the Australia biotype would be classified as race 6, but it is different because it is highly virulent in the absence of *M. incognita* (Ridgway et al., 1984). Fov DNA analysis, multigene genealogy, vegetative compatibility groups (VCG), and greenhouse pathogenicity tests started showing similarities among the classified races (Holmes et al., 2009; Kim et al., 2005; Skovgaard et al., 2001; Egamberdiev et al., 2013) as races 1, 2, and 6; races 4 and 7; and races 3 and 5 were shown to be identical or very similar genetically (Skovgaard et al., 2001). Because of these issues, new methods emerged to improve the classification system, and race classification based on host reaction was replaced by genetic classification based on DNA sequencing. Isolates with novel DNA sequences are labeled as genotypes (Holmes et al., 2009). The genetic classification is based on Fov nuclear and ribosomal regions including translational elongation factor (EF-1α), intergenic spacer (IGS) region, phosphate permase (PHO), and Beta-tubulin (BT) (Kim et al., 2005; Cianchetta et al., 2015).

By sequencing EF-1α and IGS region of isolates in Mississippi and Alabama, Bennett et al. (2013) identified an isolate that was similar to Fov race 4 in EF-1α sequence but not in the IGS region; it was initially called race 4-like and later renamed MDS-12 (Cianchetta et al., 2015). Unlike Fov race 4, MDS-12 causes greater disease in the presence of *M. incognita*. The races and genotypes of Fov known to be in Georgia are races 1, 2, and 8 and genotypes LA110 and LA108 (Cianchetta et al., 2015; Holmes et al., 2009). Fov race 4, which does not require *M. incognita* to cause devastating losses, is not found in Georgia; in the USA, it has only been found in California and Texas (Halpern et al., 2017). All races and genotypes found in Georgia so far are believed to interact with *M. incognita* in the FW disease complex.

The details of how *M. incognita* and Fov interact to cause increased FW severity are not known. Nematode inoculum density correlates positively with FW severity (Chawla et al., 2012; Garber et al., 1979). Because *M. incognita* creates puncture wounds as juveniles enter the root, it was once thought that such wounding might facilitate Fov penetration and increase FW, but that theory is incorrect (Perry, 1961; Starr, 1998). A study with *Fusarium oxysporum* f. sp. *lycopersici* resistant and susceptible tomato plants found that fungal hyphae colonized the xylem and giant cells two weeks after inoculation with the fungi (Fattah and Webster, 1983). Great deterioration of the galls was seen in resistant plants after fungal invasion. They suggested that the formation of giant cells cause chemical changes that increase fungal growth until a fungal threshold is reached and giant cells are finally invaded and deteriorated. If gall formation is what precipitates the interaction with the fungus, then resistant plant genotypes that significantly reduce gall formation may reduce Fov infection and FW.

In cotton, *M. incognita* resistance QTLs located on chromosomes 11 (*qMi-C11*) and 14 (*qMi-C14*) have been reported to have an effect on gall and egg numbers (Gutierrez et al., 2010; Shen et al., 2006), respectively. Cotton lines were developed by the UGA Cotton Molecular Breeding Laboratory to isolate *qMi-C11* and *qMi-C14* in isogenic backgrounds resulting in isogenic lines that carry either *qMi-C11* or *qMi-C14*. The parent lines (M-120 RNR and Coker 201) are themselves isogenic and contain either both QTLs (M-120 RNR) or neither QTL (Coker 201). In previous studies, we documented that *qMi-C11* inhibits an early stage of nematode development and gall formation whereas *qMi-C14* inhibited a later developmental stage but allowed normal gall formation (Silva et al., unpublished data). Cultivars with resistance to root-knot nematode decreased FW damage (Wang and Roberts, 2006). The M-315 genotype from the M-lines series (Shepherd et al., 1996) also contains the resistance QTLs *qMi-C11* and *qMi-C14* and showed resistance to FW (Scott et al., 2011). However, it is not known which resistance QTL would be responsible for the low incidence of FW. We hypothesized that the QTL *qMi-C11* causes a reduction of Fov severity by stopping root-knot nematode gall formation. The objective of this study was to characterize the effects of the *M. incognita* resistance QTLs *qMi-C11* and *qMi-C14* on cotton plants coinfected with Fov and *M. incognita*.

MATERIALS AND METHODS

Experiments were conducted in the greenhouse to evaluate the effects of Fov and RKN on six different cotton genotypes. The genotypes included four isogenic lines with all possible combinations of the resistance QTLs plus two additional controls. The isolines were M-120 RNR (with both *qMi-C11* and *qMi-C14*), CH11 (with *qMi-C11*), CH14 (with *qMi-C14*), and Coker 201 (neither QTL). Clevewilt-6 (with *qMi-C11* and moderately resistant to FW) and Rowden (neither QTL and highly susceptible to FW) were included as additional controls. The factorial arrangement of treatments applied to each cotton genotype with *M. incognita* inoculation alone, Fov inoculation alone, combined *M. incognita* and Fov, and a control with neither pathogen. Seven replications per genotype per treatment were arranged in a randomized complete block design. Four cotton seeds per pot were directly sown into 10.6 cm × 10.6 cm × 12.4 cm pots filled with approximately 500 ml of steam-pasteurized soil (Tifton loamy sand).

Nematode inoculum from greenhouse cultures was obtained from eggplant roots that were washed free of soil and then eggs were harvest using 0.06% NaOCl. For treatments receiving *M. incognita* inoculum, soil was infested at planting with 10,000 *M. incognita* eggs evenly distributed in each planting hole (1.5 cm deep).

The Fov isolate used was obtained in 2015 from a single plant showing symptoms of FW in a cotton field in Lowndes County, GA. The pathogen was isolated from stem pieces that were surface sterilized (0.875% NaOCl for 1 min then rinsed 3 times in sterile water). Stems were cut open longitudinally and placed on acidified PDA for 5 days. Race identification was done as described by Cianchetta et al. (2015). The isolate was identified as Fov race 1.

For use as inoculum, the Fov isolate was grown on sterile wheat seeds for 15 days (Klisiewicz and Thomas, 1970). Wheat seeds were soaked in water overnight, autoclaved, allowed to cool, and then five 1-cm-diameter agar plugs with the Fov isolate were added to the 1 L flask containing the sterile wheat seeds. For treatments receiving Fov, soil for each individual pot was mixed with 10 ml of infested seeds prior to filling the pot.

The experiment was conducted three times, and two experiments (trials A and B) were evaluated at 14 and 28 days after inoculation (DAI), whereas one experiment (trial C) was evaluated only at 28 DAI. At 14 DAI, the number of root galls was counted, and at 28 DAI, plant height, total plant weight, root weight, and shoot weight were measured. Trial A had very low Fov infection compared to trials B and C at 28 DAI, so trial A was only evaluated for gall numbers.

Leaf discoloration as a symptom of FW was evaluated on a 0 to 5 scale according to McFadden et al. (2004) and Ibrahim and Nirenberg (1993) where 1= no symptoms (0%), 2 = chlorosis and/or wilt restricted to cotyledons or first leaf or ≤25% of leaves infection, 3= chlorosis and/or wilt extended beyond the first leaf or (>25%- ≤50%), 4 = severe chlorosis and/or wilt with whole plant affected or (>50%- ≤ 90), and 5 = almost dead or dead (>90%). Stems were cut longitudinally from the root crown to the apical bud to assess the degree of discoloration on a 0 to 5 scale where 1= healthy (0% discolored), 2 = ≤25%, 3 = >25%- ≤50%, 4 = >50%- ≤75%, and 5 = >75%. The number of dead plants per pot was also recorded.

Data were analyzed for differences among cotton genotypes and also among treatments within a genotype using the PROC MIXED procedure in SAS 9.3 software (SAS Institute Inc., Cary, NC). Treatments within each genotype were analyzed as a 2×2 factorial to determine whether there was a synergistic interaction between Fov and *M. incognita* on that genotype (a significant nematode×Fov interaction [$P \leq 0.05$]). Trials B and C were combined for analysis because the cotton genotypes reacted similarly in the two trials and the treatments had similar effects in the two trials (no significant trial×genotype or trial×treatment interaction [$P \leq 0.05$]).

RESULTS

Total plant weight in all the genotypes decreased significantly compared to the control for plants inoculated with both Fov and *M. incognita*, and the Fov+*M. incognita* treatment had the lowest weight for all genotypes (Fig. 5.2). The combined treatment also reduced total plant weight compared to the Fov alone treatment for all of the genotypes except Rowden and CH14. Neither *M. incognita* nor Fov alone reduced total plant weight on any genotype except Rowden. Plant weights differed among genotypes for the nontreated controls, with Rowden having greater weight than the other genotypes (Fig. 5.1). Total plant weight did not differ among genotypes for plants receiving both Fov and *M. incognita* (Fig. 5.1). In the pooled analysis with both trials, the treatments had a similar effect on the total plant weight of all genotypes (no treatment×genotype interaction [$P = 0.2271$]). When genotypes were analyzed individually, the effect of Fov was influenced by *M. incognita* (a significant Fov×*M. incognita* interaction) on CH11 (*P* = 0.019) and M-120 RNR (*P* = 0.0039).

The combined Fov and *M. incognita* treatment resulted in the numerically lowest root weight for all genotypes, however, the combined treatment was only different from the control for Rowden, M-120 RNR and Clevewilt-6 (Fig. 5.2). The combined treatment had lower root weight than Fov alone only for CH11 and M-120 RNR. For nontreated plants, Rowden had greater root weight than the other genotypes. However, for the Fov, *M. incognita*, and Fov plus *M. incognita* treatments, there were no differences in root weight among the genotypes. When trials were pooled for analysis, the treatments elicited similar responses in all the genotypes (no treatment×genotype interaction [*P*= 0.3512]). The effect of Fov was influenced by *M. incognita* infection (an Fov×*M. incognita* interaction) on CH14 (*P* = 0.046) and on M-120 RNR (*P* = 0.0009) when the genotypes were evaluated individually.

Plant height was numerically greatest in the nontreated control and numerically lowest in the Fov plus *M. incognita* combined treatment for all genotypes (Figure 5.3). Plant height was reduced by the combined Fov plus *M. incognita* treatment compared to the control for all genotypes (Figure 5.3). Compared to the Fov alone treatment, the combined treatment reduced plant height of Coker 201, CH11, M-120 RNR and Clevewilt-6, but not of Rowden or CH14. The *M. incognita* alone treatment reduced plant height of Rowden, Coker 201, and CH11. When trials were combined for analysis, the genotypes reacted differently to the treatments (a treatment×genotype interaction [*P* = 0.03]). Infection by *M. incognita* influenced the effect of Fov (Fov×*M. incognita* interaction) on Clevewilt-6 (*P*=0.0196) and M-120 RNR (*P*= 0.0217).

The combined Fov plus RKN treatment resulted in the lowest numerical shoot weight in all genotypes, and the combined treatment was statistically lower than the nontreated control for all genotypes except CH11 (Figure 5.4). Infection by *M. incognita* reduced shoot weight compared to the control on Rowden and Coker 201, and Fov alone reduced shoot weight compared to the control only on Rowden. Treatments affected all the genotypes similarly (no treatment×genotype interaction [$P = 0.277$]). The effect of Fov was influenced by *M. incognita* (a significant Fov×*M. incognita* interaction) on CH11 ($P = 0.0239$) and M-120 RNR ($P = 0.0078$).

Gall numbers at 14 DAI were low in trial A compared to trial B (Figure 5.5). In trial A in the *M. incognita* alone treatment, the level of galling was greater in Coker 201 and CH14 than in Rowden or CH11. In trial A, the combined Fov and *M. incognita* treatment had similar numbers of galls to the *M. incognita* alone treatment for most genotypes, however, there was an increase in galling on Rowden and a decrease on Coker 201. In trial B in the *M. incognita* alone treatment, the level of galling was greater on Rowden, Coker 201, and Clevewilt-6 than on CH11 or M-120 RNR. In trial B, the combination of Fov and *M. incognita* resulted in similar levels of galling to *M. incognita* alone on all genotypes except Rowden, which showed a decrease in galling with the combination treatment. The treatments did not have consistent effects on the genotypes (a treatment×genotype interaction [$P = 0.0447$]) in trial A, but they did in trial B ($P = 0.1000$).

At 28 DAI, trial A had numerically fewer galls than trials B and C (Figure 5.6). In trial A, the *M. incognita* alone treatment had greater galling on Coker 201 than on CH11, CH14, M-120 RNR, and Clevewilt-6. In trial A, the combination of Fov plus the nematode reduced galling compared to the nematode alone treatment only on Coker 201. In the nematode alone treatment in trial B, Rowden and Coker 201 had the greatest galling, but they differed only from Clevewilt-6. The addition of Fov compared to the nematode alone treatment in trial B reduced galling on

Rowden but had no effect on the other genotypes. In trial C in the nematode alone treatment, the only difference in galling among genotypes was that Coker 201 had more galling than CH11. When Fov and the nematode were both present, the level of galling was not different on any genotype than the treatment with the nematode alone. The effects of treatments on root galling were not consistent among the genotypes (a treatment×genotype interaction) in trial A (*P* = 0.0472) or Trial B (*P* =0.017), but the effects were consistent in trial C.

Trial A was not considered in the analysis of the percentage of dead plants. The treatment with the numerically greatest percentage of dead plants at 28 DAI was the Fov plus *M. incognita* treatment on all genotypes in both trials B and C, and the difference was statistically significant on most of the genotypes in both trials (Fig. 5.7). Fov alone increased plant mortality compared to the nontreated control on Rowden in both trials and on Coker 201 in trial C, but Fov did not increase mortality on the other genotypes. Infection with *M. incognita* alone generally did not cause plant death. The treatment had similar effects on all genotypes (no genotype×treatment interaction).

Foliar damage ratings were numerically greatest for all genotypes in the Fov plus *M. incognita* combined treatment, and those values were statically greater than for all the other treatments except for Rowden in trial A and CH11 in trial B (Fig. 5.8). In those two cases, foliar damage ratings for the combined treatment was numerically but not statistically greater than the Fov alone treatment. Fov alone increased foliar damage ratings relative to the control treatment only for Rowden in both trials and Clevewilt-6 in trial C. Nematode infection alone increased foliar damage ratings relative to the control treatment for Coker 201 in trial A and Clevewilt-6 in Trial C. The effect of the treatments differed among the genotypes (a genotype×treatment interaction [*P* = 0.0046]). For individual genotypes, the effect of Fov was influenced by nematode infection (a significant Fov×*M. incognita* interaction) on CH11 ($P = 0.0018$), CH14 ($P = 0.0015$), Clevewilt-6 ($P \leq 0.0001$), Coker 201 ($P \leq 0.0001$), and M-120 RNR ($P = 0.0011$), but not on Rowden.

Vascular discoloration for the combined Fov plus nematode treatment was greater than for any other treatment for all genotypes in both trials (Fig. 5.9). Fov alone increased vascular discoloration ratings relative to the nontreated control on most genotypes in both trials, except for CH14 and Clevewilt-6 in trial B and Ch11 and CH14 in trial C. Vascular discoloration also was observed in the nematode alone treatment, resulting in ratings that were greater than the nontreated control for Coker 201, CH11, and Clevewilt-6 in trial C. The treatment had similar effects on all genotypes on trial B and C (no genotype×treatment interaction).

DISCUSSION

Although there are no cotton cultivars with high levels of resistance to FW in cotton, some moderately resistant cultivars are available (U.S. Department of Agriculture, 2015, U.S. Department of Agriculture, 2017). QTL mapping on resistant cotton lines for race 1 and 4 uncovered major candidate genes in Pima-S7 and Pima 3-79 located on chromosome 16 with additive effects and on Pima-S6 on chromosome 14 with a dominant effect (Ulloa et al., 2013; Ulloa et al., 2011). It is not known whether the QTL on chromosome 14 that contains gene(s) for resistance to RKN (Gutierrez et al., 2010) is the same QTL for Fov resistance. In fact, an issue that must be considered when developing a FW-resistance cultivar is the greatly increased severity of FW associated with *M. incognita*-infected plants (Castillo et al., 2003; Wang and Roberts, 2006). The goal of our study was to evaluate the effects of *M. incognita* resistance QTLs on the interaction between Fov and the nematode. We documented a reduction of plant growth and an increase in FW severity on all genotypes regardless of nematode resistance QTLs when coinfected with Fov and *M. incognita*. None of the genotypes in our study, which means neither of the nematode-resistance QTLs alone or in combination, significantly affected root, shoot, or whole-plant weight or foliar or vascular symptoms of FW. Similar results were obtained on different cotton genotypes in previous studies (Holmes et al., 2009; Wang and Roberts, 2006).

The root-knot nematode inoculum level can influence the FW-RKN interaction in FW resistant cotton genotypes breaking the resistance and increasing FW severity (Castillo et al., 2003). In studies using nematode eggs as inoculum, the percentage egg hatch can be a significant factor affecting the degree of nematode parasitism in the plant. Commonly, the percentage egg hatch averages around 20% (Ingham et al., 2015), therefore, the hatch percentage in our studies can be estimated to be around 2,000 J2 per pot (from 10,000 eggs/pot), which is a relatively high population density compared to typical *M. incognita* levels found in cotton fields in Georgia (Timper et al., 2006). Our high RKN inoculum levels could be the reason why all of the resistance QTLs to RKN appeared to be susceptible. Our choice of Fov and *M. incognita* inoculum levels may have influenced the level of FW severity we observed, and it may also have influenced the interaction of Fov and the nematode.

We observed some vascular discoloration in treatments that did not receive Fov, however, the unexpected discoloration was more common in the *M. incognita* alone treatment than in the nontretated control. Because Fov can be a seed borne pathogen (Bennett et al., 2008), surface sterilized seeds of each cotton genotype in our study were plated onto APDA medium, however, no Fov was recovered, which is evidence that our seed lots were not infected. Our nematode inoculum was not tested for the presence of Fov. The source of the Fov contamination in our study remains unknown.

Fov has been classified into two pathotypes: root-rot types that causes wilting independent of nematode infection with severe root damage and vascular competent types that are dependent on the nematode to cause severe disease and do not cause severe root damage (Bell et al., 2016). Vascular competent pathotypes cause disease when using stem-puncture inoculation but not in infested soil assays whereas root-rot pathotypes cause disease in the opposite way (Bell et al., 2017, Bell et al., 2016). In this experiment we used soil infestation assays to observe the Fov-*M. incognita* interaction in different cotton genotypes. Although race 1 has been described as a vascular competent pathotype (Bell et al., 2017), we obtained severe disease in all our cotton genotypes with and without the nematode infection. We did not test stem-puncture methods in our experiment, however, based on the results our isolate behaved as a root-rot pathotype.

Controlling *M. incognita* in cotton is recommended as the best means to control FW (Scott et al., 2011). Until the recent availability on *M. incognita*-resistant cultivars, control of *M. incognita* was typically accomplished by applying nematicides (Weingartner and Shumaker, 1990), and nematicide application was effective in reducing FW (Brodie and Hauser, 1970). Controlling *M. incognita* through the use of resistant genotypes also was expected to provide adequate control of FW. However, our results shows that for the inoculum levels used in our study the resistance QTLs used in developing *M. incognita*-resistant cotton cultivars do not affect the disease severity of FW. However, the use of host-plant resistance to reduce nematode population levels may help suppress FW in subsequent years or may allow moderately effective nematode control options to more effectively reduce FW. Therefore, the best tactic for managing FW in cotton may involve pyramiding resistance to *M. incognita* and FW in the same cultivar.

In recent years, Fov virulence levels have changed in southern Georgia (Bell et al., 2017). Race 1 that used to present mild virulence and increase virulence in the presence of RKN (Chawla et al., 2012; Kim et al., 2005) showed, in this study, to be highly virulent in all of our lines with and without nematode inoculation. The genotype M-120 RNR was developed from a cross between Auburn 634 RNR (the donor of QTLs *qMi-C11* and *qMi-C14*) and the recurrent parent Coker 201 (Shepherd, 1974). In contrast to our results with M-120 RNR, Auburn 634 RNR is very resistant to FW with a mortality rate <1% (Kirkpatrick and Shepherd, 1989). A possible reason for that discrepancy is that the genes responsible for FW resistance in Auburn 634 RNR were not transferred to M-120 RNR. An alternative explanation is that the genes were transferred but the aforementioned changes in Fov virulence (Bell et al., 2017) have affected the interaction of Fov and *M. incognita*. We did not obtain lower galling from our resistant lines as expected, however, nematode reproduction presumably would have been reduced (Shen et al., 2006; Gutierrez et al., 2010). Thus, the high galling production may have influenced in the amount of Fov infection.

Pathogen invasion of plants elicits defense responses that protect against subsequent infections (Vallad and Goodman, 2004). Biotrophic pathogens may activate the salicylic acid (SA) pathway which protects against further infections by biotrophs, and necrotrophic pathogens may activate the jasmonic acid (JA) pathway which protects against further infections by necrotrophs (Liu et al., 2013; Spoel et al., 2007). If the SA and JA pathways are both being activated in a plant at the same time, the SA response will prevail and the JA response will be suppressed (Liu et al., 2013). Infection of cotton by *M. incognita* induces systemic acquired resistance (SAR), presumably by activating the plants SA pathway, and SAR is induced even in nematode-resistant genotypes including M-120 RNR (Aryal et al., 2011). Fov is considered a hemibiotrophic pathogen that starts infecting the plant as a biotroph but at some point in the disease process becomes a necrotroph (Jones and Dangl, 2006). When acting as a necrotroph, Fov activates the plant's JA pathway and suppresses pathogen attack (Thatcher et al., 2009). However, if infection of the plant by *M. incognita* elicits the plant's SA defenses and suppresses the JA defenses, the plant may be rendered more susceptible to the necrotrophic phase of Fov infection. A similar scenario was documented on *Arabidopsis thaliana* when infection by *Pseudomonas syringae* (a biotroph) induced SA defenses and suppressed JA defenses thereby making the plants more susceptible to *Alternaria brassicicola* (a necrotroph) (Spoel et al., 2007).

Our research shows that the QTLs *qMi-C11* and *qMi-C14*, which impart resistance to *M. incognita* in cotton, do not affect the interaction of Fov and *M. incognita* under the conditions of our tests. Therefore, controlling *M. incognita* in cotton through host plant resistance utilizing those QTLs may not provide control of FW. Our results were obtained with fairly high inoculum levels of Fov and *M. incognita,* and additional research is needed to determine whether similar results would be obtained with lower inoculum levels or with other isolates of Fov.

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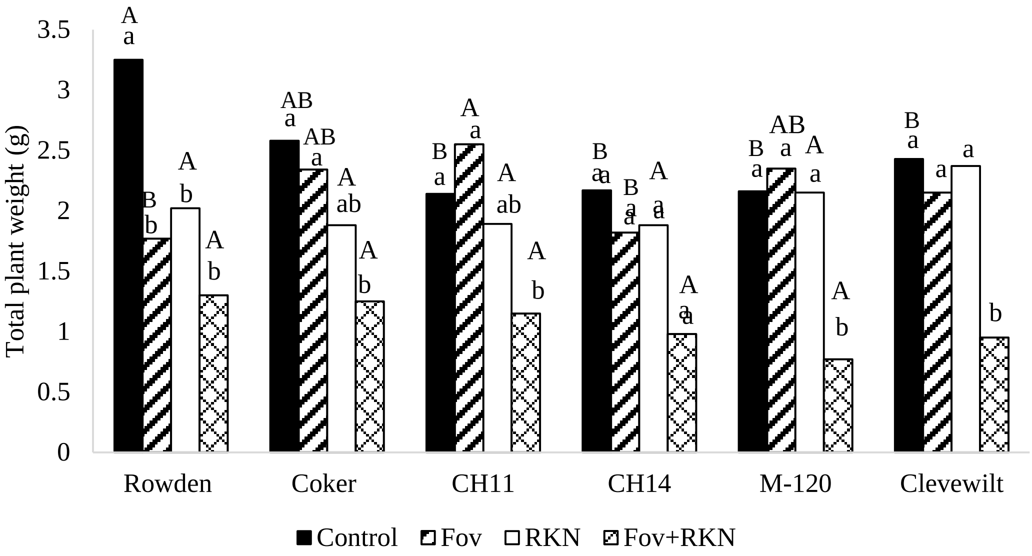
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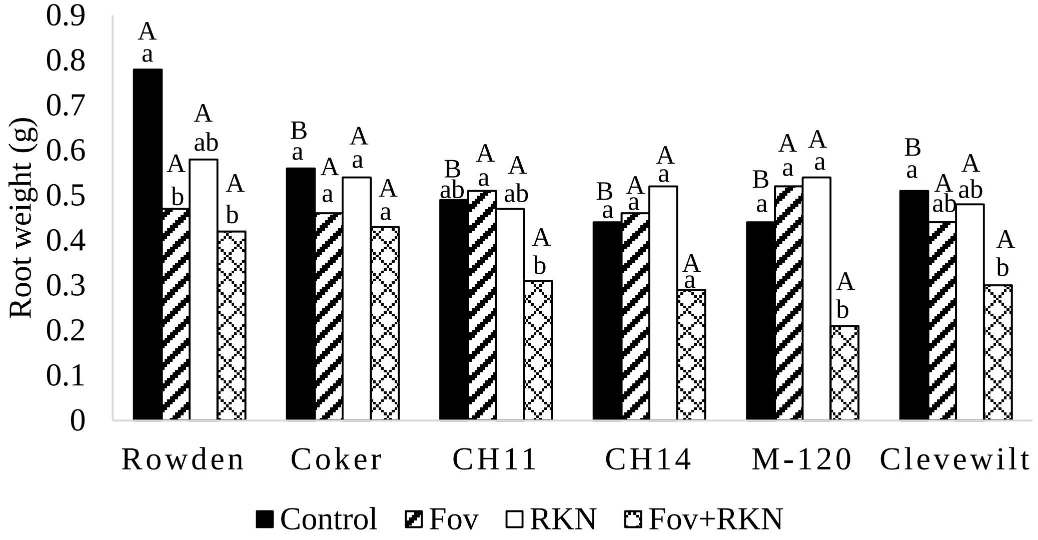
3
2.5
2
1.5
1
0.5
0
A
a
B
b
A
b
A
b
AB
a
AB
a
A
ab
A
b
B
a
A
a
A
ab
A
b
B
a
a
B
a
a
A
a
a
A
a
a
B
a
AB
a
A
a
A
b
B
a
a
a
b
Rowden
Coker
CH11
CH14
M-120
Clevewilt
■ Control
▨ Fov
□ RKN
▩ Fov+RKN

Figure 5.1.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0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Root weight (g)
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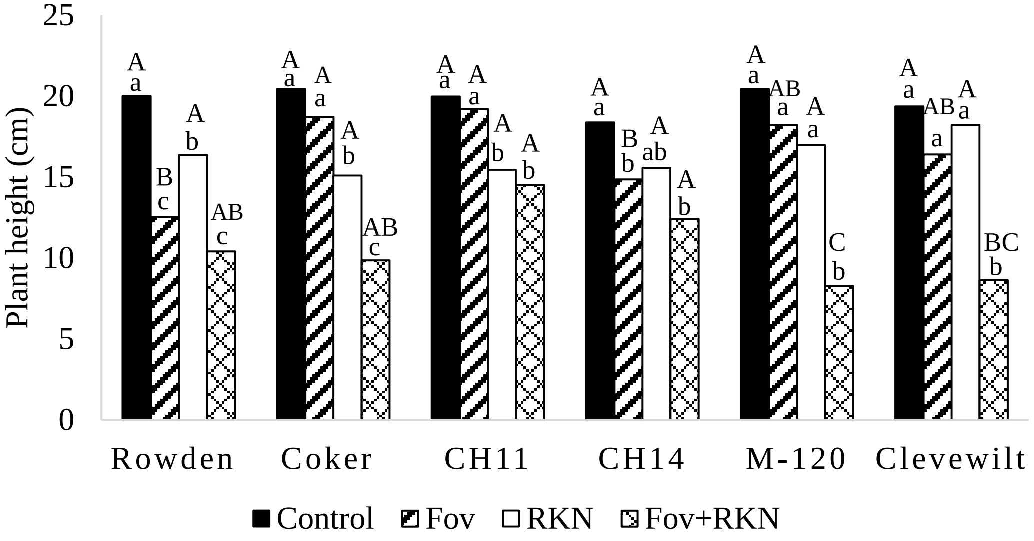
A
b
A
ab
A
b
B
a
A
a
A
a
A
a
B
ab
A
a
A
ab
A
b
B
a
A
a
A
a
A
a
B
a
A
a
A
a
A
b
B
a
A
ab
A
ab
A
b
Rowden
Coker
CH11
CH14
M-120
Clevewilt
■ Control
▨ Fov
□ RKN
▩ Fov+RKN

Figure 5.2.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15
10
5
0
Plant height (cm)
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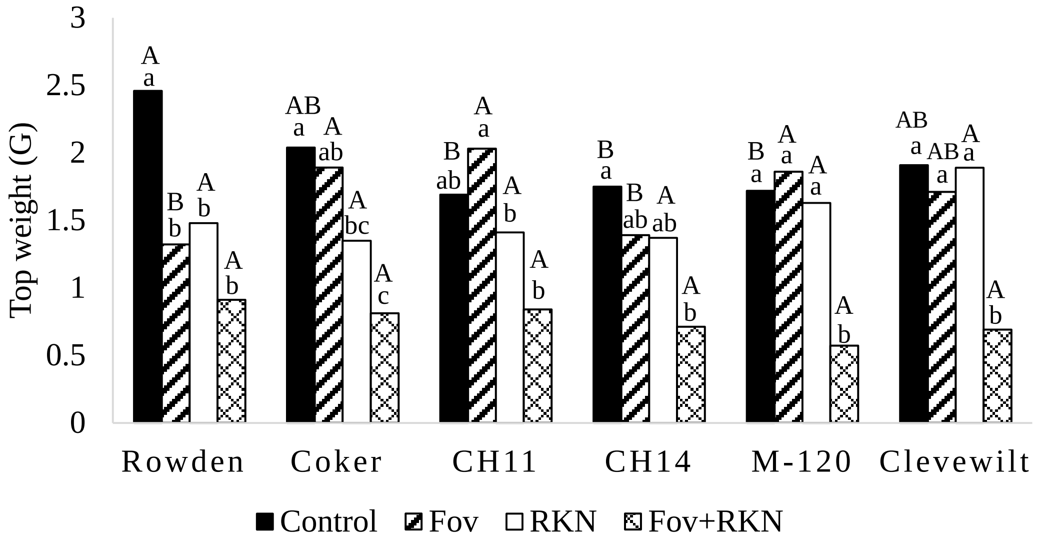
B c
A b
AB c
A a
A a
A b
AB c
A a
A a
A b
A b
A a
B b
A ab
A b
A a
AB a
A a
C b
A a
AB a
A a
BC b
Rowden
Coker
CH11
CH14
M-120
Clevewilt
■ Control
▨ Fov
□ RKN
▩ Fov+RKN

Figure 5.3.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1
0.5
0
Top weight (G)
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B
b
A
b
A
b
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a
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ab
A
bc
A
c
B
ab
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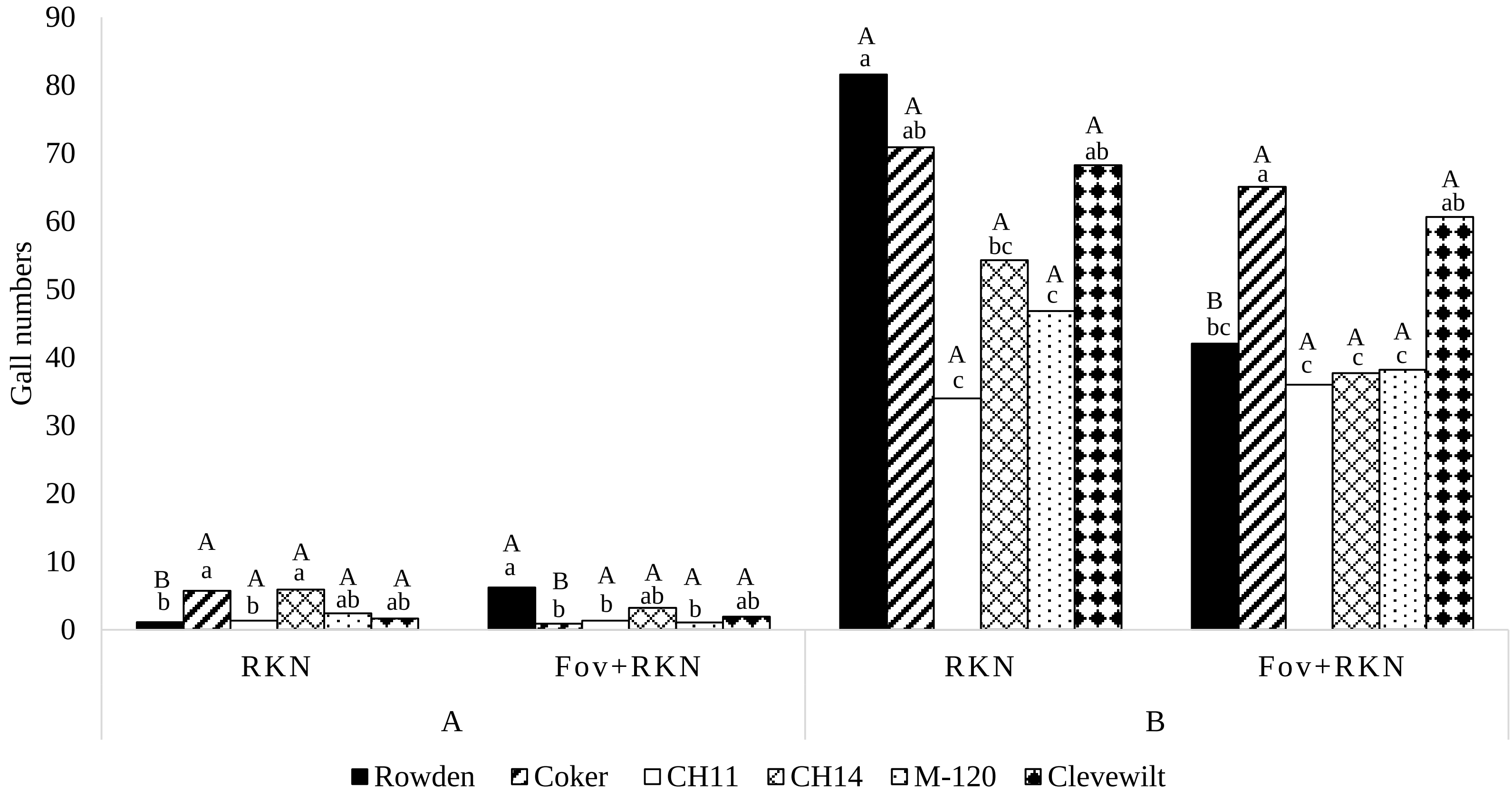
A
b
A
b
B
a
B
ab
A
ab
A
b
B
a
A
a
A
a
A
b
AB
a
AB
a
A
a
A
b
Rowden
Coker
CH11
CH14
M-120
Clevewilt
Control
Fov
RKN
Fov+RKN

Figure 5.4.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50
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A a
A ab
A ab
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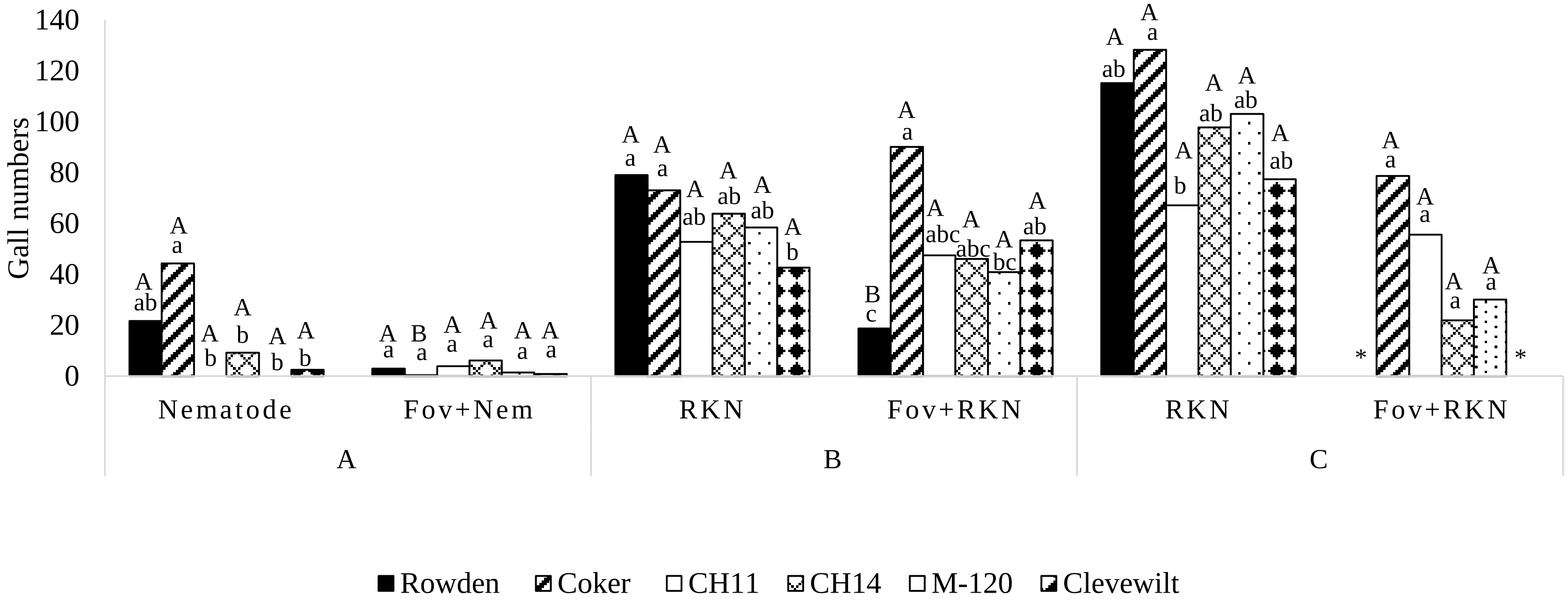
A b
A ab
A b
A ab
A a
A ab
A c
A bc
A c
A ab
B bc
A a
A c
A c
A c
A ab
RKN
Fov+RKN
RKN
Fov+RKN
A
B
Rowden
Coker
CH11
CH14
M-120
Clevewilt

Figure 5.5.


Gall numbers
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20
40
60
80
100
120
140
Nematode
Fov+Nem
RKN
Fov+RKN
RKN
Fov+RKN
A
B
C
Rowden
Coker
CH11
CH14
M-120
Clevewilt
A ab
A a
A b
A b
A b
A b
A a
B a
A a
A a
A a
A a
A a
A a
A ab
A ab
A ab
A b
B c
A a
A abc
A abc
A bc
A ab
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*
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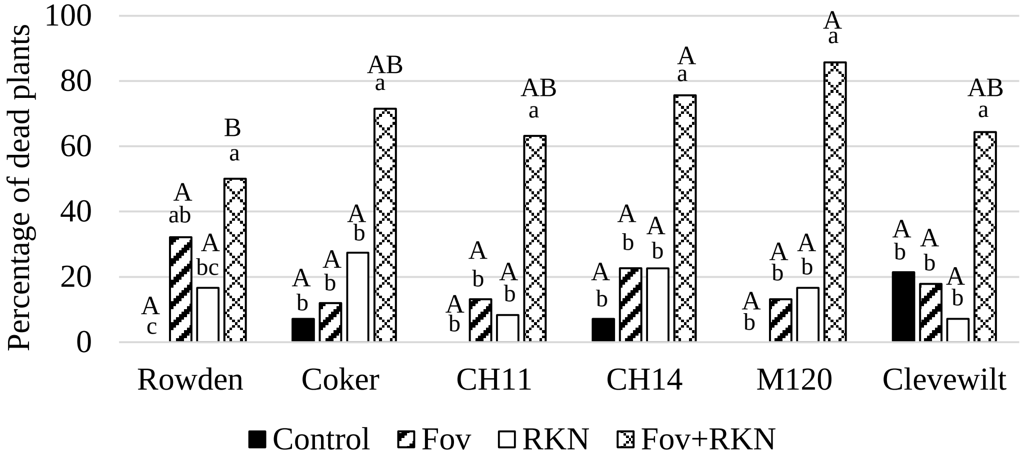
A a
A a
A a
*

Figure 5.6.

Trial B



Trial C

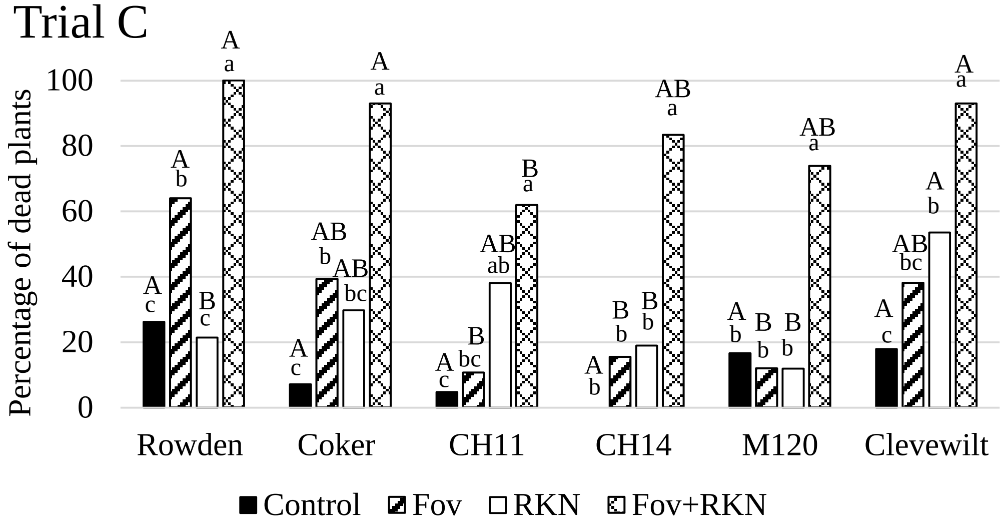
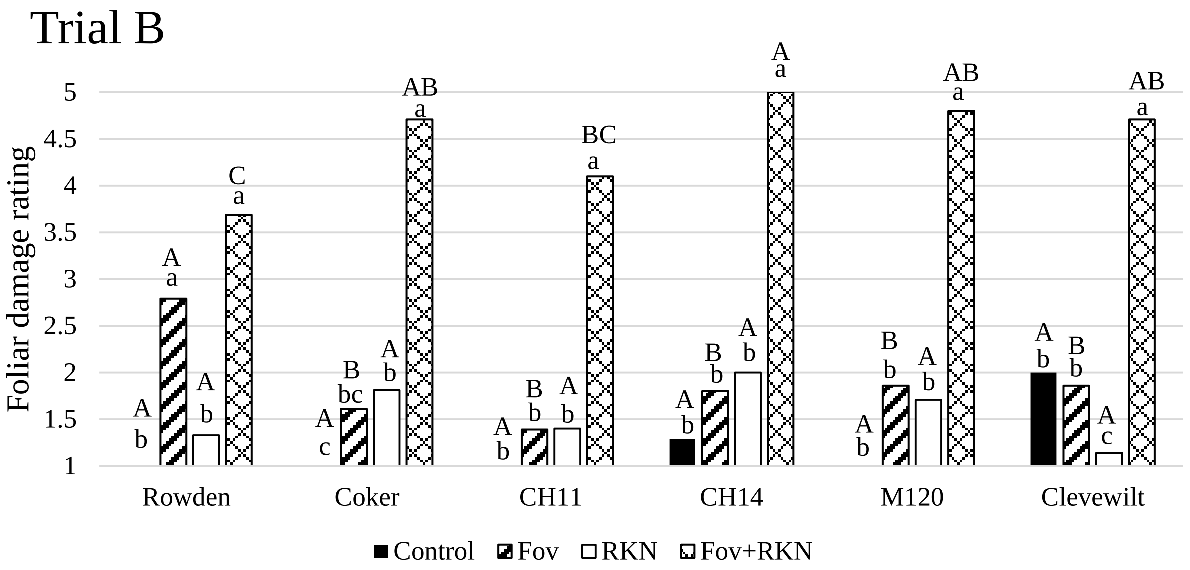


Figure 5.7.


Trial B

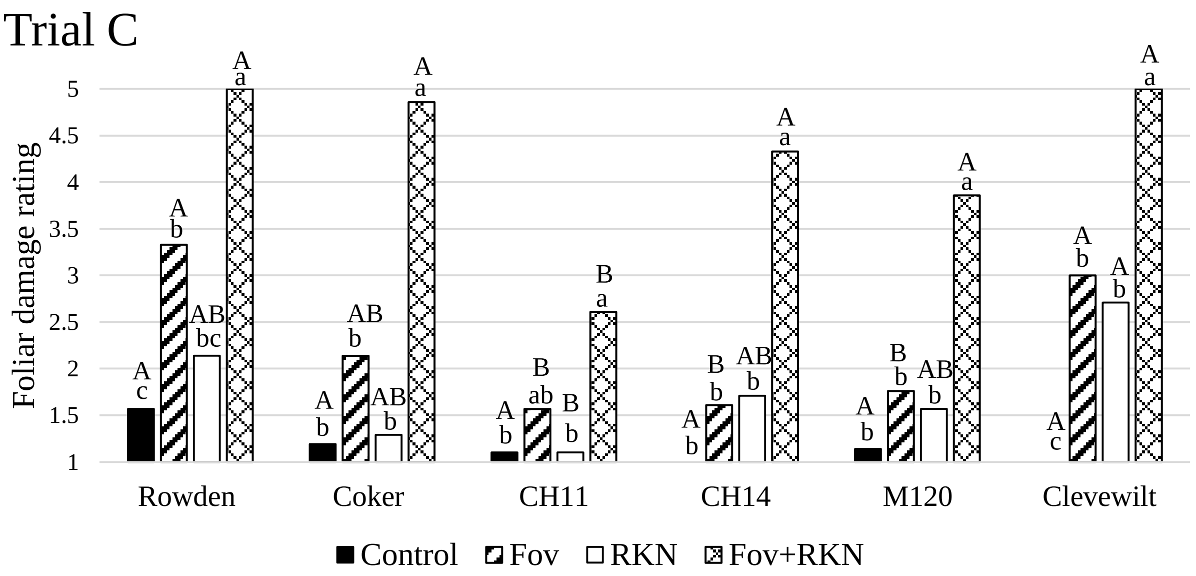

Trial C

Figure 5.8.

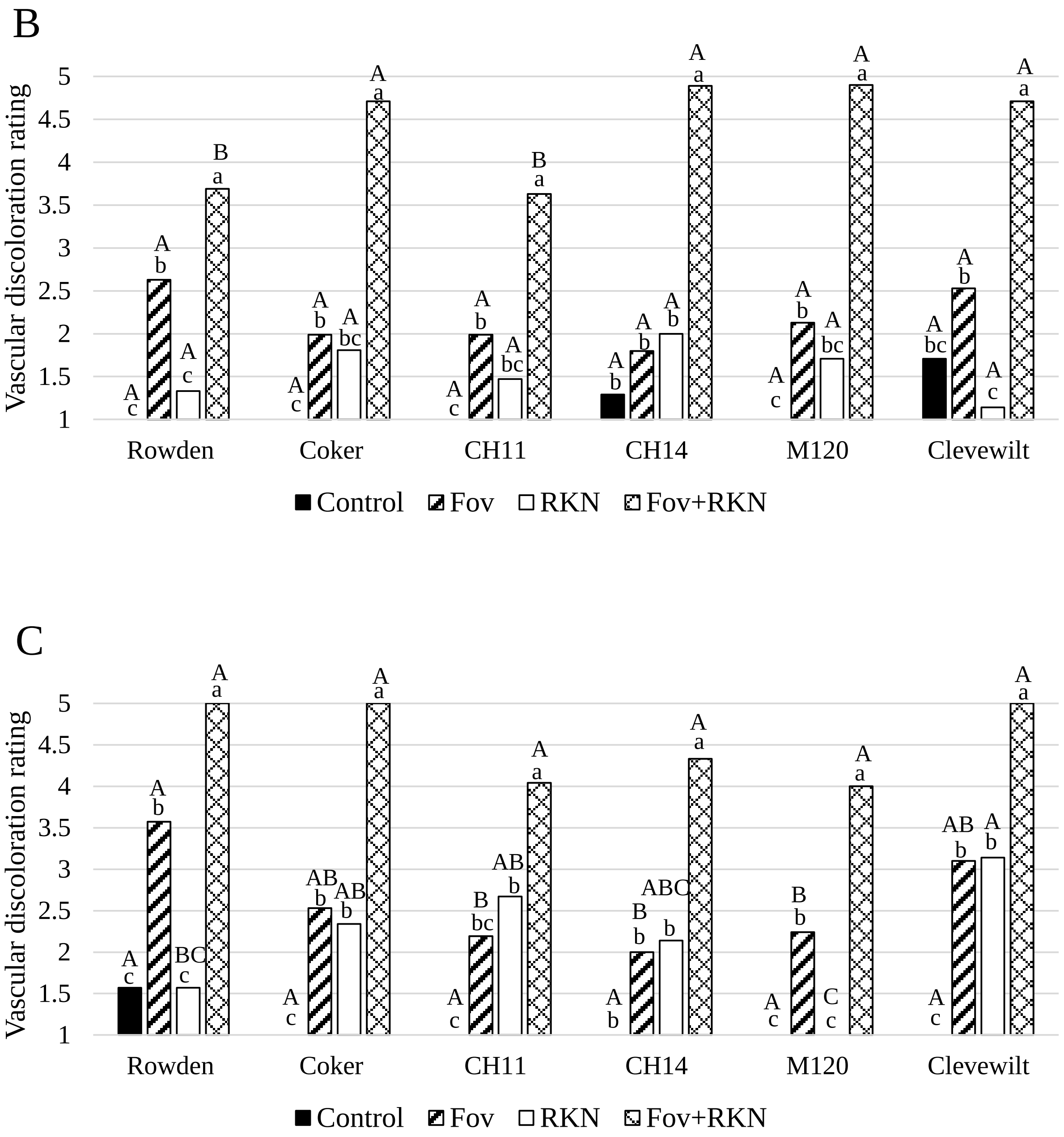

B
Vascular discoloration rating
5
4.5
4
3.5
3
2.5
2
1.5
1
A c
A b
A c
B a
A c
A b
A bc
A a
A c
A b
A bc
B a
A b
A b
A b
A a
A c
A b
A bc
A a
A bc
A b
A c
A a
Rowden
Coker
CH11
CH14
M120
Clevewilt
■ Control ▨ Fov □ RKN ▩ Fov+RKN
C
Vascular discoloration rating
5
4.5
4
3.5
3
2.5
2
1.5
1
A c
A b
BC c
A a
A c
AB b
AB b
A a
A c
B bc
AB b
A a
A b
B b
ABC b
A a
A c
B b
C c
A a
A c
AB b
A b
A a
Rowden
Coker
CH11
CH14
M120
Clevewilt
■ Control ▨ Fov □ RKN ▩ Fov+RKN

Figure 5.9.

Figure 5.1. Effect of the treatments with and without Fov or *M. incognita* on plant weight. Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among treatments and caps letters mean differences among genotypes.

Figure 5.2. Effect of the treatments with and without Fov or *M. incognita* on root weight. Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among treatments and caps letters mean differences among genotypes.

Figure 5.3. Effect of the treatments with and without Fov or *M. incognita* on plant height. Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among treatments and caps letters mean differences among genotypes.

Figure 5.4. Effect of the treatments with and without Fov or *M. incognita* on top weight. Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among treatments and caps letters mean differences among genotypes.

Figure 5.5. Effect of Fov on gall numbers in different cotton genotypes in two different trials 14 days after inoculation. Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among genotypes and caps letters mean differences among treatments.

Figure 5.6. Effect of Fov on gall numbers in different cotton genotypes in three different trials (A, B, and C) 28 days after inoculation. Different letter over bars indicate significant differences

at α=0.05. Small letters mean difference among genotypes and caps letters mean differences among treatments.

*missing data due to plants being all dead by this point.

Figure 5.7. Percentage of dead plants 28 days after inoculation in different cotton genotypes in two different trials. Different letter over bars indicate significant differences at α=0.05.

Figure 5.8. Foliar damage ratingon the scale 1 (no discoloration) to 5 (>75%) 28 days after inoculation in different cotton genotypes in two different trials (B and C). Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among treatments and caps letters mean differences among genotypes.

Figure 5.9. Vascular discoloration on the scale 1 (healthy leaves) to 5 (>90% or dead) 28 days after inoculation in different cotton genotypes in two different trials (B and C). Different letter over bars indicate significant differences at α=0.05. Small letters mean difference among treatments and caps letters mean differences among genotypes.

CHAPTER 6

CONCLUSIONS

This study confirms that *qMi-C11* and *qMi-C14* act at different times and have different effects on the development of *M. incognita* and therefore, have different modes of action. In fact, *qMi-C11* reduces gall production by stopping early development of *Meloidogyne incognita* and *qMi-C14* reduces nematode reproduction by stopping fourth stage juvenile (J4) development into females. However, we did not obtain consistent differences in egression of the nematode from roots due to effects of the QTLs. We conclude that *qMi-C11* and *qMi-C14* do not significantly stimulate nematode egression from cotton roots, but both *qMi-C11* and *qMi-C14* reduce the number of *M. incognita* egg masses and eggs/egg mass. Neither *qMi-C11* nor *qMi-C14* alone affects gall size, but both QTLs together appear to slow the rate of gall development.

In our studies with Fusarium wilt in cotton fields, the races and genotypes previously described were found in most of our cotton fields. However, genotypes such as MDS-12, LA 127, and LA140 were found in Georgia for the first time. Surprisingly, Fusarium Wilt was found to be commonly associated with *B. longicaudatus* and not *M. incognita*. Although *B. longicaudatus* was known to be able to interact with the Fusarium Wilt pathogen, it was not believed to do so commonly.

Finally, the test of the *M. incognita* resistance QTLs on the interaction between *M. incognita* and *Fusarium oxysporum* f. sp. *vasinfectum* showed the QTLs to be ineffective against Fusarium wilt. The *Fusarium* f. sp. *vasinfectum* and *M. incognita* disease complex reduced plant growth and increased plant mortality regardless of the QTLs. Thus, there is not enough evidence in our studies to conclude that nematode resistance QTLs that reduce gall formation and nematode development reduce FW severity. Additional study is needed to determine the factors involved in the interaction of these two pathogens.