RESISTANCE IN UPLAND COTTON TO THE SILVERLEAF WHITEFLY TRANSMITTED COTTON LEAF CRUMPLE DISEASE

E. T. Natwick[1], C. Cook[2], R. Gilbertson[3]
Young-Su Seo[3] and T. Turini[1]
[1]University of California Coop. Ext., Holtville, CA
[2]United Agri Products, Santa Rosa, TX,
[3]University of California, Davis, CA

Abstract

Eight cotton, *Gossypium hirsutum* L., cultivars or breeding-lines were evaluated in the field for resistance to the silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, transmitted cotton leaf crumple (CLCr) disease caused by cotton leaf crumple geminivirus (CLCrV) in Imperial Valley, CA. The cultivars were Texas 121, AP 4103, AP 6101 and Stoneville 474 and the breeding-lines were DG 2165, DG 2108, DG 2383 and DG 2387. Cotton entries were rated for severity of CLCr disease symptoms. Leaf and petiole samples from each entry were used to confirm the presence of CLCrV by squash and dot blot hybridization with a general DNA probe, which detects the presence of whitefly transmitted geminiviruses. DNA sequencing of a PCR amplified fragment from an infected plant was used to confirm that the geminivirus was CLCrV. Results showed differences in whitefly infestation levels and virus disease symptoms among cotton entries. The variety Stoneville 474, with hirsute leaves, had more silverleaf whitefly adults and nymphs than any of the other entries. The breeding-lines DG 2383 and DG 2387, with Cedix parentage, had a lower CLCr disease rating than other entries.

Introduction

Whitefly-induced economic losses to cotton occur as a result of reduced cotton yield (Mound 1965) and contamination of lint with honeydew and sooty molds (Davidson et al. 1994). The whitefly-transmitted cotton leaf crumple (CLCr) disease, caused by cotton leaf crumple geminivirus (CLCrV), can also cause extensive reduction in yield (Dickson et al. 1954, Duffus and Flock 1982). Cotton plant height and yield are significantly reduce by CLCrV infections at early and mid-growth stages (Brown et al. 1987). Insecticides provide temporary control of silverleaf whitefly (Chu et al. 1993 and Natwick 1993). However, CLCr disease is still common and a long term solution that offers economical and environmental advantages is needed. Therefore, development of pest-resistant cultivars is a goal that warrants increased attention (Painter, 1951, Khalifa and Gameel 1983). Cotton susceptibility to whitefly colonization increases with increasing leaf pubescence (Mound 1965), is lower for okra-leaf varieties (Natwick et al. 1997), and is greater for *G. barbadense* than for *G. hirsutum* (Natwick et al. 1995). The objectives of this study were to: 1) Use a rating system to evaluate cotton lines for resistance to CLCr disease; 2) Confirm that the virus associated with CLCr symptoms in the field plots in Imperial County is CLCrV; 3) Use molecular tools to evaluate resistance to CLCrV in upland cotton.

Material and Methods

The study was conducted in 1999 at the University of California Desert Research and Extension Center in Holtville, CA. The experimental design was a randomized complete block with four replicates. Each plot was 14 m long and 8 m wide. Rows were 1 m apart. Seeds of eight cotton genotypes were sown and irrigated on 30 March 1999. The cultivars were Texas 121, AP 4103, AP 6101 and Stoneville 474 and the breeding-lines were DG 2165 and DG 2108 and with Cedix parentage were DG 2383, and DG 2387. There were no insecticide spray treatments applied to the plots.

Whitefly adults were sampled from ten plants in each plot on each sampling date using the leaf turn method (Naranjo & Flint 1995). Leaf samples were taken on 28 May and then weekly from 24 June through 6 September 1999. Whitefly nymphs were counted on single leaf disks of 1.65 cm^2 from each of ten 5^{th} node leaves per plot weekly from 12 July through 6 September.

The following rating scale for CLCr disease symptom was used on 23, 27 and 30 August and on 6 September: 1 = leaf smooth, few if any bumps or blisters; 2 = some obvious blisters and crumpling, but less than 50% leaf with symptoms; 3 = Obvious crumpling, blisters, vein clearing from more than 50% to close to 100%, leaf not rolled; 4 = severe crumpling, blisters, leaves noticeably rolled and distorted. Leaf and petioles from each plot were used to confirm the presence of CLCrV by squash blot hybridization with a general DNA probe, which detects the presence of whitefly transmitted geminiviruses (Gilbertson et al. 1991). DNA sequencing of a polymerase chain reaction (PCR) amplified fragment from an infected plant was used to confirm that the geminivirus was CLCrV.

DNA extracts were prepared from leaf tissue and PCR analysis was conducted using degenerate geminivirus primers (pAL1v1978 and pAR1c496) (Rojas et al. 1993). To evaluate resistance to CLCrV, ten representative leaves from each variety were given a visual CLCr disease rating and then squashed onto nylon membranes and DNA extracts prepared using the Dellaporta extraction method for PCR analysis. The membranes with the squash blots were probed with two types of probes, a general probe for whitefly-transmitted geminiviruses and a CLCrV-specific probe (a 1.4 kb DNA-B fragment of CLCrV that was cloned in 1997). The general

Reprinted from the *Proceedings of the Beltwide Cotton Conference*
Volume 1:164-166 (2000)
National Cotton Council, Memphis TN

probe gave high levels of background, and even the positive controls (CLCrV-infected) gave hybridization signals that were just above background. The CLCrV-specific probe gave better results, although squash blot hybridization signals were generally low, even for obviously infected (i.e., having strong CLCr symptoms). The following rating scale was used to assess the results with the CLCrV-specific probe: 0 = signal (no infection); 1 = faint and uneven signal (questionable infection); 2 = obvious and generally uniform signal (infected plant; low to moderate levels of virus); 3 = dark uniform signal (infected plant; moderate to low levels of virus) 4 = dark black uniform signal (infected plant; very high level of virus).

Seed cotton was hand picked from 4-m sections of row in each plot on 5 October. Seed cotton weights were recorded and samples were ginned. Lint weights were recorded, and lint turnout percentages were calculated.

Seasonal whitefly density and CLCr disease ratings were analyzed using ANOVA (MSTAT-C 1989). Student-Neuman-Keul's Multiple Range Test (SNKMRT) was employed for means separations.

Results and Discussion

The Stoneville 474 cultivar had more silverleaf whitefly adults than all other entries and there were no other differences for whitefly adults among the entries; $P \leq 0.05$ (Table 1). Stoneville 474 had more silverleaf whitefly nymphs than all other entries with a seasonal mean of 15.1 per cm^2. DG 2108 had a seasonal mean for whitefly nymphs of 8.7 per cm^2 which was more than all entries except Stoneville 474. DG 2165 (3.5 per cm^2) had fewer whitefly nymphs than all entries except and DG 2383 (3.8 per cm^2). DG 2383 had fewer whitefly nymphs than all entries except DG 2165, AP 4103 (4.9 per cm^2) and AP 6101 (5.1 per cm^2).

The cultivar AP4103 had more severe CLCr disease symptoms than all other entries with a CLCr disease rating seasonal mean of 3.6; $P \leq 0.05$ (Table 2). The cultivar AP6101 had a CLCr disease rating seasonal mean of 3.0; more severe CLCr symptoms than all other entries except AP 4103. Stoneville 474 had a CLCr disease rating seasonal mean of 2.5 which was more severe CLCr disease symptoms than all other entries except AP 4103 and AP 6010. Texas 121 had a CLCr disease rating seasonal mean of 2.1 which was more severe CLCr disease symptoms than all of the experimental breeding-lines DG 2108 (1.5), DG 2165 (1.4), DG 2383 (1.1) and DG 2387 (1.1).

To confirm that the virus associated with cotton leaf crumple virus symptoms in the field plots in Imperial County is cotton leaf crumple geminivirus, three leaves showing representative CLCr symptoms were sent to UC Davis for detection of CLCrV. DNA extracts were prepared from leaf tissue from all three samples and PCR analysis was conducted using the degenerate geminivirus primers pAL1v1978 and pAR1c496. From all three samples, an approximately 1.1 kilobase (kb) DNA fragment was amplified, whereas no such fragment was amplified from an uninfected tobacco sample. These results indicated that there was a geminivirus infection in all three samples.

The sequence of a portion of the 1.1 kb DNA fragment was determined. This was accomplished by recovering the DNA from an agarose gel and direct sequencing the amplified fragment using the primer pAL1c1978. With this primer we obtained a sequence of 782 base pairs. This sequence was compared with a sequence that was obtained from cotton plants showing CLCr symptoms in 1997 from this same location. The 1997 sequence was presumed to be CLCrV as there was no homologous. The 1999 sequence was presumed to be that of CLCrV due to it coming from a geminivirus with CLCr symptoms. There is currently no homologous CLCrV sequence available for comparison in the GeneBank. The 1999 sequence was 96% identical to the 1997 sequence (565/584 nucleotides were identical). Thus, on the basis of these results we can conclude that the geminivirus associated with the CLCr symptoms in 1999 was indeed CLCrV and that the virus had not changed dramatically over the past 2 years.

The CLCr disease ratings from ten representative leaves from each variety and the CLCrV-specific probe ratings are included in Table 3. The PCR membranes with the squash blots were probed with two types of probes, a general probe for whitefly-transmitted geminiviruses and a CLCrV-specific probe (a 1.4 kb DNA-B fragment of CLCrV that was cloned in 1997). The general probe gave high levels of background, and even the positive controls (CLCrV-infected) gave hybridization signals that were just above background. The CLCrV-specific probe gave better results, though the hybridization signals, even from leaves with strong symptoms, were fairly low. This suggests a fairly low titer virus in these relatively older leaves. However, there was a correlation between the leaf ratings for CLCr and the hybridization signals (Fig 1). The varieties AP 4104 and AP 6101, with the highest CLCr disease ratings, also had the highest CLCrV-specific probe ratings (Table 3) suggesting that these two materials were most susceptible. Moderate susceptibility was found for Stoneville 474, Texas 121, DG 2108, and DG 2165 (Table 2 and Table 3), but only DG 2108 showed elevated hybridization signal. Resistance was found in only DG 2387 based on all the rating and hybridization signals (Table 2 and Table 3).

PCR experiments yielded inconsistent amplification from the DNA extracts prepared from cotton leaves. Experiments were conducted in which geminivirus plasmid DNA was spiked

into these extracts and the PCR repeated; in these experiments, the expected DNA fragment was amplified. This indicates that it was not PCR inhibitors that were preventing amplification. The amount of virus in these older leaves may have been too low for efficient detection of the virus by PCR. However, this needs further investigation to determine the best DNA extraction methods and the optimal time to sample leaves for such experiments.

The Stoneville 474 seed cotton yield (1250 lb/acre) was lower than all other entries in the study except DG 2108 (1616.2 lb/acre), $P \leq 0.05$, (Table 4). The AP 6101 seed cotton yield (2743.3 lb/acre) was greater than all other entries in the study except AP 4103 (2454.5 lb/acre) and DG 2383 (2301.3 lb/acre). Stoneville 474 had a lower lint yield (499.5 lb/acre) than all other entries in the study except DG 2108 (660.7 lb/acre). The AP 6101 lint yield (1069.2 lb/acre) was greater than all other entries in the study except AP 4103 (950.2 lb/acre) and DG 2383 (961.4 lb/acre). Texas 121 had the lowest percentage lint turnout (37.9 %) which was lower than DG 2383 (41.8) DG 2387 (41.1) and DG 2108 (40.9) , $P \leq 0.05$.

Differences in cotton susceptibility to whitefly colonization have been reported for different cotton species, differences in leaf pubescence and leaf shape . Butler et al. (1991) suggested that glabrous, small leaf area and open canopy cottons, and gossypol content were important traits to investigate for developing whitefly-resistant cottons. The entries in this study were glabrous, normal-leaf cottons, except for Stoneville 474 with hirsute leaves. Adult silverleaf whitefly vector CLCrV. There were small differences for adult whitefly density among varieties, with the exception of Stoneville 474.

The breeding-lines DG 2165, DG 2383 and DG 2387 have heritable traits for CLCr disease resistance that should be investigated for development and release of CLCrV-resistant cotton varieties.

References Cited

Bellows, T. S., Jr., T. M. Perring, R. J. Gill, and D. H. Headrick. 1994. Description of a species of *Bemisia* (Homoptera: Aleyrodidae). Ann. Entomol. Soc. Am. 81: 195-206.

Brown, J. K., J. D. Mihail, and M. R. Nelson. 1987. Effects of cotton leaf crumple virus on cotton inoculated at different growth stages. Plant Dis. 71: 699-7032.

Butler G. D., Jr., D. F. Wilson, and G. Fisher. 1991. Cotton leaf trichome and populations of *Empoasca lybica* and *Bemisia tabaci*. Crop Protection 10: 461-464.

Chu, C. C., T. J. Henneberry, and D. H. Akey. 1993. Results of field studies with insecticides for sweetpotato whitefly *Bemisia tabaci* control on cotton in the Imperial Valley, CA, P. 960. *In* D. J. Herber and D. A. Richter [eds.] Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.

Davidson, E. W., B. J. Segyra, T. Steel and D. L. Hendrix. 1994. Microorganisms influence the composition of honeydew produced by the silverleaf whitefly, *Bemisia argentifolii*. J. Insect Physiol. 40: 1069-1076.

Dickson, R. C., M. McD. Johnson, and E. F. Laird. 1954. Leaf crumple, a virus disease of cotton. Phytopathology 44: 479-480.

Duffus, J. E., and R. A. Flock. 1982. Whitefly transmitted disease complex of the desert southwest. Calif. Agric. 36: 4-6.

Gilbertson, R. L., S. H. Hidayat, R. T. Martinez, S. A. Leong, J. C. Faria, F. Morales, and D. P. Maxwell. 1991. Differentiation of bean-infecting geminiviruses by nucleic acid hybridization probes and aspects of bean golden mosaic in Brazil. Plant Dis. 75: 336-342.

Khalifa, H. and O. I. Gameel. 1983. Breeding cotton cultivars resistant to whitefly *Bemisia tabaci* Genn. Nato. Adv. Stud. Inst. Ser. A. Life Sci. 55: 231-180.

Mound, L. A. 1965. Effect of leaf hair on cotton whitefly populations in the Sudan Gezira. Empire Cotton Growing Rev. 42: 33-40.

MSTAT-C. 1989. MSTAT-C users' guide: a microcomputer program for the design, management, and analysis of agronomic research experiments, version 1.3 ed. Michigan State University, East Lansing, MI.

Naranjo, S. E., and H. M. Flint. 1995. Spacial distribution of adult *Bemisia tabaci* (Homoptera: Aleyrodidae) in cotton and development and validation of fixed-precision sampling plans for estimating population density. Environ. Entomol. 24: 261-270.

Natwick, E.T. 1993. Silverleaf whitefly control in cotton using various insecticides in the Imperial Valley of California. pp. 722-729 *In* D. J. Herber and D. A. Richter (eds.). Beltwide Cotton Conf., Nashville, TN.

Natwick, E. T., C. C. Chu, H. H. Perkins, T. J. Henneberry, and A. C. Cohen. 1995. Pima and upland cotton susceptibility to *Bemisia argentifolii* under desert conditions. Southwest Entomol. 20 (4): 429-438.

Natwick, E. T., C. C. Chu, A. C. Cohen, G. S. Simmons, and D. E. Brushwood. 1997.Silverleaf whitefly infestation levels and percentages of parasitism in relation to cotton variety and insecticide treatments, pp. 915-918. *In* R. Dugger, and D. A. Richter [eds.] Proc. Beltwide Cotton Prod. Res. Conf., Natl. Cotton Council of Amer., Memphis, TN.

Painter, R. M. 1951. Insect resistance in crop plants. University press of Kansas, Lawrence, KS.

Rojas, M. R., R. L. Gilbertson, D. R. Russell, and D. P. Maxwell. 1993. Use of degenerate primers in the polymerase chain reaction to detect whitefly transmitted geminiviruses. Plant Dis. 77: 340-347.

Table 1. Silverleaf whitefly seasonal means for adults per leaf and nymphs per cm^2 of leaf for cotton varieties, Holtville, CA, 1999.

Variety	Adults	Nymphs[a]
Stoneville 474	22.1 a	15.1 a
DG 2108	12.5 b	8.7 b
AP 6101	11.2 b	5.1 cde
AP 4103	10.9 b	4.9 cde
DG 2387	10.8 b	5.4 c
Texas 121	10.6 b	5.3 cd
DG 2165	10.2 b	3.5 f
DG 2383	9.1 b	3.8 ef

[a]Log transformed data used for analysis; reverse transformed means reported. Mean separations within columns by Student-Newman-Keul's Multiple Range Test, P≤0.05.

Table 2. CLCr disease ratings on cotton varieties and breeding-lines, 1999.

Variety	23 Aug	27 Aug	30 Aug	6 Sep	Mean
AP 4103	3.5 a	3.6 a	3.6 a	3.8 a	3.6 a
AP 6101	2.5 b	3.0 b	3.0 b	3.1 b	3.0 b
Stoneville	2.4 b	2.6 c	2.4 c	2.4 c	2.5 c
Texas 121	1.9 b	2.1 d	2.1 c	2.1 c	2.1 d
DG 2108	1.2 c	1.5 e	1.5 d	1.6 d	1.5 e
DG 2165	1.3 c	1.5 e	1.4 d	1.3 de	1.4 e
DG 2383	1.0 c	1.1 f	1.1 d	1.1 e	1.1 e
DG 2387	1.0 c	1.1 f	1.1 d	1.1 e	1.1 e

Mean separations within columns by Student-Newman-Keul's Multiple Range Test, P≤0.05.

Table 3. CLCr disease ratings versus CLCrV specific probe ratings.

Variety	CLCrV disease rating	CLCrV specific probe
AP 4103	3.10	1.75
AP 6101	2.50	1.85
Stoneville 474	1.65	0.95
DG 2108	1.35	1.40
Texas 121	1.30	1.00
DG 2383	1.20	1.05
DG 2165	1.00	1.00
DG 2387	1.00	1.00

Table 4.Pounds per acre seed cotton and lint, and lint turnout percentages.

Variety	Seed cotton	Lint	Lint % Turnout
AP 6101	2743.3 a	1069.2 a	39.0 ab
AP 4103	2454.5 ab	950.2 ab	38.7 ab
DG 2383	2301.3 ab	961.4 ab	41.8 a
DG 2165	2014.4 bc	804.5 bc	39.9 ab
Texas 121	2000.7 bc	757.6 bc	37.9 b
DG 2387	1899.8 bc	782.4 bc	41.1 a
DG 2108	1616.2 cd	660.7 cd	40.9 a
Stoneville 474	1250.0 d	499.5 d	39.9 ab

Mean separations within columns by Student-Newman-Keul's Multiple Range Test, P≤0.05.

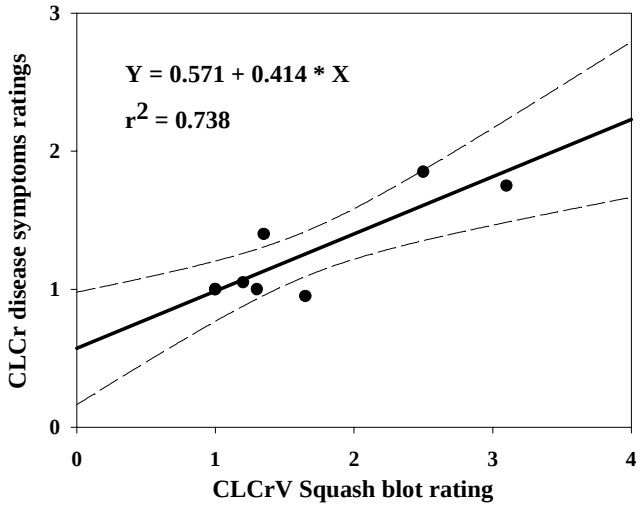

Figure 1. Comparison of a CLCr disease rating scvale with a rating scale for a CLCrV specific-probe on cotton leaf tissue extracts. Regression line with 95% CI and n=8.