Management of *Aphis gossypii* Populations and the Spread of *Cotton leafroll dwarf virus* in Southeastern Cotton Production Systems

by

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Abstract

Cotton aphid, *Aphis gossypii* Glover is a highly polyphagous pest known to cause both direct and indirect damage to cotton. Management of *A. gossypii* is a concern in Alabama due to two emerging problems, insecticide resistance, and transmission of *Cotton leafroll dwarf virus* (CLRDV). The overall objective of this research was to evaluate management tools of *A. gossypii* in cotton. The objective of Chapter 2 was to quantify the susceptibility of *A. gossypii* populations collected from different cotton production regions across Alabama by calculating LC_{50s} from dose-response curves to quantify susceptibility to imidacloprid. On average, field collected populations were 69.71 and 81.16 times more resistant than the susceptible colony at 48 and 72 h respectively. These results indicate variable levels of susceptibility among cotton aphid populations with some exhibiting high levels of resistance. Insecticide applications targeting *A. gossypii* should be minimized to reduce selection for insecticide resistance.

The Chapter 3 objectives were to investigate the efficacy of cultural and aphid management strategies on reducing final CLRDV incidence and monitored aphid population dynamics in relation to timing of CLRDV spread. In this study, chemical and cultural practices evaluated did not reduce final incidence of CLRDV. Final CLRDV incidence was nearly 100% in all plants sampled in GA, and incidence ranged from 60 – 100% across plots in AL. Although insecticide use did reduce aphid populations in the field, it did not reduce the proportion of plants infested with aphids. Aphid monitoring showed that *Aphis gossypii* and *Protaphis middletonii* Thomas were the dominant species collected from pan traps. Results from the sentinel plants objectives to monitor spread of CLRDV into the field identified three distinct time periods of spread concurrent with aphid trapping efforts, and two of them coincide with aphid dispersal

events. Further research is needed to understand the relationship between aphid population dynamics and CLRDV spread, in addition to investigation of other management strategies to reduce CLRDV in cotton.

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List of Abbreviations

IPM	Integrated pest management
IRM	Insecticide resistance management
RR	Resistance ratio
CLRDV	*Cotton leafroll dwarf virus*
%	Percent
°C	Degrees Celsius
h	Hour
g	Gram
mm	Millimeter
cm	Centimeter
m	Meter
ml	Milliliter
PPM	Parts per million
PCR	Polymerase chain reaction

Chapter 1

Literature Review

1.1 Economic Importance of Cotton

Agriculture makes up a large constituent of the world economy; a role that the southeastern United States plays a major part in. Cotton (*Gossypium* spp.), is a crop that is primarily grown for fiber, but the seeds are an important food source for both humans and livestock (Luttrel et al. 1994). In the 2018/2019 season, cotton production was estimated at 18.4 million bales in the U.S., with approximately 4.3 million produced in the southeast and 4.6 million in the Mississippi delta region. The U.S. is the world's leading exporter of cotton and contributes billions of dollars a year to the economy (Dohlman et al. 2019).

Cotton is grown south of the 36th parallel (USDA NASS 2010), and high concentrations of cotton can be found in the Texas high plains, the Mississippi delta region, coastal plains of Georgia, and North Carolina (USDA NASS 2010). Most cotton produced in the U.S. is upland cotton (*Gossypium hirsutum* L.), which is planted in Alabama, Arizona, Arkansas, California, Florida, Georgia, Kansas, Louisiana, Mississippi, Missouri, New Mexico, North Carolina, Oklahoma, South Carolina, Tennessee, Texas, and Virginia whereas the remaining cotton produced is American pima cotton (*Gossypium barbadense* L.) which is grown in Arizona, California, New Mexico, and Texas (USDA NASS 2010). Planting and harvesting dates can vary in each state and in each region of a state based on climatic zones (USDA NASS 2010). Local weather influences crop management decisions, yield potential, and time to maturity (Luttrel et al. 1994).

Cotton faces many insect pest problems that can impact maturity and yield. Common insect pests of cotton found throughout the southeastern U.S. include: tobacco budworms *Helicoverpa zea* Boddie, *Heliothis virescens* Fabricius (Lepidoptera: Noctuida), *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae), thrips including *Frankliniella fusca* Hinds, *Frankliniella tritici* Fitch, *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), and *Aphis gossypii* Glover (Hemiptera: Aphididae) (Luttrel 1994). Contemporary challenges with cotton aphid (*Aphis gossypii*) management are the subject of this thesis, and include investigating reports of reduced efficacy of an insecticide commonly used for population management in Alabama, and whether or not aphid management can reduce incidence of *Cotton leafroll dwarf virus* (CLRDV) (genus, *Polerovirus*), which is an emerging problem across the cotton belt.

1.2 Aphid morphology

There are approximately 4700 species of aphids worldwide. Of these, 450 have been recorded on crop plants, and approximately 100 are pests. Aphids belong to the order Hemiptera and the sub-order Sternorryncha, along with Aleyrodoidae (whiteflies), Coccoidea (scale insects and mealy bugs), and Psylloidea (psyllids or jumping plant lice). All of these insects are phytophagous and sap-sucking. Most of the economically important species of aphids are found within the subfamily Aphidinae (includes *A. gossypii*), which is also the largest subfamily within the family Aphididae. (van Emden and Harrington 2007).

Aphids are small soft bodied insects, most ranging from 1 – 3 mm in size. Aphids have piercing-sucking mouthparts, and feed on phloem sap from the sieve tubes of higher plants through their specially adapted mouthparts known as stylets. Aphids possess unique dorsal tube-like appendages located on their abdomen called cornicles (or siphunculi), which are part of their modified digestive system, and aid in excretion of phloem sap. Cornicles also have specialized

functions in some species that include secretion of specialized fluid that may act to glue appendages and the cephalic parts of natural enemies, and secreting alarm pheromone that induces behavioral responses in conspecifics (Picket and Griffiths 1980).

Aphids produce winged (alate) and wingless (apterous) adult morphs. Apterous adults are an anomaly among insects, but apterous aphids are common in rapidly increasing populations when habitat and host quality are optimal. Alates are produced when poor host conditions begin (e.g., crowding, poor host quality) and individuals need to disperse to seek a new host plant. Some aphid species show high polymorphism due to an assortment of factors (e.g. temperature, host quality, etc.). *Aphis gossypii*, is well known to show phenotypic plasticity in size and color to an unusual degree compared to other insects and aphids. The large morphs are dark green/black in color, whereas the "yellow dwarfs", as commonly termed in the literature (Wool et al. 1995, Watt and Hales 1996), grow to be about ¼ of the typical adult size. Yellow dwarfs never attain the usual dark green/black coloration found in the large morphs and remain a pale yellow throughout their lives (Kring 1959, Wool et al. 1995, Watt and Hales 1996). These morphs are thought to represent a distinct, developmentally programmed morph that results from suboptimal conditions (Wool et al. 1995, Watt and Hales 1996). Previous observations have often attributed development of yellow morphs to higher temperatures (Setokuchi 1981, Gu et al. 2013), however, when aphids collected from the field were placed on cotton in good or bad condition, at various temperatures, regular morphs, and yellow dwarfs were produced, respectively (Wool et al. 1995, Watt and Hales 1996). Similar to alate production, *A. gossypii* on crowded or poor-quality hosts will also produce yellow dwarfs and will continue to do so as long as the host is kept in such conditions. The suggested adaptive value behind dwarf production is that by being small, having a low rate of population increase and developmental rate, a

population of yellow dwarfs inflicts less nutrient drain and conserves the host resource (Watt and Hales 1996).

1.3 *Aphis gossypii*

Aphis gossypii will produce either viviparous alate or apterous females via a form of parthenogenesis known as thelytoky (their most frequent mode of reproduction), but can also exhibit alternating reproductive modes, and undergo periods of sexual reproduction. Offspring produced via sexual reproduction will begin as an egg, which will then undergo four nymphal immature stages, followed by the adult stage. Clones reproduced asexually will follow the same life cycle, except they will be born as a first instar from their mother; aphids are one of the few insects that give live birth The rate at which *A. gossypii* populations reproduce is highly variable and dependent on a suite of factors including host plant, temperature, and other environmental conditions. Degree days can be used to measure the growth and development of plants and insects during the growing season. On cotton, the optimal temperature range for population growth is reported between 25-30°C , e.g., average reproduction rate: 51.5 individuals, mean generation time: 10.4 days (Kersting et al. 1999).

Aphids undergo two major types of life cycles based on reproductive mode and how host plants are utilized: non-host alternating (monoecious or autoecious) and host alternating (heterorecious). *Aphis gossypii* have a worldwide distribution due in part to its broad host range (over 92 plant families) (Ebert and Cartwright 1997). However, research has indicated that there are many different biotypes of this aphid that exhibit preference for specific plants within the organism's reported host range (Guldemond et al. 1994). In warmer environments *A. gossypii* exhibits an anholocyclic (asexual reproduction) life cycle, while in cooler areas they may exhibit a holocyclic (asexual and sexual reproduction) life cycle (Slosser et al. 1989, Zhang and Zhong

1990). Aphids with holocyclic lifecycles produce overwintering eggs on the primary host in the fall to survive the below freezing temperatures during the winter. *Aphis gossypii* have multiple hosts they may utilize as primary hosts during the winter in the U.S., including *Hibiscus syriacus* L. and *Catalpa bignonioides* Walter (Kring 1959). Common crops that are fed on during the remaining seasons of the year in the southeast include, but are not limited to: cotton, many species of Cucurbitaceae (e.g., melon, pumpkin, *Cucurbita pepo* L., cucumber, *Cucumis sativus* L.), and various vegetables (e.g., pepper, okra, *Abelmoschus esculentus* L., eggplant, *Solanum melongena* L.) (Ebert and Cartwright 1997).

1.4 Economic Importance of *Aphis gossypii*

Aphis gossypii is an economically important pest of over two dozen crops worldwide. Populations may cause direct feeding damage, but the greatest impact of this species is as a plant virus vector because *A. gossypii* is reported to transmit over thirty viruses to several crops worldwide (Ebert and Cartwright 1997). There are eight species of aphids reported to feed on cotton in the southeastern US (Stoetzel et al. 1996). Of these, *A. gossypii* is the only one that colonizes cotton and is reported to cause economic damage. Nymphs and adults may be found on the underside of cotton leaves as well as the tips and shoots of growing points. Cotton is most susceptible to aphid feeding damage at the seedling stage. Heavy populations on seedlings can cause curling and cupping of leaves (which in turn may hinder photosynthesis), defoliation, and severe stunting of growth (Abney et al. 2008). Although direct feeding does not damage mature cotton, indirect damage caused by honeydew produced from large populations can contaminate exposed lint, which is termed “sticky cotton”. Honeydew may also act as a nutrient source for fungi that can further decrease photosynthesis and lint quality (Stoetzel et al. 1996, Ebert and Cartwright 1997, Hequet et al. 2007, Abney et al. 2008). There are two economically important

viruses that *A. gossypii* transmits to cotton, *Cotton bunchy top virus* (Genus: *Polerovirus*, Family: Luteoviridae), reported in Australia, and CLRDV found in Africa, India, and South America (Cauquil and Vaissayre 1971, Reddy and Kumar 2004, Correa et al. 2005, Michelotto et al. 2007, Mukherjee et al. 2012), and recently confirmed in plant samples collected from the US from 2017-2019 (Avelar et al. 2019). Prior to the discovery of CLRDV in the U.S., cotton viruses had not been reported in the southeastern region of the cotton belt. There is currently no information available about management of CLRDV, and major knowledge gaps in epidemiology remain. Understanding the timing of virus spread to the crop, and the significance of transmission events on final virus incidence is needed to reduce the number of unnecessary insecticide sprays for *A. gossypii*.

1.5 Insecticide Resistance

In the 1960s and 1970s worldwide efforts were made to change from a unilateral dependence on chemical insecticides to a more systematic approach that takes into account ecological relationships and the implementation of multiple, strategic pest-control tactics (i.e., integrated pest management - IPM). Even though IPM has made substantial breakthroughs over the years, alternatives to pesticides are not always available for pest population suppression (Luttrel 1994). A component of IPM is insecticide resistance management (IRM), which has the goal of delaying the evolution of resistance to insecticides. Effective insecticides are a necessary component of IPM in many cropping systems, and costly to replace if efficacy is lost because insect populations become resistant to them. Insects developing resistance to insecticides is a major issue, because the production of new alternative insecticides can’t keep up with the resistant affective strains that are being found and in turn costs billions of dollars in crop damage a year (Bottrell 1979, Gould et al. 2018).Thus, an effective IRM strategy in place will result in

increased longevity of the product (Phillips et al. 1989).

Genetically acquired resistance can be defined as a genetically conferred trait that results in reduced sensitivity toward abiotic or biotic agents to which an earlier generation was susceptible (Bottrell 1979). When management tools and tactics are used to manage populations of organisms there will likely be a number of individuals that survive because some will avoid exposure and others will have a random genetic mutation that confers resistance to the selected agent. In response to selection over time, the frequency at which resistance alleles occurs will increase (Bottrell 1979, Gould et al. 2018). The buildup of resistant individuals in a population depends on genetic, biological, and ecological characteristics of the population including the initial resistance allele frequency, dominance, number of genes involved, cross-resistance, population size, gene-flow/dispersal ability, generation time, habitat preference, and range. The selection pressure also influences the evolution of resistance and includes the proportion of the population's range receiving an insecticide treatment, dose, spatial and temporal intensity of insecticide use (Bottrell 1979, Taylor and Georghiou 1982).The selection of resistance against widely used control agents e.g., herbicides, insecticides, and antibiotics, are commonly observed in pest populations (Onstad et al. 2002, Jacobson et al. 2009, Head and Greenplate 2012, Gore et al. 2013, Gao et al. 2014, Huseth et al. 2016, 2017, 2018). While much of the focus of resistance management is on chemical control, evolution of resistance against other widespread control tactics (e.g., crop rotation, hand weeding, and biological control) is also a challenge.

It is critical to understand the ecology and life history of insect pests, when trying to design and implement effective IPM and IRM strategies. Some factors to include are host utilization of crop and non-crop plants as well as their distribution, generation timespan, number of generations per growing season, mating behaviors, reproduction, and genetics of resistance.

Additional components of the management system that need to be understood include history of insecticide resistance, current and previous management strategies used, and economic thresholds (Head and Greenplate 2012). An understanding of this information will aid in integrating the appropriate technical applications (e.g. planting dates, crop rotation, insecticide rotation, type of control application, timing of application, etc.). Integrating several management techniques, instead of relying on just one, aids in combating resistance, not only to insecticides, but other forms of pest control as well (Sparks and Nauen 2015). Management of *A. gossypii* has typically relied on insecticides, and as a result they have evolved resistance to a wide range of insecticide classes (Gong et al. 1964, Furk and Vedjhi 1990, O'Brien and Graves 1992, O'Brien et al. 1992, Grafton-Cardwell et al. 1992, Ahmad and Iqbal Arif 2008, Shi et al. 2011, Gore et al. 2013, Koo et al. 2014, Bass et al. 2015, Hirata et al. 2015, Kim et al. 2015).

1.6 Conclusion

Two emerging problems related to *A. gossypii* in Alabama are resistance to insecticides used for population management, and transmission of CLRDV. Although insecticide resistance in *A. gossypii* has been reported in other areas of the U.S. it has not yet been documented in the southeast. The second chapter of this thesis quantifies the susceptibility of *A. gossypii* to imidacloprid across the state of Alabama to identify whether the reduced efficacy observed by stakeholders is due to the evolution of resistance in aphid populations. The emergence of CLRDV in the southeast is so recent that little is known about its epidemiology and management. The third chapter investigates the efficacy of cultural and chemical management tactics targeting *A. gossypii* on reducing final CLRDV incidence, and reports aphid population dynamics in relation to timing of CLRDV spread in these trials.

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Chapter 2

Susceptibility of *Aphis gossypii* (Hemiptera: Aphididae) to imidacloprid in Alabama

2.1 Introduction

The cotton aphid (melon aphid), *Aphis gossypii* Glover, is a highly polyphagous pest with a long history of insecticide resistance in productions systems where chemical control methods are heavily used. This pest causes direct damage to seedling cotton *Gossypium hirsutum* L. by feeding, which can stunt plant growth if heavy populations are present (Hequet et al. 2007). Indirect damage is also caused by honeydew excreted from large populations, which can contaminate exposed lint, and act as a nutrient source for sooty mold that can decrease photosynthesis and lint quality (Hequet et al. 2007).

In the southeast, insecticide applications are not recommended in normal years because naturally occurring fungal entomopathogen *Neozygites fresenii* (Nowakowski) Batko is usually effective in controlling *A. gossypii* before honeydew contamination is a problem. Other natural enemies such as *Pandora neoaphidis* Remaudiere and Hennebert (Humber), coccinelids and parasitic wasps may also assist with population management (Weathersbee and Hardee 1994, Marti and Olson 2006, 2007, Abney et al. 2008). In years where natural epizootics are delayed, or in areas where management recommendations are not followed, insecticides are applied to manage this pest.

Historically, organophosphate insecticides were used to manage *A. gossypii*, however resistance has been documented against this insecticide class (Furk and Vedjhi 1990, Grafton-Cardwell et al. 1992, Ahmad and Iqbal Arif 2008). Negative effects of organophosphates, carbamates, and pyrethroids are that they reduce beneficial arthropod populations and may trigger secondary pest problems. Pyrethroids have also been reported to increase *A. gossypii* fecundity (Slosser et al. 2001). Due to these concerns, neonicotinoids largely replaced these products in the 1990s because of their efficacy, they are less harmful to natural enemies, and are more affordable than many other chemical products (Tomizawa and Casida 2003). Imidacloprid became the first compound available in 1991, and has been widely used in Alabama for aphid management. In the ten years that followed, six more commercially marketed neonicotinoids were launched: nitenpyram, acetamiprid, thiamethoxam, thiacloprid, clothianidin, and dinotefuran (Tomizawa and Casida 2003, Elbert et al. 2008). Acetamiprid and thiamethoxam became available for aphid management in 1995 and 1999, respectively, and at least four of these active ingredients are used for management of other pests of cotton.

In Alabama, consultants have reported reduced efficacy of imidacloprid (Jacobson, personal communication). These reports, combined with the historic ability of *A. gossypii* to rapidly develop resistance to insecticides (Gong et al. 1964, Furk and Vedjhi 1990, O'Brien and Graves 1992, O'Brien et al. 1992, Grafton-Cardwell et al. 1992, Ahmad and Iqbal Arif 2008, Shi et al. 2011, Gore et al. 2013, Koo et al. 2014, Bass et al. 2015, Hirata et al. 2015, Kim et al. 2015) are the impetus for this research. The objective of this study was to quantify imidacloprid susceptibility of *A. gossypii* in Alabama by calculating LC_{50}s and resistance ratios (RR) for a susceptible and field collected populations of *A. gossypii*.

2.2 Material and Methods

Data collection was conducted in June and July of 2019. Samples were collected from 24 cotton fields across Alabama. GPS coordinates of the collection locations were retrieved from Google Earth. *Aphis gossypii* populations were collected from cotton fields that had not received an insecticide spray targeting aphids. One infested leaf from multiple plants across a field were collected to obtain a representative sample of the aphid population present at each location. Infested leaves were placed in a gallon sized plastic bag lined with a paper towel and transported back to the laboratory. Once in the laboratory, aphids remained in the plastic bags at room temperature, approximately 26 ± 2°C, for up to 48 h until bioassays were performed. A susceptible population of *A. gossypii* was obtained from Corteva (Indianapolis, IN) to serve as a reference population in the LC_{50} trials. This colony was originally collected from squash *Cucurbita* spp. grown in a greenhouse in 2019 and has since been raised in the laboratory on squash and cotton without any exposure to insecticides.

Bioassays were performed to test the susceptibility of the neonicotinoid insecticide imidacloprid (Admire Pro, Bayer CropScience, Research Triangle Park, NC) in 2019. Bioassays were conducted following the protocol established by IRAC (IRAC No. 019). Bioassay containers consisted of 26 (bottom diameter) by 42 (height) by 40 (top diameter) mm plastic cups filled with 5 ml of 1% plant agar solution (Research Products International, Mount Prospect, IL). Containers were modified to allow for ventilation by removing 18 mm diameter holes from bioassay lids using a soldering iron and covering them with 100% polyester ultra-fine mesh screen (Skeeta, Bradenton, FL) using hot glue. Serial dilutions of formulated product were performed to create a standard range of 7-8 concentrations of the active ingredient, and water was used as a control. The range of imidacloprid doses were determined in preliminary experiments and were designed to facilitate high throughput testing of field collected

populations. Formulated product was diluted in water to acquire 500 ml solutions. Within each solution, a non-ionic surfactant (ProSolutions 80:20, ProSolutions LLC, Springfield, TN) was added at a 0.5% rate to evenly spread the chemical across the surface of the leaf disc.

Fully expanded cotton leaves were removed from non-treated, insect-free DP1646 (DeltaPine®, Dekalb Genetics Corporation, Dekalb, IL) plants during the 1-8 true-leave stage. A steel metal cork borer was used to cut 25.65 mm diameter leaf discs from each leaf. Leaf disc desiccation was minimized by temporarily storing cut discs in a plastic cup lined with a moist paper towel until they were submerged into solutions. Each dose of insecticide was applied to groups of leaf discs by submerging them into one of the insecticide solutions for ten seconds, and were then air-dried abaxial side up. Once leaf discs were completely dry they were placed abaxial side up into an individual bioassay container with plant agar; discs were then gently pushed into the agar with a piece of soft foam to minimize desiccation. Ten late instar aphids were transferred individually to each leaf disc using a paint brush and confined using the ventilated lid. A total of five leaf discs were tested for each concentration, and this design was replicated two times for bioassays of each field-collected population and four times for the susceptible colony. Bioassay cups were held in an environmentally controlled room at 26 ± 2°C, with a photoperiod of 14:10 (L:D) h. An assessment of mortality (dead, moribund, and live aphids) was made after 48 and 72 h of exposure to the leaf disc treatment. A paintbrush was used to gently prod the aphid to aid in confirming mortality status. When prodded with a paintbrush, dead aphids showed no movement, live aphids showed coordinated movement, and moribund aphids showed uncoordinated movement. Moribund aphids were classified as dead for final analysis. Although not common, aphids that were found dead due to fungal entomopathogens or parasitized by braconids were excluded from final analysis.

Data were log transformed and analyzed using Probit analysis (PROC PROBIT, version 9.4, SAS Institute, Cary, NC) in SAS. LC_{50} values and 95% fiducial limits (F.L.) were reported as untransformed values. The ratio of the LC_{50} values from field tested colonies compared to the susceptible colony were calculated as resistance ratios (RRs). Differences between field and susceptible LC_{50}s were considered significantly different if the 95% C.L. for the given RR did not include 1.0 (Robertson et al. 2007). Pearson X^2 values were used to test the null hypothesis that the model is a good fit to the data; values that were not significant ($P > 0.05$) indicated the data was a good fit to the model.

2.3 Results

Bioassays were performed on 26 populations (Table 2.1), one of which was from a natural infestation that was collected and reared from the Auburn, AL laboratory greenhouse in 2018. Populations with control mortality ≥ 10% were excluded from final analysis, as a result, 23 populations are presented (Robertson et al. 2007). The LC_{50}s at 48 h for field collected populations ranged from 3.91 – 74.98 ppm, and the RRs ranged from 11.33 – 217.09 (Table 2.1). All field collected populations had significantly higher LC_{50}s than the susceptible population. The average LC_{50} and RR for field collected populations was 28.03 ppm and 69.71 ppm, respectively, meaning that on average field collected populations were 69.71 times more resistant to imidacloprid than the susceptible colony. The X^2 values were not significant in 20 populations (Table 2.1).

Each population's LC_{50} decreased from the 48 h trial to the 72 h trial. The LC_{50}s at 72 h for field collected colonies ranged from 0.31 – 19.99 ppm (Table 2.2). RRs of field collected colonies ranged from 4.26 – 277.64 ppm. Although all LC_{50}s declined from the 48 h analysis to the 72 h analysis, some colony's RRs increased. All but three field collected populations had

significantly higher LC_{50}s than the susceptible population. The average LC_{50} and RR for field collected colonies at 72 h was 7.36 ppm and 81.16 ppm, respectively, which showed that on average field collected colonies were 81.16 times more resistant to imidacloprid than the susceptible colony. The X^2 values were not significant in 21 populations (Table 2.2).

2.4 Discussion

The results from this study confirmed imidacloprid resistance in *A. gossypii* populations across Alabama cotton production regions. LC_{50}s for all populations during the 48 h analysis, and all but three (Prattville, Atmore, and North Eufaula, AL) for the 72 h analysis were significantly higher than the susceptible colony (i.e., RR's CLs that did not contain 1) (Robertson et al. 2007). This study documents LC_{50}s as high as 74.98 and 19.99 ppm, and RR as high as 217.09 and 277.64-fold at 48 and 72 h, respectively. *Aphis gossypii* resistance to imidacloprid has been reported in Korea, China, and Japan (Shi et al. 2011, Koo et al. 2014, Hirata et al. 2015, Kim et al. 2015), and resistance to thiamethoxam has been reported in the U.S, (Gore et al. 2013). Studies of field evolved resistance to thiamethoxam in the mid-south documented LC_{50}s as high as 1,234 and 122.42 ppm, and RR as high as 562.6 and 29.1-fold at 48 and 72 h, respectively (Gore et al. 2013). Gore et al. (2013) collected populations from fields that were treated with a foliar application of a neonicotinoid insecticide as well as non-treated fields, and found that treated fields had a significant effect on decreasing cotton aphid susceptibility to thiamethoxam at 48 and 72 h.

The number of studies documenting insecticide resistance in *A. gossypii* show the propensity of this pest to evolve resistance to widely used chemical control measures. Resistance to a wide range of insecticides, including organophosphates, organochlorines, pyrethroids, carbamates, flonicamid, phenylpyrazoles and neonicotinoids has been documented (Gong et al.

1964, Furk and Vedjhi 1990, O'Brien and Graves 1992, O'Brien et al. 1992, Grafton-Cardwell et al. 1992, Ahmad and Iqbal Arif 2008, Shi et al. 2011, Gore et al. 2013, Koo et al. 2014, Bass et al. 2015, Hirata et al. 2015, Kim et al. 2015). Biological factors that accelerate evolution of resistance in *A. gossypii* include: high fecundity, short generation time, and reproductive modes. Neonicotinoid insecticides target insect nicotinic acetylcholine receptors, and mechanisms of resistance have been attributed to an R81T mutation in loop D of the beta 1 subunit in resistant clones of *A. gossypii* (Koo et al. 2014, Bass et al. 2015, Hirata et al. 2015, Kim et al. 2015). Kim et al. (2015) found that L80S was an additional point mutation in the beta 1 subunit in imidacloprid resistant individuals. A study in China showed imidaclorpid resistant *A. gossypii* populations exhibited cross-resistance to some neonicotinoids (i.e., acetamiprid, thiacloprid, nitenpyram), but not others (i.e. dinotefuran, thiamethoxam, and clothianidin) (Shi et al. 2011). In South Korea, an imidacloprid resistant *A. gossypii* strain exhibited cross resistance to all tested neonicotinoids (acetamiprid, clothianidin, dinotefuran, thiacloprid, and thiamethoxam) (Koo et al. 2013). Cross resistance among different neonicotinoid active ingredients has not been evaluated in the US.

The results of this study indicate variable but overall high levels of imidacloprid resistance among *A. gossypii* populations across different cotton production regions of Alabama. Currently, only three other active ingredients are available for management of *A. gossypii* (i.e., acetamiprid, thiamethoxam, flonicamid), and two of them are neonicotinoids. Due to the propensity of *A. gossypii* populations to evolve resistance to widely used insecticides, and the potential for cross-resistance to occur among other neonicotinoids (Shi et al. 2011; Koo et al. 2013), chemical tools should only be used to manage this pest when natural management by annual epizootics fail. Although additional insecticide exposure may also occur as a result of

actions made to manage other pests (e.g., tarnished plant bugs, *Lygus lineolaris* Palisot de Beauvois) (Snodgrass and Scott 2000). Avoiding applications of these active ingredients to manage other insect pests of cotton when *A. gossypii* is present should also be a consideration of season-long IPM programs. Additional knowledge about *A. gossypii* ecology and selection pressure across southeastern agroecosystems will help inform development of sound IPM and IRM strategies for this pest.

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Table 2.1. Leaf-dip bioassays with imidacloprid (Admire Pro) against *A. gossypii*, 48 h after treatment

Population	**Number of Aphids**	**Ln Slope (SE)**[a]	**LC_{50} ppm (FL)**[b]	**X^2** (*P*-value)	**RR**[c] **(CL)**[d]
Prattville, AL	787	1.46 (0.17)	3.91 (2.21-6.32)	102.01 (0.01)	11.33 (2.43-52.85)*
West Auburn, AL	791	1.38 (0.17)	5.09 (2.72-8.22)	57.40 (0.82)	14.74 (3.69-58.88)*
Piedmont, AL	689	1.69 (0.20)	6.33 (3.99-9.23)	53.19 (0.91)	18.35 (5.28-63.76)*
Fairhope, AL	787	1.26 (0.12)	7.43 (4.65-11.32)	71.01 (0.38)	21.51 (7.15-64.69)*
North Eufaula, AL	797	1.44 (0.20)	9.10 (5.02-14.05)	41.96 (0.99)	26.33 (8.20-84.53)*
Brown, AL	708	1.41 (0.14)	11.71 (7.60-17.41)	56.31 (0.84)	33.89 (11.92-96.41)*
Atmore, AL	785	0.91 (0.10)	12.99 (6.56-23.66)	81.18 (0.12)	37.60 (13.38-105.67)*
Laurel Hill, FL	786	1.24 (0.17)	14.77 (7.44-25.68)	94.45 (0.02)	42.77 (14.37-127.31)*
Brewton, AL	781	2.24 (0.26)	15.72 (11.21-21.93)	85.79 (0.07)	45.51 (15.41-134.35)*
Shorter, AL	792	1.11 (0.14)	16.00 (8.52-26.69)	63.52 (0.63)	46.33 (16.48-130.23)*
Brundidge, AL	783	1.27 (0.12)	17.01 (10.44-26.22)	51.16 (0.99)	49.24 (17.92-135.28)*
Headland, AL	779	1.43 (0.17)	17.43 (10.77-26.43)	61.40 (0.67)	50.47 (18.06-141.03*
East Auburn, AL	784	1.02 (0.10)	17.70 (10.26-29.57)	61.28 (0.71)	51.24 (19.02-138.08)*
Tyler, AL	766	1.48 (0.17)	29.51 (18.53-44.77)	51.76 (0.93)	85.44 (31.61-230.95)*
Cedar Bluff, AL	601	1.10 (0.17)	40.41 (19.49-73.85)	41.49 (1)	116.98 (43.21-316.67)*
Alexis, AL	616	1.28 (0.18)	43.58 (23.38-74.61)	38.03 (1)	126.17 (46.61-341.51)*
Marion Junction, AL	700	1.17 (0.13)	45.00 (27.13-73.95)	59.25 (0.77)	130.28 (49.92-340.01)*
South Eufaula, AL	790	1.29 (0.15)	46.25 (28.12-73.46)	53.35 (0.9)	133.91 (51.00-351.59)*
Andalusia, AL	664	0.97 (0.12)	56.42 (29.05-100.41)	70.58 (0.68)	163.34 (62.88-424.34)*
Black, AL	815	0.86 (0.09)	60.63 (31.77-110.04)	64.16 (0.87)	175.53 (68.52-449.67)*
Excel, AL	791	1.09 (0.12)	64.77 (38.91-108.27)	57.40 (0.96)	187.54 (73.02-481.64)*
Tallassee, AL	775	1.53 (0.21)	74.98 (44.50-125.55)	96.51 (0.01)	217.09 (81.38-579.10)*
Susceptible	1592	0.78 (0.05)	0.35 (0.20-0.56)	132.85 (0.61)	-

[a]The slope of the dose-response regression line and standard error.

[b]The concentration of imidacloprid that kills 50% of the population with 95% fiducial limits.

[c]LC_{50} Resistance ratios of the of field-collected population: the susceptible population.

[d]Resistance ratio's 95% confidence limits. RRs marked with an asterisk differed significantly from the susceptible population. Significance was determined using methods from Robertson et al. (2007). RRs were not significant if their CLs contained 1.

Table 2.2. Leaf-dip bioassays with imidacloprid (Admire Pro) against *A. gossypii*, 72 h after treatment

Population	Number of Aphids	Ln Slope (SE)[a]	LC_{50} ppm (FL)[b]	X^2 (*P*-value)	RR[c] (CL)[d]
West Auburn, AL	791	0.98 (0.09)	0.31 (0.14-0.57)	66.97 (0.51)	4.26 (1.10-16.87)*
Prattville, AL	787	1.12 (0.13)	0.81 (0.35-1.55)	95.92 (0.01)	11.15 (0.00-63025.38)
Atmore, AL	785	0.92 (0.11)	1.51 (0.56-3.14)	66.41 (0.53)	20.93 (0.27-1597.58)
East Auburn, AL	784	1.23 (0.11)	1.89 (1.15-2.94)	79.96 (0.15)	26.20 (3.07-223.44)*
North Eufaula, AL	797	1.23 (0.17)	1.90 (0.74-3.52)	43.32 (1)	26.30 (0.94-739.16)
Fairhope, AL	787	1.76 (0.23)	2.92 (1.65-4.41)	82.88 (0.11)	40.47 (6.31-259.50)*
Piedmont, AL	689	1.97 (0.29)	4.32 (2.46-6.39)	60.39 (0.73)	59.87 (13.49-265.75)*
Brown, AL	708	1.55 (0.17)	4.34 (2.66-6.46)	56.78 (0.83)	60.11 (18.94-190.75)*
Laurel Hill, FL	786	1.50 (0.30)	4.74 (1.46-8.79)	87.71 (0.05)	65.62 (10.85-396.67)*
Shorter, AL	792	1.56 (0.26)	6.60 (3.04-10.73)	60.75 (0.72)	91.37 (27.16-307.41)*
Marion Junction, AL	700	1.49 (0.24)	6.67 (3.06-11.08)	59.57 (0.76)	92.42 (28.10-303.95)*
Brewton, AL	781	2.04 (0.28)	7.21 (4.60-10.20)	80.32 (0.15)	99.88 (35.80-278.64)*
Andalusia, AL	664	0.88 (0.10)	7.31 (2.93-14.89)	77.21 (0.47)	101.18 (39.71-257.75)*
Headland, AL	779	1.22 (0.28)	7.45 (1.48-16.27)	112.49 (0)	103.22 (23.80-447.79)*
South Eufaula, AL	790	1.58 (0.24)	8.04 (4.09-12.76)	44.09 (0.99)	111.39 (39.06-317.68)*
Brundidge, AL	783	1.55 (0.19)	8.55 (4.69-13.48)	50.90 (0.99)	118.41 (44.92-312.08)*
Cedar Bluff, AL	601	1.17 (0.18)	9.77 (4.07-18.35)	44.00 (0.99)	135.32 (51.36-356.57)*
Tyler, AL	766	1.80 (0.28)	9.88 (5.39-15.47)	86.78 (0.06)	136.80 (50.13-373.35)*
Alex, AL	616	1.22 (0.22)	10.79 (3.73-20.89)	44.41 (0.99)	149.43 (52.22-427.55)*
Tallassee, AL	775	1.36 (0.19)	17.07 (9.08-29.00)	94.92 (0.05)	236.34 (110.59-505.09)*
Excel, AL	791	1.13 (0.14)	19.87 (10.61-33.32)	66.97 (0.98)	275.14 (141.54-534.82)*
Black, AL	815	0.99 (0.12)	19.99 (8.97-37.35)	53.40 (0.99)	277.64 (138.16-554.88)*
Susceptible	1592	1.03 (0.70)	0.07 (0.04-0.11)	121.47 (0.82)	–

[a]The slope of the dose-response regression line and standard error.

[b]The concentration of imidacloprid that kills 50% of the population with 95% fiducial limits.

[c]Resistance ratios of the LC50 of field-collected population: the susceptible population.

[d]Resistance ratio's 95% confidence limits. RRs marked with an asterisk differed significantly from the susceptible population. Significance was determined using methods from Robertson et al. (2007). RRs were not significant if their CLs contained 1.

Chapter 3

Planting Date and *Aphis gossypii* Management on Final Incidence of *Cotton leafroll dwarf virus*

3.1 Introduction

The cotton aphid, *Aphis gossypii* Glover, has been reported to transmit over thirty viruses to crops worldwide (Ebert and Cartwright 1997), and is the primary vector responsible for transmitting *Cotton leafroll dwarf virus* (CLRDV, Genus: *Polerovirus*, Family: Luteoviridae) to cotton, *Gossypium hirsutum* L (Cauquil J and Vaissayre M 1971, Michelotto and Busoli 2003, 2007, Mukherjee et al. 2016, McLaughlin et al. 2020, Heilsnis et al. 2020). This virus has been reported from Africa, Asia, and South America with reported losses up to 1500 kg · ha^{-1} in South America (Corrêa et al. 2005, Silva et al. 2008, Distéfano et al. 2010). CLRDV is an emerging cotton virus in the U.S. and is the first virus reported to infect cotton and reduce yield in the southeast (Avelar et al. 2019). CLRDV was first reported from Alabama in 2017, and is currently distributed across North Carolina (Thiessen et al. 2020), South Carolina (Wang et al. 2020), Georgia (Tabassum et al. 2019), Mississippi (Aboughanem-Sabanadzovic et al. 2019), Louisiana (Price et al. 2020), Texas (Alabi et al. 2020), and Kansas (Ali and Mokhtari 2020). In the U.S., symptoms are highly variable among locations and include stunting, leaf distortions, drooping of leaves, petiole and vein reddening, abnormal top growth accompanied by shortened internodes, yellowing of leaves, and shedding of squares and bolls. Virus incidence ranges from 2-100% (Aboughanem-Sabanadzovic et al. 2019, Avelar et al. 2019, Tabassum et al. 2019, Alabi

et al. 2020, Ali and Mokhtari 2020, Brown et al. 2020), and it is unknown whether variation in disease among locations is due to varietal, environmental, crop age, or insect vector related factors. Management of CLRDV in Brazil, and closely related poleroviruses in Australia, is conducted using resistant varieties, and some studies report aphid management as a component of disease management (Fang et al. 2010, Reddall et al. 2004, Cascardo et al. 2015, Ellis et al. 2016, Galbieri et al. 2017). CLRDV has been detected in all commercially available cotton varieties in the U.S., and management tactics for reducing CLRDV have not been investigated in the U.S.

Using aphid management as a component of CLRDV disease management would increase the season-long cost of cotton pest management by requiring additional insecticide applications for both aphids and secondary pests that are commonly flared when insecticide use increases. *Aphis gossypii* is an annual pest of cotton in the southeast and mid-south regions of the U.S. cotton belt, but direct injury to cotton occurs only when high *A. gossypii* populations feed on young or stressed plants (Marti and Olson 2006, 2007, Abney et al. 2008). Indirect damage caused by honeydew contamination on exposed lint is the primary concern due to the potential reduction in lint quality and cotton productivity (Hequet et al. 2007). Current management recommendations suggest avoiding insecticide sprays to manage *A. gossypii* populations because yield reductions caused by *A. gossypii* feeding are not generally observed (Layton et al. 1999, Johnson et al. 2002). Under normal conditions *A. gossypii* populations in the southeast and mid-south are naturally reduced in July by annual fungal epizootics caused by the entomopathogen *Neozygites fresenii* Nowakowski (Batko) before bolls open (Pena 1993, Weathersbee and Hardee 1994, Marti and Olson 2006, 2007, Abney et al. 2008). Unnecessary insecticide applications should also be avoided to reduce selection for insecticide resistance, which has been reported for

insecticides used to manage this species in cotton (Gore et al. 2013).

Cultural and chemical practices reduce virus incidence in other pathosystems in the southeast, but their efficacy is impacted by seasonal dynamics of reservoir hosts and vectors in the cropping landscape, and how quickly the vector can acquire and transmit the plant virus. Adjusting the planting date may decrease virus incidence when younger crops are temporally isolated from vectors because older plants are generally less susceptible to virus infection (Beaudoin et al. 2009, McMechan and Hein 2016, Kone et al. 2017, Srinivasan et al. 2017). Timing insecticide sprays to reduce primary spread of viruses, infection that occurs as a result of vectors moving into the crop from the surrounding landscape, reduces incidence of some persistently transmitted viruses by increasing plant health or reducing feeding behaviors that are responsible for virus transmission (Pappu et al. 2000, Groves et al. 2001, Jacobson and Kennedy 2011, 2013, Chappell and Kennedy 2018, Li et al. 2019). Managing the size of colonizing vector populations with insecticides has been shown to reduce secondary spread of the virus that occurs as populations of vectors within the crop grow and spread throughout a field (Swenson 1968, Momol et al. 2004, Reitz and Funderburk 2012). Studies from Brazil investigating the impact of aphid management for reducing the incidence of CLRDV in cotton reported a 200-300 kg/ha increase in cotton yield, and a 1.5 - 2 point decrease in disease severity rating (1 - 5 scale), on susceptible cotton varieties when insecticides were applied at a threshold of 5, 20, 40, or 60% of plants infested with colonies of 5-10 aphids (Galbieri et al. 2017). Insecticides have only been reported to reduce primary spread if they have antifeedant properties and it takes longer periods of feeding for virus transmission to occur (Pappu et al. 2000, Groves et al. 2001, Jacobson and Kennedy 2011, 2013, Chappell and Kennedy 2018, Li et al. 2019). CLRDV is reported to be transmitted by alate aphids in less than one minute of feeding (Michelotto and Busoli 2007),

which suggests insecticide applications reduce secondary spread of CLRDV caused by colonizing populations.

The efficacy of vector population management for reducing virus incidence also depends upon the magnitude of primary spread into a crop and the relative contribution of primary versus secondary spread to final virus incidence. Initial analyses of virus incidence in AL and GA have shown that up to 80-100% of plants test positive for CLRDV using PCR-based diagnostic methods (Brown et al. 2020). This high incidence suggests that the majority of plants in a field become infested with aphids during the growing season. Previous studies examining *A. gossypii* populations in the U.S. cotton belt quantified population size, but not the proportion of plants infested, and knowledge of the timing and magnitude of season-long aphid dispersal into cotton is limited. The high incidence of CLRDV could also be caused by the presence of multiple vectors. The primary vector in Africa, India and South America is *A. gossypii* (Cauquil J and Vaissayre M 1971, Michelotto and Busoli 2003, 2007, Mukherjee et al. 2016), but the cowpea aphid, *Aphis craccivora* Koch, and the green peach aphid, *Myzus persicae* Sulzer, have also been reported to transmit CLRDV in India (Reddy and Lava Kumar 2004, Mukherjee et al. 2016). A study from China reported detecting CLRDV in *Aphis glycines* Matsumura collected from soybean, however, this species has not reported to feed on cotton and vector competence has not been confirmed (Feng et al. 2017). *Aphis gossypii* is the only known vector of CLRDV in the U.S. (McLaughlin et al. 2020, Heilsnis et al. 2020), but seven other aphid species are reported to colonize cotton in the U.S. (Stoetzel et al. 1996) including: *A. craccivora* (Blackman and Eastop 2000); bean aphid, *Aphis fabae* Scopoli (Blackman and Eastop 2000); potato aphid, *Macrosiphum euphorbiae* (Thomas) (Blackman and Eastop 2000); *M. persicae* (Kennedy et al. 1962, Blackman and Eastop 2000); corn root aphid, *Protaphis middletonii* (Thomas) (Blackman

and Eastop 2000); rice root aphid, *Rhopalosiphum rufiabdominale* (Sasaki) (Blackman and Eastop 2000); and the bean root aphid, *Smynthurodes betae* Westwood (Blackman and Eastop 2000). All of these species or at least one of their junior synonyms (e.g., in *P. middletonii* as *Aphis armoraciae* Cowen Chan et al. 1991 lists five viruses associated with *A. armoraciae*) are known to transmit at least one plant virus. The status of these aphid species as vectors of CLRDV is not referenced in Chan et al. (1991) and is currently unknown in the U.S.

The impetus of this study was to identify short-term strategies for CLRDV management by investigating the efficacy of cultural and chemical aphid management practices to reduce the final incidence of CLRDV in cotton. Another objective was to understand how seasonal dynamics of vector dispersal and timing of virus spread may impact the efficacy of the management strategies evaluated. To investigate this, aphid population dynamics and the timing of virus spread were monitored concurrent with these experiments to identify when the crop is becoming infected with CLRDV, and which aphid species are dispersing through the landscape when virus spread occurs. Two-site years of data are presented from replicated small plot field trials conducted in south AL and south GA where high incidence of CLRDV was observed in the preceding year. Aphid dispersal events responsible for colonization events in cotton were monitored weekly with pan traps and virus spread was monitored using weekly cohorts of healthy sentinel plants placed around the perimeter of the fields. Two planting dates and four aphid management regimes were evaluated for reducing aphid populations and CLRDV in cotton. Results on the proportion of plants infested with aphids, aphid population size, final incidence of CLRDV, yield and lint quality are presented.

3.2 Methods and Materials

3.2.1 Small Plot Experiment

Field trials were performed at the Brewton Agricultural Research Unit in Brewton, Escambia County, Alabama (31.141700, -87.050000) and in Tifton, Tift County, Georgia (31.489738, -83.519721). Each plot was 4-rows wide (0.91 m centers) and approximately 9.14 m long. Plots were separated by a skip row on each side and a 2 m alley on each end to minimize aphid spread between plots via movement across and down rows, respectively. Two replications of this experiment were performed using variety DP1646 (DeltaPine®, Dekalb Genetics Corporation, Dekalb, IL) in Brewton, AL and Tifton, GA. This experiment was conducted using a split plot design with four replications; planting date was the main effect and aphid management regime was the subplot effect. There were two planting dates, May 2 and June 4, 2019 in AL, and May 2 and June 3, 2019 in GA. Four aphid management regimes were evaluated: 1. No insecticide applications; 2. Weekly applications of insecticide beginning at the 1-true-leaf stage; 3. Weekly applications after the first detection of aphid colonies in the plots; 4. One calendar-based application of insecticide the first week of July. Aphids were managed with acetamiprid (Assail 70 WP United Phosphorus, Inc., King of Prussia, PA) at a rate of 175 g a.i. ha^{-1} to reduce the risk of flaring whiteflies late-season. Dates for first spray treatment applications by planting date and location are listed in Table 3.1. All plots were over-sprayed with acetamiprid at the same rate on 16 July 2019 in AL and 12 July 2019 in GA when populations of aphids were observed to decline in control plots, due to fungal epizootics.

Seed planted for the field trial contained an imidacloprid seed treatment (0.375 mg a.i./seed) (Admire Pro, Bayer Crop Science, Research Triangle Park, NC) that was included for thrips management. In both locations two spotted spidermites, *Tetranychus urticase* Koch were managed by spraying all plots with abamectin (Agri-Mek, Syngeta, Pensacola, FL) at a rate of

385 g a.i. ha^{-1} on 6 June 2019 and 13 August 2019 in AL, and 27 July 2019 for GA. Stink bugs were managed by spraying all plots using dicrotophos (Bidren8, AMVAC, Axis, AL) at a rate of 560 g a.i. ha^{-1} on 16 July 2019 in AL, and 12 July 2019 and 31 July 2019 in GA. Weeds, pathogens and fertility were managed based on standard local practices (Whitaker et al. 2019, Alabama Coorperative Extension System 2020).

Stand counts were recorded during the seedling stage in rows two and three of each plot by counting the total number of plants in each row. To determine the proportion of plants infested with aphids plants in the middle two rows of each plot were inspected for the presence or absence of aphids. In AL, these were ten consecutive plants in each of the middle two rows (20 total), whereas in GA, 10 plants were randomly selected from rows two and three. Population size of aphids was monitored by recording the total number of live aphids present in a sample. In AL, counts were performed separately on one upper, middle, and lower fully expanded leaf of each of the ten random plants in the middle two rows, whereas in GA, counts were only performed on the upper leaf. During the early growth stages when fewer than three true leaves were present, counts were only performed on the uppermost expanded leaf. On larger plants, the upper position was standardized as the 4^{th} fully expanded leaf below the terminal at both locations.

Final incidence of CLRDV in each plot was determined using PCR-based methods below. In AL each of the ten consecutive plants in row two that were monitored for aphid presence were sampled 12 August to test for virus infection. In GA, ten randomly sampled plants from rows two and three were sampled 31 July 2019 for CLRDV testing.

Plots were machine harvested from the middle two rows of each plot. Plots were harvested on 25 September for the first planting date and 11 October 2019 for the second

planting date in AL. In GA, plots were harvested on 24 September for the first planting date and 4 November 2019 for the second planting date. In GA, lint from the middle two rows were used to calculate yield for both planting dates, but fiber quality analysis was performed on rows one and four for the early May plant date, and two and three for the early June plant date. In both locations, seedcotton yield for each plot was weighed after harvest, and then plot samples were sent to the University of Georgia Microgin (Tifton, GA) for quality analysis (Toews and Shurley 2009). Classing procedures followed the USDA Official Grades for Cotton procedures using the Uster High Volume Instrument (HVI) system, and differences in length, strength, uniformity, and micronaire were analyzed (United States Department of Agriculture-Agriculture Marketing Service 2001).

3.2.2 Monitoring aphids and CLRDV Spread

Pan traps constructed using 3.79 L (volume), 21 cm (diameter) buckets cut to a 7.5 cm height, and painted yellow with Krylon® "Gloss Sunbeam" yellow spray paint (Sherwin-Williams, Cleveland, OH), were used to monitor aphids following previously described aphid trapping methods (Heathcore et al. 1969, Nielson and Wolfenbarger 1970, Kring 1972). Four yellow pan traps were placed around the perimeter of the small plot trial at each location, with one pan trap located in the middle of each of the four field edges, and surrounded by bare soil season-long to increase alightment by alate aphids (Kennedy et al. 1961, Doring et al. 2004). Each trap was filled with 50% propylene glycol to preserve aphids, and a drop of liquid dish soap to reduce the surface tension of the liquid. Every seven days aphids from each pan trap were collected, stored individually in 70% ethanol, and transported back to the laboratory. In the laboratory, aphids were separated from other insects, and adult alate aphids were counted. Adult

alate aphids were identified to species using existing identification keys (Stoetzel et al. 1996, Stoetzel and Miller 2001), and morphological characters of individuals were examined in ethanol using an Olympus SZX12 microscope with an Olympus DR PLAPO 1X PF objective (Olympus Corporation of the Americas, Center Valley, PA). In this study the eight aphids reported from cotton in the United States were targeted for identification: *A. craccivora*, *A. gossypii*, *A. fabae*, *M. euphorbiae*, *M. persicae*, *R. rufiabdominale*, *S. betae*, and *P. middletonii*. Aphid species other than these eight were counted and listed as "other" due to the difficulty in identifying aphid species from yellow pan traps, with the exception of the rusty plum aphid, *Hysteroneura setariae*, which was one of the dominant species collected in AL, and a first report in AL. Voucher specimens of each species identified in this study were slide-mounted using the protocol of the Systematic Entomology Laboratory – USDA ARS (USDA) and deposited at Auburn University Museum of Natural History (AUMNH).

Sentinel plants were used to monitor the timing of virus spread. Healthy cotton (DP 1646) that did not have a field rate of seed-applied insecticide was planted in 3601 standard plant tray inserts (BWI, Nash, Texas) using ProMix MX General Purpose (Premier Horticulture Inc., Quebec Canada) soil and grown in virus and insect-free incubators. When cotton had reached the 3–4 true-leaf stage individual plants were transplanted to 15.24 cm Blow-Molded Classic Line; C600 (Nursery Supplies Inc., Chambersburg, PA) pots, fertilized with 20–10–20 Peat-Lite Special, Base Formulation, M-77 Chelating Formula (Peter Professional, Summerville, SC), and covered with a 60.5 (height) by 34 (diameter) cm sleeve cage made out of 100 micron thrips-proof screen. Eight individually potted sentinel plants were held for 1–2 days in the greenhouse until they were transported to the field, and four sentinel plants remained in the greenhouse to serve as control plants in future virus testing, and to monitor for unintended virus spread in the

greenhouse. Two plants were placed on each field border (eight total), uncovered so that aphids could freely locate and access them, and were surrounded by bare soil season-long to increase alightment by alate aphids (Kennedy et al. 1961, Doring et al. 2004). Seven days after being placed in the field, the cohort of eight sentinel plants were replaced with a new cohort of healthy 3–4 true leaf plants. After collection, sentinel plants were transported back to Auburn University greenhouses, sprayed with Flupyradifurone (Sivanto™ Prime, Bayer CropScience, Research Triangle Park, NC) at a rate of 980 g a.i. ha^{-1} to remove aphids, and were grown insect-free in a greenhouse for at least 1 month (Galbieri et al. 2010) before being tested for infection with CLRDV. Sentinel plant monitoring was conducted concurrently with aphid trapping at Brewton, AL. Sentinel plant monitoring was not conducted in GA because the current cost of CLRDV diagnostics was cost prohibitive.

3.2.3 PCR confirmation of CLRDV

CLRDV infection was confirmed in samples collected from field plots and sentinel plants using nested RT-PCR. The nested-PCR assay targeting the CLRDV partial coat protein gene is often the best approach for increased sensitivity and reduced non-specific binding. The coat protein gene is encoded on a sub-genomic RNA and is at a higher copy number relative to most of the virus genome making it a good target for a low titer virus such as CLRDV. Two petioles were collected from each plant, one from old growth and one from new growth, and combined into one sample. RNA was extracted from the petiole tissue of each sample using Qiagen RNeasy Plant Mini kits (Qiagen, Germantown, MD) following the manufacturer's recommendations. The cDNA was synthesized using SuperScript IV first-strand synthesis system (ThermoFisher Scientific, Waltham, MA) and amplified with polerovirus-specific PCR

primers Pol3628F/Pol4021R (Table 3.2) targeting a 395 bp genome segment of ORF3-5, partial coat protein gene.

The first round PCR product generated using Pol3628F/Pol4021R primers was diluted (1:10) and amplified with CLRDV-specific primer CLRDV3675F and polerovirus primer Pol3982R (Table 3.2; Sharman et al. 2015) targeting a 310 bp section of the coat protein gene located within the first round PCR target. Both rounds of PCR were set up in 25 µl reaction volumes with 1 unit Platinum *Taq* polymerase (Invitrogen, Carlsbad, CA), 1.75 mM $MgCl_2$, 200 mM dNTPs, 200 nM of each primer and 2 µl of cDNA template. Temperature cycling parameters for both rounds of PCR consisted of an initial denaturation of 95°C for 60 s, then 35 cycles of: 95°C for 15 s, 62°C for 20 s, 56°C for 10 s and 72°C for 40 s; followed by a final denaturation of 72°C for 3 min. Positive controls (plants that had previously tested positive) and negative (plants that had been grown in a controlled environment in the absence of aphids) were included in each run, and PCR products were examined by gel electrophoresis.

3.2.4 Statistical Analyses

Planting date and insecticide treatment were organized in a 2 x 4 factorial arrangement in a split plot design with four replications, for a total of eight treatment combinations. Data were analyzed with generalized linear models using the GLIMMIX procedure in SAS (Version 9.4; SAS Institute, Inc., Cary, NC, USA). All aphid incidence and count data were analyzed separately for each location. Aphid count data was analyzed using a negative binomial distribution. The first analysis compared the total number of aphids on the upper leaf using planting date, aphid management regime, and their interaction term as main effects, and main plot and subplot were used as random effects. Separate analyses were conducted to compare

aphid numbers among the three leaf positions (lower, middle, upper) using planting date, aphid management regime, and their interaction term as main effects in AL; main plot, subplot, and plant were used as random effects. Aphid and CLRDV incidence data were analyzed using a binary distribution with main plot and subplot as random effects. Yield and lint quality data from AL and GA were analyzed together using a Gaussian distribution with location and main plot as random effects. In these analyses if the interaction between the main effects was not significant this term was removed from the final model. When interaction terms were significant between the main effects the SLICE option was used to examine the simple effects.

3.3 Results

3.3.1 Monitoring aphids and CLRDV Spread

3.3.1.1 Seasonal aphid dynamics

A total of 7,296 aphids were captured at both locations, of which 6,434 were one of the eight species reported to infest cotton that were targeted by this study (Table 3.3). *Aphis gossypii* was the most abundant species collected at both locations and accounted for 60% and 86% of the individuals collected at Brewton and Tifton, respectively. *Aphis gossypii* were captured each week of trapping, and a large increase in numbers was observed late-June and early-July at both locations (Table 3.3). At both locations *M. persicae* and *A. craccivora*, were observed in low numbers, with *M. persicae* primarily present in May, while *A. craccivora* was captured throughout the collection period. *Protaphis middletonii* were observed every week at Brewton, and in higher numbers than at Tifton, where this species was captured May–July, but not in August. One or fewer *M. euphorbiae* individuals were collected in each trap at Brewton during

July and August, but were not captured in Tifton. *Rhopalosiphum rufiabdominale* were present in low numbers May–July at Brewton, and only sporadically May–June in Tifton. *Smynthurodes betae* were present in low numbers in May–August at both locations (Table 3.3). Of the eight targeted species, *Aphis fabae* was the only species not collected at either location.

The rusty plum aphid, *Hysteroneura setariae* (Thomas), was the third most abundant aphid species collected in Brewton (Table 3.3) and represents a new state record for Alabama. *Hysteroneura setariae* host alternates between *Prunus* spp. and species of Poaceae such as corn (Blackman and Eastop 2000, Stoetzel and Miller 2001, Nasruddin 2013). It has not been recorded from cotton as its host, and we did not observe species other than *A. gossypii* in cotton plots during this trial. This species is considered a pest of corn, rice, sugarcane, wheat (Blackman and Eastop 2000, Stoetzel and Miller 2001), and soybeans, *Glycine max* L. (Jahn et al. 2005). The rusty plum aphid is known to transmit numerous plant viruses (e.g., Chan et al. 1991, Saleh et al. 1989, Blackman and Eastop 2000, Masumi et al. 2011), but is not currently known to transmit any viruses that infect cotton.

3.3.1.2 Timing of CLRDV spread

Leaving cohorts of laboratory grown 2–3 true-leaf cotton in the field for one week proved to be an effective method of monitoring weekly CLRDV spread. None of the control plants that remained in the greenhouse throughout the course of this study tested positive for CLRDV, suggesting that virus spread in the greenhouse did not occur. CLRDV was detected in cohorts of sentinel plants that were in the field the first two weeks of monitoring when *Protaphis middletonii* was the most abundant species captured, three weeks later during peak flights of *A.*

gossypii in June–July, and three weeks later at the end of August when captures of all aphid species present were low (Table 3.3).

3.3.2 Small plot experiments

3.3.2.1 Proportion of plants infested with aphids and aphid counts

Understanding the proportion of plants infested with aphids is important to determine the proportion of plants in a field that are at risk of becoming infected with CLRDV during primary spread events. Data collection for aphids first began on 17 June for both the early May and early June planting date in AL, and on 30 May and 20 June for May and June plant dates, respectively, in GA. In these trials the May-planted cotton had a higher proportion of plants infested with aphids (Tables 3.4 and 3.5) and more aphids present (Tables 3.6 and 3.7) on June 17 when aphid colonization events began. At both locations 100% of the plants were infested with aphids on the two evaluation weeks that generally corresponded with peak flight activity detected with the pan traps, regardless of plant date or insecticide treatment regime. After this time, but before populations declined due to epizootics, the proportion of plants infested and average number of aphids were significantly higher in the June-planted cotton. The only date a significant reduction in the proportion of plants infested was observed in AL on 24 June for weekly insecticide applications beginning at the first true leaf stage. No significant difference in the proportion of plants infested was observed among treatments on any other evaluation date in AL or GA. Initiation of weekly insecticide sprays significantly reduced the number of aphids in insecticide-treated compared to the non-treated control plots, but never eliminated aphid populations.

Aphid population size is related to colonization, persistence, and the potential for secondary spread within the crop field. In both locations, total aphid count means were greater

on the early May planted cotton than the early June cotton before peak dispersal was detected in the pan traps, while means were higher for early June planted during and after peak dispersal. Aphid count means were highest on the non-sprayed plots across insecticide treatments for each date. Average number of aphids counted across the lower, middle, and upper leaves were low the first two weeks, 17 June and 24 June, averaging less than one aphid per leaf. In the following two weeks, 1 July and 8 July, aphid counts increased to 19-20 and 27-28 across leaves, respectively. Aphid count means across planting dates were similar on 17 June and 24 June, but were higher on the early June planting date than the early May plant on 1 July and 8 July. Across all dates, aphid count means were lowest for the first true leaf weekly insecticide treatment, while the non-sprayed plots had the highest. The upper leaf had higher average aphid counts than the middle and lower leaf for each date. A significant interaction occurred between planting date and treatment on 24 June and 1 July. Aphid count means were higher for each early June plant date treatment interaction than the early May plant date treatment interactions for each date. A significant interaction occurred between planting date and leaf position on 1 July and 8 July; aphid count means were greater across sampled leaves of cotton from the early June plant date for both evaluation dates. There was a significant interaction between insecticide treatment and leaf position on 1 July and 8 July. Cotton in the insecticide treatment control group had the highest aphid means counts across leaf positions for both dates (Table 3.8).

3.3.2.2 Proportion of plants infected with CLRDV

In AL and GA, there were no significant differences in final virus incidence among plots (Table 3.9). In AL, CLRDV was confirmed in 60-100% of the plants tested in each plot. In GA, incidence ranged from 90-100%; all plants tested from plots that received one calendar-based

application of insecticide the first week of July came back positive, while the others plots had at least one that came back negative.

3.3.2.3 Yield and lint quality analyses

In both locations, planting date significantly affected fiber length, micronaire, uniformity, and strength, but insecticide treatment did not result in differences among these values. Means for length, uniformity, and strength were higher for the June planted cotton than the May planted cotton, while micronaire was higher in early May than early June planted. Planting date significantly affected yield, but was not significantly affected by insecticide treatment. The average lint was higher in the early May planted cotton than early June planted (Table 3.10). Based on the incidence data and absence of clear disease symptoms among plots (data not shown) we do not believe the difference in yield among early and late plant dates is due to CLRDV.

3.4 Discussion

The final incidence of CLRDV in these experiments was not significantly different or reduced by adjusting the planting date or intensively managing aphid populations. Virus spread to a crop is determined by the amount of inoculum in the environment, transmission efficiency of the vectors, distance between inoculum and crop, number of vector species, seasonal population dynamics, vector dispersal behavior, and susceptibility of the variety to the virus (Jacobson 2019). Aphid monitoring during this study identified seven of the eight species of aphids reported to infest cotton at both locations, and *A. gossypii* was the most abundant species at both locations. Although *A. gossypii* was captured every week, CLRDV was detected in the sentinel

plant cohorts during three distinct time periods, and not randomly throughout the monitoring period. The timing of *A. gossypii* flights in relation to virus spread during four consecutive weeks June–July suggests that *A. gossypii* was transmitting CLRDV during this time because large flights of *A. gossypii* occurred, and large colonizing populations were observed in the cotton plots. The role of this vector in spreading CLRDV during the other two time periods is less clear. CLRDV was detected in the first two cohorts of sentinel plants when *P. middletonii* populations were highest at both locations (Table 3.3). At Brewton *P. middletonii* comprised 81% and 71% of the total aphids collected during the two weeks CLRDV was detected in sentinel plants there, and the number of *P. middletonii* individuals captured were up to 9-fold higher than numbers of *A. gossypii*. The captures of all other species were also low during this time. These results provide rationale for investigating the vector competency of *P. middletonii* to transmit CLRDV. CLRDV was also detected in the sentinel plants during the last four collection dates, however, numbers of all species were low during this time, including those not identified. Cotton plots were not monitored for aphid populations late-season, but it is possible that virus spread occurring in August was due to secondary spread of virus from the cotton plots caused by low populations of aphids that persisted in them.

The aphid insecticide management regimes were designed with the intent to monitor the relative contribution of primary versus secondary spread if the weekly insecticide applications were effective at reducing colonization. It is unlikely that primary spread of the virus can be suppressed if CLRDV is transmitted in under 40 s. Secondary spread may be reduced if dispersal of resident aphids through the field after colonization events is responsible for increasing the proportion of plants infested with viruliferous aphids, and the proportion of plants infected with CLRDV. The insecticide applications used for aphid population management in this study did

reduce the total numbers of aphids, but did not prevent infestations. Aphid counts in pan traps and on plants at both locations showed large populations of dispersing and colonizing aphids at both locations. This resulted in 100% infestation of plants with aphids in our field experiment, including the plots receiving weekly foliar sprays of insecticide. It is not possible to distinguish between primary and secondary spread in this study, but the increase in infected sentinel plants and the proportion of field plants infested suggests the risk for primary spread during peak flights was high. To better understand the relative contribution of primary spread to final virus incidence the proportion of colonizing aphids that were viruliferous would need to be monitored in future studies. To our knowledge, the roles of primary spread verses secondary spread have not been researched in this pathosystem, and it is unclear why insecticides were effective at reducing disease in previous studies (Galbieri et al. 2017). Whether or not insecticide applications can reduce secondary spread would need to be investigated at locations where initial infestations of *A. gossypii* result in an aggregated distribution in cotton fields, and not 100% infestation.

Insecticide treatment regime did not have a significant effect on lint quality or yield, which supports previous research documenting no significant yield increase when *A. gossypii* populations are managed (Layton et al. 1999, Johnson et al. 2002). Aphid counts were higher on the early May planted cotton on the first three evaluation dates, but were higher in the early June planted cotton for the remainder of the experiment. It is not clear whether this was due to dispersing aphids alighting more readily to the smaller cotton, or due to host selection preference for the early June planted cotton (Döring 2014). Neonicotinoid seed treatments used in the plots for thrips management may have suppressed colonization initially. Similar reductions in populations on the first evaluation days were not observed in GA, but this was likely observed

due to a combination of overall low aphid populations, and the relative timing of aphid flights/colonization and insecticide applications. Aphid count means were highest across leaf positions in plots that were not sprayed with an insecticide, indicating that insecticide use reduced aphid populations across the plant. Aphid count means were also higher across leaf position in the early June planted cotton indicating that infestations were occurring at a higher rate in the early June planted cotton. The reduction in aphid populations observed in GA on 11 July were likely caused by naturally occurring epizootics which have been shown to effectively reduce *A. gossypii* populations in July in the southeast (Pena 1993, Weathersbee and Hardee 1994, Marti and Olson 2006, 2007, Abney et al. 2008). In AL, aphid counts ended on 8 July with large aphid populations still present, indicating that epizootics had not yet reduced aphid populations.

Adjusting planting date can reduce virus incidence by either increasing the age of the crop to increase mature plant resistance, by temporally isolating crops from vectors, or altering attractiveness of the crop if there is a preference for a specific phenological stage. Although it is currently not known if cotton is more susceptible to CLRDV during the seedling stage, mature-plant resistance (i.e., decreasing susceptibility of a plant to a pathogen as age increases) is a common interaction observed in various insect-borne plant disease systems (Beaudoin et al. 2009, McMechan and Hein 2016, Kone et al. 2017, Srinivasan et al. 2017). Although it is impossible to know when field plants were infected, or how many were infected each week, temporal isolation was not achieved in AL where virus spread was detected in sentinel plants beginning the first two weeks after the May plant-date. Colonizing aphids were not observed in the field plots during this time, however, the neonicotinoid seed treatment used for thrips management may have suppressed colonization. The effects of neonicotinoid seed treatments on

transmission of CLRDV are unknown, but it is unlikely that transmission is disrupted if CLRDV is transmitted in less than 40 s. Another reason the absence of aphids does not equal the absence of transmission includes the role of potential transient vectors that feed on but do not colonize crops. Transient vectors are reported to contribute significantly to virus spread in other pathosystems (Halbert et al. 1981, Kalleshwaraswamy et al. 2007).

This study serves as a starting point for developing short-term management solutions for CLRDV in the U.S. In this study plant date and aphid management did not reduce the final incidence of CLRDV at locations where high incidence of this virus was observed during the 2018 and 2019 cropping seasons. Aphid dispersal events coincided with most weeks virus spread was detected in the sentinel plants, and other species of aphids that should be tested for vector competence were identified in this study. As has been reported previously in multiple U.S. states, visual disease symptoms and yield losses due to CLRDV were not apparent at either location. Future research is needed to better understand the biotic and abiotic interactions underlying disease caused by CLRDV that results in yield loss-outcomes. Future monitoring of aphid species composition and CLRDV spread is needed to understand seasonal patterns of virus spread across the cotton belt. Longer trapping periods that extend beyond the beginning and end of the cotton cropping season will also provide insights into patterns of virus spread among weed hosts that serve as reservoirs of CLRDV in the landscape, especially as more alternate hosts are identified. Understanding the timing and magnitude of virus spread by vectors is needed to develop integrated disease management strategies for reducing incidence and yield losses caused by CLRDV.

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Table 3.1. Dates weekly foliar insecticide applications were initiated in the small plot experiments.

Location	Date	Planting Date[‡]	Chemical Treatment[§]
Alabama	5/24/2019	Early May	First True Leaf
Alabama	6/19/2019	Early June	First True Leaf
Alabama	6/21/2019	Early May, Early June	First Colonization
Alabama	7/2/2019	Early May, Early June	Early July
Georgia	5/10/2019	Early May	First True Leaf
Georgia	6/14/2019	Early June	First True Leaf
Georgia	6/14/2019	Early May	First Colonization
Georgia	6/21/2019	Early June	First Colonization
Georgia	7/3/2019	Early May, Early June	Early July

[‡]Planting Date: Early May - cotton planted first week of May, Early June - cotton planted first week of June.

[§]Insecticide treatment indicating when weekly foliar sprays were initiated

Table 3.2. Primer sequences used for detection of *Cotton leafroll dwarf virus* in cotton plants.

Primer name	Primer direction	Sequence (5' – 3')	Round[1]	Product size	Reference
Pol3628F	Forward	TAATGAATACGGYCGYGGSTAG	1	395 bp	Sharman et al., (2015)
Pol4021R	Reverse	GGRTCMAVYTCRTAAGMGATSGA			
CLRDV3675F	Forward	CCACGTAGRCGCAACAGGCGT	2	310 bp	Sharman et al., (2015)
Pol3982R	Reverse	CGAGGCCTCGGAGATGAACT			Sharman et al., (2015)

[1]Designates which primer pair is used for the first (1) and second (2) amplification for the nested PCR.

Table 3.3. Number of aphids collected in pan traps at Brewton, AL and Tifton, GA, and the number of sentinel plants from weekly cohorts deployed at Brewton, AL that tested positive for *Cotton leafroll dwarf virus.*

Trapping Start Date	***Aphis gossypii*[a]**	***Myzus persicae*[a]**	***Aphis craccivora*[a]**	***Protaphis middletonii*[a]**	***Smynthurodes betae*[a]**	***Rhopalosiphum rufiabdominale*[a]**	***Macrosiphum euphorbiae*[a]**	***Aphis fabae*[a]**	***Hysteroneura setariae*[a*]**	**Other[a]**	**Total[c]**	**CLRDV[d]**
Brewton, AL												
5/13/2019	6.5 (1.3)	0.8 (0.8)	0.5 (0.3)	51.3 (1.5)	0.5 (0.5)	0.3 (0.3)	0.0	0.0	0.0	3.5 (0.7)	253	2/8
5/20/2019	5.0 (1.1)	0.0	0.3 (0.3)	33.0 (5.4)	0.5 (0.3)	0.5 (0.5)	0.0	0.0	0.0	3.5 (1.6)	171	1/8
5/27/2019	3.8 (1.6)	0.0	0.0	17.3 (2.8)	0.5 (0.5)	0.5 (0.3)	0.0	0.0	0.3 (0.3)	6.0 (1.4)	113	0
6/3/2019	4.8 (1.7)	0.0	0.0	7.3 (2.5)	1.5 (0.3)	2.3 (1.4)	0.0	0.0	0.5 (0.3)	5.3 (1.6)	86	0
6/10/2019	7.0 (1.6)	0.0	0.8 (0.8)	12.5 (0.9)	0.8 (0.5)	0.8 (0.3)	0.0	0.0	0.8 (0.3)	5.5 (1.7)	112	0
6/17/2019	6.0 (1.7)	0.0	0.0	4.8 (1.8)	1.0 (0.4)	0.5 (0.5)	0.0	0.0	2.5 (0.7)	3.5 (1.2)	73	1/8
6/24/2019	142.8 (51.7)	0.0	0.3 (0.3)	14.3 (1.7)	5.3 (2.8)	2.0 (1.1)	0.0	0.0	3.0 (1.2)	19.5 (4.4)	748	8/8
7/1/2019	238.8 (40.1)	0.0	0.0	22.8 (2.0)	3.0 (1.1)	0.5 (0.3)	0.0	0.0	11.3 (4.0)	5.8 (1.7)	1128	5/8
7/8/2019	117.8 (19.5)	0.0	0.0	8.0 (1.7)	1.5 (0.3)	0.3 (0.3)	0.0	0.0	13.3 (1.3)	13.8 (5.3)	618	4/8
7/15/2019	20.3 (4.0)	0.0	0.0	2.0 (0.8)	3.5 (1.0)	0.3 (0.3)	0.3 (0.3)	0.0	10.8 (4.2)	1.0 (0.7)	152	0

7/22/2019	11.8 (2.8)	0.0	0.0	9.5 (2.5)	0.5 (0.3)	4.5 (1.9)	1.0 (0.7)	0.0	11.5 (4.7)	0.3 (0.3)	156	0
7/29/2019	2.0 (0.7)	0.0	0.5 (0.3)	7.3 (1.7)	1.8 (0.5)	0.0	0.8 (0.5)	0.0	4.3 (1.5)	2.5 (0.9)	76	0
8/6/2019	0.8 (0.5)	0.0	1.0 (0.4)	8.5 (2.4)	4.0 (2.0)	0.0	0.3 (0.3)	0.0	1.0 (0.7)	2.0 (0.0)	70	1/8
8/12/2019	1.8 (0.9)	0.0	0.8 (0.5)	2.3 (0.9)	2.5 (0.3)	0.0	0.0	0.0	0.8 (0.8)	1.5 (0.5)	38	2/8
8/19/2019	1.0 (0.7)	0.0	1.0 (0.6)	1.3 (0.5)	1.0 (0.4)	0.0	0.0	0.0	1.0 (0.4)	1.5 (1.2)	27	2/8
8/26/2019	7.5 (4.0)	0.0	1.3 (0.5)	2.5 (1.0)	3.5 (0.3)	0.0	0.0	0.0	0.3 (0.3)	2.3 (1.4)	69	4/8
Total[c]	2,309	3	25	817	125	49	9	0	244	309	3890	-
Tifton, GA												
5/13/2019	2.5 (1.6)	4.0 (2.4)	0.0	7.5 (2.9)	0.0	0.5 (0.3)	0.0	0.0	0.0	0.3 (0.3)	59	-
5/20/2019	0.5 (0.3)	0.3 (0.3)	0.0	8.5 (2.6)	0.3 (0.3)	0.5 (0.3)	0.0	0.0	0.0	1.5 (0.3)	46	-
5/27/2019	0.8 (0.3)	0.0	0.3 (0.3)	5.3 (1.0)	0.5 (0.3)	0.0	0.0	0.0	0.0	0.8 (0.5)	30	-
6/3/2019	0.3 (0.3)	0.0	0.0	1.0 (0.4)	0.5 (0.3)	0.0	0.0	0.0	0.0	0.0	7	-
6/10/2019	1.5 (1.5)	0.0	0.0	0.0	0.0	0.3 (0.3)	0.0	0.0	0.3 (0.3)	0.5 (0.5)	10	-
6/17/2019	21.5 (6.6)	0.3 (0.3)	1.8 (0.8)	0.8 (0.8)	1.3 (0.5)	0.0	0.0	0.0	0.3 (0.3)	5.3 (1.8)	124	-
6/24/2019	349.3 (29.8)	0.0	2.0 (0.7)	1.3 (0.8)	1.3 (0.8)	0.0	0.0	0.0	0.8 (0.5)	15.3 (2.6)	1479	-
7/1/2019	336.8 (131.7)	0.0	0.8 (0.3)	1.3 (1.0)	0.8 (0.3)	0.0	0.0	0.0	0.3 (0.3)	41.3 (9.7)	1524	-

7/8/2019	12.8 (5.1)	0.0	0.5 (0.3)	0.8 (0.5)	0.3 (0.3)	0.0	0.0	0.0	0.3 (0.3)	4.5 (1.7)	76	-
7/15/2019	1.3 (0.6)	0.0	0.0	0.0	0.5 (0.5)	0.0	0.0	0.0	0.0	2.0 (0.4)	15	-
7/22/2019	0.3 (0.3)	0.0	0.0	0.0	0.3 (0.3)	0.0	0.0	0.0	0.0	0.3 (0.3)	3	-
7/29/2019	0.8 (0.5)	0.0	0.0	0.3 (0.3)	0.5 (0.3)	0.0	0.0	0.0	0.0	0.8 (0.5)	9	-
8/6/2019	1.0 (1.0)	0.0	0.0	0.0	0.3 (0.3)	0.0	0.0	0.0	0.0	2.8 (2.1)	16	-
8/12/2019	0.3 (0.3)	0.0	0.3 (0.3)	0.0	0.3 (0.3)	0.0	0.0	0.0	0.0	0.3 (0.3)	4	-
8/19/2019	0.5 (0.5)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3 (0.3)	3	-
8/26/2019	0.3 (0.3)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1	-
Total[c]	2920	18	22	106	26	5	0	0	7	302	3406	-

[a]Mean (standard error) of alates counted in four traps per location.

[c]Total number of alates counted in four traps per location (all species).

[d]Proportion of eight sentinel plants that tested positive for CLRDV.

*Not reported to infect cotton or transmit CLRDV. First record of species in AL and present in high numbers.

Table 3.4. Means comparison of the proportion of cotton plants infested with aphids among planting dates (PD) and insecticide treatments (IT) in Alabama.

		Evaluation Dates					
Main Effects		**6/17/2019**[†]	**6/24/2019**	**7/1/2020**[Y]	**7/8/2020**[Y]	**7/15/2019**	**7/22/2019**
PD[‡]	**IT**[§]						
May		0.47 (0.041) a[¶]	0.78 (0.07) a	1	1	0.34 (0.06) b	0.63 (0.10) b
June		0.12 (0.02) b	0.52 (0.10) b	1	1	0.78 (0.05) a	0.89 (0.04) a
	Control	0.30 (0.05) a	0.75 (0.08) ab	1	1	0.56 (0.09) a	0.87 (0.07) a
	First True Leaf	0.22 (0.04) a	0.49 (0.11) c	1	1	0.61 (0.09) a	0.74 (0.11) a
	First Colonization	0.32 (0.05) a	0.57 (0.11) bc	1	1	0.63 (0.09) a	0.88 (0.07) a
	Early July	0.20 (0.04) a	0.80 (0.07) a	1	1	0.49 (0.09) a	0.61 (0.13) a
PD x IT	**Insecticide treatment least square means grouped by planting date**						
May	Control	0.58 (0.07) ab	-	1	1	-	-
May	First True Leaf	0.24 (0.06) b	-	1	1	-	-
May	First Colonization	0.63 (0.07) a	-	1	1	-	-
May	Early July	0.45 (0.07) ab	-	1	1	-	-
June	Control	0.12 (0.04) a	-	1	1	-	-
June	First True Leaf	0.20 (0.05) a	-	1	1	-	-
June	First Colonization	0.12 (0.04) a	-	1	1	-	-
June	Early July	0.07 (0.03) a	-	1	1	-	-
Significance of Main Effects	**(Num df, Den df)**						
PD	(1, 608)	F = 48.77, *P* < 0.0001	F = 22.8, *P* < 0.0001	-	-	F = 27.5, *P* < 0.0001	F = 9.07, *P* = 0.003
IT	(3, 608)	F = 1.8, *P* =	F = 7.66,	-	-	F = 0.49,	F = 0.16,

		0.15	$P < 0.0001$			$P = 0.69$	$P = 0.16$
PD x IT	(3, 608)	F = 5.52, P = 0.001	NS[Z]	-	-	NS	NS

[†]Dates when aphid presence was recorded

[‡]Planting Date: cotton was planted 2 May and 4 June.

[§]Insecticide treatment indicating when weekly foliar sprays were initiated.

[¶]Values are presented as mean ± Standard error mean (SEM). When the interaction term is not significant ($P > 0.05$) main effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$). When the interaction term is significant ($P \leq 0.05$) simple effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$).

[Z]NS = not significant. Interaction terms were excluded from analysis when they were not significant ($P \leq 0.05$).

[Y]No statistical analyses were performed because all plants were infested.

Table 3.5. Means comparison of the proportion of cotton plants infested with aphids among planting dates (PD) and insecticide treatments (IT) in Georgia.

		Evaluation Dates						
Main Effects		**5/30/2019[†w]**	**6/6/2019**	**6/13/2020**	**6/20/2020**	**6/27/2019[Y]**	**7/3/2019[Y]**	**7/11/2019**
PD[‡]	**IT[§]**							
May		-[x]	-	-	0.99 (0.05) a	1	1	0.80 (0.07) b
June		-	-	-	0.99 (0.06) a	1	1	0.98 (0.01) a
	Control	0	0.10 (0.05) a[¶]	0.17 (0.07) a	0.99 (0.02) a	1	1	1.00 (3.095E-6) a
	First True Leaf	0	0.03 (0.02) a	0.05 (0.03) a	0.98 (0.02) a	1	1	0.87 (0.07) a
	First Colonization	0.05	0.18 (0.06) a	0.22 (0.08) a	1.00 (0.000003) a	1	1	0.86 (0.07) a
	Early July	0.05	0.10 (0.05) a	0.14 (0.06) a	1.00 (0.004) a	1	1	0.87 (0.07) a
Significance of Main Effects								
PD		-	-	-	df = 1, 288, F = 0.00, $P = 0.96$	-	-	df = 2, 288, F = 15.82, $P < 0.0001$
IT		-	df = 3, 144, F = 1.38, $P = 0.25$	df = 3, 144, F = 1.44, $P = 0.23$	df = 3, 288, F = 0.79, $P = 0.50$	-	-	df = 3, 288, F = 0.02, $P = 0.99$

[†]Dates when aphid presence was recorded

[‡]Planting Date: cotton was planted 2 May and 3 June.

[§]Insecticide treatment indicating when weekly foliar sprays were initiated

[¶]Values are presented as mean ± Standard error mean (SEM). When the interaction term is not significant ($P > 0.05$) main effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$).

[Y]No statistical analyses were performed because all plants were infested.

[X]No aphid counts were conducted for the Early June planting date from 5/30/2019 - 6/13/2019 because the first true leaf was not present, as a result there were no planting date comparisons made for this date.

[W]No statistical analyses were performed because aphid presence was only detected on two plants.

Table 3.6. Means comparisons of the average total number of aphids on the upper most fully expanded cotton leaves among insecticide treatments (IT) and planting dates (PD) in Alabama.

		Evaluation Dates			
Main Effects		**6/17/2019**[†]	**6/24/2019**	**7/1/2019**	**7/8/2019**
PD[‡]	**IT**[§]				
May		0.91 (0.34) a[¶]	1.13 (0.32) a	17.5 (3.34) b	27.82 (4.95) b
June		0.15 (0.07) b	1.01 (0.26) a	68.75 (13.00) a	57.55 (10.20) a
	Control	1.36 (0.68) a	3.36 (1.24) a	55.02 (9.90) a	95.08 (15.28) a
	First True Leaf	0.06 (0.04) b	0.40 (0.15) c	18.98 (3.45) b	23.89 (3.90) b
	First Colonization	0.72 (0.37) a	0.62 (0.23) bc	25.31 (4.59) b	34.12 (5.51) b
	Early July	0.33 (0.18) ab	1.59 (0.56) ab	54.79 (9.91) a	33.09 (5.35) b
PD x IT	**Insecticide treatment least square means grouped by planting date**				
May	Control	-	-	-	90.31 (20.54) a
May	First True Leaf	-	-	-	11.44 (2.66) c
May	First Colonization	-	-	-	21.26 (4.87) b
May	Early July	-	-	-	27.30 (6.24) b
June	Control	-	-	-	100.00 (22.72) a
June	First True Leaf	-	-	-	49.88 (11.38) b
June	First Colonization	-	-	-	54.78 (2.67) b
June	Early July	-	-	-	40.10 (9.17) b
Significance of Main Effects	**(Num df, Den df)**				
PD	(1, 288)	F = 10.02, *P* = 0.002	F = 0.09, *P* = 0.77	F = 25.96, *P* < 0.0001	F = 8.39, *P* = 0.004
IT	(3, 288)	F = 4.63, *P* = 0.004	F = 6.4, *P* = 0.0003	F = 14.96, *P* < 0.0001	F = 26.12, *P* < 0.0001

PD x IT	(3, 288)	NS[z]	NS	NS	F = 6.64, *P* = 0.0002

[†]Dates when aphid presence was recorded

[‡]Planting Date: cotton was planted 2 May and 4 June.

[§]Insecticide treatment indicating when weekly foliar sprays were initiated.

[¶]Values are presented as mean ± Standard error mean (SEM). When the interaction term is not significant ($P > 0.05$) main effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$). When the interaction term is significant ($P \leq 0.05$) simple effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$).

[Z]NS = not significant. Interaction terms were excluded from analysis when they were not significant ($P \leq 0.05$).

Table 3.7. Means comparisons of the average total number of aphids on the upper most fully expanded cotton leaves among insecticide treatments (IT) and planting dates (PD) in Georgia.

Main Effects		Evaluation Dates						
		5/30/2019[†w]	6/6/2019	6/13/2020	6/20/2020	6/27/2019	7/3/2019	7/11/2019
PD[‡]	**IT[§]**							
Early May		-[x]	-	-	11.40 (2.74) a	25.94 (5.15) b	33.43 (4.76) b	1.14 (0.25) b
Early June		-	-	-	8.48 (2.30) a	52.69 (10.42) a	81.62 (11.58) a	5.81 (1.04) a
	Control	0	0.15 (0.13) a[¶]	0.09 (0.23)	11.28 (3.09) a	47.77 (7.68) a	77.61 (10.15) a	5.39 (0.64) a
	First True Leaf	0	0.03 (0.03) a	0.002 (0.006)	7.00 (1.90) a	21.66 (3.73) b	38.13 (5.03) b	2.00 (0.42) bc
	First Colonization	1 aphid	0.20 (0.17) a	0.03 (0.09)	11.63 (3.14) a	40.22 (6.89) a	34.49 (4.55) b	2.62 (0.37) b
	Early July	1 aphid	0.08 (0.07) a	0.41 (0.88)	12.72 (3.43) a	44.87 (7.68) a	72.92 (9.54) a	1.56 (0.31) c
PD x IT	**Insecticide treatment least square means grouped by planting date**							
Early May	Control	-	-	-	-	-	58.59 (10.85) a	4.64 (0.90) a
Early May	First True Leaf	-	-	-	-	-	17.40 (3.27) b	0.73 (0.45) bc
Early May	First Colonization	-	-	-	-	-	23.49 (4.40) ab	1.21 (0.27) b
Early May	Early July	-	-	-	-	-	52.13 (9.67) a	0.41 (0.12) c
Early June	Control	-	-	-	-	-	102.81 (18.98) a	6.24 (1.00) a
Early June	First True Leaf	-	-	-	-	-	83.58 (15.44) ab	5.45 (1.55) a
Early June	First Colonization	-	-	-	-	-	50.65 (9.39) b	5.64 (1.15) a
Early June	Early July	-	-	-	-	-	102.00 (18.83) a	5.93 (1.24) a
Significance of Main Effects								
PD		-	-	-	df = 1, 288, F = 0.29, $P = 0.59$	df = 1, 288, F = 6.40, $P = 0.01$	df = 1, 288, F = 19.72, $P < 0.0001$	df = 1, 288, F = 22.57, $P < 0.0001$

IT	-	df = 3, 144, F = 0.74, *P* = 0.53	df = 3, 144, F = 0.99, *P* = 0.40	df = 3, 288, F = 1.22, *P* = 0.30	df = 3, 288, F = 10.01, *P* < 0.0001	df = 3, 288, F = 18.90, *P* < 0.0001	df = 3, 288, F = 27.13, *P* < 0.0001
PD x IT	-	-	-	NS[z]	NS	df = 3, 288, F = 5.50, *P* = 0.0011	df = 3, 288, F = 21.26, *P* < 0.0001

[†]Dates when aphid presence was recorded

[‡]Planting Date: cotton was planted 2 May and 3 June.

[§]Insecticide treatment indicates when weekly foliar sprays were initiated

[¶]Values are presented as mean ± Standard error mean (SEM). When the interaction term is not significant ($P > 0.05$) main effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$). When the interaction term is significant ($P \leq 0.05$) simple effects means for each treatment level were followed by lower case letters that indicate significant differences between treatments using the Tukey method for multiple comparisons ($\alpha = 0.05$).

[Z]NS = not significant. Interaction terms were excluded from analysis when they were not significant ($P \leq 0.05$).

[X]No aphid counts were conducted for the June planting date from 5/30/2019 - 6/13/2019 because the first true leaf was not present, as a result there were no planting date comparisons made for this date.

[W]No statistical analyses were performed because aphid presence was only detected on two plants

Table 3.8. Results of a split-plot design to examine the effect of plant date (PD) (2 May, 4 June) (Main plot effects), insecticide treatments (IT) (time weekly insecticide applications were initiated; sub-plot effects), and leaf position (LP) sampled on the average total number of aphids (± Standard error). Aphids were counted separately on one randomly selected leaf from the lower 1/3, middle 1/3, and the upper most fully expanded leaf of ten cotton plants per plot. Means comparisons were performed using Tukey's method at *P* =0.05 level; lower case letters following means indicate significant differences between/among treatments.

			Evaluation Dates‡			
	Main Effects		**6/17/2019**	**6/24/2019**	**7/1/2019**	**7/8/2019**
PD	**IT**	**LP**				
May			-Q	0.50 (0.11) a	4.85 (0.96) b	15.16 (2.65) b
June			-	0.41 (0.09) a	39.83 (7.78) a	30.18 (5.26) a
	Control		0.06 (0.04) b	1.11 (0.27) a	37.94 (6.14) a	81.36 (13.52) a
	First True Leaf		0.003 (0.002) b	0.20 (0.05) b	5.56 (0.93) b	9.90 (1.68) c
	First Colonization		0.15 (0.08) a	0.25 (0.06) b	6.15 (1.03) b	12.15 (2.04) c
	Early July		0.004 (0.006) b	0.78 (0.19) a	28.71 (4.67) a	21.38 (3.57) b
		Lower	0.02 (0.009) b	0.33 (0.07) b	6.67 (0.99) c	10.78 (1.44) c
		Middle	0.02 (0.01) ab	0.48 (0.10) ab	12.65 (1.84) b	23.49 (3.09) b
		Upper	0.06 (0.03) a	0.59 (0.12) a	31.79 (4.57) a	38.67 (5.03) a
PD x IT	**Insecticide treatment least square means grouped by planting date**					
May	Control		-	-	15.94 (3.67) a	78.28 (18.38) a
May	First True Leaf		-	-	1.53 (0.37) b	4.46 (1.08) c

May	First Colonization	-	-	2.06 (0.50) b	7.15 (1.71) bc
May	Early July	-	-	10.96 (2.53) a	21.18 (4.99) b
June	Control	-	-	90.31 (20.59) a	84.57 (19.87) a
June	First True Leaf	-	-	20.20 (4.63) b	21.98 (5.19) b
June	First Colonization	-	-	18.36 (4.2) b	20.66 (4.87) b
June	Early July	-	-	75.19 (17.15) a	21.59 (5.10) b

PD x LP **Leaf position least square means grouped by planting date**

May	Lower	-	-	1.93 (0.42) c	8.63 (1.62) c
May	Middle	-	-	3.91 (0.82) b	15.46 (2.88) b
May	Upper	-	-	15.01 (3.07) a	26.12 (4.81) a
June	Lower	-	-	22.97 (4.68) c	13.45 (2.51) c
June	Middle	-	-	40.87 (8.30) b	35.68 (6.60) b
June	Upper	-	-	67.31 (13.63) a	57.25 (10.50) a

IT x LP **Leaf position least square means grouped by insecticide treatment**

Control	Lower	-	-	27.65 (5.01) c	54.36 (10.14) b
Control	Middle	-	-	37.58 (6.77) b	110.08 (20.51) a
Control	Upper	-	-	52.55 (9.41) a	90.01 (16.63) ab
First True Leaf	Lower	-	-	2.15 (0.43) c	4.04 (0.80) c
First True Leaf	Middle	-	-	4.50 (0.86) b	10.43 (2.01) b
First True Leaf	Upper	-	-	17.79 (3.23) a	23.03 (4.32) a
First Colonization	Lower	-	-	1.76 (0.36) c	3.73 (0.73) c
First Colonization	Middle	-	-	5.47 (1.04) b	14.21 (2.70) b
First Colonization	Upper	-	-	24.20 (4.38) a	33.86 (6.31) a
Early July	Lower	-	-	18.94 (3.47) c	16.45 (3.11) b
Early July	Middle	-	-	27.69 (4.99) b	18.66 (3.49) b
Early July	Upper	-	-	45.11 (8.08) a	31.86 (5.92) a

Significance of Main Effects				
PD	-	df = 1, 638, F = 0.57, *P* = 0.45	df = 1, 628, F = 57.36, *P* < 0.0001	df = 1, 630, F = 7.79, *P* = 0.005
IT	df = 2, 318, F = 8.43, *P* = 0.0003	df = 3, 638, F = 14.3, *P* < 0.0001	df = 3, 628, F = 98.01, *P* < 0.0001	df = 3, 630, F = 53.34, *P* < 0.0001
LP	df = 2, 318, F = 3.61, *P* = 0.03	df = 2, 638, F = 4.25, *P* = 0.01	df = 2, 628, F = 206.03, *P* < 0.0001	df = 2, 630, F = 135.79, *P* < 0.0001
PD x IT	-	NS	df = 3, 628, F = 3.22, *P* = 0.02	df = 3, 630, F = 8.69, *P* < 0.0001
PD x LP	-	NS	df = 2, 628, F = 25.95, *P* < 0.0001	df = 2, 630, F = 3.77, *P* = 0.02
IT x LP	NS	NS	df = 2, 628, F = 20.75, *P* < 0.0001	df = 6, 630, F = 17.07, *P* < 0.0001
PD x IT x LP	-	NS	NS	NS

[†]Dates when aphid presence was recorded.

[Z]NS = not significant. Interaction terms were excluded from analysis when they were not significant ($P \leq 0.05$).

[Q]No aphid counts were conducted on the middle and lower leaf for the June planting date because these leaves were not present yet, as a result there were no planting date comparisons made for this date.

Table 3.9. Final *Cotton leafroll dwarf virus* incidence in small plot field trials conducted in Alabama and Georgia. Means comparisons were performed to analyze the main plot effect of planting date (PD) and sub plot effect of insecticide treatment (IT) on the average proportion (± Standard error) of ten plants per plot that were confirmed to be infected with CLRDV using PCR-based diagnostics. Means comparisons were performed for Alabama data using Tukey's method at P =0.05 level. No statistical analyses were performed on Georgia data because only 6/320 plants came back negative for CLRDV.

	Alabama	**Georgia**
PD		
May	0.79 (0.05)	0.98
June	0.83 (0.05)	0.99
IT		
Control	0.82 (0.05)	0.99
First True Leaf	0.84 (0.05)	0.95
First Colonization	0.70 (0.07)	0.99
Early July	0.85 (0.05)	1
Significance of Main Effects		
PD	df = 1, 288, F = 0.26, P = 0.61	-
IT	df = 3, 288, F = 1.78, P = 0.15	-

[‡]Planting Date: May - cotton planted first week of May, Early June - cotton planted first week of June

[§]Insecticide Treatment: Insecticide treatment indicating when weekly foliar sprays were initiated.

Table 3.10. Lint yield and quality metrics in small plot field trials conducted in Alabama and Georgia. Means comparisons were performed to analyze the main plot effect of planting date (PD) and sub plot effect of insecticide treatment (IT) on the average (± Standard error) HVI length, Micronaire (Mic), uniformity, strength, and yield of plots. Means comparisons were performed using Tukey's method at P =0.05 level, and lower case letters indicate significant differences between treatments.

		HVI Length (mm)	**Mic**	**Uniformity** (%)	**Strength** (grams per textile)	**Lint Yield** (kg ha^{-1})
PD[‡]						
	Early May	29.92 (0.14) b[¶]	4.58 (0.04) a	82.02 (0.16) b	29.49 (0.15) b	1642.16 (113.68) a
	Early June	31.07 (0.15) a	4.32 (0.04) b	83.26 (0.17) a	31.42 (31.11) a	1356.17 (113.68) b
IT[§]						
	Control	30.51 (0.16) a	4.42 (0.04) a	82.33 (0.19) a	30.12 (0.20) a	1565.07 (112.39) a
	First True Leaf	30.51 (0.16) a	4.47 (0.04) a	82.86 (0.19) a	30.45 (0.21) a	1467.27 (112.39) a
	First Colonization	30.67 (0.16) a	4.44 (0.04) a	82.66 (0.19) a	30.11 (0.20) a	1496.85 (112.39) a
	Early July	30.27 (0.16) a	4.47 (0.04) a	82.70 (0.19) a	30.51 (0.20) a	1467.46 (112.39) a
Significance of Main Effects	**(Num df, Den df)**					
PD	(1, 44)	F = 31.54, P < 0.0001	F = 23.63, P < 0.0001	F = 28.26, P < 0.0001	F = 54.26, P < 0.0001	F = 21.29, P < 0.0001
IT	(3, 44)	F = 1.46, P = 0.24	F = 0.43, P = 0.73	F = 1.65, P = 0.19	F = 1.14, P = 0.34	F = 2.38, P = 0.08

[‡]Planting Date: May - cotton planted 2 May (AL and GA), Early June - cotton planted 4 June (AL) and 3 June (GA)

[§]Insecticide treatment indicating when weekly foliar sprays were initiated.