Bemisia argentifolii Adult, Nymph and Egg Densities and Egg Distribution on Selected Upland Cottons[1]

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Abstract Five upland cotton, *Gossypium hirsutum* L., cultivars, Deltapine (DPL) 50, 5415 and 5432, Fibermax 832 and Siokra L23, were studied in relation to silverleaf whitefly, *Bemisia argentifolii* Bellows and Perring, oviposition and colonization. Deltapine 5415 and 5432 had the highest numbers of eggs and nymphs and Siokra L-23 the lowest. Siokra L-23 also had the lowest number of adults compared to the other four cultivars. Over 75% of eggs were oviposited on leaf surfaces between veins and 23% were oviposited in veins that were ≤4 cells wide. Few eggs were found on veins that were five or more cells wide. No eggs were inserted into leaf stomata.

Key Words Silverleaf whitefly, egg distribution, upland cotton cultivars

Oviposition of whitefly, *Bemisia argentifolii* Bellows and Perring (= *B. tabaci* strain B), has been used as an indicator of adult behavior (Chu et al. 1995, Simmons 1994), host plant preferences (Blua et al. 1995, Gergis 1994, Yee and Toscano 1996), host plant resistance (Decanini et al. 1995, Lambert et al. 1997), leaf age preference (Byrne and Draeger 1989), and effectiveness of insecticide treatments (Liu and Stansly 1995, Toscano et al. 1997). *Bemisia argentifolii* oviposition has been reported to be affected by various leaf characteristics including trichomes (Heinz and Zalom 1995), leaf age, position on the stems (Liu and Stansly 1995), leaf moisture (Skinner 1996a), temperature (Skinner 1996b), and nutritional condition (Bentz et al. 1995, Skinner 1996a).

Yellow-green leaf color has been reported to attract whitefly adults to potential host plants (Lenteren and Noldus 1990). After landing on cotton leaves, *B. argentifolii* adult females immediately begin stylet probing activities on leaf surfaces (Chu et al. unpublished data). Oviposition may occur before stylets penetrate into phloem tissue (Walker 1987). *Bemisia argentifolii* egg distributions are aggregated (Tonhasca et al. 1994) and, in cotton, eggs generally are oviposited on or within approximately 30 µm of vascular bundle-associated elongated epidermal cells (Cohen et al. 1996b).

We report here on the densities of *B. argentifolii* adults, nymphs, and eggs on cotton leaves. *Bemisia argentifolii* egg distribution and mechanisms of ovipositional

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site selections in relation to underleaf surface features of selected cotton cultivars are also examined.

Materials and Methods

Adult, egg and nymph densities on cotton leaves in the field. Five cotton cultivars were selected from those grown at the University of California Desert Research and Extension Center at Holtville, CA in 1998. Three were normal-leaf Deltapine (DPL) cultivars, DPL 50, 5415, and 5432, and two were Australian okra-leaf cultivars, Fibermax 832 and Siokra L23. A randomized complete block experimental design with four replicates was used; plots measured 14 m × 8 m with a 1-m row spacing. Cotton seeds were planted and irrigated for germination on 20 March. No insecticides were applied to the plots. On each sampling date, *B. argentifolii* adults were counted on one fifth main node leaf below each terminal on five plants in each plot (Naranjo and Flint 1995). An additional fifth main node leaf was selected from each of five plants for counting numbers of eggs and nymphs. Nymph and egg counts were made on two 1.25-cm^2 leaf disks from each leaf with the aid of a microscope (Naranjo and Flint 1994). Sampling dates were 10 and 17 June, 7, 14, 21 and 28 July, and 4 and 12 August.

Egg and nymph densities on cotton leaves after fixation in a laboratory. Additional fifth main stem node leaves were randomly selected from each field plot. For each variety, three replicated plots were sampled, one leaf from each plot. Leaves including petioles were detached from plants at dawn on 5 August, fitted into water-filled plastic floral tubes and shipped overnight under cool conditions to the North Dakota State University Electron Microscopy Center at Fargo, ND, for egg and nymph counts. Fifteen photographs of the underleaf surface (Naranjo and Flint 1994) of each variety were obtained at 50× magnification using an Olympus SZH dissecting microscope and Kodak Elitechrome slide film (ASA 200). This included five photographs (37.2 mm^2 leaf area each) of each leaf for each of three replicates. The number of eggs and nymphs were determined from projected images of 13 to 16 leaf area for each variety. Each slide was examined and counted at least twice. After each leaf was photographed, segments of leaves were prepared for examining egg distribution pattern on leaves.

Egg distribution on cotton leaves. One leaf segment of approximately 1 cm^2 in area was randomly selected from the center portions of each of three leaves of each variety and placed in 2.5% glutaraldehyde in Millonig's phosphate buffer (pH 7.4) for 3 h at room temperature (22°C), dehydrated in an ethanol series (30%, 50%, 70%, 90%, 95% and three changes of 100%) for 30 min in each solution, dissected and dried in a Tousimis Autosamdri-810 critical point drier using CO_2 as a transitional fluid. Dried leaf samples were mounted on aluminum stubs using silver paint and coated with gold/palladium in a Balzers SCD030 sputter coater. Four replicates of 4 mm^2 leaf area from each prepared 1-cm^2 leaf segment were examined for egg distribution on the underleaf surface using a JEOL JSM6300 Scanning Electron Microscope. Positions of the eggs were classified as being inserted into leaf veins of $\leq$4-cells or $\geq$5-cells wide (Fig. 1b and 1c); between veins, or into stomata (Fig. 1a). Eggs were classified as occurring in clusters when at least 5 eggs were oviposited $\leq$20 μm distance from each other; otherwise, they were classified as occurring singly.

Area measurements for areoles and veins. Seventh main stem node leaves below terminals from three plants were randomly sampled from two cultivars, DPL

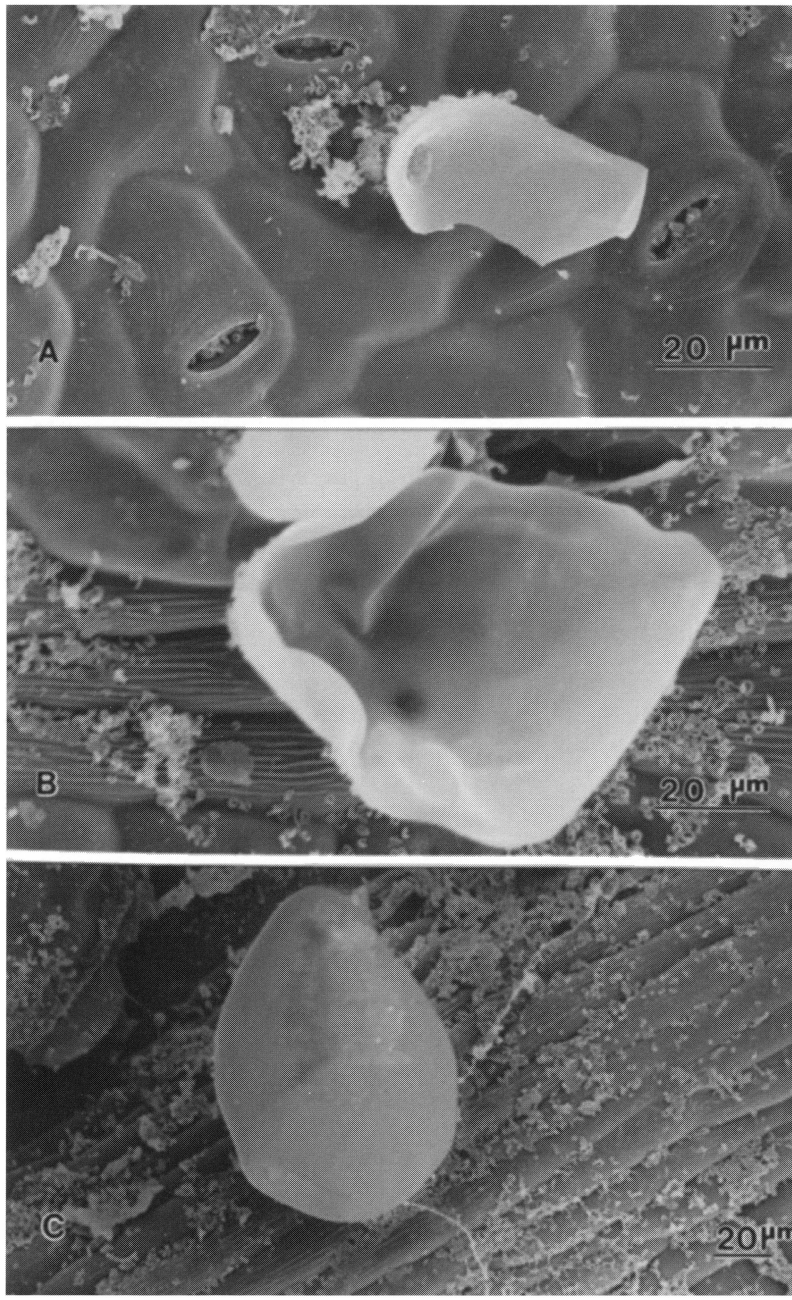

Fig. 1. Scanning electron micrograph of *Bemisia argentifolii* eggs laid in various locations on underleaf surface of cotton: (A) a ruptured egg laid between veins, (B) a ruptured egg on 3-cell wide vein, and (C) an intact egg laid on >5-cell wide vein (magnified 800×).

Table 1. Mean (±SEM) numbers of silverleaf whitefly adult, eggs and nymph densities on underleaf surfaces of five cotton cultivars from field and laboratory at Holtville, CA, 1998

Variety	Normal (N)-/ Okra (O)-leaf	Field measurement			Laboratory measurement	
		Adults/leaf	Eggs/cm^2	Nymphs/cm^2	Eggs/cm^2	Nymphs/cm^2
DPL 5415	N	65.4 ± 12.5 a	196.0 ± 36.5 a	218.4 ± 44.9 a	105.9 ± 18.4 b	70.5 ± 12.5 a
DPL 5432	N	73.1 ± 13.8 a	158.8 ± 26.1 ab	127.4 ± 26.9 b	216.7 ± 22.9 a	46.3 ± 7.7 b
DPL 50	N	65.4 ± 11.4 a	121.5 ± 24.2 bc	90.1 ± 15.0 b	117.5 ± 11.8 b	25.7 ± 4.7 bc
Fibermax 832	O	66.9 ± 12.3 a	129.5 ± 18.2 bc	132.0 ± 26.1 b	124.8 ± 13.7 b	33.1 ± 5.0 bc
Siokra L23	O	45.2 ± 10.9 b	84.8 ± 15.7 c	135.5 ± 32.4 b	28.4 ± 4.8 c	14.3 ± 3.3 c

Means (±SEM) within a column followed by same letters are not significantly different (Student-Neuman-Keul's Multiple Range Test, $P = 0.05$).

Table 2. Mean numbers (±SEM) of eggs and percentages of *Bemisia argentifolii* egg positioning on underleaf surfaces on five cotton cultivar at Holtville, CA, 1998

	% Eggs in clusters			
	Inserted in veins			
Cultivar*	≥5-cell wide	≤4-cell wide	Between veins	Inserted in stomata
DPL 5415	0.0 ± 0.0 (0.0) h**	2.3 ± 1.9 (5.1) e-h	11.5 ± 5.4 (19.3) cde	0.0 ± 0.0 (0.0) h
DPL 5432	0.0 ± 0.0 (0.0) h	5.3 ± 1.9 (8.6) d-h	8.0 ± 3.1 (13.2) d-g	0.0 ± 0.0 (0.0) h
DPL 50	0.3 ± 0.3 (0.6) g-h	1.8 ± 1.0 (3.7) e-h	20.5 ± 4.2 (42.4) bc	0.0 ± 0.0 (0.0) h
Fibermax 832	0.5 ± 0.3 (0.7) g-h	3.8 ± 1.7 (5.2) d-h	14.0 ± 5.2 (19.9) cd	0.0 ± 0.0 (0.0) h
Siokra L-23	0.0 ± 0.0 (0.0) h	0.8 ± 0.8 (4.7) gh	1.3 ± 1.3 (7.8) fgh	0.0 ± 0.0 (0.0) h
Average	0.2 ± 0.1 (0.3) D	2.8 ± 0.7 (5.9) C	11.1 ± 2.2 (23.3) B	0.0 ± 0.0 (0.0) D

	% Eggs singly				
	Inserted in veins				
Cultivar*	≥5-cell wide	≤4-cell wide	Between veins	Inserted in stomata	Total eggs counted
DPL 5415	0.0 ± 0.0 (0.0) h	8.5 ± 2.6 (14.7) def	34.8 ± 6.5 (60.9) a	0.0 ± 0.0 (0.0) h	57.1 ± 6.3 A
DPL 5432	3.8 ± 1.3 (6.8) d-h	12.3 ± 3.7 (20.3) cd	29.0 ± 4.4 (51.1) ab	0.0 ± 0.0 (0.0) h	58.3 ± 3.3 A
DPL 50	0.5 ± 0.5 (1.2) gh	5.3 ± 1.1 (11.0) d-h	19.8 ± 2.5 (41.1) bc	0.0 ± 0.0 (0.0) h	48.0 ± 2.5 A
Fibermax 832	0.8 ± 0.3 (1.5) gh	9.8 ± 3.0 (17.4) cde	32.5 ± 4.1 (55.3) ab	0.0 ± 0.0 (0.0) h	61.3 ± 9.9 A
Siokra L-23	0.0 ± 0.0 (0.0) h	3.8 ± 1.0 (34.2) d-h	7.8 ± 2.5 (53.3) d-g	0.0 ± 0.0 (0.0) h	13.5 ± 2.2 B
Average	1.0 ± 0.4 (2.1) D	7.9 ± 1.2 (16.6) B	24.8 ± 2.8 (52.1) A	0.0 ± 0.0 (0.0) D	47.6 ± 4.6

* Siokra L-23 and Fibermax 832 are okra-leaf cottons and all Deltapines cultivars are normal-leaf cottons.

** Mean number ±SEM (percentage) of four 4 mm^2 leaf areas in five cultivars and eight oviposition sites or the total eggs in the column or average of eight oviposition sites over the five cultivars not followed by the same letters are significantly different (Student-Neuman-Keul's Multiple Range Test, $P = 0.05$).

5415 and Stoneville 474 at Maricopa Agricultural Center, AZ, at dawn on 6 August 1998, and shipped to the North Dakota State University Electron Microscope Center, ND, for processing. Leaf segments of approximately 1 cm^2 in area were randomly sampled from center portions of leaf blades and placed in clearing agents chloroform-methanol to incubate leaf segments for 1 h at room temperature (23°C). The cleared leaves were then stained in aqueous 1% safranin O and destained as required in acidified ethyl alcohol. Stained leaf segments were examined and photographed using an Olympus BH-2 light microscope and mounted on 35-mm slides. Images of leaves on each slide were 37.2 mm^2 of leaf area. Images of cleared leaves were captured using an image scanner and imported into a computer using Adobe Photoshop® (Version 5.0 Adobe System Inc., San Jose, CA) and the TWAIN_32 cross-platform interface and saved as Tagged-Image File Format files. Image analysis software (OPTIMAS™, Media Cybernatics, Silver Spring, MD) was used to calibrate, obtain measures and format data. Areas of aeroles and of vascular bundles, including bundle sheath extensions that surrounded xylem and phloem vessels, were measured. An areole is defined as an area surrounded by vascular bundles in a plane of leaf tissue. Three replicated areas each from a sampled leaf were measured from each cultivar.

Data analyses. Data of field counts of whitefly densities were first pooled over 10 leaf disks each plot for each sampling date and then pooled over eight dates. A single randomized complete block ANOVA (MSTAT-C 1989) was performed. Data of laboratory counts of whitefly densities over 15 slides, areas of areoles and vascular bundles and vascular bundle area% over three replicates, and numbers of egg distributions (square root [x + 1] transformation) on leaves were averaged over four leaf segments were analyzed with completely randomized ANOVA. Means were separated with Student-Neuman-Keul's Multiple Range Test, $P = 0.05$. Percentages of each egg position were calculated based on 100% for total number of eggs counted.

Results and Discussion

Whitefly adult, egg, and nymph densities on cotton leaves in the field. Seasonal mean numbers of adults/leaf were significantly lower on Siokra L23 cotton (45.2/leaf) compared to Fibermax 832 and the other three Deltapine cultivars (range = 65.4 to 73.1 adults/leaf) (Table 1). Siokra L23 had the lowest number of eggs (84.8/cm^2 leaf disk) and DPL5415 and DPL 5432 had the highest numbers of eggs (158.8 to 196.0/cm^2 leaf disk, respectively). Siokra L23 also had significantly fewer nymphs (135.5/cm^2 leaf disk) compared with DPL 5415. Laboratory counts of the numbers of eggs and nymphs/cm^2 leaf disks from slide films after fixation, dehydration and staining showed similar trends when compared with the field counts.

Egg distribution on cotton leaves. Most eggs were oviposited singly (33.7 or 70.7%). Single eggs were found between veins (24.8 or 52.0%), in veins ≤4-cells wide (7.9 or 16.6%) and in veins ≥5-cells wide (1.0 or 2.1%) (Table 2). For eggs oviposited in clusters (14.0 or 29.3%), most (11.0 or 23.1%) were oviposited between veins, with some laid in veins ≤4-cells wide (2.8 or 5.5%) and in veins ≥5-cells wide (0.2 or 0.3%). For all eggs, 35.8 (75.1%) were oviposited between veins and 11.9 (24.9%) in veins. None of the eggs oviposited either singly or in clusters were inserted into leaf stomata. There were varietal differences with respect to oviposition sites between veins, but not in veins. Deltapine 5415 and Siokra L-23 had the highest and the least numbers of eggs oviposited singly in veins, respectively. Siokra L-23 also

Table 3. Mean (± SEM) areas of areoles and veins in leaf blades of field grown DPL 5415 and ST 474 at Maricopa, AZ, 1998

	Area (mm^2)		
Cultivar	Areole	Vein	% Vein
DPL 5415	0.758 ± 0.090	0.113 ± 0.018	14.7 ± 0.8
ST 474	0.793 ± 0.048	0.111 ± 0.012	14.2 ± 1.9

Means (± SEM) of leaf number 7 within a column are not significantly different (F – test).

had the least number of eggs oviposited in clusters compared with all the other cultivars tested.

Area measurements for areoles and veins. Average areole areas were 0.758 and 0.793 mm^2 for DPL 5415 and ST 474, respectively, and vascular bundles 0.113 and 0.111 mm^2. For both cultivars, the vein area in a plane of leaf tissue was approximately 14%. A large number of small vascular bundles was seen after cellular contents were cleared exposing the vascular bundle networks in the leaf tissues.

Cotton is a preferred host for *B. argentifolii* when compared with broccoli and lettuce but is less preferred to melons (Chu et al. 1995). Susceptibility to *B. argentifolii* colonization was reported greater for pima than for upland cottons (Natwick et al. 1995), and differences in susceptibility also have been reported among upland cultivars (Chu et al. 1998a). Differences in the seasonal mean numbers of adults/leaf and eggs and nymphs/cm^2 of leaf disk for the Deltapine cultivars in this study confirmed our earlier observations (Chu et al. 1998b). Siokra L-23 had fewer adults/leaf and fewer eggs and nymphs/cm^2 leaf disk compared to the other four cultivars (Table 1). These differences appear to be related to Siokra L23's okra-leaf shape and depths of vascular bundles within leaves (Chu et al. 1999). Reduced whitefly (Sippell et al. 1993) and mite (Wilson 1994) infestations have been reported on okra-leaf shaped cottons compared with normal-leaf cultivars. The reason appeared to be the reduced leaf area for oviposition and protection sites. In contrast, reduction in nymph (Flint and Parks 1990) and adult (Butler and Wilson 1984) populations on okra-leaf shaped cotton WC-12NL compared to normal-leaf shape DPL 61 were inconclusive. Thus, in addition to the okra-leaf characteristic, our results suggest that many other factors may influence whitefly resistance and need to be examined. For example, we found that *B. argentifolii* adult and nymph densities were negatively associated with depth of the nearest vascular bundles from underleaf cotton surfaces in Deltapine cultivars ($r = -0.79$ and -0.71, respectively, $n = 8$) (Chu et al. 1998b) or Deltapine and Australian cultivars ($r = 0.81$ and 0.61, respectively, $n = 13$) (Chu et al. 1999).

Cohen et al. (1996b) suggested that *B. argentifolii* adults may use lamina trichomes and elongated cells as cues for oviposition because eggs were generally oviposited near elongated cells associated with leaf veins. In our studies, approximately 75% of the eggs on average were laid between leaf veins and 25% were oviposited in veins on the underleaf surfaces. In most cases, whitefly adult females lay eggs while feeding (Walker 1997). Our studies did not clearly indicate that adults used trichomes or elongated cells as cues for oviposition. The area of veins (14%) in leaves is much less than the area between veins (86%) which may explain the high percentage of eggs oviposited between veins.

Egg pedicels of several whitefly species have been reported to be inserted into host plant stomata. *Bemisia tabaci* (Gennadius) and *Trialeurodes vaporariorum* (Westwood) insert their egg pedicels into non-stomatal cells (Paulson and Beardsley 1985). This also appears to be the case for *B. argentifolii* (Lauritsen and Paulson 1998).

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