Okra-Leaf and Normal Leaf Cotton Resistance to Silverleaf Whitefly

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ABSTRACT

Okra-leaf and normal leaf upland cotton cultivars and experimental breeding-lines were evaluated for susceptibility to silverleaf whitefly, Bemisia argentifolii Bellows and Perring and cotton leaf crumple disease caused by cotton leaf crumple geminivirus (CLCV). In March 1997, 16 cotton cultivars and breeding-lines were sown into a randomized block experiment, with four replications at Holtville, CA. The normal leaf genotypes included Deltapine Seed (DP20, DP50, DP90, DP5415, DP5432, DP5461 and DP9057), ChemBred (CB1135), South Texas Planting Seed Co. (Texas 121), USDA-ARS (C118-2-93) and CSIRO (CS50). Okra-leaf genotypes included CSIRO cultivars and a breeding-line Siokra L23, Siokra V-15, Siokra 1-4, Siokra S-101 and 89230-341-907. Silverleaf whitefly adults were counted weekly between June and August, from 10 plants per plot via leaf turn using the 5th main stem leaf from the terminal. Leaves were removed and whitefly eggs and nymphs were counted on 1.54-cm^2-leaf disks. CLCV disease symptoms were rated on a scale of 1 to 4. Results showed differences among cotton entries for whitefly infestation levels and virus disease symptoms. The okra-leaf breeding-line 89230-341-907 had the lowest CLCV geminivirus disease rating and fewer silverleaf whitefly adults, eggs and nymphs than normal leaf cultivars. The okra-leaf cultivar Siokra L23 had fewer whitefly adults and eggs than the normal leaf cultivars. Leaf trichome density was positively correlated to densities of whitefly adults, eggs and nymphs and was significantly lower for 89230-341-907 than for all other entries except Texas 121.

Introduction

Sweetpotato whiteflies, *Bemisia tabaci* Gennadius, were sporadic pests of cotton in southern California and Arizona (Gerling, 1967) until 1981 when severe economic losses to cotton occurred (Johnson *et al.*, 1982). In the Imperial Valley, CA, high sweetpotato whitefly densities in 1981 resulted in an estimated loss of $100 million to growers and consumers (Duffus and Flock, 1982) and $4 million loss to the cotton crop (Natwick *et al.*, 1984). The newly described silverleaf whitefly, *B. argentifolii* Bellows and Perring (Bellows *et al.*, 1994), caused severe economic losses to cotton and other crops in the United States in 1991. Conservative estimates exceeded $200 million and the loss to cotton producers in the Lower Rio Grande Valley of Texas exceeded $80 million (Henneberry, 1993). Losses to cotton in Arizona in 1992 exceeded $100 million (Henneberry, 1993). Whitefly-induced economic losses to cotton occur as a result of reduced cotton yield (Mound, 1965) and contamination of lint with honeydew and sooty moulds (Davidson *et al.*, 1994). The whitefly-transmitted cotton leaf crumple disease, caused by cotton leaf crumple geminivirus (CLCV), can also cause extensive reduction in yield (Dickson *et al.*, 1954; Duffus and Flock, 1982).

Chemical applications provide temporary control of this pest (e.g. Chu *et al.*, 1993; Natwick, 1993) but long-term solutions that offer economical and environmental advantages are needed. With the concept of integrated pest management, breeding and selection to develop insect-resistant cultivars is a goal that warrants increased attention (Painter, 1951; Khalifa and Gameel, 1983). The objectives of this study were to evaluate cotton lines for resistance to silverleaf whitefly and CLCV.

Material and Methods

Sixteen cotton cultivars and breeding-lines were sown into plots of a randomized block design experiment replicated four times and irrigated for germination on March 28 1997 at the UC Desert Research and Extension Centre, Holtville, CA. The seed companies, cultivars and breeding-lines from the U.S. included Deltapine Seed (DP 20, DP 50, DP 90, DP 5415, DP 5432, DP 5461, DP 9050 and DP 9057), ChemBred (CB 1135), South Texas Planting Seed Company (Texas 121) and USDA-ARS (C 118-2-93) and from Australia, CSIRO cultivars and breeding-lines included CS 50, Siokra L23, Siokra V-15, Siokra 1-4, Siokra S-101 and 89230-341-907. Individual plots measured 14 m in length with 4beds on 1-m centres. No insecticides were applied to the cotton plots.

Silverleaf whitefly adults were counted weekly from 19 June through 13 August, 1997 from 10 randomly

selected plants in each plot with the leaf turn method (Naranjo and Flint, 1995), using the 5th main stem leaf from the terminal. Ten 5th position main stem leaves down from the terminal were removed from randomly selected plants in each plot sampled weekly from 6 June to 19 August 1997. Silverleaf whitefly eggs, nymphs and parasitism by *Eretmocerus* spp., were counted on 1.54-cm^2-leaf disks cut from one side of the midrib of each leaf sampled. Percentage parasitism was calculated by dividing the total number of parasitised whitefly pupae by the total number of parasitised plus non-parasitised whitefly pupae, including exuvia left by emerged whitefly.

Disease symptom ratings for CLCV (1= no symptoms, 2= mild leaf crumpling, 3= moderate leaf crumpling and 4= severe leaf crumpling) were recorded on 8 and 12 August. Leaf and petiole samples from each plot were sent to the Plant Pathology Department at UC Davis to confirm the presence of CLCV by squash and dot blot hybridization with a general DNA probe, which detects the presence of whitefly transmitted geminiviruses (Gilbertson *et al.*, 1991).

Ginstar® EC Cotton Defoliant, (AgrEvo USA Company), was applied at 102-gm ai/ha on 19 August to defoliate the plants. Seed cotton was hand picked from 4-m sections of row in each plot on 3 September 1997. Weights of seed cotton sample were recorded and the samples were ginned at the USDA-ARS, Western Cotton Research Laboratory in Phoenix, AZ. Lint weights were recorded and percentages of lint turnout were calculated. Cotton lint samples were sent to the USDA-ARS Cotton Quality Research Station in Clemson, SC for analysis of stickiness using the thermodetector technique (Perkins and Brushwood, 1995) and will be analyzed for percent reducing sugars (Perkins, 1993).

Statistical analysis procedures for whitefly adults, eggs and nymphs and for yield data included Bartlett's test for homogeneity of variance, Tukey test for additivity and two-way analysis of variance for a randomized block design. For analysis, percentage of parasitism was transformed using the arcsine transformation. Where significance was found, means were separated using Student-Newman-Keuls' test mean comparison procedure. Analysis was performed using Michigan State University software MSTAT-C (MSTAT-C 1989).

Results and Discussion

Silverleaf whitefly nymphs were being parasitised by *Eretmocerus* spp. by mid-July. There were no differences among the cotton lines for seasonal means of percentage parasitism except that parasitism percentages of 20.1 and 22.6 for Texas 121 and DP 50 respectively, were significantly lower than Siokra S-101, Siokra 1-4, Siokra L23 and 89230-341-907 with percentages ranging from 37.1 to 39.1, $P \leq 0.05$ (Table 1).

Among the Australian cultivars, all but Siokra S-101 and Siokra V-15 had the lowest seasonal means for whitefly adults of all the entries in the trial. The cultivar CB 1135 had more whitefly adults than all other entries in the trial and Siokra 1-4, Siokra L23 and 89230-341-907 had seasonal means for adult whitefly that were significantly lower than all other entries in the trial, $P \leq 0.05$ (Table 1). The cultivars DP 5461 and DP 90 had more whitefly adults than CS 50 and DP 5432.

The cultivar CB 1135 had significantly more whitefly eggs than all other entries and Siokra L23 and 89230-341-907 had significantly lower seasonal means for whitefly eggs than all other entries in the trial with the exception of Siokra 1-4 and Siokra S-101, $P \leq 0.05$ (Table 1). Cultivar DP 5461 had significantly more eggs than Siokra S-101, Siokra 1-4, Siokra L23 and 89230-341-907.

More whitefly nymphs were observed on CB 1135 than all other entries and the breeding-line 89230-341-907 had a seasonal mean value for whitefly nymphs that was lower than all other cultivars, $P \leq 0.05$ (Table 1). DP 5461 and the USDA-ARS breeding-line C118-2-93 had significantly more nymphs than DP 20, Siokra L23 and Siokra 1-4 and Texas 121 had significantly more nymphs than DP 20 and Siokra L23.

Leaf trichome density for the breeding-line 89230-341-907 (0.18/cm^2) and the cultivar Texas 121 (0.35/cm^2) were lower than all other entries (Table 1). Leaf trichome densities were positively correlated to densities of whitefly adults ($r=0.318$), egg density ($r=0.577$) and nymph density ($r=0.621$), $P \leq 0.05$ (Table 2).

There were no significant differences among the cotton cultivars for seed cotton yield ($F=1.856$, $P=0.056$ and CV=14.02%; ANOVA). The ANOVA for lint yield was significant with $F=1.923$, $P=0.046$ and CV=13.96%, but there were no mean separations via SNKMRT, $P \leq 0.05$ (Table 3). Seed cotton yields ranged from 4342.4 kg/ha (DP 9057) to 3086.4 kg/ha (Texas121) and lint yields ranged from 1737.0 kg/ha (DPL 9057) to 1219.9 kg/ha (Texas121). Lint turnout percent ranged from 43.25 (CS 50), significantly higher than all other entries, to 36.50 (DP 50), significantly lower than all other entries.

Squash and dot blot analysis of leaf and petiole samples, collected in August, verified that cotton plants in the plots were infected with a whitefly-transmitted geminivirus (DNA sequencing of a polymerase chain reaction amplified fragment from an infected plant confirmed that the geminivirus was CLCV). On 8 August, C118-2-93 had a CLCV rating of 2.38 which was greater than the CLCV ratings for Siokra 1-4, DP 5415, DP 5461, Siokra L23 and 89230-341-907. On 12 August, Texas 121 and Siokra S-101 each had CLCV ratings of 3.00 which were greater

than the CLCV ratings for DP 5415, DP 5461, Siokra L23 and 89230-341-907, P≤ 0.05 (Table 4).

Thermodetector ratings ranged from light stickiness to moderate stickiness. The ANOVA for the thermodetector ratings was significant with F=2.311, P=0.015 and CV=28.40%, but there were no mean separations via SNKMRT, P≤0.05 (Table 4). The breeding-line 89230-341-907 had the lowest densities of and the lowest thermodetector rating, but overall, thermodetector ratings were poorly correlated to whitefly population densities (Table 5).

Differences in cotton cultivar susceptibility to whitefly colonization have been reported for different cotton species (Natwick *et al.*, 1995), differences in leaf pubescence (Norman and Sparks, 1997) and leaf shape (Butler and Wilson, 1984). Smooth leaf or semi-smooth leaf cotton cultivars and breeding-lines were used in this study. Some differences in whitefly densities were related to leaf pubescence. Butler *et al.* (1991) suggested that glabrous, small leaf area and open canopy cottons and gossypol content were important traits that should be investigated for developing whitefly resistant cottons. The cultivar Siokra L23 and breeding-lines 89230-341-907 may have some heritable traits for whitefly resistance that should be investigated for developing whitefly-resistant cotton cultivars.

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Table 1. Silverleaf whitefly seasonal means for adults per leaf and eggs, nymphs and trichomes per cm2 of leaf and percent parasitism of whitefly nymphs by *Eretmocerus* spp., Holtville, 1997.

Cultivar	Adults	Eggs	Nymphs[a]	Trichomes/ cm2 [a]	% Parasitism[b]
CB 1135	7.63 a	4.73 a	4.84 a	4.49 a	33.2 ab
DP 5461	6.06 b	2.91 bc	2.19 b	1.58 bc	32.8 ab
DP 90	5.50 bc	2.03 bcd	1.26 bcdefghi	1.67 bc	31.6 ab
Siokra S-101	5.16 bcd	1.42 de	1.33 bcdefg	2.41 abc	37.1 a
DP 5415	5.13 bcd	2.08 bcd	1.70 bcde	1.66 bc	33.0 ab
C118-2-93	5.05 bcd	2.47 bcd	2.09 b	2.17 abc	31.8 ab
Texas 121	4.55 cd	2.22 bcd	1.79 bc	0.35 d	20.1 b
DP 50	4.28 cd	2.17 bcd	1.33 bcdefgh	2.62 abc	22.6 b
Siokra V-15	4.22 cd	2.11 bcd	1.15 bcdef	3.13 ab	33.2 ab
DP 20	4.22 cd	1.76 cd	0.90 defghi	1.45 bc	30.0 ab
DP 9057	4.16 cd	1.95 bcd	1.06 cdefghi	1.77 bc	32.3 ab
CS 50	4.02 d	3.25 b	1.96 b	1.76 bc	25.4 ab
DP 5432	4.00 d	2.44 bcd	1.72 bcd	1.01 c	29.8 ab
Siokra 1-4	2.48 e	1.41 de	1.04 cdefghi	2.75 ab	37.4 a
Siokra L23	2.01 e	0.68 e	0.67 ghi	1.42 bc	37.8 a
89230-341-907	1.92 e	0.54 e	0.30 j	0.18 d	39.1 a

[a] Log transformed data used in analysis; reverse transformed means reported.
[b] Arc-sine transformation for percent data used in analysis of percentage of parasitism.
Mean separations within columns by Student-Newman-Keuls' Test, P≤0.05.

Table 2. Relationship between silverleaf whitefly colonization and density of trichomes of sixteen cotton cultivars in Holtville, CA in 1997.

Whitefly stage	Correlation coefficient	Intercept	Slope	Standard error	Student's t value	Probability
Adults	0.318	3.721	0.357	0.135	2.645	0.010
Eggs	0.577	1.177	0.504	0.091	5.557	<0.001
Nymphs	0.621	0.668	0.513	0.082	6.241	<0.001

Table 3. Seed cotton and lint as kilograms per hectare and percentage of lint turnout for cotton cultivars and breeding-lines at the University of California desert research and extension centre, Holtville, CA, 1997.

Cultivar	Kg seed cotton/ha	Kg lint/ha	Lint % turnout
DP 9057	4342.4 a	1737.0 a	40.00 cde
DP 5415	4277.1 a	1745.0 a	40.75 bcd
Siokra L23	3901.7 a	1609.6 a	41.25 bc
89230-341-907	3823.3 a	1442.9 a	37.75 g
C118-2-93	3821.6 a	1471.5 a	38.50 fg
DP 20	3752.0 a	1453.5 a	38.75 efg
Siokra V-15	3746.0 a	1479.6 a	39.50 def
DP 50	3744.5 a	1364.6 a	36.50 h
CB 1135	3732.5 a	1502.2 a	40.25 cd
DP 5432	3639.9 a	1464.6 a	40.25 cd
Siokra 1-4	3557.1 a	1468.2 a	41.25 bc
DP 5461	3441.8 a	1360.9 a	39.50 def
CS 50	3376.6 a	1457.3 a	43.25 a
DP 90	3371.4 a	1340.9 a	39.75 def
Siokra S-101	3106.0 a	1294.2 a	41.75 b
Texas 121	3086.4 a	1219.9 a	39.50 def

Mean separations within columns by Student-Newman-Keuls' Test, P≤0.05.

Table 4. Cotton leaf crumple virus ratings[a] and thermodetector ratings[b], Holtville, CA, 1997.

Cultivar	8 Aug. CLCV rating	12 Aug. CLCV rating	Thermodetector rating
C118-2-93	2.38 a	2.75 abc	14.00 a
Texas 121	2.25 ab	3.00 a	19.00 a
CB 1135	2.25 ab	2.88 ab	18.50 a
CS 50	2.13 abc	2.50 abcd	9.50 a
DP 5432	2.13 abc	2.50 abcd	14.25 a
DP 50	2.13 abc	2.25 abcd	17.25 a
Siokra S-101	2.00 abc	3.00 a	18.75 a
DP 90	2.00 abc	2.50 abcd	15.75 a
DP 20	2.00 abc	2.50 abcd	13.25 a
Siokra V-15	2.00 abc	2.38 abcd	11.75 a
DP 9057	1.88 abc	2.25 abcd	14.25 a
DP 5461	1.75 bc	2.00 bcd	16.00 a
Siokra L23	1.75 bc	1.88 cd	11.75 a
Siokra 1-4	1.63 c	2.38 abcd	13.25 a
DP 5415	1.63 c	1.88 cd	13.25 a
89230-341-907	1.63 c	1.75 d	8.75 a

[a] 1= no symptoms, 2= mild leaf crumpling, 3= moderate leaf crumpling and 4= severe leaf crumpling.
[b] Thermodetector spots are used to rate stickiness as follows: less than 5 = non-sticky, 5 to 14 = light stickiness, 15 to 24 = moderate stickiness and above 24 = heavy stickiness.
Mean separations within columns by Student-Newman-Keuls' Test, P≤0.05.

Table 5. Relationship between lint stickiness, via thermodetector ratings and silverleaf whitefly population density, Holtville, CA in 1997.

Whitefly stage	Correlation coefficient	Intercept	Slope	Standard error	Student's t value	Probability
Adults	0.421	7.774	1.490	0.421	3.652	0.001
Eggs	0.234	12.062	1.061	0.560	1.894	0.063
Nymphs	0.283	12.071	1.361	0.587	2.319	0.024