

**COTTON LEAFROLL DWARF VIRUS INCIDENCE AS IMPACTED BY PLANTING DATE AND
COTTON CULTIVAR IN ALABAMA**

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Abstract

In 2020 and 2021, research trials were conducted at the Brewton Agricultural Research Unit (BARU) and Prattville Agricultural Research Unit (PARU), AL to assess the impact of planting date and cultivar selection on the incidence of Cotton leafroll dwarf disease (CLRDD) caused by the *Cotton leafroll dwarf virus* (CLRDV). The experimental design was a split plot with planting date as the main plot and cotton cultivar as the split plot treatment. Cotton cultivars included Deltapine 1646 B2XF (DP 1646), PhytoGen 480 W3FE (PHY480), PhytoGen 500 W3FE (PHY500), DynaGro 3615 B3XF (DG 3615), Deltapine 359 (Pima variety; DP359), and an experimental breeding line (EXP1). Cotton plants were rated in 2-wk intervals for CLRDD beginning at 30 days after planting (DAP) and ending at 120 DAP. One mature leaf from a symptomatic and an asymptomatic plant in each plot were collected in 4-wk intervals and tested for CLRDV using PCR. Incidence was highest in BARU in 2020, but highest in PARU 2021. CLRDV incidence was higher in late planted cotton when compared to the early planted cotton at both locations in 2020 and 2021, but only significantly at BARU in 2020 and PARU 2021. For cotton cultivar, PHY 480, CLRDV incidence was significantly lower in the early planted vs. late planted cotton in both years. At both locations, PHY480 and DP1646 had the highest incidence of CLRDV and DG3615 had the lowest incidence of CLRDV in 2020 and 2021. In terms of the impact of CLRDV on lint yield, it is still difficult to draw conclusions from this data set as planting date alone can also impact yield. However, these results do indicate that planting cotton early could reduce the incidence of CLRDV and potentially yield in high-risk areas.

Introduction

Cotton (*Gossypium hirsutum*) is among the most important fiber crops worldwide, with the United States (U.S.) being the third largest cotton producer and leading cotton exporter. In 2020, the U.S. harvested 8.3 million acres of cotton valued at approximately \$4.7 billion (USDA-NASS 2020). In the U.S., Alabama is ranked 5th in cotton production and harvested 446,000 acres valued at approximately \$223 million in 2020 (USDA-NASS 2020). In 2017, cotton leafroll dwarf disease (CLRDD), caused by the *Cotton leafroll dwarf virus* (CLRDV, genus *Polevirus*, family *Luteoviridae*), was first identified in the U.S. in Alabama (Avelar et al. 2019a). In 2017, CLRDD incidence and yield losses occurred on approximately 50,585 ha of CLRDV infected cotton in south Alabama. Based on symptoms alone, incidence ranged from 3-30%, and yield losses were estimated at an average of 560 kg/ha across this area, which was valued at \$19 million dollars (Avelar et al. 2019a). CLRDV is transmitted by the cotton aphid (*Aphis gossypii*), which is ubiquitous in the U.S. The virus is persistent and circulative in the vector, is reported to be transmitted by viruliferous aphids in as little as 40 sec and can be transmitted for up to 12 days (Michelotto and Busoli 2003, 2009). The widespread abundance of aphids and rapid transmission of CLRDV, combined with the propensity for aphids to disperse long distances on wind currents appear to have facilitated the spread of CLRDV across the southern U.S.

cotton belt in subsequent years (Aboughanem-Sabanadzovic et al. 2019; Alabi et al., 2019; Ali and Mokhrari, 2020; Ali et al., 2020; Faske et al. 2020; Iriarte et al. 2020; Price et al. 2020; Tabassum et al. 2019; Thiessen et al., 2020; Wang et al. 2020).

Cotton blue disease (CBD), caused by a closely related, but geographically different strain of CLRDV, was initially described from infected cotton originating in Africa around 1949 and more recently in Brazil (Correa et al. 2005), Argentina (Distefano et al. 2010), India (Mukherjee et al., 2012), Thailand (Sharman et al. 2015), and Timor-Leste (Ray et al. 2016). Cotton plants infected with this virus exhibit stunting due to internodal shortening, leaf rolling, petiole and vein reddening, distorted new growth, reduced flower and boll size, and sterility (Mukherjee et al. 2016). In 2006, a new strain of the virus was observed in fields of CBD-resistant cotton in Brazil. Virus infected plants exhibited mild symptoms of CBD such as red, withered leaves and accentuated verticality (Silva et al. 2015). The new strain was identified and subsequently named as the “atypical” CLRDV strain. Another outbreak was reported in 2009-2010 in Argentina, when CBD-resistant cultivars showed severe disease symptoms. Whole genome sequences of isolates from Alabama show that the U.S. strain of CLRDV is different from the typical and atypical strains found in Brazil and Argentina (Avelar et al. 2019b; Ramos-Sobrinho et al. 2021; Tabassum et al. 2021). In the U.S., it is currently unknown when infection occurs, the length of latency periods, disease progression and symptom development, the effect of environmental factors on disease, and yield impacts.

In terms of management, CBD has been controlled in Brazil through use of resistant cotton cultivars, excessive insecticide applications, and strict sanitation practices (Agrofoglio et al. 2019). There is currently no source of resistance to the CLRDV-US strain in commercially available cotton cultivars in the U.S, and research conducted in 2019 at Auburn University has demonstrated that resistant sources available in Brazil are susceptible to the CLRDV-US strain U.S (Hagan, *unpublished*). Insecticide applications targeting the aphid vector are not expected to reduce virus transmission as it occurs in under a minute and increasing the number of insecticide sprays is not economically viable in the U.S. (Hagan et al. 2019; Jacobson, *personal communication*; Greene, *personal communication*). In Alabama, planting earlier in the cotton production window has been recommended to producers in areas at high risk for infections as higher CLRDV incidence and disease severity has been associated with late planted Alabama cotton (Hagan et al. 2019). Surveys in Alabama and Georgia have found CLRDV in 23 different winter weed species and overwintering cotton stalks (Conner, *personal communication*; Sedhain et al. 2021). In the absence of resistant cultivars, management practices have focused on planting early, cotton stalk destruction, and winter weed control in and around fields slated to be cropped to cotton (Hagan et al. 2019). However, additional research is needed to better understand yield impacts of CLRDD and establish effective management strategies. Thus, two trials were established at two AAES outlying research units in southwest and central Alabama to determine the impact of planting date and cultivar on CLRDD incidence and cotton yield-related parameters.

Materials and Methods

In 2020 and 2021, CLRDV sentinel plots were established at the Brewton Agricultural Research Unit (BARU) in Brewton, AL and the Prattville Agricultural Research Unit (PARU) in Prattville, AL. The experimental design was a split plot with planting date as the main plot and cotton cultivar as the split plot treatment. In 2022, cotton cultivars included Deltapine 1646 B2XF (DP1646), PhytoGen 480 W3FE (PHY480), DynaGro 3615 B3XF (DG3615), Deltapine 359 (Pima variety; DP359), and an experimental breeding line (EXP1). In 2021, cotton cultivars included DP1646, PHY480, PhytoGen 500 W3FE (Phy500), and DG3615. Planting dates were approximately May 1 and June 1 at BARU and PARU. Individual split plots consisted of four 20 ft rows on 3 ft centers arranged in four replications. Cotton was maintained according to the recommendations of the Alabama Cooperative Extension System. Beginning at 30 days after planting (DAP) and continuing at 2-wk intervals until 120 DAP, plants displaying symptoms of CLRDD were marked with a numbered and dated tag. The first or second mature leaf on the central leader terminal of each tagged plant was collected, individually bagged, transported on ice, and later tested using PCR for CLRDV. In addition, one mature leaf in the main terminal from a randomly selected plant in each plot was collected at each sampling date and tested for the presence of CLRDV using PCR. Cotton plants in a 3 ft section in one of the outside rows of each plot were marked to record the number of open and unopen bolls, locked bolls, and rotten bolls immediately before harvest. Cotton was mechanically harvested, and samples collected for grading. Significance of planting date x PCR results and cultivar x PCR results, respectively, were determined using analysis of variance (ANOVA) followed by a pairwise comparison using Fisher’s protected least significant difference (LSD) test with a *P* value of 0.05. All statistical analyses were complete using the IBM SPSS Statistics (version 26; IBM Corp., Armonk, NY).

Results and Discussion

At BARU and PARU, CLRDV was confirmed in symptomatic cotton within 45 DAP and 60 DAP in 2020 and 2021, respectively. All cotton cultivars tested positive for CLRDV and CLRDD incidence (% symptomatic plants confirmed by PCR) varied by location and cultivar in both 2020 and 2021 (Fig. 1). In 2020, CLRDV incidence was greatest in southwest Alabama at BARU and declined as moved toward central Alabama at PARU (Fig. 1A). Surprisingly, the opposite was observed in 2021 (Fig. 1B). This variation in incidence by location and year is likely due to the low aphid pressure observed at BARU (data not shown). In both 2020 and 2021, PHY480 had the highest incidence of CLRDV and DG3615 had the lowest at both locations.

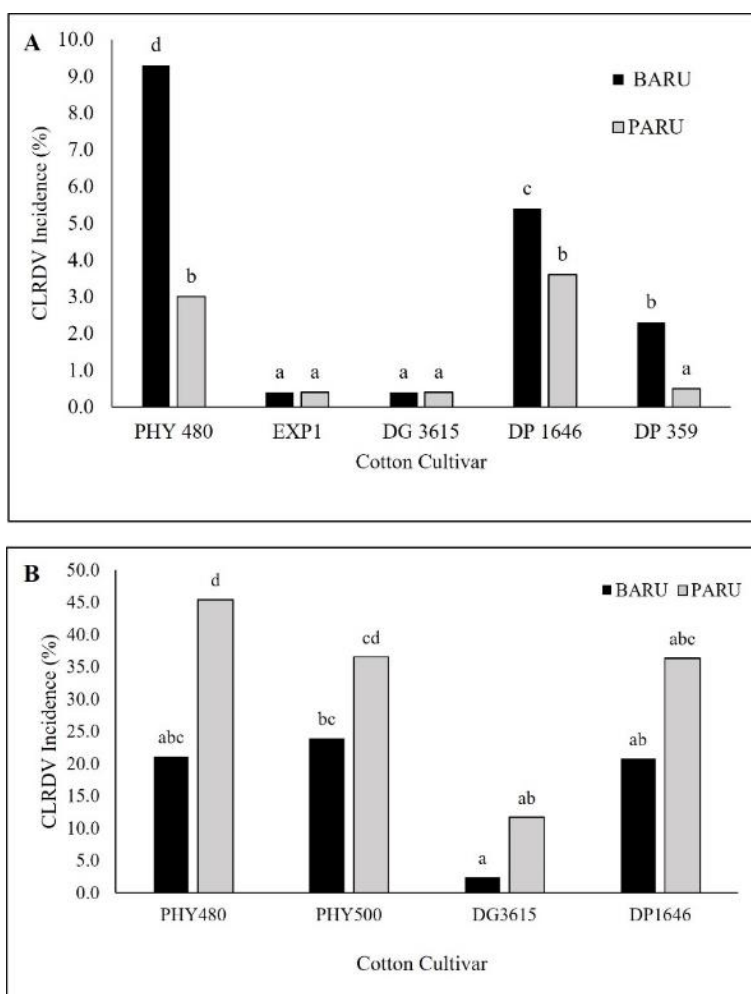


Figure 1. Cotton leafroll dwarf disease (CLRDV) incidence (% symptomatic plants confirmed by PCR) varied by location (Brewton Agricultural Research Unit- BARU; Prattville Agricultural Research Unit- PARU) and cotton cultivar (Deltapine 1646 B2XF (DP1646); PhytoGen 480 W3FE (PHY480); PhytoGen 500 W3FE (PHY500) DynaGro 3615 B3XF (DG3615); Deltapine 359 (Pima variety; DP359); and an experimental breeding line (EXP1)). A *P* value of 0.05 was used in the IBM SPSS least significant difference statistical analysis. **A.** 2020, and **B.** 2021.

At BARU, planting date had significant impact on CLRDV incidence in 2020, but not in 2021 (Figure 2). In 2020, the later planted cotton (June 1st) had significantly higher CLRDV incidence when compared to the early planted cotton (Fig. 2A). Although of numerical increase in CLRDV incidence was observed the second planting date in 2021, the difference was not significant (Fig. 2B). At PARU, planting date had significant impact on CLRDV incidence in 2021, but not in 2020 (Figure 2). In 2021, the later planted cotton (June 1st) had significantly higher CLRDV incidence when compared to the early planted cotton (Fig. 2B). Although of numerical increase in CLRDV incidence was observed the second planting date in 2020, the difference was not significant (Fig. 2A). In both 2020 and 2021, planting data had the most impact at the locations with the highest CLRDV incidence, which was BARU and PARU, respectively.

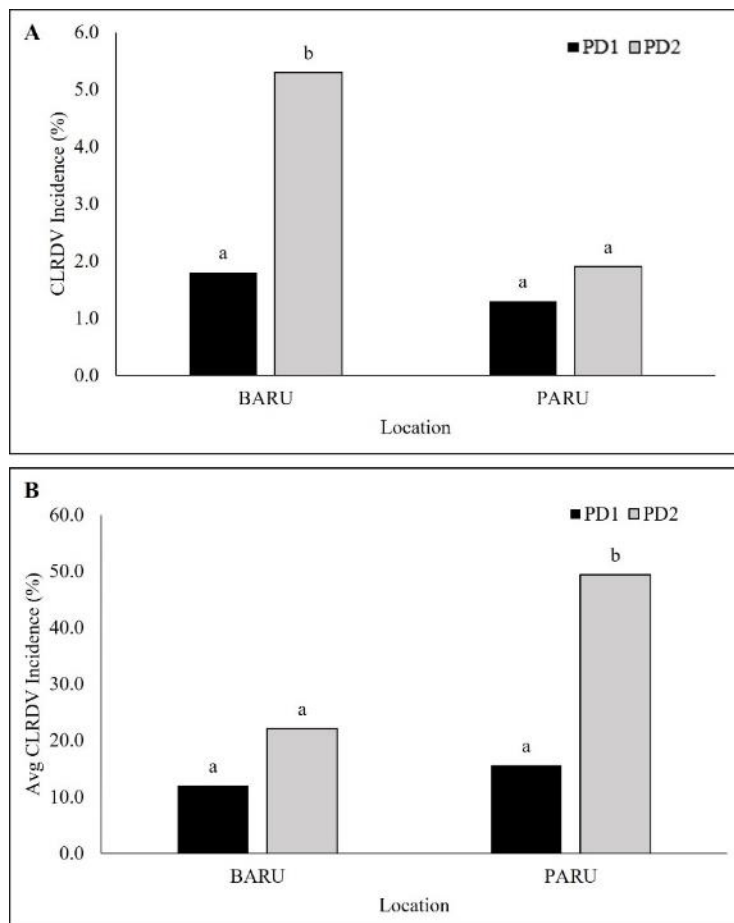


Figure 2. Cotton leafroll dwarf disease (CLRVD) incidence (% symptomatic plants confirmed by PCR) as influenced by planting date at Brewton Agricultural Research Unit (BARU) and Prattville Agricultural Research Unit (PARU). A *P* value of 0.05 was used in the IBM SPSS least significant difference statistical analysis. **A.** 2020, and **B.** 2021.

In 2020, the impact of planting date and CLRDD on cultivar on yield-related parameters at BARU and PARU can be seen in Tables 1 and 2, respectively. When comparing planting dates, a decrease in the number of open bolls and yield was observed for all cotton cultivars as the total number of positive CLRVD plants increased in the June-planted cotton at both locations. Although planting early can reduce the yield impacts of CLRDD, it has been known to increase the occurrence of boll rot and hardlock in South Alabama as seen in the BARU trial (Table 1). However, this is typically not the case in central Alabama as seen in the PARU trial (Table 2). However, the increase in hardlock and boll rot did not negatively impact yield in the first planting date at BARU.

In terms of overall cultivar performance, DP359 performed poorly in terms of yield and the number of open bolls at both locations (Tables 1 and 2). This is unsurprising as PIMA cotton cultivars are not typically grown in Alabama for this reason. EXPI and DG3615 had the highest yields in the first planting date at BARU and PARU. Despite having significantly higher CLRDD incidence, DP1646 still performed well in terms of yield in the first planting date at both locations. However, this was not the case for PHY480, which had significantly lower yields compared to EXP1, DG3615, and DP1646 in the first planting date at BARU (Table 1). At PARU, PHY480 also had significantly lower yields when compared to DG3615 and DP1646 in the first planting date (Table 2).

Table 1. Impact of CLRDD (as indicated by CLRDV virus presence) on the number of open, unopen, locked, and rotted bolls and yield (lbs./A) by planting date and cultivar at the Brewton Agricultural Research Unit in Brewton, AL in 2020.

Planting Date	Cultivar	Total # of Positives	Open Bolls	Unopen Bolls	Locked Bolls	Rotten Bolls	Yield (lbs./A)
May 1 st	PHY480	24.0	50.3 bc ^z	1.0 a	20.3 b	2.8 ab	1296 c
	EXP1	1.0	68.5 d	0.0 a	16.3 ab	1.3 a	1805 e
	DG3615	1.0	53.5 cd	0.0 a	11.0 ab	0.5 a	1807 e
	DP1646	7.0	49.3 bc	0.5 a	15.3 ab	1.0 a	1765 e
	DP359	2.0	39.8 bc	0.0 a	12.3 ab	4.8 b	901 b
June 1 st	PHY480	50.0	37.0 abc	1.0 a	13.0 ab	1.0 a	1170 c
	EXP1	2.0	42.8 bc	2.3 a	7.8 a	1.8 a	1532 d
	DG3615	2.0	34.5 ab	5.0 b	8.0 a	1.0 a	1244 c
	DP1646	36.0	46.3 bc	2.0 a	9.5 a	0.8 a	1345 cd
	DP359	16.0	19.5 a	7.5 b	17.0 ab	3.8 b	450 a

^z Means followed by the same letter are not significantly different according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).

Table 2. Impact of CLRDD (as indicated by CLRDV virus presence) on the number of open, unopen, locked, and rotted bolls and yield (lbs./A) by planting date and cultivar at the Prattville Agricultural Research Unit in Prattville, AL in 2020.

Planting Date	Cultivar	Total # of Positives	Open Bolls	Unopen Bolls	Locked Bolls	Rotten Bolls	Yield (lbs./A)
May 1 st	PHY480	8.0	56.5 bc	0.0 a	18.5 ab	1.3 a	1697 c
	EXP1	1.0	65.0 c	0.0 a	8.3 a	2.5 a	1924 cd
	DG3615	3.0	57.5 bc	0.3 a	16.5 ab	2.3 a	2173 d
	DP1646	7.0	67.3 c	0.3 a	16.3 ab	2.3 a	2151 d
	DP359	5.0	63.3 c	1.0 ab	22.3 b	3.5 a	953 a
June 1 st	PHY480	35.0	50.5 abc	1.5 ab	14.8 ab	3.0 a	1107 b
	EXP1	3.0	53.3 abc	0.5 a	20.0 ab	4.5 a	1134 b
	DG3615	4.0	38.0 ab	1.3 ab	15.5 ab	3.8 a	1284 b
	DP1646	26.0	52.5 abc	3.3 ab	16.8 ab	4.0 a	1239 b
	DP359	5.0	35.5 a	13.0 b	8.5 a	4.3 a	626 ab

^z Means followed by the same letter are not significantly different according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).

In 2021, the impact of planting date and CLRDD on cultivar on yield-related parameters at BARU and PARU can be seen in Tables 3 and 4, respectively. When comparing the planting dates at BARU, a significant increase in the number of open bolls was observed in PHY480 and PHY500. However, planting date did not significantly impact the number of unopen bolls, locked, or rotted bolls. It also did not significantly impact yield. At PARU, planting date only had a significant impact on the number of locked bolls (Table 4). The later planted cotton had significantly lower number of locked bolls for PHY480, PHY500, and DG3615 than the early planted cotton. However, planting date did not have a significant impact on the number of open, unopen, or locked bolls, or yield.

Table 3. Impact of CLRDD (as indicated by CLRDV virus presence) on the number of open, unopen, locked, and rotted bolls and yield (lbs./A) by planting date and cultivar at the Brewton Agricultural Research Unit in Brewton, AL in 2021.

Planting Date	Cultivar	Total # of Positives	Open Bolls	Unopen Bolls	Locked Bolls	Rotten Bolls	Yield (lbs./A)
May 1 st	PHY480	3	9.25 ab	1.5 a	45 b	1.75 a	1123 cd
	PHY500	3	5.75 a	0.5 a	50.5 b	2 a	1063 cd
	DP1646	3	16.25 bc	1.75 a	56 b	1.25 a	1441 e
	DG3615	0	7.5 ab	0.25 a	44.75 b	1.5 a	967 bc
June 1 st	PHY480	8	7.5 c	0.25 a	44.75 a	1.5 a	967 cd
	PHY500	9	25.5 bc	1.5 a	14.5 a	3.5 a	1184 ab
	DP1646	4	16.25 c	0.75 a	12 a	3.5 a	818 de
	DG3615	1	22.75 ab	0.75 a	8.75 a	1.25 a	1266 a

^z Means followed by the same letter are not significantly different according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).

Table 4. Impact of CLRDD (as indicated by CLRDV virus presence) on the number of open, unopen, locked, and rotted bolls and yield (lbs./A) by planting date and cultivar at the Prattville Agricultural Research Unit in Prattville, AL in 2021.

Planting Date	Cultivar	Total # of Positives	Open Bolls	Unopen Bolls	Locked Bolls	Rotten Bolls	Yield (lbs./A)
May 1 st	PHY480	2	38.25 a	2.75 a	25.25 c	2.5 a	3694 a
	PHY500	1	49.5 a	2.5 a	16 bc	1.5 a	3358 a
	DP1646	1	45.5 a	4.25 a	17 bc	1.5 a	4182 a
	DG3615	3	53.25 a	4 a	24 c	1.25 a	3756 a
June 1 st	PHY480	11	55.5 a	4.75 a	5.25 a	1.75 a	3817 a
	PHY500	5	57 a	3.75 a	5 a	2.5 a	4563 a
	DP1646	8	44.75 a	4.25 a	8.5 ab	3.75 a	4484 a
	DG3615	6	43.5 a	4 a	6.75 ab	2 a	3924 a

^z Means followed by the same letter are not significantly different according to Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).

Summary

CLRDV is now considered part of the environment and is widely distributed across the southern U.S. cotton belt. However, there is little research-based information on when infection occurs, the length of latency periods, disease progression, symptom development, the effect of environmental factors on disease, management strategies, and yield impacts. Yield losses from the typical CLRDV strain have been estimated between 68% and 80% in susceptible cultivars (Santos et al. 2004; Silva et al. 2008). In cotton cultivars resistant to the typical strain of CLRDV, yield losses of 13.4 to 21.5% were reported in those cultivars infected with the atypical strain of CLRDV (Galberi et al. 2017). In general, yield impacts caused by the U.S. strain (CLRDV-AL) have been difficult to quantify, except in extreme cases. In the absence of resistant cultivars, management practices have focused on early planting, cotton stalk destruction, and winter weed control in and around fields slated to be cropped to cotton (Hagan et al. 2019). In 2018, June-planted cotton fields displayed more severe symptoms of and had greater incidence of CLRDD when compared to May-planted cotton fields at multiple locations across Alabama (Hagan et al. 2019). However, additional research was needed to assess the impact of planting date on CLRDD incidence and yield impacts.

Thus, the goal of this study was to establish research trials at two different locations in southwest and central Alabama to determine the impact of planting date and cultivar on CLRDD incidence and cotton yield-related parameters. In 2020 and 2021, CLRDD incidence (% symptomatic plants confirmed by PCR) varied by location, cultivar, and year. Disease incidence in 2020 was highest in southwest Alabama and declined as you moved towards central Alabama. However, the opposite was observed in 2021. In both years, CLRDV incidence was highest in PHY480 followed by DP1646 at both locations. Disease incidence was significantly lower for DG3615 at both locations in 2020 and 2021.

However, no source of resistance to CLRDV-AL was identified as all six cotton cultivars tested positive for the virus at both locations. CLRDV incidence was higher, but not always significantly, in late-planted cotton when compared to early-planted cotton, which is consistent with the 2018 observations mentioned previously. Furthermore, higher number of open bolls and lint yield in the May-planted cotton when compared to the June-planted cotton. Although an increase in hardlock was observed in the early-planted cotton in southwest Alabama in 2020 and in central Alabama in 2021, this did not translate to yield losses. In terms of the impact of CLDRV on lint yield, it is still difficult to draw conclusions from this data set as planting date alone can also impact yield. However, these results do indicate that planting cotton early could reduce the incidence of CLRDV and potentially yield in high-risk areas.

Acknowledgements

Funding for this project was provided by Cotton Inc., the Southern IPM Center, and the Alabama Cotton Commission.

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