OCCURRENCE OF COTTON LEAFROLL DWARF DISEASE AS IMPACTED BY COTTON CULTIVAR AND PLANTING DATE

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Abstract

In 2017, Cotton leafroll dwarf disease (CLRDD), caused by the *Cotton leafroll dwarf virus* (CLRDV), was first identified in the U. S. in Alabama during 2017. Yield losses were estimated at an average of 560 kg/ha valued at $19 million dollars. In subsequent years, CLRDV has spread across the cotton belt as far west as Texas and north to Virginia. Trials were conducted at the Brewton Agricultural Research Unit (BARU) and Prattville Agricultural Research Unit (PARU) to assess the impact of planting date and cultivar selection on the incidence of CLRDD caused by CLRDV. Experimental design was a split plot with planting date (approximately May and June 1st) as the main plot and cotton cultivar as the split plot treatment. Cotton cultivars included Deltapine 1646 B2XF (DP1646), PhytoGen 480 W3FE (PHY480), DynaGro 3615 B3XF (DG3615), Deltapine 359 (DP359), and an experimental breeding line (EXP1). Beginning 30 days after planting (DAP) and continuing at 2-wk intervals until 105 DAP, samples were collected from plants displaying symptoms of CLRDD tested via PCR for the presence of CLRDV. One mature leaf from the main terminal from a randomly selected plant in each plot was also collected for PCR testing. At both locations, CLRDD incidence (% symptomatic plants confirmed by PCR) was significantly higher in PHY480 and DP1646 in both planting dates when compared to EXP1, DG3615, and DP359. CLRDD incidence was significantly higher in the 2nd planting date than the 1st planting date at BARU, but only numerically higher at PARU. Significantly higher yields were recorded for EXP1, DG3615, and DP1646 in the 1st planting date than the 2nd planting date at both locations. These results indicate that adjusting planting dates may have impact on CLRDD incidence and yield losses.

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 2019, the U.S. harvested 4.7 million hectares of cotton valued at approximately $5.8 billion (USDA-NASS 2019). In the U.S., Alabama is ranked 5th in cotton production and harvested 532,000 acres valued at approximately $396 million in 2019 (USDA-NASS 2019). In 2017, cotton leafroll dwarf disease (CLRDD), caused by the *Cotton leafroll dwarf virus* (CLRDV, genus *Polerovirus*, 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 (Tabassum et al. 2019; Aboughanem-Sabanadzovic et al. 2019; Alabi et al., 2019; Iriarte et al. 2020; Ali et al., 2020; Ali and Mokhrari, 2020; Price et al. 2020; Faske et al. 2020; Wang et al. 2020; Thiessen 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 (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). 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 virus commercially available 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-AL strain in the 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). 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 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). 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, 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. 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). 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 105 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 PROC GLIMMIX in SAS. Statistical analyses were done on rank transformations for non-normal values. Non-transformed data are reported. Means were separated using Fisher's protected least significant difference (LSD) test ($P \leq 0.05$).

Results and Discussion

At BARU and PARU, CLRDV was confirmed in symptomatic cotton within 45 DAP (data not shown). All five varieties tested positive for CLRDV and CLRDD incidence (% symptomatic plants confirmed by PCR) varied by location and cultivar (Fig. 1). Unsurprisingly, CLRDD Incidence was greatest in southwest Alabama at BARU and declined as moved toward central Alabama at PARU. Two cotton cultivars, PHY480 and DP1646, had significantly higher CLRDD incidence at both locations when compared to the other three cultivars at both locations. EXPI and DG3615 had the lowest incidence of CLRDD at both locations.

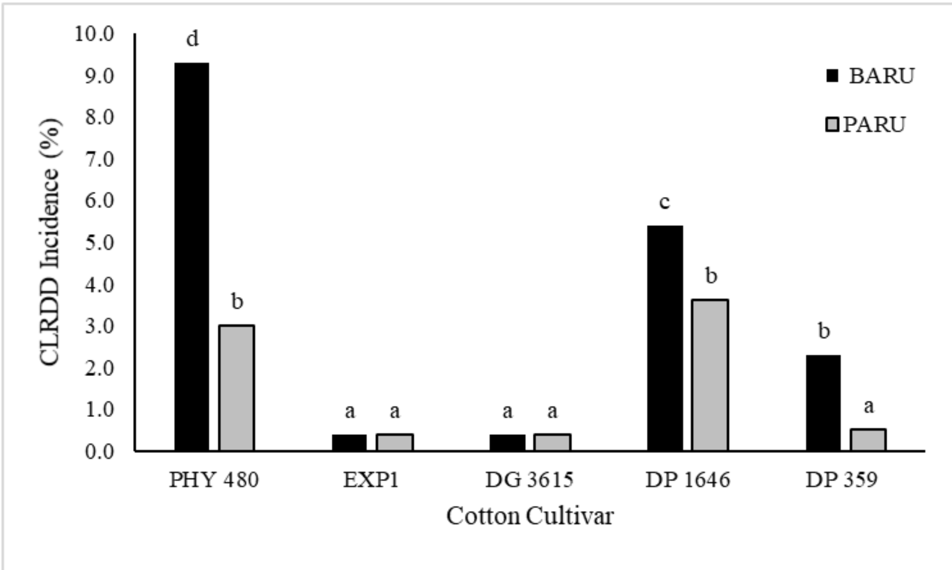

Figure 1. Cotton leafroll dwarf disease (CLRDD) incidence (% symptomatic plants confirmed by PCR) varied by location (Brewton Agricultural Research Unit- BARU; Prattville Agricultural Research Unit- PARU) and cotton cultivar (Deltapine 1646 B2XF (DP1646); PhytoGen 480 W3FE (PHY480); DynaGro 3615 B3XF (DG3615); Deltapine 359 (Pima variety; DP359); and an experimental breeding line (EXP1)).

At BARU, a significant planting date × cultivar interaction, which was recorded at all sampling dates except for August 26, indicated that CLRDD incidence (% symptomatic plants confirmed by PCR) differed by cultivars across planting dates (Table 1). In contrast, there was not a significant interaction between planting date and cultivar for any of the sampling dates at PARU, which could be due to reduced disease pressure at PARU (Table 2).

Table 1. CLRDD incidence (% symptomatic plants confirmed by PCR) as influenced by planting date, cultivar, and planting date x cultivar at the Brewton Agricultural Research Unit in Brewton, AL.

	Sample Date				
Source of Variation	28 Jul	12 Aug	26 Aug	8 Sep	% Dis
Planting Date	0.06[z]	18.69***	5.94*	14.11**	18.02**
Cultivar	0.92	12.46***	10.13***	5.31**	36.28***
Planting Date x Cultivar	1.69	8.31***	1.73	4.15*	6.95***

[z] Cumulative CLRDD incidence (% symptomatic plants confirmed by PCR) based on the total number of positive symptomatic plants at the end of the trial.
[y] Significance of *F* values at the 0.05, 0.01, and 0.001 levels is indicated by *, **, or ***, respectively.

Table 2. CLRDD incidence (% symptomatic plants confirmed by PCR) as influenced by planting date, cultivar, and planting date x cultivar at the Prattville Agricultural Research Unit in Prattville, AL.

	Sample Date			
Source of Variation	9 Jul	19 Aug	1 Sep	% Dis[z]
Planting Date	22.75***,[y]	1.03	0.01	0.92
Cultivar	5.15**	10.69***	9.72***	9.55***
Planting Date x Cultivar	1.60	0.55	2.03	2.29^

[z] Cumulative CLRDD incidence (% symptomatic plants confirmed by PCR) based on the total number of positive symptomatic plants at the end of the trial.
[y] Significance of *F* values at the 0.10, 0.05, 0.01, and 0.001 levels is indicated by ^, *, **, or ***, respectively.

As indicated by a significant planting date interaction, CLRDD incidence varied by planting data at BARU (Table 1). CLRDD incidence was significantly higher in the second planting date than the first planting date (Figure 2). Although there was not a significant planting date interaction at PARU, CLRDD incidence was numerically higher in the second planting date than in the first planting date (Table 1 and Figure 2). This indicates that planting date does impact CLRDD incidence as previously observed.

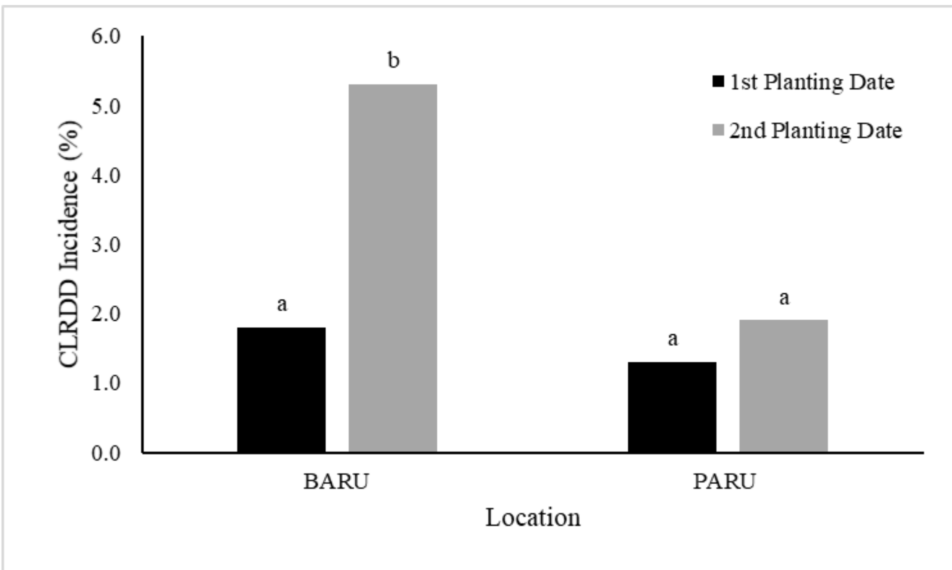

Figure 2. Cotton leafroll dwarf disease (CLRDD) incidence (% symptomatic plants confirmed by PCR) as influenced by planting date at Brewton Agricultural Research Unit (BARU) and Prattville Agricultural Research Unit (PARU)

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 planting dates, a decrease in the number of open bolls and yield was observed for all cotton cultivars as the total number of positive CLRDV 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 3). However, this is typically not the case in central Alabama as seen in the PARU trial (Table 4). 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 3 and 4). 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 3). At PARU, PHY480 also had significantly lower yields when compared to DG3615 and DP1646 in the first planting date (Table 4).

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.

Planting Date	Cultivar	Total # of Positives	Open Bolls	Unopen Bolls	Locked Bolls	Rotten Bolls	Yield (lbs/A)
May 1st	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 1st	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 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.

Planting Date	Cultivar	Total # of Positives	Open Bolls	Unopen Bolls	Locked Bolls	Rotten Bolls	Yield (lbs/A)
May 1st	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 1st	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 ($P \leq 0.05$).

Summary

Although CLRDD is now widely distributed across the southern U.S. cotton belt, 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 the U.S. strain (CLRDV-AL) have been difficult to quantify, except in extreme cases. However, Galberi et al. (2017) reported that U.S. cotton cultivars are highly susceptible to CDB. 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 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, CLRDD incidence (% symptomatic plants confirmed by PCR) varied by location and cultivar. Disease incidence was highest in southwest Alabama and declined as you moved towards central Alabama. CLRDD incidence was highest in PHY480 followed by DP1646 at both locations. Disease incidence was significantly lower for EXPI and DG3615 (<1% CLRDD incidence) at both locations. However, no source of resistance to CLRDV-AL was identified as all five cotton cultivars tested positive for the virus at both locations. CLRDD incidence was also higher 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 and boll rot numbers was observed in the early-planted cotton in southwest Alabama, this did not translate to yield losses. Overall, cotton cultivars EXPI and DG3615 had the highest yields at both locations. Despite the higher number of CLRDV positive plants, DP1646 had lint yield amounts comparable to EXPI and DG3615. In contrast, CLRDD incidence had a greater impact on yield for PHY480. Thus, these results indicate that planting cotton early in high risk areas could reduce the yield impacts caused by CLRDV. However, these trials will need to be repeated in 2021 to confirm these results.

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