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PLANT PATHOLOGY & NEMATOLOGY

Investigating the Interaction of Plant Age and Timing of Cotton Leafroll Dwarf Virus Infection on Yield Loss

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ABSTRACT

Cotton leafroll dwarf virus (CLRDV) is an aphid-transmitted virus recently identified across the Cotton Belt region of the U.S. Yield responses to CLRDV have been inconsistent, and asymptomatic infections can occur in which no losses are apparent. Conditions underlying the observed variation in yield responses are not understood. A three-year field study was conducted to examine whether yield loss caused by CLRDV is influenced by the age of cotton plants at the time of infection. Timing of infection was investigated by infesting caged plots of cotton with viruliferous *Aphis gossypii* to transmit CLRDV at different seedling growth stages, beginning after seedling emergence and continuing weekly for three to four weeks. Timing of infection impacted yield only in one out of three years suggesting that plant age is not a primary factor contributing to CLRDV-related yield loss. Future studies are needed to identify the environmental factors influencing disease severity and yield loss.

Cotton leafroll dwarf virus (CLRDV) (Family: Solemoviridae; Genus: *Polerovirus*) is a phloem-limited virus (Parkash et al., 2021) transmitted by the cotton aphid, *Aphis gossypii* Glover, in a persistent and circulative manner (Heilsnis et al., 2022, 2023; Michelotto and Busoli, 2003, 2007). CLRDV was first detected in cotton, *Gossypium hirsutum* L., in the U.S. in 2017 and has since been reported across the Cotton Belt (Aboughanem-Sabanadzovic et al., 2019; Alabi et al., 2020; Ali and Mokhtari, 2020; Ali et al., 2020; Avelar et al., 2019; Faske et al., 2020; Iriarte et al., 2020; Olmedo-Velarde et al., 2025; Price et al., 2020; Tabassum et al., 2019; Thiessen et al., 2020; Wang et al., 2020). CLRDV is the first virus reported to cause yield loss in the southeastern portion of the Cotton Belt (Avelar et al., 2019). Virus-like symptoms observed in cotton fields infected with CLRDV include stunting, leaf curling or rolling, leaf rugosity, reduced boll set, red stems, red petioles, bronzing, and shortened internodes (Edula et al., 2023). Identification of infected plants based solely on symptoms can be inaccurate because asymptomatic infections occur (Brown et al., 2020; Edula et al., 2023; Mahas et al., 2022; Tabassum et al., 2020). To date, widespread yield losses have not been reported (Lawrence et al., 2019, 2020, 2021, 2022; Roberts, Conner, Jacobson, personal observations) even though high rates of CLRDV incidence based on molecular testing have been reported in some areas of Alabama and Georgia (Mahas et al., 2022; Sedhain et al., 2021). Specific abiotic and biotic factors that influence symptomology, disease severity, and yield are not understood.

Plant age at time of virus infection is one factor that has been shown to influence disease severity and yield loss caused by plant viruses (Hu and Yang, 2019). Age-related resistance refers to plants becoming less susceptible to infections and pathogens as the plants mature due to changes in pathogen perception and defense activation (Hu and Yang, 2019). It often occurs as the plant goes through different developmental stages (Develey-Rivière and Galiana, 2007) such as when a plant transitions from the embryonic stage to the juvenile vegetative stage, from the juvenile vegetative stage to adult vegetative stage (Hu and Yang, 2019), during transition to the flowering stage, or at the start of senescence (Develey-Rivière and Galiana, 2007). This phenomenon has been observed for other insect-transmitted viruses in both cotton and other crops, and for other cotton pathogens. Two whitefly-transmitted viruses, cotton leaf curl virus (CLuV) (Family: Geminiviridae; Genus: *Begomovirus*) and cotton leaf crumple virus (CLCrV) (Family: Geminiviridae; Genus: *Begomo-*

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virus), have been shown to cause greater yield loss in cotton when the plant is exposed during early vegetative stages (Brown, 1987; El Nur and Abu Salih, 2009). Similarly, symptoms are more severe when younger plants are infected with CLCrV (Brown, 1987). Another study found that five-day-old cotton developed symptoms faster than 12-day-old cotton when inoculated with the fungus *Rhizoctonia solani* J.G. Kühn (Hunter et al., 1978). CLRDV symptoms expressed by cotton have been reported to vary depending on the plant growth stage (Bag et al., 2021; Edula et al., 2023), and plants infected late-season in Argentina exhibited symptoms only in the upper part of the plant (Agrofoglio et al., 2017). Knowledge about age-related resistance is important for devising management strategies during specific times when crops are most susceptible to disease.

The objective of the current study was to examine whether age-related resistance influenced yield losses caused by CLRDV infections in cotton. Cotton was inoculated at different seedling growth stages to compare the effect of plant age at the time of CLRDV infection on yield under field conditions.

MATERIALS AND METHODS

Experimental Design and Treatments. A three-year small plot field experiment was conducted because no CLRDV symptoms have been observed in greenhouse conditions, and the aggressive growth habit of cotton plants makes them difficult to grow under greenhouse conditions. Small plots were established from 2019 to 2021 at the E.V. Smith Agricultural Experiment Station in Shorter, AL (32°26′32.2″ N, 85°53′51.0″ W). Experimental units were two rows wide by 6.10 m in length. DP 1646 B2XF (DeltaPine®, Bayer Crop Science, St. Louis, MO) without an insecticide seed treatment was planted on 30 May 2019, 26 May 2020, and 18 May 2021 at 9.84 seeds m^{-1}. To examine the influence of age-related resistance on yield, six treatments were arranged in a randomized complete block design with four replications: no aphids released (control), release of nonviruliferous aphids (aphid control), aphid transmission of CLRDV one week after emergence at the cotyledon stage (week 1 release), aphid transmission of CLRDV two weeks after emergence at the one-to-two true-leaf stage (week 2 release), aphid transmission of CLRDV three weeks after emergence at the three-to-four true-leaf stage (week 3 release), and aphid transmission of CLRDV four weeks after emergence at the five-to-six true leaf stage (week 4 release). Weekly releases of putatively viruliferous aphids were conducted over four weeks in 2020 and 2021; in 2019 there were only three release dates, beginning at the one-to-two true-leaf growth stage (week 2 release to week 4 release).

Small Plot Cages to Exclude Insects. Infection timing to cotton plots was controlled by covering plots with insect-proof screen the day after planting. This excluded natural infestations of insects and enabled controlled releases of putatively viruliferous *A. gossypii* (see below). The cages were constructed by stretching mesh 50 anti-insect screen (Green-Tek, Baldwin, GA) over a PVC pipe (2.50 cm diameter) frame (622.30 cm L x 142.24 cm W x 129.54 cm D). Hobo RX3000 data loggers (HOBO®, LI-COR Environmental, Lincoln, NE) with sensors that measured air temperature, photosynthetically active radiation (PAR), relative humidity (RH), and soil moisture content were used to monitor environmental conditions inside and outside the cages. EC5 Soil Moisture Smart Sensors (LI-COR Environmental, Lincoln, NE) were installed at 15 cm depth. Two data loggers were placed between the two rows of cotton inside two cages. A third data logger with sensors was placed between two border rows of cotton outside the cages; four border rows of cotton were planted along two sides of the research plots, parallel to the caged plots/block design and planted the same date with the same variety. Weather data was collected from 19 June to 22 July 2019, 2 June to 20 July 2020, and 20 May to 21 July 2021.

Aphid Colony. An *A. gossypii* colony was initiated using multiple apterae individuals collected from a cotton field in Tallassee, AL, in 2019. This colony was reared on healthy one-to-two true-leaf DP 1646 B2XF seedlings. Each week, two adult apterae were transferred to new seedlings and allowed to reproduce for one week before repeating the process. This produced a large number of adult apterae available for experiments and minimized production of alatae in the colony. Adult apterae were preferred because alatae are less prone to settle and colonize on plants due to their dispersal behaviors (Johnson, 1958), which can interfere with virus acquisition during experiments.

CLRDV-Infected Plants. CLRDV-infected plants were collected from a cotton field in Tallassee, AL, in 2018, and maintained in the greenhouse with annual transmission to healthy DP 1646 B2XF seedlings using *A. gossypii* according to Heilsnis et al. (2022, 2023). Greenhouse conditions included weekly fertilization and supplemental light in early spring and fall to maintain a minimum 14-h day. Temperatures were held between 18 and 43 °C. Infection status of CLRDV-infected plants was tested each spring using reverse transcription-polymerase chain reaction (RT-PCR) according to the methods of Mahas et al. (2022) before experiments began.

Transmitting CLRDV to Plants in Field Cages. Putatively viruliferous aphids released into the caged field plots were generated by infesting CLRDV-infected source plants with mixed age apterous and alatae aphids (3-7 d old) for a three-to-five-day acquisition access period (AAP) prior to infesting experimental plots. This provided a minimum AAP of 72 h, which should maximize transmission rates (Heilsnis et al., 2023; Michelotto and Busoli, 2007). Transmission did not increase after 72 h AAP, but sometimes a longer time was needed to increase the population of aphids available to use for transmission. CLRDV was transmitted to plots by placing leaves infested with 20 to 40 putatively viruliferous aphids from the CLRDV-infected source plants onto every healthy cotton plant in cages. This minimized handling effects that cause aphid mortality and facilitated colonization and transmission of CLRDV throughout each plot. For the aphid control plots, cotton leaves from the greenhouse colony with 20 to 40 mixed-age aphids were used to infest plants. Due to space constraints in the field and colony rearing limits, we could not replicate non-viruliferous aphid releases on each of the four weekly release dates. Therefore, one of the four plots in the aphid control treatment were infested each week from emergence to the five-to-six true-leaf growth stage. One week after aphids were released into a cage (7 d inoculation access period), plants were sprayed with 204 g ai ha^{-1} flupyradifurone (Sivanto® Prime, Bayer Crop Science, St. Louis, MO) to kill aphids.

Plot Maintenance. Plants in each caged plot were inspected weekly for unintended aphid and other insect pest infestations until cages were removed. First, plots that had not been infested were checked to ensure that unintended infestations did not occur in the cages. Then, plots sprayed the week before were checked to ensure aphids were killed. Last, aphids were monitored in cages infested the week prior to ensure that aphids colonized every plant. No unintended infestations were observed in cages during these experiments, indicating cages were successful at preventing natural infestations of pests prior to aphid release and after insecticide applications. Therefore, no additional management interventions were needed while plots were caged.

Cages were not large enough to grow cotton season-long and were removed approximately eight weeks after planting when cotton growth reached the top of the cage on 23 July 2019, 21 July 2020, and 22 July 2021. Removal timing occurred approximately two weeks after the week 4 release, and after natural infestations of aphids in the area had crashed due to natural epizootics of entomopathogenic fungi. Aphid infestations are not typically observed after the epizootics decrease populations, and this timing should prevent natural infestations of aphids from occurring after cage removal. Season-long management was conducted according to standard local recommendations (Alabama Cooperative Extension System, 2025).

Data Collection. Final CLRDV incidence was determined by individually testing 10 randomly selected plants from each plot (40 total per treatment) on 13 Sept. 2019, 14 Sept. 2020, and 22 Sept. 2021 with RT-PCR (Mahas et al., 2022). CLRDV was not tested prior to cage removal because it takes up to eight weeks after transmission to detect CLRDV in newly infected plants (Mahas et al. 2022). It was also cost prohibitive to test for CLRDV multiple times during the growing season, therefore tests were performed after cutout. Cutout is defined as the growth stage when flowers developed after this point have a low probability of producing harvestable bolls and when the terminal node on the main stem is five nodes above the uppermost white flower in the first position (Bourland et al., 2001).

To better understand any yield loss that might occur, plant mapping was performed on plants tested for CLRDV after cutout, on 13 Sept. 2019, 18 Sept. 2020, and 22 Sept. 2021. Data were recorded for plant height, total number of nodes, retention of first and second position bolls, the sum of retention for first and second position bolls, and symptoms (leaf tenting, drooping and rugosity, reddening of stems, leaf veins and petioles, leaf bronzing, stunted plant growth and senescence, brittle stems and petioles, whip on the top of the mainstem, and downward folding of leaves).

Lint was harvested from all plants in both rows of each plot on 24 Oct. 2019, 27 Oct. 2020, and 17 to 19 Nov. 2021. A JD 9920 cotton picker (John Deere, Moline, IL) was used to harvest the lint in 2019, and a Case IH 2555 cotton picker (Case Corporation, Racine, WI) was used in 2020. Lint was hand harvested in 2021 due to weather and equipment issues. Plot samples were sent to the University of Georgia Micro-Gin (Tifton, GA) for processing and to obtain weight of seed cotton and lint (Li et al., 2011). Fiber samples were sent to the USDA Classing Office in Macon, GA, for the 2019 season, and to the USDA Classing Office in Memphis, TN, for the 2020 and 2021 growing seasons to obtain HVI measures of fiber length, strength, uniformity, and micronaire (USDA-AMS, 1993).

Statistical Analyses. Data were analyzed using the GLIMMIX procedure in SAS (version 9.4; SAS Institute, Inc., Cary, NC). Prior to analysis, data were tested for normality and homogeneity of variance, and the assumptions of the model were satisfied. Data from aphid control plots were analyzed as one treatment because there were no significant differences among these plots with different release dates. Three sets of analysis were performed in this study. CLRDV incidence, lint yield, and all response variables related to lint quality and plant mapping were compared among treatments in separate analyses run by year. To evaluate the occurrence of differences in yield and weather variables caused by cages, these variables were compared among locations (outside or inside cages), across all years. Lastly, temperature and soil moisture content inside cages were compared among years. Treatment and location were included in their respective models as fixed effects. Block was also included in the models as a fixed effect, or as a random effect when preliminary analyses showed no significant differences between blocks. All data were modeled using the gaussian distribution, except for CLRDV incidence, which was modeled using the binomial distribution. Means comparisons were performed among treatments using Tukey-Kramer's method at the $p = 0.05$ level.

RESULTS

CLRDV Incidence. CLRDV was detected in all plots (Table 1). A higher proportion of infected plants were observed in the plots where viruliferous aphids were released (56-98%), compared to the control (2-58%) and aphid control plots (7-78%), except in 2021, when there were no statistical differences among the aphid control and release weeks 1, 2, and 4. CLRDV incidence in both control treatments in 2021 was notably high, especially when compared to values found in 2019 and 2020. Overall, these results suggest CLRDV was successfully transmitted to caged plants during the prescribed time treatment. Detection of CLRDV in both control treatments indicated additional virus spread occurred late-season after cages were removed even though aphid infestations were not observed during the study. The timing and magnitude of natural spread cannot be determined with more frequent testing because there are several weeks of variation when new infections can be detected. This is a current limitation in CLRDV research that was not anticipated due to our lack of knowledge regarding the timing of CLRDV spread at the time of this study (discussed below).

Table 1. Final cotton leafroll dwarf virus incidence reported as the average proportion (± standard error) of plants infected per plot, by year and treatment

Treatment	Final Virus Incidence		
	2019[z]	2020	2021
Aphid Control	**0.10 (0.05) c**	**0.07 (0.04) d**	**0.78 (0.07) bc**
Control	**0.02 (0.02) c**	**0.24 (0.07) c**	**0.58 (0.08) c**
Week 1	**N/A[y]**	**0.76 (0.07) b**	**0.81 (0.06) ab**
Week 2	**0.56 (0.08) b**	**0.84 (0.06) ab**	**0.86 (0.06) ab**
Week 3	**0.98 (0.02) a**	**0.96 (0.03) a**	**0.95 (0.03) a**
Week 4	**0.73 (0.07) ab**	**0.91 (0.04) ab**	**0.86 (0.06) ab**
Significance of Main Effects[x]			
Treatment	$F_{4,190}=12.31, p<0.0001$	$F_{5,231}=14.47, p<0.0001$	$F5{,}231=3.40, p<0.0055$
Block	$F_{3,190} = 4.12, p=0.0073$	$F_{3,231} = 2.47, p=0.0624$	$F3{,}231 = 1.40, p=0.2427$

[z]Means with the same letter in the column are not significantly different ($p = 0.05$, Tukey-Kramer's method).

[y]Plots were not infested before the one to two true-leaf stage in 2019.

[x]Abbreviations: F: F statistic; p: p-value associated with the F-statistic.

Plant Mapping and Symptoms. Significant differences among treatments were only observed in plant height and retention of first position bolls, in 2019. Plants from week 2 release were smaller than plants from all other treatments, except week 3 release. The number of bolls in the first position of plants from week 2 release were also greater than in plants from week 3 release (Table 2). At the time plant mapping occurred, no apparent foliar symptoms or visual differences among plots were observed (data not shown).

Lint Yield and Quality. Significant difference in yield was observed only in 2019, when week 2 release plots produced less lint than the control, with an average reduction of 633 kg ha^{-1} (Fig. 1A; $F_{4,13.04} = 1.50$, $p = 0.2598$). Although not statistically significant, a numerical trend for yield increase was observed between release weeks 2 and 4 in 2019. In

Table 2. The average (± standard error) of plant mapping measurements per plot, by year and treatment

Treatment	Plant Height (inches)[z]	Total Number of Nodes	1st Position Bolls	2nd Position Bolls	Sum of 1st and 2nd position bolls
			2019		
Aphid Control	45.34 (1.01) a	19.37 (0.41)	7.17 (0.51) ab	5.04 (0.56)	11.84 (0.98)
No Aphids	47.77 (2.14) a	19.78 (0.87)	6.56 (1.08) ab	5.34 (1.19)	11.71 (2.08)
Week 2	41.15 (0.67) b	19.01 (0.27)	8.44 (0.34) a	4.83 (0.37)	13.18 (0.65)
Week 3	43.67 (2.14) ab	20.31 (0.87)	6.52 (1.08) b	3.81 (1.19)	10.24 (2.08)
Week 4	44.61 (0.73) a	19.09 (0.30)	6.99 (0.37) ab	4.18 (0.42)	11.00 (0.71)
Significance of Main Effects[y]					
Treatment	$F_{4,185} = 5.54$, $p = 0.0003$	$F_{4,185} = 0.72$, $p = 0.5796$	$F_{4,185} = 1.85$, $p = 0.0243$	$F_{4,174} = 0.69$, $p = 0.5967$	$F_{4,185} = 1.52$, $p = 0.1993$
Block	$F_{3,185} = 18.58$, $p = 0.0001$	$F_{3,185} = 9.62$, $p = 0.0001$	$F_{3,185} = 0.75$, $p = 0.5258$	$F_{3,174} = 3.50$, $p = 0.0168$	$F_{3,185} = 2.28$, $p = 0.0806$
			2020		
Aphid Control	57.01 (1.47)	23.19 (0.58)	10.73 (0.67)	8.08 (0.81)	18.79 (1.38)
No Aphids	59.27 (0.72)	21.77 (0.29)	10.09 (0.33)	7.27 (0.40)	17.16 (0.68)
Week 1	59.03 (0.89)	21.41 (0.35)	9.60 (0.41)	6.21 (0.51)	15.42 (0.84)
Week 2	60.70 (1.01)	22.39 (0.40)	10.27 (0.46)	7.56 (0.56)	17.82 (0.95)
Week 3	62.09 (1.80)	22.08 (0.71)	9.05 (0.83)	6.54 (0.99)	15.41 (1.69)
Week 4	59.46 (1.26)	21.61 (0.50)	9.40 (0.58)	6.86 (0.70)	16.20 (1.19)
Significance of Main Effects					
Treatment	$F_{5,233} = 1.35$, $p = 0.2435$	$F_{5,233} = 1.79$, $p = 0.1165$	$F_{5,233} = 0.96$, $p = 0.4428$	$F_{5,227} = 1.16$, $p = 0.3307$	$F_{5,233} = 1.42$, $p = 0.2188$
Block	$F_{3,233} = 6.44$, $p = 0.0003$	$F_{3,233} = 2.34$, $p = 0.0745$	$F_{3,233} = 2.62$, $p = 0.0514$	$F_{3,227} = 0.89$, $p = 0.4450$	$F_{3,233} = 2.12$, $p = 0.0981$
			2021		
Aphid Control	44.24 (1.28)	24.74 (0.53)	7.82 (0.48)	4.63 (0.50)	11.28 (0.89)
No Aphids	40.43 (1.10)	23.54 (0.45)	7.09 (0.41)	3.86 (0.49)	9.61 (0.77)
Week 1	43.89 (1.40)	23.40 (0.58)	7.91 (0.56)	4.12 (0.54)	10.50 (0.98)
Week 2	41.42 (1.50)	23.19 (0.62)	6.36 (0.56)	2.72 (0.52)	8.81 (1.05)
Week 3	44.96 (2.43)	24.32 (1.00)	8.03 (0.91)	3.20 (0.85)	10.94 (1.70)
Week 4	42.35 (1.51)	24.16 (0.62)	7.47 (0.56)	4.45 (0.58)	11.62 (1.06)
Significance of Main Effects					
Treatment	$F_{5,218} = 1.60$, $p = 0.1622$	$F_{5,212} = 1.10$, $p = 0.3615$	$F_{5,216} = 1.23$, $p = 0.2968$	$F_{5,173} = 1.82$, $p = 0.116$	$F_{5,218} = 1.15$, $p = 0.3345$
Block	$F_{3,218} = 8.62$, $p = 0.0001$	$F_{3,212} = 5.71$, $p = 0.0009$	$F_{3,216} = 4.24$, $p = 0.0061$	$F_{3,173} = 2.86$, $p = 0.0385$	$F_{3,218} = 4.80$, $p = 0.0029$

[z]Means with the same letter in the column are not significantly different (*p* = 0.05, Tukey-Kramer's method).

[y]Abbreviations: F: F statistic; *p*: *p*-value associated with the F-statistic.

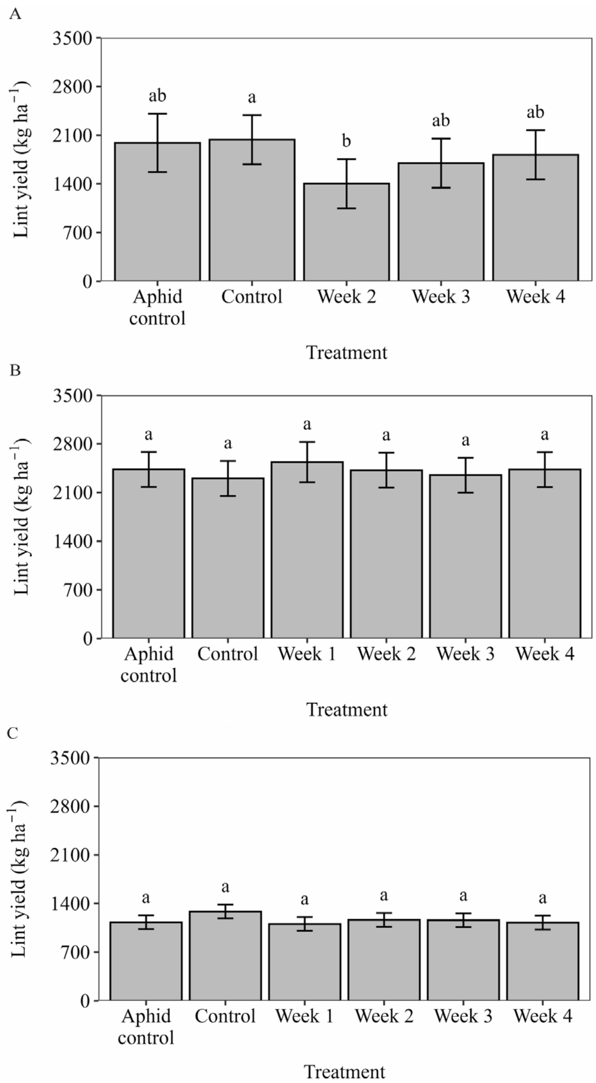

Figure 1. The average (± standard error) of lint yield (kg ha-1) produced per treatment in 2019 (A), 2020 (B), and 2021 (C). Means with the same letter are not significantly different (p = 0.05, Tukey-Kramer's method

2020 and 2021, there were no significant differences in yield among treatments (Fig. 1B; $F_{5,17} = 0.09$, p = 0.9929 and 1C; $F_{5,15} = 0.41$, p = 0.8351).

There were no significant differences among treatments observed for any lint quality metric in 2019 and 2021 (Table 3). In 2020, fiber length of the control treatment was significantly greater than all other treatments. The average micronaire of the aphid control treatment was significantly lower than all release weeks, whereas the control treatment differed only from the week 2 release, with a reduction of 4% (Table 3). No differences were consistent with any treatment effect.

Cage-Related and Environmental Effects. Cages have been reported to alter growing conditions of crop plants in research studies. In this study, some differences were observed between the environments inside and outside the cages. The caged environment was significantly warmer and had reduced PAR in all three years, whereas lower RH was observed in 2020 and 2021 (Table 4). Soil moisture, measured at 15 cm depth, showed no significant differences between environments (Table 4). Compared to the border rows, cotton generally grew taller in the cages until plants were removed, but by harvest there were no visual differences between cotton in caged plots and cotton in the four border rows. Differences in yield due to cages were observed only in 2020, with a significant average increment of 53.8% for cages, compared to the yield of border rows (1497.48 kg ha^{-1}) (2019: $F_{5,16.04} = 2.35$, $p = 0.0879$; 2020: $F_{7,23}$ = 2.02, $p = 0.0968$, and 2021: $F_{6,18} = 0.69$, $p = 0.6569$).

Weather variables also were examined to quantify variation among the three years of this trial. The average daily temperature in the caged plots was significantly higher in 2019, whereas no differences were observed between 2020 and 2021 (Fig. 2A; $F_{2,281} = 22.03$, $p = 0.0001$). Soil moisture content differed significantly among the three years, with the lowest value observed in 2019, followed by 2020 and 2021 (Fig. 2B; $F_{2,281} = 103.21$, $p = 0.0001$). There were no significant differences in PAR ($F_{2,136} = 1.93$, $p = 0.1489$) and RH ($F_{2,143} = 0.38$, $p = 0.6824$) across the three years of the trial.

DISCUSSION

With rising input costs and increasing pressure to improve water-use efficiency, irrigation management has become a critical factor in sustaining cotton profitability. Optimizing irrigation not only influences yield potential but affects crop maturity, production costs, and pest management decisions. This research provides insight into how soil moisture sensor-based irrigation strategies can improve efficiency and support more economical cotton production in Mississippi.

Based on soil moisture sensors, soil moisture was adequate for growth and development of cotton during early to mid-season. In both years, irrigation was not triggered until the third week of bloom with two events in 2022 and one in 2023. However, noticeable yield differences were found between irrigated and

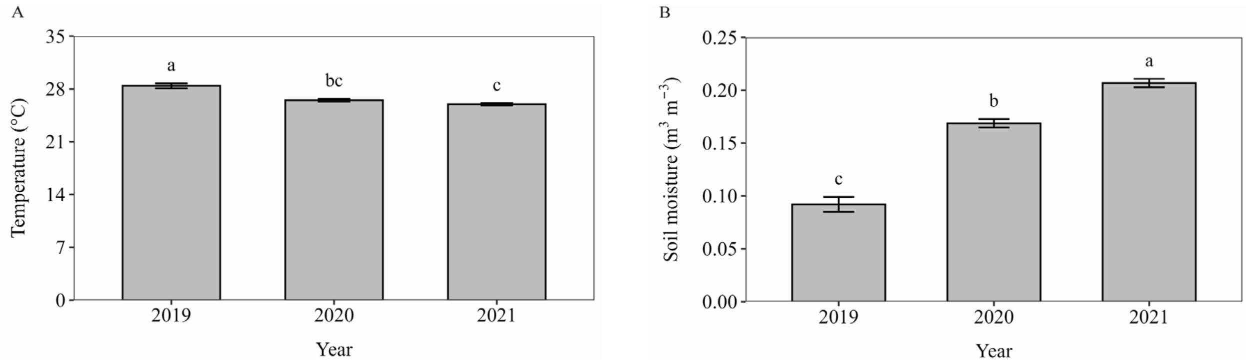

Figure 2. The average (± standard error) of daily temperature (A) and soil moisture content (B), measured inside cages. Numbers are averaged across cages. Means with the same letter are not significantly different (p = 0.05, Tukey-Kramer's method).

Table 3. The average (± standard error) of lint quality measurements per plot, by year and treatment

Treatment	Length (mm)[z]	Micronaire	Uniformity (%)	Strength (g per textile)
		2019		
Aphid Control	1.21 (0.01)	4.56 (0.17)	82.46 (0.69)	31.73 (0.45)
Control	1.22 (0.01)	4.62 (0.14)	83.24 (0.58)	31.98 (0.39)
Week 2	1.21 (0.01)	4.52 (0.14)	83.14 (0.58)	31.70 (0.39)
Week 3	1.21 (0.01)	4.65 (0.14)	83.04 (0.58)	31.80 (0.39)
Week 4	1.22 (0.01)	4.60 (0.14)	82.64 (0.58)	32.20 (0.39)
Significance of Main Effects[y]				
Treatment	$F_{4,14} = 0.42$, $p = 0.7918$	$F_{4,13.07} = 0.45$, $p = 0.7726$	$F_{4,13.06} = 0.97$, $p = 0.4549$	$F_{4,14} = 0.28$, $p = 0.8885$
		2020		
Aphid Control	1.23 (0.01) b	4.03 (0.05) c	82.14 (1.67)	30.93 (0.23)
Control	1.29 (0.01) a	4.08 (0.05) bc	82.72 (1.67)	30.65 (0.23)
Week 1	1.25 (0.01) b	4.23 (0.06) ab	82.67 (1.82)	30.42 (0.27)
Week 2	1.25 (0.01) b	4.25 (0.05) a	82.54 (1.67)	30.38 (0.23)
Week 3	1.24 (0.01) b	4.18 (0.05) ab	82.02 (1.67)	30.70 (0.23)
Week 4	1.25 (0.01) b	4.20 (0.05) ab	82.19 (1.67)	30.95 (0.23)
Significance of Main Effects				
Treatment	$F_{5,17} = 6.20$, $p = 0.0019$	$F_{5,17} = 2.99$, $p = 0.0410$	$F_{5,16.01} = 0.13$, $p = 0.9822$	$F_{5,14} = 1.02$, $p = 0.4419$
Block				$F_{5,14} = 7.84$, $p = 0.0026$
		2021		
Aphid Control	1.22 (0.01)	3.53 (0.10)	83.03 (0.26)	31.35 (0.43)
Control	1.24 (0.01)	3.83 (0.10)	83.45 (0.26)	31.83 (0.43)
Week 1	1.23 (0.01)	3.83 (0.10)	83.40 (0.26)	32.18 (0.43)
Week 2	1.23 (0.01)	3.58 (0.10)	83.23 (0.26)	32.20 (0.43)
Week 3	1.24 (0.01)	3.93 (0.10)	83.93 (0.26)	31.65 (0.43)
Week 4	1.23 (0.01)	3.80 (0.10)	83.30 (0.26)	32.00 (0.43)
Significance of Main Effects				
Treatment	$F_{5,18} = 1.11$, $p = 0.3895$	$F_{5,18} = 2.67$, $p = 0.0566$	$F_{5,18} = 1.49$, $p = 0.2506$	$F_{5,1}8 = 0.58$, $p = 0.7136$

[z]Means with the same letter in the column are not significantly different ($p = 0.05$, Tukey-Kramer's method).

[y]Abbreviations: F: F statistic; *p*: *p*-value associated with the F-statistic.

Table 4. The average (± standard error) daily temperature (°C), photosynthetically active radiation (PAR, µmol m^2 s^{-1}), relative humidity (RH, %) and soil moisture content (m^3 m^{-3}) evaluated outside (border) and inside (caged) cages, by year

Treatment	Temperature[z]	PAR	RH	Soil Moisture
		2019		
Border	27.50 (0.23) b	520.60 (15.6) a	80.07 (0.96) a	0.071 (0.00480)
Caged	28.52 (0.23) a	410.05 (15.6) b	78.52 (0.96) a	0.070 (0.00480)
Significance of Main Effects[y]				
Treatment	$F_{1,66}$ = 9.99, p = 0.0024	$F_{1,66}$ = 25.10, p = 0.0001	$F_{1,66}$ = 1.32, p = 0.2547	$F_{1,66}$ = 0.04, p = 0.8339
		2020		
Border	25.72 (0.25) b	495.84 (22.05) a	80.80 (0.92) a	0.172 (0.00443)
Caged	27.08 (0.22) a	334.80 (22.05) b	77.20 (0.92) b	0.171 (0.00443)
Significance of Main Effects				
Treatment	$F_{1,84}$ = 16.48, p = 0.0001	$F_{1,86}$ = 26.67, p = 0.0001	$F_{1,66}$ = 9.99, p = 0.0024	$F_{1,96}$ = 0.04, p = 0.8392
		2021		
Border	25.25 (0.2) b	464.84 (16.75) a	81.74 (1.08) a	0.204 (0.00651)
Caged	26.70 (0.2) a	374.67 (16.75) b	78.15 (1.08) b	0.212 (0.00651)
Significance of Main Effects				
Treatment	$F_{1,124}$ = 27.38, p = 0.0001	$F_{1,120}$ = 14.49, p = 0.0002	$F_{1,124}$ = 5.47, p = 0.0212	$F_{1,124}$ = 0.73, p = 0.3936

[z]Means with the same letter in the column are not significantly different (p = 0.05, Tukey-Kramer's method).

[y]Abbreviations: F: F statistic; p: p-value associated with the F-statistic.

non-irrigated plots. A two-year average of 265 kg ha^{-1} increase was shown using a soil moisture threshold of 90 centibars. Results from this study agree with Bryant et al. (2023), suggesting producers not using soil moisture sensors to trigger irrigations in cotton are likely applying excessive water with irrigation.

In the current experiment, the effect of irrigation, level of fruit removal, and week of removal differed between NAWF measurements taken among treatments. Prior to removal, the previous irrigation event likely drove the increase in NAWF in the irrigated plots compared to non-irrigated plots. However, it is important to note that both irrigated and non-irrigated plots had approached cutout with less than or equal to five NAWF before square removal was initiated in week four. In weeks five and six, NAWF in irrigated plots was significantly greater than in non-irrigated plots. However, in both weeks of bloom, all plots remained below five NAWF regardless of treatment. The amount of heat units accumulated in previous weeks most likely promoted cutout in both years (Fig. 1). Although plants remained cutout, the increase in nodes resulting from 100% removal during week four paired with an irrigated environment indicates plant maturity was impacted. However, 50% square removal during both weeks did not affect maturity compared to 100% square removal. Using NAWF counts to compare 0 to 50% square removal, limited maturity delays were observed regardless of the week of bloom square removal was imposed.

At first cracked boll, plant characteristics differed among irrigation practice. Irrigated plots had greater plant heights, total nodes per plant, and NACB than non-irrigated plots. Neither week of removal nor level of removal affected plant height, total nodes, or NACB when measured at first cracked boll. Because NACB measurements were taken upwards along the mainstem to the uppermost harvestable boll, this indicates that there were no significant differences in presence of harvestable bolls between removal levels.

Despite delays in maturity, no significant yield penalty was observed with any square removal level imposed during the fourth or fifth week of bloom. Results from this experiment indicate that terminating insecticides at the fourth week of bloom can result in similar yields and reduced expenses compared to season-long control. Additionally, monitoring soil moisture with sensors in this study demonstrated the potential to reduce irrigation expenses while maintaining optimum yields.

When incorporating these farming practices, it will be important to consider the environment and pest population year to year. In a study conducted during 2001, 2002, 2004, 2005, and 2007 using drip irrigation and manual infestations of laboratory-grown tarnished plant bugs, Teague et al. (2008) found significant yield penalties during 2004 from damage to upper canopy and outside bolls in high-yielding cotton after cutout. These results contradict findings from other years but are important to consider when evaluating current insecticide termination timings. Ultimately, with the current cost for tarnished plant bug control, $28.70 per hectare, these results indicate losses after cutout contribute small amounts to yield and insecticide applications might not pay for themselves beyond the fourth week of bloom.

Results from this research demonstrate that soil moisture sensor-based irrigation at a 90-centibar threshold increased lint yields by an average of 265 kg ha^{-1} compared to non-irrigated cotton, while reducing unnecessary water applications. Square removal treatments during bloom affected plant growth and maturity but did not result in yield penalties, supporting current insecticide termination guidelines for tarnished plant bug management. Taken together, these findings suggest that adopting soil moisture sensors can improve water-use efficiency, reduce input costs, and maintain profitable yields. Producers are encouraged to incorporate sensor-based irrigation strategies as a practical tool for optimizing both economic and pest management decisions in Mississippi cotton production. For further validation of this study, large scale experiments need to be conducted across the midsouthern U.S.

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