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## PERFORMANCE OF *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE) ON HEALTHY AND COTTON LEAF CURL VIRUS INFECTED COTTON

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### ABSTRACT

*Cotton leaf curl virus* (CLCuV) is a group of whitefly-transmitted Geminiviruses that cause extensive damage to cotton in India and Pakistan. Begomoviruses have a complex association with their whitefly vector. To further understand these relationships, fecundity and life history parameters of *Bemisia tabaci* (Gennadius) were compared on 5, 20, 35 d post inoculated (DPI), CLCuV infected and healthy cotton plants to determine the effect of virus on its vector. *Cotton leaf curl virus* infection increased percent egg viability of *B. tabaci*. Whiteflies deposited significantly fewer eggs on virus infected plants compared to healthy plants. The development time of whiteflies from egg to adulthood was significantly reduced on CLCuV infected plants with shorter nymphal and pupal duration. Male and female whiteflies had shorter longevity on CLCuV infected plants compared with healthy plants. The egg duration was similar on CLCuV infected and healthy plants regardless of DPI of plants.

Key Words: Whitefly, developmental stages, fecundity, longevity, cotton

### RESUMEN

*El virus de encrespamiento de la hoja de algodón* (CLCuV) es un grupo de virus Gémini transmitidos por moscas blancas que causa un daño extensivo al algodón en la India y Pakistán. Los virus Begomo tienen una asociación complicada con su vector aleirodido. Para entender mejor estas relaciones, la fecundidad y los parámetros del ciclo de vida de *Bemisia tabaci* (Gennadius) estos fueron comparados en plantas sanas de algodón y plantas infectadas con CLCuV a los 5, 20, 35 días después de ser inoculadas (DDI) para determinar el efecto del virus sobre su vector. La infección del *virus de encrespamiento de la hoja de algodón* aumento el porcentaje de viabilidad de los huevos de *B. tabaci*. Los aleirodidos depositaron significativamente menos huevos sobre plantas infectadas con el virus en comparación con las plantas sanas. El tiempo de desarrollo de los aleirodidos del huevo al adulto fue significativamente reducido sobre las plantas infectadas con CLCuV con el tiempo del estadio de la ninfa y pupa mas corto. La longevidad de los machos y hembras aleirodidos fue mas corta en plantas infectadas con CLCuV en comparación con las plantas sanas. La duración del estadio de huevo fue similar en plantas infectadas con CLCuV y en plantas sanas sin importar los DDI de las plantas.

Plant pathogen-vector systems are characterized by complex direct and indirect interactions (Byrne & Bellows 1991a; Blair et al. 1995). The interactions between plant-pathogenic viruses and their insect vectors range from the insect functioning as a casual carrier to intimate molecular interactions (Nault 1997). The Geminiviruses are a large and diverse family of viruses that infect a wide range of important crop species. This family is divided into 3 genera: *Mastrevirus*, *Curtovirus* and *Begomovirus*. Begomoviruses are transmitted by the whitefly, *Bemisia tabaci* (Gennadius).

Earlier studies suggest that viruses can have positive, neutral or negative effects on their vectors (Costa et al. 1993; Colvin et al. 2006; Jiu et al. 2007). Virus-infected plants undergo changes that affect the biology of insect vectors. Insect vectors feeding on infected plants have been reported to differ in

growth rates, longevity, and fecundity compared with those vectors feeding on uninfected plants (Kennedy 1951; Baker 1960). Some vectors preferentially colonize infected plants (Castle et al. 1993). Barley yellow dwarf virus RPV-NY (BYDV-RPV-NY) infection increased the rate of population growth of *Schizaphis graminum* (Rondani), relative to that on healthy oats. The proportion of *Rhopalosiphum padi* (Linnaeus) developing to alatae on BYDV-infected oats was significantly greater than on healthy oats (Montllor & Gildow 1986). Virus infection can increase plant suitability for insect vector survival and reproduction by improving the nutritional quality of the plant (Kennedy 1951; Baker 1960). However, increased survival may lead to virus proliferation in the field (McElhany et al. 1995).

In the past decade, Cotton leaf curl disease (CLCuD) has become a major threat to the cotton

industry in Pakistan (Briddon & Markham, 2000) and more recently has spread to India (Briddon et al. 2006). Characteristic symptoms of the disease include upward and downward leaf curling, vein thickening, and occasionally enation formation on the underside of leaves (Khalid et al. 1999). Cotton leaf curl disease has a complex etiology but is believed to be caused by *Cotton leaf curl virus* (CLCuV) (Mansoor et al. 2003; Varma & Malathi, 2003). *Cotton leaf curl virus* is transmitted by *B. tabaci* in a circulative, persistent manner, and can be retained from a few d to the entire life period of the whitefly (Liu et al. 1998; Mansoor et al. 2003; Mann & Singh 2004a). Khan and Ahmad (2005) detected CLCuV DNA in host plants within 17 d of inoculation and in *B. tabaci* within 4 h of feeding on infected host plants. The acquisition and transmission of CLCuV by *B. tabaci* as well as effects of the virus on host plants have been studied in detail (Nateshan et al. 1996; Khan & Ahmad 2005; Mann & Singh, 2004b, 2004c). However, the interactions between this geminivirus and *B. tabaci* are still poorly understood. In the present studies, we compared the performance of *B. tabaci* on CLCuV infected and healthy cotton plants to determine the effect of CLCuV on its vector.

#### MATERIALS AND METHODS

##### Maintenance of Whiteflies, Virus Source, and Experimental Plants

###### Whiteflies

Viruliferous and non-viruliferous whiteflies were maintained on American cotton, *Gossypium hirsutum*. Whiteflies were obtained by collecting adults from untreated *Desi* cotton, *Gossypium arboreum*, which is resistant to CLCuV (Nateshan et al. 1996; Briddon et al. 2001; Rahman et al. 2002). The adults were released on cotton plants in insect rearing cages for oviposition. The adults were removed and killed manually after 3 d to retain eggs only. Newly emerged whitefly adults from these eggs were released and maintained on new cotton plants for breeding. Whitefly adults also were obtained from leaves bearing puparia of *G. arboreum* collected from the field. These adults were released on healthy plants for verification of virus infection. Adults from plants with no symptoms in 40 d were considered non-viruliferous. These adults were reared on *G. hirsutum* plants in separate insect rearing cages for further studies.

###### Virus Source

Cotton plants, *G. hirsutum* CV F846 (CLCuV susceptible) exhibiting typical symptoms of CLCuD described by Kapur et al. (1994) and Kang et al. (2003a) were obtained from the field and transplanted in earthen pots containing a mixture of loamy soil and farm yard manure in a 1:1 ratio.

New plants were raised and infected with CLCuV through whitefly mediated transmission. Whiteflies were given an acquisition access period of 24 h on virus source and were confined on new healthy plants for 8 h (inoculation access period). The new plants were raised and maintained in 1.2 × 1.2 × 1.2-m cages and used as virus source after the appearance of typical CLCuD symptoms.

###### Experimental Plants

The experimental cotton plants, *G. hirsutum* CV F846, were planted in earthen pots (30x23 cm) with 3 to 4 seeds per pot. The plants were raised and maintained in separate cages following the standard procedures for growing cotton crop. Plants were made CLCuV infected by inoculating healthy plants through whitefly mediated transmission. Inoculations were made on 20-d-old plants, and the experiments were conducted at 5, 20, and 35-d Post Inoculation (DPI) on these groups of plants with different virus infection status. These groups were defined as CLCuV infected with no symptoms, initiation of CLCuD symptoms, and with well defined CLCuD symptoms. The greenhouse experiments comprised 3 replications with 15 test plants in each replication with 2 clip cages on each plant.

##### Performance of *B. tabaci* on Virus Infected and Healthy Cotton Under Greenhouse Conditions

###### Egg, Nymphal, Pupal Stage Duration and Adult Longevity

Performance of *B. tabaci* was compared on CLCuV infected and healthy cotton plants of same age from Aug to Oct 2005. Four pairs of male and female adult whiteflies were confined on the underside of leaves with clip cages for egg laying. The adults were removed after their first oviposition. Number of eggs laid under each clip cage was observed with the help of a binocular microscope. Twenty eggs per clip cage were retained, and the remaining were removed carefully with a moist cotton swab. The clip cages were monitored twice daily to observe offspring development with regard to duration of egg, nymphal, and pupal stages. Nymphal duration was defined as completion of the 3<sup>rd</sup> instar, when the nymph is elliptical in shape and the eyes are not completely divided (Sharaf & Batta 1985; Brar et al. 2005). The 4<sup>th</sup> instar was defined as the pupa, as many authors consider 4<sup>th</sup> instar and pupal stages to be morphologically distinct but difficult to delineate (Husain, 1931; Gill 1990; Byrne & Bellows 1991b; Salas & Mendoza 1995).

###### Adult Longevity

Ten newly emerged male and female non-viruliferous whiteflies were separately confined on the underside of cotton leaves. Insects were transferred

to new leaves every week to avoid emergence of new adults on the same leaves. Insect mortality was recorded daily until all the insects died.

Fecundity

Two pairs of newly emerged male and female whiteflies were confined on the underside of leaves of test plants with clip cages for oviposition. Female whiteflies were serially transferred daily to new test plants after their first oviposition until all insects died. Number of eggs laid and hatched on each plant was recorded.

Field Studies

Colonization of *B. tabaci* on CLCuV Infected and Healthy Cotton Plants

Colonization of *B. tabaci* on CLCuV infected and healthy cotton plants were studied under natural field conditions. Cotton plants, *G. hirsutum* CV F846, were raised on a sandy loam soil following the protocol of Punjab Agricultural University, Ludhiana, India. Each plot consisted of six 6 m long rows with 8 plants in each row. The plants were allowed to grow under natural conditions until maturity. Total number of whitefly adults per leaf on CLCuV infected and healthy plants were recorded. Whitefly populations were sampled on 3 leaves of 15 plants upon initiation, and 15 d after appearance of CLCuD symptoms. Plants not showing CLCuD symptoms after 20 d were considered healthy. Double the number of plants were sampled in the case of healthy plants. Experimental design was a Randomized Complete Block with 4 replications of each treatment.

Data Analysis

A two-factor ANOVA was conducted with CLCuV infection and post inoculation period as factors. When the factors or their interactions were significant at  $\alpha = 0.05$ , the means were separated with LSD by SAS, Version 9.1 (SAS Institute 2003).

RESULTS

Egg, Nymphal, Pupal Stage Duration and Adult Longevity

Egg

There were no significant differences in the duration of egg stage at 5, 20 and 35 DPI between CLCuV infected and healthy cotton plants (Table 1). Egg duration increased as post inoculation period increased from 5 to 35 d (25- and 55-d-old). Egg duration did not change with severity of symptoms at 20 and 35 DPI (40- and 55-d-old). Egg duration increased with plant age in 20- and 55-d-old healthy plants. The overall mean duration of egg stage varied from 3 to 6 d to 4 to 7 d on CLCuV infected and healthy plants, respectively.

Nymph

The overall nymphal duration was significantly reduced on CLCuV infected plants when compared with healthy plants at all stages of infection (Table 2). The nymphal duration did not change significantly with the severity of symptoms on CLCuV infected plants. The nymphal duration did not change with age of healthy plants. The overall nymphal duration ranged from 11 to 21 d on CLCuV infected plants compared to 11 to 18 d on healthy plants.

Pupa

The pupal duration was significantly lower on 20 and 35 DPI plants (Table 3). There were no significant differences at 5 DPI. The pupal duration did not change significantly with symptom severity on virus infected plants. The pupal duration did not change with age of healthy plants. Overall the pupal duration varied from 3 to 7 d on CLCuV infected plants and 5 to 8 d on healthy plants.

Adult Longevity

Life expectancy of male and female adults of *B. tabaci* developing on CLCuV infected plants was significantly reduced compared to adults develop-

TABLE 1. EGG DURATION OF *B. TABACI* ON CLCUV INFECTED VERSUS HEALTHY COTTON PLANTS (*G. HIRSUTUM* CV F846).

Treatments	Mean egg duration <sup>1</sup> (days)				
	5 DPI <sup>2</sup> Mean ± SE	20 DPI Mean ± SE	35 DPI Mean ± SE	LSD ( $\alpha = 0.05$ )	Range
CLCuV infected plants	3.58 ± 0.13 b	3.97 ± 0.07 ab	4.26 ± 0.14 a	0.41	3-6
Healthy plants	3.88 ± 0.10 b	3.96 ± 0.10 b	4.30 ± 0.07 a	0.31	4-7
LSD ( $\alpha = 0.05$ )	NS <sup>3</sup>	NS	NS	—	—

<sup>1</sup>Means within rows followed by same letter are not significantly different at  $\alpha = 0.05$ , based on LSD means.  
<sup>2</sup>DPI = Day Post Inoculation.  
<sup>3</sup>NS = Not significant at  $\alpha = 0.05$ , based on LSD means.

TABLE 2. NYMPHAL DURATION OF *B. TABACI* ON CLCUV INFECTED VERSUS HEALTHY COTTON PLANTS (*G. HIRSUTUM* CV F846).

Treatments	Mean nymphal duration (days)				
	55 DPI <sup>1</sup> Mean ± SE	20 DPI Mean ± SE	35 DPI Mean ± SE	LSD ( $\alpha = 0.05$ )	Range
CLCuV infected plants	13.74 ± 0.24	13.91 ± 0.76	14.66 ± 0.66	NS <sup>2</sup>	11-21
Healthy plants	15.38 ± 0.42	16.17 ± 0.39	16.16 ± 0.68	NS	11-18
LSD ( $\alpha = 0.05$ )	1.34	1.33	1.40	—	—

<sup>1</sup>DPI = Day Post Inoculation.  
<sup>2</sup>NS = Not significant at  $\alpha = 0.05$ , based on LSD means.

ing on healthy plants. Both male and female whiteflies survived longer on 35 DPI plants (55-d-old) compared to 5 and 20 DPI plants. Both male and female whiteflies survived longer on 55-d-old plants than 25- and 40-d-old healthy plants (Table 4). Females survived longer than males on healthy as well as virus infected plants. Overall, male and female longevity ranged from 6 to 10 and 8 to 12 d, respectively, on CLCuV infected plants compared to 7 to 12 and 8 to 14 d, respectively, on healthy plants.

Fecundity

*Cotton leaf curl virus* infection had a significant effect on fecundity of *B. tabaci*. Total number of eggs laid on 35 DPI plants was significantly lower than on healthy plants of the same age (Table 5). There was no significant effect of symptom severity on whitefly fecundity on CLCuV infected plants. There was no effect of plant age on whitefly fecundity on healthy plants. Egg viability was significantly higher on 20 and 35 DPI virus infected plants compared to healthy plants of same age. Egg viability was not influenced by the symptom severity on CLCuV infected plants or the age of healthy plants.

Colonization of *B. tabaci* on CLCuV Infected and Healthy Cotton Plants

The mean number of adults colonizing per leaf on CLCuV infected and healthy plants was not

significantly different under field conditions at initiation or complete development of symptoms (data not shown).

DISCUSSION

The effects of CLCuV on its vector appear to be mixed and complex with both positive and negative affects on life history parameters. Mean nymphal duration was significantly reduced on virus-infected plants of all ages. However, reduction in pupal duration and adult longevity was observed on 20 and 35 DPI plants only. Assuming that shorter developmental period indicate good host suitability (Muniz 2000), CLCuV showed positive effects on the development of immature stages.

Improved whitefly performance on CLCuV infected plants appears to be the result of altered nutritional status of host plants, and the suppression of plant defense against the vector (Belliere et al. 2005; Stout et al. 2006). Virus infection in cotton plants has been shown to increase peroxidase activity, catechol, phenols, carotenoids, proteins, total sugars, chlorophyll, oil content, lipase enzyme contents (Kaur et al. 1998; Ashraf et al. 2004; Kang et al. 2003b) and reduced Ca<sup>++</sup> and K<sup>+</sup> content (Nadeem et al. 2006). Increased total sugar and reduced K<sup>+</sup> content of cotton leaves has been shown to attract higher number of whiteflies, (Dominick & Sundaram 1992; Abdullah &

TABLE 3. PUPAL DURATION OF *B. TABACI* ON CLCUV INFECTED VERSUS HEALTHY COTTON PLANTS (*G. HIRSUTUM* CV F846).

Treatments	Mean pupal duration (days)				
	5 DPI <sup>1</sup> Mean ± SE	20 DPI Mean ± SE	35 DPI Mean ± SE	LSD ( $\alpha = 0.05$ )	Range
CLCuV infected plants	4.57± 0.16	4.47 ± 0.14	4.09 ± 0.03	NS <sup>2</sup>	3-7
Healthy plants	5.24 ± 0.22	5.55 ± 0.25	5.33 ± 0.16	NS	5-8
LSD ( $\alpha = 0.05$ )	NS	0.98	1.01	—	—

<sup>1</sup>DPI = Day Post Inoculation.  
<sup>2</sup>NS = Not significant at  $\alpha = 0.05$  based on LSD means.

TABLE 4. LONGEVITY OF *B. TABACI* ADULTS ON CLCUV INFECTED VERSUS HEALTHY COTTON PLANTS (*G. HIRSUTUM* CV F846).

Treatments	Male					Female				
	Mean adult longevity <sup>1</sup> (days)									
	5 DPI	20 DPI	35 DPI	LSD	Range	5 DPI <sup>2</sup>	20 DPI	35 DPI	LSD	Range
	Mean ± SE	Mean ± SE	Mean ± SE	( $\alpha$ = 0.05)		Mean ± SE	Mean ± SE	Mean ± SE	( $\alpha$ = 0.05)	
CLCuV infected plants	6.58 ± 0.26 b	6.73 ± 0.45 b	9.48 ± 0.17 a	1.22	6-10	8.43 ± 0.16 b	9.03 ± 0.72 b	10.87 ± 0.61 a	1.78	8-12
Healthy plants	7.99 ± 0.20 b	8.27 ± 0.40 b	11.57 ± 0.11 a	0.84	7-12	9.46 ± 0.03 b	10.66 ± 0.36 b	12.18 ± 0.38 a	1.45	8-14
LSD ( $\alpha$ = 0.05)	NS <sup>3</sup>	0.64	0.83	—	—	NS	0.98	1.19	—	—

<sup>1</sup>Means within rows followed by same letter are not significantly different at  $\alpha$  = 0.05, based on LSD means.  
<sup>2</sup>DPI = Day Post Inoculation.  
<sup>3</sup>NS = Not significant at  $\alpha$  = 0.05, based on LSD means.

TABLE 5. FECUNDITY AND EGG VIABILITY OF *B. TABACI* ON CLCUV INFECTED VERSUS HEALTHY PANTS OF COTTON (*G. HIRSUTUM* CV F846).

Treatments	Mean number of eggs per female					Mean per cent eggs hatched				
	5 DPI <sup>1</sup>	20 DPI	35 DPI	LSD		5 DPI	20 DPI	35 DPI	LSD	
	Mean ± SE	Mean ± SE	Mean ± SE	( $\alpha$ = 0.05)		Mean ± SE	Mean ± SE	Mean ± SE	( $\alpha$ = 0.05)	
CLCuV infected plants	49.17 ± 0.58	45.60 ± 0.71	44.83 ± 2.36	NS <sup>2</sup>		53.73 ± 0.91	59.78 ± 1.04	59.42 ± 1.03	NS	
Healthy plants	51.93 ± 1.55	50.00 ± 0.63	53.20 ± 1.09	NS		48.85 ± 0.79	53.80 ± 0.92	43.50 ± 0.67	NS	
LSD ( $\alpha$ = 0.05)	NS	NS	5.93	—		NS	3.93	5.09	—	

<sup>1</sup>DPI = Day Post Inoculation.  
<sup>2</sup>NS = Not significant at  $\alpha$  = 0.05, based on LSD means.

Singh 2004) while chlorophyll, phenols, carotenoid and peroxidase are associated with decreased whitefly populations (Ilyas 1991; Butter et al. 1992; Ravi et al. 2006).

Virus infection had no effect on egg duration but reduced that of the nymph in 5 DPI plants. As symptom severity increased egg, nymphal, and pupal duration, as well as adult longevity, remained unaffected. This further strengthens the supposition that changes in insect development are not due to altered plant morphology, but to the improved nutritional suitability of cotton plants. The effect of virus in the absence of symptoms on pupal duration could not be determined as symptoms appeared before pupation occurred on 5 DPI plants.

The differences observed in egg duration, longevity, fecundity, and viability within virus infected plants appears to be related to plant age, as similar differences were observed in healthy plants of the same age. In general, the duration of egg, nymph, pupa, and adult longevity tended to increase with plant age. This general trend may be due to decreases in temperature from Sep to Oct in Punjab state. Abdullah & Singh (2004) demonstrated longer whitefly adult longevity with decreased temperature under Punjab conditions.

Reduction in longevity, and fecundity may be due to the direct negative effect of progressive tissue invasion by the virus as suggested by Pesic-Van Esbroeck et al. (1995) in Squash leaf curl virus (SqLCV) infected whiteflies, and Rubinstein & Czosnek (1997) in TYLCV infected whiteflies. Pesic-Van Esbroeck et al. (1995) reported that invasion SqLCV into whitefly tissues and organs resulted in gross and ultra structural abnormalities of the reproductive, digestive and excretory systems. Rubinstein and Czosnek (1997) demonstrated 40 to 50 percent reduction in mean number of eggs laid by viruliferous whiteflies infected with TYLCV compared to healthy whiteflies. However, Stansly & McKenzie (2007) in preliminary studies attributed the reduced fecundity of *B. tabaci* on TYLCV infected plants to reduced host suitability.

Differences were not significant in mean number of adults colonizing CLCuV infected and healthy plants under field conditions. Thompson (2002) reported similar results for total number of *B. tabaci* nymphs and pupal cases on East African Cassava mosaic virus (EACMV) infected plants. However, Colvin et al. (1999) positively correlated the symptom severity of EACMV and UgV to whitefly density.

In general, our results extend previous findings of mixed effects of viruses on their insect vectors. The patterns of virus effects on their insect vector appear to be unique depending upon the type of plant-virus-vector relationship. The virus appears to have direct negative effects on its vector, but benefits the vector indirectly by improving nutritional suitability of plant.

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